Studies on the Metabolism of Vitamin A: the Effect of Vitamin A Status on the Content of some Steroids in the Ovaries of Pregnant Rats

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Ganguly *et al.* (1971) have reported that the ovaries of pregnant rats raised on a vitamin A-deficient diet supplemented with retinoic acid secreted progesterone and 20 α -hydroxypregn-4-en-3-one at a somewhat lower rate than did those of rats given the same diet supplemented with retinyl acetate. The lowered ovarian steroid secretion rate was considered as a possible reason for the foetal resorption in such animals.

To investigate further the nature of this apparent ovarian disorder, the content of pregnenolone as well as that of progesterone and 20 α -hydroxypregn-4-en-3-one were determined in ovarian tissue from the 80 rats (40 on each diet) that had previously provided the pooled blood extracts, each pool being from ten rats (Ganguly *et al.* 1971). The ovaries, freed of most of the adhering adipose tissue, were allocated and pooled in the same way as that previously used for the blood samples. Thus each tissue pool was from ten rats all of which had received one diet, each pool being paired with a pool from animals that, apart from the dietary difference, were as similar as possible.

Each ovarian tissue pool was ground in a Ten-

Broek-pattern tissue grinder with three successive 5 ml portions of ethyl acetate, the ethyl acetate phases being combined after separation by centrifuging. Each combined ethyl acetate extract was evaporated to dryness, 30 ml of 1% sodium hydrogen carbonate added to the residue, and the procedure continued as for blood extracts (Ganguly *et al.* 1971). At the stage of the separation of the neutral steroids on t.l.c. plates, the area containing pregnenolone was removed in addition to those areas containing progesterone and 20 α -hydroxypregn-4-en-3-one and extracts containing the three steroids were prepared. That of pregnenolone was further purified by chromatography on Whatman no. 50 paper, as described by Holzbauer & Newport (1967) with the E₁ solvent system of Eberlein & Bongiovani (1955). The three steroids were then determined by g.l.c. (Ganguly *et al.* 1971). The g.l.c. of the pregnenolone extract showed the presence of two other steroids, one partly and the other well separated from pregnenolone.

Table 1 shows that, when comparison is made within each pair of pooled tissue extracts, the contents of progesterone and 20 α -hydroxypregn-4-en-3-one

Table 1. *Content of progesterone, 20 α -hydroxypregn-4-en-3-one and pregnenolone in the ovaries at around days 9 and 15 of pregnancy of rats given a vitamin A-deficient diet supplemented with retinoic acid or retinyl acetate*

Experimental details are given in the text. Each set of results refers to a group of ten rats.

Supplement	Day of pregnancy		Progesterone (ng/two ovaries)	20 α -Hydroxy- pregn-4-en-3-one (ng/two ovaries)	Pregnenolone (ng/two ovaries)
	Mean	Range			
Retinoic acid	8.2	8 to 9	970	510	31
Retinyl acetate	8.2	8 to 9	1610	810	50
Retinoic acid	10.3	9 to 11	1750	290	37
Retinyl acetate	9.8	9 to 11	2240	520	71
Retinoic acid	15.3	15 to 17	1030	2060	40
Retinyl acetate	13.8	13 to 15	1890	4240	33
Retinoic acid	15.4	15 to 16	700	1310	20
Retinyl acetate	15.3	15 to 16	1680	1770	31

without exception, and of pregnenolone with one exception, were lower in the ovaries of retinoic acid-fed rats.

The new results give further support to the conclusions that at both times in pregnancy studied the ovaries of rats maintained on a vitamin A-deficient diet supplemented with retinoic acid are defective in the synthesis and secretion of progesterone and 20α -hydroxypregn-4-en-3-one. This may be due to the lowered activity of ovarian 3β -hydroxy- Δ^5 -steroid dehydrogenase (Juneja, Moudgal & Ganguly, 1969), but the possibility now arises that inadequate ovarian pregnenolone production is also involved. These authors reported that pregnenolone replacement therapy in retinoate-fed rats prevented foetal resorption.

Two steroids were found by g.l.c. in the pregnenolone extract; these probably are the pro-

gesterone metabolites 3β -hydroxy- 5α -pregnan-20-one (partly separated from pregnenolone) and 5α -pregnane- $3\alpha,20\alpha$ -diol (Holzbauer, 1969). The ovarian content of each was about equal to that of pregnenolone, but less than 5% of that of progesterone. The content of the pregnanes was not influenced by vitamin A status.

Eberlein, W. R. & Bongiovani, A. M. (1955). *Archs Biochem. Biophys.* **59**, 90.

Ganguly, J., Pope, G. S., Thompson, S. Y., Toothill, J., Edwards-Webb, J. D. & Waynforth, H. B. (1971). *Biochem. J.* **122**, 235.

Holzbauer, M. (1969). *J. Physiol., Lond.*, **204**, 8P.

Holzbauer, M. & Newport, H. M. (1967). *J. Physiol., Lond.*, **193**, 131.

Juneja, H. S., Moudgal, N. R. & Ganguly, J. (1969). *Biochem. J.* **111**, 97.