Influence of Dietary Fat on Growth of Mammary Cancer in Rats: Especially Delayed Turnover of Fatty Acid Composition in Tumor Tissue

Yoshikiyo BANDO

Department of Pathology, Kansai Medical University, Moriguchi, Osaka 570
(supervised by Prof. Sotokichi MORII)

Since Tannenbaum reported in 1942 the high incidence of spontaneous mammary cancer and the short period until its occurrence in mice fed on a high fat diet, many excellent studies have been carried out on the relationship between the quantity of dietary fat and incidence of the mammary tumors including spontaneously occurring and chemically induced ones in men and rodents. It has been proved by my coworkers that a high corn oil diet enhanced the growth of mammary cancer induced by 7, 12-Dimethylbenz(α)anthracene (DMBA) in female rats, while that a coconut oil diet inhibited it. The effect of quality, especially fatty acid composition, of dietary fat upon the promotion of mammary cancer was pointed out by us. Although there were many papers on the metabolism of fatty acids in Ehrlich ascites tumor cells, few literatures have been described so far on the fatty acids or their turnover rate in mammary tissues.

Mammary cancer with DMBA in young adult female rats should be employed as the most suitable model for human breast cancer, and acino-ductular architecture of the mammary parenchym is surrounded by abundant adipose tissues in female rats. In order to investigate the influence of dietary fat upon the promotion stage of mammary cancer, gas chromatographic analyses of fatty acid compositions in both mammary fatty pads and chemically induced mammary tumors were performed in rats fed on 4 different semisynthetic fat diets, respectively, 0-13 weeks after the exchange from the special feeding to the control diet. And also, in present paper, autoradiographic observations on the tritiated thymidine uptake were done on mammary parenchym of the rats supplied with the same fat diets during 2 months.

MATERIALS AND METHODS

1) Experimental animals and their environment

Sprague-Dawley JCL female rats aged 6 weeks were purchased from Japan Clea
Laboratory in Osaka. Throughout the experiment they were kept in metal cages setting at an air-conditioning room (22±2°C, 60±5% of humidity) illuminated artificially 14 hours a day, took a commercial diet, CMF type of pellet of Oriental Yeast Company, except the special feeding period below-mentioned, and a tap water *ad libitum*.

2) **Special fat diets and way of administration**

The 4 different semisynthetic fat diets were made by Oriental Yeast Company in Tokyo. Their compositions were indicated in Table I., and they were isocaloric (3.95 Cal/g) from each other. The high fat diets contained some purified oils in rate of 20 weight percentage, and both the other fat diets and CMF pellet did some fats in rate of 8%. Every morning 15-20g of a special fat diet powder was administered to a rat after making a paste with a little tap water.

<table>
<thead>
<tr>
<th>Composition</th>
<th>20% fat diet</th>
<th>8% fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Dextrose</td>
<td>35</td>
<td>62</td>
</tr>
<tr>
<td>Corn or coconut oil</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>Vitamine mixture</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The diets are isocaloric (3.95 Cal/g) from each other.

3) **Induction method of mammary cancer**

According to Morii and Kitajima's method17), 0.6ml of the emulsion containing 2.5mg of DMBA; Eastman Chemicals, was prepared. The doses were injected into caudal veins of the rats weighing about 200g twice on 55 and 58 days of age. Daily palpation for the mammary regions of the experimental rats started 3 weeks after the first intravenous injection. The time of appearance of the first palpable mammary tumor was dated from the first injection, and the location and the size of tumors detected were recorded every day. Mammary carcinomas, some of which were certified by the histologic examinations, were suspected by the hardness of tumor, and some bean-sized cancers without necrosis were applied to the chemical determination.

4) **Experimental design**

The control animals were fed on CMF pellet throughout the experiment, and a half of
them was administered with DMBA. The rats of 4 experimental groups were fed on 4 different fat diets, respectively, in the period between 30 and 90 days after the first intravenous injection, and then they were given again CMF pellet until their autopsies were done. The autopsies in the experimental groups were performed for 6 groups, respectively, on 0, 2, 4, 7, 10, and 13 weeks after the exchange to CMF pellet, and those in the control rats were done on the same days of age as fat diet groups (20, 22, 24, 27, 30 and 33 weeks of age). Two–three rats were sacrificed for each different group, and total 84 animals were used.

5) Pathological observation

All animals were weighed daily from 50 days of age to the day of autopsy. On autopsy, the rats were killed by decapitation and all mammary tumors were removed, counted and weighed in a balance. The pituitary, adrenals, ovaries, uterus, liver and kidneys were weighed on a torsion balance. Some of the mammary tumors, main organs and abdominal mammary fatty pads were fixed in 10% neutral formalin and examined by histologic methods (Hematoxylin–Eosin stain, Sudan III stain, etc.).

6) Analysis of fatty acid composition

The mammary carcinoma analyzed was in gross solid hard, pinky-colored and free from both necrosis and hemorrhage. The tested mammary fatty pad was taken usually from the abdominal region and it colored pinky white. Both 1g of wet weight of the mammary carcinoma and 100mg of wet weight of the mammary fatty pad were harvested for a rat, and they were stocked in -20°C until the initial step of chemical analysis; homogenization at 4°C. The fatty acids were extracted basically by Folch et al.’s procedure. They were analyzed as their methylesters by gas–liquid chromatography using Yanagimoto GCG 5 DH instrument equipped with a hydrogen flame ionization detector and 2 columns packed with 15% Diethyleneglycol succinate on Chromosorb W at 180°C. One column was for the sample and another for reference. Nitrogen was used as a carrier gas. The methylesters of fatty acids were prepared by interesterification by heating some amounts of lipid with 20ml of the solvent; benzene:methanol:concentrated sulfuric acid=10:20:1 in volume ratio, under a nitrogen atmosphere in a sealed glass tube 1 hour in a boiling water bath. The fatty acids were identified by comparison of retention times with authentic methylester standards. The quantitative analysis was tried on the basis of proportionality of the peak areas by triangulation, in comparison of weight percentage of a certain known weight of 17 carbon–chain length fatty acid mixed with each sample in a chromatographic instrument. The semisynthetic diets used were also analyzed by the same procedure.
7) Radioautography for tritiated thymidine uptake

Immediately after the special feedings of 2 months, 1μCi of tritiated thymidine per g of body weight was injected into a caudal vein of the rats 2 hours before their decapitations. Two animals were observed for each experimental group. Both abdominal mammary fatty pads and induced mammary cancers were taken, and fixed in 10% neutral formalin. The paraffine sections, 5μ in thickness, were applied to the dipping method of microradioautography (Emulsion; Sakura NR-M2, Exposure; 4 weeks, Development; Konidol X). Hematoxylin–Eosin stain was carried out after development. On the examinations of the radioautograms, the nuclei determined to be labeled had more than 5 grains. The actual numbers of the labeled nuclei per 3000 parenchymal cells were counted by 3 different workers according to the random field method reported by Fitzgerald et al14), and then the average for the each groups were compared from each other.

EXPERIMENTAL RESULTS

1) General condition, body weight curve and autopsy finding

Almost all rats observed developed well and kept good conditions in general throughout the experiment, except for the deaths from pneumonia in few ones. In all groups, a smooth elevation of the body weight was observed during the period from 7 to 22 weeks of age, although a little downward deviation of it was noticed in few days after the intravenous injection of DMBA. The curves of average body weights for 4 experimental groups were indicated in Fig. 1... In few days after the exchange from CMF pellet to the fat diets, more amounts of the fat diets were ingested, but almost equal volume (11-20g per rat a day) of them was taken up thereafter in every experimental group. After the exchange from the fat diets to CMF pellet, their body weight run around 300g. Average body weights for 4 experimental groups were hardly different from each other at the time of the first injection of DMBA (Age 55 days), of the diet exchange to the fat diets (Age 88 days), and of the first autopsy (Age 148 days), respectively, as shown in Table I... At autopsy, the main organs in each group did not weigh so much differently from each other, as shown in Table II... Neither any significant histopathological changes in the main organs nor hyperplastic features in the mammary fatty pads could be observed. No any prominent difference from each other experimental group could be recognized on their histologic examinations. Such a similarity above-mentioned on the 4 different experimental groups suggested that the 4 different semisynthetic fat diets ingested induced neither caloric nor hormonal influences
upon the hosts bearing the analyzed mammary carcinomas.

**Fig. I.** Body Weight Curves of Experimental Animals

**Table II.** Average Body Weights of Experimental Animals

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No of rats</th>
<th>Time of first DMBA -injection (Age 55 days)</th>
<th>Time of Diet exchange (Age 88 days)</th>
<th>Time of first autopsy (Age 148 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% Coconut oil diet</td>
<td>11</td>
<td>175.5 ± 12.78</td>
<td>254.4 ± 16.90</td>
<td>316.3 ± 40.67</td>
</tr>
<tr>
<td>8% Corn oil diet</td>
<td>12</td>
<td>177.6 ± 12.17</td>
<td>255.0 ± 18.58</td>
<td>294.8 ± 26.37</td>
</tr>
<tr>
<td>20% Coconut oil diet</td>
<td>12</td>
<td>166.3 ± 18.83</td>
<td>233.8 ± 25.62</td>
<td>285.0 ± 40.52</td>
</tr>
<tr>
<td>20% Corn oil diet</td>
<td>12</td>
<td>175.2 ± 13.81</td>
<td>244.8 ± 27.25</td>
<td>298.2 ± 32.60</td>
</tr>
</tbody>
</table>

All data are Means±S.D. (gram).

_Supplement to J. Kansai Med. Univ., Vol. 27, Dec. 1975_
2) Detection and morphologic examination for induced mammary tumor

The palpable mammary tumors could be detected in almost all carcinogen-treated rats 2-3 months after the administration. The same influences of the fat diets upon the promotion of mammary tumors, e.g. incubation time, active center and total weight per rat, as previously reported by my coworkers, could be also noticed. Most of the histologically examined mammary tumors revealed in common an appearance of the medullary carcinoma, most of which showed papillotubular pattern of adenocarcinoma. Some mammary cancers were anaplastic carcinoma. The tumor cells exhibited often a secretory activity. No any certain relationship between the quality of the fat diets ingested and the histopathological appearance of induced mammary cancers could be recognized.

### Table III. Average Weights of Main Organs of Experimental Animals

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Organ</th>
<th>Time of autopsy</th>
<th>Pituitary (MG)</th>
<th>Adrenals (MG)</th>
<th>Ovaries (MG)</th>
<th>Uterus (G)</th>
<th>Liver (G)</th>
<th>Kidneys (G)</th>
<th>Body (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% Coconut oil diet</td>
<td>①</td>
<td>12</td>
<td>82</td>
<td>122</td>
<td>0.6</td>
<td>8.8</td>
<td>2.2</td>
<td>281</td>
<td></td>
</tr>
<tr>
<td></td>
<td>②</td>
<td>14</td>
<td>76</td>
<td>128</td>
<td>0.5</td>
<td>8.1</td>
<td>2.3</td>
<td>298</td>
<td></td>
</tr>
<tr>
<td></td>
<td>③</td>
<td>14</td>
<td>108</td>
<td>170</td>
<td>0.7</td>
<td>11.3</td>
<td>2.0</td>
<td>305</td>
<td></td>
</tr>
<tr>
<td>8% Corn oil diet</td>
<td>①</td>
<td>11</td>
<td>106</td>
<td>162</td>
<td>0.6</td>
<td>8.1</td>
<td>2.3</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td></td>
<td>②</td>
<td>13</td>
<td>72</td>
<td>142</td>
<td>0.8</td>
<td>9.0</td>
<td>2.1</td>
<td>311</td>
<td></td>
</tr>
<tr>
<td></td>
<td>③</td>
<td>16</td>
<td>68</td>
<td>168</td>
<td>0.6</td>
<td>10.3</td>
<td>2.2</td>
<td>316</td>
<td></td>
</tr>
<tr>
<td>20% Coconut oil diet</td>
<td>①</td>
<td>16</td>
<td>119</td>
<td>119</td>
<td>0.7</td>
<td>7.3</td>
<td>2.0</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td></td>
<td>②</td>
<td>16</td>
<td>79</td>
<td>135</td>
<td>0.6</td>
<td>10.9</td>
<td>2.2</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td></td>
<td>③</td>
<td>15</td>
<td>87</td>
<td>113</td>
<td>0.7</td>
<td>9.9</td>
<td>2.0</td>
<td>318</td>
<td></td>
</tr>
<tr>
<td>20% Corn oil diet</td>
<td>①</td>
<td>10</td>
<td>78</td>
<td>115</td>
<td>0.4</td>
<td>8.1</td>
<td>1.9</td>
<td>273</td>
<td></td>
</tr>
<tr>
<td></td>
<td>②</td>
<td>16</td>
<td>77</td>
<td>124</td>
<td>0.7</td>
<td>8.8</td>
<td>2.0</td>
<td>308</td>
<td></td>
</tr>
<tr>
<td></td>
<td>③</td>
<td>19</td>
<td>104</td>
<td>141</td>
<td>0.7</td>
<td>12.1</td>
<td>2.4</td>
<td>318</td>
<td></td>
</tr>
</tbody>
</table>

① first autopsy day (Age 20 weeks)  
② Age 27 weeks  
③ last autopsy day (Age 33 weeks)

All results are Means in 2 or 3 rats in each experimental groups.

2) Detection and morphologic examination for induced mammary tumor

The palpable mammary tumors could be detected in almost all carcinogen-treated rats 2-3 months after the administration. The same influences of the fat diets upon the promotion of mammary tumors, e.g. incubation time, active center and total weight per rat, as previously reported by my coworkers, could be also noticed. Most of the histologically examined mammary tumors revealed in common an appearance of the medullary carcinoma, most of which showed papillotubular pattern of adenocarcinoma. Some mammary cancers were anaplastic carcinoma. The tumor cells exhibited often a secretory activity. No any certain relationship between the quality of the fat diets ingested and the histopathological appearance of induced mammary cancers could be recognized.
3) **Fatty acid composition**

a) Fat diets used

The semisynthetic fat diets included variable amounts of some fatty acids, which were analyzed to be composed of 12-18 carbon-chain length fatty acids, as shown in Table IV. The diets mixed by coconut oil had a large amounts of both lauric and myristic acids, 51 and 21% in the rate of weight percentage, respectively, while those with corn oil included a great quantity of linoleic, oleic and palmitic acids, 50, 26 and 13% in the rate of weight percentage, respectively.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>8% Coconut oil diet</th>
<th>8% Corn oil diet</th>
<th>20% Coconut oil diet</th>
<th>20% Corn oil diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capric</td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Lauric</td>
<td>51</td>
<td>3</td>
<td>52</td>
<td>3</td>
</tr>
<tr>
<td>Myristic</td>
<td>21</td>
<td>2</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Palmitic</td>
<td>9</td>
<td>13</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Stearic</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Oleic</td>
<td>5</td>
<td>27</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Linoleic</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>51</td>
</tr>
<tr>
<td>Linolenic</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total of others</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

b) Mammary fatty pads

As shown in Fig. I, sample I of the mammary fatty pads, taken immediately after the special feeding period, indicated that the pattern of fatty acid composition reflected prominently to that of dietary fat ingested. Namely, the coconut oil diet group had more amounts of lauric acid, while the corn oil diet group hold more linoleic acid. In fact, the weight percentages of fatty acids were 13.9 in lauric, 8.4 in myristic, 24.4 in palmitic, 31.0 in oleic, and 8.1 in linoleic acids on 8% coconut oil diet group, but 1.0 in lauric, 2.0 in myristic, 19.1 in palmitic, 30.4 in oleic, and 36.8 in linoleic acids on 20% corn oil diet group. And also, sample II, taken 13 weeks after the exchange to CMF pellet, revealed roughly similar pattern in every experimental group. It was distinct that sample II of 20% coconut oil diet group had more linoleic acid and less lauric acid than those of sample I in the same group. On the weight percentages of 2 kinds of fatty acid in 20% coconut oil diet group, a downward deviation of lauric acid crossed
an elevation of linoleic acid around 7 weeks after the exchange to CMF pellet, as shown in Fig. IV. The alteration of lauric acid in the stage between 0-13 weeks after the exchange to CMF pellet was compared with that in each other group, as shown in Fig. IV.

c) Mammary tumor tissue

The weight percentages of fatty acids in the mammary tumor tissues were in general about 20 in palmitic, around 25-30 in oleic, and few in arachidonic acids, respectively. It was interesting that the weight percentages of arachidonic acids found just in the mammary tumor tissues run in parallel to the indices of tumor growths. It could be also detected that pattern of fatty acid composition in tumor tissue reflected moderately to that of the dietary fat administered. In fact, immediately after the special feeding period the weight percentages of fatty acids in the mammary tumor tissues were 26.6 in lauric, 11.7 in myristic, and 8.5 in linoleic acids on 20% coconut oil diet group, while they were 1.3 in lauric, 1.7 in myristic, and 28.7 in linoleic acids on 20% corn oil diet group. The reflection of dietary fat in the special feedings seemed to vanish.
slowly after the exchange to CMF pellet, as shown in Figs. I., II., and IV.. A little deviation of linoleic acid could be seen, but the percentage of lauric acid hardly changed. On the weight percentage of fatty acid, the fatty acid composition of mammary tumor tissues would retain unchanged even at 13 weeks after the dietary exchanges. Absolute values of total weight of methylated fatty acids, especially lauric, linoleic and arachidonic acids, were calculated (Figs. V. and VI.). As shown in Fig. V., absolute volumes of lauric acid in each group changed less after the dietary exchange, although those of linoleic acid increased distinctly at the same stage.

4) Uptake of tritiated thymidine As shown in Table V., the incorporation rate of tritiated thymidine into the mammary parenchym in 20% corn oil diet group was much higher than those in the other groups, especially in neoplastic cells. In general, the greater was the concentration of fat in the diet, the higher was the incorporation rate.

of tritiated thymidine into the nuclei of mammary parenchymal cells. As a matter of fact, the tumor cells incorporated more thymidine on every group. It was interesting that the uptake of tritiated thymidine into the nuclei of mammary parenchymas was higher in the corn oil diet groups than that in the coconut oil diet groups, and that this higher uptake would be independent of estrous cycle.

DISCUSSION

As for the effects of dietary fat upon the lipid metabolism in mammary tumor tissues, many factors of both exogenous and endogenous fats should be concerned. In present paper, the following factors would be taken into account: (a) lipid class and fatty acid composition of both the dietary fat and the lipid in the mammary tumors and their surrounding mammary fatty pads, (b) the enzyme system related to lipid metabolism, the activities of which were proved in the intestine, pancreas, liver, adipose tissue, and mammary tissue including tumors, (c) hormonal control of lipid metabolism and versus the influence upon hormones with lipid material as their carrier.

---

**Fig. IV.** Alterations of Weight Percentages of Lauric Acids after Exchange to CMF-Diet
The mammary fatty pads in rats are composed of 3 tissue components; epithelial, adipose and connective. The proportions of 3 different tissues vary greatly under the hormonal state of host. Connective and adipose components predominate in the breast of young virgin rats, while epithelial one does in lactating rats. The alteration under the endocrinological condition of host in proportions of carcinoma cells to stromal tissue would be smaller than that of epithelial component in mammary fatty pads, even though most of the DMBA-induced mammary tumors might be hormone-dependent. According to present observations, the proportion of tissue components in both the mammary fatty pads.

Fig. V. Changes of Methylated Fatty Acids per Weight of Mammary Tumor after Exchange to CMF-Pellet in Female Rats Fed on Coconut Oil Diets

(d) direct and indirect effects of the specially prepared fat diets.

pads and the induced mammary cancers did not differ prominently from each other group.

It was mentioned by Hoshino\textsuperscript{20,21)} that at the histological level an adipose tissue was necessary as an essential environment for growth of the mammary gland. The dependency of mammary parenchym upon adipose tissues might be related to the lipid-solubility of steroid hormones, which play many important roles in the development of mammary gland. Therefore, the hormone-dependent tumors of mammary gland would be dependent on the lipids in tumor and its surrounding adipose tissues. Hormonal balance in the hypophyseal-ovarian system could influence profoundly on differentiation and growth of

\textbf{Fig. VI. Changes of Methylated Fatty Acids per Weight of Mammary Tumor after Exchange to CMF-Pellet in Female Rats Fed on Corn Oil Diet}
the mammary gland in all mammals, and it has also prominent effects on the mammary fatty tissue. Other hormones as glucocorticoids, thyroxine and insulin might play an important role in fat metabolism generally. So, lipids in the induced mammary cancer could be influenced with the hormonal milieu of host. On the other hand, a low fat and restricted calorie diet caused the severe atrophies of the mammary gland, ovary, uterus, etc., which looked like those after hypophysectomy, and low incidence of spontaneously occurring mammary tumor followed to the changes in mice.

Pathologic findings in present experiment, as the same as previously reported by my coworkers\(^7\)\(^8\), indicated that 4 different isocaloric fat diets ingested could induce neither any different caloric effect (suspected by the weights of body and main organs) nor any different hormonal milieu (suspected by the findings on endocrine organs) upon the hosts from each other group, that they did not evoke any different state of the enzyme system in the main organs (speculated by the histologic observations) from each other, but that the low coconut oil diet inhibited the mammary tumorigenesis by DMBA while the high corn oil diet enhanced the growth of the induced mammary cancers. Therefore, these interesting effects of the fat diets employed might be developed through either quality or quantity of fats in the diets.

The adipose tissue might be a main site in lipogenesis\(^22\). It would be important in supply of the deposited fat to other tissues in the form of fatty acids as well as in stock of exogenous dietary fat in the esterified states. The quality of lipids in the

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No of rats</th>
<th>Sample</th>
<th>Average numbers counted by 3 different workers</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% Coconut oil diet</td>
<td>2</td>
<td>M P 5</td>
<td>3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M C 17</td>
<td>21</td>
<td>17.3</td>
</tr>
<tr>
<td>8% Corn oil diet</td>
<td>2</td>
<td>M P 19</td>
<td>31</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M C 43</td>
<td>41</td>
<td>41.7</td>
</tr>
<tr>
<td>20% Coconut oil diet</td>
<td>2</td>
<td>M P 27</td>
<td>24</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M C 330</td>
<td>369</td>
<td>334.0</td>
</tr>
<tr>
<td>20% Corn oil diet</td>
<td>2</td>
<td>M P 49</td>
<td>70</td>
<td>59.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M C 401</td>
<td>327</td>
<td>388.6</td>
</tr>
</tbody>
</table>


Two diestrous rats for each groups were injected intravenously with tritiated thymidine (1\(\mu\)Ci/g of body weight) 2 hours before their decapitations.
adipose tissue was generally thought to reflect that of dietary fats. The lipid in the mammary fatty pads should be influenced by the dietary fats, because it is one of the adipose tissues. In fact, Gammal et al.\textsuperscript{6}) reported that the fatty acid composition in the mammary fatty tissue was reflecting the fat intake of host. My coworkers\textsuperscript{7) 8}) and I reconfirmed the dependency of fatty acid composition in the mammary fatty pads upon the dietary fats. Present experiment indicated also that fatty acid composition of the abdominal mammary fatty pads begun to change 2 weeks after the diet exchange and transferred completely to the new one 7 weeks after, on the rats fed on the diets which were exchanged from 20% coconut oil diet to CMF pellet. This timing corresponded with the rat carcass fat examined with half life of isotope\textsuperscript{23}). According to Bortz and Lynen\textsuperscript{24}), the activity of a purified preparation of acetyl Co A carboxylase obtained from rat liver had been shown to be strongly inhibited by the in vitro addition of long-chain acyl Co A derivatives. This inhibition appeared to be due to the competition with acetyl Co A for the active enzyme site. It was also mentioned by Kinsella et al.\textsuperscript{25}) that the fatty acid synthetase in bovine mammary gland was progressively inhibited by increasing concentration of long-chain acyl Co A, palmitoyl Co A and myristoyl Co A. Lipogenesis increased to the highest levels in the rats on a high carbohydrate, fat-free diet, while fasting or feeding of a high fat diet abolished lipogenesis in adipose tissue markedly\textsuperscript{22}). The content of protein in diet did not exert a major influence on lipogenesis\textsuperscript{26}). Therefore, it might be suggested that fatty acid synthesis de novo remains stopped in the high fat diet groups, as there are enough long-chain fatty acids in the tissue. These papers would probably support the inhibition of fatty acid synthesis de novo in great degree from glucose and protein in present experiment.

Neoplasms have generally low ability to synthesize cholesterol and fatty acids from acetate\textsuperscript{22}). It was proved by Spector et al.\textsuperscript{14}) that exogenously supplied free fatty acid was a major source of fat for Ehrlich ascites tumor cells. They might show, therefore, the greater dependence of tumors upon the exogenous fatty supply than normal tissues. Strong reflection of the dietary fat in the mammary tumor tissues of present experiment might be understood on the high fat diet groups. Pattern of fatty acid composition in each corresponding lipid was relatively similar between the mammary fatty pads and the mammary tumors induced, except for more linoleic and arachidonic acids in tumor tissues. My data upon the rats fed on 8% corn oil diet indicated the following weight percentages: palmitic 20.8, palmitoleic 10.7, stearic 2.3, oleic 33.0, linoleic 30.0, linolenic 1.0, and arachidonic none in the mammary fatty pads, on the other hand, 21.4, 8.3, 4.7, 26.9, 20.8, 8.1 and 3.2 in the mammary tumor tissues. The tumor tissues
included polyunsaturated fatty acids more. Moreover, arachidonic acid could be detected just in the tumor tissues. It would be synthesized de novo in animals, especially active mammary parenchym. It was interesting that the weight percentage of arachidonic acid run in parallel to the indices of tumor growth of each experimental group. Tominaga described the presence of arachidonic acid in the hyperplastic lactating mammary tissue, as well as the induced mammary tumor tissues. The synthesis of the fatty acid from linoleic acid might be speculated.

Both the proportion of lipid class and their fatty acid compositions in the induced mammary cancers with DMBA were compared by Tan et al. with those in the mammary fatty tissues of Sprague-Dawley female rats fed on an ordinary diet; neutral lipid 98.6±1.0, glycolipid 0.4±0.2, phospholipid 1.1±0.8 in the mammary fatty tissue, and neutral lipid 64.0±15.7, glycolipid 2.5±1.5, phospholipid 33.5±15.1 in the mammary cancer. These values were, of course, weight percentages, and they indicated that phospholipid in tumor was prominently more abundant than in mammary fatty tissue, although the fatty acid composition of both tissues was resemble. According to Veerkamp et al., neutral lipid fraction seemed to possess animal specificity, for example, their fatty acid patterns differed from animal to animal, but resembled one another different tissues of one animal. On the other hand, phospholipids showed some tissue specificity, since their fatty acid patterns differed within one animal but showed similarity in homologous tissue (including tumor) of different animals. Fatty acid compositions of phosphatide fraction and of lipid from mitochondrial membrane preparation of the same tissue showed a greater similarity. Therefore, the fatty acid composition of mammary tumor cells might relate more to phospholipid of biomembrane system of the cells.

According to present analyses, the corn oil diet used had long-chain fatty acids mainly, while the coconut oil diet ingested included medium-chain fatty acids in relatively large amount. Long-chain fatty acid are different from short- or medium-chain fatty acids in absorption by the intestine and in transportation from the intestine to each tissue, perhaps to mammary tumor tissue. Long-chain fatty acids absorb mainly as triglyceride in chylomicron via the thoracic duct, but short-chain fatty acids as fatty acid-albumine complex by way of the portal vein. In addition, long-chain fatty acids require bile acid for micel formation in absorption. As for the concentration of fatty acids in the serum, there is a difference in the state of existence between long-chain fatty acid and short-chain one. The former exists as tryglyceride in 85% and the latter as fatty acid-albumine complex in most part. In general, adipose tissues take up fatty acids from triglyceride in chylomicron in the serum by work of lipase as well as those
from fatty acid–albumine complex in the serum. In the experiment using Ehrlich ascites tumor cells, tumor cells failed to utilize the lipid esters contained in Ehrlich ascites tumor fluid. There was also a fact that the radioactive palmitate contained in rat chylomicron was utilized very slowly to unesterified palmitate, but that fatty acids were rapidly taken up by tumor cells in an unesterified form. In a series of the experiments using Ehrlich tumor cells, the uptake of fatty acids by tumor cells enhanced as the molar ratio of fatty acid to albumine increased. The increased molar ratio of fatty acid–albumine complex might result in the high fat diet groups. Frederick and Begg showed that the force-fed rats bearing tumor exhibited a gross lipemia with neutral fat as a major lipid component in the blood. In tumor-bearing rats fed on a high fat diet might develop a hyperlipemic state. In brief, almost parallel relationship between dietary fat and lipid in both the pads and the tumors might be supported in absorption, concentration in the blood, and uptake of fatty acids by the cells.

Fatty acids mobilize usually from the adipose tissues including the mammary fatty pads. Under physiological conditions net release of fatty acids would not occur in Ehrlich tumor cells. As substrate in Ehrlich tumor cells, linoleic acid was oxidized at a somewhat greater rate than the other long-chain fatty acids, whereas lauric acid was metabolized at considerably lower rate than free longer-chain fatty acids. The mammary cancer cells would be similar to Ehrlich tumor cells. The difference in metabolism of fatty acids between mammary tumors and mammary fatty tissues might be also related to the ratio of phospholipid per neutral fat.

It was interested that the turnover rate of fatty acids in the induced mammary tumors with DMBA delayed in comparison of that in the mammary fatty pads of the same host. The higher weight percentages of lauric and arachidonic acids in the neoplastic tissues of rats on 20% coconut oil diet still remained 10-13 weeks after the exchange to CMF pellet, although the fatty acid composition in the mammary fatty pads shift completely to that of CMF pellet 5-7 weeks after the diet exchange. Absolute values of the methylated lauric and arachidonic acid per wet weight of the tumor tissue kept in high levels 13 weeks after the exchange to CMF pellet in 20% coconut oil diet group. The similar delay or retardation should be observed on the high corn oil diet group, but it could not be detected prominently because of the similarity of fatty acid pattern between corn oil diet and CMF pellet. It is uncertain whether the delay or retardation might be induced by a neoplastic nature, e.g. dysdifferentiation, or not.

Present radioautographic analyses indicated that the high corn oil diet might evoke to accelerate DNA synthesis in the parenchymal cells of both mammary gland and mammary
cancer. Some dietary fat factor induced the alteration in the mammary parenchym, which was followed by an increase in thymidine uptake. Recently, Banerjee et al.\textsuperscript{34}) stated that by using tritiated thymidine an increase in its incorporation into the nuclei or nucleoli of the mammary gland cells during early lactation was followed by not only replication for acinar cell proliferation but also partial replication for functional purpose in the gland. In the experiment using rat liver, Haeffner and Privett\textsuperscript{35}) stated that the enzymic activities for oxidation and reduction in mitochondria were more enhanced by supplement of corn oil diet than coconut oil diet, probably through an increased unsaturation of fatty acid supplement. The activity of ATPase of mitochondria in rat liver was also influenced by both the degree of unsaturation and the content of essential fatty acid in the fatty acid supplement. The enzymic activity of mitochondria would appear to alter a biomembrane permeability. As for the relationship between membrane permeability and fatty acid composition of phospholipid\textsuperscript{36}), it was reported that the ratio of palmitic acid to oleic acid in erythrocytes might play a role in hemolysis time related in some respect to the membrane permeability. In short, in proportion to some other saturated medium-chain fatty acids, polyunsaturated fatty acids and essential fatty acids might be concerned with membrane permeability.

In conclusion, influences of the high fat diets upon the growth of DMBA-induced mammary cancer would not be developed through any hormonal or caloric factors, but they might be caused by both more reflection of dietary fats and slower turnover of fatty acids in the neoplastic tissues than in the mammary fatty tissues.

**SUMMARY**

Young adult Sprague-Dawley female rats, given CMF pellet and tap water \textit{ad libitum}, were injected twice with DMBA around 8 weeks of age. The rats of 4 experimental groups were administered daily a definite amount of 4 different semisynthetic isocaloric diets (8 and 20\% coconut oil diets and 8 and 20\% corn oil diets), respectively, in the stage between the 12th and 20th week of age, and then they were given again CMF pellet. The control rats, either with DMBA or without the carcinogen, were fed on CMF pellet throughout the experiment. Both the induced mammary cancers and the mammary fatty pads were harvested either 0, 2, 4, 7, 10, and 13 weeks after the exchange to CMF pellet in the experimental groups or on the same ages as experimental groups in the controls, and they were investigated by histopathologic examinations, gas-chromatographic observations for fatty acid composition, and radioautographic determinations for thymidine uptake. Fatty acid compositions in both tissues of the experimental
rats reflected those of the diets ingested, respectively, and the reflection was greater in the higher fat diet groups. On the mammary cancer tissues, polyunsaturated fatty acids, probably related to phospholipids, could be detected more. After the exchange to CMF pellet, the reflection of the former diet, e.g. lauric acid in coconut oil diet group, on the neoplastic tissues remained in more prolonged duration than on the mammary fatty pads. Thymidine uptake into both the mammary fatty pads and mammary cancers increased on the corn oil diet group, especially higher fat diet group. DNA synthesis within the tumor tissues might be accelerated by altered membrane permeability owing to increased polyunsaturated fatty acids. Influence of the high fat diet on promotion of mammary cancer might be concerned mainly with more reflection of dietary fats and slower turnover of fatty acids in the tumor tissues.

Acknowledgements

The author is greatly indebted to prof. S. Morii, in the Department of Pathology for his kind directions and to prof. K. Saito, in the Department of Biochemistry for his courteous advices during this work. This investigation was supported in autoradiographic section by Dr. Y. Kawakita, my coworker. The author is also grateful to all investigators associated with this work for their elaborate cooperations.

References


