A VIRUS-INDUCED MAMMALIAN GROWTH WITH THE CHARACTERS OF A TUMOR (THE SHOPE RABBIT PAPILLOMA)

II. Experimental Alterations of the Growth on the Skin: Morphological Considerations: the Phenomena of Retrogression

By J. W. BEARD, M.D., and PEYTON ROUS, M.D.

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

Plates 41 to 44

(Received for publication, August 9, 1934)

The effects on the papilloma of transfer from the skin to the interior of the host have been described in Paper I. The influence of some chemical and physical agencies upon cutaneous growths will now be considered.

Stimulation with Scharlach R

The papilloma when implanted within the host grows progressively, invading and replacing normal tissues and killing the animal. Yet on the skin, its natural habitat, it invariably ceases to extend after a time, though its cells continue to proliferate, heaping up its mass. An observation recorded in Paper I suggests that this secondary limitation of growth is not due to the loss of an essential power on the part of the cells but to local conditions. Where incisions had been made tangential to established, stationary papillomas the latter grew into and for some little distance along the newly healed wounds, though elsewhere their borders remained unchanged. We have made numerous attempts to obtain aggressive skin growths by experimental means.

The subepidermal injection of a saturated solution of Scharlach R or of Sudan III in olive oil causes the epithelium of the rabbit's ear to burrow downwards and exhibit for a brief period the histological appearance and invasive tendency of carcinomatous tissue (1). Werner found the growth of a transplantable mouse carcinoma to be

greatly stimulated by local injections of the dye (2). The same holds true of the rabbit papilloma.

Repeated injections of Scharlach R into the tissue immediately under and around the growth cause it to extend downwards and sideways instead of outwards, with result in fleshy, subepidermal masses (Fig. 22, *B*). The non-papillomatous epithelium next it partakes of the stimulating effect, but there the hair follicles are principally affected. Their epithelium grows downwards and they become distended, tending soon to round up into pearls lined with normal looking epidermis and filled with dead, concentric layers of keratinized cells (Fig. 23). The papilloma does not arise from the hair follicles but from the tissue between them (3); and its dye-stimulated epithelium retains a distinctive character, through becoming very irregular, thrusting long processes here and there into the tissue, and not infrequently entering the blood vessels. (See Figs. 15 and 16 of Paper I for vascular invasion in the absence of dye stimulation.) If Scharlach R is injected at one side only of a papilloma, the latter extends in this direction. Its effect upon old stationary growths can be exemplified by the findings in a domestic rabbit carrying, as result of broadcast inoculation on the abdomen, a large "pancake" of papillomatous tissue that had ceased to extend, and several smaller growths along one side where the virus had been tattooed into the skin. Repeated injections of the dye were made about these latter, and some found its way down through the tissues to the border of the "pancake." As result the mass underwent a pronounced secondary thickening, due to epithelial penetration into an underlying reactive connective tissue; but this took place only on the side where the dye was present.

The skin over the large, fleshy, subepidermal growths that form as result of repeated dye injections under and around developing papillomas, tends to break down after a time, owing to pressure necrosis; infection supervenes; and the mass becomes foul and sloughing. But at the periphery, where Scharlach R still is present, an active proliferation and subepidermal extension continues.

No enduring increase in the activity of the papilloma has been brought about by Scharlach R: as the latter disappeared the growth became quiescent. And no certain instance of induced metastasis formation has resulted from its use, though a possible one is reported in Paper I. Repeated injection of the dye causes papillomas located on the ears of domestic rabbits to become huge, conical, cutaneous horns, as much as 5 or 6 cm. high, with fleshy bases and dry, brown, vertically striated peaks. The proliferating epithelium under the middle of one such horn (Fig. 25) penetrated to the under surface of the ear (Fig. 26) through a pre-existing lacuna in the cartilage, and formed a secondary nodule there (Fig. 27). This happened after

the coloration indicating the presence of Scharlach R had long since disappeared from the tissue. One may suppose that the epithelium took the way of least resistance from its confinement beneath the horny mass. This supposition seems the more reasonable because experiments that will now be described have shown that when a skin papilloma is prevented from extending outwards it will burrow into the tissue under it.

The Consequences of Preventing Outward Growth of the Skin Papilloma

To determine the effect of altering the mechanical conditions of growth a number of papillomas were covered with collodion.

It proved essential to apply the collodion when the first papule or papules appeared after virus inoculation, for if it was done later they either pushed out between the layer and the skin, like a flat, many petalled chrysanthemum, or, when they tended to broaden at the base, they undermined the adjacent normal epithelium, killing it by cutting off its blood supply, with result in a broad ulcer with thick, fleshy edges. If, however the collodion was put on when the first punctate roughening of the epidermis was noticeable after intradermal injection of the virus, and if the layer was flexible and was not soon loosened by desquamation and growth of the hair, as too often happened, the epithelium affected by the virus, instead of projecting in elongated papillae, continued its primary extension into the connective tissue, and formed in the course of some weeks a thick, discoid mass between the fibrous layer of corium and the epidermis (Fig. 22, *C*). Often several centimeters in diameter, it continued to enlarge after the collodion had been stripped away. The epidermis over the center of such masses became tense and after a time broke down, but elsewhere it was only stretched and thinned. Like the implantation growths described in Paper I, they consisted of a rind of translucent, grayish pink tissue, about a dense, tough, dry, creamy to sooty, necrotic material which separated more or less readily into papilliform processes when cut across. Under the microscope the rind was found to consist of proliferating epithelium and reactive connective tissue, in papillomatous arrangement, with processes everywhere pointing toward the growth's center (Fig. 24). Here pressure necrosis had occurred. A more or less well defined capsule was present, consisting of newly proliferated connective tissue with, as a rule, almost no lymphocytes and no polymorphonuclear cells. Into this new tissue the epithelium had pushed forth in more or less orderly, close-ranked processes, incorporating some of it as cores of the papillae in process of formation. Thus the growth enlarged by progressive invasion of its surroundings as well as by expansion.

The papillomas prevented by collodion from extending outwards proved not only capable of enlarging beneath the epidermis but

they did so at the expense of the normal tissues. Though their encroachment was orderly it took place in a way suggestive of malignancy, and it was progressive. The stimulation of Scharlach R rendered the papilloma highly irregular and invasive, and from its results one derived the impression that sustained malignancy would result from continued stimulation. Such stimulation has been accidentally provided in not a few implantation growths through the influence of contaminating bacteria (see Paper I).

Variations Due to the Influence of the Host

The character of the papilloma is influenced not only by local conditions but by the individuality of the host. In some rabbits the growth retrogresses, a phenomenon which will be considered further on; and even in favorable hosts it varies considerably. The differences in gross aspect of the papillomas produced experimentally on the skins of wild and domestic rabbits have already been described. They would appear to be largely due to differences in the skin itself as a supporting fabric, that of wild individuals being thin and rather dry whereas in domestic ones it is often thick and succulent. When it is thin in the latter the growths tend to resemble those in wild rabbits. Some differences observed in the response of the normal skin to injections with Scharlach R in olive oil are not without significance in this relation. Thick, succulent skins thicken still further and their epidermis proliferates markedly, taking on an appearance like shagreen and later becoming scurfy; thin skins on the other hand appear almost unaffected. The papilloma is most fleshy and exuberant in precisely those skins which respond most pronouncedly to Scharlach R. The maximum variations in hosts favorable to the growth are well illustrated by the findings in two litter mates inoculated shortly after birth.

The rabbits, of gray-brown, domestic breed, were inoculated when 8 days old with the same specimen of virus fluid, by rubbing it into the scarified epidermis over a broad expanse on the abdomen. The growth appearing in one was low and confluent, and in its superficial portion dry, friable and sooty, brittle, and porous, like a mass of coke (Fig. 28). In the other little rabbit it consisted of numerous separate, brown, radish-shaped cutaneous horns, several centimeters high, vertically

striated, tough and somewhat elastic, with bulging, fleshy bases. As these broadened the skin was forced into folds to make room for them all (Fig. 29). They consisted, as sections showed, of very numerous, thin papillae, some of which were alive far up into the brown horns (Fig. 31). The living tissue of the low growth was almost entirely restricted to a basal layer with but poorly developed, infrequent and blunt, papillomatous processes (Fig. 30). Yet this growth, though apparently stationary at the time when both rabbits were killed, 111 days after inoculation, had at several points invaded the lymphatics beneath it.

There was no histological evidence that bacterial infection was responsible for the differences noted. The animal with the low, dry papilloma had somewhat the thinner skin and had not increased quite so rapidly in weight as the other.

The animals of this experiment seemed to be in much the same bodily state and their skins differed little in the gross. The pronounced contrast in the character of the papillomas may well have been due to differences in reactivity of the host tissues. If the epithelium stimulated by the virus is to assume the papillomatous form, the skin must provide for it a supporting fabric of connective tissue with the necessary vessels,—in short a stroma. The behavior and morphology of the growth cannot but depend largely on the extent to which this stroma is forthcoming. A like dependence exists in the case of many tumors.

Morphological Considerations

The influences which determine the differing forms that the papilloma assumes on the skin and in the interior of the animal can be discussed conveniently at this point.

Irrespective of where the growth is situated the division of its cells goes on most actively in the deeper layers of epithelium, lessening and ceasing in proportion as the maturation into keratinized elements takes place. The growth rate may be considered in terms of a cone with its apex corresponding to the level in the epithelium where proliferation wholly ceases. The differentiating, keratinizing cells beyond this apex do not desquamate but remain firmly attached, a peculiarity largely responsible for the height of skin papillomas. The result for fragments of the growth that have been placed in the inner organs is that the proliferating layer of epithelial cells, extending laterally as result of its multiplication, yet tethered by the keratinized mass, spreads around the latter, the base of the hypothetical cone broadening until it has formed a sphere. Doubtless this encircling and enclosing would take place in uninvasive growths even if the keratinized cells did tend to come loose. Its consequence is that rounded nodules develop from

the bits of papilloma implanted within the animal, living epithelium enclosing dead squamous tissue, the apparent reverse of the state of affairs on the skin, where the latter is the covering layer. The way in which the two forms of the papilloma are produced finds direct illustration in those cases in which cutaneous growths have been forced to extend beneath the surface by covering them with collodion (Fig. 24). The conditions on the skin surface do not enable the proliferating layer of epithelium to enclose the overlying keratinized tissue: instead it buckles into papillae. A secondary outward protrusion, with buckling and more papillae, occurs as result of proliferation within the confines of the surface mass.

The epithelium of interior growths frequently sends out processes into the surrounding tissue, but these tend to become spherical later for the reasons just given. An extreme instance of the phenomenon can be observed at the surface of the globoid implantation nodules which sometimes practically replace the spleens of wild rabbits. Here numerous, blunt, papillary outgrowths of proliferating epithelium may exist side by side, covered and held together by a film of peritoneum only; yet they never thrust forth separately into the abdominal cavity with result in a frankly papillomatous mass, because inevitably under the conditions each alters toward the spherical. Only once have we found an arrangement like that on the skin, this where an implantation in the kidney gave rise to a growth lying partly on the surface of the renal pelvis, where it took the form of a long, slender horn of keratinized cells (Paper I).

It is plain that the organoid appearance of the papilloma is largely due to the interplay of two factors,—one a progressive lessening in the rate of proliferation as the epithelium differentiates, the other a failure of the keratinized cells to loosen and come away from those beneath; but the importance of these factors must not be overrated. Finding expression independently of them is an innate tendency of the cells affected by the virus to assume the papillomatous arrangement.

These are the findings in growths which behave in an orderly way. But as the photographs show, growths resulting from implantation have often the morphology of epidermoid carcinomas. Where the epithelium has become aggressive, invading tissues actively as happens in many cases, it is no longer constrained to surround the differentiated, dead cells nor is its multiplication conditioned by the need for a stroma. It grows where it will, irregularly. Though pearl formation is frequent (Figs. 7 and 12), so too is keratinization on one side of rapidly advancing epithelial processes (Fig. 17). At some parts of the border of growths that appear orderly the phenomena associated with malignancy can often be detected. The benign looking papil-

loma of Fig. 30, for example, had penetrated at several points into the lymphatics.

The epithelial cells increase rapidly by mitosis in favorable hosts, and those which are dividing attract attention by reason of their great size and pallor, a pronounced stippling of the cytoplasm with granules that stain deeply with methylene blue, and a sharply defined chromatin arrangement. In very rapidly enlarging, malignant growths the division figures may be highly irregular, asymmetric, and hyperchromatic (Fig. 9), and many of the cells die before division is completed or shortly afterwards.

A frequent complication in the pattern and development of interior growths comes about through a swelling of the necrotic material that they contain. The importance of this factor increases directly with the amount of included, dead material.

Large growths are always tense on palpation though usually elastic, and where unsupported by the surrounding tissue they often bulge abruptly (Fig. 5) and may even rupture (lung growths, mesenteric nodules), with result in a form like a bud (Fig. 32). The broken epithelial layer may grow around the extruded squamous material secondarily, or when in a favorable situation, as in the lungs, it may extend edgeways into the normal tissue (Fig. 14), proliferating so rapidly as to keep ahead of differentiation, and hence of the influence of attached, keratinized cells to cause rounding up. Under these circumstances it appears highly malignant.

Ordinarily the pressure exerted by the necrotic, central material does not suffice to rupture the nodule, but acts to flatten and thin its peripheral layer of living epithelium, especially where this finds no support. Such a state of affairs is well seen where kidney or liver growths project from the organs (Fig. 4), the living epithelium here being often reduced to a layer one or two cells thick, flattened, and with no hint of the papilliform arrangement that is present near by, where it is backed by parenchyma. The changes consequent on gradually increasing pressure of the necrotic material are often clearly discernible in the markings of the latter. Its central portion may show the papilliform structure everywhere on cross-section, evidencing the character of the early growth, while further out the dead, keratinized layers have a concentric arrangement, especially where there has been little mechanical resistance to the expansion of the mass, as where it protrudes into the abdominal cavity. Here in extreme cases the epithelium becomes entirely necrotic because its blood supply has been cut off. Where it has merely been thinned and flattened as result of the interior pressure mitoses are rare and there is an orderly differentiation to the keratinized state (Fig. 33).

Watch has been kept for variations in the underlying type of the papilloma. A single example only has been found thus far. In a

domestic rabbit dying of implantation growths in the liver, kidney, stomach, axilla, and extensor muscles of the hind legs,—an especially favorable host as shown by the occurrence of nodules in a lymph gland (Fig. 17) and in the lungs,—not a few of the implants showed unusually high and crowded, columnar basal cells, with processes extending into the connective tissue from the primary papillae at short intervals, rendering the growth a papilloma of the second order so to speak, with result in an unique appearance (Fig. 10). This state of affairs may have been the consequence merely of a stepping up of the proliferative activity of the basal epithelium.

The Phenomena of Retrogression

Not infrequently an induced papilloma retrogresses. The possibility that it may do so in a distinctive way, perhaps indicative of its virus cause, has led us to study the process in detail.

Wild rabbits resistant on experimental inoculation with the virus are not infrequently encountered. Shope found none with complete immunity, though three which were carrying the growth when received proved partially resistant (4). We have found four with complete immunity against our virus amongst 25 inoculated, two of them having papillomas of "natural" origin, the only ones that had. Our animals came from the same source as Shope's. Those that showed themselves resistant have been inoculated repeatedly with active virus fluid, always ineffectually.

Gross Manifestations.—In an occasional cottontail the papilloma appears promptly and grows for several weeks, drying up and falling off later, leaving a smooth, healthy-looking skin. This has happened in several animals inoculated with virus procured from fresh material and of but slight pathogenic capabilities, as control inoculations on domestic rabbits showed. Thus far in our experience wild rabbits with visceral implantations of their own vigorously proliferating papilloma have regularly succumbed to the resulting growths, though the skin papillomas may have ceased to extend.

Adult domestic rabbits are without exception primarily susceptible to the virus, as Shope noted. Of the 64 thus far inoculated by us none has failed to develop a papilloma. The course of the latter has been found to vary greatly though. As already stated it grows best in

individuals with thick, succulent skin and may enlarge rapidly during the first week; but it regularly ceases to encroach later, remaining to all appearance stationary in most instances. Occasionally it undergoes retrogression. The mass dries everywhere, its base becomes thinner, and a gradual flaking away exposes healthy skin; or else the area of proliferating tissue beneath the mass dwindles gradually, with result in a tassel-like, pedunculated growth. The weight of this may act to elongate its pedicle (Fig. 34).

Retrogression occurs occasionally of one of several growths arising as result of implantations within a wild rabbit; but the process, in our experience at least, is restricted to nodules in unfavorable situations. This is the case as well in some domestic rabbits; but in others with papillomas caused by the same virus fluid the enlargement of all of the interior growths slackens after the first months and they assume the globular form (Figs. 36 and 37), becoming well encapsulated (Fig. 38) and notably firm. Complete necrosis of individual nodules sometimes occurs, especially of those in the subcutaneous tissue, kidney, stomach, and such as are precariously nourished from the mesentery or peritoneal lining through a pedicle (Fig. 36, *P*); but in no case thus far studied has the growth failed to progress elsewhere (Fig. 37) and kill the animal. It is reasonable to expect that instances of general retrogression will be encountered when the skin papilloma utilized for implantation has not been doing well. Thus far we have implanted only material from robust growths.

The Histology of Retrogression.—The retrogressive changes are best studied in sections from interior nodules, since here the complicating effects of trauma, drying, and infection can be excluded.

The influence of the pressure exerted by the necrotic material within large growths to cause localized retrogression at their periphery has already been mentioned. It should be discriminated from retrogression proper, and this can usually be done. Mention has also been made of cell death following upon abnormal mitosis. Necrosis may abruptly overtake individual groups of the epithelial cells of invasive, highly malignant growths, either because their own rapid proliferation has deprived them of nourishment or because they are not suited to survive. But none of these happenings constitutes retrogression in the proper sense. This takes two forms. The epi-

thelial cells may suddenly cease to proliferate, and all differentiate to the squamous form, their death occurring as result of maturation. Such retrogression is usually very local. Or there may be a more widespread retrogression which is gradual and follows a definite order. It resembles that brought about by pressure, as above described, save that there is no unusual flattening of the epithelial cells.

The first changes noted microscopically are a blunting and broadening of the processes at the border of the growth, and an encapsulation of it with dense connective tissue in which are a few round cells. In the gross one notes a rounding of the mass and a sharpening of its contours. Soon the epithelium no longer pushes out here and there, but becomes a smoothly curving rind about a necrotic mass which has the papillomatous arrangement at its center but is increasingly concentric toward the periphery. Mitosis becomes infrequent, the epithelial layer takes on an appearance of special orderliness, its cells and their nuclei become smaller and stain less deeply,—in a word the tissue reverts more or less completely to the normal in its appearance. Occasionally even where much thinned it may still have characters indicative of the presence of the virus, both cells and nuclei being abnormally large and staining darkly. As result of cell differentiation in the absence of multiplication the mass eventually comes to consist wholly of dead, keratinized elements. Where these first come in contact with the surrounding connective tissue some polymorphonuclear leukocytes may gather, followed later by granulation tissue and giant cells which, together with connective tissue elements, penetrate very slowly and ineffectually amidst the dead cell masses, breaking up the outer ones (Fig. 44).

Our method of implantation, with shavings from surface growths, must often have involved some introduction of normal epithelium with the pathological; and it might be supposed that survival of this, or a gradual replacement by it of the papillomatous epithelium, not true retrogression, was responsible for the thin, maturing layer of more or less normal looking epidermal cells found at the periphery of most stationary or dwindling nodules. In general, however, the pattern of the necrotic material at the center of such nodules proves that their growth was everywhere papillomatous at first; while furthermore each step in the retrogressive process has been studied without discovery of any replacement of the sort outlined. No hair follicles or sebaceous glands have been noted in association with the reordered, epithelial layer.

Surface growths in hosts developing resistance show few papillae, and these are usually short and necrotic nearly to their bases. The wide spaces between them contain dead, keratinized tissue save for a smooth, underlying layer of epithelium. In appearance this epithelium has reverted more or less to the normal, and its connection with the hair follicles and sebaceous glands is now once again evident, as was not the case when it was in active papillomatous proliferation. The underlying connective tissue is dense and contains some makrophages and scattered accumulations of lymphocytes. Where pedunculation of the growths is accentuated by their weight (Fig. 34) the infrequent papillae may remain alive even though much attenuated (Fig. 35). Their appearance and the general conditions suggest that they and their nourishing cores have been pulled out by gravity.

The Behavior of Growths on the Skin

In the skin of even the most favorable rabbits the extension of the papilloma ceases after a time. Though it enlarges rapidly at first,—many scattered papules sometimes broadening and coalescing to form one mass, or a single papule developing into a cutaneous horn or warty growth several centimeters across,—its extension at the expense of its surroundings slows after some weeks and stops, though its cells continue to proliferate actively, the mass often becoming so redundant as to be thrown into thick folds and to lop over at the sides. Beneath it there is now a layer of dense connective tissue. Some of the epithelial processes which penetrate here and there into the latter are cut off by it from the surface and round out into pearls, often several millimeters in diameter, with a firm, yellowish white, spherical center of dead, concentric, squamous layers which shell out readily from a smooth, enclosing rind of living epithelium. Other processes not only maintain themselves but proliferate and burrow in irregular tongues amidst the dense connective tissue. Though the growth has ceased to enlarge, its appearance now suggests a developing malignancy. It is always heavily contaminated with bacteria from the skin and one may suppose that, as in the case of infected muscle implants, these bacteria sometimes, by influencing the epithelial cells or by the changes they bring about in the connective tissue underlying the

growth, or by both combined, induce a more aggressive behavior. None of our papillomas has been followed for more than 6 months.[1]

COMMENT

The findings here reported, like those of Paper I, demonstrate the ability of the papilloma to grow progressively and destructively on favorable occasion. Why then when situated on the skin does it ordinarily cease to enlarge after a time? Its cells continue to multiply, but everywhere surrounding it a layer of dense connective tissue gradually forms. Our experiments have shown that the growth encroaches upon its surroundings before this appears, as also when it is cut through later, and when the new tissue does not have time to become dense before the epithelium pushes into it,—as in instances in which extension of the papilloma takes place beneath the surface as result of covering it with collodion. The new layer of connective tissue evidently holds in check the epithelial cells which, in the lack of special stimulation, have no considerable aggressive ability. Numerous observations attest to this last fact. When fragments of the papil-

[1] Dr. Shope has generously permitted us to study and report upon a skin papilloma from the abdomen of a domestic rabbit killed recently, 393 days after inoculation. The animal had previously recovered from a fibroma also induced by a virus (Shope, R. E., *J. Exp. Med.*, 1932, **56**, 803). The papilloma now under consideration had long ceased to extend and had been gnawed close, but there remained an indurated base nearly 1 cm. thick. Sections of this show an irregular, thin, surface layer of proliferating epithelium, manifesting the papillomatous arrangement in some places, and beneath it to a depth of 3 to 4 mm. irregular processes, cords, and groups of proliferating epithelial cells, amidst an edematous connective tissue in which the fibrillar bundles of the corium can be recognized, now largely fragmented. The epithelial cells show some differentiation to the squamous form, but are both invasive and infiltrative. The picture is that of an epidermoid carcinoma of unequivocal malignancy (Fig. 39). The regional lymph glands of the animal were not strikingly enlarged, nor were nodules seen in the lungs.

In this instance a secondary breaking up of the connective tissue barrier beneath the growth may have enabled it to penetrate deeply. Or conceivably an independent carcinomatous change may have supervened in epithelial tissue prepared by long standing injury referable to the virus. The latter explanation can scarcely be advanced, however, to account for the prompt invasive and destructive growth from fragments of early skin papillomas implanted in the internal organs of the host. The causative virus has been recovered repeatedly from the nodules developing as result of such implantation in wild rabbits.

lomatous tissue are distributed by the arterial blood, as in the experiments of Paper I, they give rise to growths in the lungs only; and when brought to the lungs by the venous blood they cause few nodules in comparison with the number of fragments introduced. The tattooing of individual papules that have just appeared on the skin after intradermal virus inoculation, as in some tests not mentioned heretofore, does not result in subepidermal growths from cells dislodged and driven under the surface, but instead the papilloma is wholly destroyed. Though the actively proliferating epithelium frequently enters the blood and lymph vessels, metastasis formation does not ordinarily ensue.

It is instructive to compare the findings with those on record for tumors. Many of the skin epitheliomata of man and of tarred rabbits and mice remain stationary and benign in appearance unless liberated by operation, or stimulated by infection, by renewed tarring, or in other ways. So too with some spontaneous chicken tumors. Many human carcinomata fail to cause metastases although cells from them are distributed in abundance by the blood and lymph (5). Not a few mouse tumors fail entirely to yield growths on intravascular and intraperitoneal injection of pieces of them,—in which respect the papilloma is relatively successful. There is a large literature on the restraining effects of the connective tissue associated with stationary and retrogressing epidermal tumors. Authorities hold it to be largely responsible for the behavior of the growths (6). Several instances are on record in which, according as the tissue barrier changed from time to time, becoming denser or thinner, the histological appearance of the tumor altered toward the benign or the malignant respectively (7).

In the phenomena of its retrogression the papilloma gives no hint of a virus cause. The gross and microscopic changes resemble those occurring in the case of epidermoid tumors. As with these, retrogression may be general or may take place at some situations while growth continues at others. In both instances individual cells or groups of cells may suddenly necrose as result of their own abnormality. Generally though when retrogressing the epithelium of the papilloma, like that of epidermoid carcinomas, ceases to proliferate and comes to its death as result of a process of orderly differentiation

into keratinized cells. This process occurs in man, though it is rare (8); it has been observed in spontaneous mouse tumors (9); and it is frequent in the case of the tar cancers of mice and rabbits. The numerous published pictures of the changes in these last (10) show a detailed resemblance to those occurring in the papilloma. The retrogression of tar cancers, proven such by biopsy, is accompanied in many cases by a papillomatous rearrangement of the superficial masses, with reappearance of the connection of hair follicles and sebaceous glands with the epithelial layer, and eventually a complete reversion of the latter to the normal. A pedunculation may develop precisely like that illustrated in Fig. 34 of the present work. The retrogression of tar tumors that have penetrated beneath the surface leads to the formation of rounded, keratinized pearls lined by normal-looking squamous epithelium. All of these alterations have been noted in the case of the papilloma.

The changes occurring in the tissue about retrogressing papillomas are like those about retrogressing tissues in general, irrespective of whether they are neoplastic. New connective tissue forms and lymphocytes accumulate in greater or less quantity. Where the dead, keratinized cells of epidermoid carcinomas first come in contact with their surroundings polymorphonuclear leukocytes may gather, to be succeeded by granulation tissue with giant cells, which slowly enters the dead mass (11). This is true of the papilloma as well (Fig. 44).

SUMMARY

The injection of Scharlach R into the skin about rabbit papillomas resulting from virus inoculation causes them to invade the underlying tissue and form large, fleshy masses beneath the surface. Histologically these appear malignant, and they frequently invade the blood vessels. Covering young papillomas with a layer of collodion causes them to burrow downwards with result in discoid masses which enlarge progressively, both by expansive growth beneath the epidermis and by invasion. Such masses, like the nodules resulting from implantation, have the papillae turned toward their interior, the apparent reverse of the condition of affairs when the growth is situated on the skin surface. The reasons for this are analyzed. The peculiarities of the host influence skin papillomas not a little, as is plain from

the forms they assume; but the epithelial changes induced by the virus take a single direction, and no significant variations from type have been encountered.

Local or generalized retrogression of the experimentally induced papilloma is not uncommon. The histological alterations that take place are identical with those attending retrogression of the epidermoid tumors, and the reactive changes taking place in the surrounding tissue are also like those about such tumors. The slowing and cessation of growth that occur secondarily in the case of virus-induced skin papillomas are associated with the formation under them of a dense layer of connective tissue, and to this their behavior is attributable. Similar findings have been often recorded for tumors, notably for the epidermoid cancers produced in rabbits by tarring.

BIBLIOGRAPHY

1. Fischer, B., *Münch. med. Woch.*, 1906, **53,** 2041. Helmholz, H. F., *Bull. Johns Hopkins Hosp.*, 1907, **18,** 365. Haga, I., *Z. Krebsforsch.*, 1913, **12,** 529.
2. Werner, R., *Münch. med. Woch.*, 1908, **44,** 2267.
3. For a general review of the changes induced by Scharlach R and other dyes see Ewing, J., Neoplastic diseases, Philadelphia, W. B. Saunders Co., 3rd edition, 1928.
4. Shope, R. E., *J. Exp. Med.*, 1933, **58,** 607.
5. Schmidt, M. B., Die Verbreitungswege der Karzinome und die Beziehung generalizierter Sarkome zu den leukämischen Neubildungen, Jena, Gustav Fischer, 1903.
6. Ribbert, H., *Deutsch. med. Woch.*, 1916, **42,** 278. Yamagiwa, K., and Itchikawa, K., *Mitt. med. Fak. Univ. Tokyo*, 1918, **19,** 483. Itchikawa, K., and Baum, S. M., *Bull. Assn. franç. étude cancer*, 1924, **13,** 257.
7. Leroux, R., *Bull. Assn. franç. étude cancer*, 1927, **16,** 16.
8. Orth, J., *Z. Krebsforsch*, 1904, **1,** 399. Schwarz, L., *Virchows Arch. path. Anat.*, 1904, **175,** 507. Kimura, K., *Gann*, 1908, **2,** 75. Ribbert, H., *Deutsch. med. Woch.*, 1916, **42,** 278.
9. Woglom, W. H., *J. Cancer Research*, 1922, **7,** 379.
10. Yamagiwa, K., and Itchikawa, K., *Mitt. med. Fak. Univ. Tokyo*, 1918, **19,** 483. Itchikawa, K., and Baum, S. M., *Bull. Assn. franç. étude cancer*, 1924, **13,** 257. Roussy, G., Leroux, R., and Peyre, E., *Presse méd.*, 1922, **30,** 1061; *Bull. Assn. franç. étude cancer*, 1924, **13,** 164. Leroux, R., *Bull. Assn. franç. étude cancer*, 1927, **16,** 16.
11. Becher, J. A., *Virchows Arch. path Anat.*, 1899, **156,** 62. Orth, J., *Z. Krebsforsch.*, 1904, **1,** 399. Fischer, W., *Beitr. path. Anat. u. allg. Path.*, 1912, **55,** 1. Ribbert, H., *Deutsch. med. Woch.*, 1916, **42,** 278.

EXPLANATION OF PLATES

PLATE 41

FIG. 22. The papilloma as influenced by physical and chemical factors. The virus was tattooed into the skin at four spots, each about 2 mm. across, on the side of a domestic rabbit (D.R. 1-22), and 22 days later when papules were just perceptible at sites *A* and *B* the first of several injections of Scharlach R around and beneath them was carried out. The growth did not appear at sites *C* and *D* until the 28th day. The papules at site *C* were covered with collodion on the 43rd day and those at *D* were left as control. On the 84th day the collodion was stripped away, with some petechial hemorrhage into the tense skin, as the photograph taken 24 hours later shows. The growths at *A* and *B* were for a long time almost completely subepidermal, ulcerating shortly before the picture was taken. They have coalesced. The discoid, subepidermal growth at *C* shows a crater containing dry tissue, the result of pressure necrosis. The control papilloma at *D* is much smaller than the others, wholly superficial, and with a somewhat constricted base. × ⅔.

FIG. 23. To show the influence of Sudan III and Scharlach R. The growth was produced by a punctate inoculation of virus fluid into the ear of a domestic rabbit, and when a solitary papule had appeared, 33 days later, the tissue under and around it was injected with Sudan III in olive oil. Several similar injections of Scharlach R were made afterwards. There resulted a fleshy mass, largely subepidermal but ulcerating at its summit. The section, taken through its margin, shows irregular downgrowths of papillomatous epithelium with pearl formation. There are lacunae underneath, where the olive oil dissolved out. The epidermis covering the outer side of the mass shows some extension downwards and enlargement of the hair follicles, a characteristic effect of the dyes on normal skin. × 7.

FIG. 24. Cross-section of a part of the growth under collodion shown in Fig. 22, *C*,—from a biopsy specimen removed on the 85th day. The growth lies in the corium. The living papilliform processes have their base at its periphery and extend into its necrotic interior. × 7.

PLATE 42

FIG. 25. Cutaneous horn developing from a solitary papule induced by punctate inoculation of the skin of the ear of an adult domestic rabbit (D.R. 9). Scharlach R in olive oil had been repeatedly injected beneath and around the papule soon after it appeared. The other papillomas on the ear can be disregarded, as pertaining to other experiments. The great conical horn was dry, brown, and vertically striated. Picture taken 96 days after inoculation and 63 days after the appearance of the growth. × ½.

FIG. 26. A picture taken on the same day to show the extension to the under side of the ear. × ½.

FIG. 27. Cross-section of the growth, made 7 days later when the ear was amputated. Most of the pale, papillomatous tissue is dead but still moist. Direct extension to the under side of the ear has taken place through a small natural opening in the cartilage, occupied in part by a vein and artery. Natural size.

FIGS. 28 and 29. Differing character of the growths in two domestic, gray-brown litter mates. The skin of the abdomen was scarified and inoculated with virus fluid when they were 8 days old. The photographs were taken 97 days later, at which time both rabbits were in excellent condition and of nearly the same weight. The hair had been removed from about the confluent mass of Fig. 28 so that its low, dry, cindery character could be seen. The many separate cutaneous horns of the other (Fig. 29) were so large and crowded that a stretching and folding outwards of the skin had occurred to make room for them. The reductions are different: Fig. 28, × ½; Fig. 29, × ⅔.

FIGS. 30 and 31. Cross-sections of two discrete papillomas removed from the agglomerates of Figs. 28 and 29 respectively on the 70th day after inoculation. The differences in the lesions are described in the text. The tip of the growth of Fig. 31 was cut away to facilitate sectioning. × 7.

PLATE 43

FIG. 32. Periphery of an omental growth found in a wild rabbit (W.R. 18) killed 49 days after implantations into the abdominal viscera. The swelling of the necrotic material inside the nodule has caused it to rupture and "bud." The epithelium is flattened as result of pressure, and has reverted far toward the normal in appearance. Many of the other implants had grown actively (see Figs. 3, 4, and 5) and invaded their surroundings. × 125.

FIG. 33. Local retrogression, induced in part at least by pressure, at the periphery of a nodule in the liver of a wild rabbit. The animal was killed 48 days after implantation because the broad papilloma on the skin was growing smaller. The nodules that had developed in the muscles proved completely necrotic, but those in the abdominal viscera showed in general only localized regions of retrogression, with proliferation elsewhere in them. This was true of the growth furnishing the figure. In the part shown there is a pronounced flattening of the epithelium with reversion toward the normal. × 620.

FIG. 34. Ear of a domestic rabbit (D.R. 1-24), photographed 153 days after the virus had been tattooed into a number of spots across the tip, middle, and base. 5 days prior to inoculation some of the spots had been infiltrated with Scharlach R in olive oil (see Fig. 49). The growths became large and heavy, and their bases underwent a secondary constriction such that some of them came away. Only those at the middle and tip of the ear now remain, all notably pedunculated. The growths *A*, *B*, and *C* formed at one time a single mass, and *A* and *B* are still united by dead tissue though they have separate pedicles. Natural size.

FIG. 35. Portion of the base of papilloma *C* of Fig. 34,—to show the infrequent, attenuated, papilliform processes, and the exceptionally benign appearance of the growth. × 11.

FIG. 36. Implantation growths in the abdominal organs of a domestic rabbit (D.R. 1-21) dying of them 133 days after skin inoculation and 115 days after implantation. There are large, coalescing masses in the liver, a projecting nodule (*S*) in the wall of the stomach, and others (*P*) on the omentum and mesentery,

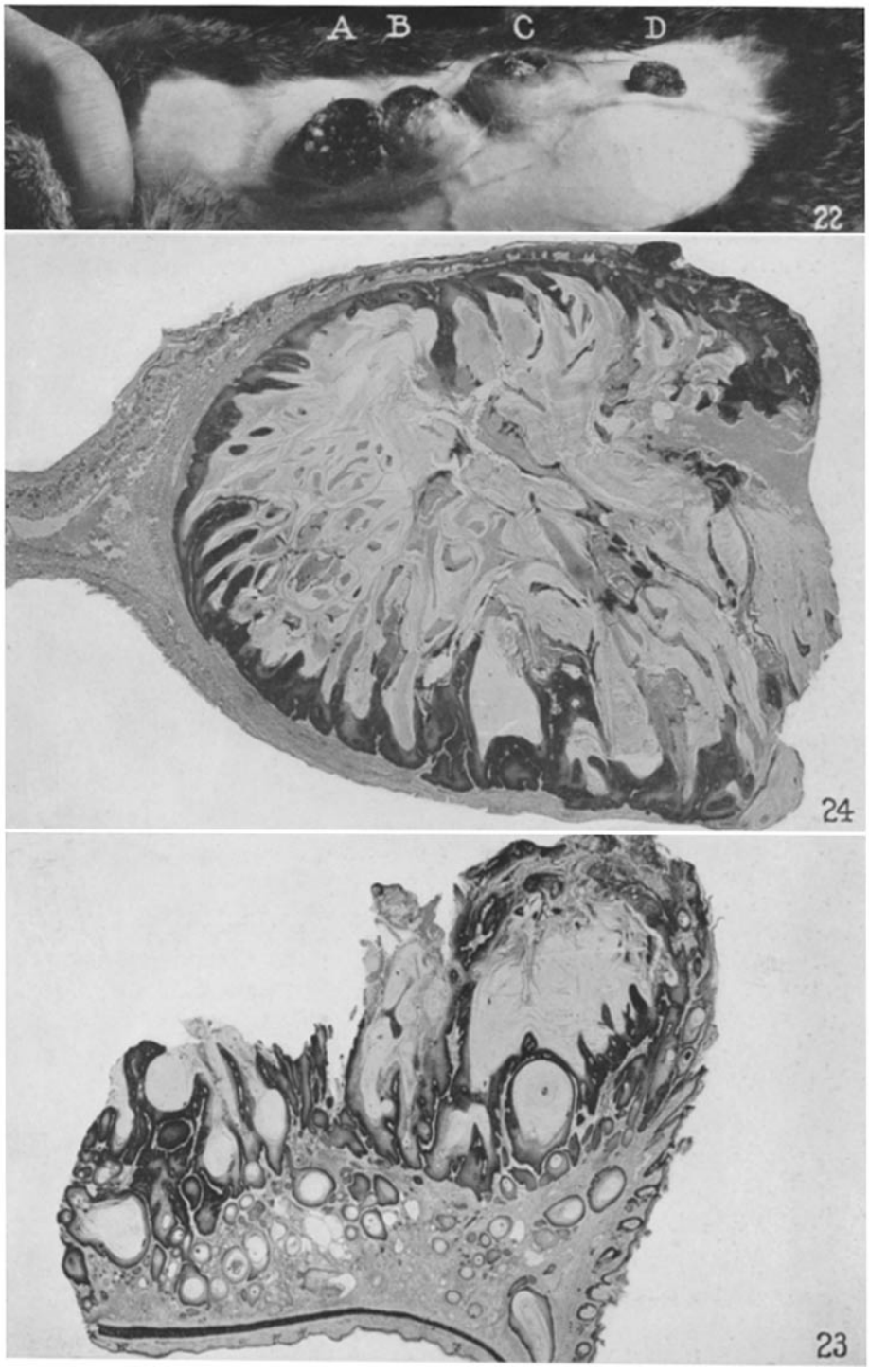
resulting from accidental dissemination. Some resistance to the papilloma had developed (see comment on Fig. 37), and the nodules tend to be spherical and well encapsulated. At *A* is an implantation growth protruding from the capsule of the kidney. Natural size.

PLATE 44

FIG. 37. Growths resulting from implantations in the extensors of the upper forelegs of the domestic rabbit, D.R. 1-21. That in the right leg almost entirely enclosed the humerus and had caused toe-drop. Some resistance had developed in the host as shown by a falling off in the rate of enlargement of the masses, and by their smooth, rounded form and definite encapsulation. × 7/10.

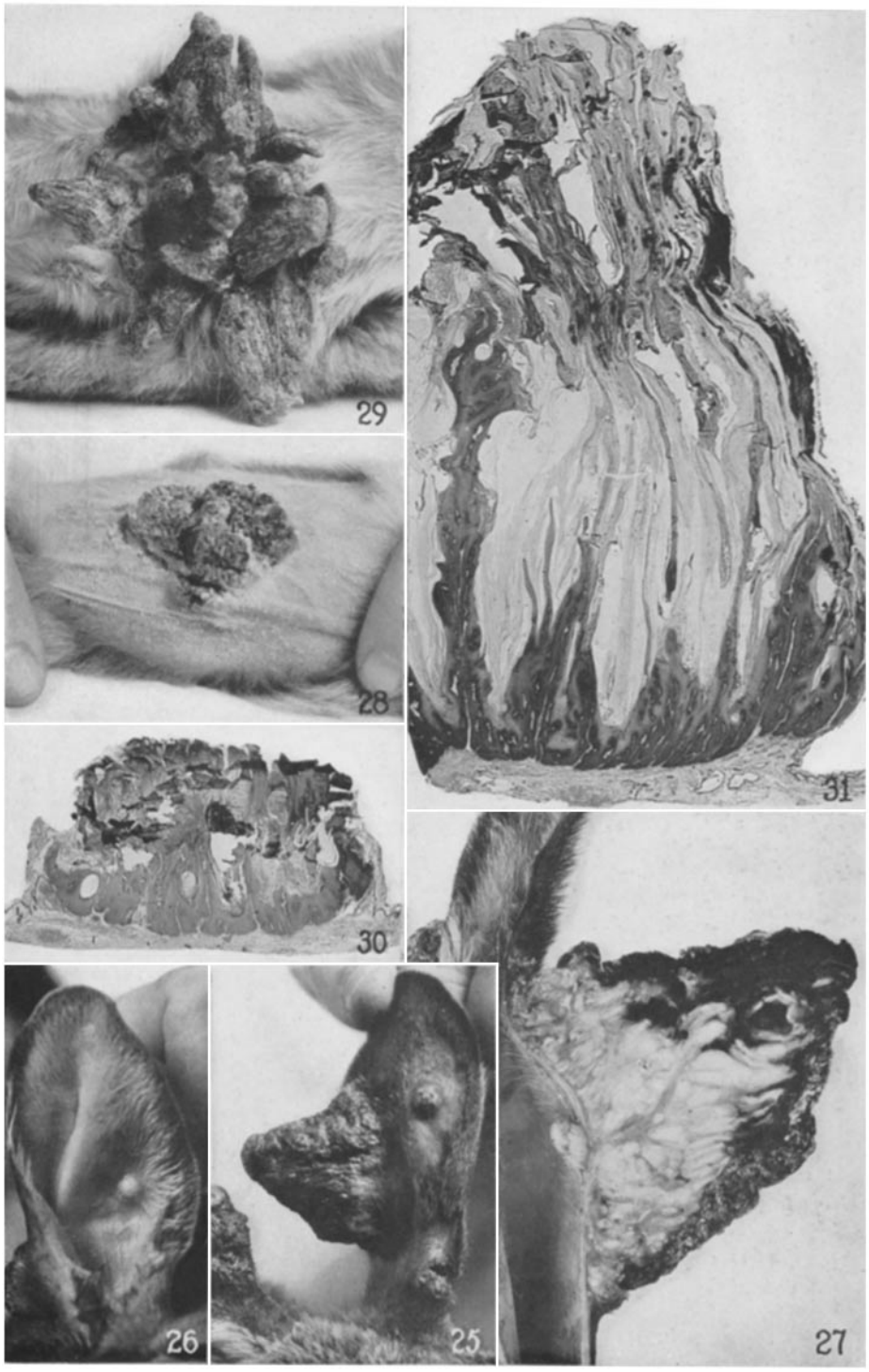
FIG. 38. The mass in the right leg. It was encapsulated and necrotic save for a thin rind of the characteristic epithelium. Compare with Figs. 1 and 2, muscle growths in a favorable host. × 7/10.

FIG. 39. Section from the thick, gristly base of a growth that had endured more than a year. It was situated on the abdomen of a domestic rabbit killed 393 days after inoculation, and had been gnawed flat. Microscopically it had lost almost everywhere the papillomatous character and looked like an epidermoid carcinoma, as the picture shows. In the region photographed, which was several millimeters beneath the surface, proliferating epithelial strands and groups of cells have penetrated amidst a disorderly connective tissue which is dense in some portions, edematous in others. The section was taken vertically to the surface, the uninvaded tissue on the left of the photograph being its deepest portion. (Block from Dr. Shope.) × 42.



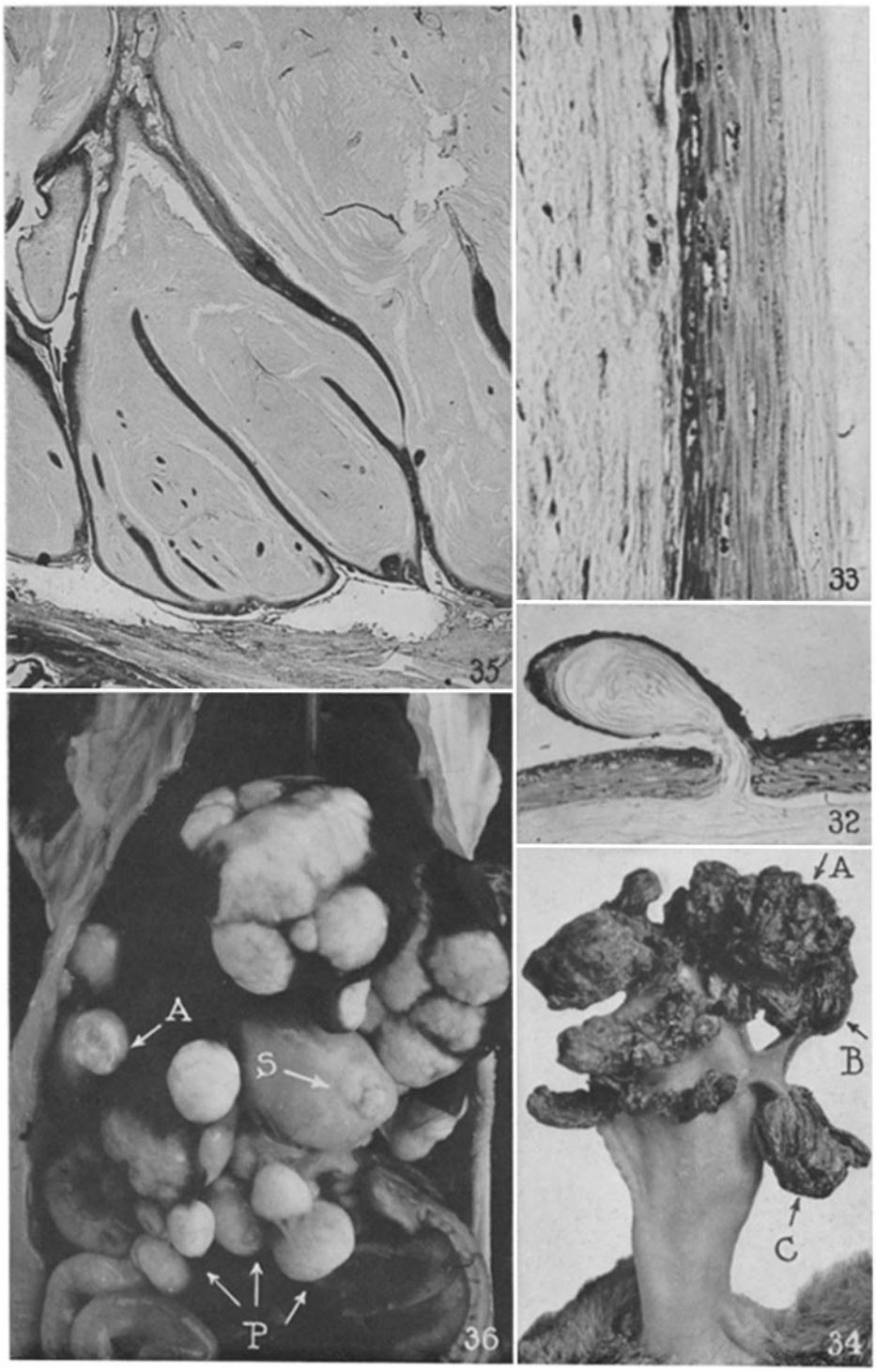
Photographed by Louis Schmidt

(Beard and Rous: Virus-induced mammalian growth. II)



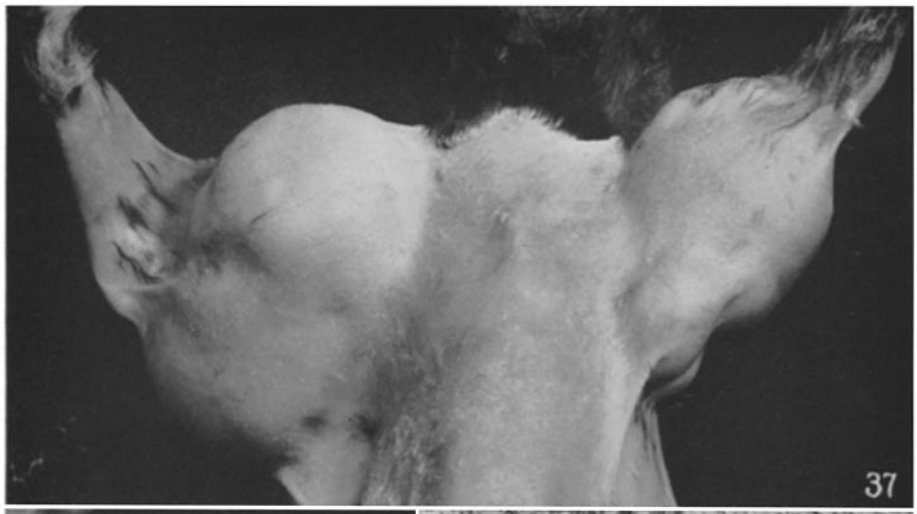
Photographed by Louis Schmidt

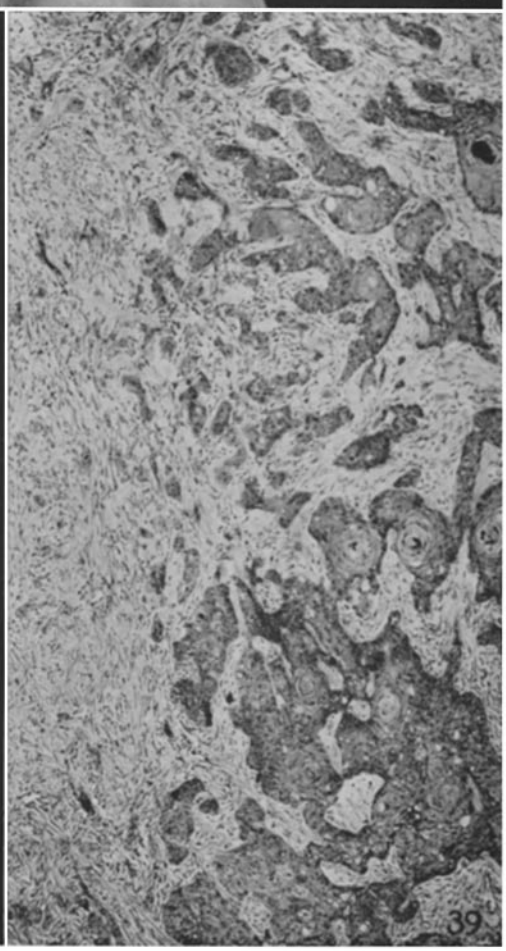
(Beard and Rous: Virus-induced mammalian growth. II)



Photographed by Louis Schmidt

(Beard and Rous: Virus-induced mammalian growth. II)





Photographed by Louis Schmidt

(Beard and Rous: Virus-induced mammalian growth. II)