[CANCER RESEARCH 49, 1447-1451, March 15, 1989]

Effect of Dietary Palm Oils on Mammary Carcinogenesis in Female Rats Induced by 7,12-Dimethylbenz(*a*)anthracene[1]

K. Sundram, H. T. Khor,[2] Augustune S. H. Ong, and R. Pathmanathan

Palm Oil Research Institute of Malaysia, Bangi, Selangor [K. S., A. S. H. O.], and Departments of Biochemistry [H. T. K.] and Pathology [R. P.], Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

ABSTRACT

Female Sprague-Dawley rats, 50 days of age, were treated with a single dose of 5 mg of 7,12-dimethylbenz(*a*)anthracene intragastrically. 3 days after carcinogen treatment, the rats were put on semisynthetic diets containing 20% by weight of corn oil (CO), soybean oil (SBO), crude palm oil (CPO), refined, bleached, deodorized palm oil (RBD PO) and metabisulfite-treated palm oil (MCPO) for 5 months. During the course of experiments, rats fed on different dietary fats had similar rate of growth. Rats fed 20% CO or SBO diet have higher tumor incidence than rats fed on palm oil (PO) diets; however differences of mean tumor latency periods among the groups were not statistically significant. At autopsy, rats fed on high CO or SBO diets had significantly more tumors than rats fed on the three PO diets. Our results showed that high PO diets did not promote chemically induced mammary tumorigenesis in female rats when compared to high CO or SBO diets. CO and SBO differ greatly from the palm oils in their contents of tocopherols, tocotrienols, and carotenes. But further experiments would be required to determine whether the observed differences in tumor incidence and tumor numbers were due to the differences in these minor components or due to the unique triglyceride structure of the palm oils.

Analysis of the fatty acid profiles of plasma total lipids of tumor-bearing rats and of the tumor total lipids showed that, with the exception of arachidonic acid, the fatty acid profiles reflect the nature of the dietary fats. At autopsy, there were no differences in the plasma total cholesterol contents among rats fed on different dietary fats, but rats fed on palm oil diets had a significantly higher plasma triglyceride level than that of rats fed CO or SBO diets. As for the tumor lipids, there were no significant differences in the triglyceride, diglyceride, and phospholipid levels when the CO or SBO groups were compared to the palm oil groups.

INTRODUCTION

The ability of high fat diets to enhance chemically induced mammary carcinogenesis in female rats has been widely reported (1-7). Different types of dietary fats show different magnitude of tumor promotion (3, 4). Polyunsaturated oils promote mammary tumorigenesis much greater than saturated fats (3, 8, 9). PO[3] is an oil obtained from the mesocarp of the oil palm fruits and is often confused with PKO which is obtained from the nuts of the oil palm fruits. PO and PKO have distinctly different fatty acid composition (10).

PO has approximately 50% saturated and unsaturated fatty acids (11-13). PO can be refined, bleached, and deodorized as RBD PO and fractionated into palm olein and palm stearin. PO has been an important source of dietary fat for many people in the tropical countries for centuries. However, the impact of PO as a dietary fat on the development of atherosclerosis and chemically induced mammary tumors in laboratory animals is still largely unexplored. In the present study, the effect of different high PO diets on mammary carcinogenesis in female rats treated with DMBA is being investigated.

MATERIALS AND METHODS

Animals, Diets, and DMBA Administration. Female Sprague-Dawley rats were obtained from the Animal Breeding Unit, University of Malaya at 45 days of age. They were allocated into groups of 20 each, put on laboratory chow diet, and housed in an experimental room with 12-h light and 12-h darkness. At 50 days of age, all rats were given a single dose of 5 mg of DMBA in 0.25 ml of CO intragastrically. All rats were continued on laboratory chow diet for another 3 days after DMBA administration; thereafter, they were fed semisynthetic diets containing 20% by weight of SBO, CO, CPO, RBD PO, and MCPO. The diets (Table 1) were formulated according to Newberne *et al.* (14). Water and feed were given ad libitum. The rats were weighed weekly. 3 weeks after DMBA administration, palpation for mammary tumor was started and the appearance of each tumor was recorded. The experiments were terminated 5 months after DMBA administration.

Just before autopsy, blood was taken from ether-anesthetized tumor-bearing rats by heart-puncture technique and collected in centrifuge tubes containing EDTA (1 mg/ml) as the anticoagulant. Plasma was prepared by centrifugation at 2000 × *g* for 10 min in a refrigerated centrifuge and stored at −20°C.

At autopsy, the presence of mammary tumors in each rat was carefully examined and suspected tissues were preserved in 10% formalin and sent for histopathological examinations. Solid mammary tumors were excised, frozen, and kept at −20°C.

Lipid Extraction. Plasma and tumor lipids were extracted with chloroform:methanol (2:1, v/v) and washed with dilute salt solution (15). The lower chloroform layer was dried and quantified gravimetrically as described (16). Aliquots of the total lipids were spotted on thin-layer plates (silica gel G-coated) and the lipid components were separated in a solvent system of hexane:diethyl ether:formic acid (80:20:2, by vol) in a thin-layer chromatographic tank previously saturated with vapor of the solvent system. Lipid spots were visualized by transient exposure to iodine vapor or by spraying with 2,7-dichlorofluorescein. Neutral lipids were quantified by acid-charring method (17) and phospholipids were quantified by phosphorus estimation according to Rouser *et al.* (18).

Fatty Acid Analysis. Various dietary fats, plasma total lipids, and tumor total lipids were transmethylated with methanolic HCl at 120°C for 2 h (3) and the fatty acid methyl esters were extracted and purified as described (19). The fatty acid methyl esters were analyzed by gas chromatography (Shimadzu GC 8A) using a glass column (2 mm x 2 m) packed with 10% SP 2300 (Supelco). The analysis was carried out by temperature programming from 200 to 230°C at 2°C/min. Peak areas were quantified by an electronic data processor (Shimadzu C-R3A) and expressed as a percentage of the total fatty acids.

Analysis of Tocopherols, Tocotrienols, and Carotenes. The tocopherol and tocotrienol contents of CO, SBO, CPO, RBD PO, and MCPO were analyzed by high-performance liquid chromatography (13), and expressed as parts per millions (ppm). The carotenes were determined by measuring their absorbance in hexane at 446 nm (20).

RESULTS

The fatty acid composition of the different dietary fats used in this study is shown in Table 2. CO and SBO both contain

Received 6/24/88; revised 11/14/88; accepted 11/28/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a research grant from the Palm Oil Research Institute of Malaysia.

[2] To whom requests for reprints should be addressed, at Department of Biochemistry, Faculty of Medicine, University of Malaya, 59100 Kuala Lumpur, Malaysia.

[3] The abbreviations used are: PO, palm oil; PKO, palm kernel oil; RBD, refined-bleached-deodorized; CO, corn oil; SBO, soybean oil; CPO, crude palm oil; MCPO, metabisulfite-treated palm oil.

Table 1 *Composition of semisynthetic diets*

Ingredients	Percentage (by weight)
Casein, vitamin-free	22.0
Dextrose	45.0
Fat	20.0
Alphacel	7.0
Vitamin mixture (AIN-76)	1.0
Salt mixture (AIN-76)	4.5
DL-Methionine	0.3
Choline bitartrate	0.2

Table 2 *Fatty acid composition of the five dietary fats (% of total fatty acids)*

Fatty acids	Corn oil	Soybean oil	RBD palm oil	Crude palm oil	MC[a] palm oil
12:0	0.1	0.1	0.3	0.2	0.2
14:0	t[b]	t	0.8	0.8	0.9
16:0	9.7	8.9	39.5	39.4	39.5
16:1	0.3	0.3	0.3	0.4	0.5
18:0	2.7	3.6	4.3	4.2	4.2
18:1	37.0	20.6	43.1	43.1	43.0
18:2	48.7	57.2	10.7	11.0	10.8
18:3	1.1	8.4	0.5	0.4	0.4
20:0	0.4	0.3	0.5	0.4	0.5
22:1	ND	0.6	ND	ND	MD
Saturates	12.9	12.9	45.4	45.0	45.3
Monounsaturates	37.3	21.5	43.4	43.5	43.5
Polyunsaturates	49.8	65.6	11.2	11.4	11.2

[a] MC = metabisulfite-treated.
[b] t = trace, <0.1%.
[c] ND, not detected.

Table 3 *Contents of tocopherols, tocotrienols, and carotenes of corn oil, soybean oil, crude palm oil, RBD palm oil, and metabisulphite-treated palm oil. [Expressed as parts per million (ppm)]*

	Corn oil	Soybean oil	Crude palm oil	RBD palm oil	MC[a] palm oil
α-T[b]	126	28	143	97	64
γ-T	446	235	ND[d]	ND	ND
δ-T	25	145	ND	ND	ND
Total T	597	408	143	97	64
α-T3[c]	ND	ND	188	161	33
γ-T3	ND	ND	296	203	74
δ-T3	ND	ND	81	51	80
Total T3	nil	nil	565	415	187
Carotenes	ND	ND	584	ND	326

[a] MC, metabisulphite-treated.
[b] T, tocopherols.
[c] T3, tocotrienols.
[d] ND, not detected.

about 12% saturated fatty acids of which palmitic acid constitutes about 9–10%. The unsaturated fatty acids of CO are made up of about 37% oleic, 49% linoleic, and 1% linolenic acids; whereas the unsaturated fatty acids of SBO are consisted of about 21% oleic, 66% linoleic, and 8% linolenic acids. On the other hand, the fatty acid composition of CPO, RBD PO, and MCPO are broadly similar. Palmitic acid constitutes about 40% of the saturated fatty acids and oleic acid constitutes about 43% of the unsaturated fatty acids in all three types of palm oils. About 11% of the unsaturated fatty acids are linoleic acid. The above results indicate that PO has four times higher content of palmitic acid and five times lower linoleic acid content than CO or SBO. PO also has 6 and 23% higher oleic acid contents than CO and SBO, respectively.

In addition to the differences in fatty acid composition, PO also differs greatly from CO and SBO in its content of tocopherols, tocotrienols, and carotenes (Table 3). CO and SBO have high levels of tocopherols but are devoid of tocotrienols and carotenes. All the tree types of palm oils have lower level of tocopherols when compared to the CO and SBO; but they have high levels of tocotrienols and carotenes, except for RBD PO which has lost all its carotenes during physical refining. The metabisulfite treatment of CPO has retained 56% of the carotenes and, in turn, lost about 32% of the tocopherols and 67% of the tocotrienols present in the CPO. The RBD PO, on the other hand, retained 67% of the tocopherols, 73% of the tocotrienols, but lost all the carotenes as compared with CPO.

When female rats were treated with DMBA and fed semisynthetic diets containing the five different fats and oils for the 5-month experimental period, mammary tumors appeared at different rate in different groups. Fig. 1 shows the percentage incidence of female rats with palpable mammary tumors in the various dietary groups for the 5-month experimental period. Rats fed 20% CO and SBO diets appeared to develop mammary tumors earlier and faster than rats fed on the PO diets; but the differences in the mean latency periods were not statistically significant. The latency periods for CO, SBO, CPO, RBD PO, and MCPO groups were 102, 98, 104, 102, and 105 days, respectively. The final incidences of palpable mammary tumors were 85, 90, 65, 70, and 70%, respectively, for the CO, SBO, CPO, RBD PO, and MCPO groups.

The cumulative number of mammary tumors in the different dietary groups are shown in Fig. 2. It is clearly seen that the 20% CO and 20% SBO groups had significantly more mammary tumors than the groups fed PO diets. At autopsy, the groups fed 20% CO diet and 20% SBO diet had 71 (palpable, 64; nonpalpable, 7) and 57 (palpable, 52; nonpalpable, 5) mammary tumors, respectively, whereas the groups fed 20% RBD

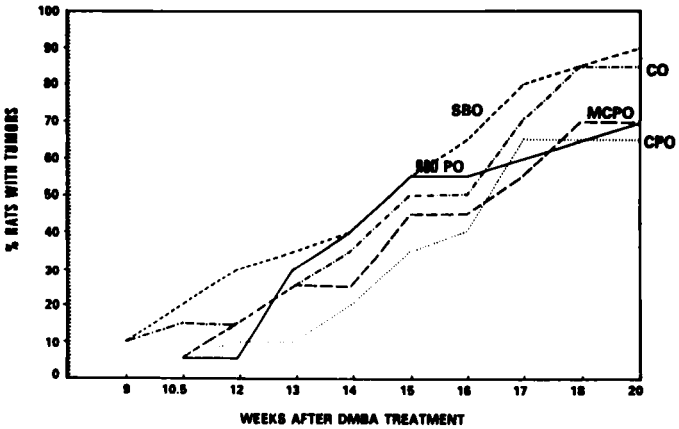

Fig. 1. Percentage incidence of female rats with palpable mammary tumors in the course of the experiments. Rats were fed with laboratory chow diet before and continued for 3 days after DMBA administration; thereafter the rats were fed with semisynthetic diets containing 20% by weight of CO, SBO, CPO, MCPO, and RBD PO. There were 20 rats per group.

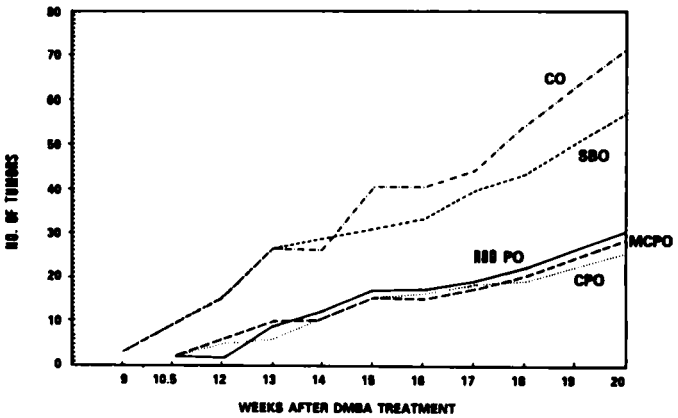

Fig. 2. The cumulative number of palpable mammary tumors in female rats treated with DMBA and fed with semisynthetic diets containing different fats and oils as described in Fig. 1.

PO diet, 20% CPO diet, and 20% MCPO diet had 30 (palpable, 27; nonpalpable, 3), 25 (palpable, 23; nonpalpable, 2) and 28 (palpable, 23; nonpalpable, 5) mammary tumors, respectively. The number of mammary tumors per rats in the CO group (4.18) and in the SBO groups (3.17) were significantly higher than that of the RBD PO group (2.14), CPO group (1.92), and MCPO (2.00). The mammary tumors in all dietary groups were mainly adenocarcinomas with only 4, 4, 1, 2, and 2 adenomas in the CO, SBO, CPO, RBD PO, and MCPO groups, respectively.

The fatty acid composition of plasma total lipids of tumor-bearing rats from different dietary groups were analyzed and the results are shown in Table 4. The plasma from tumor-bearing rats fed on PO diets has significantly higher levels of palmitic and oleic acids and a significantly lower level of linoleic acid than that of rats fed on CO and SBO diets. However, arachidonic acid is the major plasma fatty acid of tumor-bearing rats in all the dietary groups and its level in the plasma of tumor-bearing rats fed on CPO diet is significantly lower than that of tumor-bearing rats fed on SBO and RBD PO diets.

The fatty acid profiles of mammary tumor total lipids were also investigated and the results are shown in Table 5. In general, the mammary tumor fatty acid profiles reflect closely the nature of dietary fats consumed by the rats. Tumors from rats fed on PO diets have higher palmitic acid and oleic acid contents and lower linoleic acid content compared to those from rats fed on CO and SBO diets. There is no consistent effect of dietary fats on the level of arachidonic acid; the levels of arachidonic acid in the CPO and MCPO groups are significantly lower than that in the RBD PO group. The arachidonic acid level in the SBO group is significantly higher than the MCPO group.

Table 4 *Fatty acid profiles of plasma total lipids of tumor-bearing rats (Expressed as % of total fatty acids)*

Mean of 10 samples ± SD. Means with superscript *a* are significantly different from those of *b*, and means with superscript *c* are significantly different from those of *d* ($P < 0.05$). MC, metabisulphite-treated; t, trace (<0.1%).

Fatty acid	Corn oil	Soybean oil	Crude palm oil	RBD palm oil	MC palm oil
14:0	t	t	0.7 ± 0.5	0.3 ± 0.1	0.3 ± 0.1
16:0	16.2 ± 0.9[a]	15.3 ± 0.8[a]	23.0 ± 2.2[b,c]	20.7 ± 1.2[b,d]	21.8 ± 2.7[b]
16:1	t	t	t	t	t
18:0	16.8 ± 1.6[a]	17.3 ± 0.7[a,c]	14.3 ± 2.9[b]	16.5 ± 1.6	14.6 ± 1.9[d]
18:1	10.4 ± 1.2[a]	7.7 ± 0.5[a]	19.3 ± 4.8[b]	17.6 ± 2.0[b]	18.9 ± 3.0[b]
18:2	19.6 ± 2.9[a]	21.6 ± 3.2[a]	11.6 ± 2.2[b,c]	8.4 ± 0.7[b,d]	11.4 ± 1.0[b,c]
18:3	t	t	t	t	t
20:0	0.6 ± 0.1	0.2 ± 0.1	0.8 ± 0.2	t	0.8 ± 0.2
20:1	0.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.5	0.7 ± 0.2	0.4 ± 0.2
20:2	t	t	t	0.2 ± 0.1	t
20:4	30.4 ± 2.4	32.9 ± 3.1[b]	25.9 ± 6.0[a]	31.1 ± 1.8[b]	27.1 ± 3.8
22:0	t	0.8 ± 0.2	t	t	t
22:1	t	0.2 ± 0.1	t	t	t
20:5	0.7 ± 0.1	t	t	t	t
24:0	1.6 ± 0.2	t	2.0 ± 0.3	2.4 ± 0.5	2.6 ± 0.4
24:1	2.7 ± 0.9	0.4 ± 0.9	1.6 ± 0.5	1.8 ± 0.3	1.4 ± 0.7
22:5	0.9 ± 0.3	t	t	t	t
22:6	0.1 ± 0.1	t	t	t	t

Table 5 *Fatty acid profiles of mammary tumor total lipids (Expressed as % of total fatty acids)*

Mean of six samples ± SD. Means with superscript *a* are significantly different from those of *b*; and means with superscript *c* are significantly different from those of *d*. ($P < 0.05$). t, trace, < 0.1%.

Fatty acids	Corn oil	Soybean oil	Crude palm oil	RBD palm oil	MC palm oil
14:0	0.6 ± 0.1	0.7 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.2
16:0	15.4 ± 0.1[a]	17.8 ± 1.8[a]	24.2 ± 1.0[b]	25.2 ± 1.8[b]	22.9 ± 6.5[b]
16:1	1.5 ± 0.5	2.1 ± 0.4	2.2 ± 0.3	2.4 ± 0.3	2.0 ± 0.5
16:2	0.4 ± 0.1	0.6 ± 0.3	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2
18:0	6.7 ± 3.4	7.3 ± 3.4	6.0 ± 1.4	7.5 ± 1.0	5.9 ± 2.3
18:1	33.1 ± 4.9[a]	28.7 ± 3.7[a]	43.3 ± 4.5[b]	41.3 ± 3.9[b]	43.0 ± 7.8[b]
18:2	29.5 ± 5.4[a]	28.1 ± 1.4[a]	11.9 ± 3.5[b,d]	7.1 ± 2.0[b,c]	16.2 ± 1.3[b,d]
18:3	0.5 ± 0.2	1.6 ± 0.8	0.4 ± 0.2	t	0.4 ± 0.3
20:0	0.2 ± 0.1	t	t	t	t
20:1	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	0.8 ± 0.3	0.9 ± 0.2
20:2	0.6 ± 0.5	0.6 ± 0.2	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
20:4	7.1 ± 4.2	8.1 ± 2.1[a]	5.9 ± 2.6[d]	10.1 ± 3.4[c]	4.7 ± 0.9[b,d]
22:0	t	t	t	0.1 ± 0.0	t
22:1	0.2 ± 0.1	t	t	t	t
20:5	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
24:0	1.9 ± 0.4	1.8 ± 0.2	1.6 ± 1.0	1.6 ± 0.8	1.1 ± 0.6
24:1	0.8 ± 0.4	1.1 ± 0.5	1.0 ± 0.6	1.2 ± 0.6	0.8 ± 0.3

The distribution of the major classes of plasma lipids in the tumor-bearing rats of the different dietary groups is shown in Table 6. At autopsy, there was no significant difference in the total plasma cholesterol contents in the different dietary groups; but the triglyceride level in the group fed the CPO diet were significantly higher than that of the groups fed the CO and SBO diets. The triglyceride level in the groups fed the RBD PO diet was significantly higher than that of the group fed the CPO diet. There were no significant differences in the total plasma phospholipid contents among the groups fed CO, SBO, and CPO diets; but the total plasma phospholipids of the group fed the RBD PO diet was higher than that of the groups fed the SBO and CPO diets.

Table 7 shows the distribution of major lipid classes in the mammary tumors at autopsy in the different dietary groups. There is no apparent trend in the distribution of major lipid classes in the mammary tumors between the CO- and SBO-fed groups and the PO-fed groups and the differences were mostly insignificant. However, mammary tumors from the CO- and RBD PO-fed groups had significantly higher cholesterol content than those from the SBO-fed group. Mammary tumors from the RBD PO group also had higher phospholipid content than those from the MCPO group.

DISCUSSION

In the present study, the growth of the female rats treated with DMBA and fed on semisynthetic diets containing 20% by weight of CO, SBO, RBD PO, CPO, and MCPO was comparable throughout the experimental period (Fig. 3) indicating that there was no significant difference in energy intake and utilization among the various dietary groups. The CO and SBO used in this study were comparable to those used by other investigators in terms of their fatty acid composition (3, 21) but they differ significantly in fatty acid composition from the palm oils (Table 2). In addition, CO and SBO also differ greatly in their minor components such as tocopherols, tocotrienols, and carotenes from that of the palm oils. CO and SBO have high levels of tocopherols but are completely devoid of tocotrienols and carotenes. CPO is very rich in tocopherols, tocotrienols, and carotenes. However, conventional refining processes have removed practically all the carotenes and parts of the tocopherols and tocotrienols. The metabisulfite treatment was meant to retain the carotenes in the palm oil while reducing the content of free fatty acids and peroxides which could otherwise promote oxidative degradation of the oil.

PO is a relatively new source of dietary fat in many developed countries and very few studies on its effect on chemically induced carcinogenesis have been carried out in the past. Our results (Figs. 1 and 2) show that CO and SBO enhanced mammary carcinogenesis in female rats induced by DMBA much more than the three types of PO used in this study. Ip (22) showed earlier that addition of PO to a high CO diet effectively reduced the tumor-enhancing effect of the high CO diet. Therefore, our results (Figs. 1 and 2) confirm previous findings that high CO and SBO diets promote chemically

Table 6 *Major plasma lipids of tumor-bearing rats (Expressed as mg/100 ml plasma)*

Mean of 14 samples ± SEM. Means with superscript *a* are significantly different from those of *b*; means with superscript *c* are significantly different from those of *d* ($P < 0.05$). MC, metabisulfite-treated.

Dietary fats	Free cholesterol	Esterified cholesterol	Total cholesterol	Triglycerides	Phospholipids
Corn oil	20.7 ± 2.4[a]	63.4 ± 3.5	82.5 ± 4.4	31.5 ± 5.8[a]	128.7 ± 6.8
Soybean oil	25.7 ± 0.9[b]	66.4 ± 2.5	92.1 ± 3.2	29.2 ± 1.1[a]	125.2 ± 6.3[a]
Crude palm oil	25.7 ± 1.4[b]	62.2 ± 4.4	88.3 ± 7.0	60.4 ± 6.4[b,c]	125.5 ± 4.2[c]
RBD palm oil	28.2 ± 2.4[b]	65.2 ± 4.4	94.6 ± 6.8	44.3 ± 4.0[b,d]	146.5 ± 6.9[b,d]
MC palm oil	26.6 ± 0.6[b]	60.7 ± 3.1	86.0 ± 1.6	45.0 ± 11.4[b]	140.5 ± 5.1

Table 7 *Major mammary tumor lipids (Expressed as mg/g tumor)*

Mean of six samples ± SEM. Means with superscript *a* are significantly different from those with superscript *b*; and mean with superscript *c* is significantly different from that with superscript *d* ($P < 0.05$).

Dietary fats	Total cholesterol	Triglycerides	Diglycerides	Phospholipids
Corn oil	3.3 ± 0.2[a]	11.9 ± 1.5	1.2 ± 0.1	9.5 ± 1.2
Soybean oil	1.6 ± 0.1[b,c]	8.4 ± 0.3	1.1 ± 0.1	8.0 ± 2.0
Crude palm oil	2.3 ± 0.4	11.7 ± 1.3	1.2 ± 0.1	10.8 ± 1.7
RBD palm oil	2.9 ± 0.2[d]	9.9 ± 0.6	1.5 ± 0.2	12.3 ± 1.6[a]
MC palm oil	2.3 ± 0.5	11.5 ± 1.9	1.2 ± 0.1	5.8 ± 1.5[b]

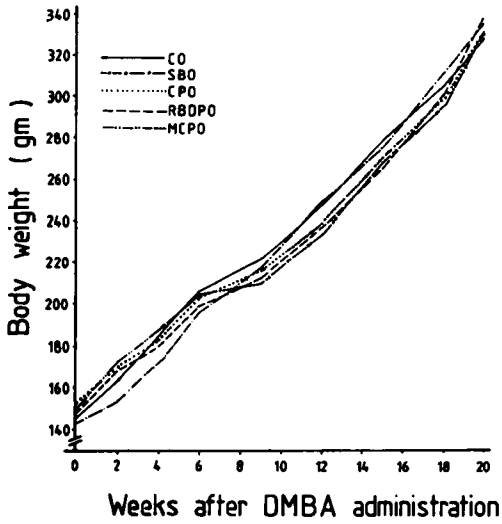

Fig. 3. The growth curves of female rats treated with DMBA and fed on different high fat semisynthetic diets. There were 20 rats in each group and their body weights were monitored at weekly interval. The average body weights of the animals were plotted against weeks after DMBA administration.

induced mammary carcinogenesis in female rats (2, 3) and indirectly support the findings of Ip (22) that palm oil has a nonpromoting effect on chemically induced mammary carcinogenesis in female rats. The reason that PO does not promote mammary carcinogenesis as the CO and SBO do is not immediately known. However, it has been previously shown that polyunsaturated oils (CO, sunflower seed oil, and SBO) promoted, whereas saturated fats (coconut oil, lard, beef tallow, and butter) did not promote chemically induced mammary carcinogenesis in female rats (3). In the present studies, PO gave results similar to that produced by the saturated fats which were reported previously (3). In another study on the action of dietary fats in the initiation phase of mammary carcinogenesis, Sylvester *et al.* (21) showed that high PO diet showed the least stimulatory effect on the initiation of mammary carcinogenesis when compared to high lard, beef tallow and CO diets. On the basis of the incidence of palpable mammary tumors, the high PO group gave results comparable to that of the 5% CO group (21). Honstra *et al.* (23) in their study on thrombosis also showed that PO did not behave like a saturated fat because it possesses antithrombotic effect that is not seen in saturated fats. Hence, from the response of PO in various studies, PO is neither a saturated nor a polyunsaturated fat.

From the chemical standpoint, PO has about 50% saturated and 50% unsaturated fatty acids. Palmitic and stearic acids account for around 40% and 4% of saturated fatty acids, respectively; whereas oleic and linoleic acid account for 43% and 11% of the unsaturated fatty acids (Table 2). Several investigators (8, 24) has confirmed the requirement for linoleic acid in the promotion of mammary carcinogenesis and the critical level of linoleic acid is around 4–5% of the diet, above which this acid exerts a promoting effect on mammary carcinogenesis. In the present study, linoleic acid contributes only about 2% in the PO diets; thus it is below the critical level mentioned above but sufficient to prevent essential fatty acid deficiency. In addition, the molecular structure of PO triglycerides was also strikingly different from that of SBO (25, 26). Whether or not this unique chemical composition and structure of PO triglycerides have contributed to its unusual property in thrombosis and mammary carcinogenesis remains unknown.

On the other hand, it is noted that there is a distinct difference in the contents of tocopherols, tocotrienols, and carotenes in these dietary fats and oils (Table 3). CO and SBO have high levels of tocopherols but are totally devoid of tocotrienols and carotenes. In contrast, CPO is extremely rich in tocopherols, tocotrienols, and carotenes (Table 3) and our results agree with previous reports (12, 13). Since CO and SBO have, in fact, higher level of tocopherols than the palm oils, it is unlikely that the tocopherols were the cause of the nonpromoting effect of palm oils in mammary carcinogenesis. On the other hand, tocotrienols were recently shown by Kato *et al.* (27) to prolong the lifespan of tumor-bearing rats following its administration. Furthermore, β-carotene and vitamin A have been considered to have anticancer property (28, 29) and experimental evidences showed that dietary supplementation of β-carotene and vitamin A inhibits the development of several chemically induced cancers in laboratory animals (30–34). Whether or not the observed nonpromoting effect of PO on mammary carcinogenesis is due to its minor constituents such as tocotrienols and carotenes or due to its specific triglyceride structure remains to be determined.

The effect of dietary fats on the pattern of distribution of fatty acids was more apparent on the mammary tumor fatty acids than on the plasma fatty acids of tumor-bearing rats. The tumor fatty acid profiles essentially reflect the nature of dietary fats consumed by the rats. This finding agrees with previous reports (35, 36). With regard to the serum and mammary tumor lipid profiles, there is no consistent change among the groups fed on polyunsaturated CO and SBO diets and those fed on PO diets (Tables 6 and 7). Therefore, it is not possible to correlate the changes in lipid profiles to the nonpromoting effect of palm oil on chemically induced mammary carcinogenesis.

ACKNOWLEDGMENTS

Technical assistance was provided by Selvarajan Dorasamy. We thank the Director-General of Palm Oil Research Institute of Malaysia for permission to publish this manuscript.

REFERENCES

1. Carroll, K. K., and Khor, H. T. Effect of dietary fat and dose level of 7,12-dimethylbenz(*a*)anthracene on mammary tumor incidence in rats. Cancer Res., *30:* 2260–2264, 1970.
2. Carroll, K. K., and Khor, H. T. Dietary fat in relation to tumorigenesis. Prog. Biochem. Pharmacol., *10:* 308–353, 1975.
3. Carroll, K. K., and Khor, H. T. Effect of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats by 7,12-dimethylbenz(*a*)anthracene. Lipids, *6:* 415–420, 1971.
4. Hopkins, G. J., and Carroll, K. K. Relationship between amount and type of dietary fat in promotion of mammary carcinogenesis induced by 7,12-dimethylbenz(*a*)anthracene. J. Natl. Cancer Inst., *62:* 1009–1012, 1979.
5. King, M. M., Bailey, D. M., Gibson, D. D., Pitha, J. V., and McCay, P. B. Incidence and growth of mammary tumors induced by 7,12-dimethylbenz(*a*)anthracene as related to the dietary content of fat and antioxidant. J. Natl. Cancer Inst., *63:* 657–663, 1979.
6. Rogers, A. E., and Wetsel, W. C. Mammary carcinogenesis in rats fed different amounts and types of fat. Cancer Res., *41:* 3735–3737, 1981.
7. Cohen, L. A., Thompson, D. O., Maeura, Y., Choi, K., Blank, M. E., and Rose, D. P. Dietary fat and mammary cancer. I. Promoting effect of different dietary fats on *N*-nitrosomethylurea-induced rat mammary tumorigenesis. J. Natl. Cancer Inst., *77:* 33–42, 1986.
8. Carroll, K. K., and Hopkins, G. J. Dietary polyunsaturated fat versus saturated fat in relation to mammary carcinogenesis. Lipids, *14:* 155–158, 1978.
9. Hopkins, G. J., Kennedy, T. G., and Carroll, K. K. Polyunsaturated fatty acids as promoters of mammary carcinogenesis induced in Sprague-Dawley rats by 7,12-dimethylbenz(*a*)anthracene. J. Natl. Cancer Inst., *66:* 517–522, 1981.
10. Rossell, J. B., King, B., and Downes, M. J. Composition of oils. J. Am. Oil Chemists Soc., *62:* 221–229, 1985.
11. Clegg, A. J. Composition and related nutritional and organoleptic aspects of palm oil. J. Am. Oil Chemists Soc., 50: 321–324, 1973.
12. Tan, B. K., and Oh, F. C. H. Malaysian palm oil: chemical and physical characteristics. PORIM Technol., *3:* 1–5, 1981.
13. Jacobsberg, B., Deldima, P., and Gapor, A. B. Tocopherols and tocotrienols in palm oil. Oleagineux, *28:* 25–32, 1978.
14. Newberne, P. M., Bieri, J. G., Briggs, G. M., and Nesheim, M. G. Control of diets in laboratory animal experimentation. Inst. Lab. Animal Resour. News, *21:* A1–A12, 1978.
15. Folch, J., Lees, M., and Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., *226:* 497–509, 1957.
16. Khor, H. T., and Oo, K. C. Changes in lipid profiles in the developing haustorium of oil palm seeds. Phytochemistry, *23:* 1577–1581, 1984.
17. Marsh, J. B., and Weinstein, D. B. Simple charring method for determination of lipids. J. Lipid Res., *7:* 574–576, 1966.
18. Rouser, G., Siakotos, A. N., and Fleischer, S. Two dimentional thin layer chromatographic separation of polar lipids and determinasion of phospholipids by phosphorus analysis of spots. Lipids, *5:* 494–496, 1970.
19. Khor, H. T., and Tan, H. L. The lipids of young cassava (Manihot esculenta, Crantz) leaves. J. Sci. Food Agric., *32:* 399–402, 1981.
20. Britton, G., and Goodwin, T. W. Biosynthesis of carotenoids. *In*: D. B. McCormick and L. D. Wright (eds.), Methods of Enzymology, Vol. 28, pp. 654–700. New York: Academic Press, 1971.
21. Sylvester, P. W., Russell, M., Ip, M. M., and Ip, C. Comparative effects of different animal and vegetable fats fed before and during carcinogen administration on mammary tumorigenesis, sexual maturation and endocrine functions in rats. Cancer Res., *46:* 757–762, 1986.
22. Ip, C. Ability of dietary fat to overcome the resistance of mature female rats to 7,12-dimethylbenz(*a*)anthracene induced mammary tumorigenesis. Cancer Res., *40:* 2785–2789, 1985.
23. Honstra, G., Hennissen, A. A. H. M., Tan, D. T. S., and Kalaufusz, R. Unexpected effect of dietary palm oil on atherothrombosis (rats) and atherosclerosis (rabbits). Comparison with other vegetable oils and fish oil. *In*: C. Galli and E. Fedelli (eds.), Fat Production and Consumption: Technology and Nutritional Implications, pp. 69–82. NATO-ASR Series A: Life Sciences. New York: Plenum Publishing Company, 1987.
24. Ip, C., Carter, C. A., and Ip, M. M. Requirement of essential fatty acid for mammary tumorigenesis in the rat. Cancer Res., *45:* 1997–2001, 1985.
25. Takano, S., and Kondoh, Y. Triglyceride analysis by combined argentation/nonaqueous reverse phase high performance liquid chromatography. J. Am. Oil Chemists Soc., *64:* 380–383, 1987.
26. Philips, F. C., Erdahl, W. L., Schmidt, J. A., and Privett, O. S. Quantitative analysis of triglyceride species of vegetable oils by high performance liquid chromatography via a flame ionization detector. Lipids, *19:* 880–886, 1984.
27. Kato, A., Yamacka, M., Tanaka, A., Komiyama, K., and Umezama, I. Physiological effect of tocotrienol. Yukagaku, *34:* 375–376, 1985.
28. Peto, R., Doll, R., Buckley, J. D., and Sporn, M. B. Can dietary β-carotene materially reduce human cancer rates? Nature (Lond.), *290:* 201–208, 1981.
29. Wolf, G. Is dietary β-carotene an anticancer agent? Nutr. Rev., *40:* 257–261, 1982.
30. Paganini-Hill, A., Chao, A., Ross, R. K., and Henderson, B. E. Vitamin A, β-carotene and the risk of cancer: a prospective study. J. Natl. Cancer Inst., *79:* 443–448, 1987.
31. Suda, D., Schwartz, J., and Shklar, G. Inhibition of experimental oral carcinogenesis by topical β-carotene. Carcinogenesis (Lond.), *7:* 711–715, 1986.
32. Aylsworth, C. F., Cullum, M. E., Zile, M. H., and Welsch, C. W. Influence of dietary retinyl acetate on normal rat mammary gland development and on the enhancement of 7,12-dimethylbenz(*a*)anthracene induced rat mammary tumorigenesis by high levels of dietary fat. J. Natl. Cancer Inst., *76:* 339–345, 1986.
33. Temple, N. J., and Basu, T. K. Protective effect of β-carotene against colon tumors in mice. J. Natl. Cancer Inst., *78:* 1211–1214, 1987.
34. Zile, M. H., Cullum, M. E., Roltsch, I. A., Dehoong, J. V., and Welsch, C. L. Effect of moderate vitamin A supplementation and lack of dietary vitamin A on the development of mammary tumors in rats treated with low carcinogenic dose levels of 7,12-dimethylbenz(*a*)anthracene. Cancer Res., *46:* 3495–3503, 1986.
35. Hillyard, L, Ananda Rao, G., and Abraham, S. Effect of dietary fat on fatty acid composition of mouse and rat mammary adenocarcinomas. Proc. Soc. Exp. Biol. Med., *163:* 376–383, 1980.
36. Cohen, L. A., Thompson, D. O., Choi, K., Karmali, R. A., and Rose, D. P. Dietary fat and mammary cancer. II. Modulation of serum and tumor lipid composition and tumor prostaglandins by different dietary fats: association with tumor incidence patterns. J. Natl. Cancer Inst., *77:* 43–51, 1986.