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Effect of Vitamins on Acetylcholine Synthesis. The Apparently Specific Action of Vitamin E.*

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In search of substances that modify the synthesis of acetylcholine the effect of vitamins on the acetylcholine synthesis *in vitro* was investigated.

Method. The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[1] with minor modifications.[2] Varying amounts of the substances were added to mixtures containing 100 mg minced fresh frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, and 3 cc Ringer's solution. The pH of the mixtures was adjusted to 7.4. Identical mixtures without the substances served as controls for the water soluble substances and mixtures containing an adequate amount of sesame oil for the oil-soluble substances. The mixtures were shaken and incubated aerobically for 4 hours at 37° C. After incubation the amounts of total acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog. The amount of acetylcholine synthesized was calculated by subtracting from the acetylcholine content of the incubated mixtures the acetylcholine content of identical nonincubated mixtures. By adding the substances in varying concentrations to incubated control mixtures after incubation it was ascertained whether the substances modified the sensitivity of the rectus abdominis muscle to the acetylcholine content of the mixtures during the 2 minutes of immersion for the biological assay. If so the changes were taken in account by the calculation.

Results. The amounts of acetylcholine synthesized in the presence of the substances used are given in Table I. A marked decrease of synthesis was found in the presence of low and increasing concentrations of vitamin A and K,[3] and high concentrations of B_1[4] and D.[5] A marked increase of synthesis was found in the presence of low and increasing concentrations of α-tocopherol, and of high concentrations of some of the members of the vitamin B group and vitamin C.

Discussion. An adequate explanation of the mechanism through which the substances used modify the synthesis of acetylcholine cannot yet be offered. Most of the substances used have a positive oxidation-reduction potential, and vitamin K may modify the activity of —SH containing substances, an active group probably contained in the enzyme involved in the synthesis of acetylcholine.[6]

The decrease of synthesis of acetylcholine in the presence of vitamin A may be significant in the body since stimulation of the sympathetic nervous system or injection of epinephrine[7-10] are known to be followed by an increased concentration of vitamin A in the serum because of an increased mobilization from its stores.

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TABLE I.
Effect of Vitamins on Synthesis of Acetylcholine.

	Amt of total acetylcholine synthesized in % of control*					
	Amts of the substances added to 100 mg frog brain (mg)					
Substance	3	0.3	0.03	0.003	0.0003	0.00003	0.000003
Vit. A†		12	34	67	81	90	99
Thiamine chloride		46	64	87	118		
Riboflavine	136	126	101	100	101		
Nicotinic acid	145	144	119	98	111		
Nicotinamide	125	120	110	107	106		
Calcium pantothenate	148	137	123	99	100		
p-Amino benzoic acid	123	110	104	104	105		
Pyridoxine	123	115	104	101	100		
Vit. C	140	119	98	96	97		
Vit. D‡		30	75	97	101	103	99
α-Tocopherol (SMACO)			225	185	162	130	110
Vit. K (menadione)		28	66	74	83		

* The S.E. of the mean for each value was less than ±5%. Each value represents the average of 8 separate experiments. The amount of acetylcholine synthesized in μg per 100 mg frog brain followed by the S.E. of the mean was 1.50 ± 0.044.

† Each mg contained 500 I.U. vitamin A.

‡ Each mg contained 400 I.U. vitamin D.

α-tocopherol even in minute concentrations induced a striking increase of the synthesis of acetylcholine, suggesting that it has a specific action on this process. It is known that vitamin E and α-tocopherol modify the metabolism of lipids, phospholipids,[11-15] and may enhance phosphorylation.[11, 16-20] These processes may contribute to the increase of the synthesis of acetylcholine since choline occurs in the body as a constituent of phospholipids, and the energy required for the synthesis of acetylcholine may be supplied by energy rich phosphate bonds. However, it is possible that vitamin E is a part of the coenzyme involved in the synthesis of acetylcholine.

A close relationship between vitamin E and synthesis of acetylcholine in the body is suggested by the consideration that the placenta, an organ without nerve supply but rich in acetylcholine,[21] is also rich in vitamin E;[19] vitamin E deficiency is followed by a decrease of choline esterase content of tissues,[22, 23] a decrease known to occur in denervated muscles;[24,23] and deficiency of vitamin E may result in the degeneration of the end-plates in striated muscle.[25]

Summary. 1. The effect of vitamins on the synthesis of acetylcholine was investigated. 2. Vitamin A and K decreased the synthesis of acetylcholine in low and increasing concentrations. Vitamin D did not modify the synthesis in low concentrations and decreased it in higher ones. Vitamin B_1 slightly increased the synthesis of acetylcholine in low concentrations and decreased it in higher ones. 3. Ribo-

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flavin, nicotinic acid, nicotinamide, calcium pantothenate, p-amino benzoic acid, pyridoxine, and vitamin C did not modify the synthesis in low concentrations and increased it in higher ones. 4. Vitamin E increased the synthesis in low and increasing concentrations.

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Phlebostatic Axis and Phlebostatic Level, Reference Levels for Venous Pressure Measurements in Man.*

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In the determination of venous pressure both in the clinic and in the laboratory it is often necessary to make the measurements with the subjects in positions (sitting, intermediate sitting, with parts under study in many positions relative to the level of the heart) other than supine. The levels of reference available at the present time fail to fulfill the requirements of practicability and accuracy necessary for clinical and experimental studies. At least 9 different reference levels have been given for the measurement of venous pressure.[1-8] Some have been related to anatomic parts of the body and some to the horizon or examination table. The lack of consistency in the reference or heart levels makes it difficult to undertake studies on venous pressure. The present studies were conducted to find a point of reference for venous pressure measurements which may be applicable to subjects of any build, in many positions, and with the vein under study in any positions necessary for the study of clinical problems concerned with venous pressure measurements in any superficial vein.

Method and Materials. The apparatus, the Phlebomanometer described elsewhere, is accurate to ± 1 mm of water, and checked satisfactorily at frequent intervals with a water manometer.[9] Approximately 265 determinations were made on 165 normal young adults (ages 16-34 years) of both sexes and negro and white races who rested prior to and during measurements on a firm plywood table with an adjustable head. The veins studied are indicated below.

Results. In 99 subjects of both races and sexes it was found that when a plane passing longitudinally through the body parallel to its anterior surface and midway between the dorsal surface of the thorax and the base of the xiphoid process was used as the reference level the venous pressure measurements in the median basilic veins varied relatively little even though the thickness of the chests varied considerably (78-135 mm, mean 97 mm). That the plane described is a good reference level was further substantiated by the fact that in 10 selected subjects with very thick chests (mean reference level 126 mm from dorsum of trunk) and 10 with very thin chests (mean reference level 89 mm from dorsum of trunk) the venous pressure

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