from the rest of the DNA in sequence-specific DNA-protein interactions, in addition to the discrimination resulting from selective interactions involving the bases.

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SOME RECENT OBSERVATIONS ON THE SYSTEMIC MODE OF ACTION OF VITAMIN A

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ABSTRACT

Some of the important observations made in recent years regarding the systemic mode of action of vitamin A are summarised. It is pointed out that regeneration of liver following partial hepatectomy is markedly less in vitamin A deficient rats. Similarly division and differentiation of the primitive epithelial cells of the oviduct of oestrogen-treated chicks are significantly arrested on deprivation of vitamin A. It is discussed that vitamin A is required for controlled division and differentiation of cells.

UNLIKE many of the water-soluble vitamins, proper understanding of the mode of action of the fat-soluble vitamins has been rather challenging, and vitamin A has not proved to be an exception in this respect. Although it was one of the earliest vitamins to be discovered, its systemic mode of action is still not properly understood, and completely new approach has become necessary for unravelling the prerise mechanism through which it functions. Recently Ganguly[1] made such a radically new approach and pointed out that the classical symptoms of deficiency of vitamin A described by numerous workers can be explained on the basis of a general interpretation that vitamin A is required for division and differentiation of cells of higher animals. While arriving at such an interpretation it was assumed that a particular compound should act at a single fundamental point rather than at several areas of physiology. According to Ganguly[1] such an interpretation would readily explain the diverse effects of deficiency of vitamin A described in the literature. Since then we have made several attempts to produce *evidence in support of* such a hypothesis by using regenerating rat liver and oestrogen-primed chick oviduct as typical model systems for rapid growth and differentiation of cells and are summarising here some of our salient observations in this regard.

Regenerating rat liver: Regenerating rat liver, following partial hepatectomy, has been widely recognised as a typical model system for rapid cell division[2]. By using vitamin A-depleted and normal rats Jayaram *et al.*[3] have shown that regeneration of the liver in terms of net increase in tissue weight as well as in terms of DNA, RNA and protein contents is markedly lower in the vitamin A-deprived rats which could be restored to near normal levels by supplementation of the deprived rats with retinyl acetate *immediately after* partial hepatectomy.

Examination of the *in vivo* incorporation of radioactive predursors into the DNA, RNA and proteins of the regenerating liver at different time intervals after partial hepatectomy revealed that the rates of synthesis of these macromolecules were appreciably slower in the vitamin A-deprived rats. It was also demonstrated that the activity of the RNA polymerase, which normally shows marked increase in its activity during the early phases of regeneration, showed significantly less increase in its activity during the same phase of regeneration of the liver of vitamin A-deprived rats[4].

At the same time histological examination of the regenerating liver of the vitamin A-deprived and normal rats revealed that the mitotic index was lower and the appearance of the endoplasmic reticulum was finer in the deprived rats, as compared to the corresponding normal controls[4].

Oestrogen-primed chick oviduct : Due to several reasons the oestrogen-primed chick oviduct has proved to be an ideal system for the study of the possible role of vitamin A in division and differentiation of cells. Firstly, the magnum portion of the oviduct of the immature chicks consists of a single layer of stratified columnar epithelial cells. Following oestrogen administration these primitive epithelial cells undergo rapid division after which they differentiate into three types of cells namely, tubular gland cells, goblet cells and ciliated cells[5,6]. Secondly, the newly hatched chick contains some amounts of vitamin A in its liver and yolk sac, and draws upon this supply of vitamin A for its growth during the initial stages after the hatch[7], so that if it is put on a vitamin A-deficient diet immediately after the hatch it can be made deficient of vitamin A within two to three weeks.

By using chicks made deficient of vitamin A in this manner Joshi *et al.*[8] showed that, following administration of oestradiol 17-β for six consecutive days the growth of the magnum portion of the oviduct in terms of increase in its length and weight as also in its total DNA, RNA, protein and phospholipid contents is perceptibly less in such birds. At the same time it was observed that such effects of the deficiency can be reversed by supplementation of the depleted birds with retinyl acetate. These results thus showed that the overall growth of the oviduct during stimulation by oestrogen is dependent upon availability of adequate amounts of vitamin A.

Incorporation, in vitro, of the radioactive precursors into the DNA, RNA and proteins of the oviduct magnum :

DNA and RNA : When the oviduct magnum of the deficient and normal birds treated with oestrogen was incubated with [^{3}H]–thymidine or [^{3}H]–uridine in the incubation medium, incorporation of these radioactive precursors into the respective nucleic acids, when expressed on the basis of μg. DNA, was markedly less in the depleted tissue, as compared to the control tissue. It was most significant that addition of retinol to the incubation mixtures restored the incorporation of both precursors to near normal values in the deficient tissue, with retinol showing no such effects on the normal tissue[9].

Proteins : The bulk of the increase in the weight of the oestrogen-primed oviduct is due to rapid increase in the number of tubular gland cells which are responsible for the syntheses of several egg-white proteins like ovalbumin, conalbumin, ovomucoid, lysozyme, etc. The goblet cells, on the other hand, synthesize ovomucin and avidin. Since depletion of vitamin A exerted marked effects on the synthesis of DNA and RNA in the oviduct, attempts were made to study the effects of vitamin A deficiency on the incorporation of ^{14}C–*Chlorella*–protein–hydrolysates into the different proteins of the oviduct similarly incubated Here, the synthesis of ovalbumin, which represents about 50 to 55% of the total cytosol proteins of the oviduct, was markedly lower in the deficient tissue, while that of the other proteins like conalbumin, ovomucoid and ovomucin remained unaffected.

Glycosylation of proteins : In recent years there has been extensive work on the role of polyprenols in sugar-transfer reactions, particularly in micro-organisms[10]. Being a tetraprenol, retinol has been considered to take part in similar sugar-transfer reactions, especially in glycosylation of proteins in mammalian systems[11]. The oestrogen–primed chick oviduct is an ideal system for the study of such glycosylation reactions of proteins, because this particular tissue actively synthesizes several glycoproteins of the egg, under the influence of oestrogen. Therefore the contents of the free and protein-bound sugars (both hexose and hexosamines) were compared in the oviducts of the vitamin A-deficient and normal chicks, when the deficient tissue was found to contain lower amounts of the protein bound sugars, with a concomitant increase in the free sugar contents. But, following incubation of the oviduct with [^{3}H]–mannose in the medium, while mannosylation of the total oviduct proteins was not significantly affected, that of the cytosol proteins showed considerable decrease in the deficient oviduct. Like the incorporation of ^{14}C–amino acids, here also, the effect of the deficiency was more marked in the case of the major glycoprotein of the oviduct, namely ovalbumin,

which obviously indicated that lower incorporation of the labelled sugar might be due to reduced synthesis of the protein component. In sharp contrast, mannosylation of ovomucins was significantly higher in the deficient tissue. These results thus did not lead to any definite conclusions regarding any role of retinol in the glycosylation of the oviduct proteins.

Histological studies : Several interesting observations were made from histological examinations of the magnum of the deficient and normal oviducts[9]. Thus, while the number of lobules per section of the oviduct remained more or less unchanged in the two types of tissues, the average number of acini (tubular gland cells) per lobule was drastically reduced in the deficient magnum, in that, whereas in the normal tissue the average number of the acini was $88 \cdot 7 \pm 3 \cdot 6$, the corresponding number in the deficient tissue was only $31 \cdot 6 \pm 4 \cdot 2$. It was equally significant that the gland cells were poorly developed and appeared elongated in the deficient samples, while they were well formed and round in the normal tissue. Moreover, disproportionately large number of goblet cells was visible in the deficient oviduct, which also showed the presence of large amounts of alcian blue-positive materials. It should be obvious that the appearance of increased amounts of free and bound sugars found in the deficient magnum, as described above, should explain the presence of such large amounts of the alcian blue-positive matetials in the same samples, and thereby establish that the histochemical observations and the analytical values are in fairly good agreement.

Electronmicroscopic studies : These histological observations were further extended by electronmicrosdopic examination, which not only confirmed these findings, but in addition, revealed further striking differences between the two types of tissues. Thus, under normal circumstances large number of protodifferentiated cells appear at the epithelial region of the oviduct magnum at the very early stages of oestrogen administration, after which they slowly move down and fill up the lobules in the form of acinar gland cells. But, in our experiments, while there were few protodifferentiated cells in the magnum of the normal birds, these cells were present in large numbers in the epithelial region of the depleted tissue. Such a situation clearly suggested that growth and development of the protodifferentiated cells were markedly less in the depleted tissues and such an interpretation was fully substantiated by the appearance of strikingly less number of tubular gland cells per unit area, with stromal oedema still persisting in the deficient tissue, while in contrast stromal oedema was preceptibly less and the gland cells were markedly higher in the normal tissue.

Several remarkable differences were noticeable in the gland cells also. Thus the size of these cells, as measured from the acinar lumen to the farthest part of these cells, was appreciably smaller in the depleted tissue. The morphology of these cells with respect to the development of the rough endoplasmic reticulum (RER) and polysomes seemed to be poor, so that it would appear that the machinery for protein synthesis was not properly formed, both in quality and quantity. Yet another interesting aspect of the morphology of these cells was the striking difference in the nature of the secretory granules Thus, while both condensing vacuoles (which eventually become mature granules) and the mature secretory granules were found in the cytoplasm of the normal birds, essentially mature secretory granules were seen in the depleted birds.

Another highly significant finding was the appearance of large number of globlet cells throughout the surface epithelium of the depleted magnum even after the administration of the oestrogen for six consecutive days, while in the normal tissue these cells were visible only occasionaly. According to Kohler *et al.*[5] the tubular gland cells are the first to differentiate from the primitive epithelial cells, while the goblet cells and ciliated cells do not appear in appreciable number until the maturation of the tubular gland cells is complete and these processes take place around 7 to 8 days after the oestrogen treatment has been started. It is thus clear that the control of the programmed differentiation of the epithelial cells of the oestrogen-treated oviduct is lost in the absence of vitamin A.

Isolation of a receptor protein for retinol from the oviduct :

In recent years several receptors for steroid hormones have been isolated from various animal tissues[12], one of them being a specigc receptor for progesterone isolated from hen oviduct[13], and it is generally believed that the cellular receptors for the steroids deliver the steroids to the nucleus of the target tissues for their physiological function. Similarly receptors for various retinol derivatives have been described in different animal tissues[14,15,16]. We have also isolated from the oviduct of laying hens a receptor for retinol in fairly pure form. Its molecular weight was 13,180 daltons and it could bind specifically with retinol, retinyl acetate and retinyl palmitate. It had two types of binding sites with Ka of $3 \cdot 7 \times 10^7$ M^{-1} and $3 \cdot 9 \times 10^6$ M^{-1}, respecty and it represented about 0·16% of the total cytosol proteins of the oviduct magnum. We should however mention it here that the function of the plasma retinol-binding protein (RBP) is quite different from that of the cellular

receptor protein in that, while the former transports retinol from the storage site and delivers it to the cell where it is required, the latter makes retinol available to the actual site inside the cell where it is ultimately used. It would be interesting to speculate that both steriod hormone and retinol participate through the respective receptor proteins at the chromatin level of the cell nucleus in division and differentiation of cells. These considerations have led us to suggest that retinol should be called a co-steroid hormone[9].

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DISCOVERY OF THE CYANOPHYCEAN ALGAL REMAINS AND MICROPLANKTONS IN THE LATE PRECAMBRIAN SCHISTOSE PHYLLITES AND ITS BEARING ON THE AGE OF THE AMRI UNIT, GARHWAL HIMALAYA, INDIA

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ABSTRACT

The late Precambrian schistose phyllites (Amri Unit) of the Gahrwal Himalaya at north-eastern Dogadda yielded high concentration of spheroidal algal remains (*Myxococcoides minor*) of living Cyanophycean (Chroococcaceae) affinities. In addition, they consists of microplanktons (*Granomarginata primitiva, Protosphaeridium volkovae*) and fungal (?) remains (*Eomycetopsis septata*). The general morphological characters of the microfossils are described. Their discovery in the rocks of the area is the first find of these microorganisms known to-date. The occurrence of microflora (microfauna) in the rocks assists in the reconstruction of the paleo-environments of the ancient Garhwal Himalayan ocean, and lating of the Precambrian phyllites of the Amri Unit.

INTRODUCTION

THE occurrence of fossil spheroidal algal remains, of living cyanophycean (Chroococcaceae) affinities, and organic- walled microplanktons (acritarchs) from the late Precambrian schistose phyllites of Dogadda (Garhwal Himalaya, India) have not been reported so far. In India, however, acritarchs and cyanophycean algal remains (spheroidal, filamentous) have been recorded from North India (Kumaun and Garhwal Himalayas, Son Valley), South India (Kaladgi Basin, Karnataka State, Andhra Pradesh) and Madhya Pradesh (Rampura), ranging in age from late Precambrian to Cambrian (Shukla *et al.*[12], Saluja *et al.*[13,14], Venkatachala and Rawat[19]; Venkatachala *et al.*[20], Prakash[9], Vishwanathiah *et al.*[21], Maithy and Shukla[3], Nautiyal[5-7]). Furthermore, a considerable amount of information on the spheroidal algal remains and microplanktons from the late Precambrian rocks of the U.S.A., Canada, Australia, Africa; and Russia has been published (Vologdin and Drozdova[22]; Shopt[15]; Schopf and