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Differences in Vitamin E Levels in Tissues of the Spontaneously Hypertensive and Wistar–Kyoto Rats (41560)

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Abstract. Tissue vitamin E levels were significantly lower in spontaneously hypertensive rats (SHR) than in the normotensive, genetically related Wistar/Kyoto (W/K). This difference was also observed when animals were given identical oral doses of vitamin E. The possible relationship of lower vitamin E tissue levels to lower immune responses in the SHR is discussed.

The spontaneously hypertensive rat (SHR) strain was derived from the Wistar–Kyoto (W/K) by Okamato and Aoki (1). It is characterized by a progressive increase in the systolic blood pressure in male animals from 130 mm Hg at weaning to 200 mm Hg by 12–15 weeks of age. In addition, the SHR has recently been characterized as immunosuppressed compared to the W/K, having depressed thymic rosetting and splenic lymphocyte mitogen responses (2–7).

An initial report (8) has suggested that vitamin E levels are lower in plasma and platelets of the SHR compared to the W/K rat. Since vitamin E has been shown to stimulate the immune system by increasing mitogen responses and antibody production (9), and to enhance peripheral circulation (10), the vitamin E concentration in several tissues of the male SHR and W/K fed identical diets at two different ages were determined.

In this paper, we extend our earlier findings of significantly reduced levels of tocopherol in the spleen, thymus, and liver of the SHR as compared to W/K consuming a chow diet (11). Additionally, plasma and tissue levels of vitamin E were measured following the administration of identical oral doses of vitamin E to SHR and W/K.

Materials and Methods. Groups of 5- and 10-week-old male SHR (n = 6) and W/K (n = 6) were purchased from Taconic Farms.[2] The animals were maintained on Purina rodent feed[3] and tap water *ad libitum* on a 12-hr light/dark cycle. Blood pressures were taken by indirect tail cuff method (12) and body weights recorded. The 5-week-old group was sacrificed at 6 weeks of age, and the 10-week-old group at 16 weeks of age. Blood pressures and body weights were measured prior to sacrifice. The animals were anesthetized with Metofane[4] and blood collected by cardiac puncture. Spleen, thymus, heart, and liver were removed from 6-week-old animals, and these organs, as well as testes, kidneys, adrenals, cerebrum, spinal cord, and perirenal fat were removed from the 16-week-old animals.

In order to determine whether the difference in tissue tocopherol levels observed in the SHR and W/K were due to tocopherol intake (absorption), weanling male SHR and W/K were fed a vitamin E-deficient diet (described by Draper (13) and modified by substituting 10% stripped lard for corn oil) for 6 weeks. The rats were then dosed orally with 4, 10, or 40 mg/kg body weight *dl*-α-tocopheryl acetate dissolved in stripped corn oil for 4 days. On the fifth day the animals were sacrificed and plasma and liver vitamin E levels were determined.

Vitamin E analysis. Plasma, heart, testes, and kidneys were analyzed fluorometrically by a modification of the method described by Taylor *et al.* (14). During the course of the analysis we developed a method using high-pressure liquid chromatography (HPLC) which proved to be very specific and reliable, with excellent recoveries and reproducibility. We therefore decided to analyze all the re-

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[4] Methoxyflurane inhalation anesthetic, Pitman–Moore, Washington Crossing, N.J.

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TABLE I. BODY WEIGHTS AND BLOOD PRESSURE OF SHR AND W/K AT 6 AND 16 WEEKS OF AGE[a]

Strain	Age	Body weight (g)	Blood pressure (mm Hg)
W/K	6	135 ± 4	94 ± 12
SHR	6	96 ± 3	173 ± 10
W/K	16	320 ± 8	126 ± 6
SHR	16	324 ± 10	197 ± 2

[a] Data represent the mean ± SE of all measurements made.

maining tissues by HPLC as follows: A 0.5- to 1.0-g sample of each tissue was homogenized and extracted two times with 10–20 volumes of HPLC grade acetone.[5] After centrifugation, the supernatant was removed and evaporated to dryness under nitrogen in a 30°C water bath. The residue was resuspended in redistilled ethanol and 20 μl was applied to a Waters μ Bondapak C_{18} 3.9-mm × 30-cm column. The HPLC method was a modification of one described by Hatam and Kayden (15). The solvent system was 97% methanol in water. The column effluent was monitored at an excitation of 205 nm with an emission filter of 340 nm using a model GM 970 fluorometer.[6]

We compared the results of both methods and found that fluorometry consistently gave higher values than HPLC probably due to nonspecific fluorescing substances in the sample. However, the differences in vitamin E levels between the SHR and W/K were of the same magnitude. We therefore feel that although the absolute numbers may differ, the general conclusion that SHR have lower vitamin E tissue levels than W/K consuming rodent feed is valid.

Results. At 6 weeks of age, the SHR were already hypertensive, having an average blood pressure above 150 mm Hg. By 16 weeks of age, the pressure had increased to 197 mm Hg. At both ages, the W/K remained normotensive (Table I).

In all tissues analyzed, with the exception of plasma, the level of vitamin E of the SHR was lower than that of the W/K at both ages (Tables II and III). Differences in vitamin E levels of the lymphoid organs were most striking at 6 weeks of age, when the levels of vitamin E in the thymus and spleen of the SHR were approximately one half that of the W/K.

Following the oral administration of 4, 10, and 40 mg/kg body weight *dl*-α-tocopheryl acetate for 4 days to SHR and W/K fed the vitamin E-deficient diet (13) for 6 weeks, the plasma and liver tocopherol levels were lower in the SHR than in W/K at all three dosage levels administered (Fig. 1). The increase in plasma tocopherol levels in both strains was directly proportional to the logarithm of the dose administered. This relationship was not completely linear in the liver.

Discussion. The results in this study raise two interesting questions. (1) Why is there a difference in vitamin E content in the tissues of the SHR and W/K even though they are fed identical diets? (2) Is the lower tocopherol content in the tissues of the SHR related to the hypertension and/or impaired immunocompetence in these animals?

When vitamin E-depleted SHR and W/K rats were given identical doses of vitamin E orally (Fig. 1), the levels of vitamin E in the plasma and liver of the SHR were significantly lower than those found in the W/K rats. This observation rules out the possibility that the difference in vitamin E tissue levels were a consequence of differences in intake of vitamin E in rats fed *ad libitum*. It also suggests that there may be some defect in absorption of vitamin E in the SHR compared to the W/K. However, differences in blood or tissue levels can not unequivocally establish an absorptive defect. In addition, since a difference in plasma tocopherol levels was not

TABLE II. VITAMIN E CONCENTRATION IN THE TISSUES OF SHR AND W/K AT 6 WEEKS OF AGE

Tissue	α-tocopherol (μg/g) W/K	α-tocopherol (μg/g) SHR	
Plasma	4.7 ± 0.1[a]	3.6 ± 0.3[a]	N.S.
Spleen	26.5 ± 0.6	17.0 ± 0.9	$P < 0.001$
Thymus	9.7 ± 0.8	5.1 ± 0.3	$P < 0.001$
Heart	17.9 ± 0.9	13.0 ± 0.5	$P < 0.05$
Liver	10.2 ± 0.7	7.4 ± 0.2	$P < 0.001$

[a] μg/ml.

[5] Csallany, A., personal communication.

[6] Schoeffel Instrument Corp., Westwood, N.J.

seen when SHR and W/K were not depleted of vitamin E, interference with vitamin E absorption may not be the only explanation.

Other possible explanations for this difference may be the rate of transport of tocopherol through the plasma membrane, the subsequent transport through the cytosol and deposition into the subcellular membraneous structures, or rate of utilization. For example, tocopherol-binding proteins, which facilitate transport of tocopherol into mitochondria (16), microsomes (17), and nuclei (18), have been identified. Perhaps the SHR have a defect in such transport proteins. The retention of α-tocopherol in membranes could also influence the total tocopherol concentration in the cell. Alterations in the fatty acid composition of membrane phospholipid might influence uptake and retention of α-tocopherol (19). Thus, the nature of lipid in the cells of the tissues of the SHR might also influence the total tocopherol concentration.

Administration of vitamin E had no effect on hypertension in one study (20), but prevented the blood pressure increase in salt-loaded SHR animals in another (21). In neither report was there any effort to relate vitamin E tissue levels to the observed effects. It is of interest that administration of high levels of vitamin E to adult-onset diabetics did result in a small reduction in blood pressure (22).

TABLE III. VITAMIN E CONCENTRATION IN THE TISSUES OF SHR AND W/K AT 16 WEEKS OF AGE

Tissue	α-tocopherol (μg/g) W/K	SHR	
Plasma	4.2 ± 0.3[a]	3.5 ± 0.3[a]	N.S.
Plasma/lipid[b]	1.66 ± 0.1	1.64 ± 0.2	N.S.
Spleen	17.9 ± 0.8	14.6 ± 0.9	$P < 0.01$
Thymus	15.9 ± 1.6	9.2 ± 0.5	$P < 0.01$
Heart	19.8 ± 0.7	14.2 ± 0.7	$P < 0.001$
Liver	10.5 ± 0.4	7.1 ± 0.3	$P < 0.001$
Testes	13.9 ± 0.8	8.1 ± 0.2	$P < 0.001$
Kidney	11.5 ± 0.5	6.9 ± 0.4	$P < 0.001$
Adrenals	180.1 ± 20.4	134.4 ± 18.7	$P < 0.01$
Fat	25.8 ± 2.4	14.1 ± 0.9	$P < 0.01$
Cerebrum	12.5 ± 0.5	8.8 ± 0.2	$P < 0.001$
Spinal cord	6.2 ± 0.3	4.9 ± 0.2	$P < 0.01$

[a] μg/ml.
[b] mg α-tocopherol/g lipid.

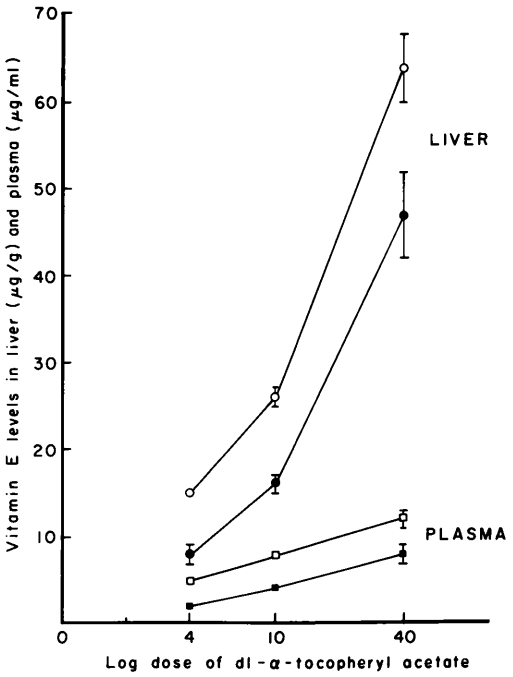

FIG. 1. Vitamin E levels in liver and plasma of SHR and W/K after 4 days of oral dosing with *dl*-α-tocopheryl acetate in stripped corn oil. Values represent mean of six animals/group ± SE. W/K liver (○), SHR liver (●), W/K plasma (□), SHR plasma (■).

The finding of a depressed immune mechanism in the SHR animal model in conjunction with lowered vitamin E levels in the lymphoid tissues is consistent with the known immunodepression resulting from vitamin E deficiency (23). It is also of interest that increasing the amount of vitamin E in the diet of rodents significantly increases the humoral antibody formation (9). Prostaglandins increase the levels of cyclic AMP in lymphocytes, resulting in depression of cell mediated as well as humoral immune responses (24). Adherent cells from the spleen of SHR produce approximately twice as much prostaglandin of the E series as those of W/K (5). Endogenous prostaglandin levels in the bursa and spleen of the chicken are suppressed by administration of vitamin E (25), and, as we have shown in this report, the spleen of the SHR contains approximately half the vitamin E found in the spleen of the W/K. Whether there is a direct relationship between the low levels of vitamin E in the tissues of the SHR and the increased production of prostaglan-

din, possibly resulting in hypertension and/or depressed immune response, is yet to be determined.

In summary, tissue levels of vitamin E are much lower in the SHR than in the W/K. This difference was manifest at both 6 and 16 weeks of age. The possible relationship of lower vitamin E tissue levels to lower immune responses in these animals is discussed.

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