Early Changes in the Experimentally Produced Adenomas and Adenocarcinomas of the Stomach*

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(Received for publication November 1, 1947)

Until the work of Stewart and Lorenz in 1942 (7), attempts at producing adenocarcinomas of the stomach by means of the carcinogens had failed. Klein and Palmer reviewed these efforts in 1941 (3). Carcinogens had been administered with the diet mixed with roughage, placed in the mouth by dropper and kept within the lumen of the stomach for some time by means of paraffin pellets. Squamous-cell carcinomas were produced in the lower end of the esophagus and in the forestomach of rats and mice, but not adenocarcinomas of the glandular portion. The forestomach is lined with squamous cell epithelium and theoretically is not protected by mucin as is the rest of the stomach.

Stewart (6) placed the carcinogen within the wall of the stomach of mice by means of threads, oil, and horse serum. The greatest production of adenocarcinomas was obtained with a suspension of methylcholanthrene in horse serum. Of 47 mice injected with this suspension, 6 developed adenocarcinomas; 12, mixed adenocarcinomas and sarcomas; 6, adenocanthomas; 2, mixed adenocanthomas and sarcomas; 7, adenomas; and 3, sarcomas. The lesions were produced within 19 to 46 weeks.

In this investigation, threads impregnated with methylcholanthrene were used to produce adenocarcinomas and adenomas, and the early changes occurring about them were studied in an attempt to disclose the mechanism of formation of new growths in the stomach. The reactions of the cells of the stomach wall to unimpregnated threads and to threads containing a similar but noncarcinogenic anthracene compound were also studied in order to distinguish unusual changes about the threads containing the carcinogen. Previously, a comparison was made of early tissue reactions about carcinogenic and noncarcinogenic threads in muscle and skin in the formation of sarcoma and epithelioma (2).

METHOD

Untreated Corticelli silk thread, size B, was placed over methylcholanthrene and the powder was melted by heating. The thread was stirred into the liquid, which was subsequently allowed to recrystallize by cooling. Melting and stirring were repeated until the thread became swollen and yellow with the carcinogen. The noncarcinogenic compound was put into the thread by the same method. 1,2-Benzanthracene was used as the similar anthracene but noncarcinogenic compound. Nine investigators have failed to produce malignant tumors with this compound (1).

Rats of the Wistar strain were employed. Under ether anesthesia, an incision was made through the midline of the anterior abdominal wall and the stomach was delivered into the wound. By exerting tension on the pylorus, a fine straight needle could be pushed under the serosa towards the esophagus for a distance sufficiently great to allow the insertion of 1 cm. of thread. Its ends were cut just as they pierced the serosa; no long ends were left because they produce sarcomas outside the stomach. Threads placed in this manner lay between the serosa and mucosa, under the central or acid glandular portion of the stomach. The abdominal wall was closed with silk sutures.

The rats were fed on equal parts of Rockland rat pellets and Purina dog chow. Three rats were killed at weekly intervals for 35 days in order to study the early changes, then at 14 day intervals for 120 days. Others were killed from the 200th to 590th days when tumors could be palpated. A companion rat exposed to the carcinogen for the same length of time but without a palpable tumor was killed each time to ascertain why a tumor had not developed. In all, approximately 150 rats were used.

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* This work was financed in part by a grant from the National Cancer Institute.
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RESULTS

Figs. 1 and 2 illustrate adenocarcinomas and adenomas produced by the carcinogenic threads. The early changes preceding the formation of these tumors were as follows: Within a week after implantation, a ridge of heaped-up epithelium overlay the course of the threads. These ridges tended to ulcerate as a result of an inflammatory change. Within 72 hours after insertion of a carcinogenic thread, a heavy infiltration of round cells took place about it; blood vessels became dilated and more numerous at the periphery of the area of infiltration, edema occurred, and finally ulceration resulted (Fig. 3). Within 2 or 3 weeks, epithelial-lined clefts or sinuses formed in these ridges. Sinuses formed in the following manner: a small amount of thread became exposed as the result of ulceration in a ridge, or one end of a thread sloughed out into the lumen, or a thread perforated the mucosa during insertion. In either case, epithelial cells grew back along the portion of the thread that remained embedded, down along its course through the muscularis mucosae, and filling the tract. Often both perforation and ulceration worked together to produce sinuses.

Some ulcerations developed as early as 10 days after a thread was embedded, and eventually exposed all carcinogenic threads. For example, after 150 days, silk fibers were rarely found in any of the microscopic sections, indicating that either the threads had sloughed out or they had been easily removed by the microtome during cutting and therefore were not well encapsulated in fibrous tissue.

Clefts formed whenever the mucosa was destroyed over a long length of thread and yet it remained long enough within the stomach wall to allow the defect to become lined with new epithelium. Grossly, clefts were paralleled on either side by ridges of hypertrophied mucosa, and when these ridges were pulled apart in some of the early specimens, the thread could be seen within the depth of the cleft. When a cleft did not extend for the entire length of a thread, sinuses formed on either end of it.

The new epithelium that grew into and lined these sinuses and clefts came from the surrounding mucosa. As soon as a break occurred in the muscularis mucosae, the superficial mucus-secreting cells extended downward in a single row. They quickly formed acini in the sinuses and completely filled the tube-like defect with a cylinder of acini by the 26th day, as shown in Fig. 4. Around the circumference of the cylinder a very thin rim of reticulin was deposited, but no reticulin was deposited between the acini. These mucous cells, extending in a single row, first relined the inner circumferences of the clefts and then proliferated to form a new mucosa, less thick than the original and with rudimentary crypts (Fig. 5). Beyond it a fairly deep layer of reticulin was deposited. The morphology of this new epithelium that now existed below the muscularis mucosae resembled more that of the prepyloric antrum of the stomach than that of the acid pepsin-secreting epithelium of its origin. Special stains for pepsin failed to color this epithelium, indicating that this enzyme was not secreted. No eosin-staining acid cells were seen.

Instead, in both the sinuses and clefts, the epithelial structures were composed of cuboidal cells that took a basophilic stain. The cells were not irregular in size nor contour, nor did they contain an unusual number of mitotic figures. Many of them stained with mucicarmine and some contained pink droplets, indicating the presence of mucus. Occasionally they formed mucous cysts. No further change occurred in the morphology of these misplaced epithelial structures until after the 35th day.

Ulcerations sometimes occurred about the control threads impregnated with 1,2-benzanthracene but sloughing did not take place as rapidly, as frequently, or as completely. In fact, about half of these threads finally became encapsulated. When ulceration occurred, however, or when one of these threads perforated the mucosa, the superficial mucosal cells likewise grew downward in the manner just described to form epithelial-lined clefts and sinuses. In the sinuses acini formed and inside the clefts mucus-secreting cells later proliferated to form a new epithelium. Both architectures persisted thereafter without subsequent change. Neither pepsin nor acid-secreting cells ever reappeared among these cells that were observed to continue to exist below the muscularis mucosae for as long as 150 days. In some instances they did become slightly atrophic.

The downgrowths of epithelial cells around the 1,2-benzanthracene threads also became encapsulated with reticulin. On cross section a mass of acini could be seen surrounded with a capsule of reticulin and in one quadrant of the circle, fibers of silk were found separately encapsulated with reticulin. Giant cells were present between them. This arrangement represented the final quiescent stage of reorganization. Even when a downgrowth of epithelium was present, encapsulation of the thread containing 1,2-benzanthracene was complete in one month.
Clefts did not form about untreated control silk threads although sinuses occasionally formed when a length of thread perforated through the mucosa or an end extended into the lumen. Then epithelium grew back along the untreated silk in exactly the same manner as it did along the carcinogenic and non-carcinogen threads. Sinuses containing acini formed and both the thread and the epithelial tube finally became encapsulated. The process was completed about 10 days earlier than about the 1,2-benzanthracene threads. Usually the untreated silk threads became completely encapsulated without a downgrowth of surrounding epithelium. The timing of the process and the arrangement of reticulum about each fiber has been described in a previous report (2).

In other words the mucus-secreting cells extended downward early around all threads under the stimulus of injury or when a break occurred in the muscularis mucosae. However, they continued to proliferate and to form adenomas or adenocarcinomas only in the presence of methylcholanthrene. The invasive tumors formed in the sinuses while adenomas formed in the clefts.

The transformation in the sinuses will be described first. Until the 35th day the tubes of epithelium extending downward about the methylcholanthrene threads were somewhat larger than those about the controls, possibly as the result of a larger amount of tissue destroyed by the carcinogen. Save for this difference, the epithelium could not be distinguished from that seen about the chemical control. By the 50th day, on the other hand, differences were becoming apparent. Whereas each fiber of the thread with 1,2-benzanthracene was encapsulated separately and 8 to 10 acini of epithelium appeared in a perfect circle on cross section, with regular and heavy strands of reticulin deposited around the periphery of the group; by contrast, the thread containing methylcholanthrene was not encapsulated at all. Many more acini were present and there were many mononuclear cells between the acini and about their periphery but not between the fibers of the thread. The acini were not arranged in a perfect circle. They were distributed irregularly and some jutted out into an imperfect reticulin barrier of very fine fibrils about the periphery. In these areas where the epithelial cells extended, the fibrils were edematous and infiltrated with mononuclear cells.

Grossly, there was considerable thickening of the stomach wall for some distance about the thread. The increase in the number of acini and their extension outward suggested that by the 50th day the epithelial cells were beginning to proliferate again and that by some mechanism, recently established reticulin barriers were destroyed and new ones were not being deposited.

Thereafter with the passage of time, the masses of acini enlarged. They became 10 or 12 times their original size at 35 days, they extended through zones of weakened reticulin clear out to the liver and in areas, the cells grew in compact groups. Some of them also became irregular in size and shape and mitotic figures became more numerous. In two animals large tumors grew outside the stomach and there were peritoneal implantations locally, but no metastases were found in the liver or lungs.

The adenomas formed in the clefts as the result of further proliferation of the epithelium that lined them. Papillomatous growths began to appear after the 50th day and later; they not only filled the cleft but pushed open its mouth and grew by long stalks into the lumen of the stomach. Normal mucosa on either side was pushed far apart by growths bulging through the mouth of a cleft (Fig. 7). The reticulin barrier, previously deposited outside the lining epithelium of the cleft, was undamaged by the new growth. In other words the growth of the adenoma was outward into the lumen of the stomach and not into the tissues. In some of the largest adenomas, however, the component cells were irregular in size and shape and this change, combined with a wide base, suggested the appearance of a fungating carcinoma.

In summary, then, 3 distinct changes were observed in these experiments in the formation of the new growths of the stomach. First, static tissues: muscle, reticulin, fat and certain types of epithelial cells—the chief and parietal cells were destroyed by the carcinogen; second, mucus-secreting cells survived this destruction and extended from the surface of the mucosa down through the normal connective tissue barrier into the space left by the destruction, and continued to exist without organized blood supply. Lastly, under the continuing influence of the carcinogen, these cells proliferated further to form large masses of epithelial tissue. When adenocarcinomas developed, newly formed reticulin barriers were destroyed and attempts to form new barriers failed. On the other hand, if the reticulin barrier remained intact, an adenoma formed.

Of course, not all epithelial-lined clefts or sinuses formed by methylcholanthrene threads developed adenomas or adenocarcinomas. In the first group of animals, for example, only 6 out of 22
that were allowed to run long enough developed new growths. In these, size B silk was impregnated with the carcinogen. In a second group, a larger silk thread, size C, was used and no new growths developed because all threads sloughed from the stomach wall within 3 weeks. In a third group, B silk was again used, but the ends were not impregnated with chemical so that the untreated portions could become encapsulated and thus keep the entire thread in contact with the ingrown epithelium for a longer period of time. In this lot 14 out of 40 had invasive epithelial growths and 9 had adenomatous growths, illustrating the importance of persistence of contact of the carcinogen with the misplaced or buried mucous cells. Seven sarcomas were found in the three series and only 2 sarcomas were combined with epithelial growths. In the stomachs of 2 animals large growths of acini were found within the stomach wall and each acinus was separately surrounded by heavy fibers of collagen. So many acini were present that the thread must have remained for a sufficient length of time to cause further proliferation of the acini, yet the effect of the methylcholanthrene was eventually lost and the entire area became filled with dense connective tissue fibers, as may be seen in Fig. 8.

**DISCUSSION**

Certain tissues and cells composing the stomach wall were destroyed by the carcinogen. Muscle, leukocytes, parietal and chief cells, reticulin and collagen were destroyed. So extensive was the damage that the carcinogenic threads sloughed from the tissues. On the other hand, mononuclear cells and fibroblasts survived at a distance from the thread and, more important still, the supermucous-secreting cells not only survived, but moved downward into the area containing the carcinogen and proliferated there.

Similar extensions of mucosal cells also were found growing downward below the muscularis mucosae; occasionally around untreated threads and more frequently around threads containing a similar but noncarcinogenic anthracene compound. Because these downgrowths occurred with injury alone—but always with one producing a defect in the muscularis mucosae—we conclude that even when the carcinogen was present the process was initiated by non-specific injury and that removal of the barrier of the muscularis mucosae was the important factor allowing the mucus-secreting cells to move downward and to organize themselves in a misplaced growth.

The initial movement of these cells in a single row recalls the outward movement in a single row of epithelial cells occurring in the basement layer of the skin after injury. In the wound of the skin this movement is attributed to amoeboid motion and is combined with hyperplasia of the old uninjured epithelium on either side of the defect. Hyperplasia and hypertrophy of the surrounding superficial mucosal cells also occurred in the stomach, accounting for the two parallel ridges of mucosa overhanging either side of a cleft. Because of these similarities, the initial movement of mucous cells must likewise be attributed to amoeboid motion.

When no carcinogen was present, these mucous cells continued to exist below the muscularis mucosae and showed no tendency to proliferate further. Instead, they achieved some degree of differentiation and became encapsulated. The sinuses formed anatomically resembled those described originally by Aschoff-Rokitansky in the stomach, duodenum and gallbladder of man. The persistence of mucous cells under these circumstances illustrates their capacity to survive, for then they normally are farthest removed from blood channels and therefore receive their nutrition tenuously, while at the same time acting as buffer cells against injury. Conversely, the relative incapacity of the acid and pepsin-secreting cells to survive injury and to proliferate thereafter is demonstrated.

Displacement of mucous cells beneath the muscularis mucosae in the presence of a carcinogen seems essential for the formation of a new growth in the stomach. In this new environment mucous cells could alter their function of secreting highly protective mucus, surrounding reticulin barriers could be further destroyed and persistent contact with the carcinogen becomes possible.

Neither these three alterations nor the change in blood supply could have been present in earlier experimental attempts to produce gastric neoplasms with the carcinogens. Their absence may explain why feeding and placing carcinogens in contact with normally secreting mucous cells have repeatedly failed to produce gastric tumors. The mucus-secreting cells lining the innermost surface of the stomach are ordinarily continuously subjected to injury and they, in response, desquamate and regenerate. In the presence of the carcinogen within the lumen and increased surface destruction, these normal activities would only be intensified. Destruction of reticulin barriers seems to be particularly important because this destruction opens pathways for cells to move, i.e., to become invasive...
and autonomous in growth. Lastly, the thesis that destruction within the stomach wall is necessary to produce new growths has historical confirmation—the first successful attempt to produce carcinoma of the stomach was obtained by Stewart and Lorenz (6) when the carcinogen was placed within the wall, in contrast with the many previous failures resulting from the maintenance of carcinogen in the lumen. Surface application of a carcinogen, in fact, produces neoplasms in only one tissue, the epidermis, yet in this structure the carcinogen dissolves in the sebum and penetrates into the skin so that changes are instituted deep within the tissue (5). When the carcinogen fails to penetrate, neoplasms do not form (4). Acceptance of the thesis that destruction within the wall is necessary focusses attention on the important corollary that the carcinogen must institute changes other than causing cells to proliferate.

The experiments furnished some clue to the duration of contact required between the tissues and the carcinogen. If the carcinogenic thread remained within the stomach wall for a period less than 35 days, no attempt at tumor formation was observed. Stomachs of rats have been reopened and the threads removed if they had not sloughed away by the 35th day and no growths have resulted. Threads removed after the 60th day, on the other hand, yielded adenomas.

Lastly, the mucous cell of the gastrointestinal tract must be added to the list of those cells that can survive the destructive action of carcinogens. Like the fibroblast and the epithelial cell of the basement layer of the skin, it is capable of ameboid motion and can, therefore, move away from a focus of destruction. Like them also it possesses unusual proliferative capacities normally, and is constantly regenerating in response to destructive forces. By contrast, and equally as important, is the fact that the static elements of the tissues, particularly reticulin and collagen, cannot move away from destruction and can be elaborated only secondarily after cells have matured and would not regenerate despite continuing specific destruction.

Naturally, after the completion of these experiments, we were curious whether the evolutionary changes that had been observed in the experimental production of new growths of the stomach could also be traced in neoplasms evolving spontaneously in the human stomach.

Loss of the capacity to secrete acid and pepsin, or mucous transformation, atrophy or "intestinalization" is a common precancerous change. Its presence in the mucosa of stomachs resected for cancer and for peptic ulcer has been statistically correlated by Stout (8). Through his courtesy, approximately 250 microscopic sections from resected stomachs were reviewed to discover if there were more changes at a distance in the muscularis mucosae in carcinomatous stomachs than in those resected for ulcer.

The following observations were made: (a) The muscularis mucosae is nearly always completely destroyed at the site of the carcinoma. (b) Lymphoid infiltration into the muscularis mucosae is not uncommon, but the epithelial cells do not transgress through these areas. (c) Aschoff-Rokitansky sinuses are sometimes found but are not frequent, and the epithelial cells that inhabit these sinuses are mucous cells only. (d) In superficially spreading carcinoma, small cells that tend to grow in sheets above the muscularis mucosae seem to drop right through the muscularis mucosae and into the loose areolar connective tissue beneath, but no obvious break in the muscularis mucosae can be detected.

CONCLUSIONS

Silk threads containing methylcholanthrene produced new growths when embedded in the wall of the rat's stomach. The early changes have been studied. Shortly after implantation beneath the serosa, round cells infiltrated and edema appeared. Ulceration occurred in the mucosa and then the superficial mucous cell on either side grew into the defect to form an epithelial-lined cleft. If a thread perforated the mucosa or the ulceration occurred directly over a thread that remained in position, the superficial mucous cells grew back along the thread to form an epithelial-lined sinus. Acid and pepsin cells were destroyed and did not grow downward. In the clefts these cells formed a new mucosa composed entirely of mucous cells while the sinus was filled with a cylinder of these cells that arranged themselves in acini. This process took approximately 2 weeks and the cells showed no malignant change.

After 35 days, the cells that were able to continue their existence beneath the muscularis mucosae began to grow again. Adenomas formed in the clefts while adenocarcinomas formed in the sinuses. Adenomas grew outward toward the lumen of the stomach, pushed open the mouth of the cleft, and sometimes filled the lumen of the stomach. This type of growth did not disturb the reticulin boundary that had been formed beyond the new mucosa lining the cleft. Adenocarcinomas grew within the wall of the stomach and the re-
ticulin barriers already formed about the sinus were destroyed. These mucus cells transposed beneath the muscularis mucosae required prolonged contact with the carcinogen or else they remained in the state typical of their orientation in the sinus or cleft. This contact in the rat must exist at least for 35 days. The reasons why placing the carcinogen in the wall of the stomach is successful in producing new growths in contrast to failures obtained by keeping it in the lumen is discussed. In the wall of the stomach long contact can be maintained with mucus cells, existing in a new environment where perhaps their protective secretion may be lost and where damage can be done by the carcinogen to the surrounding reticulin barriers and blood vessels. Carcinogens in the lumen by contrast only make contact with normal mucus cells that desquamate and increase in their rate of proliferation.

The mucus cells must be added to the list of cells able to survive destruction caused by the carcinogens. Like the fibroblast and the epithelial cell of the basement membrane of the skin, it possesses amoeboid motion and an inherent high rate of proliferation in response to injury. Microscopic sections of the human stomachs resected for carcinoma and ulcer were examined for the presence of mucus transformation, Aschoff sinuses and destruction of the muscularis mucosae to ascertain whether a correlation could be obtained with the experimental results. Mucus transformation and destruction of the muscularis mucosae directly over the tumor were commonly found in stomachs resected for cancer. Aschoff sinuses were rare at a distance from the tumor. Defects in the muscularis mucosae at a distance from the tumor could not be found, but on the other hand, the small cells of the superficially spreading type of carcinoma seems to drop right through an apparently intact muscularis mucosae.

REFERENCES
DESCRIPTION OF FIGURES 5 TO 8

Fig. 5.—Cleft, 4 weeks old, made by methylcholanthrene thread. The area of ulceration through the mucosa can be seen as well as the transition in the epithelium.

Fig. 6.—Methylcholanthrene thread sinus, 50 days. Note absence of reticulin barrier along bottom and irregularity of the growth of the epithelial cells.

Fig. 7.—Methylcholanthrene thread, 60 days. Epithelial cells in cleft beginning regeneration. Note papillomatous growth has been cut across in center of cleft. Mouth of cleft is beginning to separate and the epithelium lining the wall has a more disordered growth than is seen in Fig. 5. In some areas they are growing in sheets. The reticulin capsule is not as well demarcated as usual around a cleft.

Fig. 8.—Methylcholanthrene thread, 200 days. Dense connective tissue surrounding numerous acini. On left is large cystic structure, partially cut off. Whether these epithelial cells are continuing their growth in spite of "scirrhous" connective tissue is not known.
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Cancer Res 1948;8:419-427.

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