FURTHER EXPERIMENTS ON THE CAUSE OF SEQUENTIAL NEOPLASTIC CHANGES

The Effects of 20-Methylcholanthrene on Transplanted Epidermal Mouse Papillomas and the Derivative Carcinomas

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Plates 21 to 25

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Several major neoplastic phenomena must be understood if cancer and its course are to be comprehended. At this writing numerous investigators are trying to determine the nature of primary neoplastic change, many of them using oncogenic viruses as agents to induce it rapidly. In contrast to this activity few workers have concerned themselves with the discontinuous, step-like, irreversible alterations whereby tumor cells go from bad to worse. Have these changes the same character as the primary alteration? If so, then the oncogenic agents inducing this might be expected to bring them on. Recent experiments on the theme (1) have shown that direct exposure of the pulmonary adenomas of mice to urethane or 20-methylcholanthrene (MC)—agents which rapidly induce these benign growths in animals of strains liable to them—does not hasten in the least the superimposed cancerous changes often undergone by their cells or render them more frequent.

It is common knowledge that 20-methylcholanthrene, when applied to most epithelial tissues, induces carcinomas that are such from the beginning, not due, that is to say, to secondary changes in benign growths; for growths of this sort seldom arise. Some of the cancers MC evokes from mouse epidermis seem to be of this primary kind but one cannot be sure. For MC also brings into existence, more often and usually earlier, benign papillomas (paps.) as well, consisting of cells that are liable to become cancerous (2). The aim of the work here reported has been to learn whether MC—which readily induces benign paps.—is capable of hastening or increasing the number of carcinomas deriving from them during their maintenance by transplantation.

GENERAL PLAN OF THE EXPERIMENTS

The Papillomas Used.—The first need was to obtain for comparative tests numerous epidermal paps. consisting of material from the same tumor and running the same course. It had been the general experience that paps. induced by the chemical car-

cinogens dwindle and disappear unless aided, and hence they were regarded as "conditional" (3). Nevertheless efforts were made some years ago to propagate those induced with tar or MC, and grafts of them were found to "take" and grow progressively after implantation at deep sites in sucklings and young weanlings (4, 5). The cells of some had lost more or less completely the ability of normal epidermal elements to spread laterally on bare connective tissue. In consequence they failed to line the graft pocket in which they were implanted and gave rise to solitary cauliflower, or pedunculated, growths projecting into cysts walled with connective tissue and full of watery exudate. These crippled papillomas (Type C) were manifestly unfit for the present work. At the other extreme were those composed of able-bodied cells, (Type A), cells that is to say which lined the graft pocket completely by lateral spread, and differentiating inwards, formed a solid, spherical growth consisting of a continually enlarging core of keratin, enclosed in a shallow, even layer of benign, papillomatous tissue which—unless replaced by derivative cancers—continued to proliferate in nearly all instances until it killed the host through its nutritive demands. Several such tumors have now been long maintained by repeated transfer. They have provided an ideal material for the present work. The solid core of keratin can be shelled out, the shallow peripheral layer of papillomatous tissue spread on glass, and many grafts of the same size cut and transferred, each to a different mouse, with result in spherical or ovoid tumors that can be matched for test.

The Carcinogen Employed.—Many reports testify to the fact that tar and MC induce epidermal paps. of similar sort and that their oncogenic effects can be superimposed. MC is far more effective as a carcinogen than the related polycyclic hydrocarbons contained in tar, and it has considerable promoting power; but tar has much more of this, rendering exceedingly vigorous the papillomas it induces (6). Hence it was used to obtain the primary papillomas for transfer. It could not be brought to bear however on the growths resulting from deep implantations. This could readily be done with MC in a suitable solvent.

Character of the Tests.—Carcinomatous changes can be rapidly induced in the epidermal cells of mouse embryos by injecting fragments of their skin, suspended in serum-Locke's solution, 1:19 (s-L), into the thigh muscles of weanling mice together with MC dissolved in droplets of olive oil (7 *a*). At first this method of test was used to learn the effects of the carcinogen on pap. tissue, but more satisfactory results were obtained by the subcutaneous implantation in weanlings of trochar grafts, together with crystals of MC, a method whereby Greene (8), in noteworthy experiments, procured highly various carcinomas from the tissues of embryo guinea pigs and mice many years ago.

Materials and Methods

The Animals.—Matched weanlings were used, weighing 12 to 17 gm, and of the same sex in each experiment. They were C mice of our long inbred, homogeneous strain.

The Papillomas.—Paps. I and V of previous work (4) and Roof Pap. 41 (5) (generously given us by Dr. Betty Roof) provided the tissue for test. All three tumors had been transferred repeatedly, I and V in several concurrent lines, and carcinomas had repeatedly taken off "spontaneously" from each of them. Pap. I was in its 4th Gen. when first tested, Pap. V in its 9th, and 41 in its 5th. Pap. I has already been pictured in its early generations as has also the primary Pap. V (4). This latter is shown again in Figs. 1 and 17 of the present paper. Pap. 41 is now figured for the first time.

The Materials.—As in the work with embryo skin, crystalline MC (Edcan[1]) was employed, and Grübler's Scharlach R. The olive oil was of the commercial kind used previously. 1 per cent of MC was dissolved in it for Exp. 1 and approximately 2 per cent of Scharlach R, and then s-L was added to the amount of one-third the oil volume. The control mice received merely oil droplets with the dye.

The Implantations.—The intramuscular injections were into the posterior thigh muscles of one leg, with the needle thrust through a slit above and back of the knee, and they were made forcibly to scatter the material. The subcutaneous implantations were made by trochar into the tissue behind a shoulder, through a slit over the haunch. Groups of 5 controls, and 5 "MCs" were implanted alternately, and a hole was punched through the ear of each MC animal to designate it. During all tests except Exp. 4 the control weanlings and those receiving MC grew at the same rate.

Appraisal of the Findings

All of the paps. were well encapsulated. Those in the muscle sometimes extended into the overlying subcutaneous tissue but never involved the skin. They were usually multiple spheres at first and so remained in the control instances, whereas in nearly all cases the corresponding growths containing MC coalesced as time went on. At autopsy they were dissected out and traced in their largest dimension on a celluloid sheet.

The tumors arising after the subcutaneous implantation of grafts were nearly always solitary. They too were spherical or ovoid, and from time to time the maximum diameter of each was measured through the skin, without knowledge of the group of mice to which it belonged; and when these were killed the growths were exposed and profile tracings made. After they grew big the overlying skin became attached to the cyst wall because of ischemic necrosis due to pressure from the keratin core and here some flattening occurred. The areas thus affected are hatched in the charts. In these latter the growths are ranged according to their final size.

Nearly all of the cancers deriving from the pap. layer were small excrescences. Some made themselves known by an outward protrusion, but more often they were visible only on the inner side of the layer. When this was spread they could then be seen as discrete mounds, protuberances, or thickenings, often scarcely a millimeter across. Many of them failed to form keratin, or that which overlay them was moist or pultaceous, and occasionally fluid accumulated over the largest growths.

Frozen sections and the Terry stain were used to learn the morphology of each cancer and the character of the tissue at all questionable spots. Slices from these as well as the cancers were fixed in "Zenker", together with the adjacent pap. layer, and were sectioned and stained with eosin and methylene blue. The cancers were all epidermal carcinomas and they proved highly various.

Serial sectioning of the entire living layer for hidden malignancies was not feasible, and only such cancers as were visible in the gross have place in the findings, save when the microscope had disclosed more than one cancer in a single nodule. Then their number was recorded in brackets. Every pap. in which cancer was found is charted in black save those of Exp. 2.

FINDINGS

Results of the Intramuscular Implantation of Tissue Fragments with MC and Scharlach R Dissolved in Oil

In most of the experiments already mentioned as carried out with fragments of embryo skin and droplets of olive oil mixed with s-L (7 *a*), Scharlach R as

[1] Edcan Laboratories, South Norwalk, Connecticut.

well as MC had been added to the oil because of its remarkable power to attract epidermal cells (9). Within a few days after the injection of the suspension thin strands of them rapidly extended from the fragments toward the droplets and soon enclosed these in small cysts which later coalesced, with result in a single, bigger one, lined with epidermis that differentiated inwards. It contained all the oil and MC. From the lining, which had only abortive hair follicles, was devoid of a stratum granulosum, and formed keratin poorly, owing to its direct exposure to the carcinogen, paps. and carcinomas arose within a few weeks. Later tests showed Scharlach R to be unnecessary because MC itself attracted the epidermal cells strongly. Nevertheless the dye was used in the following test not only as an adjuvant but as a tell-tale to where the droplets containing MC were.

Experiment 1, Chart I.—All was done by the same technic as had been used with embryo skin, and with the same amount of MC and Scharlach R in oil, together with Locke's solution. The neoplastic tissue was procured from a growth of Pap. I in its 3rd Gen. The tumor was 25 mm across, subcutaneous and spherical, with a shallow peripheral lining of living tissue and the usual keratin core. Frozen sections of this lining showed an even layer of orderly benign pap. By means of a knife blade pushed sideways some of this layer was separated from the connective tissue capsule, hashed fine, suspended in s-L, and distributed equally in two dishes, to be used with two 0.25 cc syringes. For each injection 0.08 cc of the thin suspension was drawn up into the appropriate syringe, and then 0.02 cc of oil in which Scharlach R had been dissolved (OS), or MC as well (OSM). It seemed best not to mix the oil with the suspension so that the MC might persist longer, but the forcible injection resulted in some dispersion of it.

The 11 matched weanlings of each group were killed in batches on the 51st or 84th day.

Chart I depicts the gross findings. In all save one of the OS controls the tumors were multiple and discrete, whereas those of the OSM had, with one exception, undergone secondary fusions as attested by their irregular shape and by the remains of raphes within them. The growth of the OSM tumors was in several instances slower at first than that of the controls, as early palpation disclosed, yet despite this and the fusions they nearly equalled these in bulk at final examination.

No red globules were found at autopsy outside of the growths, nor was any seen in four of the OS tumors; but OSM was present in the cysts of all the mice receiving it—a fact readily understandable since Scharlach R and MC, when together in oil droplets, have a greater power to attract epidermal cells and be encysted by them than either has alone (7 *a*). All the tumors of both groups had large cores of close-packed keratin, and most of the dye-stained oil lay near their centers, continual keratinization having removed it farther and farther away from the peripheral pap. layer. But the keratin was reddened for as much as 7 mm around the encysted oil, thus making plain that the spread outwards of soluble substances had not been barred. Usually the coloration was far removed from the living pap. but in several instances a big globule of OSM was found lying next this at the periphery of the growth, and at such spots the neoplastic tissue was scanty or missing, though farther away the layer gradually attained to the same thickness as elsewhere. It follows that in such instances all gradations of exposure to the MC existed for as long as this latter was present in the oil globules; though for how long one could not tell.

None of the mice killed after 51 days had cancerous growths amidst the papil-

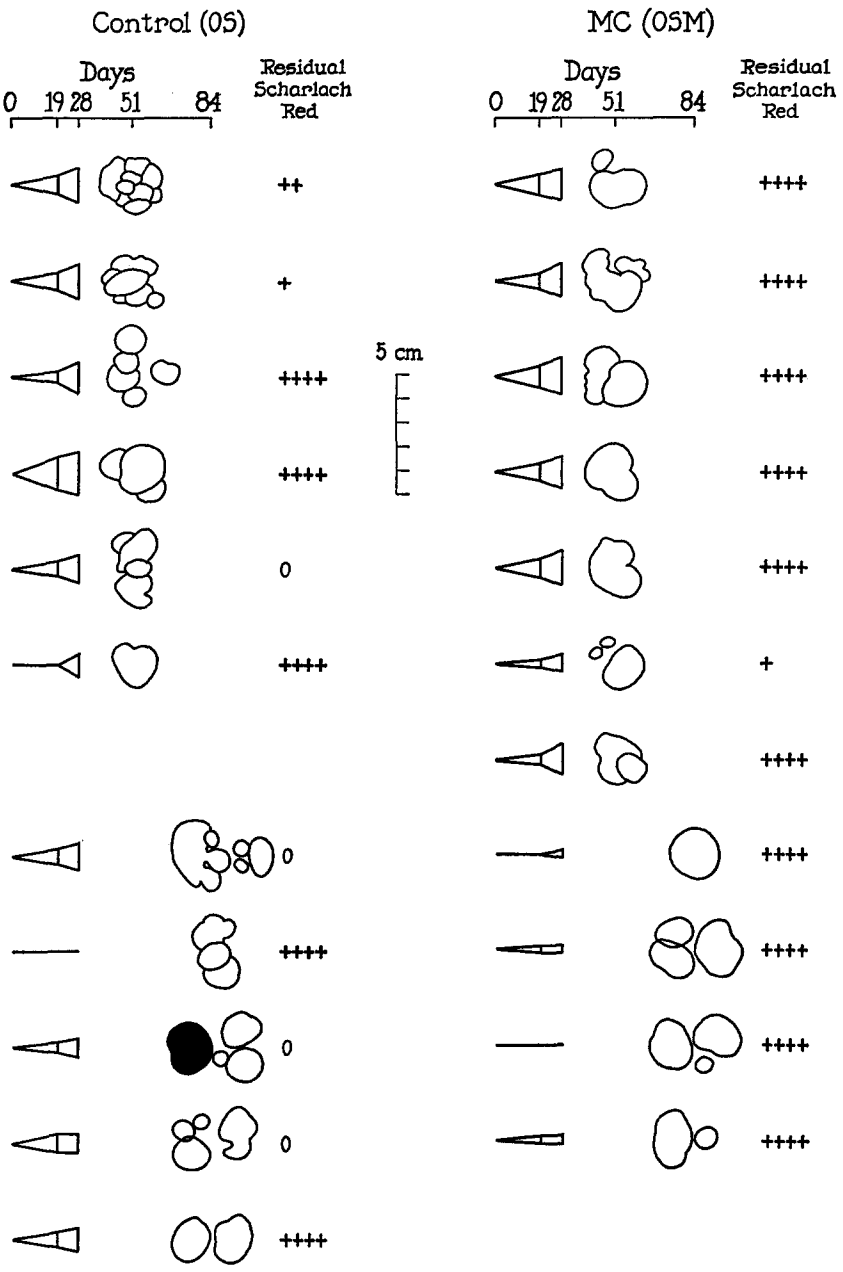

CHART I (EXP. 1). The tumors resulting from intramuscular injection in suspension of fragments of Pap. I in its 4th Gen., together with oil containing either Scharlach R alone (OS) or with MC (OSM).

The one tumor found to contain a cancer visible in the gross is shown in black.

lomatous tissue, nor were any found in the OSM paps. examined after 84 days—nearly thrice the time required for it to elicit tumors from embryo epidermis (7 *a*). But in a control growth, now devoid of OS—if indeed it had ever contained this—a tiny carcinoma was present.

Several similar experiments with tissue fragments were ruined by the presence in the implanted material of preexisting cancers, and the tests as a whole were unsatisfactory in another respect: there was no telling how long the MC had lasted. Obviously some means had to be found to determine its presence, gauge its amount, and learn its situation in relation to the layer of living pap. tissue. This means was provided by its brilliant blue fluorescence when dissolving in tissue fluid and viewed in ultraviolet light (UV). The use of Scharlach R had to be discontinued because it masks this fluorescence; but new tests made plain that MC could be brought to bear directly on pap. tissue when introduced in crystalline form together with it.

Results of the Intramuscular Implantation of Tumor Tissue in Suspension with MC Crystals

A scouting test showed that when 0.12 cc of a thin suspension in s-L of MC crystals which had been lightly triturated was forcibly injected into the thigh muscles of mice, and the site of injection bared at once and viewed in UV, the crystals lay scattered along the muscles for about a centimeter, shining out as lemon yellow dots on a black background. During the next few days, as they began to dissolve, a shallow, blue zone had formed around each, as viewed in UV, and soon all were enveloped in a single blue cloud.

Experiment 2.—A tumor of Pap. V in its 11th Gen. furnished the material for test. A thin suspension was made of its finely minced tissue in 0.9 cc of s-L, and to this 1.5 cc of s-L was added containing 2.25 mg of lightly triturated MC crystals. 0.12 cc of the resulting mixture was injected into the thigh muscles of each of a group of weanling mice. For the control group 1.5 cc of the same MC suspension was added to 0.9 cc of s-L devoid of tissue, and 0.12 cc of the mixture was injected into each mouse.

The tissue had been procured from what appeared to be a typical, keratinizing, pap. sphere, and no malignancy could be discerned in fresh, Terry-stained sections, but in a later Zenker-fixed specimen an uninvasive carcinoma was found at one spot in the shallow pap. layer (Figs. 1 and 17).

The mice receiving only MC in s-L were killed after 17 to 31 days. On the 17th the scattered crystals lay amidst a blue cloud, but within the next week this became pale and more widely diffused, and after 31 days it was gone and a few yellow dots surrounded by thin blue aureoles was all that could be discerned.

The findings were very different in the mice that had received MC together with tumor tissue. After 14 days a considerable number of small, scattered, thin-walled cysts had arisen, which shone brilliantly blue in UV though outside them no such coloration was visible. Obviously most of the MC had been enclosed in the cysts and was now being conserved within them. Sections showed that they had been formed by the implanted neoplastic tissue and contained keratin, surrounded in a few in-

stances by a shallow layer of benign pap. but in most cases by much more vigorous carcinomatous tissue (Figs. 2 to 4).

The cysts were fewer but larger after 17 days, owing to their confluence, and in an animal killed after 23 days only a solitary, much bigger cyst was present. It was well encapsulated but a cut revealed in UV an interior intensely blue. Only a trace of blue was visible amidst the tissue outside.

Three of the mice were let live for 36 days, and in two of them a single medium-sized cyst was found, while in the third a small one lay adjacent to a similar growth. Each had a keratinized core and all were of the same general character. Their interior shone blue in UV, as in the earlier instances, but no such fluorescence could be seen in the surrounding tissue. One of the growths will be described in detail as representative:—

The solitary, spherical 11 mm cyst, when cut across, did not differ in daylight from those due to ordinary trochar grafts of pap. tissue, being neatly encapsulated and full of close-packed, ivory keratin; but in UV it looked markedly different (Figs. 7, 8, and 13). The center of the keratin core had now a brilliant, blue fluorescence (Fig. 13) with a stippling of white, owing to undissolved MC crystals, as disclosed by scrapings (Figs. 9 and 10), and some small, rounded, dead-white patches where these lay close-packed. Toward the periphery of the growth much dissolved MC was present, the keratin shining in a lurid blue which faded away toward the irregular layer of living tissue, and was almost or quite lost before reaching it. Yet in many places this latter shone in a blue rivalling that of the core where most intense. Obviously not a little of the dissolved carcinogen, on its way toward escaping through the wall of the cyst, had been retained by the living neoplastic cells. Some of the living layer of tumor had been torn away, between the arrows of Fig. 13, and blue fragments of it can be seen below the growth amidst the dark of the muscles. Much of it looked too thick to consist of ordinary benign pap. and in one region it projected far toward the center of the growth, a finding confirmed when the keratin core of the tumor was shelled out and the layer inspected in daylight. The largest inward protrusion proved to be a papilliferous carcinoma which overhung an irregular layer of benign pap. tissue (Fig. 12) and had extended out into the adjacent muscle. Elsewhere there was an even layer of malignant papilloma (Fig. 11) several times thicker than the benign layer of Fig. 12.

The only crystalline MC that had escaped encystment lay some distance away in the muscles. It is visible in Fig. 13 as two lemon-colored dots with a blue zone of dissolved MC around each.

In this experiment the scattered crystals of MC, on beginning to dissolve, were soon enclosed by benign and malignant neoplastic cells from the tissue fragments scattered with them. Numerous small cysts resulted, some of them papillomatous, others carcinomatous, and they subsequently coalesced, forming a single big one that was lined with benign and malignant tissue and contained practically all of the remaining MC. The end-result was like that after fragments of mouse embryo skin are injected into the muscles together with OSM (7 *a*).

The only way in which the MC could escape from the tumor was through the living wall of living neoplastic tissue and here a considerable quantity was retained (Fig. 13).

As already stated, a carcinoma had been found amidst the pap. layer providing the material for test (Figs. 1 and 17). The character of most of the cysts examined 14 days after the implantation made plain that cancerous cells must have been present in many of the implanted fragments.

In another similar test the tissue of Pap. V was exposed to MC for a longer time.

Experiment 3, Chart II.—The tissue for implantation was procured from the shallow, even lining of a keratinizing cyst of Pap. V, 10th Gen., belonging to a line separated from that of Exp. 2 in the 8th Gen. A tiny mound, which later proved to be a squamous cell carcinoma, was avoided, and frozen sections of a slice from next the test material showed what was taken to be benign pap.; but staining after Zenker fixation later showed that it had actually been a malignant papilloma (Fig. 16) no thicker than the benign and closely mimicking it.

The tissue was hashed fine, suspended in 1.2 cc of s-L, and to half of it 1.0 cc was added of a suspension containing 1.5 mg of lightly triturated MC crystals. To the remaining half 1.0 cc of s-L was added as such. Two groups of weanlings were injected in the right, posterior thigh with 0.12 cc of one suspension or the other, and after 49 days, they were killed.

Chart II shows that all but one of the mice receiving the suspension which contained no MC developed several, more or less spherical tumors at the site of implantation. This one had only a single growth. The tumors of nearly all of the MC group must for a while have been multiple too, as indicated by their final shape, but had mostly been rendered solitary through fusion, as attested by incomplete raphes within them. In both these respects the findings resembled those of Exp. 1. MC was still present in three of the growths but was confined in one to a small, completely keratinized cyst intensely blue in UV.

In every animal save one of the MCed group, cancers had arisen as discrete mounds, nodules, or thickenings of the pap. layer. They were, without exception, epidermal carcinomas yet widely various in their morphology. Some of those within the MCed tumors were considerably larger than those of any in the controls, and in several instances the keratin overlying them was pultaceous and light brown or pink from extravasated blood. This was also the case in two controls containing solitary cancers that had extended laterally.

Despite the stimulating effect of MC in this experiment the number of cancers on the pap. layer exposed to it was no greater than in the control growths.

A further experiment of similar sort was carried out with Roof Pap. 41 in its 5th Gen. At each of the previous transplantations of this tumor it had yielded what appeared to be typical benign paps. forming keratinized cores, but derivative cancers had been present much more often than in Paps. I and V during their early maintenance.

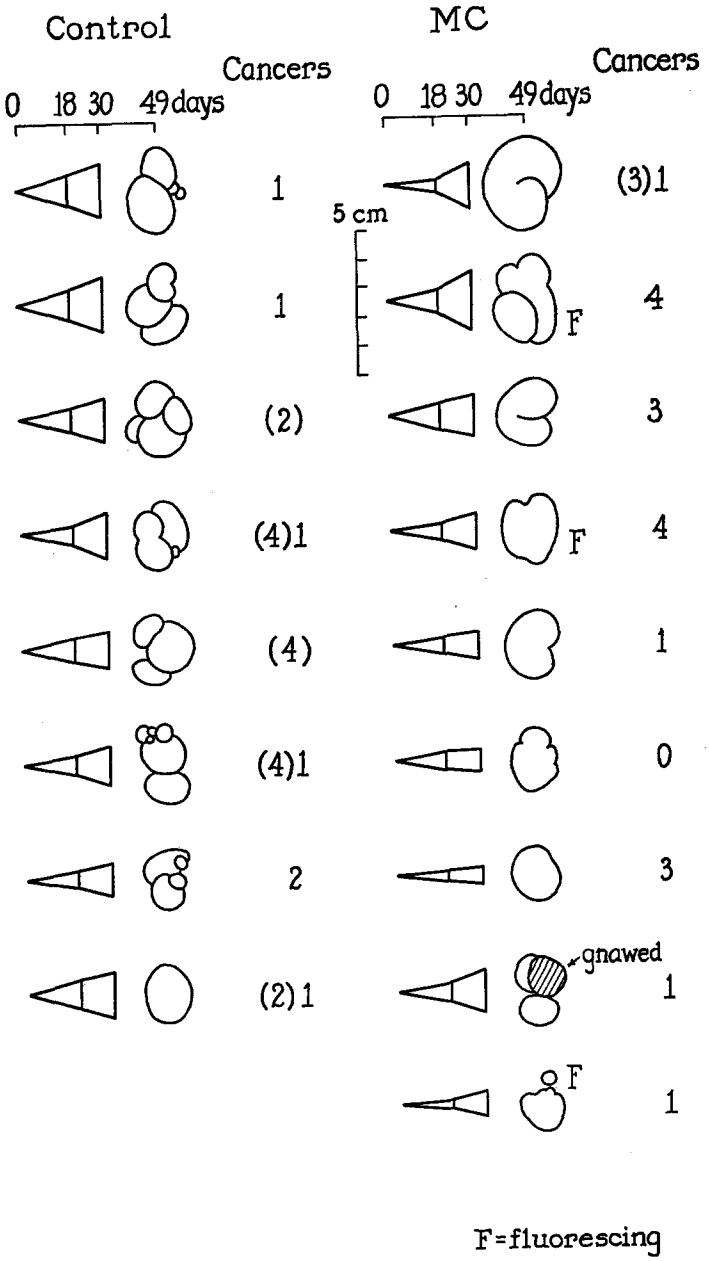

CHART II (EXP. 3). The tumors of another test resembling Exp. 1 except that MC was in crystalline form and the tissue used was that of Pap. V in its 10th Gen. The growths were all characteristic paps. but none of them, save one of the MC group, was free from grossly visible cancers, as the numerals show. When the microscope disclosed more than one in sections made for routine examination of what appeared to be a single growth the total number found is bracketed.

To have shown the tumors in black would have obscured their multiplicity in the control animals.

Experiment 4, Chart III.—The growth furnishing the material was a typical pap. sphere 3 cm across, but with living tissue only ½ mm thick enclosing a dense white core. Each of 10 weanlings was given an intramuscular injection of 0.12 cc of a suspension made like that of Exp. 2 and hence containing both tumor fragments and MC. The 10 controls received the same amount of suspension containing the fragments only.

Stained fresh sections of a slice from next the material implanted, showed merely a shallow layer of benign pap., but Zenker-fixed sections examined later (Fig. 18) revealed three tiny cancers as well. One was a keratinizing, squamous cell carcinoma (Figs. 18 and 19) and another was anaplastic (Fig. 20). The third was a malignant papilloma, very like that of Fig. 11.

The largest tumor of each group was taken for sagittal section after 27 days. Both were then single cysts (Figs. 5 and 6) but the MCed growth was several times as big as the control and exhibited signs of cleavage. It contained much dissolved MC, deep blue in UV; yet the only sign of MC outside it was a pale blue spot on its capsule. The control tumor was a keratinizing, squamous cell carcinoma and, save for a short stretch of shallow, benign pap. (Fig. 6, arrows), the MCed growth consisted wholly of this latter, in a layer about three times as thick as that of the control (Figs. 14 and 15). Small areas of malignant pap. and anaplastic carcinoma could be discerned at high magnification of other sections.

Five of the nine control mice had no tumor when killed (Chart III) though a small nodule had formed early in four of them. All five had been kept for 76 days. The four controls with tumors were killed after 62 days, that is to say, a day later than the MC animals. Each had by then a solitary growth 0.7 to 1.0 cm across. One of these contained firm, creamy keratin, but in the others this was soft or pultaceous. Sections showed some pap. tissue in them but they consisted almost entirely of one or more of three different cancers, the keratinizing, squamous cell carcinoma of Figs. 5, 6, 14, and 15, an anaplastic carcinoma such as that of Fig. 20, and a malignant papilloma.

The MCed tumors, in contrast to the controls, were mostly multiple and relatively huge when their emaciated hosts were killed. They were composed of the same three cancers, and a little benign pap. tissue was also present; but the proliferation of the cancers, notably that of the squamous cell carcinoma had so far outstripped this latter as to render it almost negligible. Much necrotic tissue had accumulated amidst the cancers, together with some yellow or pink keratin, rendered moist or pultaceous by exudate. In only one tumor was MC still present, a patchy, blue trace. None existed anywhere else.

In this experiment groups of mice had, as usual, been injected in alternate batches of five, and the only difference in the suspensions was that one contained MC crystals. Yet the results were astonishingly different. All of the MCed growths had become enormous as compared with the controls, and this though the cancers exposed to the carcinogen had in three instances got off to a slow start. The growths were all multiple or showed signs of having been so. It was as if the malignant tissues present in the fragments scattered in the muscles together with MC had lacked that tendency to coalesce which fragments of benign pap. had so strikingly exhibited under its promoting influence in the earlier tests.

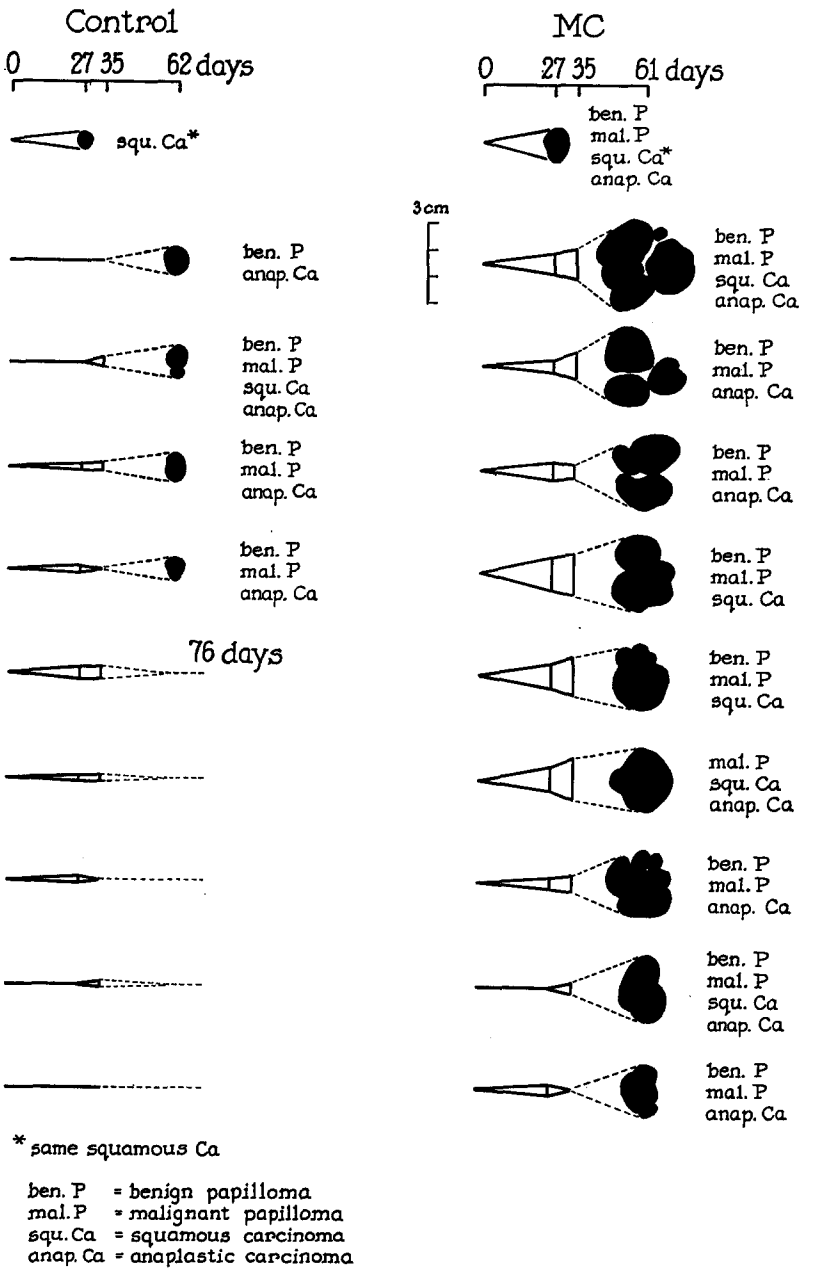

CHART III (EXP. 4). Another experiment like that of Chart II but with tissue of Roof Pap. 41, Gen. 5, yielding tumors that in every instance were composed almost entirely of cancers. For this reason all are shown in black.

The failure of half of the control implants of this experiment justifies the assumption that about half of those exposed to MC would also have failed in its absence. The malignant cells were so responsive to it as to raise the question whether cancerous components hidden in the implanted tissue and unperceived in the control growths, might not have been stimulated by the MC to manifest themselves. Many blocks from the MCed tumors were searched in this relation but no such component was found.

The Results of Implanting Crystalline MC Sandwiched between Pap. Grafts

The possibility that cancer cells already present in pap. tissue and scattered by hashing it might mar the tests with fragments of such material, had been realized early, as also that the risk could be lessened by the use of small, discrete implants. Hence we now resorted to trochar grafts such as were employed for routine maintenance of the paps. It seemed not unlikely, in view of the experiments already described, that pap. cells proliferating from grafts of this sort would extend around and encyst MC crystals placed immediately next them, and this turned out to be the case.

The following procedure was used to make sure that the MC would be closely apposed to the neoplastic tissue:—

Three grafts were implanted together in the subcutaneous tissue behind a shoulder of weanling mice by means of a needle of 1 mm bore, thrust through a slit made over the haunch. The grafts were of the size used for ordinary transplantation, and they all were taken from the layer lining the side of a medium-sized, spherical, subcutaneous pap., not from its deepest, oldest part where cancers are most frequent. Each graft was separately washed in s-L before it was placed in the needle, in order to free it from any cancer cells which might have accidentally become attached to it. A sterilized needle and plunger were used for each implantation.

The first graft thrust into the tip of the needle (with the plunger already placed for extrusion) was cut to fit its bore snugly so that, as the last graft to be expelled, it would sweep out any MC remaining within. The next graft inserted had been touched to lightly triturated crystals of the carcinogen with result that some of these adhered to it. The third graft was like the first. The control mice received three grafts with no MC. The plunger protruded about a millimeter beyond the needle tip—which had a short bevel—and the two were withdrawn together, between a compressed thumb and forefinger so that none of the grafts would follow. The fact that the tumors arising were practically always solitary, spherical, and devoid of raphes showed that the grafts had indeed proliferated as one.

The amount of MC adhering to the middle graft could be gauged only approximately, and the early experiments were failures, either because so much was present as to kill all the implanted tissue or to damage it so greatly that the resulting tumors were not comparable with the controls in size. Occasionally one ceased growing after a while, and on cross-section was found to consist entirely of keratin, brilliantly blue in UV, its living tissue having wholly succumbed. Not infrequently the crystals caused

local death or only thinning of the pap. layer near which they lay, and little keratinization occurred there, but farther away the layer gradually attained to the same thickness as elsewhere and formed keratin as actively. At spots where the layer was absent or thinned an intense white or blue glow prevailed in UV, but this gradually lessened farther away and was lost.

In many instances keratinization took place at the same rate everywhere around the inside of the tumor, and so displaced the MC that as time passed this lay near the center of the keratin core. Here, as in Exp. 1, a diffuse blue glow in UV, sometimes 7 to 8 mm across, made plain that the carcinogen had been gradually dissolving; but when the tumors had become large this glow no longer reached the living pap. layer, or did so only when the crystals lay considerably off center.

Not a few experiments were lost because cancers unperceived at the time of implantation replaced most of the pap. tissue within a few weeks.

Our aim was to employ the largest amount of MC that would be tolerated by the graft tissue yet not interfere markedly with its growth, so that the exposure of the pap. lining to it would be long. The charts show that this aim was accomplished often, a relatively slow enlargement of the new tumors taking place at first, as compared with that of the controls, because of the well known repressive influence of MC when in quantity (10); but later on, as its concentration waned, they grew the faster, stimulation having succeeded repression (6). Often no repression took place and the tumor did well from the first. It follows that the amount of carcinogen brought to bear on the growing pap. tissue ranged from much to little in the groups of mice receiving grafts implanted together with it.

Experiment 5, Chart IV—The grafts came from a tumor of Pap. V in the 9th Gen. of the line used for Exp. 3—a line so prone to derivative carcinomas that it seemed certain they would already be present in some of the grafts now used, though none was come upon with the microscope in the neoplastic tissue next to that providing them.

One of the MC tumors (not charted) ceased to enlarge when 8 mm across and on examination it proved to be wholly keratinized and deep blue in UV. Of the remaining mice three from each group were killed 43 days after implantation and the rest after 46 or 47 days. Examination at this time under UV showed MC inside 7 of the 14 tumors resulting from tissue implanted with it. In five it was brilliantly visible but restricted to one stretch of the layer. Here the pap. was thinned and had formed almost no keratin. In the other two growths fluorescent blue patches disclosed MC near the center of their cores. None was present outside any tumor.

The chart shows that 12 of the 15 controls had visible carcinomas amidst their pap. lining, whereas only 4 of the 14 MCed tumors had them. Nearly half of these latter had enlarged more slowly during the first 31 days than any of the controls, but most of them had grown so fast later as to equal these in size. Four of the MCed tumors had done as well from the first as the most vigorous controls and three of these still had the carcinogen within them. Nevertheless, they contained no visible cancers. Nor did several others that had shown little sign of early repression and had later reached the size of the controls.

Despite what seemed to be conditions favoring carcinogenesis in this experi-

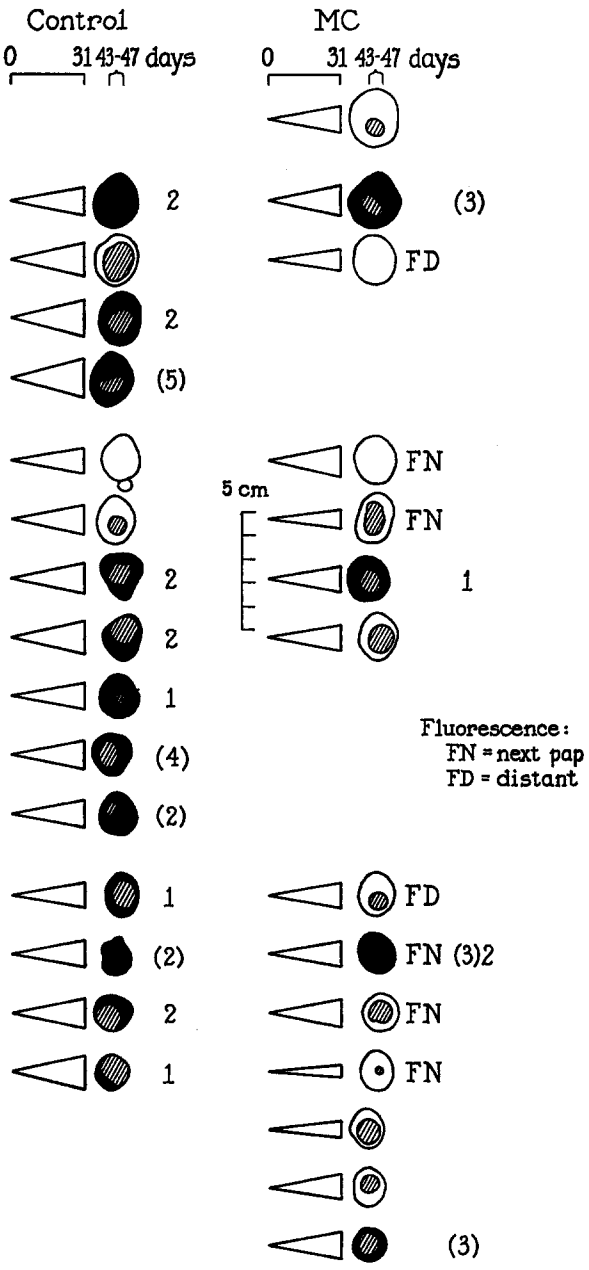

CHART IV (EXP. 5). Tumors resulting from the subcutaneous implantation of grafts of Pap. V in its 10th Gen., with and without MC. The hatching shows where they had undergone superficial, ischemic necrosis, owing to the pressure of the keratin core. Every growth containing a cancer visible in the gross is depicted in black. All of them were small.

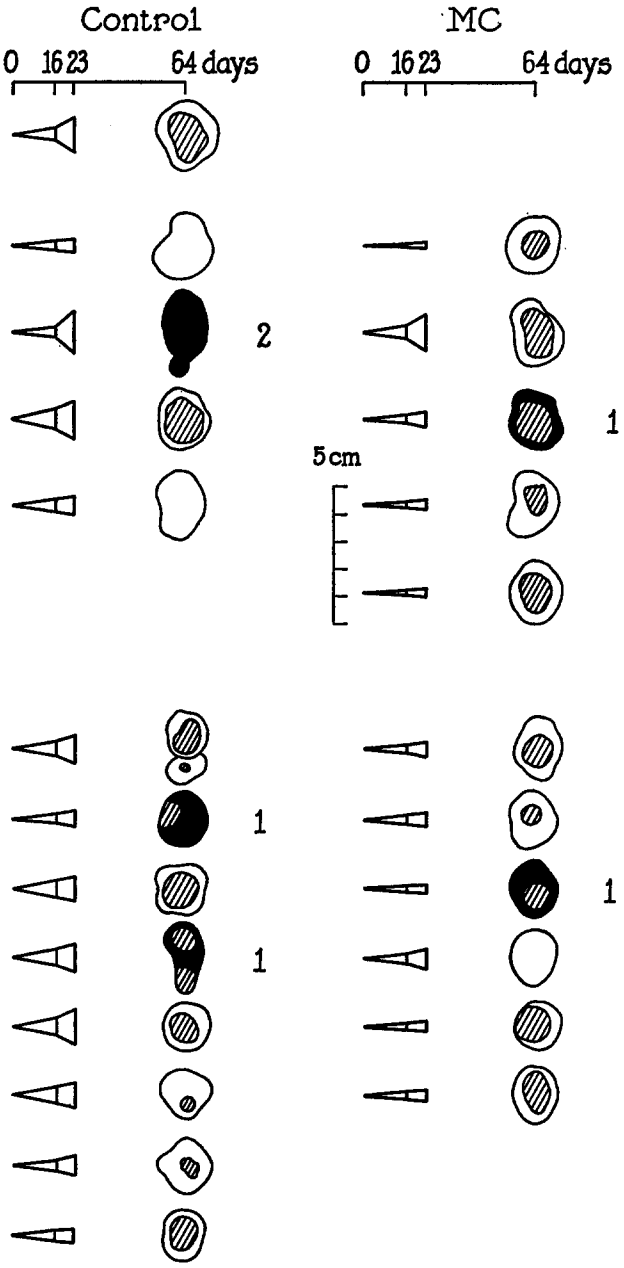

CHART V (EXP. 6). An experiment like that of Chart IV but made with grafts of Pap. V, 8th Gen.

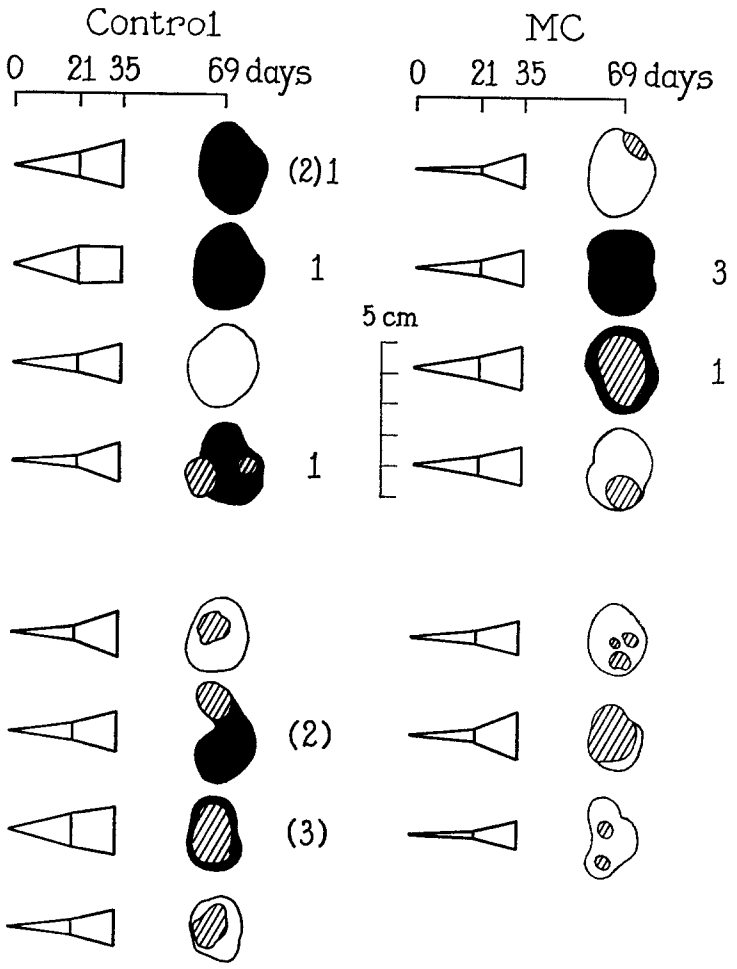

CHART VI (EXP. 7). Another similar experiment, this time with grafts from a growth of the 9th Gen. of Pap. I.

ment, the over-all effect of the MC was to lessen markedly the number of visible cancers.

Experiment 6, Chart V.—Again the grafts were from a tumor of Pap. V but of its 8th Gen., when it was much less liable to contain cancers than in Gen. 12 of Exp. 2. Sections taken from nearby showed only benign pap. The tumors were examined after 64 days. No MC was found in any.

The slow early growth of most of the MC tumors, as compared with that of the controls, indicated that a repressive amount of the carcinogen must have been present in many of them; and their later rapid growth as its quantity grew less bespoke stimulation. A single, tiny, carcinomatous nodule was present on the lining of two of them, as also on that of two controls; and a third control growth had two cancers. On first sight the findings seem equivocal. They will be discussed further on.

Experiment 7, Chart VI.—The grafts were taken from a growth of the 9th Gen. of Pap. I, line D. Again the microscope disclosed no cancer in the neighboring tissue. All was done as before and again no MC was found in any pap. after 69 days.

Some of the MCed tumors of this test enlarged more slowly during the first 3 weeks than did most of the controls, but after another 2 weeks they were almost or quite as large as the biggest of these.

The early repressive effect of MC had been slight and brief, and it appeared to be followed by stimulation, yet cancers were definitely fewer in the tumors exposed to it.

DISCUSSION

Pap. I was so nearly free from derivative cancers during its early generations that Exp. 1 was not marred by them, though intramuscular pap. fragments were tested and the resulting tumors were let grow for nearly 3 months. MC had been encysted by all the growths of its group, as proven by the inclusion of Scharlach R as well, and enough of the carcinogen was present to retard the early enlargement of a few of the tumors and to bring about in most instances a coalescence of the multiple growths that had arisen. Yet no cancer appeared in response to the carcinogen, though one was found in a control growth. The test would have been satisfactory had the length of exposure to MC been known.

An unexpected obstacle was met when, after a considerable interval, the intramuscular experiments were resumed. Many carcinomas were present by that time amidst the fragments of pap. tissue. They had been so infrequent in the early generations of Paps. I and V that it had seemed unlikely they would accumulate within the paps. if, at every transfer for their maintenance, trochar grafts were carefully selected. But this was to reckon without the occurrence, as time went on, of cancers forming an even layer of the same thickness and

gross aspect as that of the pap., keratinizing like it, and neither proliferating any faster nor encroaching on it but remaining self-contained. Just such a cancer is shown in Fig. 1, lying next the pap. layer with which Exp. 2 was done, and others were obviously responsible for what happened in Exps. 3 and 4, which yielded tumors that had been virtually taken over by carcinomas.

Appraisal of the "sandwich" experiments is not easy because cancers were already present in the implanted materials. Twelve of the 15 control tumors of Exp. 5 contained them after only 43 to 47 days of growth, and because of this the findings at first seemed devoid of significance save as indicating that the MC had so repressed the growths exposed to it that cancers seldom appeared. But three of the tumors which still contained MC at the end of the test had grown as fast from the first as had the controls, and yet they contained no cancers. The cells of epidermal carcinomas are exceedingly responsive to stimulation by MC, (Exp. 4), and hence it seemed possible that they would have been markedly sensitive to its repressive influence. But the fact that the cancerous MCed tumor of Exp. 4, which was examined after 27 days contained much of the carcinogen yet had already become several times as big as the biggest controls speaks against this, and so too does the prodigious activity of the MCed cancers followed longer. Those that had encysted MC crystals in Exps. 2 and 3 also showed no signs of repression but, on the contrary, stimulation.

The incidence of cancer in the two groups of Exp. 6 did not differ significantly, but it will be noted that the only MCed tumors in which cancers arose were amongst those that had been markedly repressed by the carcinogen during their first weeks. Most of the paps. were so stimulated by the MC later as to equal the controls in size after 2 months, yet cancers had not become more numerous than they were in these latter.

The early retardation of growth by MC was relatively slight in Exp. 7, and again most of the tumors exposed to it eventually equalled the controls in size. Nevertheless, they had definitely fewer cancers.

MC injured the grafts of some experiments so greatly, as already stated, that these had to be discarded, and it must frequently have destroyed a considerable portion of the implanted tissue in the tests that were successful. This portion, like that which survived, may well have contained preexisting cancerous cells which had not as yet asserted themselves, as also others that were only on their way to malignant change. Their deletion by MC would have meant an eventual yield of fewer cancers than in the controls. Yet this will not account for the relative infrequency of cancers in those tumors of Exps. 5, 6, and 7 on which the carcinogen had little or no repressive effect.

It will be recalled that when crystalline MC was scattered in the muscles together with the pap. fragments of Exps. 2, 3, and 4, it markedly enhanced the growth of such carcinomas as happened to be already present and in Exp. 4

stimulated them prodigiously. This did not happen in the sandwich experiments: the cancers that attained to perceptible size in the MCed growths were no bigger than those of the controls. But there was an important difference in the conditions of test. The tissue that was fragmented for the suspensions of Exps. 2, 3, and 4 had been finely hashed which doubtless freed many preexisting cancerous cells to a greater or lesser extent from the pap. tissue enclosing them, with result that they were directly exposed to the MC. This must have happened much less often in the sandwich tests. The carefully selected grafts used in these to produce the tumors were firm, close-textured, and had been well washed. Such cancerous elements as may have lain within them, or within the pap. tissue resulting from their growth, had to compete with the adjacent pap. tissue if they were to assert themselves after implantation. Many of the pap. and cancer cells resulting from the application of carcinogenic hydrocarbons to the skin of mice are so held in check by the normal epidermis round about them as to lie latent for long periods (11).

To sum up, neither in Exp. 1 nor in the three sandwich tests was any evidence obtained that MC increased the number of cancerous changes or hastened their occurrence. Actually the evidence was all the other way.

The question must now be asked whether the exposure to MC was sufficiently long to have yielded valid tests of its carcinogenicity:—

The period of test was limited to the life of individual tumors, and these had to be examined after little more than 2 to 3 months at most, to avoid complications due to their size. MC often persisted in them for more than 6 weeks (Exp. 5), and during much of this period the conditions would appear to have been ideal for it to have proved carcinogenic in the definitive sense of this term; for it could escape from the growths only through the layer of living pap., which retained it in quantity (Fig. 13). In not a few instances the crystals of MC lay close to the pap. layer which, in consequence, was exposed to the carcinogen to an extent ranging from the barely tolerable to the negligible, as already pointed out; and somewhere along this range the optimum amount to induce further neoplastic changes must have been present. Yet though 2 months were let elapse in Exps. 6 and 7 before the tumors were examined cancers visible in the gross were not as numerous as in the controls.

Our experience has been that when MC in Crabtree's solvent is applied only twice to the shaved skin of adult mice of our inbred stock some paps. arise within 21 days (7 *b*), their frequency increasing later and cancers visible in the gross deriving from many of them. When suspended fragments of embryo skin are injected into the thigh muscles together with droplets of olive oil containing MC, epidermal paps., and primary cancers as well, appear in less than 4 weeks (7 *a*). Greene (8) obtained them within 35 days by implanting embryo skin together with MC crystals. Yet these findings with tissues previously normal do not justify any decision concerning the length of time required for

MC to induce secondary cancerous changes in cells that are already in a benign papillomatous state.

One might have to conclude that the exposure to MC had been too brief to yield conclusive findings were it not that the carcinogenic effects of the polycyclic hydrocarbons on mouse epidermis are gradual and cumulative, and that their influences can be superimposed. Cells must have been present often in the tar-induced papillomas of the present work that were on their way to malignancy; and an ample opportunity was provided for methylcholanthrene to have consummated the change.

Nevertheless one cannot conclude forthwith that MC is unable to induce or hasten secondary carcinomatous changes, because it has been found to do this when applied in oil to expanses of rabbit skin during the few days of healing after it has been abraded with sand paper and inoculated broadcast with the Shope papilloma virus (12). Though the growths that arise are to all appearance typical virus paps. cancers derive sooner and oftener from their cells than from those of control paps. appearing on inoculated skin to which oil had been applied alone. Yet this does not mean that the virus and MC act in the same carcinogenic way:—

The cells of Shope virus papillomas differ distinctively in aspect, differentiation, and proliferative activity from those of paps. induced by the chemical carcinogens and they are easily recognized by their morphological stigmata (13). When suspended virus is thrown into the bloodstream of rabbits that have papillomas due to tarring just appearing on their ears, it localizes in these growths, changes some of them into characteristic Shope papillomas, and in other instances acts in concert with the unknown actuating cause of the tar papillomas, with result in "anomalous tumors" that are expressive of the mingled yet differing morphological traits of them both (14).

Findings of similar import are obtained when fragments of paps. induced by tar and of the carcinomas also arising are exposed to a suspension of Shope virus *in vitro*, and then implanted in the muscles of their rabbit hosts— which receive in addition, at corresponding sites, control fragments of the same tumors that have been merely exposed to serum-Locke's solution. Under such conditions the virus has a transforming effect upon the cancers when they happen to be of a differentiating sort, altering their morphology toward that of virus paps. and abruptly rendering them much more vigorous (15). On anaplastic cancers its influence is confined to the abruptly synergistic: acting together with their unknown cause it makes them suddenly grow with great vigor and become much more aggressive.

These facts justify the conclusion that the chemical oncogens and the Shope virus act on epidermal cells in differing ways when inducing cancerous changes and that when they are working in concert their effects, though often merged, continue to differ in nature.

The Compulsive Keratinization of Papilloma Cells

The enlargement of Type A papillomas is wholly due to their intrinsic cell proliferation, but the resulting tumors are so unaggressive that they get bigger

only because of their continual differentiation and keratinization. The growths "shove outwards by pushing inwards" (4) as their cores accumulate, and methylcholanthrene does not bring this process to a stop unless it well-nigh kills the cells. Very different are its effect on fragments of normal embryo mouse skin implanted in muscle together with it (7 *a*). True, it then causes the epidermal cells to form numerous small keratinizing cysts which coalesce secondarily as do those composed of pap. tissue (Exp. 2); but the epidermal lining of the resulting, solitary cyst soon ceases to have a stratum granulosum and produces almost no keratin. Later it enlarges only as paps. and carcinomas arise on its walls and keratin from these growths accumulates together with extravasate. The cells of Type A paps., on the other hand, when exposed to MC, continue to do the normal duty of keratinization but with abnormal vigor even when the carcinogen is present in well-nigh lethal quantity. Theirs is compulsive functioning like the keratinization of the epidermal cells of Shope papillomas. One is reminded of the activity of certain tumors of the ductless glands, which results in an overproduction of hormones.

The Relative Responsiveness to MC of Papilloma and Carcinoma Cells

As the concentration of MC diminished in the paps. of our experiments they grew much faster than did the controls yet on final inspection their living layer was but little thicker (Figs. 1 and 12), a fact which seems to indicate that their differentiation to the keratinized state took place at the same rapid rate as their proliferation. The cells of the carcinomas, on the other hand, which formed relatively little keratin, were not diverted from multiplying by this task, and, under the influence of MC they quickly formed tumors that were sometimes greatly larger than those of the controls and contained much more living tissue (Exp. 4). But there was another and more potent reason why they enlarged so rapidly, namely the extraordinary responsiveness of their cells to the growth-promoting influence of MC. This far exceeded its effect on papillomatous elements (*vide* Figs. 11 and 12). In Exp. 4 not only did the cancerous implants exposed to the carcinogen form huge tumors as compared with the controls, but half of these latter failed to yield growths in the absence of its help. Their failure was not unique; many of the epidermal carcinomas consequent upon the action of MC on embryo skin and appearing highly malignant, do not survive transplantation(7*a*).

Few clinical studies have been made as yet of the response of human cancers to intercurrent promoting influences, although there are some familiar instances, for example the enhanced malignancy that follows upon ulceration and bacterial infection.

Can Methylcholanthrene Bring about Sequential Cancerous Changes?

During previous work with the present aim (1) tests were made to learn whether the exposure of mammary mouse cancers to MC would bring on the

progression towards anaplasia by discontinuous, sequential, cell changes that frequently takes place spontaneously in tumors of this kind. The carcinogen was brought to bear on two mammary cancers that were forming acini and tubules, and though the exposure to it was maintained for 10 and 11 months respectively by repeated transplantation of the growths together with more MC, no change in their character took place. Nor did a shorter exposure of the epidermal cancers of Exp. 4 cause any new malignant components to appear in them although their growth was stimulated prodigiously. Foulds, who has studied intensively the progression of mammary mouse cancers, came to the conclusion that this "is probably inherent in the nature of tumors" and "is independent of specific carcinogenic stimuli" (16).

Significance of the Ability of MC to Attract Epidermal Elements

In 1944 Coman proved that the cells of human squamous cell carcinomas have lost much of the adhesiveness of normal cells (17). The phenomenon has attracted much attention of late (18) and it has become one of the criteria for assuming that malignant changes have occurred in tissue cultures of normal cells. Zeidman of Coman's laboratory demonstrated that exposure to MC crystals *in vitro* reduces the adhesiveness of normal epidermal elements (19). Doubtless this change aids the positive chemotaxis that MC exerts on epidermal mouse embryo cells, and on those of the derivative paps. and carcinomas as now demonstrated. When its attracting effect on embryo epidermis was first noted (7 *a*) the question whether this might have to do with its pronounced ability to cause neoplastic change was considered, and comparative tests in this relation were made later by Smith (20), using 3,4-benzpyrene (BP) and 1,2,5,6-dibenzanthracene (DBA). These showed that BP had much less attracting power than MC, and that DBA had but little, findings which accord with what is known of the relative carcinogenic power of the three hydrocarbons. But Scharlach R stimulates and attracts normal epidermal elements so strongly that for years it was used to hasten the healing of wounds in human epidermis; yet it has never in more than a half century of highly various tests caused the epidermal cells exposed to it to become cancerous.[2] Injection of the dye into Shope papillomas causes them to look and act like vigorous, malignant carcinomas for the nonce, yet fails to hasten in the least the occurrence of the carcinomas often deriving from them (22). There could be no better proof that temporary stimulation and attraction from without, resulting in pseudocarcinogenesis, is very different from sustained impulsion from within.

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[2] The sole instance reported was in a rabbit which had received arsenic as well (21).

SUMMARY

When crystalline 20-methylcholanthrene (MC) and the cells of tar-induced mouse papillomas (paps.) are injected together into the thigh muscles of mice the carcinogen exerts a marked promoting and chemotactic influence upon the cells while it is dissolving in the tissue fluid. Under such circumstances it strongly stimulates and attracts them, with result they surround and include the scattered crystals in small cysts that later coalesce to form a larger one from which the MC only very gradually escapes.

Because of these findings intramuscular tests were made to learn whether MC would hasten the occurrence or increase the number of cancers that now and again derive from paps.; but the tests were repeatedly marred by the extraordinary behavior of such cancerous cells as happened to be already present in the implanted material. They responded far more actively to MC than did the pap. cells and soon took over the growths. Some carcinomas which failed to grow when transplanted alone, or only gradually formed small, regressing nodules, gave rise rapidly to huge growths of similar sort when exposed to MC.

To exclude cancerous cells so far as possible from the later tests small grafts of pap. tissue with MC crystals adhering to them were implanted subcutaneously. The pap. cells promptly lined the graft pockets, encysting the crystals incidentally, and formed tumors that enlarged progressively by keratinizing inwards. While they did this their living layer of pap. tissue was continually bathed in dissolved MC throughout many weeks. Despite these apparently favorable conditions the carcinogen neither hastened the occurrence nor increased the number of visible epidermal cancers deriving from the paps. It also failed to bring about sequential malignant changes in the carcinomas.

These negative results accord with those already obtained through long exposure of the benign pulmonary adenomas of mice to urethane or methylcholanthrene, agents which rapidly induce these benign tumors yet which were found to be incapable of furthering the cancerous changes to which such growths are prone. They accord also with another previous finding, namely that MC fails to bring on the malignant changes of discontinuous, sequential sort that mammary mouse carcinomas often undergo "spontaneously."

Taken together these facts indicate that the change or changes whereby normal cells are converted into benign tumor cells differ in nature from those taking place when they become cancer cells, as also from those occurring when cancer cells undergo further, step-like, malignant changes.

A study has been begun to learn whether the widely various carcinomas deriving from benign papillomas differ from these latter and from one and other in their chromosomal content.

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EXPLANATION OF PLATES

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

PLATE 21

FIG. 1. Part of a slice from next the neoplastic tissue of Pap. V that was used for Exp. 2. It consists largely of benign pap. but an uninvasive carcinoma occupies the bracketed stretch (see also Fig. 17). × 33.

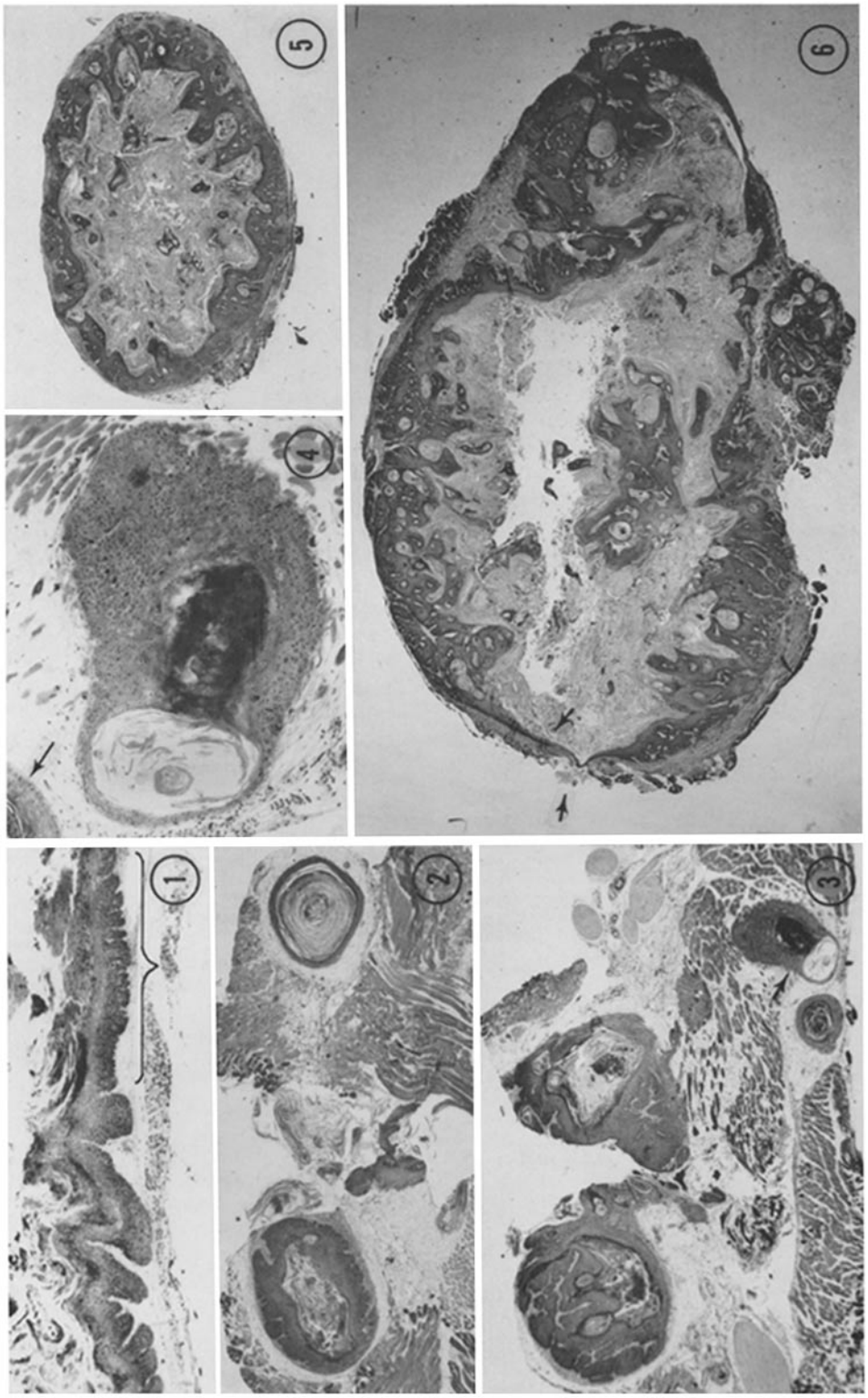
FIGS. 2 and 3. Some of the scattered neoplastic cysts of Exp. 2, found 14 days after the forcible injection into the thigh muscles of methylcholanthrene crystals together with fragments of the pap. layer of Fig. 1. Both specimens are from the same animal (Exp. 2). × 21.

Fig. 2 shows two intact cysts in cross-section, a concentrically keratinizing benign pap. on the right and an epidermal carcinoma on the left, with a thick irregular wall. Between them, lower down, are fragments of another cancerous cyst. In Fig. 3 there are two carcinomatous cysts of similar sort, and a third (arrow) that is lined with cancerous tissue save at its smaller end where a thin layer of benign pap. covers the wall (Fig. 4). A fourth cyst (below the shaft of the arrow) has a much thicker lining of pap. All had been blue in UV.

FIG. 4. The complex cyst of Exp. 2 that is designated by the arrow in Fig. 3. The shallow layer of pap. tissue lining its smaller end has formed keratin, but the nature of the dark mass enclosed in active cancerous tissue is problematic. At the left upper corner of the figure a part of the adjacent pap. of Fig. 3 can be seen (arrow). × 85.

FIG. 5. Sagittal section of the control tumor of Exp. 4 that was 27 days old. Its irregular wall consists entirely of keratinizing, squamous cell carcinoma (see Fig. 14). × 10.

FIG. 6. Sagittal section of the corresponding MCed tumor 27 days old of Exp. 4. It is several times bigger than the control and its wall is much thicker though consisting of the same cancer save for a short stretch of benign pap. (between the arrows). Like the tumor of Fig. 5 it is full of loose keratin which, in the growth here shown, appeared deep blue in UV (see Fig. 15). × 10.



(Henderson and Rous: Sequential neoplastic changes)

PLATE 22

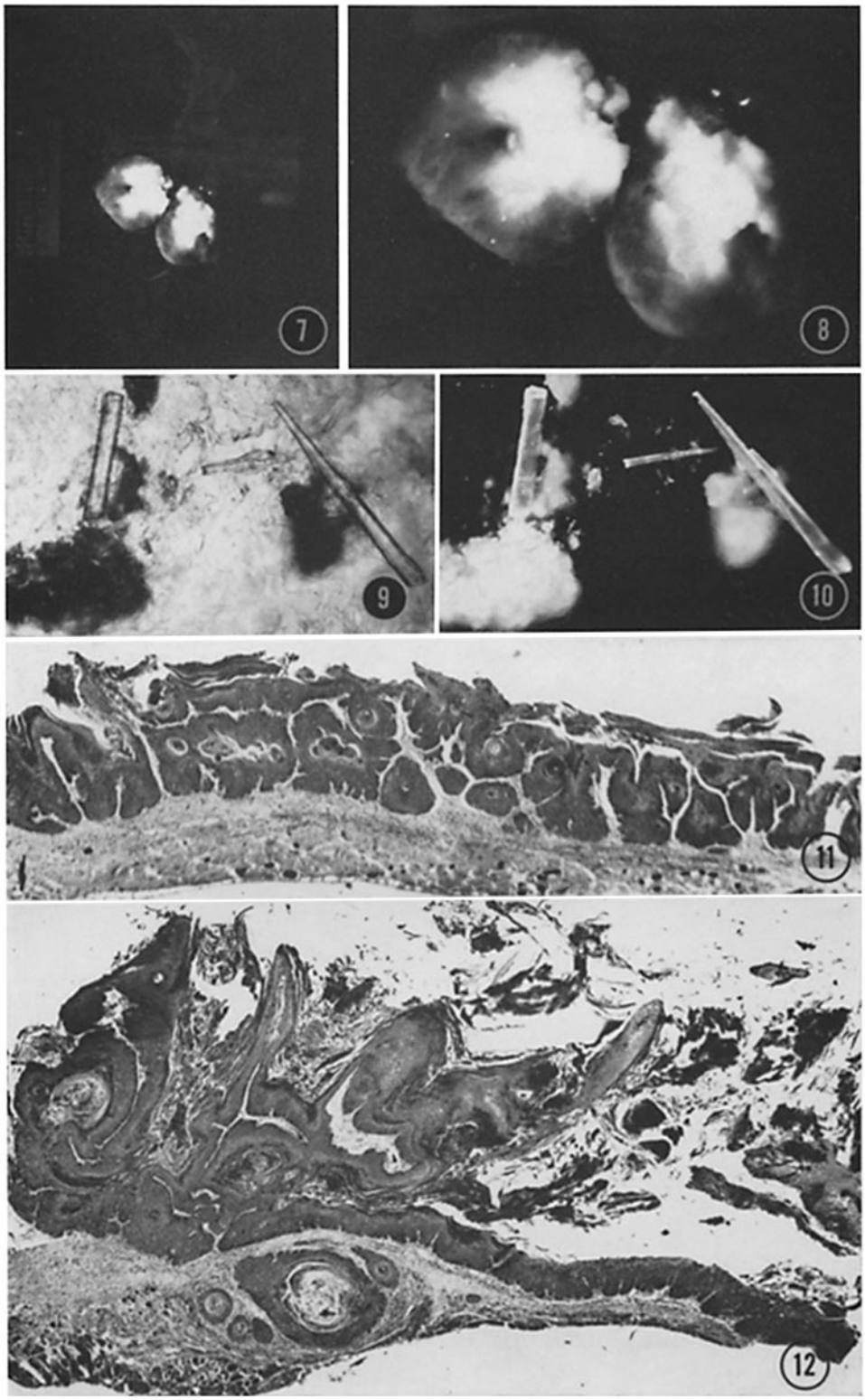
FIG. 7. A solitary, spherical cyst resulting from the intramuscular injection 36 days previously of fragments of the tumor layer shown in Fig. 1, together with MC crystals (Exp. 2). It has been halved by a sagittal cut and photographed by UV. One half is still embedded in the thigh muscles, (as can be dimly perceived when the picture is looked at from the left). Its dense, fluorescing, keratin core appears white because of its content of MC, and much of the peripheral, living layer is visible because containing some of the fluorescing carcinogen, notably along its base next the invisible connective tissue capsule. The two tiny, white dots lying amidst the dark of the muscle, outside and above the lower half of the tumor, consist of crystalline MC that had escaped encystment. Natural size.

FIG. 8. The same tumor at higher magnification. In opening it the knife tore away the tissue on the right lower side of its right-hand half. Much living tumor had been present there as indicated by the fluorescence of the corresponding region in the intact half of the growth. Between it and the brilliant white core is a zone that does not fluoresce at all. × 3.

FIGS. 9 and 10. MC crystals from the core of the cyst of Figs. 7 and 8, as viewed in daylight and UV respectively. They show almost no erosion. The nature of the amorphous material—which fluoresces because of its MC content—is unknown. × 168.

FIG. 11. Malignant papilloma on the wall of the tumor of Figs. 7, 8, and 13, at the same magnification as the layer that had provided the tissue for Exp. 2 (see Fig. 1). It is much thicker than the benign pap. present on the wall of the same cyst (Fig. 12) and its disorderly layer is forming but little keratin. × 33.

FIG. 12. The papilliferous, squamous-cell carcinoma of Figs. 7, 8, and 13. It has extended through the encapsulating tissue into the adjacent muscle, and it overhangs on the right a layer of benign pap. scarcely thicker than that wherewith the tumor of the present experiment was produced (Fig. 1). × 33.



(Henderson and Rous: Sequential neoplastic changes)

PLATE 23

FIG. 13. Color photograph of the cyst of Figs. 7 and 8 as viewed in UV. White spots can be seen where many of the MC crystals lie close packed amidst the keratin core—which is blue because containing the carcinogen in solution. Elsewhere it is stippled with white where the crystals are more scattered. Toward the periphery of the growth the amount of dissolved carcinogen becomes so slight that here the blue fades away, but further on the left it becomes intense again because much MC has been retained by the peripheral layer of living tissue.

Below and outside the half of the growth that lies on the right, some of the tumor tissue torn away in the region between the arrows is visible in blue amidst the black of the muscles. The two discrete, lemon yellow dots above the half on the right are MC crystals lying free because too far off to have been encysted. They have begun to dissolve and are surrounded and overlain by a shallow zone of fluorescing blue. × 4.

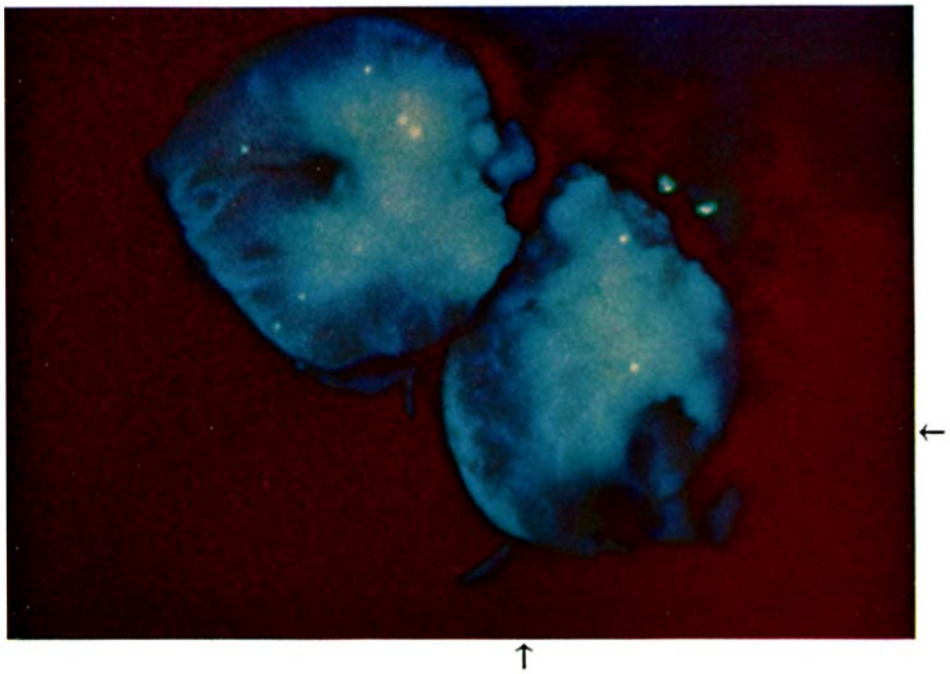


FIG. 13

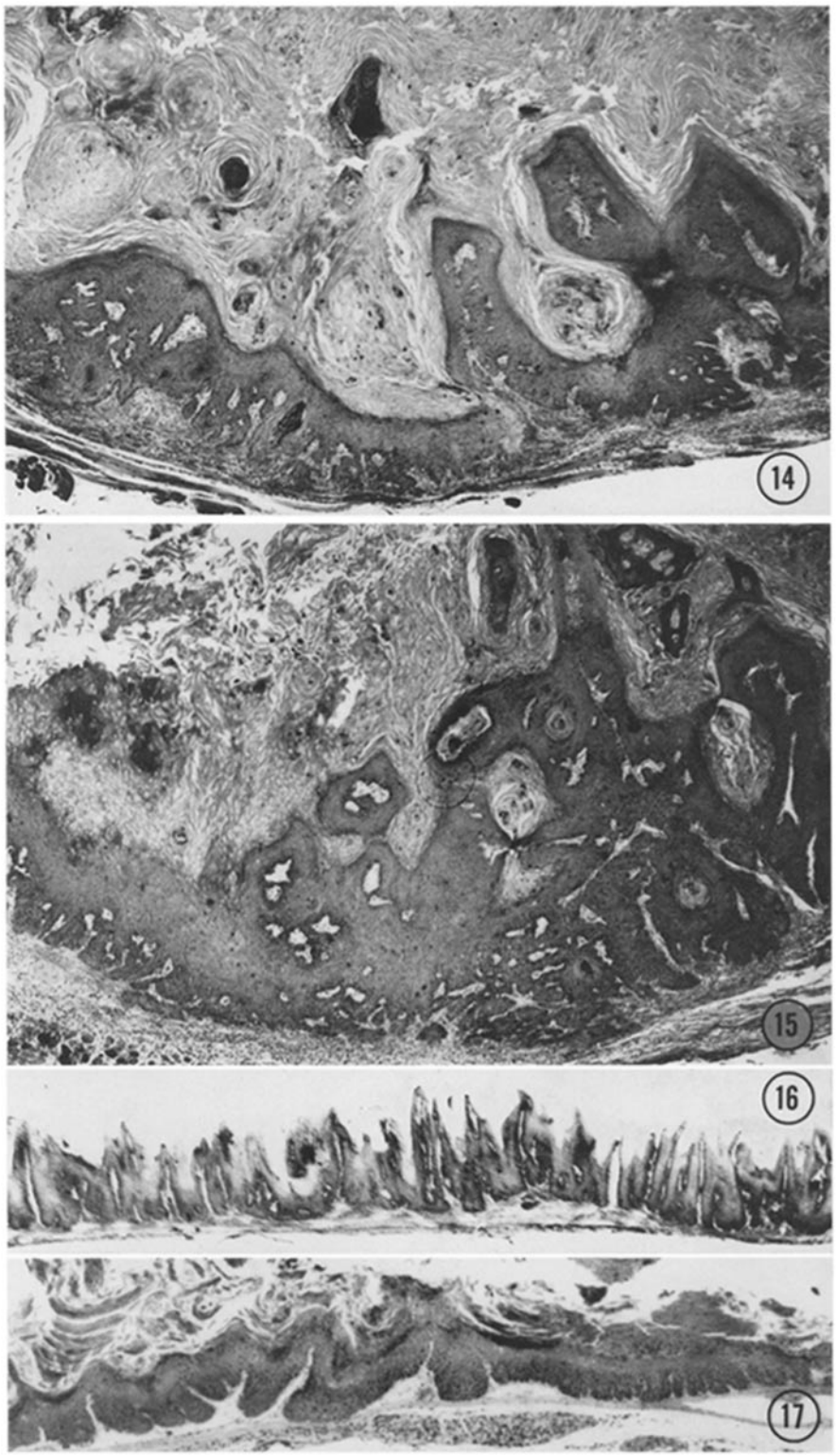
(Henderson and Rous: Sequential neoplastic changes)

PLATE 24

FIGS. 14 and 15. The carcinomas of Figs. 5 and 6 (Exp. 5) at higher magnification. They are identical in character but the walls of the cysts they enclose differ greatly in thickness. × 41.

FIG. 16. Section through an expanse of malignant pap. from next the material of Pap. V used in Exp. 3. It had the aspect of benign pap. in the gross and had formed a layer of the same thickness. × 28.

FIG. 17. Higher magnification of Fig. 1, to show that the pap. keratinizes and the carcinoma does not. × 57.



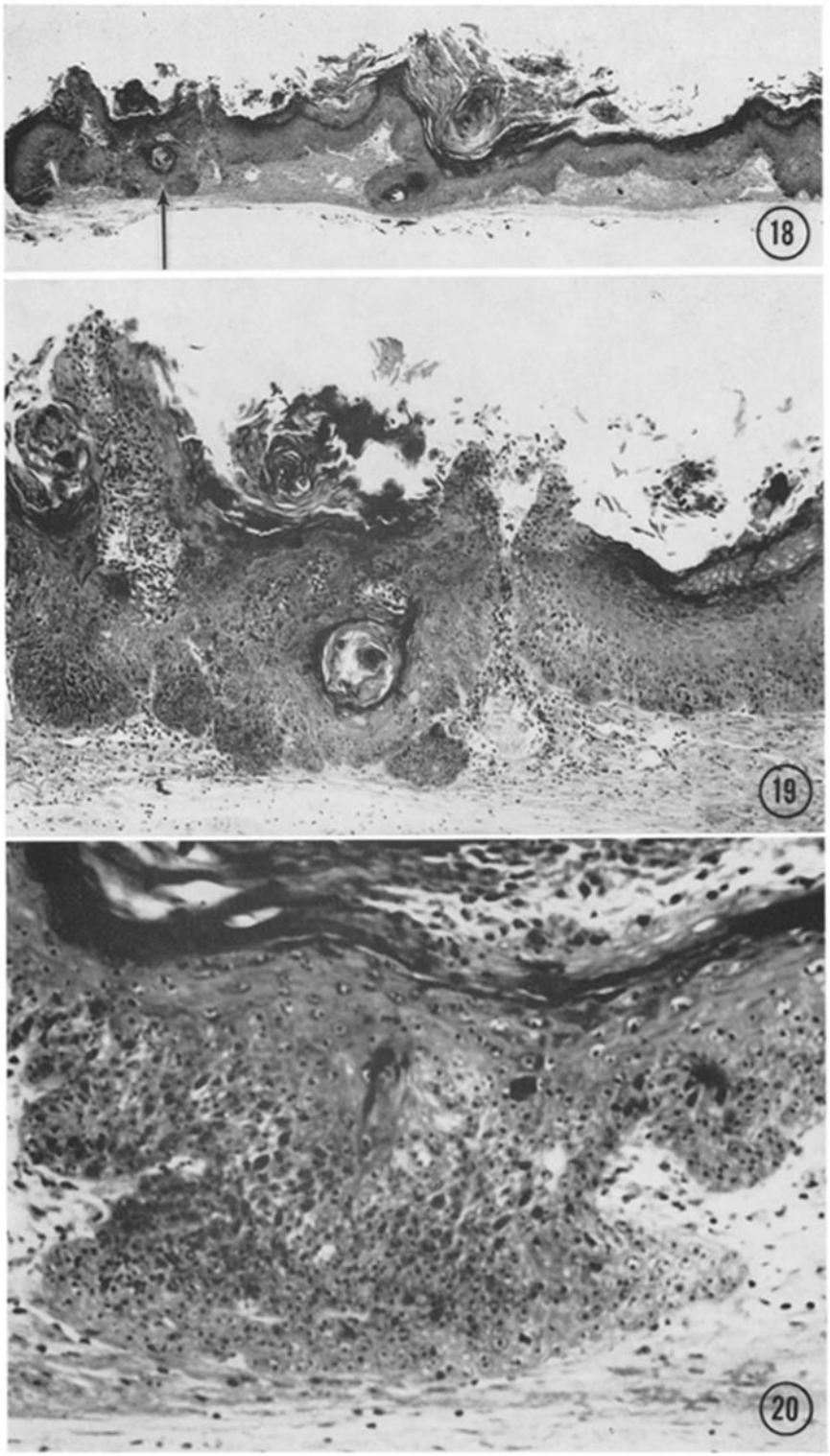
(Henderson and Rous: Sequential neoplastic changes)

PLATE 25

FIG. 18. Section from next the tissue of Roof Pap. 41 that was used for Exp. 4. The pap. layer is about as thick as that which furnished the material for Exp. 2 and at one spot amidst it (arrow) a keratinizing, squamous cell carcinoma has arisen (see also Fig. 19). In other sections the pap. layer has undergone cancerous changes along its base (see Fig. 20). × 31.

FIG. 19. The squamous cell carcinoma of Fig. 18 at higher magnification. × 103.

FIG. 20. Anaplastic carcinoma extending downwards from another part of the layer of Fig. 18. × 285.



(Henderson and Rous: Sequential neoplastic changes)