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

I. Description of the Phenomenon

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

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

Plates 13 to 18

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Virus diseases involve reciprocal activities on the part of the infected cells, no matter how soon these die, and their opportunity to influence the situation becomes greater the longer they endure. When they proliferate in series, the resulting growths must be looked upon as the expression of a working partnership with the virus, a state of affairs evident in various tumors of the domestic fowl, and in the infectious papillomas of several mammalian species. Ordinarily these growths, as propagated in the laboratory, are the outcome of infection of the cells of an acutely damaged tissue; and all those caused by the action of any one virus are essentially alike. In the present paper and others to follow it will be shown that tumors of widely differing character will result from the action of a single virus if the cells are appropriately altered before it comes into association with them.

The virus employed for our experiments was that which causes the cutaneous papillomas of western cottontail rabbits (1). On inoculation into domestic rabbits it gives rise to similar growths which often undergo malignant change, carcinomas arising directly from their epithelium (2). This change has never occurred until after several months, in our experience; but various interferences which enhance the cellular proliferation and render it disorderly act to shorten the precancerous period, and they may elicit cancer forthwith when the papilloma has been growing for a long time. Such occurrences have led us to ask whether cancer will develop at once if the virus is brought

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into association with epidermal cells that are already in a pathological state. This is the case, as will be demonstrated.

Rationale

Many agents causing epithelial disturbance and proliferation fail to bring about the tissue alterations preliminary to cancer. For our purpose some substance was needed that would prepare the cells suitably, yet that could be utilized without likelihood that it would itself elicit cancers under the conditions of the experiments. Such a substance was available, namely a tar with which we had done much work, the horizontal retort tar of the Oster-Gasfabrik of Amsterdam.[1] It elicits cancer within a few months in mice (3), and benign "warts" in domestic rabbits (4); yet cancer appears in these latter only after a very long time and then rarely. Our rabbits were tarred on the ears until a few small warts had appeared on some of them, and then were inoculated with virus. Indications had already been obtained of slight differences in the outcome of individual cell-virus associations, as expressed in the growths consequent upon them (5), and hence it seemed important to scatter the virus entities to individual cells altered in varying degree by the tarring. With this in view the virus was injected intravenously. It localized in the tarred skin and here promptly elicited carcinomas as well as a variety of papillomas.

Local Effects of the Tarring

The effects of tarring have been often described, yet some account of them as observed in our rabbits is essential. The animals were all agouti (brown-gray) adults weighing about 2500 gm. More than 30 had been utilized previously in an attempt to obtain tar cancers for serological purposes (4), but only one such growth had been got, and this after nearly 2 years, although the tarred skin in most cases underwent the well known changes preliminary to malignancy, numerous "warts" arising, that is to say papillomas and "carcinoids" (6),—growths which look like cancers both in the gross and microscopically, but which remain local, fail to grow on implantation elsewhere in the host (7), and disappear or become mere papillomas if tarring is left off. The experience of others with other tars gives every reason for the supposition that cancer would eventually have arisen in some instances had not nearly all of the animals died of tar intoxication after 6 to 15 months.

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[1] The gift of Dr. Karl Landsteiner.

In the tarred controls of the present work no cancers ever arose. They bring the total number of control animals to more than 90.

The inner surface of the ears was tarred twice a week. In most instances they soon became thickened, warm, and hyperkeratotic, scurfy or macerated; and after 2 to 4 months a few small warts appeared in more than half of the animals. The diffuse histological changes were those often recorded (8), and the growths were the familiar tar papillomas and carcinoids. Not infrequently tumors of the one kind graded into the other.

The later course of events varied widely. The ears of some rabbits remained free from warts, while those of others at the opposite extreme developed numerous growths which, rapidly enlarging, filled the aural shells. Some of the large tumors were predominantly epithelial, and took the form of cauliflowers, cones, or cutaneous horns, but the generality owed their size to proliferation of the connective tissue supporting a thickened, papillomatous epidermis. They often rounded out into tangential spheres, which, as time passed, became pedunculated and underwent retrogression to fibrous tags. Both the papillomas and carcinoids sometimes invaded the connective tissue soon after their appearance, and, extending through lacunae in the cartilaginous plate, formed mounds on the outside of the ear, which occasionally ulcerated; but later they ceased to enlarge and either disappeared or became indolent cones or horns. Often, though growing swiftly at first, they ran a brief course, disappearing despite the continued application of tar. Leroux (9) has well described the retrogression of such tumors.

The rabbits stripped the ears between the paws to remove the tar, and in this way transferred some of it to the outer side, with result that here the skin lost its hair, became hyperkeratotic, and occasionally developed papillomas. Their rarity in this situation during the first 4 months of tarring deserves stress because the introduction of the virus into the blood stream during this period was often followed by the appearance of hosts of papillomas on the outsides of the ears.

In most of the experiments the virus was injected after tarring had been done for 2 to 4 months, and it was kept up for a few later weeks, though this was not essential to the carcinogenesis, as recent findings have shown. When it was finally discontinued the skin of the ears of most of the control rabbits, which had often been macerated, thickened, and furry, rapidly dried down, and a more or less pronounced desquamation took place, revealing at length a smooth, normal-looking surface. Most of the growths dried down too, and some came away; but others, after persisting for a few weeks as mere scabs, began to grow again, becoming horns or dry cauliflowers, or fleshy cones or onion-shaped masses; and sometimes one or more new ones appeared. When they had been large, crowded, and macerating, as rarely happened, they tended to keep on growing, aided by the maceration, and occasionally reached a diameter of several centimeters in the absence of any further tarring. But these large growths were pedunculated, fibrous, and wholly benign.

Very important for the interpretation of the findings after virus injection was the lack of pronounced pigmentation of the tar tumors, and the situation of nearly all on the inside (tarred side) of the ears. In agouti rabbits an occasional small, indolent tar wart is light or medium gray, or very rarely dark, owing to included melanoblasts; but it regularly loses this color in proportion as it proliferates more actively; and vigorously growing tar tumors are never gray but creamy, buff, or pink. Many of the papillomas induced in tarred skin by the virus were by contrast slaty brown, or coal black; and one could be sure that the virus had a hand in any deeply pigmented, yet actively enlarging papilloma which appeared after its injection. This is not to say that virus was absent from such pink, buff, or creamy growths as also appeared, for it elicited many. Furthermore some virus-induced growths that were at first deeply melanotic often became pink later, as happens with such growths on scarified normal skin (2). The appearance during the 3rd to the 5th week after virus injection of numerous papillomas on the outsides of the ears, many of them slaty, was proof positive of the action of the virus.

Our tar was of moderate "carcinogenic" potentialities, to judge from its ability to elicit papillomas. The one cancer it induced, a squamous cell carcinoma with some papillomatous features, appeared after 21 months in an animal tarred for two periods of 5 and 6½ months. When it was killed, after 656 days, a cystic metastasis with the same papillomatous features was present in an auricular gland.

The aim of the tarring after injection of the virus was to prevent reversion of the epidermal cells to the normal state before it had had time to take effect. In skin directly inoculated with a potent virus material 10 days to 3 weeks ordinarily elapses before the first roughening preliminary to papillomatosis can be noted in the gross; but when the individual virus entities are scattered to the tarred epidermis by way of the blood, the growths often appear later and sometimes not until 2 months or more have gone by.

The Virus Materials and Their Effects

Most of the virus materials were generously given us by Dr. Shope, as glycerolated papilloma tissue from cottontails. Attempts to maintain by passage the pathogenicity of active strains of virus from "natural" growths are not very successful, an inoculum of diminished potency being usually obtained from cottontails, while from domestic rabbits the virus is either not recovered at all or in greatly attenuated form. Large amounts of material of high titer were essential to the work, both because of the dilution inevitable to dispersion of the virus on the blood, and because weak strains of it have little carcinogenic capacity, malignancy seldom supervening upon the papillomatosis that they induce (5). The material principally employed consisted of the natural growths from a single cottontail (1211), and it was exceptionally active, as proven by checkerboard titration (4). In the tarred

skin it promptly elicited carcinomas as well as papillomas in a considerable proportion of the injected animals. Another material, nearly as active, was injected in much smaller amount and the virus localizations and cancers were correspondingly fewer. A third material consisted of the pooled papillomas from 7 cottontails experimentally inoculated with a potent virus. It localized in considerable quantity in the ears of the 15 rabbits receiving it, all susceptible and many with tar warts; yet it gave rise only to slowly-growing, ordinary papillomas. This experiment need not be mentioned further.

The Virus Tumors Arising in Scarified Normal Skin

The growths produced by the virus on direct inoculation into the normal skin of agouti rabbits are all papillomas of a single characteristic sort (1,10), some pink but many gray owing to included and stimulated melanoblasts, elements not rendered neoplastic, however. The individual growths take the form of high cones or fleshy onions, more rarely of cutaneous horns or cauliflowers, and their keratin builds high in dry, vertically ribbed or striated layers. In the gross the papillomas resemble some of the tar tumors (11), notably those which continue to enlarge after tarring has been stopped; but as a group they proliferate much more vigorously and are often distinguishable by a pronounced melanosis, a fact already brought out. The cancers arising from them in the ordinary course of events are never pigmented though. They are often multiple, and range in morphology from complicated papillomas of slight aggressive power to the most anaplastic of metastasizing, squamous cell carcinomas.

General Method

In most of the experiments the tar was applied to the central two-thirds of the inner surface of the ears, whence it spread to the edges. Before every third application as much of the old layer was stripped off as possible. A day or two prior to the virus injection all tar was removed, the warts were drawn to size on standard forms, note taken of the general state of the ears, and on the basis of the findings the animals were separated into comparable groups, one serving as control. The charting was frequently repeated later and all significant changes were noted. In most instances tarring was resumed for 14 to 30 days after virus injection, and then the layer was permanently removed and immediate charting done. The controls were similarly treated save that they received no virus and were kept isolated.

The virus suspensions were made by grinding the glycerolated papilloma tissue with sand, suspending in Tyrode (pH 7.0–7.4), centrifuging briefly, and passing the supernatant fluid through a Berkefeld filter. The filtrate was slowly injected into a vein on the outer side of the thigh.

When biopsy specimens were required from the ears, sharp cork-borers were employed and a blow of the mallet.

THE EXPERIMENTS

For the first experiment three groups of tarred rabbits were employed. Into one a virus filtrate was injected, another was set aside for control, while the third received an incubated mixture of virus suspension and heated cancer extract. This was used because Berry has shown that rabbit fibroma virus incubated with heated rabbit myxoma tissue gives rise to disease of the latter type (12). Ours was an attempt, collateral to the main experiment, to convert the papilloma virus into a carcinoma virus. To learn the effect of tarring on ordinary epidermal cells infected with the virus materials, the latter were tattooed into the insides of the ears of several normal rabbits. Tarring was then begun for the first time.

Experiment 1.—The virus-containing fluid was a 4 per cent Tyrode extract of the papillomas from W.R. 1211, which had been passed through one or another of 3 Berkefeld filters, V or N, and been pooled. The cancer extract was made by grinding with sand and Tyrode the tissue of several large, squamous cell carcinomas that had arisen from virus papillomas. The malignant tissue had been frozen and dried some months previously, after separation from all gross remains of the papillomas. A 7 per cent extract of it by dry weight was heated in a water bath at 65°C. for 30 minutes, centrifuged to throw down gross particles, and the murky, supernatant fluid was mixed with an equal portion of virus fluid and incubated at 37°C. for 3 hours prior to injection, while another portion of the virus fluid, mixed with the same amount of Tyrode, was similarly incubated.

Five rabbits were injected intravenously with virus mixed with Tyrode, 4 with the mixture with cancer extract, while 5 more were kept as controls. All had been tarred over the entire inner surface of the ears during periods of from 42 to 89 days, with result in small warts. On the day after the injections, tarring was resumed and repeated twice weekly during the next 25 days.

Two of the rabbits with more numerous and larger warts than any of the others had been placed in the *control group* (Chart 1). The warts of one dwindled during the later tarring but those of the other (rabbit 6) enlarged even after it had been stopped, and new ones appeared. Maceration furthered their course. When this rabbit was killed after 9 months, its ears bore crowded masses of pedunculated, fleshy papillomas, with a few cutaneous horns, all benign. This instance has proved unique in our experience. Little change occurred in the ears of the other controls during the later tarring, and thereafter most of their growths disappeared. In 2 of them a subepidermal mound had formed on the outside of the ear, opposite an active growth on the inside, as if by extension from the latter, but it dwindled and vanished together with this after tarring had been left off.

In a *subsidiary control test* the two virus mixtures were tattooed, with an electric machine, into 2 strips about 5 mm. wide, extending nearly the entire length of the inside of the ears of 5 normal rabbits. The virus with Tyrode was introduced into the left ear, the mixture with cancer extract into the right, and tarring was then begun for the first time and kept up twice weekly until death. Semiconfluent and confluent papillomatosis developed along all the tattooed strips after about 18 days, and the growths enlarged with a rapidity unprecedented in the case of untarred ears (Chart 3). At first predominantly gray, as when no tarring is done, they soon became high, pink, macerating, cauliflower masses. Their foul state led to early death in 3 cases. All of the growths remained ordinary, virus-induced papillomas throughout the 55 to 84 days of tarring, though sections showed their epithelium to have extended down into the profuse reactive tissue, forming cysts, as often happens in the case of vigorous virus papillomas induced in scarified normal skin (2, 5). Elsewhere on the ears the tarring caused the usual hyperplastic thickening, but no warts. In one animal (No. 2), 2 discrete papillomas appeared off the line of tattoo inoculation, but their slaty color marked them as due to the virus.

From Chart 3, of the growths due to *tattoo inoculations* into normal ears which were tarred later, it will be seen that the incubation with cancer extract had greatly cut down the pathogenicity of the virus. It led to no qualitative difference, though, in the growths engendered, all remaining papillomas of the characteristic sort despite great stimulation by the tar.

The course of events in the *animals injected with virus incubated with Tyrode* (Chart 2) differed notably from that in the controls (Chart 1).

During the 3rd week after the injection many new, rapidly enlarging warts appeared, and most of the old ones began to grow at an unprecedented rate (*vide* the records of the 22nd day). In addition, a diffuse change took place in the ears of rabbits 11, 12, and 13. During the 3rd week after the injection they suddenly became much swollen, stiffened, brawny, and hot, and within the next few days papillomatosis appeared over large areas on both their outer and inner surfaces. It took the form of gray and pink, rugose expanses, or of multitudes of minute growths, mostly gray. Some larger, discrete, rapidly growing tumors appeared as well, and many of the preexisting tar warts suddenly began to grow with unprecedented rapidity. The discontinuance of tarring resulted in no slowing of the proliferation, and the ears were soon almost entirely occupied by growths, some of them malignant in behavior. Only the large tumors were charted: there were too many small ones. The time of the first changes after virus injection corresponded roughly with that when papillomas became noticeable in the tattooed

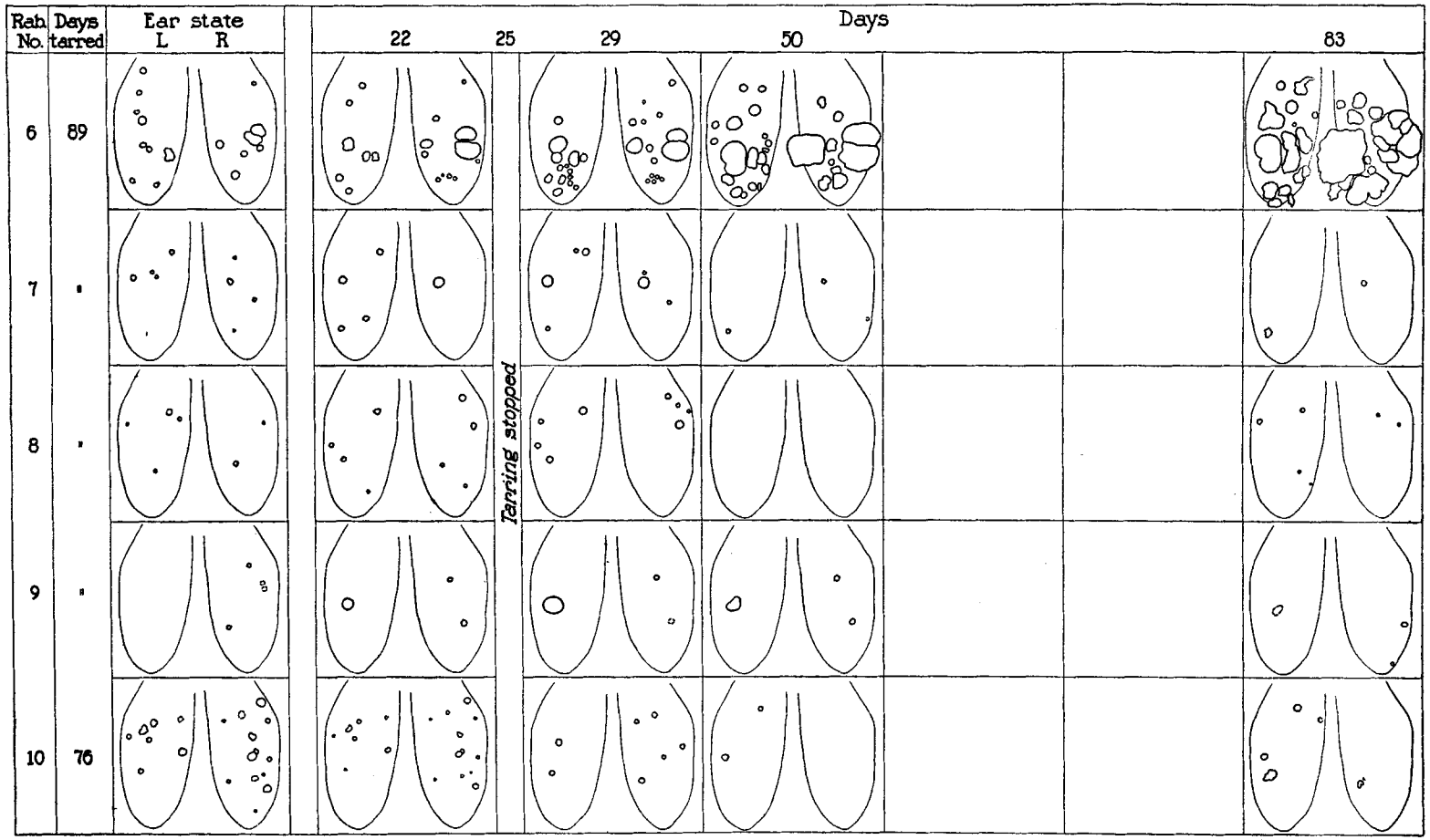

CHART 1. Tarred controls.

CHARTS 1 to 4. Experiment 1. Growths on the inner surface of the ears, before and after virus injection. Minute ones are not charted. Hatching shows areas of confluent proliferation. Along the middle of the ears of rabbits 12 and 13 maceration kept it low, and no charting has been attempted here. Growths of carcinomatous morphology, as determined by biopsy specimens or at autopsy, are shown in solid black from the time when their character was first demonstrated microscopically.

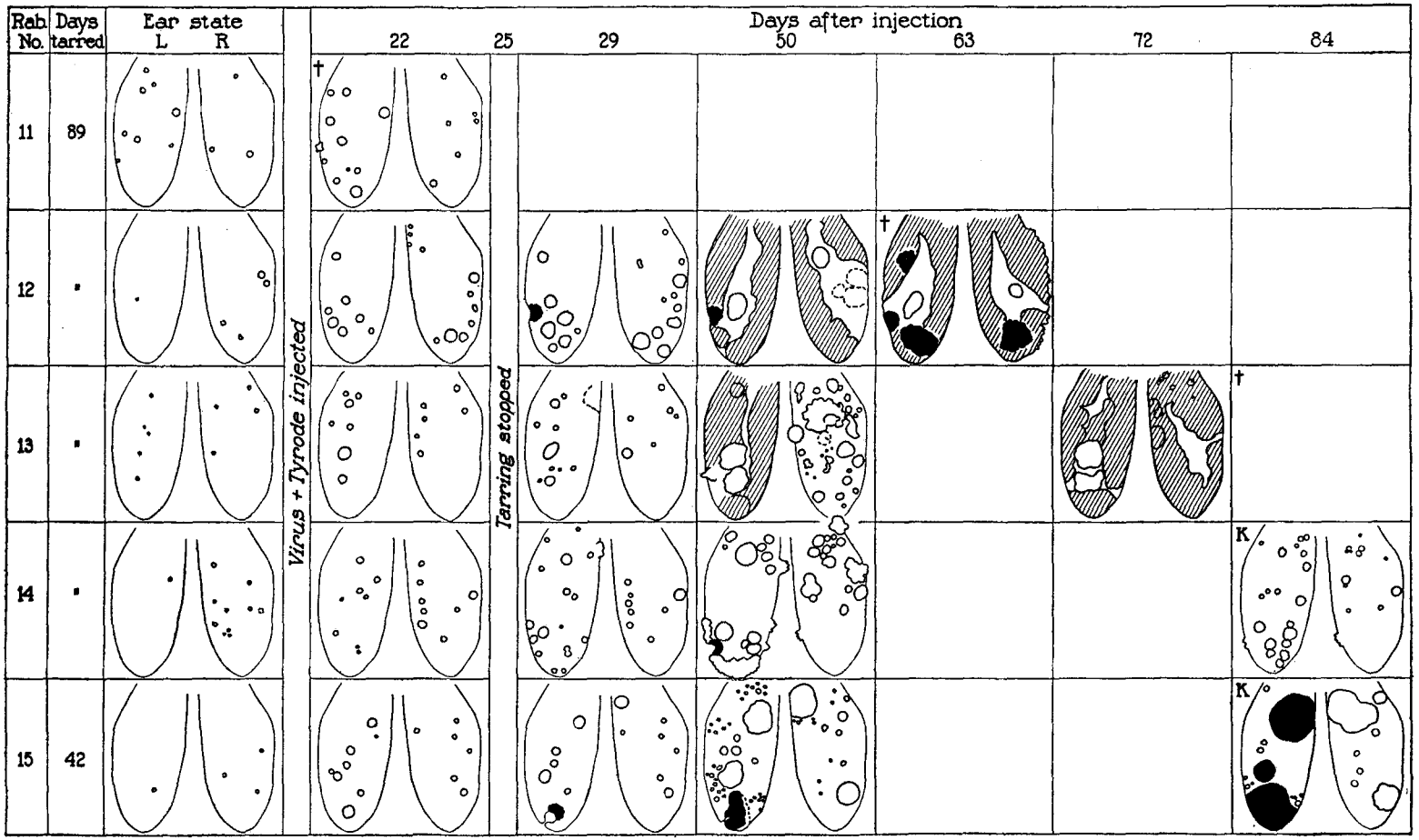

CHART 2. Virus + Tyrode.

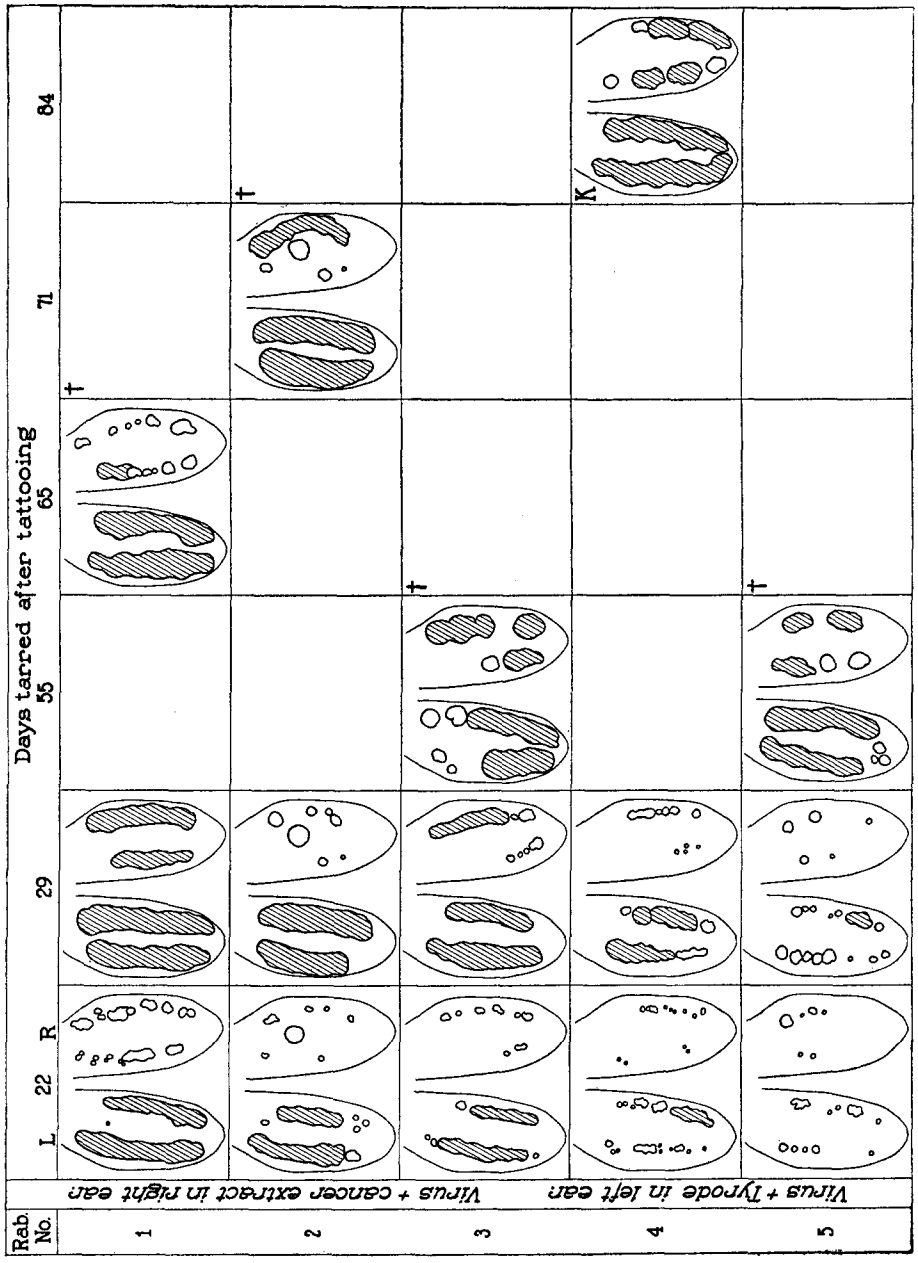

CHART 3. Tattoo inoculation into normal ears that were tarred later. Left ear, virus + Tyrode; right ear, virus + cancer extract.

group. The charts made between the 29th and 50th days have not been reproduced.

Rabbit 11 had thickened, hyperkeratotic ears at time of injection. It died soon after, of intercurrent causes, yet provided much informative material. On the 18th day the ears had suddenly become greatly swollen and brawny; and 2 small, gray, subepidermal mounds had appeared on their outer surface. The swelling increased, more growths appeared, the old grew rapidly (Chart 2), and at death on the 22nd day the ears were from 5 to 10 mm. thick, covered inside with a furry, macerating sheet of fungoid tissue amidst which the old tar warts and some new ones stood forth as slightly higher, discoid growths. Only the latter are indicated in the chart. On section the sheet varied in thickness from 1.5 mm. near the tip of the ear to 7 mm. toward the base, was vertically striated, creamy, streaked and spotted with gray, and consisted microscopically of confluent papillomatous tissue, as if from broadcast, epidermal infection with the virus. The embedded warts, old and new, were also of papillomatous character. Some appeared to be breaking up into squamous cell carcinomas along their base, a frequent finding in ordinary tar warts. Blocks taken at random disclosed occasional localized downgrowths of carcinomatous morphology where no tar warts had existed prior to the injection (Fig. 1).

The skin of the outer side of the ears was irregularly raised, and a cut disclosed numerous separate, discrete, gray or creamy, subepidermal, acorn or onion-shaped growths from 0.5 to 1.5 mm. in diameter. The microscope showed these to be discrete papillomas, deriving from the epithelium of hair follicles and not yet erupted. Some were dark gray.

The early changes in rabbits 12 and 13 were of like sort. The sheets of new tissue which formed on the insides of the ears during the 3rd week after injection soon thickened to 1.0 to 1.5 cm., enveloping the warts previously present or newly appeared, and filling the ears with a high, foul, scabbed mass, save along a central strip where maceration and pressure necrosis kept the tissue low. (Fig. 16 of Experiment 2, illustrates this state of affairs.) Scattered, gray, subepidermal mounds appeared on the outer surface during the 3rd week; and rapidly increasing in size and number these broke through the stretched epidermis, in the case of rabbit 13, and became vigorous, conical or onion-shaped growths with fleshy, bulging, gray bases and dry, sooty, vertically striated peaks,—characteristic virus-induced papillomas in short. Often they coalesced. They were most numerous where the skin was hyperkeratotic and hairless. On the back of the neck, where transferred tar had caused similar, but slighter, skin changes, many discrete or semiconfluent papillomas also arose.

On the 78th day after injection *rabbit 13* died of sepsis. Ruddy fungoid growths up to 2.5 cm. in diameter, some deriving from tar warts, were then present on the inner side of the ears, amidst a thick sheet of foul, vertically striated pink and gray tissue. The microscope disclosed only non-malignant papillomatosis, though the papillomas were of highly various character. Their histology will be

considered in a succeeding paper. Many small abscesses were present in the auricular glands.

The events in *rabbit 12* warrant more detailed description. The ears were slightly thickened and mildly hyperkeratotic at time of injection, and they bore 3 small warts and 2 dubious rugosities. These underwent little change until the 3rd week when some had become raised, granulating discs, while other similar discs had appeared and also ruddy, subepidermal mounds, with scattered gray ones on the outsides. On the 25th day, that of the final tarring, the ears had recently undergone a turgid, hot stiffening. Many new, gray, subepidermal mounds were now present on their outsides, and 4 larger, pink ones had developed there opposite 4 raw, ruddy discs on the inner surface, as if by extension from these. 3 of the inner discs had first become noticeable between the 18th and 22nd days, while the fourth had been present at time of injection, as a small tar wart. Other discrete, ruddy discs or hassocks were also to be noted now on the insides of the ears. On the 29th day the pink, outer mounds had enlarged greatly and 2 were ulcerating. The corresponding discs lay amidst a thick sheet of new tissue covering most of the inner surface of the ear, which sheet is not indicated on the chart. A piece was punched from the disc and mound nearest to the ear margin. It showed what appeared to be an ulcerated, anaplastic, squamous cell carcinoma (Fig. 2), which had extended beneath the adjacent skin, through the ear cartilage, and under a nearby papilloma. During the later weeks, until death on the 63rd day, this growth enlarged but little. The other ruddy, discrete tumors continued to grow however, though obscured by the thick sheet of proliferating tissue covered with heavy, brown scab that rose about them. Under this latter some of them extended widely (Chart 2), while the pink mounds on the outside opposite certain of them became deeply ulcerated. Only the large scabs and the growths discernible through it could be recorded at the late chartings. The numerous, gray, subepidermal mounds on the outside of the ears rapidly enlarged (Figs. 26 and 27) and fused into irregular plateaus covered with breast-shaped, subepidermal protrusions (Fig. 3), each with a nipple-like, dry, black cone at its top. Later sections showed the plateaus to consist of a multitude of keratinizing papillomas of virus type.

On the 50th day a firm, spherical nodule 4 mm. across was felt in a lymph gland at the base of the left ear, the one which carried the biopsied growth. 4 similar growths, with irregular, raised ulcerations opposite them on the outer side, had enlarged progressively; and near the tip of the left ear 2 of these had fused into a thick, fungoid mass.

The animal was sacrificed when moribund, on the 63rd day. The fused growths just mentioned had eaten a foul, transverse furrow, and the ear tip hung limp, attached only by cartilage. The gland nodule had reached 8 mm. in diameter.

Microscopically the 5 aggressive, destructive growths were carcinomas, some anaplastic, others with papillomatous features (Fig. 4). Their extension through lacunae in the cartilaginous plate accounted for the ulcerating mounds on the outer

side of the ears. The numerous other, more or less discrete growths lying amidst the inner masses of fungating, scabbed tissue proved to be papillomas of highly various sorts, none certainly malignant. The greater part of these masses consisted of confluent papillomatosis of ordinary virus type, some of it gray; and the mammillated plateau on the outer side consisted entirely of growths of this kind. Sections from many places were searched for malignant growths that were just arising, but none was found.

The nodule in the basal gland had the morphology of an actively invading squamous cell carcinoma, cystic and with papillomatous features (Figs. 5 and 19). No other secondary growths were found.

The remaining 2 rabbits of the group exemplify the effects of a less abundant localization of the virus in the ears.

Rabbit 15, tarred 42 days when injected, had ears much changed, carrying 3 minute warts. In the 3rd week the growths suddenly increased in number and size. No diffuse thickening or confluent papillomatosis developed. Several of the newly appeared growths were raised, raw discs with depressed centers; and by the 22nd day a pink, subepidermal mound had formed on the outside of the left ear opposite one of them, first noted 4 days previously. On the 29th day, when the mound had ulcerated, a punch biopsy was done, and the growth was found to be a squamous cell carcinoma histologically, which had extended through the cartilage. Later it enlarged rapidly into a broad, fungoid, weeping, lenticular mass on the inner surface of the ear, and a raised ulceration without. So also did 2 similar, discoid growths dating from the 22nd day (Figs. 8, 12). The successive pictures (Figs. 6 to 13) show how quick were the changes. 2 fimbriated, pink growths on the right ear, one of them derived from a preexisting tar wart, also enlarged (Figs. 6, 10). The other, which was new, extended through the cartilage to form a big mound that remained subcutaneous (Fig. 11). In the gross these were mere papillomas. A scattering of discrete, small, sooty or pink growths of the same sort also appeared, notably on the outside of the left ear.

After the 50th day a progressive dwindling took place of many of the smaller growths just mentioned (Chart 2). Few were left by the 84th day, when the rabbit was killed, though the 5 large tumors already described had continued to grow, those of the left ear destructively. The skin between the growths had long since become to all appearance normal. The microscope showed the 3 destructive growths to be squamous cell carcinomas in histology, whereas the 2 on the other ear, though aggressive and somewhat disorderly, were benign papillomas. Many round cells and makrophages were present under and about the small, retrogressing growths, and dark gray spots due to phagocytes crammed with melanin marked where some had disappeared that had been dark gray. There were no metastases.

The ears of *rabbit 14* had been little changed by the tarring. The early events after inoculation were like those in rabbit 15, and soon after tarring was stopped a pink growth appeared which rapidly extended through the ear. Biopsy on the 32nd day showed the growth to be histologically a squamous cell carcinoma. Un-

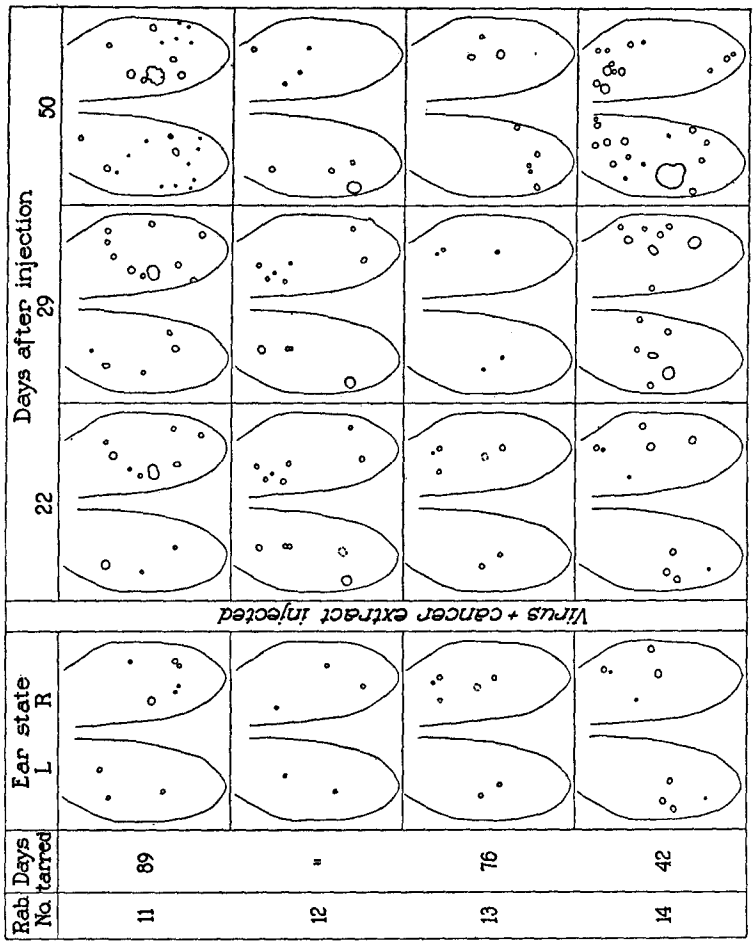

CHART 4. Virus + cancer extract.

fortunately nearly all of it had been taken, and local purulence destroyed the rest. Later some of the fairly numerous growths disappeared, and most of those that persisted to the 84th day, when the animal was killed, were in some degree pedunculated. All were papillomas, and nearly all were dark gray.

In these rabbits, the injection of *virus incubated with Tyrode* was followed by remarkable changes in the ears. After a few weeks, papillomas developed, often in immense number, and in 4 of the 5 animals destructive growths also appeared that behaved like carcinomas and had the morphology of such at early biopsy. They rapidly extended through the cartilage, frequently penetrated into the blood and lymph vessels, and continued to proliferate after tarring had been stopped and even while papillomas on the same ears were retrogressing (rabbit 15). In one animal a secondary growth developed in a lymph node.

The changes were almost negligible by comparison in the animals which received *virus incubated with heated cancer extract.*

In the 3rd week (Chart 4) a few papillomas appeared that were obviously due to the virus, being dark gray, vigorous, rapidly growing, and situated in some instances on the outside of the ears. Others also developed of which there could be no certainty as to cause, since they were creamy or pink. No malignant tumors had arisen by the end of the experiment (84th day), nor did the existing growths alter significantly after the 50th day. Hence the later chartings are omitted.

These findings confirmed the outcome of the tattoo inoculations in showing that the incubation with tumor extract had greatly lessened the pathogenicity of the virus. Indeed the injected animals served as additional controls, testifying to the absence of cancers consequent on the tarring as such.

Sufficient of the virus material of Experiment 1 was available for several more tests. In one the effect was noted of incubation with a cancer extract devoid of inhibitory effect on the virus. The rabbits came from the same batch as those tarred for Experiment 1.

Experiment 2.—A Berkefeld filtrate was made of a 5 per cent extract of material W.R. 1211, and part was mixed with twice its bulk of a heated 8½ per cent extract in Tyrode of a squamous cell carcinoma derived from a virus-induced papilloma. The cancerous tissue had been kept frozen for 16 months. It was ground with sand, extracted with Tyrode, spun to remove particles, and the central portion of the murky, supernatant fluid was taken off through a long needle, and heated at

65° for 30 minutes. The mixture with virus was incubated at 37°C. for 3½ hours and then 13 cc. was injected into a leg vein of rabbits 1-31 and 1-35 N.

In a collateral test of the effect of the cancer extract on the virus filtrate, some of the latter, as such and diluted to 1 per cent, 0.2 per cent, and 0.04 per cent, was mixed with twice its bulk of Tyrode, and with heated and unheated cancer extract respectively, after which it was incubated as above and inoculated into checkerboard squares on the skin of 3 normal rabbits. All of the inocula yielded growths, the heated and unheated cancer extracts having no more effect upon the outcome than the Tyrode. After 9 days papillomas began to appear where the mixtures with 5 per cent virus had been inoculated, and after 16 days and 21 days where 0.2 per cent and 0.04 per cent had been introduced. The number of growths varied directly with the virus dilution.

D.R. 1-31 N, tarred 46 days, had 7 warts, 1 to 6 mm. across when injected with the mixture of virus and cancer extract. Tarring was kept up for 30 days more. During the first 2 weeks after the injection the warts enlarged slowly and no new ones appeared; but within the next 2 weeks a great increase in their size and number occurred (Chart 5). Most of the bigger ones, both old and new, became raised, fungating, ruddy, discoid masses, covered with foul secretion which caused maceration. By the 26th day the largest was 2 cm. across and 5 mm. high. The ears did not dry down after tarring was stopped; the tumors continued to enlarge rapidly, more appearing; and on the 38th day an ulcerated mound was present on the outside of the right ear opposite one of the oldest growths, which had been noted as a slight thickening of the skin at the time of virus injection, and had since become a fungating disc with ill defined borders. Biopsy of it showed a growth with the histology of an anaplastic, squamous cell carcinoma (Fig. 20), which had extended through a lacuna in the cartilaginous plate. It soon caused ulceration on the outer side. Opposite two other, similar discs, first noted on the 24th day after the injection, and now with depressed centers, mounds had appeared, one of which ulcerated in the next few days (Fig. 17). In a region about 2 cm. across, toward the base of the ear, a deep thickening (Y) developed on both sides of the cartilage. A few old growths on the inner surface,—tangential, fleshy spheres or pedunculated cauliflowers, such as tarring frequently elicits,—did not alter noticeably; but elsewhere over this surface great numbers of small, rugose mounds and obvious papillomas arose after the 26th day, and on the outer surface a scattering of subepidermal, breast-shaped mounds, mostly gray, the larger with a central, dark, nipple-like protrusion, consisting of keratinized tissue.

Proliferation continued at a rapid pace, the growths on the inner surface becoming confluent and macerating; and more ulcerating mounds appeared on the outside. Biopsy through one of these latter on the 40th day disclosed another anaplastic, squamous cell growth that had originated on the inner aspect of the ear and extended through the cartilage. The animal, by now very thin, was transfused with 51 cc. of citrated blood on the 38th day. By the 43rd day the shells of the ears were filled with high, fungating masses of growth, blocking the auditory

canals, and covered with thick, dirty brown scab, save along a macerating, longitudinal fold (Fig. 16). The ears were distorted, and nodular on their outer sides

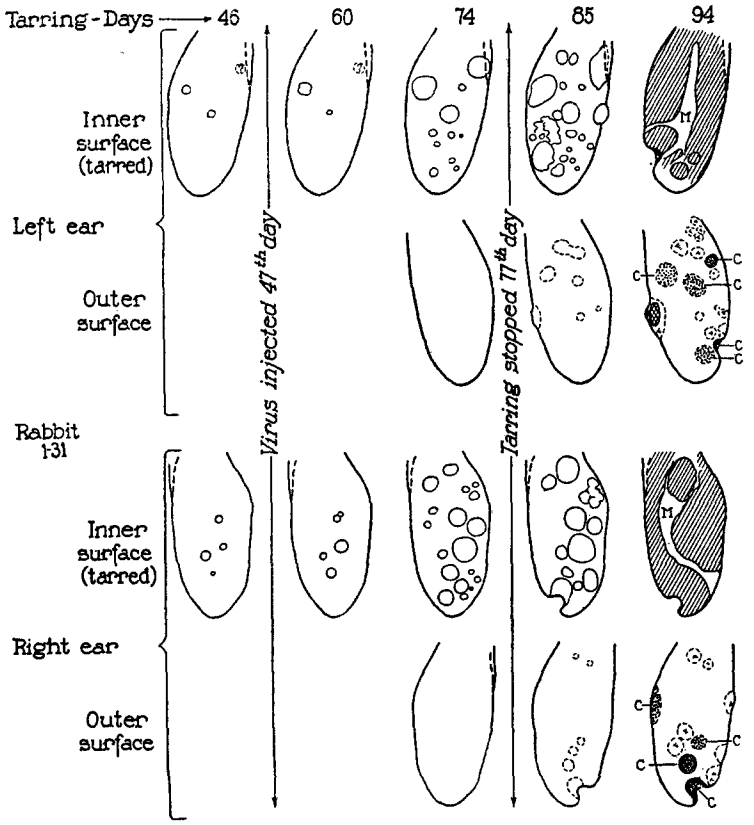

CHART 5. The course of events after virus injection into D.R. 1-31 (Experiment 2). Hatching shows areas of confluent proliferation. The broken lines indicate the ill defined edges of the mounds appearing on the outsides of the ears, cross-hatching there means ulceration, and stippling, subepidermal malignant growths. No attempt has been made to designate the malignant growths on the inner surface, because these were largely hidden in the confluent, fungoid masses. C, C = cancers on the outer surface, as disclosed by sections. M = furrow due to maceration.

(Fig. 17), and here the thickening (Y) was greater, and an ill defined bulge on the outside of the right ear near its base (Cs) first noted on the 28th day, had become prominent, 2½ cm. across, and fluctuating.

The animal was chloroformed on the 48th day, when moribund. On cutting through the ears a thick layer of soft tissue was disclosed, covering the insides, mostly of pinkish cream color but with gray streaks and patches (Fig. 18 *a*). There was some vertical striation but much diversity of texture, owing to imbedded growths, once discrete and still discernible by reason of their irregular markings and serpiginous or dotted necrosis. Many had extended through the cartilaginous plate,—especially near the ear tips where lacunae were frequent,—giving rise to the mounds and ulcers on the outer side. Also present here were scattered, discrete, acorn-shaped growths, creamy or dark gray, with a fine, vertical striation (Fig. 18 *b*),—later stages of the breast-shaped, subepidermal mounds previously noted. They were papillomas such as are caused by the Shope virus, and had originated deep in the skin and dried into blunt, dark cones at their summits. The deep thickening (Y) consisted of papilloma tissue of similar sort that had never erupted.

Blocks were fixed from 20 different situations, mostly where growths had extended through the cartilage. At 12 of them one or more destructive, infiltrative growths were found, squamous cell carcinomas histologically (Figs. 21, 23). All were of considerable size, 1.5 to 4 cm. in diameter, search disclosing none that was just beginning. The bulge on the outside of the left ear (Cs, Figs. 16 and 17) proved to be a lenticular cyst (Fig. 22) full of thin fluid containing necrotic fragments. Its ragged walls were lined with anaplastic tissue of carcinomatous character (Fig. 23), and similar tissue, largely destroyed by purulent infection, was present on the inner surface of the ear opposite it (Fig. 22).

The thick layer covering the inside of the ears consisted for the most part of papillomatous tissue, predominantly of ordinary virus type, though with discrete "papillomas of the second order" (2), cystic papillomas, malignant papillomas, and growths with the morphology of frank carcinomas incorporated in it, as were also a few old, fibrous, more or less pedunculated tar papillomas.

In one of the swollen, auricular lymph glands 2 minute epithelial growths were found, one keratinizing, and of squamous cell type, the other wholly anaplastic. There were many small abscesses in these glands and in the lungs.

D. R. 1-35 N, previously tarred 46 days, had at injection one wart 4 mm. across which did not alter later, though another small growth appeared by the 28th day. Not until nearly 40 days had elapsed did more develop. Then they arose rapidly as numerous, scattered cauliflowers, cones, or onions on the inner surface, and mounds or acorns on the outer side. Nearly all were slaty gray. Tarring was kept up until the 64th day. Under its influence many of the growths became large, and a few on the inner surface discoid and ruddy. On the 81st day the rabbit was killed because moribund from sepsis. The growths had continued to prosper, yet none had extended through the cartilage and only papillomas were found in the numerous blocks taken.

In one of the rabbits of this experiment the injection resulted in a profuse localization of virus in the ears, as attested by the develop-

ment of innumerable papillomas together with many growths that appeared to be cancers and behaved as such during the few weeks before death. Were it not for the results of Experiment 1 and for the findings in the other animal receiving the inoculum, one might be tempted to suppose that the preliminary incubation of the virus with heated cancer extract had resulted in a carcinogenic material of singular potency. In this animal, however, only papillomas occurred, although tarring was kept up for more than 2 months after the virus injection, with the aim of inducing secondary cancerous changes in some of the many, vigorously proliferating growths.

The Changes Caused by the Virus in Skin Long Tarred

It seemed well to extend the observations with the same virus material (W.R. 1211) to rabbits tarred for much longer periods. 2 were available that had been tarred over the entire inner surface of the ears for 40 weeks, and carried in consequence numerous, very large, cauliflower growths and fleshy, rounded tumors with more or less pedunculation, all appearing benign.

Experiment 3.—The rabbits were injected with 15 cc. of 2 per cent filtered virus fluid and two weeks later tarring was stopped. *D.R. 6-46* had at injection 15 warts up to 3 cm. across and more than 2 cm. high. None was gray. 17 days later a few gray, subepidermal mounds had appeared on the outer sides of the ears, and the biggest growth on the inside, previously a smooth, ruddy, almost tangential sphere, showed several protruding bosses on its surface, which in another 3 days had become large, rugose, and patched with gray (Fig. 30). Many small, gray papillomas had appeared elsewhere on the inner side of the ears, and on the outer side at one edge a scabbed thickening had developed, more than 1 cm. away from any other lesion. After another 4 days this had become a raised, ulcerated disc, 1.3 cm. across, with a smaller, slightly raised scab opposite it on the inside. It looked cancerous.

During the next 7 weeks the new growths on the insides of the ears enlarged rapidly, became crowded and in some regions confluent. Where discrete they were cone- or onion-shaped, gray or infrequently pink. They encroached upon and obscured all of the tar warts except the large pedunculated sphere studded with bosses. This was snipped off on the 36th day, and was found to consist mainly of fibrous tissue, covered almost everywhere with thick, papillomatous epithelium, some of it melanotic. There were some gray or creamy "acorns" embedded in the growth, and in a few places its covering was mere hyperplastic

epidermis. Many gray papillomas had appeared in the hyperkeratotic skin on the back of the neck.

The rabbit was killed on the 73rd day after injection, while still in excellent condition. The growth that looked cancerous had ulcerated further, causing necrosis of the cartilage and a deep nick in the ear. It had extended to a distance of about 1.3 cm. from this nick on either side of the cartilage, and was erosive, not fleshy. Microscopically, it was a squamous cell carcinoma, anaplastic in some regions. No malignant changes were evident elsewhere. Both ears were almost completely covered on the inner surface with pink or gray growths, and many of the latter were present on the outer surface as well. The regional lymph glands were not examined.

D.R. 6-51 had six large, pink, tar tumors at time of injection, several of them 3 to 4 cm. in diameter, fleshy and pedunculated. When tarring was stopped 14 days later, some scattered, small, gray mounds had just appeared on the outer surface of the ears; and in another 3 days these organs were noted to have undergone the brawny, hot thickening and stiffening already described. A raised, scabbed disc had formed in the hyperkeratotic skin of the outside of one ear, more than 2 cm. away from the nearest growth, and a mound had appeared opposite a tar tumor on the inside. The surface of several of the larger tar tumors had become nodular, and elsewhere on the inner surface many new, pink, discoid tumors had arisen. All enlarged rapidly. By the 21st day the ears were greatly thickened, distorted, hot, heavy, and pendulous. The changes continued; the ears became several centimeters thick; nodular bulgings appeared on their outer surface, subepidermal growths along their edges (Fig. 25); their orifices became choked with pultaceous matter, and their concavities full of confluent growth, partly obscuring the tar tumors (Fig. 24). On the hyperkeratotic back of the neck numerous gray papillomas appeared.

The animal died of seropurulent pleural effusions on the 32nd day. The ears weighed 220 gm. (Fig. 25). On the inner side of the cartilage was an irregular sheet of vertically striated tissue 8 to 12 mm. thick; and a similar sheet, mostly subepidermal, existed outside. Most of the old tar tumors had been destroyed by maceration. The scabbed disc had grown smaller: it proved due to a papilloma of virus type. No malignant growth was found elsewhere on extensive sectioning.

The virus localized abundantly in the ears of these rabbits. In view of the marked general changes, the numerous, large, tar warts, and the outcome of the previous experiments, it had seemed reasonable to suppose that many malignant growths would be elicited. On the contrary, only one appeared after the injection. Yet the conditions were so favorable to the virus, that the ears of one animal reached enormous size within a few weeks, owing to confluent papillomatous proliferation.

Later Course of the Induced Malignant Tumors

Most of the rabbits in which the virus caused confluent or coalescing growths died early from sepsis incidental to their presence. Metastases had already developed in the lymph nodes of 2, but they might have arisen from cells thrust into the lymphatics when the ears were biopsied. In the next experiment the inoculum was reduced to elicit fewer growths; and these were allowed to run their course without interference. The virus material W.R. 1211 was by now exhausted, and recourse was had to that from W.R. 1183.

Experiment 4.—The 42 rabbits had been tarred for 89 days over about half of the inner surface of the ears. At injection they were separated into four comparable groups. The ears of some were but little changed, and carried no warts or only one or two; but those of more than half of each group were thickened, hyperkeratotic and hot, and bore several warts.

Eight rabbits were injected intravenously with 15 cc. of 0.5 per cent filtered virus fluid, and some of it was tattooed into an area 2 to 3 cm. across on the shaved, left side of the body. Tarring was kept up for 25 days more. All of the tattoo inoculations yielded papillomas, and in 5 of the 8 animals virus localized in the tarred skin as proven by numerous sooty papillomas on both aspects of the ears. In 3 of the 5 many rapidly enlarging, ruddy tumors also appeared on the inner surface, and in 2 of the 3 some of the growths here were malignant. A second group of 9 rabbits were similarly injected but tarred no longer. Though they were susceptible, as shown by growths at the tattoo sites, relatively few sooty papillomas developed on their ears, only occasional ruddy growths, and no malignant tumors. A third group, of 11 animals, received 0.2 per cent virus and were tarred later, like the first lot. Though they all developed tattoo growths very little virus came out into the ears, few gray papillomas arising, only an occasional ruddy growth, and none that was malignant. This group can be thought of as furnishing accessory controls. The 10 controls proper were tarred like the first and third lot and most of them were kept for months after the termination of the experiment. Their warts remained small and either disappeared later on, persisted as such, or very slowly enlarged.

In a succeeding paper, the findings will be scrutinized in detail. Here only those animals of the first group that developed malignant growths need be considered. A staphylococcus meningitis caused death of one (No. 28) on the 71st day. It had no warts when injected, nor any 2 weeks later, but during the 3rd week, when virus papillomas were appearing on the side, many discrete growths arose on the ears, and after another 2 weeks more than 50 were present, mostly sooty and still small. At the charting of the 36th day a new, pink, subepidermal mound attracted attention. It was 6 mm. across, had encroached upon a neighboring, sooty papilloma, appeared to be infiltrating laterally, had a scabbed top,

and was recorded as probably malignant. A week later a firm mound had developed opposite it on the outside of the ear. It grew steadily, infiltrating and ulcerating on both aspects of the organ, and when the animal died was 2 cm. in diameter (Fig. 28). On the outer side several fleshy, subepidermal prongs, not visible in the photograph, extended from it toward the base of the ear. Such prong-like extensions have never been found extending from the tar carcinoids of our many control rabbits, nor have these grown after tarring was stopped. The microscope showed a growth with the histology of a squamous cell carcinoma with elongated, cystic extensions such as are encountered in many cancers derived from ordinary, virus-induced papillomas (5). It will be pictured in a later paper.

The other rabbit developing malignant tumors (No. 27), had 3 small warts at injection. During the 3rd week thereafter growths suddenly appeared in considerable number on the ears, nearly all on the left, some pink, the majority gray; and papillomas were now visible at the tattoo site. During the next 2 weeks many growths developed on the left ear and a few on the right. On the 36th day a firm thickening could be felt on the outer side of the right ear opposite a fleshy disc with ill defined margins that dated from the 3rd week after injection. The disc soon became a broad, weeping mound which encroached upon and undermined the nearest growth, a sooty papilloma originally more than 1 cm. away (Fig. 31); and the extension to the outer side of the ear, elsewhere wholly devoid of tumors, underwent ulceration. The ulcer had raised, infiltrating edges (Fig. 32). At the 123rd day the malignant growth had long since destroyed the neighboring, sooty papilloma on the inside, as well as others further off and a considerable part of the ear itself (Fig. 33). The foul, granulating expanse was stippled with yellow dots, suggestive of keratinization. The ear had reverted to the normal in the region not occupied by growths.

The enlarging tumors on the left ear soon became crowded, and maceration took place along its middle. Here, where the tar had been directly applied, no gray growths arose but instead several weeping, pink discs that rapidly grew large and fleshy. Owing to later distortion these came to occupy the bottom of a deep, longitudinal fold, with many high, crowded, gray papillomas fencing them from close inspection. On the 70th day a mound had appeared outside the cartilage, opposite one of them, and a week later 2 more mounds opposite others. By now many large, conical or jagged papillomas, more or less confluent and mostly sooty, were present on both sides of the ear.

On the 93rd day the lymph glands at the base of both ears were enlarged and firm, and 2 weeks later a nodule nearly 1 cm. across was palpable in a gland on the left, now 3 cm. long, and another on the right, 0.5 cm. in diameter. The nodules enlarged rapidly and others appeared in neighboring glands. Figs. 14, 33, and 34 tell the state of affairs on the 123rd day. The ears had undergone extensive destruction. By the 156th day about half of the right one was gone (Figs. 35, 36), and only the stump of the left remained, thickened, brawny, and edematous. Enormous metastatic masses had replaced the auricular nodes, and others

existed lower down in the neck. A thick cord could be felt connecting the growth on the right ear with the nearest glandular mass. The skin over the most prominent of those on the left side was attached, and fluctuation could be felt immediately beneath it.

The thin, weak animal was killed on the 159th day. Microscopically the destructive growths on the ears had the morphology of more or less anaplastic, squamous cell carcinomas (Fig. 37), and the masses in the neck consisted of similar tissue, with remnants of the lymph nodes and of the adjacent salivary glands (Figs. 15, 29), amidst abundant, reactive connective tissue. The fluctuation was due to an abscess amidst the neoplastic tissue. The cord connecting with the growth on the right ear was carcinomatous. No visceral metastases were found, but much amyloid change in liver and spleen.

In this experiment reducing the amount of virus injected had one of the desired results, premature death from sepsis being much less frequent; but the reduction was carried too far in one group of animals, with the result that very few virus localizations occurred in their ears. When many took place the papillomas still appeared relatively late and grew rather slowly as compared with those of the previous experiments. 5 of the 8 rabbits receiving the largest inoculum and tarred for 25 later days developed sooty growths on the ears in moderate number, with some pink ones; and concurrently malignant tumors appeared in 2 of the 5 individuals. One died early of intercurrent causes. A notably invasive growth arose on the right ear of the other, as also a few sooty papillomas, while on the left ear several malignant growths of the same sort appeared, together with numerous papillomas. Both ears underwent progressive destruction and immense metastatic masses formed in the regional glands. The associated papillomas remained merely such while this was happening.

The Effect on Tar Warts of Virus-Induced Fibromatosis

Castiglioni (13) has reported that rabbits rendered syphilitic by the intravenous route are notably responsive to tarring, although the ears show no signs of syphilitic infection. He describes both papillomas and carcinoids as appearing early, but no cancers arose. It has seemed possible that some of the growths elicited in the present work might be tar warts stimulated to factitious malignancy (14) by connective tissue disturbances referable to the virus, although no such disturbances have been found, and the hypothesis will not explain the

metastasizing tumors or those which continued malignant while round about them the ears were reverting to normal. Nevertheless tests were undertaken with the virus causing rabbit "fibromas" (15) to find what effect a vigorous connective tissue proliferation would have upon tar warts.

A 5 per cent extract of glycerolated "fibroma" tissue[2] in Tyrode was cleared with the centrifuge, and 0.1 to 0.3 cc. was injected at each of 4 to 16 situations in the skin of the inner surface of the ears of 4 rabbits. The ears of 3 had been tarred twice a week for 165 days, and carried 4 to 7 warts from 3 to 25 mm. in diameter, while those of the fourth had been tarred thrice weekly for 5 months, and, after 6 months' intermission, for 5 months more twice weekly, with result in 29 warts 3 to 10 mm. across. The virus fluid was injected directly under or next to several warts of each animal, and the tarring was kept up afterwards for 4 weeks. Early in the 3rd week "fibromas" developed as ruddy mounds or discs, but no new warts were evoked. Repeated punch biopsies were made. Often the fibromatous proliferation took place immediately under the epithelium of the tar tumors, yet the latter did not extend downwards, the only effect upon them being pressure distortion or occasional local necrosis. Frequently the "fibromas" extended through the cartilaginous plate, with result in low mounds beneath the outer skin. Here too no epithelial downgrowth was induced.

The Findings as a Whole

The present paper is mainly concerned with the gross changes taking place in the tarred skin after the virus lodged in it, and with those tumors which appeared to be malignant. A variety of other growths were also elicited in addition to the papillomas characteristic of the virus, namely papillomas of complicated pattern and problematic malignancy, cystic papillomas, and frankly malignant papillomas. They will be considered in a later paper. The unavoidable employment of several virus materials added a complicating factor to the many implicit in the experiments; yet the results are consistent.

In the individuals most susceptible to the virus, its localization at numerous situations in the tarred ears was signalized by a sudden, brawny, warm thickening which occurred in the 3rd week after the injection, an incubation period roughly corresponding with that when a virus fluid of moderate pathogenicity is rubbed into scarified normal skin. During the next few days a more or less confluent

[2] Strain D, D.R. 1514, kindly provided by Dr. Shope.

sheet of papilloma tissue formed on the inside of the ears, and low, scattered, subepidermal mounds, mostly gray but occasionally pink or creamy, appeared in the hyperkeratotic skin of the outer side. At the same time some of the pre-existing tar warts, previously indolent, began to enlarge with great rapidity, and fleshy, pink or cream-colored growths also arose where no warts had been present. That these were nearly all referable to the action of the virus was plain from the findings in the controls; yet all resembled in their gross appearance one or another of the various tumors that follow merely upon tarring, though they proliferated much more vigorously. Some were mounds or discs, fleshy cones or dry, cutaneous horns; others were of cauliflower, onion, or hassock shape; while yet others became fleshy, fibrous spheres that underwent pedunculation secondarily.

In many cases the mounds forming on the outside of the ear rapidly became breast-shaped, with nipple-like, dark, keratinized protrusions which later heightened into vertically striated cones, the growths then taking on the aspect of ordinary, virus-induced papillomas. In some animals, though, they remained predominantly subepidermal, and, coalescing, formed plateaus covered with rounded protrusions (Fig. 3). Many growths on the inside of the ears were lost to view in the sheet of papillomatous tissue that rose about them, but others maintained their identity until the aural shell filled up with foul, macerating tissue and its whole interior became a fleshy, scabbed mass (Figs. 16, 17). Then, on cutting through the ear, the sheet of new tissue showed some vertical striation, perhaps streaked or mottled in gray, the pink growths amidst it presenting a diversity of patterns (Fig. 18 *a*).

Special significance attaches to these rapidly enlarging tumors which took the form of low mounds or raw, beefy discs dotted with yellow necroses; for many of these proved malignant. They had ill defined margins, or a rim of raised and infiltrated skin, and sometimes a depressed, crateriform center. Some were derived from tar warts, but others arose where none had been visible. As a rule they enlarged progressively, even though tarring was stopped; invaded and replaced the tissue about them; and soon extended through the cartilage, causing ulceration on the outer side.

Death occurred early, owing to the septic state of the ears, which were full of foul, fungating growth, yet metastasis had already occurred in some cases. In one of these the secondary nodule was recognized only 32 days after the parent growth on the ear had first declared itself, 18 days after virus injection. Both grew rapidly.

When less virus localized in the ears, as attested by a smaller number of sooty papillomas, the growths of other sorts were less numerous. They arose concurrently with the crop of papillomas, and at about the same time some of the tar warts started to grow at an unprecedented rate. The appearance of one or more gray, subepidermal mounds on the outer surface of the ears often served as a tell-tale to the presence of the virus when it was proving effective at but few situations. In such instances some gray or almost black growths usually arose on the inner

surface as well, but here the melanoblasts responsible for their hue (16) were less frequent and the epidermal alterations were greater. The more pronounced these alterations the more frequent in general were non-pigmented growths.

Generally speaking, malignant growths were few as compared with ordinary virus-induced papillomas; and in proportion as the amount of virus acting upon the ears was diminished, they became rare. One noteworthy exception to the rule was met, however, several cancers arising in rabbit 15 of Experiment 1 at the same time as did thinly scattered papillomas (Figs. 6 to 13). Only animals favorable to the virus, as evidenced by the induced papillomatosis, developed malignant tumors also; yet they failed to occur in some individuals so favorable that their ears were largely converted into papillomatous masses (Figs. 24 and 25). The malignancy was often multiple, and involved both ears.

The stimulating effect of tar upon the growths that it elicits is one of the truisms of cancer research. Virus-induced papillomas are also very responsive to its influence, often burrowing, extending through the cartilaginous plate, and ulcerating, though remaining essentially benign, as shown by the fact that they build up later into discrete conical or onion-shaped growths of the characteristic sort. Needless to say the tarring after virus injection must have elicited some tumors referable merely to it, though few such developed in the controls. The contrast afforded by these latter animals was remarkable.

A secondary resistance to the sooty papillomas occasionally developed, as evidenced by the retrogression of most of them; and under its influence some of the pink growths that had been growing rapidly also disappeared, or reverted to their previous indolent state. This happened in rabbit 15 of Experiment 1. Yet while most of the papillomatous growths elicited by the virus in this animal were disappearing the malignant tumors continued to proliferate and invade, and a few papillomatous growths also went on enlarging rapidly (Chart 2).

Some tar usually reached the base of the neck where the ears rested, producing alopecia and hyperkeratosis; and many growths sometimes

appeared there after the virus injection. They were always gray or pink papillomas of the characteristic sort.

DISCUSSION

Were the malignant growths which arose in the tarred skin referable to the action of the virus? In only one of more than 90 tarred but uninoculated rabbits, of the breed experimented upon, many of them tarred for long periods, has any such growth arisen. It was a metastasizing squamous cell cancer consequent upon tarring for two periods of 6 months and 5 months respectively, and it appeared after 21 months in all. When the virus failed to localize in the ears of the injected rabbits no malignant growths developed; and the more abundant its localization as evidenced by gray papillomas engendered, the more often, generally speaking, did they occur. They appeared at the same time as these papillomas, that is to say a few weeks after injection of the virus; and they arose only when conditions were favorable to the latter, as demonstrated by the behavior of the gray growths. They occurred with a frequency unparalleled in the recorded experience with tarred rabbits, and were often multiple, and frequently numerous, facts which will find further exemplification in a succeeding paper. In sum, the facts leave no doubt that the virus called them forth.

Were the malignant growths the expression of an unique disease, simulating cancer but to be discriminated from it? A categorical answer can be given to this question, owing to the fact that tumors as a class inscribe themselves upon the organism with a minute elaboration. The malignant growths of the present work exhibited all those histological features which typify carcinomas of squamous cell origin; yet such features do not suffice in themselves for a diagnosis of cancer in the case of growths arising in tarred skin. These may be highly anaplastic and invade rapidly, penetrate through lacunae in the cartilage and cause ulceration, yet disappear after a time or undergo a transformation into indolent papillomas, even when tarring is kept up (9). Some of the tar tumors of our control animals were of this sort, incapable of independent malignancy, as shown by their disappearance or reversion to the benign state when tarring was left

off. Final proof that the malignant growths evoked by the virus were true carcinomas rests upon those cases in which the growths not only had the morphology but manifested the independent activity of such tumors. This happened in every instance save one, when the animal lived long enough, and in this one the growth was destroyed by purulence after biopsy (Experiment 3).

Since the publication of a preliminary report of the work here set forth, Lacassagne and Nyka (17), using benzpyrene instead of tar, have confirmed its findings, and Andrewes, Ahlström, Foulds, and Gye (18) have studied the alterative effects of tar upon the outcome of infection with the virus which gives rise to rabbit "fibromas." Ordinarily this virus causes connective tissue growths which are restricted to the immediate site of inoculation and regularly retrogress after a few weeks of active proliferation. This was the case with the strain employed by the authors mentioned. But when the animal had received an intramuscular injection of tar, and the virus was thrown into the blood stream, not only did it elicit in some cases widely distributed growths with the character of "fibromas," which enlarged progressively and caused death, but in certain instances growths arose which the authors describe as of neoplastic character. They appeared at the site of the tar injection, that is to say, where the connective tissue had undergone most change. In this general relation it is important to recall the demonstration of Teague and Goodpasture (19) that the virus of herpes simplex, when acting upon tarred skin, induces lesions resembling those of herpes zoster.

SUMMARY

The Shope papilloma virus elicits carcinomas forthwith, as well as papillomas in great variety, when it is distributed by way of the blood stream to the tarred epidermis of domestic rabbits.

The phenomenon will be analyzed in succeeding papers with the aid of additional instances.

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EXPLANATION OF PLATES

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

PLATE 13

FIG. 1. Early malignant downgrowth in rabbit 11 of Experiment 1: 22nd day after inoculation. The surface epithelium from which the anaplastic proliferation derives, though still intact, stains much lighter than that adjoining it. ×44.

FIG. 2. Ulcerating, destructive growth at the ear margin of rabbit 12: biopsy specimen of the 29th day. The anaplastic proliferation has extended beneath the skin, past the cartilage, and under an adjacent, newly appeared papilloma at the ear edge. ×12½.

FIG. 3. Ears of rabbit 12 on the 43rd day after virus inoculation: to show some of the malignant growths (C, C), and the mamelonated plateaus of papillomatous growth, largely subepidermal. The malignant ulceration has eaten deep into the tip of the left ear. (The aural shells were full of coalesced, fungating tissue,—*vide* Fig. 16 of another animal.) ×2/5.

FIG. 4. Autopsy specimen from rabbit 12: Part of a large, ulcerated, malignant growth with papillomatous features. Extension has taken place through many lacunae in the cartilage. At the right there is heavily pigmented, benign papillomatosis. The keratin overlying it has been cut away. ×11.

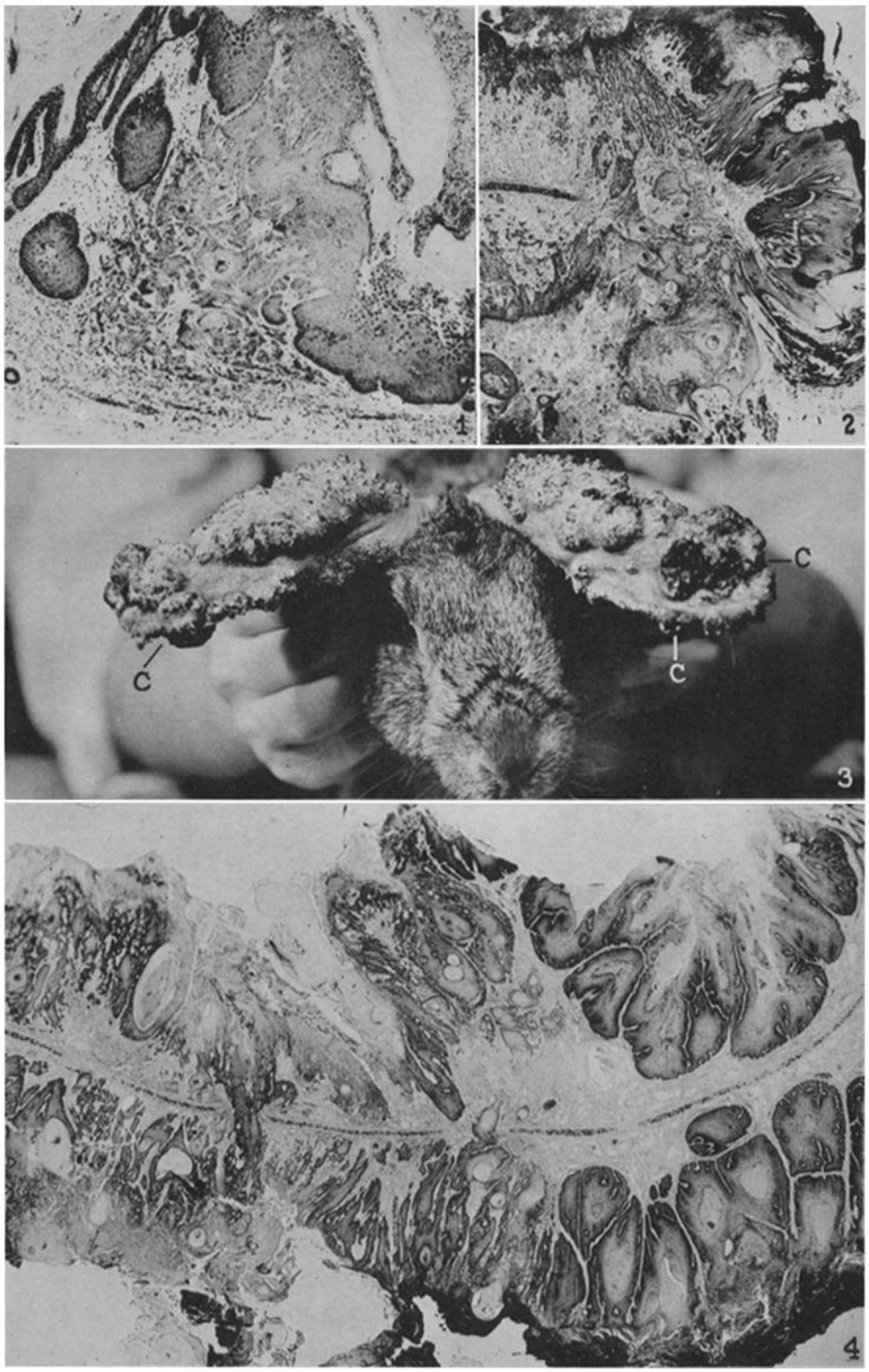

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PLATE 14

FIG. 5. Edge of a cystic, metastatic growth in an auricular node of rabbit 12. (See also Fig. 19.) × 78.

FIGS. 6 to 13. The ears of rabbit 15. Figs. 6 and 7, 8 and 9 show the inside and outside of the right and left ears respectively, on the 41st day. Figs. 10 and 11, 12 and 13 show the state of affairs 16 days later. The hole in the left ear (Fig. 8) was due to a biopsy on the 29th day. It later filled with growth (Fig. 12). C, C = carcinomas, as determined microscopically. P, P = the aggressive papillomas described in the text. The scattered small growths were all ordinary papillomas, mostly dark gray.

FIG. 14. Ears of rabbit 28 (Experiment 4) on the 123rd day (see also Figs. 33 and 34). The ulcerated, coalesced, malignant growths on the left ear, which have extended through and destroyed its central portion, are surrounded by numerous, discrete, sooty papillomas. A similar malignant tumor has perforated the right ear and extended to the outer side which is devoid of growths elsewhere. There is metastatic enlargement of the auricular lymph nodes (arrows). × 1/3.

FIG. 15. Metastasis in an auricular lymph node of the same rabbit. × 41.

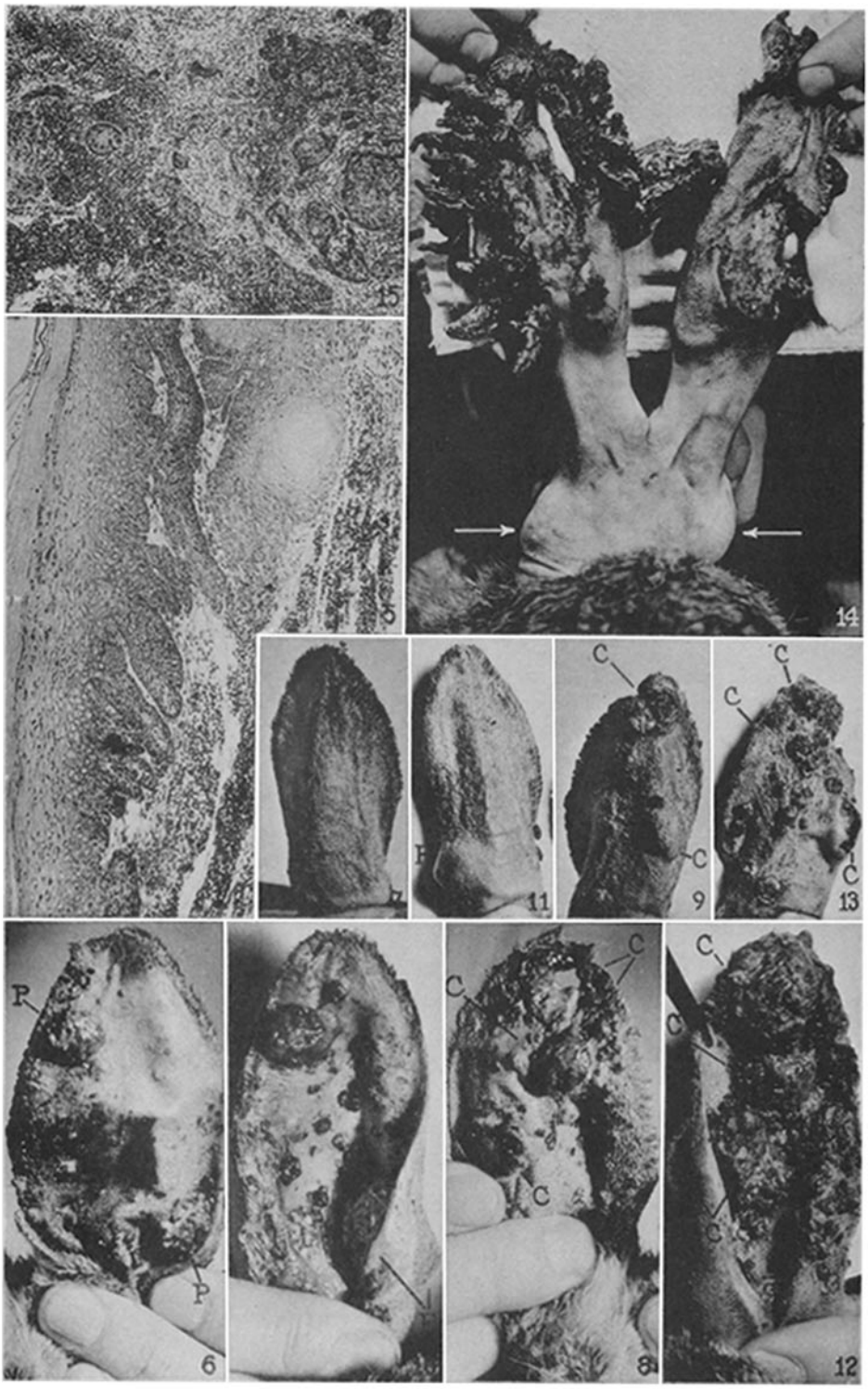

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PLATE 15

FIGS. 16 and 17. The ears of D. R. 1-31 (Experiment 2) on the 47th day. The aural concavities are full of confluent, scabbed, fungating growth, and great distortion exists. On the outer side are scattered mounds and bulgings, some being deep lying, benign papillomas capped with dark keratin, while others are malignant (C,C) and in some cases ulcerated. Two biopsies (Cx) had been made at the ear margin. At Y is the diffuse thickening mentioned in the text (page 415), and at Cs the bulge caused by a deep lying, cystic, malignant growth (*vide* Fig. 22). × 2/5.

FIG. 18. (*a*) Longitudinal slice through an ear of Figs. 16 and 17. The diversified markings of the fungoid tissue indicate the variety of the growths. At several places (C,C) they have extended through the cartilage and formed mounds on its outside, especially near the ear tip. (*b*) Slice through several of the embedded, acorn-shaped papillomas on the outer side of the ear. 2 are medium and dark gray respectively, and all are vertically striated. × 4/5.

FIG. 19. Invasion by the glandular metastasis shown in Fig. 5. × 400.

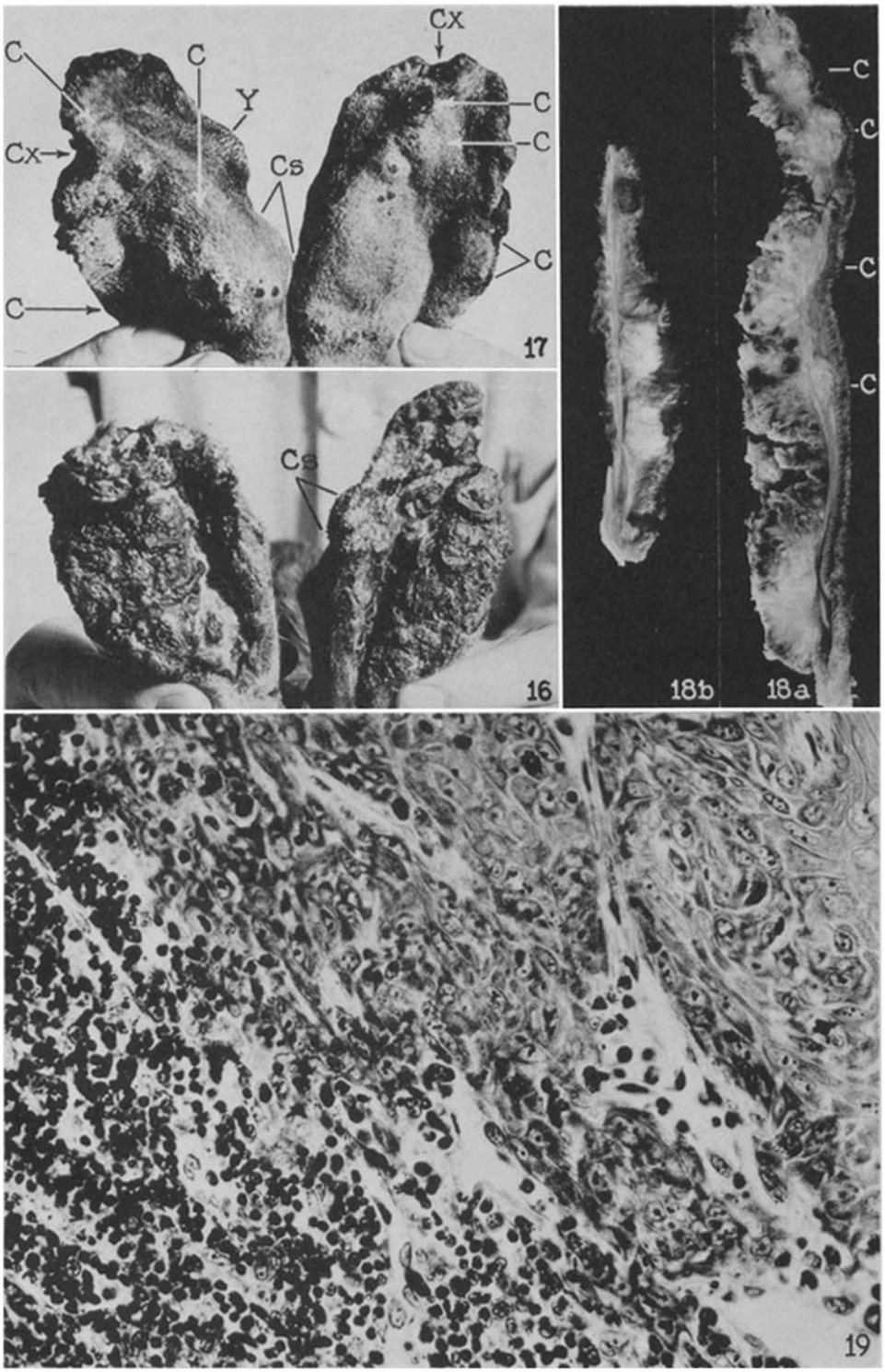

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PLATE 16

FIG. 20. Extension of an anaplastic growth through a lacuna in the ear cartilage: biopsy on the 38th day, rabbit 1-31 (Experiment 2).

FIG. 21. Random section through the edge of an ear, at autopsy of the same animal. A highly anaplastic, ulcerating growth has extended through the cartilaginous plate. Nearby is a deep lying, heavily pigmented, acorn-shaped papilloma. × 10.

FIG. 22. Cross section of the bulging cyst, Cs, of Fig. 17. It is lined with malignant growth (Fig. 23), which can be seen also in a broad, ulcerated expanse opposite it on the inner side of the ear. × 4.

FIG. 23. Part of the cyst wall. × 40.

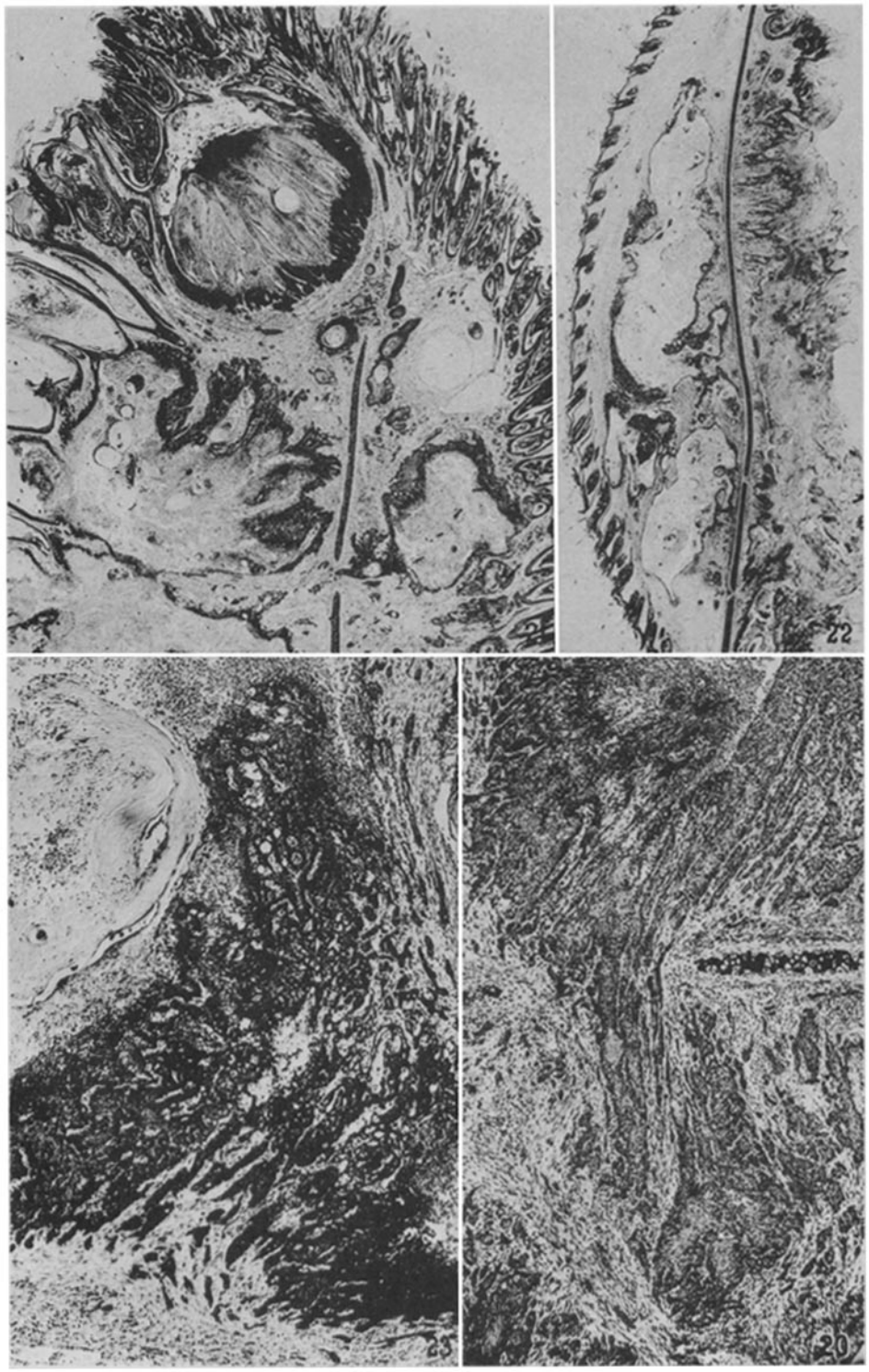

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PLATE 17

FIG. 24. Results of a profuse virus infection of skin tarred for many months previously (rabbit 6-51, Experiment 3: 23rd day after virus injection). Within the greatly thickened aural shells 2 large tar tumors can still be seen amidst much new-formed, fungating tissue. × 2/5.

FIG. 25. Same ears on the 28th day. They are distorted and rendered enormous by diffuse, papillomatous proliferation, which is almost entirely subepidermal on their outer side and along their edges. They weighed 220 gm. Nowhere did malignancy exist. × 2/5.

FIGS. 26 and 27. Ears of rabbit 12 on the 29th and 42nd days: to show the rapid progress of the changes. × 2/5.

FIG. 28. Ulcerated, malignant growth on the outside of the ear of rabbit 27 (Experiment 4): 67th day. The bracket indicates its situation. The other growths are gray papillomas. Shadows complicate the picture. × 4/5.

FIG. 29. Extension to a salivary gland of the metastatic tumor at the base of the right ear of rabbit 28 (Experiment 4). × 42.

FIG. 30. Virus-induced, bulging, papillomatous excrescences on an old, pedunculated tar wart. Some of them are patched with gray. The surface of the wart had previously been smooth (D. R. 6-46, Experiment 3: 36th day). × 2/5.

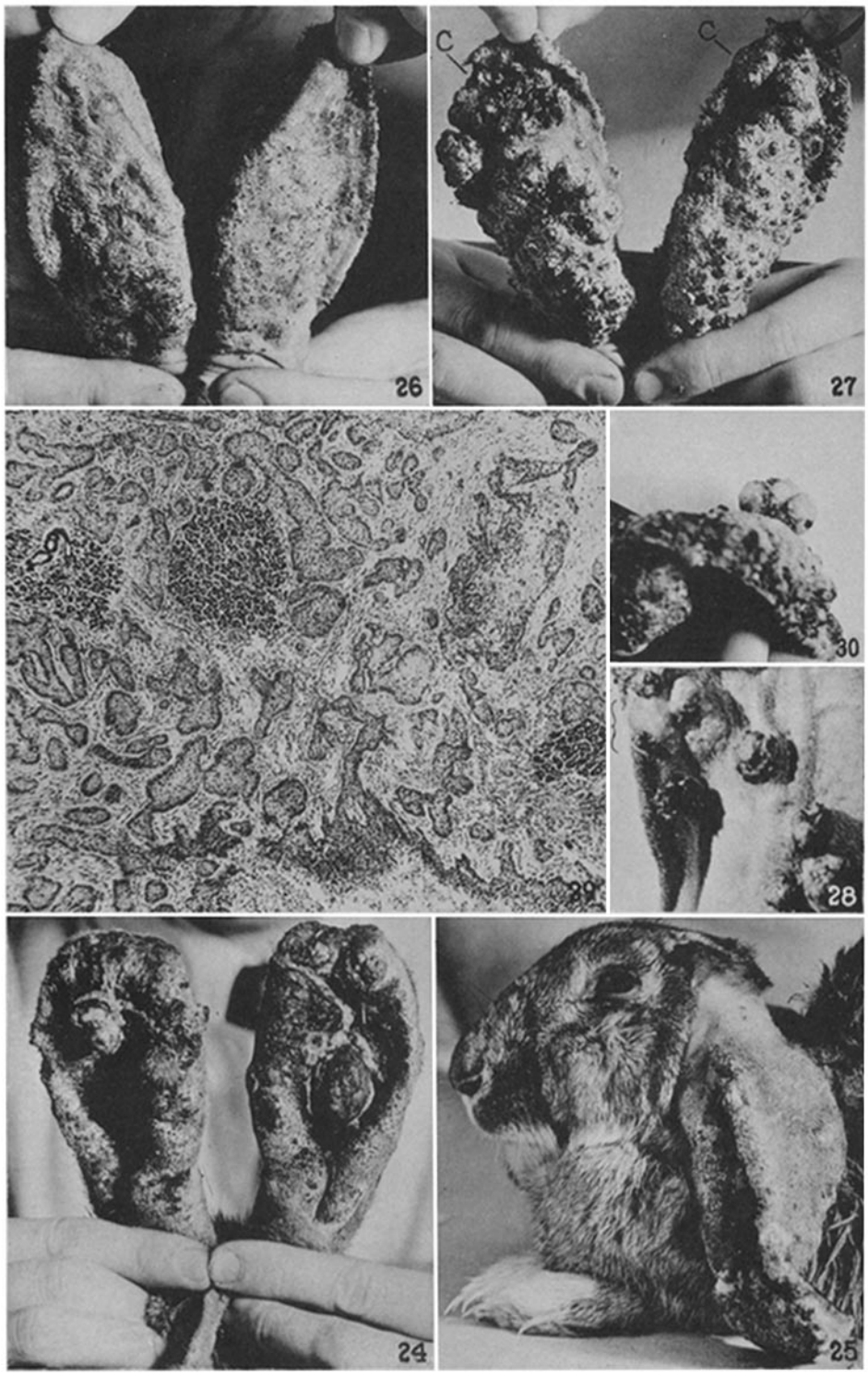

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PLATE 18

FIG. 31. Malignant growth on the inner surface of the right ear of rabbit 28: 67th day. It has encroached upon a nearby papilloma of dark hue. × 2/5.

FIG. 32. Extension of the growth of Fig. 31 to the outer surface of the ear: 107th day. × 2/5.

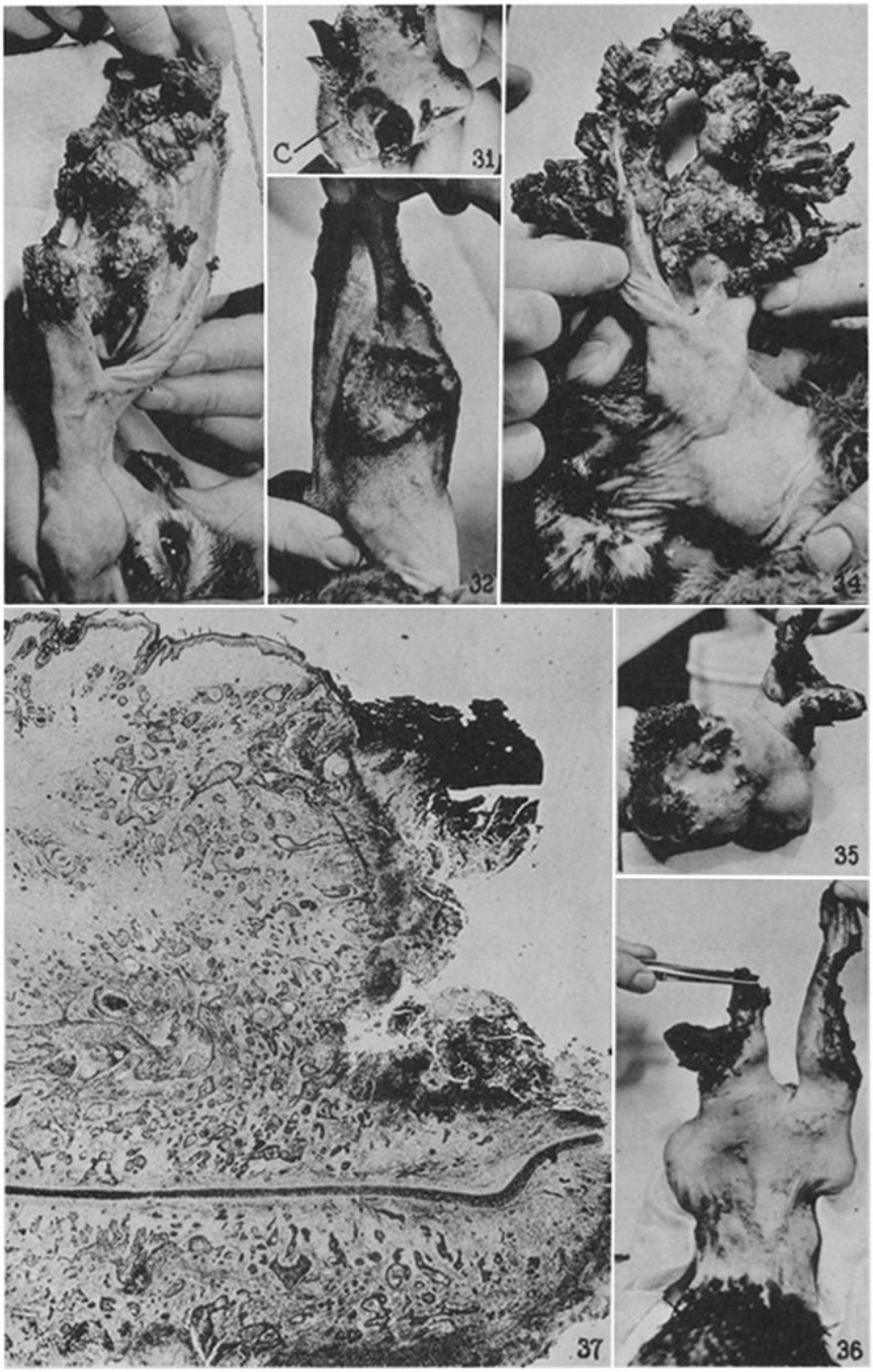
FIG. 33. Same growth on inner surface, with metastases in the auricular glands: 123rd day. (See also Fig. 14.) × 2/5.

FIG. 34. Destructive growths on the other ear of the rabbit, with glandular metastases: 123rd day. (See Fig. 14.) × 2/5.

FIG. 35. Left ear of Fig. 34 on the 156th day. Only an edematous, ulcerated stump remains, covered with dry blood. There is swelling round about and a large metastatic mass at the angle of the jaw. × 1/5.

FIG. 36. The ears and metastatic masses from behind: 156th day. × 1/5.

FIG. 37. Section through stump of the left ear: 159th day. × 11.



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