INFLUENCE OF VITAMIN A DEFICIENCY ON THE BIOSYNTHESIS OF CHOLESTEROL, SQUALENE AND UBIQUINONE

U. Gloor and O. Wiss

Department of Vitamin and Nutritional Research of F.Hoffmann-La Roche & Co.Ltd. Basle, Switzerland

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In previous work done in this laboratory it has been shown that ^{14}C-labeled mevalonic acid is incorporated into the ubiquinone (coenzyme Q) molecule by the rat liver (Gloor and Wiss, (1958)). The rate of incorporation was found to be depending on the nutritional supply with vitamin A. An increase could be stated with progressive vitamin A-depletion. Moreover strong indications were found that in addition the cholesterol and squalene synthesis is influenced by vitamin A-deficiency (Gloor and Wiss, (1959)). Using the same technique as described before (Gloor and Wiss, (1959)), new evidence for vitamin A to be involved in the biosynthesis of these terpenoid substances could be obtained.

All rats, despite their body weight differences, were supplied with the same dose (500 µg) of ^{14}C-labeled mevalonic acid (575,000 c/mg) by intraperitoneal injections. Table I shows that the total rate of incorporation into the unsaponifiable material of rat liver does not depend on body weight. The rate of mevalonic acid incorporation into the total unsaponifiable material lies between 11 % and 18 % of the applied dose of racemic mevalonic acid. It seems, however, that after prolonged vitamin A-depletion the incorporation of mevalonic acid is reduced. In these experiments all the animals were sacrificed twenty hours after administration of mevalonic acid. In the former experiments (Gloor and Wiss, (1959)), however, this period lasted three days. The inconsistency concerning the total amount of mevalonic acid incorporation must probably be attributed to these different time intervals.

Despite these differences in the total amount of mevalonic acid incorporation, the influence of vitamin A-depletion on the ubiquinone, cholesterol and squalene synthesis (squalene has now been identified) could be fully confirmed. The cholesterol synthesis was found reduced from 94 % to 45 %; the squalene synthesis increased from 2 % to 31 %, and that of ubiquinone from 2 % to 14 % (table I).

Table I

Days of vitamin A-application	Body weight in g (duplicates)		Total activity in the unsaponifiable, cpm (pooled livers)	% Incorporation of radioactivity (total unsaponifiable material = 100 %)		
				squalene	ubiquinone	cholesterol
0	37	35	99,400	2.0	2.4	94.5
7	59	53	98,900	8.8	10.2	80.4
14	87	79	102,300	20.0	14.1	52.8
21	101	106	68,200	26.0	10.4	58.2
24	90	74	64,800	30.9	13.4	44.5
← Single dose of 500 I.U. vitamin A orally per animal →						
20 hr after vitamin A application	105	85	96 000	14.3	9.9	75.0

Whereas mevalonic acid incorporation into squalene was continuously increasing during the vitamin A-depletion period, the incorporation into ubiquinone reached a plateau value after about two weeks (table I). This agrees with the assumption that the benzoquinone part of the molecule, being essential for the animal body, becomes limiting for its synthesis.

A marked increase of mevalonic acid incorporation into fatty acids of the liver during the vitamin A-depletion period was also observed. The data available indicate that the activity was mainly present in the branched but not in the straight chain fatty acids.

A problem of primary interest is whether these effects of vitamin A-depletion are secondary ones, due for instance to morphological alterations,

or whether vitamin A is acting on a metabolic level. In this respect it is interesting to note that already 20 hours after a single dose of vitamin A, given to animals depleted during three and a half weeks, a clear trend towards normalization of the cholesterol and squalene synthesis was observed. Mevalonic acid incorporation into cholesterol rose from 44 % to 75 %, that into squalene decreased from 31 % to 14 % (table I). The ubiquinone synthesis was found only little changed. These values correspond quite well with those obtained after one week of vitamin A-depletion. The early biochemical alterations, observed after such a short deficiency period, and their rapid reversion after a single dose of vitamin A, suggest an action of vitamin A on a metabolic level. It is assumed that vitamin A or a metabolic derivative is involved in one of the last steps of the cholesterol synthesis. By such an interaction it is reasonable that squalene, other precursors, and its metabolic derivatives are being enriched in the vitamin A-deficient state.

From pooled livers of vitamin A-deficient rats 318 mg of crystalline ubiquinone were isolated. It turned out to be a mixture of ubiquinone(50) and ubiquinone(45). By chromatography on polyethylene powder only 13 mg of pure ubiquinone(50), but 191 mg of pure ubiquinone(45) were obtained. The intermediate fractions contained a mixture of both. Approximately 90 % of the total amount were found to be ubiquinone(45). These ubiquinones were identified by comparison with samples obtained by partial synthesis (Rüegg et al.,(1959)) (mp., UV.-spectrum - $E^{1\,\%}_{1cm}$ at 272 mμ - and paper chromatography). The investigations of Bruce et al. (1959) indicate that also in the liver of normal rats ubiquinone(45), in addition to ubiquinone(50), is present. Further work is under way to elucidate whether the high percentage of ubiquinone(45) is related to vitamin A-deficiency.

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