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Increase in Alkaline Phosphatase Activity in the Liver of Mice Bearing Ehrlich Ascites Tumor

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SUMMARY

In mice bearing Ehrlich ascites tumors, alkaline phosphatase activity was increased fivefold in the liver and by 50% in the kidney. In mice bearing solid tumors caused by inoculation of tumor cells into the axillary region, the activity of this enzyme in the liver was increased 11-fold, whereas the activity in the kidney did not change. Alkaline phosphatase activities in the liver and kidney were not altered by administration of adrenal steroids. Adrenalectomy, fasting, and pregnancy did not affect the activity of alkaline phosphatase in the liver and kidney.

Treatment with tumor extracts or ascites fluid of normal mice increased liver alkaline phosphatase activity.

These findings suggested that the elevation of liver alkaline phosphatase activity was caused primarily by the tumor itself, and not by hormonal imbalance provoked secondarily by the presence of the tumor.

INTRODUCTION

There have been many investigations on metabolic abnormalities of tumor-bearing animals (4, 6, 9, 13, 16, 20), but the factors responsible for these abnormalities have not been fully elucidated. In a previous paper (14) we reported that activities of several enzymes including alkaline phosphatase changed in the kidney of Ehrlich ascites tumor-bearing mice. These findings prompted us to investigate the metabolic changes in the liver. In the liver of these mice, alkaline phosphatase activity increased more than that of the other enzymes so far examined.

The effects of hormones and tumor extracts were also investigated, in order to gain some additional information that might elucidate the mechanisms of this rise in enzyme activity. Tyrosine aminotransferase and glucose-6-phosphatase were studied as markers, because the control mechanisms of these enzymes are to some extent understood.

MATERIALS AND METHODS

Chemicals. Cortisol succinate was purchased from Japan Upjohn Limited, Tokyo, Japan. Deoxycorticosterone acetate was purchased from Nakarai Kagaku Co., Kyoto, Japan. Adrenocorticotropic hormone was purchased from Daiichi Kagaku Co., Tokyo, Japan. Other chemicals used were commercially available and of reagent grade quality.

Animals and Diet. Male mice of inbred strain DD or DDY weighing 20 to 25 g were used, unless otherwise described. Mice were maintained on standard laboratory chow (Oriental MF) and water *ad libitum*. In experiments on administration of hormones, extracts of tumor cells, or ascites fluid, littermates of male mice bred in our own laboratory were used. In order to minimize the effects of diurnal fluctuation of enzyme activities and of food intake on these activities, all the mice were fasted for 4 hr preceding sacrifice, which took place at 1:00 p.m.

Tumor Implantation. About 10^8 Ehrlich ascites tumor cells obtained from ascites of tumor-bearing mice on the 8th day after implantation were inoculated i.p. into normal mice. Solid tumors were produced by the s.c. implantation of 10^8 cells in the axillary region of mice. The mean survival time of ascites tumor-bearing mice was about 19 days. Of the tumor-bearing mice, those with solid tumors that had no necrotic region macroscopically were used.

Hormone Administration. Littermate animals were divided into 2 groups, experimental and control. Animals in the experimental group were given hormones i.p., doses of which are presented in the text and tables; control animals received i.p. vehicles only.

Adrenalectomy. Adrenal glands were extirpated by dorsal incision under ether anesthesia. After adrenalectomy, animals were kept on standard laboratory chow and 0.9% NaCl solution instead of water. These animals survived for another 1 to 2 weeks.

Preparation of Tissue Extracts. Livers and kidneys were minced and homogenized for 2 min in Potter-Elvehjem all-glass homogenizers with 19 volumes of 0.25 M sucrose containing 10^{-4} M EDTA. After centrifugation at 2500 rpm (600 × *g*) for 10 min, supernatants were used as enzyme samples. As for alkaline phosphatase, the activity in the 600 × *g* supernatant was about 50% of that in the whole homogenate.

Determination of Enzyme Activities. Alkaline phosphatase activity was determined by a modification (19) of the method of Kind and King (10). One-tenth ml of liver homogenate or 50 μl of kidney homogenate diluted 40-fold with distilled water were used as the enzyme sample. Reaction was started by adding the enzyme sample to 2.0 ml of 5 mM disodium phenylphosphate in 0.05 M bicarbonate buffer, pH 10.15, containing 2.25 mM 4-aminoantipyrine. After 15 min

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of incubation at 37°, reaction was stopped by adding 2.0 ml of 5.8 mM potassium ferricyanide containing 0.21 M boric acid. After centrifugation at 2500 rpm for 30 min, the supernatant was read at 500 nm.

Glucose-6-phosphatase activity was determined by the method of Swanson (17). Reaction was started by adding 0.1 ml of liver or kidney homogenate and, after the reaction was stopped with 1.0 ml of 10% trichloroacetic acid and centrifugation, a 0.5-ml aliquot of the supernatant was used for determination of inorganic phosphate by the method of Fiske and Subbarow (3).

Tyrosine aminotransferase activity was determined by the method of Hayashi *et al.* (5). Five μl of liver homogenate or 10 μl of kidney homogenate were used as enzyme sample.

One unit of alkaline phosphatase activity was defined as the activity that produced 1 mg of phenol, and the activity was expressed as munits/min/mg of protein. Tyrosine aminotransferase activity was expressed as nmoles of substrate transformed per min per mg of protein, and glucose-6-phosphatase activity was expressed as μmoles/min/mg of protein.

Protein was measured by the method of Lowry *et al.* (11).

RESULTS

Activities of Enzymes in Different Tumor-bearing States. In mice bearing Ehrlich ascites tumor, liver alkaline phosphatase activity increased gradually after implantation and reached a level 5-fold that of normal mice by the 10th day, as shown in Chart 1. The 50% rise in kidney alkaline phosphatase activity was also observed within 7 days after implantation (Chart 2; Table 1).

In solid tumor-bearing mice, the rise in liver alkaline phosphatase activity was observed when tumor weights exceeded 1 g, and thereafter the activity was gradually increased and reached a level 11-fold that of normal mice after tumor weights exceeded 4 g (Chart 3; Table 1). However, no significant change was observed in kidney alkaline phosphatase activity (Table 1).

Accidentally, solid tumors that contained liquid were found in the course of the experiments. When mice bearing tumors that contained 1.0 to 1.5 ml of clear fluid were examined, liver alkaline phosphatase activity was found to be lower than that of compact tumor-bearing mice (Table 1). In these mice, kidney alkaline phosphatase activity was slightly higher than that of compact tumor-bearing mice (Table 1).

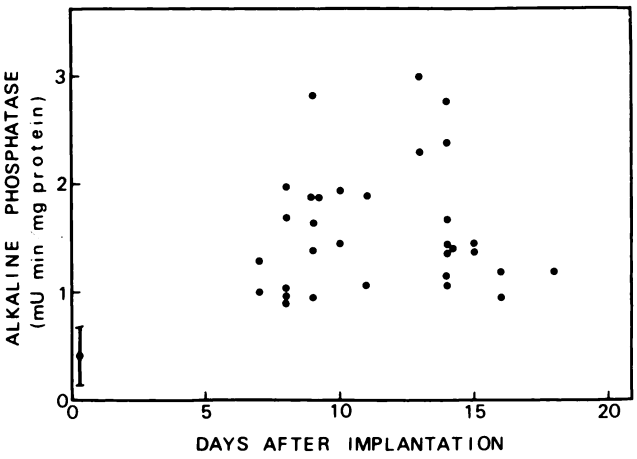

Chart 1. Liver alkaline phosphatase activity of Ehrlich ascites tumor-bearing mice. ●, individual value of a tumor-bearing mouse; ○ and *vertical bar* at *left*, mean and S.D. of control mice, respectively. *mU*, milliunits.

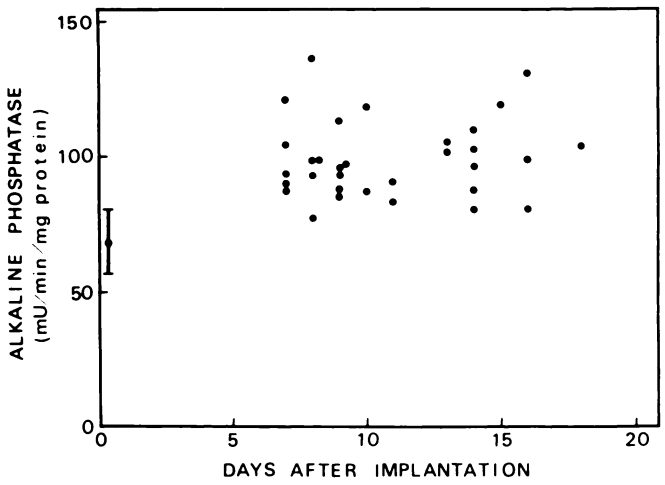

Chart 2. Kidney alkaline phosphatase activity of Ehrlich ascites tumor-bearing mice. ●, individual value of a tumor-bearing mouse; ○ and *vertical bar* at *left*, mean and S.D. of control mice, respectively. *mU*, milliunits.

Glucose-6-phosphatase activity increased by 30% in the liver and by 25% in the kidney of ascites tumor-bearing mice, when they were examined 7 to 16 days after implantation (Table 1). In mice bearing solid tumors, glucose-6-phosphatase activity increased slightly in the kidney, but no significant change was observed in the liver (Table 1).

The rise in tyrosine aminotransferase activity was observed in the liver of ascites tumor-bearing mice without significant change in the activity of the kidney enzyme. On the other hand, in mice bearing solid tumors, kidney tyrosine aminotransferase activity was decreased (Table 1).

Enzymes during Fasting and Pregnancy. When mice were starved for 48 hr, tyrosine aminotransferase activity increased 7-fold, and glucose-6-phosphatase activity increased slightly in the liver. In the kidney of these mice, tyrosine aminotransferase activity was decreased and glucose-6-phosphatase activity was not altered (Table 2). Changes in alkaline phosphatase activity of the liver and kidney and in the activity of glucose-6-phosphatase of the kidney were negligible (Table 2).

In pregnant mice, there was no significant change in the activities of the enzymes, except for liver tyrosine aminotransferase activity, which was increased slightly.

Effects of Hormone Administration on Activities of Enzymes. Various doses of deoxycorticosterone acetate, cortisol succinate, or adrenocorticotropic hormone were administered to normal mice, and the activities of the enzymes in the liver and kidney were assayed at various time intervals. Only liver tyrosine aminotransferase activity responded to these hormones. A single i.p. injection of deoxycorticosterone acetate (7 mg/capita) caused a 6-fold increase in the activity of liver tyrosine aminotransferase (72.5 ± 7.3

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Table 1

Activities of enzymes in tumor-bearing states

The values indicate mean ± S.D., except in Experiment 3, in which these indicate mean ± S.E. In Experiment 1, mice bearing ascites tumors 7 to 16 days after implantation were used as hosts. In Experiment 2, mice bearing solid tumors, whose weights exceeded 4 g, were used. In Experiment 4, mice bearing solid tumors that contained 1.0 to 1.5 ml of fluid were used as hosts. Five mg DOCA[a] were administered 6 times at intervals of 12 hr to solid tumor-bearing mice, and these mice were sacrificed 4 hr after last administration.

Experiment	Liver			Kidney		
	Alkaline phosphatase (milliunits/min/mg protein)	Tyrosine aminotransferase (nmoles/min/mg protein)	Glucose-6-phosphatase (μmoles/min/mg protein)	Alkaline phosphatase (milliunits/min/mg protein)	Tyrosine aminotransferase (nmoles/min/mg protein)	Glucose-6-phosphatase (μmoles/min/mg protein)
1. Controls	0.31 ± 0.16 (34)[b]	27.3 ± 11.4 (10)	0.139 ± 0.008 (9)	67.4 ± 11.8 (35)	2.32 ± 0.48 (8)	0.193 ± 0.014 (10)
Hosts of ascites tumors	1.59 ± 0.58[c] (31)	119.3 ± 27.5[c] (10)	0.182 ± 0.010[c] (10)	98.2 ± 14.2[c] (31)	1.80 ± 0.57 (10)	0.239 ± 0.018[c] (10)
2. Controls	0.34 ± 0.10 (17)	19.9 ± 8.5 (16)	0.109 ± 0.018 (19)	79.1 ± 16.4 (18)	0.855 ± 0.404 (11)	0.186 ± 0.028 (19)
Hosts of solid tumors	3.79 ± 0.91[c] (13)	20.8 ± 5.0 (11)	0.113 ± 0.018 (14)	90.4 ± 22.3[d] (14)	0.200 ± 0.081[c] (9)	0.206 ± 0.022[e] (14)
3. Controls	0.33 ± 0.02 (12)		0.116 ± 0.005 (14)	62.3 ± 2.7 (13)		0.225 ± 0.008 (14)
Hosts of solid tumors without liquid	5.56 ± 0.58 (5)		0.105 ± 0.011 (5)	79.6 ± 5.2 (5)		0.225 ± 0.012 (5)
Hosts of solid tumors with liquid	2.53 ± 0.13[f] (4)		0.107 ± 0.006 (3)	92.2 ± 4.8[g] (4)		0.198 ± 0.016 (3)
4. Hosts of solid tumors	2.84 ± 0.64 (8)	31.8 ± 11.7 (10)	0.097 ± 0.016 (10)	79.2 ± 16.0 (10)		0.199 ± 0.018 (10)
Hosts of solid tumors injected with DOCA	1.82 ± 0.54[h] (11)	64.7 ± 33.2[h] (16)	0.109 ± 0.016 (15)	73.5 ± 13.8 (16)		0.194 ± 0.029 (16)

[a] DOCA, deoxycorticosterone acetate.
[b] Numbers in parentheses, number of animals.
[c] Difference between controls and hosts is statistically highly significant ($p < 0.01$).
[d] Difference between controls and hosts is statistically not significant ($p > 0.1$).
[e] Difference between controls and hosts is statistically significant ($p < 0.05$).
[f] Difference between hosts of solid tumors with liquid and those of solid tumors without liquid is statistically highly significant ($p < 0.01$).
[g] Difference between hosts of solid tumors with liquid and those of solid tumors without liquid is statistically not significant ($0.05 < p < 0.1$).
[h] Difference between hosts of solid tumors and those injected with DOCA is statistically highly significant ($p < 0.01$).

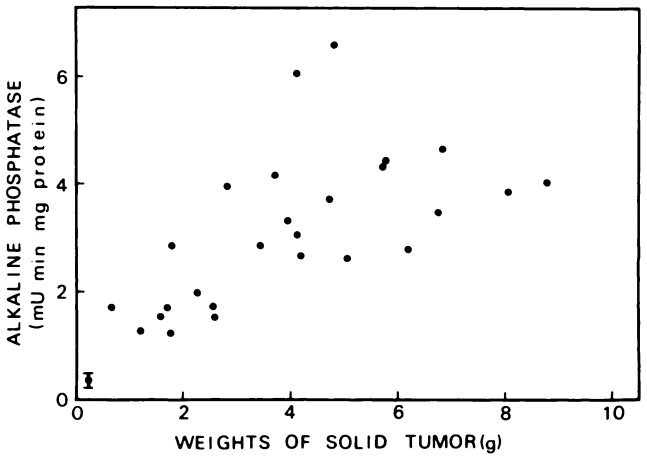

Chart 3. Liver alkaline phosphatase activity of solid tumor-bearing mice. ●, individual value of a tumor-bearing mouse; ○ and *vertical bar* at *left*, mean and S.D. of control mice, respectively.

nmoles/min/mg of protein) compared with that of normal mice (13.0 ± 4.0 nmoles/min/mg of protein). Similar results were obtained when cortisol succinate and adrenocorticotropic hormone were used in place of deoxycorticosterone acetate.

Activities of glucose-6-phosphatase and alkaline phosphatase in both the liver and kidney, as well as the activity of tyrosine aminotransferase in the kidney, were not changed under these experimental conditions.

On the other hand, liver glucose-6-phosphatase activity decreased when assayed 8 days after adrenalectomy, as compared with the activity in sham-operated mice, and administration of cortisol succinate (equivalent to 2.5 mg cortisol per capita) to the adrenalectomized mice caused 60% increase in liver glucose-6-phosphatase activity (Table 2). The activities of alkaline phosphatase in the liver and kidney were found not to be affected by these treatments. Similar results were obtained by treatment with deoxycorticoster-

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one acetate, in place of cortisol succinate.

When deoxycorticosterone acetate was injected into solid tumor-bearing mice, liver alkaline phosphatase activity decreased to 65% of that of solid tumor-bearing control mice (Table 1). By the same treatment, liver tyrosine aminotransferase activity was increased, but liver and kidney glucose-6-phosphatase activity and kidney alkaline phosphatase activity were not affected.

Effects of Administration of Ascites Fluid and of Tumor Tissue Extract on Liver Alkaline Phosphatase Activity. When ascites fluid was injected into normal mice, as described in the legend to Chart 4, liver alkaline phosphatase activity was increased (Chart 4*A*). When ascites fluid that was concentrated 5-fold by ultrafiltration with Diaflo Membrane UM-2 was injected into normal mice, liver alkaline phosphatase activity rose markedly (Chart 4*C*), indicating an effective factor of more than 1000 daltons. Injection of the filtrate did not cause any elevation of the enzyme activity. Extracts of solid tumors were tested as described in Chart 4, and liver alkaline phosphatase activity was also increased (Chart 4*B*).

DISCUSSION

Nakata *et al.* (12) reported that liver alkaline phosphatase activity in rats bearing AH130 ascites tumor increased slightly. In this paper, we describe the significant change in the activity of this enzyme in the liver of mice bearing Ehrlich ascites tumor. The change in kidney alkaline phosphatase activity was also reported in a previous paper (14). Factors causing these metabolic deviations may be divided into the presence of tumor itself and the presence of ascites. Effects of these 2 factors might be distinguished from each other by comparing enzyme activities in ascites tumor-bearing mice with those in solid tumor-bearing mice. Such a comparison shows that liver alkaline phosphatase activity was increased much more in hosts of solid tumors than in those of ascites tumors, whereas kidney alkaline phosphatase activity was increased only in ascites tumor bearers.

In this regard, it is interesting that, in mice bearing solid

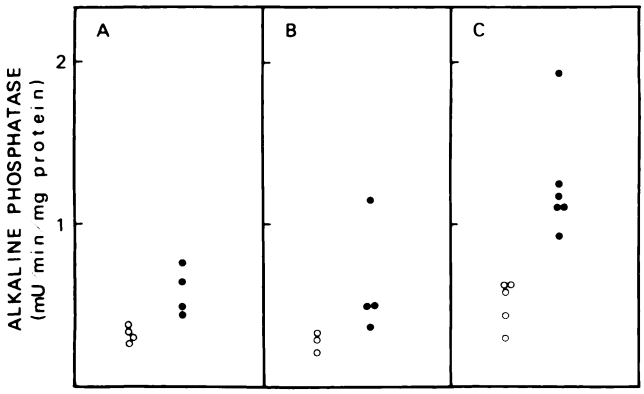

Chart 4. Effects of administration of ascites fluid and of extracts of solid tumor on liver alkaline phosphatase activity. *A*, ascites fluid; ascites was obtained from ascites tumor-bearing mice 7 to 14 days after implantation and centrifuged at 700 × *g* for 25 min. Two-tenths-ml aliquots of the supernatant (53 mg protein per ml) were injected i.p. every 12 hr 6 times to normal mice. *B*, extracts of solid tumor; solid tumor was homogenized with 9 volumes of 0.9% NaCl solution. The homogenate was centrifuged at 8,000 × *g* for 30 min. Two-tenths-ml aliquots of the supernatant (6.4 mg protein per ml) were injected in the same way. *C*, concentrated ascites fluid; ascites fluid was concentrated by ultrafiltration using Diaflo Membrane UM-2, and 0.1 ml of the concentrated fluid (285 mg protein per ml) was injected in the same way. ●, activities in treated mice; ○, activities in control mice treated with 0.9% NaCl solution, *mU*, milliunits.

Table 2

Enzyme activities in mice administered with cortisol and starved mice

In Experiment 1, mice were adrenalectomized and given 4 doses of cortisol acetate (equivalent to 2.5 mg cortisol per capita) at intervals of 12 hr from the 6th day after adrenalectomy, and enzyme activities were assayed on the 8th day. In Experiment 2, mice were starved for 48 hr. The values indicate mean ± S.E.

	Liver			Kidney		
Experiment	Alkaline phosphatase (milliunits/min/mg of protein)	Tyrosine aminotransferase (nmoles/min/mg of protein)	Glucose-6-phosphatase (μmoles/min/mg of protein)	Alkaline phosphatase (milliunits/min/mg of protein)	Tyrosine aminotransferase (nmoles/min/mg of protein)	Glucose-6-phosphatase (μmoles/min/mg of protein)
1. Sham operated	0.382 ± 0.153 (4)[a]	18.9 ± 2.4 (4)	0.091 ± 0.001 (4)	57.4 ± 4.8 (4)	0.777 ± 0.074 (4)	0.153 ± 0.012 (4)
Adrenalectomized	0.408 ± 0.102 (6)	15.2 ± 1.2 (6)	0.073 ± 0.003 (6)	63.2 ± 6.3 (6)	0.470 ± 0.122[e] (6)	0.147 ± 0.003 (6)
Adrenalectomized + cortisol	0.304 ± 0.046 (6)	111.2 ± 11.0[b] (6)	0.117 ± 0.010[b] (6)	57.1 ± 3.3 (6)	0.513 ± 0.067 (6)	0.157 ± 0.008 (6)
2. Normal	0.423 ± 0.052 (3)	18.4 ± 2.30 (4)	0.104 ± 0.004 (4)	82.4 ± 8.5 (4)	0.803 ± 0.233 (4)	0.200 ± 0.007 (4)
Starved	0.616 ± 0.079[d] (5)	117.0 ± 18.1[c] (7)	0.172 ± 0.008[c] (6)	71.5 ± 3.6 (5)	0.144 ± 0.072[c] (7)	0.188 ± 0.009 (5)

[a] Numbers in parentheses, number of animals.
[b] Difference between adrenalectomized and cortisol injected mice is statistically highly significant ($p < 0.01$).
[c] Difference between starved and normal mice is statistically highly significant ($p < 0.01$).
[d] Difference between starved and normal mice is statistically not significant ($p > 0.05$).
[e] Difference between adrenalectomized and sham operated mice is statistically not significant ($p > 0.05$).

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tumors that contained liquid, the rise in liver alkaline phosphatase activity was smaller than that in compact tumor bearers, and a rise in kidney alkaline phosphatase activity was also observed. These findings were similar to those observed in ascites tumor bearers.

In tumor-bearing animals, there may be an imbalance of endocrine systems, which is provoked by nonspecific stresses and/or unusual nutritional conditions. In reports concerning changes in endocrine systems in tumor-bearing animals, the role of adrenal cortical functions has been intensively investigated (1, 7, 15). Moreover, a close relationship between alkaline phosphatase activity in rat liver and adrenal functions has been reported (18). In addition, it is well known that bile duct ligation causes elevation of rat liver alkaline phosphatase (2, 8), and Boernig *et al.* (2) concluded that this elevation was mediated by cortisol. In ascites tumor-bearing mice, it is probable that bile stasis is occurring. Therefore, it could be assumed that the rise in alkaline phosphatase activity in ascites tumor bearers might be caused by adrenal steroids. However, as shown in Table 2, the injection of adrenal cortical hormones did not affect alkaline phosphatase levels in the liver and kidney of intact and adrenalectomized mice, while the same treatment raised liver tyrosine aminotransferase and glucose-6-phosphatase activities. This discrepancy might be ascribed to species differences.

In mice bearing ascites tumors, secondary hyperaldosteronism may possibly be present because of retention of liquid, and hence, administration of deoxycorticosterone acetate to mice bearing solid tumors was studied. A decrease in alkaline phosphatase activity by 40% and an increase in tyrosine aminotransferase activity in the liver were observed. These changes were comparable to those found in the ascites tumor-bearing state, and they could be explained by hyperaldosteronism.

In the later stages of the tumor-bearing state, food intake is decreased and animals are close to a fasting state (9). However, this cannot explain the change in liver alkaline phosphatase activity, which was shown to be negligible in starved mice (Table 2). In pregnant mice bearing physiologically proliferating tissues, liver alkaline phosphatase activity was not altered. Therefore, it may be assumed that elevation of enzyme activity in tumor-bearing mice is caused specifically by the presence of the tumor itself and not by secondary effects. Consistent with this assumption is our finding that the factors effective on liver alkaline phosphatase activity were in the higher-molecular-weight fraction from ascites fluid and tumor tissue extracts.

In conclusion, it has become clear that liver alkaline phosphatase activity increased markedly in the tumor-bearing state, and the presence of ascites caused the decrease of this effect to a certain degree. The latter finding may be attributed, at least in part, to mediation by mineralocorticoids. It is probable that there may be some factor(s) produced in and released from tumor tissue, which causes induction of liver alkaline phosphatase. This factor (or factors) is now being purified from tumor tissues.

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