In vitro Incorporation of Glycine-1-^{14}C into Total Muscle Protein of Normal and Vitamin E-Deficient Rabbits.* (30539)

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Heretofore it has not been possible to conclude from *in vivo* studies that the observed increase in incorporation of glycine-1-^{14}C into total muscle protein of vit E-deficient rabbits (1,2) is caused by an abnormality in skeletal muscle *per se*. On the contrary, the multiple metabolic pathways available to glycine in the intact animal, the increased incorporation of glycine-1-^{14}C into plasma proteins of vit E-deficient rabbits(3) and the exceptional metabolism of glycine in vit E deficiency(4) strengthened the possibility that the cause for the increased glycine incorporation into muscle proteins was located outside of skeletal muscle. In addition, *in vivo* studies may be complicated by variations in blood supply to the tissue. Therefore, we have used slice and homogenate preparations of skeletal muscle to study the incorporation of glycine-1-^{14}C into protein. Both preparations exhibited increased oxygen consumption and increased incorporation of glycine-1-^{14}C into total muscle protein as a result of vit E deficiency.

Methods. Four-week-old White New Zealand rabbits of mixed sex were placed on a vit E-deficient semi-synthetic diet. Composition of the diet(4), procedure for addition of sodium sulfaquinoxaline to the drinking water(2) and supplementation of the control animals with vit E(2) were the same as previously described. When the vit E-deficient rabbits showed distinct signs of muscular weakness, they and the control animals which had been on the diet for a comparable length of time (3-4 weeks) were sacrificed for *in vitro* studies. The criterion for muscular weakness was the difficulty the rabbits had in assuming an upright position after being placed on their sides. Only those animals which could right themselves less than 20 times were considered to be dystrophic.

Skeletal muscle was placed on ice immediately after excision and slices were prepared with a Stadie-Riggs tissue slicer. Homogenates were prepared in Krebs-Ringer phosphate buffer (pH 7.4) by passing minced skeletal muscle through an Emanuel-Chaikoff hydraulic homogenizer equipped with a 100 millimicron rod. The incubation flasks for the slice preparations contained 5.1 μmoles (15 μC) of glycine-1-^{14}C in 3.0 ml Krebs-Ringer phosphate buffer and approximately 500 mg of muscle slices. For the study of homogenates, 3.0 ml of a 15% (w/v) homogenate was used and to this was added 0.3 ml of a glycine-1-^{14}C solution containing 5.1 μmoles (15 μC) of glycine. Incubations were carried out in a Warburg apparatus at 37°C so that oxygen consumption could be measured.

Incubations were terminated by addition of 10% trichloroacetic acid (TCA) and protein was isolated by the Schneider procedure(5). To verify that the radioactivity in the protein samples was due to incorporation and not to adsorption, some of the protein samples were dissolved in sodium hydroxide, reprecipitated with TCA and recounted. Several repetitions of this process did not alter the specific activity of the protein samples.

Results and discussion. Oxygen consumption was increased in the muscle slice preparations from vit E-deficient rabbits (Fig. 1a), as previously reported by Houchin(6). In contrast to the observations by Houchin(6), increased oxygen consumption was also found with muscle homogenates (Fig. 1b), but different homogenizing techniques were used and it is possible that our homogenates contained significant numbers of relatively intact cells. The increase both for the slice and for the homogenate preparations was significant ($P<0.025$).

Although increased oxygen consumption has been one of the earliest recognized abnormalities in vit E deficiency, the nature of this defect is still uncertain. It may be due

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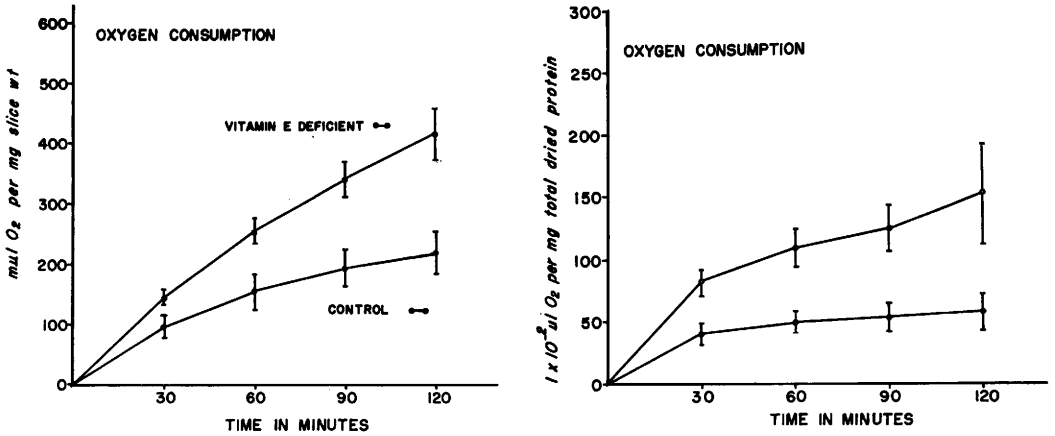

FIG. 1. Effect of vit E deficiency on oxygen consumption of muscle slices (left) and homogenates (right). Points represent mean values obtained from 5 vit E-deficient and 5 control animals for experiments with slices, and 4 vit E-deficient and 4 control animals for experiments with homogenates. Vertical lines represent the standard deviation of the mean, calculated according to the following equation:

$$\text{Standard deviation of mean} = \frac{\sqrt{\frac{\Sigma d^2}{n-1}}}{\sqrt{n}}.$$

to the lack of the antioxidant function of vit E. However, the observation that $^{14}CO_2$-formation from ^{14}C-labeled amino acids is increased in vit E-deficient rabbits, while $^{14}CO_2$-formation from labeled glucose, acetate and palmitate is not(7) indicates that the problem is more complex. A direct relationship between oxygen consumption and membrane transport has been shown in frog tissues(8,9) and a good correlation between oxygen consumption and thyroxine-stimulated protein synthesis has been demonstrated(10, 11). The increased oxygen consumption of muscle from vit E-deficient animals could possibly be related to more active transport processes, or to more rapid protein synthesis or to both.

Increased amino acid transport into muscle of vit E-deficient rabbits has been found *in vivo* and *in vitro*(12), and it has not been clear from previous *in vivo* studies(1-4) whether increased glycine incorporation into proteins is a reflection of changes in glycine transport, or of increased muscle protein synthesis, or of other abnormalities, such as increased blood supply. The possibility of abnormal glycine metabolism outside skeletal muscle is indicated by the finding of low urinary excretion of glycine in comparison to other amino acids(4) and of increased incorporation of glycine-1-^{14}C into plasma proteins(3). In vit E-deficient animals there might be increased blood flow to the muscle, as in denervated muscle(13), or larger blood pools in muscle, as reported for mice with hereditary dystrophy(14). It follows that the increased appearance of glycine-1-^{14}C in protein isolated from muscle of vit E-deficient rabbits could be due to plasma proteins trapped in the muscle. These complications have been eliminated in the present studies by using *in vitro* preparations.

The incorporation of glycine-1-^{14}C into total muscle protein was significantly increased ($P<0.05$) in muscle slices and in homogenates from vit E-deficient rabbits (Fig. 2a and 2b). The difference between means of the two experimental groups was somewhat greater with slices than with homogenates. The finding that intactness of cellular structures enhances the difference in glycine incorporation rates is in agreement with the concept that differences in glycine transport into muscle cells are at least partly responsible for the higher rate of incorporation of glycine into muscle proteins of vit E-de-

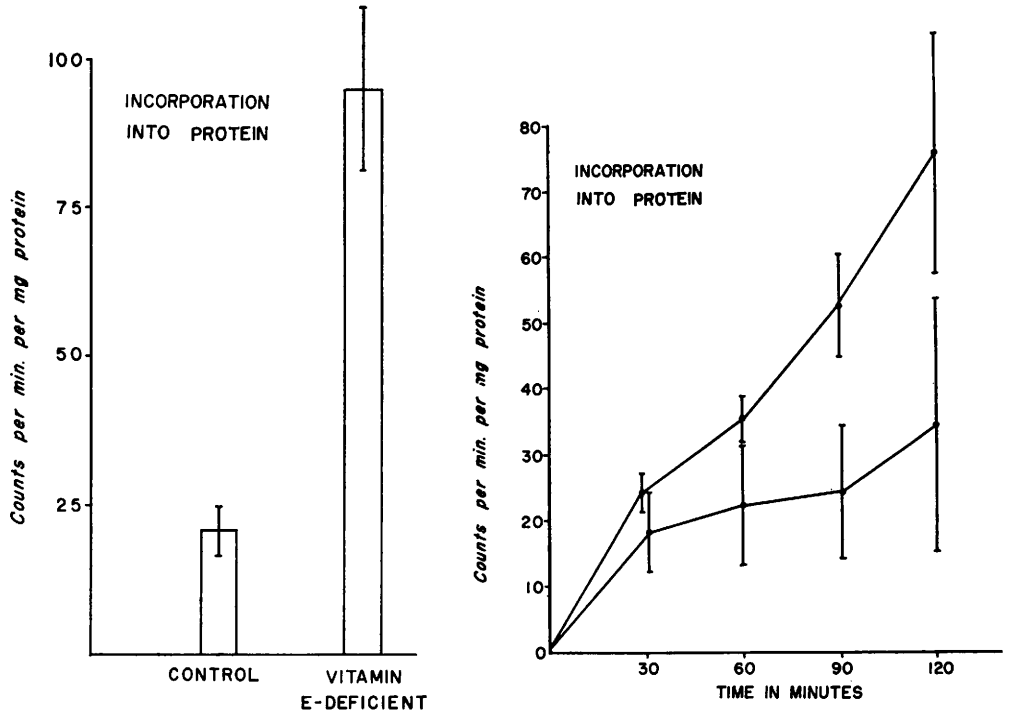

FIG. 2. Effect of vit E deficiency on glycine-1-^{14}C incorporation into total muscle protein of muscle slices (left) and homogenates (right). In the experiments with slices (left) protein radioactivity was determined after 120 min of incubation. No. of animals used and designation of vit E-deficient and control groups as in Fig. 1.

ficient animals. Whether the increased incorporation observed with homogenates of dystrophic muscle is due to the presence of intact cells or to a higher rate of protein synthesis remains to be established.

The results of these *in vitro* experiments constitute conclusive evidence that the increased glycine incorporation into muscle proteins of vit E-deficient rabbits cannot be due to an abnormality of glycine metabolism outside the muscle tissue or to differences in blood supply.

Summary. Skeletal muscle slices and homogenates from control and vit E-deficient rabbits were incubated in the presence of glycine-1-^{14}C. In both preparations vit E deficiency resulted in an increased glycine incorporation into total muscle protein which was accompanied by increased oxygen consumption.

1. Dinning, J. S., Sime, J. T. and Day, P. L., J. Biol. Chem., 1955, v217, 205.

2. Diehl, J. F., Arch. Biochem. Biophys., 1960, v87, 339.

3. ———, Z. Naturforsch., 1963, v18b, 168.

4. ———, Proc. Soc. Exp. Biol. and Med., 1959, v100, 657.

5. Schneider, W. C., J. Biol. Chem., 1945, v161, 293.

6. Houchin, O. B., ibid., 1942, v146, 313.

7. Diehl, J. F., Arch. Biochem. Biophys., 1962, v99, 148.

8. Zerahn, K., Nature, 1956, v177, 937.

9. Leaf, A., Renshaw, A., ibid., 1956, v178, 156.

10. Hanson, R. W., Lindsay, R. H., Barker, S. B., Endocrinol., 1961, v69, 883.

11. ———, Biochim. Biophys. Acta, 1963, v68, 134.

12. Diehl, J. F., Biochem. Z., 1963, v337, 333.

13. Hudlická, O., in The Denervated Muscle, E. Gutman, ed., Prague, 1962, p175.

14. Rabinowitz, J. L., Burns, J., Chase, G. D., Allen, H. C., Jr., Atompraxis, 1959, v5, 487.

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