CANCERS DERIVING FROM THE VIRUS PAPILLOMAS OF WILD RABBITS UNDER NATURAL CONDITIONS

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(Received for publication, January 23, 1940)

The cutaneous papillomas experimentally produced by the inoculation into domestic rabbits of a virus native to western cottontails (1) frequently become carcinomatous (2). The malignant growths arise directly from the virus-infected cells as result of changes which often appear to be slight, and many of them continue to exhibit the morphological characters distinctive of the virus action until anaplasia supervenes. Other facts also bespeak an intimate relation of the virus to the cancers (3), yet whether it is their essential cause remains uncertain. And since domestic rabbits are foreign hosts the growths it induces in them, both papillomas and carcinomas, must be looked upon as the outcome of artificial conditions. Several authors have noted (4) that cancers sometimes originate from the virus papillomas of cottontails, but thus far only one of the malignant growths has been described in detail, and the relation of the papilloma to this was not wholly clear (4 *b*). Obviously the phenomenon deserves careful study; and there is the more reason to undertake it because the conditions in cottontails would seem favorable to tests for the presence of the virus, as is not the case in domestic rabbits. In these latter the virus, though demonstrable by indirect test (5 *a* and *b*), is usually "masked" (6): it cannot ordinarily be recovered in active form from even the most vigorous papillomas that it directly induces, and it has never been got from the derivative cancers. From most cottontail papillomas, on the other hand, it can be obtained in abundance. The present paper deals with twenty cancers which originated in the naturally occurring virus papillomas of cottontails and with experiments bearing upon the relation of the virus to the cancerous change.

Materials and Methods

The animals with cancer were procured through the generous interest of Mr. Earl Johnson who annually traps many thousand cottontails in Kansas and Oklahoma. Virus papillomatosis is exceedingly frequent in this region, and Mr. Johnson has sent

us every rabbit passing through his hands which had "warts" that appeared to be extending deep or had ulcerated. Some carried mere vigorous papillomas which had grown down into the subcutaneous tissue and formed keratinized cysts there (7). The cancers were fleshy, destructive growths, often ulcerated, and sometimes with palpable metastases.

The tumors were traced in outline on cellophane and described within 24 hours after receipt of the animal. Not infrequently blood was taken for the complement fixation reaction, which has proved highly specific in disclosing the antibody elicited by the virus and specifically neutralizing it (8). All operations were performed under ether anesthesia. Transfusions to prolong life were done with the citrated blood of normal cottontails.

To procure tissue for virus tests and for implantation, the skin was shaved about the cancer and swabbed with iodin, followed by 95 per cent alcohol, care being taken that neither reached the growth. This was everted by a cut part way around the encircling zone of sterilized skin, and with a separate set of sterile instruments portions of the base of the tumor were sliced off transversely for virus tests. Another slice, just overlying, was then taken for section. The material to be tested for virus was placed in a mixture of glycerin and Locke's solution in equal parts, and kept in the ice box until required.

The cancer was now cut free and one or more vertical slices directly across its mass were fixed for section. Often its outer portion was necrotic and foul: in such instances the necrotic material was trimmed away. The living tissue was diced and glycerinated, in most instances separately from the material first procured. Any papillomas present were treated in the same general way, save that their basal tissue was seldom saved separately. Gland and lung metastases were procured and glycerinated with due precaution for asepsis. Zenker was the fixative, and eosin and methylene blue the stains used.

For implantation purposes some of the fresh tumor tissue was hashed in a mixture of Tyrode and the animal's own serum, and suspended in more Tyrode. The implantations were done by forcibly injecting 1 cc., containing 5 to 10 suspended tumor fragments, into the anterior and posterior thigh muscles, muscles of the upper forelegs, and sometimes into the testes, through skin slits made to exclude the carrying of epidermis in on the needle. Individual fragments of certain tumors were implanted in the anterior chamber of the eye according to the method used by Greene (9).

Abstracts of the Case Histories

W. R. means wild cottontail rabbit; R. and L. mean right and left; ant. and post., anterior and posterior; pos. and neg., positive and negative; pap., papilloma.

All the cancer animals were adult males and females.

W. R. 1-55—when received had over R. haunch a fleshy, raised, discoid growth (G) 3.5 × 5 cm. across, 2 cm. high. Its flat, necrotic top showed no paps.; its bulging sides were covered with tense, pink skin; a blunt tongue extended 1 cm. laterally from its deep, nodular base. On back and haunches were 4 smaller, discrete growths, dark gray "onions" or craggy masses, typical virus paps., vertically striated, dry almost to skin. One (A) had a fleshy base. Several other gray, craggy paps. up to 1.5 cm. high were present on under side of animal and on legs. On chest was a gray pap. (D), 2.5 cm. across, lower than the others, and covered with flaky, irregularly striated keratin over a thick base that had extended laterally involving skin. Through it opaque yellow dots could be seen in the neoplastic tissue.

G was removed, save for a small piece. Its nodular, fleshy base contained several small cysts with ragged walls of soft, fine-textured tissue 0.8 cm. to 1.5 cm. thick, enclosing clear, glairy fluid and yellow, grumous lumps. Some of the tissue was passed through three changes of Locke's solution to minimize bacterial contamination, and bits were implanted in both forelegs and L. ant. and post. thighs of the host. Much clear, stringy fluid had come away into the Locke's. Rest of the basal tissue and all the superficial were glycerinated separately.

Blood serum taken just prior to the operation gave strongly pos. complement fixation in mixture with an extract containing the virus.

4th day: Transfused 20 cc. *14th day*: Wound completely healed: firm nodules at implantation sites. *24th day*: A pap. in L. axilla (E) now has, like D, a fleshy, extending base dotted with yellow. The dry material overlying some paps. on haunches and back is lower and flakes away. At implantation sites in legs are fusiform or zeppelin-shaped growths, firm, somewhat nodular. Today those in R. foreleg and L. post. thigh, 3.5 and 1.8 cm. long respectively, were almost completely removed. They were unencapsulated, fleshy, close-textured, grated under the knife, showed serpiginous necroses, and exuded stringy, clear fluid like the primary growth. Bits were implanted in legs of 20 cottontails. (No tumors resulted.)

41st day: Growth A is now ulcerated, ruddy, weeping. A pap. in L. groin has become a fleshy, weeping disc (H), 2.5 cm. across, 0.8 cm. high. *44th day*: Zeppelin-shaped, implantation growth, 2.5 cm. long, in L. foreleg removed almost entirely, and pieces implanted in legs, testes, ant. chambers of eye of 10 cottontails. (No tumors resulted.) Remainder stored. Serum taken today yielded still more strongly pos. complement fixation.

55th day: Several new paps. now, onion-shaped, dark gray, such as virus causes. Beneath healed incision to remove tumor G are 2 smooth, globular, subcutaneous nodules, 1.5 cm. across, one of them where a fragment was purposely left. Of paps. previously present on back, 3 (E, F, B) are now capped, like A, with brown coagulum instead of gray crags, and have basal extensions. Similar changes are taking place in a pap. on L. leg (J). Ulcerated growth H in groin is larger, has extended deep; brown scab overlies growth D on chest. In R. foreleg and L. post. thigh are nodules now where fragments were left at operations of 24th day. The implantation mass in muscles of L. ant. thigh is $3 \times 2.5 \times 2$ cm., with a nodular protrusion into subcutaneous tissue.

56th day: Growth in L. ant. thigh largely removed for glycerination: contains a cyst full of glairy fluid and grumous yellow material. Pap. J, now 1.5 cm. across, 1 cm. high, has an ulcerated spot with local downgrowth. It was excised and this part taken for section, remainder seeming to be typical virus pap. Basal tissue of remainder was implanted in R. ant. and post. thigh, and rest glycerinated in two lots (J^1, J^2). Transfused 27 cc.

63rd day: Died. At autopsy 5 typical benign paps. were glycerinated individually: several more present on skin. Already small, shotty, partly gray nodules had resulted from intramuscular implantation of pap. J on 56th day. They were glycerinated, as were also local recurrences of G and the recurrences of 3 implants of latter, now 1.5, 1.7, and 2 cm. across. The 5 surface paps. which had become partly malignant (A, C, D, E, F) were glycerinated individually, as was also the deep tissue of B, which seemed everywhere cancerous. The ulcerated disc H, 3×5 cm. across, was underlain by an abscess and hence was not glycerinated. R. lung contained a tumor mass 2 cm. across,

a sphere flattened on pleural aspect but protruding 5 mm. above surface. It had a living rind ½ cm. thick, enclosing a mass of keratinized, necrotic stuff like stiff putty.

Microscopic.—The large, discoid growth, G, on haunch was everywhere a wholly anaplastic squamous cell carcinoma (Fig. 1), as were the recurrences and implantation growths (Fig. 2). In the cross-section of mass G the microscope showed a minute pap. of virus type on skin 1 mm. away from the cancer.

The benign paps. were all of the sort the virus causes, as were the unulcerated portion of J^1 and the implantation nodules, J^3. Several other paps. had seemed partly malignant, and areas of convoluted or cystic pap., malignant pap., and squamous cell carcinomatosis were found in them (Figs. 9, 11, 21). Growth H was composed of malignant pap. and two sorts of keratinizing squamous cell carcinoma. Tumor B was everywhere of the latter sort, as was the growth in lung. One could not tell from which keratinizing primary cancer the latter had derived.

Summary.—This animal had a large, discoid cancer when received, as well as 2 paps. just becoming malignant and many scattered benign paps. The discoid cancer was nearly all excised and bits were implanted in the legs of the host, where they grew rapidly. Transplantation of the material thus provided to 30 normal cottontails on two occasions proved unsuccessful, even though the testes and ant. chamber of the eye were utilized.

As time passed new benign paps. appeared and several of the old became malignant. At death after 63 days' observation, 9 independent, cancerous masses were present on the skin. A large keratinizing lung metastasis had formed, which might have derived from any one of several cutaneous carcinomas. The tissue of a benign pap. implanted only a week before death had given rise to nodules. Complement fixation tests on two occasions showed that the blood had a high titer of antibody directed against the virus.

W. R. 1-56—had a fleshy hassock, A, on belly, 5 cm. × 4 cm. across, 1.6 cm. high, and B, similar, 4 cm. across, in groin. Both had bulging sides with adherent pink skin, a nodular base, and a dirty brown crust.

30th day: B is now 9 cm. long, fungoid, has extended around to back next tail. Close by on back is a creamy, vertically striated pap. (D), 0.9 cm. across, with weeping, macerated surface but shallow base. On rump is C, a raised, moist, fungating disc, 4.2 × 5.5 cm., with small nodules beneath. Growths C and D were doubtless overlooked at the first examination, which was hasty.

All growths removed, bits from base of B implanted in legs. Its soft, friable, pink, close-textured tissue showed faint, vertical striation and serpiginous necroses as well. Rest of base and superficial tissue glycerinated separately.

Growth C, similar in gross, showed when sliced a gray area, 6 mm. across, near edge; here and in some other regions were traces of vertical striation. Basal and superficial portions glycerinated separately. Growth A was fungoid, raised 1 cm., with a fleshy nodular downgrowth. It was patched with gray spots up to 1 cm. across, and in places vertically striated. The pigmented portions were cut away and remainder glycerinated. D proved to be a creamy, vertically striated pap. with shallow, sharply demarcated base. It was not saved.

37th day: Died. Wounds purulent, several minute nodules at one implantation site, abscesses at others; no metastases.

Microscopic.—Growth D was a mixture of benign and malignant pap.; B showed gradations to squamous cell carcinoma (Figs. 7, 8), multicentric and of several types,

as did C also, though a basal extension from it consisted entirely of convoluted papillomatosis (Fig. 10). Growth A proved to be everywhere a malignant pap. (Fig. 22), patched with gray.

Summary.—2 growths were noted on receipt of the animal and 2 more found a month after, when it was first examined carefully. 3 showed cancers amidst ordinary pap. tissue, while the fourth was wholly cancerous, though papillomatous in form.

W. R. 1-99—had on L. flank an ulcerated disc (A), 5.5 cm. across, 1 cm. high, fleshy, with bulging sides and flat top, raw and sanguineous because eaten down (Fig. 5). In L. groin, 3 cm. away, was a truncated cone (B), 2.5 cm. across, mostly dry and gray, and over sternum a similar cone (C), 2 cm. across, patched with gray. In L. groin was a mass of enlarged, partly coalesced lymph glands.

All growths were removed, as also the enlarged glands, and bits of latter implanted in ant. and post. thigh muscles. On section A was pink, fleshy, with keratinized yellow plugs in a nodular base. The enlarged glands were soft, friable, pink, with scattered serpiginous necroses and yellow plugs. Tumor B, capped with dry, sanguineous scab, was partly gray, mostly pink, with traces of vertical striation, yellow plugs in basal tissue, and a nodular, deep extension. C was similar, gray-patched, dubiously striated, capped with brown scab. Bits of its basal tissue were implanted in forelegs. With the blood serum a strong complement fixation reaction was got. Transfusion, 24 cc.

7th day: Died. Wounds purulent, abscesses in forelegs, in hind ones small tumor nodules. A gland in L. axilla was 1.2 cm. across, firm, pink, close-textured, with serpiginous necroses. In L. lung 8 fleshy lumps, 0.1 to 1.8 cm. across, roughly spherical, somewhat flattened on pleural surface, consisting of tissue like axillary nodule. Obstruction of a bronchus by one growth had caused atelectasis of a lobe. R. lung almost completely replaced by 2 partially coalesced masses, together 3 × 2 × 2 cm., mostly necrotic, in places purulent, with a rind 2 to 4 mm. thick of pink, living tissue. It contained several smaller growths as well. Tumors from this lung glycerinated separately.

Microscopic.—Tumor A consisted of a squamous cell carcinoma, everywhere forming keratinized cysts (Fig. 12) save at one spot where was a very different squamous cell carcinoma with some pap. features. Groin metastasis, axillary nodule, implantation growths, and some of those in L. lung (Fig. 13) all had the cystic character of A. Tumor B (Figs. 14, 15) consisted largely of malignant pap., in part pigmented; but near its center was a squamous cell carcinoma and, next one edge, convoluted and malignant pap. Most lung metastases, including great mass in R. lung, had character of the cystic squamous cell carcinoma of growth B, though not all had (Fig. 16). C consisted wholly of convoluted pap., obviously malignant, in many places pigmented.

Summary.—Animal carried a large, ulcerated, discoid squamous cell carcinoma and 2 malignant paps. All were removed and bits of 2 tumors implanted. Death 7 days later. Numerous lung metastases from 2 of the growths, and axillary and groin metastases in lymph glands; implants of one tumor had grown.

W. R. 1-53—died 2 days after arrival: immediate autopsy. On belly, just ant. to vulva, was a fleshy hassock 5 cm. across, 2.5 cm. high, with slightly constricted base and no sign of pap. Its flat top was covered with brown scab, its sides bulged, and here skin was fixed and thickened. In L. groin 4 fleshy, subcutaneous nodules, the largest 1.5 cm. across, and in R. groin a similar nodule 6 mm. across. Main growth proved close-textured, with serpiginous necroses and some ragged-wall cysts, full of

glairy fluid as in tumor G of W. R. 1-55. Beneath its irregularly nodular base were several small discrete nodules of similar tissue (glycerinated together).

Microscopic.—The primary growth consisted entirely of 2 squamous cell carcinomas, one mostly keratinizing, but in its newer portions breaking up into anaplastic growth (Fig. 19), the other (Fig. 18) with unusually small cells having an arrangement suggestive of derivation from a benign pap. The groin metastases consisted of cancer of the first type mentioned, as did the single, minute, pulmonary metastasis that was found (Fig. 20).

W. R. 1-54—died just after arrival: immediate autopsy. On belly at almost same place as primary growth of W. R. 1-53, was a fleshy disc 7 cm. across, ulcerated down to skin level near center, elsewhere covered with creamy, necrotic tissue, and with rolled edges 5 to 8 mm. high. It extended toward L. groin, and here a separate, flattened sphere 1.8 cm. in diameter was felt beneath it. Primary growth and sphere were removed and glycerinated separately.

Microscopic.—The tumor on skin was a malignant pap. (Fig. 23) which had broken up into squamous cell carcinoma at many places; the deep sphere consisted entirely of latter with remnants of a lymph node.

W. R. 66—had 5 typical, gray paps. when received. A few weeks later one, next penis, had changed to a raised, ulcerated, fleshy disc with weeping surface. There was a characteristic gray pap. not far away.

13th day after change first noted: Disc has grown fast (Fig. 6), now 3.5 cm. across and 1 cm. high. Neighboring pap. mass is larger, consists of dark gray crags and "onions." Several mm. of normal skin separate the growths, but they rub against each other when rabbit crouches. Animal bled for serum, killed. Disc is sharply demarcated, raw, lower toward center, surface medium gray toward pap., non-pigmented in its deeper tissue, and non-striated: glycerinated as usual. Its deep, nodular base had reached and become attached to a corpus spongiosum. Neighboring pap. also glycerinated. No metastases. Bits of base of disc implanted in legs of 6 cottontails and in testicles of one. (No growths resulted.)

Microscopic.—Disc everywhere consisted of squamous cell carcinoma (Fig. 17) with some pap. traits and melanosis. Pap. was a typical, pigmented, virus pap.

Summary.—Animal had several benign paps. when received, one of which changed to a squamous cell carcinoma. Bits of the cancer failed to grow in other rabbits.

W. R. 1-39—kept several months as normal and then tumor found on abdomen. It was a flattened, raw dome, with slightly constricted base, 2.4 cm. across, 9 mm. high, fleshy, pink, with irregular vertical striation and some minute cysts in the basal tissue. Microscopically it was everywhere a squamous cell carcinoma.

The Progression to Cancer

All of the malignant growths were typical carcinomas both in form and behavior. They grew progressively, invaded and destroyed, and frequently metastasized. Those implanted in the leg muscles gave rise to nodules, when not infected with pus-producing bacteria. Attempts to transfer two of them to other individuals (W. R. 1-55, 66) were unsuccessful, as is usually the case, though not always, with the cancers deriving from the virus papillomas of domestic rabbits (5 *b*).

Some of the cottontails carrying the cancers had benign cutaneous growths as well, with the characteristic morphological features of virus-induced papillomas (1, 10), and virus was recovered from every such growth that was tested (Table I). Several became malignant while under observation, and the cancers were seen to originate within the papillomatous mass. Malignancy was already present in some papillomas when the animals came to hand, though a large proportion of the tumor mass still consisted of ordinary virus papilloma tissue. In other instances none of this tissue was present though the cancers had morphological traits reminiscent of it. In certain instances in which these indications were lacking the presence of benign papillomas elsewhere on the skin, or of papillomas just becoming malignant, bespoke an origin of the cancers from such growths. And in instances in which all such signs were lacking the cancers proved to be of precisely those sorts which derive from papillomas experimentally induced with the virus in domestic rabbits. As a whole the evidence is conclusive that the malignant cottontail tumors originated in benign papillomas caused by the Shope virus.

To describe the morphological changes which took place as the cancers developed is to recite over again the happenings when malignancy originates in papillomas experimentally induced with the virus in domestic rabbits (2).

The papillomas of cottontails are usually the more pigmented, being often almost coal black; but otherwise they are nearly similar to those of domestic rabbits. They take the form of superficial "onions," or cones, or irregular, craggy masses, and consist of vertically striated, dry, keratinized squames which have built high over a well demarcated, shallow, living base. The first gross changes which suggest that malignancy may appear are an increasing irregularity of striation of the dried material, with flaking away of it so that the growth becomes lower. Concurrently its base becomes more fleshy, it bulges more at the sides, and the color lightens or alters from gray toward brown. Downward protrusions from the base can now often be felt, or there may be lateral extensions in the subcutaneous tissue with fixation of the overlying skin. Through the latter yellow plugs may be seen in the new, fleshy tissue. Ulceration follows, most often near the center of the tumor mass and deepest there, but soon extending toward the edge with result that the papillomatous tissue progressively comes away or is replaced. These changes may result in saucer-shaped growths with raised edges (Fig. 5), or in raised, beefy discs with flat tops (Fig. 6), either raw and sanguineous because eaten back, or covered with foul, pultaceous tissue or brown coagulum.

Once malignancy has appeared, the course of events is usually more rapid than in domestic rabbits. In them the first changes in the papilloma, the flaking, lowering, and the thickening at the base, do not connote the existence of cancer but may be visible months before it appears. In cottontails on the other hand cancer is actually present or very soon follows. In both species the gross changes mentioned are the expression of an increased activity and disorder of the papilloma (Figs. 9, 21). Microscopically

the first manifestation of malignancy is usually, though far from always, a papillomatosis of the "second order" (2), or what might better be called convoluted papillomatosis (Figs. 10, 11). The thickened epithelial layer differentiates and keratinizes, much as in benign papillomas, but is folded "every which way" not vertically. Its component cells are no longer arranged in graded ranks but higgledy-piggledy. They and their nuclei may now vary considerably in size and tinctorial capacity. Giant cells, giant nuclei, pathological mitoses, and multinuclear cells are frequent, and differentiation into keratinized scales is imperfect or often not completed prior to cell death. The basal epithelial layer has no longer a well defined, almost linear boundary, but extension takes place from it here and there into the connective tissue. The changes are expressive of no mere increase in the disorder of a benign papilloma but of significant qualitative differences.

Metastasis of the convoluted papillomas has not been observed. They are usually soon superseded by frankly malignant papillomas, tumors more markedly disordered, having a layered epithelium that usually extends down irregularly as tubes or solid prongs (Fig. 21) but they may for a while retain the form of an ordinary papilloma (Figs. 14 and 15). Such growths tend to break up soon into squamous cell carcinomas at many spots where they are invading. The carcinomas are generally of keratinizing type (Figs. 12, 16, 17, 18), though occasionally anaplastic (Figs. 1, 2). Metastases may exhibit either character. As time passes the entire tumor becomes everywhere a squamous cell carcinoma.

As a rule, soon after one cancer appears others do so elsewhere in the papilloma, and all tend to become more malignant by a progression of the sort just described. Malignancy generally develops first in the oldest parts of the papillomas of domestic rabbits, beginning near the center of masses which have enlarged by peripheral extension. Cross-sections of some of the larger tumors of cottontails show that the same holds true of them as well. In several instances the successive changes from one form of papillomatosis to another and ultimately to squamous cell carcinomatosis were almost diagrammatically evident. At the edge of the mass there was benign papillomatosis, a little further in convoluted papillomatosis, still further in frankly malignant papillomatosis, while squamous cell carcinomatosis occupied the central regions (Figs. 7, 8). Evidently the form of malignancy assumed was a function of time. This was not always so, however. Squamous cell carcinomas sometimes appeared to originate directly from the base of benign papillomas (Fig. 9). A diagram already published to illustrate the course of events and the various possibilities in domestic rabbits sums these up for cottontails as well (2).

Within the several forms of cancer mentioned considerable variety may be encountered, not a few of the growths being highly individual in their details. They are often, indeed usually, multicentric, as already stated; and the occurrence of many tumors in a single mass, together with the secondary union of one form of malignant epithelium with another, frequently results in a complex intermingling of neoplastic types, those of notably different character appearing to grade into one another.

The pigmentation of benign papillomas is due to melanoblasts and phagocytic clasmatocytes. It sometimes persists in convoluted papillomas, pigment-laden cells of both sorts flourishing in such growths (Fig. 11) and rendering them gray. This does not happen in domestic rabbits, only the benign growths showing melanosis. But, just as in such animals, the pigmented cells give no sign of neoplastic change, and they

are left behind as the tumors become more malignant, though often a little residual pigment persists in clasmatocytes and may even patch squamous cell carcinomas with gray. This was noted of a cottontail cancer already described (4 *b*).

In domestic rabbits the cancerous change is often preceded by downgrowth of the benign papilloma as such, with result in large, keratinized "pearls" beneath it, formed by a rind of living epithelium. The local conditions in cottontails are notably favorable to such downgrowth and it frequently takes place during the first few months of papillomatous proliferation, the epithelial tongues occasionally extending within lymphatics as far as the nearest gland, or even directly invading and replacing voluntary muscle (7). Yet, curiously enough, this active aggression is not usually followed by cancer. We have kept a number of cottontails showing it, in the hope of eventually witnessing a malignant change, but downgrowth ceased as the months passed and the cysts originally formed were eventually resorbed, or no longer enlarged, or ruptured outwards (7), and in the end the growths became indolent or disappeared. In this general relation it may be noted that cystic, keratinizing carcinomatosis, a type of malignancy frequent in domestic rabbits, was encountered only once in our cottontail material (Fig. 12). The metastases in this case were also cystic (Fig. 13), as usually happens in the domestic species.

The cancers originated directly from papilloma cells in every case in which the material provided opportunity for study of the point. And in not a few instances in which the relation of the benign growth to the malignant could no longer be directly traced, the influence of the papilloma virus was still cytologically evident.

When the virus induces papillomas it causes the differentiating epidermal cells to become much larger than normal, and to have much larger vesicular nuclei with marginated chromatin (1, 10). Little flattening takes place in the stratum granulosum, only a few granules forming there, of greatly differing size, while as keratinization takes place coarse parakeratotic lumps may appear in the cytoplasm next the nucleus. During completion of this process the cells undergo some ballooning and afterwards their outlines persist, loosely reticulating the dead tissue. All these changes may be encountered individually in tar cancers, yet when found together they are highly characteristic of the action of the virus, both in cottontails and domestic rabbits, and in the latter species they enable the papillomas due to virus to be readily discriminated from those due to tarring, despite pronounced superficial resemblances (11). Recently we have found that the same holds true of the virus and tar papillomas of cottontails. Some of the distinctive cytological traits which enable virus papillomas to be identified as such are still perceptible in not a few of the derivative cancers. This is notably true of many which retain the general architecture of virus papillomas (convoluted and cystic papillomas, malignant papillomas) and sometimes holds even for the metastases of squamous cell carcinomas (Fig. 16). But in proportion as anaplasia develops the virus stigmata tend to disappear.

Conditions Influencing the Origin of the Cancers

Cancers frequently arise from the virus-induced papillomas of domestic rabbits whereas in cottontails they are exceedingly rare. Not that papil-

lomas are infrequent in these latter: they are so common in Kansas and Iowa that one or two cottontails out of every twenty sent us as free from them has been found to carry them hidden in the fur. The cancers of the present work came from the region mentioned. They kill the animal soon, which will go some way to explain why they are not encountered oftener. But a larger reason exists in the resistance which most cottontails offer to the continued growth of virus-induced papillomas (4 *b*).

Only after papillomas have flourished for months do cancers appear in domestic rabbits, the likelihood of malignancy varying directly with the proliferative vigor of the benign growths. Few of the papillomas of cottontails continue vigorous, many disappearing after two or three months even though they may have enlarged rapidly at first, and those that persist mostly become indolent. During the last five years we have inoculated more than a hundred cottontails with a view to procuring cancers, using the most pathogenic virus materials available and in some instances submitting the growths to repeated injection with Scharlach R, a procedure which markedly stimulated them. Yet in nearly every instance the papillomas eventually disappeared, and in only one was malignancy attained, this after nearly two years and under circumstances which failed to exclude the possibility that the cancer might have originated from non-papillomatous epithelium (4 *b*). Several animals which had vigorous papillomas when trapped have been kept under observation for two years or more, but none has developed cancer though in some instances the growths continued to proliferate actively.

Our success in procuring instances of cancer from the wild population of cottontails can be easily understood. Nature does the sieving out of unpropitious material. In domestic rabbits it has been found that the more highly pathogenic the virus inoculum, as evidenced by the papillomas produced, the more likely is cancer to ensue (4 *b*). Highly pathogenic strains are the most likely to be perpetuated in cottontails under the conditions of chance transfer of the virus by way of scratches and wounds. The virus titers much higher in "natural" papillomas than in the generality of growths experimentally induced in cottontails.

Various intercurrent disturbances hasten the appearance of cancer in experimentally induced papillomas, notably inflammation due to bacterial infection or incision, and the proliferation excited by Scharlach R (2). In view of this it is not surprising to find that the cancers of cottontails mostly arose from papillomas situated near the genitalia or anus, on the buttocks or in groins, at places that is to say where the growths were especially liable to abrasion, maceration, and bacterial infection. Papillomas are frequent on the cheeks, head, and neck of trapped cottontails but here they have remained benign, in our experience.

Though malignancy in cottontails is rare, when it occurs it tends to be multiple. Not only does it usually originate at more than one spot in a papilloma, often at many, but when the animal carries a number of papillomas cancer may appear in several of

them at nearly the same time.[1] Our seven cottontails carried 20 distinct cancerous growths, one having 9. This was not because their papillomas proliferated more vigorously than those of other cottontails held for years without malignancy eventuating; nor can it be laid to a special carcinogenic effect of the strain of virus responsible for the papillomas. We have inoculated numerous cottontail extracts into domestic rabbits in the search for such strains but have found none in which the ultimate carcinogenesis could be preferred to any ability on the part of the virus beyond that needed to produce long-persisting, vigorous papillomas. The tendency to develop cancer, so marked in an occasional cottontail, must be laid to some peculiarity of its organism or tissues. The existence of such peculiarity is very evident in the case of domestic rabbits inoculated with the virus under controlled conditions (2, 7). In some of them multiple cancers develop at an early period, whereas in others, with growths due to the same inoculum and apparently of equal vigor, malignancy does not supervene until many months later.

Cancers Deriving from Virus-Induced Papillomas in Jack Rabbits and Snowshoe Rabbits

The frequency with which cancers arise from papillomas experimentally induced with the virus in domestic rabbits has led us to inoculate jack rabbits (*Lepus californicus*, Gray) and snowshoe rabbits (*Lepus americanus*, Erxleben), species of proved susceptibility to the virus (12). It was rubbed into scarified areas several cm. across, on both sides of the animals, and sometimes was also tattooed into the skin at nearby points. The rabbits did badly in captivity yet in several of the small number surviving more than a few months the papillomas underwent cancerous change.

The jack rabbits came from the trapper who furnished the cottontails, and one of them had, when received, a small papilloma of virus origin (12). Since no other such growth has been noted on the thousands of jack rabbits which have passed through the trapper's hands, it may conceivably have been due to virus transferred from a papillomatous cottontail after capture. The general susceptibility of jack rabbits to the virus, as disclosed by inoculation, also indicates the rarity of papillomatosis in this species under natural conditions. A considerable proportion of inoculated cottontails fail to develop growths, as if they had previously had the disease; but of 31 jack rabbits inoculated, 29 have developed papillomas, and one of the two resistant individuals carried the growth just mentioned. Only 16 survived for 3 months, the least time in which cancer has ever arisen in domestic rabbits, and only 5 for 4 months. In several of those dying near the end of this period the form of the papillomas had undergone note-

[1] No certainty exists that the cottontails with multiple papillomas that became cancerous within a few weeks of each other owed their growths to infection on a single occasion and with one virus material; but this seems likely in view of all the circumstances. The age of the cottontail papillomas was not known, but our experience with domestic rabbits and inoculated cottontails which were held to develop cancer gives reason for the supposition that they had existed long.

worthy alterations. Along the base of the vertically folded epithelial layer numerous tongues now extended into the connective tissue (Figs. 24, 25); yet the growths keratinized as before and showed no new cytological abnormalities. They were distinctly different from the original papillomas, and deserved the term papillomas of the "second order" (2) by reason of their complexity.

Squamous cell carcinomatosis developed in one jack rabbit. It had been inoculated on the sides with two virus materials, one of which gave a far more vigorous papillomatous growth than the other. This latter was still benign when the animal died after 7½ months, whereas the more vigorous growth had been entirely replaced by cancer (Fig. 26). The other 4 rabbits still carried at death, after 5 to 9 months, only ordinary papillomas or papillomas of the "second order."

No naturally occurring virus papilloma has been reported in snowshoe rabbits, and every one of the 16 we inoculated developed growths. 8 died before 3 months were up, and only 5 were left after 4 months. In 2 of these multiple carcinomas subsequently developed in the papillomas. The most malignant occurring in snowshoe 2 became ulcerated, extended in long strands along the lymphatics (Fig. 3), and gave rise to numerous secondary nodules in the axillary lymph nodes and lungs, rendering the host moribund after 12½ months. The neoplasm was like none thus far encountered in other species of rabbits (Fig. 4): special stains were required to demonstrate that it was not a sarcoma. On either side of it were 2 convoluted papillomas, differing in morphology but obviously malignant, while the peripheral part of the tumor mass consisted of ordinary benign papillomatosis. The growth had been produced with a virus material known to cause in domestic rabbits very vigorous papillomas which frequently became cancerous. Bits of one of the lung metastases were implanted in the leg muscles of 3 snowshoe rabbits at 6 situations and in the subcutaneous tissue of their axillae as well. No growths resulted.

The second snowshoe rabbit with cancer had likewise been infected with virus of a strain of high initial pathogenicity, but this had just been passed through a snowshoe rabbit, with result in such loss of potency that a 10 per cent extract of the glycerinated snowshoe tissue gave rise merely to 5 scattered papillomas on the broad area into which it was rubbed on the animal now under consideration. They appeared late and grew slowly, though persistently. The largest was only 2.3 cm. across at death after 2 years, but, like the next largest, 2 cm. across, it had recently changed to a raised, fleshy disc with weeping surface, wholly different from the craggy, dry papillomas nearby. Sections of the discs disclosed multiple cancers in both (Fig. 27). They were still superficial. The other growths were ordinary papillomas microscopically.

In the most susceptible of the jack and snowshoe rabbits the papillomas failed to grow as fast or become as fleshy as the papillomas of the most favorable domestic rabbits, those in which cancer occurs frequently and early, while furthermore the malignant change was not preceded, as in these, by increasingly exuberant proliferation. The papillomas exhibited peculiarities of detail, apparently characteristic of the host species (12), and the carcinomas differed somewhat from those occurring in cottontails and domestic rabbits. The very malignant snowshoe cancer, which metastasized to the lymph glands and lungs, was like none thus far met in other species: its cells were fusiform, with very little stroma (Fig. 4), and in many spots it resembled a sarcoma. In this connection it should be said that cancer G of cottontail 1-55 (Figs. 1, 2) has had no parallel in domestic rabbits. But such differences would appear to be essentially minor.

The papillomas of jack and snowshoe rabbits are not notably vigorous, like those of domestic rabbits, yet as in them, secondary cancerous change is frequent,—far more frequent than in cottontails. The cancers originate from the papilloma cells, and tend to change from one form to another, each more malignant than the last.

The Presence of Virus

Comprehensive tests were made to learn whether virus was present in the cottontail cancers. The technique already described to obtain uncontaminated tissue for glycerination had been devised with this end in view.

The papilloma virus is remarkably resistant, no attenuation occurring in glycerinated materials kept for a year or more in the ice box. The specimens of the present work were tested after days or weeks in glycerin, and the tissue of W. R. 66 was utilized while fresh as well. All of the materials from any one animal were tested at the same time, with few exceptions. The blocks of glycerinated material were passed through several changes of Tyrode's solution, ground in a mortar with sterile sand, Tyrode was added to make a 10 per cent suspension (5 per cent and 3 per cent in the case of the two tests with the cancer of W. R. 66), the suspension was briefly centrifuged to clear it of fragments, and then rubbed into freshly scarified areas on 3 normal domestic rabbits. Each of these received 20 to 24 inocula, into checkerboard squares of scarified skin with bands of fur between (5 *a*). After drying in a blast of warm air, the squares were covered with separate pieces of gauze, held in place with adhesive and a many-tailed binder, and this dressing was not removed until 10 days later, when healing had been completed. Thus all possibility of accidental transfer of virus from one spot to another was ruled out. The findings were recorded at frequent intervals during the next 42 days, after which no more growths appeared. The situation of the inocula was varied from animal to animal in each group of 3; and the results within each group proved consistent. In those few instances in which some of many tumor materials from a single individual were tested on one group of domestic rabbits and some on another, a second set of comparative tests was carried out later to control the findings.

The results as a whole are set forth in Table I, with the multiple tumors of each animal listed in order of increasing malignancy. Many of the growths contained both benign and malignant tissue, the latter often of several sorts. Stars are used to indicate the proportion of the various tumor forms present in a representative, microscopic cross section of each mass. Its entire content is represented by four large stars, one star standing for approximately a quarter of it, and a small star for one-eighth of it. Since the appraisal was two-dimensional, and any benign papillomatosis found was usually situated at the periphery of the growth, it follows that far more tissue having this character was often present than the amount recorded.

Titration experiments have shown that the quantity of virus present in an inoculum finds expression both in the time elapsing before growths appear and in their number and character (5 *a*). As in previous work, the following symbols have been employed: ++++ = confluent papillomatosis, +++ = semiconfluent papillomatosis, ++ = many discrete papillomas, + = a few papillomas, ± = 2, 3, or 4 discrete papillomas, ± = 1 papilloma.

TABLE I

Tests for the Presence of Virus in the Naturally Occurring Growths of Cottontail Rabbits

Cottontail rabbit No.	Test material No.	Tumor	Greatest diameter of growth	Gross character	Microscopic findings on cross-section: Virus papilloma	Convoluted or cystic papilloma	Malignant papilloma	Squamous cell carcinoma	Virus yield	Remarks
			cm.							
1-55	1	M^1	1.0	Gray, craggy, superficial mass	****				++++	Typical virus papilloma
	2	M^2	1.5	Another part of same	****				++++	" " "
	3	N, O	0.5, 1.5	2 gray "onions"	****				++++	" " "
	4	K	0.8	Gray "onion"	****				++++	" " "
	5	J^1	1.5	Gray, craggy, superficial mass	****				+++±	Typical virus papilloma. Both materials tested on 2 sets of animals, with same results each time
	6	J^2		Another part of same	****				+++	
	7	J^3		Intramuscular nodules	****				±	Typical virus papilloma. Autoimplant of basal tissue of J^1
	8	C^1	2.0	Striated gray crags with fleshy extending base	***		*	*	0	
	9	C^2		The basal extension of C^1	**		*	*	0	
	10	A	2.5	Ulcerating, gray, craggy mass with fleshy base	**	*		*	0	
	11	E	1.2	Brown, crusted crag	**		*	*	0	
	12	F	2.2	Scabbed, fleshy disc	*	*	**		0	
	13	D^1	4.0	Ulcerated fleshy disc			**	**	0	
	14	D^2		Deep extension of D^1				****	0	
	15	B	2.5	Scabbed, fleshy disc extending down				****	0	Tested deepest tissue
	16	G^1	5.0	Foul, fungating, fleshy disc	?			****	0	Tested deepest tissue twice, on 2 sets of animals
	17	G^2		Fleshy intramuscular growths resulting from autoimplantation of G^1				****	0	Extreme anaplasia
	18	G^3		Recurrence of G^1 after operation				****	0	" "
	19	G^4		Another recurrence of G^1				****	0	" "
	20	?		Lung metastasis				****	0	Not tumor G

See text for explanation of symbols.

TABLE I—*Concluded*

Tests for the Presence of Virus in the Naturally Occurring Growths of Cottontail Rabbits

Cottontail rabbit No.	Test material No.	Tumor	Greatest diameter of growth	Gross character	Microscopic findings on cross-section: Virus papilloma	Convoluted or cystic papilloma	Malignant papilloma	Squamous cell carcinoma	Virus yield	Remarks
			cm.							
1-56	1	B^1	9.0 × 4.0	Fungoid, ulcerated, discoid mass	**		**	**	±†	All gradations to squamous cell carcinoma
	2	B^2		Basal tissue of B^1				****	0	
	3	C^1	5.5	Fungoid, ulcerated, discoid mass, patched with gray	*		***	*	0	
	4	C^2		Lateral extension and basal tissue of C^1		****			0	
	5	A	5.0	Ulcerated, fungoid mass, patched with gray	?		****		0	
1-99	1	B^1	2.5	Truncated, fleshy cone, partly gray and striated, partly scabbed	?	*	**	*	±†	Virus papillomatosis had seemed present in the gross
	2	C	2.0	Fleshy, scabbed, truncated cone, partly gray		****			0	" "
	3	A^1	5.5	Raised, ulcerated, sanguineous disc		*		****	0	
	4	A^2		Metastasis in a lymph node of groin				****	0	
	5	A^3	1.2	Metastasis in an axillary lymph node				****	0	
	6	A^4	1.8	Lung metastasis				****	0	
	7	A^5	3.0	Lung metastasis with abscess				****	0	
1-54	1	A^1	7.0 × 5.0	Raised, ulcerated disc with rolled edge			**	**	0	
	2	A^2	1.5	Metastasis in a groin lymph node				****	0	
1-53	1	A^1	5.5	Basal portion of ulcerated, fleshy disc				****	0	Materials extracted together for test
	2	A^2		Metastases in groin lymph nodes						
66	1	A	3.5	Gray, craggy mass	****				++++	Typical virus papilloma. 3 per cent extracts of both growths inoculated into 6 test rabbits
	2	B	3.5	Weeping ulcerated disc				****	0	
	1	A			****				++++	5 per cent extracts used of both growths
	2	B						****	0	
1-39			2.4	Fleshy disc			**	**	0	

† The extract caused 3 papillomas, in 1 of 3 test animals. They appeared very late (35th day).

From each of 7 benign papillomas present on the cancer animals an abundance of virus was got (Table I). Yet none at all could be obtained from any of the 23 materials which consisted wholly of cancerous tissue coming from 12 primary tumors, the metastases of these and the implantation nodules. The glycerinated tissue from several of the cancers was gray with melanin, which usually meant that they had only recently derived from papillomas, a point already considered. There were available for test 15 materials from 12 growths still consisting in greater or less part of benign papilloma tissue. Almost as consistently negative results were obtained with these, only one material (W. R. 1-56, material 1) yielding virus,—in exceedingly small amount although at least half of the growth consisted of benign papilloma.

The presence of benign papilloma tissue has been queried in the case of 3 materials of the table. Microscopic study of a cross section of tumor G, material 16, W. R. 1-55, showed a minute papilloma on the skin next the large anaplastic cancer. It was so small as to have been overlooked in the gross, and others might also have escaped attention and been glycerinated with the cancer. The growth providing material 5, W. R. 1-56, was patched with gray and had an ill-defined vertical striation. Though the gray tissue was discarded at glycerinization and a cross section of the growth disclosed only malignant papilloma, the presence of benign papilloma tissue somewhere in it cannot be ruled out. Neither of these materials yielded virus on test. It was present in very small amount, however, in an extract from another growth (material 1 of W. R. 1-99) which had appeared in the gross to consist largely of benign papilloma yet showed none in microscopic cross-section. The tumor was a superficial, truncated cone, like many ordinary papillomas, gray in about one-third of its living substance, with ill-defined vertical striation, but covered with a thin, dry scab at one spot. Unexpectedly the section showed malignant tissue everywhere, although more than half of the mass had the papillomatous structure (Figs. 14, 15), the malignant cells in some regions clothing vertical folds like those of a benign papilloma. Several other forms of cancer were also present, ranging from convoluted papillomatosis to squamous cell carcinomatosis where the growth was scabbed. The extract yielded a mere trace of virus. The gross character of the tumor makes it seem likely that benign papilloma made up part of the material utilized for extraction and hence not available for microscopic examination.

Had our tests been limited to papillomas which were everywhere benign and to growths wholly cancerous, their results might have been taken to support the assumption that the development of malignancy entailed disappearance of the virus; but the results with tumors of mixed composition cast a different light on the possibilities. Even when these tumors consisted largely or preponderantly of papilloma tissue precisely like that of benign growths yielding virus in quantity, little or none could be recovered from them (materials 8, 9, 10, 11 of W. R. 1-55, material 1 of W. R. 1-56).

These findings could mean only that the papilloma tissue within mixed growths had ceased to contain virus while still to all appearance typical, or else that the associated malignancy had brought with it conditions adverse to demonstration of the virus. In an attempt to learn more, some extracts of glycerinated cancer tissue, made as usual, were mixed *in vitro* with potent papilloma virus and inoculated into domestic rabbits. In this way it was discovered that the very cancers in which virus had been sought by extraction frequently yielded an "inhibitor" instead, capable of inactivating the virus in considerable quantity (Table II). No such neutralization tests were made with extracts of wholly benign papillomas because it was assumed that they would contain virus in abundance, as turned out to be the case (Table I).

The cancer extracts were mixed with an equal amount of papilloma extract that had been passed through Berkefeld filters and was known to contain active virus. After 2 hours' incubation, the mixtures were inoculated as usual into checkerboard squares on 3 normal rabbits. The extracts from W. R. 1-99, 1-55, 1-54, and 1-56 were all tested against the same virus preparation, and inoculated into the same animals. The glycerinated materials from which they came had been kept 7 or 8 days in the ice box since the tests for infectivity of Table I. The extract of the cancer of W. R. 66 had been tested for infectivity on the day before. It was mixed with a virus material of only moderate pathogenicity and inoculated into a different set of animals from those already named.

Table II shows that most of the cancer extracts examined had some neutralizing effect on the virus.

No effort had been made to control some important variables. The tissues had been placed in differing proportions of glycerin and kept in the ice box for widely different periods of time, a circumstance not without bearing on the outcome of the tests, in view of the observation that glycerinated tissue sometimes yields more virus than does fresh. It may be no accident that amongst the extracts tested against the same virus filtrate those procured from tissues which had been glycerinated for the shortest time exerted most neutralizing effect.

The extract of material B^1 of W. R. 1-99 had some neutralizing effect on the virus although a previous extract, made with other portions of the same growth, yielded active virus in small amount (Table I). The mixed composition of the tumor, already commented upon, may account for these contradictory findings, since it gives reason to suppose that the glycerinated pieces taken for the first extraction contained more papilloma tissue. Tumor B^1 of W. R. 1-56, another growth of mixed composition, which had yielded active virus in small quantity, gave an extract devoid of definite neutralizing power.

TABLE II

Inhibiting Effect of Extracts of the "Natural" Cancers of Cottontail Rabbits

Cottontail No.	Period in glycerin	Tumor	Gross character	Microscopic	Outcome of pathogenicity tests (Mixtures of 10 per cent extract and 1 per cent virus)															Remarks
					17th day			19th day			22nd day			25th day			35th day			
					Test rabbits															
					1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
	days																			
1-55	22	C	Striated, gray crags with fleshy, extending base	Benign and malignant papilloma and squamous cell cancer	0	0	0	0	0	0	0	0	0	0	0	±	±	+	+	Extract as such caused no growths
		A	Ulcerating, gray, craggy mass with fleshy base	" "	0		0	0	0	0	0	0	±	0	±	±	0	±	±	" "
		G[4]	Fleshy, subcutaneous nodule	Postoperative recurrence of an anaplastic squamous cell carcinoma	±	++	+	±	++±	+±	+	+++	++	+±	++	++	++	++++	+++	
1-99	14	A	Ulcerated, sanguineous disc	Squamous cell carcinoma	0	0	±	0	±	++	±	±	++	±	+	++	+	++	+++	Extract as such caused no growths
		B[1]	Truncated fleshy cone, partly gray and striated, partly scabbed	Nearly all is malignant papilloma, perhaps some benign	0	±	+	0	+±	++	±	+±	++	±	++	++	+	+++	+++	
		B[2]	Fleshy, non-pigmented metastases of B in regional lymph glands	Malignant papilloma—more anaplastic than in original growth	0	0	0	0	0	±	0	±	±	±	+	±	+	++	+	" "
		C	A growth similar to B[1]	Convoluted papilloma	0	+	±	0	++	++	+	++	++	+	++	+++	+±	+++±	++++	" "
1-56	32	B[1]	Scabbed fleshy disc	Half benign papilloma; rest malignant papilloma and squamous cell carcinoma	0	+±	++	±	+++	++±	+	+++	+++	+	+++±	+++±	+±	++++	++++	Extract as such caused no growths
		B[2]	Basal tissue of same	Squamous cell carcinoma	0	++	++	±	+++	++±	±	+++	+++	+	+++±	+++±	++	++++	++++	" "
		C[1]	Fungating, fleshy disc	Benign and malignant papilloma	±	++	++±	±	+++	+++	+	+++	+++±	++	+++	++++	++	++++	++++	" "
		C[3]	Fleshy, local metastasis of C[1]	Malignant papilloma	±	+±	++	±	++±	++±	+	++±	+++	+	+++	+++±	++	++++	++++	" "
		A	Ulcerated fungoid mass	" "	±	+++	++	±	++±	++±	++	++±	++±	++	+++	+++	+++	++++	++++	" "
1-54	91	A[1]	Ulcerated, fleshy disc	Squamous cell carcinoma	+	+	++±	+	++±	+++	+±	++±	+++±	++	+++	++++	+++	++++	++++	Extract as such caused no growths
		A[2]	Fleshy, non-pigmented; metastasis in lymph node	Same cancer	±	±	++	+	++±	++±	+	++	+++	++	++	+++±	++	+++	++++	" "
Control					(Mixture of Tyrode and virus)															
					+	+++	+++±	±	+++	+++	+++±	++++	++++	++	++++	++++	++	++++	++++	
66	97	A	Weeping fleshy disc	Squamous cell carcinoma	(Mixture of 5 per cent extract and another specimen of 1 per cent virus)															Extract as such caused no growths
					0	0	0	0	0	0	±	±	0	±	±	0	±	±	±	
Control					(Mixture of Tyrode and virus)															
					+	±	±	+	±	±	+	+±	+	+±	+±	+±	++±	++	++±	

DISCUSSION

The present work has had several aims,—to learn the character of the malignant growths arising from the naturally occurring papillomas of cottontails, to find out how they originated, and to determine whether virus was present in them. The growths have proved to be true carcinomas. It follows that a virus must be added to the very numerous and heterogeneous *primary* causes for cancer which are known to be effective under natural conditions. The rabbit virus is not the first that has been observed to function in this way: squamous cell carcinoma sometimes arises from human condyloma acuminatum (13), a papillomatous growth due to a virus (14). The course of events in such cases is similar to that when virus papillomas of rabbits become malignant. In both instances the morphological changes closely resemble those occurring in human papillomas of the bladder and mouth and in tar papillomas of the rabbit and mouse when cancers arise from these (2).

The changes which take place when the virus papillomas of cottontails become malignant have proved practically identical morphologically with those already reported for domestic rabbits. Generally there occurs a progression from one form of cancer to another, each more anaplastic than the last. The result is a considerable, though limited, diversity in the character of the tumors,—a limitation scarcely surprising when one considers that the cancers deriving from the virus papillomas all spring from cells of a single sort influenced toward malignancy by an agent having highly specific effects upon them. The term "pure line" cancers, which we have applied to the malignant tumors deriving from the virus-induced papillomas of domestic rabbits, applies equally to the growths of such origin in cottontail, jack, and snowshoe rabbits.

Cottontail papillomas sometimes grow down and invade voluntary muscle at an early period in their development, appearing like squamous cell carcinomas and perhaps extending along the lymphatics (7), yet later reverting always to the ordinary, benign state. The phenomenon serves to stress a fact previously emphasized in relation to the findings in domestic rabbits, namely, that the cancers which eventually arise from virus papillomas are no mere accentuations of the papillomatous process but expressions of a qualitative alteration. Yet this often appears to be but slight, since the papilloma is itself a growth with attributes which place it at the brink of malignancy (7).

Most of the naturally occurring papillomas of cottontails yield active virus in abundance, and hence there had been reason to suppose that it

would be recoverable from the derivative cancers if present. It was readily obtained from the papillomas coexisting on the animals with cancers yet was got from only one material of 23 from growths which appeared completely cancerous on microscopic section; and the evidence in this one case was strong that benign papilloma tissue had been present in regions which could not be subjected to such scrutiny because extracted for virus test. Fortunately the tests included many growths of mixed composition, which still consisted to a large extent of ordinary papilloma tissue such as might have been expected to yield virus. From very few of them was it obtained. These puzzling results led to the discovery that extracts of the cancerous tissue instead of providing virus frequently acted to neutralize it.

At first thought the presence of an "inhibitor" in cancer extracts would seem to mean that virus was necessarily absent from the malignant tissue. But it has not this significance. For in our tests the virus was necessarily separated from the tumor cells, and the conditions under such circumstances were wholly different from those obtaining in the tumor itself. The virus is known to be effectually protected in living papillomas[2] from the action of a circulating antibody formed in response to its presence in the host and capable of quickly neutralizing it in papilloma extracts (5 *a*). A like state of affairs might very well have held as concerns the "inhibitor" now under discussion. And that it did hold, the "inhibitor" being nothing more than antibody of the sort just mentioned, which had extravasated into the tumor in excess over the amount capable of neutralizing the virus freed by extraction, many facts attest:—

One of us has recently reported that under certain conditions cottontail papillomas fail to yield virus (15), just as did the cancers of the present work. The growths of which this is true are fissured, inflamed, and disorderly, and are situated on animals which have much antibody in their blood. This antibody escapes into the growths in sufficient quantity to neutralize the virus extracted therewith, as does not happen in orderly papillomas produced with the same inoculum elsewhere on the same rabbits, extracts of these growths by contrast yielding virus in abundance. The development of cancer amidst papillomas provides conditions exceptionally favorable to extravasation.

[2] W. R. 1-55 provided several instances of such protection. Though the blood of this animal contained antibody in high titer new virus papillomas appeared on its skin during the period of observation, and enlarged rapidly. They cannot have been due to fresh infection with the virus since the presence of circulating antibody regularly prevents this, as we many times had occasion to note. Instead they must be laid to the enlargement of papillomas so tiny as to have escaped attention when the animal was first examined. A papilloma of just this sort was accidentally encountered on the bit of the skin included with the section of tumor G of W. R. 1-55, when this was examined microscopically.

In the mixed tumors of the present work, which still contained a considerable quantity of benign papilloma tissue, the latter was often disorderly (Fig. 21). We have repeatedly noted in studying the early stages of carcinogenesis in domestic rabbits that where malignancy has begun amidst a papillomatous mass a pink or red spot can usually be seen with the unaided eye, amidst the prevailing blue of sections stained with eosin and methylene blue. This is because exudation and cell necrosis have already begun in the minute cancer that the microscope discloses, providing material that takes the eosin stain. The large cancers of our cottontails were usually inflamed, often edematous, and had a weeping surface, or were covered with brown scabs of dried coagulum, or were sanguineous because bitten by the animal. The blood of cottontails that have long carried papillomas has regularly a pronounced neutralizing effect on the virus, and only in such animals does cancer arise,—judging from our experience in domestic rabbits. In the two cancerous cottontails tested for blood antibody this was found in high titer, and extracts of their carcinomas had a marked inhibitory effect on the virus (Table II, W. R. 1-55, 1-99). In this connection the fact may be significant that the several cancers of any one animal all seemed to have much the same content of "inhibitor."

If the presence of antibody derived from the blood is responsible, through its neutralizing effect, for the failure to demonstrate virus in cancer extracts, then it should be recoverable if the growths are extracted before the circulating antibody has come to effective strength. The test is not possible under ordinary circumstances because the papillomas preceding the cancers have already elicited antibody. But it can be carried out by utilizing tar cancers, since the blood of cottontails carrying these has no neutralizing power. In experiments to be detailed in a succeeding paper small pieces of cottontail tar cancers were excised, mixed with virus-containing fluid *in vitro*, and reimplanted in the host. Cancerous nodules resulted which far outstripped the control implants in rapidity of growth, frequently differed from them morphologically, as well as from the parent tumor, and often exhibited the cytological stigmata indicative of virus influence. Virus could sometimes be procured from such growths when they were extracted before antibody had appeared in quantity in the blood.

Virus cannot ordinarily be recovered in active form from even the most vigorous papillomas it engenders in domestic rabbits, although serological tests have shown that it is present in the growths and increases as they enlarge (5*a*). An "inhibitor" can often be obtained from their tissue, as Shope first noted (1), and Friedewald has lately shown this to be extravasated antibody (16). Its presence will not explain the "masking" of the virus, however, since this usually exists when the papillomas first become perceptible, that is to say before the blood has acquired any neutralizing ability. However, our failure to procure virus from the inflamed and edematous cancers of domestic rabbits might be accounted for by the extravasation of neutralizing antibody were there no other cause for "masking." For extracts of such cancers have a marked effect to neutralize virus *in vitro*, as we have repeatedly had occasion to note. In an experiment already described (17), incubation of mixed virus and cancer extract, done with the aim of producing variation of the virus, had the sole effect of greatly reducing its pathogenicity.

Occasional strains of the virus are not completely "masked" in the papillomas of domestic rabbits, but can be passed from animal to animal, as Shope showed (18). Several years ago he generously provided us with these recoverable strains for experi-

ments to learn whether virus could be got from the cancers deriving from papillomas induced with them. The latter growths appeared only after a long time and grew slowly, as Shope had already reported; yet after a year or more some underwent malignant change. Virus could not be demonstrated in extracts of any of the cancers and but seldom in extracts of the associated papilloma tissue. The cancers had become ulcerated and inflamed, providing local conditions notably favorable to the extravasation of blood antibody.

Virus can usually be recovered from papillomas induced in jack rabbits but we have made no effort as yet to test the cancerous tissue. Our one attempt with a snowshoe cancer illustrates several of the facts just discussed.

Snowshoe 2 had been inoculated on the left side with one of Shope's recoverable strains of virus (material of a 12th passage in domestic rabbits), and on the right with a strain producing in domestic rabbits much more vigorous papillomas in which, however, the virus was regularly "masked." The growths on the left appeared late and grew slowly, whereas those on the right came early, grew faster, were more fleshy, and after some months became cancerous, ulceration and metastasis rendering the animal moribund by the 381st day. The instance has already been partially described (see also Figs. 3, 4). Much papillomatous tissue of ordinary sort still surrounded the several cancers. It was glycerinated, as were the axillary and pulmonary metastases, and the benign papilloma on the animal's left side, and 62 days later 10 per cent extracts of all these materials were tested for virus, by inoculation into both the subcutaneous tissue and skin of 3 snowshoes and 3 domestic rabbits. No growths resulted from the extract of vigorous, disorderly papilloma in which cancer had arisen, nor any from those of the axillary and pulmonary cancers, whereas the extract of the slow-growing, superficial, benign papilloma consequent on infection with Shope's recoverable strain of virus gave rise anew to growths of like sort.

The demonstration that blood antibody may get out into cottontail cancers, in sufficient quantity presumptively to neutralize any papilloma virus coming away in extracts together with it, is far from meaning that virus is necessarily present in the malignant growths, much less that it is their cause. An increasing body of evidence speaks, however, for its close relation to the cancers and for its persistence and activity within them:—

The primary effect of the virus is to stimulate epidermal cells to form papillomatous growths which have themselves the immediate character of tumors (19), and from these the cancers derive secondarily by changes which often appear to be exceedingly slight. The malignant growths are not a result of any collateral effect of the growing papilloma upon non-neoplastic cells included within it or situated around its base, but of secondary changes in the epidermal elements infected by the virus and rendered neoplastic thereby. The virus has been found to persist and increase in two cancers of domestic rabbits, which have been successfully transplanted to new hosts.[3] None of the chemical or

[3] One of the cancers has now been propagated in 10 successive groups of animals. Specific virus-neutralizing antibodies have appeared in the blood of every animal in which it grew progressively.

physical agents which are called carcinogenic because they evoke tumors has any such close relationship to the neoplasms they elicit, nor does any accompany the growths enduringly and increase within them as the tumor viruses do (3).

The transplanted cancers just mentioned as harboring the virus were wholly anaplastic and devoid of any morphological signs of its presence. Not a few of the malignant growths deriving from virus papillomas, however, retain many of their traits and exhibit cytological peculiarities distinctive of the action of the virus. That this has the ability to persist in cancers of other derivation, and to influence them greatly, has been brought out in the experiments already mentioned in which the tar cancers of cottontails were infected with it. It causes even more remarkable changes in the benign tar tumors of domestic rabbits, inducing many of these to grow with unprecedented rapidity and some to become cancers straightway (17, 20, 21).

If the disease primarily caused by the papilloma virus were of any of the familiar kinds due to agents of this class, the changes in its character represented by cancer would unhesitatingly be laid to virus variation. There would be the more reason to do this because the changes are prone to take place when the virus has been subjected to unusual conditions, as after introduction into strange hosts (domestic rabbits, jack and snowshoe rabbits). Even under such circumstances the disease picture does not alter, cancer does not occur, until after a considerable time has elapsed,—a finding which falls in with general experience as concerns alteration of other viruses under such conditions (as, *e.g.*, the change of variola to vaccinia).[4] The alteration when it does take place is abrupt (7), and again the findings with other viruses may be cited; and as in their case it is qualitative, the cancers being no mere exaggeration of the papilloma process but expressive of a difference. So small, however, is this difference in many instances that a very slight shift in the pathogenic capabilities of the virus would suffice to account for them.

Cancer is exceedingly rare in cottontail rabbits, the natural hosts for the papilloma virus, yet it does occur, and when it does the cancers are frequently multiple, as if the individuals in which they developed had some tissue peculiarity rendering them unusual hosts for the virus. In domestic rabbits we have noted that experimental disturbances of the papilloma frequently precipitate the malignant change (2), and the same would seem to hold true in cottontails. Here again an unusual milieu for the virus with resulting variation of it would account for the change. The cells infected

[4] The Shope "fibroma" virus, an agent indiginous to cottontail rabbits, and causing temporary growths which have the aspects of fibromas, has been found to undergo variation after a time, when passed through a succession of domestic rabbits, with result in a strain causing inflammatory instead of proliferative lesions (Andrewes, C. H., *J. Exp. Med.*, 1936, **63,** 157; Andrewes, C. H., and Shope, R. E., *J. Exp. Med.*, 1936, **63,** 179).

with the virus, not the animal to which they belong, are its true hosts (3). For months or years, it is effectively protected by these elements from outside influences, from blood antibody for example. Under such circumstances there should be small chance for it to change. But sometimes the papillomas become crowded and disorderly after a while, or are traumatized or infected with bacteria. Such accidents may very well alter the conditions provided by the cells, with result eventually that the virus varies.

CONCLUSIONS

The naturally occurring virus papillomas of western cottontail rabbits become malignant occasionally. The cancers derive from the papilloma cells, that is to say from elements already rendered neoplastic by the virus and still infected therewith. Papillomas produced with the virus in jack rabbits and snowshoe rabbits become cancerous in the same way but much more frequently, as is the case in domestic rabbits also. To all three species the virus is foreign. The character of the cancers of the wild rabbits is described and the relation of the virus to them discussed on the basis of experimental findings. The facts support the view that the cancers result from virus variation, this in many instances being but slight.

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EXPLANATION OF PLATES

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

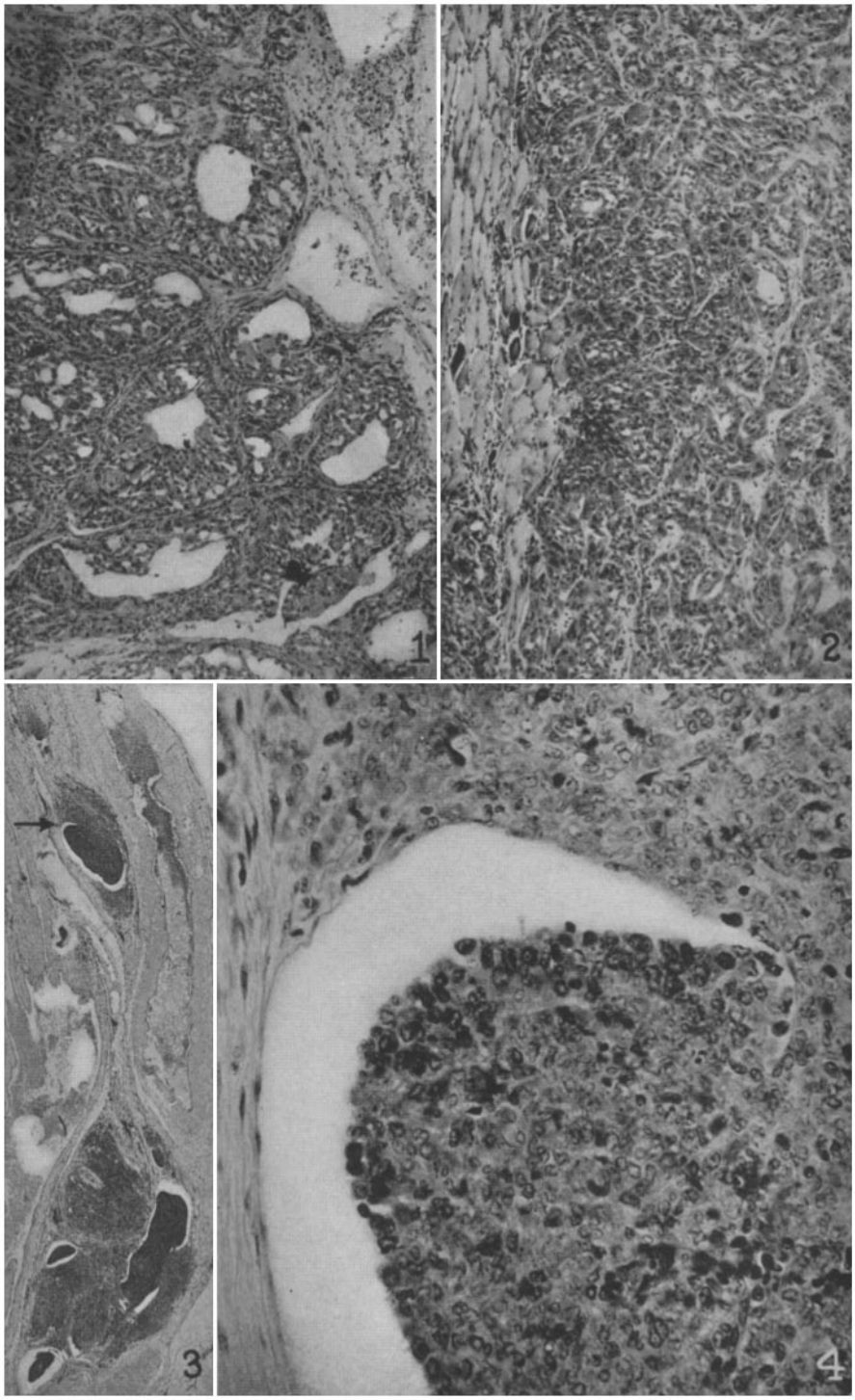
PLATE 22

FIG. 1. Carcinoma G of cottontail W. R. 1-55: part of base of the primary growth, to show alveolar arrangement and early cystic degeneration. × 70.

FIG. 2. Carcinoma G: advancing border of an implantation growth in the leg muscles. The growth is more anaplastic, but appears alveolar where it has replaced individual muscle fibers. × 70.

FIG. 3. Extension of a highly malignant carcinoma along the axillary lymphatics of a snowshoe rabbit, with invasion of the connective tissue (snowshoe 2). The growth stains so deeply with methylene blue as to appear almost black within the several lymphatics that it fills. The gray patches round about mark where tumor cells have extended out into the connective tissue (see Fig. 4). × 14.

FIG. 4. Part of the intralymphatic growth indicated with an arrow in Fig. 3. The tumor resembles a sarcoma in many ways, especially where it has extended out into the connective tissue. × 450.



Photographed by Joseph B. Haulenbeek and Louis Schmidt

(Kidd and Rous: Cancers from virus papillomas of wild rabbits)

PLATE 23

FIG. 5. Tumor A of W. R. 1-99, raw and sanguineous because gnawed by the animal. Microscopically it was a cystic squamous cell carcinoma (Fig. 12), anaplastic in a few spots. There were numerous metastases in the axillary lymph nodes and lungs (Fig. 13). × ½.

FIG. 6. Discoid cancer of W. R. 66, with an adjacent, almost black papilloma which later yielded virus as the cancer did not (see Fig. 17). × ⅝.

FIG. 7. Radial section of tumor B of W. R. 1-56, to illustrate the succession of tumor forms from periphery to center of the growth. On the extreme left is non-papillomatous skin, next comes a large expanse of ordinary, benign, virus papilloma tissue, then, under and beyond a vertical cleft, is convoluted papilloma, then malignant papilloma (bracket A), and finally squamous cell carcinoma (bracket B). All of the central region of the growth consisted of this last. It was of several types. A small amount of virus was present in an extract made from the mass as a whole. × 7.

FIG. 8. High magnification of the region about the spot X of Fig. 7. At the right of the picture is papillomatosis of the "second order," to the left malignant papilloma, and below squamous cell carcinoma. × 135.

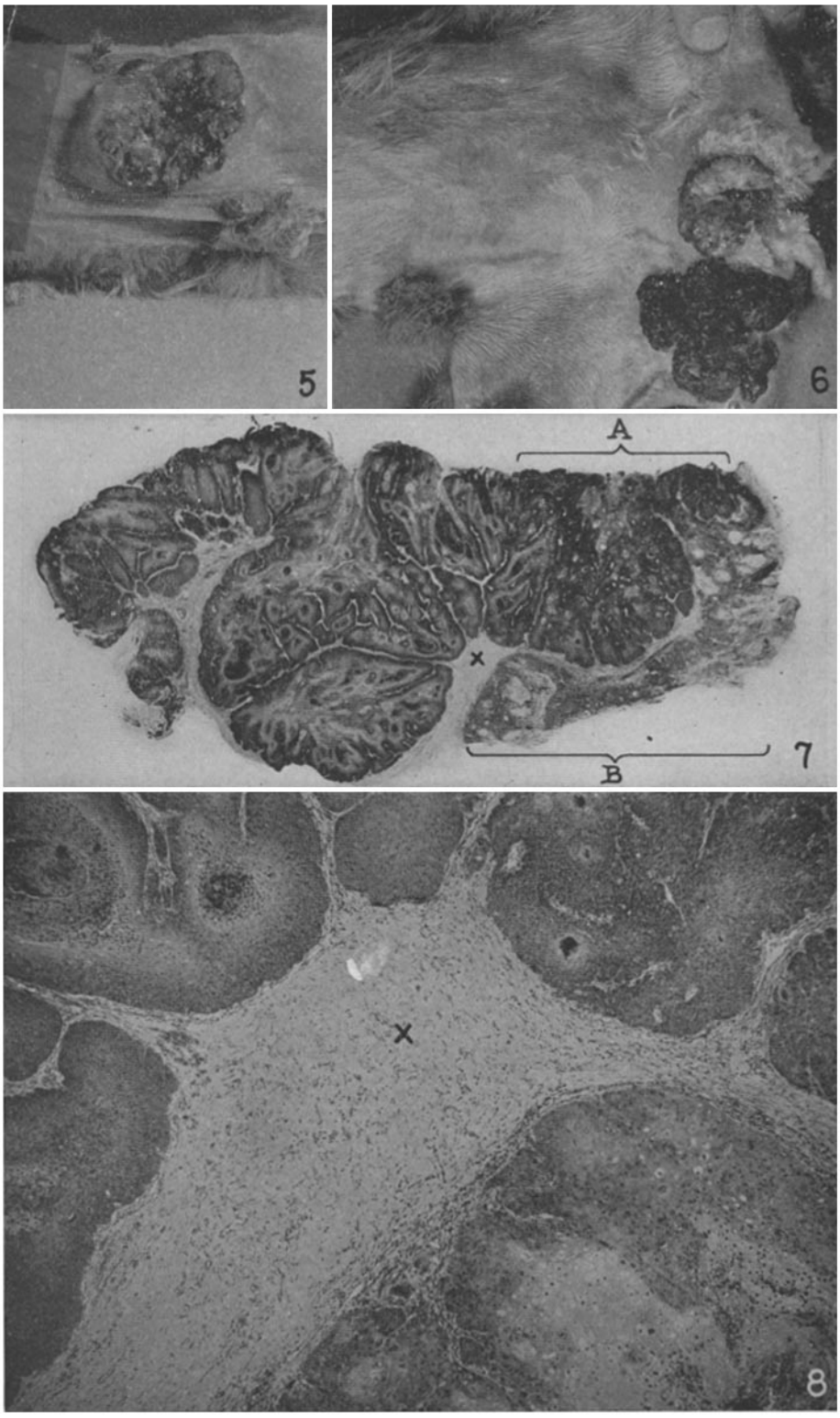


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PLATE 24

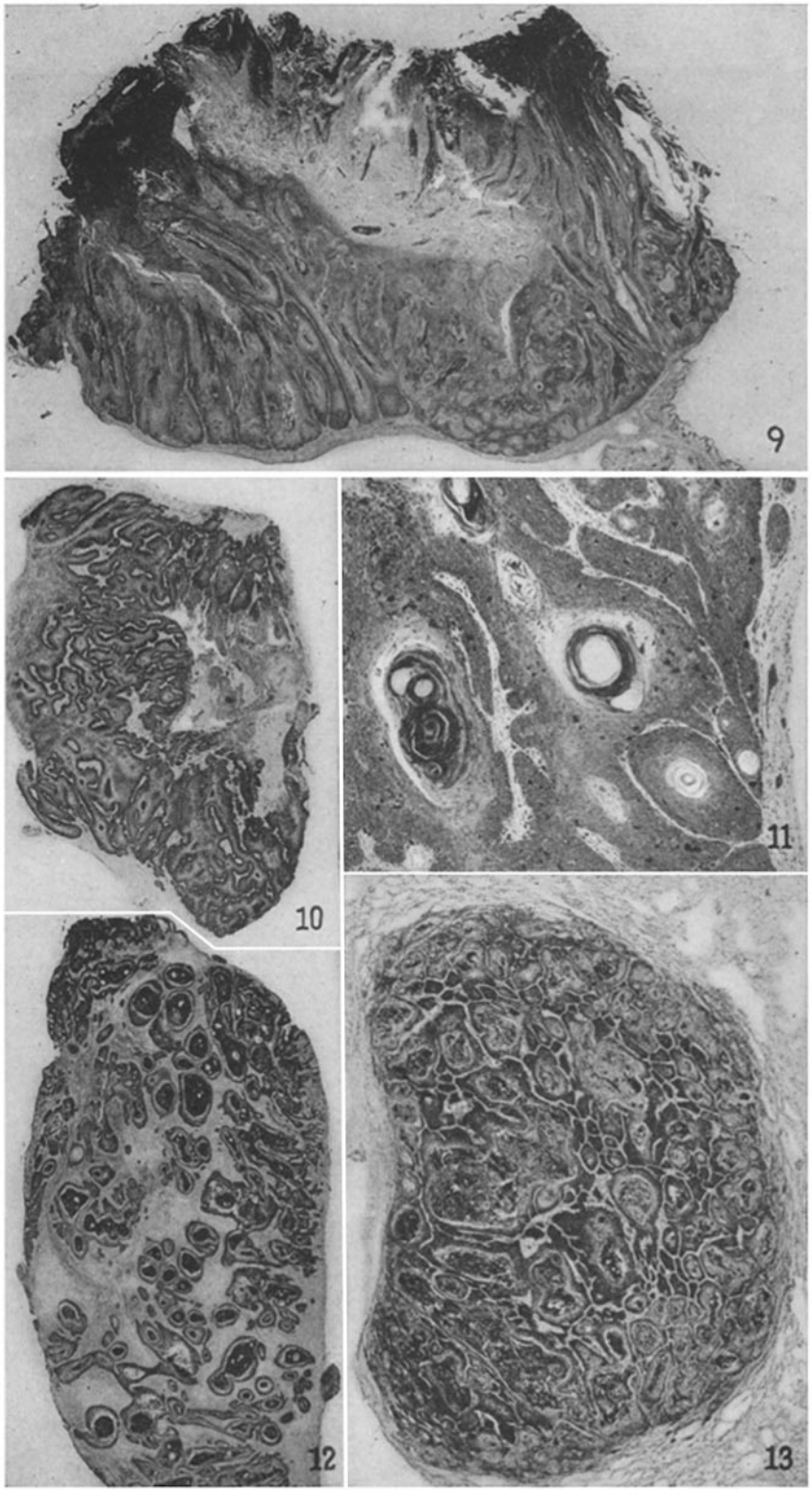
FIG. 9. Tumor C of W. R. 1-55, to show cancer just arising in the midst of a benign papilloma. The papilloma, though somewhat disorderly, is keratinizing as usual, but the cancer, a squamous cell carcinoma, is overlain by necrotic debris. The growth failed to yield virus. × 5¾.

FIG. 10. Convoluted papillomatosis: basal extension from tumor C of W. R. 1-56. The main mass showed all forms of growth from benign papillomatosis to early squamous cell carcinomatosis, whereas the extension consisted entirely of tumor tissue of the sort shown. × 7.

FIG. 11. Melanotic, convoluted papillomatosis: part of tumor A of W. R. 1-55 (see Fig. 21). Numerous melanoblasts and clasmatocytes can be seen, laden with pigment. × 112.

FIG. 12. Part of the large ulcerated tumor shown in Fig. 5 (tumor A of W. R. 1-99). The growth is an orderly squamous cell carcinoma forming keratinized cysts. × 5¾.

FIG. 13. A pulmonary metastasis of the same growth. It too is cystic. There were metastases of similar morphology in the axillary lymph nodes. × 18.



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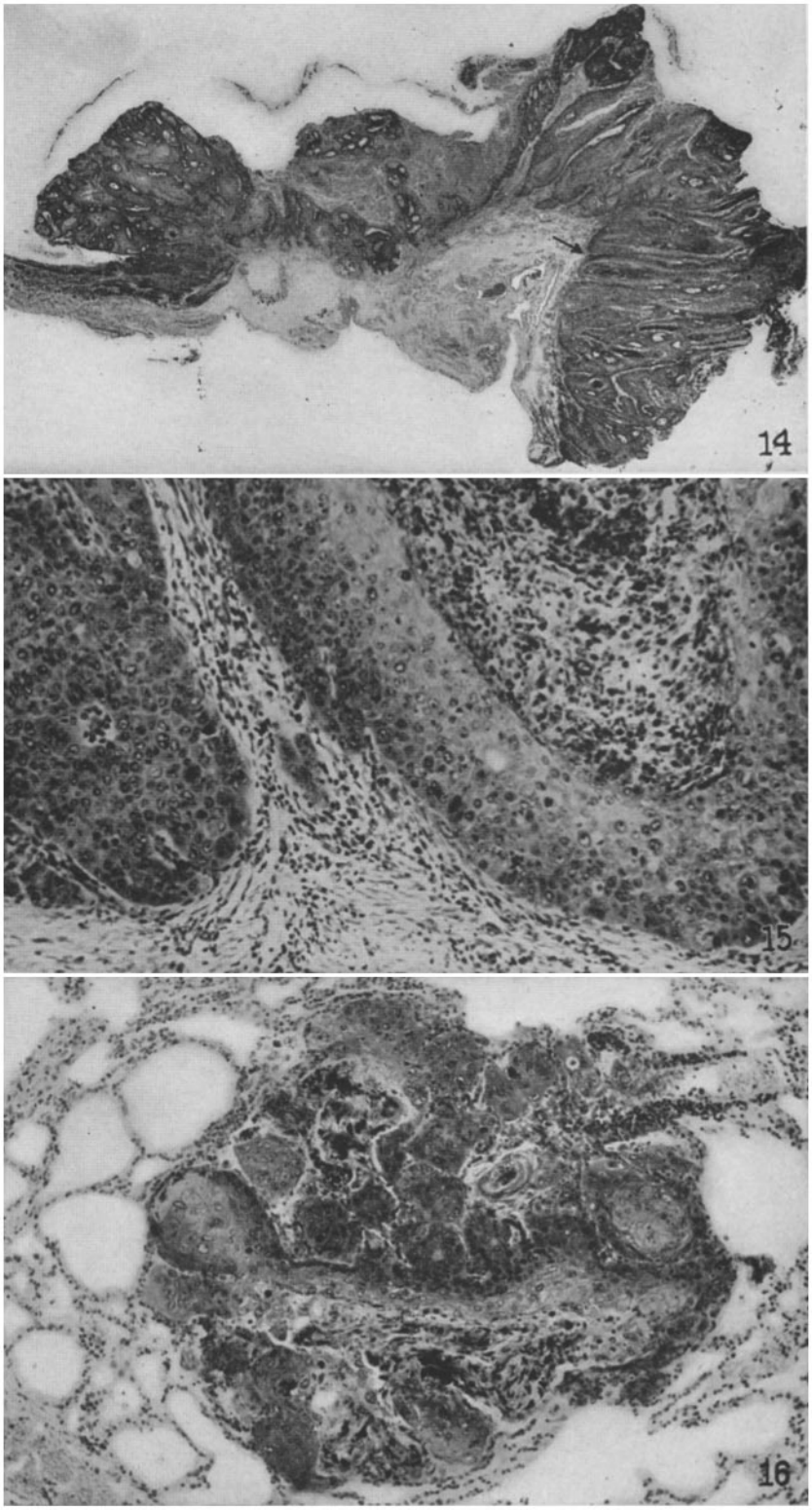
(Kidd and Rous: Cancers from virus papillomas of wild rabbits)

PLATE 25

FIG. 14. Cross-section of tumor B of W. R. 1-99. Its central portion consists of ulcerated, squamous cell carcinomatosis and its periphery of papillomatous tissue, all malignant, as higher magnification showed (Fig. 15). Even where there were orderly papillomatous foldings (arrow) the tumor cells failed to differentiate and the crypts contain necrotic debris instead of keratinized material. Ordinary virus papillomatosis had seemed present in the gross, and a little virus was found in the tumor extract. × 7.

FIG. 15. Base of the crypts indicated with an arrow in Fig. 14, to show the malignant epithelium and the debris resulting from early cell death. There are cancer cells in the connective tissue. × 155.

FIG. 16. A lung metastasis derived from another of the squamous cell carcinomas of W. R. 1-99. The differentiating cells have undergone some of the morphological changes occurring when virus papilloma cells differentiate. No virus could be got from the tumor. × 125.



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(Kidd and Rous: Cancers from virus papillomas of wild rabbits)

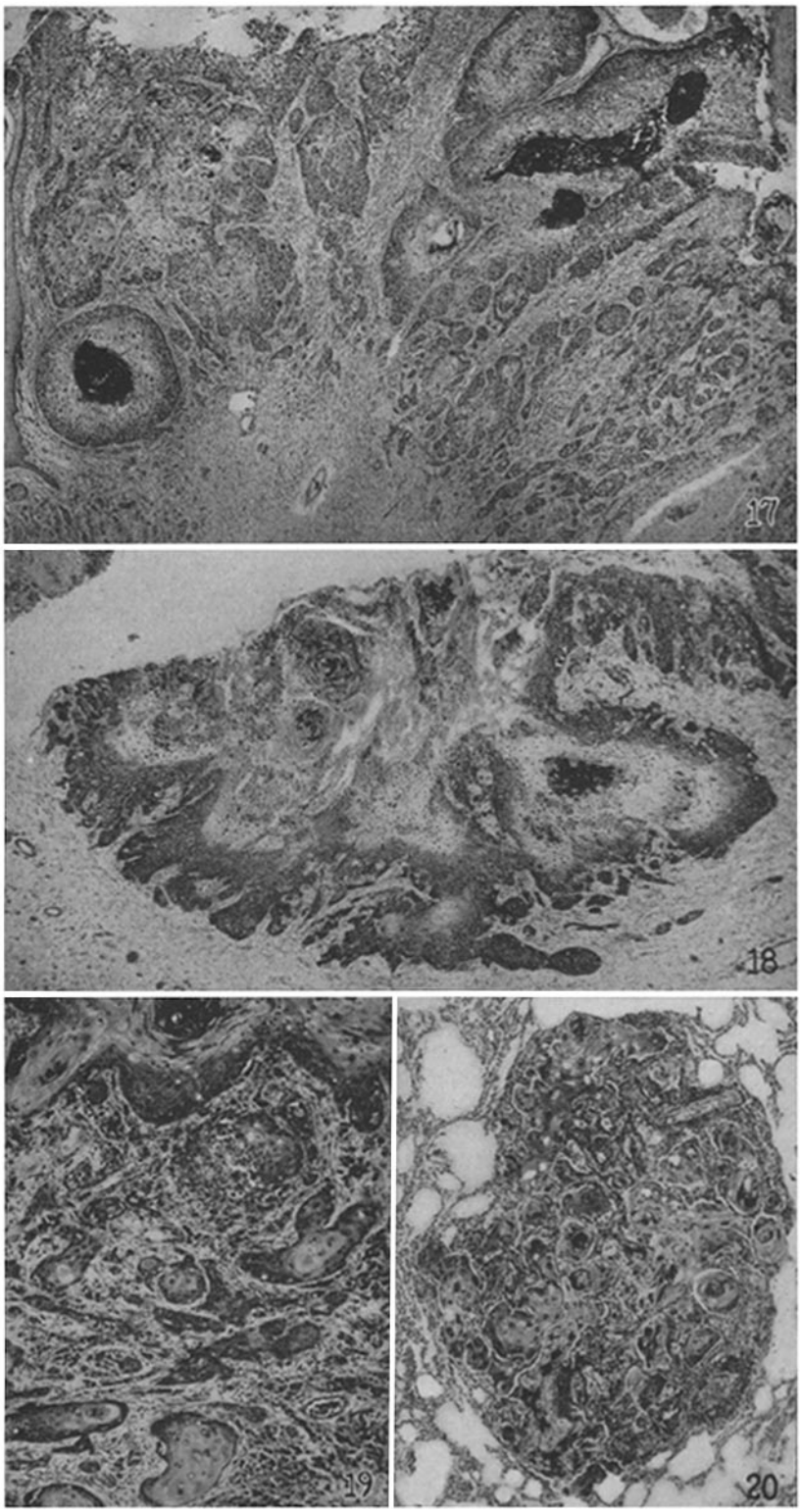
PLATE 26

FIG. 17. Margin of the discoid cancer of W. R. 66, shown in Fig. 6. The growth is a squamous cell carcinoma with traits reminiscent of a virus papilloma. The non-neoplastic epidermis along the vertical side of the disc can be seen at the extreme left. The cancer has extended laterally beneath it. × 33.

FIG. 18. One of the squamous cell carcinomas present in the main tumor mass of W. R. 153. The growth has unusually small cells. × 56.

FIG. 19. Metastasis in a groin lymph node from another of the squamous cell carcinomas existing in the same mass. The cells are much larger and keratinize differently. In the newer portions of the growth, individual tumor cells and small islands of them lie scattered amidst a reactive tissue containing many macrophages. × 61.

FIG. 20. A pulmonary metastasis which shows the same tumor faring badly amidst reactive tissue. × 65.



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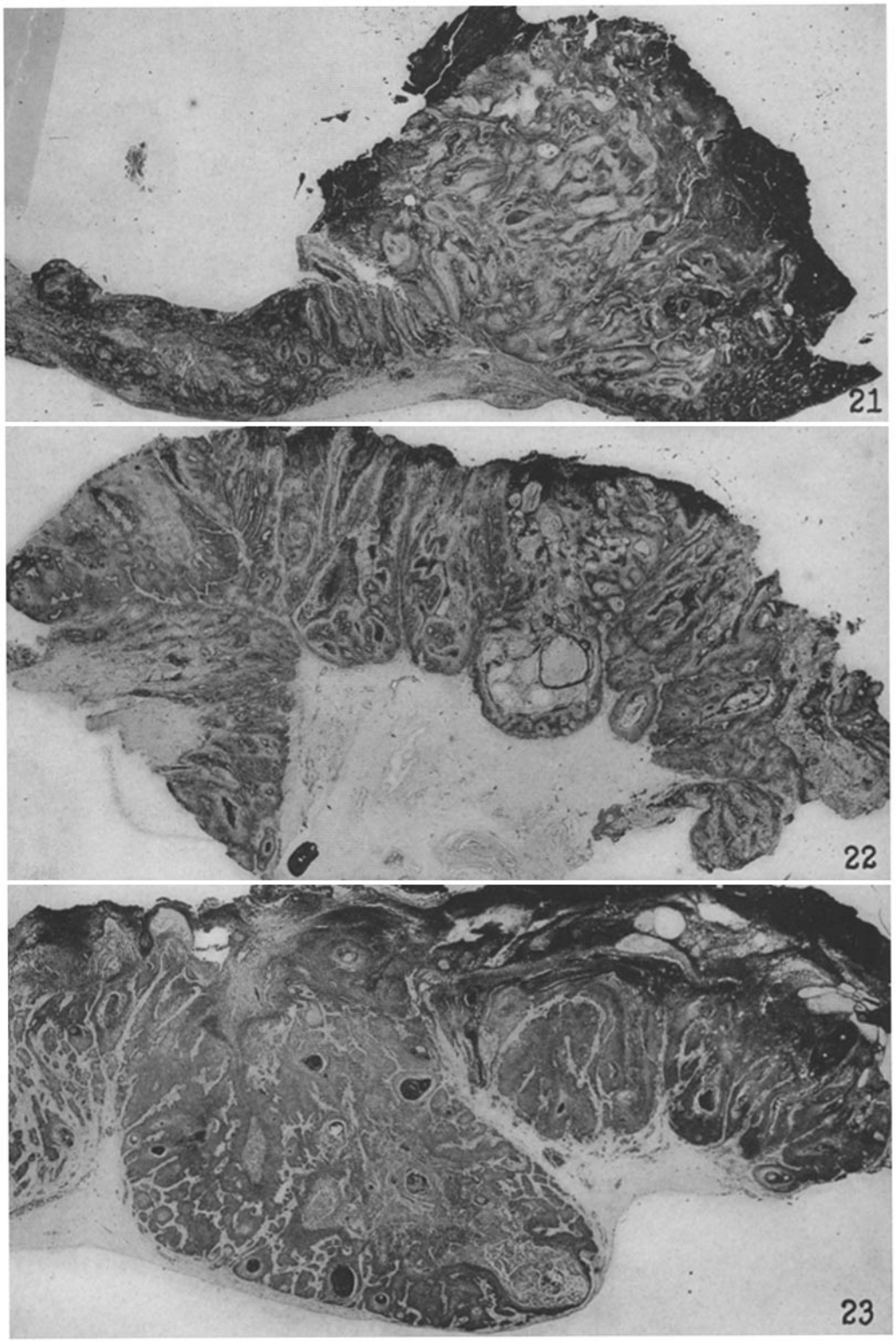
(Kidd and Rous: Cancers from virus papillomas of wild rabbits)

PLATE 27

FIG. 21. Cross-section of tumor A, W. R. 1-55. About half of the growth consists of gray, keratinizing, papilloma tissue, benign but disorderly. The dark area on the right consists of markedly melanotic, convoluted papilloma which is seen to be malignant under high magnification (Fig. 11). On the left the tumor changes to a malignant papilloma, and then to a squamous cell carcinoma which is ulcerated. The dark material along the sides of the mound of benign papilloma tissue consists of necrotic debris derived from the adjacent carcinomas and of dried blood. No virus was demonstrable in an extract of the tumor mass. $\times 6\frac{3}{4}$.

FIG. 22. Cross-section of Tumor A of W. R. 1-56. The growth was patched with gray and showed some vertical striation in the gross, but was covered with a dirty-brown scab (stripped away from the slice here shown prior to fixation). The tumor is seen to be still superficial, save for a cystic downgrowth near its middle, and it appears to consist largely of benign papilloma tissue. This was not the case, however. At higher magnifications the growth proved to be practically everywhere malignant. In the region on the right side of the picture keratinization is taking place, but toward the left the cells are dying before this happens. The cyst contains fluid instead of the keratinized material regularly found in the cysts formed by benign papilloma tissue, and its wall consists of a layer of malignant cells. No virus was got on extracting the mass. $\times 6\frac{3}{4}$.

FIG. 23. Part of the ulcerated fleshy growth of W. R. 1-54. The tumor is everywhere malignant, with regions of squamous cell carcinomatosis but consisting mostly of malignant papilloma tissue, some of which might easily be taken for benign at the magnification given. The dark plugs here and there consist of keratinized epithelium. Elsewhere the cells are dying prior to differentiation. They are much smaller than those of a virus papilloma. The mass is overlain by a scab consisting of cell debris and dried exudate and blood. No virus could be recovered from the tumor. $\times 14$.



Photographed by Joseph B. Haulenbeek and Louis Schmidt

(Kidd and Rous: Cancers from virus papillomas of wild rabbits)

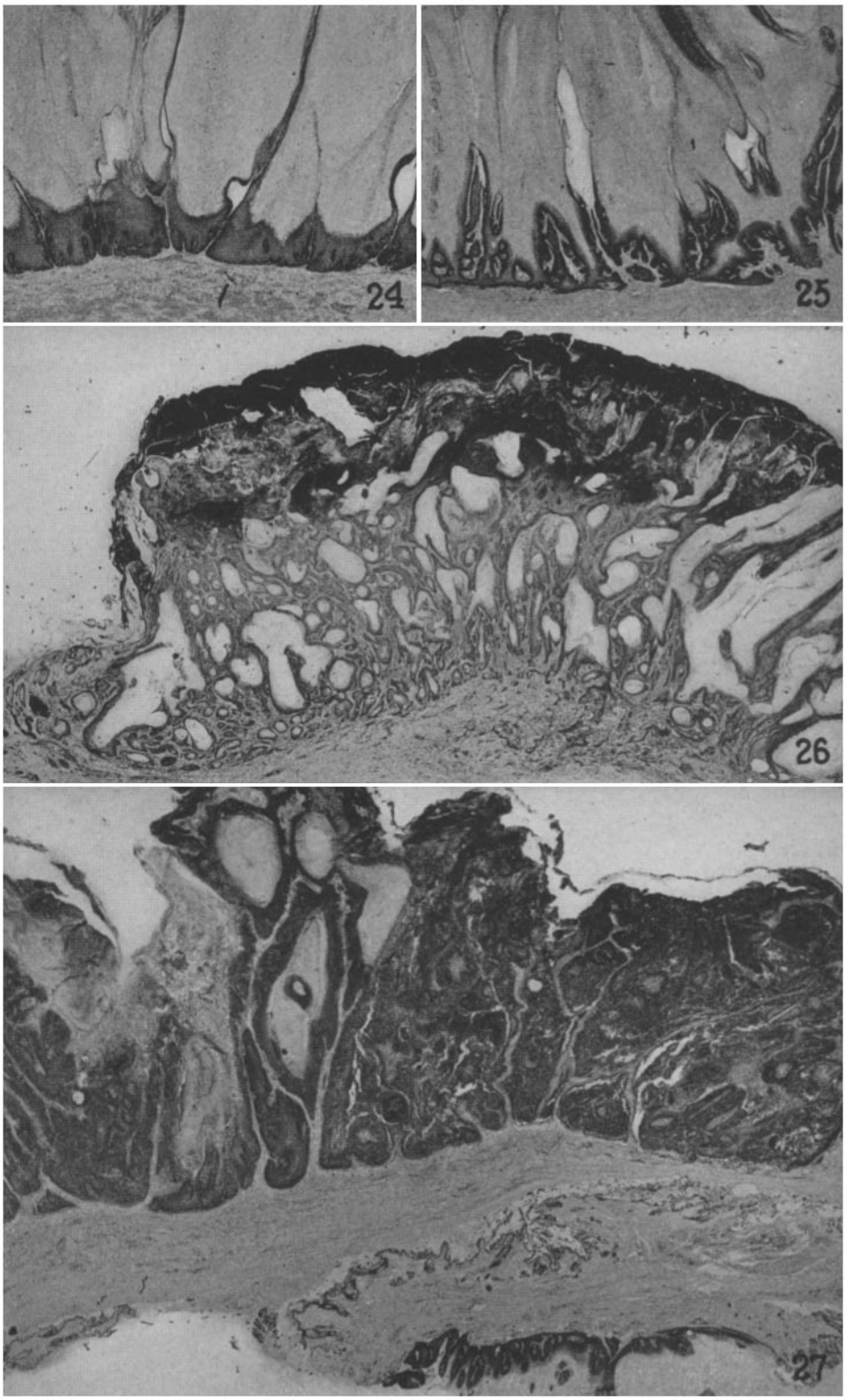
PLATE 28

FIG. 24. Papilloma of jack rabbit 9: biopsy specimen procured 30 days after inoculation of the virus. × 13½.

FIG. 25. The same growth 53 days later, at time of death. Many secondary extensions from the bases of the main papillae give it the aspect of a papilloma of the "second order." It is still benign, however. × 13½.

FIG. 26. Squamous cell carcinomatosis which has completely replaced the virus-induced papillomatosis of jack rabbit 113. The growth is extending out under the skin. × 13½.

FIG. 27. Various forms of carcinoma which have completely replaced a virus-induced papilloma of snowshoe rabbit 10. The slice of tissue was so folded for section that part of a neighboring virus papilloma was included. It and the adjacent skin can be seen, upside down, in the right lower corner. Three distinct forms of cancer are present in the main tumor mass, malignant papilloma near its center (looking like benign at this magnification), with a more anaplastic form of the same on the left and much squamous cell carcinomatosis on the right. All the growths are still superficial. × 13½.



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