[^{3}H]Formate Incorporation into Liver Nucleic Acids of Chick. Relationships Between Orotic Acid and Vitamin B_{12}

One of the most interesting aspects of the relationships between orotic acid and vitamin B_{12} concerns their common action in nucleotide metabolism. Orotic acid, in fact, was found to exert the same effects as those which had already been demonstrated for vitamin B_{12}. Orotic acid, like vitamin B_{12}, leads to an increase in the liver concentration of RNA[1] and in the free nucleotides of the liver in vitamin B_{12}-deficient chicks[2]. Under the same experimental conditions, an increased incorporation of P^{32} was also observed[3] in these structures.

In order further to investigate the role that orotic acid plays in this metabolic field, the effects of orotic acid have been studied on the turnover of acid-soluble free nucleotides and of nucleic acids of single cell fractions in the liver of vitamin B_{12}-deficient chicks. In particular, the incorporation of [^{3}H]formate into these structures has been evaluated. These results were then compared with the results obtained in animals treated with vitamin B_{12}. This work might also provide information on the role that the orotic acid can play in protein synthesis. It has been shown that orotic acid is a growth factor for chicks and rats that have been maintained under dietary conditions where only B_{12} and methionine are active[4,5].

One-day-old New Hampshire × White Leghorn chicks were divided into 3 groups. 1 group received a vitamin B_{12}-deficient diet[6]. The other 2 groups received the same diet supplemented with either 10 μg of vitamin B_{12}, or with 10 mg of orotic acid for every 100 g of diet. After 5 weeks, 6 chicks from each group were injected with [^{3}H]sodium formate in a sterile solution (20 μC/100 g body weight). 24 h later, the chicks were killed, the livers were quickly removed, placed into an ice-cold solution of 0.25M sucrose containing 3 mM $MgCl_2$, and homogenized with 4 volumes of this same medium. The homogenates, filtered through a nylon bolting cloth, were centrifuged at 800 g for 10 min to sedimentate the nuclei. The supernatant was centrifuged at 8,000 g for 15 min; the pellet discarded and the supernatant fluid was centrifuged at 105,000 g for 60 min in a Spinco model L centrifuge to sedimentate the microsomes. The nucleic acids of the nuclei and of the microsomes were extracted with 10% (w/v) $HClO_4$.

RNA was then hydrolyzed with 0.3N KOH at 37 °C for 60 min and nuclear DNA was hydrolyzed with 5% (w/v) $HClO_4$ at 90 °C for 15 min[7]. The amount of RNA and DNA present was calculated by measuring the extinction at 260 nm. The postmicrosomal supernatant was treated with 10% $HClO_4$ and centrifuged. The residue was used to determine soluble RNA according to TATA et al.[8]. The supernatant, containing acid-soluble free nucleotides, was absorbed on an ion-exchange resin column (12 · 1.2 cm diameter) of Dowex 1.8, formate form, 200–400 mesh. The column was washed with water and the total free nucleotides were then eluted with several volumes of 10N formic acid. The optical density of the eluted fluid was measured spectrophotometrically at 260 nm.

Samples of RNA, DNA and free nucleotide preparations were mixed with a scintillator solution (0.4% of 2,5-diphenyl-oxazole and 0.005% of 1,4-bis[2-(5-phenyl-oxazolil)] benzene in redistilled toluene) and counted in a Nuclear-Chicago automatic liquid scintillation spectrometer model 720.

The results of the Table indicate that in vitamin B_{12}-treated chicks, the incorporation of [^{3}H]formate into the RNA of liver cell fractions [in particular, the RNA of the nuclei ($P < 0.001$), the RNA of the ribosome ($P < 0.001$) and of cytoplasmic soluble fraction ($P < 0.001$)] is significantly higher than in the liver of vitamin B_{12}-deficient chicks. A marked increase in acid-soluble free nucleotides is also noted ($P < 0.001$).

However, the incorporation of [^{3}H]formate into the DNA does not vary between vitamin B_{12}-deficient and vitamin B_{12}-treated animals.

The incorporation of [^{3}H]formate into liver nucleic acid and the acid-soluble free nucleotide of orotic acid treated chicks shows a similar behaviour as in vitamin B_{12}-treated chicks.

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Effect of orotic acid and vitamin B_{12} on the incorporation of [^{3}H]formate into nucleic acid of liver cell fraction and into free nucleotides of vitamin B_{12}-deficient chicks

Group	Animals in experiment	Radioactivity in liver nucleic acid (cpm/mg nucleic acid)				Radioactivity in free nucleotides (cpm/mg nucleotides)
		nuclear DNA	nuclear RNA	ribosomal DNA	soluble RNA	
1	Vitamin B_{12}-deficient	450 ± 10.6	473 ± 24.0	194 ± 15.9	327 ± 12.6	880 ± 27.3
2	Vitamin B_{12}-treated	473 ± 15.5 (n.s.)	1168 ± 61.2 ($P < 0.001$)	543 ± 38.4 ($P < 0.001$)	770 ± 39.6 ($P < 0.001$)	1672 ± 77.9 ($P < 0.001$)
3	Orotic acid-treated	433 ± 20.4 (n.s.)	1248 ± 38.4 ($P < 0.001$)	429 ± 18.8 ($P < 0.01$)	449 ± 17.5 ($P < 0.05$)	1538 ± 46.1 ($P < 0.001$)

The results are expressed as means of each group of 6 animals ± S.E. of mean, P, probability of difference from vitamin B_{12}-deficient groups (student's test), n.s., non-significant.

A significant increase in the incorporation of [^{3}H]formate into nuclear RNA ($P < 0.001$), ribosomal RNA ($P < 0.01$) and acid-soluble free nucleotides ($P < 0.001$) is observed when compared with vitamin B_{12}-deficient chicks. A smaller but rather significant increase is found in soluble RNA ($P < 0.05$). No significant difference was observed in liver DNA.

The results obtained seem to indicate a further proof of the existence of a close relationship between vitamin B_{12} and orotic acid in the metabolism of nucleic acids. In fact, the increased RNA biosynthesis in subcellular fractions and the increased biosynthesis of total free nucleotides in the liver of chicks, treated with vitamin B_{12} or orotic acid, is in agreement with the results previously reported[1-3].

The variations in the nuclear and ribosomal RNA specific activity that were observed either in the group treated with vitamin B_{12} or with orotic acid, can be regarded as the expression of an increased messenger-RNA biosynthesis.

This finding could possibly lead us to see a common role of orotic acid and vitamin B_{12} at the transcription processes level. The modifications of specific enzymatic activities observed in the same experimental conditions[9-11] may confirm this viewpoint[12].

Riassunto. E' stato studiato l'effetto dell'acido orotico sull'incorporazione di formiato-H^3 nei nucleotidi liberi totali e negli acidi nucleici presenti nelle frazioni subcellulari del fegato di pulcino carente di B_{12}. La maggiore attività specifica dell'RNA nucleare e ribosomiale che si osserva negli animali trattati con acido orotico come in quelli trattati con vitamina B_{12} potrebbe essere considerata l'espressione di una più elevata sintesi di RNA messaggero determinata da queste due sostanze.

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Glycoside Effect upon Membrane Enzymes of Erythrocytes and Muscle in Duck Myopathy

Myopathies frequently have been associated with muscle-membrane abnormality. The suggestion that these abnormalities are themselves indications of a generalized and probably hereditary defect and might therefore have reflection in non-muscle cells[1] has awaited confirmation. Heretofore, the test applied was relatively insensitive. In an attempt to examine this at higher level of resolution, we have made reference to the pattern of membrane-catalyzed adenosine-triphosphate hydrolysis.

Ducks (*Anas platyrhynchos*) of the white Pekin strain, known to spontaneously developing myopathy[2-4] and Mallards, generally free of the defect, were used in this study. Birds of varying age were used for specimens of blood and of striated muscle from the legs. Individual animals were distinguished as myopathic or as 'normal' (Table). The clinical and pathologic characteristics of the myopathy occurring in the white Pekin duck have been described[2-4].

In each case, five ml of blood was collected in a Vacutainer tube containing 25 mg of Na_2EDTA and stored refrigerated. The blood samples were used within 2 days.

Blood samples were haemolyzed with aqueous *Tris* buffer, 0.002*M*, pH 7.4, with 0.005*M* Na_2EDTA. In each instance, a 1 ml sample was treated with 10 ml of the solution and then refrigerated for 5 min. The preparation was then centrifuged at 20,000 *g* for 15 min. The pellet was washed and recentrifuged 4-5 times in the same buffer and 0.002*M* NaCl. Before each wash the pellet was homogenized. The supernatant was discarded each time. The yellowish-white final pellet was collected and used as the source of ATPase activity.

Membrane fractions from muscle tissues (removed from the leg) were isolated by differential centrifugation.

Summary of experimental results; the effect of ouabain $10^{-4}M$ upon ATPase activity[a]

Bird	Strain	Muscle	Age	Red-blood cell ghosts	100,000 × *g* muscle fraction	80,000 × *g* muscle fraction
3024	Pekin	Normal	Mature	−		
3090	Pekin	Normal	Mature	−		
3041	Mallard	Normal	Mature	−	−	−
3052	Mallard	Normal	Mature	−	−	−
3056	Mallard	Normal	Mature	−	−	−
3192	Pekin	Normal	Mature	−		
3198	Pekin	Normal	Mature	−		
2202	Pekin	Normal	Mature	−		
3238	Pekin	Normal	Mature	−		
3246	Pekin	Normal	Mature	−		
3222	Pekin	Normal	Mature	−		
3221	Pekin	Normal	Mature		−	−
3205	Pekin	Normal	Mature		−	−
3149	Pekin	Myopathic	4 weeks	+	+	
3150	Pekin	Myopathic	4 weeks	+	+	
2928	Pekin	Myopathic	Mature	+	+	+
2945	Pekin	Myopathic	Mature	+	+	+
3102	Pekin	Myopathic	Mature	+	+	+
3140	Pekin	Myopathic	Mature	+	+	+

[a] − = inhibition of ATPase activity; + = stimulation.

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