THE EXPERIMENTAL PRODUCTION OF PRECANCER AND CANCER OF THE STOMACH

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Much work has been devoted to the experimental production of malignant growth of the glandular epithelium of the stomach, but success has only rarely been achieved [15, 16]. The only carcinogen which has so far proved capable of inducing malignant growth is methylcholanthrene. The use of 9,10-dimethyl-1,2-benzanthracene (DMBA) for this purpose, irrespective of its mode of administration, led to the appearance of squamous-cell carcinoma of the fore-stomach [3, 9]. Most of the work has been carried out on mice and rats, in which the stomach consists of a fore-stomach, lined with stratified squamous epithelium, and a glandular stomach.

The numerous facts obtained by research workers point to the extraordinary degree of resistance of the glandular epithelium of the stomach of animals to the development of spontaneous and induced malignant neoplasms [10, 11, 12, 14, 16]. Analysis of the data in the literature also shows that the local action of the carcinogen alone is clearly insufficient to disturb this resistance. We have examined the data on this subject more minutely in a survey on experimental carcinoma of the stomach [1].

In the present investigation we set out from the assumption that in order to produce malignant growth in the glandular portion of the stomach the action of the carcinogen must be supplemented by other factors, disturbing the general and local resistance of the organism and modifying the functional state of the organ. Of particular interest is the disturbance of the gastric function which can be produced without any direct action being exerted on the stomach wall.

For this purpose we ligated the pancreatico-duodenal vein. In small laboratory animals (mice, rats) this procedure leads to a disturbance of the liver function similar to that observed after the formation of an Eck's fistula in dogs [6]. According to S. I. Lebedinskaya [2], the formation of this fistula causes a prolonged increase in gastric secretion besides disturbing the liver function.

DMBA was given as paraffin-wax pellets, in an attempt to create a depot of carcinogen and thereby to ensure its prolonged administration to the surrounding tissues in small doses, and on the assumption that introduction of the carcinogen in this way would lead to the appearance of heterotopic proliferation of the glandular epithelium as a reaction to a foreign body. Moreover, the administration of a carcinogen in oily solutions and suspensions is accompanied, as a rule, by its penetration into the fore-stomach, which is particularly sensitive to the action of DMBA [3, 4, 5, 7, 8, 13].

EXPERIMENTAL METHOD

The experiment was conducted on 30 rats, 8 of which were controls. Under ether anesthesia the anterior abdominal wall was incised in the midline. DMBA was implanted in the form of a 10% paraffin-wax pellet weighing 5 mg beneath the serosa of the anterior wall of the pyloric portion of the stomach. The pancreatico-duodenal vein was ligated with silk thread and the wound was closed in layers. The stomach of sacrificed or dying rats was opened up along the greater curvature and fixed in distended state in a 12% solution of neutral formalin. Celloidin-paraffin or frozen sections from strips of the stomach wall, cut to correspond to the position of the neoplasms along the long axis of the stomach, were stained by the ordinary methods and also by special methods: the Dominici-Kodrovskii method, Best's carmine and Meyer's mucicarmine, in order to reveal the secretory granules in the glandular cells of the stomach.

EXPERIMENTAL RESULTS

As a result of the experiments conducted in the manner described above, neoplasms were produced in the gastric mucosa, always situated in the glandular portion on the anterior wall in the prepyloric region, where the main glands are still predominant in the mucous membrane. No changes were observed in the fore-stomach.

The neoplasms were of different sizes – from simple thickenings of the mucosa hardly visible with the naked eye to well defined nodules 2-5 mm in diameter. In one case (rat No. 5) a nodular growth occupied nearly the whole glandular part of the stomach, covering the passage into the duodenum.

Corresponding to the macroscopic appearances, in all the animals heterotopic proliferation of the glandular epithelium was found at the site of the growths, differing in depth and extent of spread. When the changes were hardly visible to the naked eye, the proliferating tissues occupied small areas of the mucous membrane, penetrating only into its muscular coat. Larger epithelial proliferations usually penetrated into the deeper layers of the stomach wall – into the submucosa and muscularis mucosae and as far as the serosa. The deeper it was found, the more extensive its area of spread.

Whereas in the normal mucosa the glandular tubes were arranged parallel to each other, in the heterotopic proliferations they differed in their shape and length and were oriented in different directions, sometimes lying close together, sometimes some distance apart. The lumina of these tubes varied in size, and they sometimes had the appearance of cystiform dilations..

In the course of the spread of the heterotopic proliferations, the morphological and functional characteristics of the glandular cells lining the epithelial tubes underwent modification.

In the first stages of the process, when the wax pellet implanted beneath the serosa, containing the carcinogen, was still present 24 days later in the subserous membrane and was penetrating into the muscularis mucosae, and when inflammatory infiltration and necrosis were observed in the stomach wall, changes also began to affect the main glands situated near the pellet. These changes took the form of dedifferentiation of the glandular cells. The first cells to disappear were the principal cells, in which the pepsinogen granules were replaced by a mucous secretion, then followed the parietal cells and, lastly, the typical accessory cells. New forms of cells appeared in the glandular tubes, proliferating deeply. They differed from the typical glandular cells in the shape and size of the cell body and in the position of the nucleus within the cytoplasm.

At the 4th and 6th months of the experiment, after elimination of the pellet containing the carcinogen (Fig. 1), signs of inflammation were no longer found. Although the foci of necrosis and inflammatory infiltration had disap-

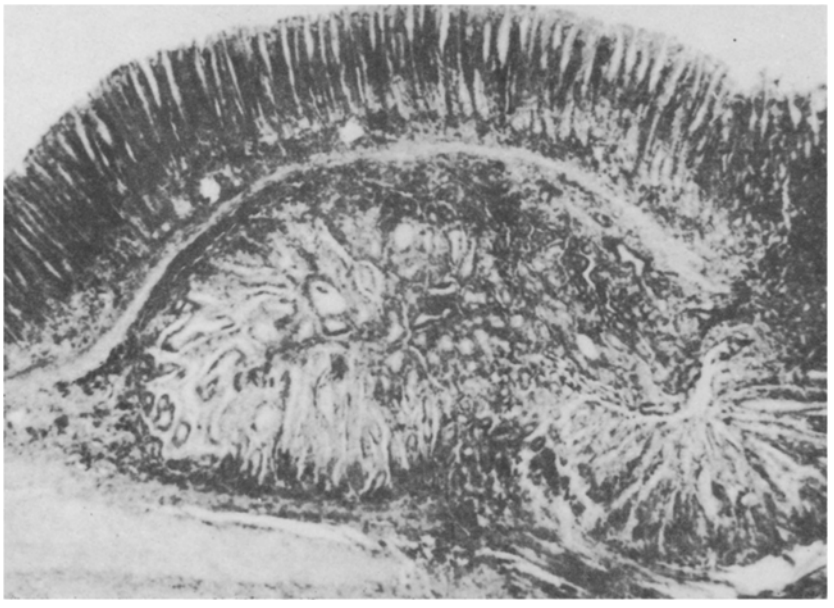

Fig. 1. Focus, adenomatous in structure, situated in the submucosa (4 months after implantation of carcinogen). Objective 3, eye-piece 6.

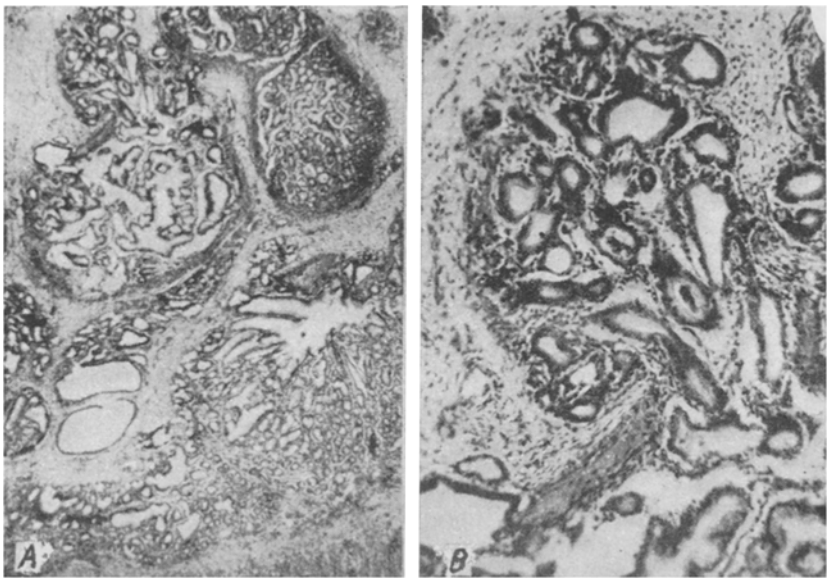

Fig. 2. Malignant adenomatous proliferation of epithelial tubes (18 months after implantation of carcinogen). Marked infiltrating and destructive character of growth. A) Objective 3, eye-piece 6; B) objective 10, eye-piece 6.

peared, the heterotopic areas of epithelial proliferation for which they were responsible persisted. They consisted of circumscribed foci, adenomatous in structure and of different sizes, situated in the submucosa and associated with areas of atypical glandular structures in the mucous membrane. These areas of epithelial proliferation occurred in two principal variants, each of which was characterized by the appearance of new cell forms. One variant was distinguished by the diversity of its glandular elements, having several features in common with the surface epithelium or with the accessory cells, and sometimes with the cells of the pyloric glands. Finally, goblet cells of the intestinal epithelium were observed among them, and cubical or flatter cells without secretion granules were always present. The second variant was characterized by a greater degree of homogeneity of its cells, and by predominance of one of the generations of atypical glandular elements.

In the later stages of the experiment (after 16-18 months) activation of the growth of the individual glandular foci was observed, infiltrating and destructive in its character (Fig. 2). The polymorphism of the cells lining the glandular tubes, observed in the earlier stages, gave way to the predominance of a new, and more homogeneous generation of cells. These cells have lost their secretory function but possess high mitotic activity. Heaping up of the cells developed in the newly appearing glandular tubes with a single layer of epithelium, passing close to each other, giving the impression of nuclei arranged in rows of two or three. These nuclei differed in size and situation within the cell. The glandular tubes penetrated through the dense scar tissue (Fig. 3, B), formed at the site of implantation of the carcinogen, as far as the serosa. In one rat they invaded the mucous membrane of the glandular part of the stomach throughout nearly its whole extent, thereby revealing the malignant nature of the intensively growing epithelial cells. For this reason the neoplasm can be regarded as a true adenocarcinoma (Fig. 3, A).

Hence, by the use of the method we have described, the pathophysiological mechanism of which requires further analysis, we have for the first time induced malignant epithelial growths of adenocarcinoma type in the stomach, developing after very long intervals (1.0-1.5 years) by means of 9,10-dimethyl-1,2-benzanthracene. From the study of the earlier stages we may deduce that the process of carcinogenesis in the stomach in these particular experimental conditions begins with inflammatory atypical heterotopic proliferation of the epithelium. This proliferation persists after elimination of the carcinogen and cessation of the inflammation in the form of circumscribed foci, in which the cells undergo modification although, admittedly, they show only a very limited tendency toward growth. These foci may be regarded as precancerous in the true sense of the word. After a relatively long interval of time the cells

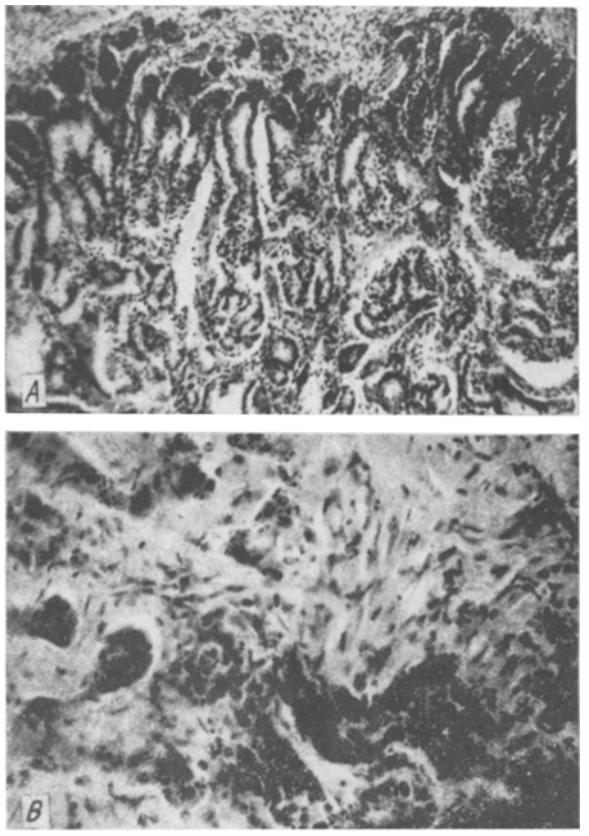

Fig. 3. Changes in the glandular epithelium of rat No. 5 (18 months after implantation of the carcinogen). A) fragment of tumor of adenocarcinoma type. Objective 10, eye-piece 6; B) invasion of dense connective tissue by individual epithelial tubes. Objective 24, eye-piece 6.

of these foci acquire new morphological and biological properties and begin to show activity expressed as infiltrating and destructive growth through all layers of the stomach wall. They can thus be regarded as the cells of a malignant neoplasm.

SUMMARY

The pancreatico-duodenal vein was ligated in albino rats simultaneously with the introduction of a paraffin capsule with 9,10-dimethyl-1,2-benzanthracene into the anterior wall of the pyloric region of the stomach. Thickenings and mucosal nodules of various size were observed at the site of administration; histologically they represented heterotopic proliferations of the glandular epithelium penetrating to different depths, even to the serous membrane. Notwithstanding the cancerogen elimination, arrest of inflammation and absence of necroses, heterotopic proliferations persisted in the submucosa in the form of foci of adenomatous structure, characterized either by a multiformity of glandular cells, or by a prevalence of one of the generations of atypical glandular elements. Activation of growth of the glandular foci exhibiting a marked destructive and infiltrating nature of growth and a high mitotic activity at late experimental periods (in 16-18 months) points to the malignant nature of these proliferations. Thus, by employing an additional factor for changing the functional state of the organ, the possibility of inducing malignant epithelial proliferations of the adenocarcinoma type by means of 9,10-dimethyl-1,2-benzanthracene was demonstrated for the first time. The rumen was always devoid of any alterations.

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