downward but moving toward the meniscus (Fig. 4).

For any particular polymer it is often quite difficult to find appropriate solvents to illustrate the four cases. An example of another system which we have found is atactic polypropylene oxide in methanol, benzene, chlorobenzene, and $CCl_2F—CClF_2$ (*2*).

The existence of peaks above and below the baseline leads to some interesting possibilities in the ultracentrifugation of solute mixtures. For example, if two solutes in a single solvent correspond to the cases (+ +) and (− −), sedimentation peaks should form at both bottom and meniscus, both upright and moving toward each other; if it is assumed that the peaks are stable for a long time they should meet near the center of the cell, forming a single large peak which then splits into two again. So far, unfortunately, we have not been able to find the right combination of solvent and solutes to demonstrate this case experimentally. Similarly, if one starts with peaks on opposite sides of the baseline and moving toward each other, a canceling out should occur, and separate peaks would emerge again as they pass each other. A possible system for this case would be polyvinyl acetate and polyisobutylene in chlorobenzene, but the peaks we observed do not move rapidly enough (in relation to diffusion) to show the expected cancellation and emergence phenomena.

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1. For example, inverted peaks are obtained with certain lipoprotein fractions: see H. K. Schachman, *Ultracentrifugation in Biochemistry* (Academic Press, New York, 1959), pp. 126–127. As pointed out by a referee, similar phenomena can be observed in diffusion; L. G. Longsworth, in *Electrochemistry in Biology and Medicine*, T. Shedlovsky, Ed. (Wiley, New York, 1955), pp. 235–236, and in electrophoresis: H. A. Hoch and A. Chanutin, *J. Biol. Chem.* **200**, 241 (1953).
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## Carcinogenic Aromatic Hydrocarbons: Special Vulnerability of Rats

Abstract. *Compared with other species, the rat is unusually vulnerable to polynuclear aromatic hydrocarbons. In the rat, relatively small amounts of carcinogenic aromatics (i) profoundly depress incorporation of thymidine in DNA, (ii) greatly induce menadione reductase in liver, and (iii) then kill the rat.*

Mammary glands of young adult female rats of the Sprague-Dawley (S-D) strain are foremost among cells of living creatures in their susceptibility to induction of cancer by irradiation (*1*) or by hydrocarbons and also in the speed with which cancers become evident. Mammary glands of the rat are surpassed in these respects only by cells of chickens inoculated in vivo with Rous sarcoma virus I.

The cell-free filtrate of an avian sarcoma injected in other fowls leads to a palpable tumor within 10 to 21 days (*2*). We have now found that a single large but tolerable dose of any of a number of polynuclear aromatic hydrocarbons or aromatic amines invariably elicited mammary cancer in S-D female rats aged 50 days (*3*); the first palpable cancer was detected in 20 days after administration of the hydrocarbon, and a large proportion of the tumors was manifest before the 30th day. Of these carcinogens, 7,12-dimethylbenz[a]anthracene (7,12-DMBA) is more efficient than all others by ten times. Whereas a single feeding of 7,12-DMBA under simple conditions (*4*) always induces breast cancer, intravenous injection of a concentrated lipid emulsion of 7,12-DMBA is equally effective, less hazardous to laboratory personnel, more convenient, and less costly in that smaller doses are required.

Cancers elicited by 7,12-DMBA differ between rat and mouse. In newborn mice, subcutaneous injection of 7,12-DMBA increased the incidence and the rate of development of both lymphomas and pulmonary tumors (*5*), whereas cancer of the breast was not elicited. In the newborn rat, 7,12-DMBA evoked mammary cancer (*6*) in high incidence whereas lymphomas were seldom induced. In adult S-D rats, a single feeding of 7,12-DMBA (133 mg/kg) induced mammary cancer in every female (*3*) but ovarian tumors did not develop; the dosage causing death (after a single feeding) of one-half the rats ($LD_{50}$) was 270 mg. Morii and Kuwahara (*7*) fed a single meal of 7,12-DMBA (500 mg/kg) to mice of two different strains without inducing mammary cancer; 60 percent of their mice of each strain developed cancers—predominantly ovarian in $C_3H$/HeJ mice and mainly lymphomas in $C_{57}$ black mice. Accordingly there are two central problems in the vulnerability of animals to 7,12-DMBA: differences in (i) species and (ii) target areas.

Pathologic changes induced in mice by large doses of 7,12-DMBA have been described (*8*), but the minimum dosage causing death has not been determined. The $LD_{50}$ in rodents was derived by the probit method of Gaddum (*9*) after a single intravenous injection of a lipid emulsion of 7,12-DMBA. Rats of two strains succumbed to much lower doses of 7,12-DMBA than did mice or *Citellus tridecemlineatus* (Table 1). For female mice of Carworth Farms No. 1 (CF1) strain,

Table 1. Toxicity of 7,12-DMBA in rat, mouse, and *Citellus*. The animals were given a single intravenous injection of lipid (15 percent) emulsion of 7,12-DMBA (0.5 percent) and observed for 21 days. S-D, Sprague-Dawley; CF1, Carworth Farms No. 1.

| Strain | Sex | Age (days) | Number | $LD_{50}$ (mg/kg) |
|---|---|---|---|---|
| | | *Rat* | | |
| S-D | ♂ | 25 | 71 | 58 |
| S-D | ♀ | 25 | 100 | 54 |
| Long-Evans | ♀ | 25 | 54 | 73 |
| | | *Mouse* | | |
| CF1 | ♂ | 44 | 44 | 382 |
| CF1 | ♀ | 120 | 34 | 443 |
| $C_{57}$ black | ♂ | 57 | 51 | 340 |
| | | *Citellus* | | |
| | ♂ | >1 yr | 11 | >227 |
| | ♂, ♀ | 65 | 20 | >227 |

Table 2. Incorporation of tritium (microcuries per gram of tissue, fresh weight) in the DNA fraction (perchloric acid–insoluble) of tissues of rats and mice 24 hours after injection with $TdRH^3$. Groups were of four or five females: Sprague-Dawley rats, about 130 g, aged 43 days; CF1 mice, about 20 g, aged 56 days. Intravenous injection with a lipid emulsion of 7,12-DMBA (0.5 percent) preceded by 4 hours the $TdRH^3$ (0.5 μc/g) injection. Adrenals were pooled; other tissues were processed individually, and the tritium content of washed perchloric acid-insoluble residues was determined (*10*).

| 7,12-DMBA (mg/kg) | Rat | | Mouse | |
|---|---|---|---|---|
| | Ileum | Adrenal | Ileum | Adrenal |
| None | 1.39 (1.01–1.79) | 0.12 | 0.88 (0.7–1.2) | 0.11 |
| 30 | 0.04 (.03–.05) | .05 | 0.82 (0.6–1.1) | .09 |
| 300 | | | 0.31 (.2–.5) | .07 |

Table 3. Menadione reductase and dehydrogenases in livers of rats and mice. Each group comprised eight females. Lipid emulsion (15 percent) containing 7,12-DMBA (0.5 percent) was injected intravenously 24 hours before autopsy. Livers were centrifuged at 11,000*g* for 15 minutes at 4°C, and the enzymes in the supernatant were assayed (*13*, *14*). One unit of menadione reductase, LDH, or MDH is defined as the enzyme activity that oxidizes $1\mu M$ of reduced nicotinamide adenine dinucleotide per minute under the stated conditions. For ICD, G-6-PD, or 6-PGD, one unit is the activity that reduces $1\mu M$ of nicotinamide adenine dinucleotide phosphate per minute. The results (in units) are for 1 g of liver, fresh weight; plus or minus standard deviation.

| Menadione reductase | Dehydrogenase | | | | |
|---|---|---|---|---|---|
| | LDH | MDH | ICD | G-6-PD | 6-PGD |
| | | *S-D rat: control, no hydrocarbon* | | | |
| 27.2 ± 5.3 | 140 ± 57 | 283 ± 80 | 31.5 ± 6 | 4.0 ± 2.5 | 3.4 ± 1.8 |
| | | *S-D rat: 7,12-DMBA, 30 mg/kg* | | | |
| 65.7 ± 13.1 | 188 ± 68 | 319 ± 82 | 30 ± 6 | 3.0 ± 1.2 | 2.2 ± 1.0 |
| | | *$C_{57}$ black mouse: control, no hydrocarbon* | | | |
| 10.7 ± 1.7 | 83 ± 12 | 445 ± 55 | 28 ± 2 | 0.6 ± 0.1 | 0.6 ± 0.1 |
| | | *$C_{57}$ black mouse: 7,12-DMBA, 300 mg/kg* | | | |
| 14.4 ± 3.7 | 81 ± 13 | 400 ± 22 | 22 ± 1 | 0.9 ± 0.1 | 0.9 ± 0.1 |
| | | *CF1 mouse: control, no hydrocarbon* | | | |
| 3.4 ± 1.6 | 85 ± 13 | 471 ± 33 | 23 ± 5 | 1.2 ± 0.5 | 0.7 ± 0.3 |
| | | *CF1 mouse: 7,12-DMBA, 30 mg/kg* | | | |
| 5.3 ± 1.5 | 88 ± 13 | 484 ± 39 | 25 ± 5 | 1.1 ± 0.3 | 0.7 ± 0.2 |
| | | *CF1 mouse: 7,12-DMBA, 300 mg/kg* | | | |
| 5.2 ± 2.3 | 95 ± 18 | 374 ± 72 | 19 ± 4 | 1.2 ± 0.3 | 1.0 ± 0.2 |

the $LD_{50}$ was eight times larger than for S-D rats. The $LD_{50}$ for guinea pigs was greater than 130 mg/kg; each of ten survived this dosage given by vein. For controls in each experiment, the lipid emulsion alone (devoid of hydrocarbons) was similarly injected in amounts similar to those used in the experimental animals; the emulsion alone was not toxic.

A pulse dose of a lipid emulsion of 7,12-DMBA was given 4 hours before administration of tritium-labeled thymidine ($TdRH^3$); both doses were intravenous. Twenty hours later organs were harvested, and the tritium content of the washed DNA fraction (perchloric acid–insoluble) was measured (*10*). Radioactivity in animals which had received 7,12-DMBA and $TdRH^3$ was related to that of controls which had received the nucleoside alone (Table 2). Spectacular differences between rat and mouse were found. In animals injected with 7,12-DMBA (30 mg/kg), incorporation of tritium was (percent): in the ileum, rat 3, mouse 93; in the liver, rat 73, mouse 98; in the adrenal gland, rat 42, mouse 82. A huge dose of 7,12-DMBA (300 mg/kg) killed all rats within 15 hours; this dose was well tolerated by mice, in which incorporation was (percent): in the ileum, 35; in the liver, 102.

Aromatic compounds foreign to the organism set in motion synthesis of selective and diverse enzyme systems. Compounds of this sort include 3-methylcholanthrene (3-MC) and 7,12-DMBA. Among enzymes induced by these carcinogens are (i) those in the ascorbic acid pathway (*11*), (ii) microsomal enzymes (*12*) requiring for activity $O_2$ and reduced nicotinamide adenine dinucleotide phosphate, and (iii) a soluble diaphorase, menadione reductase (*13*). Both rats and mice were injected with 7,12-DMBA or 3-MC, and 24 hours later concentrations of soluble enzymes in their livers were determined; we measured menadione reductase and five dehydrogenases: lactic (LDH), malic (MDH), isocitric (ICD), glucose-6-phosphate (G-6-PD), and 6-phosphogluconic (6-PGD). In no case was the concentration of any of the dehydrogenases influenced significantly by 7,12-DMBA (Table 3). But statistically significant ($p < .01$) increases of menadione reductase occurred in the livers of animals injected with hydrocarbons —very pronounced increases in the rat, less dramatic rises in mice of two strains (Table 3). In animals injected with 7,12-DMBA (30 mg/kg), the increases in hepatic menadione reductase were: in rats, 142 percent; in mice 56 percent. Intravenous injection of a lipid emulsion of 3-MC (20 mg/kg) similarly increased menadione reductase in the liver: in rats, 191 percent; in mice, 19 percent.

In rats after administration of 7,12-DMBA, depression of DNA synthesis and increase in menadione reductase occur concurrently. In earlier experiments (*10*) we showed that 7,12-DMBA must react with cellular constituents for a few hours before incorporation of thymidine in DNA is depressed; the relation of time, $t$, to depression of incorporation is 1 hour $< t < 4$ hours. The same time relation (*14*) prevails for induction of quinone reductase in the liver. That 3-MC causes increase in uptake of orotic acid into nuclear RNA (*15*) suggests that the carcinogenic hydrocarbons also increase RNA synthesis.

In summary, a pulse-dose of 7,12-DMBA rapidly and severely depressed synthesis of DNA in rats while remarkably and selectively inducing synthesis of menadione reductase in their livers. Such changes did not occur in mice given equivalent doses of aromatic carcinogens.

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