INTRODUCTION

Tumor cells have a very distinctive metabolism. Metastasis and unlimited growth are distinguishing characteristics of tumor cells not seen in normal cells, and these characteristics result in widespread fatal malignancies in humans. Several previous studies have considered the metabolic changes that take place in the host after tumor implantation. Metabolic abnormalities arising from malnutrition and malignancy are seen in animals and in patients with cancer cachexia, and various cancers affect lipid metabolism.1-7) It was found that rats subcutaneously implanted with the ascites hepatoma cell line AH109A show hyperlipidemia with a notable decrease in the high-density lipoprotein (HDL) fraction and an enormous increase in the very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) fractions5-7) during the growth of the solid tumor.5) AH109A is a hepatoma that originates from the liver. The liver carries out major lipid metabolism, including cholesterol, fatty acid, and triglyceride biosyntheses, as well as VLDL production. It is thus suspected that hepatoma cells affect lipid metabolism in the host body and that the cancer state affects lipid metabolism.

RCN-9, a transplantable rat colon adenocarcinoma, was induced in the colon of a male Fisher F344 rat by the subcutaneous administration of 1, 2-dimethylhydrazine. RCN-9 metastasizes spontaneously, and when RCN-9 cells were injected subcutaneously or into the cecal subserosa of syngeneic rats, carcinomas with progressive growth were obtained and the development of lung and liver metastases, respectively, ensued.8) In the present study, to investigate the effects of RCN-9 implantation on lipid metabolism, I examined the changes in lipid levels in the serum and liver of RCN-9-implanted rats. RCN-9 is a colon cancer that originates from the colon; the effects of the implanted RCN-9 tumor cells on lipid metabolism may be small compared to those of a hepatoma,5-7) which originates from the liver and is active in various types of lipid metabolism. In addition, since decreases in food intake and body weight are seen in the tumor-bearing state as a result of a cancer-induced cachexia as mentioned above, I conducted a pair-feeding study to investigate the effects of food restriction on tumor bearing in RCN-9-implanted rats.
Masashi KAWASAKI
Effects of Tumor Bearing on Serum and Liver Lipid Levels in RCN-9-Implanted Rats

(Oriental Yeast Co.), 1 % vitamin mixture (AIN 93 composition)\textsuperscript{9} (Oriental Yeast Co.), 0.25 % choline bitartrate (Wako Pure Chemical Industries, Osaka, Japan), and 0.3 % L-cystine (Wako Pure Chemical Industries). After preliminary feeding, the rats were divided into three groups (n=7 each) of similar body weights. The first group received a subcutaneous implantation of 5×10^6 RCN-9 cells (provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan) suspended in phosphate-buffered saline (PBS(−)) (0.5 ml/rat) in the back to produce a solid tumor (tumor-bearing group). The second group received a sham injection of PBS(−) alone (0.5 ml/rat) and were designated the normal rats (control group). The rats of the third group were given the same amount of diet that the tumor-bearing rats ate the day before, and this group was designated the pair-fed group. The RCN-9 cells were prepared by means of a cell culture system. RCN-9 cells were cultured in RPMI-1640 medium supplemented with 10 % fetal calf serum. The rats of each group were maintained for a further 21 days on the basal diet. The diameter of the solid tumor on the backs of the tumor-bearing rats was measured to estimate the tumor volume every day. Water and diet were offered at 7:00 p.m. every day and remained available at all times. On the 21st day, all of the rats were deprived of their diet at 9:00 a.m. but allowed free access to water until sacrifice, which was performed 4 hours later. Blood was collected and left to clot at room temperature to obtain serum. The liver, epididymal adipose tissue, and solid tumor were quickly removed, washed with cold 0.9 % NaCl, blotted on filter paper, and weighed. The serum and liver were stored at −30 °C until the lipid concentration analyses were performed. Aliquots of the liver were also preserved in methanol and stored at 4 °C until the analyses of the lipid contents were performed.

**Lipid analyses.** The lipoprotein separation of serum was as follows, HDL was separated from VLDL and LDL by precipitation method using sodium phosphotungstic acid and MgCl\textsubscript{10} and VLDL was separated from LDL and HDL by ultracentrifugation.\textsuperscript{11) Serum total, HDL-, and (LDL+HDL)-cholesterol, free cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations were determined by an enzymatic method using sodium phosphotungstic acid and MgCl\textsubscript{10} and VLDL was separated from LDL and HDL by ultracentrifugation.\textsuperscript{11)}

Serum total, HDL-, and (LDL+HDL)-cholesterol, free cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations were determined by an enzymatic method using a Cholesterol E-test, Free cholesterol E-Test, Triglyceride E-test, Phospholipid C-test, and NEFA C-test (Wako Pure Chemical Industries), respectively. The difference between the total cholesterol concentration and HDL-cholesterol concentration was regarded as the (VLDL+LDL)-cholesterol concentration. The difference between the (LDL+HDL)-cholesterol concentration and HDL-cholesterol concentration was regarded as the LDL-cholesterol concentration. The difference between the (VLDL+LDL)-cholesterol concentration and LDL-cholesterol concentration was regarded as the VLDL-cholesterol concentration. The ratio of the (VLDL+LDL)-cholesterol concentration and LDL-cholesterol concentration was regarded as the atherogenic index. The difference between the total cholesterol concentration and free cholesterol concentration was regarded as the esterified cholesterol concentration, and the ratio of the esterified cholesterol concentration to total cholesterol concentration is designated as the cholesterol ratio.

**Total lipids** were extracted according to the procedure of Folch et al.\textsuperscript{12} from the liver. After portions of the chloroform phase had been dried under nitrogen, the contents of cholesterol,\textsuperscript{13} triglyceride,\textsuperscript{14} and phospholipid\textsuperscript{15} were determined.

The serum and liver thiobarbituric acid-reactive substances (TBARS) values were measured according to the method described by Yagi\textsuperscript{16} and Mihara et al.,\textsuperscript{17} respectively.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>Tumor-bearing</th>
<th>Pair-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>81.7 ± 1.1</td>
<td>81.7 ± 1.2</td>
<td>86.0 ± 1.3</td>
</tr>
<tr>
<td>Food intake (g/21d)</td>
<td>242.9 ± 4.3</td>
<td>236.5 ± 6.1</td>
<td>229.9 ± 2.1</td>
</tr>
<tr>
<td>Body weight gain (g/21d)</td>
<td>177.8 ± 3.5</td>
<td>174.4 ± 3.6</td>
<td>171.2 ± 1.6</td>
</tr>
<tr>
<td>Liver weight (g/whole body)</td>
<td>7.48 ± 0.26</td>
<td>6.99 ± 0.25</td>
<td>7.10 ± 0.09</td>
</tr>
<tr>
<td>Epididymal adipose tissue weight (g/whole body)</td>
<td>2.86 ± 0.13</td>
<td>2.66 ± 0.12</td>
<td>2.82 ± 0.18</td>
</tr>
</tbody>
</table>

Values represent the means ± standard errors for seven rats.

Fig. 1. Estimated solid tumor volume in tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors.
Statistical analysis. Results were expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance followed by Bonferroni test using the SPSS Statistics, version 21 (IBM Japan, Ltd., Tokyo, Japan). Differences were considered significant at $p < 0.05$.

RESULTS

Table 1 shows the initial body weight, food intake, and body weight gain for the 21-day duration of the experimental period, and the weights of the liver and epididymal adipose tissue at the end of the experimental period. There were no significant differences between the control and tumor-bearing groups with regard to the food intake, body weight gain, and liver and epididymal adipose tissue weights. In the pair-feeding study, the food intake, body weight gain, and weights of the liver and epididymal adipose tissue in the pair-fed group were not significantly different from those of the control group. The food intake, body weight gain, and weight of the liver and epididymal adipose tissue in the pair-fed group were not significantly different from those of the control group.

Fig. 2. Serum cholesterol concentration, atherogenic index, and cholesterol ratio in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Bonferroni test. C, normal rats fed ad libitum group (control); T, tumor-bearing rats fed ad libitum group; P, normal rats pair-fed with tumor-bearing rats.

Fig. 3. Serum triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. C, normal rats fed ad libitum group (control); T, tumor-bearing rats fed ad libitum group; P, normal rats pair-fed with tumor-bearing rats.
Masashi KAWASAKI
Effects of Tumor Bearing on Serum and Liver Lipid Levels in RCN-9-Implanted Rats

The absolute and relative weights of the solid tumors at the end of the experimental period were 0.33 ± 0.11 g and 0.19 ± 0.06 % of body weight, respectively.

Figure 1 shows the estimated solid tumor volume in the tumor-bearing rats after RCN-9 implantation. Solid tumors were observed 6−7 days after RCN-9 implantation, and they continued to grow with time.

The serum cholesterol concentrations are shown in Figure 2. The total cholesterol concentration in the tumor-bearing group was not significantly different from that of the control group. Regarding the lipoprotein cholesterol, the VLDL-, LDL-, and HDL-cholesterol concentrations were not significantly different between the control and tumor-bearing groups. The atherogenic index in the tumor-bearing group was not significantly different from that of the control group. The serum free and esterified cholesterol concentrations were not significantly different between the control and tumor-bearing groups. The cholesterol ratio in the tumor-bearing group was significantly higher than that in the control group. In the pair-feeding study, the total cholesterol concentration in the pair-fed group was not significantly different from that of the control group. The VLDL-, LDL-, and HDL-cholesterol concentrations in lipoprotein cholesterol and the free and esterified cholesterol concentrations in the pair-fed group were not significantly different between the control and pair-fed groups. These cholesterol concentrations in the pair-fed group were not significantly different compared to those of the tumor-bearing group. The atherogenic index and cholesterol ratio in the pair-fed group were not significantly different from those of the control group.

The serum triglyceride, phospholipid, and NEFA concentrations are shown in Figure 3. There were no significant differences between the control and tumor-bearing groups with regard to the serum triglyceride,

Fig. 4. Liver lipid contents in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. C, normal rats fed ad libitum group (control); T, tumor-bearing rats fed ad libitum group; P, normal rats pair-fed with tumor-bearing rats.

Fig. 5. Serum and liver thiobarbituric acid-reactive substances (TBARS) values in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at p < 0.05 by one-way analysis of variance followed by Bonferroni test. C, normal rats fed ad libitum group (control); T, tumor-bearing rats fed ad libitum group; P, normal rats pair-fed with tumor-bearing rats.
phospholipid, and NEFA concentrations. In the pair-feeding study, the serum triglyceride, phospholipid, and NEFA concentrations were not significantly different between the control and pair-fed groups. These serum lipid concentrations in the pair-fed group were not significantly different compared to those of the tumor-bearing group.

The liver lipid contents are shown in Figure 4. The liver cholesterol, triglyceride, and phospholipid contents were not significantly different between the control and tumor-bearing groups. In the pair-feeding study, there were also no significant differences between the control and pair-fed groups with regard to the liver cholesterol, triglyceride, and phospholipid contents. These liver lipid contents in the pair-fed group were not significantly different from those of the tumor-bearing group.

The serum and liver TBARS values are shown in Figure 5. Both the serum and liver TBARS values in the tumor-bearing group were significantly higher than those in the control group. In the pair-feeding study, there were no significant differences between the control and pair-fed groups with regard to the serum and liver TBARS values. The serum and liver TBARS values in the pair-fed group were not significantly different from those of the tumor-bearing group.

DISCUSSION

The present study examined the effects of tumor bearing on serum lipid concentrations and liver lipid contents in RCN-9-implanted rats. The results revealed that RCN-9 implantation to rats significantly enhanced their serum and liver TBARS values as compared to the control group. TBARS values are an index of lipid peroxidation in the blood or tissues. Lipids peroxidize at double bonds between carbon atoms of fatty acids. Unsaturated fatty acids have double bonds between carbon atoms, and thus these fatty acids are easily oxidized. Srivastava et al. reported a significant increase in the plasma levels of TBARS in patients with oral cancer compared to control subjects. In cancer patients, the unsaturated fatty acids in the cell membranes of the erythrocytes in blood are thought to be highly susceptible to oxidative attack. Thus, in plasma, the erythrocytes’ cell membrane becomes the major substrate for reactive oxygen species-mediated damage. This mechanism might account for the enhanced serum and liver TBARS values in the present study’s RCN-9-implanted rats compared to the control group.

The study also reported that the levels of superoxide dismutase, reduced glutathione, glutathione peroxidase, and catalase, which are antioxidant enzymes, were significantly decreased in the oral cancer patients compared to healthy subjects, and that the cancer patients also showed increased plasma TBARS level. It is likely that, in the present study’s RCN-9-implanted rats, the antioxidation of unsaturated fatty acids in the cell membranes of the erythrocytes and liver might be reduced by the suppression of these antioxidant enzyme activities, and thus the serum and liver TBARS values were enhanced. A suppression of these antioxidant enzyme activities might have been the cause of the significant increases in the serum and liver TBARS values of the RCN-9-implanted rats.

The effects of diet restriction in tumor-bearing rats was also investigated in the present study. Although the RCN-9 implantation significantly enhanced the serum and liver TBARS values compared to the control group, these TBARS values were not changed by the diet restriction. The enhancement of the serum and liver TBARS values might be due, at least in part, to the tumor-bearing effect itself and not to the suppression of food intake accompanying the tumor-bearing state.

It is well known that some cancers affect lipid metabolism. For example, serum total cholesterol and (VLDL+LDL)-cholesterol concentrations were increased in ascites hepatoma cell line AH109A-bearing rats, whereas the serum HDL-cholesterol concentration decreased. Another study showed that in Lewis lung carcinoma-implanted mice, the serum total cholesterol and free cholesterol concentrations were increased, as was the serum (VLDL+LDL)-cholesterol concentration. In the present study, the RCN-9 implantation did not change most of the parameters related to lipid metabolism. In the tumor-bearing state, the disorder of lipid metabolism occurred during the growth of the solid tumor. In a previous study of tumor-bearing rats, an increase in the plasma triglyceride concentration and a suppression of the activities of tissue lipoprotein lipase, the hydrolytic enzyme in serum triglyceride, were seen with increased tumor burden, and tumor removal completely reversed these changes. These findings provide evidence that the tumor-induced changes in lipid concentrations in blood are stimulated by the presence of the tumor. In the present study, the solid tumors were observed within a short time, and the growth of the solid tumors was rapid after the RCN-9 implantation but slow during the late period. On the 21st day after RCN-9 implantation, the solid tumor weights were only less than 1 % of the body weight. Therefore, most of the parameters related to lipid metabolism examined in the present study might not be changed by RCN-9 implantation. Tumor cells have a very distinctive metabolism with the potential for infinite growth. The reason why the growth of the implanted RCN-9 tumor cells was slow is unclear. RCN-9 cells originate from F344 rats; rejection of the cells is unlikely in the present experimental animals because they too were F344 rats.

In conclusion, RCN-9 implantation in rats enhanced the serum and liver TBARS values. The mechanisms responsible for the observed changes in the TBARS values that occurred after RCN-9 implantation were not identified, and they remain to be investigated in a future study.

ACKNOWLEDGMENTS

This study was supported by a grant from Iwate Prefectural University.

REFERENCES

1) Hirasawa, A., Makita, K., Akahane, T., Yokota, M., Yamagami, W.,
Effects of Tumor Bearing on Serum and Liver Lipid Levels in RCN-9-Implanted Rats

Masashi KAWASAKI


