Effect of Vitamin A Nutritional Status on the Growth of Oestrogen-Primed Chick Oviduct

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Growth and development of the oestrogen-primed oviduct of immature chicks in terms of weight, length, total protein, total RNA, total DNA and total phospholipids are markedly arrested on deprivation of vitamin A; supplementation with retinyl acetate reversed the effect of the deficiency almost fully, whereas retinoic acid was only partly effective.

After reviewing the existing information on the effect of vitamin A deprivation on animal physiology, Ganguly (1974) pointed out that vitamin A is probably required at some step(s) in cell division. By using regenerating rat liver, after partial hepatectomy, as a model system for rapid cell proliferation, Jayaram *et al.* (1975) clearly demonstrated that regeneration of the liver of vitamin A-deficient rats is markedly arrested, but can be restored to near-normal values by supplementation of the deficient animals with retinyl acetate immediately after the hepatectomy. Yet another system where rapid cell proliferation and differentiation can be induced is oestrogen-primed oviduct of immature chicks. Within 24 h after administration of oestrogen the surface epithelial cells of the left oviduct of immature chicks start undergoing rapid division and by day 3 or day 4 the cells begin to differentiate into tubular gland cells, which is essentially completed by day 6 of oestrogen administration. The growth of the oviduct is so pronounced under such treatment that within 6 days its total weight increases from about 20 mg to about 1.5 g (Kohler *et al.*, 1969; O'Malley *et al.*, 1969). Using such a system we show here that on deprivation of vitamin A the growth of the oestrogen-primed oviduct is markedly arrested, but can be restored to normal by supplementation with retinyl acetate.

Materials and Methods

White Leghorn female chicks (1 day old) obtained from the Central Poultry Farm at Hesarghatta (Bangalore) were kept on the vitamin A-deficient diet of Joshi *et al.* (1973) until they ceased to grow. At this stage the birds were distributed in several groups as follows. One of the groups continued to receive the deficient diet only, whereas two other groups received, in addition to the deficient diet, supplements of 40 µg of retinyl acetate per bird per day, or of 10 µg of retinoic acid per g of diet. At the same time birds in another group simultaneously received the deficient

diet together with 40 µg of retinyl acetate daily from day 1 until the end of the experiments, and they are referred to here as normal control birds. At 2 days after the supplementation with vitamin A was started, the birds began to receive by intraperitoneal injection 2 mg of oestradiol-17β benzoate (dissolved in 0.1 ml of groundnut oil) per bird per day for 6 days. The deficient and normal groups also received similar injections of the steroid at comparable age and for 6 days. At the end of the oestrogen treatment for 6 consecutive days all birds were killed by decapitation, after which their oviducts were speedily removed.

The excised oviducts were kept chilled over crushed ice, washed with cold 0.9% NaCl and blotted free of blood, after which their weight and length were recorded. The magnum portions of the oviducts were carefully cut, weighed and homogenized in cold 0.9% NaCl (1:10, w/v) in a Potter-Elvehjem homogenizer fitted with a Teflon pestle, after which suitable portions of the homogenates were used for the determination of proteins, RNA, DNA and phospholipids. Proteins were measured by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard, and the RNA and DNA were determined as described by Jayaram *et al.* (1975). The phospholipids were extracted as described by Bligh & Dyer (1959), and the lipid P was measured by the method of Bartlett (1959) as modified by Marinetti (1962).

Results and Discussion

Three independent sets of experiments with a large number of birds were carried out with considerable degree of reproducibility, and the results are assembled in Table 1. After oestradiol treatment the development of the oviduct in terms of increase in weight and length was markedly less in the deficient animals than in the corresponding normal controls. In sharp contrast supplementation of the deficient birds with retinyl acetate restored the growth of the

Table 1. Effect of vitamin A nutritional status on the growth and development of the oviduct of young chicks, after stimulation with oestrogen

Experimental conditions were as described in the text. All birds received by intraperitoneal injection 2 mg of oestradiol-17 β benzoate in 0.1 ml of groundnut oil per bird per day for 6 consecutive days. Values obtained in three separate sets of experiments are assembled together and are expressed as averages $\pm$ S.E.M.

Total number of animals used	Treatment	Magnum											
		Oviduct		Protein		RNA		DNA		Phospholipids*			
		Weight (g)	Length (cm)	Weight (g)	Total (mg)	(mg/g of tissue)	Total (mg)	(mg/g of tissue)	Total (mg)	(mg/g of tissue)	Total (μmol)	(μmol/g of DNA)	
17	Deficient	0.75 ±0.034	8.9 ±0.13	0.36 ±0.023	26.04 ±1.84	72.77 ±4.68	85.38 ±6.72	8.93 ±0.28	3.22 ±0.19	10.47 ±0.52	5.12 ±0.29	14.36 ±0.62	15.66 ±0.58
17	Retinoic acid after depletion	1.12 ±0.042	11.0 ±0.14	0.60 ±0.014	47.40 ±1.11	79.40 ±1.12	90.29 ±5.57	9.47 ±0.16	5.66 ±0.16	10.68 ±0.33	10.53 ±0.34	17.72 ±0.59	19.91 ±0.70
14	Retinyl acetate after depletion	1.35 ±0.031	12.50 ±0.18	0.72 ±0.02	63.41 ±2.52	86.71 ±1.84	91.80 ±2.85	10.19 ±0.20	7.40 ±0.16	10.66 ±0.26	14.64 ±0.52	20.09 ±0.57	20.90 ±0.70
11	Retinyl acetate from day 1 (normal)	1.49 ±0.053	12.60 ±0.32	0.81 ±0.03	78.15 ±1.89	96.61 ±2.94	105.19 ±4.26	10.32 ±0.36	8.47 ±0.28	11.34 ±0.53	17.98 ±0.62	23.17 ±1.13	25.08 ±1.12

* Expressed as μ mol of lipid P.

oviduct almost to that of the normal controls, whereas the growth in the retinoate-treated birds was markedly greater than in the deficient ones but appreciably less than in those given the supplements of retinyl acetate.

Statistical analysis of the differences in the length and weight of the oviducts of the normal, retinyl acetate- and retinoate-supplemented birds against those of the deficient ones on the one hand, and of the normal and retinyl acetate-supplemented birds against the retinoate-supplemented ones on the other, revealed that the differences were quite significant ($P < 0.01$ in all cases). But the differences were not significant ($P > 0.05$) when the values for the normal and retinyl acetate-treated birds were compared, which clearly showed that supplementation with retinyl acetate after depletion of vitamin A restored the growth of the oviducts to normal values.

Analysis of the magnum portions of the oviducts for weight, total proteins, total RNA, total DNA and total phospholipids revealed that these parameters were significantly less in the deficient birds ($P < 0.01$) than those of the normal controls, and of the retinyl acetate- as well as retinoate-supplemented ones. Here also, these values were significantly less in the birds receiving retinoic acid ($P < 0.01$) than in the normal controls or in those given retinyl acetate after depletion. Further, the differences in the protein, RNA and phospholipid contents of the acetate-supplemented and normal control groups were statistically significant ($P < 0.01$), but in contrast that of the DNA values was not significant ($P > 0.05$). When expressed per g of tissue or per mg of DNA the differences between the deficient group and the normal and retinyl acetate-supplemented groups were significant for proteins and phospholipids ($P < 0.01$), but those for the RNA and DNA were not significant ($P > 0.05$).

These preliminary results therefore clearly demonstrate that under oestrogen stimulation the growth of the oviduct in terms of length, weight, DNA, RNA, proteins and phospholipids is markedly arrested in the birds depleted of vitamin A and that it can be restored to normal values by supplementation with retinyl acetate. It is, however, more pertinent in this context that, when expressed per g of tissue the DNA and RNA values were not significantly different in any of the groups, which makes it obvious that the increase in length and weight of the tissue reflects an actual increase in the cell population, and consequently cell division is grossly affected in the deficient group. On the other hand the significant differences in the protein and phospholipid contents in the deficient birds from those in the retinyl acetate-supplemented as well as in the normal controls when similarly expressed per g of tissue would appear to suggest that the formation of membranes (probably including the rough endoplasmic reticulum) as well

as protein synthesis is also affected in the deficient birds. In fact histological investigations of these oviducts (P. S. Joshi, S. K. Murthy, K. Bhargava & J. Ganguly, unpublished work) clearly show that the size of the lobules which enclose the albumin-secreting tubular glands, and also the number of these glands per lobule, are strikingly less in the oviduct of the deficient birds. At the same time the development of the smooth muscles of the oviduct wall is equally arrested in the same birds.

Yet another point that deserves some comments here is the question of the ability of retinoic acid to replace retinol in the normal growth of animals. Ever since Wald (1960) demonstrated that retinoic acid can support growth of vitamin A-deficient rats, it has been widely accepted that it can replace retinol in such processes. But we (Jayaram *et al.*, 1975) have shown that in regenerating rat liver, after partial hepatectomy, retinoic acid is less effective than retinol in reversing the effects of vitamin A deficiency. The present results show that in yet another system of induced cell proliferation retinoate is less effective than retinol in this respect. Such effects of the retinoate seem to be similar in other systems also

where rapid cell proliferation is involved, e.g. spermatogenesis, steroid secretion in the ovaries, foetal development etc. in rats (Ganguly, 1974).

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