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# Study models for inducing tumors by 7,12-dimethylbenz (A) - Antracene in rat mammary gland

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**Summary**

*Five study models (A, B, C, D, E) were used to investigate the effect of 7,12-dimethylbenz(a)anthracene in inducing tumors in the mammary tissue of Spreague-Dawley rats. Each group was subjected* ad libitum *to the following diets: group A - commercial ration; groups B/C - a semipurified linoleic acid-rich diet; group D - a semipurified saturated fatty acid-rich diet; group E - a semipurified fat diet composed of 50% soybean oil (rich in essential fatty acid), 25% olive oil (rich in oleic acid) and 25% coconut oil (rich in saturated fatty acid). The animals were fed through intragastric instillation, one or two doses of DMBA dissolved in 1 ml of soybean oil. Group A animals received a single 10 mg-DMBA-doses 60 days after birth. Group B animals received two-20 mg-DMBA-doses, the first dose 45 days after birth and the second 60 days after birth. Groups C, D and E animals were treated in the same way as group B animals. The control-groups were fed 1 ml-placebo-soybean oil. DMBA induced tumors in the treated rats as visualized in the following results:*

*1) Groups A, B, C, D and E developed 52%, 76%, 88%, 60% and 67% tumors, respectively;*

*2) In the test groups A, B, C, D and E the first palpable tumor was reported at 200 ± 52, 103 ± 36, 48 ± 1, 133 ± 62 and 183 ± 45 days after DMBA administration, respectively.*

*3) 14.8%, 40.7%, 97.5%, 50.0% and 41.7% of all tumors were classified as adenocarcinoma.*

*The results suggest that study model "C" was the most effective one, inducing higher incidence of adenocarcinomas in DMBA-treated-rats, the symptons being evidenced in the shortest experimental time.*

**Key words:** *breast cancer; DMBA; rat mammary gland; study models*

## Introduction

The compound 7,12-dimethylbenz (a) anthracene has been used for over 30 years as a very potent chemical carcinogen of animal mammary tissue [1-4]. Upon administration of DMBA, hyperplastic lesions are found shortly after treatment, and those lesions may be classified into two groups: benign or malignant tumors [5-7]. It is well known that the effectiveness of DMBA as a tumor inducer depends on several factors or experimental conditions: the age of animals during DMBA administration [1, 2, 5], nutritional lipids [8-17] and hormone status [18]. Huggins et al. [1, 2] and Russo et al. [5, 18] have clearly demonstrated that DMBA is more effective when administrated prior to mammary gland maturation (up to 60 days of age). Older virgin female rats showed significant lower incidence of tumors induced by DMBA [5, 18]. Dietary lipids and fatty acids (FA) may be considered as co-carcinogen [10] because high fat diets and linoleic acid-rich diets strongly stimulate the incidence, malignancy, number of tumors per rat and the yield of tumor-bearing rats due to the action of DMBA in mammary tissue [8-18]. On the other hand, dietary lipids

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rich in saturated FA or in n-3 polyunsaturated FA have an opposite effect [9, 12, 13, 17, 18].

In the present work five DMA study models were investigated with the objective of identifying the most effective method of inducing adenocarcinomas in rats in the shortest experimental time.

## Material and Methods

Weaning female Sprague-Dawley rats were used. Groups of five animals were housed in plastic cages, fed with their particular diets and maintained in temperature controlled room at 22 ± 1°C and 12-hour light/dark cycle. Study model A was fed a commercial ration ("Probiotério G", Anderson Clayton Co.). Study models B, C, D and E were fed with semipurified diets prepared in our laboratory [19]. Diets B and C contained sunflower seed oil (rich in linoleic acid), diet D coconut oil (rich in saturated fatty acids), and diet E 50% soybean oil (rich in essential fatty acids), 25% olive oil (rich in oleic acid, a monounsaturated FA) and 25% coconut oil (rich in saturated FA). At appropriate times, the animals received one or two 10/20 mg-DMBA doses (SIGMA Co.) dissolved in 1 ml of soybean oil administrated through intragastric instillation, the animals being fed with diets according to Table 1. The control-group animals received a placebo of 1 ml of soybean oil by intragastric instillation, the feeding treatments presented in Table 1. The animals were weighed and palpated weekly. By the end of the experiment, the animals were anesthetized with either, the tumors surgically removed, their size and weight determined, and sent for histopathological study. The rats were sacrificed after 350 days (groups A and B) and between 190 and 350 days (groups C, D, E) after DMBA administration. Some rats from groups C, D and E were sacrificed prior to the end of the experiment due to symptoms of weakness or imminent death.

**Table 1.** Type of diets and the amount of one or two doses of DMBA administrated through gastric instillation after 45 and/or 60 days birth in female rats of study models A, B, C, D and E

| Models | Diets (source of fats) | 45 days | 60 days |
|---|---|---|---|
| A | Chow | none | 10 mg |
| B | semipurified (SO) | 10 mg | 10 mg |
| C | semipurified (SO) | 20 mg | 20 mg |
| D | semipurified (CO) | 20 mg | 20 mg |
| E | semipurified (MIX) | 20 mg | 20 mg |

SO = sunflower seed oil; CO = coconut oil; MIX = 50% of soybean oil, 25% of olive oil, 25% of coconut oil

## Results and Discussion

The results in Table 2 show the incidence of tumors DMBA-treated rats of each study model.

**Table 2.** Percentage of tumors and tumor-bearing rats induced by DMBA in female rat mammary gland in study models A, B, C, D and E

| | Study models | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Number of rats | 25 | 25 | 25 | 10 | 15 |
| Total number of tumors* | 27 | 27 | 40 | 12 | 16 |
| Number of tumor-bearing rats | 13 | 19 | 22 | 6 | 10 |

*Benign plus malignant tumors

By the end of the experiment, the percentage of tumor-bearing rats was higher in group C (Figure 1) as well as the palpable-tumor-detection-time (Figure 2). The incidence of tumors found in tumor-bearing rats was a little higher in group A (Figure 3). However, most of the tumors found in groups A, B, D and E were benign while most of the tumors present in group C were adenocarcinomas (Figures 4 and 5). Group A reported the only *in situ* carcinoma.

The results described above suggest that model C (linoleic acid-rich diet and two-20 mg-DMBA-doses) was the most effective in inducing the largest number of tumors in rats, in the shortest experimental time. Nearly 100% of the tumors in model C were adenocarcinomas. When the dietary lipids were poor in linoleic acid (groups D and E), the rats (two-20 mg-

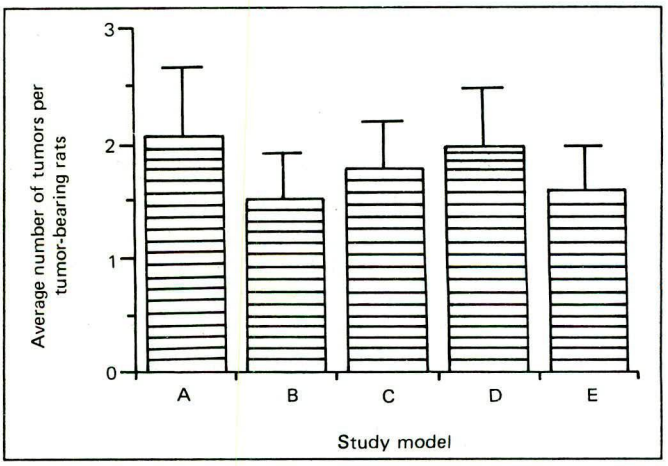


**Figure 1** - The average number of mammary tumors found (benign plus malignant) in tumor-bearing rats treated by DMBA in the five study models A, B, C, D and E.

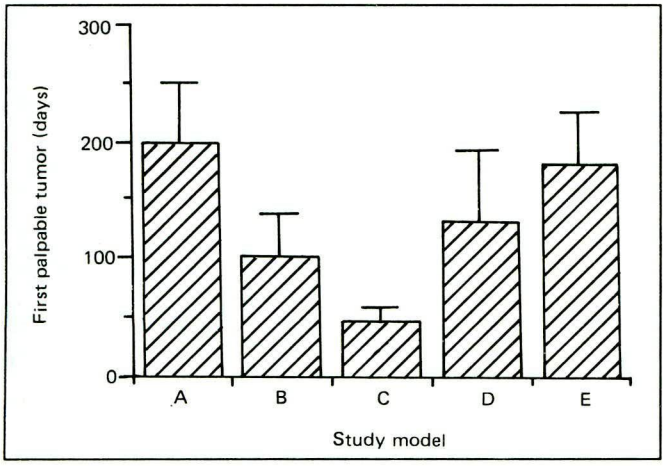


**Figure 2** - The average time of detection of the first palpable mammary tumor of rats treated by DMBA in the five study models A, B, C, D and E.

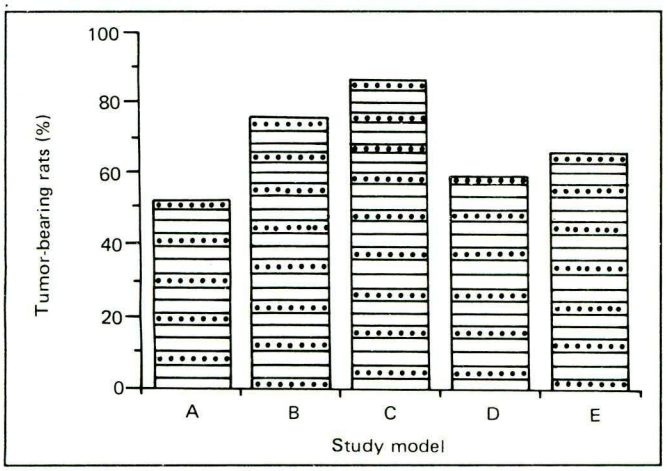


**Figure 3** - Percentage of mammary tumor-bearing rats treated by DMBA in the five study models A, B, C, D and E.

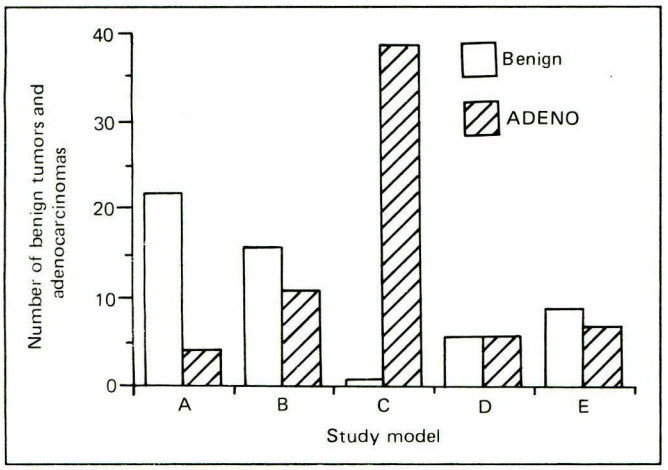


**Figure 4** - Total number of mammary tumors in tumor-bearing rts treated by DMBA in the five study models A, B, C, D and E.

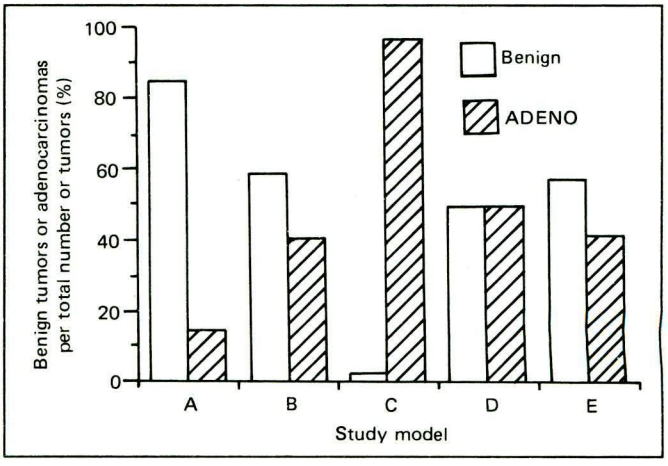


**Figure 5** - The percentage of benign tumors (adenocarcinomas) in relation to the total number of mammary tumors of tumor-bearing rats treated by DMBA in the five study models A, B, C, D and E

DMBA-doses) reported a much lower indidence of adenocarcinomas when compared to model C. These results are supported by a number of literature reports [8-13, 15, 17]. It was observed in addition, that 20 mg of DMBA induced highly toxic effects. Therefore, additional studies have yet to be carried out in order to further improve this study model.

## Resumo

*Cinco modelos de estudo (A, B, C, D, E) foram usados para investigar o efeito do 7,12-dimetilbenz(a)antraceno em induzir tumores no tecido mamário de ratas Sprague-Dawley. Cada grupo foi submetido* ad libitum *às seguintes dietas: grupo A - ração comercial; grupos B e C - uma dieta semipurificada rica em ácido linoléico; grupo D - uma dieta semipurificada rica em ácido graxo saturado; grupo E - uma dieta gordurosa composta de 50% de óleo de soja (rico em ácido graxo essencial), 25% de óleo de oliva (rico em ácido oleico) e 25% de óleo de coco (rico em ácido graxo saturado). Os animais receberam, através de instilação intragástrica, uma ou duas doses de DMBA dissolvidas em 1 ml de óleo de soja. Os animais do grupo A receberam uma dose simples de 10 mg de DMBA, 60 dias após o nascimento. Os animais do grupo B receberam duas doses de 20 mg de DMBA, a primeira dose 45 dias após o nascimento e a segunda 60 dias após o nascimento. Os animais dos grupos C, D e E foram tratados da mesma maneira que os animais do grupo B. Os grupos-controle receberam 1 ml de placebo, óleo de soja. O DMBA induziu tumores nos ratos tratados como visualizado nos seguintes resultados:*

*1) Os grupos A, B, C, D e E desenvolveram 52%, 76%, 88%, 60% e 67% de tumores, respectivamente.*

*2) Nos grupos-testes A, B, C, D e E, o primeiro tumor palpável foi visto a 200 ± 52, 103 ± 36, 48 ± 1, 133 ± 62 e 183 ± 45 dias após a administração do DMBA, respectivamente.*

*3) 14,8%, 40,7%, 97,5% e 41,7% de todos os tumores foram classificados como adenocarcinomas.*

*Os resultados sugerem que o modelo de estudo "C" foi o mais efetivo, induzindo, o maior, incidência de adenocarcinomas nos ratos tratados por DMBA, os sintomas foram evidentes no menor tempo experimental.*

**Unitermos:** *câncer mamário; DMBA; glândula mamária de rato; estudo de modelos*

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### References

1. HUGGINS C, BRIZIARELLI G, SUTTON H. Rapid induction of mammary carcinoma in the rat and the influence of hormone on the tumors. J Exp Med 1959; 109: 25-54.
2. HUGGINS C, GRAND LC, BRILLANTES FP. Mammary cancer induced by a single feeding of polynuclear hydrocarbons and its supression. Nature 1961; 189: 204-207.
3. DIGIOVANNI J, JUCHAU MR. Biotransformation and bioactivation of 7,12-dimethylbenz(a)anthracene (7,12-DMBA). Drug Metab Rev 1980; 11(1): 61-101.
4. MILLER EC. Some current perspectives on chemical carcinogenesis in humans and experimental animals: presidential address. Cancer Res 1978; 38: 1479-1496.
5. RUSSO J, WILGUS G, RUSSO IH. Susceptibility of the mammary gland to carcinogenesis. I. Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. Am J Pathol 1979; 96: 721-736.
6. PURNELL DM. The relationship of terminal duct hyperplasia to mammary carcinoma in 7,12-dimethylbenz(a)anthracene-treated LEW/ Mai rats. Am J Pathol 1980; 98: 311-324.
7. ALVARENGA M, CAVALCANTI TC, TAHIN QS. Histopathologic grading system for epithelial abnormalities induced by 7,12-dimethylbenz(a)anthracene (DMBA) in female rat mammary tissue. Breast Dis 1989; 2: 71-79.
8. CARROLL KK, GAMMAL EB, PLUNKTT ER. Dietary fat and mammary cancer. Cand Med Ass J 1968; 98: 590-594.
9. CARROLL KK. Summation: which fat/how much fat animals. Prev Med 1987; 16: 510-515.
10. KRITCHEVSKY D. Lipids and cancer. In: Arnott MS, van Eys J, Wang Y-M, eds. Molecular Interrelations of Nutrition and Cancer. New York: Raven Press 1982: 209-217.
11. ABRAHAM S, FAULKIN LJ, HILLYARD LA, MITCHELL DJ. Effect of dietary fat on tumorigenes in the mouse mammary gland. J Nath Cancer Inst 1984; 72(6): 1421-1429.
12. SELENKAS SL, IP MM, IP C. Similarity between trans fat and saturated fat in the modification of rat mammary carcinogenesis. Cancer Res 1984; 44: 1321-1326.
13. ABOU-EL-ELA SH, PRASSE HW, CARROLL R, WADE AE, DHARWADKAAR S, BUNCE OR. Eicosanoid synthesis in 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in Sprague-Dawley rats fed primose, menhaden oil or corn oil diets. Lipids 1988; 23: 948-954.
14. TAHIN QS, CAVALCANTI TC. The effect of dietary lipids in the fatty acid composition of mammary mitochondria of female rat treated with 7,12-dimethylbenz(a)anthracene. Breast Dis 1991; in press.
15. CARROLL KK. Fish oils and cancer. In: Chandra RK, ed. Health Effects of Fish Oils. St. John's, Newfoundland: ARTS Biomedical Publishers & Distributors, 1989: 395-408.
16. JACOBSON EA, JAMES KA, NEWMARK HL, CARROLL KK. Effects of dietary fat, calcium, and vitamin D on growth and ma-mmary tumorigenesis induced by 7,12-dimethylbenz(a)anthracene in female Sprague-Dawley rats. Cancer Res 1989; 49: 6300-6303.
17. CARROLL KK. Experimental and epidemiological evidence on marine lipids and carcinogenesis. In: Less RS, Karel M. eds. Omega-3 fatty acids in health and disease, New York: Marcel Dekker Inc., 1990: 99-114.
18. RUSSO J, RUSSO IH. Biology of disease. Biological and molecular bases of mammary carcinogenesis. Lab Invest 1987; 57(2): 112-137.
19. CAVALCANTI TC, MELLO RA, De CAMARGO AM, TAHIN QS. Mammary mitochondria ATPase of female rats treated by DMBA and two lipid diets. IRCS Med Sci 1986; 14: 1161-1162.