13749 P

In vitro Effect of α-Tocopherol Phosphate on Oxygen Consumption of Muscle from Vitamin E-deficient Animals.*

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Confirming previous observations[1] on nutritionally dystrophic rabbits and rats, Houchin[2] recently reported the oxygen consumption of dystrophic hamster muscle to be much above that of normal muscle. It was also demonstrated that within 27 hours after administration of α-tocopherol acetate to the dystrophic hamster, the Q_{O_2} of the muscle was reduced toward normal value without significant regeneration of normal muscle status as indicated by a persistent high chloride content.

These observations naturally suggested that α-tocopherol may play a rôle in the normal processes of muscle metabolism. The data in this paper establish the fact that α-tocopherol phosphate† acts *in vitro* on muscle cells from vitamin E-deficient animals to lower the excessive oxygen consumption toward the normal.

Vitamin E-deficient rabbits, hamsters, and adolescent rats were studied. The oxygen consumption of muscle slices was determined in the usual manner in the Warburg apparatus. The control medium was a modified Locke-Ringer's solution; to prepare the experimental medium 5 mg α-tocopherol phosphate were dissolved in a few drops of water and made up to 100 cc with the Locke-Ringer's solution. The solution was well shaken immediately before the required amount was measured out. The results are expressed as Q_{O_2} (cubic millimeters of oxygen per milligram dry weight per hour).

As seen in Table I the oxygen consumption of muscle from E-deficient rabbits and hamsters in the medium containing α-tocopherol phosphate was 41.3% and 35.7% lower than that of slices from the same muscle in the unsupplemented medium. The oxygen consumption was thus considerably reduced toward that of normal animals.

The oxygen consumption of the muscle of control animals was

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1 Victor, J., *Am. J. Physiol.*, 1934, **108**, 229; Madsen, L. L., *J. Nutrition*, 1936, **11**, 471; Friedman, I., and Mattill, H. A., *J. Physiol.*, 1941, **131**, 595.

2 Houchin, O. B., *Proc. Fed. Am. Soc. Exp. Biol.*, 1942, **1**, 117.

† Kindly sent to us by Hoffman-LaRoche, Inc.

TABLE I.
The *in vitro* Effect of α-tocopherol Phosphate on the Oxygen Uptake of Muscle from Vitamin E-deficient and Normal Animals.

Animal and condition	No. of exper.	Q_{O_2} (averages) Control, Locke's Sol.	Exper., Locke's Sol. containing 5 mg % α-tocopherol PO_4	% decrease
E-Def. Rabbit	4	2.54	1.45	41.3
E.-Def. Hamster	5	2.89	1.86	35.7
E.-Def. Hamster, destroyed*	2	0.07±	0.06±	
Normal Hamster	1	1.74	1.78	—0.8
Normal Rabbit	2	1.34	1.40	—4.3
E-Def. Rabbit, treated†	2	1.72	1.62	6.2

*Tissue slices dropped in boiling Locke's solution, and heated 3 minutes.
†Given a therapeutic dose of 25 mg α-tocopherol acetate 48 hours previously.

not altered beyond experimental error by the medium containing α-tocopherol phosphate. When the Q_{O_2} had already been lowered by previous administration of α-tocopherol to dystrophic rabbits, the addition of α-tocopherol phosphate to the medium caused no significant change. The Q_{O_2} of muscle slices after immersion for 3 minutes in boiling Locke-Ringer's solution was negligible and was not influenced by α-tocopherol phosphate. The α-tocopherol phosphate thus participates in a biological system and does not act merely as a chemical antioxidant. The results with rats were less striking and the creatine content of the various muscles could not be correlated with the respiratory data. Results with α-tocopherol *in vitro* have been inconclusive; whether the emulsion secured was not sufficiently fine, or whether tocopherol requires phosphorylation to be active, are open questions.

Since α-tocopherol phosphate did not affect the Q_{O_2} of normal muscle or of muscle from dystrophic animals that had previously been given α-tocopherol, it may be assumed that these muscles already contained the optimum amount necessary for normal behavior. This would also indicate that some form of α-tocopherol acts directly in muscle enzyme systems to inhibit or regulate oxidation. Certain substituted phenols have been shown to inhibit respiration in fertilized Arbacia eggs and to inhibit the catalytic activity of flavoprotein,[3] and of cytochrome reductase.[4] The phenolic group in α-tocopherol may normally provide a point of delay to the progress of the oxidative process in muscle tissue. In the absence of

[3] Krahl, M. E., and Clowes, G. H. A., *J. Gen. Physiol.*, 1940, **23**, 413; Krahl, M. E., Keltch, A. K., and Clowes, G. H. A., *J. Biol. Chem.*, 1940, **136**, 563.

[4] Haas, E., Harrer, C. J., and Hogness, T. R., *J. Biol. Chem.*, 1942, **143**, 341.

α-tocopherol, oxidation would be unimpeded at this point and could continue at an excessive rate. Information on the enzyme systems concerned is necessary to an understanding of the rôle of tocopherol in muscle physiology.

13750

Plasma Volume Changes Following the Intravenous Injection of Pectin and Physiologic Saline in Man.

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The use of pectin as a blood substitute in the treatment of patients in shock has been proposed.[1] Since the rationale for the administration of pectin is to increase the plasma volume, information regarding the effect of this material upon the plasma volume is desirable. The present investigation was undertaken to determine the influence of the intravenous injection of a 0.75% solution of pectin† upon the plasma volume, and to compare the results with similar observations following the intravenous injection of physiologic saline.

Nine male patients, who were normal with respect to the cardiovascular system were studied. The plasma volume was determined by the method of Gibson and Evans[2] as modified by Gibson and Evelyn[3] using "Evans Blue" dye (T-1824). In 5 patients after the initial determinations were made, pectin solution was injected intravenously at a rate of 10 cc per minute. In 2 patients dye was reinjected and the plasma volume was redetermined immediately upon completion of the pectin infusion. In 3 patients the second plasma volume determination was made 4 hours after the end of the pectin infusion. In all cases a final determination was made at the end of 24 hours. Similar determinations at corresponding time

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1 Hartman, F. W., Schelling, V., Harkins, H. N., and Brush, B., *Ann. Surg.*, 1941, **114**, 212.

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2 Gibson, J. G., and Evans, W. A., *J. Clin. Invest.*, 1937, **16**, 301.

3 Gibson, J. G., and Evelyn, K. A., *J. Clin. Invest.*, 1938, **17**, 153.