Biosynthesis of L-Ascorbic Acid in Liver Microsomes from Mice Bearing Transplanted Tumors.* (29565)

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Tumor metabolism is known to affect the levels of enzymes in normal tissues of the host at points distant from the tumor site. The lowered activities of catalase, tryptophan pyrrolase, *D*-amino acid oxidase and arginase in the liver of rats bearing Walker tumor are typical examples(1). Begg observed that the amount of ascorbic acid in the adrenals of the rat decreases in the tumorous host and that the decrease is proportional to the growth of the tumor(2). Besides having rapid utilization of ascorbic acid in the tumorous host(3,4), this decrease in ascorbic acid content might be due to a decrease in the activity of the liver microsomal enzymes(7) responsible for synthesis of ascorbic acid. It was therefore of interest to study the influence of tumor on the synthesis of *L*-ascorbic acid by liver microsomes from mice.

Materials and methods. Male mice, approximately 3 months old, were used. Hyperdiploid Ehrlich ascites carcinoma in C57/BL mice, Gardner 6C3HED lymphosarcoma in C3H and L No. 2 lymphoma in A/He mice were transplanted and grown as described previously(5). C3H/Crgl mice with T15 hepatoma were purchased from the Cancer Research Genetics Laboratory, Univ. of California, Berkeley. The immune C57/BL mice employed were immunized against Ehrlich carcinoma by giving repeated injections of X-irradiated Ehrlich tumor cells and 2 challenges with viable cells(6).

Preparations of microsomes, assay of the enzyme systems and estimation of *L*-ascorbic acid were made by methods described elsewhere(7). Protein content of the microsomes was determined by the biuret method(8) after precipitating the protein with trichloroacetic acid.

Results. The synthesis of *L*-ascorbic acid from *D*-glucuronolactone and *L*-gulonolactone by the liver microsomes is not impaired in C57/BL mice bearing 7 days old Ehrlich ascites carcinoma (Table I). However, when tumor is grown in the mice 13 days, the weight of the liver increases considerably and synthesis of *L*-ascorbic acid per mg microsomal protein decreases significantly. This decrease in the synthesis is not directly related to tumor metabolism but to lowered food intake of the animals as is evident from the results obtained with pair-fed controls (Table I). It is noteworthy that the amount of microsomal protein per gram liver is significantly increased in immune mice. But this increase in protein content is probably a general response of the host to build up its immunity. The synthesis of *L*-ascorbic acid per mg liver microsomal protein is not increased in immune mice over and above that obtained with normal.

Table I further shows that the synthesis of *L*-ascorbic acid is highly decreased in the liver microsomes of mice bearing either T15 hepatoma or L No. 2 lymphoma. In these cases also it is very likely that the decrease is not due to any direct effect of tumor metabolism but due to lowered food intake.

In the case of C3H mice bearing Gardner lymphosarcoma, the decrease in synthesis of *L*-ascorbic acid cannot, however, be explained as a result of inanition (Table II). Histological studies indicate that unlike the situation of C57/BL bearing Ehrlich ascites carcinoma, the liver from C3H mice bearing Gardner lymphosarcoma becomes heavily contaminated by infiltration of tumor cells and this infiltration increases with increased growth of the tumor, being about 45% on the 12th day of tumor growth. At this stage about 5% necrosis of the liver cells are also observed. The possibility that decreased synthesis by the liver microsomes from C3H mice is due to contamination by the tumor

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TABLE I. Synthesis of L-Ascorbic Acid by Normal, Tumor-Bearing and Immune Mice Liver Microsomes.

Strain of mice	Condition of mice	Age of tumor growth (days)	No. of animals used	Avg† wt of liver/mouse (g)	Avg† wt of liver microsomal protein (mg/g liver)	Avg† amt of *L*-ascorbic acid synthesized (μmole /mg protein/hr in air) from *D*-glucurono-lactone	from *L*-gulono-lactone
C57/BL	Normal	—	18 (3)*	1.00	41	.131	.284
"	Ehrlich ascites carcinoma	7	12 (3)	1.10	38	.130	.272
"	*Idem*	13	8 (2)	1.60	26	.085	.187
"	Pair-fed control	—	6 (2)	.90	38	.069	.182
"	Immune	—	12 (4)	1.16	53	.136	.296
C3H/Crgl	Normal	—	4 (1)	.95	39	.125	.227
"	T15 hepatoma	56	4 (1)	1.15	37	.114	.199
A/He	Normal	—	2 (1)	1.10	36	.114	.238
"	L No. 2 lymphoma	11	2 (1)	1.60	22	.108	.228

Test system: 0.5 ml microsomes (equivalent to 0.25 g liver), 10 μmoles *L*-gulonolactone, 0.005 *M* sodium pyrophosphate, 0.001 *M* KCN, 0.02 *M* sodium phosphate buffer, pH 7.2, in a total volume of 2.5 ml; incubated for 1 hr at 37°C in air. When *D*-glucuronolactone was used, amount of substrate was 20 μmoles, cyanide concentration, 0.02 *M*, sodium pyrophosphate was omitted, other conditions were same as above.

* Numbers in parentheses are numbers of animals used per experiment.

† Variations from avg values were not significant.

TABLE II. Synthesis of L-Ascorbic Acid by Liver Microsomes from Normal and Gardner 6C3HED Lymphosarcoma Bearing C3H Mice.

Condition of mice	Age of tumor growth (days)	No. of animals used*	Avg† wt of liver/mouse (g)	Avg† wt of liver microsomal protein (mg/g liver)	Avg† amt of *L*-ascorbic acid synthesized (μmole/mg protein) from *D*-glucurono-lactone		*L*-gulono-lactone	
					Air	Absence of air	Air	Absence of air
Normal	—	8	.90	38	.147	.443	.272	.520
Tumor	7	"	1.00	36	.125	—	.262	—
"	10	"	1.40	22	.108	.301	.232	.448
"	11	"	—	—	.068	.182	.142	.256
Pair-fed control	—	6	—	—	.085	.261	.182	.368
Tumor	12	8	1.70	16	.051	.142	.102	.216
Pair-fed control	—	6	.85	33	.080	.255	.159	.362

Test system: For aerobic incubation, same as in Table I. In anaerobic incubation: 0.25 ml microsomes, 0.001 *M* KCN, 0.02 *M* sodium phosphate buffer, pH 7.2, in the main compartment of a Thunberg tube; 10 μmoles of *L*-gulonolactone and 2 mg of phenazine methosulphate in the side arm; total volume was 2.5 ml, incubated at 37°C for 15 min. When *D*-glucuronolactone was used, the conditions were the same as above except that amount of substrate was 20 μmoles and cyanide 0.02 *M*.

* Two animals were used for each experiment.

† Variations from avg values were not significant.

cells, which do not synthesize *L*-ascorbic acid, has been excluded by the fact that microsomes prepared from a mixture of 1 g C3H liver and 1 g packed Gardner lymphosarcoma cells did not give inhibited synthesis. The amount of microsomal protein obtained from 1 g C3H liver was 38 mg whereas that obtained from 1 g liver plus 1 ml packed lymphosarcoma cells was 39 mg only. Apparently the tumor cells, being hard to disintegrate under the condition of homogenization used, did not contribute any significant amount of protein to the liver microsomes. The liver from a C3H mouse bearing 12 days old Gardner lymphosarcoma looks yellow instead of the usual color of the liver and the size of the gall bladder is 2-3 times the normal size. The microsomes sedimented from such a liver are yellow in color instead of the usual pinkish color. The possibility, therefore, existed

that the inhibition in the synthesis was due to contamination of the microsomes by bile salts and pigments. This possibility was also excluded by the following experiment. A liver from a normal C3H mouse was homogenized as usual but with the addition of a gall bladder from a C3H mouse bearing a 12 days old tumor. The microsomes obtained from such a preparation were yellow. The yellow color was not removed by washing the microsomes once with 10 volumes of 0.25 M sucrose. Nevertheless, the biosynthetic capacity of these microsomes was not inhibited.

Discussion. None of the tumor cells used, including hepatoma, could synthesize any *L*-ascorbic acid from *D*-glucuronolactone or *L*-gulonolactone. This is at variance with the finding of Conney and Burns(9) who observed that C3H mouse hepatoma retained the microsomal enzyme system converting *L*-gulonolactone into *L*-ascorbic acid. Among all the cases of liver microsomes studied, only in C3H mice bearing Gardner lymphosarcoma was there a significant decrease in capacity for synthesizing *L*-ascorbic acid which could not be explained as a result of inanition, infiltration of tumor cells or contamination by bile. The decrease in the biosynthetic capacity is very probably due to a decrease in *L*-gulono dehydrogenase activity (Table II). However, this inhibition in the enzymic activity is probably a reflection of the general effect of tumor metabolism on the decreased protein synthesis by the host.

Summary. Decrease of *L*-ascorbic acid synthesis by the liver microsomes of C57/BL mice bearing Ehrlich ascites carcinoma for 13 days could be accounted for by decreased food intake of the animals. On the other hand, the decreased ascorbic acid synthesis by liver microsomes from C3H/Crgl mice infected with Gardner lymphosarcoma was due neither to inanition nor to gross infiltration of the liver with tumor cells but to a decrease in the *L*-gulono dehydrogenase activity. There was no change in the synthesis of ascorbic acid by liver microsomes from C57/BL mice made immune to Ehrlich ascites carcinoma cells, although the amount of microsomal protein per gram liver was significantly increased. Neither was there a significant inhibition in the synthesis by the liver microsomes of C3H/Crgl bearing T-15 hepatoma or of A/HC mice bearing L No. 2 lymphoma.

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