Inhibition of cyclic ovarian activity in rats treated chronically with vitamin A

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Summary. The effects of altering the dietary level of vitamin A on cyclic ovarian activity, and serum and hepatic vitamin A concentrations were studied in female rats. High but non-toxic oral doses of retinyl palmitate (5000 i.u., 3 times/week) significantly advanced the age at vaginal opening but had no effect on vaginal and ovarian cycles after 15 weeks of treatment. Hepatic stores of vitamin A were significantly elevated but serum levels were unchanged. Retinol-deficient rats given a supplement of retinoic acid had a normal vaginal opening age but displayed cornified vaginal cells for many of the days that smears were taken. Hepatic and serum vitamin A concentrations were markedly reduced. When treatment with high doses of retinyl palmitate was continued for 9 months, animals developed polycystic anovulatory ovaries which were significantly lighter than those of controls. Retinol-deficient rats given retinoic acid and demonstrating depleted hepatic vitamin A reserves had ovaries of normal weight and containing corpora lutea, indicating that retinol is not necessary for ovulatory activity. Short-term treatment (16 days) of rats with toxic doses of retinyl palmitate (50,000 i.u. daily) sufficient to raise serum and hepatic retinol levels significantly did not alter ovarian cyclicity; corpora lutea were present and ovarian weight was normal.

These data indicate that long-term treatment with high but non-toxic doses of vitamin A inhibits cyclic ovulatory activity, perhaps via alteration of a steroidogenic mechanism in the ovary or adrenal since other studies indirectly support the existence of such a relationship. Furthermore, retinoic acid is a sufficient form of vitamin A replacement to maintain cyclic ovarian activity.

Introduction

The specific role of vitamin A in reproduction has not been clearly defined. Early reports suggested that ovulation ceases as a result of vitamin A deficiency but the data were scant and difficult to interpret (Evans & Bishop, 1922; Evans, 1928). Other early work was even more inconclusive because of poorly described protocols and the failure to recognize the possibility of separate roles for retinoic acid and retinol (Truscott, 1947). More recent data suggest that rats given a retinol-deficient diet supplemented with retinoic acid have ovulatory cycles but resorb their fetuses if they become pregnant (High, Moore, Collins & Frazier, 1964; Coward, Howell, Pitt & Thompson, 1966). Oestradiol treatment of such animals prevents fetal resorption (High *et al.*, 1964; Juneja, Moudgal & Ganguly, 1969), suggesting that retinol plays a role in the secretion of oestrogen.

Other methods of studying the role of vitamin A in reproduction make use of excessive dietary levels of the vitamin. Initial reports suggested that high doses of vitamin A or carotene disrupted the oestrous cycle in rats (Sherwood, Brend & Roper, 1936; Sherwood, Depp, Birge & Dotson, 1937; Burrill & Greene, 1941; Brody & Goldman, 1941), but the data were inadequate to warrant such a conclusion and other work showed that chronic feeding of excessive levels of β -carotene to rats for several generations had no effect on fertility (Bagdon, Zbinden & Studer, 1960). Although it has often been stated that high doses of carotene are completely without toxic effects in the rat and man (Nieman & Obbink, 1954; Bagdon *et al.*, 1960; Abrahamson & Abrahamson, 1962; Moore, 1967a) the clinical association of hypercarotenaemia with reproductive disturbances is probably not fortuitous. Pops & Schwabe (1968) described 12 patients with hypercarotenaemia associated with

anorexia nervosa, a hypo-oestrogenic state with amenorrhea. In contrast, a more recent study describes anorexic patients without hypercarotenaemia and with normal serum vitamin A levels (Warren & Vande Wiele, 1973). A clinical entity described as "golden ovaries" is marked by excessive carotene in the diet, elevated carotene stores in the ovaries and menstrual disturbances (Page, 1971).

Although carotene *per se* may not be toxic or produce reproductive disorders, it is likely that carotene levels in some individuals may reflect abnormalities in vitamin A metabolism, and the present study was done to evaluate ovarian cyclic activity in animals deprived of retinol, the alcohol form of the vitamin, or fed high doses of the ester form of the vitamin, retinyl palmitate, for long or short periods of time.

Materials and Methods

Locally purchased (Tyler Labs, Bellevue, Washington) Sprague-Dawley rats were kept in an artificial photoperiod of 14 h light/day. Treatment with the special diet was initiated at 27 days of age and continued until approximately 9 months of age. Animals were randomly assigned to 4 groups: those in *Group 1* had unrestricted access to Purina Laboratory Chow pellets while those in the other groups were fed a vitamin A-free powdered rat diet *ad libitum*, supplemented with an oil vehicle gavage (0.1 ml sesame oil) three times a week containing one of the following doses of vitamin A. *Group 2*: 5000 i.u. retinyl palmitate (ICN Life Sciences Group, Cleveland, Ohio); this is approximately 7 times the reported retinol requirement for the rat (Rubin & De Ritter, 1954; Moore, 1967b; Ahluwalia & Bieri, 1970) but is considerably below toxic levels (Nieman & Obbink, 1954). *Group 3*: 3 i.u. retinyl palmitate; this is below the optimal requirements for full longevity and hepatic storage of vitamin A but is sufficient to allow growth and prevent the gross manifestations of deficiency (Moore, 1967b). *Group 4*: 50 µg retinoic acid (Eastman Kodak, Rochester, New York); this supplement is retinol-free since the acid cannot be converted to the alcohol form of the vitamin, and this dose is adequate to prevent death and to maintain growth (Kamath, MacMillan & Arnrich, 1972).

Therefore, the diets may be characterized respectively by their vitamin A content as high A, low A and no A. The Purina Laboratory Chow is a diet that has adequate vitamin A (12 i.u./g) for growth and hepatic storage. The vitamin A-free diet (ICN Life Sciences Group, Cleveland, Ohio) contained 18% vitamin-free casein, 65% starch, 5% corn oil 4% salt mix No. 2 (U.S.P. XIII), 0.5 g irradiated viosterol/100 lb diet, and 8% dried yeast.

Beginning at 31 days of age the rats were examined daily for vaginal introitus. Vaginal smears (saline lavage) were taken daily for 10 days from 14 weeks of age and were read unstained. The animals were weighed periodically throughout the 9 month period. Blood samples for retinol determinations were obtained via cardiac puncture under light ether anaesthesia. The blood was placed in ice and allowed to clot in the dark. The serum samples were then covered with aluminum foil and frozen until retinol concentrations could be estimated. Samples of liver, usually < 50 mg, were obtained under ether anaesthesia and were frozen until vitamin A could be measured. Neither the cardiac puncture nor the liver biopsy procedure was found to have any effect on reproductive cycles.

A second experiment was carried out to determine the effects of short-term treatment with very high doses of vitamin A. The vitamin (50,000 i.u. retinyl palmitate in sesame oil) was given by daily gavage for 16 days. This dose is considered toxic when given chronically (Nieman & Obbink, 1954). At the termination of both experiments ovaries and uteri were stripped free of fat and weighed. The effects of vitamin treatment on growth and final body weight made it necessary to express the organ weights as a percentage of body weights. Ovarian morphology was assessed with the use of a dissecting microscope.

Serum retinol was determined using a recently reported fluorometric method (Futterman, Swanson & Kalina, 1975) in which the lipid fraction of serum samples was extracted by mixing the sample successively with ethanol, petroleum ether and water and retaining the organic phase. Hepatic vitamin A (mainly in the form of retinyl esters) was also quantitated by a fluorometric technique (Futterman, Downer & Hendrickson, 1971). A weighed sample of liver was homogenized

in 0.1 N-NaCl and extracted successively with isopropanol and petroleum ether. The intra-assay precision (coefficient of variation) for the range of retinol values seen in our experiments was 5–9%. The inter-assay precision was 11%. The accuracy of the method was tested by recovery of known amounts of vitamin A added to serum samples. Recoveries had a range of 83–97% (mean, 90%) for samples in which 1–50 µg vitamin A had been added. All results were analysed statistically by Student's *t* test.

Results

The results of Table 1 show that there was an acceleration in the onset of puberty, i.e. first vaginal opening, in the rats in Group 2. Although the difference of 3.7 days between this group and the control animals (Group 1) is significant, the difference would have been greater had vaginae been examined before Day 31; 3 Group-2 rats had a perforate vagina on the first day of examination. When vaginal smears were examined over a 10-day period cornified cells were predominant for Groups 3 and 4 much of the time. Corpora lutea were present in all of the ovaries examined at laparotomy. Rats in Groups 1 and 2 exhibited normal 4- or 5-day oestrous cycles. At this time the animals in Groups 3 and 4 had significantly reduced serum and hepatic vitamin A concentrations, those in Group 2 showed a marked increase in hepatic storage of vitamin A but below-normal serum retinol levels compared to those in the rats in Group 1 (Table 1).

Table 1. Puberty, oestrous cycles and tissue vitamin A concentrations (mean $\pm$ S.E.M.) in rats treated with vitamin A

Group	No. of rats	Age at vaginal opening (days)	Days of cornified smears (%)†	Vitamin A conc. ‡	
				Serum (µg/100 ml)	Liver (µg/g)
1	17	41.5 $\pm$ 0.8	28.1 $\pm$ 1.6	28.7 $\pm$ 3.4 (5)	205 $\pm$ 9 (4)
2	17	37.8 $\pm$ 1.3*	25.0 $\pm$ 2.0	20.0 $\pm$ 1.5 (6)**	1491 $\pm$ 151 (4)***
3	17	42.6 $\pm$ 1.4	38.2 $\pm$ 3.8*	10.3 $\pm$ 1.2 (6)***	10 $\pm$ 3 (4)***
4	19	39.2 $\pm$ 0.8	43.9 $\pm$ 3.2***	12.0 $\pm$ 0.9 (6)***	10 $\pm$ 2 (4)***

Significantly different from Group 1 controls: * $P < 0.025$; ** borderline significance, $P < 0.05$; *** $P < 0.001$.

† At 14 weeks of age for 10 days.

‡ At 15 weeks of age; sample size in parentheses.

Table 2. Organ weights and tissue vitamin A concentrations (mean $\pm$ S.E.M.) of rats treated for 9 months with three dietary levels of vitamin A

Group	No. of rats	Body wt (g)	Ovarian wt (mg/100 g body wt)	No. of rats with anovulatory and/or cystic ovaries	Uterine wt (mg/100 g body wt)	Hepatic vitamin A (µg/g)
1	13	388 $\pm$ 10	26.2 $\pm$ 2.0	2	183 $\pm$ 9	572 $\pm$ 29
2	12	331 $\pm$ 8***	15.4 $\pm$ 2.0***	9	231 $\pm$ 13**	787 $\pm$ 83*
3	7	318 $\pm$ 10***	28.2 $\pm$ 4.0	1	154 $\pm$ 14	6 $\pm$ 1***
4	13	296 $\pm$ 5***	26.2 $\pm$ 1.9	2	171 $\pm$ 12	8 $\pm$ 1***

Significantly different from Group 1 controls: * $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$.

After the 4th week of treatment all the animals fed the powdered diet (Groups 2, 3 and 4) grew more slowly than the animals receiving Lab Chow (Text-fig. 1). The animals in Groups 3 and 4 had the lowest body weights and the lowest serum and hepatic concentrations of vitamin A.

The autopsy findings are summarized in Table 2. Because the body weights of the rats fed the vitamin A-free diet were significantly lower than those of the Lab Chow-fed animals, ovarian and

Text-fig. 1. Growth of rats treated with vitamin A: Lab Chow = Purina Laboratory Chow; High A = 5000 i.u. retinyl palmitate 3 times/week; Low A = 3 i.u. retinyl palmitate 3 times/week; retinoic acid = 50 μ g 3 times/week. Each point represents a mean of 7–13 rats.

uterine weights were expressed as a percentage of body weights. Ovarian weight of the rats in Group 2 was significantly lower than that of Group-1 rats and no corpora lutea were visible in 9 of the 12 animals. Several follicular cysts (> 1 mm in diameter) were also present. As is typical for 9-month-old rats, 1 or 2 animals in the other 3 groups were also anovulatory (Gellert, Heinrichs & Swerdloff, 1974; Gellert & Heinrichs, 1975; Huang, Marshall & Meites, 1976). Comparison of Tables 1 and 2 indicates that by 9 months of age hepatic vitamin A concentration in the control animals (Group 1) had risen, but there was a marked decline in hepatic vitamin A reserves in Group-2 animals.

The results of the second experiment are given in Table 3. The toxicity of this dose level was partly revealed by the marked reduction in body weight, red pigment around the eyes and nose, and sluggish behaviour. In contrast to the results of 9 months of vitamin A treatment (Group 2 in Exp. 1) the 16-day treatment failed to produce significant changes in ovarian weight or gross morphology; many corpora lutea were present and cystic follicles were not evident. However, uterine weight was significantly reduced in the rats fed toxic levels of retinyl palmitate.

Table 3. Organ weights and tissue vitamin A concentrations (mean $\pm$ S.E.M.) of rats treated for 16 days with 50,000 i.u. retinyl palmitate

Treatment	No. of rats	Ovarian wt (mg/100 g body wt)	Uterine wt (mg/100 g body wt)	Body wt (g)	Vitamin A conc.	
					Serum (μ g/100 ml)	Liver (μ g/g)
None (control)	5	28.5 $\pm$ 1.1	128 $\pm$ 16	211 $\pm$ 7	19.0 $\pm$ 2.4	101 $\pm$ 8
Retinyl palmitate	9	25.5 $\pm$ 1.9	60 $\pm$ 6*	155 $\pm$ 6*	48.8 $\pm$ 5.1**	3070 $\pm$ 130*

Significantly different from controls * $P < 0.001$; ** $P < 0.005$.

Discussion

Treatment of rats with high doses of retinyl palmitate produced no obvious effects on the oestrous cycle or ovulation, although vaginal opening was advanced and hepatic vitamin A levels were markedly elevated. Despite such treatment, serum retinol levels failed to rise (a non-significant reduction was noted). This is partly due to the capacity of the liver to store huge quantities of vitamin A. Toxic doses of retinyl palmitate for a short period (16 days) failed to prevent ovulation and the formation of corpora lutea, although both serum and hepatic vitamin A levels were significantly elevated. In contrast, 9 months of treatment with a high dose of retinyl palmitate caused cessation of

ovulation and the development of follicular cysts. These changes in the reproductive organs were similar to those seen in the persistent oestrous syndrome, i.e. anovulation and cystic ovaries (Gellert *et al.*, 1974; Gellert & Heinrichs, 1975). The most common method of inducing this syndrome is to administer androgen to the rat during neonatal development (Gorski, 1971). As adults such animals exhibit abnormal patterns of gonadal and adrenal steroidogenesis, the most obvious being the absence of cyclic oestrogen and progesterone secretion (Turner, 1941; Rosner, Tramezzani, Macome & Llauro, 1969; Libertun & Lau, 1972; Weisz & Gunsalus, 1973; Quattropiani & Weisz, 1973).

Although there is not complete agreement (Rogers & Bieri, 1968; Schor & Glick, 1970), a variety of studies have shown that vitamin A must be present for steroidogenesis in endocrine tissues such as ovary, testis and adrenal cortex (Johnson & Wolf, 1960; Van Dyke, Wolf & Johnson, 1960; Wolf, 1961; Grangaud, Nicol, Le Gall & Soussy, 1964; Juneja, Murthy & Ganguly, 1966; Levine, Glick & Nakane, 1967; Grangaud, Nicol & Desplanques, 1969; Singh, Singh & Venkitasubramanian, 1972; Wallace, Plopper, Bucci & Sauberlich, 1975). The argument favouring a direct effect of the vitamin on steroidogenic tissues is strengthened by the discovery of two proteins in ovary and testis which specifically bind retinoic acid and retinol (Ong & Chytil, 1975).

An explanation of our results must take into account the possibilities that steroidogenesis was disrupted. If this were the case, one would predict a more rapid onset of the persistent oestrous-like symptoms than seen in our experiments; 2 months of treatment (Table 1) should have been sufficient. The reason for the long delay in the appearance of the anovulatory state cannot be explained. The early vaginal opening in the rats treated with 5000 i.u. retinyl palmitate suggests that there is precocious secretion of oestrogen, although other steroids can also induce these changes (Moon, 1937; Nathanson, Franceen & Sweeny, 1938), indicating a vitamin A-induced alteration of a steroidogenic mechanism. It is also possible that vitamin A had a direct effect on the vaginal membrane. Vitamin A deficiency has been shown to produce a keratinized vaginal mucosa (Evans & Bishop, 1922) and local application of vitamin A to the vagina opposes the keratinizing effects of large doses of oestrogen; glycoprotein synthesis is favoured and the epithelium becomes mucified (Kahn & Bern, 1950; Kahn, 1954).

The increased incidence of the keratinized vaginal smear pattern in the animals maintained on vitamin A-deficient diets (Groups 3 and 4) was expected in view of previously reported work (Evans & Bishop, 1922), but the fact that these animals were ovulatory, as indicated by the presence of corpora lutea, demonstrates that vaginal smears cannot be used as an index of ovarian cyclicity. Despite the markedly reduced hepatic and serum vitamin A levels in these 2 groups of animals throughout the treatment period, the rats were ovulatory and uterine weights were maintained. Although nothing can be said about the regularity of ovulation, the normal uterine weights suggest that oestrogen secretion could not have been significantly affected. Our results are confirmatory of earlier reports that retinol-deficient, retinoic acid-maintained rats ovulate and are fertile (Thompson, Howell & Pitt, 1964; Howell, Thompson & Pitt, 1964; Coward *et al.*, 1966; Juneja *et al.*, 1969).

The absence of a significant change in uterine/body weight ratios in the retinoic acid- (Group 4) and 3 i.u. retinyl palmitate-treated groups (Table 2) suggests that oestrogen secretion was not significantly altered. The latter group was exposed to retinoic acid via conversion from the ester form of the vitamin. The absence of a significant change in oestrogen secretion in the present work agrees with other data showing that either retinoic acid or retinol replacement was effective in correcting abnormalities in steroidogenesis in rats deficient in both forms of the vitamin (Van Dyke *et al.*, 1960; Juneja *et al.*, 1966). If retinoic acid alone is sufficient for normal steroidogenesis, and the development of anovulation in the rats treated for 9 months was the result of a steroidogenic defect, it is likely that retinoic acid, and not retinol *per se* was responsible for the cessation of ovulation.

The possibility that high doses of vitamin A can act at the level of the pituitary or CNS to block cyclic gonadotrophin secretion must be considered. Retinol-deficient rats given retinoic acid do not exhibit normal compensatory hypertrophy to hemiovariectomy (Juneja *et al.*, 1969). Measurement of total pituitary gonadotrophins by crude assay procedures suggests that vitamin A-deficient rats store gonadotrophins because the pituitary content is increased (Mason & Wolfe, 1930; Juneja *et al.*, 1969). Other data supporting pituitary involvement show that seminal vesicle atrophy in vitamin

A-deficient rats can be reversed by treatment with HCG, suggesting that reduced androgen secretion is secondary to reduced gonadotrophin release (Mayer & Goddard, 1951).

It is concluded that a chronically elevated dietary intake of retinyl palmitate inhibits cyclic ovarian sex hormone secretion and ovulation, probably due to retinoic acid, rather than retinol *per se*, acting via alteration of ovarian or adrenal steroidogenesis. Furthermore, although ovulatory activity continues without dietary retinol, retinoic acid is required for normal activity.

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References

- ABRAHAMSON, I.A., SR & ABRAHAMSON, I.A., JR (1962) Hypercarotenemia. *Archs Ophthalmol.* **68**, 34–37.
- AHLUWALIA, B. & BIERI, J.G. (1970) Effects of exogenous hormones on the male reproductive organs of vitamin A-deficient rats. *J. Nutr.* **100**, 715–724.
- BAGDON, R.E., ZBINDEN, G. & STUDER, A. (1960) Chronic toxicity studies of β -carotene. *Toxic. appl. Pharmac.* **2**, 225–236.
- BRODY, H. & GOLDMAN, S. (1941) Relationship of vitamin A and estrogens. *Endocrinology* **29**, 164, Abstr.
- BURRILL, M.W. & GREENE, R.R. (1941) Vitamin A and the vaginal response to sex hormones in the rat. I. Estrogens. *Endocrinology* **28**, 765–766.
- COWARD, W.A., HOWELL, J. MCC., PITT, G.A. & THOMPSON, J.N. (1966) Effects of hormones on reproduction in rats fed a diet deficient in retinol (vitamin A alcohol) but containing methyl retinoate (vitamin A acid methyl ester). *J. Reprod. Fert.* **12**, 309–317.
- EVANS, H.M. (1928) The effects of inadequate vitamin A on the sexual physiology of the female. *J. biol. Chem.* **77**, 651–654.
- EVANS, H.M. & BISHOP, K.S. (1922) On an invariable and characteristic disturbance of reproductive function in animals reared on a diet poor in fat soluble vitamin A. *Anat. Rec.* **23**, 17, Abstr.
- FUTTERMAN, S., DOWNER, J.L. & HENDRICKSON, A. (1971) Effect of essential fatty acid deficiency on the fatty acid composition, morphology and electroretinographic response of the retina. *Invest. Ophthalmol.* **10**, 151–156.
- FUTTERMAN, S., SWANSON, D. & KALINA, R.E. (1975) A new, rapid fluorometric determination of retinol in serum. *Invest. Ophthalmol.* **14**, 125–130.
- GELLERT, R.J. & HEINRICH, W.L. (1975) Effect of DDT homologs administered to female rats during the perinatal period. *Biol. Neonate* **26**, 283–290.
- GELLERT, R.J., HEINRICH, W.L. & SWERDLOFF, R. (1974) Effects of neonatally-administered DDT homologs on reproductive function in male and female rats. *Neuroendocrinology* **16**, 84–94.
- GORSKI, R.A. (1971) Gonadal hormones and the perinatal development of neuroendocrine function. In *Frontiers in Neuroendocrinology*, pp. 237–290. Eds L. Martini & W. F. Ganong. Oxford University Press, New York.
- GRANGAUD, R., NICOL, M., LE GALL, J. & SOUSSY, A. (1964) Effets de la stimulation hypophysaire sur la sécrétion cortico-surrénalienne du Rat blanc carencé en vitamine A: étude *in vitro*. *C. r. hebd. Séanc. Acad. Sci., Paris* **258**, 1083–1085.
- GRANGAUD, R., NICOL, M. & DESPLANQUES, D. (1969) Effect of vitamin A on enzymatic conversion of the Δ^5 -3- β -hydroxy-into Δ^4 -3-oxosteroids by adrenals of the rat. *Am. J. clin. Nutr.* **22**, 991–1002.
- HIGH, E.G., MOORE, Y.E., COLLINS, J.D. & FRAZIER, H.M. (1964) Effects of vitamin A acid on growth, reproduction and some tissues in the rat. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **23**, 294, abstr.
- HOWELL, J. MCC., THOMPSON, J.N. & PITT, G.A.J. (1964) Histology of lesions produced in the reproductive tract of animals fed a diet deficient in vitamin A alcohol but containing vitamin A acid. II. The female rat. *J. Reprod. Fert.* **7**, 251–258.
- HUANG, H., MARSHALL, S. & MEITES, J. (1976) Capacity of old versus young female rats to secrete LH, FSH and prolactin. *Biol. Reprod.* **14**, 538–543.
- JOHNSON, B.C. & WOLF, G. (1960) The function of vitamin A in carbohydrate metabolism; its role in adrenocortical production. *Vitamins Horm.* **18**, 457–483.
- JUNEJA, H.S., MOUDGAL, N.R. & GANGULY, J. (1969) Studies on metabolism of vitamin A. The effect of hormones on gestation in retinoate-fed female rats. *Biochem. J.* **111**, 97–105.
- JUNEJA, H.S., MURTHY, S.K. & GANGULY, J. (1966) The effect of vitamin A deficiency on the biosynthesis of steroid hormones in rats. *Biochem. J.* **99**, 138–145.
- KAHN, R.H. (1954) Effect of locally applied vitamin A and estrogen on the rat vagina. *Am. J. Anat.* **95**, 309–328.
- KAHN, R.H. & BERN, H.A. (1950) Antifolliculoid activity of vitamin A. *Science, N.Y.* **111**, 516–517.
- KAMATH, S.K., MACMILLAN, J.B. & ARNRICH, L. (1972) Dietary protein and utilization of carotene or retinyl acetate in rats. *J. Nutr.* **102**, 1579–1584.
- LEVINE, S., GLICK, D. & NAKANE, P.K. (1967) Adrenal and plasma corticosterone and vitamin A in rat

- adrenal glands during postnatal development. *Endocrinology* **80**, 910-914.
- LIBERTUN, C. & LAU, C. (1972) Adrenocortical function in prepubertal rats: neonatal effects of testosterone. *J. Endocr.* **55**, 221-222.
- MASON, K.E. & WOLFE, J.M. (1930) The physiological activity of the hypophyses of rats under various experimental conditions. *Anat. Rec.* **45**, 232, Abstr.
- MAYER, J. & GODDARD, J.W. (1951) Effects of administration of gonadotrophic hormone on vitamin A deficient rats. *Proc. Soc. exp. Biol. Med.* **76**, 149-151.
- MOON, H.D. (1937) Effect of adrenocorticotrophic hormone on the sexual development of spayed rats. *Proc. Soc. exp. Biol. Med.* **37**, 36-37.
- MOORE, T. (1967a) Pharmacology and toxicology of vitamin A. In *The Vitamins: Chemistry, Physiology, Pathology, Methods*, Vol. 1, pp. 280-294. Eds W. H. Sebrell, Jr & R. S. Harris. Academic Press, New York.
- MOORE, T. (1967b) Requirements of vitamin A in animals. In *The Vitamins: Chemistry, Physiology, Pathology, Methods*, Vol. II, pp. 294-301. Eds W. H. Sebrell, Jr & R. S. Harris. Academic Press, New York.
- NATHANSON, I.T., FRANCEEN, C.C. & SWEENEY, A.R. (1938) Nature of the action of testosterone on genital tract of the immature female rat. *Proc. Soc. exp. Biol. Med.* **39**, 385-388.
- NIEMAN, C. & OBBINK, H.J.K. (1954) The biochemistry and pathology of hypervitaminosis A. *Vitamins Horm.* **12**, 69-99.
- ONG, D.E. & CHYTIL, F. (1975) Retinoic acid-binding protein in rat tissue. *J. biol. Chem.* **250**, 6113-6117.
- PAGE, S.W. (1971) Golden ovaries. *Aust. N.Z. J. Obstet. Gynec.* **11**, 32-36.
- POPS, M.A. & SCHWABE, A.D. (1968) Hypercarotenemia in anorexia nervosa. *J. Am. med. Ass.* **205**, 121-122.
- QUATTROPANI, S.L. & WEISZ, J. (1973) Conversion of progesterone to estrone and estradiol *in vitro* by the ovary of the infantile rat in relation to the development of its interstitial tissue. *Endocrinology* **93**, 1269-1276.
- ROGERS, W.E., JR & BIERI, J.G. (1968) Adrenal Δ^5 - 3β -hydroxysteroid dehydrogenase as related to vitamin A. *J. biol. Chem.* **243**, 3404-3408.
- ROSNER, J.M., TRAMEZZANI, J.H., MACOME, J.C. & LLauró, J.L. (1969) The production of androgens and estrogens by the ovaries of normal and testosterone sterilized rats. *Acta physiol. latinoam.* **19**, 97-101.
- RUBIN, S.H. & DE RITTER, E. (1954) Vitamin A requirements of animal species. In *Vitamins and Hormones: Advances in Research and Applications*, Vol. XII, pp. 101-135. Eds R. S. Harris, G. F. Marrian & K. V. Thimann. Academic Press, New York.
- SCHOR, N.A. & GLICK, D. (1970) Effect of vitamin A deficiency on 3β -hydroxy-steroid dehydrogenase activity and corticosterone in the rat adrenal. *Endocrinology* **86**, 693-695.
- SHERWOOD, T.C., BREND, M.A. & ROPER, E.A. (1936) Changes in the vaginal epithelium of the rat on an excessive vitamin A diet. *J. Nutr.* **11**, 593-597.
- SHERWOOD, T.C., DEPP, O.R., BIRGE, G.P. & DOTSON, H.B. (1937) Further studies on the effect of excessive vitamin A on the oestrous cycle of the rat. *J. Nutr.* **14**, 481-486.
- SINGH, N., SINGH, V.N. & VENKITASUBRAMANIAN, T.A. (1972) Effect of hypervitaminosis A on adrenal corticosteroids. *Ind. J. Biochem. Biophys.* **9**, 313-314.
- THOMPSON, J.N., HOWELL, J. McC. & PITT, G.A.J. (1964) Vitamin A and reproduction in rats. *Proc. R. Soc. B.* **159**, 510-535.
- TRUSCOTT, B.L. (1947) Ovarian weight and density in vitamin A-deficient rats. *Anat. Rec.* **98**, 111-126.
- TURNER, C.D. (1941) A spontaneous abnormality of the rat's ovary accompanied by masculinization of the genitalia. *Endocrinology* **28**, 729-739.
- VAN DYKE, R.A., WOLF, G. & JOHNSON, B.C. (1960) The function of vitamin A in adrenal steroid production. *Biochem. Biophys. Res. Commun.* **3**, 123-126.
- WALLACE, D.L., PLOPPER, C.G., BUCCI, T.J. & SAUBERLICH, H.E. (1975) Autoradiographic localization of vitamin A in the adrenal gland of rats. *Life Sci.* **17**, 1693-1698.
- WARREN, M.P. & VANDE WIELE, R.L. (1973) Clinical and metabolic features of anorexia nervosa. *Am. J. Obstet. Gynec.* **117**, 435-449.
- WEISZ, J. & GUNSALUS, P. (1973) Estrogen levels in immature female rats: true or spurious-ovarian or adrenal? *Endocrinology* **93**, 1057-1065.
- WOLF, G. (1961) Vitamin A in adrenal hormone and mucopolysaccharide biosynthesis. *Am. J. clin. Nutr.* **9**, 36-42.

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