THE PROGRESSION TO CARCINOMA OF VIRUS-INDUCED RABBIT PAPILLOMAS (SHOPE)*

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PLATES 19 TO 24

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The cutaneous papillomas induced by a virus procured by Shope (1) from "cottontail" rabbits possess the traits whereby tumors are recognized (2). After they have grown for some weeks they frequently thrust processes into the tissue beneath them, sometimes entering the blood and lymph vessels. If even earlier they are transplanted to favorable situations within the host they may look and behave like epidermoid carcinomas, invading, destroying, and causing death. These observations, and the malignant aspect of sections from one of our rabbits and from one of Shope's, have led us to keep animals with actively proliferating papillomas consequent on virus inoculation, to learn whether they would become carcinomatous. This has happened in 7 domestic rabbits of 10 kept more than 200 days, while in an 8th animal a new tumor has developed of problematic malignancy. The cancers have been multiple in every animal, and metastasis has taken place in 5 of them,—to the regional lymph nodes in 4, to the retroperitoneal nodes in addition in 1, and to the lungs in a 5th instance. One cancer has been successfully transplanted.

Incidence of the Cancers

The cancers have developed from the papillomas of domestic rabbits inoculated with extracts of the glycerinated growths of cottontails. Malignancy has never appeared in the latter, though 10 with induced papillomas have been under observation several months longer than the domestic rabbits. These were chosen for preservation because

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their papillomas enlarged rapidly and progressively. Others equally favorable, perhaps more so, succumbed early from complications (infection, hemorrhage, malnutrition) due to the large masses of proliferating tissue. As time passed the growths of some of the group enlarged more slowly or ceased to do so, and in one instance gradual retrogression took place. Eventually it became plain that the better the papilloma grew the more likely was cancer to occur and the more numerous were the situations of its occurrence. This held true not only of the growths in different rabbits but of those in a single individual. Breed and sex had no evident influence. All were young adults when inoculated.

The First Appearance of Malignancy

The carcinomas appeared in the midst of papillomas more than 4 months old, which in most instances were still broadening and in all were building up tissue.

The new tumors were difficult to recognize early, when within the masses resulting from inoculation of broad areas. With the animal etherized, these masses could be turned for palpation; but often the irregular bosses on their under side, indicative of early carcinoma, could not be discriminated from the keratinized pearls frequent under old papillomas, though these were usually firmer and spherical. The malignant change was readily perceptible, however, in the papillomatous masses 1 to 3 cm. in diameter (Figs. 1, 4), consequent on tattooing virus into small spots. When vigorous, they became more fleshy as months passed, bulging laterally so that their bases appeared constricted. They had a squat, onion shape, or were surmounted by several dry, jagged peaks; and cross-section disclosed the usual vertical striation, with living tissue nearly a centimeter above the skin in many places, instead of only here and there as previously. Their bases, formerly smooth and thin, except for an occasional knob-like pearl, now felt thickened and convex, sometimes almost hemispherical. When sliced vertically the tissue bulged; the sooty pigmentation, often prominent before, had largely disappeared; and the demarcation from the underlying structures, once hair-sharp, was often blurred. The basal convexity was due to active proliferation at the lower ends of the vertical folds of papillomatous tissue, with a fan-like broadening in consequence. Often no further change took place for many weeks; but when the cancerous tendency was pronounced, the gray or cream-colored, vertically striated, dry peaks underwent gradual replacement by a low, rough expanse of flaky, irregularly striated, brownish material, obviously dried exudate in part. The skin at the edge of the growth became raised, ruddy, and tense, owing to its extension beneath the epidermis; and outside its long-established borders one or more spheri-

cal swellings often appeared, which rapidly matured into large, subepidermal pearls (Fig. 16) and later sometimes became projecting papillomas. These were localized extensions into new territory such as had never previously occurred.

Often before the growth reached this state, the animal had begun to gnaw it, and a firm mass could be felt extending downwards and sideways from its base. Biopsy here disclosed a carcinoma. In other cases the superficial tissue came, or was torn, away, leaving a raised, beefy disc (Fig. 18) or an ulcer with indurated walls (Figs. 1 and 6). Less frequently cancer developed under an intact papilloma (Fig. 7). Sometimes one growth out of many, or some special part of a large papillomatous mass, was gnawed for weeks before cancer became recognizable; but generally the gnawing was done afterwards. Ordinary papillomas are not gnawed as a rule unless their situation or size causes trouble. The development of malignancy was evidently attended often by annoying sensations, as in the case of human skin cancers (3).

Sometimes in the gross the cancer appeared to have arisen everywhere from the base of a papilloma resulting from punctate inoculation; but in most cases it was more or less central, appearing where the growth had existed longest, was deepest, and had most reactive tissue underneath it. The cancers appearing in the expanses due to broadcast inoculation, though multiple, implicated but little of the growth at first. The latter had usually become confluent only secondarily; and hence the relation of the cancers to its oldest parts could not be discerned. Embedded in the thin, underlying scar tissue were scattered pearls up to a centimeter across,—containing dry, creamy, lamellated material (keratinized scales) that readily shelled out,—and in addition, under and amidst the growth, discoid, nodular or hemispherical masses of close-textured tissue diversified with smaller pearls, yellow dots, or serpiginous necroses, and devoid of the palisade striation of the papilloma, and of the gray or sooty pigmentation so usual with it. These were the early cancers.

The Malignant Tumors

At this writing more than 50 frankly malignant tumors have appeared in the 8 rabbits. Of these about 30 have been studied histologically,[1] while the nature of the others has been evident from their gross features. In addition numerous minute cancers have been procured by the biopsy of papillomas. Many of the new tumors have been malignant papillomas, the rest either squamous cell carcinomas or malignant papillomas in transition to these latter, or else and frequently, tumors representative of one stage or another of the change. Most of the new growths have enlarged steadily, though sometimes with quiescent periods; and they have caused the death of

[1] D. R. 2-53, killed recently, has provided 20 more.

3 hosts thus far, while 2 more have died of intercurrent ailments. Anemia has necessitated many transfusions; and local purulence has required drainage. In the gross two types of cancer could be discriminated,—fungoid, long localized and relatively benign, with a more or less definitely papillomatous character, and eroding, ulcerative, and metastasizing, squamous cell carcinomas.

The fungoid cancers took the form of projecting mounds or hassocks of ruddy, soft, vascular tissue, devoid of the fissures elsewhere present amongst the dry peaks (Fig. 18). When gnawed they were rapidly repaired. Slicing disclosed yellow streaks and dots and creamy pearls, amidst fine-textured tissue with few or no striae and these irregular and indistinct. The growth had penetrated the scar tissue mixed with fibrous corium, and extended laterally in the subcutaneous tissue as a firm, thick, pale layer variegated with the dots and pearls mentioned above. Sometimes it had a conglomerated aspect, like pudding stone (Fig. 22).

Instead of heaping up on the skin surface, the epithelium burrowed as irregular, tube-like processes (Fig. 19) amidst an abundant, reactive tissue in which subacute inflammation was frequent. The growth was notably desmoplastic, unlike the ordinary papilloma; but its epithelium resembled that of the latter more or less closely and tended to differentiate in the same way, with result in cores of keratinized scales. As the tubes grew down they branched or broke up into cell aggregates, some of which rounded out secondarily into pearls like those beneath an ordinary papilloma. Often instead the cells became necrotic, and a central debris accumulated, forming the dots and opaque strands noted in the gross. At the advancing edge of deep growths the invading processes were narrower, and not infrequently isolated cords and nests of cells were present as in a squamous cell carcinoma. The neoplasm penetrated amidst the cutaneous muscle and undermined, encroached upon, supplanted, and fused the neighboring papillomas. Extension tended to be slow, however, and metastasis did not take place.

The eroding type of carcinoma was sometimes recognizable early as a firm downgrowth, rounded, nodular, or shaped like a flange or prong. More often though it first came to attention when the overlying, dry peaks fell off or were torn away, leaving an ulcer with necrotic or granulating surface, which extended later (Figs. 1, 4). Occasionally a narrow fissure lined with grumous material opened amidst the peaks, subsequently forming a deep crater. Ulcer and crater had walls of gristly tissue which soon encroached irregularly on the subcutaneous structures and skin, the latter becoming thickened, raised and nodular, fixed, glistening, and tense before it broke down. Adjacent papillomas were undermined, infiltrated, and destroyed. Section of them laid bare a shallow, raw, superficial layer, soft and pink, granulating or ragged, perhaps hemorrhagic or purulent, overlying a dense plaque of tissue that grated under the knife and was pale, close-textured, and more or less distinctly dotted with yellow. The growths enlarged in the loose, subcutaneous tissue without becoming fixed on the deep body wall. Secondary contraction often led to puckering of the skin, and drew neighboring papillomas to-

gether. Sometimes the cancer destroyed all those about it without occupying much more than half their space. Retraction of an overlying nipple was once observed.

Microscopically the eroding tumors consisted of nests, cords, and strands of invasive and destructive epithelial cells of squamous type (Figs. 2, 3). Anaplasia was sometimes great, and occasionally the condition approached *carcinoma simplex*. The growth induced a profuse formation of new connective tissue. It contained, like the fungoid tumors, some makrophages, plasma cells, a greater or less number of lymphocytes,—scattered or in small accumulations,—and some polymorphonuclear leukocytes, especially when there was bacterial infection. Foci of acute inflammation so caused were frequent, as also edema, small hemorrhages, and thrombosis within the numerous, wide, thin-walled vessels. The epithelium penetrated amidst and into the fibrous bundles of the deep corium, destroying or breaking them up; and it often entered and replaced the substance of muscle fibers. Extension into the lymphatics was common. A surface spread of the neoplastic epithelium soon covered gnawed spots, when these did not become purulent;[2] but infection with pus-producing organisms was the rule. Except for repeated transfusions nearly all of the animals would have died early. Metastasis to the axillary lymph nodes has been frequent (Fig. 4). In one case it attracted attention before the primary growth did (Fig. 7). In another the retroperitoneal glands just above the pelvis were almost wholly replaced by metastases deriving from experimental implants of the growth in the thighs; and the neighboring adventitia of the aorta and vena cava were involved (Fig. 11). A lung metastasis was found in a rabbit free from local secondaries (Figs. 29, 30, 31).

All gradations between the fungoid and ulcerative, papillomatous and squamous cell, types of malignant growth were encountered (Text-fig. A), not infrequently within the same tumor (*carcinoma varia*), early biopsies disclosing tubular downward extensions which branched and broke up into the cords, strands, and cell groups typical of squamous cell carcinoma (Fig. 8).

Abstracts of the Case Histories

W. R. and D. R. mean wild and domestic rabbit respectively; R. and L., right and left side; ax., axilla; P. or pap., papilloma; malig. pap., malignant papilloma; squam. carc., squamous cell carcinoma; metast., metastasis; inoc., inoculation; and V., virus (Tyrode extract of glycerinated pap.). The paps. of multiple inocs. are numbered. All biopsies were done under ether anesthesia. Materials for implantation were cut fine, suspended in Tyrode, and injected into the leg muscles.

Dutch "show" rabbits (D. R. 2-38 and 2-39) with white hair on front half of body and black hair on rear: tattooed on sides at numerous spots 2 mm. across, with V. from W. R. 18, May 3, 1934. The paps. developing in "black" regions

[2] The papillomas offered far more resistance to bacterial infection than the cancers, their epithelium keratinizing despite it.

were sooty, and those in "white" ones non-pigmented save for an occasional, thin streak. Further tattooings carried out 25 days later with V. 5-38 + 6-38 also gave growths. Tracings made at intervals.

D. R. 2-38.—17 pigmented and 4 non-pigmented paps. Bases of 2 pigmented, of first inoc., P. 2 and 5 R., infiltrated on 34th, 41st, and 46th days with Scharlach R in olive oil to stimulate proliferation (4). *132 days*: paps. at all sites. Those treated with Scharlach R not especially big, but all of first inoc. slightly larger than of second; each pap. an oval or circular group 1.1 to 2.6 cm. in diam., of jagged peaks cleft nearly to skin level. *210 days*: further progressive enlargement. *214 days*: dry peaks of P. 5, L. gnawed away, leaving ulcer with raised, nodular edges and plaque-like, firm base (Fig. 1). Sagittal section discloses squam. carc. (Fig. 2). Bits implanted in all upper legs (abscesses resulted). P. 5, R. has united with 4 and 6 R., induration exists beneath, and a fissure discloses grumous material. Similar fissure amidst coalesced P. 1 and 2, R. *237 days*: shotty lymph node removed from L. ax. Contains carcinoma like that of 5, L. (Fig. 3). Implantations in flexor thigh muscles. (These yielded progressively growing tumors.)

249 days: P. 1, L. gnawed; mass exists under and about it, with early skin involvement. Ulceration followed. The new tumor coalesced with P. 5 and the resulting large ulcer (Fig. 4), involved adjacent paps. *271 days*: the two cancers on R. form a common mass 7 x 4.5 cm. with deep central ulcers lined by yellow necrosis. Biopsy shows squam. carc. *277 days*: new nodule in L. ax. Cancers on sides now meeting under belly. *319 days*: growths and ax. nodule enlarging. *342 days*: all paps. on L. destroyed by the extending cancer except one which is ulcerating independently. *372 days*: firm nodule in R. ax. *382 days*: malignant masses are smaller, as also implantation nodules and ax. metast. Ulcerated pap. on R. removed. Sections show ordinary pap., malig. pap., and a distinct squam. carc. *397 days*: all growths dwindling. Biopsies now of implantation growth and ax. metast. show regressive changes (malignant cells cornifying *en masse* and dying).

Comment.—Animal still alive. 3 squam. carc. noted after 214 days, 2 of them in the only paps. injected with Scharlach R. 2 other carcs. appeared later. Metast. in regional lymph nodes, and implantation growths in hind legs. All now seem regressing.

D. R. 2-39.—9 pigmented and 5 non-pigmented paps. *40th and 46th days*: base of P. 4, R. in black region infiltrated with Scharlach R. *132 days*: all paps. except 4, R. are raised, fissured discs, some closely adjoining. P. 4, gnawed nearly flat, and indurated plaque extends from beneath it into skin.

211 days: P. 4, R. now a granulating ulcer with gristly, rolled border (Fig. 6). Biopsy shows malig. pap. changing to squam. carc. (Figs. 6 A and 8). *230 days*: growth has extended under and involved neighboring paps. Biopsy (Fig. 6, B) of its furthest subcutaneous extension; same gradations from malig. pap. to squam. carc. (Fig. 9). Bits implanted in all upper legs posteriorly. *242 days*: rapidly enlarging nodules in legs; biopsy shows invasive squam. carc. (Fig. 10).

Bits implanted in ant. thigh muscles of host (squam. carc. here later), and into 3 other Dutch D. R. (negative results). Leg tumors from first implants now large. Shotty nodule in R. ax. Carc. on side has destroyed all 7 coalesced pigmented paps. save one (P. 7). This is indurated, gnawed; biopsy discloses independent squam. carc. The anterior masses resulting from coalescence of non-pigmented P.'s on R. and L. have been gnawed nearly to the thickened bases.

253 days: all P.'s on L. have thickened, bulging bases. *264 days*: cancerous mass on R. steadily enlarging. *288 days*: greatly emaciated; fleshy nodules in skin next large ulcerated mass on R., with puckering from secondary contraction. *293 days*: scrotum, hind legs, and belly edematous; very weak. *298 days*: moribund; sacrificed. Bits of cancerous mass on R. and of implantation tumors deriving from it, implanted in legs of 9 Dutch rabbits. In one a nodule appeared, 1.2 cm. across after 79 days; squam. carc. on biopsy (Fig. 27).

Post-Mortem Findings.—Large, ulcerated squam. carc. in skin and subcutaneous tissue on R., replacing pigmented P.'s. Non-pigmented P. mass is in precancerous state (Fig. 16), as is mass on L., and this contains a minute squam. carc. (No pigmented P.'s on this side.) Nodular metasts. in several R. ax. glands, and in retroperitoneal glands. Here the squam. carc. is wholly anaplastic (Fig. 11), invading walls of aorta and vena cava. At all implantation sites irregularly globoid squam. carcs. up to 7.5 cm. in diameter, some with purulent infection.

Comment.—Malignancy already advanced in one pap., when noted on 132nd day after V. inoc., and 116th day after growths appeared. Base of the cancerous pap. had been twice infiltrated with Scharlach R long before. Becoming a malig. pap. then a squam. carc., it underwent further anaplasia in the implantation nodules and metastases. At death another large and several small squam. carcs. were present.

June 11, 1934: two Dutch "show" rabbits, D. R. 2-52, -53, and an albino, D. R. 2-50, tattooed on sides with V. 5-39 + 6-48. Paps. appeared within 3 weeks, enlarging progressively: creamy or sooty in white or black regions respectively of Dutch animals, all creamy in albino.

D. R. 2-52.—*175 days*: The 15 sooty and 10 pale paps. are jagged masses 1.5 to 2.5 cm. across, some fused. P.'s 5, 6, and 7 R. recently gnawed, exposing fungoid growths about 1 cm. high, with vertical sides (Fig. 18) and bulging, deep bases. Slice of P. 7 on 176th day shows a tubular neoplasm (malig. pap.), deriving from ordinary pap. (Fig. 19). *185 days*: biopsy of subcutaneous extension under normal skin: same transitions to malig. pap. Bits implanted in hind muscles of all upper legs. *189 days*: all paps. suddenly more fleshy. *198 days*: fungoid growths enlarging: shotty lumps in legs.

210 days: Slices of P.'s 6 and 14, L. show invasive Shope pap. only (Fig. 17). Bits from P. 14 implanted in flexors of thighs. *219 days*: P.'s 5 and 7, L. gnawed. *231 days*: P. 5 L. enlarging, thick based. *242 days*: all paps. enlarging rapidly and coalescing; fungoid growths 6 and 7 R. have common, gristly base. Nodules at implantation sites enlarging. *267 days*: thick, gristly layer has extended from

base of 5 + 6 + 7, R. under nearly all paps. on R., involving them. *273 days*: subcutaneous implantation growth involving skin excised from R. thigh (mixture of ordinary and malig. pap.). *280 days*: P. 5 and 7, L., long separately fungating, now united, with deep base.

During subsequent period to death on 344th day growths of each side completely coalesced, forming masses with fungoid areas. Cancers appeared where bases of P. 6 and 14 respectively had been cut through, one a malig. pap., the other squam. carc. on biopsy. An ulcer with firm, deep base replaced P. 6 L., and a flange and a prong, both gristly, extended down from P. 3 R. and P. 1 L. respectively. Section of the prong showed malig. pap. The irregularly globular, implantation masses became 5 to 7 cm. in diameter, and one had to be drained of pus. The emaciated animal was killed when moribund.

Post-Mortem Findings.—On each side, numerous paps. and cancers, some ulcerated, fused into a mass (Fig. 22). Of 9 tumors examined histologically, 2 were small squam. carcs., 2 cystic growths (Fig. 5), and 5 large malig. paps. Bits of one of these last, implanted in legs, yielded similar growths.

Comment.—Most of the new tumors were malig. paps., of which many were recognizable besides those biopsied. The implantation growths retained this character. No metast.

D. R. 2-53.—The 5 discrete, pale paps. and 16 sooty enlarged slowly until about the 260th day, then becoming stationary, 1 to 2.5 cm. across, not fleshy, bases superficial. *285 days*: blunt forceps accidentally thrust into base of 5 R., with hemorrhage. Paps. have begun to grow again in some instances, notably 5 R. One removed on 295th day was 2 cm. across, fleshy, bulging downwards but still only a Shope pap. Shotty nodule now in L. ax. *306 days*: P. 5 R., recently injured, is now fungoid, encroaching on P. 11. *316 days*: nodule enlarging in L. ax. and primary tumor now perceptible as cone-shaped growth downward from the fleshy, newly coalesced P.'s 3 and 4. *329 days*: prong under P. 3 + 4 removed (malig. pap. becoming squam. carc.), and also L. ax. nodule (Fig. 7)—(squam. carc. with some features of malig. pap.). Bits of former implanted in upper forelegs. *336 days*: fungoid growth has replaced P. 5 R. (Biopsy shows squam. carc. arising directly from Shope pap.)

Comment.—Still living. For months paps. grew slowly, then some of them rapidly, and squam. carc. appeared late in one of these on each side, soon metastasizing to regional nodes. One pap. in which cancer appeared had been injured mechanically.

D. R. 2-50, *Albino.*—The 18 paps. grew slowly, were 2 cm. across by the 220th day, very superficial, all gnawed. They remained stationary for a few later weeks and then dwindled, about half disappearing and the rest only 0.2 to 0.7 cm. across when the rabbit was killed on the 281st day. Sections showed retrogression of the usual orderly sort (4).

On May 16, 1934, V. 5-38 + 6-38 was rubbed into a scarified skin area about 5 by 7 cm. on one side of 3 brown-gray rabbits (D. R. 2-47, -48, and -49), and Cliff-

Peck V. into a like area on the other. Confluent growths appeared very early, within 8 to 12 days.

D. R. 2-49.—The broad pap. masses became several cm. high in early months; later gnawed low, fleshy, and inflamed. They were about 7 by 7 cm., and 1 cm. high, covered with dry, serosanguineous exudate, at death from diarrhea on 212th day. Near middle of mass caused by V. 5-38 + 6-48, elsewhere vertically striated and sharply defined, was a discoid patch 3 cm. across, fungating, veal-like, with ill-demarcated base. Section showed malig. pap. grading downwards into squam. carc. 2 smaller, similar tumors, widely separate, and a 3rd large, deep and plaque-like, in mass due to Cliff-Peck V. All were squam. carcs., in some spots replacing muscle. No metast.

D. R. 2-48.—By 116th day had vigorous growths about 6 by 8 cm., 1½ to 3 cm. high, raw and beefy from gnawing. When covered with a binder they rapidly built up to 5 cm., dried, and becamed so heavy that repeated paring was necessary. *182 days*: growth on L. caused by V. 5-38 + 6-38 incised under ether anesthesia to drain pus pockets; large, underlying abscess also evacuated. *240 days*: scarring has reduced L. growth to 3.5 cm. diameter, but it is still building up actively. *251 days*: a smooth, ruddy mound, like a *glans penis*, has appeared amidst mass on L. Biopsy shows florid pap. of questionable malignancy with plexiform base. This by 275th day had nearly replaced mass; it was still encephaloid, superficial. *384 days*: tumor on L. unchanged in gross (Fig. 28) and microscopically, on new biopsy (Fig. 21). Ordinary pap. on R. somewhat smaller than before. Recent gnawings of latter, with infection and purulence, have not been followed by cancer.

Comment.—Localized purulence and incision of a vigorous pap. mass was soon followed by a new tumor of questionable malignancy. The other pap. mass stopped enlarging and has not turned cancerous during several months, though latterly purulent.

D. R. 2-47.—Paps. appeared more slowly than in others but formed confluent masses secondarily, becoming stationary after about 150 days and soon beginning to retrogress. Masses were still about 8 by 6 cm. and nearly 2 cm. high when rabbit died on 216th day. Sections showed orderly, thin-based pap., still proliferating in some regions.

D. R. 2-35.—April 30, 1934, Chinchilla inoc. on broad area of both sides with V. 5-38 + 6-38. *16 days*: beginning, confluent papillomatosis. A Tyrode extract (0.1 per cent) of rabbit testicle frozen on 4th day of infection with vaccine V., New York Board of Health strain, was injected in L. expanse, 0.1 cc. at two widely separate points, and also into normal skin nearby: excellent "takes" with local necrosis and healing. The regions of vaccinial change were charted, and on 23rd and 24th days 4 slices removed from them. The pap. masses gradually became jagged mats, about 9 by 6.5 cm. and 2 cm. high on 212th day.

228 days: rounded, smooth bosses up to 1.5 cm. diameter felt under the masses, —pearls. 5 days later L. growth gnawed over area 3 cm. across, disclosing

raised, beefy tissue, devoid of clefts. Biopsies showed squam. carc. here, and elsewhere ordinary papilloma and a pearl, respectively, with beginning invasive extension from this last (Figs. 25, 26). Prompt healing, with replacement by same tissue as before. Animal kept beefy area raw; and after several weeks it gnawed region of previous vaccinial necrosis on R. for first time. Here on 244th day it exposed a fleshy disc. 1.8 cm. across, demarcated by a fissure (Fig. 29). Biopsy showed squam. carc. *286 days*: died during operation.

Post-Mortem Findings.—In R. mass 2 large squam. carcs. and a cystic tumor (Fig. 14). In L. mass 2 squam. carcs. and a malig. pap. (Fig. 20). Numerous early malignancies (Fig. 15) elsewhere in pap. masses, and many pearls beneath. One lung metastasis with morphology of largest squam. carc. on R. (Figs. 30 and 31).

Comment.—One of the large, early cancers appeared at the site of a healed vaccinial lesion in the pap. expanse.

D. R. 2-05.—Brown-gray, inoc. with V. 5-38 + 6-48 in broad area on each side May 4, 1934. Confluent, jagged masses about 12 by 8 cm. developed, later changing to solid, cutaneous horns about 6 cm. across and 5 cm. high, building up actively. On the 190th day L. horn was gnawed, and palpation disclosed a small, gristly mass under base. This enlarged, horn was gnawed away entirely, and at death on 348th day a great ulcerated mass like a truncated cone was present, 10 cm. across, 3 cm. deep, with its base in the subcutaneous tissue and a few small pap. peaks along its border. The bulk of the mass proved to be malig. pap. tissue, with cysts, like those of D. R. 2-52 (Fig. 22), and transitions to anaplastic, squamous cell carcinoma. The superficial paps. were of the "second order" (4). The axillary nodules were metasts. of squam. carc., containing cysts (Fig. 24) reminiscent of the papilloma.

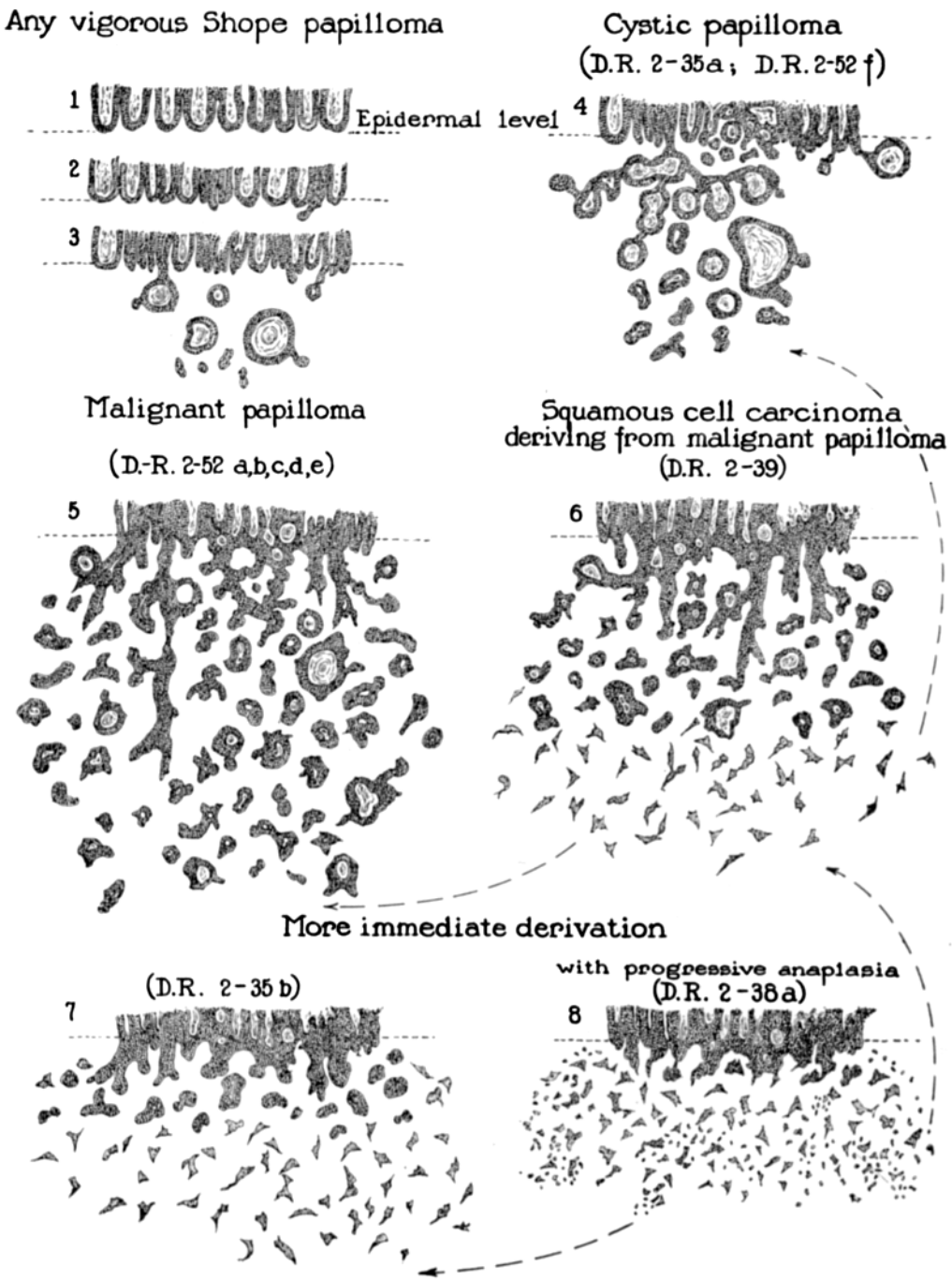
The horn on the R., long unchanged in size, had continued to proliferate, and latterly 2 subepidermal pearls had extended from its base. It was a pap. of the second order, with beginning squamous cell carcinomatosis and malignant papillomatosis at separate locations in it.

Comment.—The cancer was solitary for many weeks, but at time of death others were appearing in the mass on the opposite side.

Two instances from our previous papers (4, 5, 7) should be added, of what now appears to have been indubitable carcinosis (D. R. 1-2; Dr. Shope's rabbit).

The Progression to Malignancy

The early stages of the cancerous change cannot be comprehensively described without inclusion of the entire course of events in vigorous papillomas. These tend toward malignancy from the beginning, and attain it by a continuous series of alterations (Text-fig. A).



TEXT-FIG. A. The progression to carcinoma: schematic drawing. The numbers in brackets refer to rabbits in which tumors illustrative of one state or another of the progression were encountered; and the added letters designate the individual tumors of each animal. Many growths underwent further morphological changes after they had become malignant, a fact indicated by the arrows.

The developing papillomatosis has already been described by Hurst and by ourselves (6, 7). The epithelium grows down but soon meets the fibrous corium and a folding outwards ensues. For weeks the growth is very superficial, with an almost linear basal demarcation: the hair follicles and sebaceous glands are merely overlaid, not destroyed. Gradually it becomes bedded somewhat more deeply, and the skin appendages disappear. As early as the 2nd month blunt processes, composed of ill-ordered epithelial cells, may be pushing down between the collagen bundles (Fig. 12; Text-fig. A, 2). Their advance is slow, and the cells tend to differentiate and keratinize like those of the surface growth, with result in pearls, either separate from the surface mass or connecting by a bottle neck. They consist of concentrically arranged, exfoliated scales deriving from a layer of living epithelium that has papillomatous features (Fig. 13). Nearly all are cream-colored, even when the surface papilloma is sooty black, a difference not referable to their situation,—since implantation nodules are often pigmented,—but to the activity of the burrowing cells. (The eventual cancers were always wholly unpigmented though deriving usually from gray or black papillomas.)

As time passes scar tissue of greater or less density and thickness, depending upon intercurrent influences (trauma, bacterial infection), forms beneath the papilloma; and the fibrous corium is incorporated therein. Localized edema and focal inflammation are now frequent; and, in proportion as they occur the papilloma becomes disorderly, with thick and thin basal foldings, unevenly ranked (Fig. 13, Text-fig. A, 3). More pearls form, sometimes a centimeter down, and they may be irregular in shape, with broad epithelial processes pushing out from them. In such processes, as at some situations in the parent growth, the cells may have largely lost polarity, and they and their nuclei may be distinctly larger than before, their shape more variable, and pathological mitoses not infrequent. The picture now suggests beginning carcinomatosis. The surface mass often seems to have ceased its extension; yet it still builds up actively, material lost from its dry summits being soon replaced; and successive tracings show that it is slowly enlarging. Growths which interfere with posture and movement are gnawed and become infected and fungoid,—great beefy masses, several centimeters high, which bleed easily. If protected with a binder these dry and assume the usual aspect.

The subsequent events vary considerably. We have made numerous biopsies to study them. Sometimes the disorder of the papilloma everywhere increases, its basal foldings become crowded and still more irregular, and certain of them, extending down, anastomose and branch secondarily with result in a plexiform network two or three millimeters deep. The basal limits of the growth cannot then be made out in the gross. Occasionally the invasive processes branch almost regularly with result in a "papilloma of the second order" (4). Frank malignancy is attested by a further extension with tissue destruction. In one animal (D. R. 2-48) the new tumor, though replacing the ordinary papillomatous mass, and very different from it in the gross (Fig. 28), has remained superficial for more than 5 months and of dubious malignancy. Histologically (Fig. 21) it exemplifies the precancerous disorder.

In other cases an especially vigorous proliferation takes place at certain spots in

the papillomatous layer, with result in one or more discrete, non-pigmented "onions" in the midst of a jagged, sooty expanse. Small epithelial pearls develop in and under the "onions," and downgrowth takes place secondarily from the wall of one or several of these (Fig. 15), perhaps with later dispersion of the epithelium into the nests and groups characteristic of squamous cell carcinoma. Closely akin are cases in which subepidermal pearls form outside the long established border of a papilloma (Fig. 16), sometimes turning carcinomatous secondarily, the epithelial cells becoming large and disorderly, with big nuclei, pathological mitoses, and frequent giant or multinucleate elements. The alteration may be local or occur everywhere around the wall of the pearl, and it is followed by disorderly aggression.

In still other instances the cellular changes mentioned take place in the basal foldings of a papilloma that has not as yet encroached on the neighboring tissues, though growing vigorously, and the result is an easily recognizable cancerous focus, still within the confines of the papilloma, and grading into the latter morphologically.

When cancer has occurred it may long remain quiescent and minute, or it may rapidly replace the papilloma. Biopsy at this time of a discrete, bulging, onion-shaped growth may disclose dry, overlying papilloma and a living, basal layer composed of carcinoma, giving the false impression that the malignant change has been a general one. An earlier section would have shown its origin to be local, though often at several spots.

Fortunately for the study of the cancerous changes these find expression not only in a graded progression from ordinary papillomatosis, but in tumors representative of one stage or another of the entire process (Text-fig. A). One such instance has already been described (Figs. 21 and 28). Fig. 14 shows a vertical section through an irregular nodule found beneath a papillomatous mass: the papilloma has penetrated the scar tissue at one point and ramified, forming numerous cysts. These cysts are like the pearls found beneath papillomatous masses at an early period; but in the present instance serial sections have shown that narrow tongues of aggressive epithelium extend from them deep amidst the voluntary muscle fibres. In Figs. 5 and 22 a similar, cystic growth has extended far out under the normal skin. The way in which such growths begin is evident from one in which the downward penetration had just started at the time when a biopsy was done. A papilloma 210 days old (D. R. 2-52) had become notably fleshy, bulging downwards. Biopsy showed that at the center of the base, where pressure was presumably greatest, the growth, though still orderly, had advanced into the dense scar tissue (Fig. 17). It is questionable whether these cystic growths (Text-fig. A, 4) can be deemed malignant.[3]

Decision is easy with the tumors representing the next stage in the morphologi-

[3] In D. R. 2-53, killed since this paper was concluded, a metastasis from such a growth, with the same cystic morphology, has been found in an axillary lymph node.

cal progression. Fig. 20 shows in cross-section a persistently gnawed spot in a broad, papillomatous mass. Here a papillomatous downgrowth exists, ramifying and forming cysts filled with the debris of untimely cell necrosis. The growth has undermined and raised the neighboring, pigmented papilloma. Its initial relation to the latter can no longer be discerned.

This is malignant papillomatosis (Text-fig. A, 5). Another example has been figured with the fungoid carcinomas (Figs. 18 and 19). Here epithelial tubes are burrowing amidst new-formed connective tissue. Some of the epithelium is of the ordinary papillomatous sort, differentiating often into pearls; but much is notably anaplastic, and many of the pearls it forms contain amorphous debris. The growth eventually extended as a thick layer under some of the other papillomas on the rabbit's side, coalescing with similar growths that had arisen in these, and eventually forming one mass (Fig. 22). Bits cut from its base at an early period and implanted in the legs gave rise to large tumors, in which all gradations from benign to malignant papillomatosis were present, precisely as in the parent growth.

Here the state of malignant papillomatosis was not exceeded, despite the shuffling of opportunities effected by operation and transplantation. More often further alterations took place to squamous cell carcinoma (Text-fig. A, 6). Fig. 8 is from the fleshy border of the earliest cancer to appear in D. R. 2-39 (Fig. 6): here to all appearance a malignant papilloma is changing to squamous cell carcinoma as it grows down. But another explanation suggests itself, namely that the apparent transformation is due merely to stimulation by the cancer of the overlying papillomatous tissue. This alternative was ruled out by biopsy of the tip of an extension of the tumor far out beneath normal skin. Again the transition forms were present (Fig. 9).

Not only may benign papilloma change to malignant papilloma and this in turn to squamous cell carcinoma, but a further progression may take place to a state of ultimate malignancy, so to speak, in which every cell acts for itself (Text-fig. A, 8):—

Some of the material seen in Fig. 9 (second biopsy, D. R. 2-39), containing transitions from papilloma to carcinoma, was cut fine and injected into the leg muscles of the host. One of the resulting nodules was removed on the 12th day after implantation. It consisted of squamous cell carcinoma (Fig. 10), though with some indications (in abortive cyst formation) of its derivation from papilloma. The growth had evidently gained in malignancy. When the rabbit died metastases were found in the axillary and retroperitoneal lymph nodes, in the latter presumably as disseminations from the large implantation tumors in the hind legs, since the inguinal glands draining the skin cancers were uninvolved. The malignant epithelial cells showed little differentiation, had almost wholly replaced the retroperitoneal glands, and were individually invading the adventitia of the nearby vena cava, and aorta (Fig. 11).

In this instance the entire gamut of changes from ordinary Shope papilloma to malignant papilloma, and thence in turn to the most dishevelled and aggressive

of squamous cell carcinomas, was traversed at long last. Sometimes, on the other hand, the change to a squamous cell growth is soon over, and occupies but a few millimeters of tissue (Text-fig. A, 8). But even in such instances it comes about by graded alterations (Fig. 23).

The Influences Precipitating Carcinosis

Some virus strains induce papillomas which sooner or later retrogress, whereas others yield growths that almost always go steadily on. Since cancer develops only when this happens, one must conclude that the virus strain can be of decisive importance, though no differences in carcinogenic effect have been perceptible in our experience with those strains which induced vigorous papillomas.

Among host influences the animal species is paramount, and individuality is frequently decisive. The general and local characters of the skin are of notable importance.

The virus affects rabbits only. Cottontails are its natural hosts. Their papillomas grow more slowly than those of domestic animals, are remarkably superficial, and after some months cease to enlarge though continuing to proliferate. A shallow layer of scar tissue now underlies them and this sometimes contracts, bunching the dry peaks. Retrogression is frequent. A group of cottontails with papillomas induced by virus strains carcinogenic for domestic rabbits have now been under our observation for more than a year. In none has cancer developed; nor has it come to Shope's attention in his numerous rabbits with naturally occurring papillomas.[4]

The papillomas induced at one inoculation in domestic rabbits of a single breed may differ much in their course, some going on to cancer and others becoming stationary or retrogressing. Save in exceptional instances (D. R. 2-53) the multiple papillomas of any one rabbit all behave in the same way; and one must conclude that they are influenced by some general condition, and that this can alter (D. R. 2-53).

Those rabbits which have skins most responsive to Scharlach R are the ones in which the papilloma grows best (4), providing the greatest chance of carcinosis. The malignant change is prone to occur in pigmented papillomas. In 4 Dutch rabbits 57 discrete, sooty papillomas were induced in skin carrying black hair and 24 pale ones where it was white. Of the former growths 13 became cancerous,—but 3 had been repeatedly stimulated by the injection of Scharlach R and hence must be excluded from consideration. Cancer developed in only 2 of the papillomas of white regions; and no certainty exists that in these it did not arise from melanotic tissue, of which occasional fine streaks were present.

[4] Personal communication.

When the general disposition of the host (its species), its individual disposition, and its soil (the cells acted upon by the virus) were all favorable to papillomatosis and hence to cancer, this still occurred at some spots and not at others. One reason was occasionally evident in the differing behavior of the papillomas of the same animal (as *e.g.* D. R. 2-53, Fig. 7). Those which grew best, becoming fleshy, underwent malignant change. Here again, presumably, the factor of soil came into play, the virus at some spots affecting cells that were especially favorable to it, and hence to malignancy.

Vigorous papillomas eventually arrive at a condition in which carcinomatous changes may ensue at numerous situations within them. Yet the changes are always local at first, though the localities be many. Often the history suggests that intercurrent factors have had much to do with where and when malignancy developed.

Scharlach R was injected into 3 pigmented papillomas of the 26 induced in D. R. 2-38 and 2-39. The dye has a marked stimulating effect on such growths (4) but this is transitory; and at the time when frank malignancy developed, the injected masses were no larger than some of the others. Cancer appeared in all 3 however, whereas in only 3 of the other 23 growths; and one of the cancers arising in an injected papilloma was by far the earliest to appear.

The base of P. 5, R. of D. R. 2-53 was accidentally pierced on the 285th day. Cancer appeared 21 days later; but it was noted in another of the 15 pigmented growths at about the same time.

Vertical slices were taken on the 210th day through 2 of the pigmented papillomas of D. R. 2-52. They disclosed aggressive papilloma only (Fig. 17). The gaps soon filled up with tissue of this sort and discrete cancers developed there later.

Vaccine virus was injected early at two points in a broad papillomatous area of D. R. 2-35, causing necroses about 1½ cm. in diameter, which were duly charted. Replacement with papillomatous tissue was prompt. Months afterwards the animal tore away the papillomatous peaks at one of the charted spots, disclosing a squamous cell carcinoma.

In and under a large area of active papillomatosis on D. R. 2-48, pus pockets developed, necessitating drainage on the 182nd day. About 2 months later, a fungating tumor appeared in the mass and within a few weeks had replaced it wholly. A similar papillomatous area on the other side of the animal did not become purulent then or malignant later.

From a papillomatous area of D. R. 2-35, in which one cancer had appeared, as did several later, a large pearl was removed by exploratory operation. Everywhere save at one spot its epithelial lining appeared benign (Fig. 25); but here it was thickened and disordered, and had invaded the neighboring tissue (Fig. 26) which here and here only was inflamed and edematous, with scattered pus cells and diplococci. Elsewhere the epithelium had keratinized in concentric layers,

but here it had for some time been dying early, as the markings of the necrotic material attest.

Some of the growths of D. R. 1-22 were subjected to experiments that rendered them invasive (Scharlach R injections, layering with collodion, transplantation to the muscles, subcutaneous tissue, and viscera) (4). At death, after less than 3 months in all, many of them appeared malignant, and a large nodule with the morphology of a squamous cell carcinoma was present in a regional gland.

These examples indicate that local interferences can precipitate cancer in papillomas disposed to the change; yet such influences are far from doing so always, and cannot be deemed essential. Though locally favoring conditions bring on cancer in susceptible hosts at some spots prior to others, its rapidly increasing multiplicity as time passes shows that it would eventually occur anyhow. The effect of local factors approaches the crucial only in papillomas of slow growth, which might not become cancerous in the absence of promoting influences (*e.g.* D. R. 2-48). When the cancerous change takes place under such circumstances the growth rate may quicken so abruptly as to suggest that the malignant activity has been touched off in some way, as indeed it has.

All of the various interferences bringing on manifest cancer give rise to disturbances of the connective tissue underlying the papilloma. This association is no fortuitous one. Papillomas which remain well-ordered and to all appearances benign for long periods have always a thin connective tissue base. Those on the other hand which burrow (Fig. 13) and give other signs of beginning malignancy have inflamed, proliferating, and edematous bases. That the condition of the supporting connective tissue greatly influences the behavior of the papilloma is further indicated by experiments (7) in which implants infected with bacteria causing reactive connective tissue disturbance grew like epidermoid carcinomas, whereas in the absence of such disturbance the papillomatous aspect was retained.

It is a truism with clinicians that bacterial infection of a tumor is often followed by malignancy or enhances it; and the influence of connective tissue disturbances to further invasion has been generally recognized. The rôle of these factors must not be overstressed in the present relation, however. The diversity of the influences which precipitated malignant activity in the papilloma clearly indicates

their non-specific character. Sometimes cancerous downgrowth took place into dense scar tissue at spots where no locally favoring condition could be discerned. The trend of the papilloma toward malignancy evidently brings it at length to a state in which cancer is inevitable. Whether local influences are primarily responsible for this trend is another matter.

DISCUSSION

The progression to malignancy of papillomas is a common pathological event. The skin papillomas induced with tar and other agents in man, the rabbit, and the mouse frequently become carcinomatous, as do also papillomas of the human mouth and tongue. Laryngeal papillomas are supposed to undergo cancerous changes often, but proven instances are rare (8). The alterations leading to carcinoma in the tumors mentioned are the same, generally speaking, as in the Shope papilloma, and trauma, infection, chronic inflammation, operation, and other intercurrent influences frequently precipitate malignant activities, as in the case of this growth.

Papillomas of the human bladder, though deriving from epithelium of transitional type, provide many parallels with the Shope tumor. They trend toward carcinoma with such constancy that some authorities, notably Zuckerkandl (9), hold them all to be potentially malignant. Incision and local inflammation often bring on carcinosis. While still histologically benign the papillomas, like the rabbit growth, are readily transplantable in the host, as operative dissemination to the wall of the bladder only too often attests. Not a few cases are on record in which transperitoneal removal of an apparently benign papilloma has been followed by the appearance of implantation nodules in the healed laparotomy wound, without bladder recurrence (10). Such secondary tumors may appear benign, though they are more often malignant.

The rabbit cancers always arose from the papillomatous epithelium, not from such skin appendages and epidermal cells unaffected by the virus as underwent inclusion in the proliferating mass. This was plain not only from the way they originated (Text-fig. A) but from their morphological characters. There were no signs of a diversity of origin such as tar tumors of the skin exhibit, no growths referable to the hair follicles or sebaceous glands, and indeed no basal cell epitheliomas. The malignancy was consequent on changes in but one

kind of cell, namely that stimulated by the virus,—which is effective upon epidermal cells only (7); and the new tumors, though apparently various, were the manifestations of progress in a single direction, namely from papillomatosis to squamous cell carcinosis. From the experimenter's point of view the Shope virus is more than a notably effective carcinogenic agent.[5] By affecting cells of a single sort in the way that leads to malignancy it gives rise to what may be termed pure strain cancers. These should provide a controlled material for studies of the influence of accessory factors on the origin and manifestations of carcinosis.

The cancers developing from the rabbit papillomas were all acanthomas, tumors such as follow upon a great variety of skin irritations, most of them automatically excluded by their nature from functioning as the immediate cause for the malignancy. Tar, weak hydrochloric acid, Roentgen rays, and the bacilli of lupus can none of them be considered directly responsible for cancer, though all produce lesions in which it may develop. The Shope virus might be dismissed as acting merely in this way, did it not give rise to growths which themselves have the traits of tumors, which possess some malignant potentialities at an early period, and become carcinomatous by continuous alterations of form and behavior.

Shope could not at first recover virus from the papillomas engendered in domestic rabbits; but latterly he has done so, transmitting the disease by its means in 10 successive groups of animals (12). The rabbits of the present work were inoculated with strains known to be irrecoverable, but it has been possible by indirect means to demonstrate the presence of virus in the papillomas. A principle neutralizing it appears in the blood of rabbits carrying the growths (1). With Dr. J. G. Kidd as collaborator, we have titrated this principle,—wholly lacking in the normal animal,—and have found that its time of appearance and rate of increase vary directly as do those of the papillomatous mass. The amount of antigen, of virus that is to say, evidently becomes greater as the proliferating mass enlarges. The con-

[5] In D. R. 2-53, killed recently, after 370 days, a sagittal section was taken of each of the 20 discrete, papillomatous growths. All proved to be still Shope papillomas in greater or less part, 5 of them wholly such. Deriving from the other 15 were 20 distinct carcinomas.

clusion seems justified that the virus accompanies and is responsible for the characteristic epithelial proliferation so long as the growth remains a Shope papilloma. Attenuation experiments have shown that its state finds a direct reflection in terms of papilloma behavior; and the better the growth, the more likely is cancer to occur. The problem of causation narrows to the period when cancer begins. But when does cancer begin? Upon this point the morphology and behavior of the changing tumors yield no decisive information. In all save the most anaplastic of the cancers the influence of the Shope virus finds some expression still, in papillomatous features and cyst formation (Text-fig. A).[6] Often the alterations which lead to carcinosis do not stop when malignancy has been achieved, but go further until a state of great anaplasia has been attained. The postcancerous changes appear to be no separate course of events but only a continuation of what was long since begun.

These facts might be taken to indicate that the virus is the immediate cause for the carcinosis; yet they are compatible with the assumption that it merely provides an essential, preliminary, cell disturbance. The failure of cancer to appear when the papilloma is no longer progressing can be likened to the failure of tar cancer to develop when tarring is not kept up. The proximal cause for the carcinosis may conceivably be present, or effective, only in papillomas that are doing well. The special liability to cancer of those rabbits which are most favorable to the papilloma can be matched by the individual differences exhibited by tarred rabbits or mice as concerns papillomatosis, and the ensuing carcinosis. In them as in our rabbits cancer develops with special frequency from pigmented skin. The difficulties of telling precisely when cancer has supervened upon Shope papillomatosis are great, because it derives from a growth of neoplastic morphology; but they are considerable with irritation acanthomas in general. All authorities upon the "precanceroses" stress them.

The potential malignancy of the papillomas at an early period, as demonstrated experimentally, has not necessarily a large significance, for normal epithelium can be stimulated temporarily to malignant

[6] This is not surprising, since extraneous viruses flourish after their introduction into transplantable tumors (11), and some of them induce characteristic morphological changes (inclusion bodies).

behavior with Scharlach R or Sudan III. In the case of our young papillomas acting malignantly (7) there had been accessory stimulation. None of the growths stimulated to invasion and destruction at an early period, has given rise to secondaries, save perhaps D. R. 1-22 (*q.v.*).

The Shope papilloma frequently retrogresses after doing well for a time, whereas the cancers deriving from it and recognizable in the gross progress in most instances, though whether in all is questionable (D. R. 2-38 *q.v.*).[7] During the months before cancer appears a natural selection of individuals favorable to the papillomatosis, and in consequence to malignancy, is taking place. The longer the papillomatous growth endures the less is the incidence of retrogression; and one would expect it to be smaller still in the late period when cancer is present. In all of our animals developing cancers the uninvolved papillomas have continued to proliferate.

Whatever the immediate cause for the cancers, they are beyond question due primarily to a virus, and they develop from the papillomatous growth to which this gives rise. Are there other instances of the sort? We have been able to find none in the literature on the domestic animals, though virus-induced papillomas are frequent in dogs and cattle. Human pathology, however, provides a parallel that is remarkable in many ways.

Condyloma acuminatum is due to a filter-passing virus (13), but the occurrence of the growth under natural conditions depends both on individual susceptibility and on local irritations such as are produced by pathological secretions. When these last are done away with the growths ordinarily disappear. They are papillomas, branching as the rabbit papilloma does not, with thick-layered epithelium of squamous type, sharply demarcated from the connective tissue, and manifesting none of the early, invasive activities of the Shope tumor. The growths are not only benign but their presence is dependent on accessory conditions. Yet when these conditions are peculiarly favorable, condylomas can invade normal tissues, and be highly destructive.[8] In numerous reported instances (14) this has happened when the growth was pent beneath a phimotic foreskin, and

[7] At this writing the cancers of D. R. 2-38 are, on the average, less than one-fourth their size at one time.

[8] We are indebted to Dr. Marion B. Sulzberger for bringing these cases to our notice.

there was inflammation due to bacteria. Then it has invaded and perforated the foreskin, preceded by cellulitis, and burrowed through the *corpora cavernosa* and *spongiosum*, causing urethral fistulae. Amputation of the penis has proved the only safe course. In some cases the growth has remained a condyloma histologically (*carcinomähnlich Condyloma*, "the pathologists find nothing, yet it is cancer" (15)), and again it has given more or less outspoken morphological signs of malignant change, or has undergone the transformation to a typical squamous cell carcinoma yielding metastases. Often biopsy has shown a condyloma but the recurrence has been carcinomatous.

This progression to cancer of a notably benign growth caused by a virus dependent for its action on favoring local conditions, involves changes of far greater scope than are required of the Shope rabbit papilloma when becoming malignant. Yet for the effectiveness of even so drastic a carcinogenic agent as the Shope virus, numerous conditions must be right. The virus must find its way into animals of the proper species, into favorable individuals, into especially susceptible skin (such as responds well to stimulation with Scharlach R), into an association with the epidermis (pigmented being very favorable), and even then it may not set carcinosis in train unless local factors contribute. Malignancy is the outcome of numerous concurring influences, as holds true of cancer generally (5).

SUMMARY

The papillomas induced in domestic rabbits with virus procured from cottontails undergo progressive changes in the direction of malignancy when they grow vigorously. From the beginning they exhibit the traits whereby tumors are characterized, and they have malignant potentialities. In seven animals of a group of ten carrying papillomas for more than 200 days, cancer has developed, and in an eighth a tumor of problematic malignancy has arisen. One of the remaining two rabbits died early in the cancer period, and the papillomas of the other eventually retrogressed. Ten cottontails with induced growths of much longer duration have not developed cancer.

The malignant tumors have all been acanthomatous in type, and have arisen directly from the papillomas by graded, continuous alterations. These have often gone further after malignancy has been attained, and have eventuated in great anaplasia. Metastasis has

been frequent, and transplantation to another host has proved successful. Individual growths have occurred expressive of each stage of the transformation to cancer, as if through a stabilization at this stage; yet despite the variety thus afforded, the tumors must all be looked upon as the consequence of alterations in cells of a single sort, namely epidermal cells affected by the virus, and the alterations themselves have taken a single direction. In the morphology of many of the cancers the influence of the virus is still manifest.

The better the papilloma grew, the more likely was cancer to occur, and the greater was the tendency to multiple tumors. In the most favorable rabbits malignant changes took place at numerous locations in the papillomatous tissue, and were imminent at many others. Intercurrent factors had much to do with determining frank carcinosis; and when the tendency to it was not marked their influence sometimes seemed crucial.

Analogous instances of a graded alteration from papilloma to cancer are frequent in human pathology. The virus that gives rise to the rabbit papillomas must be looked upon as the primary cause of the cancers developing therefrom. Whether it is their proximate cause has yet to be determined.

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EXPLANATION OF PLATES

All of the sections were stained with eosin and methylene blue. S indicates the side on which the skin surface was.

PLATE 19

FIG. 1. Ulcerated carcinoma where was once a papilloma like those still present. The arrows indicate the direction of the subsequent biopsy cuts. *D. R. 2-38*: 214 days. × 5/11.

FIG. 2. Cross-section of the cancer of Fig. 1. 214 days. × 6⅔.

FIG. 3. Edge of a metastasis in an axillary node removed on the 237th day.

FIG. 4. The carcinoma of Fig. 1, photographed 78 days later. It has undermined and involved the neighboring papillomas, and coalesced with a cancer arising in one of these. The others are in a precancerous state, much less pigmented than previously and with thickened, deep bases. A second axillary metastasis has developed (arrow). × 7/11.

FIG. 5. Invasive, cystic growth deriving from a papilloma. It has extended out under the neighboring skin. *D. R. 2-52*: 344 days. Fig. 22 shows the gross specimen. The present figure has been reversed in its relation to this. (See also Figs. 14 and 15.) × 4½.

PLATE 20

FIG. 6. Ulcerating cancer that has replaced one papillomatous mass and is encroaching upon another. The arrows A and B point to the regions of successive, later biopsies. *D. R. 2-39*: 211 days. × 10/11.

FIG. 7. Cancer (A) extending from beneath a fleshy papilloma. It has raised the skin slightly. The axillary metastasis (B) attracted attention 21 days before the primary tumor was palpable. Some of the papillomas are fleshy and thick-based, whereas others have not undergone these precancerous alterations. *D. R. 2-53*: 329 days. × 5/11.

FIG. 8. Section through the edge (A) of the ulcer of Fig. 6, with adjacent skin. The growth is a malignant papilloma, breaking up into squamous cell carcinoma which has invaded the voluntary muscle. 212 days. × 7½.

FIG. 9. Section from the tip of the subcutaneous extension (B) of the same tumor. Transition forms from papilloma to squamous cell carcinoma are again found. 230 days. × 31.

FIG. 10. Part of a nodule removed from the leg muscles 12 days after implantation of the material of Fig. 9. The tumor is now almost entirely of squamous cell type. × 31.

PLATE 21

FIG. 11. Retroperitoneal metastasis from the tumor of Figs. 8, 9, 10, invading the wall of the aorta (upper part of figure). The growth is far more anaplastic than previously. 298 days. × 130.

FIG. 12. Early extension of a Shope papilloma into the corium. *D. R. 1-92*: 47 days. × 90.

FIG. 13. Later irregularity and extension downwards of another papilloma, with pearl formation. *D. R. 1-55*: 72 days. × 8.

FIG. 14. Malignant (?), cystic extension of a papilloma into the subcutaneous tissue (see also Figs. 5, 17, and 22). *D. R. 2-35*: 286 days. × 5.

FIG. 15. Early malignant changes within a papilloma. The irregularity of the uninvolved portion to right, should be noted. From the same extensive, papillomatous mass as Fig. 14. × 5.

PLATE 22

FIG. 16. Papilloma that has undergone precancerous changes. The high peaks have been replaced by flaky material, and the base of the growth is fleshy, with a raised, tense border. A large, subepidermal pearl has recently appeared (arrow) outside it. *D. R. 2-39*: 238 days. × 7/11.

FIG. 17. Localized downgrowth from the middle of the base of a long-established Shope papilloma. *D. R. 2-52*: 210 days. × 6½.

FIG. 18. Fungoid carcinomas which have replaced 3 papillomas. They have been gnawed, whereas the papillomatous masses to either side are intact. The arrow indicates where a biopsy was to be done. *D. R. 2-52*: 175 days. × 7/11.

FIG. 19. Section through one side of the growth designated in Fig. 18, with the adjacent skin. It is a malignant papilloma. 176 days. × 14.

FIG. 20. Malignant, non-pigmented papilloma extending under an ordinary papilloma (arrow) that is heavily pigmented. *D. R. 2-35*: 286 days. × 7½.

PLATE 23

FIG. 21. Section through a superficial part of the fungating tumor shown in Fig. 28. *D. R. 2-48*: 385 days. × 8.

FIG. 22. Section through the mass that eventually took the place of all of the growths shown in Fig. 18. Some of the Shope papillomas still persisted as such. The new tumors that had arisen from them, or invaded them and coalesced, were mostly malignant papillomas. The brackets indicate part of a cystic tumor, which is shown in Fig. 5. Natural size.

FIG. 23. Squamous cell carcinoma deriving directly from the base of a Shope papilloma. (From another part of the papillomatous mass furnishing Figs. 14, 15, and 20.) × 35.

FIG. 24. Metastasis in an axillary lymph node of a squamous cell carcinoma with cystic tendencies. *D. R. 2-05*: 348 days. × 6½.

FIG. 25. Malignancy developing in the wall of a pearl under an old papilloma. (See also Fig. 26.) *D. R. 2-35*: 233 days. × 7½.

PLATE 24

FIG. 26. The region indicated with an arrow in Fig 25. Bacterial infection and local inflammation are present where the epithelium has invaded the connective tissue. × 140.

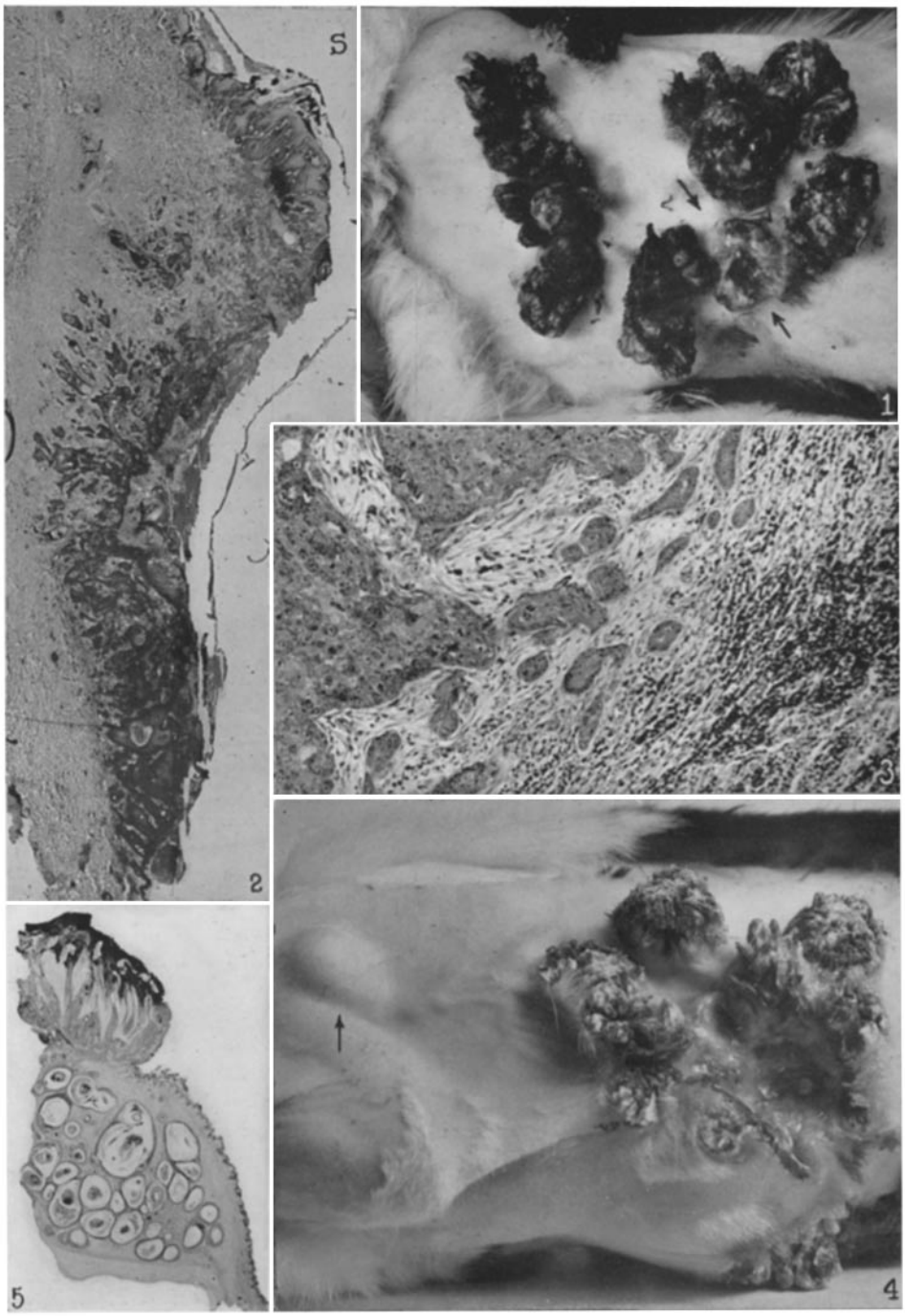
FIG. 27. Part of a tumor resulting from transplantation to another rabbit of the cancer of *D. R. 2-39*, which is shown in Figs. 8, 9, and 10. × 31.

FIG. 28. Fungating tumor of questionable malignancy, which has almost entirely replaced a papillomatous mass. Some of the latter persists along the edges as dry, sooty peaks. (See Fig. 21.) *D. R. 2-48*: 384 days. × 5/8.

FIG. 29. Cancers appearing as discoid growths in the midst of confluent, papillomatous expanses. *D. R. 2-35*: 244 days. × 5/16.

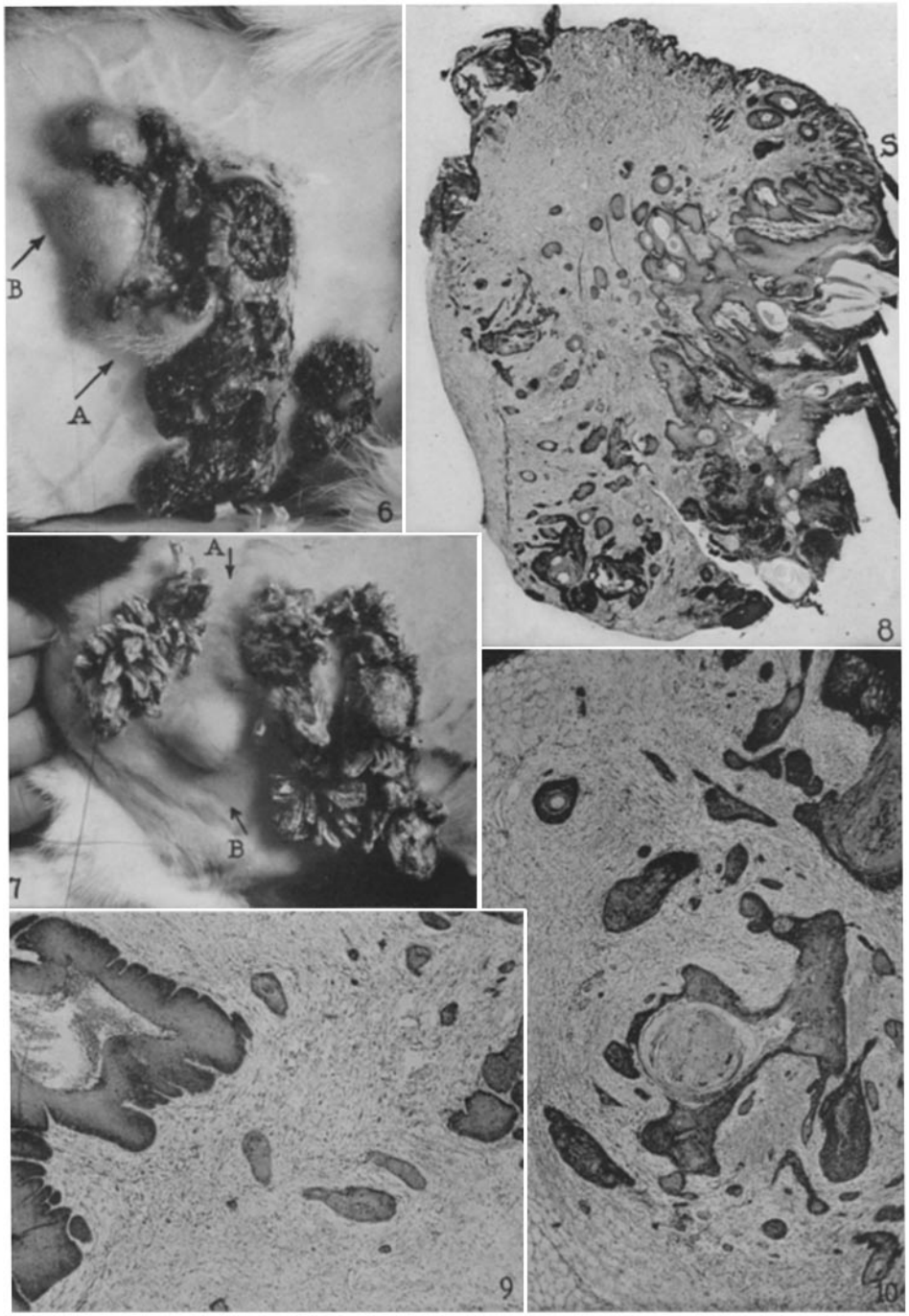
FIG. 30. One of the cancers of Fig. 29. 286 days. × 190.

FIG. 31. Lung metastasis in the animal of Fig. 29, with the morphology of Fig. 30. 286 days. × 190.



Photographed by Louis Schmidt and Joseph B. Haulenbeek

(Rous and Beard: Virus-induced rabbit papillomas (Shope))



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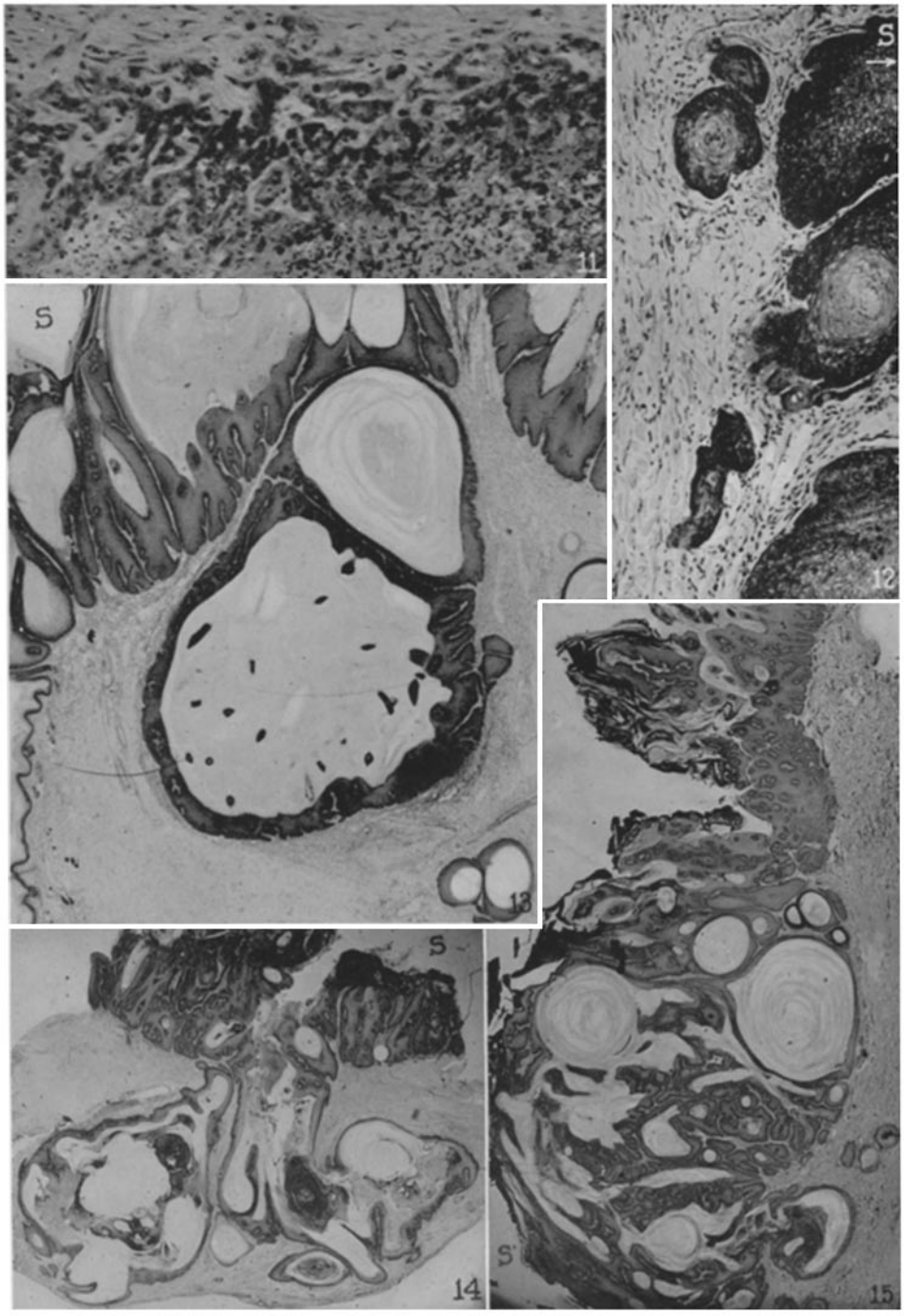

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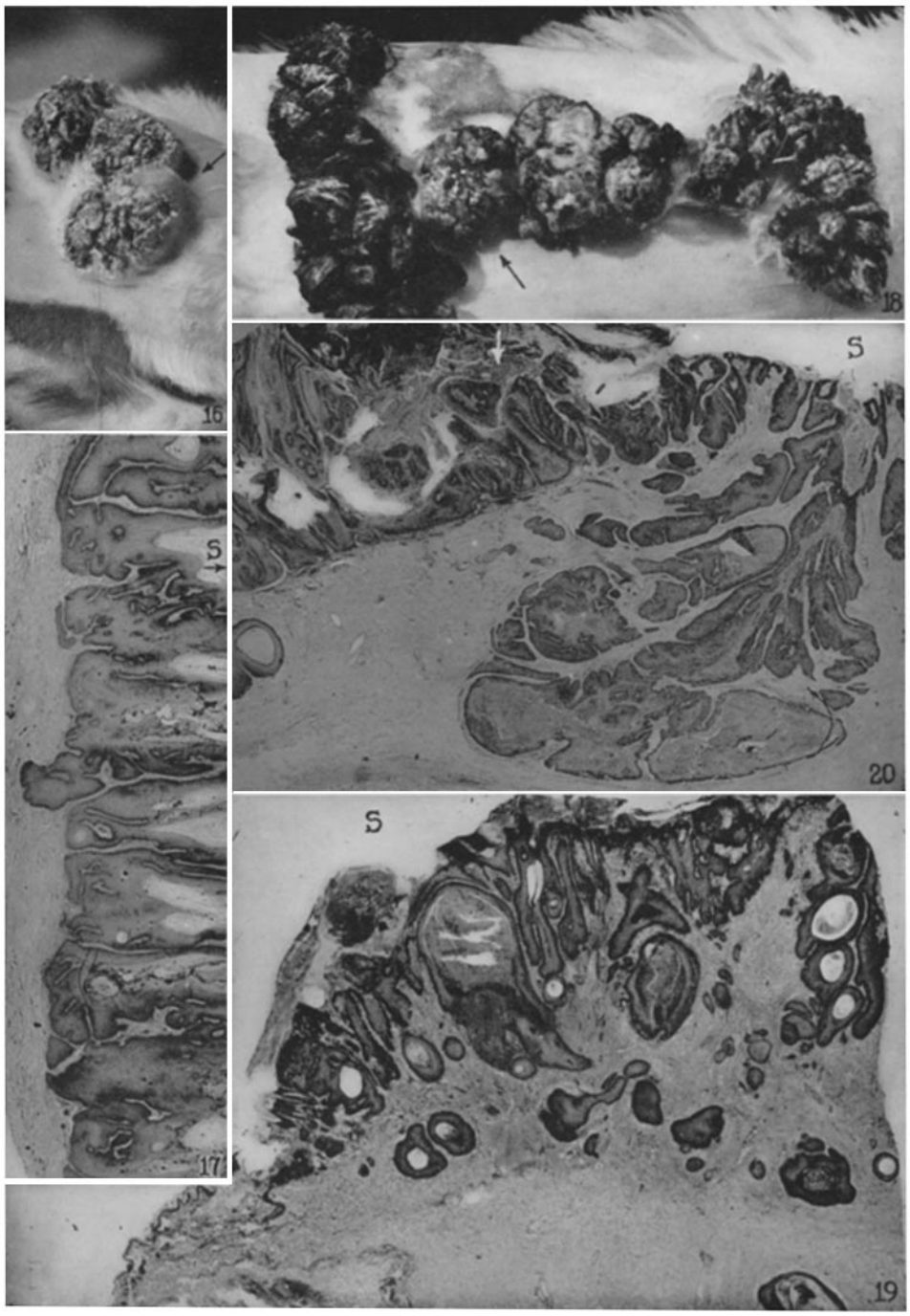
(Rous and Beard: Virus-induced rabbit papillomas (Shope))



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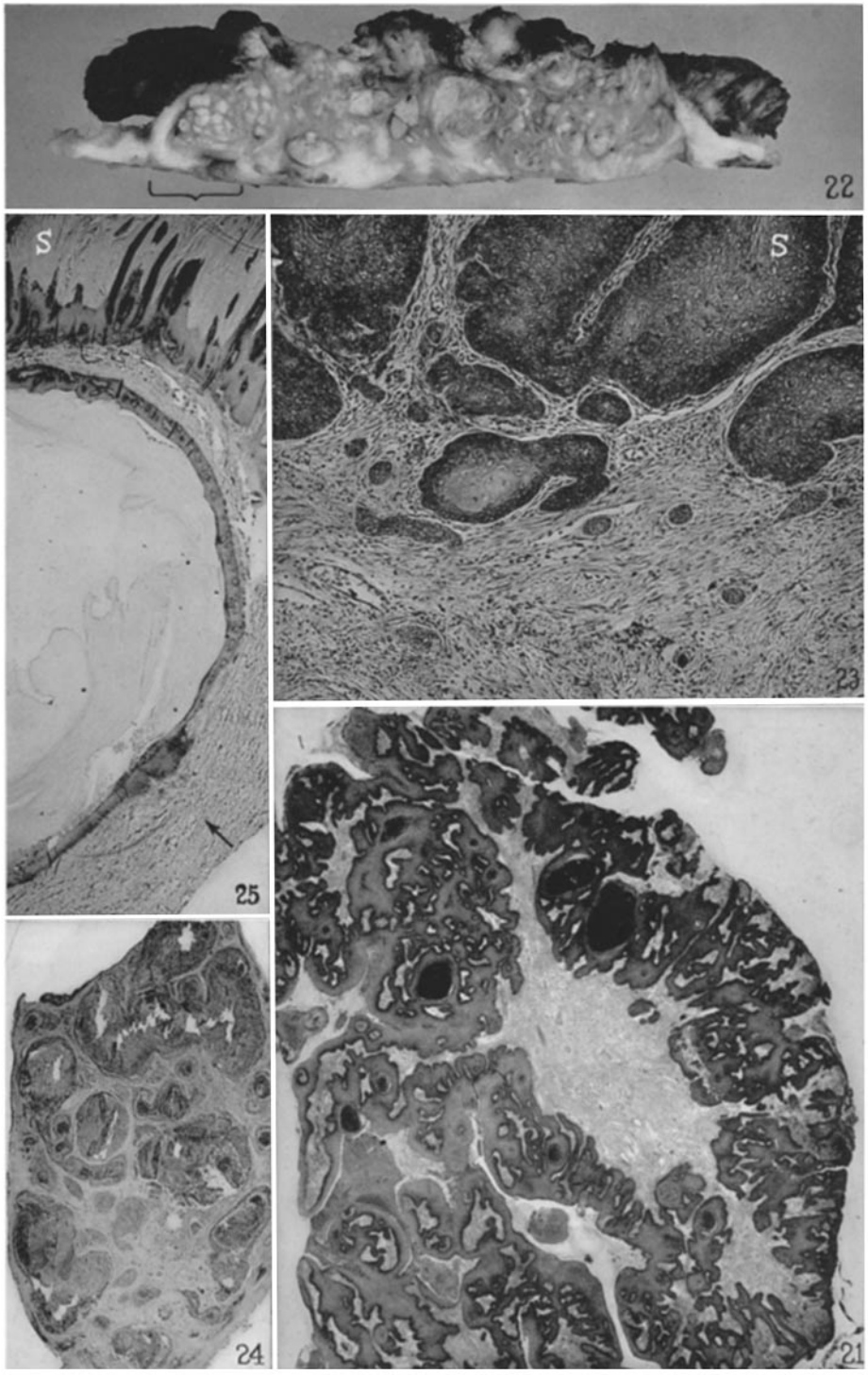
(Rous and Beard: Virus-induced rabbit papillomas (Shope))



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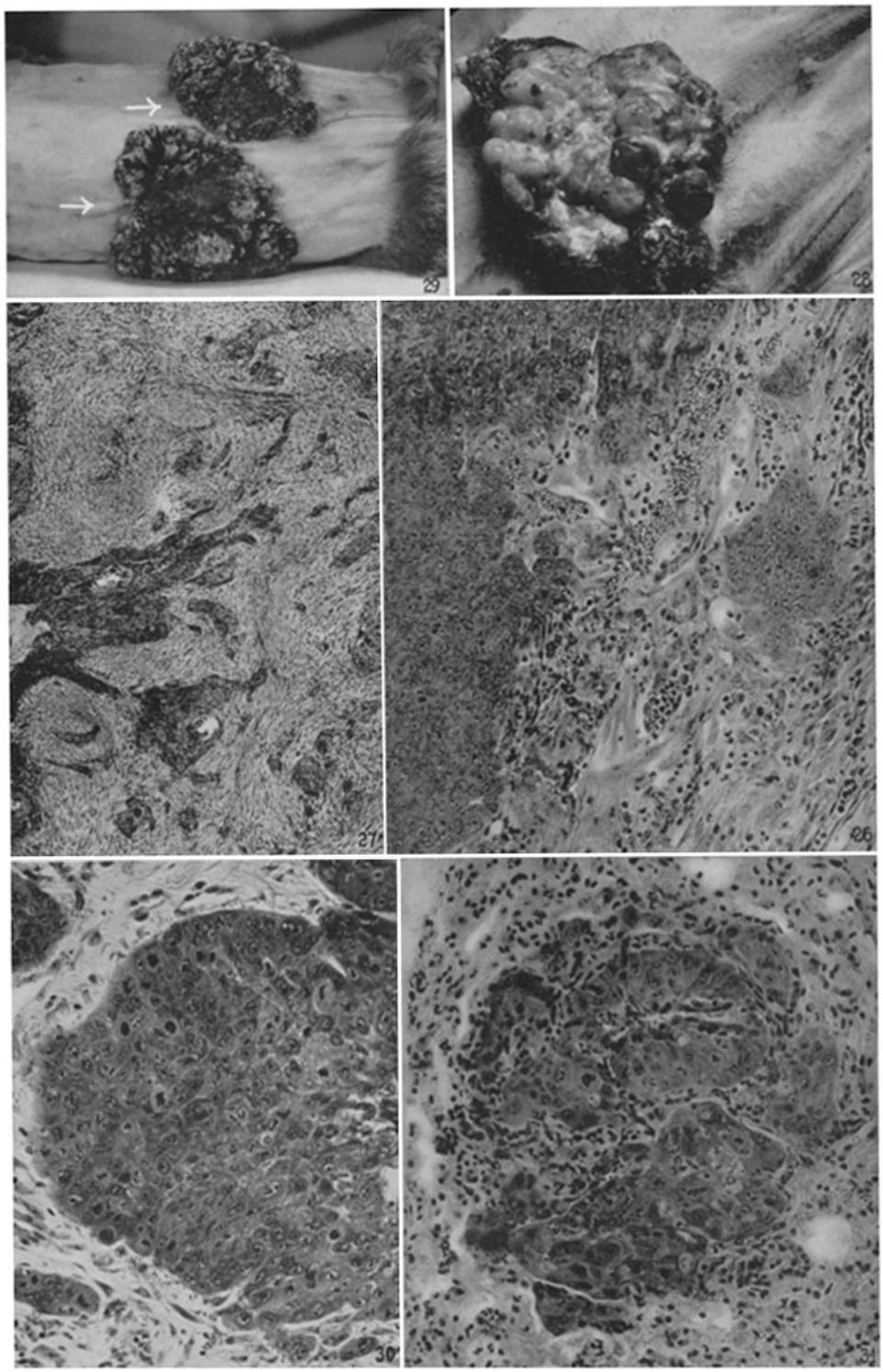
(Rous and Beard: Virus-induced rabbit papillomas (Shope))



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