INCORPORATION OF METHIONINE SULFUR INTO TISSUE PROTEINS OF GUINEA PIGS WITH VITAMIN E DEFICIENCY

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In vitamin E deficiency there is an increase in the incorporation of glycine-1-C^{14} [5, 7-9] and of methionine-S^{35} [1, 4] into the proteins of the skeletal muscles of rabbits. At the same time the incorporation of lysine-1-C^{14} and leucine-1-C^{14} does not change. The incorporation of glycine-1-C^{14} into proteins of the marrow, small intestine, spleen and liver [9] was, on the other hand, lowered.

It is known that in avitaminosis E the content of vitamin A in the liver of various animals decreases, which is explained by the antioxidant properties of vitamin E [3, 6, 11, 12].

It was expeditious to study the characteristics of protein metabolism of guinea pigs by means of methionine-S^{35} in a vitamin E deficient diet that included a high content of vitamin A.

EXPERIMENTAL METHOD

The experimental guinea pigs weighing 200-220 g were divided into 5 groups (6-10 animals in each group).

The first group included animals that had received the usual vivarium ration. The animals of the other groups were given as a basic ration the diet recommended for guinea pigs [10] with certain modifications: casein, 150 g; sucrose, 510 g; lard, 50 g; cod liver oil, 30 g, salt mixture 60 g; potassium acetate, 25 g; magnesium oxide, 5 g; cellulose, 100 g; dry brewer's yeast, 100 g; arginine, 5 g. In addition the animals received daily 0.5 ml of cod liver oil and 10 mg of ascorbic acid. Thus, the animals received 2000 international units of vitamin A weekly, which is considered sufficient to satisfy the needs of guinea pigs [10].

The animals of the second group received the indicated basic ration, those of the 3rd group received additionally a preparation of dl-α-tocopherol acetate in a dose of 25 mg twice a week; those of the 4th group received additionally twice a week 3000 I.U. of vitamin A in the form of a highly active concentrate; those of the fifth group received additionally both vitamins in the indicated quantities.

Radioactive methionine (0.1 μ Ci per 1 g of weight) was administered to the animals 2 h before decapitation. Preliminary experiments demonstrated that during this time the radioactivity of a trichloracetic extract is not less than 50% of the total radioactivity of tissues. Consequently, during this period the incorporation of the radioactive tracer into proteins still exceeds its exclusion. The specific radioactivity of tissue proteins was calculated for 10 mg of protein freed from lipids and nucleic acids.

The relative specific radioactivity was the ratio of the specific radioactivity multiplied by 100 to the total radioactivity of 1 g of tissue.

EXPERIMENTAL RESULTS

Evident signs of a deficiency of vitamin E (hyperexcretion of creatine and marked muscular dystrophy) were evidenced in the animals of the 2nd and 4th group 4-6 weeks after putting them on the experimental diet.

TABLE 1. Specific Radioactivity of Tissue Proteins of Guinea Pigs (in cpm/10 mg protein)

Tissue	Group of animals				
	1st	2nd	3rd	4th	5th
	$M \pm m$				
Brain	115±9,8	69±6,4 T=3,30	108±10,6	111±15,0	155±16,0
Skeletal muscle	81±5,8	22±4,25 T=8,30	92±18,0	172±18,0 T=4,6	107±25,5
Heart muscle	213±8,7	102±10 T=8,30	201±23,0	224±11,6	238±19,2
Liver	372±24,0	417±33,0	506±36,0 T=3,10	375±45,0	737±67,0
Plasma	484±27,8	517±16,4	683±50 T=3,50	620±45,0	662±53 T=3,0
Spleen	490±46,0	346±16,0	534±45,0	460±48	470±29
Kidney	1 029±95,0	500±43,0 T=5,0	985±54,0	905±37,0	966±48,0

TABLE 2. Relative Specific Radioactivity of Tissue Proteins of Guinea Pigs

Tissue	Group of animals				
	1st	2nd	3rd	4th	5th
	$M \pm m$				
Brain	3,70±0,16	2,70±0,19 T=4,0	4,00±0,41	4,10±0,41	3,92±0,40
Skeletal muscle	2,20±0,16	0,60±0,064 T=10,0	2,12±0,32	2,43±0,25	2,44±0,27
Heart muscle	3,20±0,16	1,78±0,17 T=6,5	2,98±0,15	3,00±0,27	2,80±0,26
Liver	1,80±0,10	1,22±0,07 T=4,8	1,64±0,10	1,95±0,22	3,02±0,56
Plasma	2,20±0,12	1,66±0,19 T=2,45	2,35±0,16	2,69±0,16	2,68±0,14
Spleen	4,00±0,32	2,64±0,17 T=3,60	4,30±0,38	4,30±0,37	4,30±0,49
Kidney	1,94±0,11	1,00±0,13 T=3,60	2,45±0,18	2,50±0,29	2,90±0,053

There were no signs of vitamin E deficiency in the animals of the 3rd and 5th groups after 5-6 weeks on the experimental ration; the same picture was observed in animals of the first and control groups.

A statistically significant decrease in the incorporation of methionine-S^{35} into proteins of the brain, skeletal muscles, heart muscle and kidneys was noted in guinea pigs of the 2nd group (Table 1). Judging by the relative specific radioactivity, the incorporation of the tracer significantly decreased also with respect to proteins of the liver and spleen (Table 2). A similar drop occurs with an insufficiency of vitamin A in the food ration [2].

The radioactivity of proteins, both specific and relative specific did not drop in the animals of the 4th group which were also on the vitamin E deficient diet and which had all signs of dystrophy but which had received additionally a concentrate of vitamin A. If we judge by specific radioactivity we note a high incorporation of S^{35} into proteins of the skeletal muscles of guinea pigs. The obtained data confirm the results of experiments on protein metabolism by means of radiomethionine that were carried out on vitamin E deficient rabbits [1]. These changes were absent on calculation of the relative specific radioactivity.

Moreover, we need point out the statistically significant increase of the specific activity of liver and blood plasma proteins of animals in the 3rd and 5th groups in comparison with those of the animals in the first group, which was not elicited in the calculation of the relative specific radioactivity.

It follows from the data obtained that to preclude secondary protein metabolic disorders indirectly associated

with avitaminosis E, vitamin A should be included in the vitamin E deficient diet in a higher quantity (for guinea pigs 8000 I. U. per week instead of 2000 I. U.). This does not remove the specific phenomenon of vitamin E deficiency, particularly the development of muscular dystrophy and the associated increase of specific activity of muscle proteins that was established in animals of the 4th group. Apparently the latter to a considerable extent is due to a change of the penetration of the labeled methionine into cells of the skeletal muscles in vitamin E deficiency, since there is no difference between muscle proteins of control and vitamin E deficient animals, according to the data of the relative specific radioactivity.

At the same time we need recognize that the incorporation of methionine S^{35} into the proteins of the brain, heart muscle, plasma, liver, spleen and kidneys in vitamin E deficiency of guinea pigs does not change. The decrease, noted in certain works, of the incorporation of a radioactive tracer into the proteins of these tissues when labeled amino acids are used is evidently a consequence of the metabolic disorders that arise in a vitamin E deficient ration and the use of vitamin A.

SUMMARY

Examination of methionine-S^{35} incorporation into the tissue proteins demonstrated that to prevent secondary metabolic disturbances, associated indirectly with E-avitaminosis, an increased amount of vitamin A (8,000 I. U. a week instead of 2,000 I. U.) should be included into the E vitamin devoid diet of guinea pigs. However, this did not eliminate distinct E avitaminosis phenomena, notably the development of muscular dystrophy. In this connection there was a rise in specific radioactivity of muscular proteins in the experimental animals. Evidently, this was largely due to changes in the labeled methionine penetration into the cells of the skeletal musculature during E-avitaminosis, since according to the relative specific radioactivity data there was no difference between the proteins of control and those of the animals with E-avitaminosis.

Reduction of methionine-S^{35} incorporation into the brain proteins of the cardiac muscle, plasma, liver, spleen and kidneys detectable in guinea pigs kept on vitamin E-devoid diet, is absent when vitamin A is given in increased amounts. Hence, this reduction cannot be considered as characteristic of E-avitaminosis.

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