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

I. The Growth on Implantation within Favorable Hosts

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Efforts to demonstrate the presence of causative agents in the recognized mammalian tumors have to the present been unsuccessful. The work to be reported here was undertaken, not as a renewed attempt in this direction, but to determine whether a growth known to be caused by a virus and of endemic occurrence, namely the Shope rabbit papilloma (1), possesses the immediate characters and the potentialities of a tumor.

The growth, as found on the skin of wild rabbits, has the appearance of a papilloma and is devoid of inclusion bodies such as indicate the presence of a virus (2). During its early extension it invades downward until it meets obstruction; and, though ceasing to enlarge after a time, it seldom retrogresses. It is readily produced in domestic rabbits by inoculation of the virus; yet from the growths in such animals this virus cannot ordinarily be recovered in active form. As Shope has pointed out, an extraneous cause can no more be demonstrated under such conditions than in the mammalian tumors thus far tested or, for that matter, in chicken tumors of filterable origin when they are growing slowly. These traits of the growth, in special, have commended it as material for investigation.

Our experimental comparison of the papilloma with the recognized neoplasms will be divided into three parts for reasons of expediency, with frequent references in one part to the material of another, and consecutive numbering of the figures. The appearance and behavior

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of implantation growths in favorable hosts are the main themes of the present paper.

Methods

The papilloma is frequently encountered on the skin of "cottontail" rabbits in Iowa and Kansas, as one or more verrucous masses or cutaneous horns. Our supply of virus-containing material was generously provided by Dr. Shope. It consisted of pieces of papillomatous tissue from two individuals, and had been kept in a single bottle of 50 per cent glycerin at refrigerator temperature for nearly a year. We have drawn upon it for virus at intervals during 7 months, always with positive results. To prepare a virus-containing extract the fragmented tissue is put through three changes of Tyrode solution, with a stay of some minutes in each; ground with sand; and made up with Tyrode to a 5 or 10 per cent extract by weight. Sometimes this has been allowed to sediment briefly before inoculation, but in most instances it was centrifuged and a portion of the slightly cloudy fluid, virus fluid as it will be termed, was withdrawn through a long needle from the midst of the supernatant column. For routine production of the papilloma it was rubbed into the shaved skin of the abdomen immediately after light scarification of a broad expanse with sterilized sandpaper; but when the effect on the growth of various factors was to be tested several widely separate inoculations were made along the sides of the animal, either by intradermal injection of 0.1 or 0.2 cc. of virus fluid at each situation, or by tattooing through a drop of it placed upon the skin. An electric tattooing machine was employed, having a group of needles about 2 mm. across that could be readily sterilized in boiling water. The sites of punctate inoculation were marked by tattooing India ink into the skin near them, and charts were made of their precise relation to the ink spots.

For most of the work, adult domestic rabbits weighing about 2 kilos, of gray-brown (agouti) breed, were utilized. The papilloma regularly develops in these when the virus is rubbed upon skin that has been scarified sufficiently to bring out a blood-tinged serum; and the growth tends to appear at about the same time in all receiving the same material. The incubation period from group to group ranged between 7 and 30 days, after broadcast inunction of the virus on the abdomen. To date we have thus inoculated 64 domestic rabbits, and the growth has appeared in every one. After intradermal injection, 1 to 2 months or more may elapse before the first papule is noticeable, and the results of some injections, perhaps a third in all, have been negative. Tattooing virus into the skin was found to yield the growth sooner and more consistently, doubtless because of the multiple inoculations effected with the needles.

The cottontail rabbits employed (genus *Sylvilagus*) came from Kansas. The growth was already present in two amongst 48 of them received, and these two proved resistant on inoculation with the virus, as did two others of the 25 thus far tested. The skin of cottontails is so thin that intradermal injections with virus-

containing fluid cannot readily be effected. Consequently tattooing has been done when multiple tests upon a single animal were desired.

Material fixed in acid-Zenker and colored with methylene blue and eosin, or with Giemsa's stain, has been used in the histological studies.

General Character of the Growths on the Skin

The histology of the growths on the skin has been described by Hurst (2). Little need be said of it at this point.

The virus causes a lively proliferation of the epithelial cells by mitosis in the basal layer and the *rete Malpighii*, with abnormal maturation and keratinization. The growing epithelium at first extends sideways under the neighboring epidermis and downwards through the connective tissue until it meets the fibrous corium, thereafter protruding in papillae that often become greatly elongated. The proliferating cells are far larger than normal, appear darker with methylene blue, have much larger nuclei, and the granules characterizing the granular layer tend to be especially abundant, large, and intensely staining. The cells flatten much less than normally during differentiation, and they fail to desquamate after keratinizing. Furthermore the papillae dry at their summits after a time because their blood supply is interfered with by lateral pressure. In consequence of these processes the growth soon becomes capped with a thick, firm layer of desiccated material, which may build up to a height of several centimeters, forming a cutaneous horn. Sometimes its living portion is pigmented, not infrequently almost coal black in the gross. The color is due to brown or black, fine or coarse granules of pigment situated in the epithelial cells and also in chromatophores scattered amongst them and in the immediately underlying connective tissue. Apart from the presence of these granules, the pigmented growths look like the others. The conditions determining pigmentation will be the subject of a future paper. The normal epithelium of the skin of cottontails and of domestic gray-brown rabbits such as we used contains brown pigment granules.

The morphology of the papilloma produced by rubbing the virus into scarified skin differs somewhat in wild and domestic rabbits. In the former it appears as pink or gray papules which darken as they enlarge, and the growth soon consists of a broad, elastic mass of nearly black, big and little, more or less fused cones with plump bases (like onion sets) resting on a thin layer of connective tissue. The mass is on the average ½ to 1 cm. high, showing far less tendency to build up than in domestic rabbits. In the latter the growth is relatively exuberant and fleshy, and may be pink or sooty, low or projecting, dry or succulent. When virus has attacked the epithelium at many neighboring points in a scarified area, with result in multiple papillomas, these soon coalesce into a mass; whereas if the initial change has been confined to one spot, as on intradermal inoculation, a single, sharply circumscribed papilloma develops. According as this broadens rapidly or slowly, or

undergoes secondary constriction at the base, it becomes verrucous or cone-shaped, perhaps acuminate, or even tassel-like with a peduncle. In masses formed by secondary fusion of many small, contiguous growths, portions of normal skin with their appendages are frequently carried far above the surrounding surface. In consequence some growths become covered secondarily with thin hair. Nipples with unaffected epidermis may occasionally persist for weeks at the summit of high growths, before their blood supply is interfered with and they undergo the general drying.

Specificity of the Virus

Though capable of affecting the cells of an alien genus of rabbit the virus produced no lesions on the skin of the rats, mice, or guinea pigs that we inoculated; and inoculations into four cats, one pig, and one goat have also yielded negative results. In susceptible animals the virus is notably specific for the epidermis.

Virus fluid was rubbed into large areas on the sides of several domestic rabbits, and small strips of the inoculated skin were removed daily, under ether, throughout the period until the growth appeared. Microscopically it could be seen that the scarification had broken through the epithelial layer, as a rule almost midway between the orifices for the hairs, and here after the break had healed the characteristic epithelial proliferation first appeared. Often, especially in experiments involving intradermal inoculation, the virus must have been brought into contact with the cells of traumatized hair follicles and sebaceous glands, yet we have never encountered a growth with morphology suggesting an origin from these structures. The epithelium of the necks of the hair follicles sometimes showed proliferation and the cellular changes characteristic of the virus, but the cells of the hair bulb were always unaffected. The typically differentiated cells of the sebaceous glands may come to be surrounded and underlain by a layer of the changed epithelium, but they are readily distinguished therefrom. Whether this layer represents an intrusion, or follows upon virus infection of the basal layer of the sebaceous gland, with result that its cells proliferate without differentiation, remains to be determined.

The virus often fails to give rise to the papilloma when tattooed into the ears of wild rabbits or does so only very slowly, though promptly causing the growth when introduced elsewhere in the skin of the same individuals. This may be due to the toughness and tenseness of the tissue, which will not admit much extraneous matter on the tattooing needles; for takes are readily got on the softer skin of the ears of domestic rabbits. Inoculations into the prepuce of a wild rabbit,—which was susceptible as shown by the results of a skin inoculation made at the same time,—and into the prepuce, tongue, mucous membrane of the cheek, and hard palate of two domestic ones resulted negatively. So, too, did direct injection of virus fluid into the liver, spleen, stomach, appendix, cecum, rectum, and kidney of an etherized wild rabbit later proven susceptible, and into the liver, kidneys, stomach, and a submaxillary gland of two domestic animals. In each case one ureter and the

duct from one submaxillary gland had been tied some weeks previously with a view to rendering the gland epithelium more susceptible. The gastric mucosa and that of the intestines were repeatedly needled while injecting the virus, to bring it into direct contact with them. In two wild rabbits the lungs and trachea were inoculated by thrusting the injecting needle into them here and there through the cleansed skin of the etherized animal. When they were sacrificed, after 66 and 67 days, no lesions were found anywhere save on the skin where the needle had been thrust through. Here in one instance the papilloma had developed. Shope found the virus ineffective when injected subcutaneously or into the peritoneal cavity; while when introduced into the blood stream it produced lesions only in the skin, at points of injury.

The agent responsible for Chicken Tumor I causes tumors most readily in young birds, the embryonic mesoderm being highly susceptible to it (3). Because of this fact we have explored the possibility that the papilloma virus might act upon embryonic epithelia of various sorts.

Three experiments were done, with material from rabbit embryos 10 to 26 days old from domestic does. The lungs, kidneys, livers, intestines and stomachs, brains, placentas, and skin were removed separately, finely hashed, and placed in suspension in Tyrode solution. A hash of the skinned embryo was also used in one case. Half of each suspension was mixed with an equal amount of freshly prepared 5 per cent papilloma virus in Tyrode solution, while the other half, appropriately diluted with Tyrode, served as control. The mixtures were allowed to stand 30 minutes at room temperature in two experiments, 2 hours in another, with frequent agitation, and were then implanted subcutaneously along the sides of twelve domestic rabbits of the same breed, 0.2 cc. at each site, the virus-containing suspension on one side, the control suspension on the other. When necessary to obtain sufficient material, organs of the same kind from several embryos were pooled.

The bits of placenta, kidney, liver, and brain did not long survive in the new hosts, but those of skin, stomach and intestines, lungs, and whole embryo proliferated, forming little nodules, and these were found to contain much living epithelial tissue of the characteristic sorts when the animals were killed after 1 to 3 weeks. The virus had had no discernible effects on this tissue, which resembled microscopically that in the control nodules on the other side of the host. In one instance two spherical lumps about 0.8 cm. in diameter developed just beneath the surface along the track of the needle used to inject embryo skin mixed with virus, but several centimeters away from the little nodule developing from the latter,—which itself showed no virus changes. They consisted of proliferating papillomatous tissue such as results from subcutaneous implantation of the papilloma, as described further on. They probably arose from epidermal cells of the host, pushed beneath the surface by the needle and at the same time inoculated with virus.

The Growths Resulting from Transplantation

There are histological indications that the papilloma can invade the surrounding structures on occasion. It not only grows downwards when first appearing but later may thrust short processes into the underlying connective tissue. This gradually thickens, often with small accumulations of polymorphonuclear leukocytes here and there in it, and after some weeks a dense layer of scar tissue has developed. The epithelium of the growth continues to proliferate but now the latter no longer enlarges. In order to determine how it will behave if placed under different conditions, as also whether the inflammatory reaction present beneath long established growths is characteristic or consequent merely on trauma and infection, we have implanted bits of papillomatous tissue in various organs of the host.

Method.—The skin of the abdomen of eight domestic rabbits and four wild ones was scarified over a large area and virus-containing fluid was rubbed in, with result in a confluent, papillomatous change. When the broad "pancake" of new-formed papillae was 1 to 2 mm. high, but still everywhere living and soft, it was vigorously scrubbed with soap and water, rinsed, and dried with sterile sponges. The surface layer was removed with a razor and discarded, and then a shaving like a Thiersch graft was taken off into Tyrode, cut into fragments small enough to pass through an 18 gauge needle, and injected in suspension into the extensor muscles of the fore or hind legs of the rabbit furnishing the material and into the liver, spleen, kidney, stomach, and the subcutaneous tissue of axilla or groin. A laparotomy was done under ether to reach the viscera.

All of the animals receiving implants of the papillomatous material have developed growths,—often enormous ones (Figs. 1, 36, 37, 38). Some of them were killed for examination; the others have become cachectic and died, usually from a terminal infection. The growths progressed more rapidly in wild rabbits, one succumbing to them in 39 days and another in 81 days. The domestic rabbits died of them after 94 to 115 days. For some time before death the "pancakes" of papillomatous tissue providing the material for implantation had been stationary in size, and indeed one was retrogressing.

General Findings.—The implantation growths had the same general structure in all the organs (Figs. 2, 3, *et seq.*). They were rounded or irregular, according as they had developed from a single focus of growth or from a number, well demarcated, creamy, gray or even

black, or patched with gray or black (Figs. 2 and 3), resistant to the knife, close-textured, and necrotic everywhere except in their outermost layer and along some inward extensions from it. Many had an evident connective tissue capsule while others had none, at least in certain regions. The papillomatous structure was faithfully recorded in the necrotic tissue, which was tough and rather dry; but the papillae did not project outwards like those on the skin. Instead they extended toward the center of the growth, with their base at its periphery (Figs. 8, 12). This arrangement had resulted, not from a buckling inwards of the proliferating epithelial layer,—for the dead interior of the mass was notably dense,—but from a direct penetration of the surrounding tissue by blunt epithelial processes which advanced in a more or less orderly fashion so close together that only a thin layer of tissue lay between adjacent ones. The proliferating basal epithelium formed the outermost layer of the growth, the dead, keratinized cells accumulating in its interior. The pattern was still papillomatous but now with papillae turned in. The septae were derived from the growth's surroundings, both by inclusion between the epithelial processes and by secondary changes in the included tissue, such that it became loose-textured, and its vessels wide, numerous, and thin-walled. The proliferating activity of the epithelial cells greatly exceeded in general their ability to thrust their columns into the surrounding structures, and in consequence most of the mass was necrotic. The papillae within it, nourished only by the vessels of their narrow cores, died *en masse* as their blood supply was cut off by the pressure of their own proliferation, though occasionally living ones persisted almost to the center.

Growths in the Subcutaneous Tissue.—The numerous implantations into the loose tissue of axilla and groin have yielded nodules that sometimes reached a diameter of 5 or 6 cm. and interfered with locomotion.

The growths were roughly spherical (Fig. 1) or like several coalesced spheres, elastic and well encapsulated, with occasional blunt protuberances. On section they showed a rind of translucent, grayish pink, living tissue about a dense, creamy to black, papilliform or concentrically striated, necrotic mass (Fig. 2). Very occasionally, as in other interior situations, irregular clefts existed between the papillae, containing a colorless, watery fluid (Fig. 10). There was a more or less well defined capsule of young connective tissue cells, with sometimes a few round cells but no

polymorphonuclear leukocytes; and the capsule and the surrounding connective tissue were far less compacted than if the growth had enlarged by expansion only. The lymph glands frequently became attached to the capsule but in general were not invaded. Sometimes as the growths became large the skin over them broke down and necrotic papillomatous tissue was exposed.

Muscle Growths.—The voluntary muscles proved a more favorable situation, the growths developing faster, with much invasion and relatively little encapsulation (Figs. 2 and 18 to 21).

The extensor muscles of the upper legs were utilized for implantation, preferably those of the forelegs since here small nodules could readily be palpated. The bundle of muscles was held between the thumb and forefingers, with the shaved skin drawn tense, and 0.5 to 1.0 cc. of a thin suspension of papilloma fragments was injected into it at a single point. After 2 to 3 weeks, one or more little nodules could be felt at the immediate site of injection, and these rapidly enlarged. The growing mass frequently replaced almost wholly the extensors in which it lay, occupying the space from elbow to shoulder; but the uninvolved muscles persisted as an overlying, thin layer. In domestic rabbits dying of the papilloma 3 to 4 months after implantation of it into the viscera and forelegs, enormous masses had developed in the latter situation (Fig. 37). They sometimes extended almost completely around the humerus (Fig. 38), causing a partial paralysis with toe-drop and a further limitation of motion due to encroachment on the axillary space.

The muscle growths had usually (Fig. 37) though not always (Fig. 1) a capsule visible in the gross. When rapidly enlarging, in wild rabbits especially, they were irregularly football-shaped with their long axis in the direction of the muscle fibres, and on cross-section they appeared to consist of many small, coalescing nodules (Fig. 2). These were the consequence of a thrusting forth everywhere at the periphery of the mass of proliferating epithelial processes which became nodular foci of growth while themselves thrusting forth new processes. Gross section showed numerous, discrete patches of dry necrosis scattered amidst grayish pink or sooty, translucent tissue that under the microscope proved to be actively proliferating epithelium, more or less encapsulated by new-formed connective tissue (Fig. 18), but invading the latter and not infrequently extending beyond its limits. The enlargement of the growth was in considerable part the result of invasion. Sometimes there was a direct penetration of the epithelium amidst the muscle fibres, with replacement of them (Figs. 19, 20, and 21). The histological appearance was very different from that due to proliferation of the sarcolemma sheath with giant cell formation, so frequently seen in injured muscle.

Growths that progressed slowly were spherical or egg-shaped (Fig. 37), or as if composed of several partially fused spheres, smooth-surfaced, well encapsulated, notably firm, and necrotic save for a thin rind of the characteristic epithelium (Fig. 38). The necrotic material, which was sometimes putty-like, shelled out

readily, leaving a smooth or velvety, living layer with small papilliform projections here and there. Such growths resembled those resulting from implantation in the relatively unfavorable subcutaneous situation.

Liver Growths.—Nodules regularly developed at the implantation sites in the liver, and sometimes nearby,—doubtless from small fragments accidentally introduced into blood vessels with the injection fluid, as will in due course appear.

The growths were spherical or irregular, often coalescing, and creamy to sooty in hue (Figs. 1 and 3). They usually protruded above the liver surface, and often reached a diameter of several centimeters (Fig. 36). On section they stood out from the parenchyma, and were firm, close-textured, necrotic save at the periphery, and relatively bloodless. The sooty regions were sharply marked from the adjacent creamy tissue by their hue (Fig. 3) but not in other ways. Microscopically the characteristic, introverted papilliform arrangement was found, with a rind of actively proliferating epithelium and an occasional living papilla far in the interior. The sooty regions showed pigmented epithelial cells and chromatophores. As in the case of growths in the subcutaneous tissue and muscle, round cells and polymorphonuclear leukocytes were notable by their absence; but there was ordinarily an irregular encapsulation with new-formed connective tissue, which was thin in some places and in others wholly lacking. Where it lacked there was no cellular reaction and the interlobular blood sinuses alone separated the liver cords from the proliferating epithelium (Figs. 8 and 11). The arrangement of the latter was orderly as a rule, growth being largely expansive, though taking place also from processes pushed here and there into the parenchyma. Not infrequently, especially in wild rabbits, narrow, disorderly tongues of epithelium extended out and directly replaced the liver cords (Fig. 11).

Splenic Growths.—These were like the ones in the liver. They grew both by expansion and by direct invasion and replacement of the splenic pulp.

In Fig. 12 two of the invading tongues of epithelium have differentiated into pearls. Mitoses were often exceedingly numerous (Fig. 9), as in the liver. There was ordinarily little or no encapsulation (Fig. 12) or other reactive change, and while sometimes the surrounding parenchyma had been compacted by the growing mass in most instances it had not. Often the short, thick spleen of wild rabbits was almost wholly replaced by the growth, only its ends remaining as tails attached to a spherical nodule 2 cm. or more in diameter (Fig. 1). In one case a thin skim of ruddy parenchyma partly covering a spherical growth 3 cm. in diameter was all that remained of the spleen. In the elongated, firm organ of domestic rabbits replacement was far less complete.

Growths in the Stomach.—The implantations in the stomach were often faulty, with escape of some at least of the fragments into the lumen of the organ or into the peritoneal cavity; and not infrequently the papilloma failed to develop at the injection site.

In positive instances an encapsulated, spherical nodule was found at autopsy. Usually it was not more than 0.3 to 0.7 cm. in diameter, even when the liver growths were several times this size. Microscopically it had the typical arrangement. Occasional epithelial processes extended into the surrounding capsule but there was no direct replacement of the gastric tissue. Sometimes the growth protruded on the peritoneal surface (Fig. 36) but more often into the lumen of the stomach. The overlying mucosa was usually unchanged, but sometimes had undergone a local thinning or showed hemorrhages or scarring.

Renal Growths.—In the relatively firm kidney cortex the papilloma grew slowly, as one or several rounded nodules (Fig. 4) surrounded by new-formed connective tissue, perhaps with some round cells. However it not infrequently put forth processes which penetrated irregularly between the tubules. A differentiation to pearls can be seen in Fig. 7.

Where the growth reached the kidney surface, and in consequence was relieved from pressure, it rapidly enlarged with result often in a projecting sphere. In one cottontail a narrow, pointed, brown horn was found projecting into the kidney pelvis. Conical and smooth, it was about 6 mm. long and 2 mm. across at its base on the medulla. Serial sections showed that it had arisen where a papillomatous nodule in the kidney substance opened out directly upon the pelvic surface. It consisted of keratinized epithelium everywhere except at its proliferating base (Fig. 49).

Enhancement of Malignancy by Bacterial Infection

The implantations into the leg muscles were carried out not only for their own sake but to provide material free from bacterial contamination for later transplantation to other individuals. The experience of one of us with ulcerated, spontaneous chicken tumors has been that if portions are transplanted to other fowls death or local necrosis is the usual consequence, whereas implantation into the muscle of the host is well tolerated, and bits of the growths to which it gives rise can be introduced into other individuals without danger. Presumably the host has developed an immunity to the organisms contaminating the tumor, which suffices to destroy most of those

introduced with pieces of it. However this may be the fact is certain that the intramuscular injection of pieces of papilloma from the skin seldom resulted in any evident infection, although innumerable bacteria must have been introduced with the material. The resulting growths enlarged by expansion, by the thrusting forth of orderly processes, and to a minor degree by irregular invasion. Occasionally, however, acute inflammatory changes took place about the implanted bits, with accumulation of pus cells and necrosis of the papillomatous tissue. Bacteria were present as stains showed. In other cases a chronic, more or less widespread, interstitial cellulitis developed, with edema. The papilloma growing amidst tissue affected in the latter way showed an active malignancy, sending out long, thin, disorderly tongues of proliferating epithelial cells which thrust between and around the individual muscle fibres, destroying them by pressure or contact (Figs. 19 and 20), and in some cases penetrating within the sarcolemma and directly replacing their substance (Fig. 21).

The rabbit of the instance figured had received implants into the liver, kidney, and spleen at the same time with those into the muscles. It died on the 23rd day after implantation, of subacute peritonitis and pleuritis. The growths in the viscera were actively invasive. Such slight cellular reaction as occurred about them did not suggest an associated infection. This has been a general finding with visceral implants of material which, in the muscle, gave rise to cellulitis.

Recurrence and Operative Dissemination of the Growth

On numerous occasions intramuscular growths have been removed from the leg for transplantation or study; and the attempt has been made to take them out completely, though with the least possible excision of the normal structures. They have nearly always recurred, not only in the muscle but sometimes in the subcutaneous tissue that was cut through; and their subsequent growth has generally been more rapid and extensive than that of the control mass in the other leg.

The laparotomy for implantation in the abdominal organs was usually made well to one side of the abdominal "pancake" of papillomatous tissue furnishing the material, but in some instances was tangential to it. Under the latter circumstances skin growths previously stationary extended for some millimeters along the newly healed incision, but not elsewhere. No independent papillomas, such

as might have resulted from secondary infection with the virus, appeared in the skin incised during the process of implantation, save in a single instance of one new growth; and the derivation of this was rendered uncertain by the development of papillomatous nodules in the underlying subcutaneous tissue and healed muscle, obviously as result of an accidental scattering of tissue bits at the time of operation. Operative dissemination often resulted in nodules on the peritoneum. During the injections into the viscera some of the papilloma fragments nearly always escaped into the abdominal cavity; and the nodules eventually found attached to the peritoneum were composed of epithelium like that introduced. Mention has already been made of the fact that intraperitoneal inoculation of the virus as such fails to cause lesions.

The peritoneal implants (Fig. 36) were usually few, never more than fifteen, and their size and condition indicated that most had been developing for a considerable period, doubtless from the time of laparotomy. Contrary to expectation, none had the frankly papilliform shape of the skin growths. They were always rounded, often with but a small base, and they had the introverted arrangement. Peritoneum, and as a rule connective tissue, covered them completely even when, as sometimes happened, they were attached by but a slender pedicle or cord. The proliferating rind of living epithelium was in many instances a smoothly curving layer, or but slightly papillomatous; but here and there it thrust processes into the connective tissue, and these were often irregular and invasive (Fig. 15).

The growths developing in the muscle along the healed laparotomy wound had the same morphology as those due to direct implantation in the leg muscles. They frequently pushed into the peritoneal cavity secondarily, and here the release from pressure caused them to mushroom out (Figs. 1 and 5); but always the broad, mushroom cap was found to be covered with peritoneum. The living layer of epithelium in the most projecting portion of the cap was frequently very thin; and occasionally both it and the peritoneum gave way before the interior pressure, and there resulted a secondary bulging sphere, with sometimes a third, smaller one upon that, the result being a conical mass made up of spheres of diminishing size, one on top of another, like a Tibetan monument. This was sometimes the case also when kidney or stomach growths had emerged on the peritoneal surface. Occasionally the connection of a secondary protrusion with the parent nodule was very narrow, like a bud (Fig. 32); but it is doubtful whether such buds ever came away and gave rise to growths.

Many mouse tumors fail to "take" when bits of them are injected into the abdominal cavity, but they will do so if introduced with an

irritant such as diatomaceous earth which aggregates here and there on the peritoneum and induces locally a connective tissue reaction favorable to the tumor cells,—as has been proved in a study of the reasons for the localization of metastases at points of injury (4). The findings just described show that the papilloma can implant itself on the peritoneal lining without such aid; but nevertheless bits of it, from the abdominal "pancakes" of domestic rabbits, were placed in suspension with diatomaceous earth and injected into the peritoneal cavity of the hosts. In some cases, though not in all, implantation growths resulted; and these had the characteristic morphology, and most of them had arisen where the diatomaceous earth elicited a reactive proliferation of the connective tissue.

Implantation by Way of the Blood Stream

Can bits of the papilloma give rise to the growth when distributed on the blood stream? Tests of this possibility were made.

Four domestic rabbits were used. The material for injection into two of them was obtained by slicing off and finely mincing in Tyrode parts of their own early skin papillomas, filtering the suspension afterwards through gauze to remove large fragments. In the case of the other two, muscle growths resulting from implantation were excised under ether and the rind of living tissue was treated in the same way. Muscle growths were used because it seemed possible that the bacteria present on skin material utilized directly might cause death of the inoculated fragments. From 2 to 4 cc. of thin suspension was injected into an ear vein of each rabbit; and from 2½ to 10 cc. into the central artery, in the direction of the heart,—after the vessel had been distended by heat and the large marginal veins had been temporarily closed with rubber-covered clamps to prevent a short-circuiting of the material into the venous blood. The arterial injections were done rapidly under high pressure, with a view to obtaining distribution by way of the aorta.

One of the rabbits was killed 13 days after injection. Its lungs contained numerous scattered, creamy, spherical nodules, from ½ to 2 mm. in diameter. None was found in the other organs. The pulmonary nodules had the characteristic morphology of the growth, some consisting of a rind of more or less orderly epithelium enclosing concentric layers of necrotic squamous tissue, while others were made up of living tissue almost throughout, and had the papillomatous form (Fig. 13). At the periphery of the latter the epithelium had pushed into the individual alveoli in disorderly aggregates, filling and destroying them (Fig. 14). There was some compression of the surrounding lung tissue, and the neighboring small bronchioles were more or less flattened; but not the least encapsulation of the growths

had occurred, nor any inflammatory reaction. There were many mitoses, and the characteristically pathological epithelial differentiation and keratinization.

The findings were negative in another rabbit, killed after 14 days; but in one of two that were allowed to live 3 months scattered pulmonary nodules from 3 to 7 mm. in diameter were found, of characteristic morphology. The other organs were negative. The rabbit had received bits of an intramuscular growth into the circulation.

Interpretation of the Results of Implantation

The morphology of the nodules developing in the interior of the host as result of the injection of tissue fragments; their frequent situation in tissues from which they could not possibly have been derived by direct virus infection, as *e.g.* muscle and spleen; the evidence for accidental implantation of the papilloma cells on the peritoneal surface, and in the muscle and subcutaneous tissue of wounds; and the specificity of the virus for epidermal cells, together provide conclusive evidence that survival and proliferation of the engrafted tissue was responsible for the growths. Final proof that the lung nodules which appear after injection of papillomatous tissue into the blood stream result from its survival and proliferation must wait upon a study of serial sections of early stages in their development. But the demonstrable ability of the papilloma cells to multiply when implanted elsewhere in the host, the rapid development of the nodules, and the failure of the virus to cause lung growths when injected as such into the blood stream or needled directly into the pulmonary tissue leave small doubt that this was the case.[1]

The liver, spleen, and voluntary muscles seem most favorable to grafts of the papilloma. When growing in the connective tissue it is well encapsulated, and pressure conditions in the kidney appear to limit it to a considerable degree. In the stomach wall it does poorly for reasons not yet plain. The nodules developing in the lungs have been few compared with the number of tissue fragments injected into

[1] Serial sections now at hand have yielded direct proof that papilloma fragments lodging as emboli within branches of the pulmonary artery survive, proliferate, penetrate the vessel wall, and rapidly invade the lung parenchyma. Nodules 1 mm. in diameter may develop within 5 days after the intravenous injection of tissue; and all are due to the growth of emboli. The lung epithelium itself undergoes no proliferation.

the circulation, and none have appeared in the other organs, though the material must have been distributed to them on the arterial blood. Even in the most favorable situation the growth's invasive power fails to equal its ability to proliferate.

Usually some new formation of connective tissue takes place about the papillomas developing from implants, its amount varying inversely with the rate of growth. When this is rapid, as in certain viscera, there may be no visible cellular reaction about the advancing epithelium (Figs. 11, 12, 13, 17). This holds true of not a few skin growths as well, at the period of their early, downward extension (Paper III). It is plain that the papilloma does not inevitably call forth reactive changes on the part of the surrounding tissues; but how far those ordinarily associated with it are due to bacterial infection is still uncertain. The scarring frequent beneath old skin papillomas is certainly due to this cause, and so too are the focal accumlautions of polymorphonuclear leukocytes. They are missing from about implantation nodules save when bacteria are present. Few lymphocytes are to be seen save when retrogression is taking place (Paper II). All in all the facts suggest that the papilloma as such elicits some connective tissue proliferation over and above that incident to the formation of a stroma, its amount depending on both the local conditions and the time available,—the epithelium sometimes advancing so fast as to outstrip any formation of it. The behavior of the growth as conditioned by the reactive tissue round about will be considered in Paper II.

The Invasion of Vessels

The cores of the papillomatous projections from the skin contain many wide, thin-walled vessels, and often the proliferating epithelium is separated from the blood by only a layer of endothelium. Direct invasion of the vessels takes place frequently, and may occur even when the growth is progressing very slowly.

The lymphatics beneath the cindery, almost stationary papilloma of Figs. 28 and 30 were invaded at several points; but the cells that had entered were differentiating and dying. In Fig. 16, taken from a growth in the abdominal wall, the epithelium within the lymphatic is also in poor condition, though the growth from

which it had extended was proliferating actively. Fig. 15 comes from the periphery of an accidental peritoneal implant; and it shows epithelium that has entered a blood vessel, covered with platelet clot. Serial sections demonstrated the fact that the invading cells represented the tip of a downgrowth from the main nodule, part of which can be seen near by.

The Possibility of Metastasis Formation

The frequency with which the papilloma invades the vessels, and the results of injecting it into the blood stream would lead one to expect metastasis formation in especially favorable hosts. Efforts have been made to induce it (for which see Paper II); and in every animal coming to autopsy search has been made, microscopic as well as gross, where this seemed advisable, for secondary localizations in the lymph nodes and lungs. A growth was found once in a lymph node (Fig. 17), but the possibility that it resulted from operative introduction of papillomatous tissue into a regional lymphatic cannot be excluded. In two instances growths were found in the lungs (Fig. 6), but also under equivocal conditions.

The domestic rabbit yielding the growth in the lymph node had been subjected to a variety of procedures. The papilloma virus was tattooed into four spots on each side and rubbed into a scarified area on the abdomen. 9 days later when the growth was first appearing in the latter situation part of it was shaved off and implanted by the usual methods in the muscles of the hind legs, the subcutaneous tissue of the left axilla, the liver, and the right kidney. Some of the papillomas appearing later where the skin had been tattooed were kept covered with a layer of collodion, and grew beneath the surface in consequence (Figs. 22 and 24); whereas the tissue under and about others was repeatedly infiltrated with olive oil containing Scharlach R in saturated solution, with result that they developed into large, fleshy, subepidermal masses (Figs. 22 and 23) which eventually fungated and became foul. One of each sort was submitted to biopsy. The "pancake" on the abdomen was redundant and fleshy at the time when the animal died, 94 days after the implantations into the viscera, which had all given rise to large growths. There was a spherical mass, 3.5 cm. in diameter and characteristically papillomatous where the graft had been placed in the left axilla; and near by but wholly separate, a lenticular growth 1.5 cm. across, which had appeared late and on repeated palpation during life had given the impression of developing in a lymph gland. Section showed this to be the case. The gland was entirely replaced by papillomatous tissue save at one end. The epithelial processes were highly irregular and very invasive (Fig. 17).

In the lungs of the animal were three papillomatous nodules, from 4 to 10 mm. in diameter, rounded, creamy, and projecting. They were all in the right upper lobe (Fig. 6). Gross and microscopic examination disclosed no others.

In this rabbit the papilloma grew especially well. Some large nodules were found at autopsy, scattered on the peritoneal surface, and there were several in the scar of the old laparotomy wound. The situation of the lung nodules in a single lobe, their large size, and the absence of any early, small ones suggest that they originated from cells accidentally introduced into the blood stream during the injection of the liver with suspended papilloma particles. The growth in the lymph gland may have been a metastasis, or may have arisen from cells introduced into the lymphatic stream when the axillary implantation was made or during biopsy of one of the stimulated, fleshy growths situated beneath the surface of the side.

In another domestic rabbit, dying 115 days after implantation into the liver and other organs, a single pulmonary nodule 7 mm. broad was found. The presence of large growths on the peritoneum and in the old wound indicated that this animal, like the one just discussed, had been favorable to accidental dissemination of the papilloma (Fig. 36).

Whatever the method whereby the growths developed in lymph gland and lungs, whether by an operative scattering or by true metastasis, there would seem to be no doubt that they arose from cells transported on the lymph and blood streams.[2]

The Recovery of Virus from Implantation Growths

No difficulty has been experienced in the recovery of virus from implantation growths in the muscle and viscera of wild rabbits. Both fresh and glycerinated tissue (50 per cent glycerin) have been employed, with grinding and extraction in Tyrode as usual, and inoculation into the scarified skin. Two experiments of the sort, with positive outcome, are described in Paper III, in connection with the work on transplantation. A third, similar experiment, but with material from a domestic rabbit, yielded negative results, as was to have been

[2] In the pulmonary alveoli of cottontail rabbits with pigmented tumors in the liver, aggregates have been found of cuboidal cells having much the same general appearance as those of the hepatic growths, and like them containing brown or black pigment. But though such aggregates are always very small, no signs of an origin from cell emboli have been noted; the cells rarely show mitoses; and they sometimes desquamate and become foamy elements like *Herzfehlerzellen*. Furthermore they can be found in the lungs of wild rabbits that are not carrying the papilloma.

expected, since the virus cannot ordinarily be recovered in active form from skin papillomas experimentally induced therewith in such animals.

Effects of the Growth on the Host

No sufficient study has yet been made of what happens to the skin papillomas eventually or of their ultimate effects on the host. Their cells certainly can proliferate for long periods: they were still doing so after 13 months in the case of some of Shope's domestic rabbits.[3] Growths of large size on the skin of such animals, resulting from inoculation of the virus into a broad area of scarification, are well tolerated but lead frequently to secondary anemia as result of repeated loss of blood from papillae that have been traumatized or gnawed away. After the first months such growths generally appear to be stationary, but in most instances this is only because the dry tissue over them is continually worn or gnawed off: if they are covered with a bandage they rapidly increase in height. Yet they no longer spread. We have followed the hemoglobin percentage and the number of circulating red and white cells, and have made differential counts on the blood of a group of domestic rabbits during the period of the papilloma's appearance and early enlargement, without finding any significant changes. Animals with large cutaneous growths capped by dead tissue usually have some polymorphonuclear leukocytosis, which the associated bacterial infection would lead one to expect. As previously mentioned the growths resulting from implantation in the viscera of favorable hosts ordinarily cause death within a few months at most. The rabbits lose weight gradually and die in extreme emaciation.

SUMMARY AND COMMENT

Rabbit papillomas developing on the skin as the result of virus inoculation can be readily transferred to the inner organs of favorable hosts by implanting bits of the living tissue. The growths thus produced proliferate actively as a rule and frequently cause death. Often they are markedly invasive and destructive; and they tend to recur after excision. Bacterial infection may greatly enhance their malignancy. Accidental dissemination may occur during operation, and

[3] Personal communication from Dr. Shope. See also Paper II.

distribution to the peritoneal surface has been repeatedly noted. There may be no cellular reaction whatever about the invading epithelium of interior growths, but usually some new formation of connective tissue takes place, its amount varying inversely with the rate of epithelial proliferation. An immediate reason exists for the inflammatory changes and scarring found beneath long-established skin papillomas, in the trauma and secondary infection to which the projecting, necrotizing masses have been subjected. In animals dying of progressively enlarging interior growths the skin papilloma may long have been stationary in size.

The growths appearing after the transfer of papillomatous tissue to the inner organs are due to the survival and multiplication of transplanted cells. However, the virus can be readily recovered from them, in the case of wild rabbits. No distinctive changes in the blood of the host have been found. The virus itself is highly specific for the epithelium of the skin, failing to act not only upon that of the other organs thus far tested but even upon embryonic skin.

The papilloma frequently penetrates into the blood and lymph vessels, especially at the edge of implantation growths. The intravascular injection of fragments of it sometimes results in pulmonary nodules of characteristic morphology. These are due to survival and proliferation of the injected cells. Secondary nodules have been encountered at autopsy in a lymph gland and in the lungs, but under conditions more suggestive of operative dissemination of the growth than of true metastasis.

Implantation growths of the papilloma in favorable hosts have the morphology of epidermoid tumors of greater or less malignancy. They behave as these do and elicit similar changes in the surrounding tissue.

The attributes and potentialities of the papilloma will be further considered in Papers II and III.

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EXPLANATION OF PLATES

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

PLATE 36

FIG. 1. Growths resulting from the implantation into a wild rabbit (W.R. 20) of bits of the animal's own papilloma. The growth appeared on the abdominal skin 7 days after virus inoculation, and 5 days later some of it was shaved off and injected into the extensor muscles of both upper forelegs, the subcutaneous tissue of the right groin, and the right kidney, spleen, and liver (at several situations). The animal died 39 days later as result of the numerous growths. The inoculated leg muscles had been almost replaced, and so too with the spleen (*A*). An irregular nodule of considerable size (*B*) was found in the healed abdominal wall. It protruded into the peritoneal cavity, as did the kidney nodule, which lay mainly in the cortex (*C*). Several large masses can be seen in the liver. At *D* is the skin growth. × ½.

FIG. 2. The groin nodule and the mass in the right leg, halved. The growth in the muscle is multicentric, with an absence of gross encapsulation (arrow). The groin nodule is partly melanotic. Natural size.

FIG. 3. Surface appearance and cross-section of growths in the liver of a wild rabbit (W.R. 18) killed 49 days after implantation of the papilloma into several of the abdominal viscera,—photograph taken to show the projecting masses and the pronounced, localized melanosis. Natural size.

FIG. 4. Implantation growth in the kidney of the same rabbit. Natural size.

FIG. 5. Growth mushrooming into the abdominal cavity of the animal, a result of accidental dissemination in the laparotomy wound. The arrow points to the growth in the abdominal wall. The mass like a glans penis projected from it into the peritoneal cavity. Natural size.

FIG. 6. Papillomatous nodules in the upper lobe of the right lung of a domestic rabbit (D.R. 1-22) that died 108 days after skin inoculation, and 94 days after implantation with bits of its own skin growth (shaved off 18 days after virus inoculation) into liver, kidney, stomach, muscles of forelegs, and subcutaneous tissue. The lung growths probably resulted from an accidental introduction of papilloma cells into the blood at the time of liver implantation. (For additional details of history, see text, page 716.) Natural size.

PLATE 37

FIG. 7. Edge of an implantation growth in the kidney cortex of a wild rabbit (W.R. 20), the same animal providing Figs. 1 and 2. There was some round-cell reaction about this growth. × 160.

FIG. 8. Edge of a growth in the liver of a domestic rabbit. On the left the advancing epithelium is completely unencapsulated. The animal (D.R. 1-23) was

killed because moribund with "snuffles" 26 days after implantation with its own growth into some of the abdominal viscera. The grafts had grown rapidly. (See Fig. 15.) × 68.

FIG. 9. Part of a growth in the spleen of the wild rabbit of Figs. 1 and 7 (W.R. 20), showing an unusual number of mitoses, many of which are abnormal. × 550.

FIG. 10. Unusual type of growth in a domestic rabbit (D.R. 1-22—see legend of Fig. 6 for history). Many but not all of the implantation growths (Fig. 17) showed this structure. The section came from a large subcutaneous mass in the axilla, containing numerous papillomatous processes in good condition, separated by rifts containing fluid, a rare finding. × 82.

PLATE 38

FIG. 11. Specimen from wild rabbit No. 18 (of Figs. 3, 4, and 5), to show the direct replacement of liver cords by an invading melanotic growth. There is no histological evidence of pressure by the enlarging mass. The melanin can be seen to one side of the nucleus of the differentiating cells. × 220.

FIG. 12. Lower magnification of the edge of the growth furnishing Fig. 9. The advancing epithelial tissue is wholly unencapsulated and actively invasive. Numerous mitoses are visible. × 130.

FIG. 13. Lung nodule from a domestic rabbit (D.R. 1-64) killed 13 days after the intravenous injection of bits of its own skin papilloma. The alveolar tissue about the enlarging growth has been stretched and pressed together, giving the appearance of encapsulation. × 42.

FIG. 14. Invading margin of another lung growth from the same animal, to show the absence of encapsulation or other reaction about the invading, differentiating epithelium. Pressure has compacted the walls of the alveoli next the growth into a spurious capsule. × 312.

PLATE 39

FIG. 15. Edge of an accidental implant on the parietal peritoneum of a domestic rabbit (D.R. 1-23),—to show direct invasion of a blood vessel by the cells of the growth. A platelet clot at one side of the vessel covers the cells completely but elsewhere it is patent. Serial sections demonstrated that the invading cells were at the tip of a slender epithelial process extending from the parent growth, a small portion of which, including a mitotic figure, is present at the upper, right hand corner of the picture. × 500.

FIG. 16. Invasion of a lymphatic by an implantation growth in the leg of a domestic rabbit (D.R. 1-22—for history see text, page 716). The epithelial cells are much degenerated. × 250.

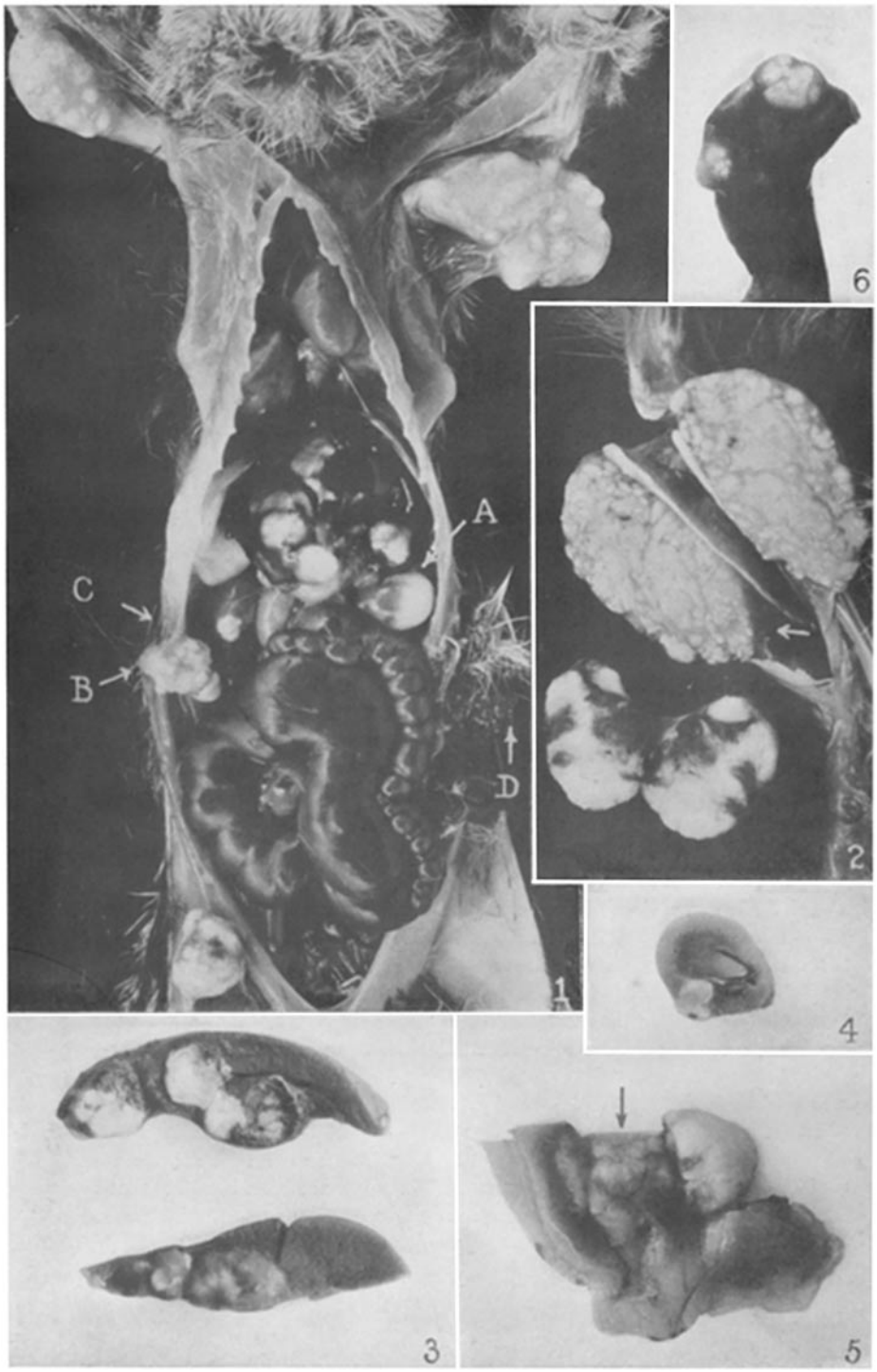
FIG. 17. Part of a secondary nodule developing in an axillary lymph node of the same rabbit. The epithelium replacing the lymphatic tissue has keratinized along one side of some of the advancing processes. The growth is unencapsulated. × 75.

PLATE 40

FIG. 18. Multicentric growth in the leg muscle of a wild rabbit (W.R. 20—see Fig. 1 for history). × 12.

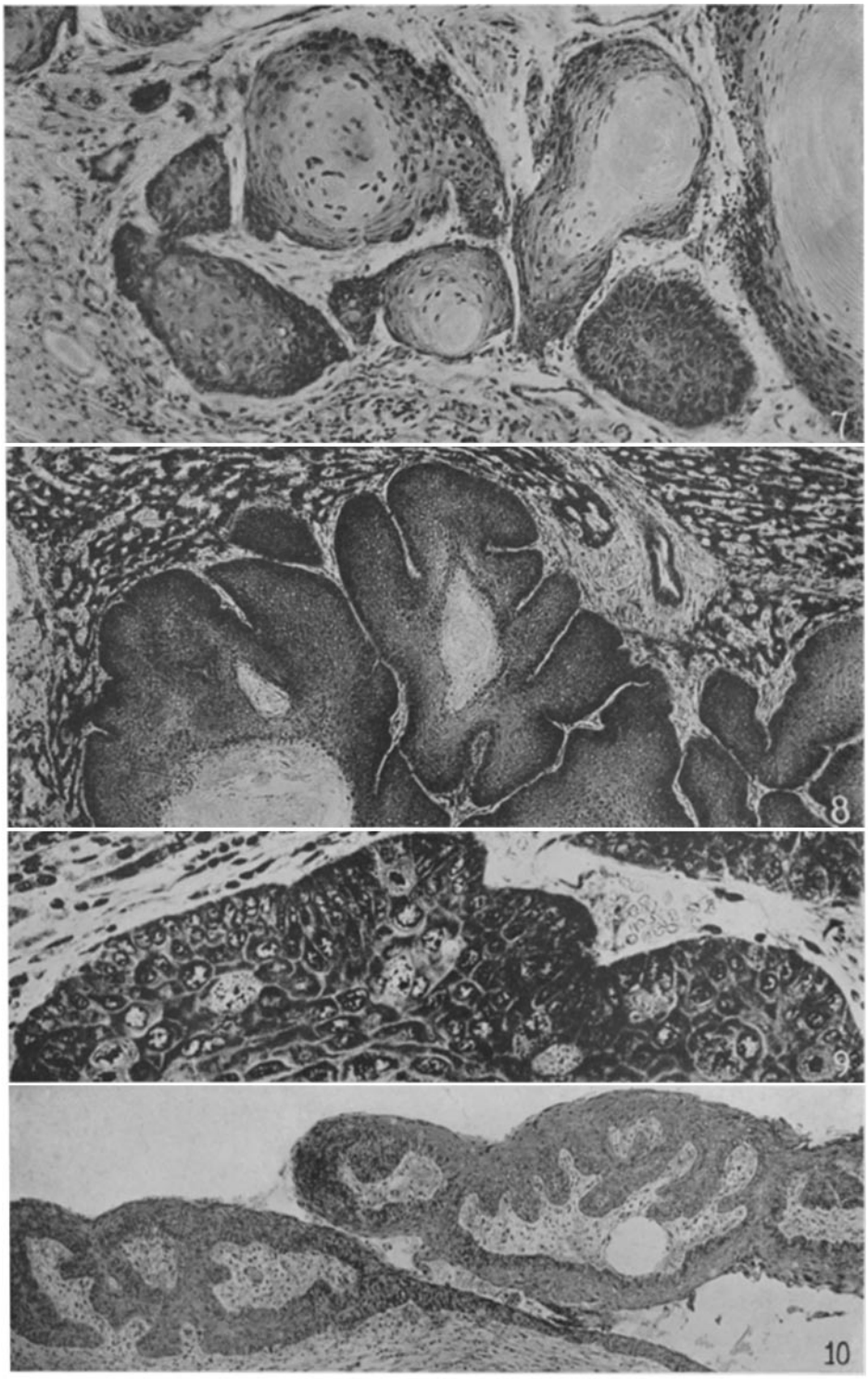
FIGS. 19, 20, and 21. Direct invasion and replacement of leg muscle by an implantation growth in a domestic rabbit (D.R. 1-20): section taken from the border of the growth. There is a cellulitis (Figs. 19 and 20) due to bacteria introduced with the implant. The animal died of general peritonitis 23 days after implantation in the muscles, liver, and kidney. Rapid enlargement of the grafts in the muscles had taken place, and so too with most of those in the viscera.

In Fig. 20 the epithelium is growing between and around the individual muscle fibres. In Fig. 21 it is directly replacing a fibre by proliferation within it. The sections all came from the periphery of a nodule that had extended far beyond the original site of inoculation. × 75, × 95, and × 270 respectively.



Photographed by Louis Schmidt

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



Photographed by Louis Schmidt

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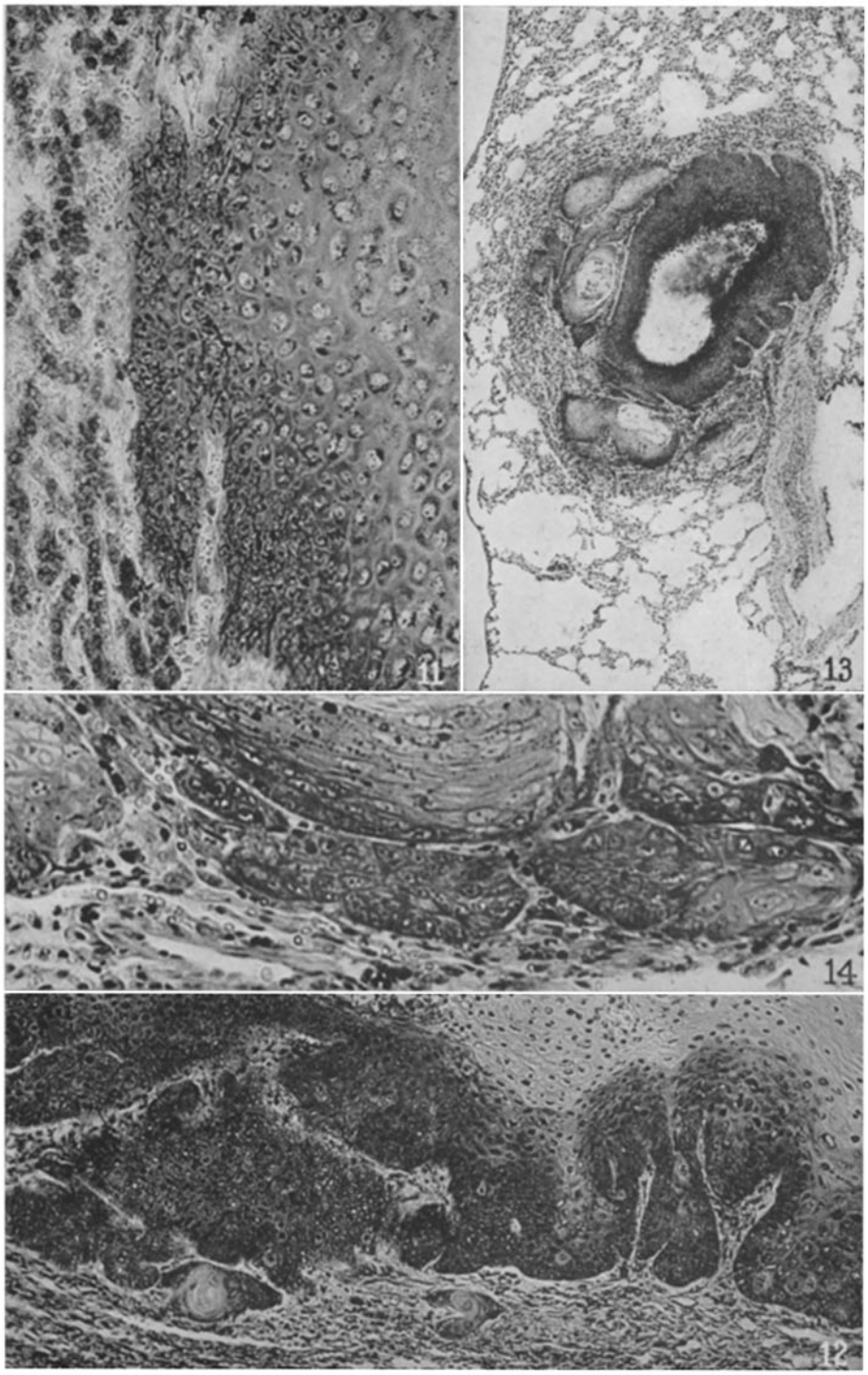

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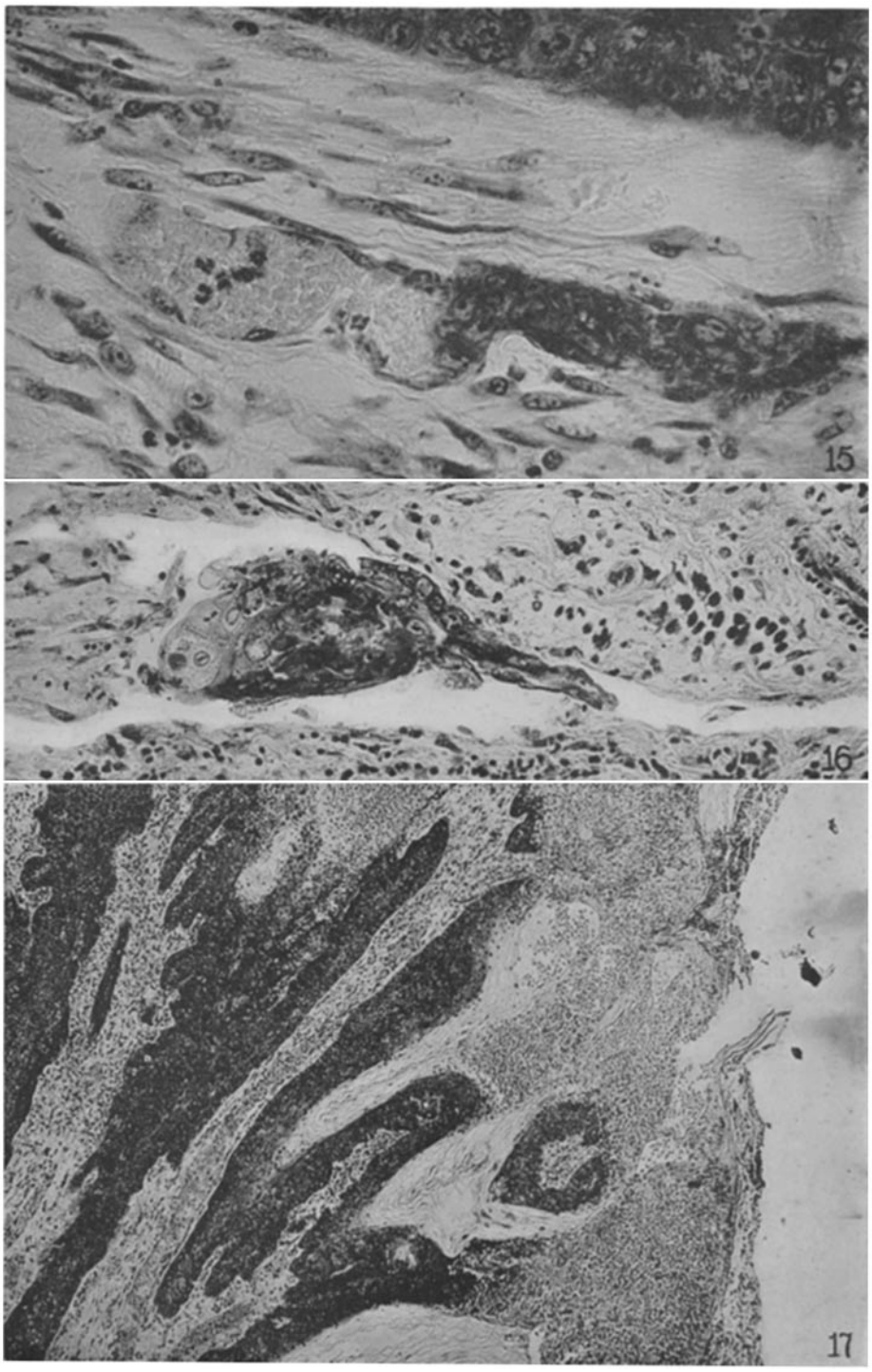

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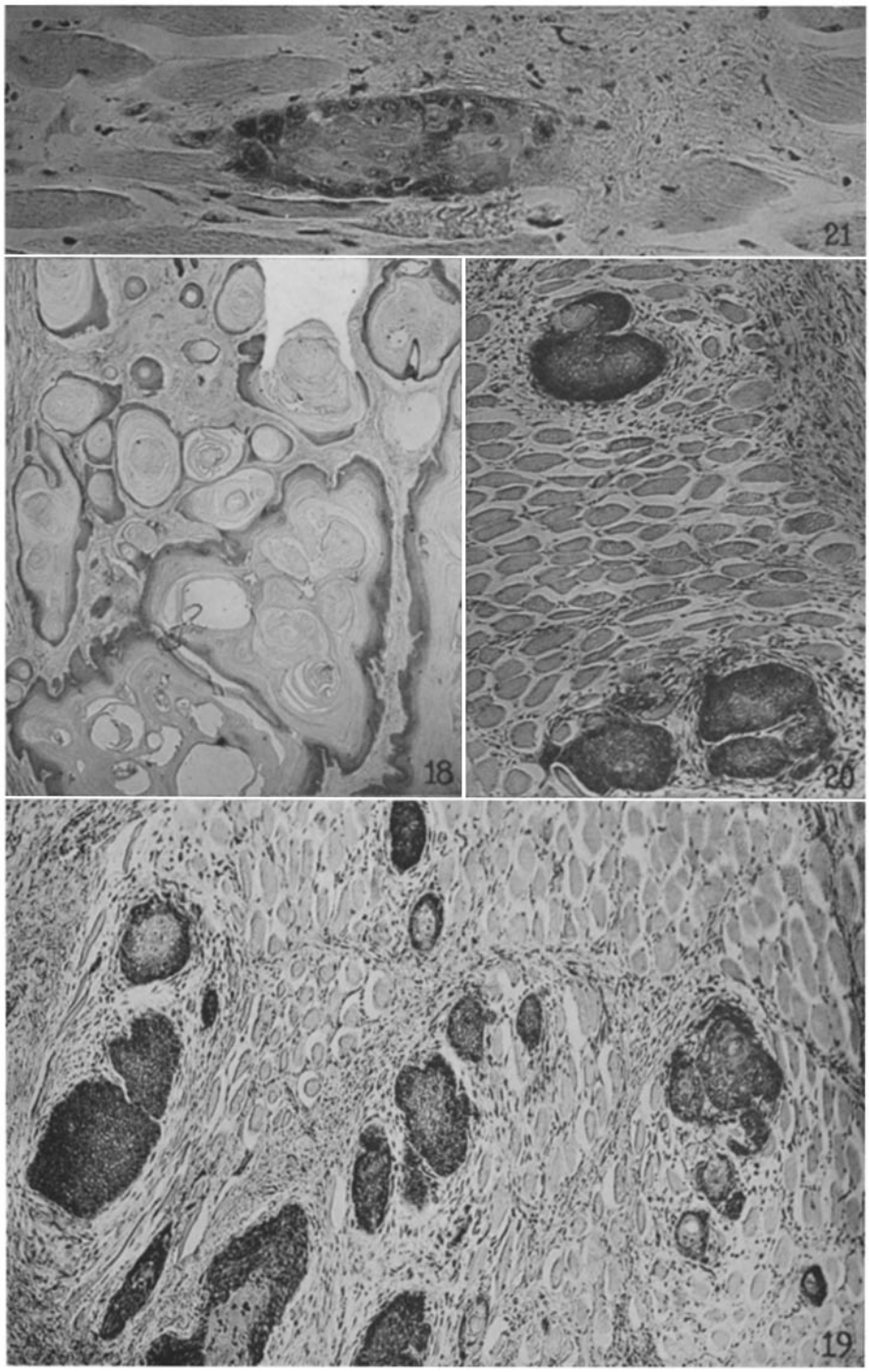

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