

The Effects of Tumor Growth on the Ascorbic Acid Concentration of Mouse Organs*

ESTHER MACULLA LEISE,† ELIZABETH K. HARVEY,
AND AUDREY B. SCHWANFELDER

(Department of Pathology, Yale University School of Medicine, New Haven, Conn.)

A relationship between ascorbic acid and tumor growth has been considered by many investigators (1). It has been demonstrated that this vitamin stimulated the growth of some tumors, depressed the growth of others, and had no influence on still other tumors. Much of this work has been carried out on the mouse, a species capable of synthesizing ascorbic acid. In these animals, the increased demand for ascorbic acid or the increased utilization of this substance may not be associated with any obvious deficiency symptoms. It seemed possible, however, that the observed variation in biological reaction might be associated with differences in tissue ascorbic acid concentration. The present study deals with the effects of transplantable tumors on the concentration of ascorbic acid in the tissues of the host.

MATERIALS AND METHODS

Five strains of mice from our own laboratory stock and a sixth, the C57L(F₁) hybrid, purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used in these experiments. Mice obtained from the breeding of C57L(F₂) hybrids were designated C57L(F_x) mice.

The tumors included MT-8, a mouse bronchogenic carcinoma (4); 755, a mouse mammary carcinoma; HC-330,¹ a hypernephroma from a human source; and the Brown-Pearce rabbit carcinoma (3). All tumors were transplanted subcutaneously into the axilla of mice of the appropriate strain. The mice were sacrificed 14–21 days after the transplantation of the tumors. Transfers of MT-8 and 755 were always successful, and tumors grew to a large size. The percentage of successful transplants of HC-330 was low, and the tumors that grew remained small. Brown-Pearce transplants grew in DBA mice, but in comparable periods of time they did not achieve the size of MT-8 or of 755 transplants.

Mice were anesthetized lightly with ether, bled by cardiac puncture, and dissected. Immediately upon dissection, the organs were placed in Petri dishes set in dry ice. Until analyses

* 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.

† Present address: Department of Medicine, Georgetown University Medical Center, Washington 7, D.C.

¹ Tumor HC-330 was obtained from Prof. Harry S. N. Greene as a growth from the anterior chamber of the guinea pig eye.

could be carried out, these were kept in sealed containers in a –60° C. refrigerator.

For the determination of ascorbic acid, the various tissues were weighed, extracted with cold 4 per cent trichloroacetic acid by grinding in a Ten Broeck tissue grinder which was kept in an ice bath, diluted to suitable volume, centrifuged, and then filtered. The analyses were carried out on aliquots of these filtrates by the method of Roe and Kuether (7). Analyses were done on individual livers and brains, but blood, adrenals, spleens and kidneys from four to six mice were pooled for more accurate analysis.

RESULTS

Table 1 contains a summary of the tissue levels of ascorbic acid in different strains of mice. Strain differences have been reported by other workers, and some are apparent here (2, 5). Except for a lower concentration of ascorbic acid in the kidneys of DBA mice, the differences in the concentrations of ascorbic acid in blood, liver, and brain were slight. An outstanding example of strain difference, however, was the concentration of ascorbic acid in the adrenal. The average values ranged from 87.2 mg/100 gm in the adrenals of C3H and C57BR mice to 231.5 mg/100 gm in C57BL mice. Leaden hybrids had adrenal values intermediate between these two. While most tissue ascorbic acid levels of C57BR mice were close to those of C57BL mice, the concentration in the adrenals of the brown mice was 37.7 per cent of that in the black mice. C57L(F₁) and F_x hybrids, which were originally derived from C57 strain mice, possessed high spleen ascorbic acid levels similar to C57 black and brown mice.

The ascorbic acid levels in comparable organs of DBA and C3H mice paralleled one another closely but were generally lower than the same organs from C57 mice. The most significant differences appeared to be the lower concentrations of ascorbic acid in adrenal and spleen.

The effects of subcutaneous transplantation of tumors on blood and tissue ascorbic acid levels are shown in Table 2. The ascorbic acid levels in all tissues of tumor-bearing DBA and C3H mice were the same or higher than those in comparable normal mice. Increases of the greatest magnitude occurred in the kidneys and livers of the DBA mice.

Received for publication October 10, 1951.

TABLE 1
TISSUE ASCORBIC ACID LEVELS IN NORMAL MICE OF DIFFERENT STRAINS
(MG/100 GM WET TISSUE)

Strain	Blood	Adrenal	Spleen	Liver	Kidney	Brain
DBA	0.94±0.07*	127.4±12.4	34.4±2.21	26.2±1.60	11.8±0.92	50.4±1.06
C57H	1.19±0.05	87.2±9.49	37.4±3.34	27.5±1.85	14.8±0.43	52.0±1.46
C57BL	1.27±0.06	231.5±8.41	45.7±3.22	30.7±1.59	19.1±0.99	54.2±0.94
C57BR	1.12±0.08	87.2±17.5	43.9±1.75	25.2±0.46	16.5±0.61	56.4±1.08
C57L (F ₁)	1.19±0.02	155.0±11.5	49.2±1.57	27.4±1.73	19.1±0.77	56.2±1.02
C57L (F ₂)	0.98	153.9	48.7	26.5±0.55	16.2	53.3±0.91

* Standard error of the mean.

TABLE 2
TISSUE ASCORBIC ACID LEVELS IN MICE WITH TUMORS

Mouse strains	Blood (mg/100 ml)	P*	Adrenal (mg/100 gm)	P	Spleen (mg/100 gm)	P	Liver (mg/100 gm)	P	Kidney (mg/100 gm)	P	Tumor (mg/100 gm)
DBA:											
Normal (no tumors)	0.94±0.07†		127.4±12.4		34.4±2.21		26.2±1.60		11.8±0.92		18.1±2.7
With MT-8 transplants	1.43±0.23	0.05-0.02	169.7±22.4	0.2-0.1	36.3±0.68	>0.5	37.0±2.24	0.01-0.001	18.4±1.31	0.01-0.001	35.9±3.3
With Brown-Pearce transplants	1.16±0.08	0.1-0.05	127.4±27.2	N.C.	35.6±1.93	>0.50	37.2±1.63	<0.001	18.4±2.46	0.02-0.01	
With HC-330 transplants	1.59	‡	173.9		38.3		33.2		17.3		
C57H:											
Normal (no tumors)	1.19±0.05		87.2±9.5		37.4±3.34		27.5±1.85		14.8±0.43		
With MT-8 transplants	1.17±0.13	>0.5	124.2±14.5	0.1-0.05	39.2±3.48	>0.5	35.4±4.40	0.2-0.1	17.4±0.66	0.01-0.001	21.0±1.1
With spontaneous mammary tumors			238.8	<0.001	33.4	>0.5	25.9	>0.5	18.7	0.02-0.01	43.4±7.6
C57BL:											
Normal (no tumors)	1.27±0.06		231.5±8.4		45.7±3.22		30.7±1.59		19.1±0.99		
With 755 transplants	1.64		98.3±8.1	<0.001	29.7	0.05-0.02	23.0	<0.001	9.2	<0.001	36.4±3.1

* Probability that deviation from normal could have occurred by chance; derived from the Student "t" test.

† Standard error of mean.

‡ Number of analyses done too few for statistical analysis.

N.C. = No change.

The adrenal levels were also often increased but with relatively wide variations. The increase of 42.2 per cent in the ascorbic acid content of the adrenals of C3H mice bearing MT-8 transplants takes on added significance, however, in light of the threefold increase observed in the adrenals of C3H mice bearing spontaneous mammary carcinomas. Adrenal ascorbic acid levels in DBA mice bearing MT-8 transplants were also increased, as were those in the group bearing HC-330. Brown-Pearce tumor transplants did not exert this effect.

The ascorbic acid levels of C57BL mice, which normally possessed the highest tissue levels, were affected differently by tumor transplants than the other two strains of mice. Except for the increased blood level of mice bearing tumor 755, the over-all decrease in the ascorbic acid concentrations of all tissues analyzed was of considerable magnitude.

The content of ascorbic acid in the different tumors varied. The tumor containing the lowest ascorbic acid concentration was MT-8, and the tumor containing the highest concentration was the spontaneous mammary tumor. There was no apparent correlation between the level of ascorbic acid in the tumor and in the tissues. Growth in DBA or C3H strain mice did not alter the ascorbic acid concentration in MT-8.

DISCUSSION

It does not appear that changes in the hosts' stores of ascorbic acid were directly associated with the nutritional demands of the tumor, for the extent of depletion or of increased production of the vitamin could not be correlated with the growth rate or size of the tumor. Tumor 755 grows as rapidly in C57BL mice as MT-8 grows in DBA or C3H mice, yet tumor 755 induced a generalized decrease in the ascorbic acid of the hosts' tissues, while the growth of MT-8 lead to increased levels.

The alteration in tissue ascorbic acid levels associated with tumor growth might have been an indirect response to nutritional or metabolic requirements of the tumor. Thus, the Brown-Pearce tumor produced increases in the ascorbic acid content of the kidney and liver but not of the adrenal. Growth in DBA mice was slow, and the tumors remained small. Greene (3) has reported that this tumor can also be transplanted to normal C3H mice but that takes are rare and growths small. In C3H mice bearing spontaneous mammary tumors, however, he obtained 100 per cent takes, and the tumors grew to a large size. In the present experiments, the adrenals of C3H mice bearing spontaneous mammary tumors contained very high concentrations of ascorbic acid. It is possible that the presence of increased amounts of ascorbic

acid in the adrenal is an indicator of changes in other factors that influence the tumor directly. From the work of Sayers *et al.* (8) and Long (6), it is now acknowledged that the level of ascorbic acid in the adrenal and possibly in other tissues is controlled by hormones from this gland and from the pituitary. The variations in tissue ascorbic acid that occur during the growth of tumors may, therefore, be the result of alterations induced in pituitary-adrenal balance.

SUMMARY

The ascorbic acid levels of normal organs of six strains of mice were compared. The concentration in the spleens of C57 mice and in related strains appeared to be higher than in DBA or C3H mice. The concentration of ascorbic acid in the adrenals of C57BL mice was the highest of the six strains examined. The concentration of ascorbic acid in the kidneys of DBA mice was lower than in the five other strains. The amounts in blood, liver, and brain were similar in all six strains.

Increases of varying degrees were observed in the ascorbic acid concentrations in kidneys and livers of DBA and C3H mice bearing transplants of a mouse bronchogenic carcinoma, the Brown-Pearce rabbit carcinoma, or a human hypernephroma. Adrenals as well as kidneys of C3H mice bearing spontaneous mammary carcinomas possessed significantly increased amounts of ascorbic acid. In C57BL mice, transplants of mammary carcinoma 755 induced decreases in tissue ascorbic acid levels.

REFERENCES

1. BURKE, D., and WINZLER, R. J. Vitamins and Cancer. *Vitamins & Hormones*, **2**:322-24, 1944.
2. CHITRE, R. G.; AMBEGAOKAR, S. D.; and JOSHI, N. G. Nicotinic Acid, Riboflavin, Ascorbic Acid and Glutathione Contents of Livers of Some Cancer-susceptible (A and C3H) and Cancer-insusceptible C57 Strains of Mice. *Ann. Biochem. Exper. Med.*, **4**:87-92, 1944.
3. GREENE, H. S. N. Heterologous Transplantation of Brown-Pearce Tumor. *Cancer Research*, **9**:728-35, 1949.
4. GREENE, H. S. N., and MURPHY, E. D. The Heterologous Transplantation of Mouse and Rat Tumors. *Cancer Research*, **5**:269-82, 1945.
5. KENNAWAY, E. L.; KENNAWAY, N. M.; and WARREN, F. L. The Ascorbic Acid Content of the Liver in Mice. *Cancer Research*, **4**:245-50, 1944.
6. LONG, C. N. H. *Recent Progress in Hormone Research*, **1**:99-122. New York: Academic Press, Inc., 1947.
7. ROE, J. H., and KUETHER, C. A. The Determination of Ascorbic Acid in Whole Blood and Urine through the 2,4-Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid. *J. Biol. Chem.*, **147**:399-407, 1943.
8. SAYERS, G.; SAYERS, M. A.; TSAN YING LIANG; and LONG, C. N. H. The Effect of Pituitary Adrenotropic Hormone on the Cholesterol and Ascorbic Acid Content of the Adrenal of the Rat and of the Guinea Pig. *Endocrinology*, **38**:1-9, 1946.