Biochem. J. (1973) **136**, 221–223
Printed in Great Britain

Effect of Vitamin A Deprivation on the Cholesterol Side-Chain Cleavage Enzyme Activity of Testes and Ovaries of Rats

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(*Received* 18 *June* 1973)

The cholesterol side-chain cleavage enzyme activity is decreased considerably at the mild stage of vitamin A deficiency in rat testes and ovaries and the decrease in activity becomes more pronounced with progress of deficiency. Supplementation of the deficient rats with retinyl acetate, but not retinoic acid, restores the enzyme activity to normal values. The cholesterol side-chain cleavage enzyme of adrenals is not affected by any of the above treatments.

Earlier nutritional work had shown that vitamin A deficiency affects the reproductive processes of both male and female rats (Moore, 1957). Later sustained work by Grangaud and his colleagues (Grangaud *et al.*, 1969) with whole living animals and with isolated enzyme systems from adrenals has produced extensive evidence showing that normal synthesis of steroid hormones in male and female rats is dependent on the vitamin A nutritional status of the animals. Independently Juneja *et al.* (1966) also have shown that even at the mild stage of vitamin A deficiency the activity of the enzyme 3β-hydroxy Δ^5-steroid dehydrogenase is affected in the adrenals, testes and ovaries of rats. In continuation of this work Juneja *et al.* (1969) demonstrated that the response of the ovaries of rats raised on a vitamin A-deficient diet supplemented with retinoic acid to unilateral ovariectomy, to pregnancy and to endogenous and exogenous gonadotrophin stimulus was markedly less than that in the controls receiving supplements of retinyl acetate. Ganguly *et al.* (1971*a*,*b*,*c*) showed that during pregnancy synthesis in and secretion from the ovaries of pregnenolone (3β-hydroxypregn-5-en-20-one), progesterone and 20α-hydroxyprogesterone were markedly less in the retinoic acid-fed rats than in the corresponding retinyl acetate-supplemented controls. Against this considerable evidence in support of requirement of retinol for normal steroidogenesis in rats, Rogers (1969) failed to observe any effect of vitamin A deficiency on steroidogenesis in rat adrenals. We have further investigated the effect of vitamin A deficiency on steroidogenesis in rat tissues and report here that even at the mild stage of the deficiency the activity of yet another enzyme, the cholesterol side-chain cleavage enzyme (cholesterol $C_{(20)}$–$C_{(22)}$ desmolase), is significantly decreased in the ovaries and testes this becoming more pronounced with the progress of the deficiency, whereas no such effect was observed in the adrenals.

Materials and methods

Male and female rats of this Institute strain were kept on the vitamin A-deficient diet after weaning, as described by Malathi *et al.* (1963), until they stopped growing. At this point some of them were killed, and others were either continued on the deficient diet (for about 4 days) or were supplemented with retinyl acetate or retinoic acid for the given time-period, after which they were killed. Immediately after the animals were killed the tissues were excised and chilled in crushed ice; they were then homogenized in 20mM-sodium phosphate buffer, pH 7.4, in 0.25M-sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenates of the adrenals and ovaries were directly used for the assay of the enzyme activity. The testes homogenate was centrifuged at 700*g* for 10min to sediment the nuclear material, after which the supernatant was centrifuged at 10000*g* for 10min to obtain the mitochondrial fraction, which was then used for the enzyme assay.

The reaction mixtures were essentially the same as described by Jungmann (1968) except that instead of NADPH an NADPH-generating system consisting of $NADP^+$, glucose 6-phosphate and glucose 6-phosphate dehydrogenase was used (Burstein & Gut, 1971). The incubations were carried out at 37°C for 30min in a metabolic shaker. At the end of the incubation the reaction mixtures were chilled in ice and, after the addition of 300μg of authentic samples of each of unlabelled pregnenolone, progesterone and cholesterol, were extracted with chloroform–methanol (to give final proportions chloroform:methanol:water, 5:5:4, by vol.). After evaporation of the chloroform layer to dryness, the residue was redissolved in chloroform and chromatographed on silica gel G plates with *n*-hexane–diethyl ether–acetic acid (12:2:1, by vol.) as the developing solvent. After the run the plates were exposed to I_2 vapour and the areas corresponding

Table 1. *Effect of vitamin A deficiency on the activity of cholesterol side-chain cleavage enzyme in testes, ovaries and adrenals of rats*

The incubation mixture contained, in a total volume of 2 ml of 20 mM-sodium phosphate buffer, pH 7.4, 10^5 c.p.m. of [4-^{14}C]cholesterol in 50 μl of propylene glycol, 10 μmol of NaCN, 10 μmol of $MgCl_2$, 3 μmol of $NADP^+$, 5 μmol of glucose 6-phosphate and 2 units of glucose 6-phosphate dehydrogenase. The amount of protein per assay mixture was 150 μg for the testes enzyme and 1 mg each for the ovary and the adrenal enzyme. Incubations were carried out at 37°C for 30 min in a Dubnoff metabolic shaker. Values are expressed as averages ± S.D. per pair of the respective organs of six animals. M and F refer to males and females respectively.

	Testes				Ovaries			Adrenals		
Treatment	Wt. of tissue (g)	Total protein (mg)	Mito-chondrial protein (mg)	Activity (pmol of product formed/30 min per mg of mito-chondrial protein)	Wt. of tissue (mg)	Total protein (mg)	Activity (pmol of product formed/30 min per mg of protein)	Wt. of tissue (mg)	Total protein (mg)	Activity (pmol of product formed/30 min per mg of protein)
Normal diet	1.87 ± 0.69	112.0 ± 30.9	23.4 ± 5.1	73.3 ± 10.0	65 ± 6	6.6 ± 0.6	16.7 ± 1.0	32 ± 5 (M) 35 ± 4 (F)	4.6 ± 1.2 4.8 ± 1.0	55.5 ± 4.2 35.5 ± 3.8
Vitamin A-deficient diet until plateau stage of deficiency	0.65 ± 0.06	37.0 ± 7.4	7.80 ± 1.07	33.6 ± 2.2	55 ± 7	5.4 ± 1.4	9.6 ± 1.4	30 ± 5 (M) 34 ± 4 (F)	4.2 ± 1.4 4.4 ± 1.2	52.3 ± 4.6 30.2 ± 4.8
Vitamin A-deficient diet until acute deficiency*	0.59 ± 0.08	34.0 ± 5.6	7.70 ± 1.05	21.4 ± 3.2	60 ± 2	5.8 ± 0.8	7.6 ± 1.5	30 ± 3 (M) 33 ± 2 (F)	3.8 ± 1.8 4.2 ± 1.6	52.5 ± 5.1 32.6 ± 4.8
Retinyl acetate-supplemented diet for 30 days after mild deficiency	1.10 ± 0.14	69.4 ± 4.9	14.0 ± 1.8	76.8 ± 7.4	68 ± 4	6.4 ± 0.5	16.9 ± 1.7	32 ± 4 (M) 36 ± 3 (F)	4.5 ± 1.3 4.9 ± 1.2	54.8 ± 3.5 34.2 ± 4.2
Retinoic acid-supplemented diet for 30 days after mild deficiency	0.56 ± 0.07	37.0 ± 3.8	10.7 ± 1.1	29.5 ± 9.9	67 ± 4	6.5 ± 0.5	9.6 ± 1.1	31 ± 3 (M) 36 ± 1 (F)	4.4 ± 1.2 4.8 ± 1.4	55.0 ± 3.8 32.5 ± 4.2

* Usually killed 4 days after the mild deficiency stage. The weight loss at this stage was about 8–10 g.

to pregnenolone, progesterone and cholesterol, and also the origin ('polar steroids'), were scraped into vials and their radioactivities counted in a Beckman LS-100 scintillation counter with 0.5% 2,5-diphenyloxazole (PPO) in toluene as the scintillation fluid. Total recovery of radioactivity from all the spots was usually 85–90%.

In expression of the cholesterol side-chain cleavage enzyme activity the radioactivities in the 'polar steroids', progesterone and pregnenolone have been added together to represent the total activity of the enzyme.

Results and discussion

A large number of male and female rats were used for these experiments, and representative values of some of them are assembled in Table 1. The results show that even at the mild stage of the deficiency the total weight, total protein content, total mitochondrial protein content and the cholesterol side-chain cleavage enzyme activity of the testes were markedly decreased and that on supplementation with retinyl acetate for 30 days the effects on total weight, total protein and mitochondria were partially reversed and the enzyme activity was fully restored; retinoic acid treatment, however, did not lead to such improvements. In the ovaries such marked changes in the weights and protein contents were not observed after any of these treatments, but the activity of cholesterol side-chain cleavage enzyme was considerably decreased during the deficiency and could be fully restored by supplementation with retinyl acetate but not with retinoic acid. In sharp contrast, none of these treatments had any effect on the activity of cholesterol side-chain cleavage enzyme in the adrenals of both male and female rats.

These results have therefore produced further evidence in support of our previous claims that retinol is essential for normal steroidogenesis in rats (Juneja *et al.*, 1966; Ganguly *et al.*, 1971*a*,*b*,*c*). The marked effects on the morphology and histology of testes after supplementation of a vitamin A-deficient diet with retinoic acid have been rather easy to demonstrate (Thompson *et al.*, 1964) and therefore well accepted. But any effect on the ovaries of such rats could be demonstrated only under stress conditions such as pregnancy and unilateral ovariectomy (Juneja *et al.*, 1969). That there is such an effect on the ovaries was obvious from the decrease in the activity of 3β-hydroxy Δ^5-steroid dehydrogenase in such ovaries (Juneja *et al.*, 1966) and from the subsequent observations on the decreased secretion rates of steroid hormones into the ovarian venous blood of retinoic acid-supplemented pregnant rats (Ganguly *et al.*, 1971*a*.*b*,*c*). The present results have clearly shown that yet another enzyme, which catalyses the first step in steroidogenesis, namely the cleavage of the cholesterol side chain, requires adequate supply of retinol for its synthesis/activity in the ovaries and testes of rats. It is rather interesting that the cells of testes and ovaries regenerate at frequent intervals whereas those of the adrenals do not do so, which might indicate that vitamin A is required at a fundamental step in cellular differentiation. Similar views have been expressed by Hayes (1971) and Corey & Hayes (1972) also.

This work was supported by the Grant no. 01-005-1 from the National Institutes of Health, Bethesda, Md., U.S.A. We also acknowledge a grant for equipment from the Wellcome Trust, London, U.K.

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