Vitamin A acid and liver levels of ubiquinone and ubichromenol

DOWLING AND WALD[1] have recently found that vitamin A acid (the carboxylic acid corresponding to vitamin A, $C_{19}H_{27}CH_2OH$) does not sustain the retinal functions of the vitamin, but they confirmed in more detail earlier reports[2,3] that it was effective in other respects. It maintained normal growth and good outward appearance of animals, and cured the overt signs of vitamin A deficiency. WODSAK[4] has reported that the acid also has activity in the test on cornified vaginal epithelium.

A readily measurable characteristic of the vitamin A-deficient rat is the rise in liver ubiquinone and ubichromenol concentrations[5,6]. (In earlier papers these have been referred to as substances SA and SC respectively.) A preliminary experiment in this laboratory by REDFEARN had indicated that animals fed on a vitamin A-deficient diet supplemented with vitamin A acid had liver ubiquinone concentrations in the normal range.

In the present work 24 male weanling rats (weights 31–43 g) were divided into three groups of eight. The first was fed on a diet[6] deficient in vitamin A. Animals in the second group received the same basal diet plus 350 μg vitamin A acid (as the sodium salt) twice weekly; those in the third group had the basal diet supplemented twice weekly with 350 I.U. vitamin A (= 105 μg vitamin A alcohol) in the form of fish liver oil (approx. 0.3 ml). Molecule for molecule, the amount of acid fed was therefore a little over three times that of the vitamin A. Reports[2–4,7] differ as to the biological potency of vitamin A acid, but most assess it as having lower activity than vitamin A itself; the 3-fold larger dose was given to allow for this. The amount was more than adequate for normal development[1], and the increase in weight of the animals fed vitamin A acid was approximately the same as of those given vitamin A. The deficient group grew similarly up to the stage of weight plateau (3–5 weeks); after a time they began to lose weight rapidly as other deficiency signs became severe. They were then killed, together with their companion animals in the other two groups.

The bodies and livers were weighed and the ubiquinone and ubichromenol contents of the livers estimated spectroscopically after saponification and chromatography[6]. Alumina columns were used for ubiquinone[6]; ubichromenol was obtained by further chromatography on magnesium oxide using increasing concentrations of methylal in ether as eluents. The livers of each group were investigated in two batches of three and one of two; the mean results are shown in Table I.

Most of the figures obtained on the separate batches did not differ from the mean shown in Table I by more than 20 %, with the exception of the ubichromenol concentration of deficient animals (up to 35 % difference). There is a clearcut distinction

TABLE I

Group *Diet*	*1* *Deficient*	*2* *Fed vitamin A acid*	*3* *Fed vitamin A*
Mean body weight (g)	67	110	111
Mean liver weight (g)	2.7	5.0	5.1
Mean liver ubiquinone concentration (μmole/g)	0.446	0.150	0.172
Mean liver ubichromenol concentration (μmole/g)	0.061	0.027	>0.013*

* See text.

between the deficient animals on one hand, and those fed either vitamin A or the acid on the other.

An accurate estimate was not possible of the mean liver ubichromenol concentration of animals fed vitamin A. At least 0.013 μmole/g was present, but the true value is higher. Some ubichromenol was lost during the lengthy chromatography needed to separate it from relatively large amounts of vitamin A.

Vitamin A acid is thus effective in maintaining normal not only the body and liver weights but also the ubiquinone levels in rat liver and it prevents a rise in ubichromenol concentration. These facts add to growing evidence that vitamin A acid can fully replace vitamin A alcohol in its "systemic" mode of action.

Vitamin A acid, unlike vitamin A, is not stored in the liver[1,3]. Our results show that the dramatic rise of liver ubiquinone concentration in vitamin A deficiency is part of the deficiency syndrome rather than an indication of the absence of stored vitamin A.

By feeding animals vitamin A acid, it is possible to study minor constituents of liver lipids, unhampered by the presence of vitamin A and its breakdown products which frequently bedevil such work, as with ubichromenol here. Our findings provide some assurance that the lipid picture in the animals will be normal. Furthermore, animals reared on vitamin A acid can rapidly be made avitaminotic, simply by withdrawing it from the diet; this would readily allow the changes in isoprenoid metabolism brought about in vitamin A deficiency[5,6,8,9] to be studied in the mature rat.

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