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Reproduction and vision in rats maintained on a retinol-free diet containing 3-dehydroretinol (vitamin A_2)

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1. Seventeen hooded female rats were given from weaning a retinol-free diet containing methyl retinoate. When 105 days old they were mated with normal bucks.

2. Six of these rats were subsequently given dietary supplements of 100 μg retinol daily and another six were given 100 μg 3-dehydroretinol daily. All twelve rats had litters.

3. The five rats that received only methyl retinoate during pregnancy resorbed their foetuses.

4. One of the litters from the female rats given 3-dehydroretinol consisted of six male pups. These young rats had never had access to retinol. They were maintained on a retinol-free diet supplemented with methyl retinoate. Two of these rats were given daily supplements of 3-dehydroretinol and another pair were given daily an equal amount of retinol.

5. The six rats were killed when 195 days old. The testes and the retina of the rats given either 3-dehydroretinol or retinol were normal. The testes and the retina of the rats given methyl retinoate only were degenerate.

It has been shown that the biological properties of retinoic acid differ from those of retinol; retinoic acid maintains growth in rats but neither vision (Dowling & Wald, 1960) nor reproduction (Thompson, Howell & Pitt, 1964). It is of interest therefore to examine whether other substances, known to replace retinol in the maintenance of growth and general health, can fulfil the special requirements of reproduction and vision.

In tissues of certain freshwater fishes, retinol is accompanied, and to some extent replaced, by 3-dehydroretinol. In rats 3-dehydroretinol is known to maintain growth and health (Shantz, 1948; Shantz & Brinkman, 1950; Sundaresan & Cama, 1961).

When dietary retinol is replaced by sources of 3-dehydroretinol the latter is gradually substituted for the former in the liver, and, within the limitations of analytical methods, there is no indication of in vivo reduction of 3-dehydroretinol to retinol (Shantz & Brinkman, 1950). However, complete replacement of retinol by 3-dehydroretinol in the rat has been claimed to be a very slow process because of tenacious retention of traces of retinol (Shantz, Embree, Hodge & Wills, 1946).

There is evidence to suggest that the aldehyde, 3-dehydroretinal, can replace retinal in the visual role of vitamin A by combining with opsin to form a new visual pigment, porphyropsin. This synthesis can take place in vitro (Wald, 1953) and evidence has been presented for its occurrence in vivo in the rat (Shantz *et al.* 1946) and in man (Millard & McCann, 1949).

During attempts to breed from rats fed on retinol-free diets containing 3-dehydroretinol, litters were obtained but the mothers failed to rear their young (Shantz *et al.*

1946). The authors suggested that 3-dehydroretinol might be unable to replace retinol in some aspects of reproduction; however no control animals given supplements of retinol were included in these experiments.

The experiments described below demonstrate conclusively the ability of crystalline all-*trans* 3-dehydroretinol (RO 4–3791/1 from S. A. F. Hoffman—La Roche and Co., A. G., Basle, Switzerland) to prevent the characteristic visual and reproductive abnormalities which occur in rats fed on vitamin A-deficient diets with or without retinoic acid.

EXPERIMENTAL

Animals and diet

Hooded rats from the closed colony maintained in the Biochemistry Department, the University of Liverpool, were used. The methods employed to study the course of pregnancy in the rat were as described previously (Thompson *et al.* 1964). The basic retinol-free diet was diet II of Thompson *et al.* (1964) either as formulated or containing methyl retinoate (10 μg/g diet).

Histological methods

Organs were fixed immediately after the animals were killed. Tissues of the reproductive tract were fixed in Bouin's fluid and embedded in paraffin wax, sections were cut at 4 μm and stained with haematoxylin and eosin. Eyes were fixed in Zenker's fluid, embedded in nitrocellulose of low viscosity, and sections were cut at 12 μm and stained with haematoxylin and eosin.

RESULTS

Reproduction in females

Female rats were reared from weaning on the retinol-free diet containing methyl retinoate. When 105 days old, seventeen were successfully mated with normal bucks. Six of the pregnant rats were given supplements of retinol and six were given 3-dehydroretinol (100 μg daily) in each instance, and the remaining five continued on the basal diet. All twelve rats given supplements had litters. Seven litters were successfully weaned and four of these were from the females given 3-dehydroretinol. The five rats that received only methyl retinoate during pregnancy showed heavy vaginal bleeding and resorbed their foetuses, as described previously (Howell, Thompson & Pitt, 1964).

Vision and reproduction in males

One of the litters obtained from the female rats given 3-dehydroretinol consisted of six male pups. These animals had thus not had access, even *in utero*, to trace amounts of retinol. They were reared on the retinol-free diet supplemented with methyl retinoate and used in further experiments. Commencing when they were 6 weeks old, four of these rats were given daily supplements: two were given 3-dehydroretinol (equivalent to 100 μg/day) and another pair were given an equal amount of retinol. The remaining pair received only the diet supplemented with methyl retinoate. The rats were killed when 195 days old and the tissues were taken for examination.

The testes from the rats given either retinol or 3-dehydroretinol were found to be of normal size and weight (Table 1) and complete spermatogenesis was observed in almost all the tubules: those from the rats given methyl retinoate only were small in size (Table 1) and the germinal epithelium showed the severe degenerative changes previously described in such rats (Howell, Thompson & Pitt, 1963). The retinas from the rats supplemented with either retinol or 3-dehydroretinol did not have detectable abnormalities (Pl. 1*a*). Those from the rats given only methyl retinoate had lost the rod-cone layer, and the number of nuclei in the outer nuclear layer was reduced. These changes were particularly marked over the central area of the retina (Pl. 1*b*). This change is similar to that previously described in retinol-depleted rats by Dowling & Wald (1960).

Table 1. *Weights (mg) of testes from six individual litter-mate rats given a vitamin A-free diet containing methyl retinoate, with or without supplements of retinol or 3-dehydroretinol*

Retinol supplement		3-Dehydroretinol supplement		No supplement	
Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
1409	1225	1658	1670	483	466
1775	1327	1338	1249	671	423

DISCUSSION

Retinoic acid is active in maintaining growth and general health in rats given retinol-free diets, but it does not maintain reproduction (Thompson *et al.* 1964) or vision (Dowling & Wald, 1960). The failure of vision in animals maintained with retinoic acid can be attributed to their inability to convert the acid into the corresponding aldehyde, retinal (Moore, 1953), which is known to be the prosthetic group of the visual pigments. The biochemical mechanism which leads to reproductive failure is not understood.

The work reported here has shown that 3-dehydroretinol, unlike retinoic acid, can maintain reproduction in female rats. It also maintains the structural integrity of the retina and the growth and morphological appearance of the testes of male animals. Previous workers (Shantz & Brinkman, 1950) have been unable to demonstrate any conversion in vivo of 3-dehydroretinol into retinol and it therefore seems probable that the 3-dehydroretinol or the corresponding aldehyde, 3-dehydroretinal, possesses biological activity *per se* in vision and reproduction. However, a small conversion into retinol could account for the maintenance of reproduction and vision, and a conclusive demonstration that such conversion does not occur in the rat may require the use of more sensitive analytical methods than have been employed hitherto.

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EXPLANATION OF PLATE

(*a*) Retina of a rat fed on the vitamin A-free diet supplemented with methyl retinoate and 3-dehydroretinol. This is a normal retina. *A*, pigment epithelium; *B*, rod-cone layer; *C*, outer nuclear layer; *D*, inner nuclear layer; *E*, ganglion cell layer. Haematoxylin and eosin.

(*b*) Retina of a rat fed on the vitamin A-free diet supplemented with methyl retinoate alone. It shows complete loss of the rod-cone layer, and the outer nuclear layer has been reduced to a single row of cells. Haematoxylin and eosin.

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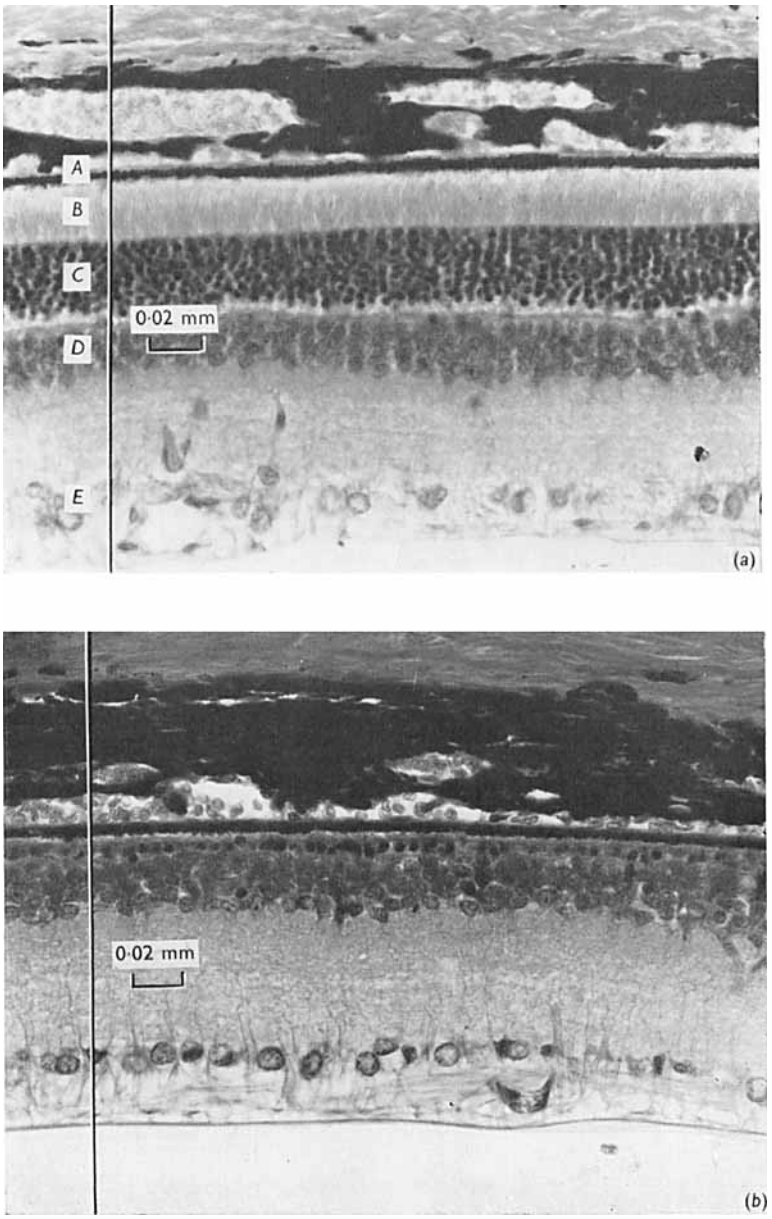

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