Aromatic Amines* III. Note on Bis(2-amino-1-naphthyl) Phosphate, a Urinary Metabolite of 2-naphthylamine. (24544)

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Recent emphasis on the study of 2-naphthylamine in relation to its ability to produce bladder cancer in man and dog has been on metabolic formation of the active carcinogen. The metabolically formed carcinogenic derivative presumably appears in the urine from the inactive primary compound and gives rise to tumor formation in the bladder. Within this concept, identificaton of this carcinogenic metabolite is an initial obligation for understanding of the etiology of this disease. Earlier papers in this series(1,2) have described some excreted metabolites of aromatic amines; the present paper is concerned with demonstration of a novel metabolite, a phosphate conjugate of 2-amino-1-naphthol in the urine of dogs. This compound appears to be a promising candidate for the role of the active bladder carcinogen.

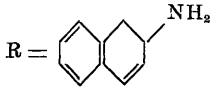
Methods. Our experiments were carried out by feeding 1 g of 2-naphthylamine per day to 3 dogs and observing the urinary metabolites of this compound. Total aromatic amine excretion was measured by the specific method employing 1,2-naphthoquinone-4-sulfonate (NQS)(1) and 2-amino-1-naphthol was determined by the color test described by Clayson(3). Our attention was drawn to the fact that 2-amino-1-naphthol appeared in increased concentration in alkaline urines. This pointed to an alkali labile precursor of 2-amino-1-naphthol. The known conjugates of 2-amino-1-naphthol, the glucuronide and the sulfate esters, both are stable in alkali. Furthermore it has been reported that no enzyme capable of splitting sulfate esters of ortho amino phenols exists in urine(4) and that the glucuronidases present have a pH optimum at pH 4.5(5). The alkali labile conjugate was readily extractable by ether and was water soluble. Chromatography with n-amyl alcohol revealed a single NQS reacting spot with an R_F of 0.8. The compound was isolated as the amine hydrochloride by passing hydrochloric acid gas through a dry ether solution. The material yielded ortho phosphate and oxidation products of 2-amino-1-naphthol on hydrolysis with strong acid, or by acid and alkaline phosphatases. The hydrolysis with acid phosphatase proceeds in 2 stages—in the first stage, one mole of 2-amino-1-naphthol was released and no ortho phosphate; in the second, another mole of amino-naphthol was released and one mole ortho phosphate (Table I). The ratio of amine to phosphate was two to one. We interpret this as evidence that we are deal-

TABLE I. Acid Phosphatase Action on Metabolite. 0.112 μm amine (NQS) of metabolite in 10 ml 0.1M 5.2 acetate buffer was incubated with 10 mg acid phosphatase (Worthington Corp.) at 37°C. After 5 min. and 17 hr 2-amino-1-naphthol was determined by method of Clayson(3) and phosphate by method of Lowry and Lopez(6).

Time	2-amino-1-naphthol (μm) Found	2-amino-1-naphthol (μm) Expected*	Phosphate (μm) Found	Phosphate (μm) Expected*	Amine (NQS) phosphate ratio Found	Amine (NQS) phosphate ratio Expected*
5 min.	.069	.056	.005	0		
17 hr	.120	.112	.055	.056	2	2

* Expected values are calculated for the following reactions.

5 min. $R_2PO_4^- + H_2O \quad ROH + RHPO_4^-$
17 hr $R_2PO_4^- + 2\,H_2O \quad 2\,ROH + H_2PO_4^-$



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ing with a bis(2-amino-1-naphthyl) phosphate. In the first stage, one mole of 2-amino-1-naphthol is released plus mono-aryl phosphate and in the second, the mono-aryl phosphate is hydrolyzed.

Phosphate conjugates have not been described previously as end products of metabolism of foreign compounds. Three features make this compound a promising candidate for the active bladder carcinogen: 1. The compound is readily split by phosphatases occurring in the dog urine to form 2-amino-1-naphthol which has been shown to be an active carcinogen(7). 2. The compound has solubility properties characteristic of compounds capable of crossing the cell wall. 3. Preliminary observations in rabbits injected with 2-naphthylamine indicate the absence of similar phosphate conjugates. Boyland and Manson(8) report the absence of 2-amino-1-naphthyl phosphate in the rat. Both these species are relatively insensitive to bladder cancer when exposed to 2-naphthylamine, while the dog, which elaborates this phosphate conjugate, is sensitive to the disease.

Summary. Bis(2-amino-1-naphthyl) phosphate has been demonstrated as a urinary end product of 2-naphthylamine metabolism. Phosphate conjugates have not been observed previously as urinary end products. The compound may be related to the occurrence of bladder cancer in this species upon ingestion of 2-naphthylamine.

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Ocular Transplants of Joint as an Experimental Method.* (24545)

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An experimental polyarthritis can be produced in rats and mice using species-specific strains of *Micoplasma arthritidis* (pleuropneumonia-like organism) in broth culture administered either intravenously or intraperitoneally. This experimental infection in rodents has served as a method for evaluating the effect of various environmental conditions and chemotherapeutic agents(1). It has been possible to segregate drugs into 3 groups: 1) those which prevent or cure the arthritis; 2) those which worsen its course; and 3) those without apparent effect. Because of the tendency for joint involvement to characterize this experimental infection, we wondered whether joint involvement would also appear in transplanted joint tissues. To approach this problem we adapted a technic previously employed for transplanting tumor(2) and ovarian tissues(3) to the anterior chamber of the rat eye. The first experiment was an effort to explore the possibility of intra-species (homologous) transplantation of rat joint tissues to the anterior chamber and to determine the possibility of "arthritic" changes in the transplanted tissue following intraperitoneal infection with broth cultures of *Micoplasma arthritidis* (pleuropneumonia-like organism). If "arthritic" changes could be produced in the

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