tained a kinase.

From our first experiment and from previous experiments(3) in which no kinase activity could be found in isolated platelets, leukocytes or erythrocytes, we concluded that if a fibrinolysokinase was responsible for the "spontaneous" activation of profibrinolysin it must be a serum component independent of cellular elements.

Experiment III strongly suggests that the Euglobulin fraction of serum contains a fibrinolysokinase which is absent in the Prolysin fraction. The isolation and action of such a substance is shown in Experiment IV. Fraction K was able to activate the Prolysin almost completely in the 6 week experimental period. The slow initiation of this activation may be due to the presence of traces of antifibrinolysin in Fraction K. Other serum fractions, prepared from whole serum and from the serum residual after Prolysin has been removed, have also proved active as fibrinolysokinases. Apparently very exact technic is necessary to separate the kinase from profibrinolysin as duplicate experiments on different serum lots will not always give a good separation. Studies are underway to elucidate further this fractionation problem.

Conclusion. Serum, independent of cellular elements, contains a fibrinolysokinase which is capable of converting profibrinolysin to active fibrinolysin.

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Relation of Vitamin E to Acute Physiological Stress.* (19015)

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According to Selye(1), the normal physiological response of the body to varied forms of stress, such as cold, heat, emotional excitement, etc., proceeds via a common pathway. The pathway seems to involve stimulation of the pituitary gland by epinephrine or other agents. The pituitary then releases adrenocorticotropic hormone (ACTH), which causes the adrenal glands to secrete various steroid hormones (*e.g.* cortisone) which mediate the physiological response of the body to stress. The entire system is designated the "general adaptation syndrome."

The nutritional status of the body has been shown to affect the capacity to respond to stress in the case of several nutrients such as pantothenic acid(2) and vit. C(3). The possibility of a causal relationship between vit. E and the stress response via the anterior pituitary-adrenal cortical pathway seemed likely in view of the known protective effect of α-tocopherol in various different types of stress, such as carbon tetrachloride poisoning (4), exposure to high altitude(5), or dietary restriction of either protein or total calories (6). Also, vit. E has been implicated as a factor in maintaining the anatomical integrity of the adrenal cortex by Tonutti(7) who found that vit. E-deficient rats showed degenerative changes in the exterior and interior zones of the adrenal cortex similar to those following hypophysectomy.

Two experiments were performed to test the possibility that vit. E is related to the initial phase (alarm reaction) of the general adaptation syndrome.

* Communication No. 177.

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TABLE I. Effect of Acute Stress on Adrenal Ascorbic Acid Content of α-Tocopherol-Supplemented and of Vit. E-Deficient Rats.

	Ascorbic acid in adrenals (mg/100 g)			
	No stress		Stress	
	(—E) rats	(+E) rats	(—E) rats	(+E) rats
Mean values (5 exp.)	396	427	254	276
S.E.	± 27	± 57	± 27	± 33

Mean % decrease due to stress:

$$\text{for (+E) rats} = \frac{427-276}{427} \times 100 = 35.2\%$$

$$\text{for (—E) rats} = \frac{396-254}{396} \times 100 = 35.9\%$$

Exp. I. Effect of vit. E nutriture on response of the rat to acute stress. Adrenal ascorbic acid content. The change in concentration of ascorbic acid in the adrenal gland is a well-known criterion of response to stress via the pituitary-adrenocortical system(8). Hence, the concentration of ascorbic acid in the adrenal glands of both stressed and unstressed rats of groups which were markedly E-deficient and of those which were identical except for continuous α-tocopherol supplementation was measured. Thirty male weanling rats of Holtzman stock were maintained on an otherwise adequate, vit. E-deficient diet No. 301(9) for 2 months, during which time one-half received d-α-tocopherol supplements, totaling 12 mg/week, by oral administration. A series of "stress" experiments was then performed on 4 rats at a time, 2 from each group, all of approximately equal body weight. Animals were used after a 6-hour fast. A tocopherol-supplemented and a tocopherol-deficient rat were each subjected to stress, while a tocopherol-supplemented and a tocopherol-deficient rat were not, as controls. "Stress" consisted of subcutaneous injection of 0.1 mg of epinephrine-HCl in 0.1 ml of distilled water in every experiment but one. In this experiment "stress" consisted of exposure to cold (5°C) for 1 hour. Controls received an injection of distilled water or in the one experiment were maintained at room temperature. The animals were killed 1 hour after injection by a blow on the head. The content of ascorbic acid in the adrenal glands of each rat was immediately determined by the method of Mindlin and Butler(10) on a 2.5% metaphosphoric acid extract of the glands made with a glass (Potter-Elvehjem) homogenizer.

Results are given in Table I. The mean decrease of ascorbic acid concentration of tocopherol-supplemented rats in response to stress was 35.2% which approximates values for normal rats, reported in the literature. The corresponding change for tocopherol-deficient rats was a decrease of 35.9%.

Thus, no difference in amount of pituitary-induced adrenal cortical activity as a result of acute stress was found between tocopherol-supplemented and tocopherol-deficient rats.

Blood concentrations of vit. A and E. Blood sera of rats in some of the experiments were analyzed for their content of vit. A and E by the micro methods of Lowry *et al.*(11,12). Results are shown in Tables II and III.

Tocopherol-deficient rats, with or without stress, had zero blood tocopherol levels. The supplemented groups had levels of 1.72 mg % (stress) and 1.68 mg % (no stress). The contrasting nutritional status of tocopherol-supplemented and unsupplemented rats with respect to vit. E is thus amply confirmed. Also is shown the lack of effect of stress on the

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TABLE II. Effect of Acute Stress on Blood Serum Vit. E Levels.

	Total tocopherols in serum (mg/100 ml)			
	Stress		No stress	
	(+E) rats	(—E) rats	(+E) rats	(—E) rats
Mean values (4 exp.)	1.72	0	1.68	0
S.E.	± .11	0	± .06	0

% change due to stress:

$$\text{for (+E) rats} = \frac{1.72-1.68}{1.68} \times 100 = +2.4\%$$

for (—E) rats = 0%

TABLE III. Blood Serum Vit. A Levels of Rats Subjected to Acute Stress.

	Blood serum Vit. A (μg/100 ml)			
	Stress		No stress	
	(+E) rats	(—E) rats	(+E) rats	(—E) rats
Mean values (3 exp.)	44	31	46	41
S.E.	± 1	± 4	± 6	± 2

Mean % change due to stress:

$$\text{for (+E) rats} = \frac{44-46}{46} \times 100 = -4\%$$

$$\text{for (—E) rats} = \frac{31-41}{41} \times 100 = -24\%$$

blood tocopherol levels of the animals.

Mean blood vit. A levels were essentially equal for 3 of the 4 groups and markedly lower for the one "stressed", unsupplemented group. The mean changes of blood vit. A levels following stress, were –4% for the tocopherol-supplemented rats and –24% for the tocopherol-deficient rats. These data are too few for conclusive results; however, they suggest that vit. E may have "protected" vit. A in the blood during stress. These blood vit. A values fail to confirm the report of Young and Wald(13) that epinephrine injection caused a pronounced increase in the blood content of vit. A. They agree with results of Goodwin and Wilson(14). Both groups used rabbits in their experiments.

Exp. II. Effect of acute physiological stress on the tocopherol content of the adrenal glands of normal rats. Mature, well-nourished male rats were used as experimental subjects. Fifteen litter-mate pairs of weanling male rats from the Distillation Products Industries' stock colony were maintained on Diet 1, complete stock breeding diet, until they reached about 400 g weight (about 5 months). One pair was used as a check on the efficacy of the particular stress used here in eliciting the pituitary-induced, adrenal-cortical response. After overnight fast, one rat received 0.1 ml of epinephrine-HCl (aq.) (1 mg/ml) by subcutaneous injection, while the control received distilled water by the same route. They were killed after one hour, by a blow on the head, and the adrenal ascorbic acid concentration was measured. The stressed rat had 213 mg/100 g of ascorbic acid in his adrenal glands and the control animal 336 mg/100 g. The decrease caused by stress, *i.e.* 36.6% of the control, was nearly identical to the mean value of a series of rats (both vit. E-deficient and sufficient) tested previously by the same technic (Exp. I). Thus, the method of physiological stress used did induce the expected pituitary-adrenal response in these rats. The remaining 14 pairs of litter-mate rats were stressed similarly, 1 or 2 pairs at a time. After overnight fast, they were injected with either 0.1 mg of epinephrine (stressed rats) or 0.1 ml of distilled water (controls). After one

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TABLE IV. Effect of Stress Upon Vit. E Content of the Adrenal Glands of Normal Rats.*

Group	Mean adrenal wt (mg)	Mean body wt (g)	Total tocopherol in the adrenals: Mean for group (mg/100 g of adrenals)	Total tocopherol in the adrenals: Mean of differences between litter pairs (mg/100 g of adrenals)
No stress	39.78	392	30.8	3.7 ± 4 (S.E.)†
Stress	38.64	380	34.6	

* One of each of 14 litter-mate pairs of male rats, raised on a complete stock breeding diet, was subjected to physiological stress (0.1 mg epinephrine by subcutaneous injection) 1 hr before sacrifice.

† This difference is not statistically significant ($p = 0.4$-0.3) by the Fisher "t" test.

hour, the animals were killed by a blow on the head and the adrenals removed and carefully dissected free of fat. They were weighed immediately to the nearest 0.01 mg on a Roller-Smith balance. Each pair of adrenals was then analyzed for content of total tocopherols. The extracts for analysis were prepared by homogenizing pairs of adrenals in all-glass apparatus (Potter-Elvehjem) in 6 ml of 1:1 ethanol-xylene, adding 3 ml of H_2O, agitating, and centrifuging. Aliquots of the xylene layer were then analyzed spectrophotometrically as in the micro method of Quaife *et al.*(12).

Results. Experimental data, including mean body weight of rats, mean adrenal weight, mean vit. E concentration in the adrenals (mg total tocopherols/100 g of adrenals) are summarized in Table IV. Also the mean of differences in adrenal tocopherol content for each pair (stressed vs. control) is given.

The apparent slight increase in tocopherol content of the adrenal glands, due to stress, was shown to be without statistical significance ($p = 0.4$-0.3).

Summary and conclusions. The nutritional status of rats with respect to vit. E had no effect on the adrenal cortical activity (as measured by change in adrenal ascorbic acid concentration) following acute physiological stress (epinephrine injection or exposure to cold). Neither the content of vit. E in the adrenal glands of normal adult rats nor the blood tocopherol content of tocopherol-supplemented or tocopherol-deficient rats was changed following physiological stress.

Thus, vit. E is apparently not a factor in the anterior pituitary-adrenal cortical response to acute physiological stress, although vit. E may be related to the manner in which the tissues utilize the adrenal cortical hormones released as a result of such stress.

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Chloromycetin Palmitate.* Its Use in Pediatrics. (19016)

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Chloromycetin is often difficult to administer to infants and children because of its bitter taste. Esters of Chloromycetin with higher fatty acids have been prepared in an attempt to produce a palatable form of the drug. One of these esters, Chloromycetin Palmitate, is now under clinical investigation. The ester is formed by replacing one of the hydroxyl groups of the propane diol side chain of Chloromycetin with Palmitic acid. Chloromycetin Palmitate has not previously been used in clinical medicine. Animal experi-

* The author is indebted to Parke-Davis and Co. which supplied the Chloromycetin Palmitate suspension and which gave permission to quote their studies of toxicity of the drug in animals.