THE PLATING OF TUMOR COMPONENTS ON THE SUBCUTANEOUS EXPANSES OF YOUNG MICE

Findings with Benign and Malignant Epidermal Growths and with Mammary Carcinomas

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A procedure is here presented whereby the differing neoplastic components present in certain composite tumors have been separated and maintained individually.[1] It was devised because of the need to free the tissue of transplanted epidermal papillomas from that of the carcinomas often deriving from the cells of these growths and hidden amidst their tissue, so that they might be carried further as such. After the procedure had proved effective, it was applied to spontaneous mammary carcinomas of "milk factor type," the most complex of all readily obtainable neoplasms, and of special interest because their occurrence is determined by a virus reaching the young through their mother's milk. Not only did it make plain that the mingled morphological components visible in stained sections of these growths could be sorted out and grown separately but it brought to light some that had not been demonstrable.

Development of the Procedure

The "benign" epidermal papillomas induced on mouse skin with tar or 20-methylcholanthrene can be readily transplanted to deep sites in suckling and weanling mice, and, in the absence of cancerous changes, they grow progressively in nearly all, proving fatal unless they are destroyed by intercurrent bacterial infection (1, 2). Those best suited to long maintenance consist of neoplastic epidermal cells which have retained the normal ability to spread laterally on denuded surfaces (type A papillomas, reference 1). After deep implantation grafts of this sort form spherical growths full of close-packed keratin surrounded by a shallow, even layer of living papillomatous tissue which differentiates inwards forming it. Any obvious extension outwards through the connective tissue smoothly encapsulating the tumor, or any local, inward protrusion of living tissue from its lining, betokens a cancer. Usually this can be left behind by transferring bits of the papillomatous layer from elsewhere; but only too often a malignant growth is so small as to escape notice, and after inadvertent transplantation it supplants the benign one.

[1] Reported in abstract at the meeting of the American Association of Cancer Research, April 8, 1961. (*Cancer Research*, 1961, **3**, 234).

The fact was early noted that when several papilloma fragments were suspended together and the suspension forcibly scattered in the posterior thigh muscles of weanling mice, the resulting discrete, papillomatous spheres remained separate long after their enlargement had pressed them against one another; whereas if one of them contained cancerous tissue, the resulting tumor was usually asymmetrical, soon became attached to those apposed to it, was frequently soft because of exudation amidst its keratin, and sometimes brownish instead of ivory-colored, owing to old ecchymoses within it. These findings suggested a way to rescue a papilloma from hidden cancers, namely to scatter many fragments of it in the same host and to select from amongst the resulting tumors one or more for transfer that appeared typically benign in the gross and on frozen section.

But where should the fragments be placed? Obviously on an expanse of readily accessible connective tissue: and it was soon found that the entire layer of such tissue on the back or belly of sucklings and weanlings could be split transversely by forcibly injecting salt solution into it followed by compressed air. When this was done the connective tissue parted along its plane of least resistance with result that the layer still attached to the skin was lifted away from the body wall, and if a suspension of tumor fragments was used they were distributed widely on the expanse thus provided. When the air was withdrawn after a few minutes, or allowed to escape, almost all of the tumor bits remained where they had lodged. No visible hemorrhages took place from the torn, web-like tissue, nor was there any later reddening, edema, exudation, or scarring in the injected area. Only during the course of many weeks did the apposed connective tissue surfaces reunite, and until this happened the cutaneous layer, when cut through, could be folded back like a sheet. Often no reunion took place for months.

Practically without exception the scattered fragments rapidly became fixed to the connective tissue covering the body wall proper, not to that overlying them, even when the injection had been ventral, that is to say when gravity was against this happening. They promptly elicited a stroma, but the tumors arising from them nearly always remained unattached to the overlying cutaneous sheet until they had become quite large. Most of the expanse of body wall was uniformly favorable to their growth.

An actual plating of the fragments had been done, analogous to the bacteriological, with the cutaneous layer helping to foster the tumor cells, and in this way serving better than the lid of any dish.

Test 1 makes plain how the procedure was used to exclude a carcinoma present in the tissue utilized for transfer of a benign papilloma.

A small mound had been noted protruding from an even layer of what elsewhere appeared to be typical papillomatous tissue (Papilloma V, Eleventh Generation). Minced fragments of them both were suspended together and plated dorsally on a weanling. Fig. 1 shows the findings 40 days later. It will be seen that the growth *C1* was larger than the others, asymmetrical, soft, and dark—obviously cancerous that is to say (Fig. 3). Tumor *C2* was soft and had partially coalesced with a typical papillomatous sphere, firm, creamy, and opaque, lying immediately next it. It too proved cancerous. All the other tumors were firm and wholly discrete, as are typical papillomas. Fig. 2 shows a section of the layer lining the largest, *P*. Though appearing benign almost everywhere it may have been becoming malignant at the small spot bracketed.

Test 2 was made by an improved technique, to be described further on.

Fragments of the same benign papilloma (Pap. V, Gen. 18) after six more trochar transplantations were suspended in Locke's solution, together with those of a transplantable mammary carcinoma (MT3, Gen. 3)—of which more further on—and submitted to two successive washings in Locke's solution, with brief sedimentations immediately after in order to free them from suspended individual cells and small clumps. They were then suspended anew and plated on the backs of weanlings. Figs. 4 to 7 show the results in a mouse killed after 20 days Tumors of three differing aspects were present (Fig. 4). Two of the small, white, spherical growths (*P*) were actually cream-colored and opaque—as was also a somewhat larger, ovoid one. Microscopically they were benign papillomas (Figs. 5 and 6). The gray growths (*M*) consisted of mammary carcinoma tissue (Figs. 5, 6, and 7). The big, ivory-yellow sphere was a malignant papilloma (Fig. 7) partly overlying another (*Cp*), such as not infrequently derive from the benign, their papillae projecting inwards like those of these latter but not keratinizing as they do.

The presence in the papilloma of cancerous tissue amidst the benign of *Test 2* had not been suspected; and the separation of them effected by the plating would have provided material for the benign growth to be carried further had this been desired. Actually Pap. V has been maintained throughout its 4th and 5th years by just such selective plating at intervals. Of late however this has become more difficult, derivative carcinomas having arisen that cannot be discerned in the gross because, unlike the generality, they grow at the same rate as the papilloma and form cysts of similar aspect, packed with firm, creamy keratin enclosed in a thin layer of living neoplastic tissue. This layer is not composed of orderly papillomatous cells, but of malignant elements based on the connective tissue encapsulating the tumor, yet never extending through it. The presence of these carcinomas *in situ*—to be figured in a later paper—can be discerned in frozen sections with the microscope, and by this means and repeated platings they have been excluded.

Material and Methods

Homogeneous C strain mice were used of a colony procured from the Jackson Laboratory 16 years ago and inbred ever since. The strain is known to carry the milk virus (3). The mammary cancers were "spontaneous" and had arisen in old females. The papillomas had previously been repeatedly transplanted and have already been described (1, 2).

Preparation of the Tissue.—The living rind of papillomatous spheres was peeled away from the keratin cores, its neoplastic tissue pushed off the encapsulating connective tissue by lateral pressure with a scalpel, and cut up in serum-Locke's solution (SL—1 part serum to 19 of Locke's). The fragments, less than a millimeter across, were sharply defined, requiring only brief washing in Locke's (L) and sedimentation prior to suspension in fresh L for injection.

Not so with the mammary cancers. They proved friable, yielding individual cells and tiny clumps in great number when minced, as well as the sizable fragments desired. Obviously these latter had to be freed from adhering cells which might complicate the issue. Selective sedimentation, washing, and screening were accordingly done.

Enough L was added to the suspension to form two or more columns some 12 cm high in ordinary test tubes, and after the grossly visible fragments had settled, the shimmering, supernatant fluid was discarded, using a Wright's pipette to remove the final drops. Fresh L was then put on in jets as the columns were built up anew, with whirling of the tubes between the hands at intervals. Direct pipetting had to be avoided because suspended fragments often adhered to the inner surface of the glass. After sedimentation had again taken place, the water-

clear, supernatant fluid was discarded and a new suspension made, but now in only enough L for plating purposes, and it was quickly poured through a metal sieve, with apertures 0.2, 0.4, or 0.6 mm across, as seemed best. The whole procedure will be termed the *sed. grid. technic.*

Method of Plating.—A syringe with a conical, metal plunger was used to ensure delivery of all the fragments. The gauge and length of the needles employed differed with whether sucklings or weanlings were injected. For plating on the back of 10 to 15 gm weanlings a No. 19 Luer needle was used, 3 cm long with a blunt bevel, and 1.0 cc of suspension containing the desired number of fragments (from 5 to 40) was drawn up into the syringe, followed by 4 to 5 cc of air. Then, with the mouse held head downward, the needle was thrust through a slit in the skin just above the back of the knee, and pushed through the thigh muscles until it had emerged in the dorsal subcutaneous tissue near the spine. Injection was now forcibly done with the animal held horizontally, belly down; and to prevent any of the suspension following the needle into the muscles while it was withdrawn, the tissue surrounding it was compressed with rubber-covered forceps. About half an hour later the air was removed from the dorsal pouch with a thin, sharp needle thrust through the skin. Any residuum was gone by next day, and no fluid had collected. So little did the plating disturb the mice that anesthesia proved unnecessary. No ventral platings were done on weanlings.

The sucklings (2 to 6 days old) were chilled until unconscious in an empty beaker set in ice water, and then injected directly into their dorsal or ventral expanse, with 0.5 to 1.0 cc of suspension according to size, followed by 1.5 to 2.0 cc of air. A No. 19 blunt Luer needle 2 cm long was used. An assistant held the mouse stretched and slanting downwards toward its head. For dorsal platings a transverse slit was made just in front of the tail and, for ventral ones, in a groin. During the injection, as well as withdrawal of the needle, the skin around it was compressed, and the compression kept up for a few moments, with the mouse now held horizontally while the scattered fragments settled in place. The air was then let escape, most of the fluid seeping out later; and the mice were placed on a bed of fine shavings in a 37°C incubator, and when warm were returned to their mother. Often they were nursing within half an hour, and this although they had looked like inflated rubber toys immediately after injection. The shavings were important as immobilizing them; for if put on a flat surface, their rolling and squirming soon pressed out fragments. These are homely, yet useful details.

Ordinarily both weanlings and sucklings were injected at each plating. Few were killed before tumors had arisen. After the skin had been thrown back, the growths were usually traced on cellophane plates for immediate transfer to cards. When they were many and small they were stripped away, grouped on cardboard for Zenker fixation, sectioned serially, and stained with eosin and methylene blue. Slices of the larger tumors were taken for section whenever advisable.

The Direct Consequences of Plating

When the right amount of fluid, backed by air, was injected, the dorsal or ventral cutaneous layer was lifted so completely away from the body wall as to be anchored only at the axillae and groins—whence came its nourishing blood vessels. The freed expanse never extended more than half-way around the sides of the body, because of some anatomical barrier, and no tumors ever arose on the legs. Much depended on where the needle point was held during the injection. The best distribution of fragments in weanlings was got when it was directly over the spine and nearly as far back as the haunches (Figs. 10 and 38). When further back, tumors seldom arose on the chest and, when further forward, care had to be taken that it did not enter the interscapular fat pad; for when it did, the plated fragments were largely confined to this, and grew more rapidly than elsewhere. Even when it was not penetrated tumors were notably frequent over it and, as time went on, formed coalescing, saddle-bag masses (Fig. 39). The growths tended to be more frequent along the sides of the body than elsewhere, and usually there were few or none over the spine, where this projected when the mouse sat humped.

The expanse freed for dorsal platings in sucklings sometimes extended to the snout, with result now and then in a tumor here or next the eye, though none ever arose on the cranium. If the injection had been far forward on the belly, the tumors were often crowded over the chest and now and then one arose under the jaw (Fig. 28) or on the back of the neck. All in all, dorsal platings proved preferable to ventral because tumors arising on the belly became abraded as they grew.

This blunderbuss method of scattering tumor fragments of differing potentialities, with the suspension as the load, compressed air as the driving force, and the entire back or belly as target, proved surprisingly effective.

General Character of the Mammary Cancers Studied and Designation of Their Components

The spontaneous mammary tumors of mice were first utilized experimentally in the heyday of morphological pathology and the descriptions accorded them then have never been surpassed.[2] They proved so complex individually, so various in their components, and so changeable, that by 1908 any systematic classification of them had been virtually abandoned. Nearly all seem to have been of what is termed "the milk virus type," as were those now studied. Our designation of their neoplastic components will be strictly objective, the immediate aspect of these determining it. Though diverse in character they were not expressive of random cell changes but were of certain kinds encountered again and again.

The terms *acinar* and *alveolar* and several others will be used in the sense in which Dunn has lately employed them (3); but for some components names were supplied, *e.g., broad-banded, hemorrhagic acinar, tessellated, patterned.* They are mere names of the moment, coined for a need.

1. Carcinoma Solidum (*S*).—The living cells form dense masses almost devoid of obvious stroma, and undergoing necrosis at their centers. In microscopic cross-section they appear as bands surrounding dead material. Usually several such bands, each enclosing an area of necrosis, are found next to and joining one another (Figs. 8, 11, 14, and 50). Hence the name *broad-banded* (*B*). Fluid often collects secondarily amidst the dead tissue and occasionally a hemorrhage takes place into it.

2. Tessellated (*T*).—On cross-section the expanse of neoplastic tissue is nearly as compact as that of carcinoma solidum but islands of stroma are present amidst it, more or less regularly disposed. When they are large the tessellation is coarse, but fine when they are small (Figs. 9, 11, 37), with all gradations between exemplified individually. Since no necrosis takes place, the stroma evidently suffices for cellular needs.

3. Acinar (*A*).—This consists of well formed, orderly acini of nearly the same size, with or without small lumina (Dunn's type A carcinoma) (Figs. 16, 52). Often the acini appear crowded and ill defined.

4. Hemorrhagic Acinar (*Ah*).—Similar in form to ordinary acinar but distinctively prone to hemorrhages, with result in purple cysts amidst the tissue (Figs. 29, 32).

[2] *Vide* the many admirable papers in the Third and Fourth Scientific Reports of the Imperial Cancer Research Fund, for 1908 and 1911 respectively, London, Taylor and Francis.

These are often large and become brown as time goes on. Some of the smaller cysts contain watery fluid only.

5. Alveolar (*V*).—Composed of "glandular" units with conspicuous free lumina often varying much in size (Figs. 51 and 55). All gradations to ordinary acinar tissue occur. The alveolar itself may grade into:

6. Tubular alveolar (*Vt*).—The tubules are frequently long and may become cystic (Figs. 22 and 52).

7. Exudative alveolar (*Vx*).—Often the alveolar tissue is prone to necrosis and fluid accumulates here with result in spaces having ragged walls (Figs. 37 and 48).

8. Cystic alveolar (*Vc*).—Sometimes this is a mere exaggeration of the tubular cystic but usually the growth consists of well defined cysts of widely various size, separated by septa of thin or thick connective tissue with an orderly layer, single to multiple, of more or less cuboidal tumor cells (Figs. 17, 18, 30, 31). The cysts contain thin fluid, colorless or amber or pink or purple, according as they are full of mere watery transudate, or colored plasma constituents, or contain red cells as well. These last are sometimes present in considerable quantity (Fig. 18).

(Tumors of categories 6, 7, and 8 all belong to Dunn's Type B, reference 3. Their cells and nuclei are generally larger than those of the other components listed.)

9. Patterned (*Pt*).—Occasionally the relation between cancer cells and stroma is such as to result in peculiar patterns (Figs. 15 and 35).

10. Desmoplastic (*D*).—The stroma so greatly exceeds the cancerous tissue in this component as to separate its cells either individually or into small islands (Figs. 8 and 12).

Components which appear similar morphologically often stain with differing intensity. Wherever necrosis with extravasation occurs hemorrhage may take place, with result in purple cysts. These should not be confused with the blood cysts which occur in hemorrhagic acinar tissue and in the cystic and exudative alveolar because of their specialized character.

Many of the tumors consequent on the platings contained several differing neoplastic components and, while small, their contours often indicated that they had resulted from the coalescence of growths each of which had consisted of but a single one (Fig. 13). Hence they will be termed *combines*. Others, usually more diverse, had shapes indicating that they had originated from a complex fragment of the growth plated. These are *sample* tumors. The derivation of most of the large growths containing several components was uncertain. They will be called *medleys*.

Most of the implanted mice were killed early to avoid the formation of combines, and hence metastasis was a rare event. The sex of animals that had become adults before they were killed had no obvious influence on the yield from the platings. Two of the spontaneous tumors studied first (MT's 1 and 2) were deemed too large for plating. Their components were of the same general sorts as in those subjected to this procedure (MT's 3 to 6). These latter will be reported upon in the order that best sets forth the findings.

Separation by Plating of the Components in a Complex Carcinoma

Mammary Tumor 5 (MT5) was a flattened, ovoid mass measuring 18 × 12 × 4 mm with many subdivisions indicated by shallow sulci and more or less separated by connective tissue.

Most of them were grayish pink and soft, not perceptibly differing in the gross; but three had small purple cysts amidst them. Some of the ragged tissue enclosing these cysts was taken for plating, together with pieces from seven of the grayish pink lobuli. About half of each sample was fixed for sectioning and what remained was pooled, prepared by the sed. grid. technic, and plated onto the backs of five weanlings.

Microscopically the samples disclosed the presence of tumor tissue of three definite kinds, broad-banded, tessellated, and desmoplastic, as also a cystic alveolar component of questionable nature (Figs. 8 and 9, *Vc?*).

The first mouse killed, after *36 days*, had numerous, scattered, pinky gray "dew-drop" growths (Fig. 10), attached to the dorsal body wall only. The largest were scraped off, grouped, and serially sectioned. Most of them (Figs. 11 and 12) consisted of but one of the components just mentioned.

After *53 days* another mouse had many tumors up to 6 mm across. Again all were sessile on the body wall with none over the thigh. Except for four containing small purple cysts they were solid and pinky gray. Most of them consisted of only one of the components already noted.

The tumors were irregularly distributed in the animal killed after *62 days* (Fig. 13). Where the needle had been withdrawn through the muscles, a big, conglomerate, gray and purple mass had arisen, and in the lumbar region were numerous discrete tumors, now coalescing. A few growths were present along the lower border of the thorax. The bracketed group, consisting of eight or more, partially fused tumors (Fig. 13), was sectioned horizontally (Figs. 14 and 15). It will be seen that they had coalesced so far as now to appear microscopically to have been a single, complex growth, only indistinct septa indicating their previous separation. The queried cystic alveolar component of the primary tumor has turned out to be really of this sort (*Vc*) and a patterned growth is also present that was not seen in the material previously examined.

The remaining two mice, autopsied after *75 days*, yielded confirmative findings. Some of the medleys were large by then.

The plating did more besides separate all of the components noted in the spontaneous cancer. It disclosed the presence of a patterned one (Fig. 14), and furthermore made plain that the queried, cystic alveolar component of Fig. 9 (*Vc?*) was really neoplastic. The growths formed by the differing individual components of the cancer all looked alike to the unaided eye, except those distinguished by purple cysts. This was a disappointing find since it showed, as did the many later platings of other tumors, that no selection could be made in the gross of any special components except the cystic.

The little tumors did not long remain separate like those due to the plating of benign epidermal papillomas. They coalesced with striking rapidity when close together, and except for the contour of the resulting combines (Fig. 13, bracketed mass) one might have thought that the latter had derived from a single sample of the original cancer. As the tumors due to plating grew larger, they became encapsulated with connective tissue, and this latter persisted after their coalescence, making plain the original separation of their components. This held true as well of the growths due to the plating of the other mammary tumors. Figs. 22 and 52 provide instances in point.

The Yield from Four Successive Platings of a Mammary Tumor Having Many Components (MT3)

The early platings of MT3, the cancer first utilized, were made with tissue inadequately prepared.

The spontaneous tumor, a pinkish gray mound 9 mm across, with a few purple cysts, really consisted of two separate and very different masses. Microscopically one appeared wholly acinar, whereas the other consisted mostly of cystic alveolar tissue, though with ordinary alveolar and hemorrhagic acinar components as well (Fig. 16).

Plating I.—Pieces from several regions of each mass were minced in SL, enough L added for plating, and the resulting suspension—which shimmered with single cells and small groups of them and also contained fragments up to nearly 1 mm in diameter—was scattered with the aid of air on the ventral expanse of several litters of sucklings.

The results of PL I were disastrous. The differing neoplastic components, well demarcated in the primary tumors, had been so closely intermingled in suspension as to give rise almost entirely to complicated medleys. Nearly all of these were mottled with purple and some had bulging purple cysts. Of the many growths sectioned only one consisted of a single component, namely cystic alveolar tissue (Fig. 17), and this one had formed such small cysts as not to have been recognized in the gross. The obvious step to take was to try and isolate the component responsible for the big purple cysts.

Plating II, Gen. 2A.—A suspension was made as previously, but only of pieces of the thin, outer walls of two large purple cysts protruding from a medley growth of PL I. Weanlings were plated on the back by way of a needle thrust through the thigh muscles.

Despite the selection of cyst walls for the plating, nearly all of the resulting tumors were medleys; but in two animals rounded, purple cysts of considerable size were present, lying separate. One was used for the next plating.

Plating III, Gen. 3A.—The cyst utilized was 12 mm across and it proved multilocular, collapsing when incised, with the release of much bloody fluid. The soft gray lining of its thin outer wall was scraped off and the remaining, almost fibrous, tissue was cut very fine in SL, Locke's added, the suspension let stand briefly to let the largest fragments come down, and then the shimmering supernatant fluid—which still contained many visible fragments—was so diluted that each mouse received only a few of these latter. Forcible injection was done, together with air, through the thigh muscles of weanlings.

Some of the suspension must have escaped into the groin of a mouse killed after *35 days*, for here a solid gray tumor, a large purple cyst, and a purple-patched medley, had arisen separately (Fig. 19). Microscopically the gray tumor consisted of ordinary acinar tissue, whereas the purple growth was of hemorrhagic acinar sort. Though appearing to be a single cyst, it actually consisted of several, all thinly lined, with a little acinar tissue between them. Both ordinary and hemorrhagic acinar tissue were present in the medley. The mice subsequently killed yielded no findings of special note.

The sed. grid procedure to obtain washed and graded tumor particles had by now been worked out, and it was used for all the later platings.

Plating III, Gen. 3B.—The second purple cyst mentioned as found in a mouse of PL II, Gen. 2A was 15 mm across. It too proved multilocular, with a little interstitial, gray tissue. Its thin, outer walls were cut very fine in SL, the washed fragments were put through a 0.2 mm grid, and scattered on the backs of weanlings.

Most of the resulting tumors were medleys, though containing purple cysts; but a separation of neoplastic components had been obtained in one instance (Figs. 20 to 22). The ovoid, purple growth Y of Fig. 20 resembled in the gross the plated one. It consisted of cysts lined with hemorrhagic acinar tissue (Fig. 21), with a little of this between them, and just, as in the case of the purple growth of Fig. 19, the peripheral cysts contained erythrocytes in greater or less quantity, whereas those deeper were devoid of them. The solid gray growth *Z* had lobular markings, and sagittal sections showed that it consisted of three distinct tumors, with narrow septa of connective tissue between them, the remains of their individual encapsulation (Fig. 22). Two consisted of tubular alveolar tissue whereas the third was of the ordinary alveolar kind.

A previous plating, done with a medley tumor of PL I, had yielded remarkable findings. Solid gray and cystic purple tissues had been hashed together and suspended, the largest fragments allowed to settle out, the supernatant fluid briefly centrifuged to throw down the next in size, and these latter were scattered on the ventral expanse of sucklings.

Some of the largest of the few tumors that arose in each mouse were purple cysts—which looked like "black raspberries" because covered with tiny, bulgings full of dark fluid. A gray layer of hemorrhagic acinar tissue was present on their under side, and some existed inside them all. Fig. 23 shows two of them in outline, together with a purple-patched medley.

No previous report of such "raspberries" has been found. Fragments of the outer wall of the larger one of Fig. 23 were plated ventrally on sucklings (PL III, Gen. 3D). The results in one instance are shown in Fig. 24. The big purple growth in the groin had irregular cystic bulgings. Figs. 26 and 27 are of a tiny "raspberry" from another of the sucklings, and Fig. 25 is from a growth half of which was rendered purple by blood cysts amidst hemorrhagic acinar tissue.

Seven 3rd. Gen. platings were made. The outer walls of purple cysts were regularly utilized and most of the resulting growths consisted of similar cysts due to hemorrhages into acinar tissue. But not all were of such origin. Tumors of extraordinary kind arose in two sucklings of the 3rd Gen. E that had been ventrally injected with small fragments of purple cyst wall, scraped free from obvious neoplastic tissue.

The mouse of Fig. 28, killed *70 days* after plating, had a huge, roughly spherical mass on the neck. It was translucent and amber, with many bulges compromising the skin. They were due to cysts distended with watery fluid of this hue. Smaller ones were present deeper in the growth, and all were so interconnected that when the mass was freed by dissection partial collapse of it took place through fluid loss. Microscopically it everywhere consisted of cystic alveolar tissue (Figs. 29, 30, and 31), this being well nigh solid toward the center of the mass.

A neighboring tumor of firm, gray tissue mottled with purple covered the left under jaw. It consisted wholly of hemorrhagic acinar tissue containing numerous scattered blood cysts (Figs. 29 and 32).

Fig. 33 shows in outline the superficial tumors on the other suckling. It had a big group of partially coalesced amber growths over the left shoulder. Several small ones of the same sort, up to 5 mm across, were come upon within its abdominal cavity. They were lightly attached to the omentum, liver or pancreas—which last had been invaded (Fig. 34); and each consisted of several tiny cysts with diaphanous walls composed of cystic alveolar tissue. Scarring made plain that the plating needle had passed through the abdominal cavity on its way to the shoulder.

One of the two subcutaneous tumors charted as on the belly of the mouse consisted of cystic alveolar tissue, but the other proved to be a patterned carcinoma (Fig. 35), such as none of the platings of MT3 had yielded previously. This was encountered again in another mouse of the same plating, but there as part of a medley.

In these instances the thin, scraped wall of the purple cyst arising from hemorrhagic alveolar tissue had yielded not only growths of such sort but also relatively huge, amber tumors composed of cystic alveolar tissue. This had been present in the larger of the masses composing the original MT3 (Fig. 16), and a growth consisting of it, but not noticeably cystic in the gross, had resulted from PL I as already mentioned (Fig. 17). Occasionally tumors of the same kind had been found in the yield from PL II. But no such immense amber growths had rapidly arisen as those just described.

Fig. 18 shows a tumor of PL III, Gen. F composed entirely of cysts lined with an orderly layer of cystic alveolar tissue, often only one cell thick. Other similar growths with larger cysts were found in other mice of the same plating. Some of the cysts were pink to purple in hue, but most of them were colorless to amber. Plasma constituents were present within these last, as shown by clotting of the amber fluid when it was let escape into the surrounding tissue. In Fig. 18 the cysts containing red cells range from gray to black as determined by their number, whereas those with fluid only appear empty to light gray, depending upon the amount of coagulum present.

The state of affairs in these cystic alveolar growths was wholly different from that when blood escaped from small, ruptured vessels into the stroma of hemorrhagic acinar tissue, tearing this apart (Fig. 32) with result in purple cysts. The red cells of the cystic alveolar tumors had not sedimented but were suspended and in good condition, never mere shadows, and none of the cysts had become brown as result of hemoglobin degradation. Obviously the difference in their individual contents was consequent on the escape into them of fluids ranging in character from salt solution to whole blood. The walls of the stromal vessels had let plasma through into certain cysts, but not erythrocytes, whereas these had passed as well into others. The excellent state of the red cells indicated that some turnover of them must have been going on through vascular connections.

Gierke (4) long ago perceived that the cells of mammary mouse tumors frequently evoke what he termed fibroplastic and angioplastic stromal responses.

Now it is plain that angioplastic responses of two sorts may take place, one of them characterized by the escape of blood into hemorrhagic acinar tissue, the other consequent on the passage of blood constituents—fluid and sometimes formed elements as well—into the orderly cysts formed by cystic alveolar tissue.

Plating IV, Gen. 4.—A tiny cystic tumor was used for this plating that had been lightly attached to the liver, near a growth of similar sort invading the pancreas (Fig. 34). Fragments of its diaphanous wall were scattered dorsally on sucklings. The numerous resulting growths all consisted of cystic alveolar tissue. Most of the cysts were amber but occasionally one was pink or purple, owing to erythrocytes. Fig. 36 shows a typical instance. The largest cysts were ruptured in stripping the skin away. Unlike most components of mammary tumors, cystic alveolar tissue is actively invasive (Figs. 30 and 34), doubtless in part because of intracystic pressure.

This fourth generation plating accomplished its aim, namely the propagation, alone and in quantity, of cystic alveolar tissue. MT3 was carried no further.

Disclosure by Successive Platings of an Incompetent Component in a Mammary Tumor (MT 4)

MT4 arose in the same old breeder as MT5, but was markedly different morphologically and grew much faster on plating. A grayish pink mound, 15 mm across, it had superficial markings indicative of six lobuli, all consisting of semimolluscoid, grayish pink tissue oozing watery fluid on incision. One of the six samples pooled for plating was found to consist of tessellated tissue whereas the others all contained only exudative alveolar (Fig. 37).

Plating I, Gen. 1.—Fragments prepared by the sed. grid. technic were scattered on the back of weanlings. One killed *25 days* later had more than 30 tumors up to 4 mm across, already coalescing (Fig. 38). Microscopically all appeared to be made up of exudative alveolar tissue, but a later, microscopic scrutiny revealed tiny discrete growths of very different character of which more will be said further on. The tumors were bigger after *39 days* with more coalescence, and now cysts containing cloudy gray fluid could be seen on the outer surface of the largest. Again they consisted of exudative alveolar tissue. The three mice killed after *54 and 55 days* had huge dorsal cuirasses consisting of coalesced growths of exudative alveolar character, with bulging cysts up to 10 mm across containing watery, milky, amber or pale purple fluid. The tessellated component was present as well in a few of the tumors, and the broad-banded in two of them (Fig. 50).

A further plating was done to learn whether the biggest cysts were due to some unrecognized neoplastic component amidst the alveolar tissue.

Plating II, Gen. 2.—The thin, outer walls of three adjacent amber cysts, nearly 30 mm across together, overlying exudative alveolar tumors on the mouse of PL I killed last, were excised; all visible neoplastic tissue was scraped from their inner surface; and the remaining stringy tissue was prepared by the sed. grid. technic, and plated dorsally on weanlings. Tumors arose rapidly: they were up to 4 mm across in a weanling killed after *35 days*. After *41 days* they were much bigger (Fig. 39) and amber or purple cysts overlay the largest. The growths of the remaining 8 weanlings, killed after *51 to 72 days*, were similar and had increased much in size. As in PL I the sole cause found microscopically for the large cysts was exudative alveolar tissue.

Scattered growths of most singular aspect were noted on the back of the weanling killed after *41 days* (Fig. 39), and were present on all of those killed later (Fig. 40). They were discrete, visible in the gross, and uniform in general character. All save a few were approximately spherical and consisted of a creamy, opaque core embedded in a colorless, almost transparent jelly (Fig. 40). The largest was boat-shaped and about 4 mm long (Fig. 48), but the others were scarcely more than 1 mm across at most and the smallest 0.5 mm. They looked as the ova of some very small frog might be supposed to look, ova recently dead and hence opaque but still surrounded by jelly. Hence they will be termed O bodies.

Microscopically the "jelly" consisted of a layer of living, close-packed neoplastic cells having little or no stroma. The central core was due to their necrosis and accumulation on the inner side of this layer (Figs. 41 to 43 and 48) which was thickest and continuous in the mice killed early (Fig. 41). It then consisted of alveolar tissue in most instances, but occasionally lumina were almost or quite lacking, and the cells were close-packed like those of carcinoma solidum (Fig. 44). Fig. 49 shows an instance in which two O bodies had partly coalesced, and Figs. 61 and 62 a similar, secondary coalescence of an O body with a growth composed of exudative alveolar tissue, each retaining its own character. In the mice killed last the O bodies were much smaller, the living zone was reduced to islands separated by connective tissue (Figs. 43 and 45), and the cores of massed necrotic elements had undergone a coagulation necrosis (Figs. 45, 49, 62). No alveolar tissue of ordinary aspect, like that of the O bodies, was present in the tumors of other sorts.

Precursors of the O bodies proved quite numerous in the slides of the growths due to PL I that were searched in the light of these findings. They had been overlooked. Fig. 46 shows one out of many in a mouse killed after 35 days, all of them separate, but next to, or under, the relatively big, exudative alveolar tumors. At high magnification the one pictured looks like a tumor graft of which only the peripheral cells have survived (Fig. 47), and this was doubtless its character. Its living islands have not yet formed a zone.

Presence of an Incompetent Component Forming O Bodies in Another Carcinoma (MT 6)

MT6, a slightly flattened sphere 13 mm in diameter, was devoid of lobulation but had a few purple cysts up to 5 mm across protruding from its grayish pink surface. A vertical slice (Fig. 51) showed tubular and cystic alveolar tissue, with a small amount of ordinary alveolar, and of acinar as well. Elsewhere in the growth a hemorrhagic acinar component was present, as disclosed by the plating.

The thin outer walls of several of the purple cysts, together with pieces of solid tissue from three widely separate spots, were pooled, prepared by the sed. grid. technic, and the resulting fragments were scattered on the back of weanlings. One killed after *18 days* had numerous tumors up to 2 mm across. All were serially sectioned. After *24 days* the growths were bigger, some were coalescing, and now many had bulging colorless to purple cysts. Further enlargement with more coalescence had taken place after *34 days*. The two mice killed after *49 days* had big composite growths behind the shoulders, containing colorless, amber, or purple cysts, and individual tumors elsewhere. Fig. 52 shows part of a sausage-shaped growth due to the recent coalescence of several. It will be seen that acinar, hemorrhagic acinar, and tubular and

cystic alveolar tissues were all present. Now for the first time O bodies were observed in the gross, several in each animal. They looked wholly like those of MT4, save that more of them were flattened and some slightly larger. The remaining five mice, killed after *56 days*, had massive saddle-bag growths, scattered tumors elsewhere, and O bodies too, as many as eleven in one instance.

Fig. 57 shows several O bodies from one of the mice killed last. It will be seen that they all have a core almost devoid of formed elements, surrounded by a layer of living cells which is continuous save in the spherical instance at the top, where it consists of islands. At this late stage in the existence of the growths their tissue resembles carcinoma solidum (Fig. 58), except for an occasional alveolus. Previously, while still doing well (Fig. 55), their tissue seemed of ordinary alveolar sort, but during the formation of their cores it underwent changes wholly unlike those in the O bodies of MT4. No frank karyoklasis took place, but a glassy, markedly eosinophilic material, resembling hyaline, accumulated between the cells, first at the center of the growths and then radiating outward. In proportion as this material increased, the alveolar tissue was compressed, ceased to have lumina, lost its pattern, and the cells, now lying in columns, became smaller as if from pressure atrophy, their cytoplasm diminishing to a mere rim and their nuclei becoming pyknotic. They might have been mistaken for lymphocytes had not many of the nuclei been oblong (Figs. 55 and 56). Eventually they became included in the glassy, central, hyaline mass and gradually faded away. Yet even during the last stage studied (Fig. 58) a few cells appeared to be alive deep within this. The whole process was orderly.

In the mouse killed only *18 days* after plating, the growths which would eventually become O bodies were readily discriminated microscopically from the many other tiny tumors, because tagged by the eosinophilic material (Figs. 53 and 54); and the course of events from then on could be closely followed. Much ordinary alveolar tissue that remained merely such was present in other growths of the same plating. Eventually the eosinophilic material ceased to be glassy, becoming cleft and usually fibrinoid (Figs. 59 and 60). The presence of a few fibroblasts amidst it in the mice killed last (Fig. 58) indicated that its organization had begun.

The character of the tumors responsible for the O bodies of MT's 4 and 6 will now be compared. They had much in common besides their early morphology and their progressive declension during a period when all other growths were continuing to enlarge. Those of both kinds proliferated for some time and seemed in excellent condition. Hence what happened later cannot be laid to cell injury at time of plating—for which the same fluids and procedures were used as in the later platings of MT3, which yielded no growths resembling them. The tissues of both formed alveoli for some while and their cells were then similar in size and aspect, though those of MT4 formed slightly larger glandular units, with bigger lumina occasionally compound. They failed to elicit more than a scanty, avascular stroma, mitoses became rare as time went on, and they underwent degenerative changes. Both gradually lost the alveolar pattern, their cells becoming crowded and eventually dying, with result in opaque cores.

Here the resemblance between the two tumors ceased. They formed O bodies in wholly different ways, the one by karyoklasis followed by coagulation necrosis, the other by the formation of a glassy intercellular material with concurrent cellular atrophy.

The gross aspect of the O bodies was striking yet none were ever seen amidst

the tumors of other sorts that arose in the hosts carrying them, nor were any morphological gradations found between them and these others. Yet to judge from their frequency cells capable of forming them must often have been present in considerable quantity. It seems likely that they were fostered by the well nourished neoplastic tissue of other sorts amidst which they were situated, yet under these affluent circumstances they did not disclose themselves by forming O bodies. Forming alveoli, as they did when faring well, they could easily have escaped notice amidst the exudative alveolar tissue of MT4 and the ordinary alveolar of MT6.

DISCUSSION

Despite its crudity the plating procedure sufficed for its purposes. Several unforeseen happenings proved crucial—the ease with which broad expanses of subcutaneous connective tissue could be exposed, the rapid attachment to the body wall of tumor fragments scattered over it, and the growth of these under the cover of an overlying cutaneous layer which long remained unattached. In these primary respects the conditions proved almost ideal for the isolation and maintenance of individual neoplastic components for study and transfer.

But now a major obstacle was encountered. The growths due to differing components were often so hard to tell apart in the gross that no offhand "fishing" for one or another of them was possible. This held notably true of the solid growths formed by most of the mammary tumor components, and hence our main endeavor was to propagate separately those which disclosed their special character through the formation of cysts. They proved to be of three sorts: hemorrhagic acinar tissue was isolated and identified as responsible for the generality of blood cysts; and both exudative alveolar and cystic alveolar tissues, where growing separately, were found to give rise to cysts containing watery, colorless to amber or ruddy fluid. No new happenings these: but only now have the components mentioned been procured and maintained individually. Success has been had inadvertently with three solid components, namely with the patterned carcinoma of Fig. 35 and with the two responsible for the differing O bodies of MT4 and MT6 (Figs. 41 to 43, 55, 56).

Sometimes the initial platings failed of their object and successive ones were required. Our efforts to propagate cystic components by themselves were not infrequently balked through the existence in the plated material of some hidden constituent that rapidly asserted itself and produced a considerable proportion of the resulting growths. In one remarkable instance (PL III, Gen. 3E of MT3) the thin outer walls of big blood cysts had been scraped free of all visible neoplastic tissue before they were cut up and washed for plating; yet the majority of the resulting tumors did not consist of the expected, hemorrhagic acinar tissue (though some there were that did) but of cystic alveolar tissue which rapidly formed huge growths composed of amber cysts that were wholly

different morphologically from the cysts in the same hosts due to hemorrhages into acinar tissue (Figs. 32, 34, and 36). The second plating of MT4 that brought the O bodies to light provided a like instance in point.

It is easy to say that less material, cut finer, should have been used for the platings just mentioned; but much smaller bits prepared by our procedure would not have grown, as experience showed. The number of tumors that arose after each scattering of fragments varied directly, as a rule, with the number of the large ones. This was the case even when mammary tumor tissue was minced in serum-Locke's, a protective fluid, and plated forthwith after only enough Locke's had been added to assure its wide distribution. The very numerous individual cells and small clumps with which the suspension shimmered under such circumstances found no expression in the findings. The tumors that arose all got off to a start at nearly the same time, with the exception of those forming O bodies: there were almost no late comers such as might have been formed by small groups of cells. The subcutaneous connective tissue provides but a poor environment for tiny grafts, as the early workers, transplanting by trochar, soon came to know. Grafts about 1 mm across, "half the size of a (Victorian) match head," were best in their view. The utilization of prepared connective tissue expanses and of balanced fluids for supension, perhaps with adjuvants as well, may lead to a truly cellular analysis of complex growths by the plating method.

In this last connection one is reminded of an experiment of the Kleins (5). Acting on Puck's demonstration (6) that cells x-rayed sufficiently to prevent their multiplication but not to kill them, provide a milieu enabling single, normal cells to proliferate *in vitro*, these workers added x-rayed cells from an ascites mammary tumor to others to which nothing was done, and injecting the mixture intraperitoneally, found that it caused a larger percentage of animals to develop growths than did unrayed cells alone.

Air has been employed previously to distribute tumor constituents in the subcutaneous connective tissue of mice, but with an aim different from ours. Hewitt (7) used it to distribute the individually suspended cells of two sarcomas, in order to learn how many were needed to yield a single growth. He first injected the air slowly, and then the cell suspension, into the backs of mice a year or more old, with result in large pouches in which the air was let stay, some persisting for more than a week. Multiple growths often arose but no differences in them were reported.

Selye has produced large pouches (8) by injecting air into the dorsal subcutaneous tissue of adult rats. He kept them open for long periods by the repeated introduction of irritants and thus showed, besides much else, that croton oil induces sarcomas (9).

No signs were come upon during the present work of host resistance directed against any of the tumors due to plating. None of the growths consisting of different components of the same mammary tumor greatly outstripped others on the same host except those of cystic alveolar tissue, and the accumulation of fluid within their myriad cysts was largely responsible for this. None under-

went any secondary dwindling except those that formed O bodies, and these did so because of innate cell peculiarities. No accumulation of plasma cells and lymphocytes, such as bespeaks host resistance, took place around O bodies that were doing badly or around any of the differing mammary or epidermal tumors which had arisen on the same mouse. The homogeneity of our C strain mice evidently extended to even the most abnormal neoplastic components of the cancers studied. It would seem worthwhile to learn whether tumors derived from the various organs of genetically homogeneous animals can be plated together without exciting deleterious interactions; for in this case chemotherapeutic tests might be made with widely different tumors on the same hosts. Fig. 7 shows an instance in which benign and malignant epidermal papillomas and a mammary carcinoma all flourished on the back of one mouse.

The spontaneous mammary carcinomas were from 9 to 18 mm across when plated, and their neoplastic components which formed growths on plating could not have fallen far behind in the competition that had previously taken place, else they would not have yielded fragments large enough to produce tumors under the new conditions obtaining. This fact enables one to understand why all of the resulting growths, save those due to the two components forming O bodies, enlarged at much the same pace. No microscopic search was made for any growths of considerable size consisting wholly of broad-banded or tessellated tissue, but these components were isolated from MT5, and so frequently held their own in the large "medley tumors" of successive platings as to make plain that they could have been maintained individually, had this been wished. The uniquely patterned carcinoma that emerged after a third successive plating of MT3 (Fig. 35) differed so distinctively from any previously observed component of this tumor that it can scarcely have been a variant of one. Most likely it had persisted unseen throughout Platings I and II. Not so with the cystic alveolar tissue which suddenly formed huge amber growths (Figs. 28 and 33) after another third plating of MT3, carried out with fragments of what was assumed, with good reason, to be its hemorrhagic acinar component. Cystic alveolar tissue had been present in the original MT3 and had formed growths after Platings I and II, but it had not asserted itself aggressively before. Foulds has called attention to the occurrence in mammary tumors of variants characterized by abrupt increases in vigor instead of by morphological change (10).

A major yield of the plating procedure was the conservation and disclosure of two neoplastic components which gave no recognizable signs of their presence until released from their tumor context and in this way enabled to proliferate separately. Though appearing to consist of ordinary alveolar tissue, they did strange things when producing the O bodies of MT's 4 and 6 respectively, and they did these in markedly different ways. The mammary mouse tumors are notoriously pleomorphic, but this is not the limit of their complexity. One

must reckon as well with differing hidden capabilities of neoplastic components that look alike.

In the early days of cancer research unavailing efforts were made to propagate tumors on the normal expanse of the peritoneal lining, but this proved refractory, seldom providing a stroma to grafts scattered on it. The injection of mechanical irritants was found to render it responsive (11), but because of its inacessibility nothing further was done. The barrier it presents has actually made possible the recent development of "ascites tumors," a richly rewarding material. The injection at one spot into mongrel new hosts of mingled bits from various parts of a single tumor has sometimes been used successfully to make sure that its component which is most likely to "take," presumably the most malignant one, is included in the material transferred (12); but trochar transplantation is still the method of choice. Much has been said of late against it as rendering many tumors artificial creations, and recourse for experimental purposes to primary growths, either spontaneous or induced, has been urged. But the heterogeneous character of the mammary tumors of mice, as illustrated by those of the present work, together with their individual complexity and inconstancy, renders this recourse dubious in their case; and they are a main reliance for experimental chemotherapy. As primary growths nearly all of them provide no standard other than that of their origin from the epithelial cells of mammary tissue. Transplantation of them by trochar has the merit that in most instances it eventually enables their fittest, and worst, component to supersede all others, with result in a stable material for test, consisting of much simplified cells, often wholly devoid of differentiation, and strikingly expressive of the neoplastic principle characterizing all tumors, whatever this may be. The three transplantable mammary growths of the mouse, recently listed as shown by experience to be best suited for research purposes (13), all possess this simplicity. Two of them attained to it during years of trochar transfer and the third was primarily simple.

SUMMARY

A procedure analogous to the plating of bacteria is described whereby some complex tumors have been taken apart and their components separately propagated. It was the outcome of finding that the forcible injection of Locke's solution followed by air can be used to split the subcutaneous connective tissue of sucklings and weanlings horizontally over the entire expanse of their backs or bellies, without inducing any complicating inflammation. Tumor fragments suspended in Locke's were widely scattered on the surfaces thus exposed. Most of them remained where they had lodged on the body wall, and rapidly becoming fixed in place, formed growths protected by the overlying cutaneous layer—which, throughout many weeks, remained unattached either to the wall or to them.

The procedure is more searching in its disclosure of tumor constituents than

those currently employed, and it has the advantage that it preserves the neoplastic components that it reveals. It has been used thus far only to rescue for experimental purposes transplantable, benign, epidermal papillomas from the hidden carcinomas deriving from their cells, and to set free and maintain the neoplastic components of complex mammary tumors of milk factor type. Success was obtained with such of the latter as were chosen for separate propagation, though successive platings were sometimes required for their isolation. Incidentally the procedure revealed two components in the mammary growths which could not have been discerned by previous methods of search. Each formed tumors peculiar to itself.

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EXPLANATION OF PLATES

PLATE 127

The sections for all the plates were stained with eosin and methylene blue.

FIG. 1. Results of plating fragments of a benign papilloma on the back of a suckling, together with bits of a derivative carcinoma. The mouse was killed 40 days later. Most of the growths were firm, ivory spheres (*P*), crowded yet discrete. The much larger, dark tumor *C1*, lying amidst them, was soft and brown. Microscopically it proved cancerous as did *C2* which also was soft and had coalesced with a smaller, firm sphere next it. × 0.95.

FIG. 2. Section of the wall of the largest firm sphere (*P*) of Fig. 1. It shows a peripheral layer of papillomatous tissue keratinizing inwards, and benign everywhere save in the bracketed area where its basal layer appears to be becoming malignant. × 22.5.

FIG. 3. Part of the wall of the big, dark tumor *C1* of Fig. 1. The cancerous tissue failed to keratinize and in some spots was almost anaplastic. Extravasation has taken place amidst its dead cells. × 22.5.

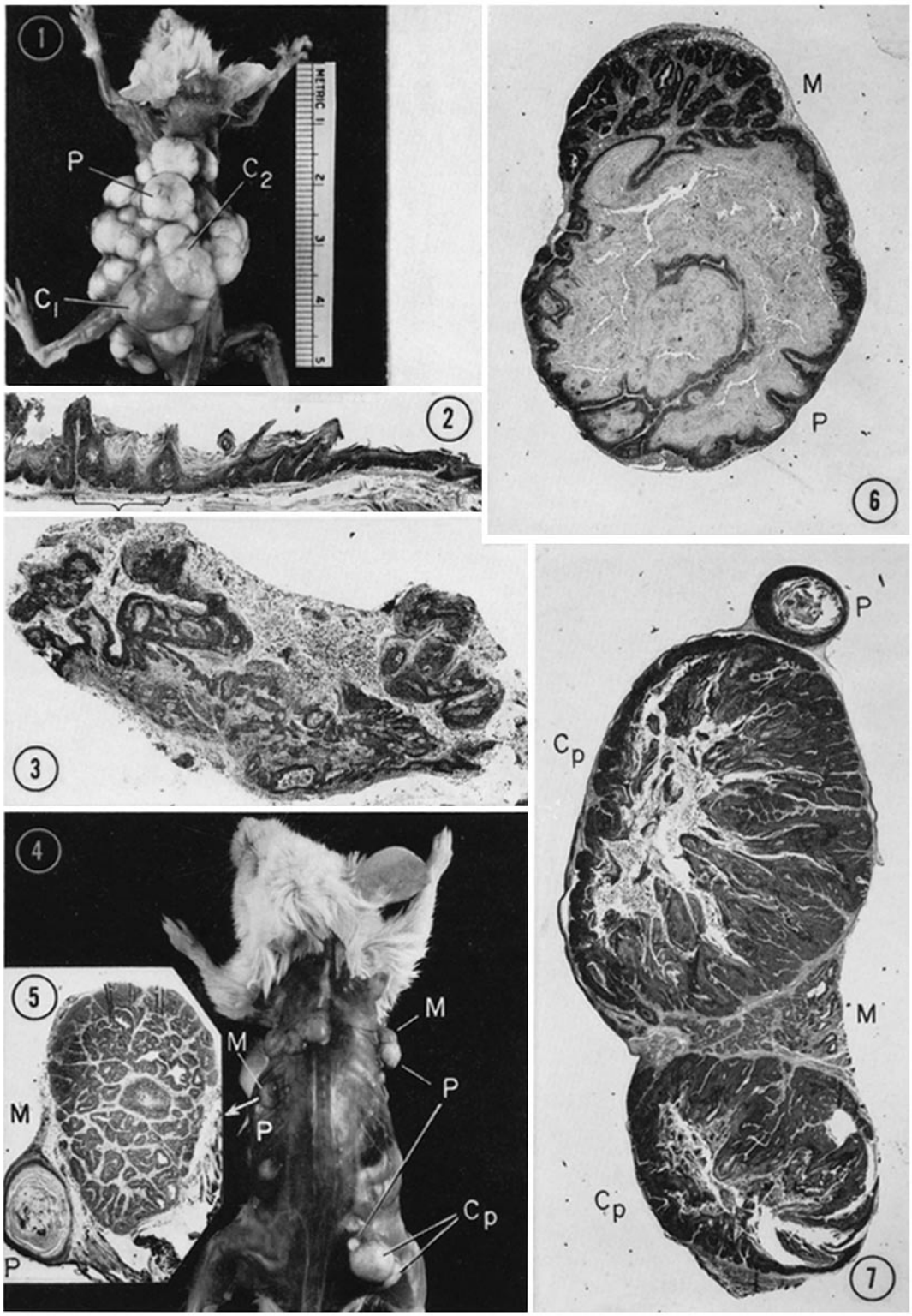
FIG. 4. Back of a mouse plated when a weanling with mixed fragments of Pap V (16th Gen. G) and a mammary carcinoma (MT3), and killed 20 days later. Most of the tumors lie along the sides of the animal, but a few are back of the shoulders and three can be seen where the injecting needle had emerged from the muscle of the right thigh.

The white, benign papillomas (*P*) and the medium gray, mammary cancers (*M*) had grown at the same rate, but some other, pale gray spheres (*Cp*) had far outstripped them. These were found to consist of malignant papillomatous tissue (Fig. 7). × 1.

FIG. 5. Character of the tumors next each other on the left side of the mouse. The smaller is a typical benign papilloma, the larger consists of mammary cancer. × 14.

FIG. 6. The closely apposed tumors back of the right axilla. *P* is an unusually active, benign papilloma, packed with keratin, and *M* is the mammary cancer. × 14.

FIG. 7. Cross-section of the tumors over the right haunch. The tiny white one (*P*) is here shown to be a benign papilloma, whereas the large spheres are papillomatous carcinomas. Between these latter and underneath them, not visible in Fig. 4, is the mammary carcinoma (*M*). × 12.



(Henderson and Rous: Plating of tumor components)

PLATE 128

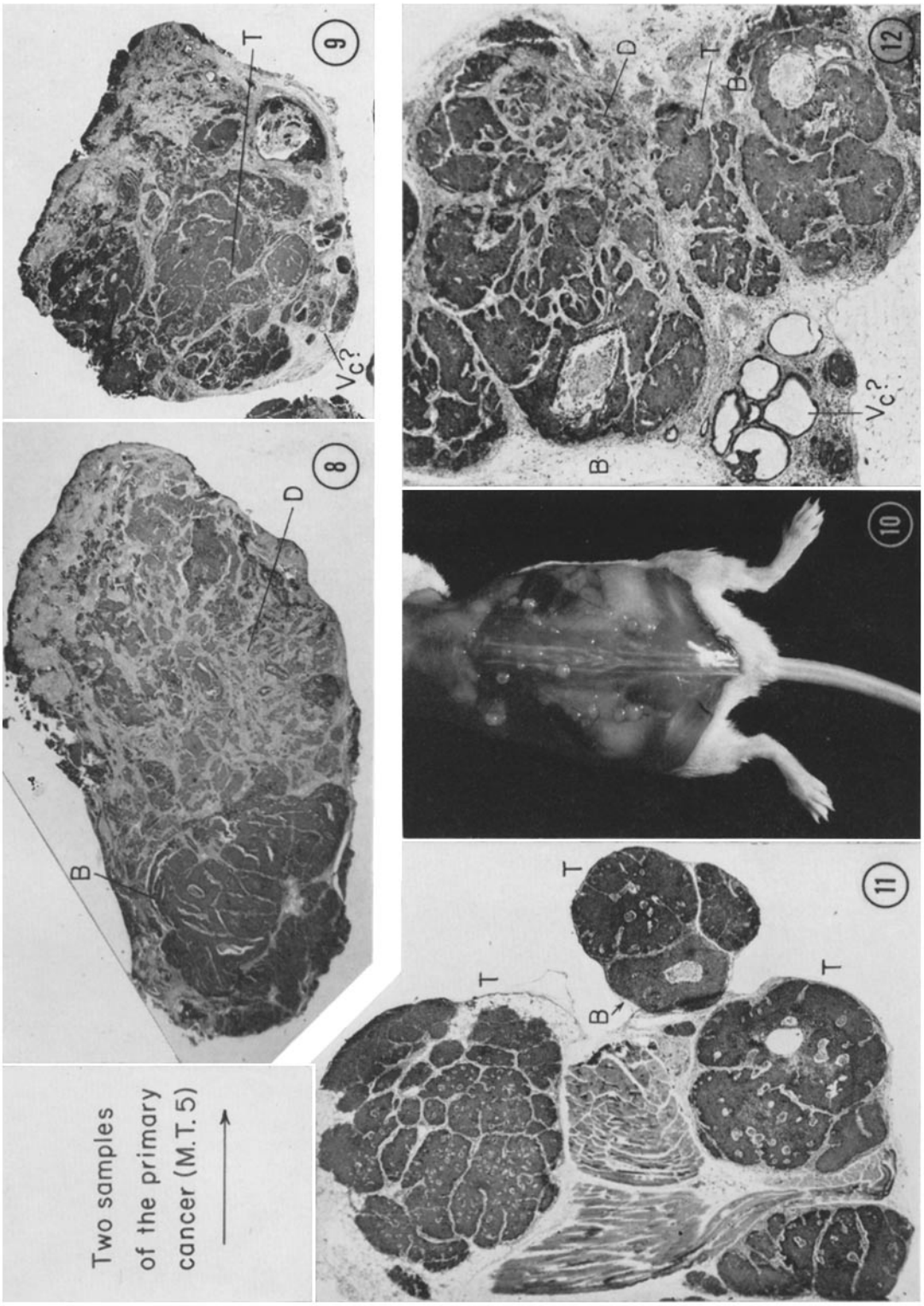
Results of plating Mammary Tumor 5

FIGS. 8 and 9. Two pieces of the spontaneous cancer. The one in Fig. 8 contains only broad-banded (*B*), and desmoplastic components (*D*), whereas that of Fig. 9 contains tessellated (*T*) and questionable cystic alveolar tissue (*Vc?*)—this poorly seen at the magnification given. × 24.

FIG. 10. Findings after 36 days in a weanling plated with washed fragments of the spontaneous cancer. The "dew-drop" growths are all attached to the dorsal body wall. Some are so small that only a bright point of reflected light bespeaks their presence. × 1.

FIG. 11. Seven of the "dew-drop" tumors of Fig. 10, together with bits of muscle scraped off with them. Most of them are composed of coarsely or finely tessellated tissue, but one is of the broad-banded sort (*B*). × 53.

FIG. 12. Five or more of the tumors of Fig. 10 and parts of some others. The largest consists of broad-banded and desmoplastic tissue, another contains cysts of dubious character (Vc?), and a third is made up of broad-banded tissue. The rest are tessellated. × 72.



(Henderson and Rous: Plating of tumor components)

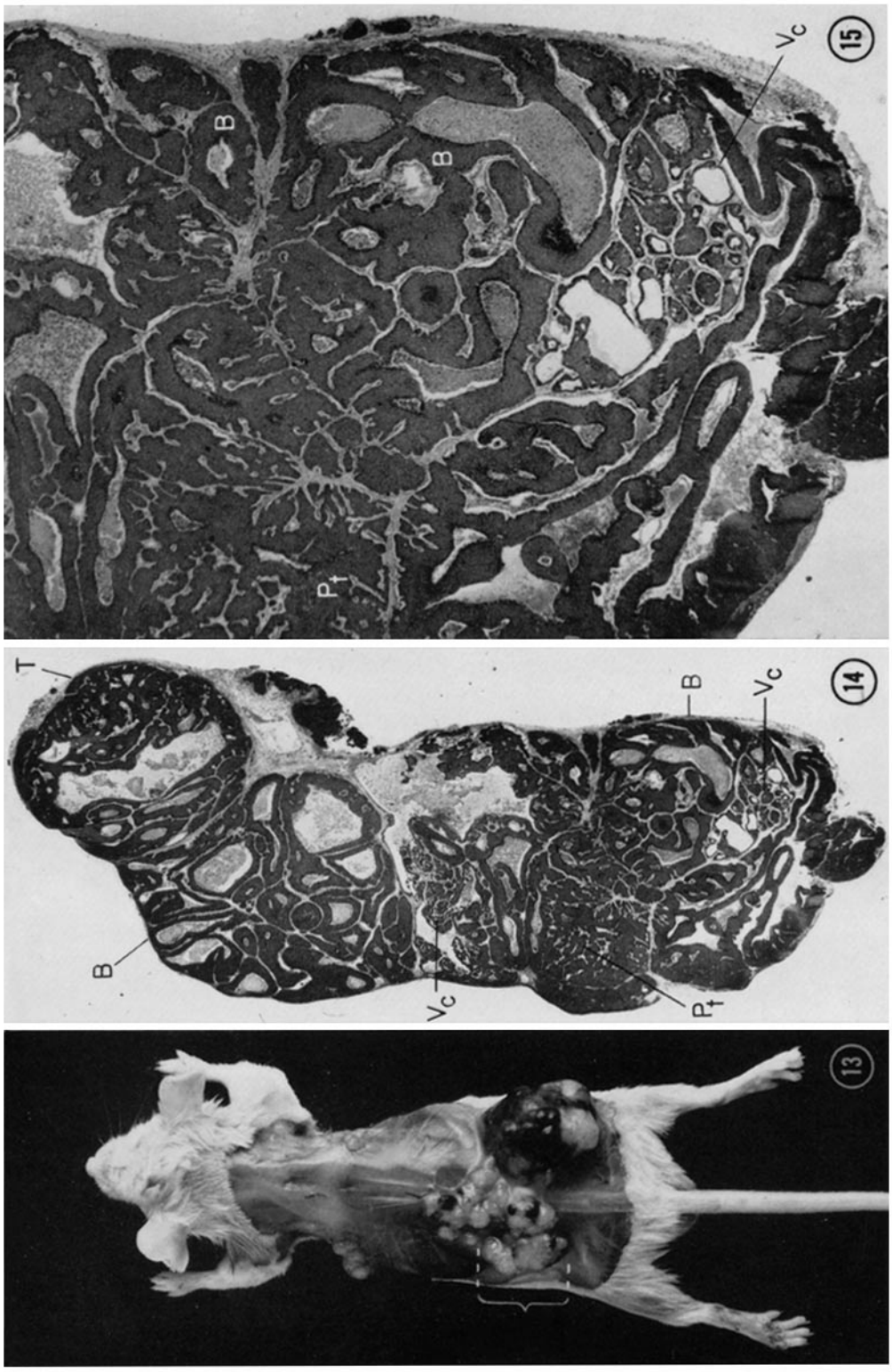
PLATE 129

Results of plating Mammary Tumor 5 (continued)

FIG. 13. A mouse of the same plating as Fig. 10 after 62 days. A big, conglomerate tumor mass topped by cysts dark with blood lies where the injection needle was withdrawn through the thigh muscles, and a few growths are present along the lower border of the thorax. The many discrete growths in the lumbar region are coalescing. A few protruding purple cysts can be seen amongst them. × 1.

FIG. 14. The bracketed mass of Fig. 13 in horizontal section. The coalescence of the individual tumors is far along yet their difference in composition can still be perceived. Fluid has collected within the broad-banded tissue. At two spots a component can be seen that is now definitely cystic alveolar (*Vc*), and at *Pt* a patterned component not previously discerned. × 10.

FIG. 15. Lowest third of the section of Fig. 14, to show in special the cystic alveolar and patterned components. The secondary cysts amidst the broad-banded tissue contain necrotic cells and coagulum. × 30.



(Henderson and Rous: Plating of tumor components)

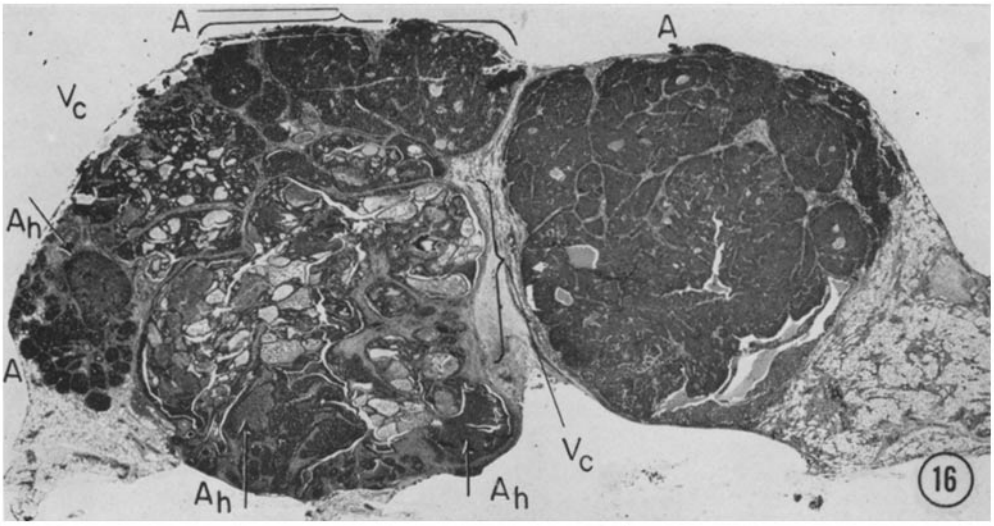
PLATE 130

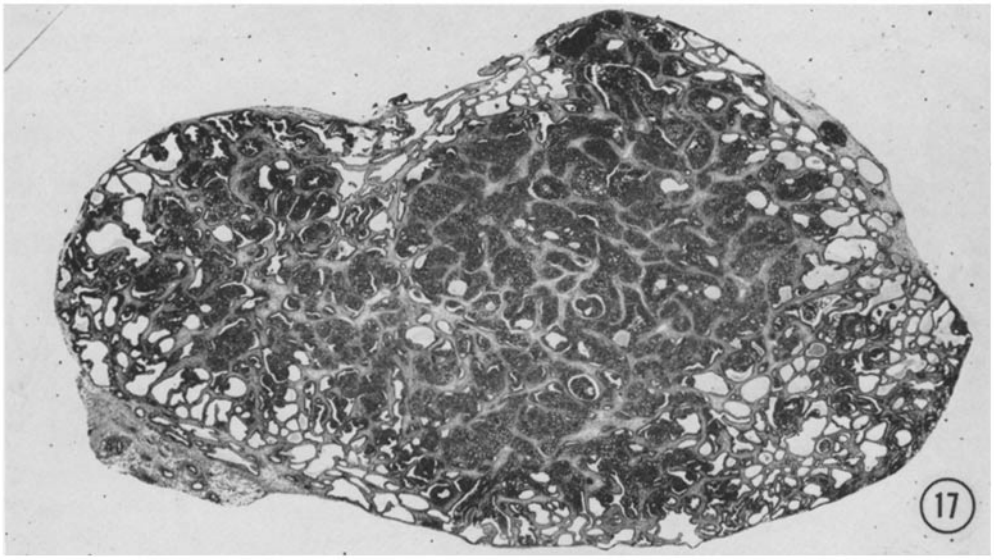
Results of the successive platings of Mammary Tumor 3

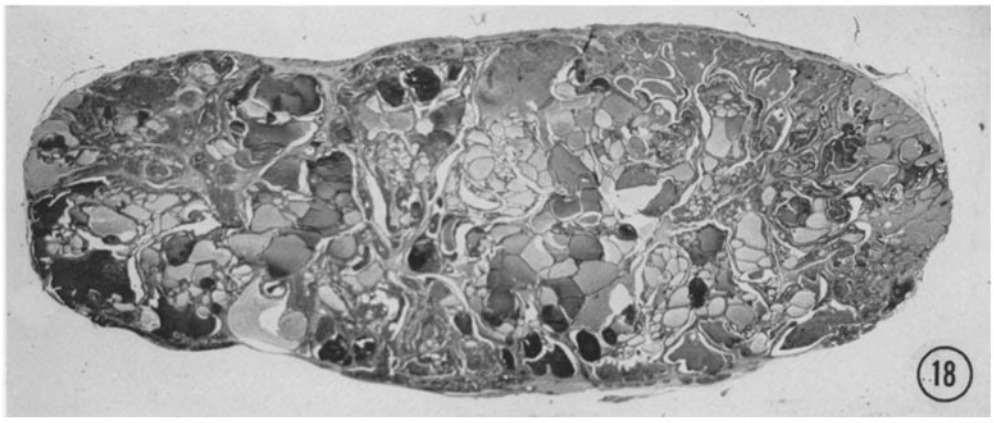
FIG. 16. Sagittal section of the spontaneous growth. It consists of two discrete and markedly different masses. The one on the right appeared to be composed entirely of acinar tissue (*A*) whereas that on the left contained a hemorrhagic acinar component (*Ah*) as well, with blood cysts in it, and a large amount of cystic alveolar tissue (*Vc*). × 14.

FIG. 17. A growth resulting from Plating I which consisted of a single component, the cystic alveolar. The largest cysts, now partially collapsed, exist at its periphery but further in the tissue is almost solid. × 13.

FIG. 18. A cystic alveolar tumor resulting from PL III of MT3 (Generation 3F). It consists entirely of cysts, some of which appear empty, whereas most of the others are of various shades of light gray, according to the amount of coagulum within them. Those that look dark gray to black owe this to red cells (see text). × 7.







(Henderson and Rous: Plating of tumor components)

PLATE 131

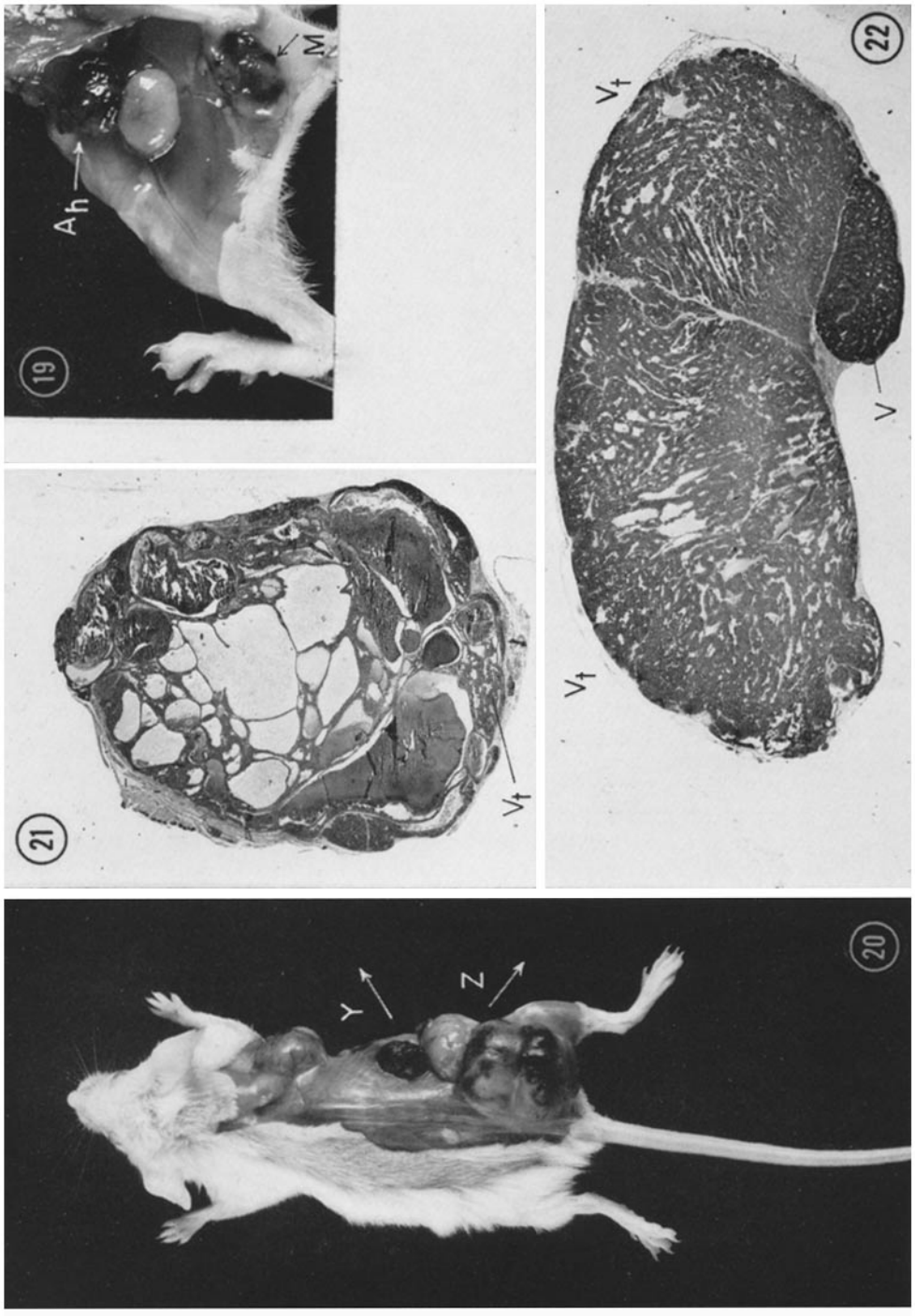
Results of the successive platings of MT3 (continued)

FIG. 19. Findings after 35 days in a weanling of PL III, Gen. 3 A of MT3. High lights mar the picture. The black growth in the anterior groin was actually purple and appeared to be a single cyst distended with blood, whereas the gray tumor was solid and looked as if it consisted of tissue of a single sort, as indeed was the case, the microscope showing this to be loosely alveolar. The purple growth, on the other hand, consisted of multilobular cysts amidst a small amount of hemorrhagic acinar tissue. The medley growth, *M*, gray mottled with purple, was purposely reflected with the skin. × 1.8.

FIG. 20. Similar findings in a mouse of PL III, Gen. 3B killed on the 39th day after plating. Again the black growth was actually purple. The gray tumor had sulci on its surface indicating it to be a composite. A big conglomerate growth on the right haunch overlies the spot where the injecting needle had been thrust through the thigh muscles. × 1.

FIG. 21. Cross-section of the black growth, *Y*, of Fig. 20. Most of the cysts around its periphery are dark with blood, whereas those in its interior are almost devoid of coagulum. Interspersed amidst them and thinly lining most of them was hemorrhagic acinar tissue, not visible here as such. At one spot a little, tubular acinar tissue (*Vt*) can be seen. × 9.

FIG. 22. The gray composite growth of Fig. 20. It consists of three tumors that have not wholly coalesced. Two are of the tubular alveolar sort, whereas the third, more compact and staining deeper, has the ordinary alveolar character. × 11.



(Henderson and Rous: Plating of tumor components)

PLATE 132

Results of the successive platings of MT 3 (continued)

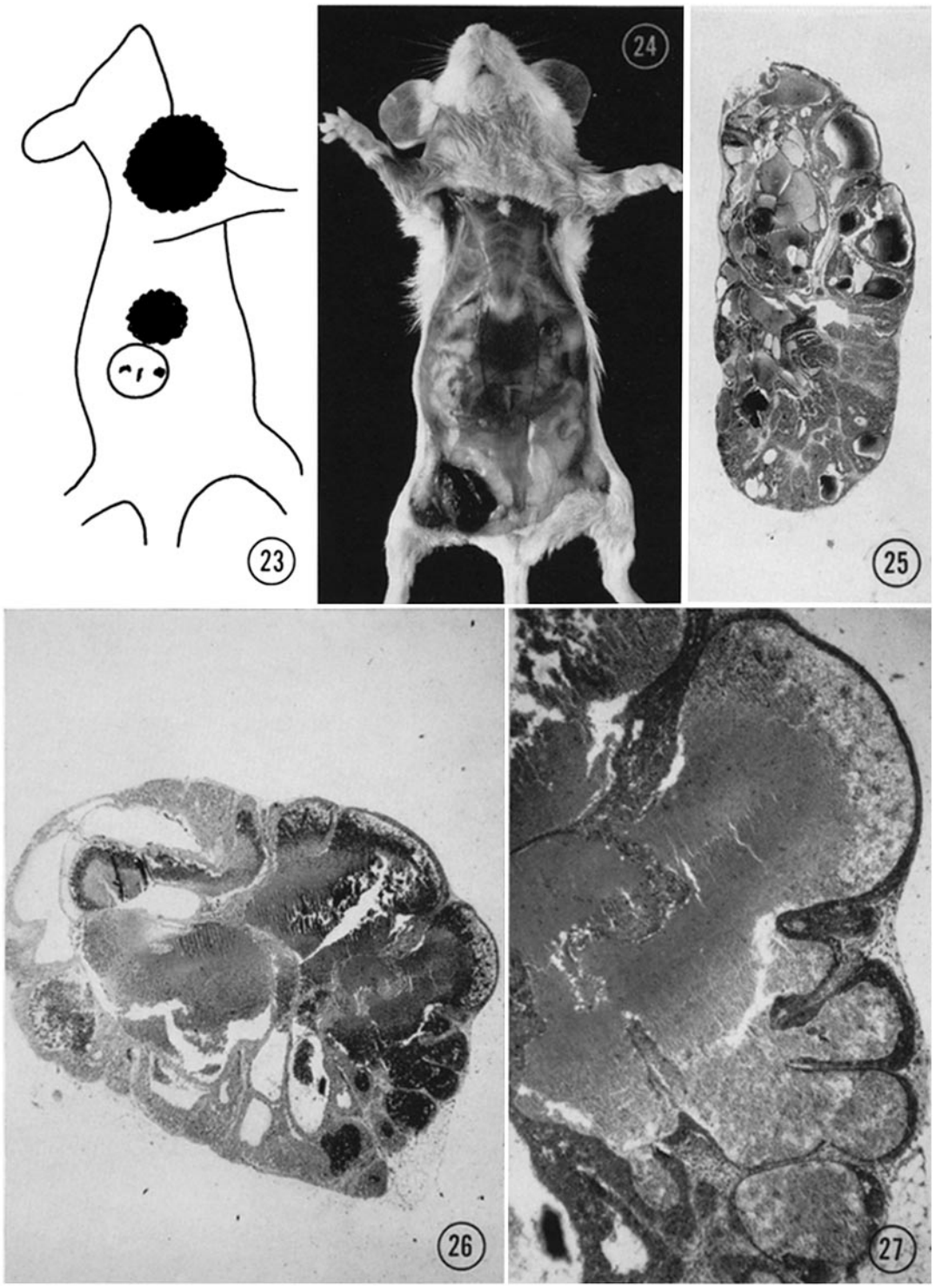
FIG. 23. Outline drawing of "black raspberry" cysts which often arose in the sucklings of PL II (Gen. 2B) implanted with small fragments of purple and gray tissues from a "medley" of PL I. The growth spotted with black on the lower abdomen was a medley. × 1.

FIG. 24. A mouse plated on the belly when a suckling (PL III, Gen. 3D) with a few fragments from the outer wall of the larger "raspberry" of Fig. 23. The irregularly cystic growth in the groin appeared everywhere purple but actually had some gray, hemorrhagic acinar tissue along its base. It is overlain along the left by fatty tissue. × 1.1.

FIG. 25. Section of a growth (PL III, Gen. 3D) consisting of hemorrhagic acinar tissue. Half of it was purple with blood cysts. × 7.5.

FIG. 26. Cross-section of a small purple growth of Gen. 3G, that had arisen after ventral plating of a suckling with small fragments from the outer wall of a "raspberry" of Gen. 2B. Some of the bulging cysts contain so many erythrocytes as to appear black whereas others, having fewer, are medium gray. The light gray tissue along the flat under side of the growth (lower left) and elsewhere within it is of the hemorrhagic acinar sort. × 25.

FIG. 27. Higher magnification of another, thinner, section through some of the bulging cysts on the upper right side of the tumor shown in Fig. 26. They are thinly lined with hemorrhagic acinar tissue. The erythrocytes they contain are well distributed, without sign of clotting. × 65.



(Henderson and Rous: Plating of tumor components)

PLATE 133

Results of the successive platings of MT 3 (continued)

FIG. 28. Tumors due to the ventral plating of a suckling 70 days previously (PL III, Gen. 3E), with fragments from the scraped outer wall of a purple cyst of a mouse of PL II, Gen. 2B. The huge, nodular mass on the under side of the neck appeared translucent and amber because of bulging, peripheral cysts full of clear fluid of this hue. In contrast the ovoid tumor next it (arrow) was opaque and gray, mottled with purple, as was a smaller growth on the belly. × 1.

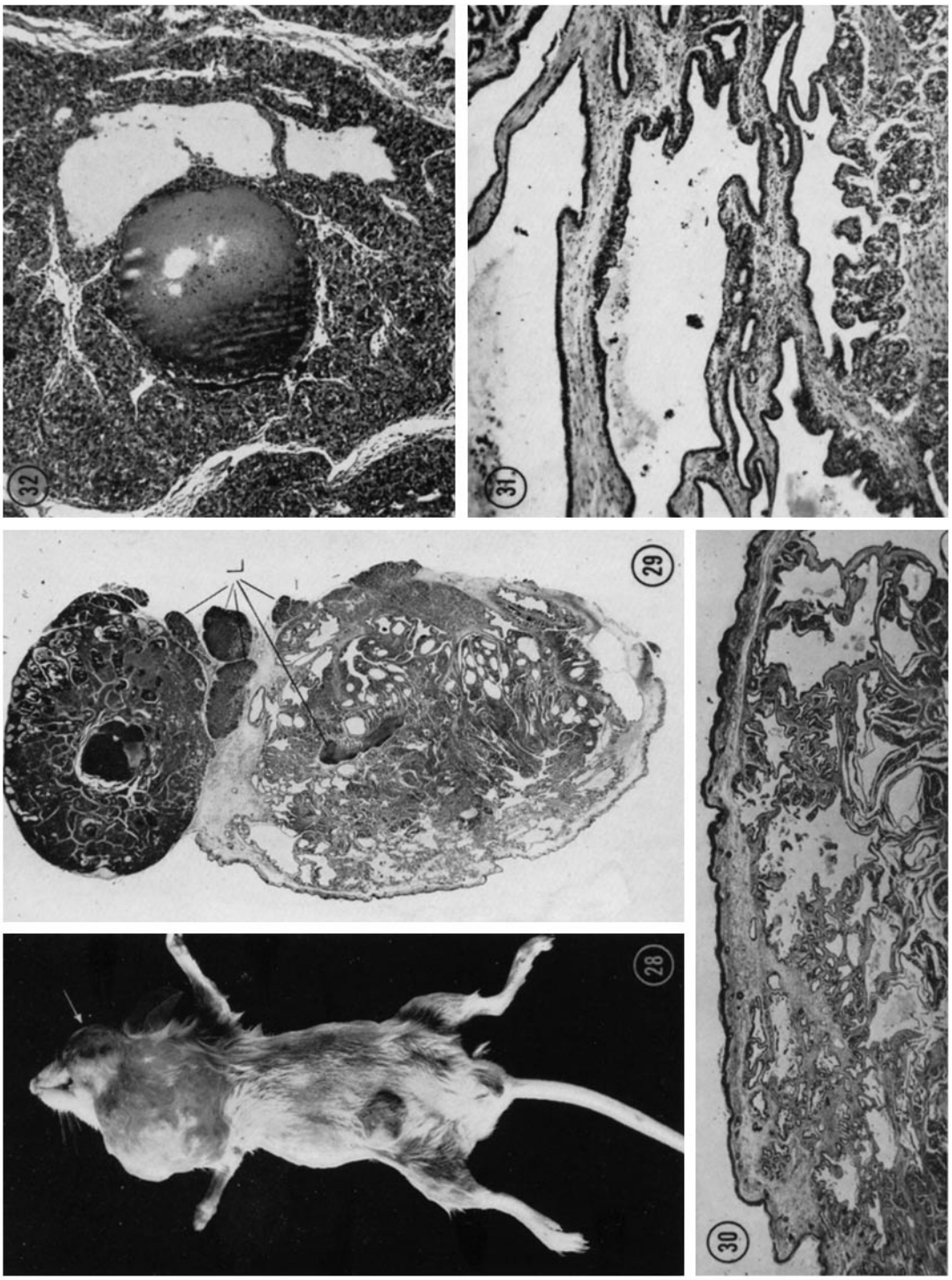
FIG. 29. Cross-section of the large tumors of Fig. 28 made in the plane of the arrow. The lines point to lymph nodes, one of which has been included in the cystic mass. The skin involved by this latter is present on the left but was stripped away from over the dark growth. The difference in the intensity of their staining emphasizes their differing character.

So much fluid was lost from the larger growth when it was dissected out that it is no longer spherical because most of the cysts beneath the skin have collapsed. For the results of similar leakage see Figs. 17 and 36. The growth consists entirely of cystic alveolar tissue—which appears almost solid in the depths of the mass and along its under side. The numerous black patches in the dark growth are due to hemorrhages into its acinar substance. × 5.2.

FIG. 30. Part of the cystic growth of Fig. 29 at higher magnification. The big cysts immediately beneath the skin have collapsed. × 17.5.

FIG. 31. To show the cystic alveolar character of the growth. Its septa have an orderly lining of neoplastic cells, mostly only one cell thick. × 84.

FIG. 32. Part of the deeply stained tumor of Fig. 29—to include one of the cysts full of blood. The cyst was due to hemorrhage into the surrounding acinar tissue. It protrudes into one containing no blood. × 84.



(Henderson and Rous: Plating of tumor components)

PLATE 134

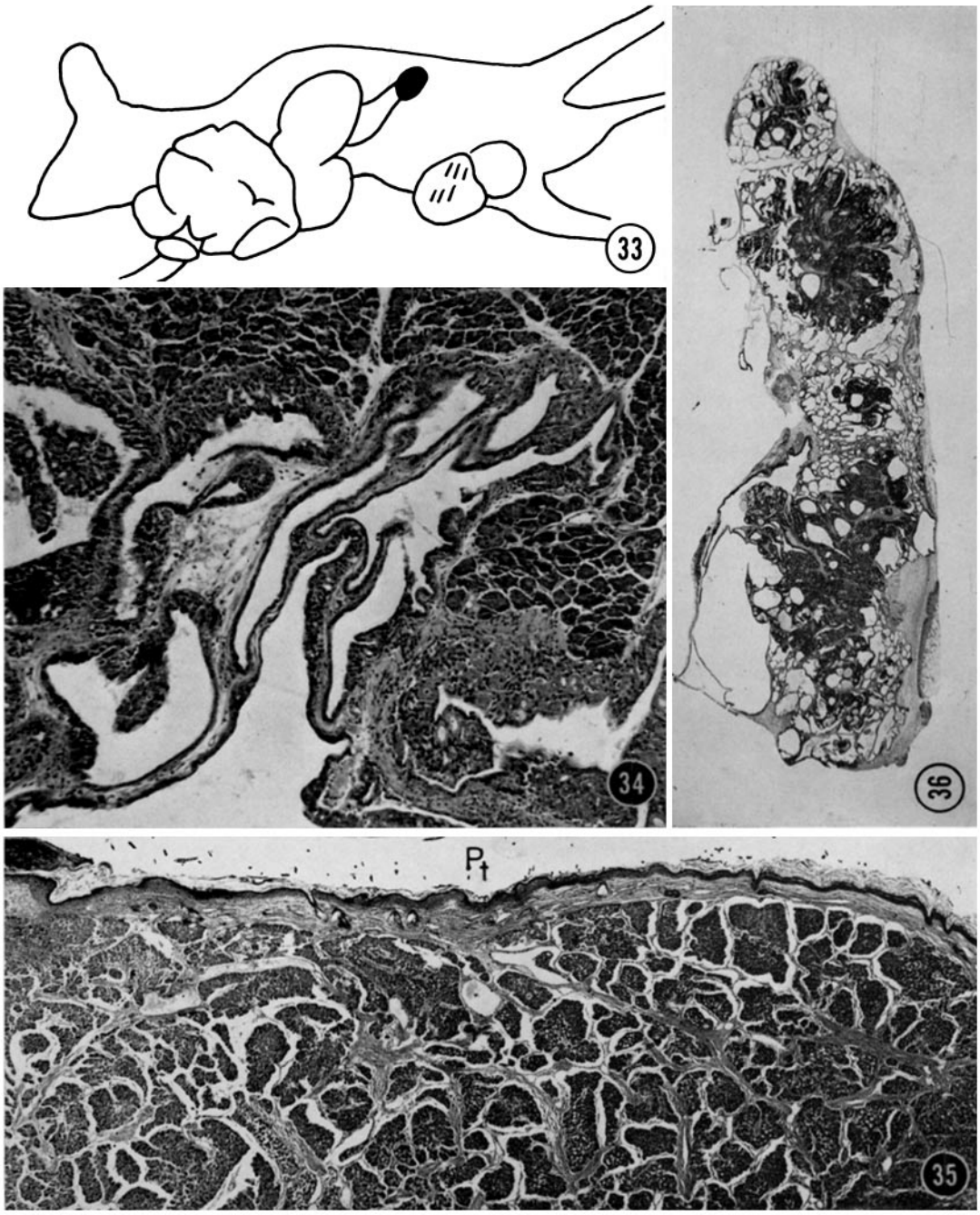
Results of the successive platings of MT 3 (continued)

FIG. 33. Outline of the tumors on another mouse of the same plating as that of Figs. 28 to 32, killed at the same time (PL III, Gen. 3E). The irregular mass over the left shoulder consisted of several big, coalescing growths, each covered on its outer surface with bulging amber cysts save at one spot (in black) where blood was present. All were lined with cystic alveolar tissue. Deeper in, the growth was more or less solid, like those of Figs. 17 and 29. One of the tumors on the belly was similar and the skin over it was scabbed (as shown by the hatching), but the other was gray and solid, and proved to be a patterned carcinoma (Fig. 35). × 1.

FIG. 34. Invasion of the pancreas of the same mouse by a cystic alveolar growth which had arisen where the plating needle was accidentally thrust through the abdominal cavity. × 91.

FIG. 35. Part of the subcutaneous, patterned carcinoma on the belly of the mouse. × 33.

FIG. 36. A growth resulting from the coalescence of four tumors of PL IV, Gen. 4 on the belly of a suckling plated 42 days previously with fragments of a tiny, cystic mass lightly attached to the liver of the mouse of Fig. 34. All four are of cystic alveolar type, but with wellnigh solid tissue deep within them. The big cysts on the outer (upper) side of the section were ruptured or largely torn away during removal of the skin. × 6.3.



(Henderson and Rous: Plating of tumor components)

PLATE 135

Results of plating Mammary Tumor 4

FIG. 37. Two of the six pooled samples of the spontaneous growth. The one on the left consists entirely of exudative alveolar tissue (*Vx*) with many ill defined spaces, the other is a tessellated carcinoma (*T*). × 25.

FIG. 38. Back of a weanling of PL I killed after only 25 days. The many tumors are already coalescing (for a section of the bracketed group see Fig. 46). × 1.

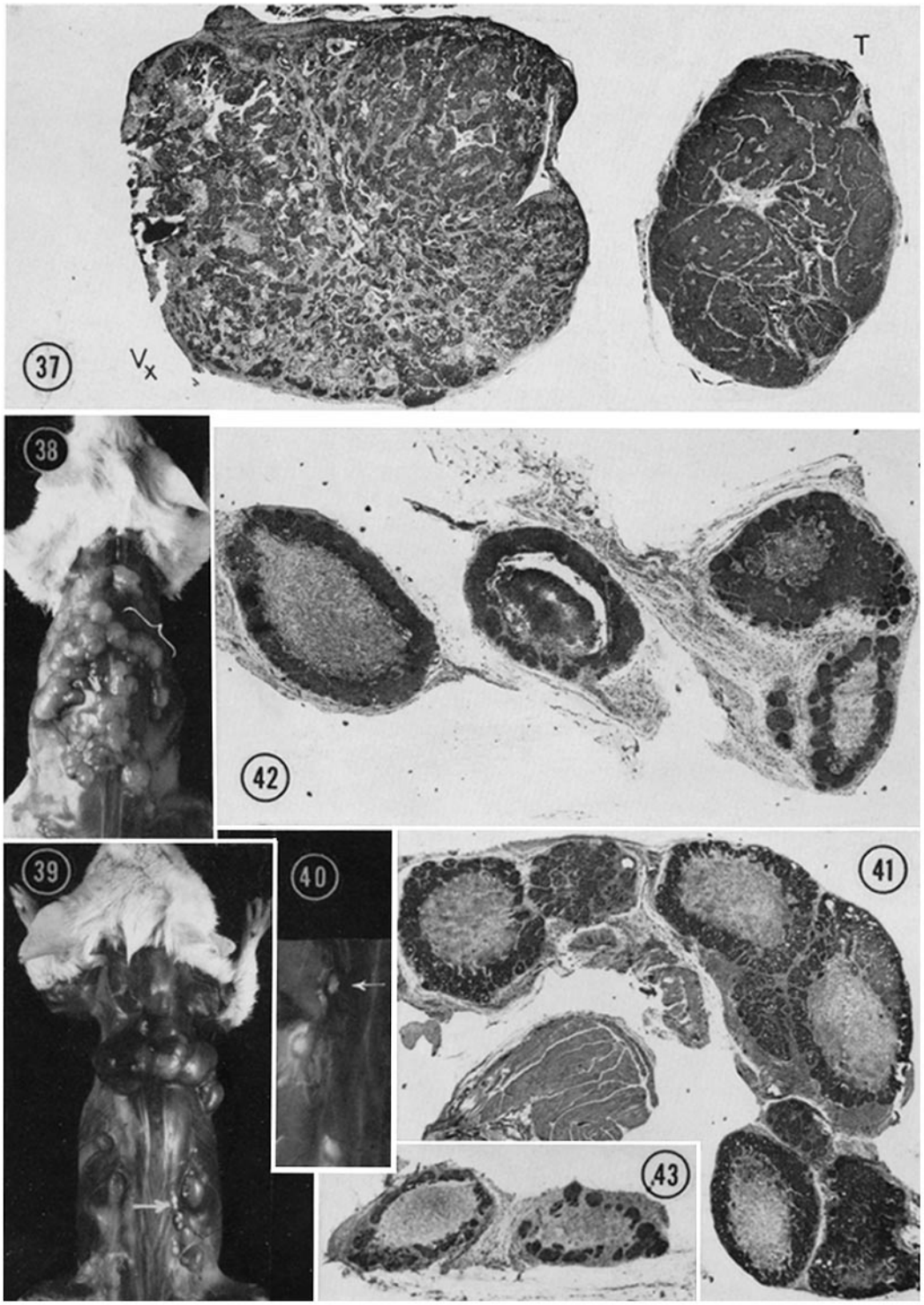
FIG. 39. Back of a weanling of PL II killed after 41 days. The abdominal viscera, dimly seen through the body wall, complicate the picture. Behind the shoulders is a saddle-bag mass of more or less confluent tumors, the largest surmounted by cysts, some of which are dark because containing blood. In the right posterior quadrant are several discrete gray tumors of medium size, and immediately next and between them are a number of tiny, wholly different growths (arrow). Though here appearing white, they actually had a creamy, opaque core surrounded by a well defined, colorless, semitransparent zone of living tissue. These are the O bodies of the text. The neighboring gray tumors (really grayish pink) consisted almost wholly of exudative alveolar tissue like that present in the primary tumor, and predominating in the growths of PL I (see Fig. 46). × 1.

FIG. 40. Three O bodies from another animal of PL II killed after 51 days. The arrow points to one seen in profile, next the blunt end of a large adjacent tumor of another sort. × 2.5.

FIG. 41. Some of the O bodies of Fig. 39 as sectioned after grouping. Four are cut through the middle, the others through their peripheral zone only—which was everywhere alveolar. The cores of the growths consist of necrotic cells. × 22.5.

FIG. 42. O bodies from another mouse of PL II killed after 35 days. The findings are similar except that few lumina are present in the living zone of tissue. × 30.

FIG. 43. O bodies from another mouse of the same plating killed after 76 days. They are less than half the size of those of Fig. 42, and their living zone now consists merely of close-packed cells in islands separated by connective tissue (see Fig. 45 also). × 30.



(Henderson and Rous: Plating of tumor components)

PLATE 136

Results of plating Mammary Tumor 4 (continued)

FIG. 44. Part of the living zone of an O body of Fig. 42. The cells are so compacted as to resemble those of carcinoma solidum, and to the right, on the inner side of the zone, they are undergoing necrosis. × 182.

FIG. 45. Part of the living zone of one of the O bodies of Fig. 43. Its islands of compacted cells, are wholly devoid of stroma and are dying on their inner (upper) side next a core which has undergone coagulation necrosis and come to contain scattered fibroblasts. No noteworthy accumulation of lymphocytes and plasma cells had occurred around the tumor. × 238.

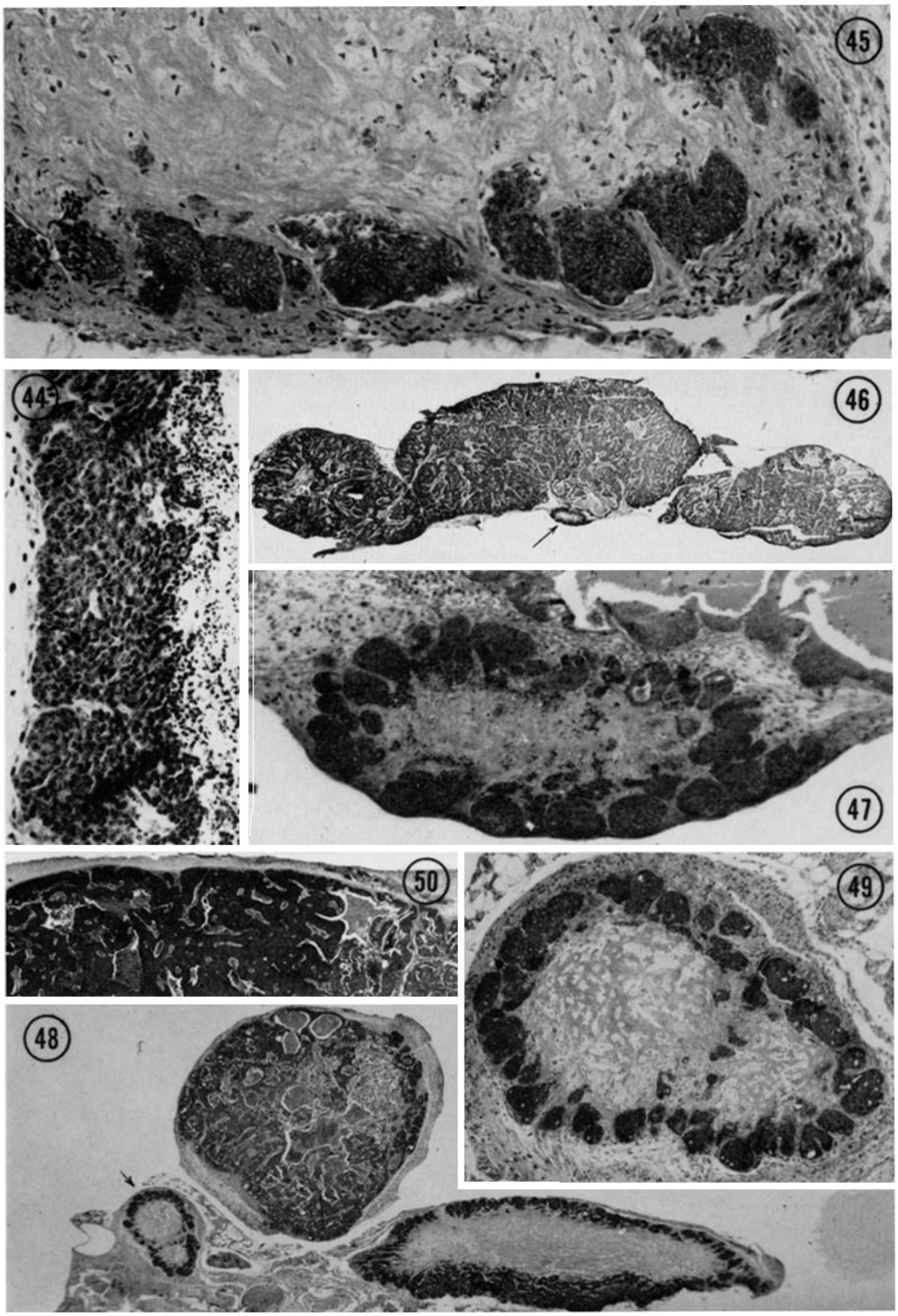
FIG. 46. Vertical section through the three exudative alveolar growths bracketed in Fig. 38. Underneath the middle one lies an O body (arrow). Its living cells stain much more deeply than those of the overlying tumor. × 7.

FIG. 47. The O body of Fig. 46 at higher magnification. At one spot its living zone shows two large alveoli, due perhaps to cells of exudative alveolar character, like those of the overlying tumor. Elsewhere they are close-packed. The dead core of the growth shows as yet no coagulation necrosis. × 88.

FIG. 48. O bodies and a small tumor of the exudative alveolar sort which lay near them: from a mouse of PL II killed after 62 days. One of the O bodies is flattened and exceptionally large (4 mm long). Two others, much smaller, are coalescing (arrow). × 15.

FIG. 49. The coalescing O bodies of Fig. 48 at higher magnification. Their living zones consist of islands of alveolar tissue, and where the growths have pressed against each other the diminished remains of such islands, originally at their periphery, lie in parallel lines. The cores of both growths have undergone coagulation necrosis. × 65.

FIG. 50. Shows adjacent broad-banded and alveolar cystic components in a medley tumor of PL I. × 18.5.



(Henderson and Rous: Plating of tumor components)

PLATE 137

Results of plating Mammary Tumor 6

FIG. 51. Sagittal section through the spontaneous MT6. It appears to consist mostly of tubular and cystic alveolar tissue but with some ordinary acinar tissue as well (*A*). × 10.5.

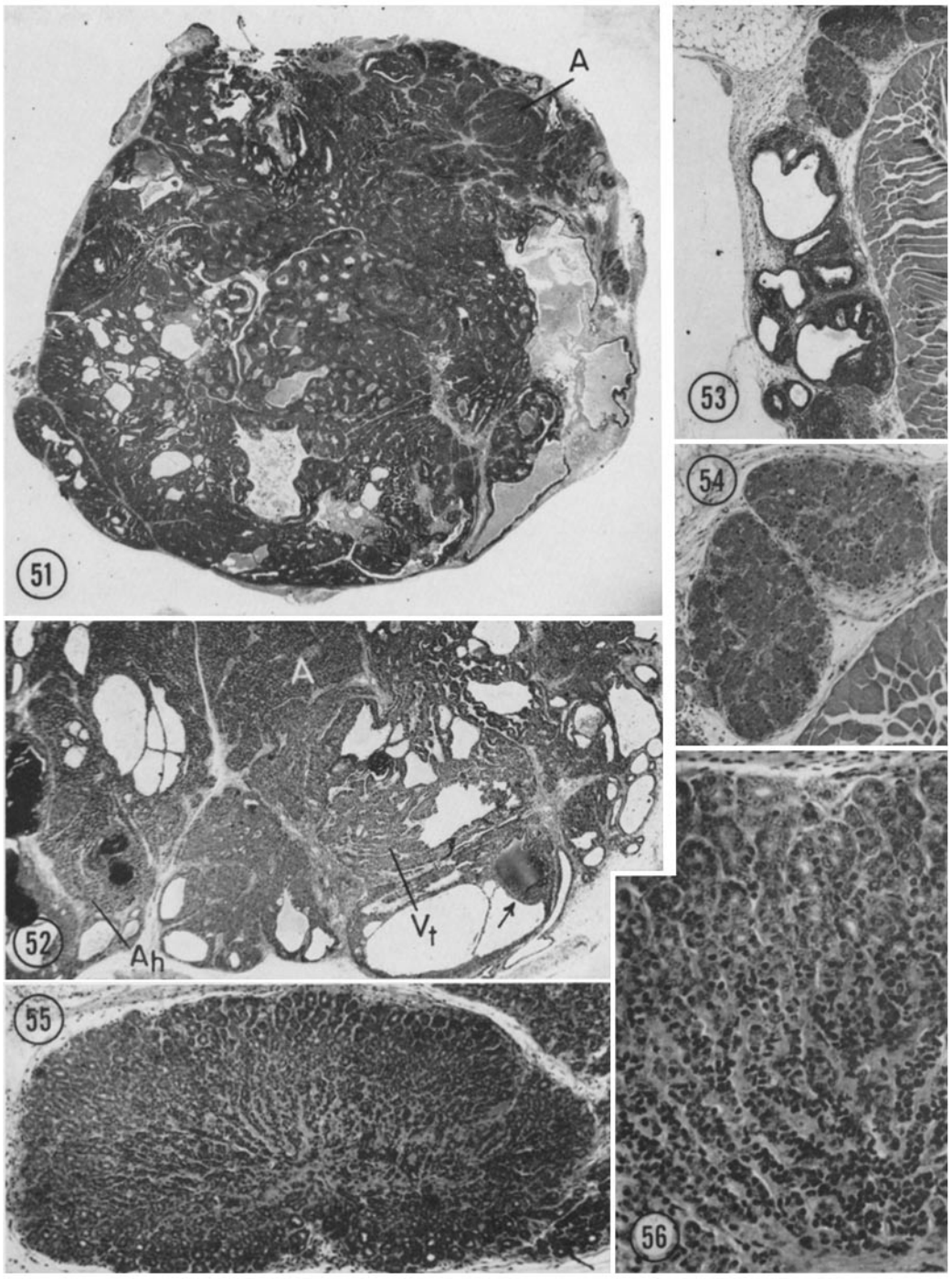
FIG. 52. Part of a sausage-shaped mass resulting from the coalescence of several discrete tumors due to the plating of MT6: from a mouse killed after 49 days. The hemorrhagic acinar tissue, seen at the left, contains several cysts black with blood and other cysts that appear empty because devoid of coagulum. Tissue of ordinary acinar pattern occupies the middle third of the section, and on the right are tubular and cystic alveolar tissues. The dark mass to which the arrow points is a separate cyst amidst this last containing red cells. × 20.

FIG. 53. Some of the tiny tumors on a mouse killed only 18 days after plating with MT6. The ovoid growths at the top of the figure, lying next voluntary muscle, are the precursors of O bodies. The growths near by are of cystic alveolar sort. × 33.5.

FIG. 54. The O body precursors of Fig. 53 at higher magnification. Their cells have a glandular arrangement and amorphous material has begun to form near the center of each. It was strongly eosinophilic. × 79.

FIG. 55. An unusually large O body precursor doing well after 56 days. It is alveolar at its periphery but further in loses the glandular pattern and the cells lie in crowded strands, with amorphous material between them, which is most abundant toward the center of the growth. It was strongly eosinophilic. × 79.

FIG. 56. Part of the O body of Fig. 55 at higher magnification, to show the orderly cellular changes. In proportion as the amorphous material increases the cells lose the glandular arrangement, their nuclei become pyknotic, their cytoplasm disappears, and they come to lie in crowded, jumbled columns. A few are vaguely visible amidst the central, amorphous material. × 280.



(Henderson and Rous: Plating of tumor components)

PLATE 138

Further results of plating Mammary Tumors 4 and 6

FIG. 57. O bodies from a mouse killed 56 days after plating with MT6: some fragments of voluntary muscle were inadvertently included when they were scraped off the body wall. One is spherical like those of MT4, whereas the others are flattened. They show the characteristic cores surrounded by a zone of living tissue. No cellular accumulation has taken place around them. × 16.5.

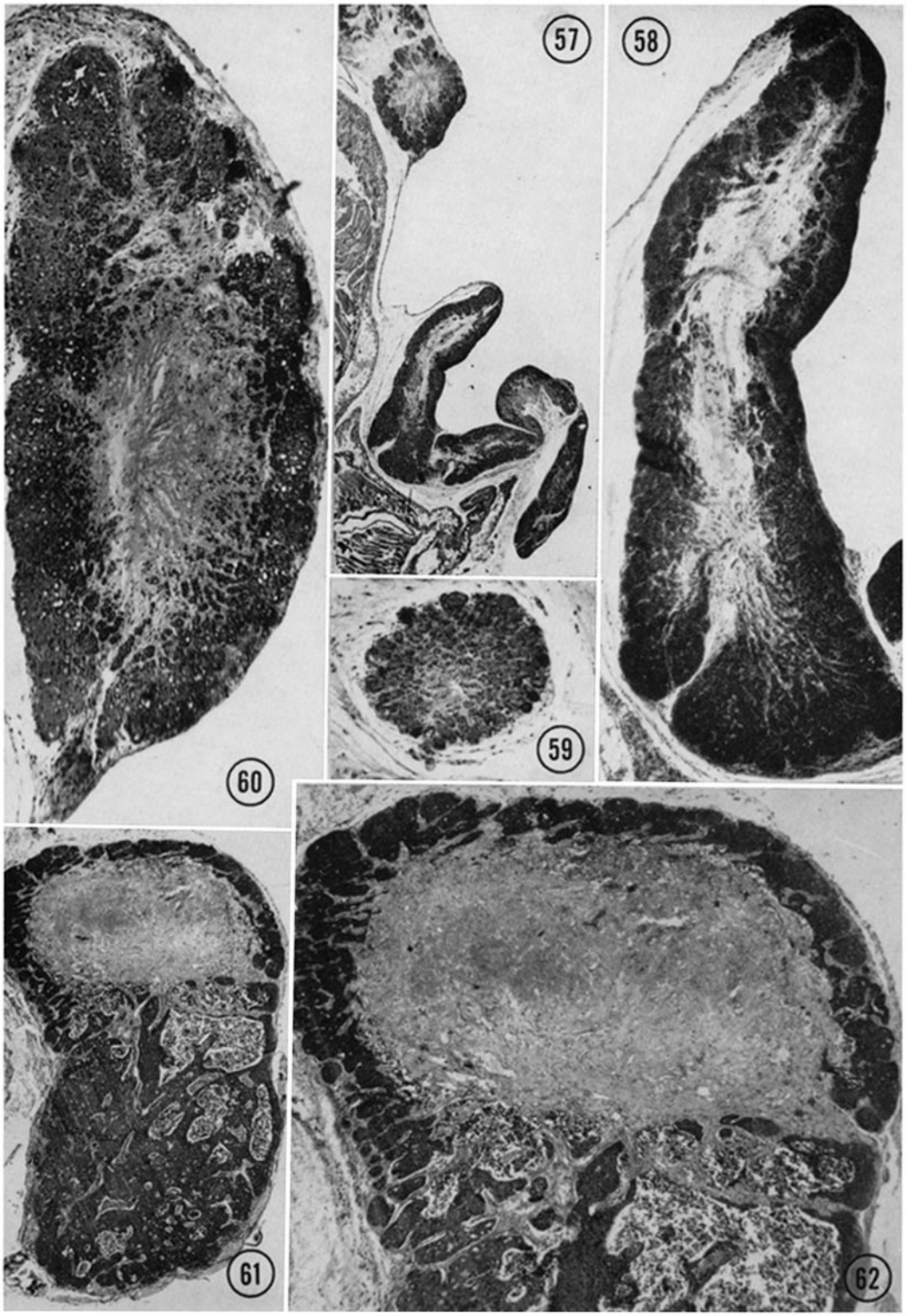
FIG. 58. The largest O body of Fig. 57. Its living zone resembles carcinoma solidum. Some of its cells have persisted amidst its amorphous core. × 58.

FIG. 59. An O body precursor of MT6 which has grown very little in the course of 56 days and is faring badly. Much more eosinophilic material is present within it than in the precursors only 18 days old of Fig. 54, and it is cleft at its center. × 79.

FIG. 60. An O body of MT6 after 49 days. Its central eosinophilic material has become fibrinoid and cleft. The upper third of the growth, demarcated on the left by a sulcus, consists of ordinary alveolar tissue forming no fibrinoid material. × 79.

FIG. 61. Coalescence of an O body of MT4 with an exudative alveolar tumor: from a mouse of PL II killed after 62 days. The living zone of the O body surrounds a core of dead material that has undergone coagulation necrosis. Most of the alveolar tumor is alive and such of its cells as are necrotic lie loose in spaces full of exudate. Its tissue is less compact than that of the O body and hence does not stain so deeply with methylene blue. × 28.

FIG. 62. Junction of the tumors of Fig. 61 at a higher magnification. The cells of the living zone of the O body are nearly everywhere as crowded as in carcinoma solidum, but an occasional alveolus can be seen. × 65.



(Henderson and Rous: Plating of tumor components)