TLC behaviour of various rimino-phenazines[a]

Compound		Rf
Isolate		0.39
B663:	3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)-phenazine	0.39
B1865:	3-anilino-7-chloro-2,10-dihydro-2-(isopropylimino-10-phenyl-phenazine	0.27
B1911:	3-anilino-7-fluoro-2,10-dihydro-2-(isopropylimino)-10-phenyl-phenazine	0.18
B1811:	3-anilino-7-ethoxy-2,10-dihydro-2-(isopropylimino)-10-phenyl-phenazine	0.02
B1739:	3-anilino-7-methoxy-2,10-dihydro-2-(isopropylimino)-10-phenyl-phenazine	0.02
B1912:	3-anilino-7-chloro-2-(cyclohexylimino)2,10 dihydro-10-phenyl-phenazine	0.55

[a] Silica gel type H plates containing 0.1 NKOH, two elutions in 10% EtOAc/Benzene.

While pigmentation of the skin of leprosy patients taking B663 has been well documented[15,16], the occurrence of crystals in human tissues has received much less attention[17,18]. It has occasionally been suggested that the red crystals seen under light microscopy, and the 'spaces' seen on electron microscopy might represent a metabolite, or an impurity. Our results in the mouse show that crystals obtained from macrophage cytoplasm are in every way identical with those in the drug used for oral feeding of the animals; it is concluded that crystals seen in the tissues represent those of the unaltered drug.

Zusammenfassung. Die Rimino-Phenazin-Verbindung (Präparat B663 – Lampren® Clofazimine) mit starker Wirkung gegen *Mycobacterium leprae* wurde $4^1/_2$ Monate an Mäuse verfüttert. Lichtmikroskopisch, dünnschichtchromatographisch und massenspektrometrisch wurde festgestellt, dass die B663-Kristalle im Cytoplasma der Makrophagen mit den in der Grundsubstanz vorhandenen identisch sind.

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27 September 1974.

[15] S. G. Browne, Lepr. Rev. *36*, 9 (1965).
[16] L. O. Levy and H. P. Randall, Int. J. Lepr. *38*, 404 (1970).
[17] A. C. Atkinson, Int. J. Lepr. *35*, 119 (1967).
[18] E. Mansfield, 10th Int. Lepra Congress, Bergen (1973), vol. 2, p. 19.
[19] Acknowledgments. We are indebted to Dr. R. J. W. Rees of the National Institute for Medical Research, Mill Hill, London for kindly supplying mice and the original B663 used in this experiment, and to Dr. M. L. Conalty for specimens of various riminophenazines from his laboratory in Trinity College, Dublin. This work is supported by grants to A. C. McDougall from the Medical Research Council and the British Leprosy Relief Association (LEPRA).

Teratogenicity Study in Rats given High Doses of Pyridoxine (Vitamin B_6) during Organogenesis

The pattern of pyridoxine or vitamin B_6 usage in women has undergone a marked change in recent years. Therapeutic doses of 40–400 mg/person/day are prescribed to counteract depressive conditions arising from schizophrenia[1] and oral contraceptives[2]. A routine administration of high doses of pyridoxine to all pregnant women with a history of depressive disorders has been suggested[3]. Since this megavitamin treatment finds an increasing application in orthomolecular psychiatry, teratogenicity studies are needed to evaluate the safety of high levels of pyridoxine exposure to pregnant women.

This report presents fetal data on rats dosed orally with 20–80 mg pyridoxine/kg on days 6–15 of gestation.

Materials and methods. Randomly bred female (Wistar) rats, 175–200 g body weight, were paired overnight with males, and the morning that a sperm-positive vaginal smear was observed, was noted to be day 1 of pregnancy. The mated females were randomly assigned to experimental groups. Pyridoxine hydrochloride obtained from Nutritional Biochemical Corporation, Ohio, was administered orally in single daily doses on days 6–15 of gestation. The doses given were 0 (distilled water), 20, 40, 60 or 80 mg/kg/day. Solutions for the 4 dose levels were prepared as 0.4, 0.8, 1.2 and 1.6%, respectively, of pyridoxine hydrochloride in distilled water. Pregnant rats were weighed daily in order to permit the volume of solution given to be adjusted to constant dose/body weight ratio for the treatment period.

All female rats were killed on day 22 of pregnancy and their viscera including uteri were examined. The fetuses were removed, weighed and examined for viability and external malformations. Early resorptions and fetuses dying late in development were recorded. One-half of the live fetuses from each litter were studied for skeletal anomalies; the remainder were fixed in Bouin's fluid and were inspected for gross visceral defects.

Normal distribution formed the basis for intergroup comparisons and probabilities were computed for values χ^2. A 5% probability level was chosen to evaluate differences.

Results. There was no evidence of maternal toxicity associated with pyridoxine treatment. Prenatal values for live fetuses, dead fetuses and resorption sites, and fetal

[1] D. Hawkins and L. Pauling, *Orthomolecular Psychiatry, Treatment of Schizophrenia* (Freeman and Co., San Francisco 1973).
[2] P. W. Adams, D. P. Rose, J. Folkard, V. Wynn, M. Seed and R. Strong, Lancet *1*, 897 (1973).
[3] F. Winston, Lancet *2*, 377 (1969).

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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Bureau of Chemical Safety, Health Protection Branch, National Health and Welfare, Tunney's Pasture, Ottawa (Ontario K1A OL2, Canada), 8 November 1974.

[4] S. Q. COHLAN, Pediatry *133*, 556 (1954).
[5] M. F. SCHUMACHER, M. A. WILLIAMS and R. L. LYMAN, J. Nutr. *86*, 343 (1965).

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).

[1] S. S. MIRVISH, L. WALLCAVE, M. EAGEN and P. SHUBIK, Science *177*, 65 (1972). S. S. MIRVISH, A. CARDESA, L. WALLCAVE and P. SHUBIK, Proc. Am. Ass. Cancer Res. *14*, 405 (1973).
[2] E. KRIEK, Biochem. biophys. Res. Commun. *20*, 793 (1965).
[3] P. D. LOTLIKAR, J. D. SCRIBNER, J. A. MILLER and E. C. MILLER, Life Sci. *5*, 1263 (1966).