

Mammary Cancer Induced by a Single Dose of Polynuclear Hydrocarbons:

Routes of Administration

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THE EXPERIMENTAL, selective, induction of cancer of the breast in albino rats by a single dose of polynuclear hydrocarbons presents a powerful analytical technic since mammary cancer arises invariably and with great rapidity. The induction of neoplasms of the mammary gland in this way occurs under highly restricted conditions but these are easily fulfilled. The inductive method is one of extreme simplicity. This communication deals with effective routes of administration of aromatic compounds *vis-à-vis* the induction of experimental mammary cancer in the rat and dosage of the compounds was studied critically.

For 25 years it has been known that the repeated administration of certain polynuclear hydrocarbons induced mammary cancer. Maisin and Coolen⁶ induced mammary cancer in mice by repeatedly painting the skin with 3-methylcholanthrene. Wilson, De Eds and Cox⁹ observed the development of cancer of the breast (and other neoplasms) in rats by incorporation of 2-acetylaminofluorene in a diet which was fed for many weeks. Mammary cancer was induced by the repeated intragastric instillation of 3-methylcholanthrene by stom-

ach tube in rats.⁷ Cancer of the breast has been evoked by multiple intravenous injections of 7,12-dimethylbenz(a)anthracene.^{2, 8}

It was found in this laboratory^{3, 5} that a single dose of polynuclear hydrocarbons sufficed to induce mammary cancer under designated conditions. Critical factors are: 1) dosage of the carcinogen; 2) species; 3) strain; 4) age; and 5) hormonal status. The mammary tubules must neither be atrophic nor excessively stimulated by steroids when the carcinogen is exhibited. The hormonal status of young adult female rats is exquisitely adapted to the induction of mammary cancer. *In normal adult female rats of the Sprague-Dawley strain, age 50 to 65 days, mammary cancer develops invariably following the single feeding of 7,12-dimethylbenz(a)anthracene, 20 mg., dissolved in sesame oil.*⁵ The earliest mammary cancer induced by this technic was detected by palpation 20 days following the single feeding, and all rats were involved with mammary cancer within a few weeks thereafter.

The sites in which cancer is elicited by polynuclear hydrocarbons are selective and the tumors arise in two discontinuous series—mammary cancer within a few weeks and other cancers after four to 12 months. Among these latter is carcinoma of sebaceous glands adjacent to the external auditory meatus and sarcomas at the site of repeated simultaneous subcutaneous or intramuscular injections of hormones (equine

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TABLE 1. *Induction of Mammary Cancer by a Single Dose of 7,12-Dimethylbenz(a)anthracene Administered by Stomach Tube*

Dose, mg.	Rats with Cancer	Appearance of Palpable Tumors, Days.			Active Centers
		Range	Median	Mean	
1.0	1	67	—	—	1
2.5	2	81-85	—	—	1
5.0	5	53-83	77	72.0 ± 12	1.1
10.0	9	37-57	51	48.8 ± 7	2.0
15.0	10	34-70	49	48.5 ± 11	3.0
20.0	10	28-59	41	42.8 ± 9	6.8
30.0	9*	31-64	36	42.4 ± 12	2.5

±, Standard deviation. There were 10 rats in each group.

* One rat died at 36 days.

gonadotrophin, estrogens, etc.). Tumors of this sort are not invariable but develop with considerable frequency. Rare tumors are renal carcinoma, leukemia, retroperitoneal lipoma; these also develop late after the administration of polynuclear hydrocarbons in contradistinction to mammary cancer.

Mammary cancer evoked in albino rat by polynuclear hydrocarbons has a unique property amongst experimental tumors. A proportion of the induced tumors are hormone dependent and their cells undergo necrosis or atrophy when the hormonal *milieu intérieur* is modified appropriately. No other experimental mammary cancers with this property are available at the present time. Hormone dependent mammary cancer occurs rather frequently in clinical cancer of the breast and advantage has been taken of this quality of the human neoplasms for therapeutic purposes. The

investigation of hormonal dependent mammary cancer was confined to clinical studies before the finding³ of hormone dependence in the experimental tumor under discussion.

Methods

7,12-Dimethylbenz(a)anthracene,* m.p. 122-123° C., was recrystallized from acetone-alcohol. Containers of this compound were protected from light by wrapping in aluminum foil.

Female albino rats of the Sprague-Dawley strain were obtained from the dealer at age 42 days and kept thereafter under controlled climatic conditions. All of the rats remained free of respiratory disease throughout the experiment.

When the rats were age 50 to 52 days a single dose of 7,12-DMBA was adminis-

* 7,12-DMBA, 7,12-dimethylbenz(a)anthracene.

TABLE 2. *Induction of Mammary Cancer by 7,12-Dimethylbenz(a)anthracene, 20 mg., Injected into the Lumen of the Stomach or the Appendix*

No. Rats	No. with Cancer	Appearance of Palpable Tumors, Days			Active Centers
		Range	Median	Mean	
1. Intra-gastric administration					
10	10	31-73	37	41.7 ± 13	2.1
2. Intra-appendiceal administration					
17	8	47-196	121	105 ± 24	1.0

±, Standard deviation.

TABLE 3. *Induction of Mammary Cancer Following a Single Intravenous Injection of 7,12-Dimethylbenz(a)anthracene*

Dose, mg.	Deaths*	No. with Cancer	Appearance of Palpable Tumors Days,			Active Centers
			Range	Median	Mean	
1	0	8	62-136	84	101.2 ± 30	1.3
2.5	0	10	33-61	44	47.4 ± 11	2.4
5	0	10	40-131	45	64.1 ± 30	2.9
7.5	6	4	42-67	45	—	5
10	7	3	45-63	—	—	6
20	10	—	—	—	—	—

* Deaths from toxic effects.

±, Standard deviation. There were 10 rats in each group.

tered; this was never repeated. Surgical operations were performed under ether anesthesia.

The animals were observed for eight months unless mammary cancer developed earlier. Necropsy was performed on all rats and the number of visible mammary cancers (active centers) was counted. In the gross, mammary tumors have a characteristic appearance which enables them to be identified in most cases; but whenever doubt arose concerning the pathological status of the tumors, histological sections were examined. Large doses of 7,12-DMBA frequently cause selective necrosis of the cortico-medullary junction of the adrenal glands. The adrenals were examined for calcification at necropsy.

Results

In our colony of Sprague-Dawley rats, mammary cancer has been observed in two rats amongst more than 20,000 untreated animals less than eight months of age. But the incidence of benign mammary tumors (fibroadenoma) is high in this strain in old age.¹

Oral Administration of 7,12-DMBA. This compound, dissolved in sesame oil, 1.0 cc., was administered by stomach tube. Mammary carcinoma was induced by 7,12-DMBA, 1.0 mg., and there was a progressive increase in the incidence of this tumor (and the number of active centers) with increment of dosage until the optimal amount was reached (Table 1). The optimal amount

of 7,12-DMBA was 20 mg. for the induction of mammary cancer by a single dose: no rat succumbed from toxic effects of the compound at this dosage level and mammary cancer developed invariably and promptly. No neoplasm other than mammary cancer was observed in this series.

Intragastric or Intracolonic Injection of 7,12-DMBA. Laparotomy was performed and 7,12-DMBA, 20 mg. dissolved in sesame oil, 0.4 ml., was injected *via* a fine hypodermic needle into the lumen of the stomach or the appendix, respectively.

All of the animals which received an injection of 7,12-DMBA in the lumen of the stomach at the time of laparotomy developed mammary cancer. In this series of ten rats, six animals had calcification of the adrenals at necropsy.

By comparison the incidence of mammary cancer, and the number of active centers, was much reduced in animals which received the carcinogen by injection into the colon, but mammary cancer did occur rather frequently (Table 2). None had calcification of adrenals. No gastric or colonic neoplasms were observed in this experiment.

Intravenous Injection of 7,12-DMBA. This compound, in the form of a finely divided fat emulsion was injected in a caudal vein. The solution contained 7,12-DMBA, 5.0 mg. per ml.**

** We are indebted to Paul Schurr, The Upjohn Company, Kalamazoo, Michigan, for preparing this emulsion.

All rats which received 7,12-DMBA, 20 mg., succumbed from acute toxicity within 72 hours. Every rat receiving 7,12-DMBA, 1.0 to 5.0 mg., survived the ordeal. All of the animals receiving an intravenous injection of 7,12-DMBA, 2.5 or 5.0 mg., developed mammary carcinoma.

A single intravenous injection of 7,12-DMBA, 1.0 mg., was more effective (Table 3) than the oral administration of the same dose of this compound in inducing mammary cancer.

Discussion

There are many advantages in the induction of mammary cancer by a single dose of polynuclear hydrocarbons: amongst these are simplicity, saving of labor, economy of rare or costly compounds, decreased exposure of personnel to carcinogens. An additional advantage is the relatively brief period of action of the hydrocarbons following a single treatment.

Effective doses of carcinogenic hydrocarbons cause selective lesions in certain tissues and in this regard they are radiomimetic. A single feeding of 7,12-DMBA, 20 mg., damaged bone marrow and lymphatic apparatus⁵ and induced adrenal necrosis with subsequent calcification in the suprarenal glands. But this dosage of 7,12-DMBA caused no critical damage, if any, to gonadotrophin production by the pituitary or to ovarian function; in the animals so treated the estrus cycle was not disturbed usually.

In contrast to radiation, carcinogenic hydrocarbons induce proliferation of the mammary epithelium with early cancer thereafter.

In the present work a systematic study was made of the mammary carcinogenic effects of a single dose of a potent polynuclear hydrocarbon, 7,12-DMBA, and the optimal dosage of the compound has been established. Both the intravenous and alimentary routes are highly effective in producing mammary cancer by a single dose.

Summary

A single meal of 7,12-dimethylbenz(a)-anthracene suffices to induce cancer of the breast under restricted conditions, but these are easily fulfilled. The hydrocarbon need not traverse the entire gastro-intestinal tract to evoke mammary cancer; absorption from the colon alone suffices for this purpose although it is less efficient than when the carcinogen is injected in the stomach or administered intravenously.

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