of the high blood pressure. With our technique the vasopressor material tended to disappear from the blood between 1 and 3 weeks after renal artery clamping and thereafter all assays of plasma for vasopressor activity were negative. Thus, in the dog as in the rat, and for reasons not clear, the acute and chronic stages of experimental renal hypertension differ sharply in respect to recovery of circulating pressor substance. While this finding does not necessarily exclude a humoral system from participating in the pathogenesis of chronic renal hypertension, it indicates that the level *per se* of circulating vasopressor material is not the critical factor in sustaining the high blood pressure in this stage.

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Serum Vitamin E Levels in a Normal Adult Population in the Washington, D. C., Area. (29515)

J. G. BIERI, L. TEETS,* B. BELAVADY† AND E. L. ANDREWS
Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

There is currently considerable interest in attempting to establish the human dietary requirement for vitamin E and, concomitantly, devising procedures for assessing the nutritional status with respect to this vitamin (1,2). The accumulation of information on the normal distribution of blood levels of vit. E (α-tocopherol) is a necessary step in this endeavor. We present here values for 132 normal adults residing in the area of Washington, D. C., which supplement data reported recently by Harris *et al*(3) for adults in Rochester, N. Y. These workers also summarized previous surveys of serum tocopherol values.

Methods. Subjects were new employees of the Nat. Inst. of Health who had passed a physical examination and were in apparent good health. Age range was 17-55 years and the division between Negroes and Caucasians was approximately 1:2. The survey was done during July and August, 1963. Blood was drawn by venipuncture in the Employee Health Service between 9 and 11 a.m.; subjects were not fasting. Eight to 10 samples daily were collected and taken to the laboratory for immediate analysis.

For the analysis of vit. E, 1.0 ml of serum was thoroughly mixed with 1.0 ml of redistilled 95% ethanol in a 15 ml glass-stoppered centrifuge tube. Three ml of petroleum ether (B.P. 40-60°C) were added and the tubes shaken rapidly by hand, or on a mechanical shaker, for 3 minutes. After centrifuging, 2 ml of the petroleum ether were carefully pipetted off and transferred to a 10 $\times$ 75 mm cuvette for the Coleman Junior Spectrophotometer. A reading at 450 mμ was made for total carotenoids. The tubes were placed in a 50°C water bath and the solvent evaporated with a stream of nitrogen. One-tenth ml of

* Guest worker; present address: Bridgewater College, Bridgewater, Va.

† Guest worker; present address: Nutrition Rerearch Laboratories, Indian Council of Medical Research, Hyderabad, India.

TABLE I. Comparison of Serum Vitamin E Values Determined by Direct or Chromatographic Procedures.

Sample	Vit. E, mg/100 ml Direct	Chromatographic
1	1.600	1.524
2	.747	.810
3	1.010	.950
4	1.137	1.079
5	1.088	1.070
6	1.288	1.220
7	.984	.947
8	1.504	1.409
9	1.072	1.044
10	.961	1.066
11	1.528	1.531
Mean ± S.E.	1.174 ± .260	1.150 ± .230

chloroform was added to dissolve the lipid residue, followed by 1.0 ml of ethanol, 0.1 ml of 0.2% bipyridyl and 0.1 ml of 0.1% ferric chloride, both in ethanol. Readings were made at 520 mμ against a water blank 30 seconds after mixing on a tube vibrator. Standard curves were made using dl-α-tocopherol‡ and crystalline all-trans β-carotene.§ The correction for carotene in the Emmerie-Engel reaction was found to be 50% of the absorbency at 450 mμ.

Results. The analytical method for α-tocopherol is a rapid one, modified from that of Farber *et al*(4), in which the interference from serum carotenoids is corrected for on the basis of the reaction of β-carotene with ferric chloride and bipyridyl. Although there is no evidence that any serum lipid constituents other than carotenoids are present in sufficient amounts to interfere in the Emmerie-Engel reaction, a comparison was made of this direct procedure with a more detailed one(5) in which the α-tocopherol is separated from carotenoids and vit. A by column chromatography. The results from 11 sera analyzed by the two procedures are shown in Table I. Although the direct method gave higher values for 8 samples, it is apparent that the possible overestimation is not sufficiently great to invalidate the method for routine survey purposes.

The distribution of serum vit. E levels for all subjects is presented in Table II according to the scheme of Harris *et al*(3). No samples were below 0.50 mg/100 ml and only 6.1% were below 0.70 mg/100 ml. Seventy per cent of the values fell between 0.80-1.39 mg/100 ml, while 9.2% were in the very high range.

In Table III are shown average values for tocopherol and carotenoids for men and women. A plot of tocopherol *vs* carotenoid values suggested a possible correlation between these blood constituents. Calculation of the correlation coefficients(6) gave values which were greater than those required at the 1% level, indicating highly significant correlations.

TABLE II. Distribution of Serum Tocopherol Values in Normal Adults (Washington, D. C.).

Range, mg/100 ml	No. of samples	% of total
.50– .59	5	3.8
.60– .69	3	2.3
.70– .79	10	7.6
.80– .89	22	16.7
.90– .99	19	14.4
1.00–1.09	24	18.2
1.10–1.19	15	11.4
1.20–1.29	12	9.1
1.30–1.39	10	7.6
1.40–1.49	5	3.8
1.50–1.59	5	3.8
>1.60	2	1.5
	132	100

Mean = 1.05 mg ± .26 (S.D.).

TABLE III. Mean Values for Serum Tocopherol and Carotene in Normal Adult Men and Women.

	Tocopherol, mg/100 ml	Carotene, μg/100 ml	r*
Men (71)	1.064 ±.25†	133 ± 47	.385 (.305)‡
Women (61)	1.035 ±.27	158 ± 62	.570 (.325)

* Correlation coefficient.
† Mean with standard deviation.
‡ Value for "r" required for significance at .01 level.

Discussion. The average serum tocopherol concentration found, 1.05 mg/100 ml, is in agreement with the average reported recently by Harris *et al*(3) for 197 adults in Rochester, N. Y. Of particular concern for nutritional survey purposes is the per cent of low values. Although no definitive evidence is available which would establish an undesirable level from the clinical standpoint, sev-

‡ Merck and Co., Rahway, N. J.
§ General Biochemicals, Chagrin Falls, Ohio.

eral investigators feel that on the basis of the *in vitro* hemolysis of erythrocytes by peroxide, a tocopherol blood level below 0.5 mg/100 ml is indicative of a deficiency(1,2). Whereas Harris *et al*(3) found 7% of their samples to be below 0.5 mg/100 ml, we found none in this range. Only 6.1% of our group were below 0.7 mg/100 ml.

A correlation between tocopherol and carotenoids in serum was also noted by Leitner *et al*(7) in their survey of British men and women. Such a relationship may be expected in a population consuming reasonable quantities of vegetables consistently, since both α-tocopherol and carotenoids occur in green and yellow vegetables.

Summary. Serum tocopherol levels in 61 female and 71 male normal adult employees of the National Institutes of Health averaged 1.06 mg/100 ml. No samples were below 0.50 mg/100 ml, and 93.9% were above 0.70 mg/100 ml. A significant correlation was found between serum tocopherol and carotenoids for both males and females.

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Serum Vitamin E Levels in the Rural Population of East Pakistan.* (29516)

M. M. RAHMAN, S. HOSSAIN, S. A. TALUKDAR, K. AHMAD, AND J. G. BIERI†
Department of Biochemistry and Nutrition, University of Dacca, Dacca, East Pakistan

Nutritional surveys in developing countries generally have not included serum vitamin E among the routine biochemical analyses. In a limited sample of Jordanian children with kwashiorkor low serum tocopherol levels were recently encountered(1). When it became apparent during the course of a recent survey in East Pakistan(2) that the rural diets in many areas were very low in fat, a special study was undertaken on serum levels of vit. E. Concomitantly, vit. A and carotene analyses were also made.

Procedure. Blood samples were taken at random during the day by venipuncture, using oxalated vacuum syringes, from villagers in 4 rural districts of East Pakistan (Khulna, Pabna, Kustia, and Sylhet). The samples were put into vials with rubber stoppers and packed in ice in an insulated chest which was shipped to the laboratory in Dacca. Not more than 24 hours elapsed between time of collection and arrival in the laboratory, by which time the ice was melted. The serum was separated and frozen until analyzed within 2 to 3 days. Studies of possible changes in the vit. E content of sera permitted to stand at room temperature (26-28°C) for periods up to 24 hours showed that no significant loss of tocopherol occurred.

Vitamin E was determined by the ferric chloride-bipyridyl reaction as described in the preceding paper(3). Vit. A was determined on an extract from 2 ml of serum prepared as above. The reaction with 1 ml of antimony trichloride in chloroform was measured in 10 × 75 mm cuvettes at 620 mμ in the Coleman Junior Spectrophotometer. A correction was made for carotenoids,

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† Nat. Inst. of Arthritis & Metab. Dis., N.I.H., Bethesda, Md.