Experimental Carcinogenesis
The Effect of 20-Methylcholanthrene Applied Directly to the Colon in Mice*

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Walpole, Williams, and Roberts (13) observed a high incidence of tumors of the colon in white rats given injections of 4-aminodiphenyl and 3,2-dimethyl-4-aminodiphenyl. By feeding radioactive yttrium (Y91) to rats, Lisco et al. (6) also reported that a high proportion of animals developed carcinoma of the colon. With other substances, cancer of the large intestine has been but seldom produced. Krebs (4, 5) reported that, of sixteen mice given alcohol by rectum, one developed carcinoma of the colon with metastasis to the liver. Spitz, Maguigan, and Dobriner (10) found seven instances of adenocarcinoma of the rectum at its junction with the colon among 385 white rats injected with benzidine. All seven occurred in male rats. Bielchowsky (1) fed acetylaminofluorene to 104 albino rats. Ninety-three of them developed a total of 105 malignant tumors, of which only five were of the intestine. One of these five was a cancer of the cecum with metastasis into the liver. The location of the other four intestinal tumors was not recorded. Among 22 rats which survived total-body irradiation longer than 6 months, Brecher, Cronkite, and Peers (2) reported that eight developed tumors and, of these eight, only one developed a lesion in the colon (350 days after irradiation with 1,000 r). Cox, Wilson, and De Eds (3), working with acetoaminofluorene in albino rats, found that among 84 animals that developed multiple tumors only two had adenocarcinoma of the colon. Lorenz and Stewart (7, 8, 11, 12) demonstrated precancerous and cancerous lesions of the fore-stomach and small intestine of mice after feeding methylcholanthrene or after injecting this carcinogenic hydrocarbon directly into the wall of the stomach, but no lesions were observed in the colon.

METHODS

Two strains of pedigreed mice (A white and C57 brown-black) were used. They were not selected as to sex and were 2-3 months old at the beginning of the experiments. The animals were divided into three experimental groups, each receiving 20-methylcholanthrene (MCA). Autopsies were performed on all animals, and all grossly abnormal or suspiciously abnormal areas were singled out and histologically studied in detail. Tissues were fixed in buffered 10 per cent formalin, imbedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

In Group 1, 20-methylcholanthrene (MCA) was administered in the diet as described by Lorenz and Stewart (7, 8, 11, 12). The experiments of these authors were repeated in 50 strain A mice in order to affirm the carcinogenic activity of this supply of methylcholanthrene. An emulsion of olive oil in water (10 per cent olive oil) was prepared so as to contain 0.4 mg of MCA/ml. This was administered with Purina Dog Chow. In the beginning all water was withheld from the diet, but the animals showed such rapid deterioration that later they were allowed water ad libitum for 1 day at weekly intervals.

In Group 2, the methylcholanthrene-olive oil mixture was fed to mice and the animals were not subjected to total-body irradiation. In Group 3, the methylcholanthrene-olive oil mixture was fed to mice which had been subjected to total-body irradiation with 1,000 r in order to determine the relative carcinogenic activity of this substance in normal and irradiated animals.

* This investigation was supported by Grant No. C-1571 (C), U.S. Public Health Service, National Institutes of Health, Bethesda 14, Maryland.

Received for publication July 7, 1955.
suspension was injected directly into the wall of the cecum. No attempt was made to calculate the exact amount of MCA injected or retained in the cecum and distal colon by the various technics used. This group was composed of 100 mice of both strains (A white and C57 brown-black), caged in groups of ten. Each animal was repeatedly operated upon to expose the cecum through a celiotomy. The wall of the cecum was then injected with MCA by means of a syringe and a 27-gauge needle. The MCA preparation consisted of olive oil containing 5 mg of 20-methylcholanthrene/ml. An amount of the carcinogen, between 0.5 and 5.0 mg. (0.1–1.0 ml. of the oil depending upon the distensibility of the tissues), was injected into the subserosa and submucosa of the cecum.

In Group 5, the MCA was introduced directly into the sigmoid colon as a pellet preparation. The pellets were prepared as follows: Beeswax (10 per cent) and carbowax (90 per cent) were liquefied by heating, and 1 part of MCA was added to 20 parts of the warm liquid wax. This mixture was taken up in a syringe and dropped through a needle onto a flat plate with powdered surface. This resulted in solidification in the form of pellets scattered on the plate. The pellets were 2–4 ml. in diameter, and it was estimated that each contained approximately 0.4 mg of MCA. This group contained 100 mice of both strains (A white and C57 brown-black). After sigmoidostomy was established in each, MCA in pellet form was introduced periodically directly into the sigmoid colon and rectum by way of the distal sigmoid stoma until the animals either died or were sacrificed.

RESULTS

Group 1.—Most of this group of 50 mice died during the first 8 weeks on the experimental diet, presumably from toxicity of the MCA. Autopsies at this interval showed no gross evidence of neoplasms or other anatomical cause of death. Six of the mice lived for 6 months and were sacrificed at the end of that time. Four of the six animals developed hyperplasia of the squamous epithelium and squamous-cell carcinoma of the forestomach. The other two showed no abnormalities. None developed lesions of the small intestine.

Group 2.—This group consisted of 100 mice which had received repeated injections into the subserosa and submucosa of the cecum at intervals of 8–19 weeks. Thirty-four of this group of 100 animals lived 20 weeks or longer (20–66 weeks) and were sacrificed at the end of that time. All received at least two injections; 24 received at least three injections; eleven received at least four injections; five received as many as five injections.

Examination of the organs of these animals revealed an inflammatory reaction at the injection sites. In three animals, there was some focal mucosal hyperplasia at or near the site of injection of MCA. In a fourth animal, which received three injections at 10- and 11-week intervals and was sacrificed 34 weeks after the start of the experiment, the wall of the cecum was firm and thickened to palpation. Microscopically, the mucosa was ulcerated at one point with acute inflammation of the underlying tissue. Nearby, the mucosa was elevated, thickened, and atypical, overlying an area of marked inflammatory reaction (Fig. 1.) The glands were distorted and were lined by one or more layers of columnar cells with basophilic cytoplasm. The cell nuclei varied in size and in staining reaction. Some of the nuclei were hyperchromatic, and many were in mitosis. Some of the mitoses were atypical. The lesion was interpreted as an early adenocarcinoma of the cecum. The three mice of this group which developed mucosal hyperplasia and the one that developed early adenocarcinoma over an inflammatory area in the cecum are the only animals that developed primary mucosal lesions of any consequence. Two animals developed squamous-cell carcinomas of the skin at the site of injection after 36 and 48 days, respectively, and in one of these the tumor extended into the cecum. Four mice developed fibrosarcomas of the soft tissues after 35–47 days. In two of these four, the fibrosarcomas involved the skin and extended into the cecum. In the two others, the fibrosarcomas were confined to the subcutaneous tissues. In these cases in which malignant changes developed outside the wall of the cecum, there was presumably leakage of the MCA emulsion from the injection site and into the abdominal wall incision.

Group 3.—This group of 100 mice was prepared with loop colostomies of the sigmoid in order to introduce the MCA in pellet form directly into the lumen of the bowel. Twenty-four animals survived this operation longer than 8 weeks. In 21, MCA pellets were inserted into the distal colostomy stoma at 3- to 10-day intervals. Five to 30 insertions were done in the 21 animals, and they lived 30–160 days. Three of the total of 24 animals which survived the sigmoidostomy were not treated by the introduction of MCA pellets. These were kept separate from the rest and served as controls.

None of this group of animals showed any evidence of neoplasia. Inflammatory changes were encountered, and in some cases, especially at the rectal squamo-columnar junction, there was moderate glandular proliferation and increased mitotic activity, but the mitoses were normal. The same
appearance was found in the three animals in which pellets were not introduced into the colostomy orifice.

DISCUSSION

Spontaneous tumors of the intestine are rare in mice. In 1938, Wells, Slye, and Holmes (14) reported their findings in 142,000 necropsies on the Slye strains of mice. They found only nineteen primary malignant growths in the large intestine. Eight were located in the sigmoid or higher; eleven were rectal lesions in mice which had chronic prolapse of the rectum. Six of the eleven were squamous-cell carcinomas, two were adenocarcinomas, and three were sarcomas. Among the other eight of the nineteen intestinal tumors, only one was a mucus-secreting adenocarcinoma of the cecum. This case also had a metastasis to a regional lymph node. These strains of mice, therefore, were selected because, if they did show carcinoma in the colon more than rarely, the lesions could reasonably be attributed to the experimental procedure.

In the present study, 55 mice survived the surgical procedures and were exposed to 20-methylcholanthrene by means of injections into the wall of the cecum (34 animals) or introductions into the distal sigmoid in the form of pellets (21 animals). Adenocarcinoma of the mucosa of the large intestine (cecum) was produced in only one of these animals (animal number 8, Fig. 1). This lesion was clearly an adenocarcinoma but had not invaded into the wall of the cecum. It overlay an area of acute inflammation resulting from the injection of the MCA.

The failure of carcinoma of the colon to develop in the experiments reported by Lorenz and Stewart (7, 8, 11, 12) could be attributed to lack of susceptibility of the colon to the carcinogen or to a possible change in the chemical nature of the methylcholanthrene as it passed through the gastrointestinal tract. The results of the experiments reported here, in which the chemical agent was applied directly to the large bowel mucosa, would indicate that the explanation for the lack of carcinogenesis in the colon when methylcholanthrene is injected is not owing to chemical changes as the substance passes along the intestinal tract, but rather to a decreased susceptibility of the colon to the carcinogenic activity of the methylcholanthrene. The possibility remains that greater concentrations or more prolonged exposure of the colon to the activity of this agent might have caused a higher incidence of colon cancer in the two strains of mice used in these experiments. It is noteworthy that, whereas sarcoma and squamous-cell carcinoma are readily produced by contact of connective tissue and squamous epithelium with MCA, adenocarcinoma is not produced readily. Lorenz and Stewart (7, 8, 11, 12) observed adenocarcinoma in the small intestine after the administration of MCA in mice but not in the colon. The single instance of adenocarcinoma of the cecum observed in the experiments reported above may have been fortuitous. Its close association with the site of injection of MCA, however, lends support to the opinion that it may, in fact, represent a carcinoma resulting from the action of MCA on the cecum.

SUMMARY

1. The carcinogenic activity of 20-methylcholanthrene for the forestomach of pedigreed mice has been confirmed.
2. The periodic injection of MCA into the wall of the cecum produced focal mucosal hyperplasia in three animals (two injections, three injections, and four injections, respectively, in 20–44 weeks).
3. One animal, sacrificed at 23 weeks after two injections of MCA into the wall of the cecum, developed a localized carcinoma of the cecum, the first adenocarcinoma to be reported after exposure of this organ to MCA.
4. The repeated introduction of MCA into the sigmoid in the form of pellets did not result in carcinoma of the sigmoid or rectum.
5. 20-Methylcholanthrene is less effective as a carcinogenic agent in the colon than it is in the stomach of the mouse, at the concentration used.

REFERENCES

3. Cox, A. V., Jr.; Wilson, R. H.; and De Eds, F. The Carcinogenic Activity of 2-Acetamidoacetophenone Car-
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