SELECTIVE DESTRUCTION IN TESTIS INDUCED BY 7,12-DIMETHYLBENZ [*a*] ANTHRACENE*

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Plates 3 to 5

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In the work to be presented it was found that a single dose of 7,12-dimethylbenz[*a*]anthracene[1] caused severe but selective damage in the seminiferous tubules of rat. Destruction occurred in young cells in the germinal series, whereas more mature germinal cells and Sertoli cells and interstitial cells were not injured. Further, no damage was demonstrated in the ovary of sisters of males exposed to a dose of 7,12-DMBA which resulted in destruction of massive amounts of the seminiferous epithelium.

In earlier experiments it was found (1, 2) that a single dose of any of a number of polynuclear aromatic hydrocarbons, under special circumstances, selectively induced mammary cancer in every female rat with great rapidity. Cancer of the breast was evoked in both adult and newborn rats (3). 7,12-DMBA was the most effective of these hydrocarbons and mammary cancer arose after a single feeding or intravenous injection. Of the carcinogenic hydrocarbons which were investigated in the present experiments, 7,12-DMBA alone induced testicular atrophy.

The methods used in the present experiments included: (*a*) determination of enzyme content of the testis, (*b*) direct anatomical observation; (*c*) histological study.

Hydrocarbon-Induced Damage of Testis.—Tuchmann and Demay (4) injected benzo[*a*]pyrene, 1 mg, directly in one testis of rats and, after 90 to 100 days, found atrophy of both testes. Haddow and Robinson (5) administered various hydrocarbons related to benz[*a*]anthracene to rats bearing transplanted tumors and observed: "The fertility of the treated animals was very definitely lowered, and in many cases the

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[1] The following abbreviations are used: 7,12-DMBA, 7,12-dimethylbenz[*a*]anthracene; 3-MC, 3-methylcholanthrene; G-6-PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconic dehydrogenase; ICD, isocitric dehydrogenase; LDH, lactic dehydrogenase; MDH, malic dehydrogenase; $NADH_2$, reduced nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; PAS, periodic acid–Schiff reagent (8).

ovary showed suppression of ovulation and the testis a variable reduction of spermatogenesis." Shubik and Della Porta (6) applied very large doses of carcinogenic hydrocarbons including 7,12-DMBA to the skin of mice; concerning mice treated with 7,12-DMBA, they stated, "There appeared to be a moderate decrease in spermatogenesis in the testes."

Methods

The experimental animals were rats of the Sprague-Dawley strain. Most of the experiments were carried out at either age 25 days or 50 to 60 days. Most of the animals were fed a commercial ration; hypophysectomized rats were kept on a synthetic high (18 per cent) protein diet.

The polynuclear aromatic hydrocarbons were recrystallized from appropriate solvents, and most of them were dissolved in sesame oil. 7,12-DMBA, m.p. 122–3°, was purified by florisil chromatography and recrystallized from acetone-alcohol; the refined compound migrated as one spot in thin-layer chromatography. In many experiments the hydrocarbons were administered as a fine emulsion[2] *via* caudal vein. The hydrocarbons were administered once only; *the day of administration was designated day* 0.

Enzyme Assays and Units.—Rats were killed by decapitation. The tissues to be analyzed were excised rapidly, weighed, and homogenized for 3 minutes in an ice-cold solution (3 ml) of 0.15 M NaCl containing 0.003 M $NaHCO_3$. The homogenates were centrifuged at 11,000 *g* for 10 minutes in a refrigerated centrifuge and the enzyme content of the supernatant was determined.

The assay methods for 6-PGD[1], G-6PD, ICD, LDH and MDH have been described earlier (7).

One unit of G-6-PD, or 6-PGD or ICD is defined as the enzyme activity which reduced 1 μmole of NADP/1 minute at 25°C under the given conditions (7). One unit of LDH or MDH is defined as the enzymatic activity which oxidized 1 μmole of $NADH_2$/1 minute. The units are expressed in terms of 1 gm of fresh tissue (wet weight).

Anatomical.—In many experiments colchicine, 2 mg/kg, dissolved in saline was injected intraperitoneally 4 hours before necropsy. The number of spermatogonia in mitosis was counted in 100 cross-sections of paraffin sections of testis tubules.

At necropsy, testis and ventral prostate were weighed on a torsion balance and fixed in Bouin's fluid; paraffin sections were stained with hematoxylin-eosin or with PAS (8).

The series of successive cellular associations that occur during the continuous renewal of the seminiferous epithelium of the rat were divided into stages using the classification of Leblond and Clermont (9). Tubules of control and 7,12-DMBA-treated animals that were in the same stage were compared.

EXPERIMENTAL

Dehydrogenases in Testis.—Determinations of the levels of five soluble pyridine nucleotide-linked dehydrogenases (Table I) were made concurrently on homogenates of liver and testis of male rats, age 50 to 60 days. All of these enzymes were present in considerably lower concentration in testis than in liver; in testis the values ranged from *ca.* one-twentieth (ICD) to one-half

[2] We are indebted to Paul Schurr, The Upjohn Company, Kalamazoo, Michigan, for preparing emulsions of 0.5 per cent 7,12-DMBA and 0.25 per cent 3-MC. The hydrocarbon was dissolved in the oil phase and emulsified in the aqueous phase of the preparation (10).

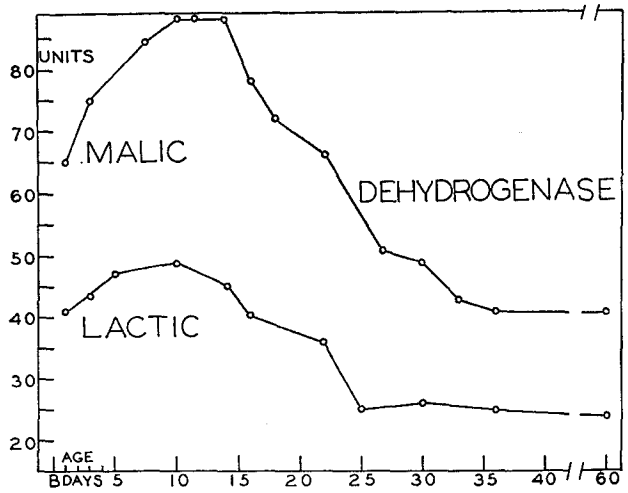
(G-6-PD) of the levels found in the liver of the same rat. In liver the concentration of 6-phosphogluconic dehydrogenase exceeded that of glucose-6-phosphate dehydrogenase, whereas in testis both enzymes were present in

TABLE I

Dehydrogenases in Liver and Testis of Adult Male Rats

The results are expressed in units per gram, wet weight.

No. of rats	Dehydrogenases				
	6-Phospho-gluconic	Glucose-6-phosphate	Isocitric	Lactic	Malic
	Liver				
15	4.51 ± 0.8	1.08 ± 0.3	26.3 ± 4.9	285 ± 31	267
	Testis				
42	0.53 ± 0.1	0.63 ± 0.2	1.15 ± 0.4	25.5 ± 2.6	39.8 ± 5



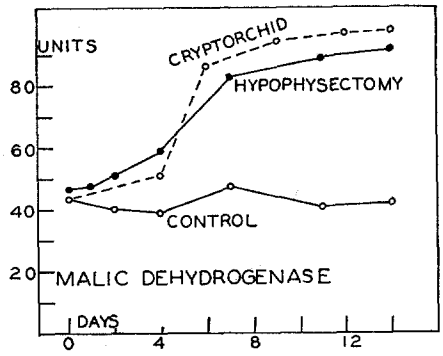
TEXT-FIG. 1. At birth the levels of LDH and MDH in testis are elevated and rise to higher levels during the first 2 weeks of life and then descend to reach the levels found in the testis of adults at age 25 to 30 days.

equal amount. In testis malic dehydrogenase had the greatest activity of the enzymes followed in rank by lactic dehydrogenase.

In testis of newborn rats, the levels of MDH and LDH were considerably higher than in adult testis (Text-fig. 1). Soon after birth the levels of both enzymes in testis rose to still higher values, attaining their maxima at age 10 days. Thereafter the values declined progressively and the levels characteristic of normal adult testis were reached at age 25 to 30 days.

In 60 rats, age 58 days, the left testis was withdrawn from the scrotum and anchored in the peritoneal cavity by surgical means, and serial estimations of concentration of MDH and LDH were made at intervals thereafter; in each animal the opposite testis in its normal site served as control. In the cryptorchid testis the levels of MDH and LDH were similar to those in the normal testis for the first 4 days after the operation. On day 4 slight, and on day 6 more pronounced, elevations of MDH (Text-fig. 2) and LDH were found, and these very considerably elevated levels were sustained throughout the period of observation lasting 74 days.

Hypophysectomy was performed on 30 rats age 25 days; 10 of their mates



TEXT-FIG. 2. The level of MDH in testis rises on day 4 in hypophysectomized and cryptorchid rats.

were not operated upon and these served as controls. Again the concentrations of MDH and LDH were determined. In the testes of control rats there was no significant change in the levels of the dehydrogenases. In hypophysectomized rats there was an increase in the levels of MDH (Text-fig. 2) and LDH, which was first observed on day 4. On days 7 to 14 the levels of MDH and LDH in the testes of rats subjected to hypophysectomy were rather similar to those found in the cryptorchid group (Text-fig. 2).

In all of the experiments, the levels of MDH and LDH were not dissociated but ran parallel to each other.

Effect of 7,12-DMBA on Adolescent Testis.—A single intravenous injection of 7,12-DMBA, 0.1 to 2 mg, was given to rats at age 25 days and with mean body weight, 58 gm. The administration of 7,12-DMBA, 0.1 mg, was not followed by a significant effect on growth of the testis. 7,12-DMBA, 0.5–2 mg, exerted increasing retardation of testis growth in parallel with the dose of the compound (Table II).

Seventy normal male rats, age 25 days and mean weight 65 gm, were given a single intravenous injection of an emulsion of 7,12-DMBA, 2 mg (31 mg/kg).

There was no change in body weight for 2 days after the injection; during the next 38 days the injected rats gained weight at the rate of 93 per cent of that of the uninjected controls. The daily gain in weight was, on average; injected rats, 5.4 gm; uninjected controls, 5.8 gm.

Uninjected control rats had a progressive increment of testis weight (Text-fig. 3) between age 25 and 65 days. At age 25 days, the seminiferous epithelium consisted of 3 classes of cells: Sertoli cells, spermatogonia, and spermatocytes (Fig. 1). At age 33 days early stage spermatids were found. At age 36 days late stage spermatids were abundant. At age 40 days a very few spermatozoa

TABLE II

Effect of Polycyclic Aromatic Hydrocarbons on Testis Weight

A single intravenous injection was given on day 0, age 25 days. There were 5 rats in each group.

Compound	Dose	Weight of testis			
		Day 0	Day 12	Day 26	Day 39
	mg	*mg*	*mg*	*mg*	*mg*
None; controls	—	209 ± 30	725 ± 82	1242 ± 60	1580 ± 62
7,12-DMBA	0.1	—	737 ± 49	1307 ± 101	1539 ± 105
7,12-DMBA	0.5	—	629 ± 30	943 ± 71	1370 ± 111
7,12-DMBA	1.0	—	597 ± 86	973 ± 160	1085 ± 90
7,12-DMBA	2.0	—	530 ± 98	679 ± 68	720 ± 68
3-MC	5.0	—	723 ± 82	1284 ± 73	1471 ± 94
Benzo[*a*]pyrene	5.0	—	635 ± 64	1225 ± 82	1500 ± 60

±, Standard deviation.

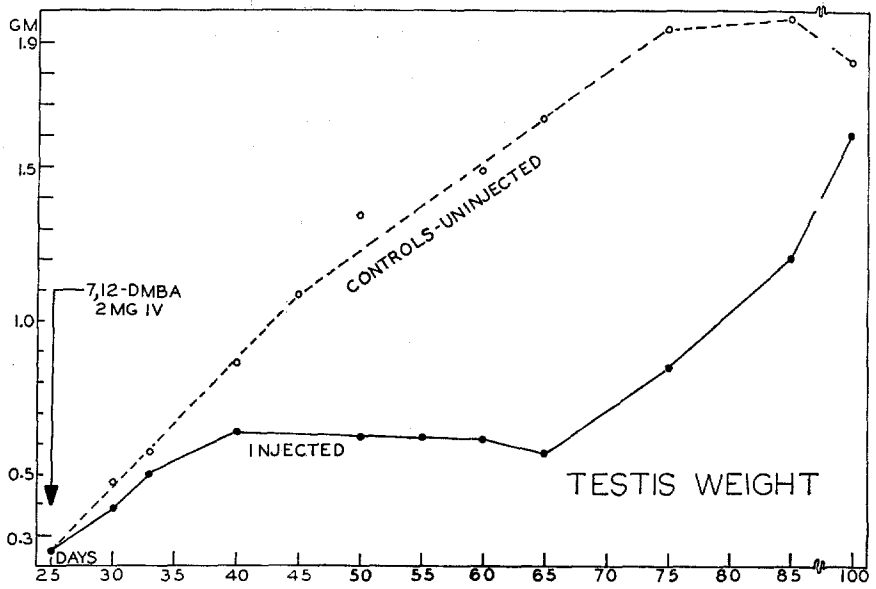
were found in epididymal secretion, and in fixed sections of testis. At age 45 days, spermatozoa were seen in many of the tubules and some of them were motile in epididymis. At age 50 days, spermatozoa were abundant in nearly all of the seminiferous tubules and were highly motile in epididymis. At this age of 50 days, testis has reached full maturity.

In the rats injected with 7,12-DMBA at age 25 days, the testis increased in size (Text-fig. 3) until day 15 (age 40 days) when the curve of testis weight entered a plateau lasting until day 40 (age 65 days). Thereafter there ensued a progressive increment of testis weight and the weights of testes of injected and uninjected rats were similar at about day 75 (age 100 days).

Cross-sections of testis tubules of control and 7,12-DMBA–injected rats were examined by light microscopy. Many types of cells of the seminiferous epithelium were not damaged by 7,12-DMBA since they progressed to more mature stages from which normal spermatozoa developed. Other germinal cells were destroyed by the hydrocarbon. No changes were observed in Sertoli or interstitial cells.

(*a*) *Normal cells in testis of rats injected with 7,12-DMBA:* The time sequence of maturation of undamaged cells in testis of rats injected with 7,12-DMBA was identical with that of the uninjected mates, namely—spermatids in early stages at age 33 days; late stage spermatids at age 36 days; the first spermatozoa at age 40 days; abundant and motile spermatozoa at age 50 days.

(*b*) *Abnormal components:* These cells of the germinal epithelium had been severely damaged by 7,12-DMBA.



TEXT-FIG. 3. Growth of testis in rats given an intravenous injection of 7,12-DMBA, 2 mg, at age 25 days, related to that of uninjected controls. Each point on the graph represents the mean value of weight of testis of 4 rats.

The earliest cytologic alteration in the seminiferous epithelium of the experimental rats was detected one day after 7,12-DMBA.

Day 1.—Intermediate spermatogonia were decreased in number.

Day 2.—Further decrease in intermediate spermatogonia, and a decrease in type A and B spermatogonia.

Day 6.—Spermatogonia were absent from some tubules and decreased in most of the other tubules. The number of resting and pachytene spermatocytes was less than that in the control.

Day 8.—Similar in appearance to day 6, but more severe depletion of spermatogonia and primary spermatocytes (Fig. 2).

Day 11.—No resting or leptotene spermatocytes were seen; spermatogonia and pachytene spermatocytes continue to be decreased (Fig. 3). There was normal maturation of spermatids.

Day 20.—All seminiferous tubules were abnormal (Fig. 4). There was an increase in spermatogonia, but marked depletion of primary spermatocytes and early spermatids; late stage spermatids and spermatozoa were present. In epididymis, spermatozoa were abundant and motile.

Day 30.—The seminiferous epithelium was severely depleted in, or lacked completely, one or more cellular components of each stage of the cycle. There was an increase in primary spermatocytes. Spermatozoa were present and motile in epididymis.

Day 40.—The extent of damage and recovery varied greatly, ranging from tubules containing only spermatogonia and Sertoli cells to tubules which were normal except for the absence of late stage spermatids (Fig. 5). Some tubules contained only Sertoli cells.

Day 50.—A few tubules were atrophic, but most showed a normal maturation of the seminiferous epithelium that had not yet reached the late spermatid stages of development.

Day 61.—Complete recovery of the siminiferous epithelium with maturation comparable to that found in uninjected mates (Fig. 6).

Spermatogonia in mitosis were counted in 100 tubule cross-sections 4 hours after injection of colchicine. In relation to normal testis of controls of the same age, 7,12-DMBA resulted in a profound depression of mitoses in spermatogonia from day 5 to day 15 (Text-fig. 4); the smallest number of mitoses was present on day 8. 20 days after 7,12-DMBA (age 45 days) the number of mitoses in the injected rats had risen to a value equivalent to the controls.

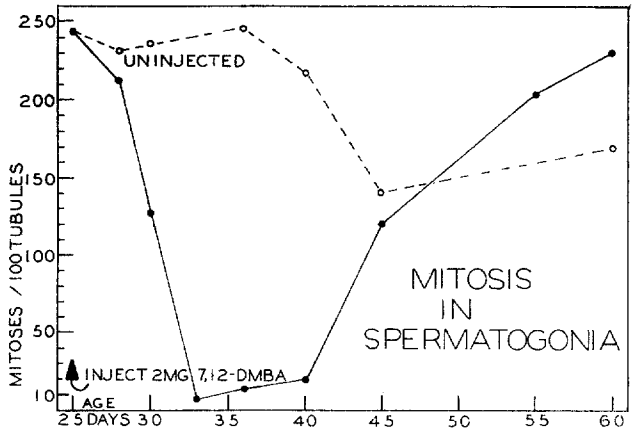
Growth of Ventral Prostate.—In rats injected intravenously with a single dose of 7,12-DMBA, 2 mg, at age 25 days, the growth of the ventral prostate was very slightly less than it was in their uninjected brothers (Text-fig. 5).

Effect of 7,12-DMBA on Adult Testis.—Seventy normal male rats, age 60 days and average weight 260 gm, were injected intravenously with an emulsion of 7,12-DMBA, 5 mg (19.2 mg/kg); 30 of their mates were untreated and these were the control group. At intervals injected and control rats were sacrificed; the testis was weighed, and level of MDH was determined and histological studies were carried out. Contents of epididymis were mixed with saline and examined for the presence and motility of spermatozoa.

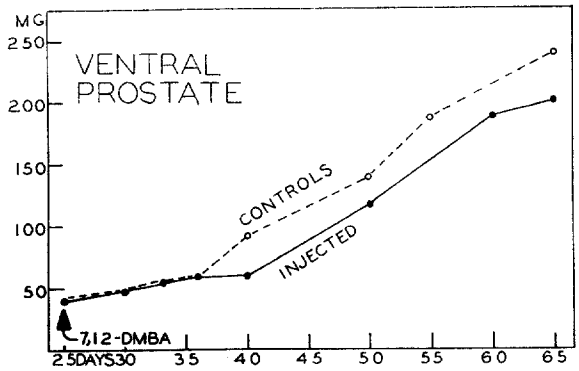
(*a*) *Weight of testis:* There were no significant changes in testis weight of the untreated and injected groups for the first 12 days of the experimental period. On day 14 (age 74 days) a decrease in weight of testis (Text-fig. 6) was observed in rats injected with 7,12-DMBA and there was a further decline in weight until a low point was reached at day 38 (age 98 days) when the weight of the testis on average was one-half that of testis of controls. The curve of weight of testis remained on a plateau from days 38 to 48; after day 48 there was a steady increment in testis weight until day 73 (age 133 days) when weight of testis was similar in both injected and control animals.

(*b*) *Malic dehydrogenase in testis:* The level of MDH in testis of control, uninjected rats was 39.8 ± 5 units throughout the experimental period. Similar values were found in testis of rats injected with 7,12-DMBA until day 12; on day 14 a significant rise

in the level of MDH was observed (Text-fig. 6) and there was a steady rise until day 38 (age 98 days); after day 38 there was a progressive decline in concentration of MDH to the levels found in testis of the control group. The level of MDH in testis of the injected rats was similar to that in untreated companions on day 73.



TEXT-FIG. 4. The numbers of spermatogonia in mitosis in cross-sections of 100 testis tubules in rats injected intravenously with 7,12-DMBA, 2 mg, at age 25 days and in their uninjected controls. Colchicine, 2 mg/kg, was injected intraperitoneally 4 hours before necropsy.



TEXT-FIG. 5. Weight of ventral prostate in rats injected intravenously with 7,12-DMBA, 2 mg, at age 25 days and in their uninjected controls.

(*c*) *Histological:* Cross-sections of testis tubules of control and 7,12-DMBA injected rats were examined by light microscopy. Changes in the seminiferous epithelium were detected 3 days after an injection of 7,12-DMBA, and abnormalities increased for some weeks. No change in Sertoli cells was observed.

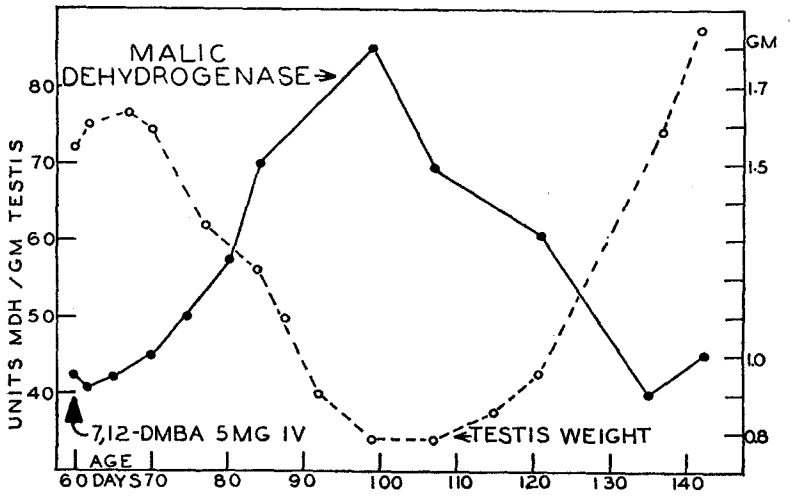
Day 3.—Type A, intermediate, and type B spermatogonia, were decreased in number.

Day 5.—Resting and pachytene spermatocytes as well as spermatogonia were fewer in the injected rats than in the controls.

Day 7.—Resting spermatocytes and intermediate spermatogonia had disappeared completely from some of the tubules.

Day 10.—Pachytene and leptotene spermatocytes were absent or decreased in number in many tubules; this continued to be true of spermatogonia and resting spermatocytes.

Day 14.—Some tubules contained only Sertoli cells, spermatids, and spermatozoa



TEXT-FIG. 6. Changes in weight of testis and its content of MDH following intravenous injection of 7,12-DMBA, 5 mg, at age 60 days. Each point on the graph represents the mean value obtained from 4 rats.

Of the primary spermatocytes, only the diplotene type were present in normal numbers.

Day 20.—Almost all tubules contained a full complement of spermatozoa and spermatids, but spermatogonia and primary spermatocytes were markedly decreased.

Day 27.—Some tubules contained only Sertoli cells and spermatozoa. Spermatogonia and resting spermatocytes had begun to return, but early spermatids and primary spermatocytes in meiosis were absent or sharply decreased in number.

Day 32.—Spermatogonia, resting spermatocytes and early primary spermatocytes continued to increase in number. Early spermatids were rare, but many tubules contained late spermatids and spermatozoa. A few tubules had no elements of seminiferous epithelium.

Day 38.—This was the period of high MDH content and the low point of testis weight. The tubules showed a return of spermatogonia and resting spermatocytes. A few primary spermatocytes in the early stages of meiosis were also present; there were no spermatids or spermatozoa.

Day 46.—Tubules contained an increasing number of the more immature elements

of the seminiferous epithelium. Early spermatids were present but the later forms were lacking.

Day 54.—Many tubules had almost recovered, containing their full complement of cell types except for spermatozoa which were lacking. A few contained only Sertoli cells, or Sertoli cells and spermatogonia.

Day 74.—The seminiferous epithelium was normal in most tubules. A few tubules contained only Sertoli cells with absence of germinal epithelium. Motile spermatozoa were abundant in epididymis.

Effect of Various Carcinogenic Hydrocarbons.—Emulsions of 7,12-DMBA, 3-MC or benzo[*a*]pyrene (B[*a*]P), in which the hydrocarbons were of submicroscopic size, were injected intravenously on a single occasion in rats, age 25 days, mean weight 56 gm. Necropsy was performed on days 12, 26, and 39; the testes were weighed and histological sections were prepared. No damage to testis was detected in rats injected with 3-MC or B[*a*]P (Table II), whereas there was severe damage in the testis of rats injected with 7,12-DMBA, 0.5 mg, or larger amounts.

In another experiment, rats age 60 days were given a single feeding of the following compounds dissolved in sesame oil: 7,12-DMBA, 20 mg; benzo-[*a*]pyrene, 100 mg; 3-MC, 105 mg; 2-acetaminophenanthrene, 40 mg. All of these hydrocarbons are strong carcinogens. There were 5 rats in each group and the testes were harvested on days 12, 24, 31, and 60 (Table II). In the group receiving 7,12-DMBA, the testis underwent degenerative changes comparable to those in rats receiving intravenously an injection of 7,12-DMBA, 5 mg. In the groups receiving other carcinogens, the testis weight and histological findings at each time period did not differ significantly from those of their untreated control brothers (Table II).

Effect of 7,12-DMBA on Reproduction Function in Female Rats.—A group of rats was injected intravenously with 7,12-DMBA, 5 mg, at age 50 days; sister rats were uninjected and maintained as controls. The rats were bred at age 66 to 96 days and the litter size and sex were determined.

Each of 9 female rats injected with 7,12-DMBA conceived and delivered a total of 68 live infants, mean 7.5; there were equal numbers of male and female offspring. 9 control uninjected rats delivered 78 live infants, mean 8.7; there were 41 male and 37 female infants.

DISCUSSION

The most significant finding in the present work is the high selectivity of lesions inflicted by 7,12-DMBA in the gonad of the male rat. The testis was severely but selectively injured, whereas ovarian function was preserved. In these experiments 7,12-DMBA alone, of the hydrocarbons which were studied, caused damage to the testis. Other powerful carcinogenic hydrocarbons, 3-

methylcholanthrene, benzo[*a*]pyrene, and 2-acetaminophenanthrene did not injure the testis.

In testis the primary site of damage caused by 7,12-DMBA was a bed of seminiferous cells near the basement membrane of the tubule; the damaged cells were spermatogonia and resting primary spermatocytes. Uninjured by the hydrocarbon were Leydig cells, primary spermatocytes in meiosis, secondary spermatocytes, spermatids, and spermatozoa.

The proof of the site of damage in testis inflicted by 7,12-DMBA was derived from the administration of the compound on a single occasion to adolescent males, age 25 days, when gonads and secondary sexual structures are immature. At this time, the ventral prostate is infantile. The growth of the prostate after an injection of 7,12-DMBA was comparable in rate to that occurring in untreated brothers. Together with histological studies, the growth of the prostate demonstrates that Leydig cells were not compromised by the hydrocarbon.

At age 25 days, the seminiferous epithelium consists of spermatogonia and spermatocytes. Primary spermatocytes in meiosis and secondary spermatocytes were not damaged by 7,12-DMBA since they underwent maturation at a rate identical with that of controls and their end stages—the spermatozoa were abundant and motile at age 50 days. This rate of maturation is in parallel to development in uninjected brothers.

The primary lesions in testis of rats injected with 7,12-DMBA were apparent on days 1 to 3 when a decrease in number of spermatogonia was observed, and on day 6 the numbers of resting and pachytene spermatocytes were smaller than in uninjected controls. On days 8 to 11 the necklace of densely staining spermatocytes near the basal cells was absent. The effects of 7,12-DMBA on testis are similar in adolescent and adult rats; at both ages the greatest atrophy after a single injection of the hydrocarbon was evident 38 to 40 days later. In both cases, the testis had returned to an essentially normal state 75 days after the single injection of 7,12-DMBA.

The incorporation of tritium-labeled thymidine is an indicator of synthesis of deoxyribose nucleic acid. In the seminiferous epithelium only spermatogonia and resting primary spermatocytes (11, 12) incorporate thymidine-H^3. These are the cells of the testis which are the site of damage of 7,12-DMBA. Subsequent atrophy, and it is of very high grade, is due to suppression of these cells which are more primitive antecedents of late-stage spermatocytes and spermatids and spermatozoa.

The testis has a high mitotic index (13) characterized by rapid renewal of cells and easily recognizable stages of differentiation. In the rat as in the mouse, in a cross-section of the seminiferous tubule, either all of the cells of the basal layer of germinal epithelium are labeled or none of this group incorporates thymidine-H^3 in that cross section (13). Yet nearly all spermatogonia and

resting primary spermatocytes were killed by a single intravenous injection of 7,12-DMBA. The cells which succumb are those in which synthesis of deoxyribose nucleic acid is occurring at a rapid rate—the spermatogonia which divide by mitosis and resting primary spermatocytes which undergo meiosis. Cells which do not incorporate thymidine-H^3 escape injury. Huggins and Yang (2) demonstrated that polynuclear aromatic hydrocarbons to produce cancer must resemble the base pairs of nucleic acids in geometrical configuration and must be able to form molecular complexes. Cancer did not arise in the present experiments, and it is inferred that the absence of neoplastic transformation is due to the lethal effects which 7,12-DMBA exerted on cells vulnerable to it.

The number of spermatogonia in mitosis did not decrease until after day 4 in rats injected with 7,12-DMBA at age 25 days. It is evident that spermatogonia can undergo at least one mitotic cycle before they succumb to the deleterious effect of the hydrocarbon. Death of the vulnerable cells is a genetic death occurring after some hours, not a sudden event.

Although the damage to the seminiferous epithelium was very severe, it was not complete, since recovery to a normal state always occurred after a single injection, because a few spermatogonia are not destroyed by the solitary injection of 7,12-DMBA.

No tubule in the testis was exempt from injury from 7,12-DMBA, although an occasional spermatogonium in most of the tubules was not destroyed. During the first 3 weeks after the onslaught of the hydrocarbon, the manifestation of destruction was uneven since degenerative changes were more advanced in some of the tubules than was observed in others. This irregularity is related to a difference in the stage of maturation at the time of exposure to the hydrocarbon.

The selective destructive effects on testis induced by 7,12-DMBA are reminiscent of those inflicted by x-rays. In a classic description of the effect of radiation on the testis of rats, Regaud and Blanc (14) found that there was extreme damage to spermatogonia, whereas spermatozoa were undamaged; this fact explains the "period of incubation" after which lesions were observed by these workers.

In rats injected with 7,12-DMBA at age 25 days, it was impressive to see continued growth of the testis for 15 days at a rate only slightly slower than that of the testis in the uninjected brothers. In fact the size of the testis increased more than twofold during the first 2 weeks after injection of 7,12-DMBA, the increase of size being due to normal maturation of uninjured cells. But after 2 weeks the normal increment of size had disappeared, due to the degenerative changes which had occurred in precursors in the germinal cell series as a result of the hydrocarbon.

Determination of the level of malic dehydrogenase was found to be a simple and useful quantitative index of changes of great magnitude occurring in testis

of rat. The normal testis, with its full complement of mature germinal cells, has a rather low level of MDH. With reference to the adult level, MDH of testis was elevated (about twofold): (*a*) in early infancy; (*b*) in hypophysectomized rats; (*c*) in cryptorchid rats; (*d*) after 7,12-DMBA. The level of MDH in testis of rats injected with 7,12-DMBA varied inversely as the weight of the testis.

SUMMARY

After a single feeding or intravenous injection of 7,12-dimethylbenz[*a*]-anthracene, the testis of rat was severely and selectively damaged, whereas the ovary of sisters was spared from injury. After many weeks, complete recovery of the testis ensued. The destructive effect of 7,12-DMBA on testis was not shared by other powerful carcinogenic hydrocarbons; 3-methylcholanthrene; benzo[*a*]pyrene; 2-acetaminophenanthrene.

The primary sites of destruction inflicted by 7,12-DMBA on testis were spermatogonia and resting spermatocytes, the only cells in testis which synthesize deoxyribose nucleic acid. No other cells in the germinal epithelium are damaged by 7,12-DMBA. The severe atrophy of testis that ensues after some weeks is secondary to destruction of precursors in the seminiferous cell line. Moreover, interstitial cells of testis are not destroyed by the hydrocarbon.

Estimation of malic dehydrogenase is a simple and useful quantitative measure of damage and subsequent repair in testis. Following administration of 7,12-DMBA, the level of MDH had an inverse relationship to weight of the testis.

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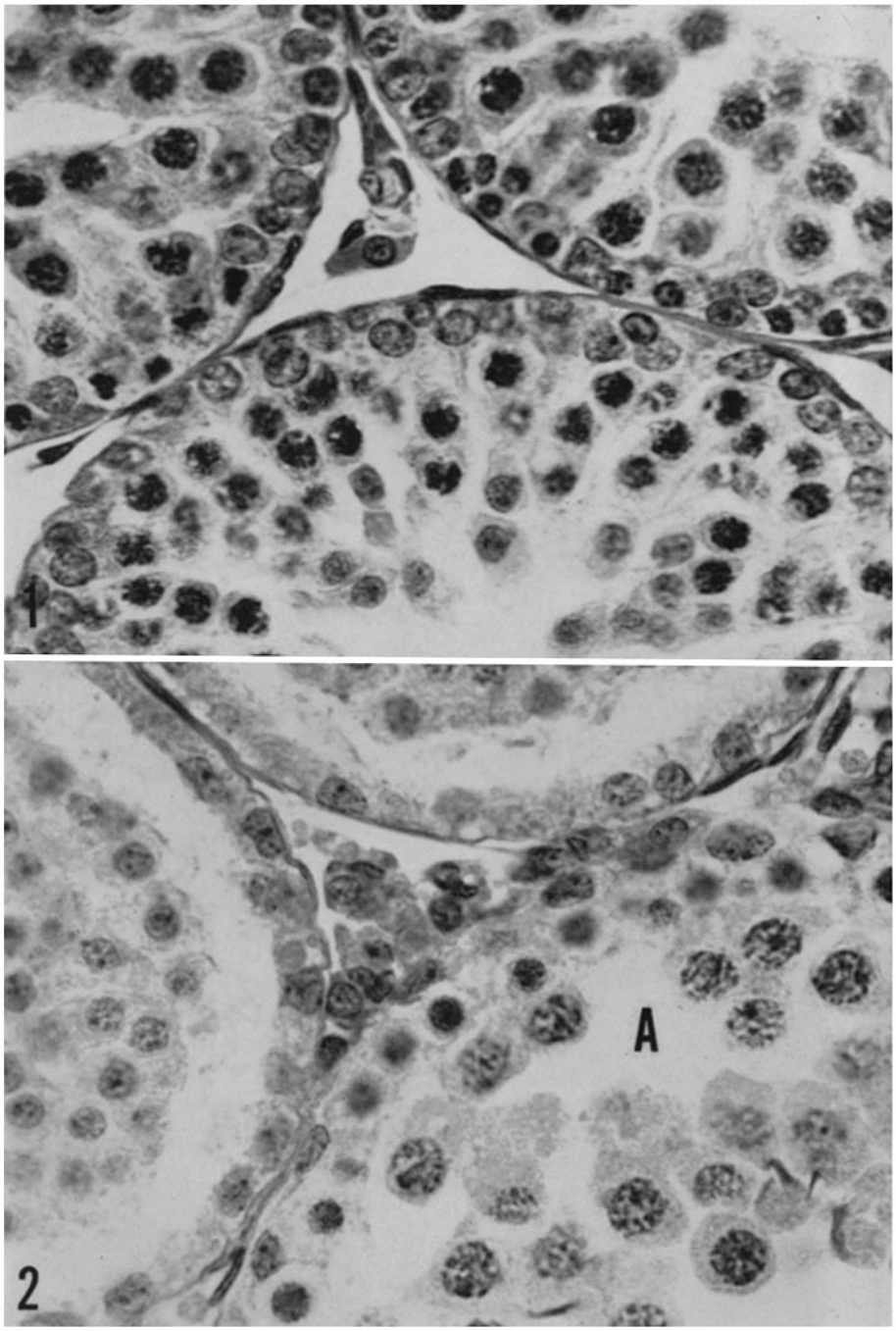
EXPLANATION OF PLATES

PLATE 3

All photomicrographs were prepared from sections stained with hematoxylin and eosin. × 875.

FIG. 1. Seminiferous epithelium of a normal rat age 25 days.

FIG. 2. Disappearance of some spermatogonia and pachytene spermatocytes from the seminiferous epithelium of a rat age 33 days, on Day 8 after injection of 7,12-DMBA. Late stage spermatids are present in one tubule (*A*).

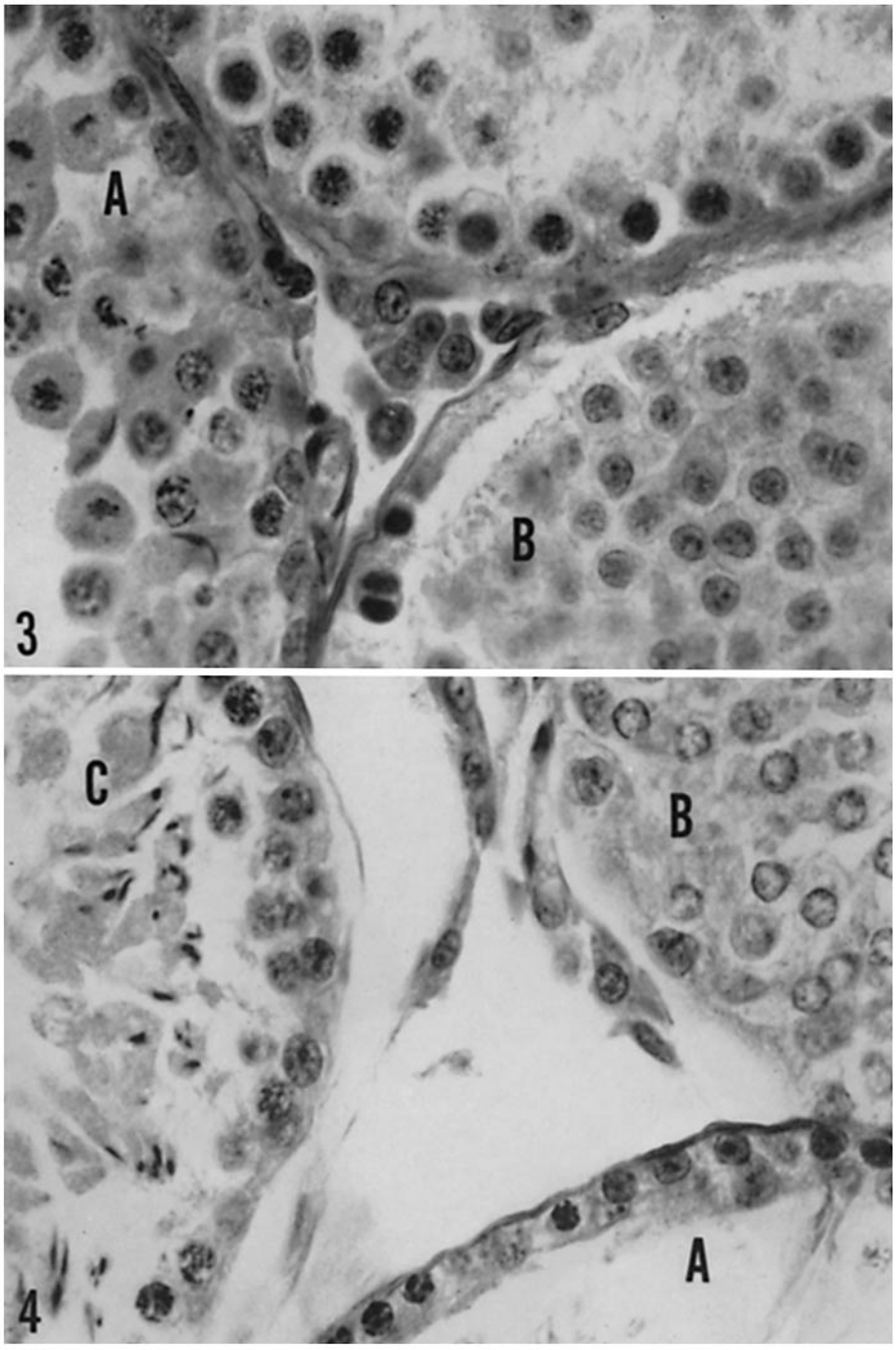


(Ford and Huggins: Selective destruction in testis)

PLATE 4

FIG. 3. Seminiferous epithelium of a rat, age 36 days on day 11 after injection of 7,12-DMBA. In one tubule (*A*) spermatids are maturing normally whereas spermatogonia and zygotene spermatocytes are decreased in number. Another tubule (*B*) has degenerating spermatogonia adjacent to the basement membrane and pachytene spermatocytes are missing.

FIG. 4. Seminiferous epithelium of a rat age 45 days on day 20 after injection of 7,12-DMBA. One tubule (*A*) contains only Sertoli cells, resting spermatocytes, and spermatozoa. In another tubule (*B*), primary spermatocytes and spermatogonia are missing. The number of spermatocytes is decreased in a third tubule (*C*).

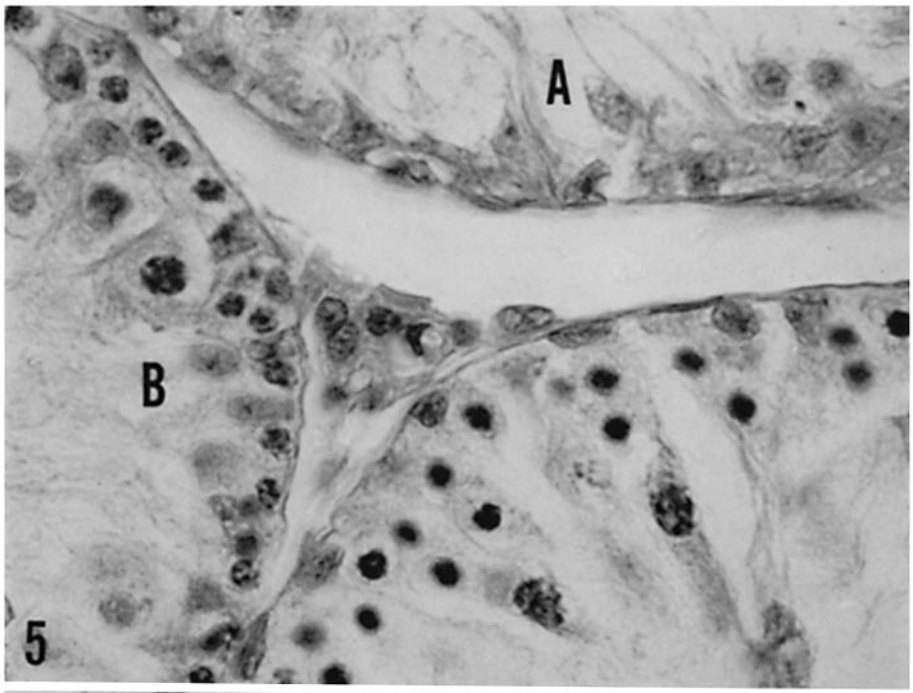


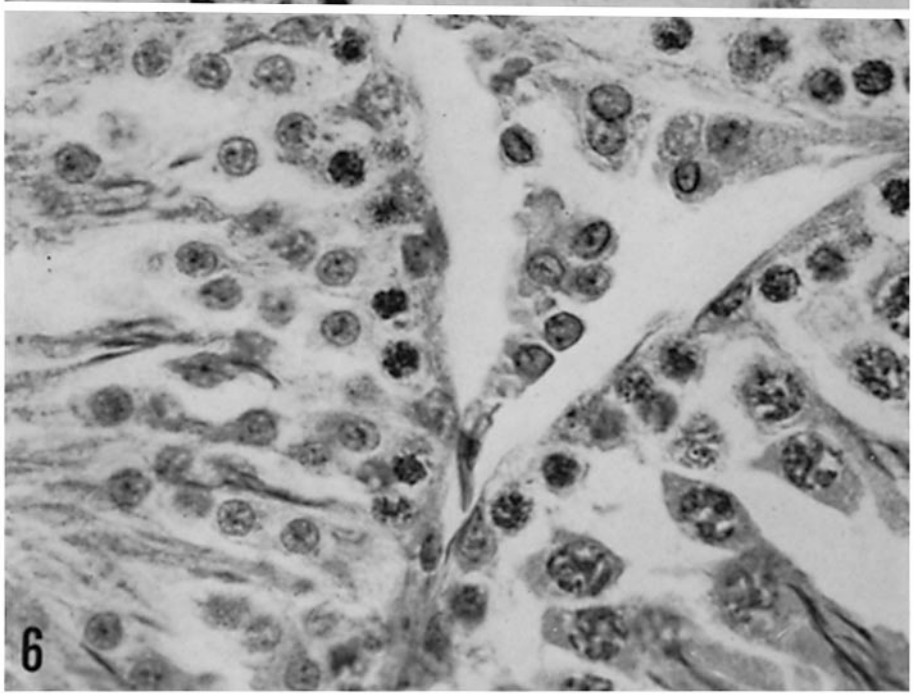
(Ford and Huggins: Selective destruction in testis)

PLATE 5

FIG. 5. Abnormal seminiferous epithelium of a rat age 65 days on day 40 after injection of 7,12-DMBA. One tubule (*A*) contains only Sertoli cells and a few spermatids. In an adjacent tubule (*B*) resting spermatocytes are abundant, but primary spermatocytes in meiosis and spermatids are much decreased in number.

FIG. 6. Complete recovery of seminiferous epithelium of a rat age 86 days, on day 61 after injection of 7,12-DMBA.





(Ford and Huggins: Selective destruction in testis)