AROMATIC-INDUCED PREVENTION OF FATAL TOXICITY OF 7,12-DIMETHYLBENZ[*a*]ANTHRACENE*

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Certain polynuclear aromatic hydrocarbons and aromatic amines are highly poisonous to the rat and death soon ensues after a single large dose. In the work now to be described, it was found that a small amount of any of a considerable number of aromatics given some hours before a massive dose of 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA)[1] which was highly toxic, induced a physiologic state which protected the recipient from the otherwise fatal amount so that life of the recipient was preserved. Protection of this sort is a spectacular effect. Its mechanism seems to be related to that involved in aromatic-induced protection of adrenal cortex against 7,12-DMBA described in the preceding paper (1).

7,12-DMBA exerts several biological effects which set it apart from all other aromatics. Among the pathologic manifestations is its effect on testis. A single dose of 7,12-DMBA causes selective destruction of testicular cells (2) specifically of germinal epithelial cells synthesizing DNA, which leads to profound, if temporary, atrophy of testis. Atrophy of this sort was largely prevented by prior administration of the protective aromatics.

Influence of Aromatics on Abnormal States.—2-Amino-5-chlorobenzoxazole induces paralysis in rats; intraperitoneal injection of 3-methylcholanthrene (3-MC) or benzo[*a*]pyrene, 24 hours before the muscle relaxant, caused the duration of the paralysis to be shortened very considerably (3).

Aromatics also influence the onset of cancer. Lacassagne *et al.* (4) repeatedly applied a solution of 3-MC to the skin of mice and cutaneous growths were evoked. The incidence of skin tumors was delayed when the solution of 3-MC also contained other hydrocarbons; *e.g.*, dibenz[*a*, *g*]fluorene.

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[1] The following abbreviations are used: DMBA, dimethylbenz[*a*]anthracene; B*a*A, benz[*a*]-anthracene; 3-MC, 3-methylcholanthrene; 3′-Me-DAB, 3′-methyl-4-dimethylaminoazobenzene; TdRH^3, tritium-labeled thymidine; and DNA, deoxyribonucleic acid.

Richardson *et al.* fed mice 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) and liver tumors arose; fewer neoplasms of the liver developed in companion groups which received, additionally, 3-MC applied to the vagina (5) or incorporated in the diet (6). Miller *et al.* (7) found that hepatic tumor induction by 3′-Me-DAB was inhibited when one of the following aromatics was added to the diet: 3-MC, benzo[*a*]pyrene, benz[*a*]anthracene (B*a*A), or dibenz[*a*, *h*]anthracene.

Methods

The experimental animals were normal rats of Sprague-Dawley strain: females age 46 to 49 days weighing 140 to 160 gm (mean 151 gm); males, age 25 days weighing 51 to 88 gm (mean 69 gm); males age 53 days (mean 230 gm). They were provided a commercial ration and water *ad libitum* and kept in air-conditioned rooms at 25° ± 1°C.

Organic compounds were recrystallized from appropriate solvents. In this paper *feeding* is the administration by gastric intubation of a solution of hydrocarbon in sesame oil. Unless stated otherwise, *injection was intravenous administration.* Lipide emulsions[2] of 7,12-DMBA, 0.5 per cent (w/w), and of 3-MC, 0.25 per cent, were injected. *dl*-Ethionine was dissolved in a minimal quantity of 0.1 N sodium hydroxide and injected in peritoneal cavity.

The aromatics to be evaluated as protectors were given prior to the massive dose of highly toxic compounds. Usually 24 hours after the protectors, the highly toxic compound, 7,12-DMBA, was injected; this point of time was designated day 0. The dosage of compounds which killed one-half of the rats, LD_{50}, was calculated by the probit method of Gaddum (8). By definition, *survival is life until day 21 or longer.* There were 8 to 10 rats in each experimental set except for studies involving tritium in which each group consisted of 4 animals.

At necropsy testis was weighed on a torsion balance and fixed in Bouin's fluid; paraffin sections were stained with PAS (9). Stages of the cycle of testis tubules were classified according to Leblond and Clermont (10).

Menadione reductase was estimated in samples of liver as described in the preceding paper (1).

In some experiments thymidine-H^3 was administered and the content of tritium was determined in washed perchloric acid–insoluble residue of testis and adrenal and in non-volatile fraction of terminal ileum. In brief, 1 ml of a saline solution of tritium-labeled thymidine ($TdRH^3$), 0.5 μc/gm, was injected intravenously at 0 hours, after the experimental animals had received hydrocarbons; control rats received $TdRH^3$ alone. The tissues were harvested at 20 to 24 hours and weighed. Testis and adrenal were homogenized in ice-cold 0.5 N perchloric acid containing non-radioactive thymidine, 0.05 per cent. The homogenate was centrifuged at 11,000 *g* for 10 minutes at 0°C, the supernatant was removed, and the precipitate washed twice by trituration with the cold perchloric acid–thymidine solution. Ileum was dried from the frozen state. Tritium was determined on the residues by the combustion technique of Jacobson *et al.* (11); the results are expressed on the basis of original weight of the samples and presence of tritium in the residues is designated *incorporation.*

RESULTS

Toxicity of 7,12-DMBA.—The LD_{50} for a single intravenous injection of 7,12-DMBA in rats age 25 days was determined: it was for males 58 mg/kg and for females 54.5 mg/kg. Earlier the LD_{50} for a single feeding of 7,12-DMBA was found (12) to be 270 mg/kg.

[2] We are indebted to Paul Schurr, The Upjohn Co., Kalamazoo, Michigan for preparing lipide emulsions. Professor M. S. Newman, The Ohio State University, Columbus, generously donated 3,9-DMBA and 6,8-DMBA.

Prevention of Loss of Body Weight in Rats Injected with 7,12-DMBA.—Without exception in 50 rats, age 46 to 50 days, injection of 7,12-DMBA, 5 mg (33 mg/kg), was followed during the 1st day by loss of weight, on average 10.6±5 gm; thereafter gain of weight ensued. Loss of weight during the first 24 hours after 7,12-DMBA, 5 mg, was prevented by a single dose, given on day −1, of any of 12 aromatics; instead of losing weight the pretreated animals gained. The aromatics which protected against loss of weight, their dosage, and the routes of administration are:

	mg
By intravenous injection:	
3-MC	0.5 to 2
Benz[*a*]anthracene	10
By feeding:	
3-Aminochrysene	1
3,9-DMBA	2
6-Aminochrysene	5
Cyclopentenophenanthrene	7.5
7,12-DMBA transannular peroxide	10
3-MC	10
6,8-DMBA	10
7-Methyl BA	10
10-Methyl BA	10
6,7,8-Trimethyl BA	10
Benz[*a*]anthracene	50
Phenanthrene	200

Signs of Toxicity in Adult Rats Injected with a Large Amount of 7,12-DMBA.—Injections of the lipide emulsion, 4 ml, without hydrocarbons were given to 10 rats age 46 days; there was no evidence of toxicity or loss of weight.

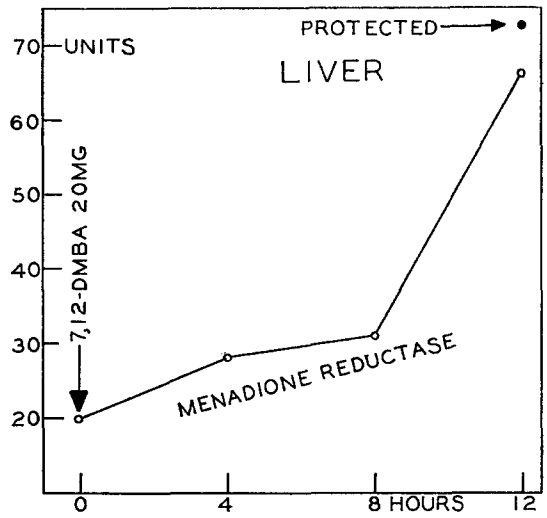
An injection of 7,12-DMBA, 20 mg (133 mg/kg), caused death of 20 consecutive rats age 46 days in 16 to 21 hours. There was a remarkable uniformity in their response. There were no pronounced changes in behavior in the early hours after injection of 7,12-DMBA. The animals had alternating periods of activity and sleep, a pattern similar to that of untreated rats in a well-illuminated room. The injected rats ate little or no food but drank much water. After 2 to 5 hours ears and paws and tail became red from vasodilatation and this persisted until death; the brown color of extensive methemoglobinemia was absent. Abruptly at 8 to 9.5 hours, there was a copious evacuation of watery stool containing much mucus; diarrhea continued at frequent intervals until death. At 10 hours the animals looked sick and the fur was untidy; they remained crouched and inactive (Fig. 1). The eyes were watery and the animals became cold to the touch. There was an agonal convulsion followed in a few minutes by death at 16 to 21 hours. At necropsy, the most obvious abnormal findings in the gross were in gastrointestinal tract; stomach was distended with fluid and food; ileum was dilated with fluid contents. The spleen was small and adrenals were slightly red but without apoplexy.

The concentration of menadione reductase in liver was estimated at intervals following injection of an overwhelming dose of 7,12-DMBA, 20 mg, in rats age 46 days.

There was an increase (Text-fig. 1) in concentration of the enzyme at 4 hours and to a higher level at 8 hours; a considerable increase (330 per cent of controls) of menadione reductase was observed at 12 hours.

Prolongation of Survival of Adult Rats Given Large Quantities of Aromatics.—Groups of females, age 46 days were injected with one of a series of gradually increasing doses of 3-MC, 24 hours prior to an injection of 7,12-DMBA, 20 mg. This dose of 7,12-DMBA caused death of all rats rapidly unless protection by aromatics had been induced.

Doses of 3-MC, 0.25 mg and 0.5 mg, did not result in prolongation of life. Each of 10 rats injected with a dose of 3-MC, 1 mg, prior to 7,12-DMBA succumbed in 1 to



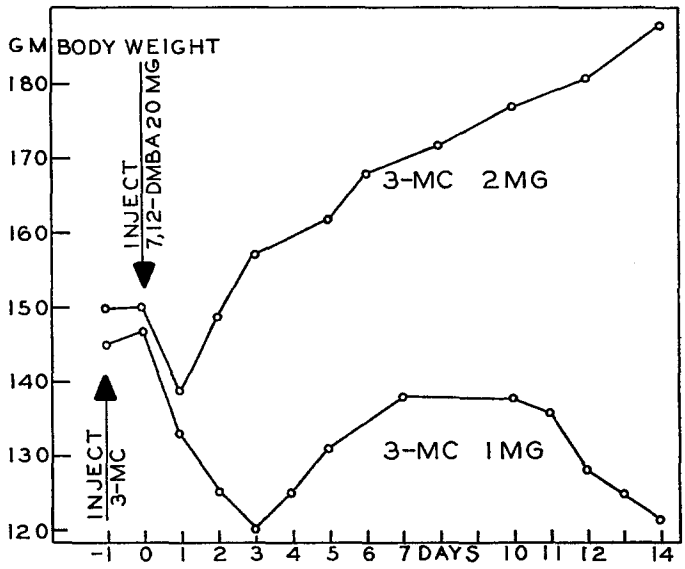
TEXT-FIG. 1. Increase in concentration of menadione reductase in liver following injection of 7,12-DMBA, 20 mg. The hepatic level of menadione reductase of rats protected with 3-MC, 2 mg, 24 hours prior to 7,12-DMBA, 20 mg, is shown in upper right hand corner. Mean values are given.

20 days, mean survival 11.5±2.3 days. Surviving animals lost weight from day 0 to day 3 and then gained weight until day 10; a secondary loss of weight ensued after day 11 (Text-fig. 2).

All rats injected with 3-MC, 2 mg (13.3 mg/kg) on day −1 survived injection of 7,12-DMBA, 20 mg, on day 0. Their course was very different from rats receiving 7,12-DMBA, 20 mg, without prior injection of 3-MC. There was decreased intake of food with increased thirst on day 0 and a loss of weight occurred (Text-fig. 2). Recovery from loss of weight was evident on day 2. There was no diarrhea or convulsion. There were no obvious signs that the animals had been given a dose of 7,12-DMBA which was overwhelming in the absence of protection established in advance. In rats given a preliminary injection of 3-MC, the level of menadione reductase in liver, 12 hours after 7,12-DMBA, was elevated but it did not differ significantly from the levels found in rats which had received 7,12-DMBA alone (Text-fig. 1).

The protective effect of 3-MC injected prior to a large dose of 7,12-DMBA was abolished in most of the animals when ethionine was given soon after 3-MC. On day −1 at 0 hours, an injection of 3-MC, 2 mg, was given to each of 24 male rats, age 53 days; ethionine, 50 mg, was given to 12 of them at +0.5 hours later and to the other 12 at +8 hours; each of them was given an injection of 7,12-DMBA, 15 mg (66 mg/kg) on day 0. In the set given ethionine at +0.5 hours, 9/12 rats died with deaths on day 1 and day 2. In the set given ethionine at +8 hours all rats survived.

Prolongation of Life of Juvenile Rats Given Large Quantities of 7,12-DMBA.—An injection of 7,12-DMBA, 6 mg (86 mg/kg), was given to each of 34 rats age 25 days; all of them died in 1 to 5 days with median survival 2±0.9 days. A group treated in the same way was given, in addition, subcutaneous injections of 0.15 N sodium



TEXT-FIG. 2. Growth curves of 2 groups of rats which were injected with 3-MC, respectively 1 or 2 mg at age 46 days and 7,12-DMBA, 20 mg, at age 47 days.

chloride, 3 ml twice daily, to lessen dehydration; all of these animals died in 2 to 5 days.

A study was made of the influence of aromatics given to males, age 24 days with mean weight 60 gm, upon their survival after injection, 1 day later, of 7,12-DMBA, 6 mg. It is convenient to assemble data of this sort as in Table I, since a shift of survival time to the right indicates at a glance that a compound has effectiveness as protector.

A single feeding, 1 mg, of each of 5 compounds on day −1 failed to influence survival time after 7,12-DMBA, 6 mg. Compounds ineffective in this way were: retene, fluorene, anthracene, acenaphthene, and phenanthrene. Likewise, feeding menadione, 1 to 50 mg, or *dl*-α-tocopheryl acetate, 12 to 50 mg, did not protect life.

7,12-DMBA itself, fed on day −1, had weak activity as an inducer of protection

against intravenous 7,12-DMBA, 6 mg. Life was not prolonged in rats which had been given a prior feeding of 7,12-DMBA, 0.5 mg or 1 mg; survival was longer in rats fed 7,12-DMBA, 2 mg (Table I), but all of the animals died within 16 days; in a group fed 7,12-DMBA, 3 mg, 7/10 rats survived.

Five compounds were highly effective in inducing protection since all members in each group fed 1 mg on day −1 survived after intravenous 7,12-DMBA, 6 mg. The effective compounds were: 3-MC, 3,9-DMBA, 6,8-DMBA, 6-aminochrysene, and benz[*a*]anthracene.

The effectiveness of 3-MC injected on day −1 (age 24 days) as an inducer of protection against 7,12-DMBA, 6 mg, was studied in some detail. Injection of 3-MC,

TABLE I

Effect of Aromatic Protectors on Survival

Survival of male rats (*ca.* 60 gm) given small amounts of hydrocarbons at age 24 days (day −1); the highly toxic dose of 7,12-DMBA, 6 mg, was injected intravenously at age 25 days (day 0). 3-MC was injected intravenously; other hydrocarbons were fed.

Hydrocarbon		Survival	Deaths ~ day																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	mg																		
None		0/34	8	21	2	2	1												
3-MC	0.05	0/8		1	1		2		2			1					1		
3-MC	0.1	0/9							1			5		1	1		1		
3-MC	0.25	17/24										3	1				1		2
3-MC	0.5	24/24	No deaths																
7,12-DMBA	1	0/8		2	4			1	1										
7,12-DMBA	2	0/8								1	2	2			1	1	1		
7,12-DMBA	3	7/10			1							1	1						
6,8-DMBA	1	7/7	No deaths																
3,9-DMBA	1	7/7																	
6-Amino-chrysene	1	8/8																	

50 to 100 μg, delayed death of many rats (Table I) but all of them succumbed; 3-MC, 0.25 mg, permitted 17/24 rats to survive; injection of 3-MC, 0.5 mg, 1 mg, or 2 mg, 24 hours prior to 7,12-DMBA, 6 mg, resulted in survival of all of the animals.

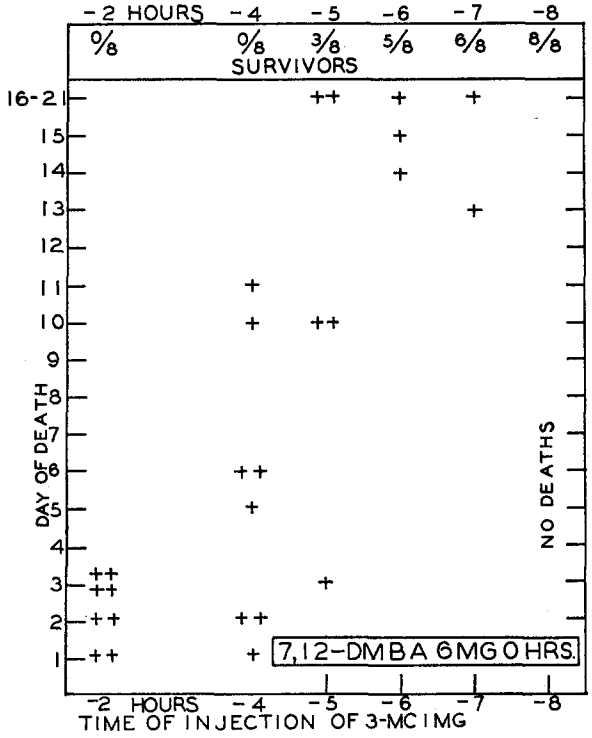
How large a dose of 7,12-DMBA can protected rats withstand? On day −1, all of the rats, age 24 days, were injected with 3-MC, 1 mg. On day 0 ten rats received 7,12-DMBA, 10 mg (166 mg/kg), in a vein; all of the animals have survived 67+ days. Another group of 10 rats was injected on day 0 with 7,12-DMBA, 20 mg (332 mg/kg), and all of these animals died, death occurring on days 9 to 11; life had been extended slightly.

On day −1 at 0 hours an injection of 3-MC, 2 mg, was given to each of 16 male rats, age 24 days; an injection of ethionine, 37.5 mg, was given to 8 of them at +0.5 hours and to the other 8 at +8 hours; all rats were given 7,12-DMBA, 6 mg, on

day 0. In the group given ethionine at +0.5 hours, there were 3 survivors; 5 rats died in 1 to 16 days, median 8 days. In the set given ethionine at +8 hours, all rats survived.

Time Dependence in Aromatic-Induced Preservation of Life.—It was found that aromatics needed to be injected some hours prior to toxic doses of 7,12-DMBA in order to cause life to be preserved. The time required to induce protection was determined.

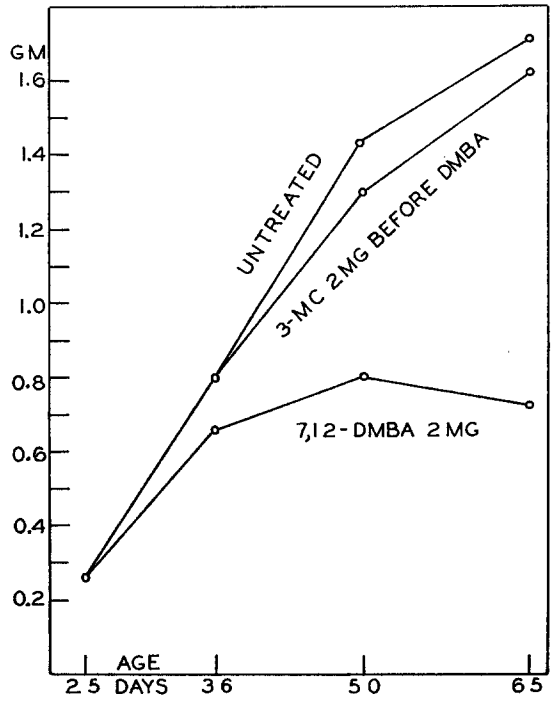
Male rats age 25 days were studied. An injection of 3-MC, 1 mg, was given to each member of a group of 8 animals at −2 hours; 7,12-DMBA, 6 mg, was injected



TEXT-FIG. 3. Survival of rats age 25 days injected with 3-MC, 1 mg, −2 to −8 hours, prior to 7,12-DMBA, 6 mg.

at 0 hours. Other groups were treated in a similar way except that the interval between 3-MC and 7,12-DMBA was longer, −4 to −8 hours. Rats which received 7,12-DMBA alone died within 5 days (Table I). Survival time was not modified in those rats injected with 3-MC at −2 hours. The first group to show the influence of protection was that injected with 3-MC at −4 hours; all of the animals died but time of their survival was prolonged somewhat (Text-fig. 3). The number of survivors and time of survival was well increased progressively with longer intervals (−4 to −8 hours) between 3-MC and 7,12-DMBA. There were no deaths in animals which had been injected with 3-MC, 1 mg, 8 hours before 7,12-DMBA, 6 mg.

Protection of Testis by 3-MC against Damage by 7,12-DMBA.—Thirty-six adolescent male rats were separated into three groups: (*a*) age 25 days, 7,12-DMBA, 2 mg, was injected intravenously; (*b*) at age 24 days 3-MC, 2 mg, was injected followed by 7,12-DMBA, 2 mg, at age 25 days; (*c*) untreated controls. Sets of these groups were sacrificed, respectively, at age 36, 50, and 65 days. At age 25 days, the seminiferous epithelium consisted of spermatogonia, resting spermatocytes, spermatocytes in meiosis, and a few early spermatids.



TEXT-FIG. 4. Growth of testis in rats injected with (*a*) 7,12-DMBA, 2 mg; (*b*) 3-MC, 2 mg, 1 day before 7,12-DMBA, 2 mg; (*c*) no hydrocarbons.

The findings in testis of rats which received 7,12-DMBA at 25 days were similar to those reported earlier (2). The testis increased in size for 11 days (the weight of testis at age 36 days was +260 per cent of that at 25 days) after which time the curve of testis weight entered a plateau lasting until age 65 days. The testis had been severely injured by 7,12-DMBA and the primary sites of destruction were spermatogonia and resting spermatocytes exclusively. The initial gain in size (for 11 days) occurred largely because of maturation of cells derived from spermatocytes which proliferate by meiosis; the subsequent plateau of growth resulted from earlier and severe damage to germinal stem cells which synthesize DNA.

The findings were very different in testis of rats of group *b* which had received 3-MC prior to 7,12-DMBA; testis had been injured by hydrocarbons but the injury

was slight. Weights of testis (Text-fig. 4) at age 36 and 65 days were not significantly different from those of untreated controls; weight of testis of rats which had received the two hydrocarbons was lower ($p < 0.01$) than that of controls at age 50 days. Histological examination of testis of protected rats revealed: at age 36 days there seemed to be a slight decrease in the number of pachytene spermatocytes in tubules in stages VI and VII; at age 50 days there was a small decrease in number of spermatids in steps 8, 9, and 10. At age 65 days cytological appearance of testis tubules of protected rats did not differ from those of normal rats. In contrast, testis tubules of rats which had received 7,12-DMBA as the sole hydrocarbon were highly disorganized and atrophic.

Effect of 7,12-DMBA and 3-MC on Incorporation of Tritium into DNA.—It was reported previously (13) that the administration of sublethal doses of 7,12-DMBA

TABLE II

Effect of Hydrocarbons upon Incorporation of Tritium in Juvenile Rats

Male rats, age 25 days weighing *ca.* 60 gm, were injected with 7,12-DMBA, 6 mg, and 3-MC, 2 mg, at various times before TdRH^3 (4.8 μg = 30 μc) at 0 hours, and organs were harvested 20 to 24 hours later. Control rats received TdRH^3 only. All injections were intravenous. There were 4 rats in each group. Tritium content of washed perchloric acid-insoluble residue of testis and adrenal and of non-volatile fraction of ileum is expressed as μc/gm wet weight of original tissue; mean values are given.

	Testis		Adrenal		Ileum	
	μc/gm	*per cent*	*μc/gm*	*per cent*	*μc/gm*	*per cent*
Controls; TdRH^3 alone	0.12	100	0.15	100	1.0	100
7,12-DMBA, −4 hrs.*	0.06	50	0.03	20	0.06	6
7,12-DMBA, −24 hrs	0.02	17	0.03	20	0.07	7
3-MC, −24 hrs	0.09	75	0.08	53	0.97	97
3-MC, −24 hrs. *plus* 7,12-DMBA, −4 hrs.	0.09	75	0.08	53	0.54	54

* There was 1 rat in this group.

4 or more hours prior to the injection of tritiated thymidine markedly decreases the incorporation of radioactivity into the perchloric acid–insoluble fraction of rat testis, adrenal, and ileum. This decrease in non-volatile radioactivity always was accompanied by a significant rise in the volatile radioactivity present in the tissue fluids. In the present experiments a lethal dose (6 mg) of 7,12-DMBA was found to cause a profound decrease in tritiated thymidine incorporation, especially in ileum (Table II). This decrease was partially prevented by the administration of 3-MC in an amount (2 mg) which also prevented fatal toxicity.

In experiments similar to that reported in Table II, using a sublethal dose of 7,12-DMBA (2 mg) the ability of 3-MC (2 mg) to counteract the lowering of tritium incorporation was even more striking, especially in ileum where complete restoration to normal levels often was observed. In all cases, the increase of volatile radioactivity in the tissue fluids caused by 7,12-DMBA was largely eliminated in the animals receiving 3-MC as well.

DISCUSSION

The administration of small doses of the protective aromatics prior to massive doses of 7,12-DMBA was found to be of crucial importance for survival of the recipient. In many ways the effect is reminiscent of hydrocarbon-induced protection (1) of adrenal. In each case, time is required for induction of protection. In each case, compounds possessing 4 or 5 condensed rings were more effective than were compounds composed of 3 aromatic rings; indeed, highly efficient inducers of adrenal protection were also remarkably efficient in causing life to be preserved. Further, protection was abolished by ethionine given soon after the small dose of hydrocarbon; protection was not influenced when the amino acid was given 8 hours after 3-MC.

While prior treatment with a small dose of an aromatic was highly advantageous in protecting life, a limit of dosage of 7,12-DMBA was found and the rat died when this amount was exceeded. LD_{50} for injections of 7,12-DMBA was 58 mg/kg in unprotected animals. When 3-MC, 1 mg, had been given 24 hours earlier, rats survived injection of 7,12-DMBA, 166 mg/kg, but succumbed after an injection of 7,12-DMBA, 332 mg/kg.

It is known that carcinogenic hydrocarbons have a strong influence on DNA. The application of 7,12-DMBA to plucked skin resulted in decreased incorporation of tritium in nuclei of growing hair follicles of mice given $TdRH^3$ sometime after the hydrocarbon (14). And there was a decrease in rate of synthesis in cells in the S phase (15). 7,12-DMBA has been found (13) to depress the incorporation of tritium in DNA of testis, adrenal, and ileum. In the present work it was found that the drastic effect of 7,12-DMBA upon DNA synthesis was considerably less in animals pretreated in an appropriate manner with 3-MC. It has been found (2) that the selective destruction of cells in rat testis caused by 7,12-DMBA arose from genetic death because the cell passed through one or a few cell divisions before it succumbed. Damage to testis was slight in rats given a small dose of 3-MC 1 day before a dose of 7,12-DMBA (2 mg) which caused atrophy of germinal epithelium of high grade in unprotected animals.

It is noteworthy that 7,12-DMBA resulted in a considerable decrease in incorporation of tritium in DNA of adrenal cortex of rats age 25 days. It will be recalled that 7,12-DMBA does not exert an adrenocorticolytic effect in animals of this age (16).

It is remarkable that synthesis of menadione reductase was stimulated in liver of rats injected with an overwhelming dose of 7,12-DMBA (lethal within 21 hours) at a time when incorporation of thymidine, hence synthesis of DNA, was considerably decreased.

From the evidence which has been presented it is deduced that synthesis of protein is of critical significance in the beneficial action of the protective aromatic which preserves life. An intravenous injection of a big dose of 7,12-

DMBA suddenly bathes cells with a very toxic compound in high concentration. In the preceding paper (1) it was shown that a previous injection of 3-MC did not cause 7,12-DMBA to be inactivated rapidly, insofar as its capacity to induce synthesis of menadione reductase in liver. It would appear that the newly synthesized protein of critical significance in aromatic protection is not exclusively concerned with degradation of 7,12-DMBA to less toxic compounds.

SUMMARY

Large doses of 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA) caused the death of rats within 1 day. A small amount of any of 5 polynuclear aromatic hydrocarbons or of an aromatic amine given before the highly toxic dose of 7,12-DMBA resulted in survival for more than 2 months and the specific atrophy of testis which follows 7,12-DMBA was largely prevented. Among the protective aromatics is 7,12-DMBA itself; a small dose of 7,12-DMBA given in advance induced protection of life against an otherwise lethal dose of 7,12-DMBA but only in a proportion of the animals, and testis was not protected from injury. The highly efficient inducers of protection were condensed aromatics composed of 4 or 5 rings.

Protection of life against toxicity of big doses of 7,12-DMBA by pretreatment with small doses of aromatics required time (*ca.* 5 to 8 hours) for its induction. Ethionine given a few minutes after a highly efficient inducer of protection, 3-methylcholanthrene (3-MC), abolished induction of protection; ethionine given 8 hours after 3-MC exerted no influence on its protective effect.

A lethal dose of 7,12-DMBA resulted in a considerable reduction in incorporation of tritium in DNA from tritiated thymidine while at the same time synthesis of menadione reductase was induced in liver. A small dose of 3-MC given prior to 7,12-DMBA was advantageous in partially protecting DNA synthesis.

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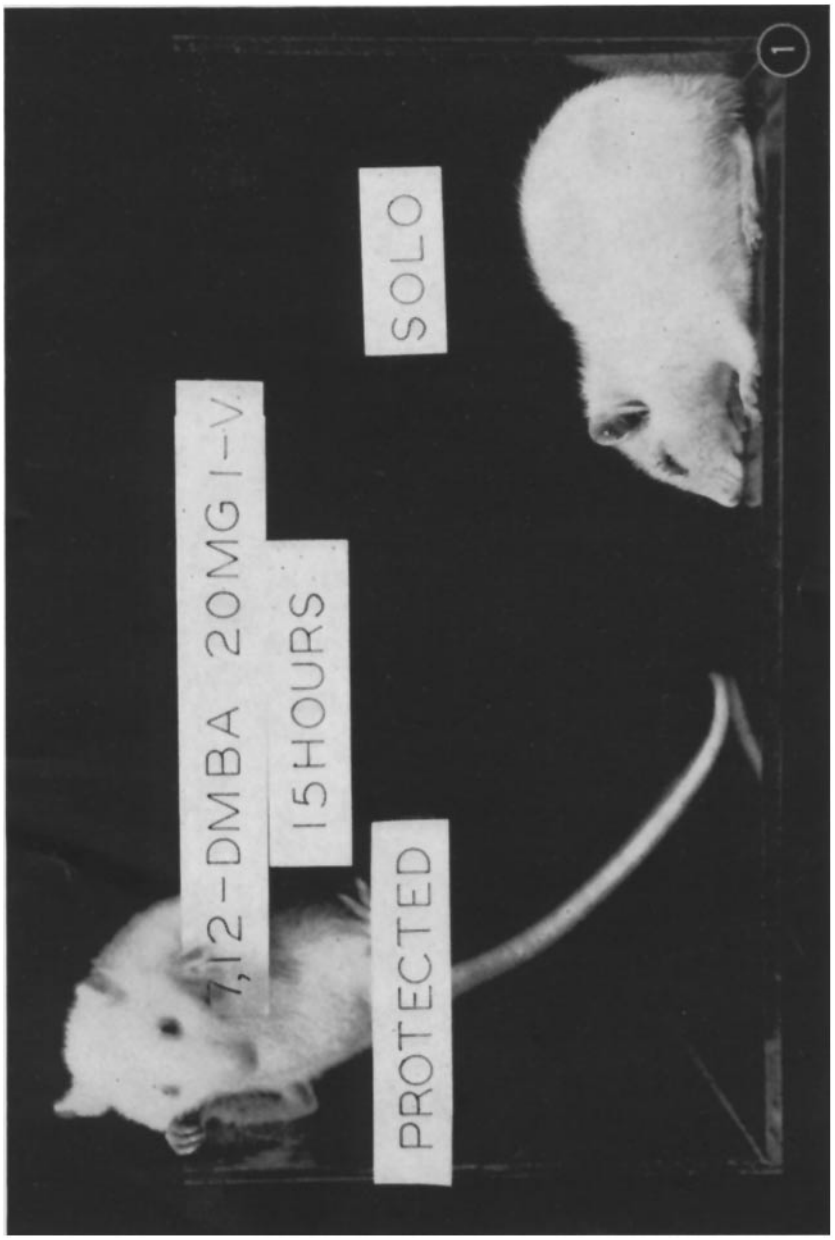
EXPLANATION OF PLATE 93

FIG. 1. Two rats, age 46 days, were injected with 7,12-DMBA, 20 mg, at 0 hours. The photograph was taken 15 hours later. One rat (left) injected with 3-MC, 2 mg, 24 hours prior to 7,12-DMBA, is active. Its sister (right) which had not received 3-MC is moribund.



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