The Effects of the Administration of Ascorbic Acid and of Rutin on the Transplantability of a Hepatoma and on the Ascorbic Acid Levels of Mouse Organs*

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Hepatoma C954 calls forth a marked vascular response and has a limited host range.[1] Since ascorbic acid plays a role in the metabolism of vascular tissue, the effect of injected ascorbic acid on the growth of this tumor in resistant strains of mice was considered. To enhance the action of ascorbic acid, rutin was also administered to certain animals (1, 3).

MATERIALS AND METHODS

Hepatoma C954 was obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and was maintained in C57L(F_1) mice from this source, prior to transfer to other strains. Tumor from these mice was transferred subcutaneously into the axilla of C57BL, C57BR, DBA, and C57L(F_x) mice (obtained from the breeding of F_1 hybrids).

In preliminary experiments, the administration of ascorbic acid in an oily base effectively maintained a prolonged high blood level of ascorbic acid. Therefore, stable emulsions of the substances to be injected were prepared by mixing equal volumes of their aqueous solutions with Pendil, a commercial preparation of peanut oil, beeswax, and oxycholesterol. Mice were injected intramuscularly with 0.1 ml. of the oily suspensions containing saline solution, sodium ascorbate (25 mg/ml), rutin[2] (25 mg/ml), or a mixture of ascorbic acid and rutin, respectively. Injections were started at the time of implantation of the hepatoma and were repeated on alternate days. Tumor incidence and latent period were recorded. The preparation and extraction of the tissues prior to the determination of their ascorbic acid content was described in the preceding paper (2).

RESULTS

Transplantation of hepatoma C954.—Of forty transplants into C57L(F_1) mice, 38 hepatomas grew successfully. A small mass became palpable in 9–14 days and continued to grow rapidly, especially in a direction parallel to the long axis. Transplantation in the C57L(F_x) mice was less successful, and only 34.6 per cent of the transplants grew. Growths were palpable in from 8 to 14 days; they had the same gross and microscopic characteristics and ran the same course as tumors in F_1 hybrids. No growths resulted in twelve C57BL or in ten C57BR mice. In eight of the fourteen DBA mice into which transfers were made, very small palpable growths appeared in 10–12 days, but these remained small and regressed about 4 days later.

The effects of the administration of ascorbic acid, or rutin, and of a combination of ascorbic acid and rutin on the growth of hepatoma C954 can be seen in Table 1. In C57L(F_1) hybrids injected with ascorbic acid, tumors were palpable 18–21 days after they were implanted. In the control groups, the latent period varied from 9 to 12 days. There were no differences in the number of successful transplants, in the gross and microscopic appearance, or in the subsequent course of the tumors in these two groups. This was also true for the group treated with the combination of ascorbic acid and rutin, although palpable tumors were frequently noted on the eighth day after transfer.

In C57L(F_x) hybrids, the incidence of tumor takes rose from 35 per cent in the control group treated only with the oily suspension of saline, and 37 per cent in the group that was treated with rutin, to 52 per cent in the group that received ascorbic acid, and 80 per cent in the group injected with the combination of ascorbic acid and rutin. Tumors in this last group also appeared to have a slightly shorter latent period.

Ascorbic acid alone, rutin alone, or the combination of these substances were without effect in altering the resistance of DBA and C57BR mice to hepatoma growths. In the group of six C57BL mice that was treated with ascorbic acid and rutin, there were four growths of hepatoma which be-

* This work was supported by grants from the American Cancer Society, the Jane Coffin Childs Memorial Fund for Medical Research, and the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

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[1] Personal communication from Dr. Elizabeth S. Russell, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

[2] Rutin used in these experiments was very kindly furnished by the Squibb Co., New York, N.Y.

Received for publication October 10, 1951.

came palpable 19 days after transfer. Transplants did not grow in any of the other groups of C57BL mice.

Tissue levels of ascorbic acid.—The ascorbic acid concentrations in the tissues of normal and tumor-bearing C57L(F_1) hybrids are recorded in Table 2. The growth of hepatoma transplants in F_1 mice appeared to have little or no effect on the ascorbic acid concentration in the adrenal or liver. Tumor growth brought about a reduction of about 15 per cent in the blood, a smaller decrease in the brain, and a decrease of 21 per cent in kidney ascorbic acid concentration. The greatest decrease (35 per cent) occurred in the ascorbic acid of the spleen. The probability that such a decrease could have occurred by chance is less than one in a thousand.

Following treatment with ascorbic acid, there was an increase in the ascorbic acid content of the blood, kidney, and liver. The administration of ascorbic acid, however, did not prevent a decreased concentration in the spleens of tumor-bearing F_1 hybrids.

Blood levels of ascorbic acid in C57L(F_1) mice with tumors were increased to the same extent in the groups treated with both ascorbic acid and rutin as in the group treated with ascorbic acid alone. An increase in the ascorbic acid content of the adrenal, however, was observed only in the groups treated with the combination of rutin and ascorbic acid. The ascorbic acid levels in tumors from both groups that received ascorbic acid were 20 per cent higher than in tumors from untreated groups. The administration of these substances did not prevent the decrease in spleen ascorbic acid concentration observed in tumor-bearing mice. This decrease of 38 per cent is statistically significant.

The tissue ascorbic acid levels of C57L(F_x) mice are recorded in Table 3. The levels in normal organs were the same as in the F_1 hybrids. In untreated F_x hybrids, growth of tumors was associated with little change in tissue ascorbic acid concentration. In those mice treated with ascorbic acid, there was a decrease in the ascorbic acid content of the spleen when tumors grew. This de-

TABLE 1

THE EFFECT OF ASCORBIC ACID AND RUTIN ON THE TRANSPLANTABILITY OF HEPATOMA C954

Strain of mice	No. injections of 0.1 ml.* (Oil suspensions) Saline	Ascorbic acid	Rutin	Ascorbic acid and rutin	Takes/no. of transplants	Latent period (days)
C57L (F_1)					23/25	9–14
	2				10/10	10–12
	6				5/5	10–12
					38/40 (total)	
		4			6/6	21
		6			4/5	18
					10/11 (total)	
				2	6/6	8–13
				4	3/3	8–13
				6	6/6	9–11
				9	6/6	8–13
					21/21 (total)	
C57L (F_x)					4/10	8–12
	2				2/10	12–13
	6				3/6	10–13
					9/26 (total)	
		2			5/6	10–12
		3			3/10	12–14
		6			6/8	14–18
		10			4/10	10–12
					18/38 (total)	
			2		6/7	9–11
			6		2/10	10–12
			10		2/10	10–13
					10/27 (total)	
				2	6/6	8–10
				3	9/10	8–10
				4	5/8	8–9
				5	6/7	11–13
				6	6/6	6–10
				8	3/9	9
				10	10/10	9–12
					45/56 (total)	

* Ascorbic acid suspension contained 25 mg/ml.
Rutin suspension contained 25 mg/ml.
Rutin and ascorbic acid suspension contained 25 mg/ml of each.

creased level was observed in animals killed 24 hours and 1 week after the last injection of ascorbic acid. In another experiment, this lowered ascorbic acid concentration in the spleen of tumor-bearing C57L(F_x) mice was observed as long as 6 weeks after the last injection of ascorbic acid. The spleens of two tumor-bearing mice in the rutin-treated group did not show this decrease. In resistant mice, the ascorbic acid content of the spleen was either normal or elevated. The average spleen level in all C57L(F_x) mice in which hepatomas grew was 34.0 ± 3.15 mg of ascorbic acid/100 gm of wet tissue; in spleens of resistant mice in all groups it was 56.1 ± 2.90 mg/100 gm of tissue. The probability that such a difference could have occurred by chance is less than one in a thousand. While the highest ascorbic acid concentration of the spleen (65.8 mg/100 gm) occurred in the one resistant mouse that was treated with ascorbic acid and rutin, the average ascorbic acid level of the spleens of eight resistant mice in the control group injected with only the oil-saline suspension was similar (64.4 mg/100 gm).

The ascorbic acid content of the livers of mice in all groups in which hepatomas grew was slightly higher than normal. When implanted hepatomas failed to grow, the administration of ascorbic acid or rutin gave rise to definitely increased levels in the liver. The ascorbic acid content of the adrenals in treated and untreated C57L(F_x) mice was variable.

Table 4 shows the results of an experiment with C57BL mice. There were four groups, consisting of six mice in each group. All received subcutaneous

TABLE 2

ASCORBIC ACID LEVELS IN TISSUES OF C57L (F_1) MICE

	Blood (mg/100 ml)	Adrenal (mg/100 gm*)	Spleen (mg/100 gm)	Liver (mg/100 gm)	Kidney (mg/100 gm)	Brain (mg/100 gm)	Hepatoma (mg/100 gm)
Normal Mice	1.19±0.02†	155.0±11.5	49.2±1.57	27.4±1.73	19.1±0.77	56.2±1.02	
Mice with hepatoma transplants for 14–18 days	1.01±0.03 (0.01–0.001)‡	162.7±30.5 (>0.5)	31.8±0.63 (<0.001)	30.4±2.68 (0.5–0.2)	15.1±1.20 (0.05–0.02)	47.8±3.08 (0.05–0.02)	8.5±0.34
Mice with hepatoma transplants also injected with ascorbic acid§	1.48±0.04 (0.05–0.02)	108.7±29.9 (0.5–0.2)	28.9±1.43 (<0.001)	40.1±1.84 (0.01–0.001)	31.2±2.01 (0.02–0.01)		10.1±0.52
Mice with hepatoma transplants also injected with ascorbic acid and rutin§	1.52±0.10 (0.05–0.02)	237.2±36.5 (0.1–0.05)	30.7±1.00 (<0.001)	29.5±2.68 (>0.5)	19.2±1.21 (N.C.)#	51.1±2.61 (0.2–0.1)	10.4±0.94

* Wet tissue.
† Standard error of the mean.
‡ Probability that deviation from normal could have occurred by chance.
§ Animals sacrificed two weeks after the last injection.
# No change.

TABLE 3

ASCORBIC ACID LEVELS OF TISSUES OF C57L (F_x) MICE

Treatment of mice	Time of sacrifice*	Blood (mg/100 ml)	Adrenal (mg/100 gm†)	Liver (mg/100 gm)	Kidney (mg/100 gm)	Spleen (mg/100 gm)	Tumor (mg/100 gm)
Normal controls		0.98	153.9	26.5	16.2	48.7	
Received hepatoma transplants:							
Transplants grew							
Injected with saline‡	24 hrs.			32.7	16.2	45.3	27.0
Injected with ascorbic acid‡	24 hrs.			31.5	18.1	29.6	18.5
Injected with rutin‡	24 hrs.		293.1	39.7	17.5	44.1	16.4
Injected with ascorbic acid and rutin	24 hrs.		123.8	31.2	15.5	31.0	11.3
Injected with ascorbic acid	1 wk.	1.14	118.9	27.3	13.1	28.4	8.2
Injected with ascorbic acid and rutin	1 wk.	1.48	172.1	35.7	16.0	25.7	8.7
Injected with ascorbic acid	6 wks.		140.0	29.6	11.0	26.8	3.2
Transplants did not take							
Injected with ascorbic acid	24 hrs.	0.99	151.2	42.9	15.4	55.1	
Injected with rutin	24 hrs.	1.62	149.4	45.9	36.8	45.8	
Injected with saline	1 wk.		75.4	30.2	12.1	64.4	
Injected with ascorbic acid	1 wk.	0.83	286.3	44.0	19.7	54.5	
Injected with rutin	1 wk.	1.14	163.7	42.4	14.6	50.6	
Injected with ascorbic acid and rutin	1 wk.		72.1	43.3	22.8	65.8	

* Time after the last injection.
† Weight of wet tissue.
‡ All materials were injected as oil suspensions. Ascorbic acid and rutin preparations contained 25 mg/ml.

transfers of hepatoma. One group received the oily suspension of saline, the second received ascorbic acid, the third received rutin, and the fourth the combined rutin and ascorbic acid in the same vehicle. Each group was given nine injections on alternate days. In the fourth group, 21 days after the transfers were made, there were four palpable tumors. The animals were all sacrificed 10 days after the last injection and the tissue content of ascorbic acid determined. In the fourth group, the tissues of only tumor-bearing mice were analyzed. In all mice, the levels of ascorbic acid in the liver and brain remained unchanged. Adrenal ascorbic acid was somewhat decreased in the oil-saline suspension and rutin groups, even though implanted tumors did not grow. In the rutin–ascorbic acid-treated group, the ascorbic acid concentrations in the adrenal, kidney, liver, and brain of mice with substance in the spleens of mice in which transplants did not take, suggests a link between the metabolic activities of this organ and the fate of tumor transplants.

The fact that rutin and ascorbic acid did not alter the resistance of DBA and C57BR mice to the growth of hepatoma transplants is further evidence that these substances did not influence the tumor directly. At least in C57L mice there appeared to be a modifying influence necessary for the growth of hepatomas which was also present in F_1 hybrids but which was lost in a large per cent of their progeny. This influence appeared to be restored by the administration of rutin and ascorbic acid. Whether the administration of these substances merely replaced a necessary intermediate or provided a specific stimulus is unanswered by these experiments.

TABLE 4

ASCORBIC ACID LEVELS OF TISSUES OF C57 BLACK MICE

Mice	Ascorbic acid (mg/100 gm wet tissue)					
	Blood	Adrenal	Spleen	Liver	Kidney	Brain
Normal controls	1.27	231.5	45.7	30.7	19.1	54.2
Received hepatoma transplants* and:						
Saline†	1.19	189.5	57.6	28.6	19.4	58.0
Ascorbic acid†	0.92		51.7	31.7	8.2	56.5
Rutin†	1.26	187.1	47.8	35.7	14.6	52.8
Ascorbic acid and rutin†	1.06	276.3	40.6	33.5	22.4	55.3

* Transplants grew only in the groups treated with ascorbic acid and rutin.

† All materials were injected as oil suspensions. Ascorbic acid and rutin preparations contained 25 mg/ml.

hepatomas were normal or slightly elevated, while the spleen levels in these mice were lower than normal. The concentrations of ascorbic acid in the spleens of resistant mice in the three other groups were higher than in normal spleens, just as in resistant C57L(F_x) mice.

DISCUSSION

The change in resistance to the growth of hepatoma transplants resulting from the administration of rutin and ascorbic acid does not appear to be brought about simply by the direct action of ascorbic acid. Their own endogenous supply of ascorbic acid, supplemented by rutin, did not alter the response of C57L(F_x) mice to hepatoma transplants. It would seem unlikely, therefore, that prolonging the action of ascorbic acid was the sole factor involved in the breakdown of tumor resistance. These substances did not appear to exert their influence directly on the transplant but did seem to affect particular organs which, in turn, might have influenced the growing tissue. For example, the pronounced decrease in spleen ascorbic acid during the growth of hepatoma transplants, even when there was an adequate exogenous supply, as well as the pronounced rise in the concentration of this

SUMMARY

The administration of ascorbic acid, combined with rutin, increased the number of takes of hepatoma transplants in a resistant group of leaden hybrids from 35 to 80 per cent. The resistance of C57BL mice to this tumor was also decreased by this combination of substances. The administration of ascorbic acid alone or of rutin alone was without effect.

In mice in which hepatoma transplants grew, there was a marked decrease in the ascorbic acid concentration in the spleen, even when additional ascorbic acid was given to the animal. In mice which resisted the growth of the hepatoma, the level of ascorbic acid in the spleen was high.

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