A COMPARISON OF VIRUS-INDUCED RABBIT TUMORS WITH THE TUMORS OF UNKNOWN CAUSE ELICITED BY TARRING

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

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

Plates 20 to 27

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The agents called "carcinogenic" act by inducing a protracted tissue disturbance, on the basis of which discrete tumors of unknown cause arise. We have encountered many such growths during experiments on the action of tar to alter rabbit skin in such a way that cancers develop (1) after infection of it with a virus which causes only benign papillomas ordinarily (2). A knowledge of the tar tumors proved essential to appraisal of this phenomenon and hence their study was undertaken. It disclosed the fact that in rabbits such as we employed the benign tumors elicited by tar were of two sorts only, and these so definite in type as to imply the action of specific causes. The commonest sort were papillomas differing distinctively from virus papillomas in certain cytological details but closely resembling them in cellular derivation, mode of development, general morphology, behavior, and fate. The results of a point by point comparison made it plain that the unknown cause of the tar papillomas had neoplastic effects like those due to an authentic virus. The present paper is concerned with all of these subjects.

Material and Methods

Our rabbits, procured from New York dealers, were hybrids of the common, brown-gray (agouti) type. The tar came from the Ostergasfabrik of Amsterdam, and was the generous gift of Dr. Landsteiner. It was smeared on the inside of the ears twice a week, for from 2 to 4 months in most instances, with stripping of the accumulated layer at every third tarring. Over one hundred animals were tarred, mostly as controls in experiments with virus, and some were kept for many later months and sometimes tarred again.

Occasionally the tarring caused tumors to appear within the first month, and they did so within 3 months in more than half of the animals. They were studied by preference soon after they arose, when only a few millimeters broad, before they had undergone the secondary alterations in structure that are frequent when tarring is kept up. Numerous biopsies were made, with a sharp cork-borer. All the material was fixed at once in acid Zenker solution, since in specimens from animals dead only a few hours the finer cytological details underwent change. The stains were methylene blue and eosin.

The Kinds of Tar Tumors

Tar was the first substance utilized in purposeful carcinogenesis, and it was applied to rabbits; but attention soon shifted to mice as a more favorable material. The large literature has been several times reviewed (3), and we have recently summed up the gross effects of the tar employed in our animals (1). It caused benign tumors early and frequently, yet gave rise to cancer only after many months and then but seldom.

Microscopically the tumors fell into four well defined categories:

1. Common Papillomas.—Yamagiwa and Ichikawa (4) termed these growths stalked and broad-based folliculo-adenomas, and noted that the latter were the more aggressive, with carcinomas sometimes originating from them. Both sorts were very frequent in our animals (Table I); and they were found to have essentially the same cytological characters and to grade into each other. They were made up of stratified squamous epithelium, thick and obviously abnormal yet keratinizing in an orderly way, supported upon narrow connective tissue cores or fingers, which occasionally branched (Figs. 5, 7, 40). They first appeared as small, subepidermal mounds which later became discrete, superficial growths of conical, cauliflower, or onion shape, or else,—when maceration had occurred beneath the tar,—raw, fimbriated discs or hassocks. The keratinized epithelium was tenacious, often building high. When tarring was kept up the aggressive growths extended sideways and often downwards in blunt epithelial foldings and tongues, with result that they had a broad base. The shape of indolent ones depended on the changes occurring in the underlying connective tissue. When it proliferated greatly they sometimes became tangential, fleshy spheres, while through its secondary contraction they often became constricted at the base (Fig. 1) or stalked or pedunculated. Persisting broad-based growths eventually underwent similar changes, save in the occasional instances in which they became malignant. Papillomas that had been many times tarred often reached a diameter of several centimeters, and then as a rule they consisted almost entirely of connective tissue. Sometimes their weight gradually pulled them out into pendulum form after tarring was left off, and not a few were torn away while others contracted into mere tabs as result of sclerosis.

If tarring was left off soon after the papillomas appeared, most of them vanished rapidly. Those that were left dried down, often to mere scabs until reconstituted from beneath, and many lost the fleshy character, becoming horns or dry cauliflowers. They tended to persist in proportion to the general changes induced in the skin, and when these had been great, new papillomas often arose. If tarring had been kept up for many months, until they were numerous and crowded, maceration and bacterial infection often persisted later, furthering their enlargement, and occasionally the aural orifice became blocked with pultaceous matter and the animal died of sepsis. Large papillomas tended to persist because their core of sclerotic connective tissue could not be resolved; but sometimes their papillomatous epithelium was ultimately replaced by smooth, non-neoplastic epidermis.

TABLE I

Relative Frequency of the Benign Tar Tumors: Dependence of the Carcinoid Form upon Tarring

Group	Number of rabbits	Period of tarring	Further period after tarring	Papillomas	Transitional growths	Apparent carcinomas (carcinoids)	Frill horns	Total growths
		days	*days*	*number*	*number*	*number*	*number*	*number*
A	18	54–118	2–40	50	10	64	5	129
B	18	61–121	57–259	93	2	0	13	108

The growths tabulated came from groups of animals tarred for the same general length of time, but differing in that the tumors were not procured from one group until long after tarring had been stopped. Only growths which were examined microscopically are listed, and in some animals they formed but a small proportion of the total number. The basis of the classification is given in the text. The frill horns could be readily identified in the gross, and every one seen was taken for section. Carcinoids also were often recognizable as such, and a greater proportion of them were examined than of papillomas. It follows that frill horns were actually much rarer than the figures indicate, and carcinoids somewhat less common.

The papillomas have often been pictured. The several hundred that we have studied microscopically differed not a little in general pattern. Some were completely superficial and regular, with widely spaced papillae and dry keratin between; others had many crowded peaks; others extended down in broad tongues that keratinized secondarily, often becoming cystic; while others still broke up along the base into what appeared to be anaplastic, squamous cell carcinomas. Yet despite this diversity the cells of all, so long as they remained benign papillomas, manifested identical *qualitative* alterations (Figs. 33 and 35), and whenever differentiation and keratinization took place it was in the same way. The minute histological findings will be described further on.

2. Frill Horns.—Though possessed of a distinctive morphology, these growths have not heretofore been reported. They were seldom more than 3 mm. across at the base, yet were readily recognizable in the gross because they built up into narrow, cylindrical or strap-shaped, curved or twisted horns (Figs. 8, 9), often 2 to 4 cm. high, of the same diameter everywhere, usually cream-colored but sometimes light or dark gray, and always dry nearly to the skin level. Here was a slightly bulging, fleshy collar covered with epidermis stretched smooth.

The frill horns arose at the same time as the papillomas, but were infrequent (Table I). Usually there were several when any. Though appearing wholly superficial, they burrowed slowly, and ineffectually, their epithelium extending downwards in a jagged, crescentic frill (Figs. 10, 11), which advanced so gradually as to be effectively barred from the cartilage by reactive connective tissue proliferation. Very occasionally a fold in the frill mimicked a papillomatous finger. Its living layer was not so thick as in papillomas, and stained deeper with methylene blue. The individual cells were irregularly polygonal, with birdseye nuclei, and they became larger when differentiating, and keratinized abruptly without preliminary granulation or flattening (Fig. 12). The keratinized material stained pink or red with the eosin and methylene blue stain, whereas that of tar and virus papillomas failed to color or took on a faint blue; and the nuclei of the cells newly dead stippled the keratinized material with dark blue, oval dots, which faded later. In the gross the horns were close-textured, horizontally laminated, sometimes with an ill-defined, vertical striation, and microscopically their keratinized material appeared dense, showing none of the loose, reticular pattern, indicative of old cell boundaries, which is visible in papillomas. The connective tissue underneath the growths, like that under papillomas, showed only chronic inflammatory changes, with a scattering of macrophages and lymphocytes. Continued tarring had little effect to render the epithelium disorderly, and when it was discontinued the horn nearly always went on growing, unlike most papillomas, and sometimes new ones appeared (compare frequency in groups A and B of Table I). Retrogression was noted only once.

3. Carcinoids.—A large proportion of the tumors appearing early had the morphology of squamous cell carcinomas (Figs. 13, 14, 16, 18), yet when tarring was left off they vanished or assumed a benign form (Figs. 17, 19). These were the growths generally termed carcinoids (5). Often they were markedly invasive at first (Fig. 15), their cells straggling down individually, or penetrating in narrow strands through a reactive connective tissue which was sometimes mucoid like that elicited by many squamous cell carcinomas; and not infrequently they extended through lacunae in the cartilage to the outside of the ear, forming mounds there which ulcerated. They could usually be recognized in the gross, being raised, raw, ragged discs with infiltrating edges (though papillomas occasionally assume this form), or else raised, discoid ulcers as much as 1.5 to 2 cm. in diameter. Yet even when proliferating within lymphatics (Figs. 15, 18) they never metastasized; and though tarring was kept up they did not continue to grow destructively but eventually took on gross forms like those of old papillomas, or else retrogressed.

Repeated biopsies showed that the supporting connective tissue had proliferated actively, walling off the growths, and that in proportion as this happened their epithelium ceased to invade, underwent differentiation, and in most instances became papillomatous (Figs. 16, 17), though cell islands of carcinomatous aspect occasionally persisted. Frequently the deeper epithelium rounded up into cysts filled with keratinized material (Figs. 18, 19) and lined with a stratified squamous layer devoid of neoplastic features; but in other instances all the deep cells keratinized, died, and were eventually resorbed. Those carcinoids which disappeared did so in these ways, save in an unique instance of a growth dying *en masse* without differentiation, as if from some intercurrent malady. Carcinoids which appeared early, before the tarring had effected great cutaneous changes, generally vanished soon after it was left off, whereas when the skin had undergone much alteration they often persisted, either as papillomas or cysts.

The earlier observers, conceiving that the carcinoids were cancers, followed with intense interest the various retrogressive changes here described (6), and these have been many times figured. Some workers attributed them to the associated connective tissue alterations whereas others held that this could not explain them.

The papillomas of animals tarred 2 to 4 months not infrequently broke up along the base into what looked to be carcinomas (Table I, Transitional Growths). Yet when tarring was left off these all underwent one or another of the changes just described. The figures of Table I show that a wholesale disappearance of carcinoids took place then. Amongst 129 growths of 18 rabbits, examined 2 to 40 days after the last tarring, 64 had the morphology of squamous cell carcinomas and 10 were transitional growths. Among 108 tumors procured 61 to 121 days after the last tarring of 18 comparable animals, there was not a single apparent carcinoma and only 2 transitional growths. All the rest were either papillomas or frill horns. Keratinized cysts have not been included in the figures.

To determine whether the carcinoids would grow in a new situation, pieces of 20 that were big and actively enlarging were punched out, hashed separately in Tyrode solution, and implanted in the voluntary muscle or connective tissue of the axilla or groin of the host. Here they behaved as did bits of 19 tar papillomas treated in the same way, their cells either differentiating, dying, and disappearing without sign of growth, or rounding up into pearls lined with stratified squamous epithelium (7). Similar results had been reported by Ferrero (8). Hair follicle epithelium, sebaceous glands, and bits of cartilage, accidentally introduced with the implants, survived in the new situations.

4. Carcinomas.—In the early work on the tar cancers of rabbits (4, 6) carcinoids were frequently taken for carcinomas. Recent authors agree that usually the latter develop only after tarring has been done for a long time. Guldberg (9) elicited papillomas after 61 to 140 days in all of 23 rabbits, but carcinomas appeared in only 7 and not until the 276th day of tarring at the earliest, and in most cases at about the 330th day. Our tar produced nearly always a fatal toxemia or liver cirrhosis, when applied regularly for more than a few months, not sufficiently long to elicit cancers. These developed, however, in 2 animals tarred again after

an interval. Tarring of one of them had been done throughout periods of 5½ and 6 months, the cancer appearing after 21 months in all. The first tarring of the other was for 4 months, with later applications for periods of 4 weeks and 3 weeks. Between the 20th and 22nd months 2 cancers appeared. In both rabbits metastasis took place to a regional lymph node.

Most tar cancers of rabbit skin are squamous cell carcinomas arising secondarily from papillomas (4, 6). The 3 we have studied had this derivation, and the metastasis of one was cystic and exhibited the papillomatous form (Fig. 20). The literature reports occasional trichoepitheliomas and carcinomas originating from sebaceous glands, as also spindle cell and polymorphous cell sarcomas.

Effects of Tarring the Tumors

In a previous paper we have dealt with the influence of continued tarring upon the morphology and behavior of virus-induced growths (10). It has even more pronounced effects on many of the tar tumors, and is often the determining factor in their fate.

Tarring causes acute inflammation of the skin, with chronic changes later if the applications are kept up (3). In some of our animals the ears were thick and hot when tumors first appeared, and not infrequently a foul maceration had taken place under the tar with result that the growths were exposed to bacterial infection, while additional opportunities for this were provided by trauma incidental to the repeated stripping of the tar. The conditions were such as are known to further the growth of tumors generally. In the loose, proliferating connective tissue most of the carcinoids grew fast and invasively; and it was frequently edematous, not only beneath them but sometimes opposite them on the outer side of the ear (Fig. 47). Thither they often extended through lacunae in the cartilaginous plate, sometimes by way of the large lymphatics. Ferrero (11) has noticed that connective tissue disturbances favor carcinoid activity. The papillomas also tended to grow rapidly and irregularly, where the connective tissue was most disturbed, and sometimes they became anaplastic along the base. The frill horns, on the other hand, proved unresponsive to stimulation, as has already been stated.

The continuance of tarring notably stimulated the activity of papillomas and carcinoids, and leaving it off had pronounced adverse effects upon them. Unless it had been long kept up the skin at once began to revert to the normal, the acute inflammation and edema disappearing, the circulation lessening, the connective tissue sclerosing, the epithelial proliferation slowing, and the epidermal surface drying down and desquamating. The tar tumors dried down too, and most of the papillomas disappeared, while the carcinoids vanished as well, or became papillomas or cysts. The more rapidly the skin returned to the normal the more sweeping was the disappearance of growths. In occasional animals tarring called forth tumors very early, in 4 to 5 weeks (Fig. 18), and sometimes then the ears

were studded with them, many being anaplastic carcinoids as biopsies proved; yet if no more tarring was done all disappeared within a fortnight. In these cases the ears had undergone but slight general changes and rapidly resumed the normal appearance.

Very different was the outcome when tarring had been kept up 3 to 4 months or had been done throughout several periods. The ears then remained thickened and indurated for many weeks, and scurfy layers formed and reformed on them, attesting to persistent epithelial activity. That the other tissues were also in an abnormally excitable state was shown by the rapid and complete filling in of holes 3 to 10 mm. across which had resulted from punch biopsies. Even though the ears presented a normal aspect when the holes were made these closed more quickly than usual, sometimes with cartilaginous thickening, while when the skin had remained thickened and scurfy not only was healing notably rapid but there was often a lumpy overgrowth of cartilage. The conditions thus disclosed were obviously favorable to the tumors, as shown by their frequent persistence and increasing disorder, and by the appearance in some instances of new ones. They proved most likely to persist along the edges and near the tips of the ears where trauma acted to prolong the abnormal state of affairs (Fig. 1); and foci of inflammation beneath them or in their stroma frequently aided their course and acted to distort their form. Some of these findings are not new, yet all deserve emphasis because they bear upon the riddles presented by the occurrence and disappearance of the carcinoids, and the eventual change of papillomas to carcinomas.

The Place of the Carcinoids as Tumors

The carcinoids arose at the same time as the papillomas, and exhibited a like tendency to disappear after tarring had been left off. They often derived from papillomas and those persisting frequently became such, and were then indistinguishable from growths primarily papillomatous. Some of the carcinoids however retrogressed to keratinized cysts, as rarely happened with papillomas; but this was only because the latter did not penetrate deep. When they did so, or were implanted within the host, the cystic form was frequently assumed; and the cysts differed in no wise from those originating from carcinoids. There was obviously a close relationship between the two sorts of tumors. In seeking to understand it certain observations on virus papillomas come to mind. These growths often assume a malignant appearance if experimentally stimulated or implanted in notably favorable locations, especially if inflammation of bacterial origin ensues (12); and yet they are not true cancers, for they revert at length to the papillomatous state. We have termed the phenomenon *factitious malignancy*. Everything that is known of the tar carcinoids indicates that they are expressions of a factitious malignancy of growths which have intrinsically the nature of tar papillomas. Their complete dependence upon tarring for their cancerous form and behavior becomes comprehensible in this light, as does also their frequent derivation from papillomas and transformation into them, or into keratinized cysts such as papillomas may secondarily form.

Many carcinoids have a malignant aspect from the first (Fig. 13). Becoming visible as small, subepidermal mounds, they grow down from an intact surface epidermis or from the epithelial cells of hair follicles; and if tarring is stopped they may die by differentiation and keratinization, and be resorbed without giving sign of any change to papillomas. The question arises of whether growths of this sort, and those carcinoids of secondary origin which disappear in the same way, do not differ intrinsically from the ones which undergo conversion into papillomas or cysts. The answer is supplied by the course of events when tarring is kept up. Then the great majority of the carcinoids keep on proliferating, though only to assume the state of papillomas when tarring is eventually left off (group A, Table I), or, if disappearing, to undergo the same changes as these. It follows that such carcinoids as die by keratinization do not differ from the rest save in having even less ability to maintain themselves.

All in all the conclusion seems justified that the carcinoids owe their malignant aspect and behavior to the effect of extraneous conditions upon growths having an intrinsic character which ordinarily finds expression in benign papillomas.

The greater the acute inflammation of ears on which tar has evoked tumors the greater was the proportion of growths exhibiting the carcinoid form. Sometimes all showed it for the time being. Yet frequently two tumors situated side by side, exposed that is to say to much the same local conditions, were the one a carcinoid, the other a papilloma (Fig. 24). The inference seems justified that papillomas differ much in their responsiveness to those local influences which result in spurious malignancy.

It has been frequently assumed that the carcinoids are carcinomas brought into being and maintained by tarring, and devoid of the power to grow independently. Carcinoids differ, however, from true carcinomas, not alone in their inability to proliferate independently as such, but in a more significant feature, namely the reversible malignancy of their cells. Real squamous cell carcinomas of man have been known to retrogress on rare occasion,—and they do so by differentiating and keratinizing completely (13), as happens with many carcinoids,—but they never, like the latter, undergo conversion into benign growths. Carcinomas they remain until the last.

A few of the small tar tumors procured from animals which survived long tarring, or which were tarred throughout several periods, were found to have the microscopic aspect of cancers, although tarring had been discontinued for many months. The general state of the ears was notably pathological, and one may assume it to have given unusual encouragement to the persistence of carcinoids; yet the development of true cancer, if only in three instances, indicates that the apparent malignancy of some of the small growths mentioned may have been real.

Comparison of the Tar Papillomas with the Virus Papillomas

The resemblance of virus papillomas to the tar papillomas has already been reported upon briefly (14). The virus growths studied had been obtained by the

direct inoculation of scarified normal skin, mostly of the animal's side, and in consequence they derived from the surface epithelium, and were subjected to none of the collateral influences that tarring exerts. Now we have studied many papillomas due to the localization of virus out of the blood stream into the skin of ears repeatedly tarred, or acutely inflamed by the application of carbon bisulfide, as also growths resulting from the direct infiltration of normal ears with the inoculum, by way of a vein, with tarring later. Tar has also been applied repeatedly to virus papillomas resulting from tattoo inoculations into normal ears. These diverse materials gave consistent findings. Previous experiments had demonstrated that when skin has been tarred for some weeks prior to infection with virus by way of the circulation a considerable variety of tumors results, some of them cancers (1). For the present comparison only those virus papillomas were utilized which arose under conditions excluding this complication, most of them from ears which had been tarred just enough to bring about localization of the virus, or from ears which were not tarred until after virus infection had taken place.

Many of the facts to be cited of the tar papillomas are truisms, and no reference will be made to the literature substantiating them. The pictures illustrating the comparison generally show growths which had not yet undergone the complications which result from continued tarring and inflammation of the supporting tissues. In other respects they are representative.

Cellular Derivation.—Tar papillomas derive mostly from the epithelium of hair follicles, whereas those produced by virus rubbed into scarified skin all spring from the surface epithelium (2), as just remarked. But if the virus is thrown into the blood stream after one or two tarrings of the ears to insure localization, most of the growths arise precisely where the tar tumors begin, namely at the lower side of the hair follicles (Figs. 21, 22). This is the case also when normal ears are directly infiltrated with virus fluid by way of a marginal vein, and are tarred a few times later to render the inoculum effective (10). As in the case of tar papillomas the growths arising from the follicles usually begin on their basal side, with a proliferation of cells in the stratum germinativum, resulting in the formation of blunt, broad processes ("elephant's feet"), and a folding inwards of the epithelium in the papillomatous pattern.

Morphology.—On the inside of the ear both the tar and virus papillomas soon erupt and become surface growths. On the outside, though, where the skin is not bound tight to the cartilage, they may reach a diameter of 4 to 10 mm. before the stretched, shiny epidermis over them dries or gives way. Here they lie embedded as more or less flattened acorns or spheres (Fig. 18 *b* of reference 15). Most of those due to tarring are creamy or pink, though some are light gray and a few dark; whereas the virus papillomas are much more frequently pigmented, often dark gray or almost black. Both are opaque toward the center, and here they consist of keratinized material formed by a proliferating rind of stratified squamous epithelium of abnormal character (Figs. 23 to 26). This is usually thickest where deepest; and its infoldings are usually somewhat more numerous

and crowded in the virus papillomas (Figs. 27 to 30),—as would follow from their greater vigor. The pattern becomes more complex as the growths enlarge, and the epithelium may thrust irregular tongues downwards (Figs. 29, 30), an invasion encouraged by continued tarring.

After they have erupted the papillomas of both sorts become superficial cones, cylinders, "cauliflowers," or "onions," or they form tall, dry horns. Their keratinized material is tenacious and builds high. They may consist of it practically to the base, or connective tissue processes covered with living epithelium may extend far up into it. The processes branch sparsely (Figs. 4 to 7). As a rule virus papillomas proliferate the more rapidly and hence are plumper, more fleshy, do not dry so far down, or split so frequently into cauliflowers, retaining instead the onion or conical shape (Figs. 2, 3). Yet when tar papillomas grow rapidly, or virus papillomas slowly on the same ear, they cannot be told apart individually in the gross unless they are melanotic, which is never the case with actively enlarging tar papillomas though frequent with those due to virus (15).

Under low magnifications the tar and virus papillomas are nearly alike (Plate 20 *et seq.*), but they differ distinctively in their finer cytology (Plate 25). In the virus growths the proliferating cells of the stratum germinativum are larger than normal, with abnormally big, vesicular nuclei (Figs. 32, 34). They get larger still as they differentiate, undergo but little flattening in the stratum granulosum, and the granules forming there are usually few, and range from small to very coarse. The cells of the tar tumors on the other hand, though unusually large in the stratum germinativum, do not increase further in size, and they flatten in the stratum granulosum, appearing fusiform or oat-shaped on cross section, with numerous small granules darkening the cytoplasm (Figs. 33, 35). The nuclei of the virus papillomas, big and vesicular from the first, increase markedly in size as differentiation goes on, and the chromatin marginates, whereas in the tar papillomas they alter little, though large primarily, and the chromatin remains central, giving a birdseye appearance. On keratinization the nuclei of the virus growths sometimes become pycnotic for a brief while, in the deep crypts between the papillae (Fig. 32), whereas those of the tar papillomas usually lose almost at once their capacity to stain. The keratinized scales, however, assume the same appearance in both instances, their outlines forming a loose reticulum indicative of some swelling. Ordinarily they fail to stain with eosin and methylene blue though they take the blue if the staining is forced.

The cells responsible for the gray or black hue of many virus papillomas, and of a few of those due to tar, are melanoblasts for the most part (Figs. 34, 35), though the epithelium in the lower part of the proliferating layer often contains brown granules. The melanoblasts are frequently black with pigment granules which tend to be larger, coarser, and darker colored in the virus growths; and such cells accumulate much more slowly in the tar tumors, only coloring those which enlarge very gradually. The exceptional tar papillomas which were almost black had become so in the course of months during which they scarcely changed in size. Gray tar tumors which begin to grow fast soon become pink. The prolifera-

tion of the melanoblasts of virus papillomas, on the other hand, frequently keeps pace with their enlargement, and in consequence they remain sooty throughout months of vigorous proliferation (2). The melanoblasts are no essential constituent of the growths, however, but are included fortuitously (16), and they may sooner or later disappear or be outstripped (17). The same holds true in more striking degree of the tar papillomas, and the melanosis is of essentially the same sort.

Any irritation of the skin of gray-brown rabbits may bring on a graying of skin previously pink, and long-continued tarring often results in black freckles (Fig. 1), due to aggregates in the corium of chromatophores stuffed with pigment. Sometimes the source of this can be traced to a distant tar tumor or patch of epidermis containing active melanoblasts or epithelial cells in which pigment is abundant. Where sooty tar or virus tumors have disappeared a dark patch may persist, due to residual melanin in melanoblasts and chromatophores. Such patches were especially frequent in the case of the virus tumors (Fig. 46), and phagocytes dark with pigment were sometimes present in the lymph nodes into which they drained.

The shape of tar papillomas is markedly affected by continued tarring. Many become pedunculated (Figs. 40, 43), the proliferating connective tissue underneath the growth lifting it away from the ear. This happens less frequently with virus papillomas, because their cells multiply faster, are more aggressive, and hence can maintain their original place near the cartilaginous plate. Yet a pedunculation of virus papillomas may occur, even in the absence of any tarring, if they become indolent (Fig. 42) (12), and it often takes place despite rapid enlargement when tarring is done (Fig. 39). The likeness to pedunculated tar tumors may then be complete at low magnifications (Figs. 39 to 43) as well as in the gross. Virus papillomas on the rabbit's side have never become pedunculated in our experience. There the fibrous corium is a thick, tough sheet which does not yield to the weight even of huge growths, while furthermore none of our animals was tarred in this situation.

The virus papillomas of some rabbits are fleshy, whereas those of others, although doing well, are dry almost to the skin (Figs. 2, 3). This is true also of tar papillomas. When growths of both origins coexist on the same ears they frequently vary together in such respects, host influences obviously affecting them in the same way.

Retrogression.—When tarring has not produced enduring effects on the skin most of the growths due to it dry down, scab, and the scab comes away leaving a smooth scar (1). Some drying down and disappearance take place in any case unless the tumors are crowded and macerating. Most of the papillomas which persist become deeply cleft, brittle cauliflowers, or petaloid excrescences with constricted bases, or else horns or narrow cones, dry nearly to the skin. A few remain fleshy, however, and these usually have an onion shape. Some growths reconstitute themselves from beneath after scabbing. These exhibit no peculiarities, nor do such papillomas as may appear later. Generally virus papillomas are

much less affected by the discontinuance of tarring, as might be expected from their great vigor; and many continue to grow rapidly, and remain of onion shape or broadly conical. Some, however, assume the cauliflower or petaloid form, or become high horns.

The retrogression of tar and virus papillomas is attended by the same microscopic changes. When it is very slow the protruding, finger-like papillae become fewer and narrower, the layer of living epithelium shallower, the underlying connective tissue denser, and eventually the mass consists almost entirely of keratin (Figs. 42, 43). These changes, largely referable to sclerosis of the supporting tissues, are especially frequent in pedunculated growths.

When retrogression is rapid the growths get lower, owing to an unrepaired loss of keratinized material, their papillae shorten, and instead of broad "elephant's feet" of living epithelium along their base one finds narrow, irregular processes, separated and underlain by a reactive connective tissue containing many macrophages and lymphocytes (Figs. 44, 45),—cells almost absent from the slowly dwindling growths just described, and infrequent in tarred skin returning to normal. The epithelial processes appear as if invasive, and mitosis is still going on in their cells (as in retrogressing mouse carcinomas also (18)); yet the growths are actually smaller and more superficial than before, connections with the sebaceous glands and hair follicles have appeared (Fig. 44), and the underlying cellular accumulation (macrophages, lymphocytes) is of the sort which accompanies the retrogression of tumors generally. At length only a smooth scar is left, covered with somewhat thickened squamous epithelium devoid of distinctive peculiarities.

After tar carcinoids and papillomas are implanted in the subcutaneous tissue or leg muscles of the host they either die by keratinization or round up into small cysts, as already stated. The same holds true of virus papillomas implanted in hosts which develop a resistance to these growths (19). In both cases the living epithelium at the periphery of the cysts ceases to have a neoplastic appearance.

Conditions of Origin.—The real cause of the tar papillomas is something other than tar. The virus on the other hand is directly responsible for the papillomas it produces. The unknown causes of the tar tumors in general, and of the growths due to other "carcinogens," act only because of some change of a peculiar nature that these agents slowly bring about in the tissues; whereas the virus, though rendered unusually effective by this change (1), can act upon epidermis which has been merely scarified or acutely injured in other ways, as by an application of sodium bisulfide (10), or by inflammation due to a subcutaneous abscess. In contrast to the causes for the tar tumors the virus needs little help to be pathogenic, and only non-specific help, while the growths it produces need no help whatever in most instances. Nevertheless one may recall, as bearing on the possible causation of tar tumors, that tarring brings about conditions exceptionally favorable to localization of the virus out of the blood stream, that it enables it to engender growths after introduction into normal skin,—when otherwise this would not happen (10),—and that it exerts a stimulating effect upon virus papillomas which

is often pronounced, though not ordinarily decisive for their fate as it so frequently is in the case of tar tumors.

The ears of some rabbits are especially sensitive to tarring; and the more markedly it changes them the more likely are tar tumors to arise. This holds also for the papillomas which result from an intravenous injection of virus, though the conditions making for localization of the virus under such circumstances must be discriminated from those determining the formation of tumors (10). When both ears have been tarred equally, tar tumors appear in approximately the same number on both. So it is also with the papillomas arising after virus has been thrown into the blood stream: the average for both ears is the same unless the circulation to one was interfered with while the virus was circulating (10).

The tumors due to tar are local epithelial phenomena, the outcome of happenings at special points in the hyperplastic epidermis. So also with the virus tumors, though when an immense quantity of virus has suddenly been placed in circulation it may infect the tarred epidermis at so many spots as to give the impression that a generalized neoplastic transformation has occurred. Theoretically one should occur if the virus reached and infected every cell in the basal layer of the stratum germinativum. Actually, however, what appears to be generalized neoplastic change after massive virus infection, is usually due to a secondary coalescence of growths arising at numerous, separate points, as sections taken at early stages have proved.

The increase in size of the papillomas due to virus is due to multiplication of the cells with which it becomes associated primarily, not to contact infection of adjacent elements (12). This holds good also for the tar tumors. Both are frequently multicentric in origin, but the tar tumors only exceptionally arise in hordes. In an unique instance, the ears of a rabbit tarred in the usual way for 110 days were practically covered with tumors, close crowded and becoming confluent. In a few small blocks taken at random 61 papillomas could be discerned, as also 2 carcinoids and 2 frill horns. These findings were not included in Table I, to avoid distortion of the figures. The ears of an animal examined after tarring throughout 13 months showed large expanses of low, diffuse papillomatosis, which might have been interpreted as the result of a generalized change in the epidermis had there not been charts proving it due to a coalescence of growths originally separate and discrete.[1]

After an intravenous injection of virus, hosts of papillomas often arose on the outsides of ears rendered hyperkeratotic by tar transferred from the insides, and on the neck as well, where similar changes had taken place for the same reason.

[1] Suntzeff, Burns, Moskop, and Loeb, studying the downgrowths of uterine epithelium in mice given an estrogen (*Am. J. Cancer*, 1938, **32**, 256), have noted that they often occur at many points, and sometimes everywhere as if by a generalized transformation. Their conclusion that these findings are scarcely compatible with the working of a virus, would seem unwarranted in view of our observations.

The growths were marked as due to the inoculum by their appearance almost simultaneously after the usual incubation period, by their rapid growth and frequent, deep melanosis, by their cytology, and by the almost complete absence of tumors from such situations in animals merely tarred, unless the tarring had been kept up for many months (15). When this was the case scattered tar papillomas eventually appeared on the neck and outsides of the ears, and they derived from the same structures and took the same forms as the virus papillomas (Figs. 21 to 30). The earlier appearance there and greater frequency of virus-induced growths were obviously referable to the relatively slight epidermal changes required for the action of the virus, to the abundance of it provided, and to the vigor of the growths it caused.

The tumors due to tarring occur at irregular intervals, and new ones may keep on appearing for months or years afterwards, if the skin has been rendered enduringly pathological. Nearly all of the growths due to virus, on the other hand, appear within a few weeks of the intravenous injection unless the skin has undergone such change. Under these circumstances characteristic virus papillomas may continue to appear sporadically for at least 4 months after the inoculation, a period double the longest observed in the case of normal skin. In one experiment a rabbit's ears were acutely inflamed by swabbing them with barium sulfide, virus was injected intravenously, and 120 days later tarring was begun. At this time there were 3 papillomas present, which had appeared shortly after the injection, but later, as tarring went on, a fourth developed, shortly before the 160th day. Like the others it was marked as due to the virus by the retention of a slaty hue despite rapid enlargement, as furthermore by its characteristic cytology.

Course.—Tar papillomas may run widely differing courses though all are markedly responsive to change in the general state of the ears. Some of the earliest to arise and the swiftest to grow may vanish despite continued tarring, and while others nearby are rapidly enlarging. Some, beginning late and growing slowly, continue to grow after tarring is left off though all their neighbors are disappearing. The majority, however, enlarge or retrogress together. So too do the virus papillomas resulting from a direct inoculation of normal skin; for they are mostly multicentric in origin and hence what they do constitutes an average response. But when the virus is distributed on the blood stream,—providing, as this does, an opportunity for its individual entities to infect single cells,—and the state of the cells has been altered by preliminary tarrings, the growths may not only appear at widely differing times and vary much in neoplastic character (10), but often certain of those which appear to be typical virus papillomas may grow vigorously while others wholly resembling them and situated on the same ear are retrogressing. They now differ in incidence and course as do the tar papillomas, though not to nearly so great an extent. This is scarcely surprising in view of the fact that the virus exerts much more compulsion on the cells than does the cause for the tar papillomas, and is far less dependent on contributory circumstances for its continued action, as expressed in behavior of the growths. What the virus does is of predominating importance for the course of neoplastic

events, once these are under way, not what attendant or intercurrent circumstances do, as in the case of most tar tumors.

The superior pathogenicity of the virus is usually evident in the behavior, as well as the form, of the papillomas engendered by it: they enlarge more swiftly than most tar tumors and appear relatively unaffected when tarring is discontinued. Nevertheless in some animals they may retrogress all at once, a host of growths which have long flourished every one vanishing, perhaps within a few days, even though tarring is kept up. This behavior is due to the development of a generalized host resistance (20), which is not always comprehensive in its effects when the papillomas are situated on tarred ears, some of them continuing to enlarge in contrast to the majority. And even when the resistance is all-inclusive it is often transient, some growths reappearing after a few weeks in their previous positions, marked by patches of pigment in the corium. Tracings were made on cellophane in a few cases while the growths were dwindling, in order to record their exact situations; and after they had vanished tarring was begun anew. Some reappeared, but one cannot be sure that the tarring was responsible since recurrence can take place without it (20). Recently we have found that renewed tarring may call forth tar tumors where they were before, months after seemingly complete disappearance.

Whether any host resistance develops to tar papillomas is uncertain. The wholesale retrogression often witnessed after tarring has been stopped is plainly due in most cases to involutionary changes in the skin of the ears; and the sensitiveness of the tumors to these latter renders it difficult to tell whether other adverse influences are working upon them. Certainly host resistance, if such there be, is much less comprehensive and effective than that provoked by the virus tumors, which is notably selective, frequently causing complete disappearance of virus papillomas while exerting no visible influence upon the tar tumors associated with them.

Spurious Malignancy.—The progressively increasing disturbance of the skin, when tarring is kept up, acts to further the growth of tar papillomas, to disorder their form and to render them aggressive. Some extend deep, forming numerous small cysts secondarily, while the pattern of others becomes irregular and complex. They may penetrate to the outside of the ear and form secondary masses there, even while retaining their original morphology (Figs. 37 and 38). Not a few of them break up along the base as if becoming carcinomas, though actually they are but carcinoids in most instances; and growths of the latter sort keep on appearing. Yet if tarring is stopped after 2 to 4 months all of the apparently malignant tumors take on the form of benign papillomas, or become cystic or disappear. It is plain that tar tumors are very prone to spurious malignancy.

Virus papillomas are much less sensitive to continued tarring, though under its influence they too may become somewhat disorderly, extend down and form cysts, or exhibit complexities of pattern. Often they become very aggressive and grow through to the outside of the ear (Fig. 36). Yet even when proliferating with prodigious rapidity they do not assume the carcinoid form, although it is seen when the growth is implanted in the interior organs, especially under condi-

tions of local inflammation (12). The virus evidently exercises a stricter formative influence upon the cells with which it is associated than does the cause of most tar papillomas. Yet the formative influence of this unknown cause is notably strict in not a few instances, the growths remaining papillomas, however much tarred, and under circumstances which lead others immediately next to them to be carcinoids (Fig. 24).

So long as tar and virus papillomas continue to be such they retain the cytological features distinctive of them, no matter how complicated or disorderly their pattern; but most of these features only become manifest as differentiation takes place, and they are lost in proportion as the cells fail to differentiate, as when they become carcinoids or carcinomas. Markedly anaplastic growths deriving from tar and virus papillomas cannot be distinguished as of differing origin. The character of the cells in the papillomatous part of the cystic metastasis shown in Fig. 20 identified the growth as a tar tumor, whereas the cells of its invasive portion yielded no information in this regard.

The Development of Carcinomas.—The course of events when tarring was discontinued after a few months proved that all of the apparent malignancy then existing was spurious; but this was not the case when tarring had been kept up for a long time or had been done throughout several periods. In 2 rabbits thus treated genuine cancers arose, as already stated, while in others malignant changes seemed under way. The 3 cancers all derived from papillomas, like the generality of these growths in rabbits (4, 6). In the present comparison we are concerned only with cancers of such derivation.[2] The changes which take place when tar papillomas become malignant have often been pictured (4, 6), and they are morphologically identical with those taking place in virus papillomas which become malignant, the carcinoma cells stemming from the papilloma cells by alterations of greater or less magnitude (21). Furthermore the tar and virus cancers exhibit precisely the same forms, some being "papillomas of the second order," some cystic, while others are frankly malignant papillomas, and yet others are more or less anaplastic squamous cell carcinomas (21). A growth may assume all these forms successively, or a form primarily or secondarily assumed may be long retained, perhaps until death of the animal. The metastases of both the tar and virus tumors may be cystic[3] or solid, exhibit papillomatous features (Fig. 20) (4), or be anaplastic.

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[2] Tar readily evokes squamous cell carcinomas in cottontail rabbits, as we have found, and some of the cancers do not derive from papillomas but have the carcinomatous form from the beginning. Yet the possibility cannot be excluded that they originated from carcinoids, themselves a manifestation of the spurious malignancy of papillomas. For carcinoids are frequently elicited in cottontails.

[3] The statement of a previous paper (*J. Exp. Med.*, 1936, **64**, 401) that the metastases of tar cancers are never cystic or papillomatous was based on information since found to be incomplete, and it is negated by the instance of Fig. 20.

The tar cancers derive mostly from those papillomas which are broad-based (4), that is to say from those which are most vigorous; and it is from the most actively growing of virus papillomas that cancers arise oftenest. Continued stimulation of tar papillomas by further tarring hastens the occurrence of malignancy; and it is hastened in virus papillomas by a variety of stimulating procedures (21). Yet these are not essential in either instance. The vigor of virus papillomas as a rule greatly exceeds that of those due to tar; and, as might be expected, they become malignant much more often, and generally after a much shorter period, while the carcinomatous change is frequently multiple and cancers sometimes appear almost simultaneously at many spots in a single growth (21). Only an occasional tar papilloma ever becomes malignant.

Tarring so stimulates neoplastic proliferation in general that it may conceivably bring into the open some cancers which would not otherwise assert themselves, and which cannot progress after it is stopped. Yamagiwa and Ichikawa elicited a tar tumor with the morphology of a carcinoma, which metastasized yet retrogressed after 630 days (4); and amongst the numerous tar tumors of cottontail rabbits that we have recently evoked squamous cell carcinomas requiring aid have been frequent, growths which enlarged so long as tarring was kept up, not infrequently destroying a great part of the ear, yet which dwindled, though retaining the morphology of cancers, after it was left off, and eventually disappeared. In domestic rabbits growths of this sort are evidently rare, though the case just cited shows that they occur. Two large ulcerating cancers which had derived "spontaneously" from virus papillomas in our domestic rabbits, and were squamous cell carcinomas on biopsy underwent secondary, retrogressive changes. One had existed for months as a large, fungoid, malignant papilloma, distinctively different from the rest of the papillomatous mass amidst which it originated. Eventually it grew smaller and was almost replaced by the surrounding papillomatous tissue. The other cancer, an anaplastic, metastasizing squamous cell carcinoma, was at one time a bulky, ulcerated growth, yet dwindled in the end to a mere puckered induration over which the skin had healed. The microscope disclosed, however, that nests of living carcinoma cells still existed amidst a sclerotic connective tissue.

DISCUSSION

The benign tar tumors here described and classified were sharp cut pathological entities. They were not local exaggerations of the epidermal hyperplasia due to tarring, nor were they random neoplastic manifestations. One did not find papillomas grading into frill horns or *vice versa*. Whatever the nature of the cause for the frill horns, it expressed itself with an exquisite particularity, making the cells do precisely thus and so, with result in highly distinctive growths. The cause for the tar papillomas, though also acting upon the cells of the

stratum germinativum, produced tumors of wholly different sort, which usually expressed themselves in a characteristic, benign form, yet were often so responsive to external conditions as to assume instead a carcinomatous aspect without undergoing real cancerous change.

A comprehensive mapping of all the neoplastic potentialities of several animal species would greatly aid thought on tumor causation. But even in man only those potentialities are known today which have become actualities as result of the "carcinogenic" accidents of life. Observation has been largely haphazard in the case of other creatures, yet it already points to remarkable species differences. Tarring the skin of dogs, for example, results in malignant melanomas, apparently with some regularity (22), growths seldom if ever evoked in the rabbit or mouse. Tarring the skin of cottontails never results in frill horns, though frequently evoking papillomas, carcinoids, and cancers, as our extensive observations have shown. Can it be that the epidermis of different species of animals has different inherent potentialities for tumor formation? Or are these potentialities not inherent but due to agents of extraneous origin? Such questions can only slowly be answered.

The study here presented was made with hybrid rabbits of one sort (agouti) and with a single tar; but recently it has been extended to another breed (Dutch show rabbits). In these tar also evoked frill horns, papillomas, and carcinoids, with no other benign tumors, and in approximately the same relative number as in agouti rabbits. Furthermore a different tar has been applied to agouti rabbits and again the same tumors have been called forth, this time in an epidermis stimulated to much greater general change. The finding might have been expected in view of the evidence that tars owe their carcinogenic action to a common constituent, 1:2 benzpyrene. The pictures in the literature show that the domestic rabbits of England, America, Italy, Scandinavia, and Japan all yield on tarring papillomas and carcinoids resembling in general those with which we have dealt. But the differing fixatives and stains used on the specimens prevent any decision on whether the papillomas had the morphology of those of our animals. Some appear unlike them in details. Orr (23) reports that the growths evoked in mice by six carcinogenic hydrocarbons resemble those due to tar. It would be worth while to know the neo-

plastic effects of a single carcinogen upon rabbits of identical sort, bred in widely separate parts of the world, living under different conditions and fed different foods. (Our rabbits, all procured in New York, were housed and fed alike.) But in any typing of the growths thus evoked it would be necessary to discriminate their essential features from those due to intercurrent influences. This has not been done in the past, but instead all of the features of the tumors have been taken to be the expression of innate, individual peculiarities, and in consequence they have been deemed much more various than they actually are.

Because of the collateral influences that tar exerts it is far more effective in producing tumors in rabbits than any of the pure carcinogenic substances (10). It evokes growths much sooner than does benzpyrene even when this is applied in relatively large quantity, and it brings about local tissue changes which enable the tumors to become established. Directly or indirectly it urges them on and produces secondary changes in them. Because of this last influence tar tumors are best studied when young. Had we not examined them then, the narrow restrictions in their types might have been overlooked and the reasons for their later complexities have been missed.

The main facts emerging from the comparison of the benign tar and virus tumors are set forth in Table II, together with certain inferences which seem unavoidable. Our study was carried out on domestic rabbits, instead of cottontails, the natural hosts of the virus, to exclude all possibility that some of the growths evoked by tar might be due to a strain of virus lying latent and of such slight pathogenicity as to require the aid which tarring gives. For it is known that the papilloma virus may lie latent after introduction into normal skin and give rise to papillomas when tarring is done (10). The virus is wholly foreign to domestic rabbits and it cannot ordinarily be got again from the growths engendered in this species. Nevertheless the tarred rabbits were isolated to rule out contact infection entirely.

Serological tests indicate that the cause for the tar papillomas is not antigenically related to the Shope virus (24), and the distinctive cytological changes that it produces accord with this finding. To account for the general likeness to the virus tumors, one might

assume that anything which stimulates proliferation of the cells of the stratum germinativum will give rise automatically to papillomatous growth, if the proliferation is superficial. One may recall in this relation the likeness of silicosis tubercles to those due to bacillus

TABLE II*a*

Comparison of the Benign Tar and Virus Tumors Deriving from the Epidermis of Domestic Rabbits

The Tar Tumors	*The Virus Tumors*
Tarring evokes the growths	Tarring results in a localization of circulating virus: it enables latent virus to produce growths
Peculiar, chronic, "carcinogenic" tissue disturbances bring the unknown causes into action	Various non-specific, acute or chronic tissue disturbances render the virus effective
The growths appear on the tarred skin at irregular intervals	After a single intravenous injection of virus the growths all appear within a few weeks unless the skin has been often tarred, when they may appear irregularly

Number and time of appearance vary from host to host, but incidence is similar on both ears

Origin punctate or focal, but often multicentric

Enlargement takes place by intrinsic cell proliferation (*aus sich heraus*)

The tumors are largely dependent for persistence and growth on further tarring or on chronic changes already induced in the supporting tissues	The growths can progress without help of tarring or of chronic tissue changes, though aided thereby
Individual rates of proliferation vary widely; implantation elsewhere in the host is unsuccessful	Proliferation usually very rapid; implantation successful
Unfavorable local conditions frequently bring about general retrogression	Local conditions seldom cause general retrogression
Growths of two distinct types,—(1) papillomas subject to spurious malignancy = carcinoids, (2) frill horns	Growths are of a single sharply defined type,—papillomas

Inclusion bodies absent: no distinctive changes in the supporting tissue

Morphology indicates that they are due to specific causes, each with characteristic effects

Causes have not been recovered from the growths	Usually the virus cannot be recovered from the growths induced with it
The unknown causes require special cell conditions for their action, and exert little compulsion	*The virus cause is very compelling and does not require special aid*

TABLE II*b*

The Tar Papillomas	*The Virus Papillomas*
Both are sharply defined pathological entities	
Arise mostly from the deeper portion of the hair follicles	Arise mostly from the deeper portion of the hair follicles when the virus localizes out of the blood stream
Arise from the stratum germinativum	
Origin frequently multicentric	
Tumors enlarge in same way, assume same gross forms; virus papillomas usually more fleshy because growth more rapid	
Superficial resemblance close but cytology distinctive	
Occasional melanosis, and melanoblasts mildly stimulated. Pigmentation lost during rapid growth	Melanosis frequent, and melanoblasts greatly stimulated. Pigmentation often retained during rapid growth
Melanosis is of similar character	
Histological phenomena similar during retrogression	
Peculiarities of individual host influence gross form in same ways	
Aspect and course markedly influenced by local conditions	Aspect and course moderately influenced by local conditions
Continued tarring renders growths aggressive, complicates morphology	
Growths highly responsive to tarring. Spurious malignancy (formation of carcinoids) is a frequent result	Growths less responsive to tarring. It fails to induce spurious malignancy but other influences do this frequently
Retrogression usual unless chronic changes have been induced in the supporting tissues	Retrogression not infrequent; chronic tissue changes aid persistence
General retrogression frequent owing to local conditions; whether an induced resistance ever brings it about is uncertain	Local conditions have relatively slight effect, but general retrogression is frequent as result of induced resistance
Growths may reappear after vanishing	
Pathogenicity of cause, as expressed in tumor behavior, varies much from growth to growth	Pathogenicity of virus, as expressed in the multiple growths of a single infection, varies little
Occasional carcinomatous change after many months	Carcinomatous change frequent after a few months
Intercurrent stimulation hastens the malignant change	
The cancers arise from the papilloma cells by similar morphological alterations and exhibit the same general characters	
The cause of the papillomas stimulates the cells mildly and exerts a formative influence upon them which is often overborne. It has a moderate carcinogenic effect	*The virus stimulates the papillomas greatly and exerts a strict formative influence upon them. It has a pronounced carcinogenic effect*

The virus seems not to be related antigenically to the tar tumor cause yet the differences in the neoplastic phenomena they induce are merely quantitative

tuberculosis. But tar and virus papillomas exhibit their characters though growing beneath the skin (Figs. 29, 30, 36, 37), while furthermore the frill horns, though submitted to similar mechanical influences and deriving from the stratum germinativum of hair follicles (Fig. 31), like the growths just mentioned, have a wholly dissimilar morphology (Figs. 8, 9).

Some of the tabulated differences in the tar papillomas and those due to virus are obviously consequent upon the great pathogenic activity of the latter, to its introduction into the test rabbits upon a single occasion, and to its almost standardized effects under ordinary circumstances. There is every reason to suppose that if we had mixed several virus materials of differing pathogenicity and injected the mixture several times, dribbling it into the animal, so to speak, the individual differences in incidence and behavior of the tar papillomas would have been duplicated.

The virus is a much more exigent formative influence than the unknown cause of the tar papillomas, the growths it produces retaining their morphology under conditions which would cause many tar papillomas to become carcinoids; but this difference would appear to be merely quantitative, since virus papillomas also become carcinoids on special occasion. In both instances the apparent malignancy means only that epithelial cells subjected to extraneous stimulation can simulate malignant cells, a fact already proven for normal epithelium by the invasive downgrowth with anaplasia which occurs after intradermal injection of Scharlach R or Sudan III in olive oil.

Though spurious malignancy is much more frequent in tar papillomas than in those due to virus, they are far less likely to undergo real malignant change, and the change generally takes place much later. The frequency with which cancer develops out of virus growths varies directly with how hard their cells are driven by the virus, as manifested by their rate of enlargement (25). The more rapid the proliferation, and the more the cells are played upon by extraneous stimuli (*e.g.* incision, Scharlach R injection, inflammation due to bacterial infection or vaccinial necrosis), the sooner and oftener does malignancy occur. The unknown cause of the tar papillomas exerts only a mild and conditional compulsion, as demonstrated by the behavior of the growths. This being so, one might expect that cancer would

arise late and infrequently from tar papillomas, as is generally the case. Yet to all appearance malignancy is the outcome of precisely the same train of events as in virus papillomas that are becoming malignant; and the resulting carcinomas are of the same kind and exhibit the same limited diversity. In a previous paper (21) we have discussed certain human cancers which arise from papillomas by changes resembling those which occur in rabbit papillomas, and have cited furthermore a virus of man which causes papillomatous proliferation out of which squamous celled carcinomatosis occasionally arises, namely the virus responsible for condyloma acuminatum (26). Like the unknown cause of the tar papillomas this virus fails to produce growths unless aided. Uncleanliness, local bacterial infection, tissue maceration, act to render it effective and to maintain the resulting condylomas, just as tar acts to evoke and maintain the tar tumors; and the cancers arise, as in their case, out of growths subjected to long-continued disturbance (27).[4]

SUMMARY

Tarring the ears of rabbits of one sort with a single kind of tar evoked epidermal tumors of a few sharply defined types, namely ordinary papillomas, carcinoids, carcinomas, and "frill horns." These last, relatively infrequent, are now recognized for the first time. The carcinoids have proved to be the expression of a spurious malignancy of papillomas, resulting from intercurrent influences, and they were wholly dependent upon these for their threatening aspect and behavior. Chief amongst such influences was continued tarring. It had the effect of establishing the papillomas, stimulated their proliferation, complicated their morphology, and rendered some of them disorderly, aggressive, and anaplastic. It brought all of the tissues of the ears into an excitable state, and often this state endured long after the skin had apparently returned to normal.

The characters of the papilloma-carcinoids and of the frill horns were so different and distinctive as to imply the action of differing, specific causes.

[4] The treatment of condyloma acuminatum has improved so greatly of late that secondary carcinomatosis has become exceedingly rare; but the conditions of its origin and development are amply documented by the numerous, well illustrated papers on the theme (27).

The papillomas were very like those induced with the Shope virus, and hence a point-to-point comparison was made of their manifestations, including the derivation of carcinomas from them. This comparison demonstrated that the unknown cause of the tar papillomas provoked neoplastic phenomena which were identical in all essential respects with those due to the virus.

To suppose, for experimental purposes, that the papillomas which tarring elicits are caused by a virus rendered pathogenic by this procedure, is to demand least of the unknown. Yet it does not follow that they must be due to a virus.

BIBLIOGRAPHY

1. Rous, Peyton, and Kidd, J. G., *J. Exp. Med.*, 1938, **67,** 399. Kidd, J. G., and Rous, Peyton, *J. Exp. Med.*, 1938, **68,** 529.
2. Shope, R. E., *J. Exp. Med.*, 1933, **58,** 607. Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60,** 701.
3. Woglom, W. H., *Arch. Path. and Lab. Med.*, 1926, **2,** 533. Seelig, M. G., and Cooper, Z. K., *Am. J. Cancer*, 1933, **17,** 589.
4. Yamagiwa, K., and Ichikawa, K., *Mitt. med. Fak. k. Univ. Tokyo*, 1915–16, **15,** 295; 1917, **17,** pt. 1; 1918, **19,** pt. 4; *J. Cancer Research*, 1918, **3,** 1.
5. Borst, M., *Z. Krebsforsch.*, 1924, **21,** 341.
6. Yamagiwa, K., and Ichikawa, K., *J. Cancer Research*, 1918, **3,** 1. Ichikawa, K., and Baum, S.-M., *Bull. Assn. franç. étude cancer*, 1924, **13,** 257. Roussy, G., Leroux, R., and Peyre, Ed., *Bull. Assn. franç. étude cancer*, 1924, **13,** 164.
7. Kidd, J. G., and Rous, Peyton, *Proc. Soc. Exp. Biol. and Med.*, 1937, **37,** 518.
8. Ferrero, V., *Arch. sc. med.*, 1926, **48,** 78.
9. Guldberg, G., *Acta path. et microbiol. Scand.*, 1931, suppl. 8, 1.
10. Kidd, J. G., and Rous, Peyton, *J. Exp. Med.*, 1938, **68,** 529.
11. Ferrero, V., *Arch. sc. med.*, 1926, **48,** 67.
12. Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60,** 701. Rous, Peyton, Beard, J. W., and Kidd, J. G., *J. Exp. Med.*, 1936, **64,** 401.
13. Orth, J., *Z. Krebsforsch.*, 1904, **1,** 399. Schwarz, L., *Virchows Arch. path. Anat.*, 1904, **175,** 507. Kimura, K., *Gann*, 1908, **2,** 75. Ribbert, H., *Deutsch. med. Woch.*, 1916, **42,** 278.
14. Rous, Peyton, and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33,** 358.
15. Rous, Peyton, and Kidd, J. G., *J. Exp. Med.*, 1938, **67,** 399.
16. Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1334.
17. Rous, Peyton, Beard, J. W., and Kidd, J. G., *J. Exp. Med.*, 1936, **64,** 401.
18. Woglom, W. H., *J. Cancer Research*, 1922, **7,** 379.
19. Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1934, **60,** 723.
20. Kidd, J. G., *J. Exp. Med.*, 1938, **67,** 551.

21. Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1935, **62,** 523.
22. Passey, R. D., *J. Path. and Bact.*, 1938, **47,** 349.
23. Orr, J. W., *J. Path. and Bact.*, 1938, **46,** 495.
24. Kidd, J. G., Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1936, **64,** 79.
25. Rous, Peyton, Kidd, J. G., and Beard, J. W., *J. Exp. Med.*, 1936, **64,** 385.
26. Serra, A., *Gior. ital. mal. ven.*, 1924, **65,** 1808.
27. Konjetzny, G. E., *Münch. med. Woch.*, 1914, **16,** 905. Buschke, A., and Lowenstein, L., *Klin. Woch.*, 1925, **2,** 1726; *Arch. Dermat. u. Syph.*, 1931, **163,** 30. Muhlpfordt, H., *Dermat. Woch.*, 1928, **87,** 1403. Israel, W., *Zentr. Urol.*, 1928, **22,** 395. Frei, W., *Arch. Dermat. u. Syph.*, 1930, **160,** 109.

EXPLANATION OF PLATES

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

V, T = virus and tar papillomas, respectively.

PLATE 20

FIG. 1. Tar papillomas. The inside of the ear had been tarred throughout several periods of some weeks each, with intervals between for recovery. Photographed on the 591st day, long after the last tarring. The skin between the growths is still somewhat scurfy in places. The tumors are far less fleshy than if tarring had been kept up, and hence do not resemble so closely the virus growths of Figs. 2 and 3. All those along the edge of the ear are papillomas, some medium gray (G), two almost black (G′). Several freckle-like patches of sooty, intracutaneous melanosis can also be seen. Near the middle of the ear is a small, recurved frill horn (H), and at one spot close to its edge is an aggregate of small subepidermal cysts (CY) resulting from the retrogression of a carcinoid. × ½.

FIGS. 2 and 3. For comparison with Fig. 1. Growths produced by directly infiltrating normal ears with papilloma virus by way of a marginal vein, and tarring them three times later, beginning after a week, in order to render the virus effective. (The uninfiltrated ears were also tarred: no growths ever appeared on them.) The papillomas of Fig. 2 were dry, cindery, and nearly all dark gray and slow growing, whereas those of Fig. 3, resulting from the same inoculum, were vigorous and fleshy, and about half of them were pigmented (G, G′). The dry tops of some of the others appear dark. Host influences have a great effect on the form of the growths. × ½.

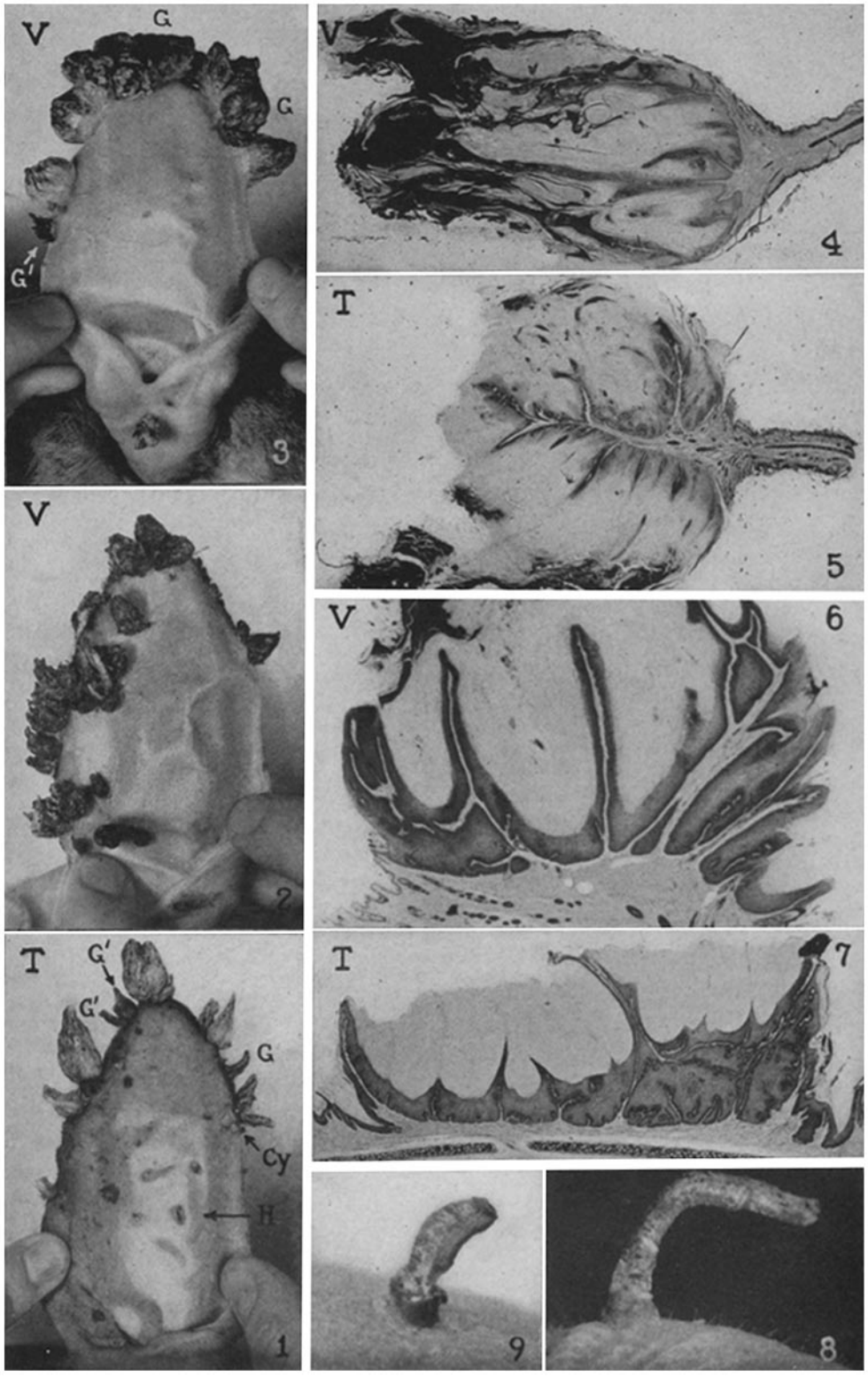
The marginal situation of nearly all of the papillomas of Figs. 1, 2, and 3 is unusual. The specimens were selected for ease in photography.

FIG. 4. The melanotic virus papilloma (G′) of Fig. 3,—for comparison with the melanotic tar papilloma of Fig. 5. The edge of the ear happens not to be incorporated in the growth. × 6½.

FIG. 5. Cross section of the melanotic tar papilloma, G′ arrow, of Fig. 1. Its outer keratinized portion has been cut away. It extends around the edge of the ear on both aspects, with result that this is incorporated as a spurious raphe. × 6½.

FIGS. 6 and 7. Sections of a virus papilloma and a tar papilloma, respectively (V, T). Most of their dry keratinized portions have been trimmed away. The virus had been directly inoculated into the untarred skin of the side. × 13.

FIGS. 8 and 9. Frill horns, showing the characteristic narrow shape and transverse striation of the dry horns, and the fleshy collar about their bases. The pictures were taken long after tarring had been stopped. × 2½.



Photographed by Louis Schmidt and Joseph B. Haulenbeek

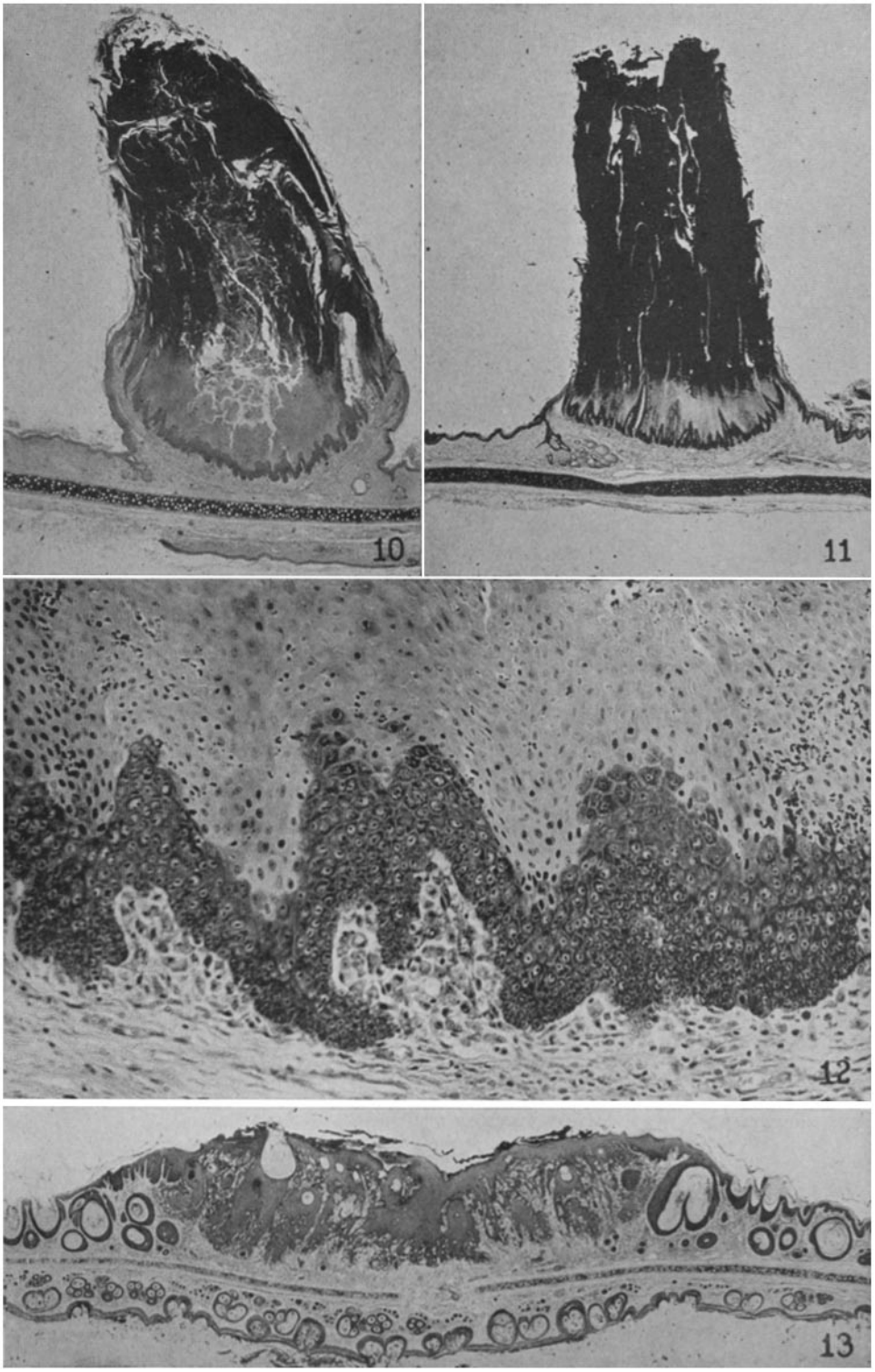
(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

PLATE 21

FIGS. 10 and 11. Small frill horns. The sharply outlined, uninvasive basal frill of living epithelium and the compact, close-textured, keratinized material are alike typical. For convenience in sectioning, the dry horns have been broken off near the base. × 14 and × 10, respectively.

FIG. 12. Part of the base of a frill horn, to show the character of the cells, the absence of a stratum granulosum, and the abrupt keratinization with transient nuclear pycnosis. The scattered, irregular granules stippling the keratinized layer in some places, especially at the right of the photograph, have come from the breakdown of in-wandered polymorphonuclear leucocytes. More macrophages lie beneath the growth than is ordinarily the case. × 190.

FIG. 13. A typical anaplastic carcinoid, selected as illustrating the fact that such tumors are not necessarily preceded by papillomatous proliferation but may extend directly down from the surface epithelium. × 14.



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(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

PLATE 22

FIG. 14. A carcinoid of more organized type. × 18.

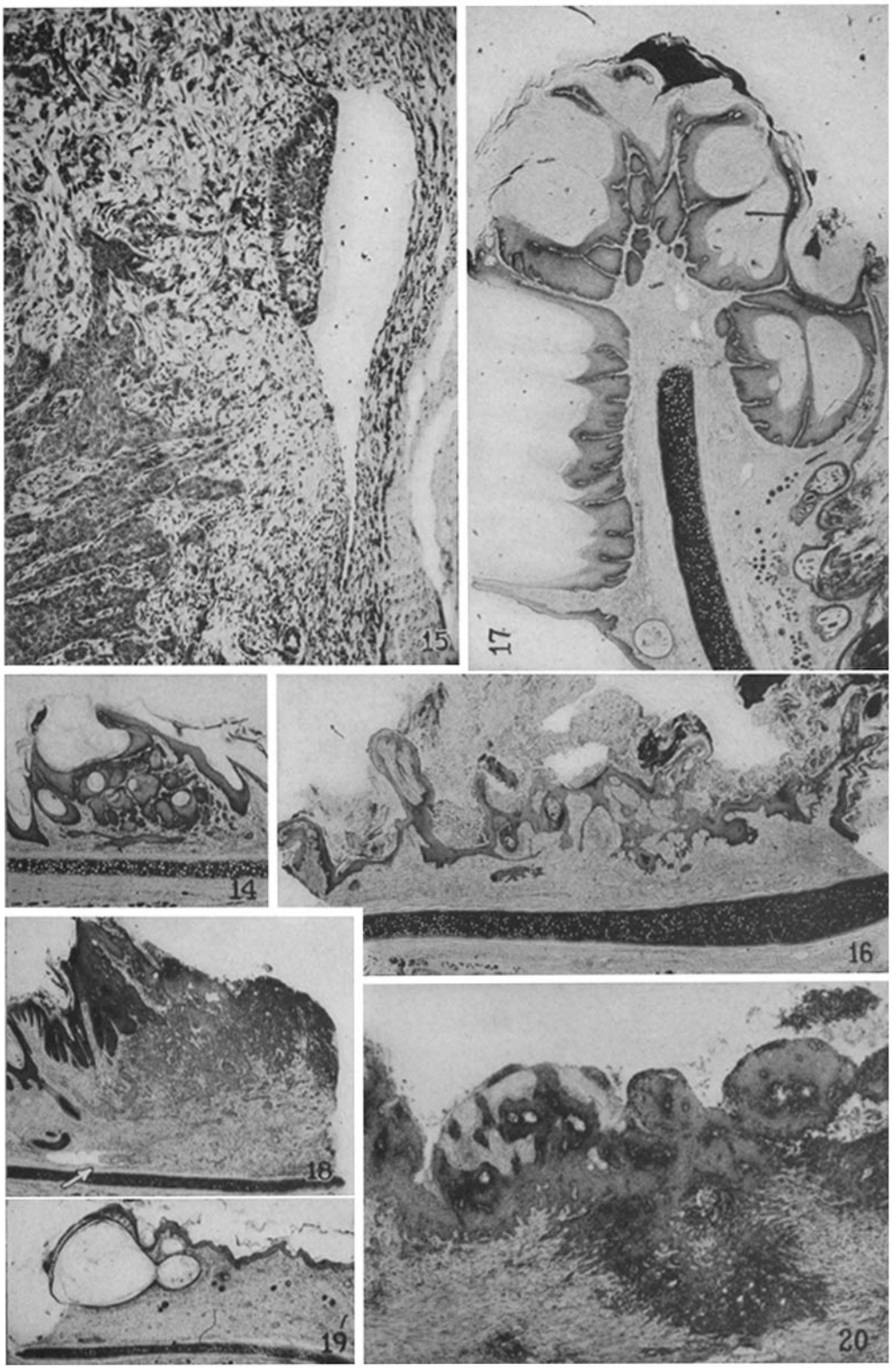
FIG. 15. Invasion of a large lymphatic by an anaplastic carcinoid which appeared after only one month of tarring and was then excised. The reactive connective tissue appears mucoid, as in the case of many squamous cell carcinomas. × 80.

FIGS. 16 and 17. To illustrate the change of a carcinoid to a papilloma after the discontinuance of tarring. Fig. 16 shows a section across a biopsy specimen punched from a large carcinoid 10 days after tarring was stopped. The ear had dried down, and most of the growths, including the carcinoid, had begun to dwindle. It was already losing its anaplastic state, but deep in the reactive connective tissue beneath it were scattered, persisting groups of epithelial cells, not visible in the photograph. Fig. 17 shows the same tumor 28 days later, at the edge of the previous punch hole. Though it has extended around the edge to the outside of the ear, it is now an orderly keratinizing papilloma. The connective tissue beneath it has become sclerotic. × 21.

FIG. 18. Part of an early carcinoid. The rabbit had been tarred only 28 days yet the growth, an ulcerated dome, was already 8 mm. across. Half of it was punched out, as shown. It had invaded a large lymphatic (arrow). At this time there were 14 other growths on the ears, all subepidermal mounds or domes up to 9 mm. in diameter, and two of medium size, as yet unulcerated, were taken *in toto*. They showed carcinoids deriving from an intact epithelium. The ears were now stripped of tar for good. 12 days later only 4 growths remained, 3 as dry scabs, while the fourth, the other half of the carcinoid pictured, was reduced to a mere subepidermal thickening. It was punched out (see Fig. 19). After 2 weeks more all of the tumors had completely disappeared. × 14.

FIG. 19. The rest of the carcinoid of Fig. 18, as it appeared 12 days later. It now consists merely of keratinized cysts lined with stratified squamous epithelium devoid of any obvious neoplastic character. The end of the cartilage at the left marks the edge of the previous biopsy wound, but the scab over the healing tissue here has been torn away, together with a little of it. × 14.

FIG. 20. Section through the wall of a cystic metastasis from a tar carcinoma (see text). The growth was situated in an auricular lymph node. The living epithelium lining the cyst is papillomatous and keratinizing, but further away is anaplastic and notably invasive. × 44.



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(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

PLATE 23

FIGS. 21 and 22. Early stages of papillomatosis due to virus and tar, respectively,—to show that the growths began in the deeper portion of the hair follicles in both instances.

Fig. 21 is from an animal with ears prepared by tarring, which died 22 days after an intravenous virus injection. Massive infection with the virus took place, as shown by a sudden brawny swelling of the ears at about the 18th day (15), followed immediately by the appearance of growths. Autopsy disclosed that they were innumerable, some creamy but many dark. The section is from the outer side of the ear, which had been devoid of tar warts.

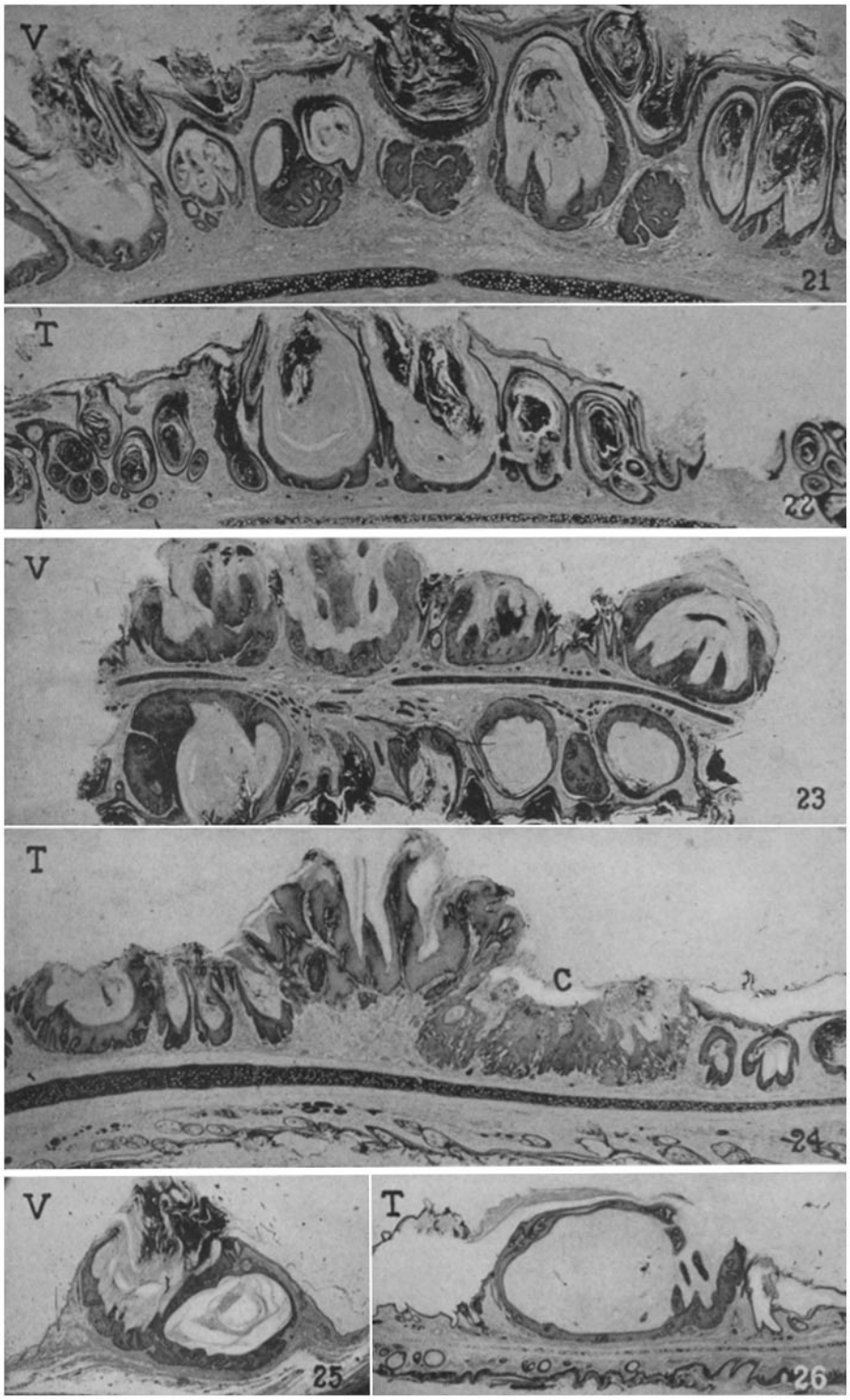
Fig. 22 is from an uninoculated animal which died after it had been tarred for 10 months, intermittently toward the end. At death it had numerous large, pedunculated papillomas on the tarred inner surface and a few small creamy-gray growths on the outside. Some that were just beginning at the latter situation are pictured. × 14.

FIGS. 23 and 24. Virus and tar papillomatosis at a slightly later stage. The resemblance is absolute at the magnification shown. A tar carcinoid (C) is included in Fig. 24. × 10.

To obtain Fig. 23, a normal rabbit ear was directly infiltrated with virus and tarred a few times later, as in the case of the ears furnishing Figs. 2 and 3. The specimen shown was punched from the ear 25 days after the infiltration, when innumerable pink or gray growths were appearing on both sides of the organ.

Fig. 24 came from the inside of an uninoculated ear tarred during a period of 80 days. Numerous growths had arisen, both papillomas and carcinoids.

FIGS. 25 and 26. A virus and a tar papilloma, situated on the outside of the ears, and just beginning to erupt. The virus papilloma was the result of a punctate inoculation into normal epidermis. The growth of Fig. 26 came from an animal tarred intermittently for 17 months. × 14.



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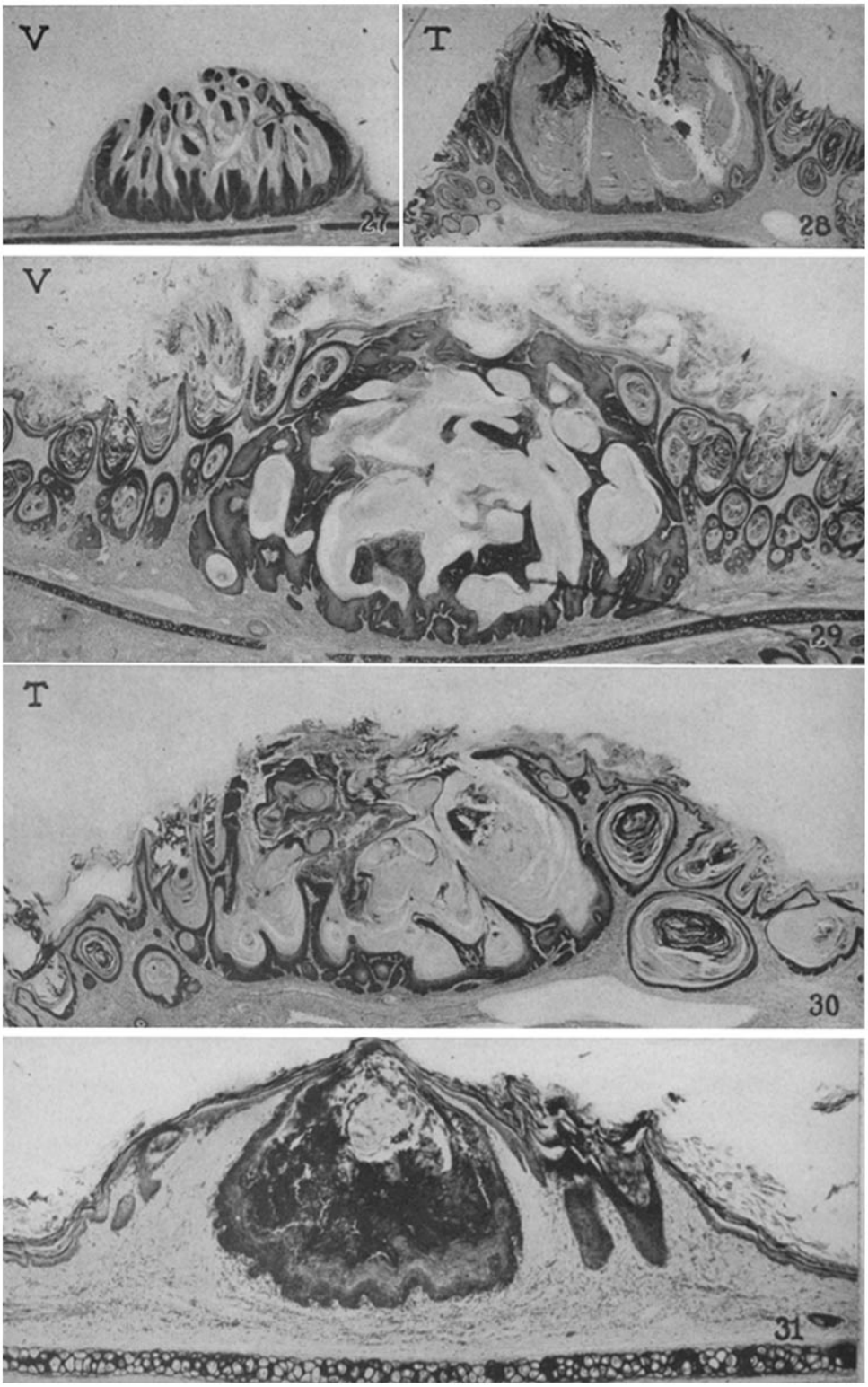
(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

PLATE 24

FIGS. 27 and 28. Further examples of erupting tar and virus papillomas. The growth of Fig. 27 was due to a punctate inoculation of virus. The tumor elicited by tar (Fig. 28) was from the same rabbit as Figs. 22 and 30. × 6½.

FIGS. 29 and 30. Somewhat larger virus and tar papillomas on the outside of the ear. The papillomatous pattern has become more complicated. The growth due to the virus resulted from direct inoculation, with tarring for 41 days thereafter. Fig. 30, of a tar papilloma, is from the rabbit, tarred for 10 months, that provided Figs. 22 and 28. × 14.

FIG. 31. Early stage of a frill horn; it is just erupting. The dense, strongly eosinophilic, keratinized material contrasts greatly with that formed by the virus and tar papillomas of Figs. 21 to 30, which stains almost not at all. The unstained whorls of keratin at the apex of the growth are the original contents of the distended hair follicle from which it arose. × 30.



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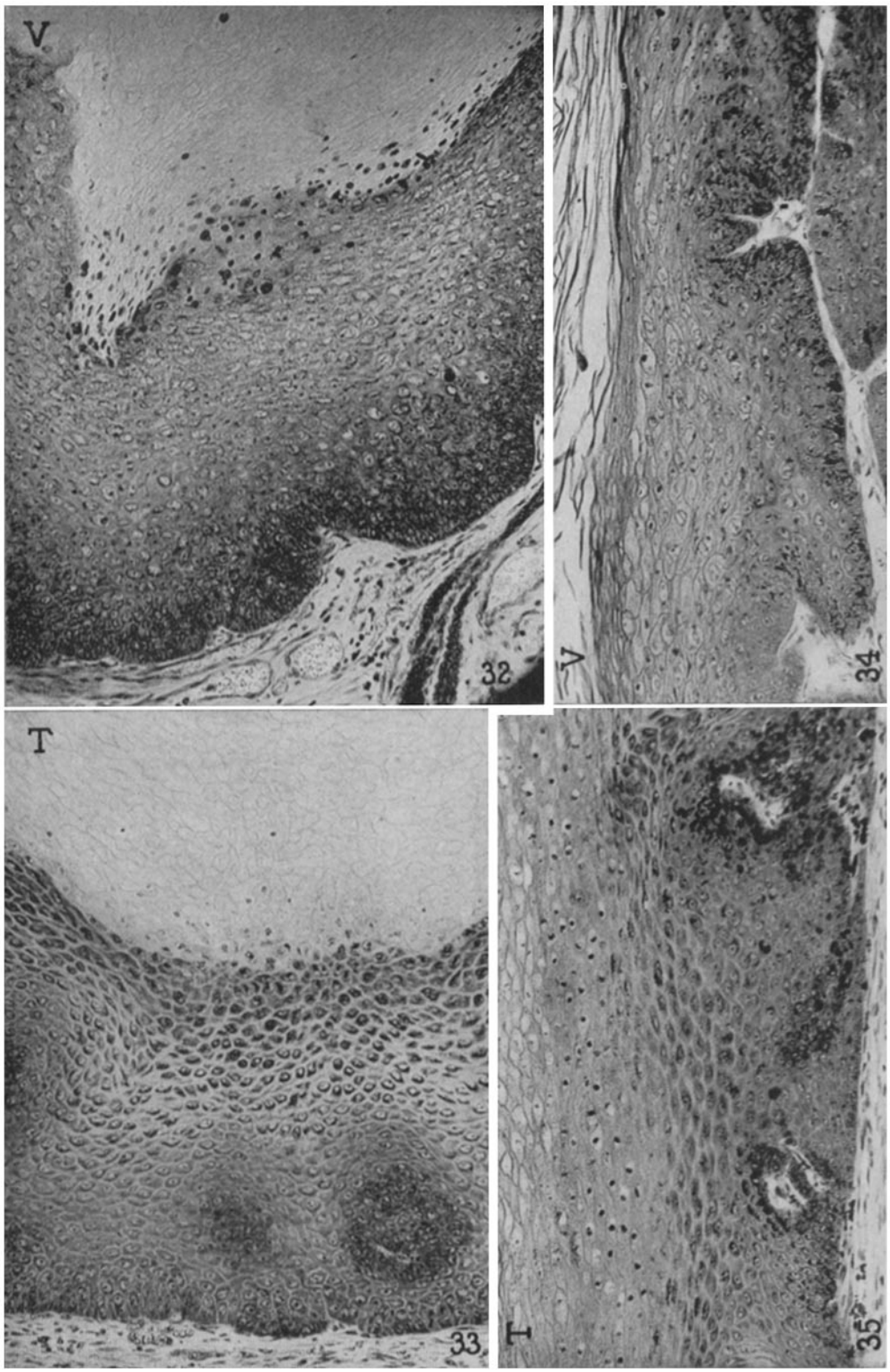
(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

PLATE 25

FIGS. 32 and 33. The living epithelial layers of a non-pigmented virus and tar papilloma, respectively,—to show the likenesses and differences described in the text. × 177.

FIGS. 34 and 35. The living layers of markedly melanotic virus and tar papillomas. The pigment-containing cells are morphologically alike in the two instances and occupy the same situations. × 168.



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(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

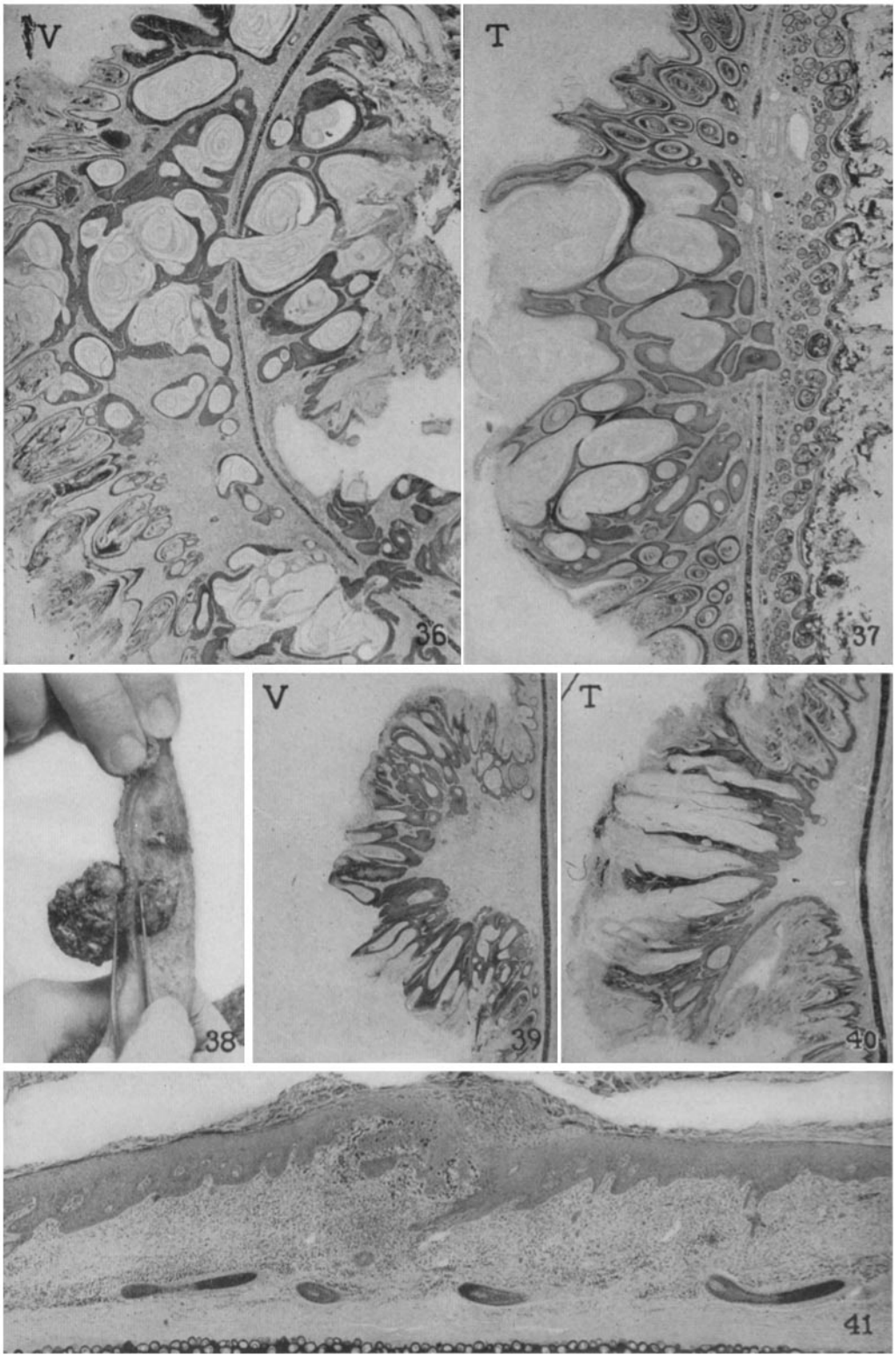
PLATE 26

FIGS. 36 and 37. Virus and tar papillomas which have extended through lacunae in the aural cartilage as result of the stimulus of continued tarring. The virus growth of Fig. 36 was the result of a tattoo inoculation into the inside of a normal ear 73 days previously, with tarring twice weekly thereafter. The growth became fungoid and foul, and extended through to the outside at several situations, of which two are seen here Fig. 36, × 9. Fig. 37, × 13.

FIG. 38. Extension of a tar papilloma to the outside of the ear. A punch biopsy disclosing the character of the growth was done early, and the hole that was left healed completely. The tumor became the large fleshy sphere that is pictured, and while doing so extended to the outside of the ear through the healed wound, with result in a fleshy "onion" there. Both growths consisted of connective tissue for the most part, covered with characteristic papillomatous epithelium, and with embedded islands of the latter. × ½.

FIGS. 39 and 40. Newly pedunculated virus and tar papillomas. The pedunculation was due to continued tarring. The virus tumor had been produced by a tattoo inoculation of a normal ear. × 6.

FIG. 41. Final stage in the retrogression of a melanotic papilloma due to localization of circulating virus in an ear long tarred (15). The growth is almost gone but its place is marked by much intracellular pigment. The skin is everywhere pathological, its epithelium thickened, and the connective tissue unusually cellular. Scattered lymphocytes are present where the papilloma was once situated. × 46.



Photographed by Louis Schmidt and Joseph B. Haulenbeek

(Rous and Kidd: Comparison of virus-induced tumors with tar tumors)

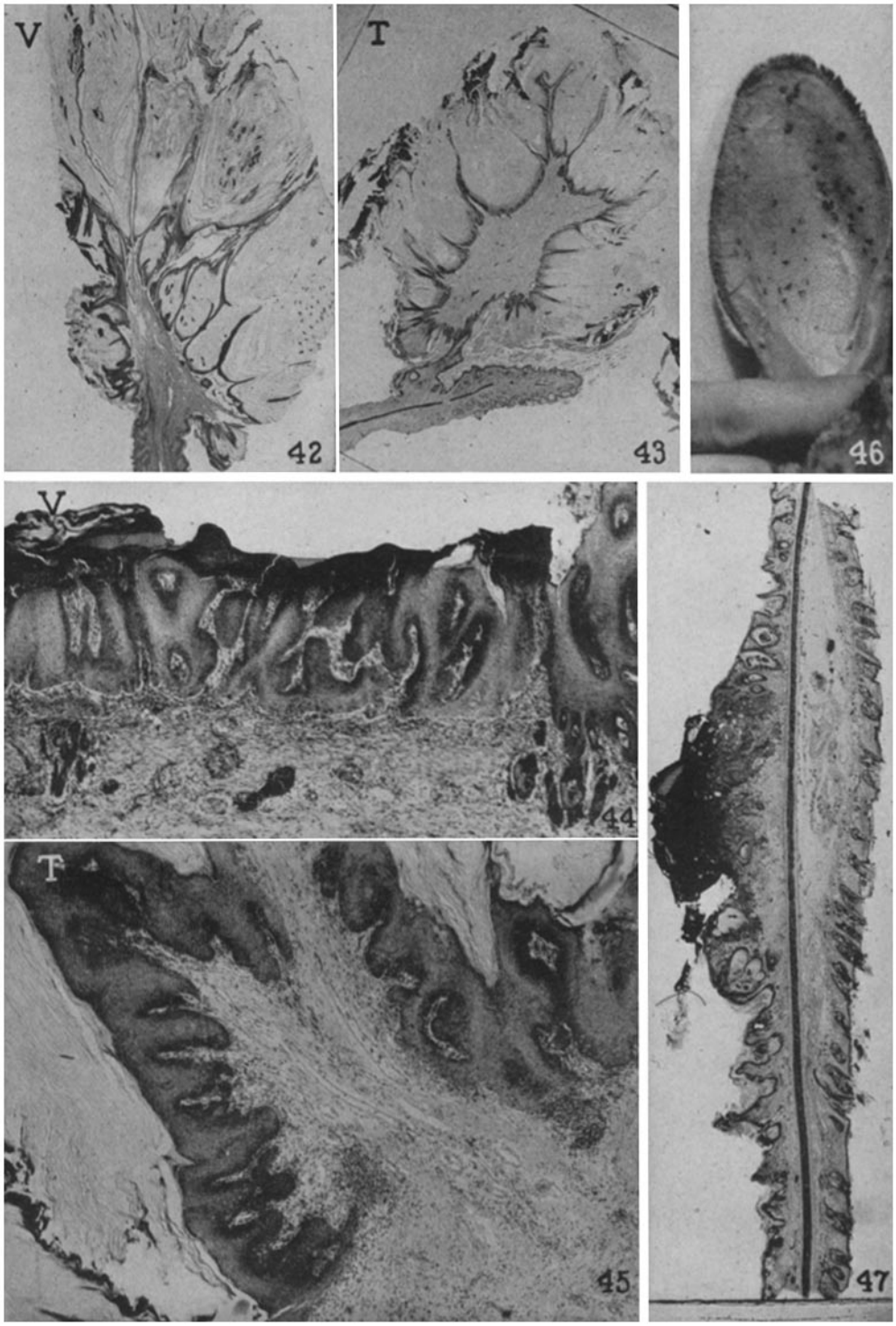
PLATE 27

FIGS. 42 and 43. Old, indolent, pedunculated virus and tar papillomas. The growth due to virus resulted from direct inoculation of a normal ear, and no later tarring was done. It had long since been stopped in the case of the tar tumor. The connective tissue cores of both growths are sclerotic, and masses of keratinized material fill the wide spaces between the infrequent papillomatous fingers. × 3½ and × 4, respectively.

FIGS. 44 and 45. To illustrate the retrogression of virus and tar papillomas. In both cases the living epithelium along the base of the growths has the form of narrow tongues into which lymphocytes have entered here and there; and such elements are fairly abundant in the underlying sclerosed connective tissue, together with macrophages. Connections can be seen between the hair follicles and the epithelium of the virus papilloma. Such connections often reappear when tar tumors retrogress (Ichikawa and Baum, *Bull. Assn. franç. étude cancer*, 1924, **13,** 257), though none is to be seen in the present instance. × 41 and × 48, respectively.

FIG. 46. "Ink spot" pigmentation where numerous melanotic virus papillomas, up to 1 cm. in diameter, had recently disappeared. The spots persisted, and later the papillomas appeared again at several of the situations they marked. × ½.

FIG. 47. Edema on the outer side of the ear opposite a tar carcinoid. There was no extension of the growth through the cartilage. × 6.



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