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PRELIMINARY COMMUNICATIONS

EFFECT OF VITAMIN A DEFICIENCY AND EXCESS ON AFLATOXIN METABOLISM IN THE RABBIT

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Interaction of vitamin A and aflatoxin was evident even at the early stages of the history of aflatoxin (1 - 3). The vitamin is known to have a protective influence against incidence of aflatoxin - induced tumour and toxicity (4 - 8). Furthermore, vitamin A has been reported to have some influence on the metabolism of a number of drugs (9 - 17) and, recently, on the metabolism of aflatoxin (18) although the structural identity of the metabolite affected was not established. Among the drugs whose metabolism were reported to be influenced by vitamin A deficiency are those metabolised by demethylation (9- 11, 18). Vitamin A influence on aflatoxin demethylation has not been investigated. For this reason and also because the influence of vitamin A on drug metabolism has been investigated in animals other than the rabbit, we decided, as a first step , to investigate the effects of vitamin A depletion and supplementation on aflatoxin metabolism in the rabbit.

Materials and Methods: Weanling male rabbits, weighing between 650 and 800g, obtained from our Pre-Clinical Animal House, were used. NADP (Sigma Grade) was purchased from Sigma Chemical Co., St. Louis. while glucose-6-phosphate (disodium monohydrate, 'A' grade) was bought from Calbiochem, San Diego, Calif.,. Crystalline aflatoxins B_1 and G_1 were obtained from Makor Chemicals, Jerusalem. The aflatoxin M_1 standard was kindly donated by Dr. R.D. Stubblefield. The silica gel used was obtained from Hopkins and Williams Ltd., England. Vitamin-free casein was purchased from ICN Pharmaceuticals, Ohio, U.S.A.

The diet used was adapted from the Cholesterol Free Rabbit Diet of Kutchevsky et al (19) by substituting vitamin - free casein, corn oil and vitamin A - free vitamin mixture (11), respectively, for casein, hydrogenated coconut oil and complete vitamin mixture (11). Male litter mate rabbits were distributed evenly into three groups of four

each. They were given ad libitum for 28 days the regimen described above (deficient group) or with either 5,000 I.U. (control group) or 60,000 I.U. retinylpalmitate/kg diet (vitamin A excess group), added. Three feeding tests each lasting 28 days were done.

At the end of the feeding the animals were killed and the vitamin A content of samples of the livers of individual animals determined as described by Bayfield (20). For enzyme assays, liver tissues for each dietary group were pooled. Portions intended for the assays of demethylase and hydroxylase activities were homogenized and fractionated as described by Stitzel et al (21) and Bassir and Emerole (22), respectively. The 9,000g supernatant was used for the enzyme assays. Protein content of this supernatant was determined by the biuret method.

The 5 ml - incubation medium contained 0.2mM NADP, 5mM glucose-6-phosphate, 2.5mM $MgCl_2$, 5mM nicotinamide, 5mM semicabazide-HCl, 50nmoles of substrate and 1 ml of 9.000g supernatant equivalent to 250 - 500mg fresh liver weight in 0.1M sodium phosphate buffer, pH7.4. Two blanks in which the toxins and 9,000g supernatant, respectively, were omitted, were used in the demethylase assay to check aflatoxin interference to formaldehyde determination(23). All incubations were done in open 25 ml Erlenmeyer flasks at 37°C for 1 hour in a Gallenkamp shaker bath.

The demethylation reaction was terminated as described by Stitzel et al (21); the precipitated protein was removed by centrifuging for 10 min. at 10,000 r.p.m. and the formaldehyde content of the supernatant then estimated as described by Schenkman et al (24) in a Perkin - Elmer Double Beam spectrophotometer. Aflatoxin B_1 hydroxylation was terminated with 2 ml ice-cold saturated NaCl (18) followed by 5 ml cold chloroform. The mixture was then extracted four times with a total of 35 ml chloroform, the operation being spread over 96 hr. or until the precipitated protein showed loss of integrity. Extracts from individual flasks were concentrated separately in a rotary film evaporator prior to spotting on thin layer chromatography plates. The plates were developed with ethyl acetate: chloroform, 2:1, since with the other solvent systems, aflatoxicol (AFRo) migrates with aflatoxin B_1 (25). AFRo (R_F: 0.65) was identified by its relative distance from the aflatoxin B_1 band (R_F 0.46)(25). Authentic standards were used for aflatoxins B_1 (AFB_1) and M_1 (AFM_1). The metabolites were eluted separately from their respective scrappings by washing four times on a sintered glass funnel with a minimum amount of methanol under reduced suction pressure. The quantities of AFB_1, AFM_1 and AFRo so

eluted were calculated from their optical densities at 363, 357 and 325nm, respectively, using the method of Rodericks and Stoloff (26)

Statistical analyses were done by student 't' test.

Results and Discussion

The results obtained (Table 1 & 2) show evidence of alteration of aflatoxin metabolism after 28 days on the test diets. During this period, liver vitamin A content , (Table 3) of the depleted rabbits decreased to 45% while that of supplemented rabbits increased to 340% of the control value. However, only AFB_1 demethylase activity was significantly reduced at the 0.01 confidence level in the marginally deficient rabbits. Nevertheless, two trends are noticeable from the results. First, in both hydroxylation and demethylation of aflatoxin, the value for the vitamin A - supplemented rabbits tended to be less than the control, an observation probably in line with the view that large doses of vitamin A *in vitro* and *in vivo* destabilize membranes (27). Secondly, the aflatoxin B_1 hydroxylase activity tended to be higher in the deficient animals compared to the control, a trend similar to the observed effect of vitamin A deficiency on benzopyrene metabolism (16, 17). The fact that vitamin A deficiency is known to enhance tumour formation by aflatoxin and benzopyrene (4, 28) brings to mind the postulation that since aflatoxin and carcinogenic polycyclic aromatic hydrocarbons have similar electronic structures, they should have similar biological activities (29). More work is being done to clarify the above observations. Meanwhile, it appears that the effect of vitamin A on AFB_1 demethylation and hydroxylation respectively, is exerted through different mechanisms and that gross vitamin A deficiency is not necessary to achieve a reduction in AFB_1 demethylation in the rabbit.

Table 1: Demethylation *in vitro* of aflatoxin B_1 & G_1 during Vitamin A depeletion and supplementation

Diet	n mole H.CHO/mg protein/hr ± S.E.M.*	
	AFB_1	AFG_1
Deficient	0.0092 ± 0.0015**	0.0146 ± 0.0028 ns
Control	0.0133 ± 0.0017	0.0169 ± 0.0017
Excess	0.0130 ± 0.0017 ns	0.0161 ± 0.0012 ns

* Represent the mean values of three experiments** signficantly lower than control values ns denotes non-significance.

Table 2: Hydroxylation *in vitro* of aflatoxin B_1 during vitamin A depletion and supplementation

Diet	mole Toxin metabolized or produced/mg protein/h ± S.E.M.*		
	AFB_1(metabolized)	AFM_1	AFRo
Deficient	0.122 ± 0.024 ns	0.031 ± 0.006 ns	0.033 ± 0.010 ns
Control	0.104 ± 0.013	0.029 ± 0.007	0.031 ± 0.011
Excess	0.081 ± 0.014 ns	0.028 ± 0.006 ns	0.026 ± 0.009 ns

* Represent the mean values of three experiments. ns denotes non-significance.

Table 3: Effect of vitamin A depletion & supplementation on liver Vitamin A content

Diet	Liver Vitamin A* (I.U. Retinol/g fresh liver)	Liver weight* (g/kg body weight)
Deficient	26.91 ± 9.07	34.71 ± 3.54
Control	59.17 ± 13.33	32.06 ± 1.32
Excess	200.17 ± 3.06	35.52 ± 0.60

* The mean of values from three experiments.

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