

## BIOCHEMICAL STUDIES OF BLOOD COMPONENTS, LIPOGENIC AND LIPOLYTIC ENZYMES IN TUMOR BEARING ANIMALS

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### = Abstract =

Attempts were made to elucidate biochemical mechanisms causing depletion and hypulipidemia of body fat in Tumor-bearing animals. The changes of blood components, lipogenic and lipolytic enzyme activities were studied in mice with Sarcoma 180 tumor cells.

The following results were obtained

1. The total carcass lipid of tumor-bearing mice was significantly decreased during the growth of tumor cells.
2. The plasma triglyceride and free fatty acid levels of tumor-bearing mice were remarkably increased, but plasma insulin and glucose levels were decreased in response to tumor cell growth.
3. At the same time, the activities of enzymes responsible for lipogenesis and lipolysis such as glucose-6-phosphate dehydrogenase and hepatic triglyceride lipase were significantly reduced in the liver and adipose tissue of tumor-bearing mice

From the above results, the observed body fat depletion and hyperlipidemia in response to tumor cell growth might in part due to the cumulative effect of insulin and decreased enzymatic activity responsible for lipogenesis in the liver and adipose tissue of tumor-bearing mice.

## INTRODUCTION

During the growth of tumor, the profound alternations of organs and functions are frequently observed in patients with various neoplastic diseases and tumor-bearing animals. The overall results are anorexia, weight loss, depletion and redistribution of host components, hormonal aberrations, hyperlipidemia and progressive alternations of vital functions. The progressive weight loss in cancer patients and experimental animals is mainly due to the depletion of body fat on the host. However, the biochemical and physiological mechanism involved in this effect of tumor on the host is unknown. Several possible mechanisms include the modifications of the rate of lipid deposition or of the rate of the lipid mobilization, which taken singly or together could result in the observed depletion of body fat. These modifications can be occurred by the several factors and the alternations of various enzymes related in fat mobilization and lipogenesis. The above suggestions were supported by some evidences. Earlier reports have shown that the free fatty acid levels of blood serum was significantly increased but markedly decreased in the total carcass lipid of tumor-bearing animals.<sup>1-4)</sup> Kralovic et al<sup>5)</sup> reported that rats bearing the Walker 256 carcinoma have increased plasma free fatty acid levels and their epididymal adipose tissue has been shown to have basal lipolytic rate 2 to 3 times higher than those of tissue from normal rats, which suggests increased mobilization of lipid. These observations have shown that during the tumor growth, there are a number of significant changes in both deposition and mobilization of lipid on the host.

In the present study, attempts were made to elucidate biochemical mechanisms partly by which body fat was depleted and hyperlipidemia was induced in tumor-bearing animals.

## MATERIALS AND METHODS

### 1. Reagents

The following chemical reagents were purchased from Sigma chemical company: bovine serum albumin, glucose-6-phosphate, oxidized nicotinamide adenine dinucleotide phosphate(NADP<sup>+</sup>), triolein, Tri(1-C<sup>14</sup>) oleoylglycerol and ACS-II were purchased from Amersham. Commercially available Kit reagents purchased from Wako Company (Japan) were used for the determination of plasma free fatty acid, glucose, insulin, total lipid, triglyceride, total cholesterol. All other chemical reagents were reagent grade.

### 2. Animals

Male ICR mice, weighing 35-40 g, were used. They were given standard laboratory diet and water ad libitum.

### 3. Inoculation of tumor cells

Six groups of 60 mice were established with each group being inoculated Sarcoma 180 tumor cells. One group was established as control and the other groups were inoculated by intraperitoneal injection with 0.5ml of Sarcoma 180 tumor cell suspension (approximately  $5 \times 10^6$  cells/mouse). Each group was weighed daily during the entire period of the experiment and sacrificed by a blow on the head. The blood was obtained by the decapitation between 10:00-12:00 AM on the 0, 2, 4, 6, 8, 10 days after Sarcoma 180 tumor

cell inoculation. When body weight was determined during the entire period of experiment, over 90% of the mice died within 13–15 days after inoculation of tumor cells.

#### 4. Measurement of total carcass lipid

Tumor-bearing mice were killed by a blow on the head and their liver, kidney, spleen, small intestine and ascites fluid were removed quickly. The remaining carcass was homogenized in a Waring blender for 5 minutes and filtered. To the 10ml of homogenate, methanol and chloroform were then added to make the volume ratio of chloroform–methanol–water of the mixture being 1 : 2 : 0.8 so that the mixture might be one phase. The mixture was allowed to stand overnight at room temperature and then centrifuged to remove the insoluble material. Chloroform and water were then added to the above supernatant to make the volume ratio of chloroform–methanol–water being 1 : 1 : 0.9 which separated the mixture into two phases, methanol–water phase (upper) and chloroform (bottom) phase. The small portion of chloroform layer was used for the measurement of total lipid.

#### 5. Measurement of glucose-6-phosphate dehydrogenase

Tumor-bearing mice were killed by decapitation and their livers and adipose tissues were removed immediately. The liver and adipose tissue were washed 3 times in ice-cold 0.15M of NaCl. The liver and adipose tissue were homogenized in 4 volumes (w/v) of Tris–HCl buffer (pH 7.4) containing 1mM of EDTA and centrifuged at 12000 rpm for 20 minutes.

The precipitate was removed and the supernatant solution was recentrifuged at 35000

rpm for 1 hour. The resulting supernatant solution was used for the measurement of glucose-6-phosphate dehydrogenase activity. All preparations were performed at 0–4 °C. Glucose-6-phosphate dehydrogenase activity was measured by the method of Rudack et al.<sup>6)</sup> Activity of glucose-6-phosphate dehydrogenase is expressed as Units ± SEM : 1. Units represents 1 nmole of NADPH formed per minute. Units are given as per mg of protein.

Protein content was determined by the method of Lowry et al.<sup>7)</sup> using the bovine serum albumin as standard.

#### 6. Measurement of triglyceride lipase

Liver homogenates (25%, w/v) were prepared in ice-cold solution of 0.25M sucrose, 10 mM Tris–HCl, 1mM EDTA, 0.25% Triton X-100, pH 7.4 in a glass–teflon pestle homogenizer. The homogenate was centrifuged at 12000 rpm for 10 minutes and the supernatant was recentrifuged at 35000 rpm for 1 hour. The resulting supernatant solution was used for the measurement of triglyceride lipase activity. All preparations were performed at 0–4 °C. Triglyceride lipase activity was measured according to the procedure of Masuno et al.<sup>8)</sup> One unit of triglyceride lipase activity was equivalent to the release of 1 umole of oleic acid per hour.

### RESULTS

During the tumor growth, the body weight and total carcass lipid change of tumor-bearing mice were studied after the Sarcoma 180 tumor cells inoculation. Total body weight of tumor-bearing mice was externally increased as shown in Fig 1. This effect was related with growth of tumor. But, when the ascites

fluid was removed from tumor-bearing mice, significant decrease of body weight was observed. Total carcass lipid of tumor-bearing mice became noticeably devoid of fat tissue(Fig.2). On day 10 after tumor inoculation, total carcass lipid of tumor-bearing mice was 30% of that of control mice.

The plasma concentrations of glucose and insulin were significantly decreased during the growth of Sarcoma 180 in mice(Fig 3 and 4). On day 10 after tumor inoculation, the plasma glucose was 43% of that of control mice. Plasma insulin level was markedly decreased between days 4 and 6 but the decrease was not significant because the values were very variable.

The changes of plasma triglyceride, free fatty acid, total cholesterol and phospholipid were observed in tumor-bearing mice. Plasma triglyceride concentrations of tumor-bearing mice reach a maximum between days 6 and 8 after tumor inoculation and then decreased. Plasma triglyceride concentrations of tumor-

bearing mice showed 3.75 times higher than that of control mice(Fig.5).

Plasma concentrations of free fatty acid was increased in tumor-bearing mice and this effect was related with growth of tumor(Fig.6) On day 10 after inoculation, the plasma free fatty acid showed 80% increase in tumor-bearing mice. Plasma cholesterol concentration showed 50% increase in tumor-bearing mice on day 8 after tumor inoculation and then decreased(Fig.7). Phospholipid level in plasma of tumor-bearing mice was nearly constant during the entire experimental period(Fig.8). But on day 6 after tumor inoculation, the content was 20% increase in tumor-bearing mice. The above results are summarized in Table 1.

The glucose-6-phosphate dehydrogenase activity in liver and adipose tissue of tumor-bearing mice were progressively decreased during the growth of tumor as shown in Fig.9 and 10. In both liver and adipose tissue of tumor-bearing mice, the glucos-6-

**Table 1.** Summary of concentrations of plasma lipids, glucose and insulin.

	Normal	Days after tumor inoculation				
		2	4	6	8	10
Triglyceride (mg/dl)	85±6.7	106±10.3	126±12.6	167±13.8	318±40.6	202±24.2
Free fatty acid (mg/dl)	47.4±4.2	48.9±4.4	44.4±2.7	51.3±3.5	76.3±8.2	83.6±8.1
Cholesterol (mg/dl)	65±4.8	72±6.5	75±8	86±6.5	101±7.6	72±8.0
Phospholipid (mg/dl)	161±9.0	1559±11.2	159±8.0	195±14	102±9.8	140.7±9.8
Glucose (mg/dl)	121±13	1117±8.6	122±3.6	114±7.1	90±9.8	52±4.1
Insulin ( $\mu$ units/ml)	7.28±0.88	6.36±1.31	6.9±2.19	3.56±0.82	3.13±1.29	2.02±0.55

phosphate dehydrogenase activity showed 40% and 35% of that of control mice on day 10 after inoculation. The hepatic triglyceride lipase activity in tumor-bearing mice was significantly decreased during the growth of tumor (Fig 11). In mice on day 10 after inoculation, the triglyceride lipase activity was approximately 80% decreased compared to that of control mice.

## DISCUSSION

The progressive weight loss is listed as one of the initial and common manifestations in various neoplastic diseases and tumor-bearing animals. It can be occurred by anorexia, decreased absorption of food from the alimentary canal and altered metabolism on host. In the present study, the overall weights of tumor-bearing mice were externally increased during the growth of tumor (Fig 1). But, when the ascites fluid in tumor-bearing mice was removed by use of syringe, significant decrease of body weight was observed. This result suggests that the increased body weight in tumor-bearing mice might be due to the increased ascites fluid as the tumor developed. Costa<sup>90</sup> suggested that weight loss in cancer patients and experimental animals can be masked by the abnormal expansion of one body compartment (most of water). The depletion of total carcass lipid in cancer patients and tumor-bearing animals can not explained only as due to anorexia. Stewart and Begg<sup>10</sup> early suggested that the depletion of body fat in tumor-bearing rats also occurred when they were force-fed on high-fat, high-carbohydrate or high-protein diet. Costa and Holland<sup>11</sup> reported that the loss of body fat in tumor-bearing mice even occurred when food intake was normal. Many researchers have

suggested that the depletion of body fat in cancer patients and tumor-bearing animals is not the result of a single event but rather the result of concerted changes in both depletion and mobilization.

When Sarcoma 180 tumor cells were inoculated to mice, the blood glucose and insulin levels were significantly decreased during the growth of tumor (Fig 3 and 4). These results were also observed in various neoplastic diseases and tumor-bearing animals<sup>12-14</sup>. Carey<sup>2</sup> et al suggested that hypoglycemia is related with tumor size and might be the result of overutilization of glucose by the neoplasm. Silverstein<sup>13</sup> suggested that insulin-like substance might be secreted from tumor tissue, but this claim remains to be elucidated.

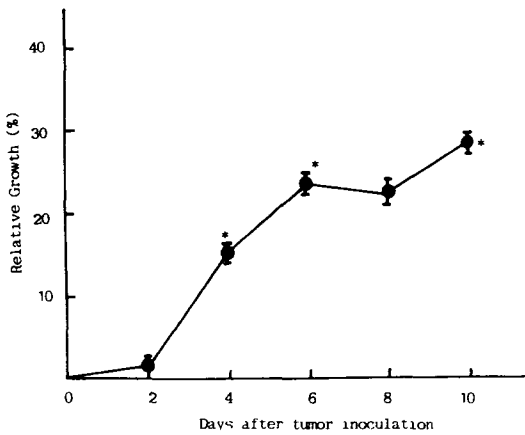
Hyperlipidemia has been observed in cancer patients and tumor-bearing animals. However, the metabolic basis for this hyperlipidemia on the host has not been fully elucidated. Barclay<sup>15,16</sup> et al suggested that the elevation of serum triglyceride might be due to the deficiency of lipoprotein lipase, the key enzyme responsible for triglyceride clearance from the blood. Kannan<sup>17</sup> et al observed that hyperlipidemia in tumor-bearing mice even occurred when dietary fat was removed. Many researchers<sup>18-20</sup> have suggested that cancer-induced hyperlipidemia might be due to the defective removal of VLDL-triglyceride and decreased lipoprotein lipase activity of adipose tissue and insulin levels. Hyperlipidemia was also found in mice bearing the Sarcoma 180 tumor cells. Plasma triglyceride and free fatty acid levels were significantly increased during the growth of tumor (Fig 5 and 6). These results, in part, might be due to the decreased levels of insulin. The changes of lipogenic and lipolytic

enzyme activity have been observed in tumor-bearing animals and cancer patients Thompson<sup>20)</sup> et al reported that endogeneous lipid synthesis and lipoprotein lipase activity were markedly decreased in adipose tissue of preputial gland tumor-bearing mice. Lanza-Jacoby<sup>21)</sup> et al showed that lipoprotein lipase and lipogenic enzymes such as glucose-6-phosphate dehydrogenase, ATP-citrate lyase and fatty acid synthetase activity were significantly decreased. But, Richard<sup>22)</sup> et al reported that glucose-6-phosphate dehydrogenase activity was increased in mice bearing the mammary adenocarcinoma. In the present study, the glucose-6-phosphate dehydrogenase activity of liver and adipose tissue in tumor-bearing mice was significantly decreased during the growth of tumor(Fig 9 and 10). The decreased activity of glucose-6-phosphate dehydrogenase activity might be reduced the supply of NADPH required in biosynthetic pathway and partly one reason of body fat depletion in tumor-bearing animals. But, the decreased activity of hepatic triglyceride lipase in tumor-bearing mice(Fig 11) remains to be elucidated. From the above results, it might be suggested that hyperlipidemia and body fat depletion observed in mice bearing the Sarcoma 180 tumor cells might be, in part, due to the decreased levels of insulin and glucose-6-phosphate dehydrogenase activity.

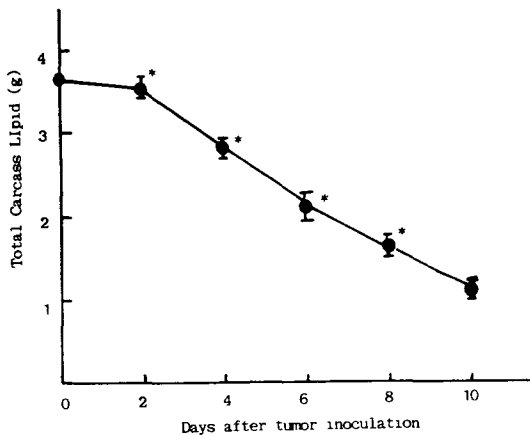
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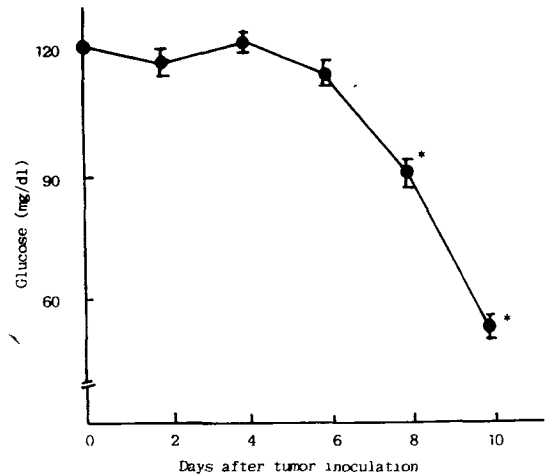
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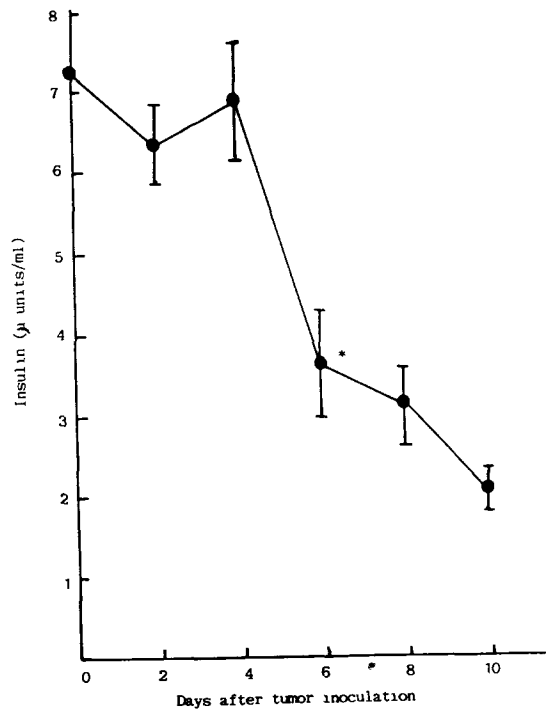
**Fig. 1.** Dependence of weight of mice with Sarcoma 180 on days. Mice, fed ad libitum, were weighted between 9:00~9:30 AM. Mean  $\pm$  SE(n=ten mice) \*p<0.01 (compare with normal mice).



**Fig. 2.** Dependence of total carcass lipid of mice with Sarcoma 180 on days. Total lipid except that of blood, liver and adipose tissue. Mean  $\pm$  SE(n=ten mice) \*p<0.01(compare with normal mice).

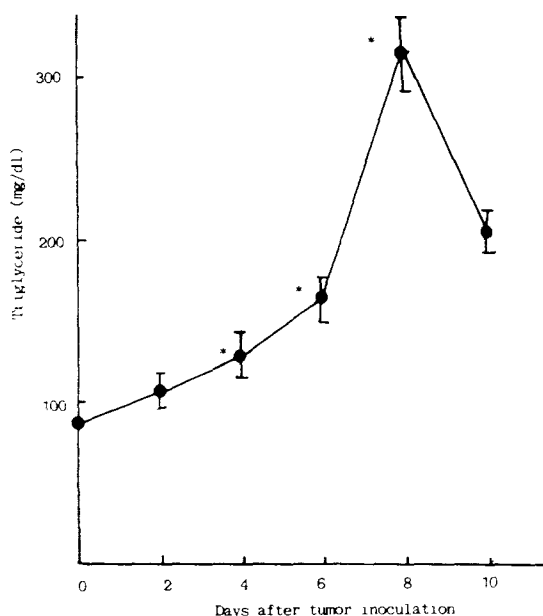


**Fig. 3.** Concentrations of plasma glucose with Sarcoma 180. Blood was obtained for the determination of plasma glucose by decapitation between 9:00~10:00 AM. Mean  $\pm$  SE(n=seven mice) \*p<0.01(compare with normal mice).

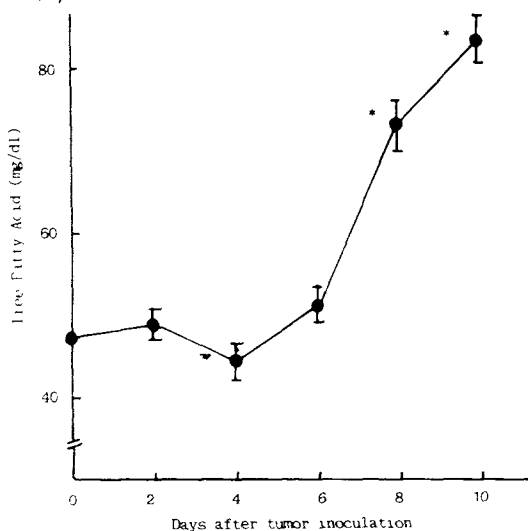


**Fig. 4.** Concentrations of plasma insulin with sarcoma 180. Blood was obtained by decapitation between 9:00~10:00 AM, Mean  $\pm$  SE(n=seven mice). \*p<0.05(compare with normal mice).

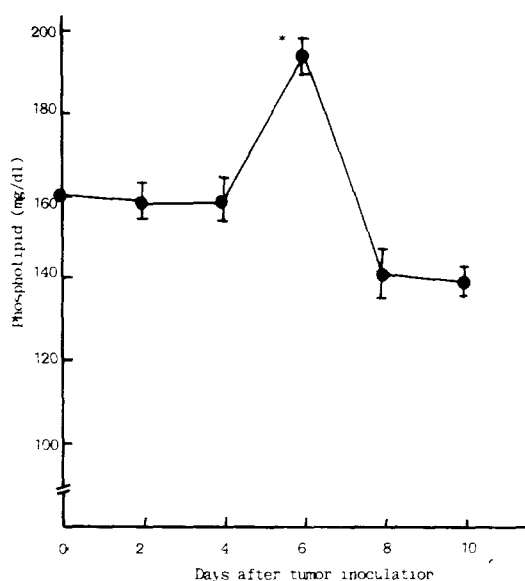




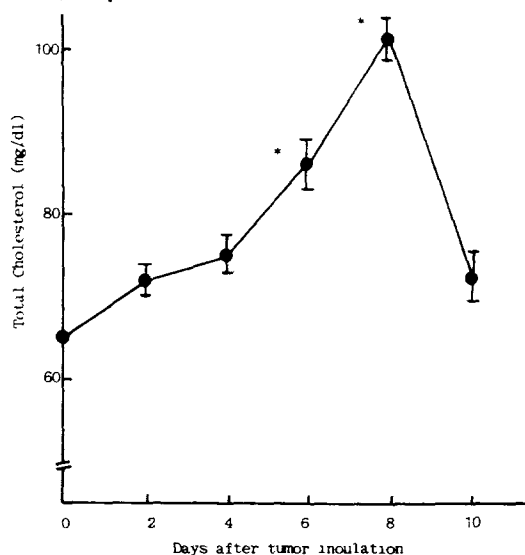
**Fig. 5.** Concentrations of plasma triglyceride with Sarcoma 180 Blood was obtained for the determination of plasma triglyceride by decapitation between 9:00~10:00 AM. Mean  $\pm$  SE (n=seven mice) \*p<0.01(compare with normal mice)



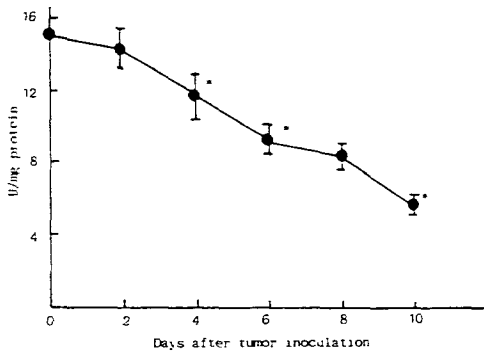
**Fig. 6.** Concentrations of plasma free fatty acid with Sarcoma 180 Blood was obtained for the determination of plasma free fatty acid by decapitation between 9:00~10:00 AM. Mean  $\pm$  SE (n=seven mice). \*p<0.01(compare with normal mice).



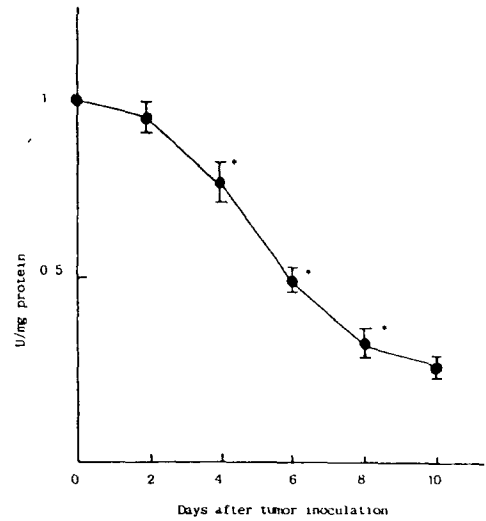
**Fig. 7.** Concentrations of plasma total cholesterol with Sarcoma 180, Blood was obtained for the determination of plasma total cholesterol by decapitation between 9:00~10:00 AM. Mean  $\pm$  SE (n=seven mice) \*p<0.01(compare with normal mice)



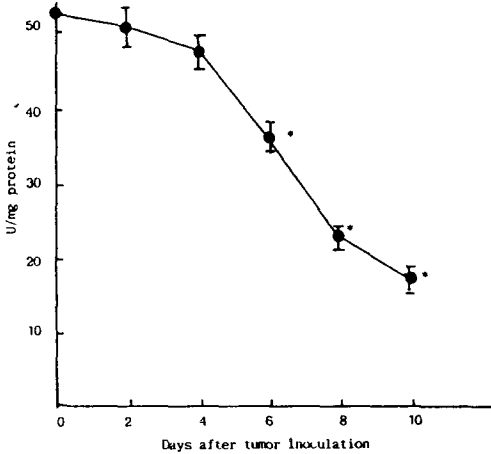
**Fig. 8.** Concentrations of plasma phospholipid with Sarcoma 180 Blood was obtained for the determination of plasma phospholipid by decapitation between 9:00~10:00 AM. Mean  $\pm$  SE (n=seven mice) \*p<0.05(compare with normal mice).



**Fig. 9.** Dependence of glucose-6-phosphatase activity in the liver of mice with Sarcoma 180 on days. Mean  $\pm$  SE (n=seven mice) \*p<0.01(compare with normal mice).



**Fig. 11.** Dependence of hepatic triglyceride lipase activity in the liver of mice with Sarcoma 180 on days. Mean  $\pm$  SE (n=seven mice) \*p<0.01(compare with normal mice)



**Fig. 10.** Dependence of glucose-6-phosphate dehydrogenase activity in the adipose tissue of mice with Sarcoma 180 on days. Mean  $\pm$  SE (n=seven mice). \*p<0.01(compare with normal mice).