100-207128
DDE/EV/03017

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Tissue Enzyme Changes in Parabiatic Rats with Subcutaneous Lymphoma^{1,2}

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Running title: Parabiatic Rats with Lymphoma

Category: Oncology

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Introduction. Reports from this laboratory have described profound biochemical changes in tissues of tumor-bearing (1) and especially of lymphoma-bearing (2) rats. Study of some 20 enzymes indicated that soon after implantation of mammary tumors the composition of the host liver became partially dedifferentiated: enzymes which accumulate late in normal development decreased in amount, while enzymes highly concentrated in normal fetal liver increased in the host liver (1). Lymphoma-bearing rats exhibited some especially rapid and conspicuous changes: the increase in hepatic thymidine kinase (EC 2.7.1.75) 4 days after implantation was not associated with tumors of renal, mammary or submaxillary gland origin; the decrease in hepatic arginase (EC 3.5.3.1), seen in hosts with any of several tumors, was more pronounced in lymphoma-bearers (2). Ornithine aminotransferase (EC 2.6.1.13) in liver decreased greatly soon after implantation of most of the neoplasms tested. These 3 enzymes were therefore measured in the present study of the systemic effect of tumors in parabiotic rats. One of the enzymes, thymidine kinase, was assayed in spleen and plasma as well as in liver; previous studies on single hosts (2, 3) showed that the splenic thymidine kinase increased in response to both lymphoma and mammary tumors whereas the appearance of the enzyme in plasma (14 days after transplantation) was associated with lymphoma-bearing specifically (2).

In the present experiments solid tumors, lymphoma and fibrosarcoma, were transplanted subcutaneously into both single rats and into one partner of a parabiotic pair. The biochemical changes found in the tissues of the latter (less striking than those in the single hosts) were also detectable, after some delay, in the tumor-free partner. The results suggest that humoral factors are responsible for mediating the effects of neoplasms on host tissues.

Materials and Methods. Male Kx rats, 40 days old, were parabiosed with appropriate surgical precautions (4) under nembutal anesthesia. Adjacent flanks were incised from just forward of the scapulae to the iliac crests. The adjacent scapulae were fixed to each other by tantalum wire. The edges of the incision were approximated and sutured to each other, layer by layer, resulting in the union of the two rats with a common peritoneal cavity. Fifteen to seventeen days after surgery, the right hand partner (parabiotic host) was implanted subcutaneously with a 1 mm³ cube of lymphoma RNC 290 (2) or of fibrosarcoma RNC 254. The tumors were also implanted into single rats of the same strain, age and sex. Animals were killed 4 to 26 days after implantation. Weights of the lymphomas ranged from < 0.05 g (invisible at 8 days after implantation) to 20-30 g at 18 to 21 days; those of the fibrosarcoma, assayed 20 to 26 days after implantation, ranged from 10-41 g. No signs of debility were noted with either tumor before the 18th day. However, only half of the lymphoma hosts survived the tumors until the 21st day; the fibrosarcoma hosts became moribund 24 to 30 days after implantation.

Aliquots of the same freshly excised tissues were homogenized in 9 vol of 150 mM KCl, or (for arginase assays (5)) in 9 vol of 50 mM MnCl₂-50 mM imidazole, pH 7.4. Whole homogenates were used for the assays of ornithine aminotransferase and arginase. Supernatant fractions obtained by centrifugation at 100,000g for 30 min were used to measure thymidine kinase. Plasma was obtained by heart puncture in heparinized syringes; it was separated from cellular material by centrifugation at 6000g for 10 min.

Assays for thymidine kinase (6), ornithine aminotransferase (7) and arginase (5) have been published previously. Activities are expressed as units (μ moles product per min, or for thymidine kinase, nanomoles per min) per g wet weight of tissue or per ml of blood plasma.

Results. The effects of subcutaneously implanted lymphoma on the hepatic activities of ornithine aminotransferase and arginase are shown in Fig. 1. The initial decreases in both activities were more pronounced in single than in parabiotic hosts. The levels of both enzymes were normal in host partners for at least 6 days after implantation but showed significant decreases by the 13th day. At 18 to 21 days, arginase was 55, 30 and 20% below its normal level in single hosts, parabiotic hosts and host partners, respectively, whereas the losses of ornithine aminotransferase were similar (60-70%) in all three types of rats.

In livers of the parabiotic hosts, as in those of single hosts, the decline in ornithine aminotransferase and arginase preceded (by 3 to 5 days) detection of the tumor by palpation; autopsy revealed no evidence of nodule formation or hyperplasia at the site of implantation (or in any tissue); host liver was cytologically normal up to at least the 14th day. The low hepatic enzyme levels in host partners were also not associated with histological changes; no lymphoma cells could be detected in these livers even 18 days after implantation. The weights of the livers in both partners (and in single hosts) remained normal throughout the 21 experimental days (Table I).

Hepatic thymidine kinase activities, in single hosts, increased 5- and 200-fold at 4 and 14 to 20 days after implantation. In parabiotic hosts no response was detectable on the 5th day. However, a 20-fold rise in the levels of hepatic thymidine kinase was seen at 14 to 20 days, and, by this time, the thymidine kinase levels in host partners had also quadrupled (Table I).

Spleen weights of parabiotic or single hosts showed no, or very small, increases 17 to 20 days after lymphoma implantation (Table I - note S.D.'s). However, splenic thymidine kinase level was strikingly elevated: in parabiotic host spleens it was almost as high as in the single hosts and about 12 times higher than normal (Table I). The enzyme activity (expressed per g)

did not correlate with spleen size: the elevations were equally striking in animals with enlarged or unchanged spleen weights. In host partners the splenic thymidine kinase remained normal up to day 14 but exhibited a 4-fold elevation at 17 to 20 days without any increase in spleen size. It has been shown previously (3) that the splenic thymidine kinase is responsive to implanted mammary tumors: thus the elevation observed here was not lymphoma-specific.

Previous studies showed that in lymphoma-bearing rats (single hosts) the rise in hepatic thymidine kinase precedes by over a week the appearance of the enzyme in plasma and that the mechanisms of the hepatic and of the plasma responses are different (2, see also Discussion). Table I shows that in single hosts, 19 days after implantation, plasma thymidine kinase activities sometimes exceeded the control values by more than 100 times. In the plasma of parabiotic hosts the enrichment at that time was only 10-fold, and in the host partner, it was 7-fold.

The fibrosarcoma evoked the same changes in hepatic enzyme levels as did the lymphoma, although the rise in thymidine kinase was less striking (compare Table II with Fig. 1 (ornithine aminotransferase and arginase) and Table I (thymidine kinase)). The three activities measured at 20 to 26 days did not correlate with tumor size. The losses in ornithine aminotransferase or arginase and the gain in thymidine kinase were similar in single and parabiotic fibrosarcoma hosts; assays at earlier time points, that may have revealed the "protection" afforded by parabiosis, were not carried out. In host partners, the mean activities of all 3 hepatic enzymes changed in the expected direction; however, only the decrease in arginase was statistically significant. A more prolonged experiment, that would amplify the responses in the host partner, was precluded by the morbidity of the rats after the 22nd day.

Discussion. Increased rates of hepatic DNA synthesis in rats cross-circulated with partially hepatectomized partners provided evidence for the humoral mediation of the stimulus for liver regeneration (8). The present observations on the liver and spleen of the tumor-free partner of parabiotic lymphoma-bearing rats suggest that the effects of tumors on host organs are also humorally mediated. The responses in the partner, as judged by the hepatic and splenic enzyme levels, occurred with delay; the effector(s), which may be unstable, probably could not attain as high concentrations as in the parabiont carrying the tumor.

It has been demonstrated by Warren et al. (9) that rats subjected to lethal doses of ionizing radiation survive for longer periods if parabiosed with an untreated individual. The present observations indicate that parabiosis also provides some protection against the systemic effects of neoplasms: the hepatic enzyme changes in single hosts of the lymphoma occurred earlier and were more intense than in parabiotic hosts. The "protection" did not extend to the rise of thymidine kinase in spleen, nor did it prolong the survival time of the lymphoma- (or fibrosarcoma-) bearing parabionts beyond that of single hosts.

The implantation of fibrosarcoma, like that of the lymphoma, caused significant decreases in the levels of hepatic ornithine aminotransferase and arginase, and increases in thymidine kinase in single or parabiotic hosts. The smaller rise in thymidine kinase may indicate that the fibrosarcoma evokes a less rapid hepatic response than does the lymphoma. Further delays were expected in the host partner; however, due to the onset of morbidity, assays could not be postponed beyond the 22nd day after implantation. Nevertheless, the increase in mean value for thymidine kinase coupled with a decrease in those for ornithine aminotransferase, and a statistically significant loss in arginase, suggest that the host partner was also under the influence of the fibrosarcoma growing in its parabiosed partner. Thus, humoral mediation of the effect of tumors on host

liver is not unique for the lymphoma. The fibrosarcoma, unlike the lymphoma, has no tendency to disseminate cells, or metastasize from its primary site. Even in lymphoma-bearing rats, during the first two weeks of the post implantation period, no tumor cells were detectable in the host livers; changes in the levels of hepatic enzymes preceded by at least 7 days the appearance of lymphoma cells in blood (2). Thus, the systemic action of solid tumors is unlikely to depend on the release of whole tumor cells but rather on that of cell-free agents. Both cytoplasmic proteins (10, 11) and chromatin (12, 13) extracted from tumors have been reported to decrease the levels of hepatic catalase (12) and of an isozyme of pyruvate kinase (13). However, it is not clear whether these observations reflect upon the phenomenon studied by us, i.e. the "undifferentiation" of host organ by losses and increases in the levels of appropriate groups of enzymes (1).

Previous studies of lymphoma hosts (2), showing that the hepatic thymidine kinase level rose 4 days after implantation while in plasma the enzymes appeared no earlier than 14 days after implantation, were confirmed. It is noteworthy, that pyrroline-5-carboxylate reductase (EC 1.5.1.2) (not measured in the present study) also rose in plasma but its hepatic level was considerably decreased throughout the 4 to 17 days after implantation (2). These opposite changes indicate that the biochemical alterations in the two tissues cannot be attributed to a common mechanism. The appearance of thymidine kinase and pyrroline-5-carboxylate reductase in plasma, unique to lymphoma hosts, is due to their being released directly from this tumor (rich in both enzymes (2)) after it has become sizable. In contrast, the increases and decreases in hepatic enzymes in rats with different types of neoplasms, too small to be palpable, must be due to complex alterations of the synthetic machinery within the liver. Thus plasma thymidine kinase is conveyed to the tumor-free partner from the parabiotic host as a necessary consequence of the common circulation, whereas the changes in hepatic (or splenic) enzyme levels involve at least two necessary steps. These are 1) the transfer of the unknown

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systemic factors from the rat carrying the tumor and 2) the subsequent impact of these factors on the metabolism of tissues in the tumor-free partner.

Summary. A solid lymphoma was implanted into normal rats and into one partner of parabiotic pairs. Appreciable decreases in hepatic ornithine aminotransferase and arginase occurred about a week earlier (4 to 6 days after implantation) in single than in parabiotic hosts. By 18 to 21 days significant decreases in both enzymes were also apparent in the host partner. The hepatic thymidine kinase showed a 5-fold elevation in single hosts 4 days after implantation; by 14 days its levels were about 200 times above normal and had also risen in the parabiotic hosts (20-fold) and the host partners (4-fold). Implantation of fibrosarcoma caused qualitatively similar but generally less pronounced changes in the above 3 enzymes in livers of single hosts, parabiotic hosts and host partners.

The splenic thymidine kinase, 14 days after implantation, was increased from control levels of about 3 units/g to 50.6, 44.8 and 13.5 units/g in single hosts, parabiotic hosts and host partners, respectively. At later stages, 17 to 20 days after implantation, of the lymphoma, appreciable amounts of thymidine kinase appeared in the plasma: the units of activity per ml were 6.2 in single hosts, 0.79 in parabiotic hosts and 0.55 in host partners (control < 0.05).

The observations on the hepatic and splenic enzyme changes in parabiotic rats suggest that effects of neoplasms on distant host tissues are mediated by humoral factors. The less pronounced responses in parabiotic than in single hosts indicate that the tumor-free partner affords some "protection" against these systemic effects.

We thank Ms. R.N. Chute for performing the parabioses, Ms. C. Peirce for the tumor transplants, and Ms. S.M. Raper for the enzyme assays.

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Table I. Thymidine Kinase Activities in Lymphoma Host Tissues^a

Tissue	Days after implantation	Thymidine kinase, units/g. (mean $\pm$ S.D.)		
		Single	Parabiotic host	Host partner
Liver	0	0.25 $\pm$ 0.19 (5)	0.26 $\pm$ 0.15 (6)	0.26 $\pm$ 0.14 (6)
	4	1.75 $\pm$ 0.50 (3)	0.46 $\pm$ 0.04 (3)	0.26 $\pm$ 0.16 (3)
	14	6.25 $\pm$ 1.50 (3)	14.2, 0.52	1.5, 0.40
	17-21	78.25 $\pm$ 55.72 (15)	7.92 $\pm$ 5.60 (4)	1.05 $\pm$ 0.12 (4)
Spleen	0	2.98 $\pm$ 1.1 (4)	3.65 $\pm$ 1.8 (3)	3.55 $\pm$ 1.5 (3)
	14	---	6.63	3.06
	17-20	50.7 $\pm$ 14.0 (3)	44.8 $\pm$ 30.0 (3)	13.5 $\pm$ 3.5 (3)
Plasma	0	0.025 $\pm$ 0.01 (6)	0.08 $\pm$ 0.02 (3)	0.08 $\pm$ 0.02 (3)
	14	0.05 $\pm$ 0.05 (3)	0.14	0.13
	19	6.23 $\pm$ 3.7 (15)	0.79	0.55
Tissue weights, grams				
Liver	0-21	11.9 $\pm$ 1.4 (15)	10.67 $\pm$ 2.05 (12)	10.53 $\pm$ 1.98 (12)
Spleen	0	0.66 $\pm$ 0.09 (4)	0.68 $\pm$ 0.10 (3)	0.67 $\pm$ 0.11 (3)
	17-20	1.02 $\pm$ 0.52 (12)	0.95 $\pm$ 0.48 (3)	0.66 $\pm$ 0.07 (3)

^a Lymphoma was implanted subcutaneously into single rats and into one member of parabiotic pairs. The number of animals tested is in parentheses.

Table II. Enzyme Activities in Livers of Rats Carrying Fibrosarcoma^a

	Hepatic enzyme activities, units/g (mean $\pm$ S.D.)			
	Control	Single host	Parabiont	
			Host	Partner
Tumor weight, grams	None	34.3 $\pm$ 6.9	25.7 $\pm$ 17.1	None
Ornithine aminotransferase	3.24 $\pm$ 0.56 (5)	0.49* $\pm$ 0.08 (6)	0.79* $\pm$ 0.33 (4)	2.80 $\pm$ 0.75 (4)
Arginase	2344 $\pm$ 360 (5)	1408* $\pm$ 115 (6)	1376* $\pm$ 207 (4)	1809* $\pm$ 291 (4)
Thymidine kinase	0.32 $\pm$ 0.25 (16)	1.88* $\pm$ 2.42 (6)	3.40* $\pm$ 2.66 (4)	0.58 $\pm$ 0.30 (4)
	[0.12-0.69]	[0.23-6.70]	[0.56-5.77]	[0.22-0.94]

^aLivers (the number of animals is in parentheses) were analyzed 20 to 26 days after implantation. The range of thymidine kinase activities is given in square brackets. The significance of differences from "Control" were assessed by the Students' T test: asterisks indicate $p < 0.05$.

Legend

Fig. 1. Hepatic ornithine aminotransferase and arginase activities in parabionts with and without lymphoma. The hepatic activities of ornithine aminotransferase (1A) and arginase (1B) in single hosts (Δ), in parabiotic hosts (Δ), and host partners (O) were measured at the indicated days after lymphoma implantations. The points refer to single livers or to means of determinations on 3-6 animals (bracket = 1 S.D.). Values are expressed as % of normal controls which were 4.18 ± 1.0 and 2535 ± 316 units/g in single animals, and 3.30 ± 0.45 and 2081 ± 640 in parabionts, for ornithine aminotransferase and arginase, respectively.

Footnotes

¹This investigation was supported by Grant No. HD 04532 from the National Institute of Child Health and Human Development, DHEW and Grant No. CA 08676 from the National Cancer Institute, DHEW; by Biomedical Research Support Grant RR 05591 from the Division of Research Resources, DHEW; and by U.S Energy Research and Development Administration Contract EY-76-S-02-3017.A001 with the New England Deaconess Hospital.

²This is article from the Cancer Research Institute of the New England Deaconess Hospital.