FUNCTION OF VITAMIN A IN THE SYNTHESIS OF 3'-PHOSPHOADENOSINE-5'-PHOSPHOSULFATE*

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Previous reports from this laboratory have presented evidence that the incorporation of S^{35}-sulfate into the mucopolysaccharide (MPS)** obtained from vitamin A-deficient rat colon segments and colon homogenates was about half that from normals (Varandani, Wolf and Johnson, 1958). This lowered incorporation could be restored specifically by the in vitro addition of vitamin A (Varandani, Wolf and Johnson, 1959; Wolf and Varandani, 1960). Vitamin A is involved neither in the amination of hexose nor in the transfer of sulfate to p-nitrophenol acceptor (Wolf, Varandani and Johnson, 1960). Evidence is now presented which indicates that the block in MPS synthesis due to vitamin A is at the sulfate activation step, that is, in the synthesis of 3'-phosphoadenosine-5'-phosphosulfate (PAPS), and that this defect can be corrected by the in vitro addition of vitamin A. Evidence is also presented that in the overall MPS-synthesizing system, when $PAPS^{35}$ was used instead of $S^{35}O_4^=$ as sulfate source, no differences were found between colon mucosal homogenates from deficient and normal animals in the transfer of S^{35} to form labeled MPS.

Vitamin A-deficient rats were prepared as described by Wolf, Lane and Johnson (1957). Vitamin A-adequate animals were obtained by feeding 6000 I.U. of vitamin A to pair-selected deficient rats of the same weight as those left deficient, 4 days prior to decapitating them for experimentation. The excised colons from the

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**The following abbreviations are used: MPS, mucopolysaccharide; ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; PAPS, 3'-phosphoadenosine-5'-phosphosulfate.

deficient and paired vitamin A-adequate rats were cleaned of feces by washing with ice-cold 0.9% saline and were homogenized in 3 volumes of homogenizing medium (Zamecnik and Keller, 1954). The debris and nuclei were removed by centrifuging for 15 minutes at 600 x g, and the supernatant homogenates were incubated as described below.

Synthesis of $PAPS^{35}$. The method of synthesis of $PAPS^{35}$ was similar to that described by Robbins and Lipmann (1957). The incubation mixture consisted of carrier-free S^{35}-sulfate, 8.4×10^5 cpm in Experiment I and 16.8×10^5 cpm in Experiment II (obtained from Abbott Laboratories, Oak Ridge, Tenn.), 10 μmoles of ATP, 5 μmoles of $MgCl_2$, 5 μmoles of cysteine, 100 μmoles of imidazole-HCl (pH 7.0) and colon homogenate containing 2 mg of protein from deficient or vitamin A-adequate rats, made up to a final total volume of 0.5 ml. The incubations were carried out at 37° under 95% O_2 plus 5% CO_2 for 30 minutes. The reaction was stopped by heating at 95° for 2 minutes and chilling in ice. The $PAPS^{35}$ synthesized was isolated by paper electrophoresis, carried out on Whatman paper No. 3 at 300 volts in citrate buffer (0.05 M, pH 5.6). $PAPS^{35}$ moved 20 cm in 6 hours. The paper was dried in air and $PAPS^{35}$ was assayed as described previously (Wolf, Varandani and Johnson 1960).

Table I

Effect of Vitamin A Deficiency on $PAPS^{35}$ Synthesis

Vitamin A Status of Rats	Addition	$PAPS^{35}$ Expt. I	$PAPS^{35}$ Expt. II
		cpm/mg protein	
Adequate	-	19,200	59,000
Deficient	-	8,440	28,700
Deficient	Vitamin A, 20 μg in Propylene Glycol, 5 μl	24,300	54,100
Deficient	Propylene Glycol, 5 μl	-	31,400

The incubation mixture is described in the text.
Activity added: Expt I, 8.4×10^5 cpm; Expt. II, 16.8×10^5 cpm.

Results in Table I show that the amount of S^{35} incorporated into $PAPS^{35}$ in the deficient homogenate is about half that of the normal. The in vitro addition of vitamin A (dissolved in propylene glycol) restored the synthetic ability of the deficient homogenates, whereas propylene glycol had no effect.

Since the vitamin A-adequate animals were deficient animals which had been given vitamin A 4 days prior to killing, the in vivo response could be due only to the administered vitamin A.

Synthesis of MPS. If vitamin A functions in the synthesis of PAPS, no differences should be expected in the MPS synthesis when $PAPS^{35}$ is used as the source of S^{35}. Such indeed is the case. $PAPS^{35}$ (52,400 cpm) was incubated with 5 μmoles of $MgCl_2$, 5 μmoles of cysteine, 10 μmoles of glucose, 1 μmole of glutamine, 3 μmoles of DPN, and deficient or normal colon homogenate containing 2 mg protein; final volume 0.7 ml and pH 7.4. The incubation was carried out at 37° for 1 hour under 95% O_2 and 5% CO_2. The reaction was stopped by adding an equal volume of 8% trichloroacetic acid. MPS^{35} was isolated by descending chromatography in 1 M ammonium acetate:ethanol, 2:5 (Wolf, Varandani and Johnson, 1960) and by ethanol: benzene, 1:1 precipitation (Varandani, Wolf and Johnson, 1958). Table II shows that the transfer of PAPS sulfate into MPS by normal and deficient colon homogenates was not affected by the vitamin A status of the animals. This confirms our previous results in which an artificial acceptor p-nitrophenol had been used (Wolf, Varandani and Johnson, 1960).

Table II
Effect of Vitamin A Deficiency on MPS Using $PAPS^{35}$ as a Source of Sulfate

Vitamin A Status	Mucopolysaccharide	
	By Chromatography	By Precipitation
	cpm	cpm
Normal	1020	795
Deficient	1020	828

The incubation mixture is described in the text.

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