Mammary and Peritoneal Tumors Induced by Intraperitoneal Administration of 7,12-Dimethylbenz[*a*]anthracene in Newborn and Adult Rats*

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SUMMARY

A lipide emulsion of 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA) was prepared which has the advantages of a high concentration of the hydrocarbon, ease of preparation, sterility, and stability. The vehicle was not toxic.

A single intraperitoneal injection of the emulsion of 7,12-DMBA induced mammary cancer preferentially in all Sprague-Dawley female rats, age 50 days, within 2 months. In the induction of mammary tumors, the emulsion was quantitatively as effective by the intraperitoneal as by the intravenous route.

No tumors of peritoneum were evoked by a single injection of the emulsified 7,12-DMBA, but peritoneal sarcomas arose in a high incidence following deposition of a compressed pellet of 7,12-DMBA in the peritoneal cavity.

A single, intraperitoneal injection of the emulsion of 7,12-DMBA in newborn female rats of Sprague-Dawley or Long-Evans strains evoked mammary tumors in high incidence. Benign mammary tumors developed in high and equal incidence in both strains following the injection of 7,12-DMBA, but the incidence of mammary cancer was rather high in Sprague-Dawley rats and very low in their Long-Evans companions. Runt disease was induced in some of the injected newborn rats.

A single intraperitoneal injection of lipide emulsion of 7,12-dimethylbenz[*a*]anthracene (7, 12-DMBA) was found to be a serviceable method of induction in rats of mammary tumors, both malignant and benign. No tumors of the peritoneum followed a single injection of the emulsified hydrocarbon, but peritoneal sarcomas developed in a large percentage of rats in which a compressed pellet of 7,12-DMBA was deposited in the peritoneal cavity.

Emulsions of polynuclear aromatic hydrocarbons have long been used in cancer research. The intraperitoneal injection of concentrated oil solutions (4) is unsatisfactory, because they elicit large amounts of fibrin and fibrous tissue (9) which cover all the abdominal viscera and cause death in many animals. An emulsion of hydrocarbon in an aqueous gelatin solution described by Boyland (1) has been used rather extensively (3) for intraperitoneal injection. Geyer *et al.* (2) prepared an emulsion of 7, 12-DMBA (0.044 per cent) which induced mammary cancer in rats after repeated intravenous injections.

The emulsion of 7,12-DMBA to be described in this paper has the advantages of a high concentration of the hydrocarbon, ease of preparation, sterility, and stability for long periods. The vehicle itself was well tolerated by the animals and was not carcinogenic. Under conditions which will be defined in this paper, mammary cancer arose in every rat within a few weeks.

MATERIALS AND METHODS

Rats of two strains, Sprague-Dawley (S-D) and Long-Evans (L-E), were bred at random *inter se* from stocks maintained in the laboratory.

Our sample of 7,12-DMBA, m.p. 122–3° C., was recrystallized from acetone-alcohol and further purified by Florisil chromatography. The hydrocarbon moved as a single spot in thin-layer chromatography.

Crystals of 7,12-DMBA were inserted in a

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drilled stainless steel block, equipped with a plunger closely fitting in the drill hole, and compressed by applying hammer blows to the plunger. The compressed pellets of 7,12-DMBA, 13.7–21.6 mg., were weighed on an analytical balance at the beginning and end of the experiment. In one experiment seventeen rats were anesthetized with ether, the abdomen was opened surgically, and a compressed pellet was deposited in the peritoneal cavity. To avoid fragmentation of the pellets, metal instruments were not used to handle them; instead, the pellets were dropped into a paper cone whose tip had been inserted in the peritoneal cavity. The incisions were closed with silk sutures. At the end of the experiment, the pellets were cleaned from adventitious tissues, dried at 100° C.

TABLE 1

MAMMARY CANCER INDUCED BY SINGLE INTRAPERITONEAL INJECTION OF AN EMULSION OF 7,12-DMBA

Recipients were Sprague-Dawley females, age 50 days, with ten rats in each group. The animals were sacrificed 180 days after the injection.

Dose (mg.)	No. survivors	No. rats with mammary cancer	Mammary cancer detected (days)		
			Range	Median	Mean*
20	2	2	42; 48		
10	8	8	39– 58	46	47.8± 6
5	10	10	36– 56	42	45.0± 8
2.5	10	10	49–165	84	85.7±33
0.5	10	1	119		
0	10	0	No tumors		

* ± Standard deviation of the mean.

for 24 hours, and extracted with benzene; after evaporation the benzene residue was weighed.

Emulsions of 7,12-DMBA were prepared in Upjohn Lipomul-IV.[1] The emulsions, protected from light in a refrigerator, have been stable for more than 18 months. In control experiments the vehicle containing no added hydrocarbon was used.

The day of administration of the hydrocarbon is designated 0-day, and the onset of tumors dates from this day. The rats were examined for tumors by palpation nearly every day beginning 3 weeks after 0-day for 180–210 days. Paraffin sections of all the tumors were subjected to microscopic examination.

Mammary glands of newborn rats were examined histologically. In addition, whole mounts of similar glands stained for the histochemical distribution of alkaline phosphatase were prepared by a method described earlier (8).

RESULTS

At age 180 days in control, untreated, virgin females in our colony the incidence (14) of mammary tumors was for S-D rats, mammary cancer, 1.2 per cent; fibroadenoma, 6.1 per cent. In similar L-E females the incidence (14) was: mammary cancer, 0; fibroadenoma, 5.3 per cent. No mammary tumors have been found in untreated males of either strain at this age.

Toxicity of 7,12-DMBA injected intraperitoneally.—Newborn S-D and L-E rats are similar in weight. On the day of birth 72 S-D rats weighed 6.4 ± 0.5 gm.; newborn L-E rats weighed 6.1 ± 0.7 gm. On their birthday, an emulsion of 7,12-DMBA was injected into the peritoneal cavity of rats of both strains in doses 0.05–2 mg.; there were five to nine rats at each level of dosage. The LD_{50} for newborn S-D rats was 0.28 mg.; for L-E rats, LD_{50} was 0.33 mg. Controls were given injections intraperitoneally of the vehicle devoid of hydrocarbon; there was no mortality when as much as 0.5 ml. of the vehicle was injected. When injected with the LD_{50} dose, deaths occurred between 8 and 19 days. A group of 65 newborn L-E rats was given subcutaneous injections in the interscapular region of 7,12-DMBA, 0.25 mg.; there were 38 survivors (58.5 per cent).

Runt disease was observed in some of the newborn rats given injections intraperitoneally of 7,12-DMBA, 0.25 mg.; there were eight runts in a group of 50 survivors.

S-D female rats, age 50 days, were given injections intraperitoneally of the emulsion of 7,12-DMBA, 0.5–20 mg.; control rats were given injections of the vehicle containing no hydrocarbon. The animals weighed 155–174 gm., and there were ten rats in each group. All rats receiving 7,12-DMBA, 0.5–5 mg., and all the controls survived. There were deaths in the group receiving 7,12-DMBA, 10 and 20 mg. (Table 1).

Tumors after intraperitoneal injection of 7,12-

[1] One of the emulsions of 7,12-DMBA, 0.5 per cent, was generously prepared by P. E. Schurr, The Upjohn Company, Kalamazoo, Michigan. The hydrocarbon was dissolved in the oil phase of Upjohn Lipomul-IV (United States patents: 2,870,019; 2,945,869; 2,977,283) and emulsified in the aqueous phase. Particles in the emulsion were submicroscopic.

Other emulsions of 7,12-DMBA (0.5 per cent) were made by an adaptation of the method of Meyer *et al.* (10). (*a*) Oil phase: cottonseed oil, 6 gm.; purified lecithin solids, 0.96 gm.; 7,12-DMBA, 0.2 gm. (*b*) Aqueous phase: dextrose, anhydrous, 1.66 gm.; Pluronic-F68, 0.12 gm.; water, 31.4 ml. Oil phase, containing the hydrocarbon in solution, was added dropwise to aqueous phase at 70° C. in a colloid mill at a rotor speed of 22,000 r.p.m. and homogenized for 2 hours at 70°. The colloid mill (Mini-Mill) was obtained from Gifford-Wood Co., Hudson, N.Y.

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DMBA in newborn rats.—There was a high incidence of mammary tumors in female rats of both strains following the intraperitoneal injection of the emulsion of 7,12-DMBA, 0.25 mg., on the day of birth. In L-E rats the tumors were predominantly fibroadenoma; in S-D rats many carcinomas of the breast were found, in addition to benign mammary tumors.

Fifteen S-D females (Table 2) received 7,12-DMBA on their birthday. Mammary tumors were found in all of them before age 180 days: mammary cancer, eight rats; fibroadenoma, six; mammary cancer and fibroadenoma, six animals. Mammary cancer was detected at age 56–146 days—mean, 88.9 ± 34 days; fibroadenoma was observed at 81–162 days—mean, 133 ± 28 days. Thirty-nine L-E females were given an injection of 7,12-DMBA on the day of their birth, and mammary tumors were detected in 31 (79.5 per cent): mammary cancer, two rats; fibroadenoma, 29 rats. Mammary cancer was detected at 72 and 128 days; fibroadenoma was observed at age 84–180 days—mean, 162 ± 21 days.

Eighteen S-D males received 7,12-DMBA on their birthday (Table 2). Mammary tumors, all fibroadenomas, were found in three of these rats. Fifty-four L-E males were given injections similarly; only one rat developed a mammary tumor, and this was a fibroadenoma.

A small number of tumors, other than those of the mammary gland, was found in rats given injections of 7,12-DMBA on their birthday. In S-D rats the only tumors in this class were squamous carcinoma of the sebaceous glands of the ear; this tumor was detected in one female and five males (Table 2). In L-E rats the following "miscellaneous" tumors were observed (Table 2): ear-duct tumors, two rats; renal adenocarcinoma, three; leukemia, two animals.

No sarcomas formed at the injection sites in these animals. No tumors of the peritoneum or of the abdominal viscera were observed.

Intraperitoneal injection of 7,12-DMBA in adult rats.—Groups of female S-D rats received an intraperitoneal injection of 7,12-DMBA, 0.5–20 mg., age 50 days; their body weight was 156–172 gm. The vehicle, 1 ml., devoid of hydrocarbon, was injected in one group of control rats. There was no mortality in this control group, and tumors did not develop in these animals.

Mammary cancer was found within 60 days after the injection in all survivors which received 7,12-DMBA, 5–20 mg. (Table 1). All rats given injections of 7,12-DMBA, 2.5 mg., likewise developed mammary cancer, but these developed at a slower rate; the time when the tumors were detected was 85.7 ± 33 days. Every rat given an injection of 7,12-DMBA, 5–20 mg., developed multiple mammary fibroadenomas before 180 days. Fibroadenoma in rats exposed to polynuclear carcinogenic hydrocarbons develops at a later date than mammary cancer does; the first of these benign tumors was detected at 139 days. In the groups given injections of 7,12-DMBA, 2.5 mg., seven of ten rats developed mammary fibroadenoma. Of 40 rats given injections of 7,12-DMBA, 0.5–20 mg., the following "miscellaneous" tumors were observed: ear tumors, three rats; sarcoma at injection site, one. There were no other tumors.

Adrenal calcification (6) was found at necropsy in four rats in a group of eight animals which survived the intraperitoneal injection of 7,12-DMBA, 10 mg. This stigma of earlier adrenal apoplexy selectively induced by 7,12-DMBA was not observed in other dosage groups.

Intraperitoneal pellets of 7,12-DMBA.—A sterile compressed pellet of 7,12-DMBA was deposited in the peritoneal cavity of seventeen S-D female rats, age 50 days, and the animals were observed for 7 months. At autopsy the pellet was found as a

TABLE 2

NEOPLASMS FOLLOWING INJECTION OF 7,12-DMBA INTO NEWBORN RATS

The rats, of Sprague-Dawley (S-D) or Long-Evans (L-E) strains, were autopsied at 180 days. 7,12-DMBA, 0.25 mg., had been injected intraperitoneally on the day of birth.

Strain	Sex	No. rats	Rats with tumors		Carcinoma		Fibroadenoma		Ear tumors		Other tumors
			No.	%	No.	%	No.	%	No.	%	
S-D	F	15	15	100	8	53.3	12*	80	1	6.6	None
L-E	F	39	31	79.5	2	5.1	29	74.4	0	0	Kidney, 2; leukemia, 1.
S-D	M	18	6	33.3	0	0	3	16.7	5	27.8	None
L-E	M	54	4	7.4	0	0	1	1.9	2	3.7	Kidney, 1; leukemia, 1.

* Six rats had mammary carcinoma and fibroadenoma.

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free-floating foreign body in two rats; it was encapsulated in the others.

Fourteen of the animals (Table 3) developed tumors at the site of encapsulation: spindle-cell sarcoma, eleven; mesothelioma, three. Two rats with peritoneal sarcoma developed mammary cancer in addition; these tumors were detected at 37 and 57 days, respectively, after implantation. There were no other tumors.

The pellets were extracted for hydrocarbon after their long sojourn. In general there was no change in their weight. In one experiment, the pellet weight was 16.0561 mg. originally, and the same amount of hydrocarbon was extracted 6 months later. An amount of hydrocarbon too small to be detected on our analytical balance had evoked peritoneal sarcoma and mammary cancer. It is appreciated that contamination of the outside of the pellets with small amounts of lipides is not unlikely, and these would be included in this determination.

TABLE 3

TUMORS OF PERITONEUM INDUCED BY COMPRESSED PELLET OF 7,12-DMBA

At age 50 days a compressed pellet of 7,12-DMBA was deposited in the peritoneal cavity of Sprague-Dawley females.

No. rats	Rats with peritoneal tumors	Peritoneal tumors detected (days)		
		Range	Median	Mean*
17	14 (82.3%)	133–213	148	155.3±25

* ± Standard deviation of the mean.

DISCUSSION

In these experiments it was found that the intraperitoneal injection of 7,12-DMBA, 5 mg., in finely emulsified state, caused no fatalities and induced mammary cancer within 60 days in every female S-D rat, age 50 days.

Under the same conditions, a single feeding of 7,12-DMBA evokes mammary cancer and in stoichiometric relationship (5) to the dose of the hydrocarbon. The incidence progressively increases when rats are fed a single meal with doses of 7,12-DMBA, 1–15 mg. A single feeding of 7,12-DMBA, 5 mg., induced mammary cancer in 50 per cent of the rats (5). In earlier experiments (7) the same batch of emulsified 7,12-DMBA used in the present work was injected intravenously into S-D female rats, age 50 days; mammary cancer was evoked in every rat following the intravenous injection of 7,12-DMBA, 2.5–5 mg., and there were no fatalities. Quantitatively the intraperitoneal injection of 7,12-DMBA resembles intravenous injection in its efficiency in evoking mammary cancer.

In mice, Pietra *et al.* (13) gave intraperitoneal injections of an aqueous suspension of hydrocarbons in 1 per cent gelatin; the usual dose of the hydrocarbon was 30–40 μg.; four hydrocarbons were investigated. They (13) observed both an augmented incidence and accelerated rate of development of lymphoma and pulmonary tumors, whereas there were no demonstrable influences upon the induction of mammary cancer. The results from the intraperitoneal injection of 7,12-DMBA in newborn rats differ from the findings in mice. In S-D females one-half of the rats developed mammary cancer, whereas leukemia was not induced. In L-E newborn rats, fibroadenoma was the predominant mammary tumor, and there was a small incidence of leukemia and renal tumors. Pulmonary tumors were not detected in either strain; Miller and Miller (11, 12) injected a suspension of *N*-hydroxy-2-acetylaminofluorene into *weanling* rats and observed the development of mammary cancer in *ca.* 50 per cent of the animals by 22 weeks.

The mammary glands of both S-D and L-E newborn rats are morphologically similar. A well developed duct system is present (Figs. 1, 2), whereas the acini consist merely of slight dilatations of terminal ducts. Yet these mammary glands of both strains are highly susceptible to induction of mammary tumors by 7,12-DMBA.

A difference in racial strain of origin is already apparent at birth in the mammary glands of newborn S-D and L-E rats in tumor formation in the mammary gland after a single intraperitoneal injection of 7,12-DMBA. Mammary fibroadenomas were evoked in high and similar incidence in both strains. The racial difference concerns mammary cancer; carcinoma of the breast occurred in moderately high incidence (53.3 per cent) in S-D rats,

FIG. 1.—Whole mount of mammary gland of S-D rat, on birthday, in which the sites of alkaline phosphatase have been demonstrated. ×90.

FIG. 2.—Histological section of mammary gland of S-D rat, on birthday. H. & E., ×180.

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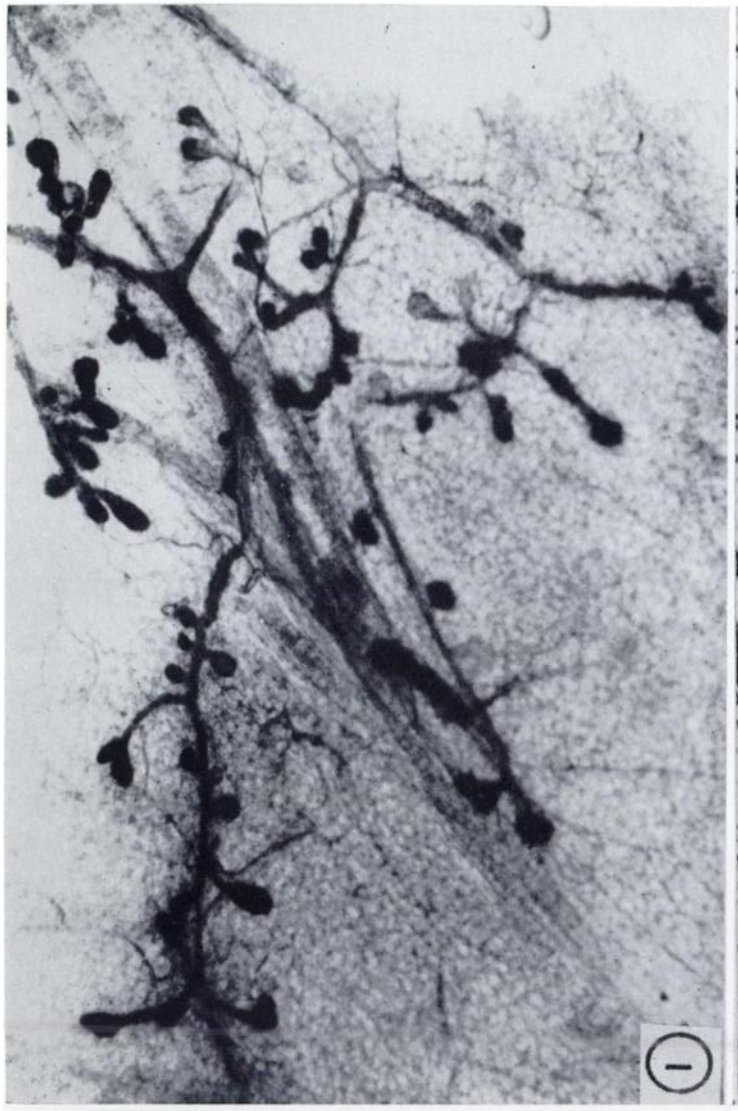

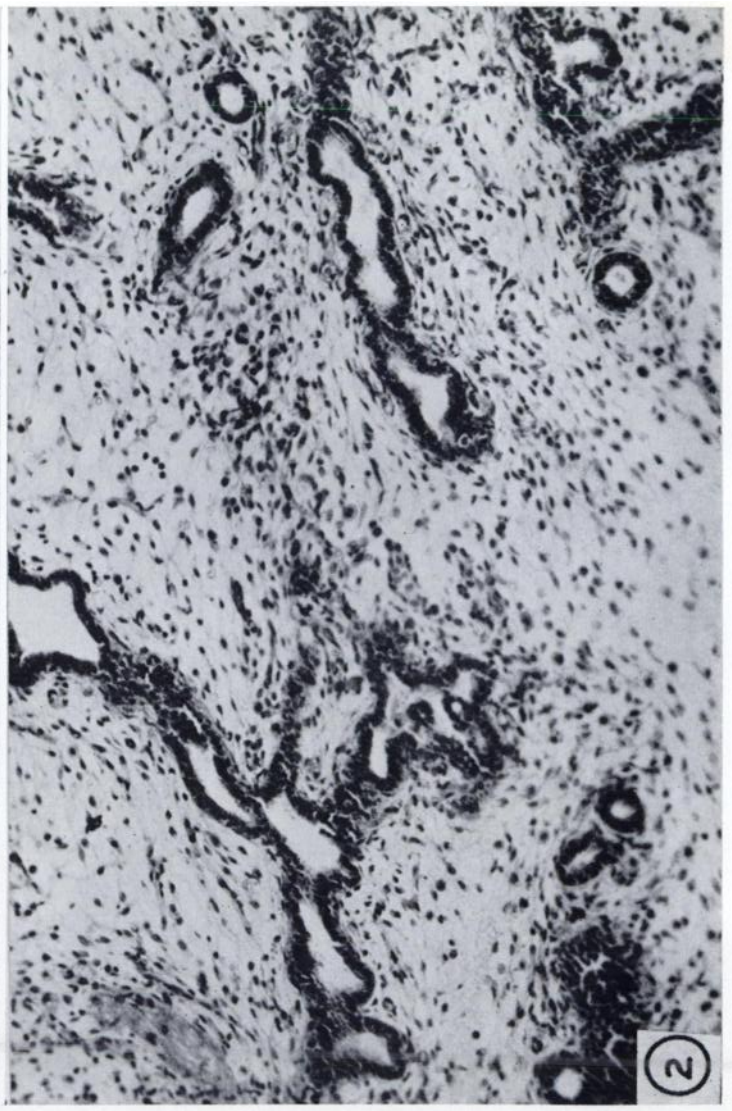

whereas in L-E rats the incidence was 5.1 per cent.

This difference in susceptibility of S-D and L-E female rats, age 50 days, to hydrocarbon-induced mammary cancer formation has been investigated (14) earlier. Whereas S-D females at this age are highly susceptible to hydrocarbon-induced mammary cancer, similar L-E females are relatively insusceptible. The insusceptibility of L-E adult females is relative, since it can be easily overcome; repeated administration of massive but tolerable amounts of hydrocarbon—e.g., the repeated intravenous injection of 7,12-DMBA, led to development of mammary cancer in 94 per cent of the L-E recipients.

An observation of interest in the present work concerned the peritoneal tumors; none occurred after injection of the emulsion of 7,12-DMBA into the peritoneal cavity. Yet the peritoneal cells are not insusceptible to the carcinogenic effects of this hydrocarbon; a compressed pellet of 7,12-DMBA deposited in the peritoneal cavity evoked tumors of the peritoneum in most rats. It would appear that not every cell of the rat is susceptible at all times to malignancy after contact with carcinogenic hydrocarbons and that a solution of 7,12-DMBA disappears from the peritoneal cavity before adequate contact with a cell in a state susceptible to the malignant transformation. In this regard, a pellet in prolonged contact with peritoneal cells sooner or later would seem to find cells prone to cancer formation.

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