choline esterase is several hundred times higher than in the fiber.

In one experiment with succinic oxidase the enzyme distribution between sheath and axoplasm was the same as that found for succinic dehydrogenase. The difference of enzyme concentration between fiber and head ganglion was also the same.

The formation of acetylcholine occurs only in oxygen and in presence of either glucose or pyruvic acid.[6] This suggests that the acetic acid is formed from pyruvic acid, a process which probably requires vitamin B_1. Mann and Quastel found that in the brain of vitamin B_1 deficient pigeons acetylcholine formation is accelerated in presence of potassium + vitamin B_1.[7] The greatest part of vitamin B_1 in living tissue seems to occur as diphosphothiamine (cocarboxylase). The concentration of this coenzyme has been determined in sheath and axoplasm. The method used was that of Lohmann and Schuster with slight modifications.[8] Table 2 gives an experiment showing that the concentration in the sheath is twice as high as that in the axoplasm. In view of the large fraction of connective tissue in the sheath these figures definitely indicate a concentration of diphosphothiamine in or near the surface many times as high as that in the axoplasm. The distribution differs from that of choline esterase as well as from that of succinic dehydrogenase and oxidase. It supports the assumption that one function of vitamin B_1 may be its participation in the formation of acetic acid for acetylcholine.

TABLE 2

COCARBOXYLASE. TIME 20 MIN. K = 0.05 μ COCARB.

	Mg fresh tissue	Cmm CO_2	μg cocarboxylase	
			Absol.	Per 100 mg
Sheath	14.0	2.66	0.0315	*225*
Axoplasm	87.0	6.89	0.0785	*90*
K		4.39		

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A COLOR REACTION FOR DEHYDROASCORBIC ACID USEFUL IN THE DETERMINATION OF VITAMIN C

WHEN the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid is treated with 85 per cent. sulfuric acid, a reddish colored product is formed which absorbs maximally at 500–550 mu and 350–380 mu. The nature of this reaction is illustrated by the following experiment. One cc of a dilute acetic acid solution of dehydroascorbic acid is mixed with one cc of a saturated solution of 2,4-dinitrophenylhydrazine in 85 per cent. H_3PO_4. After letting stand for 5 minutes, 8 cc of concentrated H_2SO_4 is added. The same red color is obtained as that observed when the crystalline 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid is dissolved in H_2SO_4. If more time is allowed for the 2,4-dinitrophenylhydrazine to couple with the dehydroascorbic acid, a deeper red color is obtained when H_2SO_4 is added. If the three substances involved in this reaction are mixed in any other sequence, the red color is not obtained. Hence, this reaction is due to the action of H_2SO_4 on the coupled 2,4-dinitrophenylhydrazine-dehydroascorbic acid compound.

The proportionality of the color obtained in this reaction is in good agreement with Beer's law. We have used this reaction to develop a simple and rapid colorimetric method for the determination of ascorbic acid. Possible interfering substances are pentoses, glucose and fructose, which in concentrations much greater than usually found in acid extracts of plant and animal tissues will form derivatives with 2,4-dinitrophenylhydrazine. The latter dissolve in 85 per cent. sulfuric acid, giving brown to yellow colors, but they do not react with the acid except when heated or upon long standing. Furthermore, the absorption curves of the sulfuric acid solutions of the xylose, glucose and fructose derivatives of 2,4-dinitrophenylhydrazine, and also of 2,4-dinitrophenylhydrazine, show practically complete transmission in the wave-lengths at which the red color obtained from dehydroascorbic acid is compared. Hence, it appears that a completely specific principle for the determination of ascorbic acid is offered.

In the proposed method, the 2,4-dinitrophenylhydrazine derivative is isolated from unknown and standard solutions of ascorbic acid according to published directions[1] and treated with 85 per cent. sulfuric acid. Colorimetric comparison is made, using a filter with maximum transmission at 540 mu. The method will be published in detail later.

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