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

II. The Evidence Provided by the Tumors: General Considerations

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

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

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In an accompanying paper the relationship of the Shope virus engendering papillomas to the cancers deriving from these growths has been approached from the side of the virus. It will now be considered in the light of certain data provided by the tumors themselves.

The papilloma is known to be characterized by those traits of appearance and behavior which distinguish neoplasms from other pathological processes (1). Much new evidence on this point has lately come to hand.

The Subcutaneous Extension of Papillomas

The papillomas produced experimentally in domestic rabbits, by the inoculation of virus into scarified areas of skin, not infrequently invade the layer of reactive tissue that gradually forms beneath them, with result in deep pearls lined by the virus-infected epithelium. An instance has been figured in a previous paper (1). When papillomas are prevented from growing outwards by covering them with a layer of collodion soon after they appear they push down until checked by the fibrous corium and form subepidermal masses that may become large (1). Three instances have been observed in which, as result of essentially similar pressure conditions, occurring naturally, the "spontaneous" papillomas of the cottontails burrowed into the subcutaneous tissue. The dealer had been requested to send us all rabbits carrying lumps under or next the papilloma.

W. R. 40 N, from Kansas, had on receipt a rounded, discrete mass in the subcutaneous tissue of the inner surface of the right thigh (Fig. 1). It was cream-colored, an irregular, somewhat flattened sphere, 4.5 by 4.5 by 3.0 cm. across, of doughy consistency, with a sooty, mat-like skin papilloma, 1 cm. high and 2 by 1.5 cm. in diameter, surmounting and attached to it. The surface growth was so situated as to be constantly pressed upon when the animal sat crouched, and it had a flat, worn top. About 3 cm. away, where the skin covering the most projecting portion of the deep mass had been stretched tense, it had undergone ischemic necrosis and separation over an area about 1.5 cm. across. When the scab thus formed was pulled away there was disclosed a cyst filled with irregularly concentric lamellae, each about ½ mm. thick, composed of a slightly moist, soap-like material which shelled out clean save at one spot at a considerable distance from the surface mass, where a nonpigmented papillomatous growth, on a base about 1.5 cm. in diameter, projected inwards from the cyst wall. The lamellae,—which had been formed by keratinization of the epithelium of the growth just mentioned,—were ranged in layers about it like the petals of a cabbage rose. Elsewhere the cyst wall was smooth. Its contents were readily shelled out and the deep papilloma was brought to the surface by pushing it through the hole left by the scab, where it retained its position, proliferating as a cream-colored, vertically striated cylinder which on biopsy had the usual, papillomatous character. In consequence of the new state of affairs the sooty, surface papilloma was turned at right angles to its former position, and, thus relieved of pressure, it built up rapidly into a high cone (Fig. 2).

Two other cottontails were found to have subcutaneous masses nearly as large as that described and capped like it with mat-like papillomas exhibiting pressure effects. One of the surface growths was situated on the lower abdomen where the thigh pressed upon it, the other just back of the axilla. In the latter instance the low, dry papillomatous peaks were worn down into a cup, where the upper joint of the fore leg pressed. Elsewhere in the case of this animal, there were several jagged, superficial papillomatous expanses of the ordinary sort on distant skin regions free from pressure. The surface papillomas were sooty in both rabbits, whereas the deep growths were creamy. In the regions where these projected most the skin had undergone a dry necrosis.

All three rabbits are still under observation.

In several instances of skin papillomas experimentally induced in cottontails deep extensions of aggressive character have developed secondarily. Two of these will be reported upon.

A 5 per cent extract of glycerinated virus material, W. R. 595 + 596, a notably active material, was rubbed into a scarified square on the right side of a cottontail (5 N). A solid mass of characteristic sooty papillomatosis developed, which soon became a high, truncated cone that occupied the entire area of inoculation

and was dry, vertically ribbed and almost black. Its living, slowly advancing, bulging, basal edge was sooty in hue practically everywhere. After 10 weeks, knobs were felt on the under side of the cone, well within its edge, and small rounded spheres, discrete or partly coalesced, had appeared outside (Fig. 3). They were situated in the subcutaneous tissue and could be moved in all directions save away from the main growth, palpation disclosing that each was connected with this by a narrow, flexible cord. Two stages in the later course of events are shown in Figs. 4 and 5. The outlying nodules, almost spherical when first noted, soon elongated, and the distance between them and the main growth gradually widened, as result of growth at their outer side combined with resorption at the inner. With one exception the nodules that traveled away most rapidly from the surface mass had rounded, creamy outer ends, as seen through the skin; but further back, the nodule was ruddy with distended vessels, and so too was the overlying skin. In the exception mentioned the growth advanced by thrusting out prong-like processes from a broad, deep extension of the surface mass, and these became thickened and somewhat blunted (Fig. 6). As time passed the cords connecting the distant nodules with the primary growth could no longer be felt, but their disappearance became progressively less complete as the nodules crept further away, owing to the continual enlargement of the latter, a process which obviously involved the leaving behind of more and more keratinized material.

The migrating nodules followed the course of the large veins in all cases, and their advance was furthest in the general direction of the axilla, though one of them curved aside after a time as if it had met an obstruction (Fig. 5). They are still advancing and enlarging. All are creamy in contrast to the melanotic, cutaneous papilloma. The latter has been lifted several centimeters above the skin level by a mass of deep growth which has gradually formed under it (Fig. 6). This is firmly attached to it, and has the shape of partially fused spheres.

In order to learn the method of advance of the growth the two prong-like extensions were excised, together with the tissue about them, and sectioned serially, one in its long axis, the other transversely through its tip. The excision was done on the 138th day, when the prongs were advancing but slowly. Like the subcutaneous masses previously studied, they consisted mostly of keratinized epidermal cells (Fig. 10) which underwent constant additions through the activities of a thick, living layer of epithelium which had the invasive character manifested by the virus-induced papilloma when growing after experimental implantation in notably favorable situations within the body (1). The main direction of advance was along and between some large blood vessels. In sagittal section the prong was wedge-shaped, but the outline of the wedge was scalloped because the epithelium had directly invaded the underlying voluntary muscle at numerous situations, the invading tongues later broadening and keratinizing (Fig. 27). Only by the secondary keratinization with cyst formation could the growth be discriminated from a squamous cell carcinoma. It was markedly desmoplastic, surrounded everywhere by new, reactive connective tissue, save in a few places where its

advance appeared to be taking place with such rapidity (Fig. 27) that it had outstripped the reactive proliferation round about.

The growth had progressed between vein and artery, where were the large lymphatics as transverse sections have shown. At the furthest point reached, however, the epithelium had ceased to proliferate, and rounded out into small cysts enclosed in a thick layer of connective tissue wherein the lymphatics were lost (Fig. 7). The main direction of its advance had been checked, as in the case of one of the large nodules of Figs. 4 and 5, which first followed the vessels and then curved sideways. At numerous situations elsewhere it was actively invading, though, and indeed in some places penetrating directly between the muscle fibres (Fig. 27).

While the findings clearly indicated that the subcutaneous extensions had taken place along the lymphatics, if not within them, it was plain that the growth could advance independently of these channels, by direct invasion of the tissues. In the following instance, of a less aggressive papilloma, the penetration along the lymphatics was especially noteworthy.

A 5 per cent extract of virus material W. R. 18, which is especially active, was tattooed into a number of scarified spots on the sides of W. R. 45. Characteristic discrete horns developed, reaching a diameter of 1 to 1½ cm. by the 55th day, but thereafter not enlarging greatly. Owing to oversight the animal was not examined again until the 134th day. Then six smooth, spherical or oblong nodules from 0.8 to 1.5 cm. in diameter were present in the subcutaneous tissue of the right side, under or near the skin papillomas, four of them completely independent of these latter. A tough, narrow cord could be felt connecting a fifth with a surface papilloma, and the sixth lay beneath one of the latter and was fixed to it.

In the subcutaneous tissues of the left side, under and attached to several of the tattoo papillomas, was a large mass rather like a lobulated kidney in shape (Fig. 8), 6 by 4 by 2 cm. in diameter, with several attached spheres or pendule-like protrusions; and there were two other deep nodules further off, one 1 cm. away from the nearest surface growth, the other 5 cm. away and in the precise position of the superficial inguinal gland. The nearer of these two seemed wholly independent of all of the other growths, but the further, which was bean-shaped and 1.5 cm. in longest diameter, was connected with the main subcutaneous mass by a rounded strand 0.15 cm. thick. It was removed for section together with the adjacent portion of this strand, and a few weeks later a "pendule" was also taken for section (Fig. 11). Both proved to be cysts walled with papillomatous epithelium of the characteristic sort and filled with keratinized scales formed by the proliferation. The strand connecting the main, deep mass with the inguinal growth,—which showed no remnant of lymph gland,—consisted of connective tissue undergoing fibrosis, with dying or dead epithelial cells along its center (Fig.

13). All of the deep nodules were creamy, in contrast to the sooty hue of the surface growths, except one which appeared to be of a dubious, pale gray as viewed *in situ*.

The skin over those subcutaneous masses which projected most dried and came away soon after they were first observed, with loss of some of the surface papillomas and of the soap-like contents of the cysts. The exposed epithelial lining of the largest of these now underwent a surface proliferation forming papillomatous peaks like those already present, save that the new ones were cream-colored. Other cysts became infected with pus-producing bacteria and eventually were resorbed *in toto*. A few small ones which had not ruptured persisted for many months as such. At death, on the 630th day, only one remained, a somewhat flattened cyst of the ordinary sort. Its epithelium showed mitoses and in one region was directly invading the reactive connective tissue.

As in the case of W. R. 5 N, the subcutaneous extensions advanced farthest in the direction followed by the main lymphatic channels. There is every reason to suppose that the nodule in the inguinal region had replaced a lymph gland. The general course of events was like that Handley has reported for cancers of the human breast (2); and the eventual death and resorption of the epithelial cord (Fig. 13) connecting the distant nodule with the main mass was such as he has described. But despite these features, and the direct invasion of the muscle that took place in cottontail 5 N, the papilloma remained merely such, at worst a locally malignant growth.

It will have been noted that with one dubious exception all of the subcutaneous extensions in cottontails were devoid of pigmentation, whereas the skin papillomas were invariably sooty. One may suppose that under the conditions of downgrowth the stimulated melanophores, passively included in the proliferating surface mass (3), were left behind. But another reason existed for the creamy hue. The downgrowths evidently derived from the most vigorously proliferating cells in the papillomas,—which were themselves essentially composites of competing cell aggregates (4),—and such aggressive aggregates usually become non-pigmented in time, even when growing on the skin surface.

In the case of W. R. 5 N, the surface papilloma was spotted with dark gray during the early days of its formation, plain evidence of its composite character. When it had become a solid, horn-like structure, the cell aggregates well within its base were inevitably pent on all sides save beneath; and downwards the more vigorous ones grew in default

of other opportunity, and then laterally in consequence of pressure conditions, until they had emerged from beneath the horn. None sprang directly from the edge of the latter, where expansive growth could take place. The deep extensions must have been narrow and cord-like on first emerging from beneath the horn, for they did not attract attention then; but with the later relief from pressure they rounded out at their further end into cysts.

The papillomas experimentally produced in domestic rabbits by inoculation with the Shope virus are far more formidable growths than those in wild rabbits, and they much more frequently go on to cancer; yet they do not penetrate into the subcutis though frequently pushing down during the precancerous period into the reactive tissue immediately beneath them, or extending laterally just beneath the epidermis with result in superficial, outlying pearls. They are checked by the stout, fibrous layer of the corium, and in consequence their proliferation is confined to the loose connective tissue immediately beneath the epidermis, unless indeed the fibrous barrier has been weakened by inflammatory changes, in which case they may go somewhat deeper. In wild rabbits no such barrier exists, the corium being a flimsy, web-like structure.

The Survival and Proliferation of Embolic Fragments of the Papilloma

The virus-induced papillomas frequently penetrate into the blood and lymph vessels but their cells adhere to one another, retaining the tenacious association that is so evident in the high, peaked surface growths. Instances of unaided metastasis formation have yet to be observed, but slight operative interferences are followed not infrequently by the development of secondary nodules in the lungs.

D. R. 4-06, an agouti rabbit employed for neutralization tests, developed papillomatosis on seven squares of skin where mixtures with virus 6-32 and serum or Tyrode had been rubbed in. After 10 weeks Scharlach R was injected into one papillomatous mass, extracts of dried and glycerinated cancer tissue into two others, and a string impregnated with a Tyrode extract of a cancer was threaded through a fourth mass and left there. No cancers ever developed; but when the animal died, after 311 days in all, a sphere 1 cm. across, consisting entirely of dead papillomatous tissue, was found in a lung (Fig. 9). The surface growths were now indolent.

In this instance the interference had been inconsiderable, yet a secondary nodule formed in the lung. It became necrotic after growing for a while, as frequently happens to the papilloma in other situations (spleen, mesentery (1)) where its enlargement is mainly expansile and eventually compromises the blood supply. The following instance shows that growth of the papilloma in the lung will continue progressively if intercurrent influences render the local conditions favorable.

Agouti rabbit 4-78 was implanted intramuscularly on the 15th, 38th, and 73rd day with bits of one of the growths resulting from the inoculation of virus W. R. 738 into seven skin areas. On the first occasion the tissue consisted of shavings from the surface of the young growth, and on the others of pieces procured from its base by operation under ether anesthesia. The tissue was suspended in Tyrode solution and injected into the muscles of the upper part of a leg. Opportunity was taken at the second and third operations to procure a slice from one of the nodules that had resulted from previous implantation in another leg.

At the 249th day, when the rabbit was killed, the seven surface papillomas had dwindled greatly and presented the microscopic picture of retrogression, whereas all of the leg growths were very large and had draining sinuses due to bacterial infection. Their tissue was proliferating actively amidst an abundant, new connective tissue containing foci of acute inflammation and necrosis. The left lung held two nodules, one a sphere, 0.8 cm. across, made up almost entirely of keratinized scales, arranged in papillomatous fashion near the center but concentrically further out, and walled with a shallow, living layer of proliferating squamous epithelium stretched and flattened by interior pressure, as often happens (1) yet having the general character of epidermis infected with the Shope virus. There was a thin connective tissue encapsulation.

A second nodule more than 2.0 cm. in diameter consisted mostly of keratinized material, but at its edge living epithelium was invading a profuse connective tissue that showed foci of acute inflammation and necrosis. In a few regions the proliferating epithelium was directly replacing the pulmonary tissue (Fig. 12). In one region it projected into the pleural cavity. Everywhere the growth had the aspect assumed by the papilloma when growing under the favoring circumstances of reactive connective tissue proliferation. The secondary cystic keratinization marked it as such a growth.

The influence of local conditions received a double illustration in this animal. When it died all the primary papillomas on the skin, a relatively unfavorable site, were retrogressing and a lung nodule situated in uninflamed tissue was almost entirely dead. A second lung

nodule, however, and the growths in the legs, were progressing actively, but all these had the aid of local inflammatory processes.

The insusceptibility of the lung tissue to infection by the Shope virus, the ease with which the papilloma can be transplanted to the interior of the host, and the circumstances under which the pulmonary nodules developed in the foregoing instances (and in others previously reported) point to cell emboli as responsible for them. It has seemed well nevertheless, to carry out an experiment whereby the fate of such emboli could be directly ascertained.

Two brown-gray rabbits were inoculated into scarified abdominal areas with a 5 per cent extract of virus material W. R. 538 + 638, and confluent papillomatosis soon appeared. A few days later some of the growth was shaved away, cut fine, suspended in Tyrode solution, and injected into an ear vein. After another 5 days the animals were killed. The lungs showed scattered, minute, translucent, gray, rounded, dot-like solidifications with, in the case of one rabbit, a few slightly larger dots of the same sort, up to 1 mm. in diameter. Numerous blocks were taken and sectioned serially.

The lung findings were the same in both animals. Many small, discrete foci of characteristic papillomatous growth were present. At the center of even the largest of these the remains of an embolus could be discerned, often to be identified as such by fragments of hair included in it (Fig. 16). Many dead or dying emboli were also present, lodged in arterioles, usually where they forked. The cells of some of these had proliferated before they succumbed, and formed syncytia (Fig. 17). Remarkably little fibrin had collected about them, but a few scattered polymorphonuclear leukocytes were present in the neighboring tissue, as generally happens where the squamous epithelium of tumors is dying.

The findings were especially informative in serial sections that happened to parallel the arteriole, so that it could be seen both proximal and distal to the cell embolus (Fig. 18). All of the latter had some platelet clot about them, and those that were proliferating had lost their angular form and rounded out by a lateral proliferation of the surface epithelium (which showed frequent mitoses), so that they had come to consist of a core of dead, keratinized material and a living rind. The arteriole was distended by their enlargement, and sometimes the neighboring bronchiole had undergone a sabre-sheath compression. The best established emboli had become more or less surrounded by new-formed connective tissue and fixed upon the arterial wall. Where this had happened, and only here, the proliferating epithelium had extended into the lung, pushing through the new connective tissue as a carcinoma does (Fig. 19), penetrating the stretched and thinned media of the vessel, and spreading in the loose pulmonary tissue, with compression or occupation of the alveoli (Fig. 16). Around some of the advancing growths there was a sparse scattering of lymphocytes, and some

of the emboli were undergoing degeneration after having done well primarily and become attached to the vessel wall. Their epithelium was dying and the material at their center had separated as if from fluid accumulation (Fig. 19).

None of the emboli that consisted of only a few cells had given rise to a growth: each of the developing nodules had at its center a piece of the surface papilloma that was of relatively large size. The new-formed connective tissue attaching the embolic masses to the vessel wall had frequently become covered by endothelium, but this had undergone no further proliferation, and the neighboring alveolar epithelium had altered no further than to assume the cuboidal form where the pressure of the developing growth had caused atelectasis.

The findings left no doubt that the lung growths arose solely by the proliferation of cell emboli. Schmidt has reported, in a paper now classical (5) that the lung metastases of visceral carcinomas arise from the larger cell emboli only, and that these become attached to the arteriolar wall by new connective tissue extending into the pulmonary tissue. Where such attachment fails to take place the embolus may live for some time, its cells often forming a syncytium; yet though in immediate contact with the vessel wall they fail to penetrate it.

The occurrences leading to death or establishment of the papilloma cells were precisely like those noted by Schmidt. Yet this parallelism in the course of events cannot be taken to mean more than that the fate of pulmonary emboli composed of epithelial cells capable of proliferation and invasion is determined by conditions and reactions that are characteristic of their situation.

Factitious Malignancy

When small pieces of a virus-induced papilloma, procured soon after it appears, are implanted in the voluntary muscles of the host, growths usually develop promptly, and, as already remarked, they may look and act as if malignant when they are proliferating in the midst of connective tissue proliferation called forth by bacterial infection. Yet they do not metastasize even when they have the aspect of squamous cell carcinomas (as determined by biopsy), and later they may retrogress if the infection is conquered by the host, their epithelium first reverting to the orderly papillomatous form, and eventually keratinizing entirely,—changes clearly recorded in the markings of the dead material. The influence of local infection to determine the fate of papillomatous nodules developing from cell emboli that reach the

lungs has been illustrated by the case of D. R. 4-78 (Fig. 12). It is not possible to tell whether the spontaneous invasion of voluntary muscle by the deep extension from the surface papilloma of W. R. 5 N (Fig. 27) was induced by local infection, though the profusion of reactive connective tissue about the growth suggests such a possibility.

The papillomas exhibiting factitious malignancy of the sort now under discussion have a feature in common which distinguishes them from the anaplastic carcinomas arising from the papilloma, though not always from the other growths that originate from the latter: they keratinize secondarily in an orderly way and form cysts filled with lamellated material. None of the various stimuli that we have brought to bear on the papilloma has caused it to become enduringly anaplastic like the more malignant of the squamous cell cancers that eventually develop from it.

The Tumors Deriving from the Papilloma

A not inconsiderable variety of tumors derive from the papilloma. As already reported, all are the outcome of changes in the epidermal cells infected with the virus, and all are expressive of progress in a single direction, namely toward anaplastic squamous cell carcinomatosis (6). The study of a large material has confirmed and extended these findings.

Many of the derivative tumors differ but little from the parent growth, some being recognizable only by their encroachments upon it and others by slight histological peculiarities in addition. Even those closely resembling the ordinary papilloma exceed it in aggressiveness: otherwise they would not attract attention. In our previous papers instances have been pictured which illustrate these facts. Discrete "onions" of aggressive, atypical proliferation may appear in the midst of a papillomatous mat, or "papillomas of the second order,"—so called because they have an unusually complicated pattern (Fig. 20),—or invasive cystic tumors. Occasional aggressive growths have been encountered which resemble ordinary, virus-induced papillomas so absolutely in their morphology that their status has remained uncertain (Fig. 21). When the alteration toward malignancy has been but slight the tumor may not become irreversible in its course. The growth like a glans penis that progressively replaced the ordinary

papilloma of D. R. 2-48 (6) never invaded the underlying tissue, and during the months before death took place it became stalked and much smaller, owing in some part to maceration of its surface. Its course was reminiscent of that of many tar papillomas. In general, however any considerable alteration toward anaplasia has been attended both by irreversibility and the tendency to metastasize. We have yet to observe a manifest, squamous cell carcinoma which did not pursue a progressive, destructive course. The cancers of D. R. 2-38, which appeared to be retrogressing when this animal was last reported upon (6) brought about death in the end and had merely become scirrhous.

Especial interest attaches to those tumors of problematic malignancy which have metastasized; for sometimes the secondary nodules differed in no essential respect from such as result from the experimental implantation of ordinary papillomatous tissue in favorable sites (Figs. 22, 23, 28, 29). More will be said upon this head further on. As might be expected retrograde local changes and necrosis are frequent in consequence of compromised vessels, and some cell nests may perish, owing to complete keratinization. A squamous cell metastasis in the lungs became almost entirely necrotic as result of pressure conditions that cut off its blood supply.

The new tumors that are expressive of but slight changes in the papilloma seldom grow large. We have never observed a "papilloma of the second order," for example, that was more than 1 or 2 cm. in diameter, nor has metastasis occurred from a growth of this sort. Such tumors tend to undergo further change in the direction of squamous cell carcinomatosis, as do the others that are relatively benign; and their enlargement is so slow as to provide ample opportunity for the change to supervene. The much more rapidly growing, malignant cystic papillomas (6) often kill the host before anaplastic changes have become advanced, though they are usually perceptible here or there. Such growths extend under the skin, forming subepidermal pearls and involving it in ulceration (Fig. 30), and they advance rapidly toward the axilla or groin along the course of the lymphatics (Fig. 31), and frequently metastasize to the regional glands (Fig. 24). The malignant papillomas which are not cystic very soon change into squamous cell carcinomas, but the papillomatous character is occasionally evident in the metastases, which are usually confined to the local glands

(Figs. 25 and 26). Squamous cell carcinomas almost regularly metastasize, both locally and often to the lungs as well, the frequency of the latter happening corresponding in general with their degree of anaplasia.

A noteworthy tendency exists for multiple cancers of the same individual to be all of one general sort, though frequent exceptions are observed. They may be mostly fungoid, malignant papillomas, cystic papillomas or eroding, desmoplastic, squamous cell carcinomas. The longer the host survives the greater is the likelihood that all will be of this last type, however they may have begun. Those animals which are relatively unfavorable to cancer, as evidenced by its late appearance, are especially the ones in which it assumes and long retains the cystic form.

While cancer is the most obvious consequence of the changes in the papilloma, the other tumors deserve equal, if not greater attention. All are expressive of the potentialities of the cells originally infected with the virus, a limitation which does not exclude considerable diversity of type, as has been seen. Multiple tumors of a single kind, occurring in the same animal, may differ slightly yet distinctively.

Induced Malignancy

It is possible to precipitate malignancy by various stimulative interferences with the papillomas. Many instances attesting to this fact have been procured since our first publication (6); and injection of Scharlach R into the base of papillomas has been repeatedly utilized to bring on cancer before its time. The success of this procedure is the more remarkable because the dye is not itself a carcinogenic agent, a fact sufficiently attested by the outcome of its clinical use. Büngeler (7) has reported the development of cancer after 15 months in a rabbit repeatedly injected with the dye; but there was an associated arsenical keratosis. The epithelium of papillomas caused by the Shope virus responds far more actively to Scharlach R than does ordinary epidermis, and under its influence large fungating and burrowing, cystic growths rapidly form; yet if stimulation is stopped retrogression or reversion to ordinary papillomatosis may ensue, even after the dye injections have been repeated at intervals during many weeks.

It is our experience that for a long time, 3 months at the least,

the virus-induced papillomas continue to be merely such no matter how stimulated, whether by transplantation, incision, vaccinial or bacterial infection, Scharlach R injections, or the permanent insertion of strings impregnated with dry or glycerinated cancer material,—procedures all that have been repeatedly utilized.

On the assumption that removal of the papilloma cells to an especially favorable milieu might disclose the existence of malignancy sooner than would otherwise be the case, we have operated repeatedly on the same papilloma at intervals of a few weeks, on each occasion implanting bits of it in the muscles of one of the legs of the host. Each time the material was taken from the same spot at the base of the growth, a procedure possible because it regenerated rapidly in the intervals. Thus cells were procured that had the additional stimulus incident to repair. But though the experiment was carried out upon several domestic rabbits with vigorously growing papillomas, no positive results were obtained. True, some of the leg nodules did eventually become cancerous, as proven by extension through the fascia, involvement of the skin, and metastasis formation, but this happened only after the lapse of several months when the surface growths had also undergone malignant changes.

The Transition to Cancer

As already reported, the development of malignancy is preceded by alterations in the aspect and behavior of the papilloma. Its enhanced vigor of growth, loss of pigmentation, tense, fleshy base, the crowded disorder of its cells, their extension downward and laterally to form "extramural" pearls, and an increasing irregularity of keratinization (6), all bespeak progress toward malignancy. Slight interferences may now precipitate this, yet often they do not, however frequently repeated, and some growths may remain merely more active and aggressive papillomas, long after neighboring ones in the same host have become cancers. The multiple papillomas produced in any one animal by tattooing virus into the skin all behave alike with occasional noteworthy exceptions (8), and all may enlarge rapidly at one period, then perhaps become stationary or dwindle, and after a while enlarge again. The observation that the more vigorously the papillomas proliferate the more likely is cancer to ensue has been abundantly confirmed; yet not infrequently it appears in some one of a number of shallow based, unpromising growths,—doubtless as a result of local stimulative occurrences. Carcinosis is obviously an outcome of

favorable conditions which may be very local, and operative during a brief period only. It is conceivable that, when they happen to be notably favorable cancer may arise even in a papilloma that is retrogressing everywhere else; but the phenomenon has still to be encountered.

Morphological Evidence of the Influence of the Virus on the Cancers

A large proportion of the tumors deriving from the papillomas exhibit some of the histological characters of the latter.

In producing papillomas, the virus acts primarily on the germinal layer of the epidermis: its cells enlarge, and often become tall and narrow, and the nucleus also enlarges, though it retains the vesicular form. Rapid multiplication by mitosis forces most of the cells toward the surface, and since proliferation still goes on in the polygonal layer this becomes unnaturally thick. The granular layer consists of elements less flattened than normal, more coarsely and irregularly granulated, and frequently containing parakeratotic lumps that are sometimes large. The cells die before they have finished flattening and keratinizing, and they heap up into tenacious, dry, scoriaceous or horny peaks.[1] The same series of events, comprising a pathological differentiation, usually takes place in the nodules resulting from implantation of the growth within the host, the cells preserving their organoid association and manifesting the usual tendency to form papillae with narrow supporting cores, though now these papillae point toward the center of the mass instead of outwards, and the keratinized material is soft.

In all save the most anaplastic of the tumors deriving from the papillomas,—those composed of elements that no longer differentiate to any noteworthy degree,—the sequence of changes just described can be more or less clearly perceived.

The fact has already been stressed that there are growths of which it is impossible to decide whether they are merely ordinary papillomas of unusually aggressive habit or new and different tumors (Fig. 21). The cystic papillomas have precisely the aspect assumed by the ordinary papilloma when growing after implantation in especially favorable sites, as *e.g.* in the leg muscles; and their metastases may be indistinguishable from the cystic nodules that result from such implantation (Figs. 22, 23, 28, and 29). The frankly malignant papillomas may give rise to secondary growths in which the papillomatous structure is retained (Figs. 25 and 26). Cystic extension along the lymphatics, such as we have en-

[1] For further details Hurst's excellent description can be consulted (*J. Exp. Med.*, 1933, **58**, 607).

countered in wild rabbits carrying the papilloma, takes place sometimes in the case of the squamous cell cancers as well, even when they are anaplastic (Figs. 14 and 15).[2]

In appraising the significance of these phenomena it is essential to remember that the potentialities of epidermal cells are many,—as might be inferred from the normal complexity of their differentiation. At any stage in the maturation from germinal epithelium, so long as the capability to divide is retained, the cells may undergo abnormal proliferation with some distinctive pathological picture as result. One need only recall in this relation the varied character of human epidermal cancers, and of the epidermoses of man,—keratosis senilis, seborrheic warts, Bowen's disease, and so forth. The proliferative diseases of the rabbit's skin find scant record in the literature, but much has been written on the growths which are consequent upon tarring and other experimental procedures. The description of these growths provides clear evidence that the character of the tumors deriving from the virus-induced papillomas is not inevitable to the cells of which they are composed. True, the virus-induced papillomas and the tar papillomas strikingly resemble one another (9), though the latter usually come to consist mostly of connective tissue after a time and become stalked, changes that are very excèptional with the virus-induced growths; but the tar tumors, while frequently forming cysts, fail to advance by cystic extension along the lymphatics (Figs. 10, 11) or to give rise to cystic or papillomatous metastases, as do many of the tumors deriving from the Shope papilloma (Figs. 24 to 26). There would seem to be sufficient reason to conclude that the virus responsible for the latter continues to exert an influence upon these growths, as well as upon those which differ less or scarcely at all from the primary papilloma (Fig. 21). The cystic character of some of the more anaplastic tumors suggests that even in their case the virus may still be effective (Figs. 14 and 15).[3]

DISCUSSION

The observations of the present paper and those preceding it show that the virus causing papillomas in rabbits directly conditions the

[2] Often the cell cords of such tumors are discontinuous, either because the malignant epithelium has been "strangled" by the reactive tissue, a frequent happening in human breast cancer, or because some of the cells have come loose and been carried a greater or less way by the lymph stream,—as does not happen in the case of ordinary, virus-induced papillomas that have invaded the vessels (1).

[3] In two recent instances inclusion bodies, of Types A and B (Cowdry) respectively, have been present in virus-induced papillomas that differed slightly yet distinctively from the general run of these growths. The finding brings up the possibility that a superinfection of tumors with viruses may sometimes occur under natural conditions, with result in alterations in the character of the growths. Our attempts to transmit to other virus-induced papillomas the agent presumably responsible for the Type B inclusions have been unsuccessful.

incidence and character of the tumors arising from these growths. The evidence concerning the etiological rôle of the virus will be briefly reviewed incidentally to a consideration of its significance.

The virus functions as the first cause of the cancers, through the papillomas that it induces. The more active it is, as evidenced by the time of appearance and behavior of these growths, and the greater the concentration of the inoculum, the sooner and oftener does malignancy develop. The cancers originate from the cells that are proliferating under the influence of the virus. But similar facts hold true of other carcinogenic agents,—dibenzanthracene for example,—though the virus is far more effective than any of these in bringing cancer about.

The papillomas caused by the virus have the immediate character of neoplasms, and, even in cottontails, which are relatively resistant hosts, they may exhibit some of the traits associated with malignancy, —as when they grow down spontaneously into the subcutaneous tissue, invade voluntary muscle, and advance along the lymphatics after the fashion of cancers of the human breast. In domestic rabbits, unaccustomed hosts for the virus and very favorable to its action, the papillomas often verge on the malignant. Sometimes a decision as to whether they have attained this state or have merely responded to fortuitous, intercurrent influences by simulating malignancy can be made only in the light of the final happenings. The presence of the Shope virus has been demonstrated indirectly, by serological test, in two animals to which a cancer deriving from a papilloma had been transplanted; but this finding does not necessarily mean that it was immediately responsible for the cancers, since wholly extraneous viruses can persist within neoplasms and undergo transfer with them. More significant is the evidence that the virus continues to exert an influence on a large proportion of the tumors originating from the papillomas.

The facts bespeak a relation of the virus to the cancers far closer than that noted of any other carcinogenic agents thus far studied. The latter bring about a chronic tissue disturbance upon which cancer develops as an essentially different pathological process; and no evidence exists that they do more than dispose to the neoplastic state. Dibenzanthracene, the only one of them that has been followed adequately, disappears from the tumors to which it gives rise (10). The

virus on the other hand directly engenders growths of neoplastic character in which it persists, increasing in amount (11); and from these growths a variety of other tumors arise, cancers amongst them, by changes which are gradual and often slight, to all appearances.

Active strains of the Shope virus are almost unconditioned in their ability to produce papillomas. They cause a frankly infectious disease, endemic in western cottontail rabbits and notably prevalent. But the cancers which are an ultimate consequence of this disease, as produced experimentally in domestic rabbits, appear only when a conjunction of favoring circumstances has come to pass. At least four sets of influences, in addition to the primary disposition of the host and the character of the virus material, act to condition the occurrence of malignancy, namely (*a*) local characters of the skin, (*b*) the peculiarities of the individual cell-virus associations, as expressed in terms of the cellular manifestations that are their outcome, (*c*) the influences of host origin but of undetermined character, which cause all the papillomas of any one animal to wax or wane together, and (*d*) those local factors which may encourage or prevent the cancerous change. Doubtless yet other influences affecting the carcinogenesis will come to light. Cottontail rabbits, the natural hosts of the virus, ordinarily become unfavorable to the papillomatous growths after a while, as manifested by their course; and cancer is rare in this species, though it does occur. In the more susceptible domestic species, the virus, though causing, accompanying, and increasing in the papillomas, is ordinarily rendered incapable of transmitting the growth. How this happens is not yet understood. But whatever the reason it in most cases wholly removes the papillomas of domestic rabbits, and the eventual cancers as well, from the sphere of the ordinary infectious diseases. The possible influence of conditioning factors to account for the statistical incidence of cancer in human and other communities has been stressed in a previous paper (1).

Is the virus, or some variant upon it, the immediate cause of the cancers deriving from the papilloma? This question should not be approached without due recognition of certain phenomena which act to confuse the issue. Prime amongst these is the occurrence of spurious cancers. The sensitiveness of the papilloma cells to external stimulation far exceeds that of normal epidermal elements. Even the latter

can mimic cancer temporarily, as when influenced by Scharlach R; and the cells infected with the Shope virus will do this in response to numerous influences as *e.g.* those provided by the intramuscular or visceral situation, by infection with bacteria that induce a proliferative connective tissue reaction, by repeated injections of Scharlach R, etc. The reversion to ordinary papillomatosis, which often takes place later, shows that the induced malignant activities, though real enough in their effects, are not an intrinsic character of the growths.

No matter how greatly the papillomas are stimulated experimentally, they do not assume the aspect of anaplastic carcinomas; yet nevertheless a possibility exists that some of the manifestations of growths of the latter sort are due to intercurrent stimulation. They are practically always infected with pyogenic bacteria, and it is reasonable to assume that stimulation from this source or another may make as greatly for anaplastic changes as in the case of the far better stabilized papillomas. The influence of bacteria to enhance the malignancy of human tumors has long been recognized. Yet one cannot suppose anaplastic squamous cell carcinomatosis in either rabbits or men to be maintained by agents that act as adjuvants to the essential cause of the neoplastic condition. The confusing influence of such agents must be reckoned with, however.

In studying the way in which the individual rabbit cancers arise, all phenomena referable to intrusive, accidental processes must be excluded if possible; and from what has been said it is clear that this cannot always be done. The only sound course is to view the process of carcinogenesis in the large. When this is attempted one perceives that the cancers derive from the papilloma by changes which appear to be continuous, and that they often undergo further alterations until a state of marked anaplasia has been reached (6). Histological examinations at an early stage in the malignancy regularly disclose a graded morphological transition to cancer. But it must be kept in mind that pictures as strongly indicative of such a transition are not infrequently encountered where a skin cancer has united with the epidermis secondarily. Neither such findings, nor the difficulty in telling whether a papilloma or cystic growth is malignant, nor the occurrence of individual tumors which exemplify every stage in the march to anaplastic squamous cell carcinomatosis can be adduced as

proof that tumors of the latter sort result from an intensification or gradually changed activity of the virus causing the papilloma. When discontinuous variations are available in sufficiently large number they can be so arranged as to produce a specious impression of continuity. It would be possible for example to assemble arrow heads in a series which might be taken as indicating that big ones had arisen from little ones. The tumors now under consideration could also be arranged in this way. But while many of them are not cancers at all, some differing remarkably little from the parent, virus-induced growth, yet in the aggregate they are the expression of a new state of affairs. They result from local modifications of the papilloma which are not accomplished until after many weeks or months of proliferation, no matter how much the growth is stimulated. Once the preliminary period has passed, new tumors can often be readily elicited by stimuli previously powerless to bring this about; and whereas previously the neoplastic process varied but little in type, the epidermal cells multiplying and differentiating in a remarkably constant way, however great the multitude of hosts, now from the growths of a single animal a variety of tumors may arise. It is not the individual character of these tumors but their unprecedented, relatively abrupt occurrence and their diversity that constitute the significant phenomena. By and large the changes represent something more than a mere enhancement of the papillomatosis.

Recent experiments have shown that when the Shope virus is brought into association with the disordered epidermal cells of tarred skin it gives rise forthwith not to ordinary virus-induced papillomas only but to tumors of all the kinds deriving from these, including the most anaplastic cancers (12). Whatever the meaning of the phenomenon it serves to emphasize the fact that the circumstances under which the Shope virus acts are far different from those obtaining in the case of any other viruses thus far studied, save those causing the chicken tumors. The papillomas are the outcome of an enduring partnership of cell and virus, and a change in either partner may react upon the other. The activities of the virus find expression in terms of the growths it causes, but their proliferating cells provide its milieu. Any considerable changes in the virus should find prompt reflection in the morphology and behavior of the papillomas, and, on the other hand,

any disturbance of the cells may alter the environment of the virus. When the latter has been introduced into domestic rabbits this environment, though strange, proves very favorable. It is doubtless stable enough under ordinary conditions; but when the papilloma has been subjected to repeated trauma, to bacterial infection or other intercurrent influences its cells may very well undergo sufficient alteration to affect the milieu upon which the virus depends. All in all the circumstances are precisely those which should lead to a formation of variant strains of the virus, as the general experience with other viruses attests.

SUMMARY

The papillomas caused by the Shope virus sometimes grow down spontaneously into the subcutaneous tissue and extend along the lymphatics in the same way as do many cancers of the human breast. They may even invade the voluntary muscle under such circumstances, taking on an aspect suggestive of squamous cell carcinomatosis, but ultimately they differentiate in the way characteristic of the papilloma. Slight operative interferences with papillomas may be followed by a development of secondary nodules in the lungs. These result from cell emboli, and the same local conditions determine their fate as are effective in the case of emboli composed of human cancer cells. The virus-induced papilloma is not only a neoplasm in its immediate aspect and habit but sometimes one that verges upon malignancy. The tumors, including the cancers, which eventually derive from it in favorable hosts, are representative of more than a mere enhancement of the activity of the growth. They develop within a relatively brief period of time but only after the papilloma has grown for a long while; and they are morphologically various whereas the parent tumor is remarkably constant in its form. Some of the new growths differ but little from the papilloma, however, even when possessed of the ability to metastasize, and many continue to be influenced by the virus.

The Shope virus is heavily conditioned in its carcinogenic activity, yet it is the nearest cause for cancer now known.

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EXPLANATION OF PLATES

The sections were stained with eosin and methylene blue except where otherwise noted.

PLATE 27

FIG. 1. Subcutaneous extension of a "spontaneous" papilloma of a cottontail rabbit (W. R. 40 N). The sooty surface growth on the inside of the thigh is flat and discoid, in contrast to the irregularly spherical, cream-colored, deep mass. The skin over the latter has dried and separated in one region (arrow). × ½.

FIG. 2. The same growths 21 days later. The keratinized material constituting most of the subcutaneous mass had been evacuated at time of the previous photograph, and the deep lying, nonpigmented papilloma (arrow) from which it had derived was brought to the surface. This growth is now covered with dried exudate. The sooty papilloma, relieved from pressure, has heaped up into a cone. × ¾.

FIG. 3. Beginning subcutaneous extensions from the base of a virus-induced papilloma in a cottontail (W. R. 5 N) (73 days). × ¾.

FIG. 4. A further stage of the extension (92 days). The discrete nodules have migrated along the large veins. The apparent connections with the primary growth are due in the main to foldings of the skin, which was stretched for photographic purposes. The cutaneous horn is raised on the side toward the abdomen by a subcutaneous mass having the form of partially coalesced spheres. × ¾.

FIG. 5. A still later stage (137 days). The direction of growth of one nodule (arrow A) has altered, and in consequence it now curves away from the vein. At

B and C are two prong-like subcutaneous extensions. These are more plainly to be seen in Fig. 6. × ¾.

FIG. 6. Lateral view of the growth (130 days). At A is the subcutaneous nodule designated with this letter in Fig. 5, and at B and C, the prongs. The independence of the outlying nodules is now evident, as is also the fact that the growths connected with the mass, and raising it, have extended from beneath it, not from its edge. × ¾.

PLATE 28

FIG. 7. Cross-section of the tip of the prong B of Fig. 6. The dark spot consists of the epithelial cells that have advanced furthest, but most of the tip consists of reactive connective tissue. It lies between an artery and a large vein (arrows). × 25.

FIG. 8. Subcutaneous extensions from virus-induced papillomas in a cottontail. At A is the scar left by excision of a nodule situated in the position of the superficial inguinal gland. It was connected with the large subcutaneous mass by a narrow fibrous cord (see Fig. 13). The "pendule" at B was later excised (see Fig. 11). Owing to the way in which the animal was held, the skin is raised into a fold between the nodule C and the main mass, but actually no deep connection existed between them. × 1.

FIG. 9. A completely keratinized papillomatous nodule in the lung of a domestic rabbit. For history see text. × 15.

PLATE 29

FIG. 10. Sagittal section of the prong C of Fig. 6 and the tissue about it. It consists mostly of keratinized material, but is irregular in outline owing to the invasive activity of the living epithelium. Near its tip small cysts have formed secondarily, and just beyond these a large vein can be seen in oblique section (V). × 7½.

FIG. 11. Section of the subcutaneous "pendule" of Fig. 8. It has been turned so that it appears to extend in a direction opposite to the real one. The growth consists mostly of keratinized epithelium and is characteristically papillomatous. Its papillae are introverted. × 4.

FIG. 12. Papilloma growing in the inflamed pulmonary tissue of a domestic rabbit. For history see text. × 24.

FIG. 13. Part of the fibrous cord connecting the large subcutaneous mass of Fig. 8 with the nodule found in the situation of the superficial inguinal gland. A profuse reactive tissue surrounds dead epithelium. × 165.

FIG. 14. Subcutaneous extensions from an anaplastic squamous cell carcinoma arising in a papilloma, as seen from the under side. The growth followed the large vessels, which have lost their blood content, however, in consequence of the excision, and hence are not visible. For comparison with Figs. 3 to 6. × 1.

FIG. 15. Longitudinal section of one of the finger-like extensions seen in Fig.

14. The growth was a squamous cell carcinoma with pronounced cystic tendencies. For comparison with Figs. 10 and 11. × 14.

PLATE 30

FIGS. 16, 17, 18, and 19. The fate of emboli of papillomatous tissue in the domestic rabbit. The animal furnishing the sections was killed 5 days after an intravenous injection of bits of a growth induced on its skin by virus inoculation. In Fig. 16 invasion of the lung is actively under way, but the position of the original embolus can be told by the fragments of hair included with it. Fig. 17 shows a dying embolus and the vessel in which it has lodged. The epithelial cells have formed a syncytium. In Fig. 18 the embolus at the fork of an artery, has proliferated and rounded out, but has not yet become attached to the vessel wall. In Fig. 19 this has happened and invasion has begun. A nearby embolus has undergone cystic degeneration and is thickly encapsulated in connective tissue. × 91.

PLATE 31

FIG. 20. A "papilloma of the second order," deriving from an ordinary papilloma induced with virus in a domestic rabbit. (All the later figures are from such animals). × 15.

FIG. 21. A deep papilloma of uncertain status. The animal died on the 191st day after the inoculation of virus into a scarified square of skin. It had then a confluent papillomatous mass several centimeters across, on the skin surface, and underlying it and extending somewhat beyond its border a larger subcutaneous nodule like a flattened sphere. The figure is taken from a section through the edge of both growths. At many points they were directly joined, but in the region shown they are separated by the thin layer of the superficial corium (arrow), marked as such by numerous sebaceous glands and more or less cystic hair follicles. They have precisely the same morphology, which is that of a somewhat irregular, virus-induced papilloma. Intact epidermis overlies the deep growth. Hematoxylin and eosin. × 15.

FIG. 22. Metastasis in a regional lymph gland from a malignant, cystic papilloma such as has been pictured in a previous paper (6). × 32½.

FIG. 23. Implantation nodule on the parietal peritoneum, resulting from the intraperitoneal injection of a fragment of an ordinary papilloma procured 23 days after virus inoculation, shortly after the growth appeared. The animal was killed 26 days later. The growth had entered a blood vessel (*vide* Fig. 15 of reference 1). For comparison with Fig. 22. × 32½.

PLATE 32

FIG. 24. Part of a metastasis of a cystic papilloma in a lymph node. × 15.

FIG. 25. Another such metastasis. The resemblance to an experimental implant of an ordinary virus-induced papilloma is complete. × 15.

FIG. 26. Still another such metastasis in cross-section, showing a living, introverted, papillomatous process and keratinized material deriving from other, similar processes. × 35.

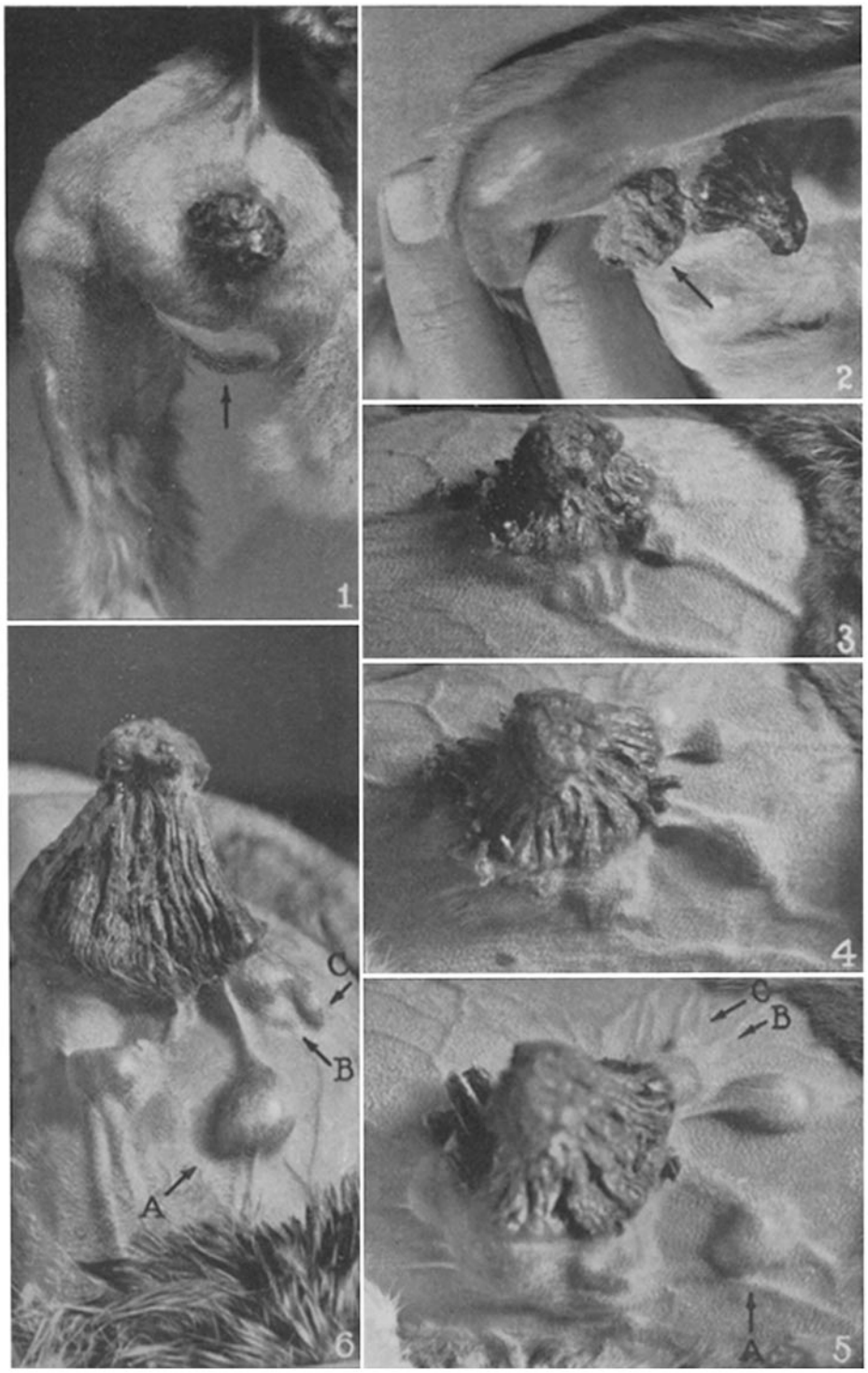
FIG. 27. The region X, of Fig. 10, as shown in an adjacent section. Save for the secondary cystic differentiation the growth resembles a squamous cell carcinoma. Much new-formed connective tissue surrounds it nearly everywhere, but at a few points it has advanced beyond this and lies amidst the voluntary muscle fibres (arrows). × 55.

PLATE 33

FIGS. 28 and 29. Parts of the growths of Figs. 22 and 23 respectively under high magnification. The cell arrangement is somewhat more disorderly in each case than in the usual virus-induced papilloma, and the entire layer of living cells is somewhat flattened (1), but the differentiation is like that occurring in such growths. × 310 and 500 respectively.

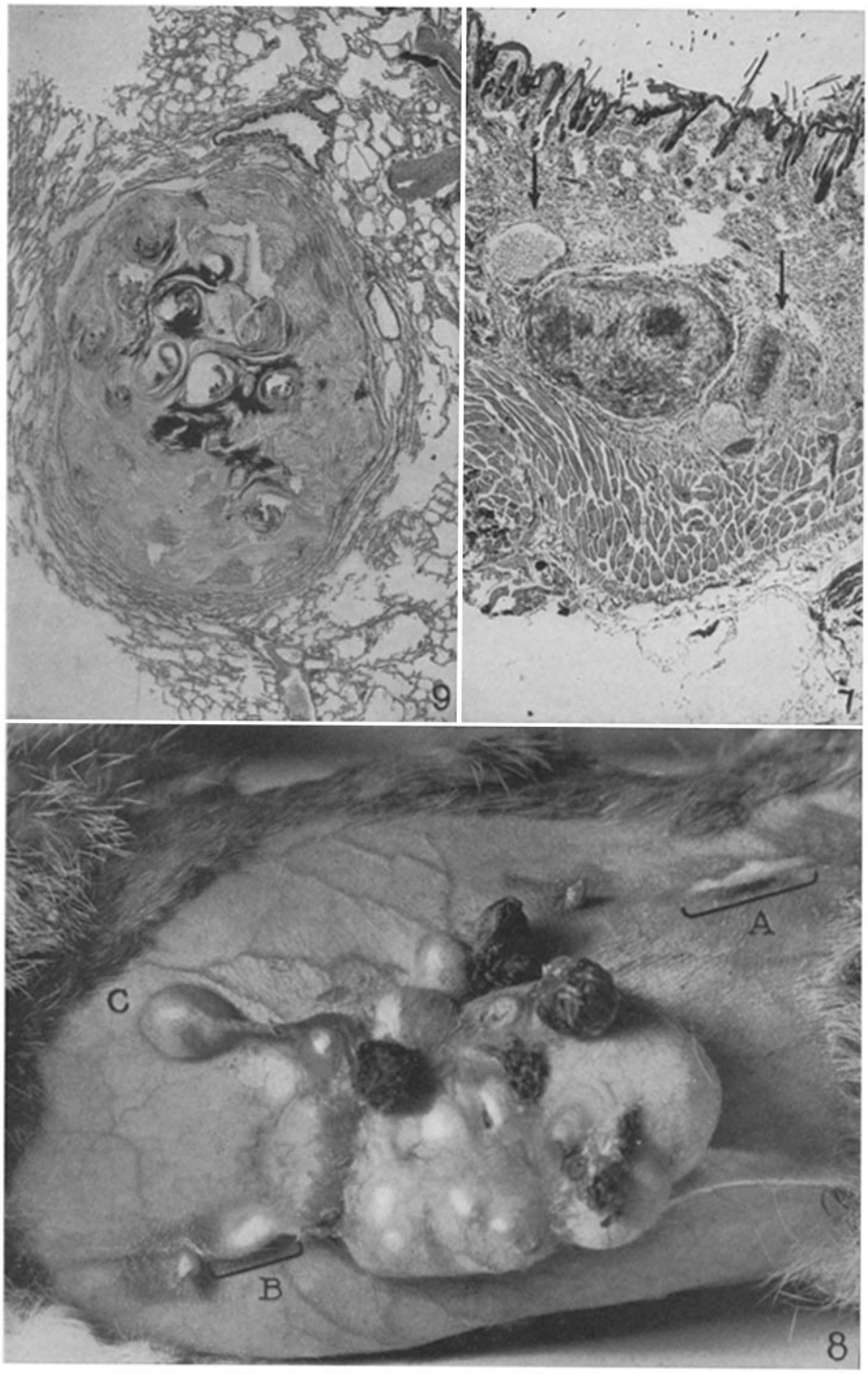
FIG. 30. A malignant, cystic papilloma that originated in papillomas due to tattooing virus into the skin low down on the side of a rabbit. Many ordinary papillomas of the same inoculation are present. All are deeply pigmented and some have coalesced. The malignant growth has extended under its neighbors and into the skin between them, forming cysts there, not a few of which are subepidermal. Secondary ulceration has taken place, and, owing to scirrhous shrinkage of the connective tissue the papillomas on the other side of the animal have been pulled over the ridge of the back-bone and hence are visible in the photograph. × 1.

FIG. 31. The same malignant cystic papilloma at an earlier date. It has advanced almost to the axilla. × ½.



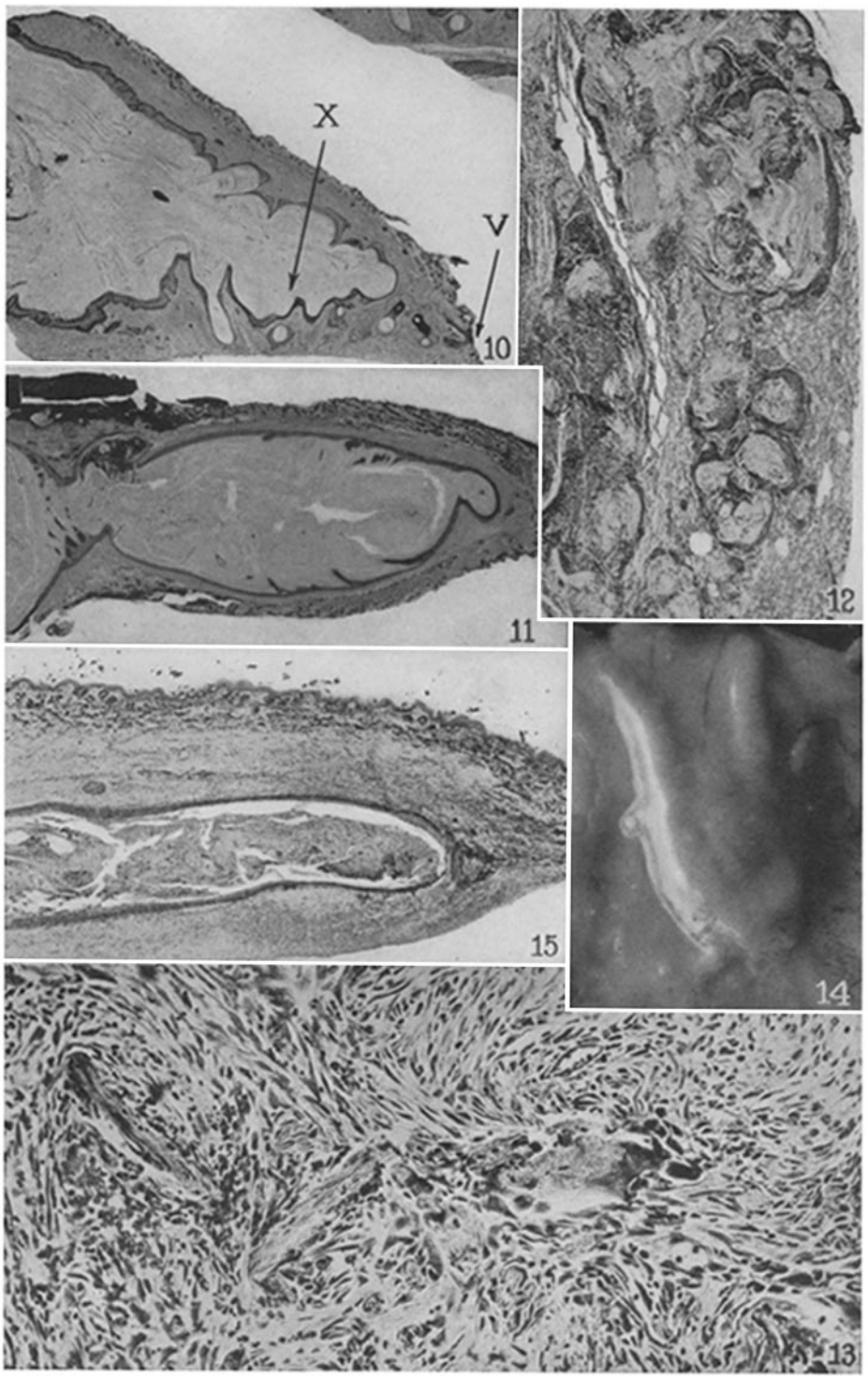
Photographed by Joseph B. Haulenbeek

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



Photographed by Louis Schmidt and Joseph B. Haulenbeek

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



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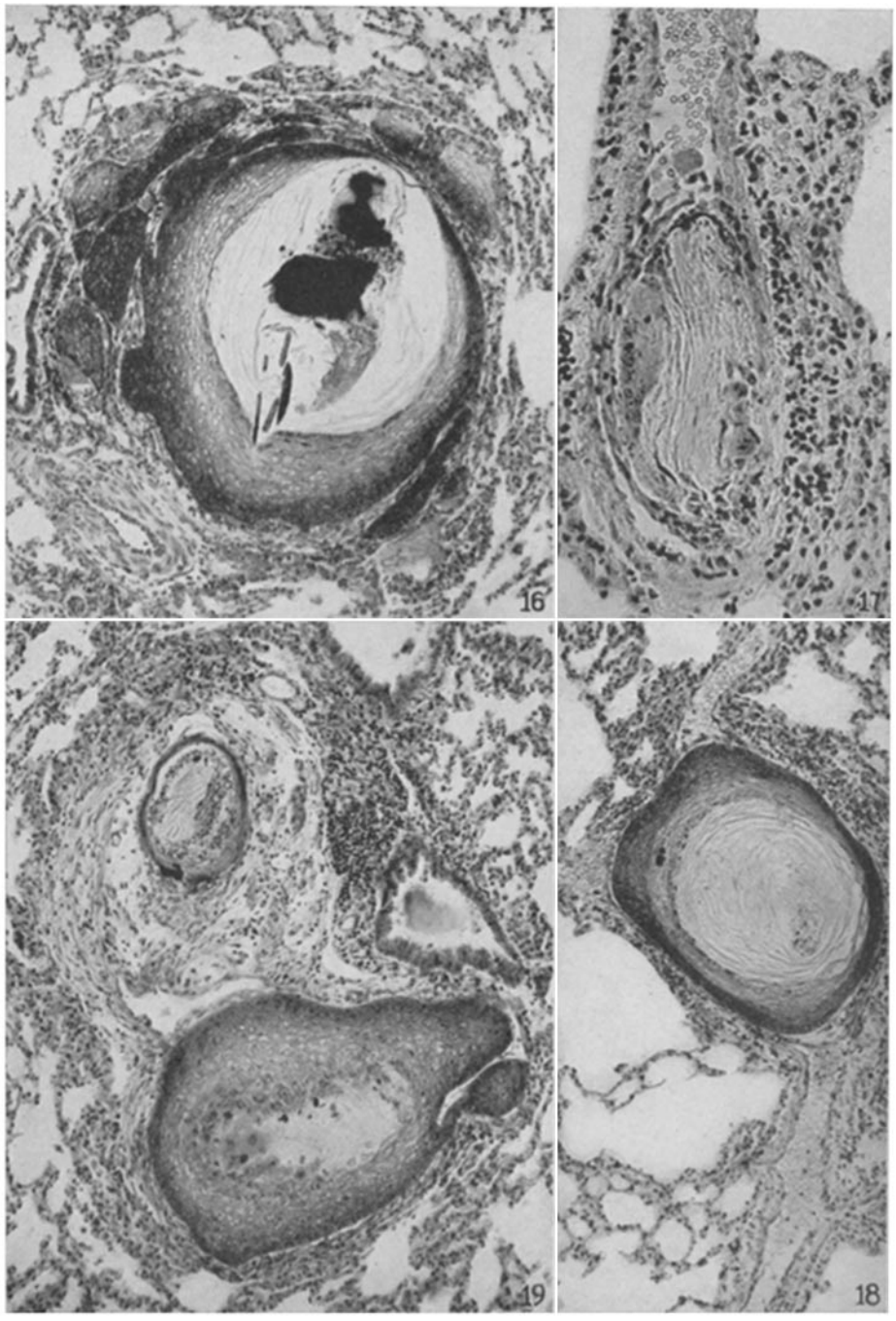

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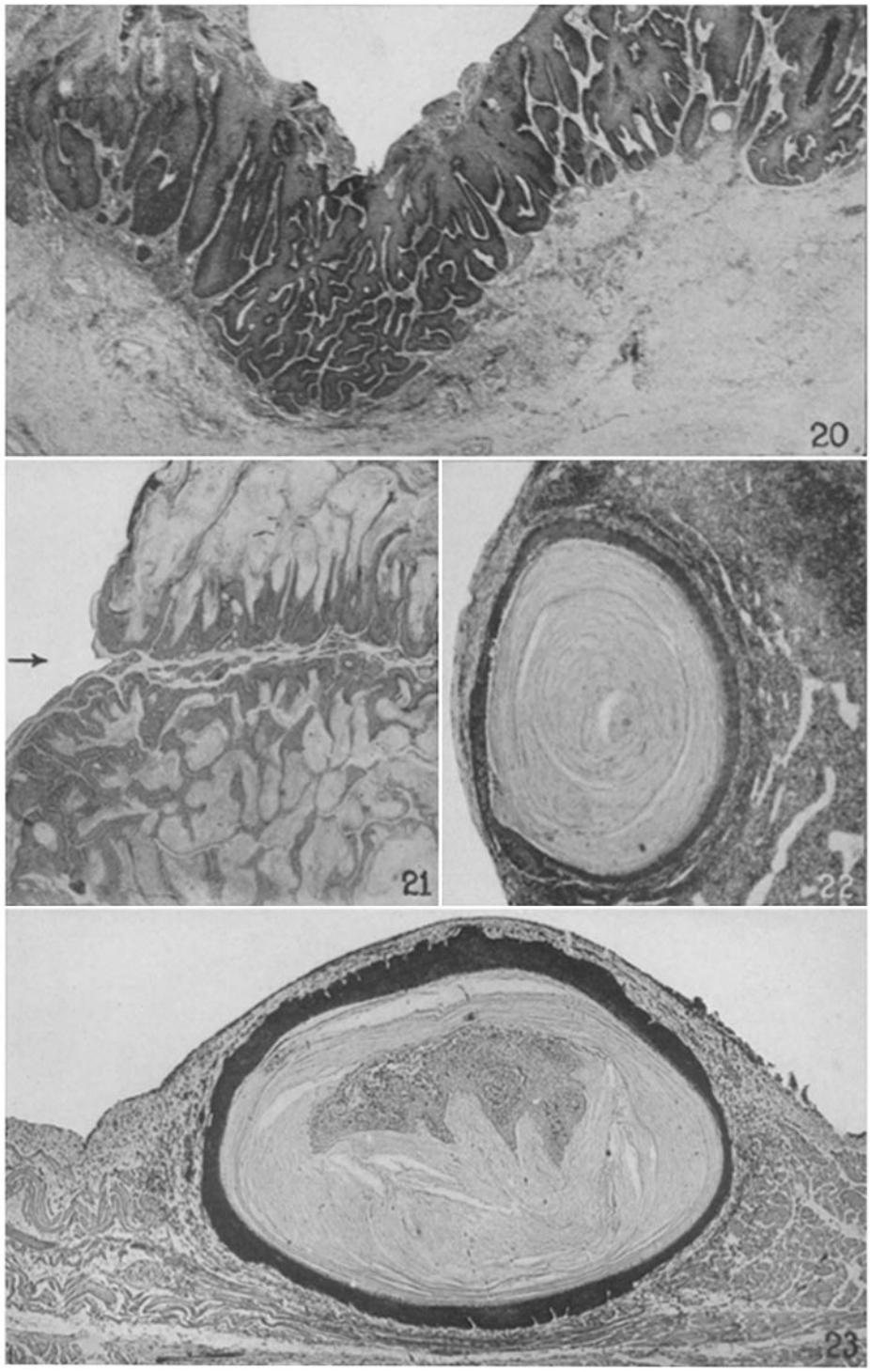

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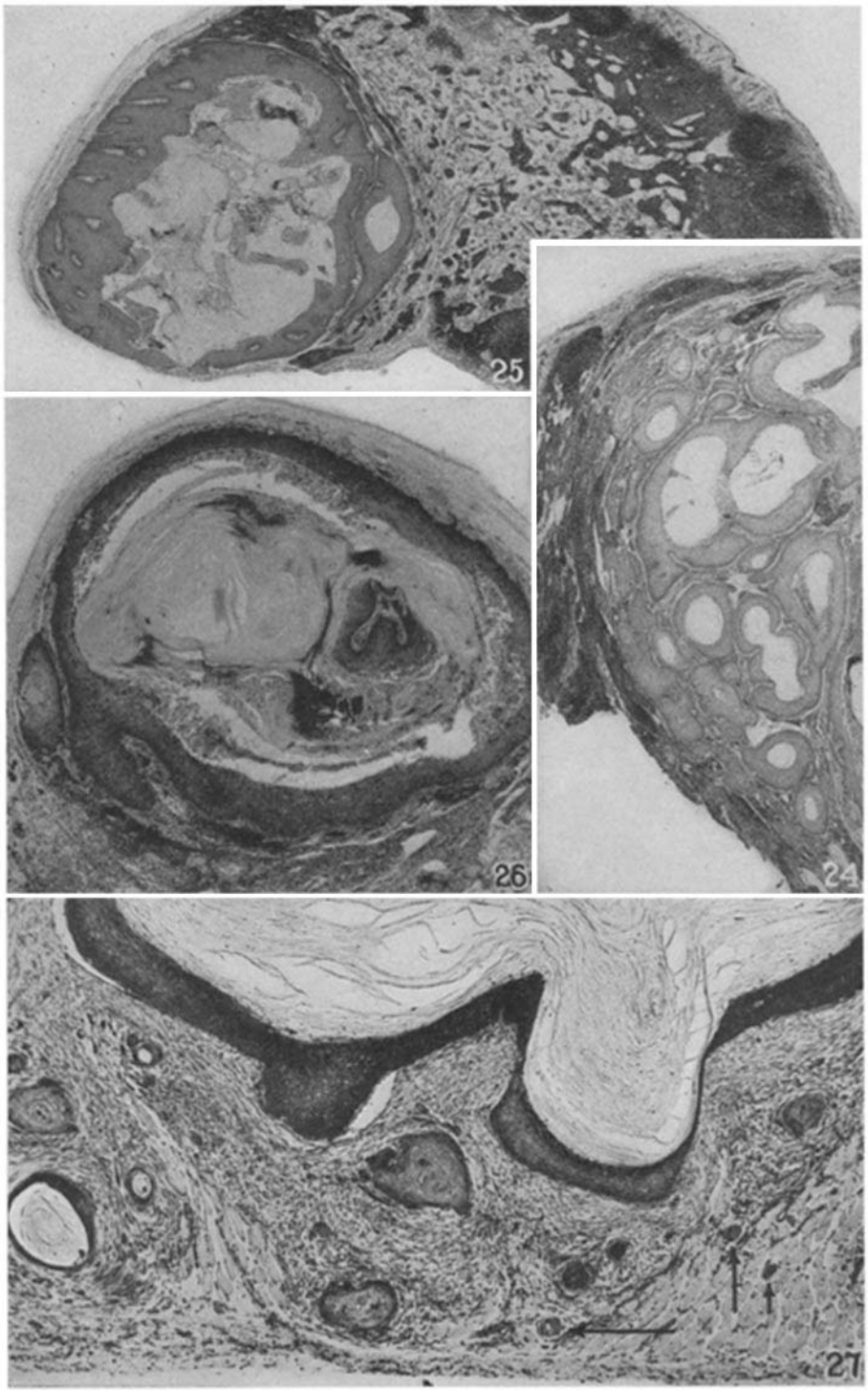

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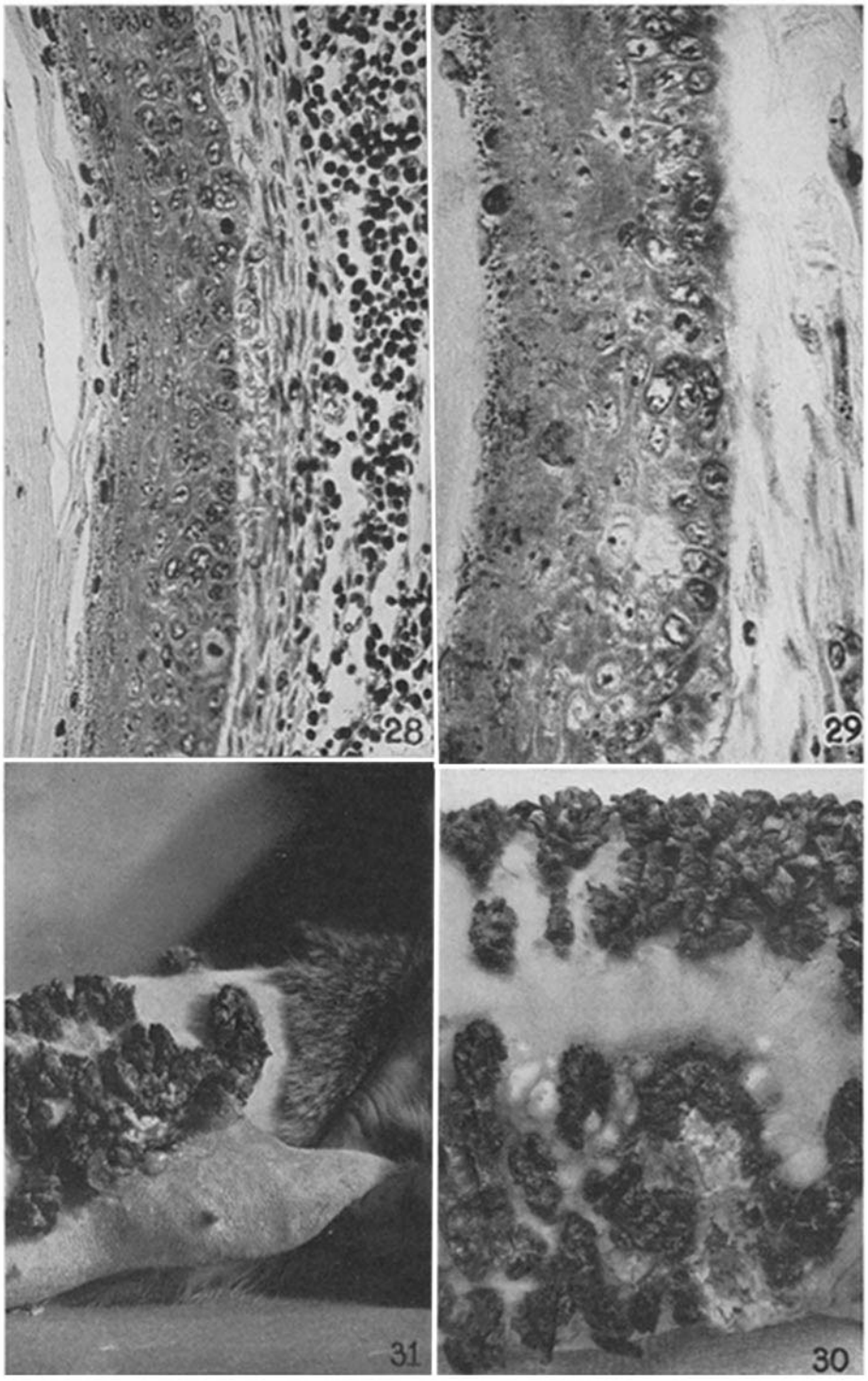

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