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Effect of hypervitaminosis A on rats: observations on growth and liver storage of vitamin A

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1. The effect of giving 14400, 28800, 57600 or 93600 i.u. retinol daily for 10 days to adult rats on their daily food consumption, body-weight and liver vitamin A content has been studied.

2. Rats given retinol lost weight and ate less food and showed toxicity symptoms dependent upon the amount of retinol fed.

3. The retention of vitamin A (as a percentage of the dose) in liver was highest (27·4 %) in rats given 14400 i.u. retinol daily for 10 days and lowest (11·6 %) in rats given 93600 i.u. daily for 10 days.

It is known that feeding massive amounts of vitamin A to experimental animals is harmful (Moore, 1957*a*). Differences in absorption, liver storage of vitamin A and the severity of toxic symptoms, due to the amount of vitamin A given, the route of administration of vitamin A and the age of the animals have been reported (Moore, 1957*b*). Thus the storage of vitamin A in liver has been reported to be less when vitamin A was given in oily solution by parenteral injection than when it was given orally or in emulsified form by intramuscular injection (Lemley, Brown, Bird & Emmett, 1947*a*; Bolin, Eveleth & Bolin, 1950). The liver is the main organ of vitamin A storage (Sherman & Boynton, 1925; Moore, 1931; McCoord & Luce-Clausen, 1934) and the liver concentration of vitamin A is usually proportional to its intake (Baumann, Riising & Steenbock, 1934). This communication describes the effects of giving various doses of retinol to adult rats on their body-weights, food consumption and liver storage of vitamin A.

EXPERIMENTAL

Male rats (Wistar strain) weighing 160–170 g, and 9–10 months old, from our Institute-maintained colony were used. The rats were housed in individual cages. They were fed on a diet of the following composition: casein 20 parts, potato starch 60 parts, hydrogenated groundnut oil 10 parts, salt mixture 5 parts (Steenbock & Nelson, 1923) and dried yeast powder 5 parts. In addition each received 4 g germinating grams (chick-pea) daily. The rats had free access to water.

Rats were divided at random into five groups of six each. To four groups retinol (Prepalin; Glaxo Pvt. Ltd., India) was given orally in amounts of 14400, 28800, 57600, or 93600 i.u. daily for 10 days. The fifth group served as the control and was given saline for the same period. The amount of retinol in the Prepalin preparation was checked (Olson, 1961) and was found to be 96–98 % of that (72000 i.u./ml) stated in the literature supplied with it. Daily records of the food consumption of the rats and

tri-weekly records of their body-weights were kept. On the 10th day rats in all groups were killed by decapitation and their livers were removed quickly, cleaned, rinsed with ice-cold saline, weighed and immersed in a mixture of chloroform: methanol (2:1, v/v). Lipids from liver were extracted and purified as described previously (Misra, 1967*a*). Vitamin A was measured in liver lipid extracts by the method of Olson (1961).

Analysis of variance was conducted to test the significance of differences in liver weights, total liver vitamin A, and liver vitamin A (μg/g tissue) between the rats given retinol and control rats.

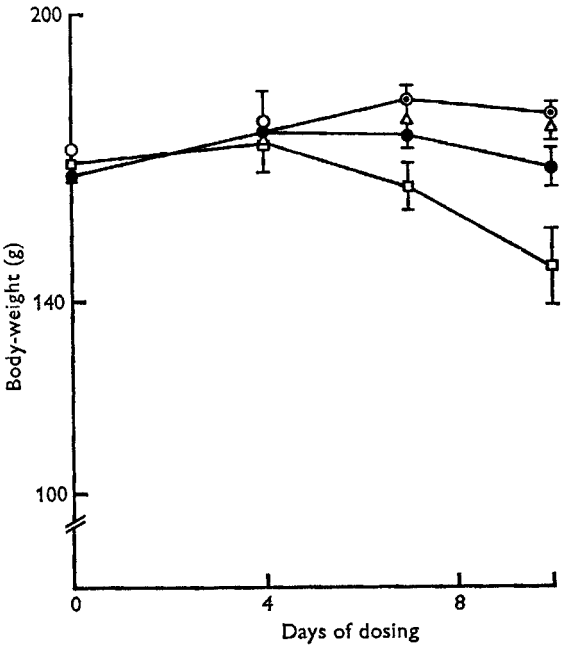

Fig. 1. Effect of giving retinol by mouth on body-weight of adult rats given daily no retinol (⊙), 14400 i.u. retinol (○), 28800 i.u. retinol (△), 57600 i.u. retinol (●) or 93600 i.u. retinol (□). Mean values for six, six, six, four and five rats respectively; standard errors are shown for the first group and the last two groups.

RESULTS AND DISCUSSION

The effect of giving different large amounts of retinol to rats on their body-weights and daily food consumption is shown in Figs. 1 and 2. When rats were given these large amounts of retinol there was a decrease in daily food consumption and body-weight, usually starting within 4–5 days. These effects were much more severe in rats given 93600 i.u. retinol daily than in those given 14400, 28800 and 57600 i.u. retinol. In addition, the rats developed signs of retinol toxicity dependent on the dose, such as epilation, bent and limping posture, bleeding around the nose and eyes and internal haemorrhages on both right and left front and back limb joints and neck. Similar signs, in rats given vitamin A orally, have been reported by Moore & Wang (1945). The toxicity of retinol depends on the route of administration. Thus, toxicity signs were

not as severe in rats given 100000 i.u. by intramuscular injection daily for 13 days (Misra, 1967*b*) as those produced by oral dosage in the present experiment.

The mean and standard error of the mean of liver weights, liver total vitamin A and liver vitamin A (μg/g tissue) are shown in Table 1. The results show that liver weights of rats given retinol were not significantly different from those of the control rats.

Total liver vitamin A and liver vitamin A (μg/g tissue) concentration in rats given retinol were significantly higher than in the control rats. Significant differences in the amounts of liver vitamin A (total and μg/g tissue) were also noted between rats given

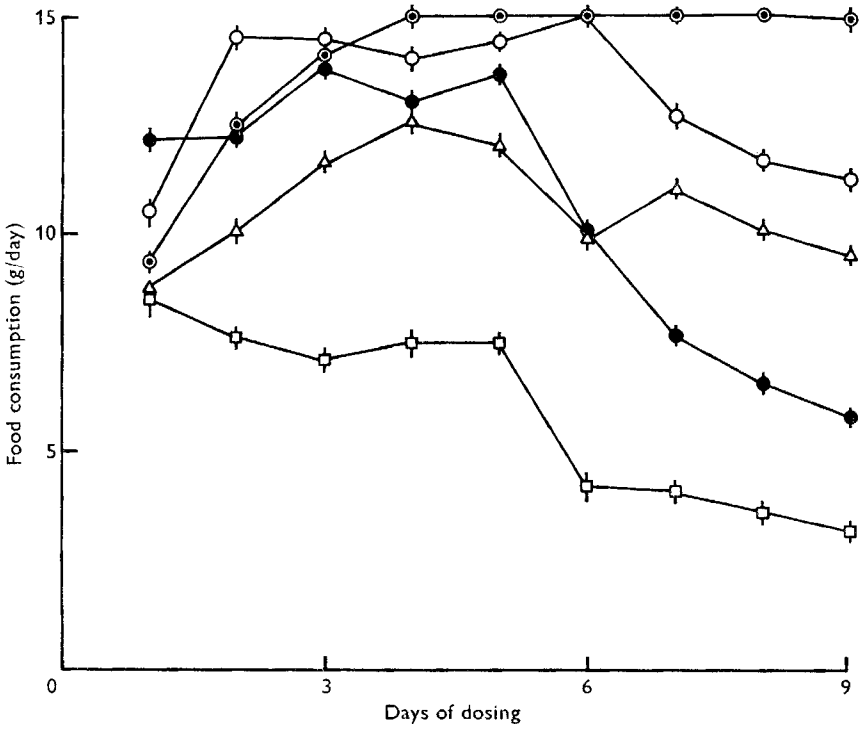

Fig. 2. Effect of giving retinol by mouth on the daily food consumption of adult rats given daily no retinol (⊙), 14400 i.u. retinol (○), 28800 i.u. retinol (△), 57600 i.u. retinol (●) or 93600 i.u. retinol (□); mean values with their standard errors for six, six, six, four and five rats respectively.

Table 1. *Effect of giving retinol by mouth for 10 days on liver vitamin A of adult rats*

(Mean values with their standard errors; figures in parentheses are the numbers of rats/group)

Retinol given (i.u./day)	Liver wt (g)	Liver total vitamin A content (μg)	Liver vitamin A storage (%)	Liver vitamin A concentration (μg/g)
None (6)	4·27 ± 0·23	324·0 ± 92·3	—	76·18 ± 21·20
14400 (6)	4·43 ± 0·17	1163·8 ± 195·2	27·4	258·15 ± 34·63
28800 (6)	4·50 ± 0·18	1893·8 ± 350·4	22·3	422·38 ± 73·84
57600 (4)	4·28 ± 0·09	3430·3 ± 548·5	20·2	808·71 ± 142·10
93600 (5)	4·85 ± 0·24	3219·0 ± 559·7	11·6	668·46 ± 118·95

various amounts of retinol. The liver vitamin A concentration increased with the amount given up to a dose of 57600 i.u., after which it decreased (Table 1). The retention of vitamin A in liver was found to be highest (27·4%) in rats given 14400 i.u. retinol, and increasing the amount of retinol resulted in a decreased retention of vitamin A in liver (Table 1). Similar results have been reported by Lemley *et al.* (1947*a*) in rats given 63–118400 i.u. vitamin A daily for 3 days. The decreased storage and retention of vitamin A in the liver of rats given these large doses may result either from its decreased absorption from the intestine or from its extensive degradation in liver, or both. The decreased absorption may result from one or more of a number of factors such as (1) a change in the epithelial lining of the intestine affecting its absorption capacity, or (2) the lack of an available carrier, e.g. palmitic acid, which has been shown to be the major fatty acid utilized for the absorption and transport of vitamin A to the liver (Ganguly, 1967), and (3) the saturation of the absorptive system, which, after a certain level, may not function in proportion to the increasing influx of dietary vitamin A. A decrease in liver tocopherol levels has been reported in conditions in which massive amounts of vitamin A are given (Pudelkiewicz, Webster & Matterson, 1964). Vitamin A is a highly unsaturated compound and is prone to oxidative degradation in the absence of tocopherol. The administration of adequate amounts of tocopherol along with a massive amount of vitamin A has been reported to increase the storage of vitamin A in liver (Lemley, Brown, Bird & Emmett, 1947*b*). Thus it is possible that the decreased retention of retinol observed in the present instance might have been caused by one or more of the factors considered above.

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