THE PATHOGENESIS OF DEFERRED CANCER

A Study of the After-Effects of Methylcholanthrene upon Rabbit Skin*

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The malignant tumors which arise in man on the basis of tissue injury sustained many years previously raise questions of prime moment for an understanding of how cancers come about. Have neoplastic cells, brought into being by the tissue disturbance, lain latent throughout all the long interval? Or did the disturbance provide a carcinogenic stimulation insufficient in itself to convert normal cells into tumor cells but succeeded by others with result in such conversion eventually? Or can it have started a process gradually, yet inevitably, consummated in the course of time? There are facts in the clinical literature for each one of these assumptions. The present paper reports experimental findings which bear upon the course of events. The ears of young adult rabbits were painted with a solution of methylcholanthrene until a few benign growths had appeared, and then the paintings were stopped and the later cutaneous happenings,—which included the ultimate appearance of cancers,—were followed throughout the animals' lives. Pieces of the ears were punched out from time to time to learn the general state of the skin and to determine the character of equivocal growths, but more especially to test whether hidden neoplastic potentialities existed in the cutaneous tissue. For where punch holes are healing in ears previously treated with chemical carcinogens epidermal tumors frequently arise which would not have asserted themselves in the absence of stimulation (1).

Material and Methods

Five young adult agouti rabbits of about 2.5 kilos weight were studied. The hairless side of their ears was swabbed all over twice weekly with a 0.3 per cent solution of methylcholanthrene (*MC* as it will be termed) throughout periods of from 215 to 250 days. Two of the animals (D. R. 1-69 and 1-72) received the hydrocarbon dissolved in benzene, and the other three (D. R. 3-37, 3-44, 3-45) in Crabtree's medium (2),—ethyl ether containing 2 per cent of light mineral oil (U. S. P. ingredients). The total period of observation ranged from 980 to

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2552 days. During it the rabbits became huge, their weight increasing to 4.5 to 5 kilos. Four of them developed one or more carcinomas. The case histories follow:—

D. R. 1-69 received methylcholanthrene in benzene for 215 days. At the end of this time the skin appeared little changed. It was thinly scurfed, somewhat pinker than ordinary, and the mouths of most of the hair follicles were widened by keratotic plugs; yet the ears were normally thin. Toward the end of the applications a few small, benign, more or less fleshy growths arose (papillomas, frill horns, carcinomatoids (3)), but within 2 weeks after the *MC* was stopped the hyperemia lessened, the tumors dwindled, and several disappeared. The general cutaneous changes persisted though, and soon new growths developed, very small and notably dry. On the 267th day after the last *MC* a *fleshy, sanguineous disc* was found to have replaced a dry, cauliflower papilloma present a few days previously; but the disc vanished within the next 6 weeks. Meanwhile new tumors continued to appear (Chart 1). The skin reverted to the normal very slowly, being still slightly scurfed and having many distended follicles on the 638th day after the last *MC*; but by the 759th day it had the normal, smooth, somewhat silvery aspect. All this while, and indeed until the 1120th day,—the last of detailed charting,—the tumors continually increased in number. On the 1217th day a raised, ulcerated disc was noted, of which there had been no sign 2 weeks before. It was a *squamous-cell carcinoma* as evidenced by biopsy, and soon it had destroyed so much of the ear that the animal was killed,—on the 1289th day.

D. R. 1-72 received methylcholanthrene in benzene during 250 days. When the applications were left off the ears were markedly changed,—somewhat thickened, covered on the treated surface with a tenacious layer of scurf, 1 mm. thick in some regions, with brightly pink, swollen skin beneath, showing much follicular enlargement; there were few tumors and these small and obviously benign. But by the 16th day after the last *MC* a subepidermal hemisphere had appeared which soon became an ulcerated disc with the morphology of an *anaplastic, squamous-cell carcinoma* as biopsy showed. It disappeared nevertheless after 9 months. During this time the skin had been returning to normal, and on the 606th day after the last *MC* application it seemed entirely so in the gross, save for a large increase in the benign tumors. These continued to accumulate (Chart 1), more and more growths appearing until shortly after the 1600th day, when the number became stationary and soon after fell off. At about the 1800th day a slow, caseous discharge began from one external auditory canal and shortly from the other. It caused little inflammation of the neighboring skin, which was frequently cleansed, and had no evident effect on the existing growths; yet since it was potentially a complicating influence the tumor incidence on the distal third only of the ears was charted during the later life of the animal. On the 1982nd day, 5 years and 5 months after the last *MC*, a purply, subepidermal mound, which had not been present 10 days earlier, was observed near the tip of an ear, and because of its threatening aspect was punched out *in toto* within a few days. The microscope showed it to have the morphology of an *anaplastic, squamous-cell carcinoma.* By now the rabbit, long well nourished, huge, and vigorous, had become gaunt, weak, blear-eyed, and apathetic, the picture of extreme old age. It died on the 2552nd day, 2302 days, or 6 years and 3⅔ months, after the last *MC.* The tumors on the distal third of the ears had become much less numerous toward the end (Chart 1). No cause of death other than senile changes was found.

The other three animals received *MC* in Crabtree's medium over a period of 246 days:—

D. R. 3-44. When the *MC* was discontinued the ears were thin, uninflamed, with no definite scurfing but with some follicular enlargement and a few small, benign tumors, which vanished soon after. During the next hundred days however the skin changed much for the worse, becoming markedly scurfed and definitely thickened; and many new tumors arose (Chart 1). All appeared benign until the 472nd day after the last *MC* when a fleshy growth was first noted which grew fast as a *malignant papilloma*, then as a *squamous-cell carcinoma*, so

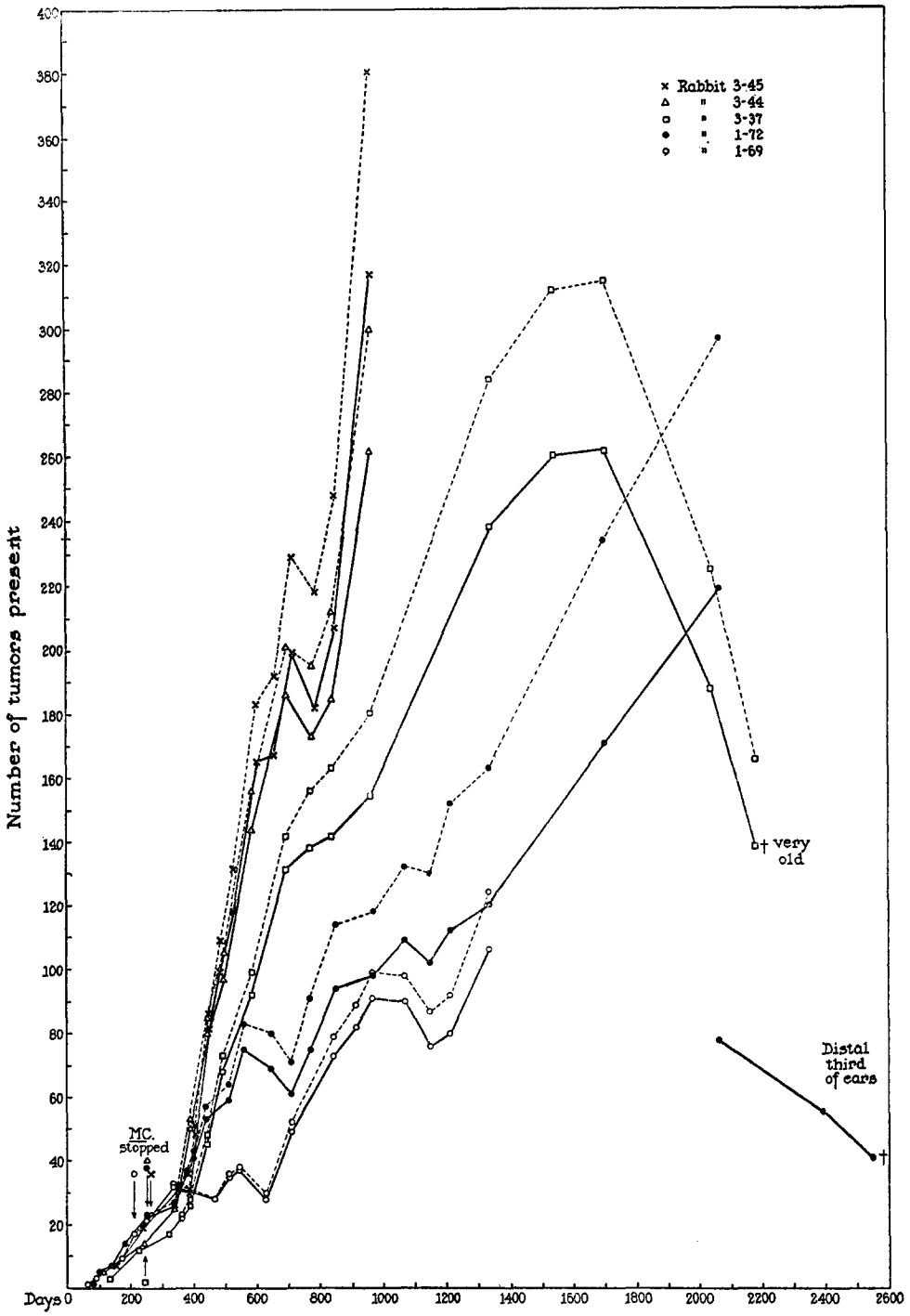

CHART 1. Incidence of tumors after methylcholanthrene.

The observed rates of accumulation are given in uninterrupted lines, the calculated rates for the entire ear surface in broken ones. The growths of rabbits 3-44 and 3-45 became so numerous and crowded toward the end of the 3rd year that no more records could be made. In a third instance (1-69) progressive destruction of an ear by a cancer brought them to a stop.

successive biopsies showed. The cancer ulcerated but became indolent, remaining so until the death of the rabbit from Ménière's disease, first evident on the 700th day after the last *MC* and proving fatal on the 734th day. By this time the skin between the growths looked almost normal. Benign tumors had steadily become more abundant.

D. R. 3-37. When *MC* was left off the ears were thin, uninflamed, slightly scurfed, with some follicular enlargement and a few tumors; but 3 months later they were far more changed, the skin bright pink, moderately scurfed, and its follicles greatly enlarged. The number of growths had increased. Scurfing and large follicles were still present in some places on the 346th day after the last *MC*, but much of the skin looked normal. It seemed wholly normal everywhere on the 1095th and 1304th days, yet tumors were still coming in (Chart 1). By the 1456th day they were no longer accumulating, and after a few more months their number began to lessen and gradually fell off until the 1957th day, when, through accident, the animal was killed.

D. R. 3-45. After the last application of *MC* the ears were thin, uninflamed, with dubious scurfing, and most of the existing growths soon dwindled or disappeared; but 110 days later the organs were thickened and much more scurfy. On the 202nd day nevertheless the skin between the growths had come to appear almost normal and on the 378th day completely so, save for some enlarged follicles. Benign tumors rapidly accumulated in large number (Chart 1), and by the 750th day a multitude of exceedingly minute ones had rendered the skin slightly rugose toward the middle of the ears. Soon afterwards the growths visible in the gross became so many that they could no longer be charted individually. *Cancer* was first noted on the 984th day after the last *MC*. It grew fast and destroyed much of the ear,—for which reason the rabbit was killed on the 1111th day. During the final 2 weeks *another cancer* appeared, on the opposite ear. Like the growth just mentioned this proved to be an *active, squamous-cell carcinoma*, but of a distinctly different morphology.

It will be seen that after the final application of *MC* the animals lived from 2 years to 6 years and $3\frac{2}{3}$ months, and that some of the cancers appeared only after long intervals,—from 15 months to 5 years and 5 months after the last *MC*.

Charting of the tumors was begun as soon as the first was noted. The outline of each ear was traced with a wax pencil on a cellophane sheet, and the situation of each growth was marked, transfer was made to cards, with the aid of transmitted light, and the tumors were drawn in their actual position, size and shape. Detailed record was made of every significant change and exceptional finding. When growths first began to be numerous after *MC* was left off, the chartings were carried out every 2 weeks, later every 4 weeks, then after 2 or 3 months, and finally after every 6 months in the case of the animals that lived very long. During the first year the ears enlarged slightly,—for the rabbits were still young adults,—but the change was so gradual that duplicate outlines could be utilized for several successive chartings. Later the same outline could be employed regularly. Prior to each charting the position of every tumor of the preceding record was marked with a red dot on a new record card, using transmitted light, and then all the growths still present were drawn in, together with any new ones, these latter carefully in relation to the old. All of the chartings were made by W. F. F. except the last few for the two rabbits living longest (D. R. 1-72, 3-37), P. R. made these.

The punch biopsies were done with sharp cork borers, 0.4 to 1.5 cm. across, sterilized by boiling. Previous experience had shown that healing was often incomplete if holes more than 0.6 to 0.7 cm. in diameter were made in the distal third of rabbit ears, but that near the middle of the organs holes a centimeter across closed nearly always, while toward the head still larger ones completely healed. The choice of cork borers was made with this knowledge,

and very few of the holes failed to close entirely. Bleeding was checked by plugging them for a few hours with sterile cotton. Healing took place without obvious infection, new tissue extending in evenly from the circumference as a rule, with result in a symmetrical new disc. At first this was hyperemic, later pale as its connective tissue contracted, but it retained its size though often gradually thickened by excess cartilage, and hence no distortion of the ear complicated the findings. While the holes were closing, or shortly afterwards, tumors often appeared at the advancing edge of the new tissue or on the completed disc. Some of these growths were punched through or removed entirely for microscopic examination; and occasionally the greater part or all of a new-formed disc was excised, together with a rim of normal tissue, by means of a cork borer bigger than the one with which the original hole had been made.

The term "discing" will be used, as in previous papers, to cover the reparative events which ended with complete closure of the holes.

Slices were taken from each punch specimen, fixed in acid Zenker's solution, sectioned, and stained with eosin and methylene blue. Often serial sections were made, both to learn the state of the skin, and to find whether microscopic growths existed where none could be seen in the gross.

The Kinds of Benign Tumors Arising

The cutaneous changes that took place during the immediate period of exposure to *MC* and the growths arising then have been described in previous papers (4). The tumors appearing later proved to be of the same sorts, all epidermal and almost all benign. The majority were papillomas and the rest frill horns, sebaceous adenomas being so rare that they can be left from consideration. No carcinomas arose during the *MC*ing. Those that appeared afterwards will be dealt with further on.

Most of the papillomas developing while *MC* was applied were fleshy and, though small, appeared vigorous (Fig. 1). The microscopic findings in similar instances have been figured previously (4). A considerable proportion of the growths had the carcinomatoid form, that is to say had the aspect of invasive squamous-cell carcinomas (4). But soon after the carcinogen was left off, these apparently malignant tumors either disappeared, or became small masses of keratinizing cysts lined with a merely hyperplastic epidermis, or changed to frank papillomas, no more of them arising. Their threatening aspect and aggressive behavior had been due to stimulation by the carcinogen, as in the case of the tumors of identical sort elicited by tar or benzpyrene (4). Yet that cells capable of forming them still existed in the treated skin was shown by the not infrequent appearance later on of carcinomatoids where new punch holes were healing, with involution of them afterwards in one or another of the ways just described, when discing had been completed. The growths of frank papillomatous form also underwent involutionary change after the *MC* was left off, either disappearing or drying down and becoming mere scabs, verrucosities, low plateaus, keratinized horns, or brittle cauliflowers with constricted bases (Figs. 2, 3, and 4 *a*), all of which forms bespeak an inactive state (4). The reasons for regarding most of the carcinomatoids as consisting merely of stimulated papilloma cells have been given in previous papers (3, 4).

Few of the frill horns actually formed horns at any time, most of them appearing to be mere tiny saucer depressions or discrete, rounded scabs in the gross, and remaining such as long as they persisted. Occasionally however one did produce a characteristic horn (Figs. 3, and 4 *a* and *b*), densely keratinized and striated transversely, instead of vertically as are the horns formed by papillomas (3).

Within 2 months after the last *MC* all of the persisting tumors had become smaller, none now being more than 2 mm. in diameter. Yet already others had arisen, and in the succeeding months and years more and more of them accumulated (Chart 1). Many were dark gray or black (Figs. 2 to 4), like the benign tumors due to tarring, when these are indolent (3). Nearly all had one or another of the forms just described, and soon ceased to grow, disappearing after several weeks or months; but some persisted for a while without increasing in size (Fig. 5). A few of the papillomas appeared suddenly as erupting mounds or fleshy cones or cauliflowers, and grew fast in their first weeks, occasionally becoming as much as a centimeter across; but in nearly all cases these dwindled gradually later or keratinized *in toto* and flaked off. Exceptionally one persisted for many months as a verrucosity, or low, dry cauliflower, or keratinized scab.

Without microscopic examination it was impossible to tell whether the many small scabs of keratin overlay papillomas or frill horns. The latter were always small and very slow growing, and their cells multiplied much less actively in response to the stimulus of discing. Instead of forming well defined, radial or segmental growths on the discs the frill horn cells then gave rise either to peripheral tumors or, failing to keep pace with the ordinary hyperplastic epithelium extending in together with them, produced at most a radial line of minute, separate aggregates (Fig. 8). These, however small, could be easily recognized microscopically because of the distinctive character of their cells, many of which differentiated as a rule into parakeratotic "bullet bodies" (Fig. 7) (4).

Throughout the years the benign tumors continued to be of the same kinds. Now and then one or several arose on the outer surface of an ear. No more need be said of these.

Rate of Accumulation of the Growths

Chart 1 gives the rates at which the tumors accumulated in the five animals. For the sake of simplicity many records have been omitted which were obtained in the intervals between those charted; they sustain the findings given. As more and more holes were made in the ears the amount of intact skin was reduced considerably,—by more than 30 per cent in D. R. 1-72. Hence two rates of accumulation have been charted for each animal, one representing the total number of growths perceptible in the gross on the skin surface unaffected by the discings, the other the calculated number present on the entire ear expanse had no holes been made.

Every growth recognizable as such with the unaided eye was counted, but no ill defined or doubtful rugosities were included nor any tumors lying within a millimeter of the holes, since healing sometimes causes growths to appear inside this zone (1, 4). For the calculated number of tumors the total area included within the ear outline was obtained with a planimeter, and the proportion that the discings had involved was ascertained.

Some of the growths that arose during the application of *MC* disappeared before it was left off, and others were punched out. Undoubtedly a proportion of those removed would have vanished had they been left *in situ*. To cover this source of error the percentage of actual

disappearances was determined and the number of tumors punched out was reduced proportionately, the figure thus obtained was added to the number of growths actually present, and the total was inserted on the chart. To take an individual case, two of six tumors removed before *MC* was stopped would have vanished if left undisturbed, judging from what happened to those left behind; so only four were added to the number actually present when the carcinogen was left off. No such allowance was made for tumors punched out later, since the accumulation soon became so great as to render the correction negligible.

It will be noted (Chart 1) that the rabbits which had received *MC* in benzene (D. R. 1-69, 1-72) developed fewer tumors than those getting it in ether and mineral oil,—a finding which extends Crabtree's observation that the ether-oil solvent renders carcinogenic hydrocarbons unusually effective on mice (2). The data on tumor accumulation were not charted until all the figures were at hand, except those of the last few examinations of the two rabbits which lived longest (D. R. 1-72, 3-37); the author making the successive records knew only that growths were accumulating more or less rapidly. Hence the major disclosure of the charting was wholly unforeseen. This disclosure was that the number of tumors increased at an essentially constant rate throughout periods of from 2 to $3\frac{1}{2}$ years after the last application of *MC*, that is to say until the end of the animal's life in three instances. In the case of the other two, which long survived (D. R. 3-37 and 1-72), the rate eventually slowed, —after 4 years and 5 years from the last *MC*, respectively—and by the time they died the growths had decreased considerably in number.

Transitory Existence of the Tumors

The steady increase in neoplasms was not the outcome solely of accessions, but represented new growths developing in excess of old ones disappearing. For vanish they did continually throughout the entire term of the observations. Not infrequently one recurred, after having been gone, to all appearance, for several months, only to vanish again later. Scarcely any of the tumors of final record dated from the early days.

Chart 2 tells what happened to the growths present on the ears of D. R. 1-69 and 1-72 at the time when the *MC* was stopped, and also to those existing rather more than 200 days later. It will be seen that most of them were gone after 5 to 8 months. Whether any persisted for 3 years was dubious, since by then it had become well nigh impossible to follow them individually. Of the 98 growths recorded as on the ears of D. R. 1-72 on the 658th day after the last *MC* only 36 were still certainly present on the 928th day, though 102 growths then existed, 66 having appeared in the interval. So great was the neoplastic turnover. Later on, when the total number of growths was lessening in the animals which had lived longest, new ones kept on appearing but now they were few. During the last year of D. R. 1-72 only nine developed on the further third of the ears, all before the terminal period in which the animal lost weight.

The microscopic changes occurring when benign cutaneous tumors of the rabbit dwindle and vanish have been described in previous papers (3, 5).

The papillomatous epithelium loses its distinctive aspect, soon appears merely hyperplastic, and later takes on the normal look; yet after so doing it may persist for months and again form a typical papilloma if appropriately stimulated (5). During the present study a hitherto undescribed retrogressive change was encountered, occurring after years, namely the formation of residual basket networks where vigorous papillomas had once grown down a little way. These "baskets" consisted of interconnecting cords, two cells thick, of orderly

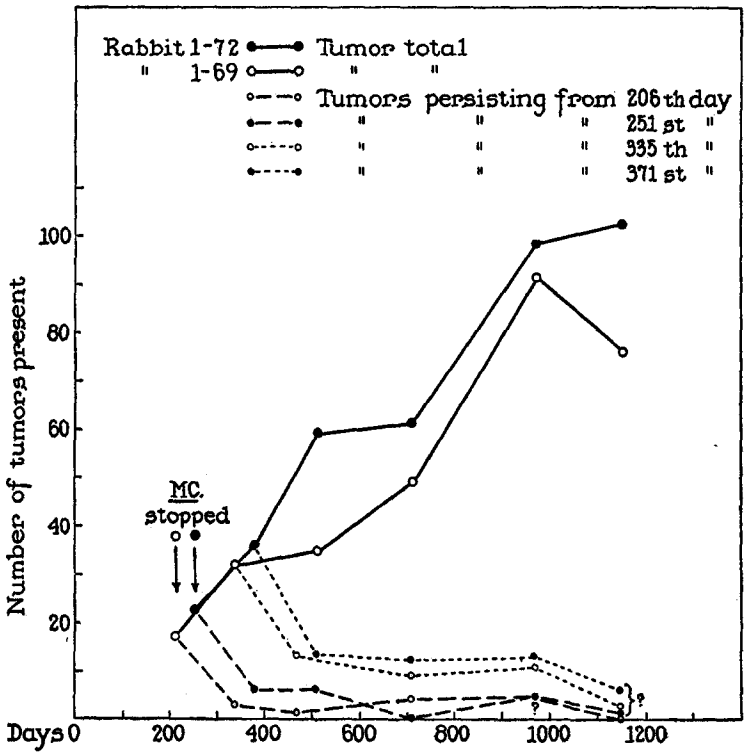

CHART 2. Rate of disappearance of the tumors present on the ears of 1-69 and 1-72 when *MC* was discontinued, and of those recorded on the 335th and 371st days. The fate of each was followed. The rates of later tumor accumulation are also indicated. ? = only dubious residua left.

epithelial elements. Every stage in their formation by the attenuation of invasive papillomatous "fingers" could be observed. The epithelial cells making up the final cords had all the appearance of ordinary basal epithelium but were devoid of mitoses and showed no tendency to differentiate (Fig. 16).

The cells of retrogressing frill horns also tend to lose their distinctive morphology, as can be well seen when only part of a growth has as yet undergone the change. The cells no longer form the distinctive parakeratotic "bullet bodies," but form a hyperplastic, keratinizing layer which cannot be told from that of an involuting papilloma. The later occurrences have not been followed.

Other Changes in the Skin

The cutaneous changes, other than tumor formation, which take place during the exposure to *MC* have already been described (4). They differed widely in degree from animal to animal.

In the present instances active hyperemia developed more or less gradually, the mouths of the hair follicles became widened with plugs of keratin, and the ear surface was covered with a tenacious layer of it. When the layer was thick, stripping it away disclosed a bright pink, definitely swollen surface.

Under the microscope the epidermis was seen to be many-layered, the sebaceous glands were much more numerous than usual, and the hair follicles were funnel-shaped or cystic, by reason of retained keratin. When the changes were pronounced scattered necrosis sometimes occurred of small groups of cells deep in the epidermal layer near the follicles (4), and there was then a local edema of the corium, with macrophages and lymphocytes in increased number. This was the case with D. R. 1-72 as biopsies showed; it had ears covered on the treated side with a layer of keratin a millimeter thick. In the other four animals, which had a thin layer of silvery scurf overlying a surface but little inflamed, the microscopic changes were still relatively mild when the *MC* was discontinued.

The after-effects of the carcinogen differed with the fluid in which it had been dissolved.

When the solvent was benzene (D R. 1-72, 1-69) the skin began at once to revert to the normal, but so gradually that in the case of 1-69 the follicles were still enlarged and there was slight, patchy scurfing more than 2 years after the last *MC* (Fig. 5). Several months more elapsed before the skin took on the ordinary pearly lustre, and pieces punched out on the 990th day (more than 2 years and 8 months after the hydrocarbon had been discontinued) showed the epidermis to be normally thin in general, with some slight, patchy hyperplasia. Sebaceous glands were still abnormally numerous however, and the corium was slightly more cellular than usual.

The skin of D. R. 1-72 seemed normal in the gross (save for tumors) by the 606th day after the last *MC*; yet on the 966th day it showed microscopic changes (Fig. 19) like those of D. R. 1-69, and when the animal died, 6 years and $3\frac{2}{3}$ months after the last *MC*, the epidermis was still three to six cells thick in a few spots, with a thin, overlying skim of keratin; the follicles were slightly widened; and the corium appeared denser than ordinary, with a few more lymphocytes and macrophages.

The course of events when *MC* had been applied in ether plus mineral oil was notably different. The changes seemed slight at the time the carcinogen was stopped, but 4 months later the skin had become markedly scurfed, was definitely thickened and indurated, and no longer hyperemic but pale. Punch biopsies showed a dense corium containing more macrophages than usual, overlain by a moderately thickened epidermis having flame-shaped, downward extensions of inactive cells along its base, apparently residual to hyperplasia (Fig. 15). These after-effects were most pronounced in D. R. 3-44 and 3-45. The healing of punch holes was now markedly pathological. The disc tissue forming to close the holes was much thicker than the rest of the ear, twice as thick in the case of D. R. 3-45, owing to excess both of connective tissue and of cartilage; and small petechiae sometimes occurred in the new tissue. Almost at once after the discs had been completed their epidermal covering on the side of the ear that had been exposed to *MC* became heavily and evenly scurfed, much more so than the

intact skin round about, and in D. R. 3-45 this change was soon followed by superficial, transitory ulceration at a few small spots on the discs. No such unusual features had been noted during closure of the punch holes made toward the end of the *MC* applications.

After another few months the general state of the skin had greatly bettered, yet in the case of D. R. 3-45 scurfing still took place of the discs filling the holes punched 235 days after the last *MC*,—this although the surrounding skin was smooth (Fig. 2). So too with the discs closing holes made later, including those of the 447th day. The discs dating from the 645th day however did not differ from the ordinary; they were no thicker than the rest of the ear, and their epidermal covering remained smooth. When the animals died,—and D. R. 3-37 survived for 4 years and 8 months after the last *MC*,—the skin showed only microscopic abnormalities like those present in D. R. 1-69 and 1-72.

In sum, the after-effects of *MC* on the skin were long lasting. Though a gradual return toward the normal took place it was still not wholly completed during periods up to more than 6 years after the last application of the hydrocarbon. The betterment began at once in the case of the animals receiving the *MC* in benzene, but when a mixture of ether and mineral oil had been the solvent the cutaneous conditions actually worsened during the first months after the applications were left off, a fact evident not only in the general state of the ears but in pathological healing where discing took place. The difference was due to a greater effectiveness of *MC* in Crabtree's medium, not to the solvent as such (4).

The Presence of Microscopic Growths

Search with the microscope showed that the pieces of skin punched out soon after the *MC* was discontinued seldom contained any perceptible tumors besides those already noted in the gross. But in the pieces taken from time to time later on, minute growths gradually became so frequent as to be present in practically every slice of tissue examined, often several of them in a single random section from a strip of skin 4 to 10 mm. in length (Figs. 5 and 6, 17 and 18).

A search with a binocular microscope magnifying 15 diameters, of one ear of D. R. 3-45 on the 505th day after the last *MC*, disclosed the presence of 65 minute growths on an area covering 10 sq. cm., amidst 25 growths perceptible to the unaided eye. This animal was one of the two with the greatest accumulation of visible tumors. Microscopic growths became still more numerous later in all of the rabbits, and at some spots caused a patchy, shagreen-like roughening of the skin. Fig. 20 shows part of the surface of a punch specimen procured from an ear of D. R. 1-69 nearly 2 years and 10 months (1020 days) after the last *MC*. Fewer visible tumors had accumulated in this animal than in any of the others (Chart 1), yet the skin surface is studded with growths imperceptible to the naked eye,—tiny, discrete, barely raised, smooth or rugose mounds, round plaque-like scabs of keratin, and shallow craters or saucer depressions, all consisting of neoplastic cell as sections showed. When the animal was killed, a few months later, the number of tumors visible in the gross was still increasing.

Only a small proportion of the microscopic growths ever became large enough to chart. Those that were punched out and searched for mitoses in serial sec-

tions proved practically devoid of them. The papillomas had the morphology bespeaking inactivity or retrogression (Fig. 17), but most of the frill horns proved wholly characteristic, even when composed of very few cells. Many lay wholly beneath the surface (Fig. 7), and could be perceived only in sections.

The Presence of Latent Neoplastic Potentialities

The hundreds of tumors visible in the gross, which gradually assembled, were the residue from thousands. When one adds to these hundred the multitude disclosed on search of the skin surface at low magnification, and those still smaller growths revealed in sections, it becomes evident that an immense number of epidermal cells, all told, underwent neoplastic change as result of the action of *MC*. Previous work has shown that tar, benzpyrene, and *MC* render many more cells capable of forming tumors than do this under ordinary conditions, as also that discing will often stimulate them to form visible aggregates (1, 4). Most of the punch holes of the present experiments were made for this purpose, and careful record was kept of the number and character of the growths elicited. Our previous experience had been that they frequently start forth and enlarge with immense rapidity during the discing of skin recently exposed to a carcinogen, although there may be few or none elsewhere on the ear.

As already remarked, the ears of agouti rabbits are notably big, and hence many holes could be punched without compromising too much of them. Thirty-four were made in the ears of the five rabbits during the period of *MC* application, and more than a hundred later on. Care was taken to place each well away from the sites of previous ones, and the skin was inspected beforehand with a lens to make sure that no perceptible tumors existed where the edge of the cork borer went through,—except when growths were purposely to be cut across. During the *MC* period much of the punching was done to procure existing tumors for examination, and the holes left were mostly 4 to 6 mm. across. Later, when the object was to test for neoplastic potentialities, they ranged from 5 to 13 mm. in diameter, and often four to six were punched at one time at widely differing situations in order to obtain representative samplings. The animal with most (D. R. 1-72) had eight holes punched before the carcinogen was left off and 38 at intervals later; and though some were more than a centimeter across all closed completely except four which nearly closed.

The course of discing in ears previously tarred has already been described (1). It took place much more rapidly when the holes were punched immediately after the last tarring than when, after the lapse of several weeks, the acute inflammation due to this agent had subsided. The same was found to hold true of the *MC*ed ears. Later on, in the case of these organs, the rate of discing became almost constant. It was ordinarily completed during the 5th week, when the holes had been 5 to 7 mm. across in the distal third of the ear, or 6 to 10 mm. across nearer the base. The rabbit that lived longest, D. R. 1-72, provided an exception however; healing was definitely slowed after it became old and gaunt. As already related, the discs formed some months after the application of *MC* in Crabtree's solvent differed considerably from the ordinary. By then the skin had become more markedly disordered than before, and the discs were unusually thick and were scurfed even when the ear surface was smooth elsewhere (Fig. 2). Nevertheless closure of the holes took place at about the usual rate.

The discings elicited numerous tumors, and their situation and course were almost diagrammatically plain. Most arose at the original rim of the hole, but many occupied segments of the disc, or formed radial ridges or strap-shaped mounds extending toward or quite to its center (Fig. 4 *a* and *b*). A few of the growths were central.

The tumors which emerged toward the end of the applications of *MC*, in response to discings, appeared soon after the holes began to close, and grew fast and were fleshy (Fig. 1). They were the usual papillomas, carcinomatoids, and frill horns. Those originating at the edge of the hole often extended in concurrently with the ordinary hyperplastic tissue next them, reaching the center of the hole when this did, or somewhat before, and not infrequently occupying much of the disc secondarily. Such a rapid response became exceptional later on, though even after years discing occasionally elicited a vigorous growth almost at once, usually a papilloma, rarely a carcinomatoid (Fig. 9). Most of the disc tumors now became visible only some while after healing had been completed, often weeks after, gradually manifesting themselves as low, dry rugosities or scabs at the periphery of the disc, or along radii or segments of it (Figs. 3, and 4 *a* and *b*). This tardy response to the stimulus of wound healing was also manifest in the behavior of such previously existing growths as were cut through, or cut next to, when punch holes were made,—a procedure utilized now and again to find out how readily they could be stimulated. Some of the growths tested in this way seemed uninfluenced, failing either to become larger or to take part in disc formation; but others did so, though their participation in the disc did not usually become perceptible until some while after it had been completed, low, segmental plateaus or rugosities, with their base on the parent tumor, gradually developing then. Sometimes biopsy and the microscope were needed to make plain that they were really neoplastic in character.

The conditions provided by the new discs encouraged the growth of the tumors only until ischemic scarring supervened, when most of them retrogressed and the remainder became quiescent, no new ones arising. In time the old discs became relatively free from growths as compared with the increasingly crowded, general surface of the ears.

The pathological discing already described as taking place some months after the application of *MC* in Crabtree's solution had no evident effect upon the character of the tumors arising.

The number of growths called forth by healing of the holes made at various times finds record in Chart 3.

The yields are calculated in terms of total centimeters of punch-hole rim from which the healing took place (irrespective of the number of holes), since it was from cells next this rim, or their proliferating descendants, that the tumors arose. There were large variables which could not be controlled. When the holes were small, as in the case of those made to remove existing growths for examination, they closed so soon that the stimulus of wound healing was relatively brief. Also, when only one hole had been made, the occurrence of a single disc tumor, or the absence of any, yielded a figure which could be widely misleading because the total healing edge was so short. For this reason the findings with rims less than 3 cm. long stand isolated in Chart 3, not joined to the generality by connecting lines. From the total rim lengths, as given, the number of holes made can be roughly inferred, over 10 cm. implying four holes usually, and sometimes six. Always, as the number of tumors on the intact skin became great, they proved most abundant near the middle of the ears, and, as might have been expected, discing in this region called forth most growths. Hence it was necessary to

punch several holes, where visible growths were few as well as where they were many, if a reliable sampling of the skin was to be obtained. After the tumors became multitudinous holes were only occasionally made.

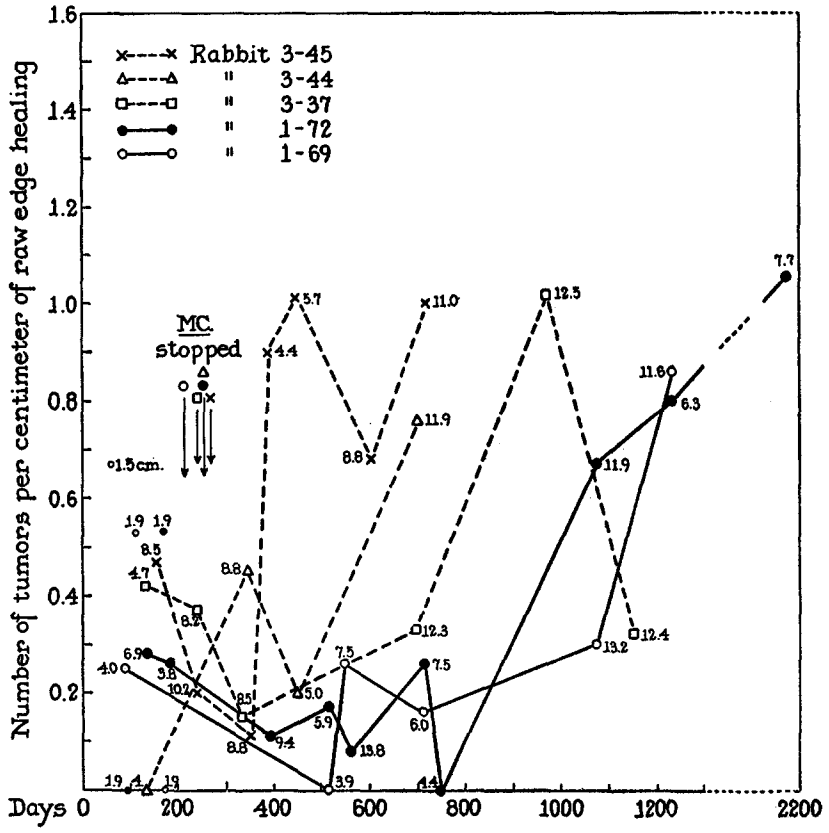

CHART 3. The neoplastic yield of discing.

The circles, squares, triangles, and X's tell when holes were punched in the ears. Their places along the abscissa mark the day. The numbers entered next each symbol give the total length in centimeters of the healing punch-hole rim from which the tumors took origin. When it was shorter than 3 cm., the symbol is smaller and lies separate,—not connected by a line with those indicative of longer rims.

It will be seen that before the *MC* was stopped, and in the months immediately succeeding, discing called forth few growths as compared with the number elicited later on.

There was one obvious reason for the increase; everywhere in the cutaneous expanse there now existed many hidden aggregates of neoplastic cells on the way toward forming visible masses, and the stimulus of wound healing was exerted upon these as well as upon solitary tumor cells. The aggregates should have formed visible growths the more readily.

That they sometimes had reached a considerable size without giving sign of their presence was evident from the not infrequent appearance of segmental tumors with a base as much as 2 mm. across at the periphery of the disc. No such broadly segmental growths had been called forth earlier.

Four holes were made in the distal thirds of the ears of D. R. 1-72 5 years and 3 months (1921 days) after the last *MC*. Careful preliminary search with a lens had disclosed no growths where the cutting edge was to go through. Nevertheless eight tumors arose from 7.7 cm. of healing rim, and this at a time when those on the unaffected ear surface were decreasing in number (Chart 1). All of the new tumors were slow to appear and indolent, yet their occurrence sufficiently proves that numerous hidden neoplastic potentialities still existed in the cutaneous tissue.

No indubitable carcinomas arose in response to discing, though vigorous carcinomatoids were occasionally got. These afterwards became dry papillomas or retrogressed.

Four holes were punched in the ears of D. R. 1-72 on the 985th day after the last *MC*; and the discing that followed elicited 10 tumors. Nine appeared late and remained tiny and dry, but the tenth arose practically at once after healing started and grew vigorously as a fleshy, peripheral mound which soon became more than hemispherical. The mound was 7 mm. across on the 20th day of healing, when a slice was taken through its middle (Fig. 9). Closure of the wound was swift, the tumor continued to enlarge, and soon it occupied about half the disc, overhanging the rest as a somewhat flattened sphere scabbed on top. It was photographed on the 47th day (Fig. 10), and, since it appeared to be no longer growing 3 days later, a hole 1 cm. across was punched next it for stimulation, and incidentally about a third of it was removed for section. This showed, like the first specimen, what seemed to be squamous-cell carcinomatosis along the base of the growth (Fig. 12), differentiating superficially into keratinized cysts lined with papillomatous epithelium (Fig. 11). As discing took place the growth enlarged further, reaching a diameter of 1.4 cm. within the next 16 days. The contrast with the tiny, dry tumors elsewhere on the ear was extreme (Fig. 13). Now the animal tore much of it away, leaving a sanguineous mound, which soon began to dwindle. With the aim of further stimulation another hole, 4 mm. across, was punched, 29 days after the previous one, partly through the mound and partly through the original disc. But this step was taken too late: sections of the piece removed showed that all the neoplastic tissue had already died, leaving merely masses of keratin surrounded by giant cells, amidst a profuse vascular reactive tissue (Fig. 14). In another 10 days the mound was gone, although discing next it had proceeded actively.

The rapidly growing, mildly aggressive tumor just described had the characteristic features of a papilloma stimulated into becoming a carcinomatoid. Its significance lies in the fact that it arose where the skin looked normal, and that it was called forth by the healing of a wound made nearly 2 years and 8 months after the last exposure to *MC*.

The Carcinomas

The true cancers arising in response to tar, benzpyrene, or *MC* are not always distinguishable from carcinomatoids, though usually this is the case. Those apparently malignant growths which become mere benign papillomas after stimulation of them is discontinued, or which then round up into inert cysts lined with a merely hyperplastic epithelium, are manifestly no cancers. The difficulty arises with such tumors as vanish utterly; some genuine carcino-

mas induced by tar or the carcinogenic hydrocarbons may do this even after they have destroyed a large part of the ear (6, 5). Yet again it is often possible to discriminate. All carcinomatoids, however active, are composed of cells like those of ordinary epidermis when extending in more or less anaplastic form amidst or under blood clot to cover a surface wound (7); and the same holds true of the spurious cancers which follow upon the subepidermal injection of Scharlach R in olive oil (8). The epithelium of the growths of both these sorts tends to differentiate after a brief period and round up into mere keratinizing cysts. When however the cells of an apparently malignant growth induced by a chemical carcinogen show bizarre division figures and other pathological abnormalities, one can be sure that here is a genuine carcinoma. Unfortunately not all show such traits, some that grow progressively having the morphology of mere carcinomatoids. Yet these can still be recognized often for what they are. Carcinomatoids are wholly dependent for their existence as such on extraneous stimulation by influences promoting cell multiplication (4, 5), and when an epidermal growth that is apparently malignant arises in the lack of such stimulation, as for example from skin that has reverted far toward the normal, months or years after exposure to a carcinogen, one is warranted in assuming that the growth is a cancer, even though it eventually retrogresses.

The tumors now to be considered, which we have classed as carcinomas, arose after *MC* has been left off, and, with one exception, while the ears were reverting to the normal, that is to say under conditions wholly adverse to the occurrence of carcinomatoids. Mention has already been made of them.

D. R. 1-72. On the 16th day after *MC* was left off a purply mound was noted to have arisen where no tumor had previously been perceptible, and soon it ulcerated and reached a diameter of 2.5 cm. It persisted 9 months, disappearing after the second of two punch biopsies which had disclosed it to have the morphology of a squamous-cell carcinoma (4).

A second similar mound suddenly developed 5 years and 5 months after the last *MC*. It underlay three of the many small, benign growths then present, a tiny cauliflower and two discrete, rugose plateaus, all papillomatous. Ulceration of the fast-growing new tumor soon destroyed these growths and it had to be punched out *in toto* to save the animal. Microscopically it was an invasive, squamous-cell carcinoma composed of markedly pathological cells.

D. R. 1-69. This cancer, like the first of D. R. 1-72, has been described previously (4). On the 267th day after the last *MC*, that is to say after the ear tissues had reverted far toward the normal, a tiny, keratinized papilloma was seen to have been replaced by a fleshy, raised, sanguineous disc 8 mm. across. The disc dwindled and disappeared within the next 6 weeks.

On the 1217th day, after a period of 3 months in which the ears had not been charted, a raised, scabbed disc nearly 2 cm. across was found to have formed, about 4 cm. distal to the previous tumor. The scab covered an area where four small, dry growths had been present, and a mound opposite it on the outside of the ear testified to its extension through the cartilage. It soon ulcerated and occupied much of the organ. Sections showed an anaplastic, squamous-cell carcinoma with many pathological features.

D. R. 3-44. On the 472nd day after the last *MC* a fleshy papilloma was noted to have arisen near the thin edge of one ear. It appeared to have originated deep in the tissue, grew fast, and within 2 months had become a cone 4 cm. high and 2.4 cm. across, consisting for the

most part of vertically striated keratin but with a bulging, infiltrating base. Successive biopsies showed an aggressive papilloma of malignant aspect which had broken up along the base into squamous-cell carcinomatosis. Soon all the keratinized material came away, leaving a raised, broad ulcer which remained indolent until the animal died, nearly a year later. Sections now showed only the carcinoma.

D. R. 3-45. On the 984th day after the last *MC* a tense, purply dome was found, covered with epidermis on which were many dry, minute growths. It soon ulcerated and when the animal was killed, on the 1111th day after the last *MC*, had destroyed much of the ear. Five days previously a similar subepidermal dome, with a dry, melanotic wart on its top and several small, discrete verrucosities on its slopes, was noted on the other ear. It too was a squamous-cell carcinoma but of very different morphology.

Cancer never appeared in D. R. 1-37.

The animals died or were killed before even the most aggressive of these seven carcinomas had run its course. The tumors were first noted 16 days to 5 years and 5 months after the *MC* was left off. One derived from a papilloma that was typical save in its great vigor, another replaced a small, benign growth of the same sort, and four more originated beneath the surface at spots where such benign tumors already existed. Only one developed where none of the latter was present. It arose in the depths of a skin from which benign tumors were continually originating.

No cancer derived from a frill horn has as yet been encountered in the many animals studied with this possibility in view.

ANALYSIS AND DISCUSSION

Conditional Nature of the Benign Tumors

The immediate effects of *MC* on rabbit skin have already been described (4). During the period of application it calls forth many more benign than malignant tumors, the latter arising but rarely. The growths are of precisely the same kinds as those due to tarring, a fact which exemplifies the rule that the tumors induced by the carcinogenic hydrocarbons have cytological characters expressive of certain neoplastic potentialities of the epidermal elements, not of any peculiarities of the hydrocarbons individually (4). Nevertheless the vast majority of the benign tumors (papillomas and frill horns), which appear during the period of exposure to tar and *MC* respectively, are so influenced by these agents as to differ greatly in both aspect and behavior. The reason is not far to seek. Tar does more besides initiate neoplastic changes; it markedly promotes the activities of the changed cells, with result in exuberant growths, whereas *MC* has but a mild influence of the sort (4) and in consequence nearly all of the tumors to which it gives rise are notably small and dry.

Papillomas and frill horns are conditional growths, not only incapable

of proliferating without aid but even of maintaining themselves (4, 5). This aid tarring provides, both by direct stimulation of the tumor cells and by the production of favoring disturbances of the supporting tissue; but if the applications are stopped soon after the first growths appear they gradually vanish as the skin reverts to the normal, and no more arise. It had seemed likely that this would be the course of events after *MC* was discontinued, but such was not the case; though the existing tumors soon became tiny or disappeared, new ones developed, and for a long while steadily accumulated. The state of the skin provided a sufficient explanation of this difference. Tar exerts so great a stimulating effect that some of the cells rendered neoplastic by it quickly form tumors, doing this while the cutaneous tissues are still so little damaged as soon to return to the normal if the tarring is stopped. *MC* on the other hand calls forth no growths until applied over a much longer period, and during it the skin becomes so seriously disordered as to remain for years in a condition favoring the multiplication of those neoplastic cells that require aid. Only in the case of the longest-lived of our animals (Chart 1, D. R. 1-72 and 3-37) did the cutaneous conditions at last so nearly approximate the normal that tumors tended to vanish more often than they arose, with result that their number fell off.

Factors Involved in the Linear Accumulation of Tumors

The steady accumulation of papillomas and frill horns after the exposure to *MC* had ceased was the more remarkable because of their transitory individual existence, all, with a few exceptions, disappearing after some weeks or months. The reason for their retrogression was not obvious: microscopically the neoplastic tissue seemed in good state while diminishing in quantity, and only very occasionally did lymphocytes and macrophages gather around it in considerable numbers. That the growths had evoked no general resistance on the part of the host was sufficiently proved by the development of new tumors as the old of identical types disappeared. It seems likely that they called forth adverse reactive changes in the tissue round about them as they enlarged, and thus brought about their own undoing; yet numerous sections colored with van Gieson's or Mallory's triple stain have failed to yield evidence supporting this assumption. No significant tissue reaction could be discerned about the many growths studied which were of microscopic size (Figs. 6, 7, 17, 18); one could not tell whether they were coming or going. The balance between success and failure must have been exceedingly delicate, as in the case of the benign tar tumors, of which many disappear even though tarring is kept up or though some other agent promoting cell proliferation, turpentine for example, is applied (5).

Throughout several years the rate of tumor accumulation was linear (Chart

1). To what can this have been due? The possibilities seem somewhat as follows:—

(*a*) *All of the tumors arose from cells rendered neoplastic during exposure to MC. Through its influence they became endowed with widely differing, evenly graded abilities to proliferate, and in consequence formed visible tumors in orderly sequence throughout the years.*

Cells undergoing neoplastic changes of identical kind, as demonstrated by the eventual tumors, frequently differ widely in proliferative vigor; and the assumption seems justified that when such cells lie scattered individually in great number throughout a tissue, the growths resulting from their multiplication will develop in a graded succession, other things being equal. Past work has sufficiently shown that latent neoplastic potentialities often exist in skin previously exposed to a carcinogen (1, 4); in D. R. 1-72 of the present work new tumors arose where punch holes were healing that had been made 5 years and 3 months after the last *MC*. Hidden neoplastic cells are indubitably responsible for some of the potentialities; for the application of non-carcinogenic agents which encourage tumor growth will not infrequently cause vanished papillomas and carcinomatoids to reappear in identical form where they were before, even though a considerable interval has elapsed (5).

As against these facts there are others rendering it impossible to suppose that a vast multitude of neoplastic elements, coming into existence while the skin was painted with *MC* or within a few weeks after,—for the purple fluorescence in ultraviolet light, bespeaking the presence of the carcinogen, lasts that long,—lay hidden in our animals until one by one, in due order throughout the years, they gave rise to tumors. As ready stated papillomas and frill horns are dependent on aid not only for enlargement but for existence. The slight to moderate cutaneous inflammation which develops during the application of *MC* in benzene or Crabtree's medium supplies such aid, but as the inflammation subsides, during the first few months after discontinuance of the carcinogen, the growths dependent upon it tend to disappear (5), a phenomenon plainly evident in our rabbits. The state of the skin of the animals which had received *MC* in benzene bettered progressively later on, and one might have supposed that in proportion as this bettering took place the papilloma and frill horn cells, forming tumors in their turn so to speak, because endowed with less vigor than their predecessors, would have been further handicapped by the diminution in the aid they received, and that existing growths would have disappeared more and more quickly. Because of both these occurrences a steadily slowing rate of tumor accumulation was to have been expected, instead of the observed constancy.

The linear increase of growths in the rabbits getting *MC* in Crabtree's

medium is even more difficult to understand on the assumption that they all derived from cells rendered neoplastic during the period of exposure to the carcinogen. After the acute inflammation due directly to the *MC* had subsided marked chronic changes took place, the skin becoming thicker and much more disordered. The changes went on for several months, and only slowly at last was the trend toward the normal resumed; yet throughout these vicissitudes, and for a long while after, tumors kept on accumulating at a constant pace.

The number of growths called forth by the healing of punch holes made shortly before *MC* was left off and during the next few months gave no hint of the presence in the skin of any considerable number of latent neoplastic cells. Repeated discings within this period elicited no more than one tumor for every 2 cm. of raw edge (or slightly less than half a tumor per centimeter, according to the chart), and in most instances only one tumor for every 5 to 10 cm. of raw edge,—this though the stimulus of discing extended at least a millimeter away from the edge of the hole, as shown by the situation of the growths it evoked. And there were reasons why discing should have been especially effective in eliciting tumors during the first months after exposure to *MC*. Observations on tarred, benzpyrened, and methylcholanthrened skin have all shown that at this early time the cutaneous tissues are in a highly responsive state (4), the epidermis proliferating practically at once where wounded. That the neoplastic cells hidden in it are then also "on their toes" is shown by the starting forth of growths almost immediately after discing begins,—and by their fleshy character, and frequent carcinomatoid form.[1]

(*b*) *The chronic cutaneous disturbance brought about by MC was itself carcinogenic, continually inducing new neoplastic changes with result that tumors kept on arising.*

In support of this hypothesis the prolonged changes preceding many cancers of the human skin can be cited; and indeed the after-effects on rabbit skin of *MC* in Crabtree's solution had for a time features in common with the x-ray alterations leading to cancer, the state of the rabbit skin for some while worsening. But the worsening went on for only a few months, and thereafter the skin trended steadily toward the normal. Throughout these swings away from and toward it the rate of tumor accumulation remained linear, as in the groups of animals which had shown cutaneous betterment from the first, those that had received *MC* in benzene. One can scarcely suppose that an evenly

[1] As time passed the neoplastic cells became much less responsive to the discing stimulus, and many cannot have responded at all. The biopsy specimen of Fig. 20, obtained nearly 3 years after the last *MC*, shows a skin thickly stippled with microscopic growths; yet healing of the hole made to obtain the specimen was attended by the formation of only four tumors visible in the gross. Most of the tiny growths that the cutting edge went near remained unaffected by the reparative stimulus. This held true also of some larger tumors purposely cut through on other late occasions to find how they would behave.

sustained carcinogenesis consequent on the pathological state of the skin would have gone on throughout these pronounced changes in its condition.

(*c*) *The MC started cells in great number toward becoming neoplastic, but at widely differing speeds, a process automatically consummated later.*

Facts recently brought forward by Kennaway and Kennaway (9) give strength to this explanation. These workers have shown by means of comprehensive statistics that carcinoma of the human penis, appearing in old age, is consequent in many instances on a phimosis surgically relieved in adolescence, and that penile cancer practically never occurs when circumcision has been done at birth. In Lenowitz and Graham's cases of deferred penile cancer the average interval from circumcision in adolescence to manifest tumor was 22 years; and these authors explicitly state that any inflammation due to the phimosis has long since disappeared (10). Dean reports that in the years prior to appearance of the cancers in his patients "none of them had cause for thinking that anything was wrong." (11) The evidence as a whole has lead E. L. Kennaway (12) to conclude that in all probability a train of cellular events is set going during the period of phimosis, which continues on throughout many years after the removal of its initiating cause, and culminates in malignancy.

The fact that the penile cancers were all long deferred tells much, in the present writers' view, as to how they arose. Tumor cells proliferate faster in young hosts, as is well known. Had the conditions prior to circumcision at adolescence caused any cells to become neoplastic forthwith, surely now and again one would have given rise to a visible growth before any long time had elapsed. Yet no cancers in recently circumcised young adults have been reported, though several have been described in which circumcision, done in late adult life to relieve chronic inflammation, was promptly followed by malignancy (13).

A continual production of new tumor cells in our rabbits as result of neoplastic changes merely started by *MC* but consummated later at widely various, evenly graded rates, will best account for the observed happenings. On this basis one can understand why the early discings elicited few growths instead of the many that should have been called forth had latent neoplastic cells been present in the multitude required to account for all the tumors arising in after years; it would appear that in the early period relatively few cells attained to the neoplastic state as compared with the eventual horde. A consecutive arrival of other elements at this state will account for the abrupt appearance now and again, under progressively less favorable cutaneous conditions, of tumors manifesting exceptional vigor. Every so often a papilloma would suddenly emerge on the skin, displaying great energy at first as if it had taken origin from a cell newly endowed with exceptional abilities, and

far outstrip the indolent generality in its enlargement, only like them to vanish in the end. Such vigorous growths could scarcely have been due to a proliferation of elements that had lain latent for years because intrinsically lethargic. It was as if they had but just gained their abilities; and so too with the cells forming those cancers which abruptly asserted themselves and grew rapidly years after the last application of *MC*,—5 years and 5 months after in the case of D. R. 1-72.

In sum, the constant accumulation of tumors throughout a long period of cutaneous vicissitudes such as are known to affect their incidence and course is best understood if new cells continually attained to the neoplastic state, elements no less well equipped by reason of it than those in which it had first been consummated, and hence as capable of producing tumors,–in so far as the local conditions would let them. The eventual diminution in the number of growths in the animals that lived longest (D. R. 1-72, 3-37) may have been due less to the influence of cutaneous conditions at last decisively during the period of phimosis, which continues on throughout many years effective against tumor formation, than to ultimate depletion of a store of cells, —or the lineal descendants of cells,—which had long previously been started toward the neoplastic goal.

Latent Neoplastic Potentialities, or Latent Tumor Cells?

Though intercurrent factors have been shown to play a large and often a critical role in determining whether any tumor forms after a cell has arrived at the neoplastic state (1, 4), it is still uncertain whether such factors can hasten or retard this event. Hence it seems well to regard the growths called forth by discing and other promoting agencies after a carcinogen has acted, as representing the realization of *latent neoplastic potentialities* present in some of the cells affected (19), and not go so far as to deem the growths due necessarily to the proliferation of *latent tumor cells* already existing as such,—unless indeed these latter are known to be present, as in the case of those rabbit papillomas and carcinomatoids which can be called forth again by non-carcinogenic stimuli months after they have vanished (5). The skin of our rabbits contained latent neoplastic potentialities throughout all their lives after the exposure to *MC*.[2]

[2] Berenblum (20) has lately reported that the application of croton oil to mouse skin 43 weeks after a single application of 9:10-dimethyl-1:2-benzanthracene elicited tumors in as large a proportion of animals as when brought to bear much earlier. He assumed, as had experimenters with rabbits (1, 4), that the growths called forth after long intervals are due to the proliferation of tumor cells already in being. His data throw no light on whether the croton oil could have hastened neoplastic changes already in gradual process of consummation, nor was this the aim of his work.

Carcinogenesis in Man and the Rabbit

Carcinomas were exceedingly rare in the rabbits as compared with the almost innumerable benign growths, hidden and visible. This fact is the more worth remark because the induced cutaneous cancers of rabbits usually, perhaps always, derive directly from one or another of the benign tumors already present.

The sequence can be easily followed in tarred skin because tar so actively promotes cell proliferation that the benign tumors soon grow big. The first experimenters utilizing tar noted that the malignant growths it induced took off from papillomas as result of superimposed carcinomatous alterations in their cells (6), as further that the cancers originated from the more vigorous of these neoplasms. Non-carcinogenic stimuli have been observed to bring on malignant change in papillomas by rendering them active (4, 5), and the existence of a vast multitude of these growths in the rabbits of the present work would seem at first thought to have provided innumerable opportunities for cancer to arise; but this is to reckon without the indolence of the papillomas, a state unfavorable to such change.

The course of events in human skin exposed to tar, soot, or shale oil nearly resembles that in the rabbit, papillomas appearing prior to cancer but vanishing if the carcinogen has not rendered the skin enduringly abnormal before the exposure to it is stopped (14). If it is kept up some papillomas grow big, new ones appear, and carcinomas may arise, either from one or another of these benign growths, or to all appearance *de novo*, just as happens in rabbits. A similar course of events has been noted in the kangri cancer of Afghans; papillomatous warts appear on a skin disordered by many slight successive burns, and carcinomas arise from one or several of these growths, or where none had been visible (15). Benign papillomas have also been noted now and again as the forerunners of malignancy in scars due to burns.[3]

Burns sustained in childhood have provided some classical instances of deferred cancer.

One group of burn cancers seen in ageing people cannot be classed as deferred, yet have interest in the present connection. In them the burn calls forth a cancer from skin which is already in a precancerous state. The patients of Arndt's series (16) averaged 49 years of age when the burn occurred; their skin was atrophic and hyperkeratotic, and the burn itself was superficial; cancer arose after an average interval of 11 months. In Treves and Pack's cases the interval was shorter, 4 months on the average, with the patients averaging 52 years (17). Stauffer has told of a man 66 years old who developed cancer within 30 days after a "flash burn" (18); a doctor,

[3] In the days when British soldiers were flogged cancers sometimes developed more than 20 years after, in the scars of the lash, and they were in some instances preceded by "warts" and derived from these latter (21).

examining him shortly before the accident, had not observed any abnormalities in the area which was burned later, but had noted precancerous changes elsewhere on his face. Several authors have realized that in cases like this,—instances of "acute wound cancer" as they have been termed (17),—the thermal injury merely stimulates cells already far on the way toward becoming cancerous, if not already so.[4]

The second group of burn cancers comprises those in which the tumors do not arise until many years after the burn. In 50 per cent of Arndt's cases it had occurred during the first year of life, and the interval to cancer averaged 33 years. In six of his 81 patients the interval was 61 to 70 years. The burns had been severe, taking long to heal and leaving deep scars which in many instances had broken down repeatedly, with the formation at last of a persisting ulcer from the healing edge of which the cancer arose, sometimes preceded by an epithelial papilloma. Now and then however the scar remained intact until the tumor came to view. Treves and Pack noted that the cancers were sometimes multiple when the burns had been extensive, and that the age of the scar was more important than the age of the individual; if he was very young when the burn took place the cancer occasionally appeared when he was scarcely more than adult.

Of all human cancers arising from the scars of long previous burns only those few in which the scars have remained intact are comparable with the deferred cancers of the penis. In the other cases marked pathological changes had taken place in the burned area before the malignant growth arose. The breaking down of the scar with subsequent repair, a process often several times repeated, may have freed and stimulated neoplastic cells long bound within dense tissue, or it may itself have been carcinogenic.

SUMMARY

The ears of young adult rabbits were painted with methylcholanthrene (*MC*) long enough to call forth a few benign tumors (papillomas, frill horns), and the animals were followed throughout their later lives. Soon after the paintings were stopped the tumors began to dwindle and vanished, yet even while they were disappearing other growths of the same kinds arose, only to vanish later in their turn. For a long while more arose than disappeared, and in consequence the number of tumors increased throughout years. They accumulated at a constant rate despite concurrent changes in the supporting skin, which might have been supposed, on previous experience, to have prevented this from happening. Only in the old age of the animals did the number of tumors eventually fall off, and by this time the skin on which they had

[4] It may be asked why wound healing but rarely evokes tumors in man since it does this frequently in experimental animals. One is likely to overlook the fact that ordinarily in man carcinogenic stimulation is weak and limited, both in scope and time. In the experiments on rabbits the conditions were forced.

arisen, long since normal in the gross to all appearance, had become nearly so microscopically. Even then latent neoplastic potentialities still existed in the cutaneous tissue; where punch holes were healing new tumors arose.

A great multitude of hidden neoplastic cells were present in the *MC*ed skin, and from them many of the growths called forth by the stimulus of healing undoubtedly derived. Yet the facts make it difficult to suppose that the long accumulation, at a constant rate, of tumors visible in the gross was due wholly to the proliferation of cells rendered neoplastic during the period of exposure to *MC*, and lying hidden afterwards for periods determined by their differing, evenly graded capabilities. Nor can the accumulation be attributed to a sustained carcinogenesis resulting from the pathological state of the skin. As a whole the findings indicate that the linear increase in growths was due for the most part to a continual arrival at the neoplastic state and subsequent proliferation of cells, or the descendants of cells, that had been no more than started on the way toward becoming neoplastic by the carcinogen. There is clinical evidence for such a course of events.

Now and again a carcinoma arose from the skin previously treated with *MC*, but they were few in all, as would follow from the presence of local conditions unfavorable to malignant change. Some appeared only after years,—in one instance more than 5 years after.

The occurrence of deferred cancer in man can be understood in terms of the findings in rabbits.

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EXPLANATION OF PLATES

The photographs were made by Mr. Louis Schmidt. The sections had been stained with eosin and methylene blue.

PLATE 17

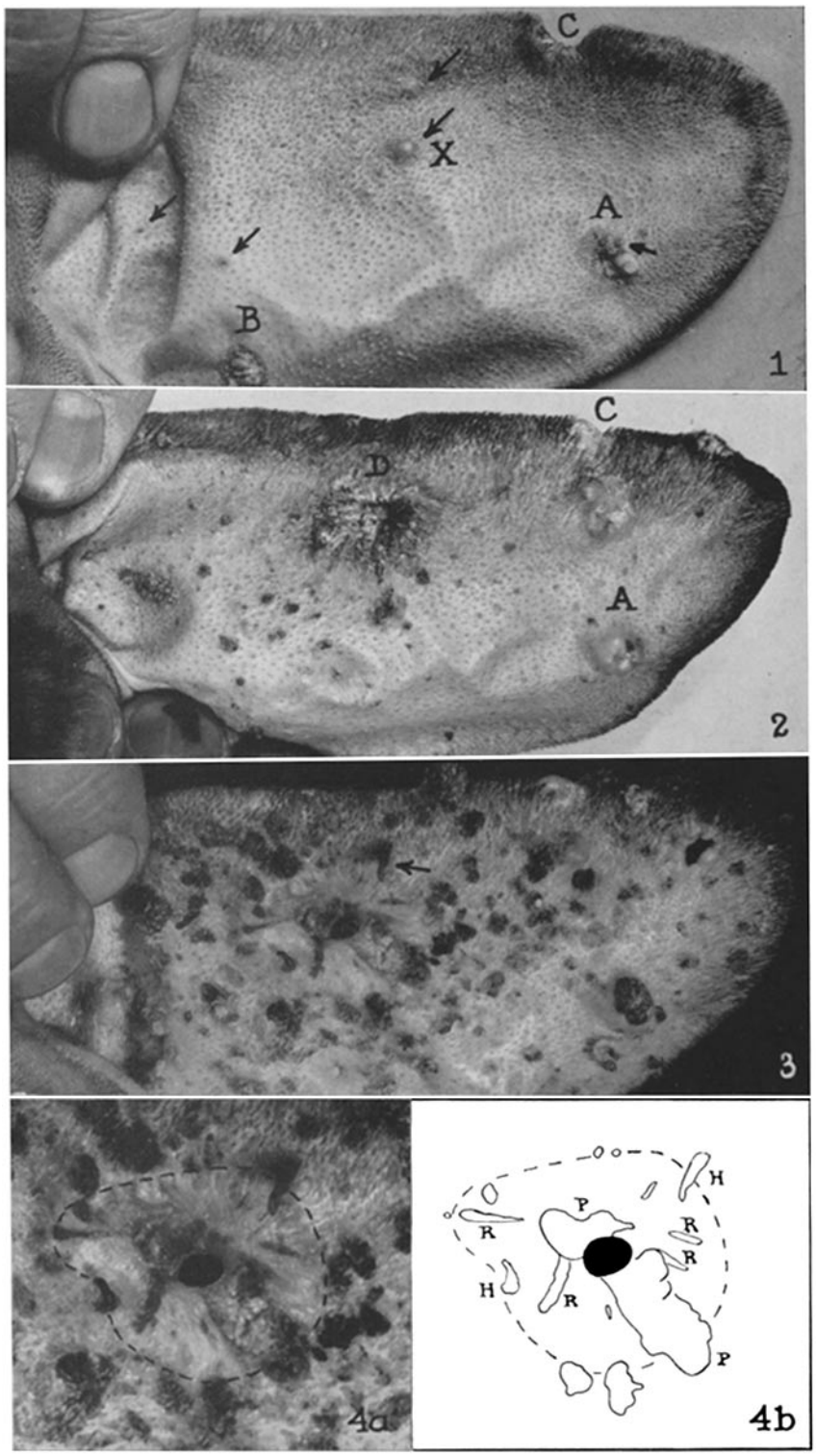
FIGS. 1 to 4. To show the progressive accumulation of tumors after exposure of the skin to methylcholanthrene.

The carcinogen had been applied to the ear of D. R. 3-45 for 161 days when Fig. 1 was obtained. By this time the mouths of the hair follicles were much widened with keratin, but only the slightest hyperkeratosis dulled the skin surface. Three punch biopsies had recently been made at *A*, *B*, and *C*, and at all of them, as healing took place, tumors had arisen from the new tissue, one growth low (at *C*), two others projecting, fleshy papillomas (at *A* and *B*). At *A* two tiny growths on the disc can also be seen (arrow). On the intact skin at *X* a fleshy papilloma is erupting, as later became plain, and elsewhere three more growths are just appearing (arrows).

The applications of *MC* were continued for 85 further days. Fig. 2 was obtained 235 days after the last of them. By then nearly all of the tumors previously present on the intact skin were either gone or had become small, dry, indolent, and in most cases melanotic. The follicle mouths were much less distended than before and the skin was again smooth and glistening; yet where healing had taken place of a huge punch hole made 49 days previously,—at the spot where the growth *X* of Fig. 1 had been present,—the newly formed epidermis was so heavily scurfed as to obscure tumors that had arisen on the disc (*D*). Elsewhere the scars of earlier biopsies can be seen, irregularly bulging because of excess cartilage formation. One of the tiny tumors present on disc *A* in Fig. 1 has persisted and become melanotic. There are many more growths than previously on the general surface of the ear, nearly all minute and most of them melanotic.

Fig. 3 was procured 705 days after the last *MC*. By now a horde of tumors has accumulated on the ear. The melanotic growth at *A*, previously tiny, now covers much of the old disc. The old disc at *D* is larger than before and irregular in shape because its proximal third was punched out during the period of scurfing, to learn more of the tumors this obscured. It is no longer scurfed, so the growths on its surface or at its edge are now clearly visible (Fig. 4 *a*). The hole made at the last punching has not completely closed. A typical frill horn 0.6 cm. high projects at the edge of the disc (*H*, Fig. 4 *b*), there is a taller one just outside it (see arrow in Fig. 3), and on the disc surface there are two rugose papillomas (*P*), one of them a broad band, as also several low, incompletely radial scabs (*R*), representative of very indolent growths of undetermined character. Nothing like these radial growths exists elsewhere on the ear (Fig. 3).

Figs. 1 to 3, × 1; Fig. 4 *a*, × 1½.



(Friedewald and Rous: Pathogenesis of deferred cancer)

PLATE 18

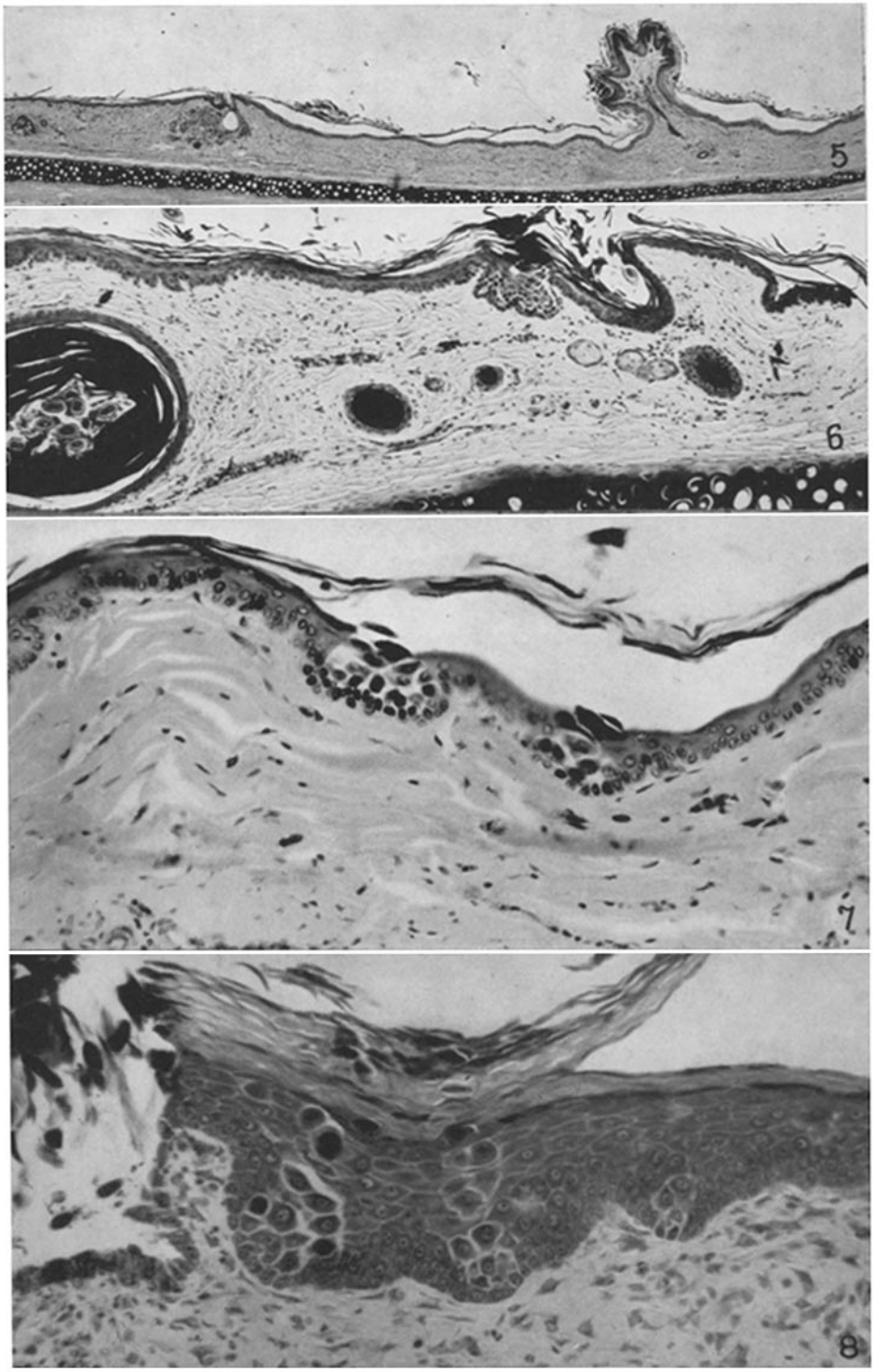
FIG. 5. Stalked papilloma punched from the ear of D. R. 1-69 on the 860th day after the last *MC*. The growth had persisted for almost a year with little alteration in size. Its epithelium is still typically papillomatous. The epidermal layer on the ear surface proper is thickened and slightly scurfed, and a distended hair follicle can be seen, with numerous sebaceous glands next it. × 22.

FIG. 6. Frill horn found in a punch specimen obtained from D. R. 3-37 on the 492nd day after the last *MC*. It lies wholly beneath the surface but is overlain by keratin denser and somewhat thicker than that round about, though not sufficiently so to have drawn attention in the gross. Serial sections of the tumor showed no mitoses. Elsewhere the epidermis is somewhat thickened and is irregular along the base. × 82.

FIG. 7. Tiny frill horns in skin removed from an ear of D. R. 1-72 three years (lacking 9 days) after the last application of *MC*. The cells are desquamating after keratinization instead of building up horns, and they have formed "bullet bodies." The neighboring epidermis is slightly thicker than normal and is covered with a thin layer of keratin. × 230.

FIG. 8. Islands of frill horn cells amidst epidermis formed where discing was under way of a hole made 37 days previously. Part of the growth from which they have derived can be seen at the right. It lies at the periphery of the disc and is of the desquamating type (4); scattered "bullets" can be seen above its basal layer, all the rest of it having come away. Toward the left, in the direction of the center of the disc, there are three separate islands of frill horn cells, diminishing in size, and a still smaller fourth one that is not in focus. Evidently some scattered cells from the frill horn were carried along with the ordinary hyperplastic epithelium as the latter grew in toward the center of the punch hole, but they proliferated so slowly as not to join up in a continuous radial growth. They are forming bullet bodies; characteristically, these lie separate from one another (see Fig. 7).

The animal came from a group not dealt with in the present paper. Its ear had been painted with 0.3 per cent benzpyrene in benzene twice weekly for 460 days, and the punch hole was made after a further 93 days. × 230.



(Friedewald and Rous: Pathogenesis of deferred cancer)

PLATE 19

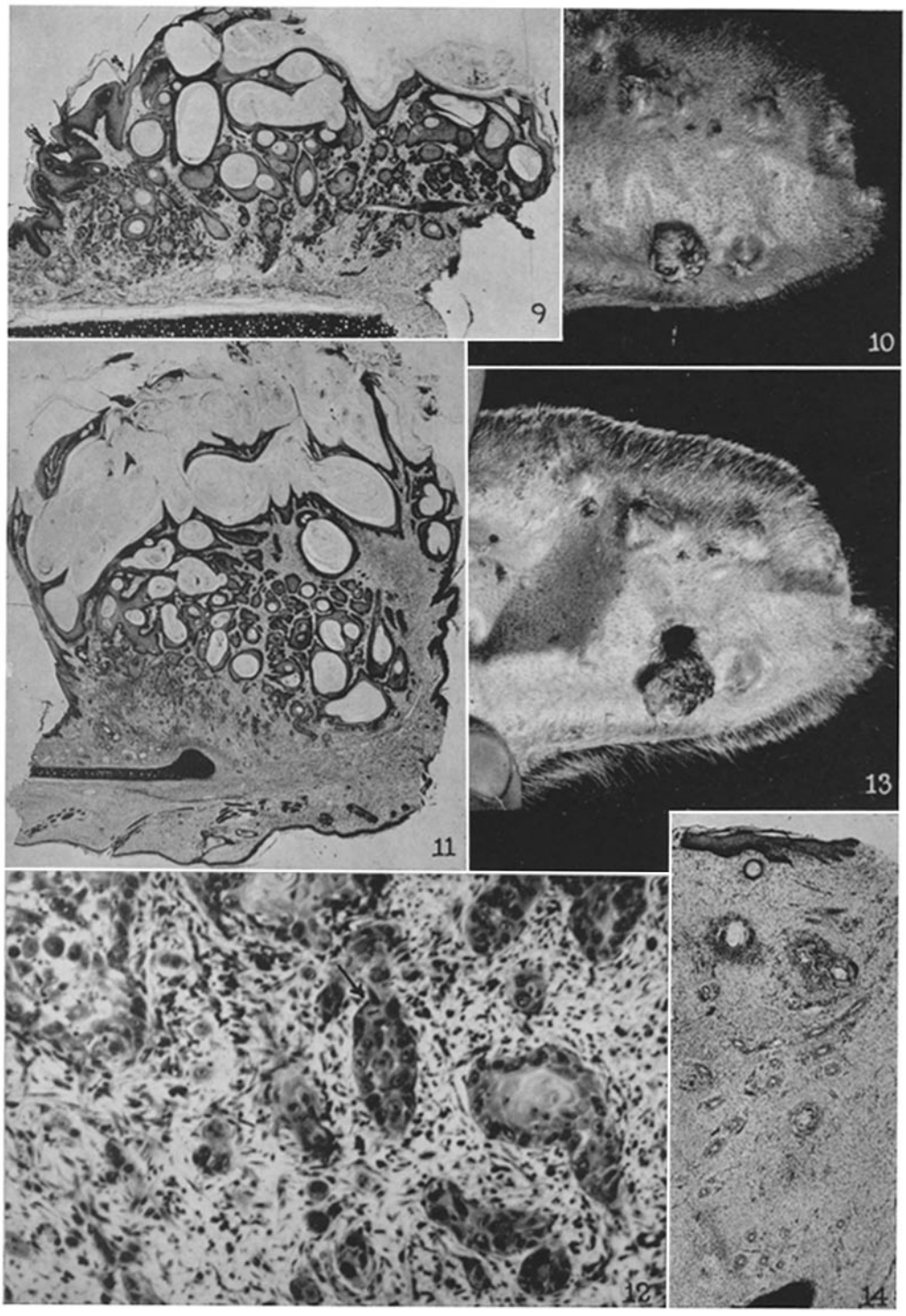
FIGS. 9 to 14. Stages in the life of a carcinomatoid which arose where a hole was closing that had been punched on the 985th day after the last *MC* (D. R. 1-72, see text).

The growth had become a high, rugose mound, 7 mm. across, when a sagittal slice, through it and the underlying cartilage, was removed on the 20th day of discing,—which had not nearly been completed (Fig. 9). It has the aspect of an aggressive, anaplastic, squamous-cell carcinoma along its base, but in its older, superficial part has differentiated and formed keratinized cysts lined with epithelium like that of a benign papilloma; and indeed characteristic papillomatous epithelium can be seen on the left covering the outer side of the mound.

The narrow gap from the biopsy rapidly filled in and by the 47th day the tumor was a fleshy, somewhat flattened sphere occupying most of the disc and hiding the rest (Fig. 10). It was cream-colored and dry toward the top and had almost ceased to grow, so on the 50th day a hole was punched next it, and incidentally a piece was removed. Sections showed it to have the same character as before (Fig. 11). The stroma along its invasive anaplastic base now contained numerous macrophages and lymphocytes (Fig. 12) as if it were beginning to fare badly, yet mitoses were numerous (arrow). The thickened cartilage is just beginning to extend into the disc.

Again the tumor enlarged rapidly and on the 66th day it had the aspect shown in Fig. 13. About a third of the mass, on the side away from the new hole, consisted of a peaked, vertically striated, creamy papilloma, whereas the rest was lower, discoid, ruddy, and topped with a sanguineous scab. The animal soon tore nearly all the tumor away, the remaining mass dwindled to a low mound, and a punch biopsy specimen obtained on the 81st day showed only some scattered keratinized masses, amidst reactive tissue (Fig. 14). Within a few weeks the residual mound had wholly disappeared.

Figs. 9 and 11, $\times 10\frac{1}{2}$; Figs. 10 and 13, $\times \frac{8}{10}$; Fig. 12, $\times$ 157; Fig. 14, $\times$ 21.



(Friedewald and Rous: Pathogenesis of deferred cancer)

PLATE 20

FIG. 15. Hyperkeratosis and the remains of extensions downward of an epidermis last exposed to *MC* in Crabtree's medium 112 days previously. The underlying corium is dense (D. R. 3-45). × 230.

FIG. 16. "Basket work" left after the retrogression of a papilloma which had extended downwards. Specimen procured 478 days after the last *MC*. The epithelial cords are two cells thick; they were devoid of mitoses. Elsewhere there is still slight epidermal hyperplasia, the corium is abnormally cellular, and some of the sebaceous glands are enlarged. × 90.

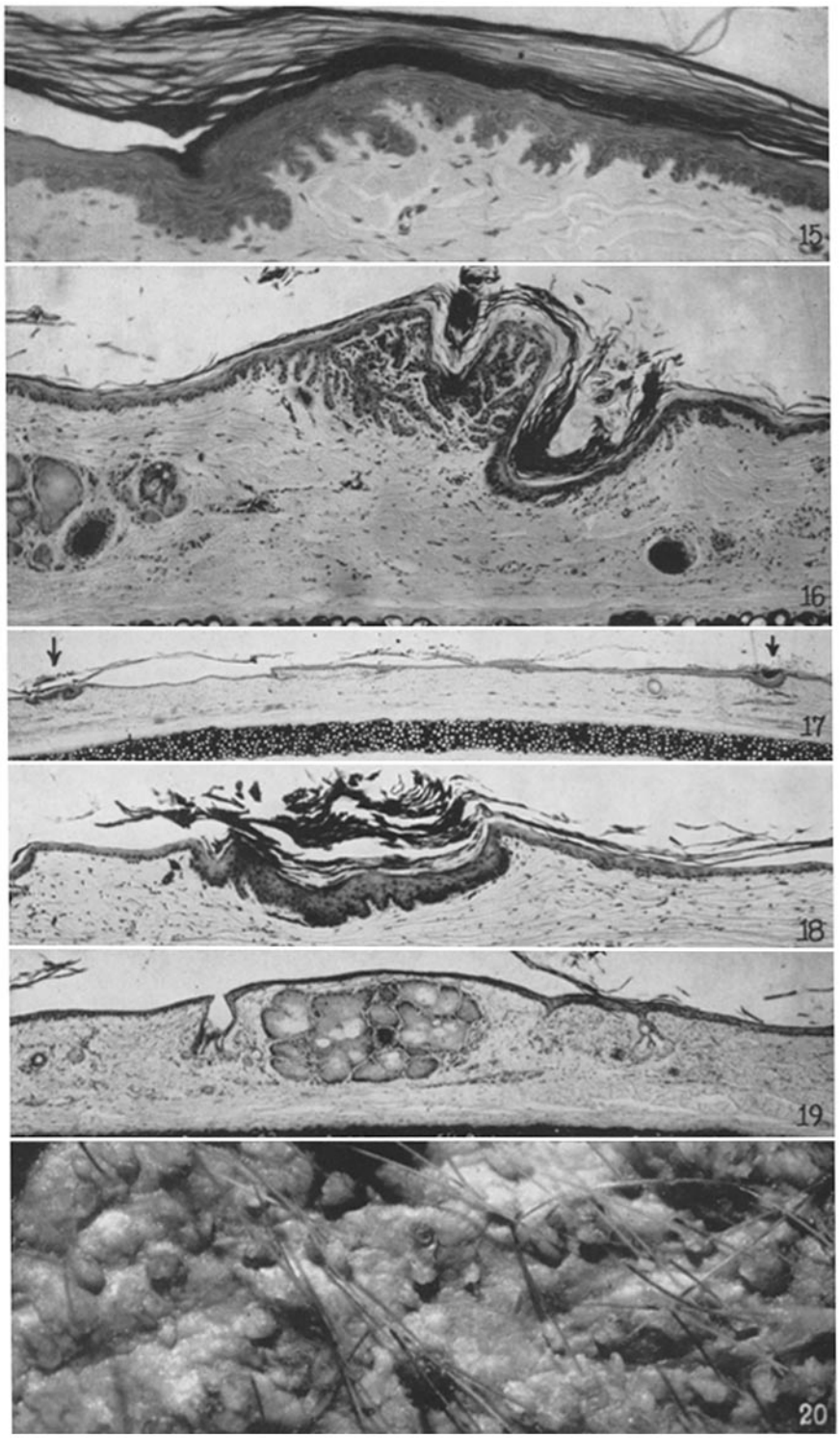
FIG. 17. Inactive microscopic papillomas (arrows) where the ear surface appeared devoid of growths; 724th day after the last *MC*. There is a slight hyperplasia of the epidermis generally. × 13.

FIG. 18. Another growth about half a millimeter across in the same piece of skin. Its morphology and staining reactions suggest a retrogressing frill horn. × 82.

FIG. 19. Enlarged sebaceous glands in skin last exposed to *MC* 966 days previously; some normal glands can be seen near by. The surface epithelium has now a nearly normal aspect, but there is still some follicular distension and the corium is abnormally cellular. × 55.

FIG. 20. Surface of a piece of skin punched out 1020 days after the last *MC*. In the gross it had appeared slightly roughened yet free from tumors.

The specimen was fixed in acid Zenker's solution and washed in water for 24 hours, which caused such swelling of the keratin that the minute growths stood forth as not previously. The photograph was taken with the specimen submerged in water. The tumors can be seen as scattered, discrete mounds, hassocks, or craters on a glistening, uneven surface. Healing of the punch hole made to procure the specimen elicited only four tumors visible in the gross. × 15.



(Friedewald and Rous: Pathogenesis of deferred cancer)