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Alteration of some toxic aflatoxin responses in Syrian hamsters fed zinc carbonate-supplemented diets

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SUMMARY

Chronic exposure to aflatoxins (AFTs) below the LD_{50} can result in reduced weight gain, hepatocellular necrosis and bile duct cell proliferation. Here, we report whether dietary zinc (Zn^{2+}) protects against both aflatoxicosis and precancer in male weanling hamsters fed either 14.6 mg/kg AFTs, 3000 mg/kg zinc carbonate, or both for 17 weeks. The AFTs (either alone or with Zn^{2+}) reduced weight gains but not feed consumption. Whereas controls possessed 172.7 ± 21.7 mg/100 ml plasma glucose, the AFTs and Zn^{2+} groups had 132.1 ± 19.5 and 122.7 mg/100 ml, respectively. For plasma cholesterol, the AFTs plus Zn^{2+} group's was 26.5 ± 4.3 compared to 32.3 ± 3.0, 31.5 ± 4.8 and 36.0 ± 2.1 mg/100 ml for control, Zn^{2+} and AFTs groups, respectively. The latter exhibited bile duct cell hyperplasia, focal liver necrosis and hemorrhage but the AFTs plus Zn^{2+} group's livers had less damage. Meglahepatocytes indicated precancerous changes. These data suggest a trend toward Zn^{2+}-induced reduction for AFTs-promoted liver damage.

INTRODUCTION

Chronic exposure to aflatoxin (AFT) levels below the LD_{50} has been shown to reduce weight gain and cause hepatocellular necrosis together with bile ductule cell proliferation [4,14,32]. Various dietary supplements have been investigated for protective, additive, or synergistic actions. Amongst these, the trace elements have received some attention. For example, Newberne and Conner [25] reported a slightly protective action for rats with increased levels of dietary selenium oxide. There was a diminution in both the extent of necrosis and the development of bile ductule cell hyperplasia. Reductions in aflatoxin-induced necrosis and cellular degeneration for gerbils fed increased sodium selenite were reported by Lalor and Llewellyn [15] also.

The possible protective action of supplemental dietary copper (250 mg/kg) to toxin-induced liver damage was investigated by Barber et al. [2] who did not observe a reduction in hepatocyte damage in swine. There was an improvement in aflatoxin-promoted loss of weight gain which was associated with increased feed efficiency. Llewellyn et al. [18]

reported a suppression in hepatic damage for hamsters which had been fed a diet supplemented with 5000 mg/kg copper acetate together with aflatoxin B_1 (AFB_1). This finding had been reported earlier for hamsters fed a diet containing 7500 mg/kg copper acetate [30].

Neathery et al. [24] fed young dairy calves diets containing either 40 or 640 mg/kg Zn^{2+} together with either 0 or 5 mg/kg AFTs for 3 weeks. The AFT calves exhibited reduced feed intake and weight gain. Upon autopsy, gross abnormalities in either the liver or other organs were not observed. The AFTs-induced toxicity was not suppressed by the addition of 600 ppm Zn^{2+} to diets. In contrast, Llewellyn et al. [17], using 5000 ppm zinc carbonate as a dietary supplement demonstrated a decrease in both liver congestion and hemorrhage in gerbils which had been fed aflatoxin. There was no change in either the number of pyknotic nuclei or in the degree of focal necrosis compared to those animals which had been fed aflatoxin alone. Dietary zinc can protect against both damage and subsequent tumor induction caused by DMBA (9,10-dimethyl-1,2-benzanthracene) [7,27]. The Zn^{2+}-promoted depressions in AFT-induced hepatic damage in Mongolian gerbils reported by Llewellyn et al. [17] indicate that zinc may be a protectant of AFB_1. The literature concerning the role of trace elements as a possible protective agent in AFB_1 — induced damage has been reviewed by Llewellyn and coworkers [19,21].

Here, we report whether dietary Zn^{2+} at a concentration other than that reported by Llewellyn etal. [17] can protect against both aflatoxins and precancer in male weanling hamsters fed 14.6 mg/kg AFTs, 2000 mg/kg zinc carbonate or both for 17 weeks.

MATERIALS AND METHODS

Rearing and maintenance of hamsters

Weanling male golden hamsters, *Mesocricetus auratus,* were housed individually within polystyrene cages (22 × 50 × 10 cm) containing 'Betta chips' bedding and fitted with plastic tops. The room containing the hamsters was maintained at 70 ± 4°F with a constant photoperiod of 12 h light and 12 h dark provided by fluorescent light.

Preparation of mixed aflatoxins

Mixed aflatoxins (AFTs) were produced upon a coconut substrate (Bakers Angel Flake) by growth of *Aspergillus parasiticus* NRRL 2999. Shredded coconut (20 g) was placed into 50 400-ml flasks fitted with cotton. Following addition of 10 ml distilled H_2O, the flasks were autoclaved for 20 min at 15 lb/in^2 and 121°C. Autoclaved coconut was cooled to room temperature and then innoculated with *A. parasiticus* spores from an active culture maintained upon rice. Flasks were incubated at 22 ± 5°C in a dark chamber. 8 days after initiation, sporulation was noted whereupon the cultures were extracted with 50 ml chloroform followed by 30 min mixing on a rotary shaker. Mixed coconut was placed into a vegetable press and squeezed into a Buchner funnel for filtration (Whatman No. 4 filter paper) under suction. A second filtering was performed using Whatman No. 2 filter paper. Aflatoxin contaminated chloroform was stored in sealed amber containers and aliquots were subjected to thin-layer chromatography and a visual dilution technique sensitive to 2 ppb [1]. Separate extracts were from several groups of cultures and the solution contained total AFTs ranging from 416 to 605 μg/ml.

Aflatoxin administration

Juvenile male Syrian hamsters were fed experimental diets for 17 weeks. The control diet was Purina Laboratory Chow (57 ppm Zn^{2+}) [1]. A high zinc (HZn) diet was prepared through addition of zinc carbonate to powdered chow which resulted in a zinc concentration of 3000 mg/kg. An aflatoxin diet was produced by spraying mixed AFTs in chloroform onto the powdered chow followed by extensive shaking within sealed bags to insure uniform AFTs distribution. This AFTs combination contains a high proportion of AFG_1 which ranks second to AFB_1 in toxicity. No toxins were present in the control diets. The AFTs were extracted from the contaminated diets with chloroform and water

and then concentrations within the chow were verified (AFTs AFB_1 = 0.1 mg/kg, AFG_1 = 14,0 mg/kg, and AFG_2 = 0.5 mg/kg) by thin-layer chromatography and a visual dilution technique sensitive to 2 mg/kg. A double-insult diet (HZn plus AFTs) containing both dietary supplements was constructed also. The total number of animals used was 24 and thus the results are preliminary in nature. Distilled water was supplied ad libitum. Both weekly water and feed consumptions as well as changes in body weight were recorded.

Assays

Animals were killed under diethyl ether anesthesia and the thoracic cavity was opened. A blood sample was removed from the left ventricle with a syringe which had been pre-treated with heparin. Organs were excised, inspected for gross damage, cleaned and weighed. For histopathology, sections of the median lobe of the liver, left kidney,and the testes were placed into 10% neutral formalin together with a cleaned section of the large intestine (see below). To obtain plasma, blood samples were centrifuged (1000 × 20 min). A glucose oxidase analysis was used to determine plasma glucose concentration [6]. Cholesterol was quantified colormetrically using the Liebermann-Burchard reagent with changes in absorbance being determined at 540 nm. Levels of pancreatic zinc were determined by Central Biological Laboratories (Richmond, VA) using atomic absorption spectroscopy [1]. The pancreas was utilized because of its capacity to store zinc-containing insulin.

Histopathology

Sections were fixed for 1.0–4.5 h at 25 ± 2°C in 5% formaldehyde saturated with $CaCO_3$. The sections were dehydrated through a graded alcohol series and progressively embedded in xylene with final embedment in paraplast. Five 10-μm sections were affixed to pre-cleaned slides. The sections were stained with hematoxylin and eosin [10] prior to viewing.

Statistical analyses

Means and standard deviations were calculated for six animals from each treatment group. For use of Duncan's Multiple Range Test [3], the degrees of freedom and the mean sum of the squares were determined using linear regressions. The statistical analyses were performed with an IBM 370/140 computer. All statistical data analyses were based on $P \leq 0.05$. Treated and control groups with differing superscripts within the Tables are significantly different.

RESULTS

Weekly data

Significant decreases in body weight gain from the second to the seventeenth week occurred within both the AFTs and the HZn plus AFTs groups (Fig. 1). A HZn diet did not alter the response. Week 6 showed the greatest reduction in weight (control = 102.3 ± 13.0 g and AFTs = 63.8 ± 7.4 g). By the 17th week, the AFTs animals weighed 110.9 ± 14.9 and the controls 144.5 ± 21.1 g. The weights of both the AFTs and the HZn plus AFTs groups were not significantly different throughout the 17th week. Neither were the weights of both the control and HZn groups. There were no significant differences in feed consumption between the groups that could account for reduction in body weight, e.g., total feed consumption during the 17th week for the AFTs and HZn plus AFTs groups were both 40g/week.

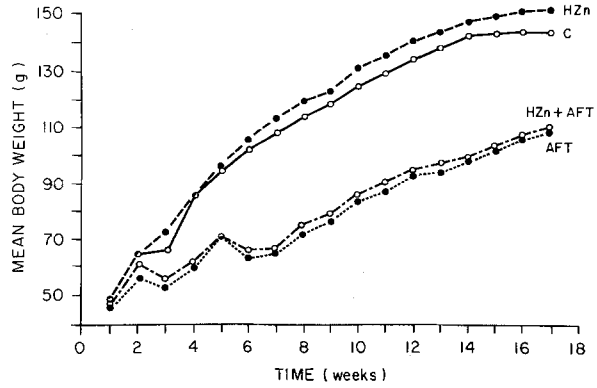

Fig. 1. Time-dependent changes in mean body weight for hamsters fed AFTs diets with and without supplemental Zn^{2+}.

Data on killing

Organ weights. An analysis of organ weights did not reveal changes in the relative weights of the liver, kidneys, lungs, heart or spleen. There were reductions in the weights of the testes in both the AFTs and HZn groups compared to the weights of the control group. Both groups displayed a diminution of approximately 50%, while the HZn plus AFTs group showed a 25% reduction (Table 1). Whereas the former were statistically different from the control, the latter was not.

Blood analyses. Plasma glucose concentrations were reduced 25 and 30% from that of the control in the AFTs and HZn plus AFTs groups, respectively, (Table 1). Whereas the reductions were statistically significant, there was no significant difference between the two groups receiving the AFTs. Plasma cholesterol levels were significantly decreased in the HZn plus AFTs group (Table 1). The control (32.3 ± 3.0) and the AFTs (36.0 ± 2.1) groups were not significantly different from each other but the reduction in the HZn plus AFTs group was.

Zinc determinations. Pancreatic zinc concentrations in the HZn plus AFTs group were significantly elevated compared to the control group (Table 1). Both the HZn and AFTs groups displayed a slight increase in zinc retention that was not significantly different from the control group.

Histopathology. The kidneys, testes and the large intestine did not exhibit signs of either lesions or toxic reactions. Neither did the liver of the hamsters fed a control diet (Fig. 2A). However, lesions and neoplastic changes developed in the AFTs group (Fig. 2B) and to a lesser extent in the HZn plus AFTs (Fig. 2C) group. There were extensive bile ductule cell proliferations and focal areas of necrosis in four of the AFTs animals. The two other hamster livers in this group possessed only slight proliferation and were without necrosis. Slight cellular infiltration (either neutrophils or polymorphs) and evidence of hemosiderin occurrence were observed in those livers which were damaged maximally. The HZn plus AFTs group contained only two livers with damage as severe as that seen within the AFTs group. Four developed a precancerous liver lacking hyperplasia but contained occasional cells which possessed enlarged nuclei with cytoplasmic inclusions (Fig. 2C). There was no histopathological evidence of liver damage within the HZn group (Fig. 2D). These results are summarized as a numerical ranking of hepatic cellular damage (Table 2).

Table 1

Mean testes weight, plasma glucose, plasma cholesterol and pancreatic zinc concentration of hamsters fed 14.6 ppm mixed aflatoxins, 3000 ppm zinc carbonate, or both for 17 weeks

Group	Testes weight (g)[d]	Plasma glucose (mg/100 ml)	Plasma cholesterol (mg/100 ml)	Pancreatic zinc (mg/kg)
Control	1.26 ± 0.32[a]	172.67 ± 21.72[a]	32.25 ± 3.02[a]	25.25 ± 6.89[b]
HZn	0.60 ± 0.53[c]	175.88 ± 22.56[a]	31.45 ± 4.77[a,b]	39.02 ± 8.86[a,b]
HZn + AFTs	0.93 ± 22.00[a,b]	122.67 ± 22.64[b]	26.50 ± 4.31[b]	50.01 ± 15.23[a]
AFTs	0.63 ± 0.43[a,c]	132.08 ± 19.47[b]	35.95 ± 2.07[a]	36.59 ± 19.87[a,b]

[a,b,c] For each parameter, means with same superscript are not significantly different ($P \leq 0.05$).

[d] Influences due to body weight are factored out using linear regressions. Values are means and S.D.

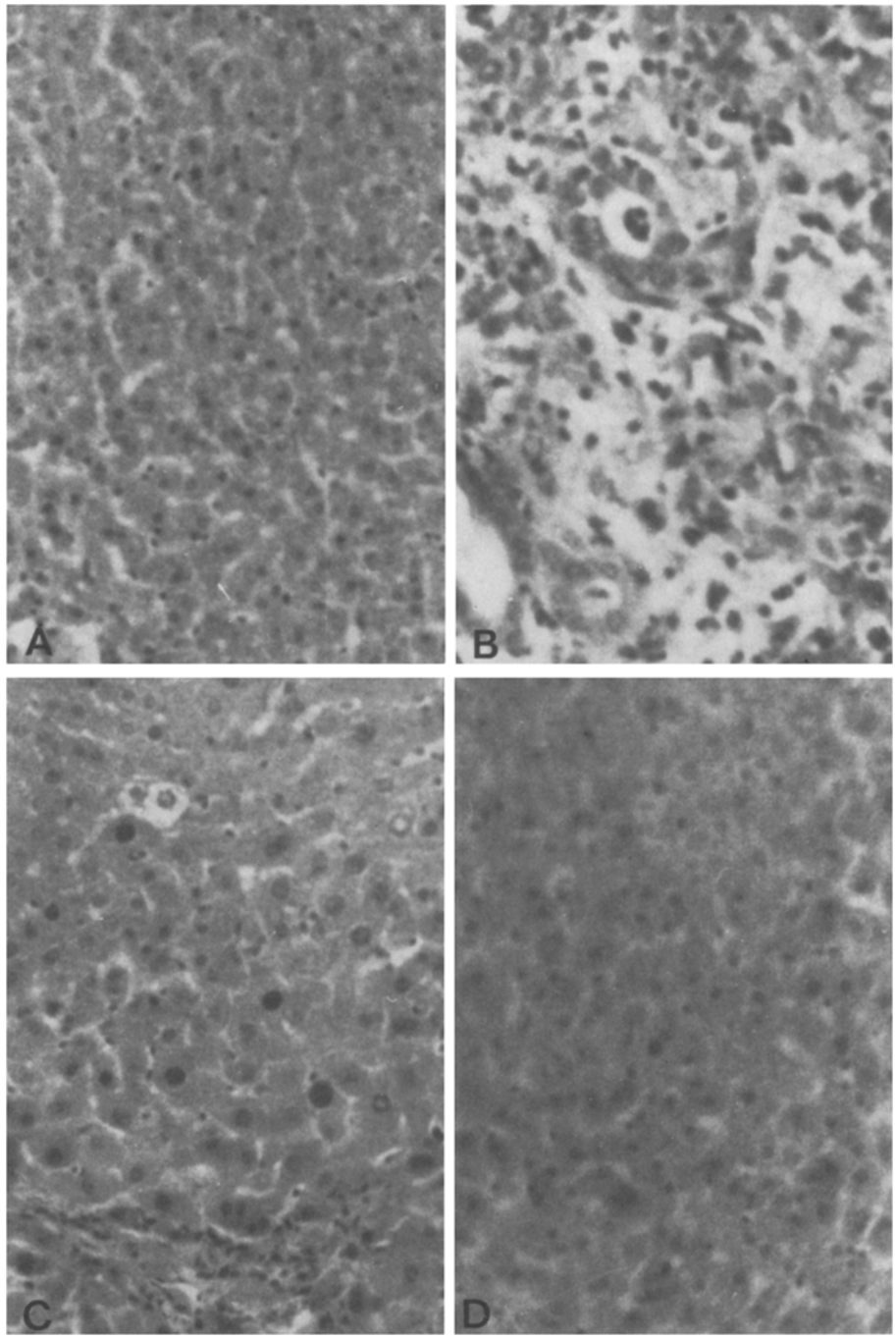

Fig. 2A–D. Photomicrographs of hematoxylin and eosin-stained sections of (A) a liver from an animal in the control group (note normal-sized hepatocytes and nuclei); (B) a liver from an animal in the AFTs group (note extensive bile ductule cell proliferation and focal necrosis); (C) a liver from an animal in the HZn plus AFTs group (evident are an increased number of meglahepatocytes, slight biliary duct cell proliferation and hemosiderin accumulation); (D) a liver from an animal in the HZn group (normal-sized hepatocytes and nuclei are apparent).

Table 2

Histopathological evaluation of liver damage in male hamsters receiving four types of diets for 17 weeks

A. Number of animals and their rating for each treatment group

No of animals	Control	HZn	HZn + AFT	AFT
0	5	6	—	—
1	1	—	—	—
2	—	—	4	1
3	—	—	—	1
4	—	—	2	4
X	0.17[b]	0[b]	2.67[a]	3.50[a]
S.D.	± 0.41	± 0	± 1.03	± 0.84

[a,b] Each treatment group with same superscript was not significantly different ($P \leq 0.05$).

B. Scale for numerical ranking of hepatic cellular damage in male hamster

Numerical value	Hepatic cells
0	Normal hepatocytes
1	Basically normal cells; signs of slight damage (not precancerous) and/or slight fatty infiltration; bacterial toxins suspect
2	Occasional enlarged nuclei (some with nuclear inclusions); mild reaction with no hyperplasia; slight fatty metamorphosis
3	Mild hyperplasia; enlarged nuclei more prevalent; inclusions of nucleus more pronounced
4	Mild to severe hyperplasia; slight cellular infiltration (neutrophils or polymorphs); focal areas of necrosis; some macrophages; evidence of hemosiderin occurrence

DISCUSSION

Body and organ weights

There were toxicity symptoms present within both the AFTs and HZn plus AFTs groups that are typical of toxic reactions produced by AFTs. For example, both groups displayed reductions in body weight gain without altering their feed consumption, a characteristic of AFB_1-treated rats [33]. Because only the testes exhibited changes in weight, the decline in body weight could not be attributed to alterations in organ weights. Both the AFTs and HZn groups were characterized by reduced testes weight (Table 1) but this was not of sufficient magnitude to account for all of the body weight loss. An AFTs-promoted diminution in testes' weights has also been observed for Syrian hamsters [18]. These reductions in testes weight have been the result of hormonal imbalances resulting from aflatoxin-induced stress [23]. Furthermore, the reduction in the HZn group is typical of animals which have received elevated dietary zinc [31]. There was no apparent change in the HZn plus AFTs group's testes weight. In contrast, Llewellyn et al. [18], using gerbils, reported decreased testes' weight in animals which had received both aflatoxin and elevated zinc.

Zinc retention

The zinc accumulation within the pancreas (Table 1) suggests that increased dietary zinc may cause a slight enhancement in pancreatic Zn^{2+} retention. Whereas a slight rise in pancreatic zinc was noted in both the HZn and AFTs groups, the HZn plus AFTs pancreatic zinc was significantly elevated. However, the increases in pancreatic zinc due to AFTs ingestion and that due to elevated dietary zinc may be unrelated. But, when both AFTs and zinc were administered simultaneously, there appeared to be an additive effect. Therefore, it is conceiveable that AFTs could promote increased uptake of either dietary zinc or mobilization of the element within the body; two alternatives which could be tested experimentally.

Plasma glucose levels

Plasma body weights (Fig. 1) appeared to coincide with AFTs-promoted reductions in the glucose plasma levels (Table 1). Both the AFTs and HZn plus AFTs groups experienced suppressions in both body weight gain and serum glucose levels. A reduced availability of glucose for cellular ATP synthesis via combined glycolysis and Krebs' cycle would lower the metabolic capabilities of the cells and possibly inhibit both mitosis and growth. In

addition, with depressed glucose levels, pancreatic zinc levels should be elevated, since the release of insulin is dependent upon blood glucose levels.

Serum cholesterol levels

Serum cholesterol levels were reduced in the double-insult group, HZn plus AFTs (Table 1) but there was a slight but not significant increase in the AFTs group. In this connection, aflatoxin has been reported to increase plasma cholesterol [22]. Cholesterol levels do not appear to be altered by either decreases or increases in dietary zinc concentrations [28,29]. It is conceiveable that alterations in zinc levels could cause copper to affect either cholesterol synthesis or transportation, since Petering et al. [26] reported that zinc interfered with copper metabolism.

Histopathology

Table 2 indicates that there was less liver damage in the double insult group, suggesting that the zinc supplementation contributed to this notable but not significant difference. Basically, 2/3 of the AFTs-treated animals possessed severe liver damage, including bile ductule cell proliferation, more enlarged nuclei and more nuclear inclusions, responses which are typical for hamsters receiving AFT [12,30].

With the addition of the zinc, these liver lesions were reversed and only 1/3 of the animals had the severe level of damage. These changes have been found for gerbils [11] fed zinc acetate (2000 mg/kg) but not in calves [24] fed zinc (640 mg/kg), suggesting organized differences in zinc utilization. As for the mechanisms by which Zn^{2+} could alter AFT-induced liver damage, it is known that substantial amounts of zinc can accumulate within the liver and that the accumulated zinc could either compete for sites on membranes with nucleic acids or could alter the metabolism of the AFTs via an action on the cytochrome *P*-450 series [13].

Limitations

The present paper represents a preliminary report since the *n* number was only six per group and since zinc levels within the liver were not quantified. An increase in *n* number would be useful in enhancing the numerical ranking of histopathology in order to more extensively assess a possible statistically-significant Zn^{2+}-promoted reversal of aflatoxin-induced liver pathology. Determination of both the plasma and liver zinc levels should be accomplished serially, since Doyle et al. [9] noted that AFB_1 can alter the distribution of trace elements in rat tissues. In addition, quantification of copper in both blood and tissues is required, since it is known that there is a close interaction in responses of laboratory animals between zinc and copper [13] and that copper may afford protection against aflatoxicosis [21]. Lastly, this investigation utilized AFTs high in AFG_1 and should be repeated with only AFB_1, also as a time-course study. Also, the possible protective action afforded by Zn^{2+} should be explored in organisms other than rodents. e.g., Avian species which are markedly sensitive to AFTs [5,8].

In conclusion, the influence of the zinc to reduce either the pathology or possibly provide for more rapid recovery is present, but both the mode and site of action are yet to be explained.

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REFERENCES

1 Association of Official Analytical Chemists. 1975. Aflatoxin: Official Methods of Analysis. Assoc. Offic. Anal. Chem. Thin-layer chromatography, Washington, D.C., Section 26031.

2 Barber, R.S., R. Braude, K.G. Mitchell, J.D.J. Harding, G. Lewis and R.M. Loosmore. 1968. Effects of feeding toxic groundnut meal to growing pigs and its interaction with high-copper diets. Br. J. Nutr. 22: 535–554.

3 Barr, A.J., J.T. Goodnight, J.P. Sall and J.R. Hewling. 1976. A Users Guide to SAS 76. SAS Institute, Inc., Raleigh, NC.

4 Butler, W.H. 1970. Liver injury induced by aflatoxins. Progress in liver disease 3: 408–418.

5 Cavalheiro, A.C.L. 1981. Aflatoxins and aflatoxicosis-a review. World's Poult. Sci. J. 37: 34–38.

6 Chick, W. L., R.L. Lavine and A.A. Like. 1970. Studies in the diabetic mutant mouse: I. Glucose tolerance in mice homozygous and heterozygous for diabetes (db) gene. Diabetologia 6: 257–262.

7 Ciapparelli, L. 1973. Effect of zinc on DMmB-induced salivary gland tumor in the albino rat. A preliminary study. S. Afr. J. Med. Sci. 37: 85-90.

8 Dashek, W.V., S.M. Barker, W.R. Statkiewicz and E.T. Shanks. 1983. Histochemical analysis of liver cells from short term aflatoxin-dosed and non-dosed *Coturnix coturnix japonica*. Aflatoxin-sensitive quail. Poult. Sci. 62: 2347–2359.

9 Doyle, J.J., W.C. Stearman II, J.O. Novman and H.D.C. Petersen. 1977. Effects of aflatoxin B_1 on distribution of Fe, Cu, Zn and Mn in rat tissus. Bull. Environ. Contam. Toxicol. 7: 33–39.

10 Galigher, A.E. and E.N. Kozloff. 1964. Essentials of Practical Microtechnique. Lea and Febiger, Philadelphia, PA.

11 Hastings, W.S. and G.C. Llewellyn. 1973. The effect of aflatoxin B_1 on growth rate and iron metabolism in juvenile Mongolian gerbils. Environ. Physiol. Biochem. 3: 213–220.

12 Harold, K. 1969. Aflatoxin-induced lesions in Syrian hamsters. Br. J. Cancer 23: 655–660.

13 Hsieh, D.P.H., Z.A. Wong, J.J. Wong, C. Michas and B.H. Ruebner. 1977. Comparative metabolism of aflatoxin. In: Mycotoxins in Human and Animal Health. (Rodnicks, J.V., C.W. Hesseltine and M.A. Mehlman, eds.), pp. 37–50. Pathotox Publ. Inc. Park Forest South, IL.

14 Kalengayi, M.M.R. and V.J. Desnet. 1975. Sequential histological and histochemical study of rat liver during aflatoxin B_1-induced carcinogenesis. Cancer Res. 35: 2845–2852.

15 Lalor, J.H. and G.C. Llewellyn. 1981. Biointeraction of sodium selenite and aflatoxin B_1 in the Mongolian gerbil. J. Toxicol. Environ. Health 8: 387–400.

16 Llewellyn, G.C. and L.E. Thomen. 1978. Body weight changes and corresponding pathological responses seen in Syrian hamsters and Mongolian gerbils fed aflatoxin B_1. Toxicon suppl. 1 (Toxins: Animal. Plant and Microbial): 779–789.

17 Llewellyn, G.C., C.E. Hastings and J.W. Hofman. 1980. Biointeraction of aflatoxin and zinc in Mongolian gerbils (*Meriones unguiculatus*). Toxicon 18: 107–112.

18 Llewellyn, G.C., L.E. Thomen and J.S. Katzen. 1981. Effects of dietary copper on developing aflatoxicosis in Syrian hamsters. J. Environ. Sci. Health. B16: 211–215.

19 Llewellyn, G.C. 1982. Aflatoxin-metal interactions and responses in laboratory and agricultural animals: a review. Dev. Ind. Microbiol. 23: 237–246.

20 Llewellyn, G.C., G. Hoke and W.V. Dashek. 1982. Alteration of aflatoxicosis in Syrian hamsters fed supplemental zinc carbonate diets. In: Fifth IUPAC Symposium on Mycotoxin and Phycotoxins IUPAC. (Krogh, P., ed.) pp. 253–256. Vienna, Austria.

21 Llewellyn, G.C., C.E. O'Rear and W.V. Dashek. 1984. Mycotoxin and copper diet review. Does dietary copper reverse aflatoxicosis in selected rodents? Dev. Ind. Microbiol. 25: 779–789.

22 Lynd, Q.R. and F.T. Lynd. 1970. Hepatic cholesterol-lipid anomalies induced by aflatoxin. Path. Vet. 7: 509–516.

23 Morgan, J.D. 1977. An evaluation of the response and recovery of Syrian hamsters exposed to copper acetate and mixed aflatoxin diets. Masters Thesis, Virginia Commonwealth University, Richmond, VA.

24 Neathery, M.W., W.H. Moos, R.D. Wyatt, W.J. Miller, R.P. Gentry and L.W. George. 1980. Effects of dietary aflatoxin on performance and zinc metabolism in dairy calves. J. Dairy Sci. 63: 789–799.

25 Newberne, P.M. and M.W. Conner. 1974. Effect of selenium on the acute response to aflatoxin B_1. Trace Subst. Environ. Health 8: 323–328.

26 Petering, H.G., L. Murthy and E. O'Flaherty. 1977. Influence of dietary copper and zinc on rat lipid metabolism. J. Agric. Food Chem. 25: 1105–1109.

27 Poswillo, D.E. and B. Cohen. 1971. Inhibition of carcinogenesis by dietary zinc. Nature 231: 447–448.

28 Roth, H.P. and M. Kirchressner. 1977. The influence of zinc deficiency on fat metabolism. Int. J. Vitam. Nutr. Res. 47: 277–283.

29 Sherman, A.R., H.A. Guthrie and I. Wolinsky. 1977. Interrelationships between dietary iron and tissue zinc and copper levels and serum lipids in rats. Proc. Soc. Exp. Biol. Med. 156: 396–401.

30 Thomen, L.E. 1979. The effects of high levels of copper acetate on aflatoxicosis in Syrian hamsters. Masters Thesis, Virginia Commonwealth University, Richmond, VA.

31 Vallee, B.L. 1976. Zinc biochemistry, a perspective. Trends Biochem. Sci. 1: 88–91.

32 Wogan, G.N. 1964. Mycotoxins in Foodstuffs. M.I.T. Press, Cambridge, MA.

33 Wogan, G.N. and P.M. Newberne. 1967. Dose response characteristics of aflatoxin B_1 carcinogenesis in the rat. Cancer Res. 27: 2370–2376.