Role of Vitamin A in Sulphate Metabolism

STUDIES ON THE ENZYMIC ACTIVATION OF SULPHATE BY VARIOUS TISSUE EXTRACTS OF NORMAL AND VITAMIN A-DEFICIENT RATS

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(Received 21 October 1966)

1. The enzymic activation of sulphate by various tissue extracts of vitamin A-deficient rats and their pair-fed controls was studied. 2. Vitamin A deficiency does not impair the enzymic activity in liver, colon and brain. However, a significant decrease in activity was observed in epiphyseal cartilage.

Reports from several Laboratories suggest the involvement of vitamin A in sulphate metabolism. The enzymic activation of sulphate to form PAPS* is a major step in the biosynthesis of most of the sulphated compounds including sulphomucopolysaccharides, and it has been shown that the synthesis of PAPS is not significantly affected in brain, liver and colon of hypervitaminotic A rats (Mukherji & Bachhawat, 1966b). However, available reports on the synthesis of PAPS in tissues of vitamin A-deficient animals are contradictory. Thus Varandani, Wolf & Johnson (1960) and also Subba Rao & Ganguly (1964) have observed that vitamin A has a direct role in sulphate activation: the decreased synthesis of PAPS in homogenates of colon and liver from vitamin A-deficient rats can be rectified *in vitro* by addition of vitamin A to the reaction mixture. But Pasternak, Humphries & Pirie (1963) were unable to detect any significant change in the enzymic activation of sulphate in rat colonic mucosa, rat cornea, rabbit colonic mucosa or rabbit corneal epithelium from vitamin A-deficient animals. However, since then Wolf and his associates (Sundaresan & Wolf, 1963; Sundaresan, Elford & Wolf, 1964) and Sundaresan (1966), as well as Subba Rao & Ganguly (1966), have confirmed their earlier observations, and also Pasternak & Pirie (1964) have again shown their inability to detect any significant decrease in the formation of PAPS in liver from vitamin A-deficient rats.

In view of this controversy and also of our previous observations of decreased incorporation of carrier-free labelled sulphate *in vivo* into various tissue mucopolysaccharides of vitamin A-deficient rats (Mukherji & Bachhawat, 1965), the present

study was undertaken. The results show that, in contrast with epiphyseal cartilage, vitamin A deficiency does not lead to any significant decrease in the activity of the PAPS-synthesizing enzyme system in rat liver, brain and colon.

MATERIALS AND METHODS

Carrier-free $\text{Na}_2^{35}\text{SO}_4$ was obtained from the Atomic Energy Establishment, Trombay, India. ATP, *p*-nitrophenol and *p*-nitrophenyl sulphate were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Vitamin A-free diet was prepared according to the method of DeLuca, Manatt, Madsen & Olson (1963). Hydrogenated groundnut oil, refined and bleached, was a gift from Hindustan Levers, Trichy, India. Inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) was prepared from baker's yeast as described by Heppel & Hilme (1951). The sulphate-transferring enzyme phenol sulphokinase (adenosine 3'-phosphate 5'-sulphatophosphate-phenol sulphotransferase, EC 2.8.2.1) was prepared from rat liver by the method of Brunngraber (1958).

Vitamin A-deficient rats were prepared as follows. Weanling litter mates matched in sex and body weight were made vitamin A-deficient as described by Mukherji & Bachhawat (1965). There was no detectable vitamin A in the livers of deficient rats as measured by the method of Ames (1954). The stage of deficiency of the rats used in the present work corresponds to the stage II avitaminosis postulated by Subba Rao & Ganguly (1966).

Preparation of tissue extracts and assay of the PAPS-synthesizing enzyme system were the same as described by Mukherji & Bachhawat (1966b).

The [^{35}S]PAPS formed was further identified by its ability to donate sulphate to *p*-nitrophenol to form *p*-nitrophenyl sulphate, as described by Balasubramanian & Bachhawat (1961). The *p*-nitrophenyl sulphate was identified by the method of Loewi & Kent (1954).

Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), with bovine serum albumin as the standard.

* Abbreviation: PAPS, adenosine 3'-phosphate 5'-sulphatophosphate.

Table 1. *Effects of vitamin A deficiency on the formation of adenosine 3'-phosphate 5'-sulphatophosphate by extracts of various tissues of rats*

Weanling litter mates matched in sex and body weight were made vitamin A-deficient as described by Mukherji & Bachhawat (1965). Values for PAPS synthesis assayed by the method of Mukherji & Bachhawat (1966b) are averages of seven pairs of rats (four pairs female and three pairs male) and are expressed in μ moles of PAPS/mg. of protein/hr.

Tissue	PAPS synthesis			P*
	Control (mean)	Deficient (mean)	Difference between pairs (mean $\pm$ S.E.M.)	
Brain	2.2	2.2	0	
Liver	17.4	16.8	0.6 $\pm$ 0.96	N.S.
Colon	2.4	6.8	4.4 $\pm$ 1.24	<0.02
Epiphyseal cartilage	5.5	3.9	1.6 $\pm$ 0.42	<0.01

* Probability (based on differences between pairs); N.S., not significant.

RESULTS AND DISCUSSION

Effects of vitamin A deficiency on the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate. The effects of vitamin A deficiency on the synthesis of PAPS by extracts of rat liver, colon, brain and epiphyseal cartilage are shown in Table 1. The results are presented on the basis of the total number of pairs since a similar pattern was observed in both sexes. There is no significant change in the synthesis of PAPS in liver and brain, but an increased synthesis was observed in colon. The formation of PAPS is decreased by about 30% in epiphyseal cartilage.

The present results show that in vitamin A deficiency there is no significant decrease in the synthesis of PAPS in rat brain and liver, but a rather increased synthesis in colon. These findings are thus in agreement with those of Pasternak *et al.* (1963) and Pasternak & Pirie (1964), who also could not detect any significant decrease in the enzymic activation of sulphate by extracts of rat liver and colonic mucosa, but observed a high synthesis of PAPS in rabbit colonic mucosa and rat cornea. However, these observations are at variance with those of Varandani *et al.* (1960) and Subba Rao & Ganguly (1964, 1966), who found a decreased synthesis of PAPS in homogenates of colon and liver from vitamin A-deficient rats that was restorable with addition of vitamin A *in vitro*, and of Carroll & Spencer (1965) and Spencer & Raftery (1966), who noted increased PAPS synthesis by liver preparations from foetal rats on the addition of vitamin A *in vitro*. Wolf and his group (Sundaresan & Wolf, 1963; Sundaresan *et al.* 1964) and Sundaresan (1966) have further shown that ATP-sulphurylase (ATP-sulphate adenylyltransferase, EC 2.7.7.4) activity is impaired in vitamin A deficiency, and that a metabolite of vitamin A,

and not vitamin A as such, can greatly restore the lost activity. Recently Hall & Straatsma (1966) have observed that in vitamin A-deficient rats PAPS synthesis is decreased in liver but not in retina.

The decreased synthesis of PAPS in epiphyseal cartilage as found in the present study is not clearly understood, but may be due to the breakdown of enzyme protein by lysosomal proteases released in vitamin A deficiency, for Howell (1965) has reported that some cartilage cells contain an abundance of lysosomes, and there are several reports of release of lysosomal proteolytic enzymes in vitamin A deficiency (Dingle, Sharman & Moore, 1966; Guha & Roels, 1965; Roels, Trout & Guha, 1965; Roels, Trout & Guha, 1964; Roxas, Sessa, Trout, Guha & Roels, 1964).

The present finding of no significant decrease in the enzymic activation of sulphate in various rat tissues in vitamin A deficiency and also in hypervitaminosis A (Mukherji & Bachhawat, 1966b), along with our previous observations of decreased incorporation of carrier-free labelled sulphate *in vivo* into various tissue mucopolysaccharides of both vitamin A-deficient and hypervitaminotic A rats with no significant decrease in total mucopolysaccharides except in epiphyseal cartilage (Mukherji & Bachhawat, 1965, 1966a), suggests that the site of action of vitamin A is somewhere else. In view of the reports that cell membrane is damaged in both vitamin A deficiency and toxicity (Brown, Button & Smith, 1963; Dingle, 1963; Dingle, Glauert, Daniel & Lucy, 1962; Dingle & Lucy, 1962; Lucy & Dingle, 1962), it is possible that there is an increased intracellular concentration of sulphate resulting in an apparent decreased incorporation of [35 S]sulphate. This intracellular concentration of sulphate may also be increased owing to formation of inorganic sulphate *in situ*,

since there are reports of release of arylsulphatases (aryl sulphate sulphohydrolase, EC 3.1.6.1) in both vitamin A deficiency and toxicity (Guha & Roels, 1965; Hsu & Tappel, 1965; Roxas *et al.* 1964).

The keen interest of Principal J. Chandy is appreciated. The work was supported by Grant no. 214-7 from the National Multiple Sclerosis Society, U.S.A., and a grant from the Council of Scientific and Industrial Research, New Delhi.

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