Effects on prenatal development of rats treated with pyridoxine in daily doses of 20-80 mg/kg/day on days 6–15 of gestation

Dose (mg/kg/day)	0	20	40	60	80
No. of rats pregnant at term	18	16	17	19	19
Mean No. of live fetuses/pregnancy	11.3	12.3	12.1	12.7	12.8
% Dead fetuses; $\frac{\text{resorbed + Dead}}{\text{total implants}} \times 100$	7.2	3.0	4.2	4.2	3.9
Mean fetal weight ± SD (g)	4.8 ± 0.4	4.8 ± 0.3	4.8 ± 0.3	4.9 ± 0.3	4.8 ± 0.4
No. anomalous/total examine	10/201	12/193	10/206	6/230	9/243
Anomalies					
Wavy ribs	8	6	2	2	3
Lumbar ribs	1	3	5	1	1
Sternal defects	1	2		1	2
Other defects (runt, hemorrhagic pericardium, edema)		1	3	2	3

Empty cells denote 0 incidence.

weight (Table) in pyridoxine treated groups were within control limits. The incidence of anomalous fetuses at the doses investigated was comparable to the control incidence (Table). Types of anomalies were generally those that occur spontaneously in this strain of rats and consisted of wavy ribs, lumbar ribs, sternal defects, runts and less commonly pericardial hemorrhage and subcutaneous edema.

Discussion and conclusion. Deficiency of vitamin A, C, D, E, folic acid, riboflavin, nicotinamide and pyridoxine resulting in fetopathy or teratogenicity has been well established. However, fetal effects of megavitaminosis during pregnancy has not received sufficient attention. Hypervitaminosis A during pregnancy was found to be teratogenic in rats[4]. Thiamine, riboflavin or pyridoxine fed in high dietary concentrations to rats before, during, and after pregnancy showed no effect on litter size, growth until weaning, and vitamin requirements of offspring[5]. Our study demonstrates lack of teratogenicity of high pyridoxine dosing during organogenesis of rats. Pre- and postnatal studies on other vitamins are needed since their high intake as dietary supplement or for therapeutic purposes is gaining popularity.

Résumé. Pyridoxine (B_6) a été administrée par gavage à des doses de 0, 20, 40, 60 et 80 mg/kg à des rates entre le 6ième et le 15ième jour de gestation. Le traitement n'a eu aucun effet apparent sur les rates pendant gestation qui ont été sacrifiées à terme afin de déterminer les effets périnataux. Les valeurs obtenues pour les fétus vivants, les fétus morts, les sites de résorption, le poids et les anomalies fétaux chez les animaux traités ne sont pas significativement différentes des valeurs obtenues chez les animaux contrôlés.

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Destruction of Triplet Nitrenium Ion by Ascorbic Acid

Ascorbic acid has already been shown by MIRVISH[1] to inhibit the formation of carcinogenic nitrosamines from secondary amines and nitrous acid, via reaction with nitrous acid. We have found that the attack of the carcinogen N-acetoxy-2-acetamidofluorene (N-acetoxy-AAF) on guanosine is also inhibited by ascorbic acid. In experiments designed to test the hypothesis that N-hydroxy-4-aminostilbene forms a nitrenium ion with reactive sites different from those of the nitrenium ion formed from N-acetoxy-4-acetamidostilbene (N-acetoxy-AAS), ascorbic acid was chosen as a proton source with which to generate nitrenium ions from the hydroxylamine[2]. The reactions of the N-acetoxy-AAF and N-acetoxy-AAS in the same medium were run for comparison, since LOTLIKAR[3] had shown that N-acetoxy-AAF reacts with methionine over a wide pH range. It appeared in this study that the level of product formed between N-acetoxy-AAF and guanosine was far below the expected level, and this particular observation was investigated further.

Materials and methods. 9 μmoles of N-acetoxy-N-arylacetamide in 0.1 ml 95% ethanol were incubated overnight at 37 °C with 0.9 μmoles of guanosine-2-^{14}C in 0.4 ml buffer (0.1 *N* citric acid or 0.028 *M* ascorbic acid). Samples from the reaction mixtures were spotted on cellulose TLC strips which were developed in *n*-butanol – acetic acid – water (50:11:25). Yields of adduct were determined by scraping the strips into vials for counting in a Beckman LS-100 scintillation counter. Yields of amide were determined by UV-quantitation of spots obtained from TLC of samples from these reactions, performed on silica gel and developed in benzene – ethyl acetate (3:1).

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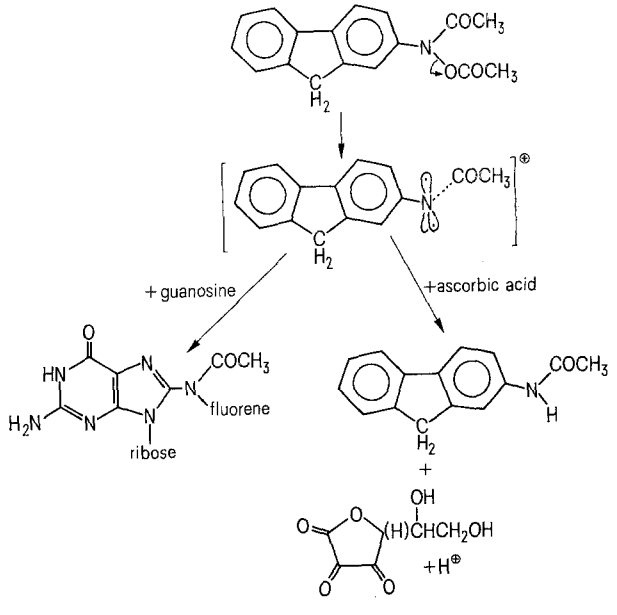
As shown in the Table, the reaction of N-acetoxy-AAF with guanosine in citric acid results in a high yield of guanosine-AAF[4,5], while the same reaction in ascorbic acid results in an 80% inhibition of guanosine-AAF formation. In contrast, the yield of product from N-acetoxy-4-acetamidostilbene and guanosine was relatively unaffected by the medium, and is comparable to that found earlier[5]. As noted earlier by LOTLIKAR[6], the reactions of esters of N-hydroxy-AAF result in small amounts of AAF, explained later as being due to hydrogen abstraction from solvent by the triplet form of the N-2-fluorenyl-N-acetylnitrenium ion (AAF^+)[7]. We observed this in the citric acid medium, but found AAF formation to be increased 5-fold in the ascorbic acid medium. When guanosine-AAF was incubated separately with ascorbic acid, no destruction of this material was seen.

Reactions of N-acetoxy-N-arylacetamides with guanosine, in the presence and absence of ascorbic acid

Compound	Medium	Yield of guanosine adduct (%)	Amide formed (%)
N-acetoxy-AAF	citric	83	6
N-acetoxy-AAF	ascorbic	17	30
N-acetoxy-AAS	citric	12	<1
N-acetoxy-AAS	ascorbic	12	<1



Reduction of N-2-fluorenyl-N-acetyl nitrenium ion by ascorbic acid.

Thus, we have noted that the reaction of N-acetoxy-AAF with guanosine is greatly inhibited by ascorbic acid, but that the reaction of N-acetoxy-AAS is not. From this, we conclude that the ascorbic acid does not act directly on unreacted N-acetoxy-N-arylacetamide. We have already shown, however, that N-acetoxy-AAF forms a triplet species, while N-acetoxy-AAS does not[7]. Thus, it appears that ascorbic acid is oxidized by triplet AAF^+ through abstraction of H from the ascorbic acid (see Figure). Such an oxidation is common for ascorbic acid, and is probably the reaction type by which ascorbic acid removes nitrous acid from a nitrosation mixture.

If this inhibition of adduct between aromatic amine and guanosine is specific for reactions involving triplet nitrenium ions, as it appears to be, the possibility of using ascorbic acid as a prophylactic against aromatic amine-induced tumors would appear to be restricted to those cases in which the nitrenium ion will fall into a triplet state. At present, it is not known how many aromatic amines would lead to such ions, nor is it known whether ascorbic acid inhibits even AAF carcinogenesis. This reaction, nonetheless, seems to be a point of control in carcinogenesis worth additional study.

Zusammenfassung. Die Reaktion des Karzinogens N-acetoxy-2-acetamidofluoren mit Guanosin wird durch Ascorbinsäure, jedoch nicht durch Zitronensäure gehemmt. Diese Hemmung bewirkt eine vermehrte Bildung von 2-Acetamidofluoren. Anscheinend reagiert Ascorbinsäure mit dem N-2-Fluorenyl-N-acetylnitrenium-triplett unter Bildung von Acetamidofluoren und oxidierter Ascorbinsäure.

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Fever Produced in Rabbits by $N^6,O^{2'}$-Dibutyryl Adenosine 3',5'-Cyclic Monophosphate

Previous experiments have shown that the fever reaction in rabbits following the i.v. injection of various exogenous or endogenous pyrogens[1,2] is associated with increased concentrations of prostaglandins of the E series (PGE) in the cerebrospinal fluid (CSF). In addition, injections of PGE into the lateral or third cerebral ventricles of different mammals were found to produce fever[3]. The hypothesis of PGE as mediators in fever genesis is supported also by the finding that inhibition of prostaglandin synthetase is the mechanism underlying the action of antipyretic, aspirin-like drugs[4]. The pharmacological effects of prostaglandins seem to be mediated in several endocrine organs via adenosine 3',5'-cyclic monophosphate (cyclic AMP) according to the 'second messen-

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