

# Effect of Arginine on Tumor Growth in Rats\*

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The experiments reported in this paper were undertaken because of the apparently conflicting findings that arginine both stimulates (1, 7) and inhibits (2-5, 13) the growth of tumor tissue.

## EXPERIMENTAL

Solutions (at pH 7.4) of L-arginine monohydrochloride<sup>1</sup> (50 mg/ml in Experiments A and B [Table 1] and 100 mg/ml in Experiments C to H [Tables 1 and 2]) were injected subcutaneously into half of the 305 rats, weighing 200-250 gm., of the Long-Evans strain bearing subcutaneous implants of either the U.C.L.A. fibrosarcoma or the Jensen sarcoma. An approximately 75-mm. cube of tumor tissue was implanted by trocar at one ventral site per animal in Experiments D to H, two such sites in Experiment C, and four sites in Experiments A and B. The administration of arginine was begun at the time of tumor implantation in Experiments A and B but was delayed until the tumor became palpable in all other experiments. The control and experimental groups were matched as closely as possible in respect to the body weight of animals and size of tumors. The rats were maintained on Rockland pellets (Experiments A and B) or a modified Ershoff (12) diet (Experiments C to H). Body weight was determined periodically, and tumor size was estimated from caliper measurements.

## RESULTS

Some of the tumors (designated as "small") grew very slowly and tended to regress, while others (designated as "large") grew comparatively rapidly with no regression. At the end of the experiments the average size of the "large" tumors was 3-25 times that of the "small" tumors. Every tumor could be classed clearly in one of these categories. In experiments (A and B) with the

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<sup>1</sup> H. M. Chemical Company's C.P. product.

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U.C.L.A. fibrosarcoma the percentage of small (measurable and nonmeasurable) tumors increased following injections (starting the day of implantation) of 50 mg of arginine/rat/day. In Experiment A the incidence of small tumors was 30 per cent (26 of 87) in the treated rats, compared to 11 per cent (9 of 83) in the control animals. In Experiment B, 95.5 per cent (84 of 88) of the tumors were classified as small in the treated animals, compared to 77 per cent (54 of 70) in the controls.

On the other hand, 35 per cent (19 of 54) of the tumors were classified as large in rats following the injection (starting 6 days after implantation) of 50 mg of arginine/rat/day, compared to 19 per cent (11 of 58) in the controls. The large tumors grew more rapidly, and the small tumors regressed more rapidly in the treated than in the control rats. The results of Experiment D (under identical conditions) were the same as Experiment C in respect to the distribution of large and small tumors, although the two large tumors of the controls grew more rapidly than the large tumors in the treated rats.

In Experiments E to H (Table 2), 200-250 mg. of arginine was injected daily, starting 4-7 days after implantation of the Jensen sarcoma. At these levels of arginine most of the tumors grew slowly or regressed. It is of interest that the same tumor tissue and rats of the same age, grown from weaning on the same diet, were employed in the experiments with males (Experiment E) and females (Experiment F).

At the 50-mg. level, arginine caused no reduction in body weight (Experiments A-D, Table 1), whereas at the 250-mg. level (Experiment G) the average weight loss was 20 gm. in 5 days compared to the average weight gain of 0.66 gm. for the controls. There was no weight loss in Experiment H, and the depression of tumor growth was less than in Experiment G.

## DISCUSSION

The authors' observations that well established tumors of the types investigated were retarded or stimulated in growth by injections of arginine and

that the tumor implants generally were most sensitive to inhibition by arginine during the early period of growth support the view that arginine may be either a growth stimulator or retarder depending upon the conditions.

The concentration of "free" (but not total) amino acids may determine the response of normal and malignant tissues to administered amino acids. The report of Roberts and Frankel (15) that the concentration of "free" arginine was relatively high in normal and hyperplastic epidermis but negligible in epidermal tumors, and the observation of Greenstein *et al.* (9, 10) that the level of arginase is generally high in tumors compared to normal tissues, indicate that a medium low in "free" arginine may be conducive to tumor growth. Conversely, high levels of arginine in the cellular fluids may exert, directly or indirectly, an inhibiting effect on tumor growth.

There is evidence supporting the concept that arginase may play a role in tumor growth by con-

trolling the cellular supply of arginine and the concentration of arginine in the cellular fluids. Bach and Lasnitzki (1) found that the arginase activity was inversely related to the rate of growth of the mouse carcinoma 63 and that (*in vitro*) arginine stimulated mitosis of this carcinoma but not normal tissue. Arginase activity may be related to increased protein synthesis and increased utilization of arginine, since there have been reports of increased protein turnover (14) and reduced arginase activity (6, 9, 18) in the livers of tumor-bearing compared to those of normal rats. According to Klein and Ziese (11) the arginase activity and the concentration of "free" arginine in muscle were markedly enhanced by the implantation of a tumor in the host. On the other hand, Greenberg and Sassenrath (8) found no statistically-significant difference in the arginase content of plasma, muscle, or liver between control and tumor-bearing mice. These workers also reported that a high-potency preparation of

TABLE 1  
EFFECT OF ARGININE ON GROWTH OF THE U.C.L.A. FIBROSARCOMA

EXPERIMENT	DAYS AFTER IMPLANTATION	No. TUMORS*		DISTRIBUTION OF TUMORS											
		Control rats	Treated rats	SMALL			Treated rats			LARGE					
				Control rats	NMT†	MT‡	Area (cm. <sup>2</sup> )	NMT†	MT‡	Area (cm. <sup>2</sup> )	Control rats	NMT†	MT‡	Area (cm. <sup>2</sup> )	
A (females)§	14	83	87	8	1	0.6	12	14	1.3	0	74	3.1	1	60	2.6
	30	66	77	8	1	0.8	20	4	0.8	0	57	3.7	0	53	3.4
	37	60	60	9	0		20	2	0.5	0	51	13.5	0	38	15.3
B (males)§	12	70	88	20	24	2.0	28	56	1.9	0	16	3.1	0	4	1.1
	25	65	87	35	14	2.4	56	27	1.5	0	16	6.4	0	4	3.6
	36	40	67	27	7	1.1	64	0		0	6	17.5	0	3	10.0
C (females)¶	6	58	54	13	24	0.37	17	18	0.23	5	6	0.33	9	10	0.17
	10	58	54	4	43	0.38	10	25	0.34	2	9	0.43	0	19	0.53
	13	58	54	9	38	0.45	16	19	0.45	0	11	0.70	0	19	0.36
	17	56	54	17	28	0.41	22	13	0.63	0	11	1.15	0	19	1.77
	20	56	54	22	23	0.46	25	9	0.59	0	11	1.54	0	19	2.73
26	56	54	31	14	0.31	29	6	0.27	0	11	5.00	0	19	5.20	
D (females)¶	8	11	14	18	2	0.25	10	6	1.06	0	2	1.86	1	6	1.00
	20	11	14	13	7	0.61	10	6	0.73	0	2	7.60	0	7	3.74
	27	11	14	15	5	0.72	13	3	0.22	0	2	14.1	0	7	6.38

\* The total number of sites (NMT plus MT) decreased with time in some experiments due to loss of animals from tumor growth or ulceration and infection of the tumor.

† Number of nonmeasurable (area less than 0.15 cm<sup>2</sup>) tumor nodules.

‡ Number of measurable tumor nodules.

§ Injected with 50 mg of arginine/day starting on day of implantation.

¶ Injected with 50 mg of arginine/day starting on the 6th day after implantation.

TABLE 2  
EFFECT OF ARGININE ON GROWTH OF THE JENSEN SARCOMA

EXPERIMENT	DAYS AFTER IMPLANTATION	No. TUMORS		DISTRIBUTION OF TUMORS									
		Control rats	Treated rats	SMALL			Treated rats			LARGE			
				Control rats	NMT*	MT†	Area (cm. <sup>2</sup> )	NMT*	MT†	Area (cm. <sup>2</sup> )	Control rats	NMT†	MT‡
E (males)‡	15	21	22	6	6	0.87	7	6	0.66	9	6.4	9	3.5
F (females)‡	15	23	21	9	5	0.66	8	13	0.72	9	3.8	0	
G (females)§	12	9	9	0	0		0	0		9	6.0	9	4.4
H (females)¶	17	7	7	0	1	1.26	0	2	1.71	6	7.2	5	6.1

\* Number of nonmeasurable (area less than 0.15 cm<sup>2</sup>) tumor nodules.

† Number of measurable tumor nodules.

‡ Injected with 200 mg. of arginine starting on the 4th day after implantation.

§ Injected with 250 mg. of arginine starting on the 7th day after implantation.

¶ Injected with 250 mg. of arginine starting on the 6th day after implantation.

arginase had no significant effect on the content of "free" arginine in tumor or liver tissue, although it induced a marked (although transient) drop in the level of plasma arginine. The increase (20 per cent) in tumor growth following the injection of arginine was considered not to be statistically significant. The further observation of Greenberg and Sassenrath that continued injection of arginase failed to affect significantly the growth of a number of tumors is contrary to that of Vrat (16, 17) that the growth of a mammary carcinoma in mice was markedly inhibited and regression induced by intraperitoneal injection of arginase. It would appear that any effect of arginase and arginine may be ascribed to induced systemic changes rather than to direct action on tumor tissue. Any final conclusion on these problems seems to depend upon a critical evaluation of the experimental variables.

#### SUMMARY

The effect of subcutaneous injections of arginine on the growth of the U.C.L.A. fibrosarcoma and the Jensen sarcoma has been studied with male and female rats. Growth-stimulating and growth-inhibiting effects of arginine on these tumors have been observed depending upon the experimental conditions.

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