Induction of Intestinal, Mammary, and Ovarian Tumors in Hamsters with Oral Administration of 20-Methylcholanthrene*

GIUSEPPE DELLA PORTA†

(Division of Oncology, The Chicago Medical School, Chicago, Ill.)

SUMMARY

Administration by stomach tube of 30–50 doses of 5 mg. of 20-methylcholanthrene to Syrian golden hamsters resulted in the occurrence of squamous-cell tumors of the forestomach, adenocarcinomas of the small and large intestine, adenocarcinomas of the mammary glands, thecomas of the ovaries, and sebaceous tumors of the skin and ear duct glands. Of these types of tumors, only a few squamous-cell papillomas of the forestomach were observed in control animals. Inflammatory lesions of the intestine with hyperplastic and atypical glands were a common finding in both experimental and control groups.

The only mammary gland tumor in untreated hamsters mentioned in the literature is a solitary cystic adenocarcinoma observed by Habermann (see [7]). Kirkman, in his monograph on estrogen-induced renal tumors in hamsters (12), stated (p. 28) that in more than 30 different types of tumors observed in his colony no mammary gland tumors were included. Fortner, who has also observed a large number of tumors in untreated hamsters, did not report mammary tumors (8). No mammary tumors were observed in large groups of untreated hamsters kept in this laboratory for their lifespan. Even if such tumors are eventually found among larger groups of females, including breeders, kept for a longer time, mammary tumors in hamsters are undoubtedly rare.

Among the studies of chemical and viral carcinogenesis in hamsters, only one (1) reported the occurrence of two mammary carcinomas in females given implants of 20-methylcholanthrene in the gall bladder and of estradiol subcutaneously. 2-Acetylaminofluorene, which in certain strains of rats increases considerably the incidence of mammary tumors (10, 14), induced only cholangiomatous tumors in the hamster (6). Kirkman, Horning, and other investigators (see [12]), obtained renal tumors in hamsters given subcutaneous implants of estrogen pellets, but neither Kirkman nor Horning observed mammary gland stimulation in their estrogen-treated hamsters.

Because of the rarity of spontaneous and induced mammary gland tumors, it was felt that it would be of interest to try to induce mammary tumors in hamsters with 20-methylcholanthrene. This chemical was selected because of the rapid induction and high incidence of mammary tumors reported by Shay et al. (13) and Huggins et al. (11) in rats after its intragastric administration. The experiment gave positive results. In addition to mammary gland tumors, intestinal and ovarian tumors were also induced.

MATERIALS AND METHODS

Syrian golden hamsters, either purchased from Abrams Small Stock Breeders, Chicago, Illinois, or bred in this laboratory from a group of hamsters originally obtained from the same commercial breeder, were used. They were housed in plastic cages with wood shavings in groups of five according to sex and were given Rockland diet in pellets and tap water ad libitum.

Three groups of animals were given 20-methylcholanthrene (MC) (Eastman Organic Chemicals) by stomach tube, dissolved 1 per cent in corn oil (U.S.P.). The first group consisted of twenty female and twenty male hamsters, 12 weeks old,
obtained from the commercial breeder. The treatment was 5 mg. of MC per dose given 3 times weekly for 12 weeks in two periods of 6 weeks separated by an interval of 2 weeks. The second group, consisting of 30 females 15 weeks old, bred in this laboratory, received up to 50 administrations, by stomach tube, of 5 mg. of MC. The treatment lasted 21 weeks and was given 3 times weekly with intervals of a week at the 7th, 8th, 15th, and 16th weeks. In the third group, there were twenty females and twenty males, 5 weeks old at the beginning of the experiment. The treatment consisted of 36 oral administrations of 5 mg. of MC spaced over 26 weeks, once weekly for 2

tissue preserved in 10 per cent buffered formalin. Hematoxylin and eosin staining technic was routinely used with the addition of special methods, when necessary.

RESULTS

The intervals in the administration of MC were caused by the occurrence of diarrhea, poor general condition, and high mortality. At the 30th week of age, that is, from 15 to 25 weeks from the beginning of the treatment, the average weight of the MC-treated animals ranged from 95 to 108 gm., while at the same age the untreated hamsters of both sexes had an average weight of 120 gm. The

TABLE 1

THE OCCURRENCE OF INTESTINAL, MAMMARY, AND OVARIAN TUMORS IN 20-METHYLCHOLANTHRENE-TREATED HAMSTERS

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>No. surv., and age of hamsters at beginning of exp.</th>
<th>No. survivors (weeks of age)</th>
<th>No. animals with intestinal carcinomas</th>
<th>No. animals with mammary carcinomas</th>
<th>No. animals with ovarian carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC, 5 mg. X 36 in 14 weeks</td>
<td>20 0 12 wks.</td>
<td>19 10 1</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>20 0 12 wks.</td>
<td>16 8 5</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Exp. 2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC, 5 mg. X 50 in 21 weeks</td>
<td>30 0 15 wks.</td>
<td>29 19 4</td>
<td>9</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Exp. 3:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC, 5 mg. X 36 in 26 weeks</td>
<td>20 0 5 wks.</td>
<td>11 10 4</td>
<td>7</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>20 0 5 wks.</td>
<td>14 3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Control 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil 0.5 ml. twice weekly for 45 weeks</td>
<td>30 0 15 wks.</td>
<td>27 27 25 22 16 12 9 5 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50 0 15 wks.</td>
<td>48 32 24 18 14 9 8 5 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control 2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated*</td>
<td>65 0 10 wks.</td>
<td>60 55 55 51 40 29 19 11 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>45 0 10 wks.</td>
<td>39 33 35 35 33 29 28 23 13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Methylcholanthrene was dissolved 1 per cent in corn oil. The treatment was given by stomach tube.

periods of 6 weeks, and 3 times weekly for 2 periods of 4 weeks, with intervals of a week at the 7th, 8th, 15th, 16th, 21st, and 22d weeks.

Two groups of hamsters obtained from the commercial breeder served as control. One group of 30 females and 50 males, 15 weeks old, was given, by stomach tube, 0.5 ml. of corn oil (U.S.P.) 3 times weekly for 45 weeks, and then was kept under observation until death. The second control group consisted of 63 females and 40 males, obtained from the commercial breeder at 10 weeks of age and kept for their life-span without any treatment. All the animals were weighed and inspected at weekly intervals. Pathological study was done on all animals, with the exception of a few lost through cannibalism. Histological study was done on corn oil-treated controls suffered from diarrhea too, particularly among the males in which many deaths occurred during the treatment. The weight of the corn oil-treated females was similar to that of the untreated females, whereas the corn oil-treated males weighed from 5 to 10 gm. less than the untreated males.

The number of survivors at 10-week intervals of age for all groups is reported in Table 1. The last few survivors of the MC-treated groups died or were killed between the 45th and 50th week of age, except for the youngest group of males (Exp. 3) which were killed at the 32d week. The few surviving animals of the control group were killed between the 105th and 110th week of age.

In Table 1 the incidence of intestinal, mam-
mary, and ovarian tumors is also shown. Because of the high mortality, it is difficult to evaluate the difference in tumor incidence among the three MC experiments. However, it seems that the group of animals which started the treatment at 5 weeks of age had a higher incidence of the three types of tumors. None of these tumors was observed in animals dying before the 30th week of age, when the treatment was either terminated or almost completed in the three experiments. At that time 50 hamsters were alive: 25 of them, or 50 per cent, developed a total of 35 intestinal tumors. They were all adenocarcinomas which had invaded the entire intestinal wall and often the mesenteric tissue (Figs. 1-3). In fifteen animals there were metastases, in fourteen to the mesenteric lymph nodes (Fig. 4), in one to the lungs. Four carcinomas were of the jejunum, six of the ileum, mostly at the jejunal-ileal junction, eighteen of the cecum, and seven of the colon. Benign polyps were seen in two animals, one in the cecum and a second in the descending colon at the site of an intussusception. Three other cases of intussusception were not associated with polyps. Inflammatory lesions of the intestinal mucosa, ulcerations, diverticulosis, and atypical hyperplastic glands, were frequently seen (Figs. 5-8). Atypical glands were more numerous and prominent in the cecum, particularly at the site of the ileocecal valve.

All the animals which survived at the end of the treatment had multiple squamous-cell papillomas of the forestomach, which were seen as soon as 13 weeks after the beginning of the treatment. These tumors were not all examined histologically, but malignant transformation was frequently seen.

Ten females of the MC-treated groups had a total of 26 mammary tumors, often multiple, up to six in one animal. Eight of the ten animals also had intestinal tumors. The average time of appearance of the first tumor in each animal was 25 weeks from the beginning of the treatment, when only twenty females were still alive. No mammary tumors were seen in the males. The size of the tumors ranged from 4 to 30 mm. at death; they had solid portions, necrotic areas, and often cystic formations filled with blood-stained fluid. Histologically, they were all carcinomas with different histological appearance in the same tumors (Figs. 9, 10). In two animals the tumor metastasized to local and distant lymph nodes (Fig. 11). Transplantations of tumors from two different donors were both successful; both tumors are growing in untreated hamsters of both sexes. The mammary glands not involved by the tumors showed dilatation and hyperplasia of the ducts and evidence of secretion. Similar lesions were also common in the females dying without tumors at least 15 weeks after the beginning of the treatment.

Nine females had ovarian tumors, in four cases bilaterally; all additionally had mammary tumors. Only one animal had tumors of the mammary glands and not of the ovaries. The ovarian tumors were whitish, solid, and firm nodules measuring from 3 to 20 mm., histologically classified as thecomas (Fig. 12). Only in one case was transplantation attempted, unsuccessfully. The uterus of the animals with thecoma was enlarged and sometimes assumed a cystic appearance with multiple endometrial polyps, often hemorrhagic and necrotic (Fig. 13). The endometrial epithelium did not show significant changes; the stroma became hyperplastic and in four cases had a sarcomatous appearance with many mitoses and cytological irregularities (Fig. 14). One papilloma and hyperplasia of squamous epithelium at the level of the portio were also observed (Fig. 15).

In neither of the control groups were mammary or ovarian tumors observed.

In addition to the tumors already described, the MC-treated hamsters developed only a few other tumors. Among the males there were three sebaceous adenomas of the dorsal sex organ (Fig. 16) and one near the tail; the females had two sebaceous carcinomas of the ear duct glands, two reticulum-cell sarcomas, one melanotic tumor of the skin, blue nevus type, and one ganglioneuroma, originating probably in the celiac plexus. No lesions of particular significance were detected in other organs.

The tumors observed in the females of the two control groups were three squamous-cell papillomas of the forestomach in two animals and one angioma of the liver. Among the males there were one papilloma of the forestomach, one carcinoma arising probably in the salivary glands, one plasmocytoma, and two reticulum-cell sarcomas. In neither control group did intestinal tumors develop. Intussusception of the colon and inflammatory lesions of both small and large intestine were a common occurrence. Small inflammatory polyps were occasionally observed in the cecum, but no adenomatous polyps, although hyperplastic and atypical glands were seen in the large intestine.

The hamsters of both sexes of both control groups frequently had hyperplastic lesions of the intrahepatic bile-duct system. These lesions consisted of bile-duct cell proliferation, formation of new bile ducts and ductules, and cystic dilatation of the newly formed ducts. The cysts were either of microscopic size, in small conglomerates with little intervening stroma, usually located in the
central parts of the lobes, or were grossly visible, from 1 to 10 mm. in diameter, mostly located at the periphery of the lobes, without a marked distortion of the liver architecture. Much more rarely the newly formed bile ducts were surrounded by fibrous and hyalinized stroma with the appearance of cholangiofibrosis. In no case was there evidence of a neoplastic proliferation. The liver parenchyma suffered atrophic changes because of compression. The older animals of the control groups commonly had thrombosis of the left atrium, less frequently of the right, accompanied by chronic stasis lesions particularly involving the lungs and the liver. Amyloidosis was common in the spleen, kidneys, liver, and adrenals.

**DISCUSSION**

Using Shay's experimental model (13), Huggins (11) has found that larger doses of MC given orally provide the most rapid method for inducing mammary carcinomas in the rat. The present experiment has shown that the hamster responds in a similar manner. In certain respects this is a more striking occurrence, since mammary tumors are particularly rare in the hamster and since in this animal prolonged estrogenic stimulation results in the formation of renal carcinomas. The mechanisms of action of carcinogenic hydrocarbons in inducing mammary tumors in other rodents are obscure, although it is thought that a hormonal mechanism is involved. In the present experiment the simultaneous occurrence of ovarian tumors, in all but one instance, and of proliferation of the endometrial stroma, seems to indicate that in the hamsters, too, MC acts through a hormonal mechanism. However, other studies of the hormonal factors involved are needed to clarify this matter. No pituitary tumors were observed, but the histological and cytological study of the pituitary has not been extensive and detailed enough to establish the role of the pituitary in the production of the mammary and ovarian tumors. All the mammary tumors were carcinomas; two of them metastasized, and two were transplanted successfully into normal hamsters of both sexes.

The relatively high incidence of intestinal tumors, both of the small and large intestine, was not expected. Stewart and his collaborators (reviewed in [17]) reported the occurrence of a high incidence of intestinal tumors, but only of the small intestine, in mice fed MC or 1,2,5,6-dibenzanthracene. 2-Acetylaminofluorene, administered by mouth, produced tumors of the small intestine in several studies (10, 14), but only in those of Bielschowsky (2) and of Cox et al. (3) were more than occasional tumors of the large intestine observed. Spitz et al. (16) produced a high incidence of adenocarcinomas of the rectum in rats given injections subcutaneously of benzenidine, whereas Walpole et al. (19) induced tumors of the small and large intestine in rats given injections of 4-aminodiphenyl and a related compound. Stewart et al. (18) have recently reported the occurrence of adenocarcinomas of small and large intestine in rats fed 2,7-diacetylflorescence. Intestinal tumors are difficult to detect if not looked for, and this might explain the lower incidence observed in our first experiment and the absence in other experiments with MC chiefly performed to study mammary tumorgenesis in the rat (4, 11). However, the primary purpose of the experiments of Shay et al. (13) performed in rats was to induce tumors of the gastric glandular mucosa. They did not observe any tumors in the gastrointestinal tract, and this should indicate once more a species difference in response to carcinogens. It is interesting to note that Fortner (9) has observed a large number of intestinal tumors considered spontaneous in hamsters. In our control groups we did not find intestinal tumors, not even polyps of the type observed by Fortner in his animals. However, inflammatory lesions accompanied by hyperplastic and atypical glands were a common finding in our control hamsters. The relationship between the MC treatment, the inflammatory lesions, and the tumor occurrence needs further investigation.

The high incidence of squamous-cell tumors in the forestomach is similar to that observed in other species with MC and other carcinoenic polycyclic hydrocarbons (10, 14). Of the other tumors found in the MC-treated hamsters, the few sebaceous adenomas of the skin and sebaceous...
FIG. 5.—Female hamster killed 20 weeks after the beginning of the MC treatment (Exp. 2). Atypical glands at the site of the ileo-cecal valve. Hematoxylin and eosin, ×80.

FIG. 6.—Female hamster killed 38 weeks after the beginning of the MC treatment (Exp. 3). Cecum with atypical glands at the surface and early carcinomatous invasion. There were metastases in the lungs. Hematoxylin & eosin, ×40.

FIG. 7.—Female hamster killed 23 weeks after the beginning of the MC treatment (Exp. 4). Atypical, dilated glands in the colon. Periodic acid-Schiff, ×80.

FIG. 8.—Female hamster killed 40 weeks after the beginning of the MC treatment (Exp. 3). Diverticulosis of the colon. Hematoxylin and eosin, ×70.
Fig. 9.—Female hamster killed 40 weeks after the beginning of the mammary gland tumors. Mammary adenocarcinoma showing irregular glandular structures with papillary projections. Hematoxylin & eosin, X250.

Fig. 10.—Same animal as in Fig. 9. Mammary adenocarcinoma showing a follicular pattern. Hematoxylin & eosin, X120.

Fig. 11.—Same animal as in Fig. 9. Lymph node metastasis from mammary adenocarcinoma showing cystic structures and papillary projections. Hematoxylin & eosin, X25.

Fig. 12.—Female hamster which died 33 weeks after the beginning of the MC treatment (Exp. 3) and had three mammary adenocarcinomas. Ovarian thecoma. Hematoxylin & eosin, X200.
Fig. 13.—Female hamster which died 32 weeks after the beginning of the MC treatment (Exp. 3) and had one mammary adenocarcinoma and one thecoma. Hemorrhagic endometrial polyp. Hematoxylin & eosin, ×80.

Fig. 14.—Female hamster killed 40 weeks after the beginning of the MC treatment (Exp. 3) which had four mammary adenocarcinomas and bilateral thecoma. Atypical proliferation of endometrial stroma. Hematoxylin & eosin, ×200.

Fig. 15.—Same animal as in Fig. 9. Squamous-cell papillomas at the vaginal-cervical junction. Hematoxylin & eosin, ×80.

Fig. 16.—Male hamster killed 38 weeks after the beginning of the MC treatment (Exp. 1). Sebaceous adenoma of the dorsal sex organ (scent gland). Hematoxylin & eosin, ×30.
carcinomas of the ear-duct glands appear to be related to the treatment and indicate a specific localization of the lipide-soluble hydrocarbon. The occurrence of a single melanotic tumor, blue nevus type, confirms the weak activity of the MC action for this type of tumor in contrast with the high specificity observed with 9,10-dimethyl-1,2-benzanthracene (6, 15).

ACKNOWLEDGMENTS

The author wishes to acknowledge his gratitude to Dr. Philippe Shubik for the many stimulating discussions and advice; to Mrs. Shelagh Maxwell and Mrs. Sushila Conger for their help in conducting the experiments, and to Mr. Manuel Sueiro for the histological preparations.

REFERENCES

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