vitamin E are, under the conditions employed, more sensitive to morphological alterations than the testes. The absence of testicular degeneration in the rabbit does not preclude the existence of vitamin E deficiency and necrosis of the skeletal muscles.

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Tocopherol Level in Serum of Normals and Patients with Amyotrophic Lateral Sclerosis.*

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Synthetic vitamin E (dl-α-tocopherol) has recently been introduced in the treatment of amyotrophic lateral sclerosis.[1] It seemed of interest to study the level of this vitamin in untreated and treated patients with this condition, and to compare it with the serum level of normal individuals. Analytical methods for the determination of tocopherol have been developed for the control of preparation and concentration of this substance from its natural sources, such as wheat germ oil. The dipyridyl method, based on the reduction of trivalent iron by tocopherol to the bivalent state and subsequent colorimetric determination of the ferrous iron as α,α′-dipyridyl complex, has been developed for the assay of tocopherol concentrates. It has been used for the study of the tocopherol level in the serum of tocopherol-deficient and tocopherol-treated rats.[2]

We have adapted this method to the analysis of human serum by means of a photoelectric colorimeter. The employment of a photoelectric method is advantageous because the pink color of the iron-dipyridyl complex is not very suitable for visual comparison.† Ten ml of serum from a fasting blood specimen are sufficient for the determination.

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1 Wechsler, I. S., *J. A. M. A.*, 1940, **114**, 948; *Am. J. Med. Sci.*, 1940, **200**, 765; *Arch. Neurol. Psych.*, 1941, in press.

2 Emmerie, A., and Engel, C., *Rec. Trav. Chim. des Pays-Bas*, 1938, **57**, 1351; 1939, **58**, 283, 895; *Vitamin E, A Symposium*, London, 1939, p. 14.

† The details of this method will be reported elsewhere.

Results. We have determined the tocopherol level in the serum of 12 normal subjects. This group comprised 9 men and 3 women in the age group from 23 to 30. The values vary between 0.59 and 1.62 mg/100 ml with an average of 0.96 mg/100 ml, the mode being 0.895. The highest values may be due to the ingestion of food especially rich in tocopherol, and we surmise that individuals on a diet with maintenance amounts of vitamin E display values of 0.6-1.0 mg %. No values for normal animals or men have come to our attention in the literature. Emmerie and Engel[2] give the values 0.064 and 0.056 mg % for the serum of deficient rats, and from 0.29 up to 1.10 mg % for the same animals upon ingestion of 1 mg tocopherol per diem, corresponding to a dose of 250 mg p.d. for a 50 kg human subject. In the organs of rats Karrer, Jaeger and Keller found tocopherol contents in the same range, namely 0.2-1.5 mg/100 g;[3] in Jensen sarcoma Euler and Euler found 0.92 mg/100 g.[4]

One female subject with duodenal ulcers, who was on a restricted diet and who is not included in the above group, showed the low values 0.39 and 0.58% at two different occasions. The lowest value (0.59 mg %) in the above series was obtained on a subject with a one-sided meat and protein diet, who also showed subnormal vitamin A and carotene values in his serum.

We have carried out 33 determinations on 13 patients with amyotrophic lateral sclerosis. These analyses are summarized in

TABLE I.
Level of dl-α-Tocopherol in Normals.

	Name	Age	Sex	Vitamin E, mg in 100 cc serum
1.	M.S.	26	m	.59
2.	H.J.	25	m	.60
3.	A.S.	28	m	.60
4.	M.E.	30	m	.73
5.	F.R.	22	f	.84
				.90
6.	J.L.	23	f	.89
7.	E.W.*	29	m	.89
8.	F.S.	26	f	.93
9.	M.S.	24	m	1.13
				1.62
10.	R.L.	25	m	1.24
11.	E.K.	25	m	1.43
12.	A.L.	28	m	1.43

*This subject, after 7 daily oral doses of 740 mg vitamin E, showed a blood level of 1.50 mg%.

[3] Karrer, P., Jaeger, W., and Keller, H., *Helv. Chim. Acta*, 1940, **23**, 464.

[4] v. Euler, B., and H., *Z. physiol. Chem.*, 1940, **265**, 147.

Table II. Four of these patients had been admitted without having previously received tocopherol treatment, and their serum values from 0.52-1.00 (average 0.76) mg % fall well within the limits of our normal controls. The majority, however, had received vitamin E treatment of one form or another before serum could be obtained. The treatment as far as it was administered in the hospital consisted of oral administration of Ephynal (Hoffmann-LaRoche), the acetate of dl-α-tocopherol. The daily and total amounts are given in the fourth column of the table. Some cases received from 100 to 200 mg α-tocopherol in sesame oil by intramuscular injections. All patients received in addition up to 2 ounces of wheat germ. A few were given 6 capsules Tocopherex (Squibb) per diem. The values during treatment vary from 1.13 to 2.26 mg tocopherol per 100 ml serum. Values around the 2 mg % level are obtained even with the smallest dosages used; hence it seems that an upper threshold obtains at 2.0-2.5 mg %, which cannot be surpassed even by the administration of liberal amounts. A normal control with 0.89 mg % showed a rise to 1.50 mg % upon daily ingestion of 740 mg of Ephynal during 6 days.

Upon discontinuation of vitamin E treatment, the blood level showed a downward trend, and the figures "off treatment" vary from the upper limit reached in the course of treatment, to low normals.

TABLE II.
Level of dl-α-Tocopherol in Patients with Amyotrophic Lateral Sclerosis.

No.	Age	Sex	Mg% tocopherol before treatment	Daily dosage in mg		Mg% tocopherol during treatment	Mg% tocopherol after treatment was interrupted for No. of days given	Mg% tocopherol on resumption of treatment
				Oral	Intramusc.			
1.	48	f	—	740	200	1.71	—	—
2.	41	m	—	740	200	1.68	—	—
3.	32	m	—	740	200	2.07	1.37 (7 days)	—
4.	58	f	—	100?	—	1.13	—	—
5.	47	m	0.69	150	—	—	—	—
6.	39	f	0.80	340	—	2.26	2.47 (2 days) 1.01 (5 days)	1.36 (3 days)
7.	58	f	—	740	200	1.79	—	—
8.	39	m	—	740	200	2.14	1.36 (4 days) 1.15 (6 days)	1.36 (1 dose of 50 mg oral)
9.	50	f	—	100	—	1.20	0.89 (6 days)	—
				100	—	1.16	0.61 (9 days)	
				—	100	0.90	—	—
10.	43	m	0.55	340	100	0.93	—	—
			0.52	—	—	—		
11.	50	m	0.69	740	—	1.70	—	0.61 (3 doses of 200 mg intramusc.)
			0.88	740	—	2.00	—	
12.	48	m	0.96	—	100	0.59	—	—
13.	38	m	1.00	—	100	0.67	—	—
				100	—	0.90		

Conclusion. From a clinical study which showed definite improvement in some cases following the administration of vitamin E, the conclusion seems warranted that the vitamin brought the results. But the above figures, although comparatively few in number, do not point to a simple vitamin E deficiency in amyotrophic lateral sclerosis. They show that the vitamin E level in the serum of untreated patients falls within the normal range. The response of the blood level to oral administration indicates that substantial amounts of 50-100 mg easily enter the blood stream. As it is known that no vitamin E is deposited in the organs of animals, it is unlikely that it accumulates in human organs. Hence, one cannot state whether the administered synthetic vitamin is excreted, destroyed or deposited as far as it is not accounted for in the circulation.

A comparison with vitamin A studies shows the following: When 100,000 I.U. of vitamin A are given, only a small quantity, never more than 3%, is recovered in the blood.[5] This vitamin is known to be stored in the liver; moreover, it is chemically less stable than tocopherol. Thus, if similar quantities of the latter are administered, a very much larger proportion appears in the circulation.

We do not know how and where vitamin E exerts its effect on nerve and muscle function. It may be that there is a barrier to the passage of tocopherol from the blood stream to nerve and muscle cells in amyotrophic lateral sclerosis, or that another obstacle of a functional nature prevents the utilization of the vitamin found in the blood at its normal level.

Patients stated that they felt better or stronger on treatment, but such subjective improvements are difficult to evaluate. Two patients in the present series showed definite objective improvement, and a third an apparent improvement, with the rise of the tocopherol level in the blood. The rest did not, and yet all responded to oral administration with an increase of serum tocopherol. This increase did not parallel the dosage ingested. That is, after 50 or 100 mg had been given, it did not matter whether the dosage was increased even to 500 mg. It is probable that the tocopherol content of the blood takes at least two or three days before reaching the maximum level; on the other hand, this level persists for a few days after administration has been stopped. In three patients who received intramuscular treatment without simultaneous oral treatment, a drop of the blood tocopherol level may be noted in the table.

[5] Peck, S. M., Chargin, L., and Sobotka, H., *Arch. Derm. Syph.*, 1941, **43**, 223; unpublished observations of Brickner, R., Kann, S., and Sobotka, H.

We wish to thank Drs. M. Ellenberg, S. Margolin and M. Sapirstein for their kind assistance with the patients.

Summary. (1) Normal serum tocopherol values determined by a new photocolorimetric method are given. (2) Untreated patients with amyotrophic lateral sclerosis show values within the normal range. (3) The blood level invariably rises on oral administration, irrespective of whether the patient does or does not show clinical improvement. (4) Intramuscular administration without simultaneous oral administration produces a drop in serum tocopherol.

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Comparison of Effects of Large Doses of Various Activated Sterols on Serum Calcium.

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When highly potent preparations of vitamin D became available, there was an immediate interest in the physiological effects of massive doses of the vitamin, and numerous studies dealing with their effects on the metabolism of calcium and phosphorus were reported. The literature has been reviewed by Schmidt and Greenberg,[1] and more recently by Reed, Struck and Steck,[2] therefore requires no extensive comment here. In general, it has been established that in normal animals large doses of vitamin D_2 result in a decreased fecal excretion of calcium, a hypercalcemia, and an increase in urinary calcium and phosphorus.

Other activated sterols might be expected to exert similar effects. Among these are vitamin D_3, dihydrotachysterol (A.T.10), and "Ertron". The last named is a form of activated ergosterol produced by the Whittier process and claimed by its manufacturers* not to have the hypercalcemic or other toxic effects of calciferol.[3]

[1] Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 297.

[2] Reed, C. I., Struck, H. C., and Steck, I. E., *Vitamin D: Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations*, Univ. of Chicago Press, 1939, p. 219.

* Nutrition Research Laboratories, Chicago, Ill.

[3] *Cf.* also: Snyder, R. G., and Squires, W. H., *N. Y. State J. Med.*, 1940, **40**, 708.