CONDITIONAL NEOPLASMS AND SUBTHRESHOLD NEOPLASTIC STATES

A Study of the Tar Tumors of Rabbits

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Plates 14 to 16

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When the chemical agents commonly termed carcinogenic are applied to the skin of man, the rabbit, or the mouse, they nearly always elicit benign growths some while before cancer appears, and the latter frequently takes origin from one or another of them. The benign growths may be very numerous, cancer being infrequent or rare by comparison, and they sometimes reach great size; yet relatively little attention has been paid to them. One reason is that the cancers outdo them in material interest, and another that in the mouse, the animal most studied experimentally, they are prone to become malignant very soon. But perhaps the prime reason for neglect of them has been their uncertain status. Many of the early students of the effects of tar regarded them as mere local expressions of a gradually heightened energy of cell proliferation, holding that only the tar cancers are true tumors. The noncommittal word "warts" is still not infrequently used in referring to them, and those investigators who term them tumors ordinarily do so without raising the question of whether they are actually neoplastic. Yet if they are genuine tumors, their scientific import should be as great as that of cancers,—or even greater at the moment, since they illustrate principles not taken into account in current thinking upon neoplastic phenomena. The present paper is concerned with the problem they present.

Are the Tar Warts Tumors?

In considering the status of the benign growths elicited by the carcinogens it will be well to take a concrete case. We have chosen the warts elicited by tar on the skin of rabbits, because they are a familiar material (1), and because rabbit warts, unlike those of the mouse, retain their initial character long, only becoming malignant after months or years, and then in but a small proportion of instances.

The warts evoked on rabbit skin by tar or the pure, chemical carcinogens

are local, epidermal outcroppings or downgrowths from a diffusely altered epidermis. They are usually punctate when first noted and always small; their morphology distinguishes them sharply from the surrounding, hyperplastic skin; and their enlargement, like that of the classical tumors, seems to take place entirely by intrinsic cell proliferation,—they grow *aus sich heraus*, in Ribbert's phrase (2). They appear more or less abruptly, as if in consequence of some discontinuous change; and they are autonomous in that they fail to conform with the laws of organism, this failure being the signature of a genuine neoplasm according to Borst (3) and Ewing (4). To the extent that they form excrescences or downgrowths they are, like tumors, "independent of the mechanical laws which govern the hereditary form of the body" (MacCallum (5)). Also they are useless, and they present no typical limits of growth, two of Ziegler's criteria (6). Their cells have an abnormal capability for proliferation, an attribute of neoplastic cells according to Ewing; but this capability is only exerted under favoring conditions. For unless tarring is kept up, or has already induced chronic changes in the supporting tissues, or unless other encouraging local conditions happen to be present,—such as are provided by inflammation, maceration, or bacterial infection,—all of the warts eventually dwindle and disappear (1) except those few which become cancerous. Here may be a distinctive difference from the true tumors. For the cells of the latter are generally supposed to be irreversibly different from the normal and it is assumed that "they do not ever return to their normal mode of life" (7).

It will be seen that the tar warts of rabbits are tumors by all of the standard criteria except two. They have no capacity for independent growth like that exhibited by most (but not all) classical tumors; and the changes in their cells may conceivably be reversible since they often become smaller and vanish. But the conception that neoplasms are endowed with the ability to grow progressively rests on experience with naturally occurring growths, and these represent the survival of the fit when man first sees them. In the current definition of a tumor no allowance is made for neoplasms which depend upon favoring factors for existence, and it cannot be used to rule them out. The idea that the neoplastic change is irreversible also rests on observations upon successful growths. To learn what happens to the cells of tar warts which disappear has been our first task.

Materials and Methods

Market rabbits of the ordinary brown-gray (agouti) variety were employed. The tar[1] was that used in our previous work with tar warts. It was applied to the inner

[1] Horizontal retort tar from the Ostergasfabrik of Amsterdam, the gift of Dr. Karl Landsteiner.

surface of both ears twice weekly, with stripping of the layer prior to every third tarring. Sometimes warts appeared after three or four applications, but usually these had to be kept up for 6 weeks to 3 months, and often longer. Cancer developed rarely, after 5 months at the earliest.

The warts have been classified and pictured in a previous paper from this laboratory (1). They fall into three well defined categories: frill horns, papillomas, and carcinomatoids, as one may call certain growths which, when tarred, mimic carcinomas. Frill horns are very infrequent, and none has been utilized in the present work, whereas papillomas and carcinomatoids are exceedingly common on recently tarred skin (1). They can in most instances be readily discriminated in the gross. The papillomas are superficial cauliflowers, horns, cones, or "onions" with broad or constricted bases, usually fleshy or dry, but often fungoid when infected with bacteria or macerating under the tar. They have a characteristic cytology, but their shape is largely determined by the amount of new connective tissue formed in support of them. Frequently it plumps them out into big, rounded or pear-shaped growths. As result of local conditions they tend to become disorderly after a while, and often the microscope shows them then to be extending down along the base as if cancerous ("factitious malignancy"); yet they still are not capable of independent growth. The carcinomatoids have the form of depressed ulcers with raised edges, or raised, raw discs having a ragged, scabbed or sanguineous surface; and they frequently penetrate into lymph vessels and extend to the other side of the ear, causing mounds which may ulcerate. Like the papillomas they have the histological aspect of tumors composed of cells of a single type supported by a more or less abundant stroma, which is frequently mucoid, increasing their superficial resemblance to squamous cell carcinomas. After tarring is stopped they undergo rapid alterations, either drying and scabbing away, like many papillomas, or taking on the character of the latter, or rounding up into keratinized cysts lined with stratified squamous epithelium as do some papillomas which have burrowed deep. Their malignancy is short lived at best; they never cause destruction which cannot be repaired; and everything goes to show that they are merely growths composed of cells altered in the same general way as papilloma cells but more susceptible to extraneous influences (1). Such influences may on occasion lead even normal epidermal cells to simulate malignant ones, as witness the effects of intradermally injected Scharlach R (8).

Not a few of the carcinomatoids and papillomas disappear, though tarring is kept up, but it has favoring effects upon most of them. It establishes the papillomas, stimulates their proliferation, complicates their morphology, and renders some of them disorderly, aggressive, and anaplastic. They may become huge through proliferation of the connective tissue forming their cores. The carcinomatoids, on the other hand, while at first enlarging rapidly do not continue malignant, however often tarred, but after some weeks or a few months become harmless through one or another of the changes just described.

Effects of the Resumption of Tarring

Our first experiments were designed to show whether tar tumors which vanish are actually gone, or leave behind cells which retain their potentialities to produce growths.

Eight rabbits were tarred twice weekly, and all warts appearing on the insides of the ears were traced in outline in their relative positions. A wax pencil and sheets of

cellophane were employed, and usually several tracings were made, at intervals of a week or more. Then the tarring was discontinued. It had never been kept up so long that the papillomas underwent the fleshy thickening with connective tissue which makes complete resolution of the growths impossible. The later course of events was recorded in further tracings, and notes were made of the character of the individual tumors and the general state of the ears. After some months, when most or all of the growths had disappeared, the ears were searched with a lens for traces of them, and sometimes, with a sharpened cork borer, pieces of the ears were punched out where they had been, for microscopic examination. Tarring was begun again later, often to be again stopped and after a while again resumed, in some instances several times. In this way we sought to learn if any of the warts reappeared at their old places, and whether in the same or a different form.

Des Ligneris has reported that a second period of tarring brings out warts sooner than the first (9). In certain of our animals it called forth such a host of growths that one could not tell whether any represented recurrences. Chart 1 depicts such an instance.

The first tarring of the ears of D. R. 3-36, which lasted 127 days, elicited few warts, but it brought about such thickening and hyperkeratosis that they vanished slowly, and some were still present after 335 more days. Except for these few the ears appeared absolutely normal on the 443rd day; yet renewed tarring for only 27 days caused them to become as markedly pathological as after 4 months of previous tarring, and warts appeared in far greater number than before.

At the end of another interval of 124 days many warts were still present, and the skin had not nearly returned to the normal. Tarring for 10 days made the growths enlarge rapidly and brought out some new ones; but they mostly disappeared in the succeeding 185 days. Then a fourth tarring, of 24 days, brought them out in great profusion. The later history of the animal is given further on.

The increasing response to the successive tarrings is clearly evident in these findings. They called forth warts in greater and greater profusion, and called them forth earlier. Several reasons suggest themselves for such phenomena. The influence of tar does not cease at once when it is no longer applied, as every worker with it knows; and hence some cells were doubtless caused to be wart cells in the intervals between applications.[2] Furthermore each tarring constituted additional carcinogenic stimulation, making more cells into potential wart cells. These elements may be present in large numbers in tarred skin, yet fail to multiply into visible growths unless

[2] Growths frequently appear on mouse skin after tarring has been left off but they seldom arise in rabbits unless the skin continues markedly pathological. We have never seen any develop when it had resumed the normal aspect. Yet then it may not be actually normal; for, as the present case shows, renewed tarring for a very brief period will bring it to a pathological state such as was only attained previously in the course of several months of tarring.

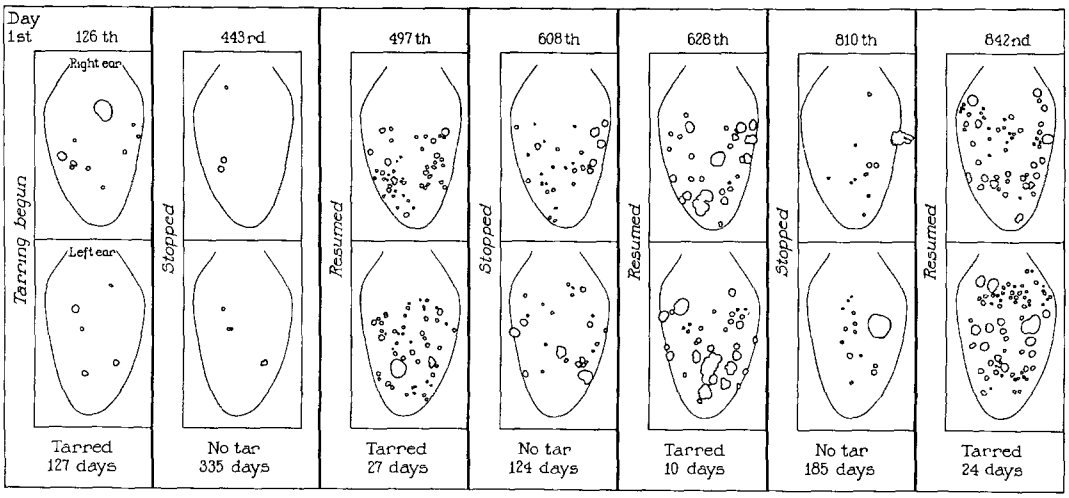

CHART 1. D. R. 3-36. Effects of renewed tarring.

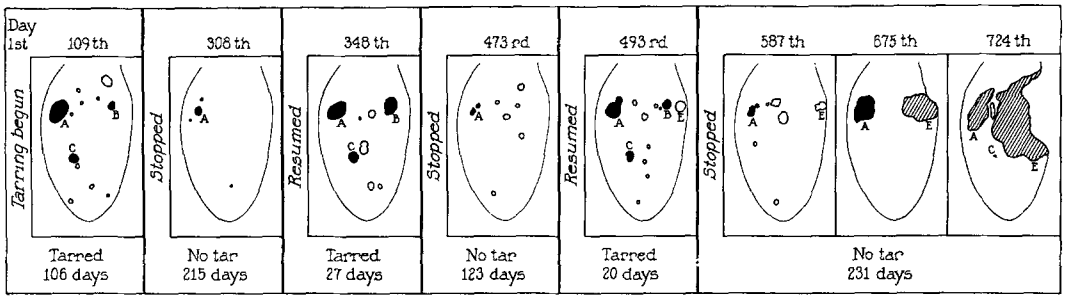

CHART 2. D. R. 4-89. Effects of renewed tarring. The warts designated in black are at sites where reappearances took place. The later cancers are represented by hatched expanses.

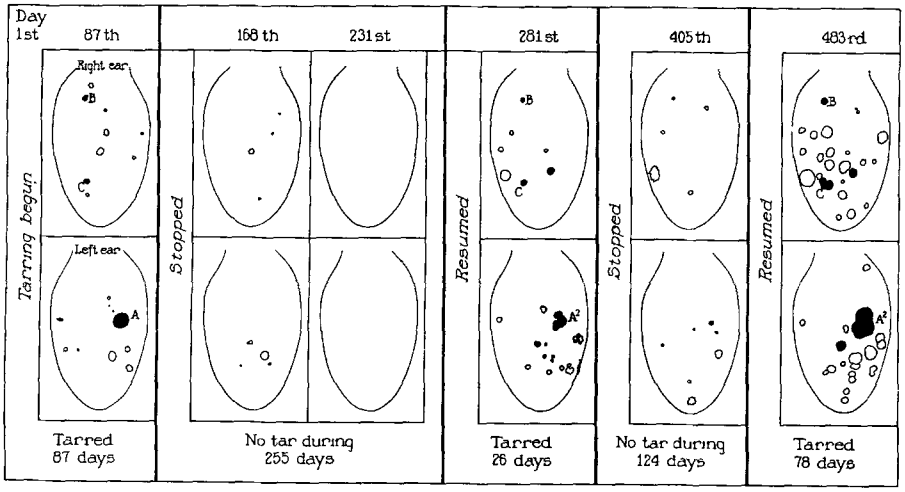

CHART 3. D. R. 4-48. Effects of renewed tarring.

encouraged, as will be shown in an accompanying paper (10). The necessary encouragement was provided in the present instance by the renewed tarrings; for tar has a marked effect to stimulate the proliferation of warts in addition to its ability to engender them (11).

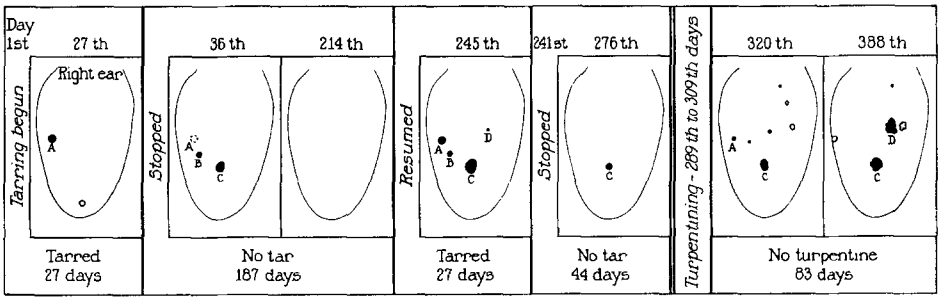

CHART 4. D. R. 4-36. Effects of tar and of turpentine.

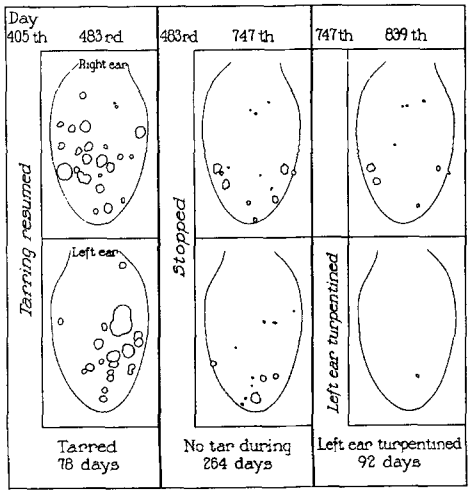

CHART 5. D. R. 4-48 (continued). Effects of turpentine.

The Reappearance of Warts

In instances like the one just given the renewed tarring brought out such hordes of warts that one could not be sure that any were recurrences. It was essential to evoke them in small numbers if this point was to be settled. Under such circumstances one could expect to chart them in their relative positions, like constellations, and follow them individually. Also the scars left where some had disappeared or been punched out for examination would aid in fixing their precise situation.

Rabbit 4-89 (Chart 2) was tarred for 106 days, at the end of which time scattered warts had appeared. Only the right ear has been charted since no reappearances were observed later on the left. During an interval of 215 days the growths almost all vanished and elsewhere the ear resumed the normal appearance. Now a new tarring of 27 days caused growths to appear at spots whence two had vanished 100 to 125 days previously (B and C); and the growth A, which had been gradually dwindling, enlarged rapidly. Again tarring was intermitted, the wart A became smaller, and those at B and C disappeared, a smooth, slightly depressed scar remaining where C had been. When tarring was done for the third time, after a shorter interval than before, warts again appeared at B and C, and the growth at C was observed to have arisen from the surface of the scar left by the previous wart. More will be said of the findings further on.

Chart 3 sums up another experiment which yielded similar results.

A first tarring of 87 days brought out scattered growths on both ears of D. R. 4-48. Shortly afterwards they began to disappear, and by the 231st day all had vanished. The large ulcerated disc A, an obvious carcinomatoid, had been gone more than 157 days when tarring was begun again on the 255th day, and so too had B and C of the other ear. The ears at this time appeared normal save for the slightest scurfiness. Nevertheless the new tarring for 26 days elicited more warts than the previous one of 87 days and the ears became as markedly altered,—thickened, hot, and heavily hyperkeratotic. Where A, B, and C had been growths again appeared. Tarring was now intermitted, though for a shorter period, A^2 became very small and the growths at B and C vanished; but with the third tarring A^2 rapidly enlarged, and growths not only reappeared at the sites of B and C but at two other situations where some had been called forth by the second tarring. The applications were kept up now for 78 days, the skin became as pathological as before, some of the existing warts grew big, especially A^2, and new warts developed. The later history of A^2 is given in Plate 14.

In these instances, as also in that of Chart 4, which has yet to be considered, the effect of renewed tarring to call forth warts at spots where some had vanished cannot be laid to chance. The expanses of skin on which growths never occurred at any time were too large for that and the total number of them too few. Furthermore, some of them appeared three times at the same situations in response to the successive tarrings.

The warts which disappeared left no discernible trace under a hand lens, except in some cases an abnormally smooth epithelium over a superficial scar; and microscopically none of their distinctive cells could be perceived. Where they once were the surface epidermis was sometimes thicker than elsewhere, as over any area of fresh repair, but in other respects it did not differ significantly from the ordinary, save on scarred spots where it overlay more or less sclerotic connective tissue and was devoid of papillae, hair follicles, and sebaceous glands.

The character of the growths appearing at situations from which warts had previously vanished indicated that they were recurrences, not new warts

referable to some special responsiveness to tar of the epithelium where warting once had been. Individually they were in every case like the growths first called forth, being in some instances papillomas and in others carcinomatoids. But the best evidence that they were genuine recurrences was got through experiments in which warts were made to reappear by means of non-carcinogenic stimuli:—

The Effect of Non-Carcinogenic Stimuli to Provoke Recurrences

Turpentine mixed with an equal part of acetone (C.P.) to lessen its effects was painted twice weekly on the insides of ears from which tar warts had disappeared, as also on normal ears. Unlike tar the turpentine mixture induced no deep, reactive inflammation of the ear and almost no thickening, but instead a superficial inflammation with the exfoliation of delicate, papery layers of epidermis, like snake skin. In some cases this inflammation was marked, superficial ulcers formed, and the turpentining had to be done less often, but in no instance did it cause the previously tarred ears to revert to the thickened, roughened, pathological state, as happens soon when tarring is begun again.

Chart 4 sums up an instance in which turpentining caused warts to reappear on skin previously tarred, and new ones to develop. In its earlier history the case illustrates again the reappearance of warts when tarring was resumed, and after an especially long time.

The first tarring of rabbit D. R. 4-36 evoked a few growths on both ears within 27 days, and a few more arose shortly after it was stopped. All soon disappeared though, and the ears quickly returned to an apparently normal condition. When tarring was resumed on the 214th day, the growth A had been gone nearly 7 months, and B and C for almost 6 months. The situation of C was marked by a scar where another wart had been punched out near it. The new tarring, for another 27 days, called forth a few more warts than previously. Of the four which appeared on the right ear, three had the situation of previous growths and resembled them in gross characters. When tarring was again stopped all except one, C, disappeared within 31 days, and C became much smaller though it was still present after 44 days. Turpentining was now begun and kept up for 20 days. During this period a growth appeared at the site of the previous recurrence at A, while C enlarged once more and five wholly new warts arose. After the turpentining was stopped most of the warts disappeared, but one again appeared at D and two new warts as well. Turpentine was not applied to the other ear,—whence all warts had disappeared,—and none arose there later.

In another animal (D. R. 4-50) a first tarring brought out many warts, most of them carcinomatoids, as proven by their form and by several punch biopsies. After tarring was stopped they either disappeared or rounded into keratinized cysts. When applied anew 5 months after the last wart had vanished it elicited scattered growths, amongst them three recurrences. When tarring was finally stopped all of the growths dwindled; but before they were completely gone turpentining of one ear was begun. It did not noticeably stimulate the growths already present yet brought out many new warts, and meanwhile those on the unturpentined ear were disappearing.

The papillomas which the turpentine called forth on skin previously tarred were less fleshy and had thinner papillae than those evoked on the same animals by tar, but otherwise were no different. None had the character of carcinomatoids, perhaps because the changes the turpentine induced in the connective tissue were so superficial. It did not always elicit warts but was sometimes devoid of this effect or even made existing growths disappear. This was notably true in the case of rabbit D. R. 4-48, the animal which had already provided the data of Chart 3 (and Plate 14, *q.v.*). No tar was applied after the 483rd day except to a small area on the left ear, between the 670th and 693rd days; and on the 747th day both organs had about the same number of dry, diminishing papillomas. Turpentining of the left ear was now begun and continued twice weekly for 92 days. It caused a fairly marked, superficial inflammation, and the growths disappeared much faster than those on the other ear (Chart 5).

Acetone is known to be non-carcinogenic and most authors have found this to be true of turpentine also, though Twort and Lyth have reported that it produces severe dermatitis and papillomas in mice (12). The mixture we employed gave rise to no growths on a group of normal, control rabbits. Some workers report that turpentine increases the effectiveness of carcinogenic agents while others state that it is devoid of such effect or is anticarcinogenic. In our own experience it has had an adverse influence on existing growths when it induced much inflammation, as in the case of D. R. 4-48 just described. The discordant findings in the literature may have been due entirely to the differing response of individual animals in this respect.

Turpentining is not the only non-carcinogenic influence which will cause tar warts to recur. Wound healing will suffice, as the following instance proves.

The first tarring of the left ear of D. R. 4-48 (Chart 3) called forth a large wart, A. It had the form of a raised, raw expanse with indurated base and sanguineous scab, was a typical carcinomatoid in other words. The second tarring brought out a similar carcinomatoid at the same situation (A^2). This growth dwindled later but was not wholly gone when tarring was instituted for a third time. Under the encouragement then received it rapidly grew big, and on the 78th day of the new tarring was more than 2 cm. across, a slightly raised, ulcerated disc, with indurated base and ragged surface. The successive changes up to this point have been recorded in Chart 3. Plate 14 tells what happened next.

On the 483rd day a piece was punched from the margin of the growth A^2 and tarring was stopped: the sections showed a characteristic, anaplastic, invasive carcinomatoid forming cysts secondarily (Fig. 1). Later the wart scabbed and dwindled, leaving a smooth scar smaller than itself, which in time became pale and slightly depressed. By the 607th day there remained only three small, pink mounds in addition to this scar.

The healing of the previous biopsy wound had left a narrow, funnel-shaped hole and this, as well as the scar, marked where the carcinomatoid had been. To get rid of the mounds prior to renewed tarring they were punched out, together with part of the old scar.

The ear was not looked at again until the 642nd day. Then it was found that the recent punch hole had healed as usual, by a symmetrical extension inwards from all around its periphery, but that the covering of the new purply-pink disc thus formed had not the usual, smooth surface. On the side where the epithelium from the old scar had extended inwards there were three adjoining rugose mounds, mostly on the surface of the new disc but also just next it on the scar. They looked like parts of a retrogressing growth, and the circumstances suggested that the stimulus of repair had brought the wart A^2 again into being. To learn their character and to test for this possibility, they were excised, together with some of the scar, by punching out a disc of tissue 1.35 cm. across. Serial sections taken through the tissue thus procured, in the long axis of the cluster of mounds, showed them to be parts of a common warty expanse in process of retrogression. Toward the center of the disc, where healing was most recent, the growth had a carcinomatoid character but was forming cysts secondarily (Fig. 2); toward the periphery,—where healing had been completed some time before,—it consisted of embedded cysts and of papillomatous fingers; while on the immediately adjacent surface of the old scar there were only such fingers, and they were small and were covered with ordinary epithelium like that elsewhere on the scar. The serial sections of the part of this last which was furthest away from the punch hole showed no warty change but merely a thin layer of ordinary epidermis devoid of papillae (Fig. 3), overlying dense connective tissue.

The healing of the large hole left by the third biopsy was followed from day to day. It took place in the usual way, from all around the periphery; but as the new tissue extended in a low mound appeared upon its surface on the side where the epithelium had derived from that of the old scar. The rate at which the hole closed fell off progressively, and after 25 days healing had become very slow and the mound just referred to seemed smaller. Consequently tarring was begun anew, on the 30th day (670th of the observations), both to encourage the growth and to render the conditions affecting it more comparable with those when the specimen of Fig. 1 was obtained. The tar was put only on and immediately around the disc, and it was applied almost daily. Within a week the mound had become higher and longer, by elevation of the adjacent epidermis, and it occupied much more of the old scar, almost to the further edge of the remaining, small portion. Its aspect after 23 days of tarring is shown in Fig. 4. It was then raised and rugose, and biopsy showed that it was an anaplastic carcinomatoid (Fig. 5) histologically identical with the original growth A^2. Nowhere in the part of the scar removed with it and sectioned serially was there any sign of warting. The hole made at the biopsy was even larger than before, but a fragment of the growth had been left *in situ*. Unfortunately infection of the wound took place, with heavy scabbing, and when the scab came away all of the wart and nearly all of the scar had been destroyed. After healing was again completed local tarring was done anew, but now it called forth no growth.

Chart 3 and Plate 14 together show that where a carcinomatoid had originally been called forth by tar a growth of the same sort was later

elicited on three widely separate occasions. On the first of these tarring evoked the growth, but on the second and third wound healing was the effective influence. The original wart had not been submitted to biopsy but was a typical, ulcerated carcinomatoid in the gross. The section taken of the large growth (A^2), which occupied its situation on the 483rd day and resembled it in the gross, showed a carcinomatoid which was becoming anaplastic but had a cystic tendency (Fig. 1). These features were evident also in the first of the growths evoked by wound healing (A^3), though this growth was retrogressing when excised and had the papillomatous form where it was oldest,—as often happens when carcinomatoids are involuting (1). The last wart (A^4), called forth by wound healing but tarred later like A^2, was histologically identical with this latter (Figs. 1 and 5). The completeness of the resemblance gains in significance when it is recalled that carcinomatoids often differ much histologically (1).

Reversion of Warty Epithelium to the Normal Aspect

In the instance just given wound healing repeatedly called forth a carcinomatoid where in the gross there had been only the old scar of a previous growth of the same sort. Portions of the scar adjacent to the carcinomatoid but outside the area involved in the healing process were removed incidentally to two of the biopsies and searched for wart cells in serial sections. None could be perceived but only a smooth, shallow layer of ordinary-looking epidermis devoid of papillae, over connective tissue that had become sclerotic (Fig. 3). Yet as healing again took place after the first of the biopsies just mentioned a carcinomatoid precisely like the previous one appeared where epithelium was extending in from the remaining portion of the scar. Bacterial infection after the second biopsy did away with any chance that this would happen again.

In considering these findings one can suppose that individual wart cells may have been incorporated here and there in the epidermal layer covering the scar, or lay scattered in the connective tissue, and hence escaped notice in the sections scrutinized; for certainly elements capable of proliferating practically at once as carcinomatoid cells must have been present somewhere for the growth to have recurred so promptly. But the alternative explanation suggests itself that the cells of the carcinomatoid, while retaining their potentialities, had resumed the normal aspect and formed the layer of epidermis over the scar. The histological findings favored this conception, for the new carcinomatoid seemed to have developed by extension downwards at many points from the surface layer (Fig. 5). To gain more data a histological study was undertaken of warts in process of retrogression;

and its results left no doubt that as these growths dwindled, and long before they disappeared, the epithelium of many of them assumed an aspect no different from that of the ordinary epidermis round about them.

The gross changes incidental to the retrogression of tar warts have been described in previous papers (13).

If tarring is stopped within a week or two after warts have arisen, and the adjacent skin is not greatly changed, they rapidly disappear; but in proportion as tarring is kept up they tend to persist, and if it produces chronic alterations about them they may grow vigorously for months, though dwindling finally as the skin approximates the normal. Those warts which continue to grow differ not a whit in their morphology from those destined to disappear, as many biopsies have proved; and the larger they are, the more do they tend to persist. As already remarked, those which have come to consist mostly of connective tissue,—the big spherical, or pear-shaped or pendule-like papillomas,—cannot be wholly resorbed: they remain as sclerotic tags, sometimes covered with normal-looking epithelium.

Many of the warts present for a long time disappear through keratinization and exfoliation, these processes taking place faster than cell replacement: they retain to the last their distinctive morphology, though gradually growing smaller. About others a reactive tissue forms containing large numbers of macrophages and lymphocytes (like that about retrogressing, transplanted tumors), and concurrently their epithelium becomes unhealthy and shallow and is replaced by normal epidermis extending in from the sides (1). By both methods of disappearance,—which have been illustrated in our previous paper,—the growths are extruded from the organism. The cells of carcinomatoids which have penetrated deep may die as such, or die by differentiation amidst a profuse, reactive connective tissue. With none of these processes which result in permanent disappearance of the growth are we here concerned, but only with those compatible with recurrence. They are most readily studied when tarring is stopped within a week or two after warts have arisen on ears not greatly changed. Under such circumstances most of the papillomas, previously moist because covered with tar, and fungoid or fimbriated, undergo a dry keratinization down to the ear level and become thick, discrete, compact scabs. In this form they may persist for many weeks, and at any time during this period resumption of the tarring will cause them to take on their previous character. Yet if they are sectioned while in the scabbed state none of the typical papilloma epithelium (Fig. 6) is found but merely a mass of keratinized scales underlain by a somewhat hyperplastic, epidermal layer devoid of distinguishing features (Figs. 11 and 12). Within the scab there may be a few living papillae covered with similar epithelium. Such instances leave no doubt that wart cells are capable of reverting (Fig. 8) to the ordinary aspect while retaining their ability to produce growths on experimental stimulation. When the skin remains pathological such stimulation may not be necessary in some instances: the scab is after a while cast off, revealing a low fleshy disc or hassock that enlarges into a characteristic papilloma which only much later dwindles gradually as cutaneous conditions become more normal.

The fate of the carcinomatoids is usually more complex. When retrogressing some, as already remarked, become papillomas indistinguishable from growths primarily of this type, and share their fate. Others form small, keratinized cysts by differentiation

as do some papillomas that have penetrated deep. These cysts may be walled with stratified squamous epithelium devoid of distinctive characters,[3] or all of the cells producing them may eventually die. Sections of superficial carcinomatoids, procured while retrogressing, show that the growths soon lose their distinctive character (Fig. 7). Renewed tarring at this stage will cause them once again to resume their original form and invasive activities, as we have many times had occasion to note. In the case of D. R. 4-48 (Figs. 1 to 5) cells with carcinomatoid potentialities persisted for months after the growth itself had disappeared, and they formed a layer with the aspect of ordinary epidermis and carried out its covering function.[4]

In sum, the findings leave no doubt that the epithelium of retrogressing tar papillomas and carcinomatoids frequently takes on the appearance of ordinary epidermis, while retaining its essential potentialities as tar wart epithelium.[5] In many cases this happens long before the growth disappears (Figs. 11 and 12). Yet the reversion to the ordinary aspect is not always accompanied by functional normality. Where growths once were the epithelium may continue for months to build up discoid, keratinized scabs (Fig. 8), though on microscopic section its cells differ from those round about only in being slightly plumper.

The protocols already given show that epithelium capable of promptly forming warts again on renewed tarring may persist for at least 6 months at spots where growths have vanished (*vide* the instance of D. R. 4-36). No tests have been made after a longer interval. The carcinomatoid which recurred on the ear of D. R. 4-48, as result of the biopsies of the 607th and 642nd days, arose from the epidermis of a scar which had been wholly devoid of any warting since some time prior to the 483rd day.

Eventual Fate of the Wart Epithelium

Even those growths which proliferate vigorously for many months and retain to the end their distinctive characters at length prove unable to

[3] A similar though less complete reversion toward the normal of the epithelium of cysts has sometimes been noted in the nodules which result from the implantation of virus papilloma tissue in the leg muscles of the host (Rous, P., and Beard, J. W., *J. Exp. Med.*, 1935, **62,** 523), and it is not infrequently to be seen in retrogressing, cystic tar carcinomas of cottontail rabbits.

[4] Rabbit papillomas due to the Shope virus occasionally reappear spontaneously at spots where they had vanished. The interval may be several months (Rous, P., and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33,** 358. Kidd, J. G., *J. Exp. Med.*, 1938, **67,** 551).

[5] Frill horns do not come into the present consideration. They retrogress by gradually dwindling, with retention of their highly distinctive morphology; and they always disappear in the end unless the general state of the ears continues to be pathological. Under such circumstances their diameter may very slowly increase.

maintain themselves. Many become smaller by retreat of their sharply defined periphery, as if unable to survive in competition with the normal epidermis, and others break up into islands which dwindle individually. When growths that were once large are induced to recur they usually appear at little spots in the area they previously occupied, as if only here had cells capable of forming them persisted. The proportion of warts that recur is small, when all are considered; most of them disappear for good. In view of these facts it seems certain that the cells of the generality are replaced eventually by normal elements.

Conditional Nature of the Tar Warts

In a previous paper (1) we have brought evidence supporting the conclusion that all of the tar warts are conditional in nature, wholly dependent on aid for enduring survival. The findings of the present work have further justified this conclusion.

In our experiments each new course of tarring rendered the cutaneous disturbance more chronic, and the longer the skin took to return to a normal aspect, the longer did the warts persist, while not a few of them enlarged. Only as tissue conditions finally approached the normal did these latter become stationary or retrogress. Portions of some which persisted longest and grew most vigorously were excised and implanted in the hosts, but none proved able to establish itself in the new situation. The following is a case in point. It corroborates previous findings (1).

D. R. 3-36, of Chart 1, had numerous warts on the 842nd day, when tarring was permanently discontinued. After another 3 months the ears were still thick and scurfy, and the warts still many, though some had disappeared or dwindled. There were five which had grown steadily larger, all typical papillomas with constricted bases. One had been present 9 months, another for 11. Two of the five were excised and pieces of the base of the others were got by punch biopsies. Each specimen was hashed, suspended in Tyrode's solution, and implanted at two situations in the leg muscles of the host according to a technique already described (14). At none did a growth arise, although sections of the biopsy material showed that some of the warts had been invading along the base. When the rabbit was killed, 4 months later, the ears were still somewhat scurfy and thickened, but more of the warts had disappeared. The three which had been partially removed for implantation were still vigorous growths, however, and as big as before.

DISCUSSION

The evidence obtained in our experiments that the cells of the tar warts are irreversibly different from the normal proves that these growths possess the attribute that alone had been wanting for their identification as tumors.

The Liabilities Entailed by the Neoplastic Change

The deviations from the normal which find expression in tar papillomas, carcinomatoids, and frill horns are sharply defined and narrowly limited, although the individual growths may behave very differently, some flourishing while others retrogress. The frill horns have a fixed and highly characteristic form, but the carcinomatoids are transitory morphological expressions of a cell abnormality which leads to the formation of papillomas in other instances (1). This abnormality, however expressed, is not infrequently lethal, the growths disappearing no matter how often tar is applied. At the opposite extreme are those warts which need but the slightest encouragement in order to flourish; and in between are the great majority, tumors which require continual aid for their survival (1). All are neoplasms artificially forced into being.

It is plain from the findings that the neoplastic state of the epidermal elements which form benign tar tumors entails disabilities as well as abilities: the cells have gained in some respects but in others they have been marred. They have been rendered capable of proliferating more rapidly than normal cells when both are tarred together, and of behaving more aggressively, and in consequence the growths composed of them enlarge at the expense of normal elements. Yet they have so far lost the abilities which enable epidermal cells to maintain themselves under ordinary circumstances that they fail to hold their own in the lack of aid, such as tarring or chronic disturbance will provide, and the tumors they have formed disappear. Even those which proliferate longest and most vigorously fail to survive transplantation to the leg muscles or connective tissue of the host, although normal elements accidentally transferred with them frequently succeed in establishing themselves, as *e.g.*, the cells of epidermis, cartilage, hair follicles, and sebaceous cysts (14).

The fact is generally recognized that the neoplastic condition is attended by a greater or less loss of functional capabilities. In view of present findings it seems certain that this condition not only entails disabilities but sometimes is lethal *ab initio*. Certainly the abnormalities which render cells tumor cells range all the way from those inducing what may be termed subthreshold neoplastic states, which give to the cells but slight advantages over normal elements and these only under morbid circumstances, to conditions which result in complete independence and vigorous activity, irrespective of favoring factors, as in the case of the cells of clinical cancers. Yet even those deviations which result in cancer do not necessarily bring with them success, as witness the frequency of hidden carcinomas in the prostates of men over 40 years old (15), growths which seldom come to clinical atten-

tion, as statistics show. Not a few malignant tumors declare themselves only because their cells proliferate more rapidly than they die. That cancer cells are often sick cells and die young is known to every pathologist.

The deviations from type represented by tumors can be exceedingly slight (3).[6] Even the cells of highly malignant cancers may organize to some extent and differentiate, as if to the best of their abilities. In the case of the tar papillomas and carcinomatoids the deviations which result in tumors are so inconsiderable that the cells, when not stimulated, may conform with the laws of organization and resume the ordinary aspect and habit of life. Yet this does not mean that they have become normal, merely that their neoplastic potentialities are in abeyance.

Malignancy as the Outcome of Step-Like Deviations

The first students of the effects of tarring rabbit skin noted that cancers appeared only after many months or years, and that they were ordinarily preceded by growths having the character of papillomas. Later there were many reports of early cancers and of retrogressing ones. That tar cancer may indeed retrogress cannot be doubted, for Yamagiwa and Ichikawa (16) observed a carcinoma which did so after 630 days of growth, with metastasis formation.[7] These authors added that many of the cancers they evoked eventually stood still and would, in their opinion, have healed if there had been "no further favorable conditions for their progression."[8] Yet the history of most of the published instances of retrogressing tar cancer shows them to have been really carcinomatoids, growths having the essential character of benign papillomas but stimulated to factitious malignancy by

[6] Foulds (*Am. J. Cancer*, 1940, **39**, 1) has recently reviewed the literature on this theme.

[7] We have ourselves recently followed a growth which must be regarded as a carcinoma that eventually retrogressed. It appeared after 5 months of tarring as a raised, ulcerated disc, and was deemed a carcinomatoid; but unlike such growths it continued to enlarge after tarring was stopped and while other, fairly numerous warts were disappearing. During the next 4 months it grew invasively, forming a discoid mass 5 cm. in diameter, which had a central ulceration 3½ cm. across with a raised, indurated border. Meanwhile the rest of the ear resumed the normal aspect, save for a few dwindling papillomas. The tumor then began to grow smaller, the ulceration started to heal, and a piece from its raised edge showed cells like those of an anaplastic, squamous cell carcinoma doing badly amidst a profuse reactive tissue which contained great numbers of macrophages and lymphocytes. Within another 4 weeks it was wholly gone, and only the changes incidental to scarring were to be seen in the tissue procured by another biopsy.

[8] Not a few of the notably destructive tumors with the morphology of carcinomas which tar elicits in cottontail rabbits retrogress and disappear after tarring is left off. They sometimes do so even after they have destroyed a large part of the ear.

local conditions. By the use of highly carcinogenic tars rabbit cancer has occasionally been evoked within 2 or 3 months (17), but in most such instances papillomas and carcinomatoids appeared earlier. That these are the forerunners of malignant change is generally recognized, as also that large numbers of benign growths are evoked, on the average, for every cancer that develops.[9] The tar with which we have worked calls forth scores of warts for each cancer elicited, and Guldberg's recent report (18) indicates that the same held true of his material.

Papillomas are more than an indicator that tar may eventually call forth malignant tumors. The cancers generally arise from them, as Yamagiwa and Ichikawa pointed out. They also noted that the change to cancer seems to be abrupt. Six of the seven tar carcinomas which we have thus far succeeded in evoking (in nearly two hundred tarred animals) arose from papillomas. The seventh growth, which appeared as such, has already been described (footnote 7). The steps in the derivation of two cancers from papillomas are summarized in Chart 2.

The growth A was a characteristic papilloma which had persisted for nearly 700 days before it underwent cancerous change. It dwindled in the intervals between tarrings and became dark gray with melanin then, as many indolent tar papillomas are wont to do. It might long have retained its primary character had not a cancer developed at the situation E (from a papilloma which appeared shortly before the 493rd day). This cancer, which grew fast and ulcerated soon, exuded an irritant secretion with which the growth A was constantly bathed; the skin round about it became inflamed and thickened; and A grew rapidly, ulcerating and invading and becoming an obvious cancer some time before the 724th day. It differed in histological type from the cancer at E. Two other papillomas also appeared on the skin inflamed by the secretion, one of them (C) at a spot where tarrings had twice previously caused a growth to recur. Both the cancers extended rapidly, huge metastases arose in the neck, and transplants of the tissue from one of the latter resulted in large carcinomatous masses in the muscles of the legs.

These findings well exemplify the way in which tar cancer usually originates in rabbits. It seldom appears at a stroke, but instead is the outcome of a secondary, step-like change in the cells of benign tar tumors which

[9] This is not to say that benign growths are always numerous in the animals in which cancer appears; for they may be but few (*vide* Chart 2, D. R. 4-89). The occurrence of malignancy seems to be conditioned by some host peculiarity, as in the case of the cancers arising from virus papillomas (Rous, P., and Beard, J. W., *J. Exp. Med.*, 1935, **62,** 523. Rous, P., Beard, J. W., and Kidd, J. G., *J. Exp. Med.*, 1936, **64,** 401). Two of our seven domestic rabbits which developed tar cancer had two cancers each. The cottontail rabbits which show them may have as many as six or seven, distinct in origin and often in morphology, and usually situated on both ears.

often have been present for a long time.[10] What happens is no mere exaggeration of the previous papillomatosis but a wholly new event, the genesis of a neoplasm distinct from its predecessor. Upon the relatively slight alteration responsible for the benign growth a second deviation from the normal has been abruptly superimposed. As a result of this further change the cells lose in capacity for organization but gain the ability to proliferate independently, and in not a few instances they prove able to metastasize.[11]

Such phenomena are not peculiar to tar tumors. A considerable proportion of human cancers arise by a step-like progression, as *e.g.* many mouth cancers from papillomas, thyroid cancers from adenomas, and mammary cancers from the papillomas of Schimmelbusch's disease. Most carcinomas of the urinary bladder originate in papillomas, and some of the latter are themselves transplantable within the host, as proven by the secondary tumors occasionally resulting from the inadvertent transfer of fragments to the abdominal wall during removal of the growths by operation (19). Induced cancers of the human skin (chimney-sweeps' cancer, mule-spinners' cancer) are often preceded by "warts." Many other examples of the step-like derivation of malignant growths could be cited.

Pathologists know that neoplastic alterations do not always cease when the cell has attained a cancerous state. By further changes the growth may go from bad to worse, its cells at last becoming wholly anaplastic. These late phenomena, as they occur in human cancers and the tar cancers of rabbits, cannot readily be assembled and analyzed; but they are almost diagrammatically evident in the carcinomas which result from the secondary malignant changes occurring in rabbit papillomas due to the Shope virus (20). An instance in point will be given.

A 5 per cent saline extract of cottontail papilloma tissue was tattooed into nine spots, 2 mm. across and widely separate, on each side of a domestic rabbit (D. R. 74). Characteristic papillomas appeared at all of the spots except one and enlarged slowly. After 11 months the growths were 2 to 4 cm. across, and several showed precancerous changes (20), while a few had begun to ulcerate at the center. 2 months later many were obviously cancerous, and when the animal was killed, after 14 months in all, thirteen of the papillomatous masses had been partially or completely replaced by fleshy,

[10] Recently Haddow has stressed this sequence of events in connection with the hypothesis that tumors are the outcome of the somatic mutation of cells (*Acta Internat. Union against Cancer*, 1938, **3**, 342).

[11] The findings in mice differ somewhat from those in rabbits. The first growths to arise in mice are usually papillomas and carcinomatoids, but progressively enlarging cancers frequently appear at about the same time, while others take origin early from papillomas. Many of the latter disappear or fail to progress after tarring is left off, but others keep on enlarging even after the skin has reverted to the normal aspect, as if they were capable of independent proliferation.

invasive tumors, some of them with foul, cup-shaped, central ulcerations. They were 2 to 5 cm. in diameter, and two on each side of the animal had become confluent by a merging of the tissue where they had touched. Numerous metastases were present in the axillary lymph nodes and lungs,—of squamous cell carcinomatosis, as the microscope showed. A slice was taken for section across the center of each tumor mass (Figs. 9 and 10).

Microscopic examination showed that every one of the growths had undergone malignant change. Cancers of four general kinds (20) could be discerned, namely: convoluted papillomatosis,—a type of growth expressive of but a slight morphological deviation from ordinary papillomatosis yet representing a new and malignant neoplasm; cystic papillomatosis, expressive of a somewhat greater change and often accompanied (in other rabbits) by metastasis formation; malignant papillomatosis, characterized by highly irregular and invasive downgrowth, though with some retention of papillomatous features; and frank, squamous cell carcinomatosis, more or less diverse in character. As in instances previously reported, cancer had appeared first in the oldest parts of the virus papillomas, that is to say at or near their centers, and it was multicentric in origin, and appeared at other spots toward the periphery as time passed. Most of the growths still consisted in greater or less part of virus papillomatosis. This was always peripheral, and on traversing the sections with the microscope one came upon tumors of greater and greater malignancy as the centers of the masses were approached. The evidence for a progressive change in the multiple growths from one neoplastic form to another, each more malignant than the last, was very striking when one considered all the tumors together. In order to appraise the findings better a camera lucida drawing was made of a representative cross-section of each mass, the regions in it occupied by the various types of tumor were outlined, and a chart was constructed to show their situation and extent schematically (Chart 6).

It is known that cancer appears earliest in those virus papillomas which proliferate most vigorously (21), and the diameters of the growths in the present instance served as an indication of their relative vigor. In the chart they have been arranged in order of diameter. It will be seen that, generally speaking, those masses which had become largest contained the greatest amount of cancerous tissue and in its most advanced form (squamous cell carcinomatosis). In a few instances secondary malignant extension into the adjacent skin was partly responsible for the size of the masses. Invasion of the underlying tissues had sometimes taken place, and where the cancers had burrowed deepest they were most anaplastic. This was notably true of the smallest growth of all, which showed only a central change to convoluted papillomatosis in the surface mass, with cystic papillomatosis deeper down and squamous cell carcinomatosis below that.

In this instance, as in less comprehensive ones previously studied (22), the carcinomas arose by secondary changes in benign papillomas,—growths induced by a virus, yet with a remarkable likeness to tar papillomas in all their manifestations (1). Generally the alterations did not stop there but one form of malignancy followed upon another with result eventually in squamous cell carcinomatosis.[12] The expressions of this last were highly

[12] This is not to say that no short cuts have been observed to this form of cancer, for it sometimes takes origin directly from virus papillomas. The various possibilities have been considered in a previous paper (Rous, P., and Beard, J. W., *J. Exp. Med.*, 1935, **62**, 523).

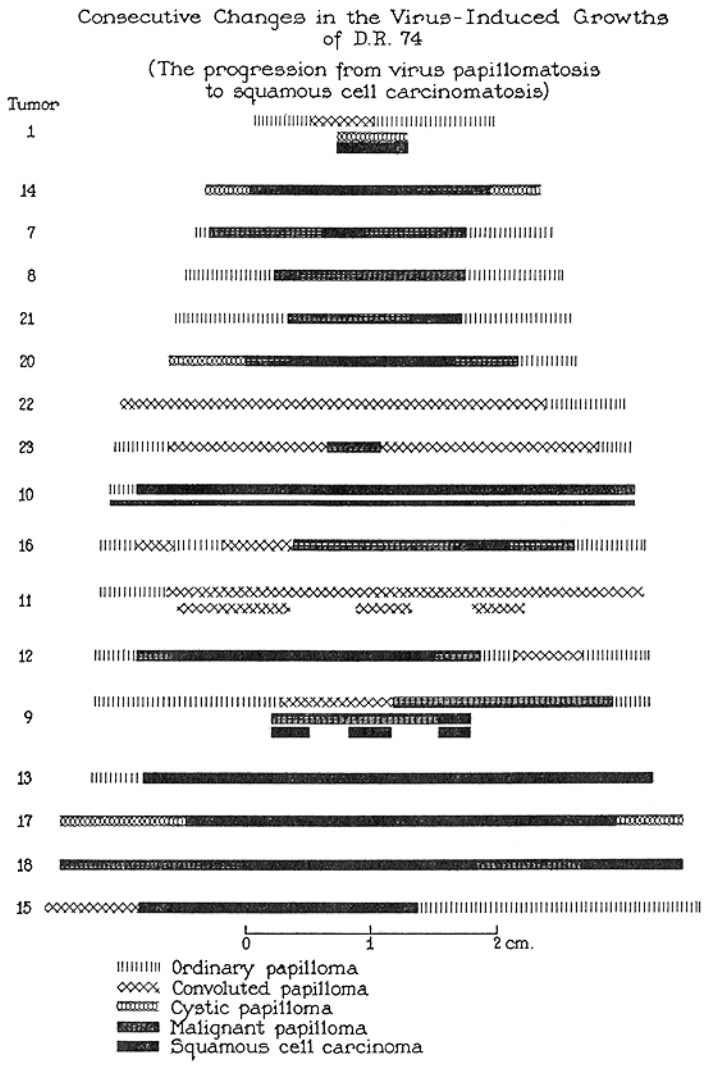

CHART 6. To illustrate the succession of changes taking place in virus papillomas which have become malignant (see text). Each of the seventeen growths which resulted from tattooing the Shope virus into small spots on the sides of D. R. 74 is represented diagrammatically in cross-section. The length of each cross-section is that of the diameter of the tumor mass at autopsy × 1.65, and they are arranged in order of size. The various types of tumor composing them, as determined microscopically, are indicated in their relative extents and positions. In a few instances (tumors 1, 10, 11, 9) the downward extensions of the growths have been charted, as exemplifying the findings; but the main purpose of the chart would have been obscured had this been done with them all. (Tumor 1 was still an ordinary virus papilloma save at its center where it had become a convoluted papilloma. Here extension downwards had taken place with a change successively to cystic papillomatosis and to squamous cell carcinomatosis. A somewhat similar progression to this last, as the growth invaded, is shown in the case of tumor 9. Tumor 11 had remained a convoluted papilloma where it had burrowed deep, while tumor 10 had become a squamous cell carcinoma almost everywhere.)

various, some of the carcinomas differentiating and keratinizing in an orderly way, others being markedly anaplastic, and those of the same general aspect often differing distinctively in details.

To account for the observed succession of neoplastic phenomena one may suppose either that the first cancers which arose underwent gradual alterations, or that changes took place in them which were discontinuous, like those responsible for the initial malignancy. Histological study of the tumor masses did not help much toward a decision. At many spots there was an apparent gradation from papilloma to carcinoma, but this should not be taken to imply a real one since it can be simulated through secondary union of the two sorts of epithelium. At other places the separate, discrete character of the malignant growths was obviously due to the destruction they had caused. The anaplasia encountered in the ulcerating cancers could have been due in some part to the influence of inflammation and bacterial infection, which not infrequently bring about factitious malignancy and may even cause virus papillomas to take on a malignant aspect temporarily (23). But these occurrences will not cover the findings in the large, these pointing directly to a succession of discontinuous changes. The most convincing evidence that some at least of the later forms of cancer arose in this way lies in observations already reported (20) which show that any one of them may derive directly from a virus papilloma, doing so by a sudden alteration after some months, while furthermore certain of the forms of cancer may retain their characteristic features for a long period, until the death of the animal. Convoluted papillomatosis is practically always succeeded by other forms of cancer, and malignant papillomatosis soon becomes squamous cell carcinomatosis. But cystic papillomatosis often undergoes no further alteration, metastasizing as such; and the various kinds of squamous cell carcinomatosis nearly always retain their original features (23). This much is wholly clear, that the virus papillomas, like the tar papillomas, are tumors expressive of a single sort of cell anomaly, but that variation enters when they become malignant, and cancers of several different types may arise directly from them. Out of the more benign of these new tumors others with greater malignancy are prone to develop, thus increasing the diversity until the cells at last become so completely anaplastic in some instances as to lose their distinguishing peculiarities almost entirely.

Possible Nature of the Neoplastic Change

Any hypothesis concerning the cause of cancer must take account of the following facts (amongst others),—the occurrence of cancer as if by a single, discontinuous change in cells subjected to chronic disturbance

(many, perhaps the majority of cancers arise in this way); its frequent occurrence as result of abrupt, secondary change in a benign tumor, itself the outcome of a change as discontinuous; the tendency for one form of cancer to be succeeded by others more malignant in character; and the spread in tumor type that may occur when benign growths alter to malignant ones and these alter further.

Present knowledge of biological phenomena has yielded two explanations for cancer which merit discussion in the light of these facts: tumors may be the result of somatic mutations of cells, or they may be due to the effect of viruses upon them. For it is known, on the one hand, that a few tumors are indubitably caused by viruses and on the other that the somatic cells of certain organisms, notably those of plants and insects, occasionally undergo discontinuous variations having the character of mutations.

The view that tumors arise by somatic mutations rests on an analogy which breaks down when the attested mutations of this sort are scrutinized, as J. S. Haldane (24) and others have pointed out. For all of the mutated cells which have thus far come to attention continue to obey the general laws of organization, and they do not proliferate at the general expense; in a word, they do not form tumors. The somatic change may find expression in colored dots on a corolla, made up of a mosaic of cells differing from the rest only in their unusual color; or a spray of delphinium may have white flowers while all the rest of the plant is blue; or a fern frond possess one ruffled pinna amongst a generality which are smooth. A guinea pig may have a patch of skin of a hue unwarranted by anything in its lineage, an obvious somatic mutation, which dies with the animal. Corolla dots, delphinium flowers, fern pinna, and skin patch they all remain, none constituting a tumor. In mammals somatic mutations are exceedingly rare.

There are yet further difficulties. Families of mice have recently been procured by inbreeding which are prone to develop tumors spontaneously in some special organ,—lungs, mammary gland, liver, or bones, to mention typical instances. Individuals with one or another of these special liabilities may develop tumors of the sort characteristic of the stock in a very high percentage of instances; yet it has been the general experience that they never manifest somatic mutations of the recognized kind. The recent discovery that the liability to mammary cancer is passed on in some mouse families by way of the mother's milk puts a further strain on the mutation hypothesis (25).

Many cancers are the outcome of successive cell changes in the direction of greater and greater anomaly, as we have brought out. No similar succession of cell mutations has been discovered thus far by geneticists,

even in germ cells,—which undergo mutation vastly more often than somatic cells,—though it is true that now and again second mutations of germ cells have been reported, taking them further away from the normal or back toward it.

To give credence to the somatic mutation hypothesis one must assume that the irreversible variations which find expression in tumors are somatic mutations of an unique sort, wholly different from any thus far recognized in nature, and that they tend to occur in sequences, also a new phenomenon.[13]

In contrast with this recourse to assumptions, the causation of some tumors by viruses is a certainty, the sole question now being whether such causation is the general rule. The difficulties of this view were found to be inconsiderable when scrutinized a few years ago (26), and since that time much new evidence has come to hand. It is now recognized that some viruses can enter the organism before birth and that some persist long without causing disease, as further that innocuous viruses can be rendered pathogenic by appropriate influences. Andrewes has reviewed the evidence in this relation (27), and we have discussed the facts indicating that the cancers which derive from rabbit papillomas due to the Shope virus are the result of virus variation (28). Kunkel (29) has recently found that tobacco mosaic virus often undergoes successive alterations, with result in new features in the disease it produces and a great broadening of its pathological manifestations.

SUMMARY

The "warts" which tar elicits on rabbit skin (papillomas, carcinomatoids, frill horns) are true tumors, benign growths expressive of slight yet irreversible deviations of epidermal cells from the normal. The neoplastic condition gives the cells a superiority over their neighbors when both are submitted to the same encouraging influences, and then they proliferate into tumors. Their state entails such disabilities, though, that they are unable to maintain themselves under ordinary circumstances, and conse-

[13] If all neoplasms were due to similar actuating principles, the virus tumors would provide an almost insuperable objection to the hypothesis of somatic mutation. For the neoplastic viruses cause tumors whenever they are brought in contact with susceptible cells, and, as Ludford has pointed out, on the mutation hypothesis one would have to suppose of such viruses that they are agents producing mutations wherever they act (*vide* Ludford, R. J., Ninth Scientific Report, Imperial Cancer Research Fund, London, Taylor and Francis, 1930, 121). The additional supposition would be necessary that the cells regularly respond in some instances with somatic mutations of peculiar type, as, *e.g.*, when influenced by the virus which regularly produces osteochondrosarcomas through alterations in the connective tissue elements of the fowl.

quently growths composed of them disappear when no longer aided. Often the neoplastic cells resume the normal aspect and habit of life long before the tumor mass is gone; and they may persist as part of an apparently normal epidermis, retaining their neoplastic potentialities for months after all signs of the growth have disappeared. In these instances it can be made to appear again, sometimes repeatedly, by non-carcinogenic stimulation of the skin (wound healing, turpentining). There is reason however to suppose that in the end the tumor cells, unless helped, die or are cast off.

It is plain that the neoplastic state does not necessarily connote independence of behavior or success in tumor formation. On the contrary it may render cells unable to survive or endow them with powers which they can exert only under favoring conditions. This is the case with the cells composing the tar warts of rabbits. In the lack of such conditions the cells of these growths do not manifest themselves but remain in a subthreshold neoplastic state, whereas if aided they form neoplasms.

The deviations from the normal represented by the benign tar tumors of rabbits are slight and limited in character, but further deviations in larger variety may be superimposed upon them, with result in malignant tumors, growths possessed of a greater, though not always absolute, independence. Tar cancers usually come about in this way, by successive, step-like deviations from the normal, and so also do the cancers which derive from virus-induced papillomas as well as many human carcinomas. After cells have become cancerous they frequently undergo further changes, some apparently step-like in character, and all taking the direction of greater malignancy.

The hypotheses that tumors are due to somatic mutations and to viruses respectively are discussed in the light of these phenomena.

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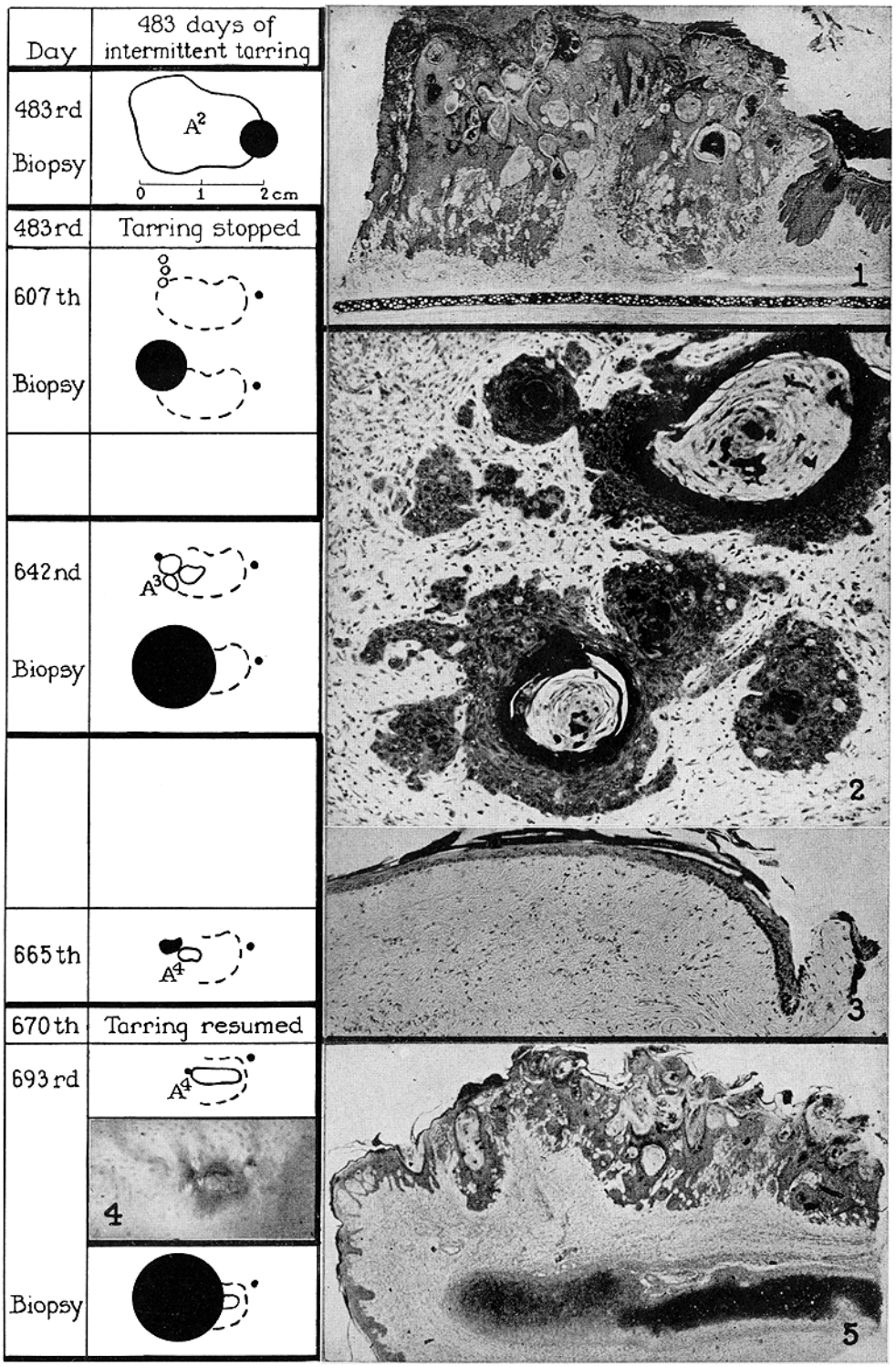
EXPLANATION OF PLATES

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

PLATE 14

FIGS. 1 to 5. Repeated recurrence of a carcinomatoid as result of wound healing. The early history of the growth is given in Chart 3. There it appears in black, but here it is given in outline and the holes left after pieces were punched out of it are indicated in black.

On the 483rd day (78th of the third period of tarring) the carcinomatoid, which had already recurred once, was more than 2 cm. across. A disc was then punched out of its margin with some of the adjacent skin, as indicated. Sagittal section of the specimen showed a growth having the morphology of an anaplastic, squamous cell carcinoma with some tendency to secondary cyst formation (Fig. 1, × 18). Later the mass on the ear became gradually smaller and by the 607th day there was left only a smooth scar (broken line), not so large as the growth had been, a tiny, funnel-shaped hole (black dot), and a few small mounds (outlined). These latter were now punched out together with some of the skin and part of the scar next them. The excised tissue was discarded, and the animal was not looked at again until the 642nd day. By this time healing had been completed save for another tiny hole, but three new growths were present. They were situated where the epithelium had extended in from the scar and on the adjacent surface of the latter. All were punched out with more of the scar. Serial sections showed an anaplastic carcinomatoid (A^3) which was becoming cystic (Fig. 2, × 90). The portion of the scar that was free from the growth showed only ordinary epithelium (Fig. 3, × 103) which was somewhat distorted and damaged near where the blade had cut through it (right side of Fig. 3). The large hole closed in rapidly and as it did so a mound appeared where the epithelium of the scar was extending in. A tracing was made of this mound on the 665th day, in its relation to the scar and to the hole, and on the 670th day tarring was resumed. Under its influence the tumor became raw, rugose, and larger, as another tracing shows. On the 693rd day it was photographed (Fig. 4, × 9/10), and then most of it, together with some of the original scar next it, was punched out. The hole of the previous biopsy was by now very small. A longitudinal section through the part of the growth taken and through the edge of this hole (Fig. 5, × 18) disclosed an anaplastic carcinomatoid, A^4, identical in character with A^2, biopsied on the 483rd day (Fig. 1).



Photographed by Joseph B. Haulenbeek

(Rous and Kidd: Neoplasms and subthreshold neoplastic states)

PLATE 15

FIG. 6. A tar papilloma. The oval pale gray areas in the deep epithelium to the right of the center of the growth are sebaceous glands, an unusual feature. × 24.

FIG. 7. A superficial carcinomatoid in process of retrogression. Tarring had been stopped 24 days previously. The deep cells of the growth (not to be seen at this magnification) had all differentiated and died, but its living surface layer had heaped up a high scab by continuing keratinization. It will be seen to differ from the surrounding epidermis not only in this respect but in being somewhat thicker and having still a few irregular tongues along its base. Nevertheless there has been a pronounced reversion toward the normal. × 24.

FIG. 8. A papilloma in process of retrogression. The growth had only very gradually dwindled after tarring was stopped, persisting as a discrete scab formed of keratinized scales. The section, taken through the scabbed area 6 months after the last tarring, shows a few narrow, low papillae covered with an epithelial layer no thicker than that round about except in a small gully toward the right hand side of the growth. Under high magnification the living epithelial cells here were slightly plumper than those of the epidermis elsewhere but in other morphological respects did not differ from them. × 24.

FIGS. 9 and 10. Cross-sections of two of the virus papillomas of D. R. 74, in which malignant changes have taken place (see page 383 and Chart 6). At the periphery of the tumor masses virus papillomatosis is still present. In Fig. 9 it occupies about one-quarter of the growth, next its left edge. All the rest is convoluted papillomatosis, as indicated by its pattern. The section was cut in a strictly vertical plane. Fig. 10 shows several areas of malignant papillomatosis and, near the center of the growth, squamous cell carcinomatosis which has extended deep. × 2½.

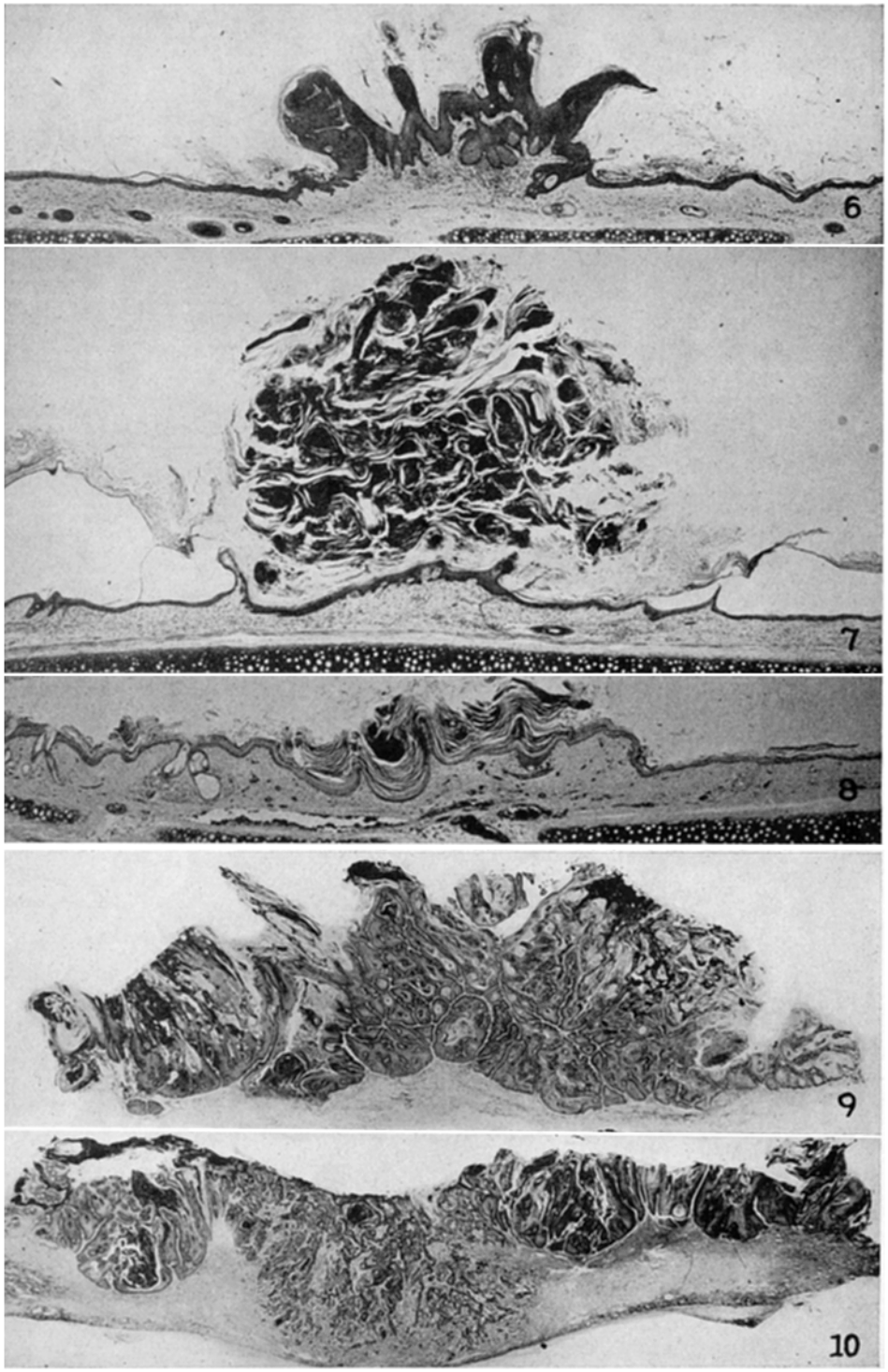


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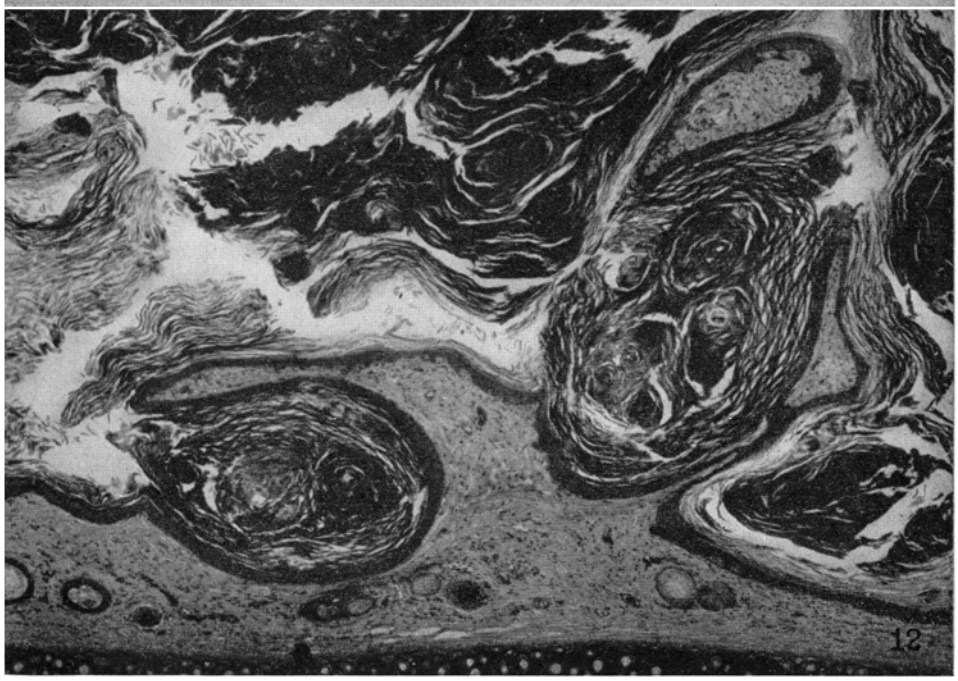
PLATE 16

FIGS. 11 and 12. A papilloma in process of retrogression: from the same animal as Fig. 7. Tarring had been discontinued for 24 days. Some living papillae have persisted amidst the scab-like mass of keratinized scales, but the epithelium covering them and the spot where the wart once was no longer differs from the hyperplastic epidermis round about. Fig. 12 shows at high magnification the left edge of this spot and some of the epidermis immediately next it. Fig. 11, × 15; Fig. 12, × 55.



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