Incorporation of [^{14}C] Amino Acids into Liver S-RNA of Chick. Effect of Orotic Acid and Vitamin B_{12}

The participation of vitamin B_{12} in protein metabolism has been suggested by various authors to explain the nutritional importance of this vitamin and of its metabolic disturbances in deficient organisms. Thus WANGLE et al.[1] found a decreased incorporation of glucose and serine into liver protein in vitamin B_{12}-deficient animals. Moreover, WANGLE et al.[2,3] observed that the incorporation of amino acids into protein in cell-free preparations from vitamin B_{12}-deficient rat liver is restored by addition in vitro of the vitamin.

Other experimental data appear to be in contrast with these results. In fact, STEKOL et al.[4], ARNSTEIN et al.[5,6] and FRASER et al.[7] showed that the incorporation of amino acids into protein was not decreased by vitamin B_{12}-deficiency. Therefore, according to these authors, vitamin B_{12} does not play a direct role in the activation of amino acids or in their incorporation into protein.

In order to investigate further relationships of vitamin B_{12} to protein synthesis, the effect of dietary vitamin B_{12} on the incorporation in vitro of L-[^{14}C] leucine, L-[^{14}C] methionine, and L-[^{14}C] serine into liver soluble RNA of vitamin B_{12}-deficient chicks has been studied. Moreover, the effect of dietary orotic acid in the same experimental conditions has been investigated. Since orotic acid is a growth factor for chicks and rats maintained in a dietary condition in which only B_{12} and methionine are active[8], it is possible that also this substance can play a role in protein synthesis.

1-day-old New Hampshire × White Leghorn chicks were divided into three groups and fed ad libitum; one group a vitamin B_{12}-deficient diet[9], the other two groups the same diet supplemented for each 100 g with 10 μg of vitamin B_{12} or with 10 mg of orotic acid respectively. After 5 weeks, the chicks were killed and the livers were removed, dropped into cold 0.03 M KCl and homogenized with 4 volumes of 0.03 M KCl. The homogenates were centrifuged for 90 min at 105,000 g. The pH of supernatant was lowered to 5.2 by ice-cold 0.02 N HCl, and after a few minutes the mixture was centrifuged at 5000 g at 2 °C. The precipitate was dissolved in 0.1 M *tris*-HCl buffer (pH 7.6) to give a final protein concentration, determined by the method of LOWRY et al.[10], of about 10 mg/ml. Aliquots of 1 ml of this solution were incubated for 10 min at 37 °C with 10 μM ATP, 10 μM $MgCl_2$, 0.033 μM L-(U-^{14}C) leucine (specific radioactivity 34.8 μC/μM), 0.508 μM L-(U-^{14}C) methionine (15.6 μC/μM) or 0.05 μM L-(U-^{14}C) serine (22.2 μC/μM); the final volume was made up to 3 ml with 0.1 M *tris*-HCl buffer (pH 7.6). The reaction was stopped by the addition of 1 ml of 5% cold perchloric acid, and the precipitates obtained after centrifugation were dissolved in 2 N NH_4OH. Suitable aliquots were plated and counted with a windowless gas flow counter.

From the Table a significant increase of [^{14}C] serine incorporation into liver S-RNA of vitamin B_{12}-fed rats appears as compared with deficient rats ($P < 0.01$). On the contrary, the [^{14}C] leucine and [^{14}C] methionine incorporation is not modified between deficient and vitamin B_{12}-treated animals.

The increased incorporation of the amino acid serine in the vitamin B_{12}-treated animals could suggest a specific effect of the vitamin on the serine metabolism. On the other hand, it has been shown in previous studies that vitamin B_{12} was able to increase the liver content of free serine[8] and the utilization of its β-carbon for the conversion of folic acid to citrovorum factor and for the biosynthesis of the methyl group of methionine[11].

The incorporation of the three amino acids into liver S-RNA of orotic acid-treated animals shows a similar behaviour to that in the vitamin B_{12}-treated animals; that is, a higher [^{14}C] serine incorporation in comparison with deficient animals ($P < 0.01$) while no significant differences are observed in the incorporation of [^{14}C] leucine and [^{14}C] methionine. Therefore orotic acid seems to display, also at this level and particularly in serine metabolism, an action similar to vitamin B_{12}[12].

Effect of dietary vitamin B_{12} and orotic acid on incorporation of [^{14}C] labelled amino acids into liver S-RNA of vitamin B_{12}-deficient chick

Group No.	Supplement to deficient diet	L-[^{14}C] leucine	L-[^{14}C] methionine	L-[^{14}C] serine
		mμM/mg of liver S-RNA		
1	None (6)	0.74 ± 0.064	1.51 ± 0.10	0.90 ± 0.098
2	10 μg vitamin B_{12} (6)	0.83 ± 0.024	1.41 ± 0.081	1.53 ± 0.190
3	10 mg orotic acid (6)	0.71 ± 0.041	1.56 ± 0.130	1.14 ± 0.044

Numbers in parenthesis denote number of rats used. Values are means ± S.E. of the mean.

Riassunto. La somministrazione dietetica di vitamina B_{12} e di acido orotico determina un aumento della incorporazione della L-serina C^{14} nello RNA solubile del fegato di pulcino carente di B_{12}, mentre non è in grado di influenzare quella della L-leucina ^{14}C e della L-metionina ^{14}C.

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