THE CARCINOGENIC EFFECT OF A PAPILLOMA VIRUS ON THE TARRED SKIN OF RABBITS

II. Major Factors Determining the Phenomenon: the Manifold Effects of Tarring

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Plates 18 to 23

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When the virus causing rabbit papillomas (1) is injected into the blood stream of rabbits repeatedly tarred on the ears it often localizes abundantly in these organs and gives rise there to carcinomas (2) and papillomas differing from the ordinary. The general course of events has been described in a preceding report (3). The present paper deals with the factors responsible for the unusual tumors, and with the influence of tarring to render the virus effective, to further proliferation of the growths and, in not a few instances, to make them anaplastic. The experiments and charts will be numbered consecutively to those already published.

The Experiments

So much virus localized in the tarred ears of our first experiments (Experiments 1 to 3) that confluent proliferation usually resulted, which soon filled the aural concavities with foul, fungoid tissue, death occurring early from sepsis. The individual growths could not readily be observed in their early stages, because the tarring was continued while they were appearing, and when it was left off later they often had coalesced or were crowded together. To obviate these difficulties, a smaller inoculum was employed in some of the present tests, to produce scattered, discrete growths, and most of the rabbits were not tarred after inoculation.

General Method of Recording the Changes.—The growths were drawn to size in their relative position, on stamped outlines. Preliminary records were made of

the tar warts, when any existed, and after the virus injection the tumors were charted every few days, the interval depending upon the rapidity of the changes. Notes were taken on all growths especially worth attention.

The size of the ears often varied from the average employed for the stamped forms, and then the tumors as drawn were crowded or scattered more than in life. Dubious growths are indicated by a broken line. The sudden appearance of rapidly enlarging, dark gray or black papillomas provided a tell-tale to infection with the virus, since pigmentation is infrequent in tar tumors and is retained only while they are indolent (3). Hence melanotic growths are charted in black. Confluent masses are merely outlined unless melanotic. Indubitable cancers are recorded in red, from the day they became visible as tumors. The stippling around the border of certain growths of Chart 11 that were included within large melanotic masses indicates uncertainty as to how far they extended.

The protocol of Experiment 4 has already been summarized (3), and the cancers described.

Experiment 4.—Brown-gray ("agouti") rabbits were used, as in every test, and tar of the Oster-Gasfabrik of Amsterdam, kindly provided by Dr. Landsteiner. The 38 animals had been tarred twice weekly for 89 days on a central area of the inner surface of the ears, about half their total expanse, whence spreading occurred to the edges. Before every third application the layer was stripped away. At the stripping 24 hours before inoculation, the "tar warts"[1] were charted, the state of the ears recorded, and the animals were separated into 4 comparable groups. 3 of these were injected into a leg vein with 15 cc. each of a Berkefeld filtrate of a Tyrode extract of glycerolated papillomas (W. R. 1183), 2 groups receiving a filtrate of 0.5 per cent strength (in terms of weight of tissue extracted), and the third the same fluid diluted with Tyrode to 0.2 per cent. An hour later this last group, 11 animals, were tarred again, as were also the 10 controls and the 8 rabbits of one of the groups receiving 0.5 per cent filtrate; and the tarring was kept up twice weekly throughout the next 25 days. The 9 animals of the second group injected with 0.5 per cent filtrate were not tarred again.

Within 15 seconds after each injection, a circular area 2 to 3 cm. across on the shaved right side was tattooed with the needles of an electrical tattooing machine, sterilized by heat, and a similar tattooing was done on the left side, but through a few drops of the inoculum. The needles brought blood. Our aims were respectively to determine whether the virus would localize from the blood in traumatized epidermis, and to produce papillomas as an index to susceptibility. At no spot injured with sterile needles did growths develop; but where the inoculum had been tattooed in they promptly appeared save in one

[1] In Paper I the morphology of the tar warts, actually tumors, has been summarized. Their histology will be dealt with subsequently.

instance in which an abscess formed instead. Susceptibility in this case was attested by the appearance of a growth where the skin had been punctured for the intravenous injection. All of the papillomas remained merely such.

The ear changes are recorded in Charts 6 to 9. The records dealing with the outsides, which had epidermis but slightly changed, are not reproduced since only ordinary virus papillomas arose there, nearly all dark gray. The ears of 3 controls and 1 animal receiving 0.2 per cent virus are omitted because they remained devoid of growths. Those of one animal receiving 0.5 per cent virus and tarred later, and of 2 not tarred again, are likewise omitted, there being no evidence of virus localization in their ears, which were little changed by the tarring and carried no warts or only one or two.

In several of the *controls* (Chart 6) the later tarring elicited a few new warts, but after it was discontinued some dwindled and disappeared, others remaining stationary, or enlarging slowly. None ever appeared on the outsides, no rapidly enlarging ones on the insides, and no cancers. Most of the animals were kept many months after the experiment ended, the majority of their tar tumors vanishing, and the others becoming indolent.

So few tumors followed *injection of 0.2 per cent virus with later tarring* (Chart 7) that the group serve as additional controls. Where the inoculum was tattooed into the body, papillomas appeared late, as always with dilute virus (4). In 5 individuals 1 or more sooty, rapidly enlarging papillomas developed on each side of the ears after a few weeks, while in two others they appeared on the insides only; but a mere scattering was obtained at best. In one individual, however (No. 9, Chart 7), most of the preexisting warts disappeared when tarring was left off, but a few began to grow rapidly when the virus took effect, as evidenced by dark gray papillomas on the outside of the ears, and they soon outstripped the growths of any control. Nothing further of significance took place and the group was discarded on the 57th day.

In several rabbits *injected with 0.5 per cent virus but not tarred later* (Chart 8), a considerable number of growths arose in the 3rd to the 6th week after injection. Those on the outside of the ears were ordinary, conical or onion-shaped, virus-induced papillomas, nearly all dark gray, whereas many on the inside were pink. Here, however, other pink growths appeared concurrently that were discoid and fungating, and in several instances these enlarged with a rapidity unexampled in the controls, and so too did some of the preexisting tar warts. In rabbit 20 which died on the 57th day, one of the latter which had taken this course reached enormous size and like 3 others in the same animal, all originally tar warts, had the histology of an anaplastic carcinoma. Yet since none metastasized or caused extensive destruction, they cannot be set down as cancerous.[2]

In 5 of the 8 rabbits *receiving 0.5 per cent virus, and tarred later* (Chart 9),

[2] The distinction to be made between carcinomas and carcinoids,—growths which look like cancers though incapable of independent progression,—is discussed in Paper I.

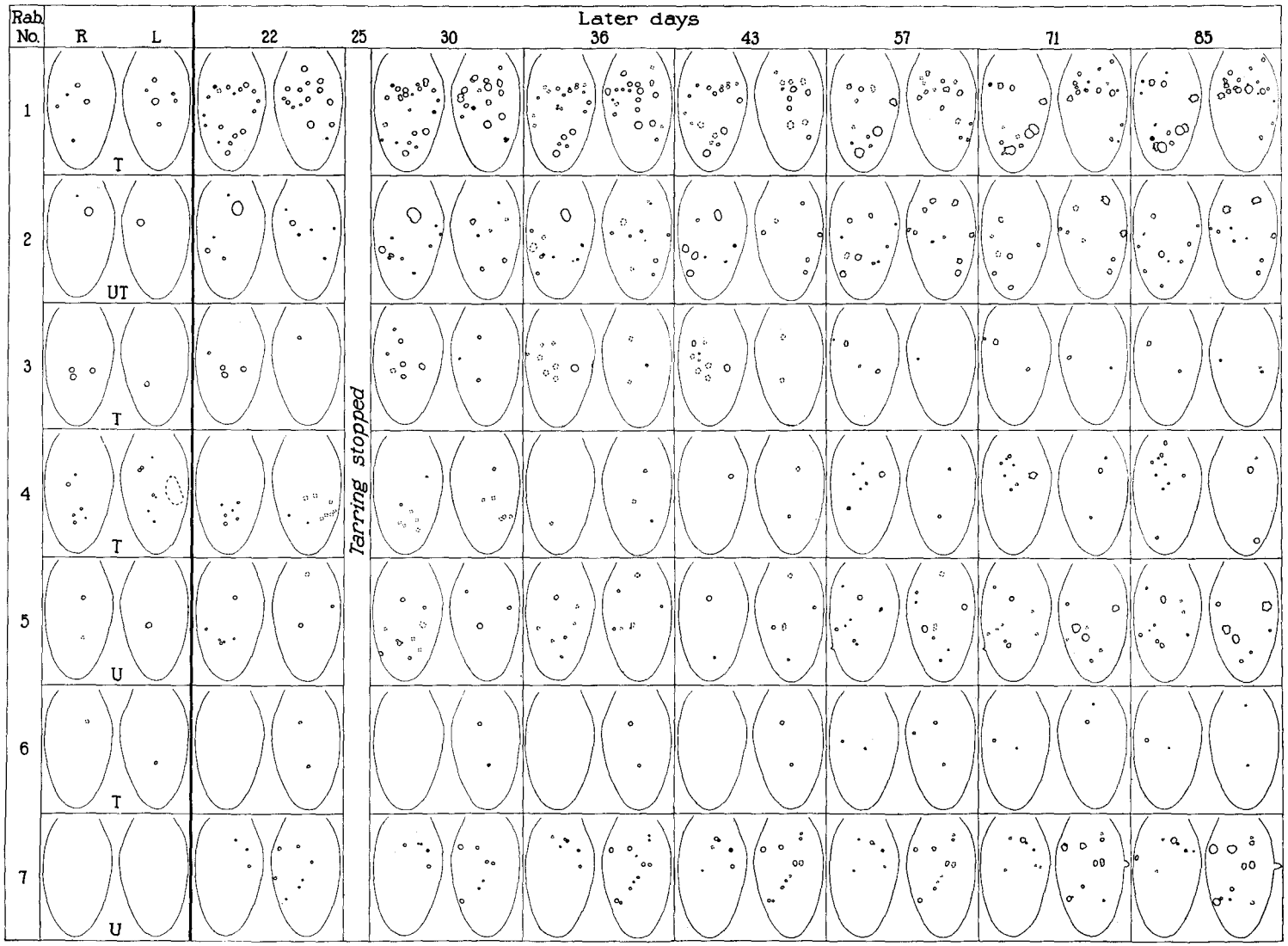

CHART 6. Tarred controls.

CHARTS 6 to 9. For description see text, and for meaning of symbols see Chart 9.

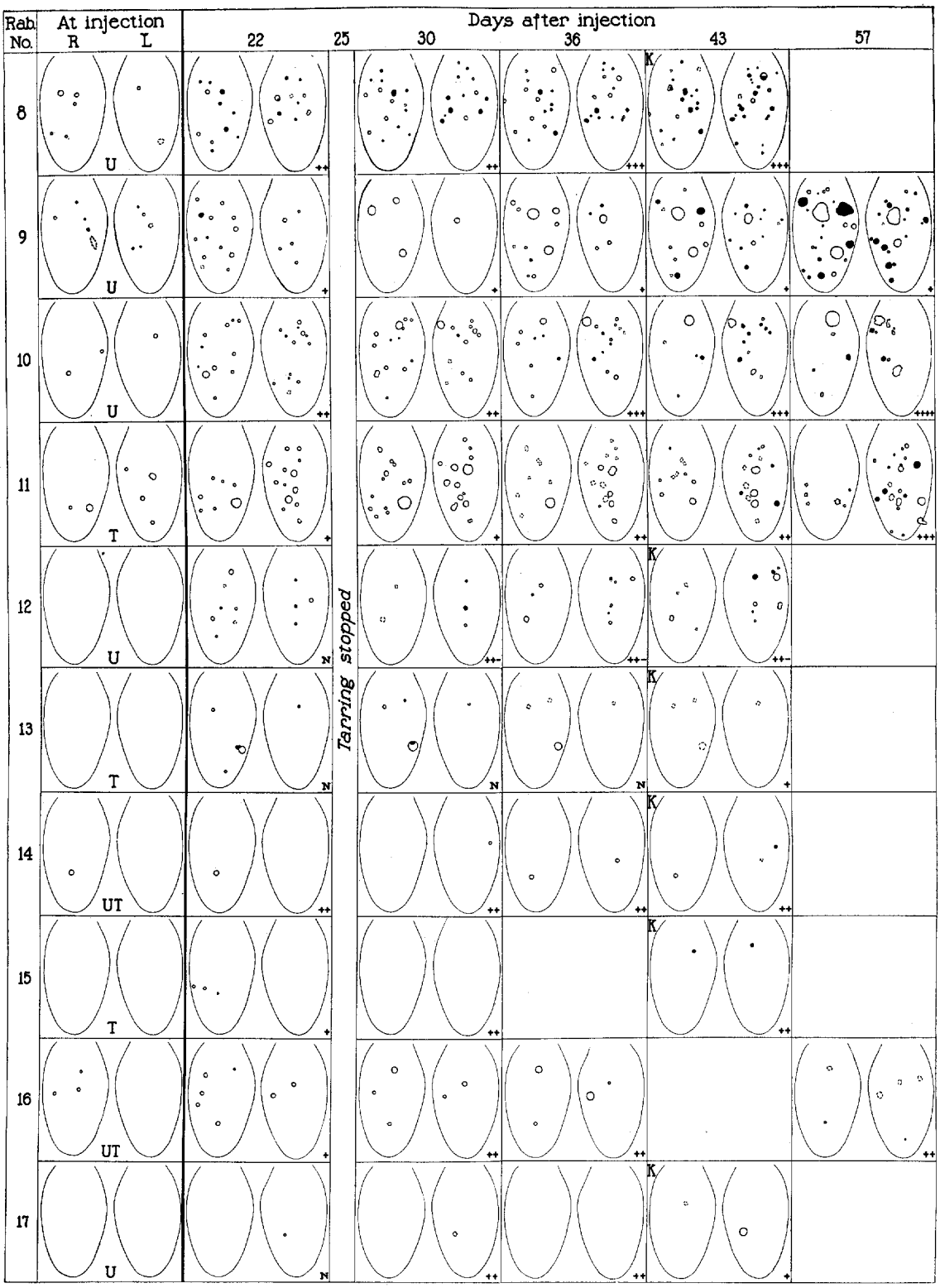

CHART 7. 0.2 per cent virus: tarring kept up.

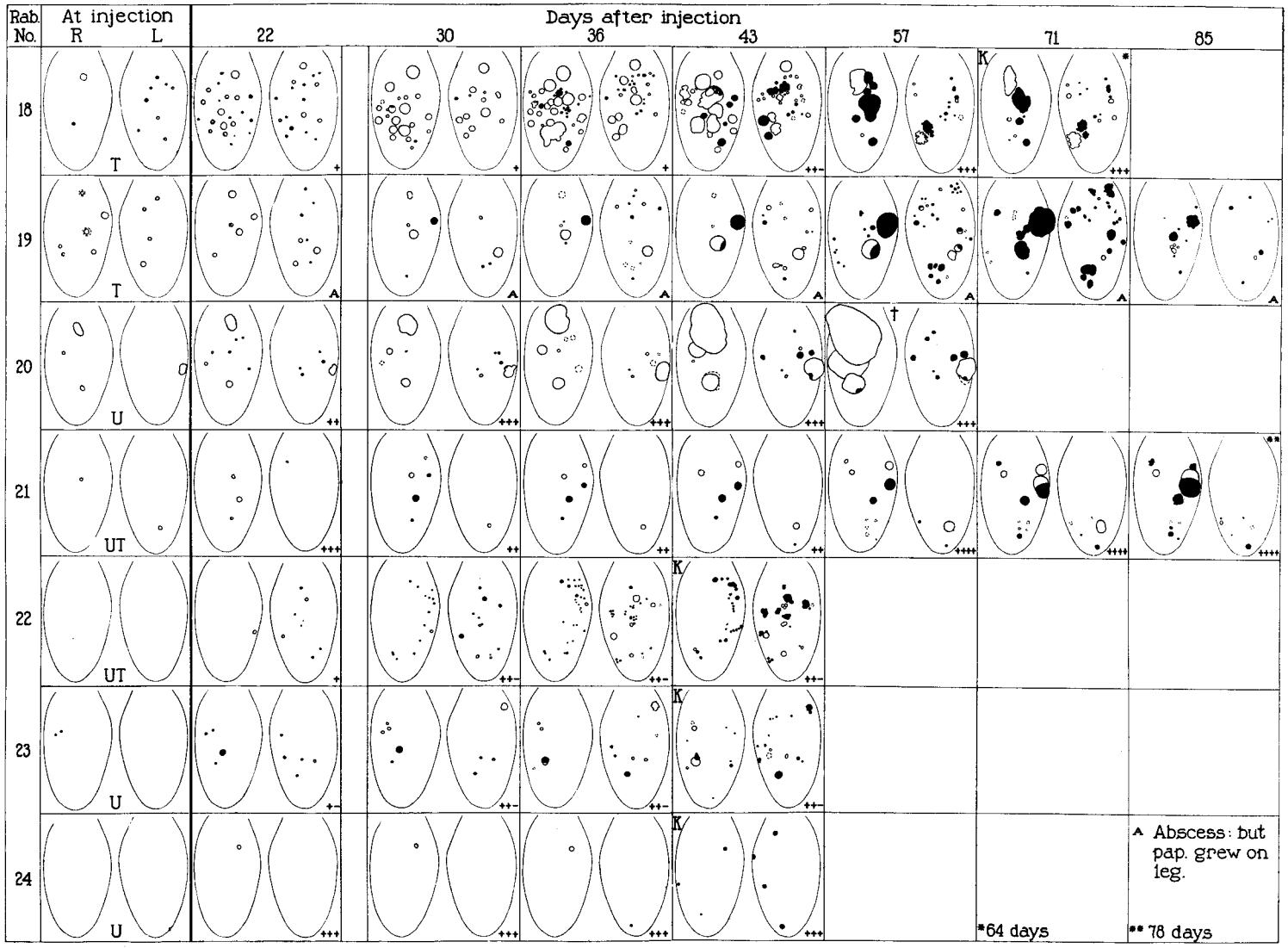

CHART 8. 0.5 per cent virus: no more tarring.

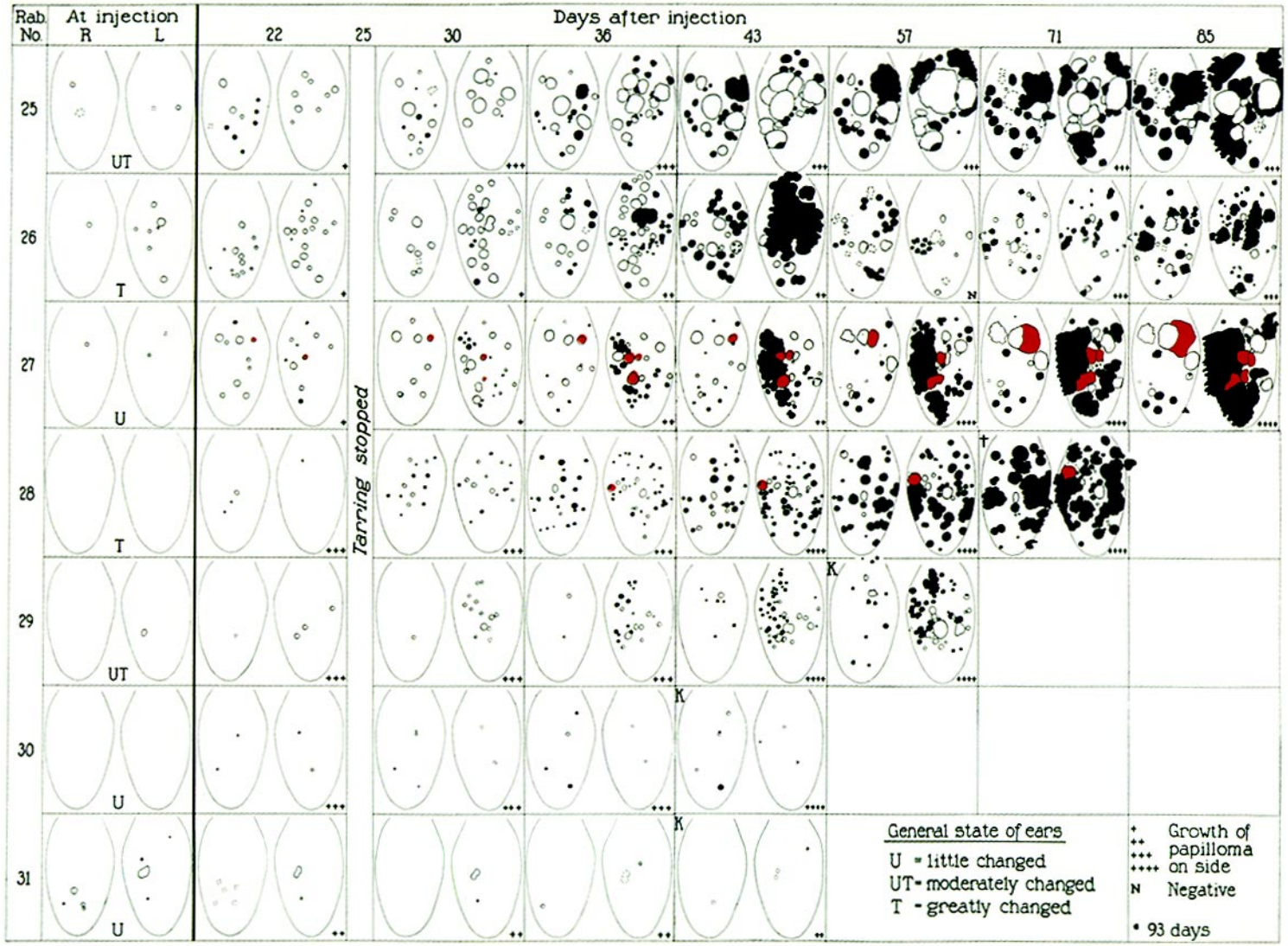

CHART 9. 0.5 per cent virus: tarring kept up.

many rapidly enlarging papillomas arose, and the influence of the virus was attested both by the great contrast with the controls and the frequency of melanosis. Vigorous pink growths of the various sorts described in Paper I appeared concurrently, and in 2 of the 5 rabbits in which the virus proved most effective, as evidenced by tumors (Nos. 27 and 28), some proved carcinomatous. Rabbit 28, with a single cancer, died early of intercurrent causes, but rabbit 29, with 4 malignant growths, lived long enough for huge metastases to form on both sides of the neck (3).

In this experiment the tar tumors of the control animals behaved as usual after tarring was left off, many disappearing and the rest remaining stationary or enlarging slowly as benign growths (Chart 6). One group of animals receiving virus got so little as to cause only a few new tumors, and these proved ordinary papillomas (Chart 7). This group were tarred for 25 days after injection. In the group receiving four times as much virus but not tarred thereafter, more growths appeared (Chart 8), and their variety was greater, but while some looked cancerous there was no certainty in the matter. In a group similarly inoculated and tarred for 25 days, the virus proved notably effective, causing a multitude of growths in many instances and carcinomas in some (Chart 9).

In the next experiment more virus was injected and the attempt was made to prevent it from localizing in one of the ears. To this end the circulation to the organ was obstructed for some minutes after the intravenous injection was begun.

Experiment 5.—Tarring was done twice weekly for 84 days before the injection. The ears of the 28 rabbits were charted on the 81st day, with no more tarring until after the injection. The animals were separated into 3 comparable groups, 2 of which received 15 cc. of virus fluid intravenously, the third serving for control. The inoculum was a Berkefeld filtrate of a 5 per cent Tyrode extract of the pooled, glycerolated papillomas of 8 cottontails. Just prior to its injection into a leg vein,—which required about a minute,—a rubber band was wound tightly around the base of the left ear and not cut away until 2 to 22 minutes later. To determine individual susceptibility virus fluid was tattooed into the left side of the body, as in Experiment 4. The ears of the controls and one injected group were tarred again after 22 hours and twice weekly during the succeeding 21 days. To the other injected group nothing further was done.

A few additional warts appeared during the later tarring of the 9 *controls* (Chart 10), but afterwards the majority vanished, though some remained sta-

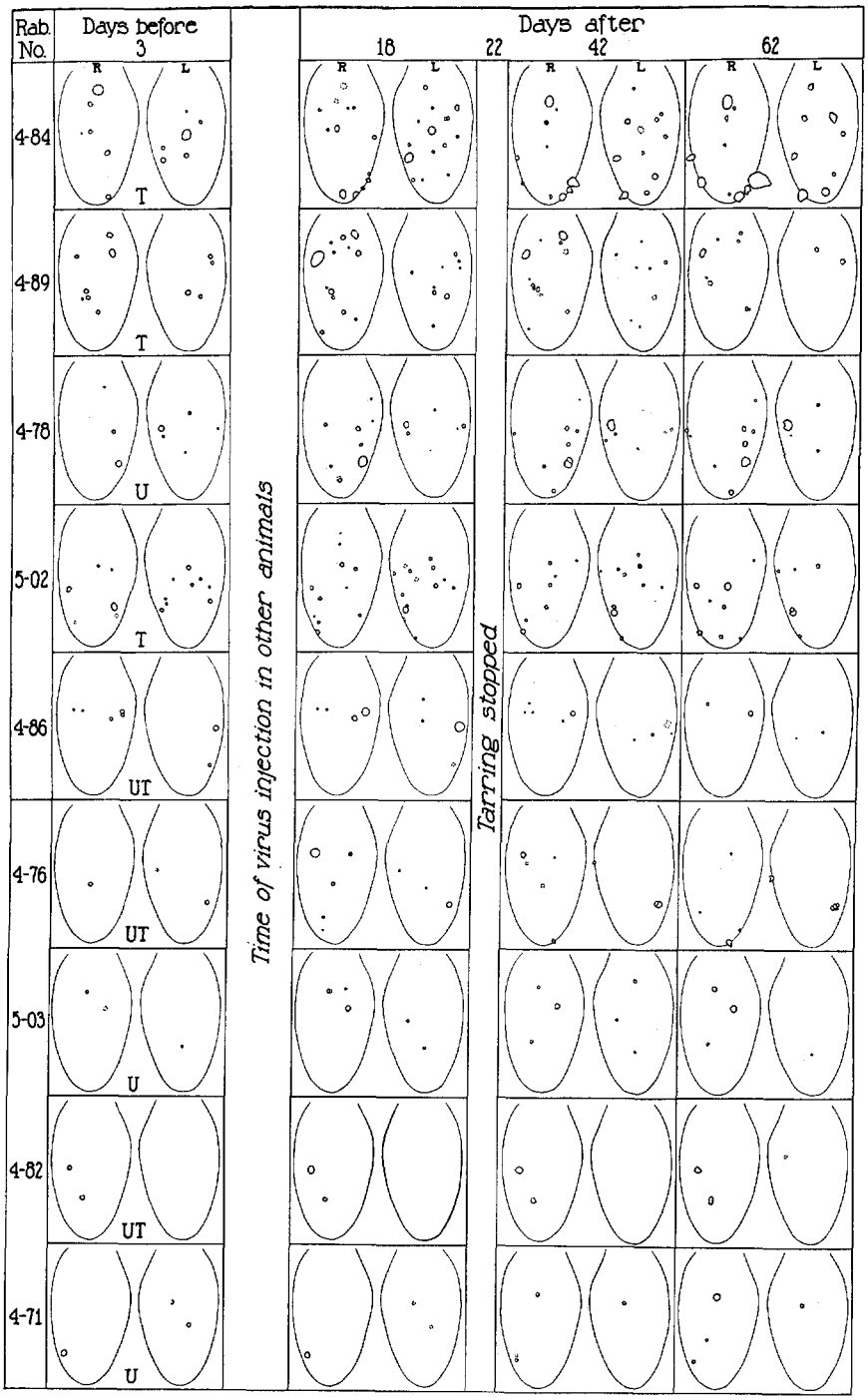

CHART 10. Tarred controls: no virus injected.

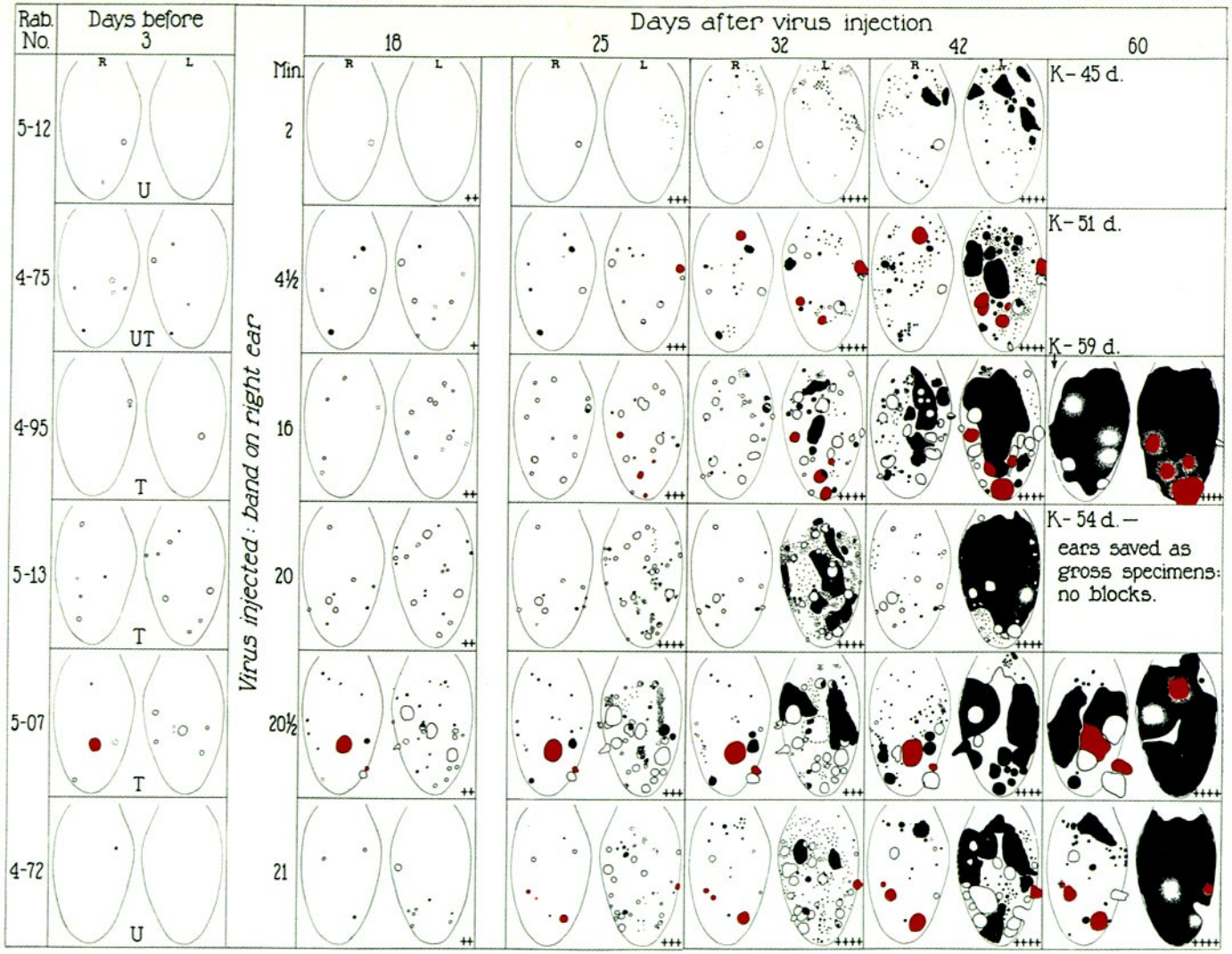

CHART 11. Virus injected: no tarring thereafter. Growths on insides of ears. Some cancers have not been charted because overlain by melanotic papillomatous tissue. This was frequently true of those which had extended to the outside of the ear.

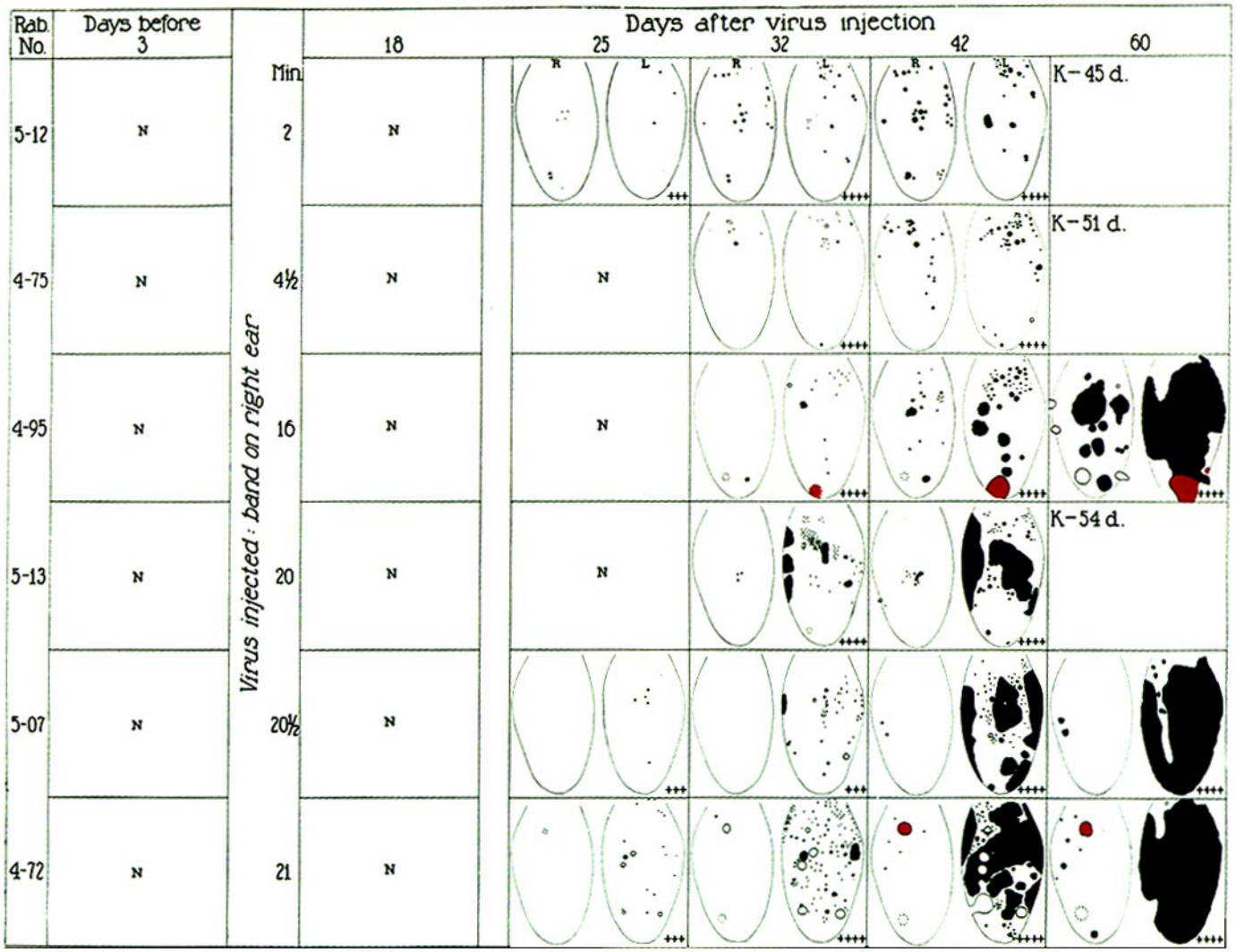

CHART 12. Virus injected: no tarring thereafter. Growths on outsides of ears.

tionary or grew slowly throughout the 62 days of observation. The rabbits with most persisting warts were kept many months, and nearly all of their growths retrogressed, a few remaining stationary or very slowly enlarging. No cancers ever developed.

All of the injected rabbits proved susceptible, papillomas developing at the tattoo site. The 8 animals *injected and tarred later* yielded such heterogeneous findings that no comparison can be made with those not tarred again. In 1 no growths indicative of virus localization arose, and in 2 only a few sooty papillomas. In 2 others more growths of this sort were elicited but most of them disappeared after some weeks, as did all the tattoo papillomas. In 1 of the remaining 3 a moderate number of progressively enlarging growths appeared, but none had a malignant aspect when the rabbit was discarded after 106 days. In the other 2 a great variety of tumors developed, cancers amongst them. They illustrated special points, to be handled in a succeeding paper.

The ears of 2 of the 11 rabbits *injected but not tarred again* were but slightly changed, and the virus caused merely a few dark gray papillomas. Several pre-existing tar warts started to grow rapidly, but none seemed malignant and the rabbits were killed early, with no blocks taken. In a third animal a sparse scattering of growths arose on the ear submitted to the tourniquet for 14 minutes, while they were fairly numerous on the unobstructed one; but again they promised little and the rabbit was discarded. Charts 11 and 12 record the changes in 6 other animals, killed between the 45th and 62nd days. The virus-induced growths were relatively few in one instance, no cancers were recognizable amongst them, and the animal was discarded early. The ears of another were saved in the gross: they carried several tumors with the aspect of cancers, but in the lack of histological confirmation none was recorded as such. Multiple carcinomas appear in all of the other 4 and a glandular metastasis in 1.

The 2 rabbits which do not appear in the charts were kept several months longer. The virus elicited numerous cancers on their ears, with a glandular metastasis in one case. Bits of the metastatic tumor were transplanted to the leg muscles with result in growths.

Cutting off the circulation for 2 minutes (Charts 11 and 12) was not followed by a noteworthy difference in number of growths elicited, but $4\frac{1}{2}$ minutes decreased it, and 20 to 21 minutes reduced it greatly. Under such circumstances the ear submitted to obstruction served usefully as a control. The number of carcinomas was considerably greater than the charts indicate, since only those are recorded which were examined microscopically, and this was undertaken with less than half of them.

The individual findings will be summarized. The pathological

changes due to tarring rabbit ears have been the theme of many investigators (7), and those noted under the circumstances of our experiments have already been described (3). There was much individual variation in hyperkeratosis, connective tissue proliferation, vascular disturbance, etc., as well as in the incidence of tar warts.

No. 5-12: Ears almost unchanged by the tarring. All of the virus-induced growths were sooty papillomas. Discarded after 45 days.

4-75: Ears moderately changed: several tar warts. After the injection a fair number of sooty and pink growths appeared on the ear submitted to the tourniquet, a scattering on the other ear. Killed on 51st day. Of 8 pink growths sectioned 6 proved cancerous, the others benign papillomas.

4-95: Ears much changed: few tar warts. Many new, rapidly enlarging pink growths appeared in the 3rd to 5th week after the injection, and toward the end of this period great multitudes of minute, sooty ones, which soon coalesced. Both were especially numerous on the ear submitted to the tourniquet. Killed on 59th day. Of 7 pink growths sectioned 4 were cancerous. One of these was primary on the outside of the ear. Another extended through to this side and became large here.

5-13: Ears much changed: many tar warts. The happenings were like those in 4-95, the ears differing even more. Killed on 54th day for gross specimens; no blocks.

5-07: Ears much changed: several large tar warts. One on the right ear began to grow fast soon after the injection and became a broad cancer. The general course of events was as in 4-95, 5-13. On the 54th day the left auricular lymph node was discovered to be much enlarged and nodular, and at sacrifice on the 61st day it was 1.8 cm. across, almost wholly replaced by a cystic, malignant papilloma. At no time had the ear been incised. One of the large tar warts became malignant after the injection. Of 7 pink growths sectioned, 4 were frank carcinomas, the others papillomas of diverse character.

4-72: Ears little changed: one small tar wart which remained stationary later. Same happenings as in rabbits just dealt with: killed on 61st day. Of 10 pink growths sectioned 6 were cancerous, 1 primary on the outer side of the ear. Several other carcinomas had been removed by early biopsies.

The 2 individuals not included in the chart were let live until the 137th day. They too developed multiple cancers, which at death occupied most of the ears. These had not been cut into at any time. A metastasis was discovered in an auricular lymph node of one of them (D. R. 4-73) on the 76th day, and portions were implanted in the muscles of all four legs of the host on the 90th day, with result in masses of invasive squamous celled carcinomatosis, 1.5 to 3.0 cm. across at autopsy.

Increasing the amount of virus had the desired effect in this experiment; it localized abundantly in the ears and rapidly elicited a variety

of growths, many carcinomas amongst them. Later it also produced hosts of ordinary gray and pink papillomas which remained benign: a large number were sectioned incidentally to study of the unusual tumors. The virus was obviously responsible for the latter, since none appeared in the controls, despite the later tarrings, and relatively few on the ears submitted to the tourniquet.

The experiment demonstrated that tarring after injection of the virus is not essential to the production of carcinomas. In the present instance as many appeared in the animals no longer tarred as in the most favorable of the tarred group.

The main object of the next test was to learn whether virus materials from different sources would give rise in the tarred skin to differing growths.

Experiment 6.—The 20 rabbits had been tarred twice weekly over a period of 27 days, once more on the 37th day, and again on the 40th day, 4 hours prior to the intravenous injection. The changes thus induced in the ears were less pronounced than in any previous test, yet in some instances these organs carried a few tar warts. The animals were separated into 4 comparable groups and injected intravenously with one or another of four highly pathogenic virus fluids deriving respectively from the glycerolated "spontaneous" papillomas of 3 wild rabbits and from the pooled material utilized in Experiment 5. 5 per cent Berkefeld filtrates were injected, 15 cc. for each animal, and some of the inoculum was tattooed into the side of the body. Here it gave rise to papillomas only.

All of the rabbits proved susceptible to infection, and, on the ears of many, multitudes of growths arose after the incubation period usual with the virus. The general course of events was precisely like that in Experiment 5, with cancers nearly as numerous. The ears had been tarred less than in any preceding experiment and were not tarred after the injection. The character of the tumors yielded no indication of qualitative differences in the inocula.

A further experiment had as its aim the microscopic study of early stages. The ears were briefly tarred, infiltrated directly with large quantities of virus, and soon after the growths had shown themselves some were punched out.

Experiment 7.—The 3 rabbits had been tarred twice weekly for 28 days, and had ears slightly to moderately changed, with no warts, one small wart, and 2 warts respectively. After the usual stripping and charting the left ear was warmed to distend its vessels, a rubber band was wound tightly around its base,

and by way of a marginal vein it was infiltrated with 10 cc. of 1 per cent virus fluid, derived from the pooled papillomas of 3 experimentally inoculated wild rabbits, and centrifuged water-clear. As the fluid went in the ear became turgid and slight oozing took place from cracks in the hyperkeratotic epidermis, and from the tar warts especially. Leakage from the wound made by the injection needle was prevented with a metal clip. 30 minutes later the rubber band was cut away, after another had been placed about the base of the other ear. This was left on 30 minutes to prevent localization of the virus flushed out of the infiltrated organ when the blood entered it again.

A profuse virus infection took place, especially in the animal with no tar warts. Its infiltrated ear suddenly became thick, hot, and brawny during the 2nd week, a change noted in previous experiments (3) when much virus had localized in the tarred epidermis, and a multitude of growths soon arose, on the injected, outer side especially. After 14 days they were fairly numerous, discrete, ½ to 3 mm. in diameter; and soon they appeared in a crowded horde, coalescing and covering the entire surface. The control ear remained thin, cool, and devoid of tumors. In the other rabbits no brawny thickening occurred, and not so many growths developed. In each case the ear injected carried the tar warts. The 2 present in one animal began to enlarge rapidly when the virus-induced tumors were appearing, and after several weeks proved cancerous. The innumerable growths elicited by the virus manifested some of the variety noted in previous experiments, but only a few were malignant. Most of these were punched out within a few days after their appearance. The control ears reverted toward the normal in one case, remaining free from tumors, while in the other two small growths arose, a pink one with the distinctive morphology (later to be described) of a tar papilloma, a second sooty, and obviously due to the virus. The rabbits were killed after 27 to 34 days.

The Anomalous Tumors

A comprehensive description will now be undertaken of the *anomalous tumors*, as one may term all those growths elicited by the virus in tarred skin, which differ from the standard productions (benign papillomas) characteristic of its ordinary action.

The papillomas which follow upon direct inoculation of the virus into the scarified normal skin of "agouti" rabbits are remarkably uniform in morphology, though some are pink and the majority gray or almost black. The difference is due to melanoblasts, passively included in the growths, which multiply and form abundant pigment, though they do not become neoplastic (5). They are most likely to be included when the papilloma is the outcome of proliferation at many neighboring points; and, since multicentric growths enlarge most rapidly, it follows that those which first become visible are most likely to be melanotic.

Nearly all produced by an inoculum of high titre are composites of cell families, each the outcome of a cell-virus association: and certain families may proliferate with special vigor and aggressiveness, pushing others to one side, extending beyond the included melanoblasts, and forming discrete, non-pigmented nodules in the midst of the mass and sometimes beneath it (6). Usually, nevertheless, all of the papillomas of any one rabbit run the same general course, enlarging together, and perhaps all retrogressing secondarily. The fewer the cell families of which they are constituted, the more likely are they to differ in small ways.

When *normal* rabbit ears are directly infiltrated with virus fluid as in Experiment 7, and then tarred several times, numerous growths may appear, all ordinary virus-induced papillomas and the generality punctate in origin. The differences they manifest when tarred do not exceed those just described, the uniformity in size, shape, and behavior being remarkable. The growths on the outside of the ears, where melanoblasts abound, are mostly dark gray, whereas those near the middle of the inside may be pink, with gray ones frequent toward the edges. Histologically the two differ only in content of melanoblasts. If a piece is punched from a spot covered with gray growths, while these are still appearing, an enlightening phenomenon may follow. Papillomas wholly devoid of melanosis arise from the epithelium that has extended to cover the raw edges of the punch hole, and enlarging with special vigor, they soon protrude from amidst the host of gray growths as pink cauliflower rosettes or hassocks (Figs. 1 and 2). Yet they have the usual histology,—though staining lighter with methylene blue,—and it is plain that they owe their lack of pigmentation to origin from an epidermis which has extended farther than the melanoblasts, and their rapid enlargement to the favorable conditions provided by the healed wound. Usually they soon become stalked, doubtless because of the ductility of the new connective tissue (Fig. 2).

Two or three tarrings of the ears prior to an intravenous injection of virus will cause it to localize in them, but only ordinary papillomatosis ensues. Many of the growths must be the outcome of individual cell-virus associations, yet their general sameness is remarkable, especially when thousands are present. If the preliminary tarrings have been many, the skin on the neck where the ears rub may have become hairless and hyperkeratotic; and here the virus may localize and cause papillomas of ordinary sort, which grow or retrogress together, with negligible exceptions.

The first evidence that preliminary tarring results in different kinds of virus tumors was obtained when the ears had been tarred during 4 weeks and were then directly infiltrated with virus fluid (Experiment 7). Pink growths arose some days before any gray ones did so,—the reverse of the usual occurrence,—and they grew much faster than the latter which usually appeared in relatively immense number. Tarring for periods up to 3 months, with intravenous inoculation of the virus and no more tar (Experiments 5 and 6), resulted in the same

occurrences in more pronounced form, the incidence of the unusual pink growths being greater. Most became visible during the 2nd to the 4th week after virus injection, almost none arising afterwards, whereas the ordinary gray papillomas kept on appearing until the 8th to 12th week, and often became a crowded host. All of the growths were subepidermal mounds when first noted, but whereas the anomalous pink tumors frequently reached a diameter of 3 to 5 mm. prior to erupting, the gray papillomas were seldom more than a millimeter across before they did so. The difference was not one of origin, for the majority of both sorts derived from the epithelium of hair follicles rendered cystic by retained keratin: it lay in the activities of the virus-infected cells. Those constituting the ordinary papillomas, though stimulated to rapid multiplication, heaped up in the direction of least resistance, which was outwards, whereas the cells of the anomalous pink growths were more aggressive, and extended laterally for some distance before the mass erupted. Yet some of the pink growths proved to be mere Shope papillomas, or variants thereof (Fig. 25), though as a rule staining lighter with methylene blue than their gray fellows. Other pink growths exhibited distinctive morphological differences from the first (Figs. 4 to 9), the folded, subsurface layers of proliferating epidermal cells being compact, cystic, or convoluted, and taking the methylene blue poorly. Nevertheless the influence of the virus was still manifest in the cytology of many. The pink growths were situated mostly near the middle of the insides of the ears, where the tar had produced greatest change, but occasionally on the hyperkeratotic, outer side also (Chart 12, rabbit 4-72); and beginning as mounds, they rapidly became hemispheres while still subepidermal, and, sometimes within a few days, took the form of deeply embedded, fleshy globes covered with tense, glistening epidermis (Fig. 3). Often, they scabbed at the summit before this happened. Some grew rapidly, some slowly, but nearly all soon dried at the top and enlarged into plump cones or "onions" or jagged masses (Figs. 10, 19), whereas the ordinary gray and pink papillomas arising later and growing nearby were, by contrast, keratinized nearly to their bases in most instances and became dry, jagged spires, horns or cauliflowers, confluent next the ear surface, or discrete even where greatly crowded. As the pink growths rose higher their dry peaks turned dirty gray, and when their bases were hidden the hue was easily mistaken for genuine melanosis. It follows that the latter was not so prevalent as some of the charts would imply.

The anomalous growths of malignant sort which arose on ears no longer tarred, appearing as pink mounds like those already described, usually enlarged into embedded spheres, scabbing or drying at the top. A few ulcerated early, and became discs with raised, infiltrating edges (see Paper I), or formed scabbed, fleshy hassocks; but the majority built up into fleshy, broad-based cones or onions, often vertically ribbed, and sometimes with such a superficial resemblance to benign papillomas that their true character was overlooked until metastasis had occurred. When cut into, though, even at an early stage, their irregular markings, punctate necroses, and extension downwards and sideways disclosed

their true nature. Frequently they broke down into ulcers and then built up again. When they extended through the ear, a frequent happening, they formed firm mounds on its outer side, which either became ulcers with thickened, infiltrated edges, or else low, fleshy cones which broke down and built again, perhaps repeatedly. Some of cystic character formed subepidermal aggregates of partially coalesced nodules from which firm prongs with keratinized cores extended toward the head, often within the large lymphatics. (*Vide* the cancer of rabbit 28, Experiment 4 (3).) Frequently the rabbits kept the malignant tumors abraded and bloody, as if they caused annoyance. As they got bigger they became broad, weeping, fungating masses, or ulcers almost at the skin level (Paper I). Some metastasized early to the regional glands (Figs. 11 and 12), but others failed to do so though occupying almost the whole ear. The metastases were frequently cystic,—in which respect they resembled some tar cancers (7), as also some carcinomas developing secondarily from ordinary virus-induced papillomas (8). The implantation of bits of the metastases in the leg muscles of the host resulted in carcinomatous masses with a tendency to greater anaplasia (Fig. 13). Many of the rabbits of Experiments 5 and 6 carried 5 to 10 or more large cancers on each ear when they were killed, 2 to 4 months after inoculation. Occasionally one arose on the hyperkeratotic outer side, as did also benign anomalous tumors. Not every threatening growth was sectioned. All of proved malignancy were non-melanotic, another point of similarity to tar carcinomas and those deriving secondarily from Shope papillomas.

When tarring was kept up after virus injection, ulceration soon took place of many of the benign growths as well as the malignant and they grew fast, and could not always be told apart for some time. Not infrequently the continued tarring also elicited tumors referable to it alone, and the longer the procedure was kept up the more often they occurred. Under such circumstances it sometimes proved impossible to say of a given tumor in the gross whether it was of tar or virus origin; yet when many new growths arose in the injected group and few or none in the controls, or on that ear of injected individuals which virus was prevented from reaching (Experiment 5), one could be certain that it was responsible for the generality.

Most of the pink, anomalous growths of ears no longer tarred retained their initial advantage over the crop of ordinary papillomas that arose later. The latter while small often resembled strewn iron filings set on edge with a magnet, the pink growths standing forth from amidst them as subepidermal globes. Later they rose into crowded, high peaks, confluent at the base; and now their competition with the pink growths affected the latter adversely, because of their great number and prodigious energy of proliferation, which often far exceeded that of the associated carcinomas, these triumphing by the destruction they caused. Frequently a jagged mass of gray, confluent papillomatosis pressed upon and overhung the malignant ulcers, obscuring them until a patch of the mass or the

entire end of the ear suddenly came away, exposing large cancers which had been burrowing and destroying all the while. Those which had become fleshy cones or hassocks were sometimes so squeezed by the surrounding papillomatous tissue that they dwindled amidst it while extending beneath. When they grew through lacunae in the cartilage to the outside of an ear covered with ordinary papillomatosis they sometimes remained invisible until the ear was sliced at autopsy. This held true of a tumor of Chart 11, which hence finds no record in the charts made during life.

The successive charts of the same ear often disclosed great individual variations in the course even of typical Shope papillomas. For example in rabbit 10 of Chart 7, 6 sooty papillomas disappeared from the left ear between the 43rd and 57th day, while a seventh grew and a wholly new one appeared and enlarged rapidly. In rabbit 9 of Chart 8 several dark gray papillomas disappeared from the middle of the right ear between the 57th and 71st days, as did also one at the injection site on the leg, whereas the other growths of this sort on the ears continued to enlarge. Later nearly all of these disappeared, a few continuing to enlarge during many further weeks, as shown in charts not reproduced. In rabbit 15 of Experiment 1 (3) most of the ordinary melanotic papillomas disappeared from the ears, yet some continued to grow, as did all of the cancers and certain pink papillomas.

The course of the anomalous growths varied notably. While nearly all appeared soon after the injection, an occasional one came late, after 2 months or more. Some of the benign ones retrogressed while others enlarged concurrently. After tarring was left off the malignant tumors generally progressed, though handicapped by the rapid reversion of the ear tissues toward the normal. Some grew fast, some slowly, and very occasionally one vanished after progressing and destroying for many weeks.

So sharp was the contrast between the pink anomalous tumors elicited in Experiment 5 and the ordinary virus-induced papillomas, gray and pink, which arose later (Figs. 3 to 10), as to suggest the presence of two differing sorts of virus in the composite inoculum. True, this had caused only ordinary papillomas where tattooed into the skin. Nevertheless some rabbits were inoculated with serial dilutions of each component of the material, these being still available. There resulted only papillomas. Comprehensive observations, made later, upon the effect of other virus materials (Experiment 6) justified the conclusion that the variety of the neoplasms was due to differing changes in the epidermal cells affected by the preliminary tarring, not to peculiar virus constituents.

Numerous early biopsies were made to learn how the anomalous tumors arose. Often these were made at a time when the ordinary papillomas had only begun to appear, and whenever possible growths of both sorts were included in the same microscopic section (Figs. 4 to 9).

The histological findings were easiest to interpret when no further tarring had been done. Under such circumstances almost all of the anomalous growths consisted in their earliest stages of irregularly folded layers of stratified epithelium from which tongues and strands of cells often extended down. From the first all were distinctively different from ordinary virus papillomas, the differences being relatively slight in the case of benign growths, finding expression ordinarily in thicker epithelial layers, more complex pattern, less tendency to keratinize, and lighter staining with methylene blue. Those malignant tumors which were least anaplastic retained the papillomatous form but had convoluted layers of epithelium, lying in a loose, perhaps spongy, disorder. The jumbled cells colored palely with methylene blue (Figs. 6, 8, 18), and often died before keratinizing. Such growths invaded the underlying tissue from the beginning (Fig. 8), as squamous cell carcinomas which sometimes formed keratinized cysts secondarily (Fig. 19). There were many minor differences in the histology of the individual growths. Very few of the untarred cancers were markedly anaplastic in their early stages, whereas this was usual with those tarred (see Paper I). The coalescence of neighboring growths often yielded complicated findings.

Many of the cancers retained their initial morphology, while others underwent further disorganization, and yet others soon became wholly anaplastic (Fig. 14). Tarring greatly hastened these changes.

The histological features were most readily interpreted when the virus had localized at scattered points, giving growths of unicentric origin. In Experiments 5 and 6 many were multicentric, owing to the large inoculum, and their composite character was frequently evident in the gross, some being spotted, streaked, or segmented in gray and pink. Microscopically in such cases ordinary virus papillomatosis was often joined to, or intermixed with, anomalous proliferation, both incorporated in one mass (Figs. 16 and 17). Sometimes the pattern of the keratinized material made clear the fact that the differing neoplastic components had long been present (Fig. 17). Such mixed growths usually became cancerous everywhere within 2 or 3 months, their malignant components destroying the benign.

The greater the preliminary changes due to tarring the more often, generally speaking, did the virus cause anomalous tumors. There were notable exceptions, however, the virus in certain instances causing many anomalous growths, some malignant, in skin but slightly

changed, and in others producing none despite marked cutaneous alterations. Most of the experiments were carried out on animals tarred from 40 to 90 days; and we have gained the impression that the longer the period, within these limits, the more frequently were the anomalous tumors malignant. Since the incidence of tumors due to tarring, as such, is greatest in skins that undergo most change, it follows that ears carrying many tar tumors were those in which anomalous tumors were most prone to develop after the virus injection. Yet once more exceptions were met, some ears devoid of tar tumors yielding many anomalous growths in response to the virus (rabbit 4-72 of Chart 11, left ear), whereas others with large ones developed only ordinary papillomas.[3]

Influence of the Susceptibility of the Host

In most of the experiments some of the inoculum was tattooed into the side of the rabbit. All thus tested proved susceptible, but in widely differing degree, the tattoo growths appearing late and sparsely in some, and in others coming early and in great numbers, soon assuming confluent form. It was in individuals of the latter sort that the cancers appeared. Yet pronounced susceptibility combined with a profuse localization of virus in tarred skin did not suffice of themselves to insure the occurrence of malignant growths.

In rabbit 6-51 of Experiment 3, injected after long tarring, the epidermis underwent a broadcast infection with the virus, and the ears reached prodigious size; yet careful search disclosed no cancers. The virus caused a multitude of papillomas in rabbits 25 and 26 of Experiment 4 (Chart 9), and by the 30th day they had outstripped those due to it in rabbits 27 and 28; yet cancers appeared only in the latter animals. Rabbit 13 of Experiment 1 bore a host of vigorous, virus-induced papillomas but no malignant tumors, whereas rabbit 15, with few papillomas, and these mostly slow to appear and enlarge, developed several carcinomas.

Not infrequently after the virus papillomas had prospered for weeks most or all retrogressed rapidly and disappeared (rabbit 26, Chart 9),

[3] The injection of a highly potent virus into the 2 animals of Experiment 3, with large tumors due to tarring for 40 weeks, resulted in but a single cancer amidst massive papillomatosis.

together with the tattoo papillomas and any that had arisen at the injection site (rabbit 19). Some generalized host influence was obviously at work (9). Many of the benign, pink, anomalous tumors likewise vanished, but others kept on enlarging, and so too did certain of the ordinary virus papillomas, in not a few instances (rabbit 15, Experiment 1). The cancers continued to progress but no new ones arose.

Influence of the Amount of Virus Infection

Very slight alterations due to tarring will bring the virus out of the blood stream, but the result is only ordinary papillomatosis.[4] This developed often on the back of the neck where the ears had rested and the skin become hairless and somewhat hyperkeratotic. The greater the ear changes at the time of the intravenous injection of virus, the more abundant did papillomas tend to be, hot, thickened, hyperkeratotic ears usually developing them in immense numbers as compared with ears little changed though tarred as often. Yet exceptions occurred. (Compare rabbits 8, 13, Chart 7; 4-72, Chart 11.) And even when the skin was markedly changed, and the animal so susceptible that the virus caused a horde of fast growing papillomas, anomalous tumors often remained completely absent (Fig. 15). Preliminary cytological changes of peculiar sort were obviously essential to their occurrence, changes always focal or punctate, a fact plainly evident no matter how abundant the virus infection. Needless to say the opportunity for the virus to reach cells changed in the appropriate way varied directly with the amount localizing in the ears; and this could be judged from the number of gray papillomas. On scrutinizing the charts it will be seen that, other things being equal, the more abundant these papillomas, the greater was the incidence of anomalous growths, with some exceptions (*e.g.* rabbit 4-72, Chart 11). Usually the virus caused many papillomas for every anomalous tumor; but again there were exceptions (rabbit 15, Experiment 1). When a dilute inoculum was employed the growths induced were relatively few (Chart 7, Experiment 4), and they appeared late and

[4] The virus will localize and cause papillomas in skin tarred but once, as Dr. R. J. Parsons has noted.

enlarged slowly, as on scarified normal skin. This was to be expected since the number of cell-virus associations formed at time of infection and producing a growth has much to do with how soon it becomes visible and its rate of enlargement.

Influence of the Virus Strain

Papillomas induced with the virus in scarified normal skin often become carcinomatous after proliferating for some months (8), and the greater the pathogenicity of the inoculum,—as evidenced in the papillomas,—and the higher its titre, the sooner and oftener does the malignant change occur (10). Because of this relationship it seemed well to employ notably pathogenic materials in the present work, and this was done except in the following test.

Experiment 8.—The ears of 31 rabbits tarred 57 days were stripped and charted, tarred again immediately, and 15 of them were injected on the 59th day with 15 cc. of Berkefeld filtrate of a 1.5 per cent extract of the pooled, glycerolated papillomas of 7 cottontails. 12 comparable individuals were reserved as controls, and 4 others had the virus tattooed directly into two strips cleaned of tar along the inside of each ear. This was reapplied to all 2 days later and twice weekly for the following 21 days.

To learn whether the virus would localize in skin inflamed by trauma, the sides of the animals destined for intravenous inoculation were tattooed with sterile needles over an area 2 to 3 cm. across, 3 to 4 hours beforehand. A marked edema developed extending for several centimeters around the traumatized area, which was swollen, ruddy, and ecchymotic by the time of the injection. Within 15 seconds afterwards a similar tattooing with sterile needles was done just outside the swelling, and then the inoculum was tattooed into the other side.

No growths ever appeared at either site traumatized with sterile needles, but the usual crowd of confluent and semiconfluent papillomas slowly formed where the inoculum had been tattooed into the side and somewhat sooner on the tattooed ears. None became malignant during several months observation. More papillomas arose on the ears of the injected rabbits than in Experiment 4, owing doubtless to the larger inoculum, yet in general they appeared only after 4 to 6 weeks, although the tarring had caused pronounced skin changes in many instances, with "warts" in some. In one animal especially susceptible to the virus, some of the latter started to enlarge rapidly when the virus papillomas appeared and later manifested malignancy; but in no other rabbits did cancer appear.

The relatively inactive virus material gave rise to few anomalous tumors, although localizing abundantly in the much changed ears.

A constant lookout has been kept for signs of qualitative differences

in the virus materials, as expressed in growths produced. None was encountered in Experiment 6, nor any since. The observation that the sooty papillomas caused by some inocula remained discrete, no matter how close-packed, whereas those due to others soon coalesced, would seem referable merely to differences in proliferative vigor.

The Manifold Effects of Tarring

Without the preliminary cell changes induced by tarring no anomalous tumors would have been got. This constitutes the most significant effect of tar, while it determines the outcome in other important respects as well.

(*a*) *Virus Localization as Determined by Tarring.*—Several tests were made to learn about the conditions determining localization of the virus. Tattooing the skin while virus circulated (Experiments 4 and 8) did not result in papillomas; but one can assume that blood clots in the puncture wounds held it fast. It is known to localize sometimes at points of actual or presumptive trauma (1) as also in epidermis proliferating in response to Scharlach R (11). In an injected rabbit of the present work papillomas appeared over a subcutaneous abscess. Yet localization in inflamed tissue is far from a regular event as Experiment 8 attests, and also Experiment 9.

Experiment 9.—A strong solution of barium sulfide in water was swabbed on the ears of 3 rabbits to produce acute inflammation. Next day, when this was at its height and the ears much swollen, 15 cc. of the highly pathogenic 0.5 per cent virus fluid of Experiment 4 was injected into a leg vein, 15 seconds later a spot on the side was tattooed with sterile needles, and then the inoculum was tattooed into the other side. Vigorous growths appeared here, but none where sterile needles had been used. Almost no papillomas developed on the ears, 2 in 2 individuals and 6 in the third.

Repeated tarring causes the cutaneous vessels to become highly pathological (12). Their state should provide larger opportunities for the virus to leave the circulation than the changes incident to acute inflammation, which might be favorable only while exudation was taking place.[4] Burrows (13) has discussed the complexities incident to localization of the viruses as a class.

The greater the changes produced by tarring, the more frequent and

profuse, generally speaking, was the localization of the virus. Most of it disappeared from the blood within half an hour (Experiment 5) unless an enormous quantity was introduced, when much was still present after an hour (undescribed tourniquet tests). Our practice was to strip the ears a day or two before the intravenous injection, and sometimes tar was not reapplied until afterwards, or never again. Even within 24 hours their condition had greatly bettered. Under such circumstances less virus should have localized in them than if their pathological state had been maintained; and certainly they developed fewer growths (Experiment 4, Charts 8 and 9). But another factor has to be considered in this relation:—

(*b*) *The Influence of Tarring to Elicit Growths of Virus Causation.*—Tarring will enable papilloma virus already ensconsed in the tissue to produce growths when otherwise it would not do so.

Experiment 10.—Both ears of 6 normal domestic rabbits were infiltrated with 10 cc. each of virus-containing fluid by the method of Experiment 7, with a tourniquet to prevent its escape, which was left on half an hour. 3 days later tarring was begun of the inside of one ear, and repeated 5 times (twice weekly). A multitude of ordinary virus papillomas, sooty and pink, soon arose on the tarred ear, whereas its fellow remained devoid of them during many weeks of observation, save where injury had been inflicted during infiltration with the virus, or hyperkeratosis had been induced by transferred tar (Fig. 21).

In a second experiment 7 days were let elapse before the first of 3 tarrings, and similar results were obtained (Fig. 22). Tests are now in progress to determine how long the virus can lie latent. It is known that under the influence of tarring papillomas which have vanished may reappear (14).

(*c*) *The Influence of Tarring on Established Growths.*—In several experiments papillomas due to virus that had localized out of the blood appeared on the tarred ears days before any arose where the inoculum had been tattooed into the side; and generally the ear growths enlarged much the more rapidly. These findings,—especially remarkable because of the virus dilution entailed by intravenous inoculation,—must be laid to the existence of highly favorable conditions in the tarred skin. For virus-induced papillomas do not fare notably well on normal ears. The effect of tarring to stimulate both established tar "warts" and virus papillomas has been remarked upon in Paper I. It had still more pronounced effects upon the anomalous

tumors. Those described in Paper I were tarred during the first weeks of their development. The photographs show how greatly they differed from the untarred tumors of the present work. The anomalous growths ulcerate much sooner when tarred, many doing so that are essentially benign, while not a few of the malignant ones invade the adjacent tissues from the first and destroy and eat deep, instead of building up into mounds, discs, or cones. Tarring notably hastens anaplasia, often rendering this complete.

The morphology and behavior of ordinary virus-induced papillomas are known to be greatly influenced by intercurrent factors (6), and it is reasonable to assume that continued tarring will sometimes cause benign anomalous tumors to progress when otherwise they might not do so. However this may be, it is certain that some of the anomalous carcinomas reverted to a more orderly though still malignant state after tarring was left off. This happened with one of the biopsied carcinomas of rabbit 15, Experiment 1 (Figs. 23 and 24); and the conclusion seems warranted that its early anaplastic condition was due to tarring. Manifestations of factitious malignancy by virus papillomas have been reported in a previous paper (6), and tar papillomas often exhibit the phenomenon. None of the malignant anomalous tumors of the present work became benign later, yet instances of the sort may be expected.

Do the Anomalous Tumors Derive from Virus Papillomas?

The conditions in tarred skin are so favorable to growth, and tarring so pronouncedly influences morphology, that the question arises of whether the anomalous cancers may not have resulted, one and all, from secondary changes in ordinary, virus-induced papillomas. On this assumption the progression to cancer,—which requires months in the case of papillomas induced on normal skin,—would be telescoped into a few days. There is the more reason to consider the possibility because disturbing influences of many sorts will hasten secondary malignancy in virus papillomas. Tarring might very well be especially potent in this respect.

To settle the point we have followed the course of thousands of ordinary virus papillomas occurring on tarred ears, and have scrutinized a large number microscopically. Contrary to expectation they retained their initial, benign character throughout the 2 to 5 months of the experiment, doing so even when next anaplastic carcinomas. In one animal only, tarred for weeks after injection

and retained unusually long (140 days), were cancers found at autopsy in the confluent, fungoid mass on the ears, which might have derived from papillomas secondarily; and in this case secondary carcinomatosis was beginning in the untarred tattoo growth on the side.

It can be stated categorically of the anomalous tumors as a group that they did not derive from ordinary virus papillomas. No proof was obtained that their number can be increased by tarring after the virus has localized in the ears. The procedure does not hasten notably the occurrence of secondary carcinomatosis. Nevertheless it is questionable whether the influence of late tarring to increase the effective localizations of the virus and to stimulate proliferation of the resulting growths will account wholly for the differences manifest in Charts 8 and 9.

The cancers deriving secondarily from ordinary virus papillomas often alter rapidly, changing within a few weeks from convoluted or cystic malignant papillomas to disorderly growths in which papillomatous features can still be recognized, and then to keratinizing squamous cell carcinomas which may finally reach a wholly anaplastic, helter-skelter state. In other instances they retain their initial form even in metastases (8), or hold to one assumed secondarily. The malignant anomalous tumors behave in all these ways,—as do carcinomas of man, for that matter.

The Virus Infection of Tar Warts

Not only did the virus evoke new growths of anomalous character, but often it spurred some of the preexisting tar tumors to unwonted activity, and in not a few cases to malignancy (see Paper I and also rabbit 5-07, Chart 11).

The greater the amount of virus localizing in the ears, as attested by new growths, the more frequently did it affect the preexisting tar tumors, causing them to grow with unprecedented rapidity. Not a few became sooty black (rabbits 18 and 19 of Chart 8; Fig. 20), and sooty spots or segments appeared in many (Chart 8, rabbits 19 and 20), sometimes spreading gradually over their entire surface. The development of secondary resistance in certain individuals led to rapid retrogression of the tattoo papillomas and of the majority of those induced on the ears. At the same time most of the tar tumors stimulated by the virus, and in some instances rendered melanotic, also disappeared (rabbit 18

of Chart 8 and 26 of Chart 9). The tar tumor of Fig. 20 (rabbit 19 of Chart 8), which had become huge and black under the influence of the virus, dwindled when the papillomas did so, and ended as a connective tissue mound covered with smooth epidermis but retaining a gray hue because of many deep-lying phagocytes crammed with the pigment of its earlier activity. Concurrently a nearby tar wart which had become black through the spread of a sooty patch, became pink again, diminishing to a small, indolent hassock such as it had been at first. In the case of rabbit 26 of Chart 9, resistance to the virus waned later, as shown by a reappearance of the tattoo papillomas, and not a few growths on the ears, both pink and sooty, reappeared concurrently. They had just begun to do so at the time of the last charts reproduced. Direct tracings of the tumors in their relative situation to one another had been made on cellophane when they were dwindling, because previous findings (9) had suggested the possibility that the resistance manifested by the host might be transient. The later tracings demonstrated that some of the growths reappeared.

Of especial interest were those numerous cases in which tar tumors stimulated by the virus remained unpigmented, grew fast, and manifested malignancy. Some that had the morphology of carcinomas became enormous and fungoid, but remained uninvasive (rabbit 20, Chart 7, right ear), while others ulcerated destructively and metastasized early. The gross aspect of such growths was the same as that of the anomalous tumors already described, and histologically many resembled the latter; but some had a hybrid morphology. The findings will be detailed in a succeeding paper. Here we are essentially concerned with those growths which appeared where the tarred skin had been devoid of any neoplastic proliferation. The great majority arose at such situations.

DISCUSSION

The findings show that the carcinogenic effect of the papilloma virus upon tarred skin is but one expression of a larger phenomenon, namely the production of a variety of tumors, all differing from those growths of remarkably uniform character which the virus causes under ordinary circumstances. The major factors which enter into the production of the anomalous tumors as a group will now be briefly considered.

Localization of the virus in the tarred epidermis is essential to the occurrence of the anomalous growths, and so too is some preparatory change of the epidermal cells which become infected with it. The

more abundant the localization of the virus the greater is the chance that it will come into association with appropriately changed cells; and the more potent the inoculum, as evidenced in the ordinary papillomas it induces, and the more susceptible the host to its action, the more likely are anomalous tumors to arise, other things being equal. The nature of the preparatory cell change is not clear. It is much more considerable than that determining an effective localization of the virus out of the blood stream; roughly speaking the skin must have been altered by tarring to nearly the extent required for the evocation of tar tumors in some of the rabbits treated. Yet anomalous tumors have often been elicited in ears which gave no sign of such growths, and which indeed had undergone little change as compared with those of other individuals of the same group, tarred to the same extent, receiving the same inoculum, perhaps carrying many tar warts, yet developing only ordinary virus papillomas.

The epidermal changes which have anomalous tumors as their outcome are always focal, the growths arising as discrete entities; and while the foci may be many and close together, when the preliminary tarring has been long, no indication has been obtained that if it were kept up the alteration of the cells would become generalized. When but little virus localizes, or at least causes few papillomas, the discrete character of the accompanying anomalous tumors can be explained to some extent by a spotty escape of virus from the blood through vascular walls that are irregularly pathological (12); but this will not cover those frequent cases in which the virus infects the epidermis almost everywhere, yet still the anomalous tumors appear as sharply defined, scattered entities amidst the sheet of confluent papillomatous proliferation. No evidence has been obtained of a predilection of the virus for cells changed in the way requisite to the production of anomalous growths. On the contrary, cutting down the amount of the inoculum usually results in a rather more than proportionate reduction in the number of such growths arising.[5] In this relation the fact may be germane that the greater the dilution of virus inoculated into normal skin the less frequently does cancer arise secondarily from the papillomatosis engendered (10).

[5] Under such circumstances the virus is prone to localize in preexisting tar tumors, as would follow from the especially favorable state of their blood vessels.

Frequently the anomalous tumors appeared with a speed that was incompatible with derivation from a single virus-infected cell. At certain spots assuredly the virus must have reached not one element but many which were ready for the production of such neoplasms; and in some instances their composite character gave proof of a multicentric origin (Figs. 16, 17). It was also evident in some of the ordinary papillomas engendered, namely those gray, raised mounds, a millimeter or more in diameter, which appeared soon after the virus injection and grew rapidly, in distinction from the great majority that came later as minute, slowly enlarging, dark points in the skin. The anomalous tumors usually arose even before the most active of ordinary papillomas, however, and for some time exceeded them in rate of growth. The tendency of the anomalous tumors to become broad-based by extension into the adjacent tissue soon after they arose must have contributed to this early superiority. Once the virus papillomas were well established, however, their relatively immense number in many instances, and their extraordinary proliferative energy resulted in masses of tissue which usually occupied much more of the ears than did the cancers, though these eventually dominated through the destruction that they wrought.

The possibility has been kept in mind throughout the work that qualitative differences in the pathogenic potentialities of individual virus entities might exist, and find expression in tumor differences. But no supporting evidence for the supposition has been found, nor evidence of any qualitative difference in the virus strains. It is conceivable, nevertheless, as accounting for the diversity of the anomalous tumors, that the condition to which the epidermal cells had been brought by tarring was such as to suit them to the disclosure of hidden qualitative differences in the individual virus entities, much as an ultrasensitive photographic plate is suited to bring out differences in rays of light that are not apparent under ordinary conditions; but this assumption seems gratuitous.

Tarring the anomalous tumors stimulated their growth markedly and also furthered the anaplasia of such of them as were malignant. Yet contrary to expectation based on these facts, tarring failed to hasten in any significant degree the secondary change of virus papil-

lomas into carcinomas. It is conceivable, indeed probable, that amongst the anomalous growths there were some which verged upon malignancy when they first appeared and were pushed over into it by the stimulation of the later tarring. No such growths came to attention though. As a group the anomalous tumors manifested their distinctive peculiarities from the first, and their number was not increased later by a secondary conversion of ordinary virus papillomas into carcinomas.

The collateral effects of tarring will explain why it evokes tumors sooner than does the potent "carcinogenic" principle, 3:4 benzpyrene, which can be extracted from it. It was chosen for our work, in preference to any of the pure "carcinogens," both because of its effectiveness in this respect and because it brings about marked general changes in the ears, a state of affairs which seemed likely to favor localization of virus out of the blood stream. Lacassagne and Nyka (15) utilizing our general method, have now successfully employed benzpyrene to obtain anomalous tumors with the Shope virus. Their protocols show that the applications to the skin prior to intravenous injection of the virus had extended over many months; yet it does not follow that a shorter period would not have proved effective.

The Shope virus exercises a strict, formative influence upon the growths it engenders in scarified normal skin, these being remarkably uniform in character, benign papillomas of a single type. In tarred skin on the other hand it gives rise to a variety of benign tumors, all with papillomatous features (Fig. 25). The carcinomas that it causes under such circumstances range from the organoid to the anaplastic, and resemble in this respect those deriving secondarily from ordinary virus papillomas, though they show a somewhat greater diversity. All in all the morphological spread of the anomalous tumors is wider, its individual expressions more various, than those exhibited by Shope papillomas and the cancers arising out of them.

SUMMARY

A considerable variety of tumors, both benign and malignant, result from the localization of the rabbit papilloma virus in skin which has

been prepared by repeated tarrings. They appear only in individuals highly susceptible to the action of the virus, and are more likely to be engendered by highly pathogenic inocula. No evidence has been found that differences in the potentialities of the virus entities are responsible for the diversity of the growths. This is referable to changes in the epidermal cells; and much more preliminary tarring is required to produce these changes than suffices to cause localization of the virus out of the blood stream with a resulting papillomatosis of the ordinary sort. The character of the individual anomalous tumors depends in some degree upon the extent of the preparatory changes in the cells, malignant growths being more frequent when the epidermis has been tarred for a relatively long period. All are focal or punctate in origin, and they exhibit their peculiar characters from the first, none being due to secondary alterations in ordinary papillomas. Tarring after the virus has localized in the epidermis does not significantly increase their number. They are the outcome of the state of the cells at the time of virus infection.

Tarring exerts important influences in addition to changing the cells in such a way that unusual tumors result from the action of the virus. The procedure is notably effective in determining localization of the virus out of the blood stream; enables it to produce growths when otherwise it would not do so though present in the tarred skin; stimulates the proliferation of the tumors engendered; makes them disorderly and aggressive; and hastens the anaplasia of such of them as are malignant. It has similar effects upon the tar tumors, as will be demonstrated in a subsequent paper.

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EXPLANATION OF PLATES

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

PLATE 18

FIG. 1. Mass of non-pigmented papillomatous tissue, originating from virus-infected epithelium after its extension to cover the raw edge of a hole in the ear. In so doing the epithelium had outstripped the melanoblasts, and hence the growth is not gray like the many papillomas induced by the virus elsewhere on the ear. It did not appear until some days after the gray growths, as would follow from the circumstances of its origin, but it enlarged far more rapidly. $\times \frac{2}{3}$.

FIG. 2. Cross-section of a raised rosette of non-pigmented papillomatous proliferation around the recently healed edge of a punch hole in an ear infected with virus. It resulted from the same conditions as the growth of Fig. 1, and was pink, whereas nearly all of the other papillomas covering the outside of the ear,—save those filling the punch hole,—were dark gray. Much new connective tissue went into its formation. $\times 2\frac{3}{4}$.

FIG. 3. Tumors of the two sorts that the virus generally elicits when it localizes in ears which have been tarred long enough for it to cause cancer (rabbit 4-72, Experiment 5, Chart 11). The large growths appeared first and were all tense, subepidermal hemispheres or globes, and bright pink, the dark hue of some of those pictured being due to dried blood from recent abrasions. These were the *anomalous tumors* of the text. On the ear to the left many minute papules can be perceived in addition, some of them black or gray. They represent the earliest stages of ordinary virus papillomatosis. Few growths of either sort are present on the other ear, owing to obstruction of its circulation for 21 minutes after the virus injection. At this time the tarred skin showed but little change, though one minute tar wart had arisen, which disappeared later. The photograph was taken 27 days after the virus injection, with no further tarring. The arrows point to the growths furnishing Figs. 4 and 5. $\times 5/11$.

FIG. 4. Growths from the ear of Fig. 3 (arrow B). They were punched out on the day after the photograph was taken which provided that figure. The larger tumor had been present about 10 days. Its epithelial layer is seen to be irregularly convoluted, and under high power it appeared to be an invasive, squamous cell carcinoma, with cells dying before keratinization was completed. It was pink in the gross and has stained poorly with methylene blue. The small, darker staining, cystic growth looked like a black dot in the gross. It can be seen to have originated from a hyperkeratotic hair follicle, and it represents an early state of ordinary, melanotic, virus-induced papillomatosis. $\times 9\frac{1}{8}$.

FIG. 5. More growths from the same ear, at a further period of their development. The arrow A of Fig. 3 points to the larger one. They were punched out 10 days after this figure was procured, that is to say on the 38th day after virus injection. Both have erupted. The larger growth is an anomalous tumor and resembles the one in Fig. 4. The small, dark growth at the right is an ordinary, keratinizing, melanotic, virus papilloma. Three other papillomas of this sort can be seen, one of them (P) underlying the anomalous tumor,—which had been present about 3 weeks,—and a second situated at its edge (P^2). $\times 14\frac{1}{2}$.

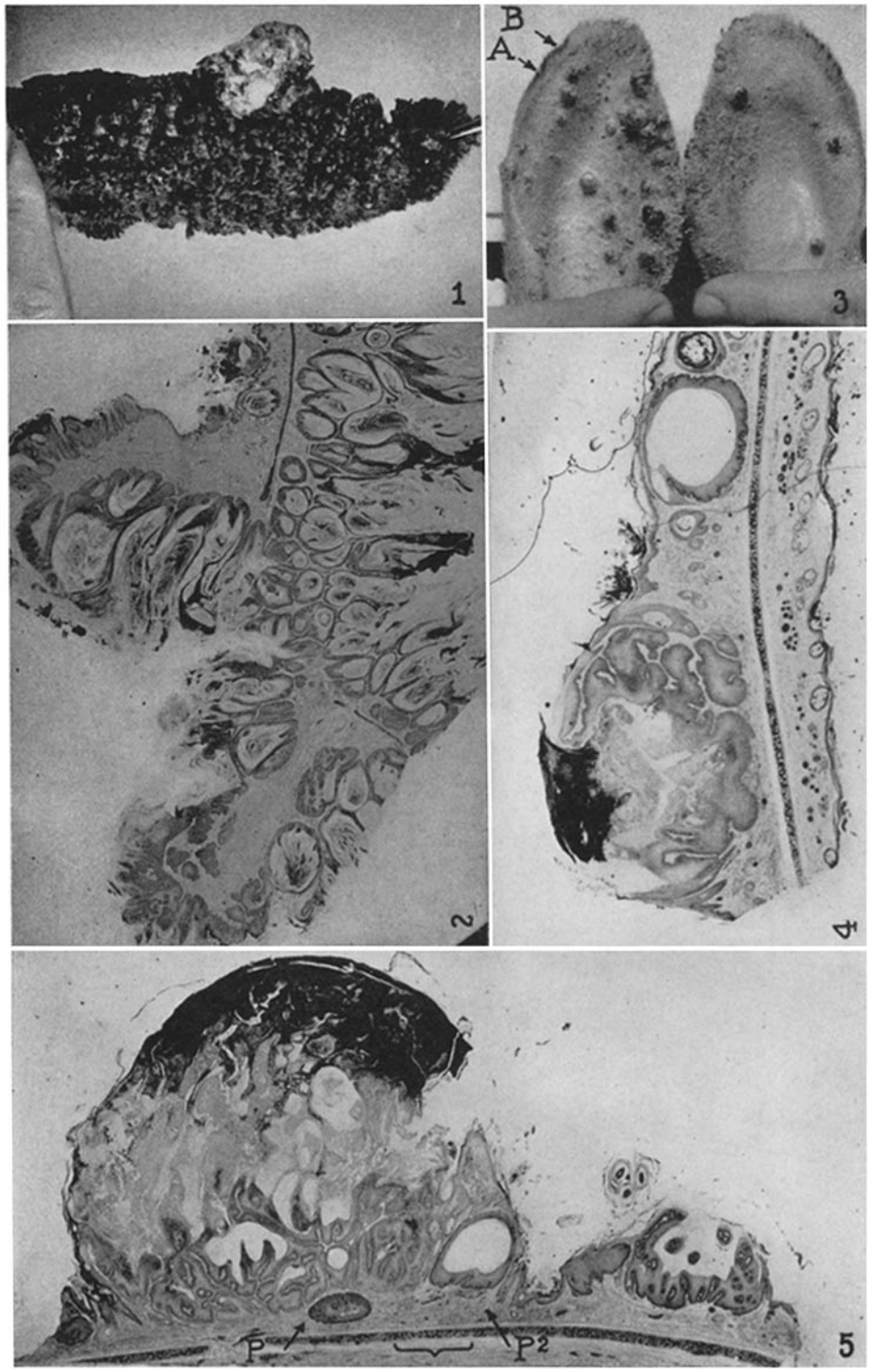

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PLATE 19

FIG. 6. A higher magnification of the bracketed portions of the large, anomalous growth of Fig. 5 and of the virus papilloma P^2. The large tumor is seen to be invading along its base (arrow). Its cells show little tendency to keratinize, and they stain lightly with methylene blue. The contrast with the adjacent, orderly, dark, keratinizing growth is great. A hyperkeratotic hair follicle separates them. × 95.

FIG. 7. Biopsy specimen procured 14 days after the infiltration of an ear with virus by way of a marginal vein (D.R. 2-33 S, Experiment 7). The preliminary tarring for 28 days had caused moderate hyperkeratosis but no warts. No more tar was applied after the injection, which was followed in the 2nd week by the appearance of numerous growths. They had been present only a few days when the biopsy was done. The growth A was sooty, B and C pink, this last still subepidermal. Microscopically A proved to be a melanotic virus papilloma, whereas B and C were both anomalous tumors. (See Fig. 8.) × 15.

FIG. 8. The adjacent growths A and B of Fig. 7, as seen in another section made of the region bracketed in that figure,—to show the pronounced differences in character and coloration existing at a spot where the two have coalesced. The cells of the anomalous tumor lie in disorder and are actively invasive (arrows). × 172.

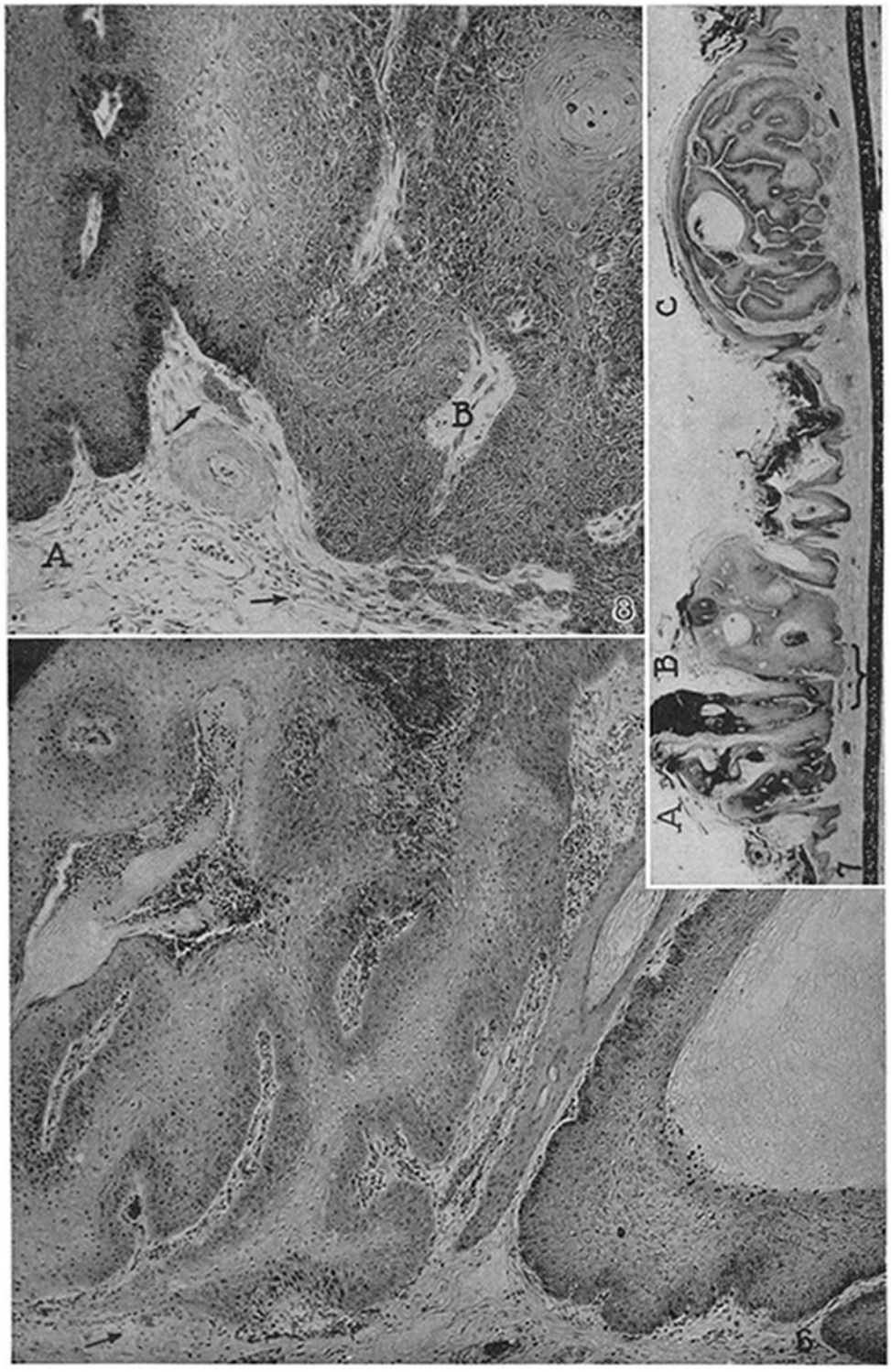

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PLATE 20

FIG. 9. Another biopsy specimen containing growths of the same sorts. It was procured from the animal of Figs. 7 and 8, 22 days after the infiltration with virus. Much the same histological differences are visible as in the previous instances. The large, globoid, pink, anomalous tumor had been present 2 weeks, whereas the several sooty papillomas (arrows) are just arising. The large growth has become anaplastic and invasive along the base. × 11.

FIG. 10. Ears of D.R. 5-12 (Chart 11, Experiment 5) photographed 34 days after virus injection,—to show a later stage of the condition pictured in Fig. 3, and from the same experiment. The flow of blood to the ear on the left was blocked for 20 minutes from the beginning of the injection. The anomalous tumors, at first subepidermal and globoid, have become fleshy or dry cones, infiltrating discs, or jagged masses. They are much larger than the sooty papillomas, which appeared later in immense number on the ear with unobstructed circulation, and now cover most of its surface. × 5/11.

FIG. 11. Portion of a metastasis in an auricular lymph node of a rabbit not tarred again after the virus injection (D.R. 4-73, Experiment 5; see also Fig. 12). The nodule was discovered on the 76th day, and was removed on the 90th, when it had almost entirely replaced the gland and was 1.3 cm. across. Bits of it implanted in the muscles of the upper legs gave rise to growths (see Fig. 13). × 11.

FIG. 12. Invasion by the metastasis of Fig. 11. In the region pictured the growth is almost wholly anaplastic. A mitosis can be seen. × 250.

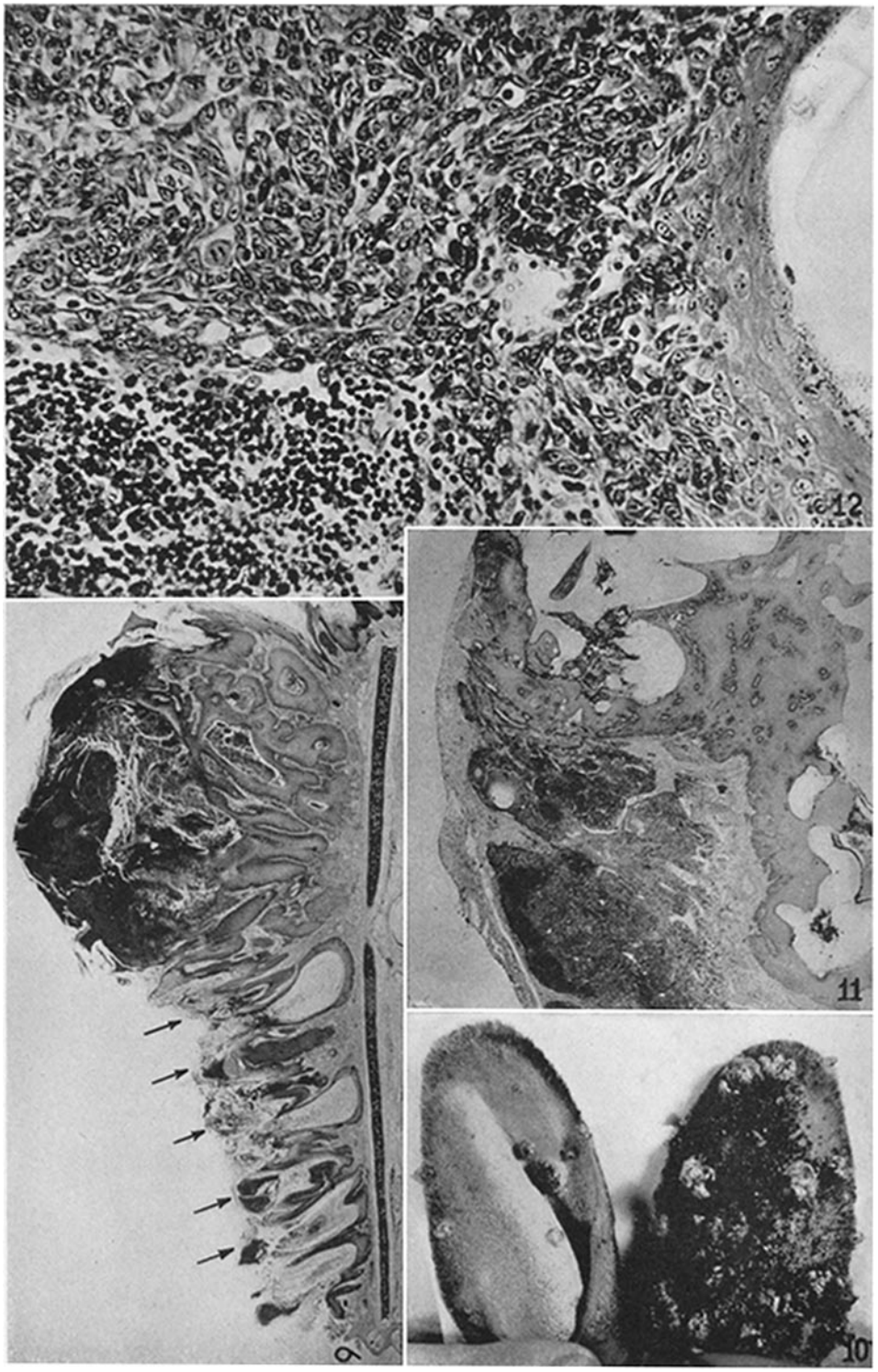

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PLATE 21

FIG. 13. A portion of one of the tumors resulting from implantation in the leg muscles of bits of the growth of Fig. 11. $\times 16\frac{2}{3}$.

FIG. 14. Two anomalous tumors with the morphology of carcinomas, from the ear of an animal killed 59 days after receiving virus intravenously, with no later tarring (D.R. 4-95 N, Experiment 5, left ear). A single tar wart had been present (Chart 11) at a distant situation. One of the cancers (A) is wholly anaplastic, the other somewhat cystic (B). There is much adjacent virus papillomatosis. $\times 6\frac{2}{3}$.

FIG. 15. The ears of an animal of Experiment 6 which illustrate the fact that an abundant virus infection of skin much changed by tarring does not necessarily result in anomalous tumors. In the instance presented only one developed amidst the innumerable papillomas. It cannot be seen in the picture. No tar warts were present at the time of injection. $\times 5/11$.

FIG. 16. A virus-induced, composite growth, from D.R. 2-31 of Experiment 6. It first appeared as a particolored, subepidermal mound which became hemispherical and then onion-shaped. Its central portion, which was gray, is seen to consist of ordinary virus papillomatosis. Its periphery was pink, and where this has been cut through is an area of anomalous growth (C), apparently malignant, joined on to the main mass, while in the region to which the arrow points is a deep, lateral extension from another component of the same sort (C), not visible in its superficial portions in this section. The growth appeared, together with many others, between the 11th and 15th day after the virus injection, and it was punched out on the 21st day. From the first the existence within it of differing neoplastic elements had been plainly visible in the gross. $\times 11$.

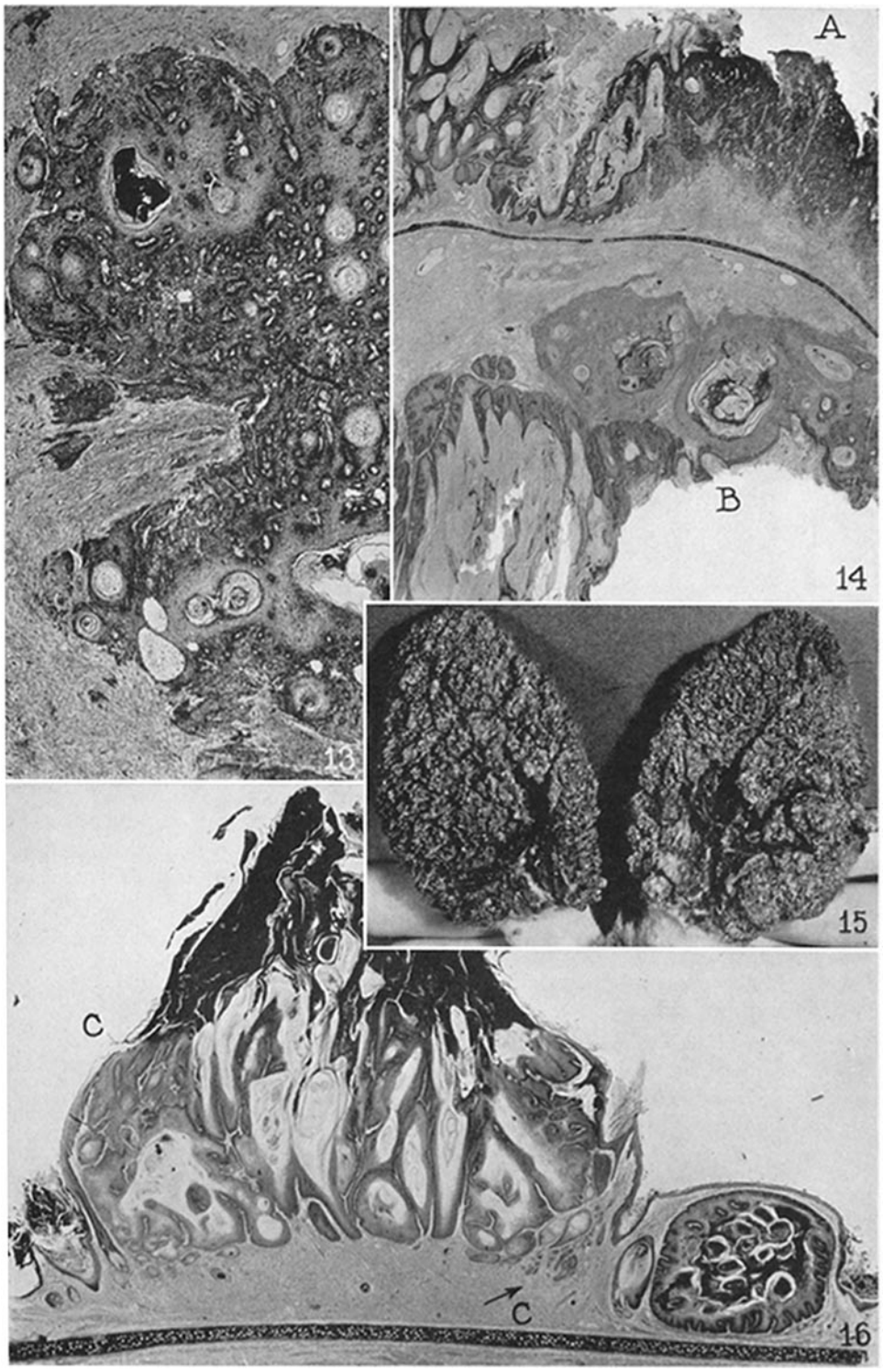

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PLATE 22

FIG. 17. A particolored, composite growth from another animal of the same experiment (D.R. 2-56, killed 57 days after virus injection). The ears were largely occupied by virus tumors of various sorts. No tar warts had been present on either at the time of injection, and no later tarring was done. A central region of the growth pictured consists of irregular, cystic tissue. This region was pink in the gross from the first, whereas the remainder of the tumor was dark gray and consisted, as the figure shows, of ordinary virus papillomatosis. × 11.

FIG. 18. Benign and malignant papillomas elicited by the virus on the ears furnishing Fig. 17 (see also Fig. 25). The benign papillomas (P, P, P) stained much the more deeply with methylene blue. The malignant tumor has extended around the edge of the ear. × $6\frac{2}{3}$.

FIG. 19. A conical growth with the morphology of a cystic, squamous cell carcinoma, which appeared after the virus had localized in a tarred ear, a fact evidenced by the concurrent appearance of many melanotic papillomas. Parts of some of the latter are included in the picture. No tar warts had been present on either ear and no tarring was done after the virus injection. (D.R. 2-23 S, Experiment 6.) × $10\frac{1}{2}$.

FIG. 20. Secondary melanosis of a tar tumor, as result of virus infection (left ear of Rabbit 19, Chart 8). Both the growths shown had been elicited by the tarring and both were pink prior to injection of the virus, but afterwards one of them became coal black all over and the other developed a dark gray segment, on the side away from the camera. Their growth rate was also markedly increased. Later most of the ordinary virus papillomas elsewhere on the ears disappeared, evidently because of a generalized secondary resistance on the part of the host, and at this time the black growth here shown disappeared entirely, while the pink one (which had now become melanotic everywhere, Chart 8) dwindled, lost its pigmentation, and assumed once again the aspect of an ordinary tar tumor. × 10/11.

FIG. 21. To show that tarring will enable ensconsed virus to produce growths when otherwise this would not happen. Both ears, previously normal, had been directly infiltrated with 10 cc. of virus fluid 3 days prior to the first of 3 tarrings of the ear on the left (Experiment 10). A multitude of papillomas arose on this ear, relatively few on the other, and these mostly where transferred tar rendered the skin hyperkeratotic. The picture was taken 53 days after the virus injection: the differences shown persisted. × 5/11.

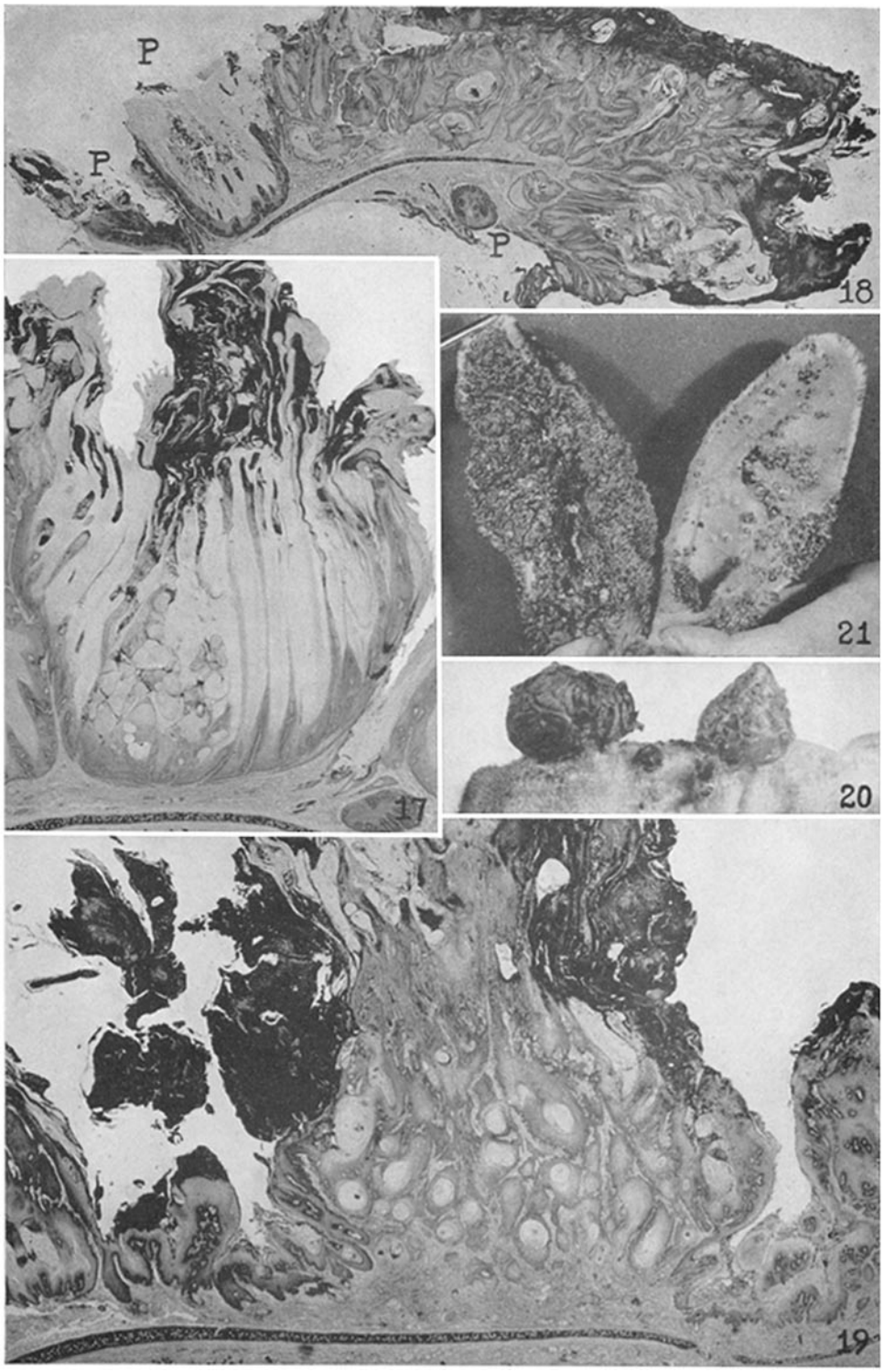

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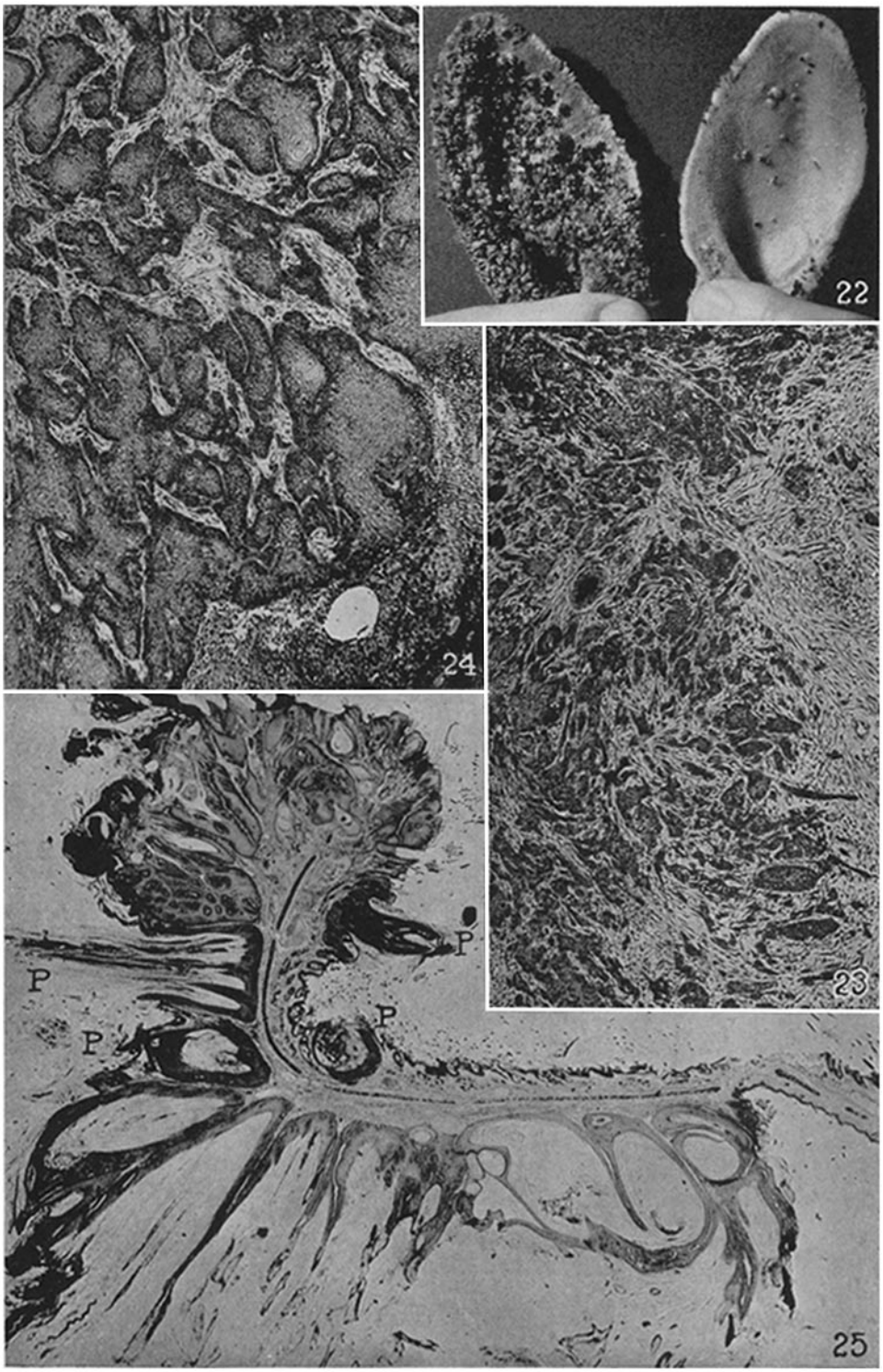
PLATE 23

FIG. 22. The ears of an animal from another experiment of identical sort save in that the tarring was started 7 days after the infiltration with virus. The picture was taken on the 29th day. No more papillomas developed on the untarred ear before the rabbit was killed, on the 70th day. × 5/11.

FIGS. 23 and 24. Portions of an anomalous tumor, as procured by biopsy on the 29th day after virus injection and at autopsy on the 84th day, respectively. Tarring was left off on the 25th day, and thereafter the general state of the ear reverted toward the normal, and the growth became much less anaplastic. × 52.

FIG. 25. Papillomatous growths of differing patterns, consequent on the action of the virus upon skin prepared by tarring (D.R. 2-56, Experiment 6, an animal devoid of tar tumors at injection and not tarred later). At P, P, P, P, are ordinary melanotic virus papillomas, stained heavily with methylene blue. At the edge of the ear, and extending around its edge, is a malignant papilloma of lighter hue, while toward the base are two benign growths of contrasting pattern, one of which has stained fairly well, the other only lightly. Fig. 17 illustrates a fifth type of neoplastic proliferation, occurring in the same animal (see also Figs. 16, 18). × $7\frac{3}{10}$.



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