# FATAL KERATOMAS DUE TO DEEP HOMOGRAFTS OF THE BENIGN PAPILLOMAS OF TARRED MOUSE SKIN

## Normal Proclivities and Neoplastic Disabilities as Determinants of Tumor Course

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Plates 5 to 15

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Epidermal papillomas are the most frequent and familiar of the mouse and rabbit growths induced by carcinogens, yet after 40 years their status is still uncertain. Their practical worth is great as betokening the effectiveness of these agents, while furthermore many cutaneous carcinomas, indeed almost all those of rabbits, originate from them. Their neoplastic character is now taken for granted and the further assumption made that they are of a single, benign kind; but they are peculiar in disappearing unless aided, and all efforts to transplant them have failed. Hence, despite their frequency, they seldom receive more than superficial attention from workers bent on procuring cancers, and the present attempts at their propagation might not have been undertaken except for a compelling motive,—to learn whether oncogens, potent in inducing them, will hasten the occurrence of the cancers deriving from them,—or will fail of such effect as in the case of the carcinomas arising from the pulmonary adenomas of mice (1). Most of the tar-induced mouse papillomas chosen for our purpose proved readily transplantable to the subcutaneous tissue and thigh muscles of other mice, almost always growing progressively and killing their hosts, if let alone. The tumors will now be described, and the results of exposing them to methylcholanthrene reported later.

### GENERAL PLAN

Mouse papillomas are more vigorous than those of the rabbit and have less tendency to regress: hence mice were used, and of a homogeneous breed,—such as was not available when the last effort at transplantation was made (2). The mongrels then used were mature, and the fact is now realized that cancers failing on transfer to other adults will often grow in the newborn (3). Even Shope virus papillomas, which regularly fail on transfer to adult rabbits, will flourish in sucklings (4). Hence

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most implantations of the present work were made in these, with weanlings as second choice.

The papillomas were induced with tar instead of a pure hydrocarbon, because it not only initiates neoplastic changes in the skin but strongly promotes tumor formation (5, 6); and the conditions were so arranged as to provide vigorous papillomas. Many adult mice were tarred until multiple growths of this sort had arisen, and then tarring was stopped and the mice were kept for nearly a year. Their skin appeared normal, yet a few papillomas had persisted, and these were utilized for transfer.

Carcinomas are often present in mouse papillomas and have destroyed most grafts of them in the past (2, 7). In the attempt to exclude them now, tiny, well-washed grafts were used, each placed by separate trochar in a different host, with many implanted at each transfer. It was hoped that thus, by casting a wide net and placing some of the right fish in new ponds, papillomas might be obtained as such; and this happened.

## *Materials and Methods*

The mice were of the notably homogeneous C strain (Rockefeller Institute stock). Landsteiner tar[1] was swabbed over the back of more than a hundred adults once or twice weekly, and after a few months the applications were left off. Many of the papillomas (paps.) regressed later, and most of the mice died, usually of cancer. The last survivors provided nine paps. for transfer, though only three were big enough to yield histological material as well. The rim of each growth was cut away, to exclude normal epidermis; its underlying connective tissue was removed; its living base was sliced off as a layer less than a millimeter thick; and by vertical cuts through this layer bits about 0.5 mm. across were procured. They were well washed in serum-Locke's solution (s-L: one part mouse serum to nineteen of Locke's), and implanted through No. 18 needles with bevels ground short. After the paps. had been repeatedly transferred cancers proved so infrequent in certain lines that millimeter grafts were used for implantation in weanlings; but no reason existed for such big ones in baby mice.

Most of the implantations were subcutaneous. A little s-L was drawn first into the needle, the graft then inserted into its tip, and it was thrust through a slit in the skin of the flank, and pushed along in the subcutaneous tissue to just behind the shoulder, where the graft was expelled. During withdrawal of the trochar the protruding end of the plunger was compressed between narrow curved forceps, together with the overlying skin, to make sure the graft stayed in place. Most of the weanlings (weight 12 to 15 gm.) were implanted in the same way, with approximately twice as much tissue, but sometimes this was put in the posterior thigh muscles, and often at both situations.

The slit in the skin was swabbed with mercuric chloride solution and mopped dry before introducing the trochar, to exclude the possibility that normal epidermal cells might be carried in together with the neoplastic. The mice were marked as soon as large enough, and only those surviving sufficiently long for a tumor to have developed find place in the protocols. Each of the hundreds of growths has been followed, repeatedly measured, often sketched, and sometimes photographed.

Frozen sections and the Terry stain were used when the character of the grafts seemed doubtful. For permanency blocks were fixed in acid-Zenker solution and stained with eosin and methylene blue. Many of the growths were big and consisted mostly of keratin so hard

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

and dry that they had to be left in "Zenker" 3 or 4 days and washed equally long to soften them before they were cut through.

### *The Primary Papillomas and Their Yield on Transfer*

Pap. I was 8 mm. by 11 mm. across, of bulging "onion" shape, somewhat constricted base, and tapering, keratinized top. Its living basal layer was typically papillomatous (Figs. 1 and 2), and grafts of it did notably well. Another big onion (Pap. IX) failed to take, though implanted in fourteen sucklings and five weanlings; but Paps. V (Fig. 3), and VI (Fig. 4), of the same shape though smaller, yielded growths in many hosts. So too did a tiny, dry cone with fleshy base (Pap. VIII), a fleshy hassock (Pap. II), and a small cauliflower (Pap. IV),—though this last yielded cancers as well. The grafts from a tiny, projecting nodule (erroneously termed Pap. III) gave rise to cancers only, and those from a fleshy disc with keratinized top (Pap. VII) were destroyed by infection.

Pap. IV was carried through only two generations because a carcinoma, present from the first, persisted amidst its tissue; and the many tumors of Pap. VIII in its 2nd Gen. (Lines A and B from different 1st Gen. tumors) were all lost through intercurrent infection, though not before it had been maintained long enough to yield findings like those of Paps. I and V. Pap. I has been carried in six distinct lines for more than 20 months, and these are now in their 7th to 9th Gen. Paps. II, V and VI are in their 9th and 10th Gens. after 18 months.

### *The Results of Implantation in the Subcutaneous Tissue*

The tumors produced by the six growths have proved them all to be paps• though remarkably various in form. Those derived from Pap. I have shown notable vigor and been studied most. Hence the description which follows will be drawn mostly from them, though with recourse to the growths derivative from the other paps. whenever needed.

Bits of Pap. I were implanted subcutaneously in four newborn mice (one litter) and in ten weanlings, these latter receiving grafts in the thigh muscles as well. Within 5 weeks discrete, spherical, subcutaneous nodules, 6 to 11 mm. across, had formed in two sucklings and five weanlings, and two of the latter had smaller but similar thigh growths as well. All of the tumors were opened between the 33rd and 63rd days and in some instances excised for transfer purposes. They proved to be firm, keratin-filled cysts, lined with a shallow, almost even layer of crowded, minute, finger-like papillae,—from amongst which in some cases a single, small "cauliflower" protruded at the deepest spot in the cyst.

Bits of the lining of the seven largest growths were implanted subcutaneously, each in many sucklings and sometimes in weanlings as well, thus starting seven lines, A to G, of Pap. I in its 2nd Gen. Sections of five of the seven tumors showed the cysts to be lined with a layer of actively proliferating and keratinizing, papillomatous epithelium like that of the primary Pap. I (Fig. 1), though with small patches of squamous cell carcinoma in two instances.

Some of the mice of Line A in its 2nd Gen. were let live for more than 6 months to get long term findings: their growths had become huge and would soon have proved fatal. All were then killed and Line A not carried further. The other six lines still flourish. As protection against loss, two or more sublines have often been started, using different tumors of the same group. For always the possibility existed,—as it still does,—that unperceived carcinomas amidst the cyst lining might take over the growths on further transfer.

The grafts of Pap. I in its later generations have yielded tumors in most instances, though with not infrequent failures to "take." For example, forty-five sucklings developed growths, out of forty-nine implanted subcutaneously with the same 2nd Gen., Line B material, and so too did fifteen of twenty weanlings. The other paps. have succeeded almost as well. Once started, the new tumors have almost always grown steadily, if at differing rates, as would follow from the differing amounts of pap. tissue they contained. The number of "takes" and rate of growth underwent little increase as propagation continued. None of the tumors regressed, and the few that became stationary were all less than 15 mm. across: they persisted because wholly keratinized (Fig. 15). Bigger growths generally killed their hosts within 3 to 5 months,—unless they erupted on the skin surface, in which case the mouse sometimes gnawed them entirely away, thus rescuing itself. But this happened rarely.

The living subcutaneous tumors deriving from Paps. I, V, and VIII were always discrete cysts, thinly encapsulated, poorly vascularized, filled with close packed, almost dry, keratinized cells and lined more or less completely with a layer of papillomatous tissue actively engaged in forming them (Figs. 7 and 8). Mitoses were numerous (Fig. 23). Some of the cysts contained solitary stalked paps. as well. The cysts enlarged so symmetrically amidst the yielding, cooperative, areolar tissue of sucklings as to remain spherical in most instances until they were 3 cm. or more across (Figs. C 1, C 2, and C 3),—when pressure from the stretched skin flattened them somewhat (Fig. C 5). As a rule they were solitary and exceedingly firm on palpation, so close packed was the keratin within them. It gave them an ivory hue which shone through the skin (Fig. C 5). Occasionally they were oblong because several graft fragments had been left lying next one another along the trochar line. In weanlings the cysts were usually oblong as also in the few adult hosts implanted.

Even in the biggest tumors the layer of living pap. tissue was seldom more than 1.5 mm. thick in its deepest, oldest region. After removal of the keratin,—which usually shelled out in a mass,—it here resembled a rough, coarse, pinky-gray velvet, consisting as it did of innumerable, narrow, papilliform protrusions, almost uniform in size and length, with almost no keratin between them. So crowded often were these papillae that when a long cut was made through the cyst wall this rolled outwards, at times almost completing a tube (Fig. 5). As more and more keratin accumulated within the cyst, thus increasing its size, the layer of pap. tissue for some while kept pace with its enlargement by lateral spread and intercalated proliferation; but as the cyst expanded toward the body surface it began to be outstripped, becoming more shallow, its papillae so diminishing in height that it resembled corduroy; then, further away, a grayish-pink shagreen (like the skin of a baby mouse with white hair just appearing); then pinkish ground glass; and at last it was a mere dull, slightly opaque skim, beyond which only glistening connective tissue lay next the keratin. All these evenly graded changes could be seen on the wall of a single big cyst from its curving base to its top.

Wherever the pap. was extending as a single layer of cells on bare connective tissue these were flattened and their nuclei too, just as in the case of virus-induced, Shope papillomas of rabbits, when forming similar cysts after deep implantation,—and of normal epidermis, for that matter, when covering a denuded surface (8). Yet the cells retained neoplastic stigmata, most plainly seen where a shallow layer of them could be directly compared with the adjacent, normal epidermis (Fig. 9).

Where the lining layer was thickest in the cysts of Pap. I it wholly resembled microscopically that of the original growth, save in one respect; it usually appeared much more orderly, indeed wholly benign, whereas this latter had had a threatening aspect, thrusting tongues into the encapsulating connective tissue (Fig. 2). So it still did in the cysts not infrequently (Fig. 14), though the tongues never got far and can have played only a negligible part in the growth's enlargement.

The dense, moist, opaque keratin was sometimes so firmly attached to the living pap. tissue

that this came away with it "by the roots," leaving little pits where giant rete pegs had been. Often it was exceedingly sticky, clinging to the knife. Its color varied, that of Pap. I ranging from ivory in most tumors to brownish yellow in a few, whereas that from some of the growths of other derivation ranged from palest ivory to lemony yellow. In the individual cyst it had the same hue everywhere. Sometimes it appeared a mere jumble but usually showed striae indicative of past happenings, like the strata of geological ages (Fig. C 3). It was odorless, save for a faint, mousy smell occasionally. Its keratinized cells, though sometimes gummed together by exudate, separated readily in salt solution, and when well washed were white. They differed widely in size and shape, none being larger than normal, but many of them smaller and angular, and often curved like tiles over an arch. An oval nucleus was frequently visible.

The keratin accumulated in such enormous quantity (Fig. C 3) that sometimes it weighed almost as much as the host. The total weight of a mouse of Pap. I, Line C, 3rd Gen., implanted when newborn and killed 122 days later, was 23 gm., of which 11.3 gm. was tumor and 9. gm. keratin.

*The Early Stages in Cyst Formation.*—Many grafts from tumors of Pap. I, 4th and 5th Gen., were implanted in sucklings, and removed at short intervals for serial sections. These showed that some had consisted mostly of encapsulating connective tissue. The first activities of the neoplastic cells after implantation was to cover this latter (Fig. 16), and extend onto the host tissue. Blunt tongues of pap. cells sometimes burrowed into the interior of the graft, when this was in good condition (Fig. 17), but not if it was doing badly (Fig. 16). Between the 7th and 14th days the pap. lined the wall of the graft pocket with a layer actively differentiating inwards, and keratin began to accumulate (Fig. 18). Papillae soon formed, each with a delicate core of stroma as it elongated (Fig. 19). The result was a cyst with pap. fingers turned in toward its center. They were longest on the deep side of the cyst, or floor as it will be termed; and they protruded vertically, showing no sign of interior pressure.

*The Stalked, Intracystic Papillomas.*—Not infrequently in vigorous tumors, opened while still small, the lining papillae on the cyst floor were uneven in height, and sometimes a small cauliflower growth with a more or less constricted base projected from amidst them. Sections of early stages showed that this had originated from a graft which had become attached at one spot only, though consisting largely of pap. tissue (Fig. 20). On cutting into a 7 mm. cyst, 33 days old, of Pap. I, 1st Gen., a ball-like, sessile cauliflower was found, surrounded by keratin and nearly filling it. Big cysts sometimes contained solitary, central, more or less globular, stalked growths, embedded in the mass of dense keratin. They were almost symmetrical and consisted of a connective tissue core connected with the floor of the cyst by a pedicle and overlain by a layer of pap. tissue thicker and with longer "fingers" than that on the cyst wall. The pedicle, thinly covered, was usually narrow, joined to the cyst floor near its middle, and the core it supported was occasionally edematous (Fig. 22).

These stalked balls were not due to penetration of the pap. into the dense keratin, but owed their place to enlargement of the cyst in all directions. They had been mere sessile cauliflowers at first, with keratinizing sides that overhung the pap. layer on the floor of the cyst. This was also forming keratin, and, as it accumulated from both directions the floor of the cyst was forced away from the cauliflower which, fixed in position, gradually became stalked. Not only did a funnel-shaped depression opposite the stalk, on the underside of the cyst floor, attest to this happening, but so too did the inclusion in the stalk, as time went on, of adipose tissue and even of muscle fibres from underneath (Fig. 28), the cyst floor having bulged down around

these structures and enclosed them. Most of the biggest blood vessels of the cyst lay in the stalk as well, some of them coursing directly to the central sphere, a state of affairs which accounts for the profusion of pap. tissue covering the latter, and its relative paucity on the cyst wall. Edema of the sphere developed when lateral pressure from the keratin compromised the veins in its stalk.

The stalked paps. arose in both weanlings and sucklings. Grafts of the vigorous pap. tissue covering their cores yielded cysts with no special tendency to form them.

*The Herniated Tumors.*—It may be recalled that where the pap. lining of cysts failed to spread laterally fast enough to contain the increasing keratin mass this was enclosed merely in compressed connective tissue (Figs. 7 and 8). This frail barrier soon broke, with result in dissecting cysts (Figs. 10 and C 4) that enlarged rapidly amidst the soft, areolar tissue. They did so for several reasons: keratin was continually forced into them from the cyst proper; it provoked a foreign body reaction (Figs. 11 and 12),—as normal keratin does when lying free, after the traumatic rupture of a dermoid cyst for example; and a copious watery exudate collected in the new cyst, enlarging this, suspending its keratin or rendering this pultaceous, and loosening that next the opening into the old cyst, so that more and more passed into the new. The pap. lining of this latter, on the other hand, proved unable to spread to the inflamed wall of the new cyst though becoming thick and vigourous next the opening into it.

Often several dissecting cysts extended away from the same tumor (Fig. C 4), and sometimes they became much the larger, 4 to 5 cm. across, and full of fluid rendered opaque and yellow by keratin. The findings on palpation were pathognomonic: next one or more fluctuating, subcutaneous bags the primary tumor could be felt as a rounded mass, hard as a marble.

The dissecting cysts were well tolerated until they became so big as to cause pressure necrosis of the skin over them. Then ulceration took place, and through the resulting hole the cyst contents exuded or else could be pressed out like tooth-paste. Bacterial infection followed and death. Transplantation of bits of dissecting cyst wall never resulted in tumors.

*The Erupted Growths.*—Instead of rupturing laterally the primary tumor more often broke through the skin, "pointing" like a boil. Pressure from its keratin shut off the overlying cutaneous vessels and here the skin mummified, becoming a brown or black scab with persisting white hair. The sharply demarcated scab could frequently be lifted off the cyst, like the lid from a pot, disclosing its ivory-pale, keratinized contents, and often the underlying keratin was partly gnawed away (Fig. C 5 and Fig. 13). But if not disturbed this lid was soon forced outwards by the continually forming keratin underneath, and this protruded, the tumor becoming more or less acorn-shaped, its kernel the exposed keratin, now dry, brown and horny, and the cup of the acorn the tumor cyst (Fig. 21). Sometimes the kernel was bluntly conical because of a gradual widening of the opening through which it was pushed out, but more often this was broad to begin with and the dry keratin mass was flat topped, like a mesa. Where it met the living skin bacterial infection was prone to occur with result in yellowish green pus.

Even when the cysts became enormous they gained no hold on the body wall proper, because their pap. lining remained encapsulated, never invading its muscle. Hence pressure conditions forced them toward the surface, and they slowly became almost tangential to it. This held notably true of the acorn growths, many becoming wholly superficial eventually.

*The Tangential Saucers and Buttons.*—The mass of dry keratin forming over erupted growths was frequently gnawed down by the mouse or its cage mates. When a broad expanse of pap. tissue was thus exposed the host mouse died, but not so if a thin, covering layer of keratin remained: then a protruding saucer- or button-shaped growth resulted, and shrinkage of the keratin that built up over it pulled its margins well above the skin level (Figs. C 6 and C 8). Remarkably symmetrical growths arose in this way, projecting out over the skin and covered with it on the underside (Fig. 24) save on their smooth, small, convex base, scarcely deeper

than the skin itself. Such saucers, once formed, retained their shape even though gnawed deep, and if the gnawing ceased they gradually filled with a brown mound of keratin which occasionally became so heavy that the saucer hung like a tassel (Figs. C 6 to C 9). Some of the saucers slowly broadened, but others remained stationary. A few of the host mice gnawed theirs off entirely, thus ridding themselves of the tumor.

Occasionally the side of a surfacing, erupted growth remained below the level of the adjacent skin, and extended laterally beneath this, the unevacuated keratin supplying the fulcrum needed for its enlargement, as in the case of deep-lying cysts. Thus huge discs formed, with dead skin, carrying white hair, overlying their borders (Fig. C 9).

*The Giant Cutaneous Horns.*—Now and then the eruption of a cyst led to the formation of a cutaneous horn on a superficial base (Fig. 26 and Figs. C 10 to C 12). The horn tended to come away, on becoming lengthy, unless fixed in a claw setting of skin (Fig. C 10) or anchored at the base by a stalked pap., present in the original cyst and gradually extruded as this surfaced, forming keratin all the while (Figs. 25, 27, and 28). Under such circumstances horns of prodigious size sometimes arose and peristed *in situ*. They were brownish black, horn-hard, and were usually topped by a layer of hairy, mummified skin. Occasionally they had a mushroom top because the skin had shrunk less on drying than the keratin under it (Fig. 25). When cut through vertically after fixation and softening in water the central, stalked pap. was disclosed (Figs. 25 and 27),—or sometimes only its stalk, the growth that topped it having died or been replaced by cancer (Fig. 28). Frequently the stalk contained fatty tissue, and even voluntary muscle fibres, incorporated from beneath the floor of the original cyst for a reason already given.

One small acorn tumor, after surfacing, came to resemble a vigorous, tar-induced, cutaneous pap. (Figs. C 13 and 29); only its claw-setting in skin suggested its deep origin.

## *The Results of Purposely Exteriorizing Subcutaneous Cysts*

Several cysts were opened, and some emptied of keratin, to learn whether superficial growths like induced paps. would result.

When the tumor was of medium size and the incision left open, its edges often united and the cyst reconstituted itself, whereas when it was large and its sides were cut away, exposing a broad expanse of pap. tissue well protected by keratin, this built up later into a dense, flat-topped, mesa-shaped mass. Occasionally, when small cysts stayed open, growths formed nearly resembling tar-induced paps. In two instances intercurrent disease killed the animals, but better luck was had with a cyst of Pap. V, 1st Gen. (Figs. 30 and 31):—

The cyst had derived from a graft implanted 34 days previously in a 2 day old mouse, and was 6 mm. across when emptied of keratin. During the next 23 days it formed a wholly superficial growth (Fig. 30), dry and vertically striated down to its base, and with a flat top having the area of the floor of the cyst when opened. Its base became constricted later, like that of many tar-induced paps., and to forestall loss of it the mouse was killed, after 61 days in all. Fig. 31 shows that microscopically it was wholly like the cutaneous paps. induced by tar and other carcinogens, save for the abundance of connective tissue lying underneath it (capsule of the original cyst).

## *The Spontaneous Formation of Surface Growths Like Tar-Induced Papillomas*

Now and then paps. arose on the skin over spots where the attempt had been made to lodge grafts subcutaneously. Figs. 32 *a*, *b*, and *c* show stages in the enlargement of such a growth.

A day old mouse was implanted behind the shoulder with tissue of Pap. II, 1st Gen., and 33 days later a conical, dry spicule with fleshy base was noted, projecting from the skin (Fig. 32 *a*). It grew bigger rapidly (Fig. 32 *b*), and in 46 days more had become a bulging onion with constricted base and dry, creamy top, now jagged because gnawed (Fig. 32 *c*). During the next month it enlarged further, but was truncated by gnawing. It remained wholly superficial, its base constricted and slightly concave on the underside. The microscope showed a typical epidermal pap. of notably benign aspect (Fig. 43). No subcutaneous growth formed at the site of implantation.

Six more mice of the same litter and four of another, also 1 day old, had been similarly implanted. Only one growth arose in each litter, a subcutaneous cyst which grew steadily.

Other instances presented themselves as time went on.

Pap. II in its 2nd Gen. yielded another onion growth on the skin. It was 3 mm. across, plump and fleshy, with constricted base and a dry, narrow top when first seen 20 days after the implantation of a 3 day old mouse. It was lost 4 days later through death of the animal. Neither this nor two litter mates similarly implanted developed a subcutaneous tumor.

A bulging onion on the skin surface (Pap. I, 4th Gen., Line B) was found 3 weeks after the implantation of a weanling. It was 2 mm. across and 3 mm. high, wholly typical, but within 2 weeks more had vanished. No subcutaneous growth formed in this mouse, but these arose in three of four other weanlings similarly implanted.

A weanling of Pap. I, 5th Gen., Line F, was found to have a tiny, superficial pap. 24 days after implantation, which, in 32 days more, became a bulging onion 5 mm. across and 5 mm. high. It was then gnawed down to a saucer. No subcutaneous cyst developed.

Two weanlings of the 6th Gen., Pap. I, Line G, developed surface paps. within 20 days after subcutaneous implantation. One had an onion 6 mm. across, which was gnawed down to a saucer soon after; the other had not only a subcutaneous cyst back of the shoulder but two smaller onions, one of them on the skin over the cyst, the other on that of the flank where it had been slit for implantation. They were taken for section 11 days later (Figs. 34 to 36).

An implanted weanling (Pap. I, 5th Gen., Line F, Subline *b*) failed to develop a subcutaneous growth but after 26 days had an onion similar to that of Fig. 32 *c* of Pap. II, though smaller,—5 mm. high, but only 1.5 mm. across at the base. It was gone after another 8 days.

Several more instances of tiny, superficial paps. that failed to persist might be cited.

These growths were remarkable not only for their resemblance to tar paps. growing *in situ* on the skin (Figs. 56, 58) but in giving no sign of secondary origin. Their neoplastic layer was joined to the adjacent epidermis, as if they had derived therefrom, and at first they grew with noteworthy rapidity but frequently regressed later, or came away,—in which traits also they resembled induced paps. Yet the failure of subcutaneous cysts to arise in most of the mice carrying them makes it likely that they originated from grafts placed just under the epidermis and breaking through it secondarily. One of the specimens taken for the study of early stages in cyst formation has provided an instance in point. The pap. lining a tiny cyst 14 days old (Fig. 20) had united with the epithelium of a hair follicle (Fig. 46), and this cyst had a hassock pap. on its floor. One cannot doubt that it would soon have surfaced.[2]

[2] Despite this evidence for a cellular origin of the surface paps. three extensive attempts have been made to procure a causative virus from the living layer of small to medium sized

### *Results of Introducing Papilloma Tissue Directly into the Skin*

In view of these findings an attempt was made to produce surface growths by tattooing pap. tissue directly into the skin.

A smaller graft than ordinary of Pap. I, 4th Gen., was implanted subcutaneously behind one shoulder of six newborn mice, and another was cut fine in just enough s-L solution to keep it moist, and the tiny tumor bits were stippled into the skin behind the other shoulder. In addition a few of the bits were inserted under the skin through slits left by the narrow sword needle used for stippling. The same was done (but without subcutaneous grafting) to five newborn mice of two other litters, to three mice 1 week old and ten weanlings. Five other weanlings got subcutaneous grafts only.

No skin growths arose on any of the mice although all six of the subcutaneous implants in newborn mice,—which were stippled ineffectually,—gave rise to cysts. These formed also in four of the five weanlings implanted subcutaneously but not stippled.

Vigorous neoplastic tissue had been used in this test. Its negative outcome makes plain that the surface growths deriving from subcutaneous implants owe their success to the well established state of their cells.

### *The Tumors Due to Grafts in the Thigh Muscles*

Grafts of mouse embryo skin fare far better in the thigh muscles than in the subcutaneous tissue, and those of pulmonary adenomas do well there (1). For this reason a graft of Paps. I, II, V, VI, or VIII was often placed in the thigh muscles of weanlings.

Solitary, keratinizing cysts resulted like those in the subcutaneous tissue though enlarging somewhat more slowly. Stalked paps. were consistently absent from them,—a finding for which pressure conditions will acount. After 2 to 4 months they erupted into the overlying subcutaneous tissue and thereafter, like cystic pulmonary adenomas (1), they enlarged much more rapidly, on the outside or inside of the thigh. Dissection cysts extended from them later and often became big, progressing along the side of the body or onto the belly wall, or occasionally down the leg. Meanwhile the intramuscular cysts kept on growing, none becoming stationary or undergoing extrusion though many erupted eventually, forming misshapen acorns. All were fatal, or on the way to becoming so, when their hosts were sacrificed. They killed either as such or through infection with bacteria that entered where the skin over them had become necrotic.

### *Results of Transfer to the Peritoneal Cavity and Viscera*

Grafts of Pap. I were placed in the spleen and liver of numerous weanling mice, and a suspension of the same material was injected into the peritoneal cavity of

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cysts of Pap. I, Pap. IV, and Ear Pap. (*q. v.*), respectively, after these had been serially transplanted. With the assistance of Dr. Betty Roof of this laboratory the scarified skin of many weanlings and young sucklings was inoculated in each instance with a 10 per cent extract in salt solution of pooled, living, pap. tissue, prepared as in the attempts to recover a virus from the cancers secondary to Shope papillomas in domestic rabbits (3); and, as in the case of the rabbits then inoculated, the skin of the weanlings had already been rendered hyperplastic,—this time by applications of croton oil, which were resumed after the skin had healed under paraffined gauze, and repeated for a month. No growths whatever arose.

many sucklings. By ill luck the pap. tissue utilized contained cells of a squamous cell carcinoma as well, and this was present together with pap. in many of the resulting growths, but others were free from it. New cancers often arose from the pap. itself during the course of months; but they were always so local as not to affect the general findings.

A fine suspension in s-L of lining tissue from a cyst of Pap. I, 4th Gen., Line C, was injected (0.07 to 0.2 cc.) into the peritoneal cavity of twenty-one sucklings 2 to 10 days old. Many developed subcutaneous cysts along the route of the injecting needle, and in not a few such instances intraperitoneal growths failed to arise. They developed in others and occasionally were found in mice having no subcutaneous tumors. Always they were cysts, ivory-colored, opaque, round or oblong, with a smooth, glistening surface (Fig. 39). Most of them had arisen where the injecting needle pierced the peritoneal wall, and were fixed here on a narrow, tangential base (Fig. 40); but a few were attached instead, by a ropy connection, to the bowel, omentum or mesentery (Fig. 41). All were poorly vascularized and some, on getting large, became necrotic, except for a thin encapsulating tissue covered with peritoneum (*e.g.* the largest tumor of Fig. 39). Those examined when small contained papilliform growths projecting into the cyst from its base and actively forming keratin (Fig. 40). The pap. tissue extended some way along the cyst wall as an almost even layer, gradually thinning, and beyond it the cyst resembled a dissecting cyst (Figs. 40 and 41). When cancer was also present very big cysts sometimes formed, profusely vascularized and almost filling the peritoneal cavity. Only then was the peritoneal fluid increased, and but slightly. The cancer was nearly always the keratinizing, squamous cell carcinoma primarily introduced. It sometimes metastasized to the liver and lungs, and occasionally to the peritoneal lining.

With ether as anesthetic a graft was placed with a trochar directly in the spleen of ten weanlings. All developed an intrasplenic cyst lined with pap. tissue, usually solitary and occasionally containing a cauliflower growth. Such cysts sometimes became several times larger than the spleen, replacing most of it; yet they remained within the capsule of the organ, which retained its general shape as if the greater part of it had undergone a firm, ivory-colored elephantiasis. The squamous cell carcinoma often complicated matters.

The findings in ten weanlings with a graft in the liver were curiously different. Tumors arose in eight, all keratinizing cysts, sometimes several which fused with result in a multilocular growth. But soon they became walled off from the hepatic tissue by a thick capsule; their advance into the liver ceased; and they extended out into the peritoneal cavity (Fig. 42), where they occasionally became large. Only in the well vascularized region next, or near to, the hepatic tissue was a living layer of pap. present (Fig. 42). Again cancer was frequent.

Some of the fine suspension used for intraperitoneal injection (0.1 cc.) was slowly injected into the spleen of four weanlings next its hilum, with the aim of starting hepatic tumors by way of the blood stream; and fragments of the pap. tissue could be seen passing along the splenic veins. Yet no growths arose in the liver during 4 to 6 months, although good-sized cysts formed in the spleens of two of the mice.[3]

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[3] Successful implantation of the paps. in the pulmonary tissue has proved difficult. Dr. Roof permits us to report that she has placed tiny grafts by trochar in the left lung of many sucklings, but only one growth resulted after the lapse of several months. This, a characteristic cyst full of dense keratin and with a low pap. lining, had replaced the entire lung. In two instances out of 15 in which she injected a fine suspension of pap. cells into the main caudal vein of many weanlings tiny, solitary nodules of pap. tissue were found 2 months later, filling a number of adjacent alveoli, but without cyst formation as yet. Bits of Shope virus papilloma, injected intravenously into rabbits, give rise to similar alveolar growths soon after lodging in the lungs (9).

Several significant facts emerged from this test. Pap. I proved incapable of extending along the peritoneal wall, though establishing itself where this had been pierced and forming the characteristic cysts. These were its regular expressions also in liver and spleen, and they were so for the same reason, namely inability of the pap. to invade the connective tissue soon surrounding it.

### *The Differences in the Tar Papillomas Disclosed by Transfer*

Maintenance in the subcutaneous tissue has brought to light significant differences in the papillomas.

Pap. I, the growth propagated longest, has done best, giving rise to steadily enlarging cysts in most mice receiving it; but Pap. V has not been far behind, though death of its cysts by keratinization while small is a less rare occurrence. The original Pap. V was an "onion" like Pap. I and its derivative tumors have been closely similar, though occasionally their keratin has been even denser, more solid, and firmly fixed to the living pap. Always on drying it has had a clouded amber hue, not dark brown like that of Pap. I. Central, stalked paps. have frequently been formed by Pap. V, and herniation, dissecting cysts, acorns, buttons, and cutaneous horns have been usual phenomena.

Pap. VIII, a tiny, dry cone with fleshy base, implanted in only four sucklings, grew in two, producing the characteristic cysts. All of the many tumors of its 2nd Gen., Line A, abruptly underwent necrosis when more than a centimeter across, through an infection that only then manifested itself. A single growth resulted from the five implantations for Line B,—the cutaneous horn of Fig. C 11, which contained a stalked pap. (Fig. 25). Since no transfers were made from the horn, Pap. VIII was lost.

What may have been a variant pap. was observed in one cyst of Pap. I (Fig. 13), with signs of it in another (Fig. 15), but it was not encountered again.

Paps. I, V, and VIII fall into a single category, which will be termed Type A because their cells are able-bodied. The neoplastic tissue of these tumors has regularly exhibited marked power of lateral spread, with result in a proliferating layer on the cyst wall, and this has produced keratin in abundance, as a solid, dense core to the cysts. The stalked paps. lying amidst this core have been solitary (Figs. 22, 25, 27, 28) and their connection with the cyst floor has been at its middle,—a fact testifying to spread of the pap. tissue from the original graft in every direction along the cyst wall. Paps. I and V have succeeded in nearly all sucklings and most weanings ever since the first transfers.

Many of the tumors deriving from Paps. II, IV, and VI have had a wholly different structure because their cells have lacked the ability to spread laterally. Such cells are more or less crippled, and hence the growths due to them will be termed Type C papillomas.

When Type C cells wholly failed to spread, they nevertheless formed progressively enlarging, subcutaneous cysts, yellow and opaque and spherical or oblong, like those of Type A but fluctuating because full of fluid. Their hue was due to

keratin as a paste or in suspension. When opened they were found to contain in addition one to several sessile or stalked paps., based here or there as if by chance, with the cyst wall everywhere else bare and glistening. It was as if these paps. were enclosed in dissecting cysts, and this proved to be the actual case. The cysts ruptured when not very large, 2 to 3 cm. across, and where this happened either a secondary dissecting cyst formed or fluid leaked out next an overlying scab of skin and death from infection followed. Fluid pressure was responsible for the break in the cyst, not keratin accumulation as in the growths of Type A. For relatively little keratin was formed, as shown by the small amounts existing in cysts several months old, containing big, cauliflower papillomas.

The course of events was obvious. The cauliflowers derived directly from the original grafts as such, or from pieces of them which failed to unite because of the crippling of their cells. For the same reason these latter also failed to line the graft pocket. But they did form keratin; and this so inflamed the wall of the pocket that extravasation took place into it, just as into the secondary dissecting cysts from growths due to paps. of Type A; and, as more and more keratin was produced, and more fluid collected about it, the pocket was converted into a steadily enlarging cyst (Figs. 33, 44, 45 *a* and *b*). Any later attempts of the pap. tissue to spread were frustrated by the keratin-induced inflammation of the cyst wall (Figs. 11, 12, 33, 44). Such inflammation checks the spread of even the paps. of Type A, as witness the bare walls of dissecting cysts (Fig. 10).

Amidst the thin fluid filling the cyst the cauliflowers had ample space for proliferation and sometimes this had singular consequences. Not only did stalked paps. form, but a second, stalked growth on top of the first, and even a third on the second (Figs. 45 *a* and *b*). The secondary dissecting cysts were at first devoid of any real wall (Fig. 44).

These were extreme instances. Most of the tumors of Type C contained numerous cells capable of spreading laterally; for many of the cysts they formed were partially lined with pap. tissue, though with scattered, bare patches amidst this, and they contained a pultaceous keratin instead of fluid; while others yet resembled in all ways those of Type A. Unfortunately the growths chosen for transfer on the first few occasions,—before the existence of Type C was realized,—were of this last sort, as providing the best material for maintenance of the tumors; and because of such selection extreme instances of crippling are now exceptional, whereas formerly common. Stalked paps. are seldom encountered now amidst the dense keratin of the subcutaneous cysts of Type A, and it seems probable that in these latter a selection by grafting has also taken place of the cells most capable of spreading laterally.

The paps. of Type C yielded relatively few tumors on first transfer to sucklings, almost none in weanlings, and those appearing grew rather slowly. The takes increased on serial passage, yet the tumors have been more difficult to maintain than those of Type A.

In corollary to the transplantation of tar-induced growths one of us (Allen) has transferred a pap. of ordinary aspect that originated deep in the outer ear of a C mouse injected into the thigh muscles with a 1 per cent solution of 20-methylcholanthrene in olive oil. This growth (Ear Pap.) was not noted until nearly 6 months

afterwards and no tumor had arisen in the thigh. It grew very slowly for 4 months more and then bits of its fleshy base were implanted subcutaneously in sucklings and weanlings. It did well in nearly all and proved to be of Type A (Figs. 47 to 51). Now, after 14 months, it is in its 5th Gen., owing to successive early transfers; but its growth is so slow, though progressive, that sometimes it is only 2 cm. across after a year. The tumor may well have arisen from the big sebaceous gland just outside the ear drum; for the pap. lining a cyst of its 1st Gen. contained some functioning glands of this kind (Figs. 37 and 38). It is notably benign, with but a slight tendency to carcinomatous change, and has the further peculiarity that its keratin, on drying after erupting, sometimes splits apart, with result in tumors of singular form (Figs. 50 and 51).

### *The Relations of Tumor and Host*

Grafts of the paps. have given rise, as a rule, either to tumors that grew steadily or to none,—when the grafts presumably consisted wholly of encapsulating connective tissue, so difficult is this to discriminate from the neoplastic. The sex of the host has had no influence on the tumors nor has their incidence differed from litter to litter of sucklings except when these were in poor condition and then the cysts enlarged slowly. Tumors arose almost as often in 7 to 10 day old sucklings as in the newborn, but were fewer in weanlings and slower growing, for which reason they were utilized often to conserve the paps.,—which still progressed after their hosts matured. Only Pap. I has been implanted in adult mice: it took less frequently than in weanlings and its growth was notably slow.

The pap. tissue never necrosed except from intercurrent infection, and no histological evidence was come upon of host resistance. No accumulation took place of lymphocytes, plasma cells, and macrophages around the small tumors that ceased growing because wholly keratinized, and it seems likely that these latter had consisted of pap. cells inherently lacking in vigor and hence failing to multiply as fast as they differentiated. The keratin content of such cysts caused them to persist, but they became lenticular (Fig. 15) and flaccid, owing to extravasated fluid. The same foreign body reaction took place along their walls as keratin calls forth around dissecting cysts.

Though growing steadily the paps. of both types took 3 to 5 months to kill implanted sucklings. These soon became wizened and scrawny, often weighing at death no more than 9 or 10 gm. exclusive of their massive tumors. Weanlings survived somewhat longer. The dissecting cysts sometimes became enormous (Fig. C 4). The great majority of the mice died of inanition.

### *The Derivative Carcinomas*

Sections of Pap. I as used for its primary transfer showed underlying nests of what looked like squamous cell carcinoma (Fig. 2); and an indubitable cancer of this sort was found in the wall of two of the five cysts of the 1st Gen. examined microscopically. By careful selection of grafts it was left behind. The first transfers of Pap. IV also yielded growths containing a squamous cell carcinoma, and this overgrew the associated pap. in the 3rd Generation.

Throughout the long propagation of the other tumors new cancers have arisen frequently,—and as often now as at first. Their incidence has not manifestly differed in the two types. Their sporadic occurrence and highly various character have alike attested to their origin through superimposed neoplastic changes in the pap. cells, and they have ranged morphologically from malignant papillomas (Fig. 52) to almost wholly anaplastic, squamous cell carcinomas. The longer the life of the tumor the oftener have they been found, almost always near the middle of the cyst floor where the pap. layer was oldest, thickest, and most crowded, that is to say where most cells existed susceptible to change.[4] Cysts 4 to 5 months old have tended to contain them; but many still older have shown none in the gross. Almost never have they caused death.

The softening of a cyst of Type A, previously rendered firm by keratin, is the first gross sign of the presence of cancer; fluctuation later on, together with rapid enlargement, testify to its dominance. The development of fluctuating cysts forthwith, where pap. tissue of this type was implanted, means that malignant cells were transferred as well and have taken over; but no such inference can be drawn in the case of Type C. The existence of a small cancer amidst a cyst lining in which it cannot as yet be discerned is often indicated by a softening of the keratin overlying it, as result of fluid exudation. Malignant papillomas may build up a firm mass of keratin, but tiny, translucent spots amidst it make plain the presence of living, cancerous tissue as well.

The pap. tissue always has a sparse blood supply, but where cancer is present in quantity it is relatively rich. Hence in selecting graft material well vascularized cysts have been avoided. In another and crucial way maintenance of the paps. has been rendered feasible, namely by the inability of the cancers to extend laterally along surfaces: they never form masses notable in the gross where the cyst lining has spread thin. Here the chances are good that the pap. cells will have far outstripped the cancerous, and grafts have been taken as routine from such regions. If all is well the lining can be stripped away from the encapsulating connective tissue by pushing it sideways with a knife, as when cleaning a raw pelt. If the scalpel meets resistance, or the bared connective tissue shows spotty opacities suggestive of keratin nests, one tries elsewhere, or preferably turns to another tumor of the same lot and if necessary to yet another. Many of the cysts of Line G, Pap. I, in its 2nd to 5th Gen., had a squamous cell carcinoma so widely distributed and well hidden amidst an even pap. lining as not to be seen in the gross, though revealed by the microscope or by lung metastases (Fig. 53); yet even Line G was at length rescued by graft selection and it is now in its 9th Gen. This selection was not possible with such Type C tumors as provided only cauliflower growths inside dissecting cysts; and it is questionable whether Paps. II and VI could have been long maintained had not advantage been taken of the ability of some of their cells to spread laterally, and outstrip such cancers as may have arisen from them secondarily.

The cancers have been notably indolent as compared with those appearing under tar papillomas *in situ*. Many of them must have been directly transferred to new hosts together with pap. tissue, in Line G for example, and hence have got off to a new start at the same time as the latter; yet none has been more than a centimeter across after months. Their growth has been

[4] The same holds true of the cancers arising in the broad expanses of papillomatosis resulting from broadcast inoculation of the Shope virus into large areas on the skin of domestic rabbits (10).

almost entirely outwards through the capsule of the cysts, and occasionally they have anchored these to the body wall of muscle. Metastasis to the lungs has been not infrequent (Fig. 53) but never to the lymph nodes, a curious fact in view of their subcutaneous situation.

As already stated, tongues of the neoplastic cells of Pap. I have often extended along a broad front into the tissue encapsulating its cysts (Fig. 14). Yet these carcinomatoid activities (11) have been regularly checked after a time and grafts taken from regions where they were manifest microscopically have yielded wholly benign growths of the usual sort.

## DISCUSSION

The epidermal papillomas induced by carcinogenic agents are justly regarded as exceptionally benign, and they are prone to disappear unless aided. Yet those of the present work have grown progressively after transfer to deep situations, and killed in most instances. The first question to arise is whether they have been typical, and if so, whether their cells did not alter on transplantation.

The cytology of the tumors has remained throughout that of ordinary paps., and subcutaneous implantation of them has now and again resulted in growths on the skin surface closely resembling those induced there by tar, if often much larger (Figs. C 13, 32 a). An uninformed observer might have supposed that they had originated directly from the epidermis of the host, and small ones (Figs. 34 and 36) have tended to regress or come away, as do the generality of small, induced paps. Carcinomas have frequently taken off from the transplanted paps.

The conclusion seems warranted that the growths studied have been papillomas such as carcinogens elicit and that they have undergone no significant changes throughout their long propagation. Everything indicates that they are neoplasms of a single kind, as has been generally assumed of induced papillomas despite their widely various gross form.

This is not to say that the cells of all induced paps. have similar capabilities. Transplantation has disclosed differences in the capabilities of the growths that have not heretofore been perceived. The cells of those of Type A retain the two prime abilities of normal epidermal cells: they form a keratinized layer protecting the skin, and they cover denuded surfaces at need by lateral spread. They are able-bodied cells in a wholly literal sense. The cells of Type C paps. are, in contrast, crippled: they have lost to a greater or less degree the ability to cover bare surfaces, and it would appear, from the small quantity of keratin elaborated by the large cauliflowers, that they fall short in this respect as well (Fig. 44).

The diametric differences in the character of the cysts produced by A and C paps., in typical instances, have followed automatically from the differing

capabilities of their cells. The A tumors, turned outside in, and massing keratin in consequence, undergo expansive enlargement because of its interior pressure. They have become introverts in a strictly material sense of this term,[5] and they enlarge because the actively proliferating and differentiating living layer of the papillomatous cyst shoves outwards by pushing inwards, adding more keratin to the unyielding lump it already encloses. Extraordinary, multiform happenings ensue which are disastrous to the host mice (see Figs. **C 1** to **C 13**). Those C tumors, on the other hand, which form fluid-filled cysts with discrete papillomatous structures projecting into them are not really introverted. Yet they too kill through the keratin they produce, though in their case because of the inflammation and exudation that it calls forth. The logic of pathology could not be more clearly exemplified than by the course of events in both instances.

The findings as a whole make plain that the pap. cells are not constrained by some interior urge to form papillomatous structures. They produce these only because of lack of room, not where they have space for lateral extension, this activity taking precedence even of differentiation (Figs. 9, 16, 17), as in the case of normal epidermal elements (8). They cannot make sufficient room for themselves by invading and supplanting normal tissues as cancers do; and since no side-slip accompanies their intercalated multiplication, foldings on a supporting stroma inevitably occur, the result being papillomas. The shape of these growths is a mere epiphenomenon. Intrinsically, the tumors are keratomas,[6] their neoplastic cells functioning as do those that form hormones or melanin but in a more immediate way; for they are themselves their own functional products, keeping the body protected by differentiating to a squamous, keratinized state, if in some respects pathological, and covering bare surfaces whenever they are still capable of doing so.

[5] *Introvert* and *extrovert* were used in the material sense, both as noun and verb, long before psychologists so revealingly took them over (*cf. New English Dictionary on Historical Principles*, Oxford, 1888–1933). They express the state of the papillomas precisely. These latter are by nature harmless extroverts, denizens of the skin surface. When those of Type A are rendered introverts by deep implantation, their structure and course are radically different, and they nearly always kill their hosts unless they attain to the skin surface secondarily, when they again lead extrovert lives. This regaining of the surface is passive on their part, a breaking through of no greater import than that of a boil, save for the curious circumstance that they themselves manufacture the inert material responsible for the break. The fact that many of the erupted growths become tangential to the body surface later on, as if practically cast forth from the organism, is obviously due merely to pressure from the underlying tissues, with the tumor passive in such relation.

[6] The word *keratoma* is in disuse save as applied to non-neoplastic skin conditions. The *Quarterly Cumulative Index Medicus* of the American Medical Association sometimes lists it, but with "see *Keratosis*" following. It has gained no place as yet in the *Current List of Medical Literature* of the National Library of Medicine.

### *Previous Knowledge of the Papillomas*

Yamagiwa and Ichikawa, who first induced cancers by tarring rabbit skin 43 years ago, noted that numerous papillomas preceded them (12). They regarded these as mere local, hyperplastic excrescences and this was the prevailing view throughout the next 10 years, both as concerns them and the tar-induced paps. of the mouse,—to which attention had soon turned (13). Their attempts to transplant rabbit paps. failed (12), as did those of Ferrero (14). In our own laboratory almost sixty active rabbit paps. have been grafted into the leg muscles[7] and subcutaneous tissue of their adult hosts without any success (11, 15).

Murray and Woglom made autografts from the paps. of tarred mouse skin and, getting only cancers, inferred that all tar-induced neoplasms are malignant (7). Kreyberg, repeating their experiments, procured no growths whatever (16). Mottram (2), utilizing many more papillomas and transplanting to other mice, obtained either cancers or small, keratinizing cysts lined with what looked like ordinary epidermis. He worked perforce with mongrel animals and implanted his material in adults.

Woglom, reviewing what was known of the papillomas in 1926 (13), reported that those induced on mouse skin so closely resembled those of the rabbit that no special description of them was necessary. Subsequent experimentation has justified his view, and for present purposes knowledge of the two can be merged almost completely.

The earliest investigators noted that many induced paps. fell off or dwindled away, and Mider and Morton reported in 1940 that all those elicited in mice by a single application of 20-methylcholanthrene regressed spontaneously, whereas if the applications were kept up some became established (17). Almost concurrently the observation was made that those elicited by tarring rabbit skin regressed or fell off if this was stopped after their appearance, and the fact was also noted that some which had regressed would reappear almost at once, even after months, if tarring was resumed (18). The firm fixation of the skin to the cartilage on the inner side of the rabbit ear enabled this fact to be discerned, because here induced cutaneous growths can be charted repeatedly in their relationships, like constellations, and identified time after time, with successive tracings of them individually and punch biopsies at will. It was further found that not carcinogens alone but various agents and conditions devoid of their specific effect yet stimulating normal epidermal cells to multiply would also elicit the paps. again (18, 19). The growths thus recalled to existence,—and often this can be done repeatedly,—are "conditional" tumors (18), made up of neoplastic cells needing aid if they are to multiply; and if this aid is maintained some of them may become permanently established. It was also shown

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[7] Dr. Roof has lately transferred to the leg muscles of newborn rabbits pieces of many well-established papillomas induced long previously with tar, but not a single tumor has resulted. Papillomas induced in domestic rabbits with the Shope virus do well after transfer to suckling rabbits (4), as already stated, whereas only autografts succeed in adults.

that the carcinogens cause numerous cells of rabbit skin to become capable of forming paps. which fail to do so ordinarily, yet which will produce such growths, even after they have long lain latent,[8] if either these agents or stimuli devoid of carcinogenic effect are brought to bear on them (18, 19). Rabbit pap. cells are frequently so far subthreshold as to need persistent urging (20), but some are so responsive to stimulation as to mimic squamous cell carcinomas temporarily (11, 18).

These various findings made plain that two distinct steps, differing basically in character, are concerned in the production of cutaneous paps.: "initiation" as it was termed, a process peculiar to carcinogens, whereby normal cells are changed to tumor cells, and "promotion" of the multiplication of the changed cells (5, 6),—which can be brought about by many non-carcinogenic agents.[9] Most carcinogens not only initiate but promote the formation of growths by the cells they have altered, and tar is remarkably effective in such relation (18). The "co-carcinogens," so called, are actually promoting agents.

Many of these findings were soon corroborated in the mouse and extended, notably by Berenblum and his associates (21, 22). The generality of the paps. induced in mice are now realized to be conditional tumors, and so too are various other neoplasms of several species (23, 24). It has become evident that the application of carcinogens to mouse skin results in numerous latent pap. cells, and the special effectiveness of croton oil in stimulating their growth in mice has been repeatedly stressed (22). The existence of two steps, initiation and promotion, in the formation of mouse paps. has also been recognized (21, 22).

The question can now be taken up of why the papillomas induced on mouse skin fare badly at this situation.

No close study has been made of the successive changes taking place when mouse paps. are in process of retrogression. Indeed the difficulty of following individual growths is great in this species owing to looseness of the skin, its lack of fixed points, distortion by scar tissue after biopsy, and the frequency with which derivative cancers mar the record. Rabbit paps. can be followed with certainty on the ear, for reasons already given, and cancer seldom supervenes.

Some of the paps. are lost through exfoliation, their cells keratinizing faster than they multiply and often desquamating completely (18). Others, when broad-based, dwindle gradually, their edges retreating; and those that are narrow-based often come away wholly (18). Mouse paps., as observed in the gross, undergo similar changes, and the assumption seems justified that these result from similar causes. The prime reason for these events is the wholly unaggressive character of pap. cells. Although

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[8] *Latent*, as telling merely that the cells do not manifest themselves, seems a more fitting term for them than *dormant*, which has special physiological implications.

[9] Initiation and promotion, while often consecutive, are not obligatory stages in the production of all tumors, as has sometimes been assumed. Initiation is crucial; without eggs no omelet: but the cells of many tumors are so well equipped primarily as to assert themselves without any promotion, and those of some do, from the first, all of which they are capable. Yet that most tumors grow faster on extraneous stimulation, clinicians know only too well.

those of the mouse are capable of multiplying endlessly, as here disclosed, they have no innate ability to invade and replace normal structures; and when proliferating on the skin surface they meet the obstacle of the epidermis to which their own layer is directly joined (Figs. 34, 36, 56, 58). Rabbit pap. cells are no match for this when the skin reverts toward the normal; they cannot then even hold their own against the ordinary epithelium but are gradually supplanted (18). And even when mouse paps. are doing well it would appear that often some local influence acts to repress their growth next the epidermis to which they are joined. When two epidermal sheets eventually meet, after advancing from either side of a superficial wound, no noteworthy piling up, due to excess proliferation, takes place; nor does this happen where deep paps., after surfacing, have united with the epidermis secondarily (Figs. 29, 31, 34, 36).

The corium also offers a formidable obstacle to paps. proliferating *in situ*. After transfer to the deep tissues they gain room for their enlargement in one or the other of two indirect ways, through pressure of the keratin continually accumulating in lined cysts or through the extravasation it calls forth in the unlined. But a dense corial layer virtually next a free surface presents conditions decisively unfavorable.

The keratin piling up over superficial paps. does not help them at all; for though it may temporarily force their living layer a little way downward at one spot or another (Fig. 54), it gains no purchase, nor can it inflame the connective tissue. Actually it often hinders the broadening of the tumors, because their living layer is tethered to it. Its shrinkage on drying sometimes pulls this layer up in an arc and lifts the adjacent skin too (Figs. 24 and 56). The saucers of the present work have raised rims because of this happening (Figs. **C** 7 to **C** 9 and 24) and it accounts to some extent for the onion shape of many paps., their abruptly narrowing, dry tops, and sides bulging out over a base that appears constricted (Figs. 34, 36, 58). Thrombosis and edema are frequent within such growths (Figs. 34, 36), and often they are literally pinched off at the base by the encroaching, normal epithelium. Active paps. frequently thrust tongues downward into the corium yet these never get far, reinforced as this is by reactive connective tissue; and no instances are on record of progressively enlarging cysts as result of such invasion. Even when papillomatous change takes place on the walls of hair follicles previously rendered cystic by concentric layers of retained keratin the resulting growths soon erupt (Fig. 59).

The contrast between the poor showing of the paps. in their native habitat and their success after transfer to deep situations finds expression in their differing fates when carcinomas originate from them. Those on the surface, "staying put" when this happens, are eroded by the malignant growth and come away, leaving an ulcer with a cancerous floor (Fig. 57). But when cancer arises from a cyst lining, the pap. outstrips it by further spread along the wall, as the cyst enlarges, and flourishes beyond the malignant growth, eventually killing the host. Only when the pap. is no longer possessed of spreading power, as in the extreme instances of Type C, *e.g.* Pap. IV (Figs. 45 *a* and *b*), is it destroyed by the cancer.

All this is well enough but it fails to make plain why induced papillomas that have got off to a good start and spread rapidly (Fig. 60) never take over large cutaneous areas, no matter how persistently urged. The promoting agent stimulating them has rendered the adjacent skin abnormally vascular, loosened its texture, and the activated cells of the pap. are replacing the merely hyperplastic epidermis (Fig. 60). The stage seems all set for further success, yet the growth comes to a halt. Why should this be? A generalized resistance elicited in the host can scarcely be invoked to explain it; for often new paps. appear and grow fast while others are dwindling, as all observers have noted. No histological signs of resistance were to be seen around the paps. of the present work. Here for the nonce the riddle must be left.

On casting about for other instances resembling the paps., in that retained normal attributes determine tumor structure and behavior, one thinks at once of those prostatic and mammary cancers which succeed because their cells are urged on by the hormones responsible for the activity of normal elements of the same sort. But these are instances of *direct promotion* of neoplastic growth and of a strikingly effective kind: the hormones not only stimulate the tumor cells to multiply but do greatly more to them, enabling them to invade and metastasize. In contrast epidermal papillomas succeed at deep sites only because of an environment so favorable as to give full scope to the innate ability of their cells to proliferate endlessly. This is *indirect promotion.* No new powers are conferred on the cells and such direct stimulation of them as may occur is incidental and episodic (Fig. 14).

These are pure instances. The stimulating influence of Scharlach R to increase the malignancy of mammary carcinomas of the mouse (25) and to cause rabbit papillomas due to the Shope virus to become huge and invasive (26) provides other examples of direct promotion. But the indirect is much more common. When tumors start forth where a hole is healing that was punched in a rabbit ear previously tarred, the hidden neoplastic cells near its edge have not only been released from their tissue context but given space in which to multiply, and provided with a succulent stroma and a profuse blood supply. Under such circumstances, they outdo the adjacent, normal epidermis despite its vigorous hyperplasia; yet one cannot suppose their cells to be directly stimulated by the tarring if this has long since been discontinued (5). But what if it is resumed? Then the papillomas on the healed area often become enormous, the activated neoplastic cells eliciting the support they need even though the tumors do not enlarge at the base (19). It would appear that many agents and influences encouraging tumor growth, some carcinogenic hydrocarbons amongst them, act both directly and indirectly, that is to say through *dual promotion.* This will often prove difficult to analyze.

Needless to say promotion plays a role not only in what primary tumors do but in the occurrence, situation, and behavior of metastases. The appraisal

of its influence on the generality of neoplasms has yet to be undertaken. That this may bring substantial rewards is obvious from the present day success in counteracting hormone with hormone, when dealing with growths spurred on by such agents. But the scientific interest of the task is broader than the practical; for only through recognition of the extraneous factors influencing neoplastic cells can one come at their innate capabilities, and fully comprehend their unnatural behavior.

## SUMMARY

Six out of eight epidermal papillomas, induced with tar in mice of homogeneous strain, have grown after transfer to the subcutaneous tissue of sucklings and weanlings. Five of them have been thus maintained for nearly or quite a year and a half, and in seven to nine successive groups of mice. The tumor studied longest has been kept going in five parallel lines since its primary implantation.

The papillomas have all grown progressively in most instances, and proved fatal. None has altered except through the occurrence of derivative cancers, but these have arisen so often as only to be excluded on transfer by a rigorous selection of grafts.

Histologically the papillomas have been of a single, completely unaggressive kind, yet transfer has disclosed great differences in their abilities. The tumors they form are of unique sorts. The cells of some are able-bodied (Type A), capable of spreading along bare connective tissue and keratinizing like normal, reparative epidermis. They line graft pockets, differentiate into the free space these provide, and form cysts densely packed with keratin. The papilloma is thus turned outside in. The cysts become huge as keratin accumulates in them, and eventually they rupture with result either in subcutaneous dissecting cysts or keratinizing surface growths that are often prodigious in size and fantastic in shape, but sometimes are completely like the cutaneous papillomas ordinarily induced by carcinogens, and tend, when small, to regress or come away as these frequently do. One growth of Type A was placed in the peritoneal cavity or in the liver, spleen or lung, and at all these situations it formed introverted cysts resembling the subcutaneous.

The cells of other papillomas are more or less crippled (Type C). In extreme instances they are unable to spread laterally, and produce relatively little keratin. They fail to line graft pockets, but their keratin inflames the exposed connective tissue, extravasation ensues, and a continually enlarging, fluid-filled cyst forms, with walls that are bare except where a stalked or cauliflower papilloma exists, projecting inwards. At last the cyst ruptures and a second dissecting cyst forms, also devoid of papilloma tissue; or else the overlying skin undergoes pressure necrosis, the cyst fluid escapes through a rent, and fatal infection ensues.

All gradations exist between Type A and Type C. The cancers derivative from both exhibit a marked disability,—though invasive they are almost or quite unable to extend along bare connective tissue. The papillomas that are possessed of this faculty spread beyond them along the cyst wall, and kill the host through their unceasing activity.

In collateral work a papilloma was transplanted that was found protruding from the external auditory canal of a mouse which had received an intramuscular injection of methylcholanthrene many months previously. The tumor is now in its 5th generation, after 15 months. The growths it forms are of Type A.

All of the papillomas are functioning tumors, with their own cells as the functioning product. Their papilliferous shape, when on the skin, is due solely to inability of their cells to gain space in other ways. Intrinsically they are keratomas.

The papillomas do well after transfer to deep situations because the growth of their cells is *indirectly promoted*, through favoring local conditions. No *direct promotion* takes place like that when the cells of prostatic and mammary tumors are stimulated to multiply by hormones. Doubtless many agents act in both ways, that is to say by *dual promotion*.

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## EXPLANATION OF PLATES

The photographs were made by Mr. J. A. Carlile.

### PLATE 5

FIG. C 1. Adjacent tumors of Pap. I, 2nd Gen., Line C, resulting from a subcutaneous implantation of graft tissue in a newborn mouse 38 days previously: to show the typical opacity, scarcity of blood vessels, and ivory hue of such growths. × 0.7.

FIG. C 2. Subcutaneous tumor, 61 days old, of Pap. I, 3rd Gen., Line B. The mouse, newborn when implanted, is abnormally small as are several others here pictured. The growth is a thin-walled cyst like that of Fig. C 5, packed with dense keratin but showing bulges on its surface that betoken its early rupture. It looks almost devoid of blood vessels. × 0.57.

FIG. C 3. Spherical, subcutaneous cyst of Pap. I, 3rd Gen., Line E, cut across its center. The mouse had been implanted when 2 days old, 113 days previously. The cyst is full of close packed, dense, almost dry keratin of yellow hue, save where marred by hemorrhage from a severed capsular vessel. Its lining of pap. tissue is so shallow as not to be visible. The tumor weighed 5.9 gm. and the mouse 9.3 gm. × 0.58.

The crimson stain on the fur of this mouse and others was put on for their identification.

FIG. C 4. Dissecting cysts much bigger than the pap. cyst from which they have extended outwards. The mouse was newborn when implanted and the tumor is now 86 days old (Pap. I, 3rd Gen., Line C). *A* = pap. cyst. *B* and *C* = dissecting cysts. In the region where *C* projects most, the skin has been rubbed and is dark because dying. × 0.47.

FIG. C 5. Cyst of Pap. I, 2nd Gen., Line D. The mouse was newborn when implanted and had just died when photographed after 83 days. The tumor, a somewhat flattened sphere, is well-nigh tangential to the body surface and the ivory hue of its keratin shines through both cyst wall and skin. Brown, dry keratin can be seen within a crater at its top; much had been eaten out by cage mates. × 1.

FIG. C 6. Saucer due to the gnawing down of an erupted growth. The mouse, 2 days old when implanted, had a big, spherical cyst 47 days later, with an overlying scab of dead skin (Pap. I, 3rd Gen., Line E). Between then and the 93rd day all but the floor of the cyst was gnawed away by cage mates, with result in this superficial saucer, having a rolled edge and projecting sides covered with skin underneath. The contents of the saucer is keratin, pale and rough where freshly gnawed. × 0.58.

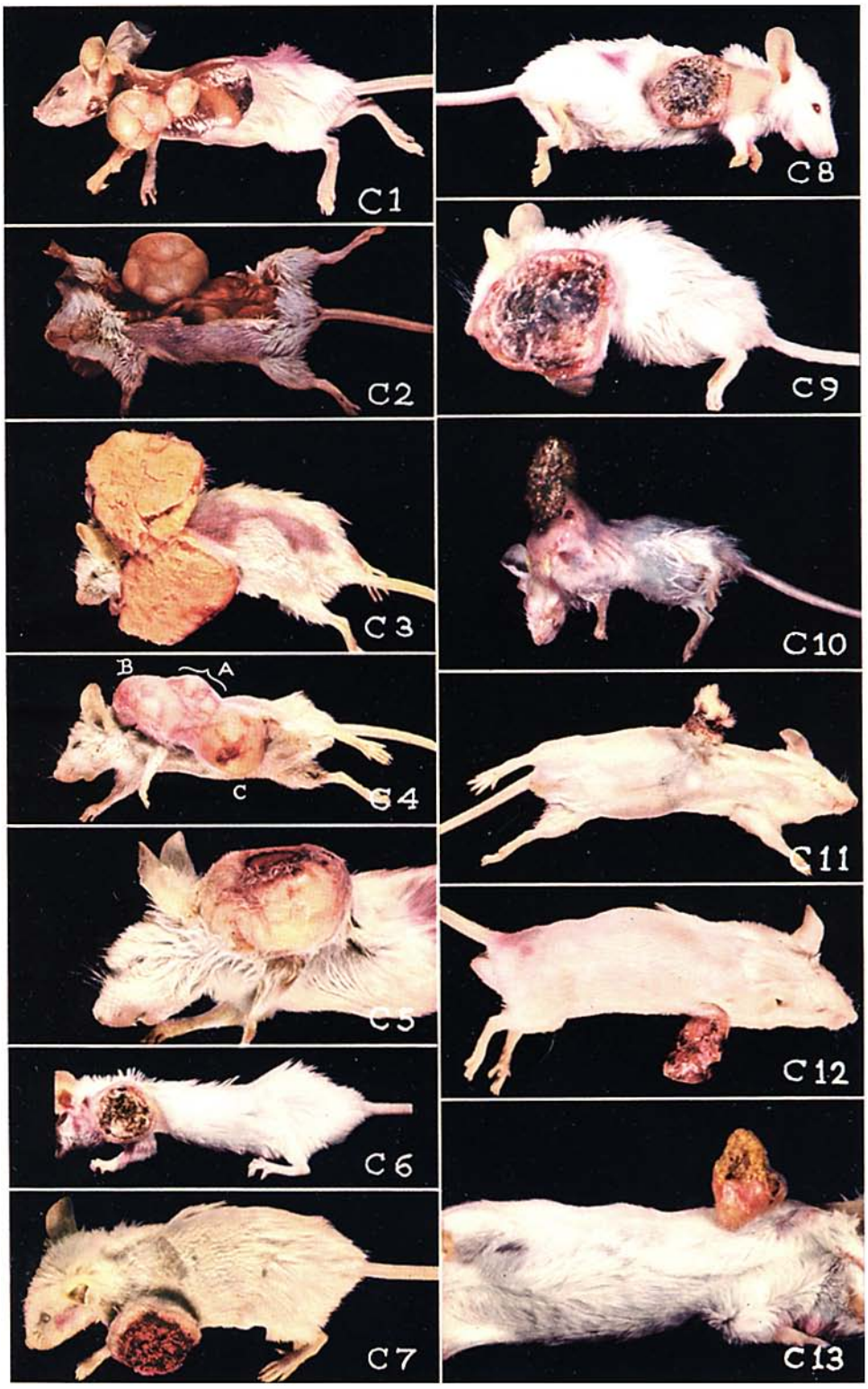
FIG. C 7. The same saucer after the mouse had been isolated for 21 days. The weight of the keratin formed since the last photograph has rendered the growth pendulous. × 0.58.

FIG. C 8. Superficial saucer resulting from a subcutaneous implant in a weanling mouse (Pap. VI, 2nd Gen.). The tumor was a subcutaneous cyst after 51 days, but erupted later and by the 91st day had become a thick, dry-topped, intradermal button. After 153 days it was the saucer shown, almost tangential to the body, full of dry keratin, and with a small smoothly convex base. (See Fig. 24.) × 0.58.

FIG. C 9. Huge saucer (Pap. I, 4th Gen., Line C) due primarily to the gnawing down by cage mates of a big acorn tumor. The saucer was 25 mm. across, 117 days after subcutaneous implantation of a week old mouse, but when the picture was taken, after 140 days in all, it had broadened to 29 mm. by extension forward just under the skin,—which here persists, blackened but carrying white hair. The rim of the growth is raised high above the body surface. × 0.7.

FIG. C 10. Horn due to the eruption of a subcutaneous cyst while still small (Pap. I, 4th Gen., Line Ca). The mouse had been one week old when implanted and the cyst erupted after 28 days, when only 6 mm. across. It was based on the deep subcutaneous tissue, and by continual enlargement and keratinization gradually formed the horn shown (185th day). This

*Concluded on reverse of Plate 5*




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 6

All sections for the microscope were stained with methylene blue and eosin.

FIG. 1. Section across the basal portion of Papilloma I (Pap. I), from which grafts were taken. It is a typical, active pap. with some downward extensions into the underlying reactive tissue. × 19.

FIG. 2. Higher magnification of central part of the same section. The islands of cells in the deep tissue are like those of a squamous cell carcinoma. × 49.

FIG. 3. Section from used portion of Pap. V,—a vigorous, wholly superficial pap. × 14.5.

FIG. 4. Section from used portion of Pap. VI,—which looks exceptionally benign. × 14.5.

FIG. 5. Fresh spec.; lining of a small cyst of Pap. I in its 3rd Gen., Line E,—to show the papillae much magnified. They are exceptionally large, glisten because washed, and are so crowded along the lower side of the cyst that here its wall has rolled outwards. × 2.7.

FIG. 6. Edge of the lining of a cyst, 97 days old and 2.5 cm. across, due to a graft in a weanling (Pap. I, 5th Gen., Line B): to show the gradual thinning of the pap. layer,—which here is devoid of papillae,—and the bare wall beyond at the right. Photographed under water after formalin fixation. × 2.7.

FIG. 7. Cross-section of a subcutaneous cyst 40 days old (Pap. I, 3rd Gen., Line B); some of its keratin has been lost. The pap. lining appears crowded along its floor (lower side of cyst), and the living layer is thicker here than elsewhere. It thins toward the body surface, the pap. foldings become less frequent and lower, and near its top on the right the wall is bare and encapsulation well-nigh lacking. An arrow marks the part shown in Fig. 23. × 6.1.

FIG. 8. Wall of the cyst of Fig. C 2 through one of the bulges there shown. Here the wall, devoid of the pap. layer covering it to either side, is exceedingly thin. The growth looks wholly benign and dense keratin still overlies it at some places. × 11.4.

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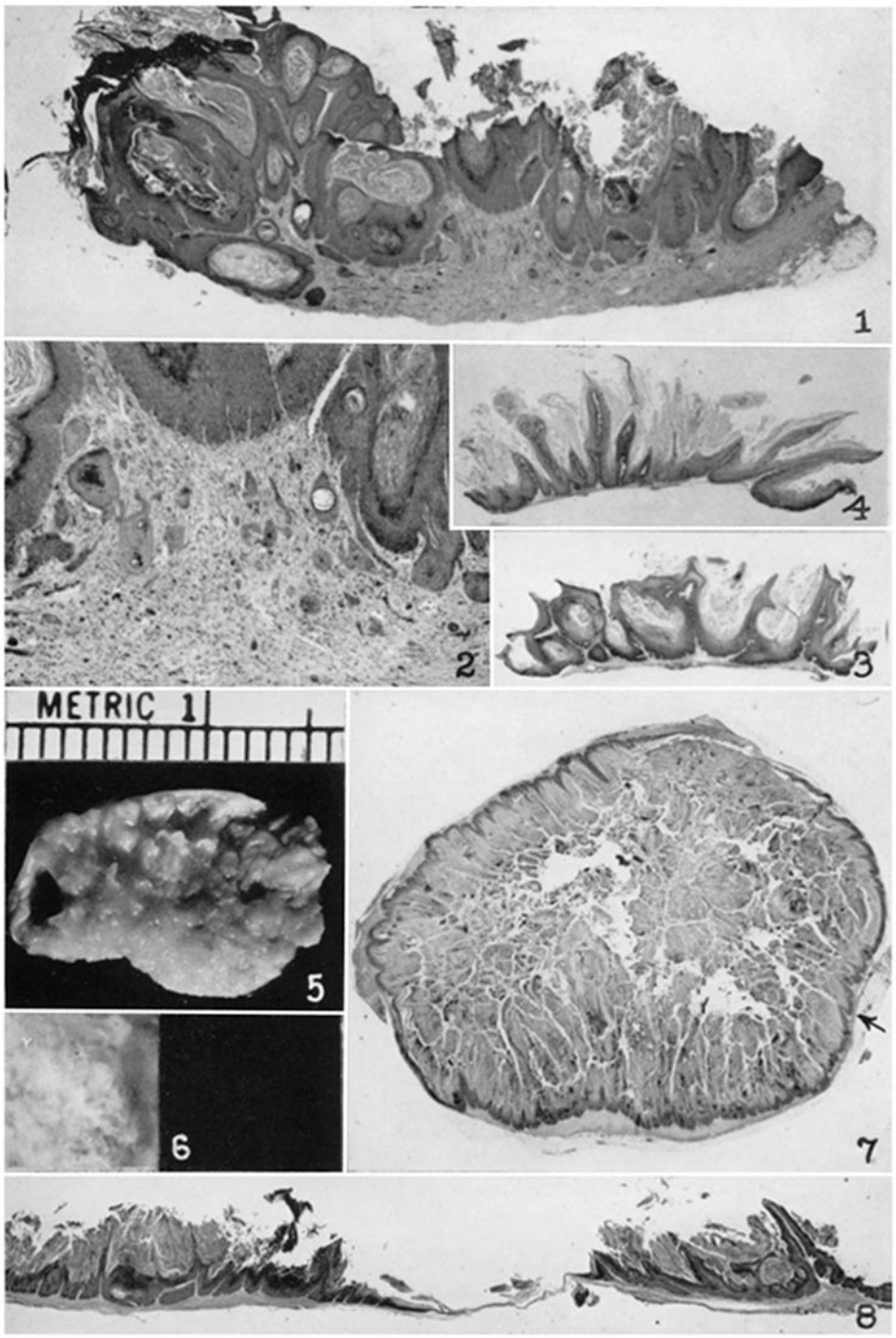
*Plate 5—Concluded*

was held in a claw-setting of skin but came away after fixation, exposing the papillomatous lining of the floor of the primary cyst. Photographed some hours after death. × 0.58.

FIG. C 11. Late state of an acorn growth of Pap. VIII, 2nd Gen., Line B. The mouse, a weanling when subcutaneously implanted, was killed 73 days afterwards. The growth had been spherical and 13 mm. across on the 43rd day, with a scab of dried skin over it, and later it became an acorn topped with dead skin carrying white hair as shown. The cup of the acorn bulges laterally. × 0.6.

FIG. C 12. Cutaneous horn due to complete extrusion of an acorn (Pap. I, 4th Gen., Line B). The implanted weanling had a spherical, scabbed cyst after 30 days, which shortly became an acorn, but with the cupped skin gradually retracting to the general level as the horn rose higher. The picture shows the horn after 70 days in all, when the mouse was killed. It was columnar, somewhat curved (see Fig. 27) and was capped with dead skin carrying a few white hairs. The fold at its base is due to torsion on the skin. × 0.6.

FIG. C 13. Superficial pap. formed by the eruption of a subcutaneous cyst (Pap. I, 4th Gen., Line Ba). The cyst was 6 mm. across within 20 days after the weanling was implanted, and it scabbed soon after, erupted, and during the next 6 weeks formed the onion growth shown. Its dry, conical peak projects from amidst vertical prongs of living skin along its plump base. A hemispherical, yellow, lateral bulge had appeared at its base by the 70th day. The mouse was killed to learn its nature (see Fig. 29). × 1.




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 7

FIG. 9. Thin pap. lining of a big cyst just under the skin (Pap. I, 3rd Gen., Line B); for comparison with the normal epidermis near by at the left. The pap. cells are the larger and have much larger nuclei. They have scarcely begun to form keratin. × 288.

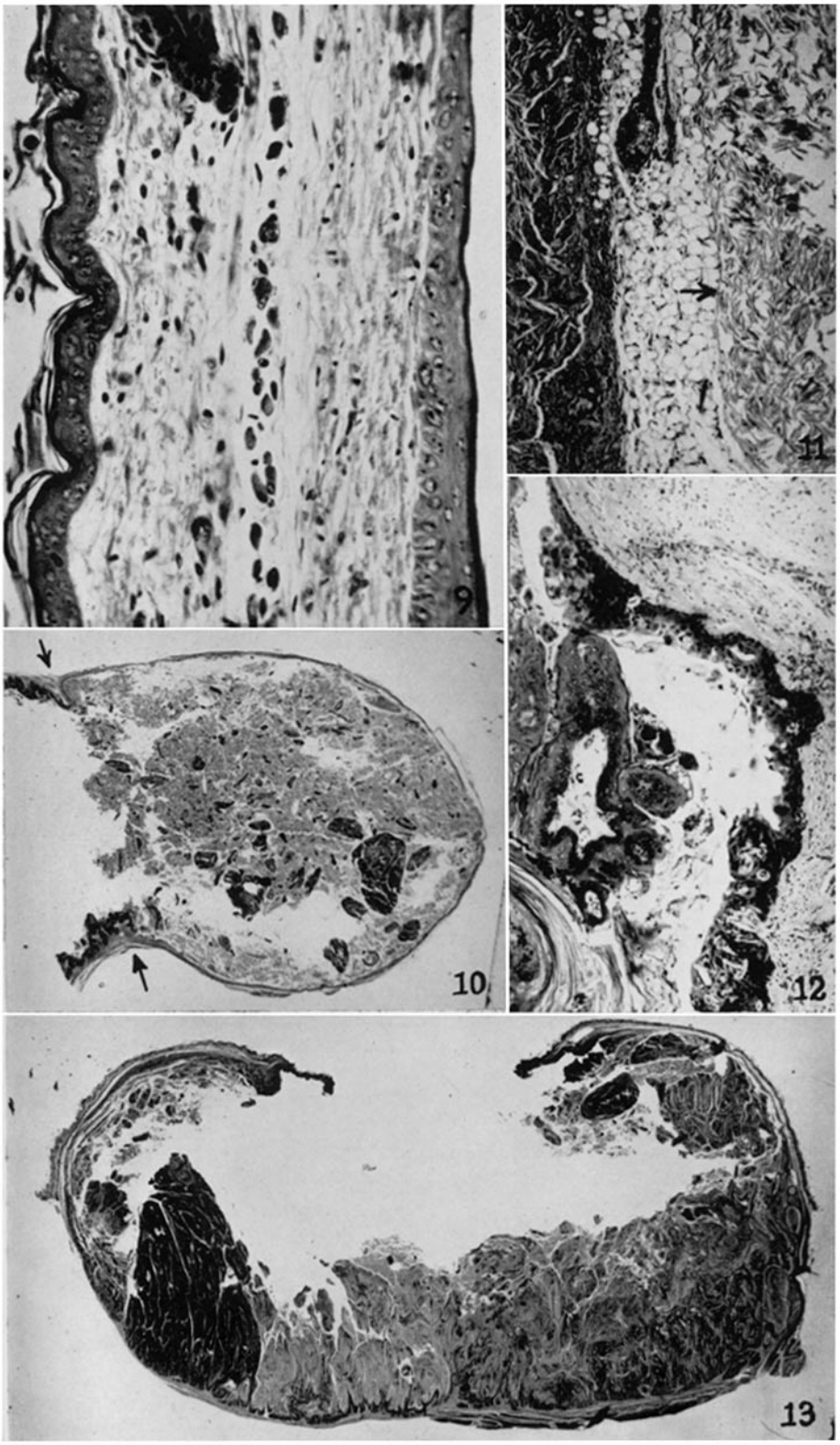
FIG. 10. Opening into a dissecting cyst (arrows). Its wall, on the right, is scarcely thickened as yet. The neoplastic lining of the pap. cyst from which it derived (Pap. I, 2nd Gen., Line G). has not extended beyond the opening between them. × 3.6.

FIG. 11. The reaction of a bare cyst wall to keratin (from specimen of Fig. 44). A thick, vertical layer of chronically inflamed tissue can be seen on the left, with keratinized squamous cells embedded in and overlying it. On the right, almost parallel to this layer, but separated by fat, is the delicate wall of connective tissue (arrow), as yet unthickened, of a new dissecting cyst containing loose keratin (Pap. VI, 2nd Gen.). × 56.

FIG. 12. Extension of a pap. layer from the base of a neighboring cauliflower (partly shown at left) onto a cyst wall already inflamed by keratin. The pap. layer (on right) has keratin amidst it and is faring ill. (see Fig. 33, arrow). × 82.

FIG. 13. Section through the cyst of Fig. C 5 (Pap. I, 2nd Gen., Line D). (Through error the skin was trimmed off half way up its sides prior to fixation.)

The skin at its top had died and come away, leaving an aperture through which much of the keratin has been lost through the gnawing of cage mates. The bottom of the cyst is lined with crowded pap. tissue, which becomes shallow along the sides and at some spots is lacking. On the floor of the cyst, at the left and in cross-section, is a sharply defined cone of keratin, much denser than that elsewhere, and appearing almost black. At higher magnification the living layer of this "variant" was more orderly and thicker than that of the neighboring Pap. I, but not different otherwise. × 5.9.




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 8

FIG. 14. Neoplastic layer along the base of the cyst of Fig. 13: to show the threatening aspect of the pap. It is invading the capsule of the tumor at several spots and at one is almost through it. × 60.

FIG. 15. Completely keratinized cyst of Pap. I in a weanling implanted 82 days previously. By the 29th day it was 8 mm. across, but then ceased enlarging and soon after became flattened and soft, undergoing no gross change later. The cyst wall is mere thickened connective tissue and encloses a mass of jumbled keratin and a conical, striated, darker mass resembling that formed by the "variant" pap. of Fig. 13. The skin appears raised by the tumor, but only because the latter was placed on cardboard for fixation. (So too in the case of Figs. 19 and 20.) × 13.

FIG. 16. Graft of Pap. I removed with the tissue about it 7 days after implantation. It consists almost entirely of dead or dying connective tissue, covered at most places with a layer of pap. cells—which is differentiating in one spot, and at another has extended to the wall of the pocket and some way into the surrounding reactive tissue. × 49.

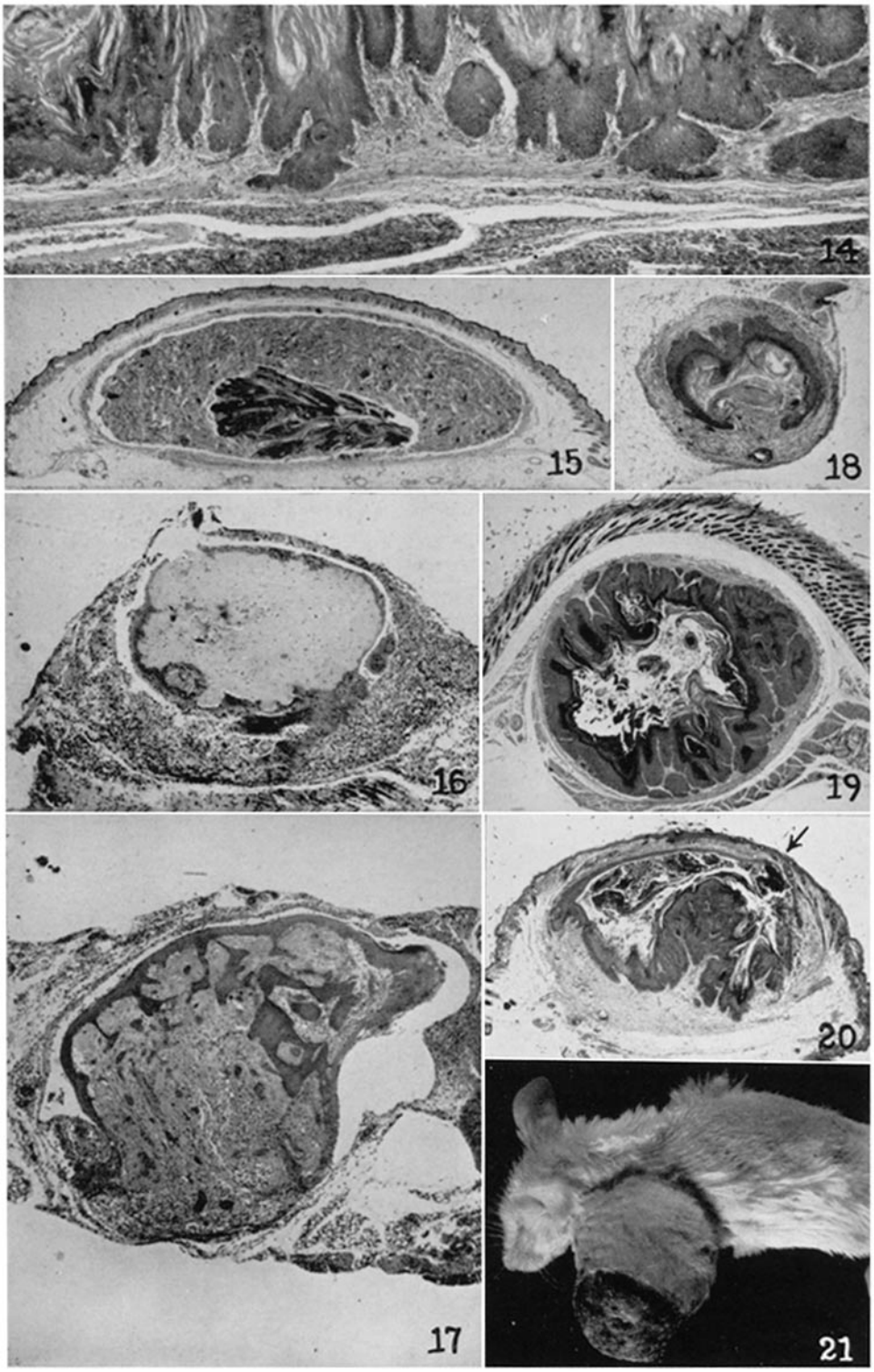
FIG. 17. Another 7 day old graft of the same material, much larger and with better preserved connective tissue into which the pap. layer has thrust deep tongues. The neoplastic cells have just begun to extend onto the wall of the pocket. × 37.

FIG. 18. A third graft of the same implantation, also 7 days old. It has become fixed along a broad base and here is now mostly dead; but the rest of the wall of the pocket is covered with a thick layer of living, differentiating pap. tissue. × 33.

FIG. 19. Cyst formed by a graft after 13 days. The pap. lining is unusually thick. × 18.

FIG. 20. Graft 14 days old. It had become attached at one end to the host tissue, like that of Fig. 17, and is now a hassock projecting into a pocket thickly lined for some distance with vigorous, actively keratinizing pap. tissue, which thins as it nears the body surface. The substance of the graft has been largely replaced by pap. and it would soon have become a cauliflower growth, as its markings show. The newly formed cyst is encapsulated save at one spot (arrow), where it connects with a hair follicle and is about to erupt on the skin (see Fig. 46). × 22.

FIG. 21. Acorn pap. (Pap. I, 3rd Gen., Line E). The mouse was newborn when implanted and the cyst was 18 mm. across and spherical after 58 days. The skin over it then began to die, forming a scab. This was gradually forced outwards by the accumulating keratin, the cyst meanwhile becoming almost tangential to the surface, and having a constricted base. Mouse killed after 105 days in all. The hair that covered the cup of the acorn has been clipped away. The light patches on the keratin are dried bread. × 0.93.




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 9

FIG. 22. Vertical section through a cyst containing a stalked pap. (Pap. I, 3rd Gen., Line E). The mouse was 3 days old when implanted, 42 days previously. The cyst, now somewhat flattened by cutaneous pressure but projecting abruptly, lies almost entirely outside the contour of the body. It is topped with mummified skin and some strands of dry, black keratin extend down from this. Its upper half is occupied by a big, edematous mass of connective tissue covered thickly with pap. except next the dead skin. A narrow stalk connects the mass with the cyst floor and here blood vessels enter it through an inverted funnel, as higher magnification showed. The cyst floor is covered with vigorous pap. tissue, but this tapers off toward its sides and they are almost everywhere bare farther up. The thin encapsulating tissue has ruptured at one spot and here a dissecting cyst is forming (arrow). Just beneath *C* is a tiny carcinoma. × 6.1.

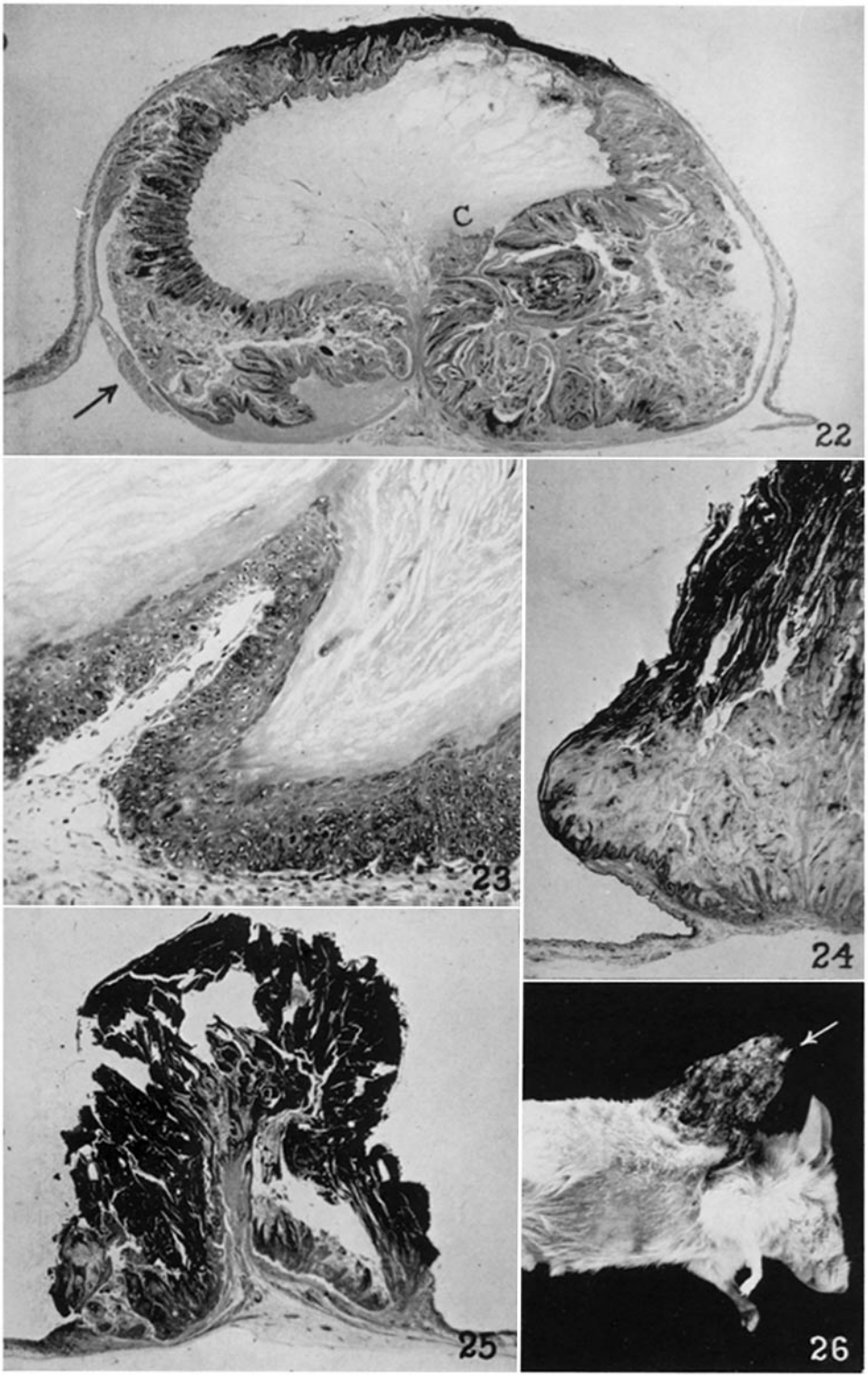
FIG. 23. Papillomatous lining of the cyst of Fig. 7 at the spot indicated by the arrow thereon. Numerous mitoses can be seen. × 174.

FIG. 24. Section through the overhanging margin of the saucer of Fig. C 8. As the saucer broadened its rim has been pulled up into an arc over the body surface by the keratin to which it is tethered,—a process furthered by shrinkage of this latter on drying. × 7.3.

In all of the sections dry keratin appears black.

FIG. 25. Cross-section of the growth of Fig. C 11 (Pap. VIII, 2nd Gen., Line B) where it is broadest. The black mass consists of keratin. Some of the dead skin topping it can be seen as a thin layer at the left. A stalked pap. was formerly present amidst it, but now only the stalk is still alive. Pap. tissue covers the base of the growth. Skin extends some little way up its right border. × 5.

FIG. 26. Cutaneous horn (Pap. VI, 2nd Gen., Line C) due to total extrusion of an acorn containing a central, stalked pap. The growth began as a spherical cyst in a weanling and was 13 mm. across after 51 days. It soon scabbed and erupted as a gradually enlarging, dry, conical mass which was 25 mm. high by the 146th day, when the mouse was killed. A tuft of white hair (arrow) had persisted on the dead skin at its top, but the living skin had retracted to the general level except for a pointed claw, as seen. × 1.




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 10

FIG. 27. Vertical section of the horn of Fig. C 12 (Pap. I, 4th Gen., Line B). The whole acorn cup, living and dead, has been extruded from the subcutaneous tissue. A narrow stalk extends up the center of the horn amidst the edematous core of the pap. it nourished, a core still covered on the right with living pap. tissue, and this in turn by keratin. The big black mass at the top of the growth consists of dry keratin, with a narrow layer of dead skin visible at one spot (arrow). × 6.

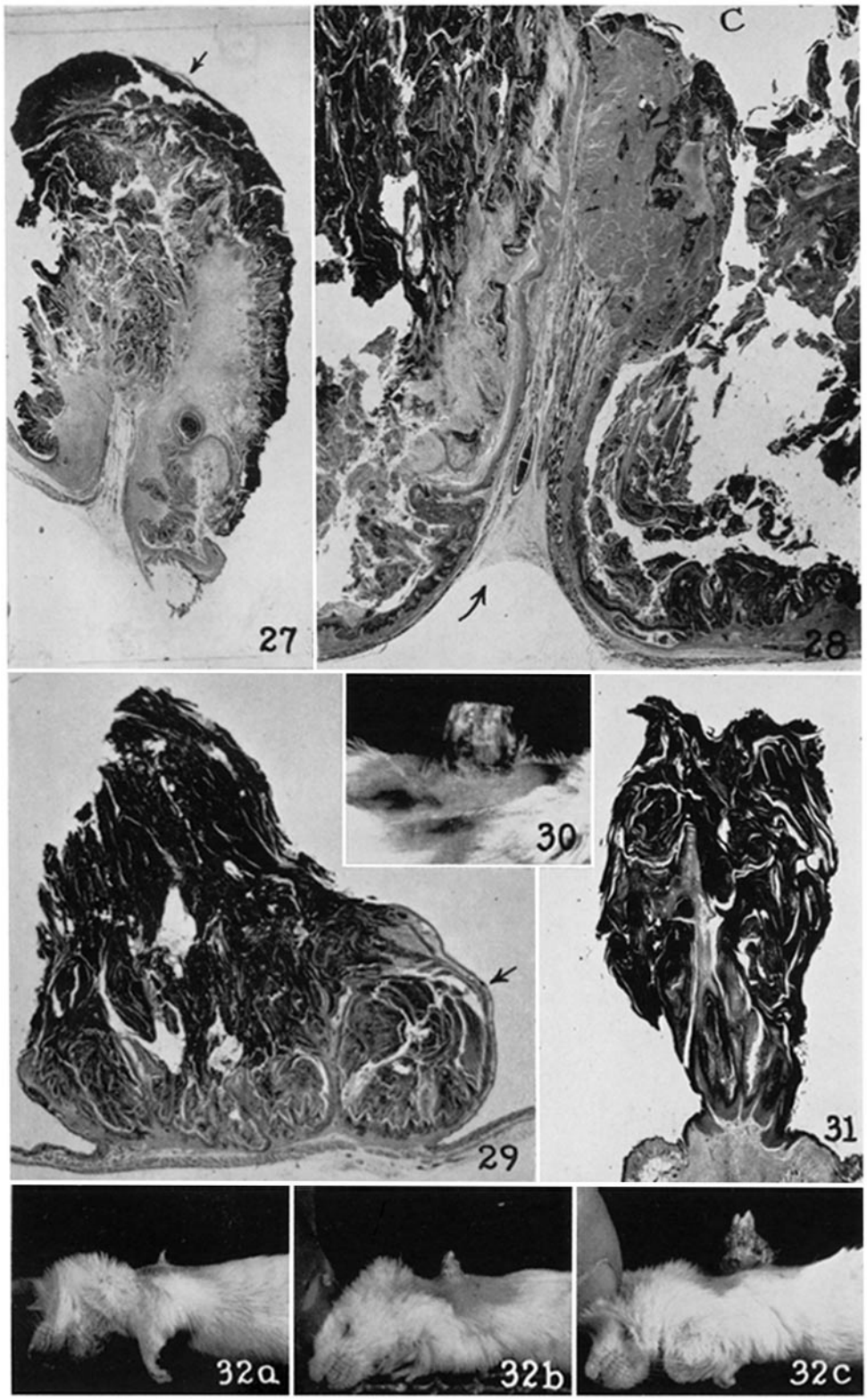
FIG. 28. Vertical section across the middle of the lower half of the growth of Fig. 26. It consists everywhere of keratin except for a layer of pap. tissue along its base and on the sides of the central stalk, and a mass of anaplastic carcinoma (*C*) near its top on the right. The pap. mass which the stalk once nourished is now gone. Numerous fibres of voluntary muscle can be seen lying vertically in the stalk, as also a big blood vessel in cross-section, and fatty tissue (arrow) fills the funnel at its base. × 10.

FIG. 29. Section through the growth of Fig. C 13 in the plane of the yellow bulge, —which proved wholly papillomatous (arrow). The growth is a typical benign, onion pap. with a peak of dry, vertically striated keratin, a markedly constricted base and plump sides on which the skin is reflected. Almost no thickening of the connective tissue beneath it has occurred. Indeed it looks as if it had arisen *in situ*. × 5.2.

FIG. 30. A superficial, flat topped, vertically striated and keratinized pap. mass which formed after the cutting open of a subcutaneous cyst of Pap. V, 1st Gen., when 34 days old and 6 mm. across (see text). Photograph taken after 23 days more. × 2.

FIG. 31. Section through the narrow diameter of the same tumor, as procured 4 days later, when it was about to come away. Its broad diameter is shown in Fig. 30. The mass of connective tissue underlying the growth was the capsule of the everted cyst. Except for it the tumor wholly resembles a tar-induced pap. × 13.

FIGS. 32 *a*, *b*, and *c*. Successive stages in the formation of an onion pap. over the site of a subcutaneous implantation of Pap. II, 1st Gen. (*a*) Superficial spicule first noted after 33 days. (*b*) Conical tumor 20 days later. (*c*) Onion pap. with bulging sides and constricted base after 26 days more. By then its dry, vertically striated top has begun to be gnawed and when the mouse was killed, after 104 days in all, it was truncated (see Fig. 43). × 1.




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 11

FIG. 33. Stalked cauliflower pap. (Pap. II, 3rd Gen.) that had projected into an unlined cyst, with another pap. superimposed upon it. Only part of this last, and of the reflected cyst wall, are shown. On the left, near the cauliflower, the wall is wholly bare but is much thickened and inflamed by keratin. On the right the pap. has extended some way along it (arrow) but is doing badly, as Fig. 12 shows. × 6.7.

FIG. 34. Superficial, onion pap. first noted on the skin of a suckling mouse 20 days after a subcutaneous implantation of Pap. I in the same locality when it was 8 days old. The growth enlarged rapidly and its host was killed after a further 11 days. Directly beneath it, and unattached to the overlying skin, was a 10 mm. keratinizing cyst. No visible connection existed between the two. × 14.3.

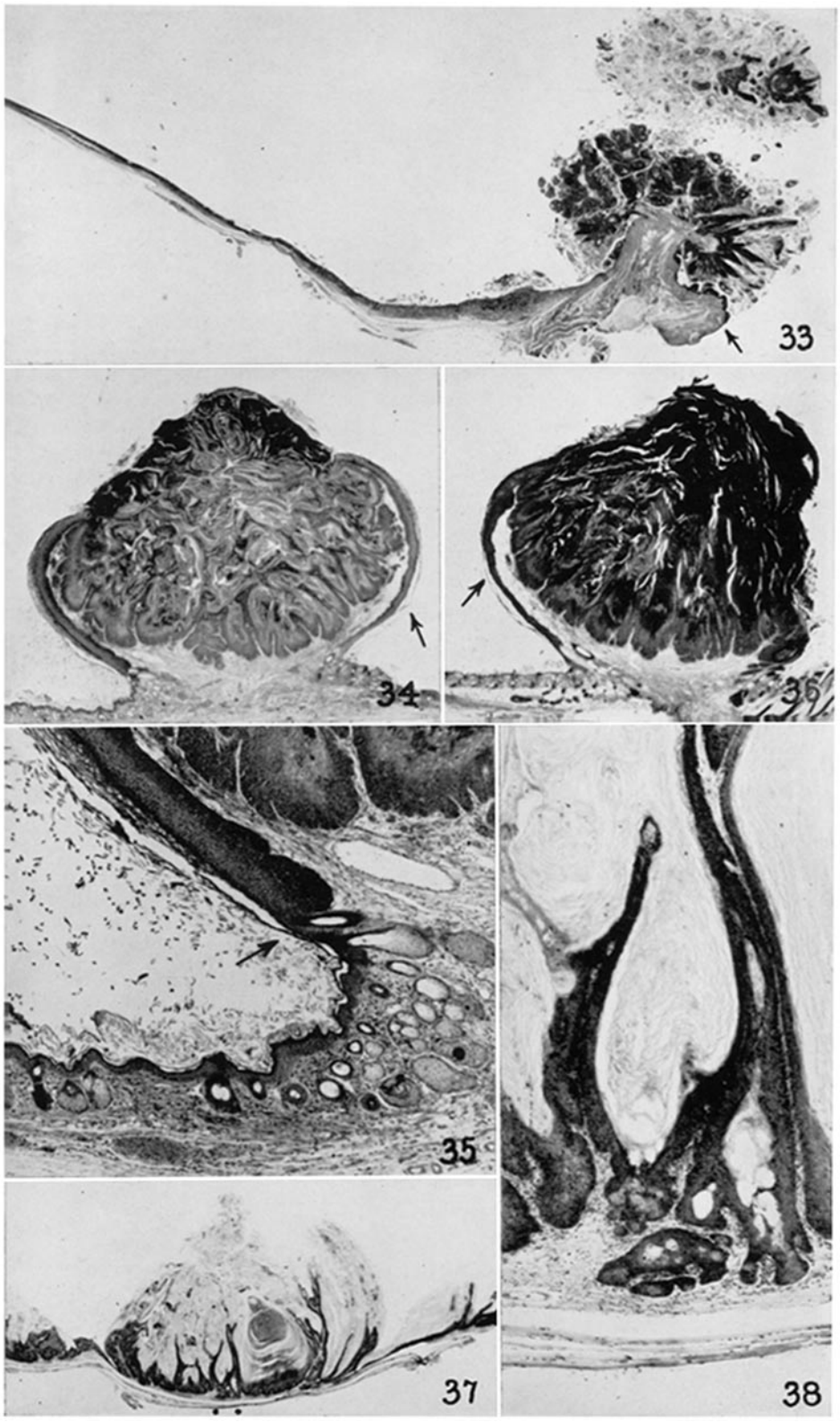
The symmetrical, onion pap. has a narrow base and is here underlain by reactive tissue. Its bulging sides are covered with a thick, smooth layer of pap. tissue which grades gradually on the right into ordinary hyperplastic epidermis, as higher magnification showed, but joins it abruptly on the left (Fig. 35). The arrow points to a markedly edematous region in the onion. A large vein here was thrombosed. × 14.3.

FIG. 35. Junction of the pap. layer on the left side of the onion of Fig. 34, with the adjacent hyperplastic epidermis (arrow). × 55.

FIG. 36. Another superficial pap. from the same animal. It was situated over the healed slit through which the trochar had been inserted for the subcutaneous implantation already mentioned, and new scar tissue extends down beneath it. The layer of pap. tissue on its sides merges with the ordinary epidermis, as in the instance already pictured, and again marked edema is present (arrow). × 14.3.

FIG. 37. Part of the floor of a cyst formed by the Ear Pap. (see text) in its 1st Gen. × 7.

FIG. 38. Higher magnification of the base of the same growth at the spot indicated by the dots. Many sebaceous glands can be seen in process of formation amidst the differentiating neoplastic cells, and secretion from them is responsible for the lacunae amidst the keratin just over them. × 57.




(Rous and Allen: Fatal keratomas from homografts of benign papillomas)

## PLATE 12

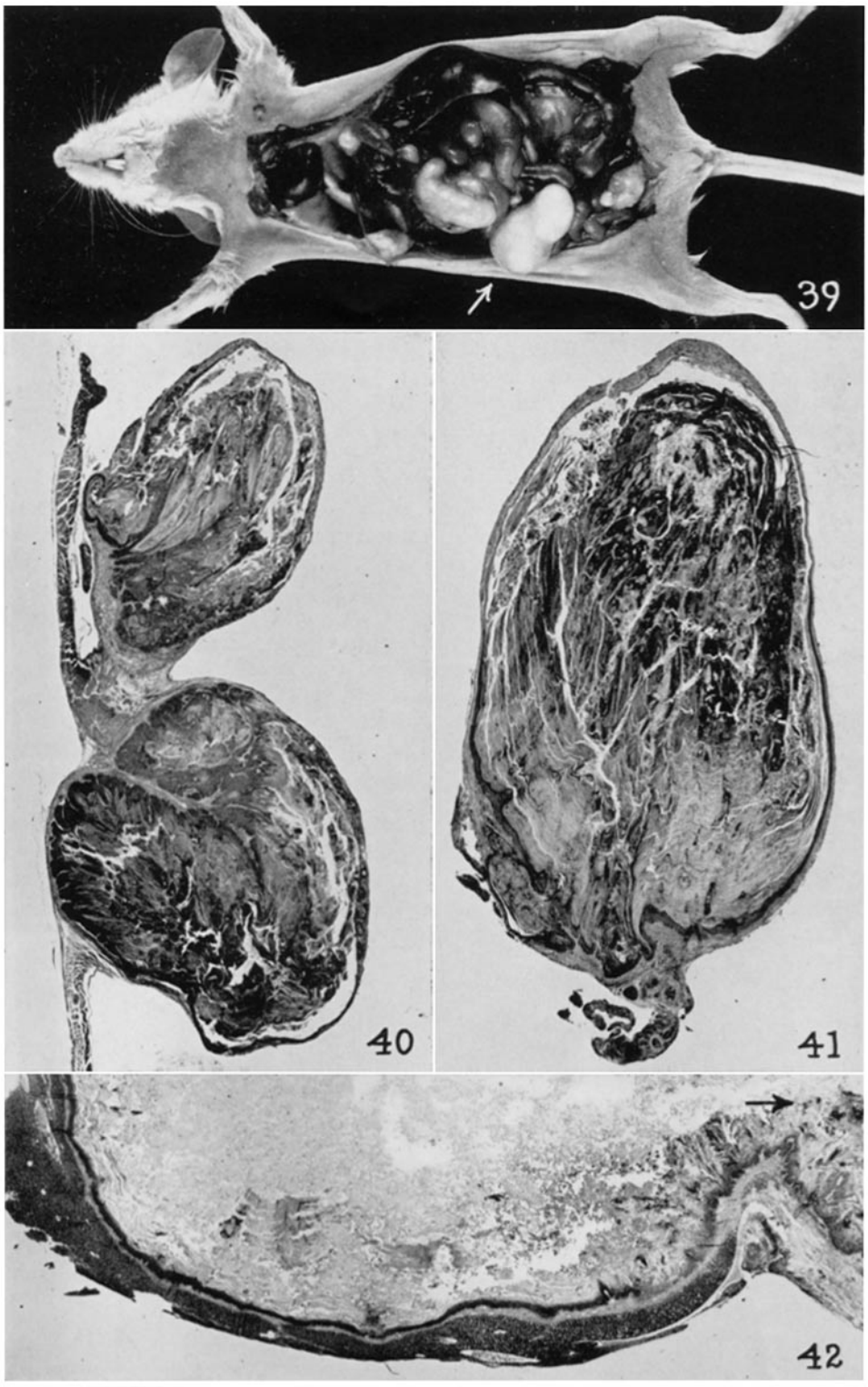
FIG. 39. Viscera of a mouse that had received, when a suckling, an intraperitoneal injection of many suspended, small fragments of pap. tissue from a subcutaneous cyst of Pap. I, 4th Gen., Line C.

Several tumors are visible amidst the abdominal organs. The largest (arrow) was a thin-walled, creamy cyst, having a ropy connection with the omentum. It contained masses of lamellated, squamous cells amidst watery fluid. Its lining layer of pap. tissue, though entirely necrotic, had remained in place, and covered little more than half of the cyst wall which consisted for the rest merely of connective tissue covered with peritoneum. Many of the other growths were largely cancerous and one had partly obstructed the bowel, which is seen to be distended over it. × 1.05.

FIG. 40. Papillomatous cysts on the peritoneal wall at the spot where it had been pierced 26 days previously for the injection of another suckling. Both cysts have living, keratinizing, neoplastic tissue near their base, but farther away their wall consists only of connective tissue covered with peritoneum. They are full of lamellated keratin. The smaller of the two cysts is pedunculated, but the larger is broad-based, not because of any invasion but as a result of the displacement of normal structures by its bulging mass. The displaced muscle extends some way up its side. Carcinomatous areas are visible in both growths. × 10.

FIG. 41. Intraperitoneal cyst, not seen in Fig. 39. It had a ropy connection at one end with the splenic mesentery and is lined here with a thick layer of pap. tissue, which thins however and is lost toward the far end of the cyst,—full of lamellated keratin. × 10.6.

FIG. 42. Wall of a cyst 5 months old, due to direct implantation of tumor bits in a weanling's liver (Pap. I, 3rd Gen., Line C). Growth within the hepatic tissue has been almost entirely expansile, and here the well-encapsulated cyst is lined with an almost even layer of pap. tissue. Its greater bulk lay in the peritoneal cavity and there, for the most part, it had no neoplastic lining but was thin-walled and full of watery fluid,—a fact indicated in the photograph by dispersion of the keratin that had come away from the intrahepatic pap. layer. The ridge and arrow on the right mark the opening into the peritoneal cavity and here the pap. had become malignant. × 12.4.

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## PLATE 13

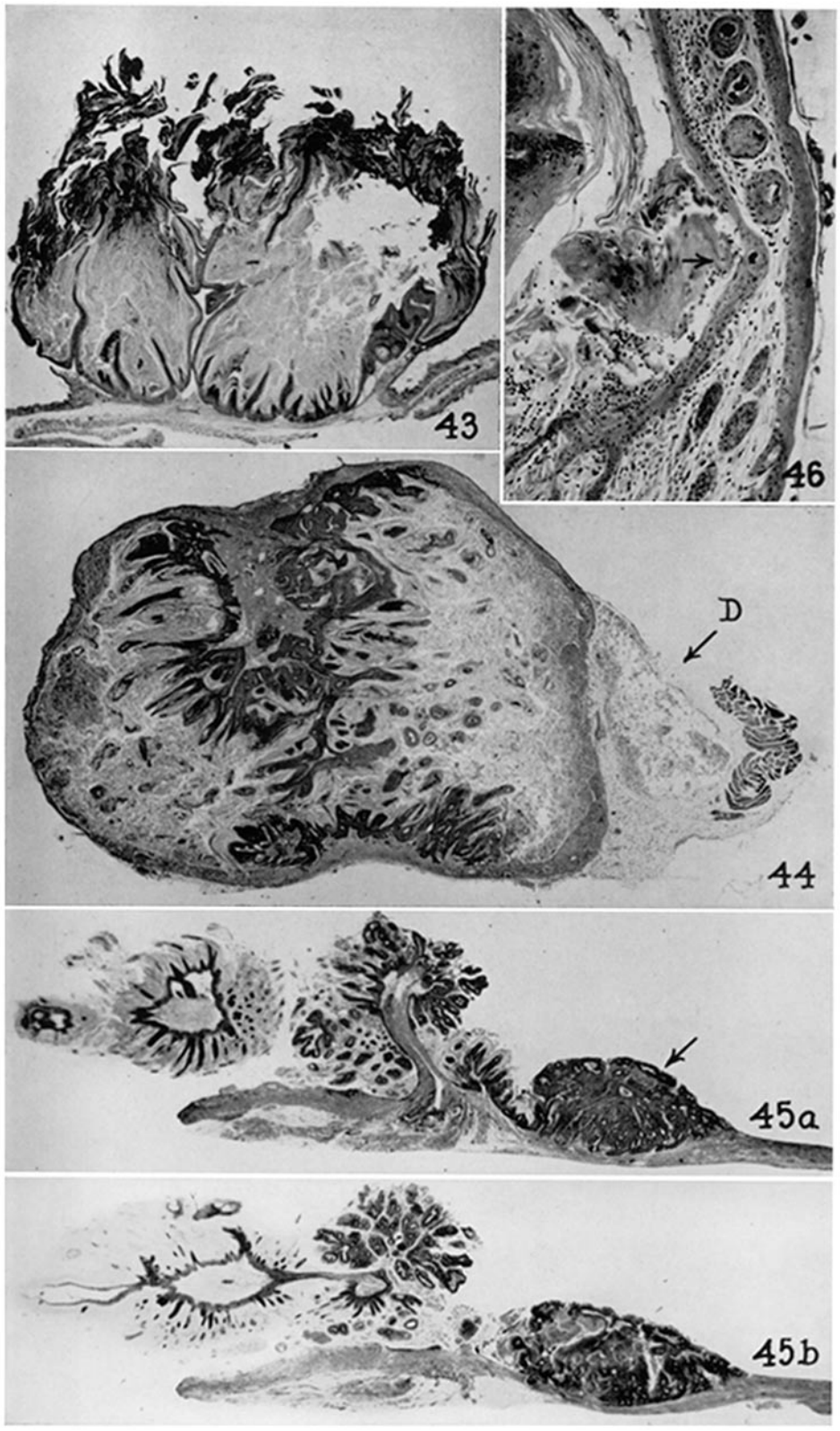
FIG. 43. Section through the tumor of Fig. 32 *c* after it was truncated; only a little dry keratin still tops it. The living pap. tissue appears notably benign and merges with the normal epidermis at one side of the constricted base of the growth. This is wholly superficial and has no reactive tissue beneath it. × 6.4.

FIG. 44. Cyst of Pap. VI, 2nd Gen., containing a sessile and a stalked cauliflower but with its walls elsewhere bare. The mouse, 15 days old when implanted, was killed 122 days later, when the cyst was 15 mm. across. A 30 mm. dissecting cyst had extended out from it. Only a small part of this latter can be seen (arrow *D*). It had no distinct wall as yet, whereas that of the main cyst was thickened by connective tissue reacting from long contact with keratin (see Fig. 11).

The big cauliflowers have formed relatively little keratin. × 10.5.

FIG. 45 *a*. Section through the wall of a 22 mm. cyst of Pap. IV, 1st Gen.,—which contained fluid rendered creamy by suspended, keratinized cells. These derived from the stalked pap. here shown, as also from another superimposed on it (like the growth of Fig. 33), and a third superimposed upon that. Nearby is a discrete squamous cell carcinoma (arrow) which, though unable to spread laterally, is invading the cyst wall. Elsewhere this wall was bare. Part of the stalked growth was transplanted, with result in cysts full of pultaceous keratin, and having cancer together with pap., at some spots on their otherwise bare walls. Fig. 45 *b*. Another section through the cyst of Fig. 45 *a*, to show the connection between the paps. superimposed on the stalked growth. × 7.3.

FIG. 46. Wall of the tiny cyst of Fig. 20 at the spot where it was about to erupt. Here its pap. lining has united with the epithelium of a hair follicle (arrow), giving the cyst direct access to the skin surface. The hair within the follicle can be seen in cross-section, as can those of other follicles near by. × 124.

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## PLATE 14

FIG. 47. 9 months old tumor due to a graft of Ear Pap., 2nd Gen. A, in the subcutaneous tissue of a weanling. The growth, a keratinized cyst of Type A, had become very large before it erupted and the huge saucer now shown was formed within the next 9 weeks. The high mound consists of dense keratin. A bulge at one side of the smooth, convex base of the saucer (arrow) suggested the presence of cancer, but a section showed only benign pap. (see Fig. 48). × 0.55.

FIG. 48. Section through the bulge of Fig. 47, viewed in reverse. × 12.2.

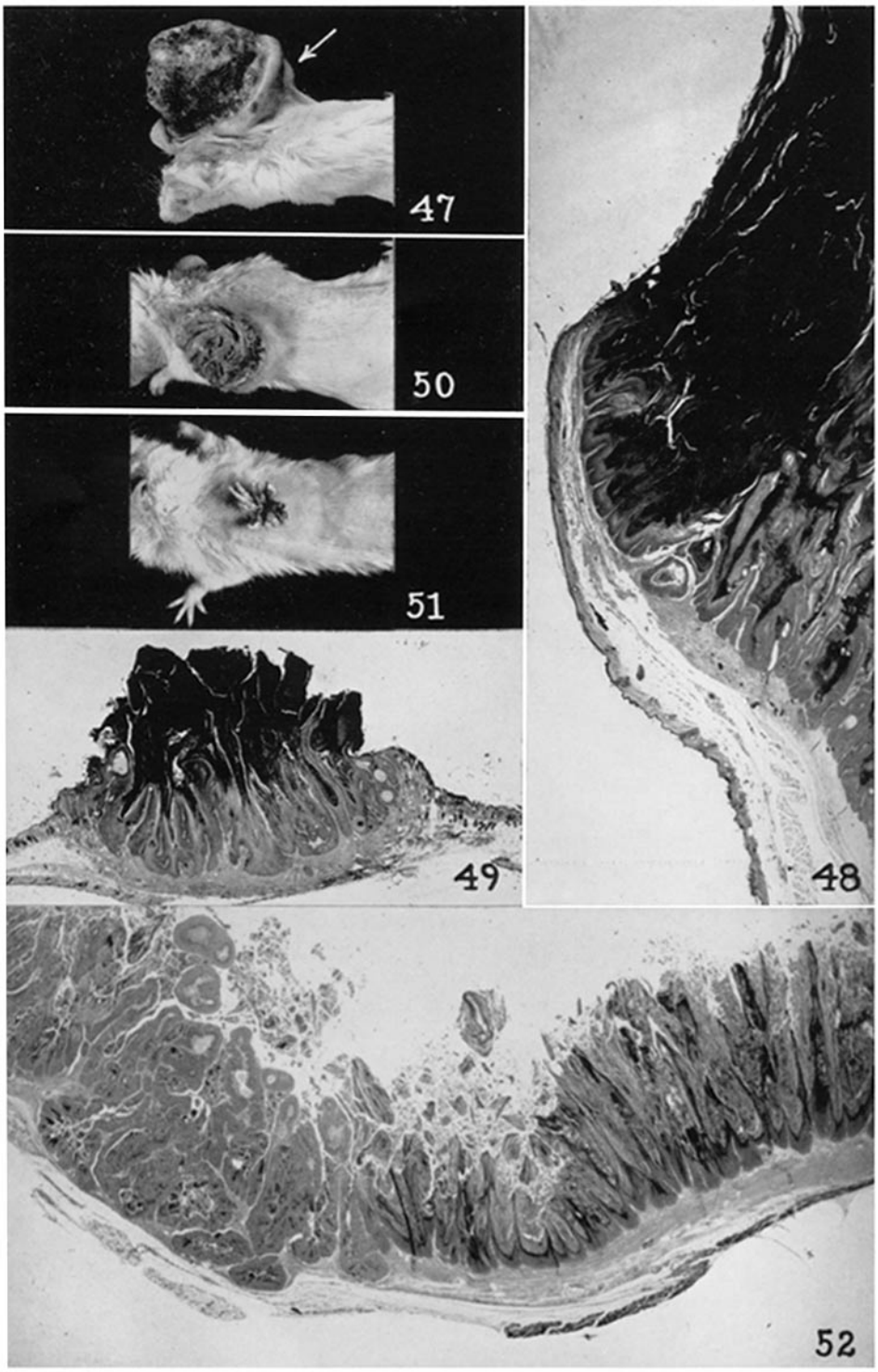
FIG. 49. Erupted subcutaneous cyst of Ear Pap., 2nd Gen. A. After 9½ months it is far on the way toward becoming a superficial pap. × 9.2.

FIG. 50. 5 months old, keratinizing growth of mesa shape, due to a graft in a suckling of Ear Pap., 3rd Gen. A. It was first a subcutaneous cyst, then an acorn, after which the skin gradually retreated from the dry mass of keratin to the body level. Photographed from above to show the concentric rifts in the keratin. A carcinoma was present at one spot along its living base. × 0.7.

FIG. 51. Growth due to subcutaneous implantation in a weanling of Ear Pap., 2nd Gen. B. The cyst formed first erupted within 2 months, with result in a saucer 15 mm. across,—which had diminished to 7 mm. when the mouse was killed after 9½ months in all. A cluster of coarse, blunt spicules protruded from it as shown. They were due to vertical splits in the keratin, and had the gross look of the gummed-together hairs of a paint brush. × 0.72.

FIG. 52. Malignant pap. on the wall of a cyst lined elsewhere with ordinary pap. (Pap. I, 2nd Gen., Line G). The cancer was not perceptible in the gross. It has extended almost through the connective capsule of the tumor, yet seems not to have invaded the pap. next it.

The spherical cyst 2 cm. across, packed with dense keratin, was due to an implant placed 119 days previously in a newborn mouse. A dissecting cyst equally large had taken off from it. The junction of the two is shown in Fig. 10. × 12.8.

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## PLATE 15

FIG. 53. Part of a spherical metastasis of a squamous cell carcinoma in the lung of a mouse killed 70 days after subcutaneous implantation when newborn (Pap. I 4th Gen., Line G). The cyst in the subcutaneous tissue was 30 mm. across and was suspiciously well vascularized but showed what appeared to be only an ordinary layer of pap. in the gross. × 42.

FIG. 54. Recently erupted pap. cyst at the edge of a superficial tar pap. and part of this latter. Pent up keratin in the cyst had caused its living layer to bulge down into the fatty tissue, compressing it. × 19.4.

FIG. 55. Three skin paps. induced by repeated applications of tar and carrying some of it on their keratinized tops. They are almost completely keratinized. The lowermost is coming away and the uppermost is constricted at its base. × 1.

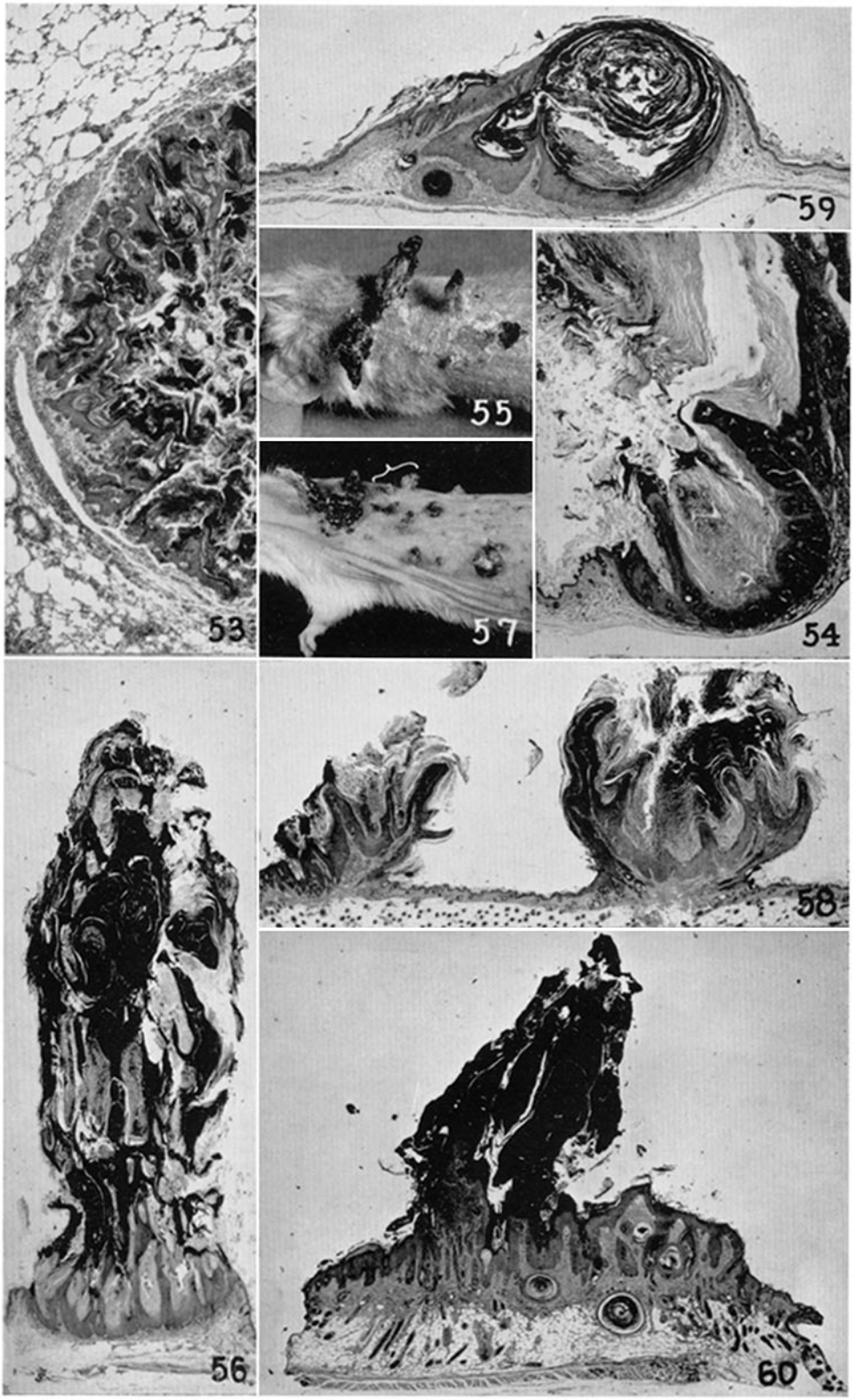
FIG. 56. The smallest of the paps. of Fig. 55 in vertical section. Its shallow living layer appears to bulge downward because its periphery, together with the adjacent skin, is pulled up by shrinkage of the dry keratin immediately over it. The growth was once broader, as shown by the keratin topping it. × 10.

FIG. 57. Tar paps. The bracket encloses two small, onion paps. A broad ulcer, black and glistening because covered with blood clot, marks where a larger pap. has been replaced by a cancer. × 1.

FIG. 58. The bracketed paps. of Fig. 57 in vertical section. The expansive growth of the larger of the two has caused the hair follicles underlying its base to slant outwards. They appear nearly horizontal. × 14.

FIG. 59. A hair follicle, rendered cystic by tar and now lined with epithelium that has undergone papillomatous change. It had become distended with concentric keratin, as often happens, and now almost the whole of its wall is covered with a thick layer of pap. cells (as higher magnification showed), which have lined a neighboring follicle as well (here shown in cross-section). The cyst lies almost entirely above the skin surface, and it is in process of erupting. × 20.

FIG. 60. Actively enlarging pap. on recently tarred skin. The growth is so broad as to occupy nearly the whole of the expanse shown but as yet has heaped up keratin only in its oldest, central region. Beneath it are several hair follicles distended with concentric keratin, one of them lying in the adipose tissue,—no strange finding, since tar stimulates follicles to extend deep into this, as here shown. × 19.4.

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