	External features (34 experimental and 34 control embryos)							Histological observations (26 experimentals and 26 controls)				
	Very good	Good	Retarded	Dead	Vesicle diameter	Body length	Tail length	CNS abnormal	Heart abnormal	Liver normal	small	absent
Experimentals	5	15	5	9	2.97 ± 0.067	1.97 ± 0.079	1.75 ± 0.077	12	5	8	12	6
Controls	24	6	1	3	3.44 ± 0.079	2.49 ± 0.070	2.32 ± 0.088	9	4	24	1	1

Vesicle, body and tail measurements are in mm, ± SE; other figures represent numbers of embryos in each category.

streptozotocin has deleterious effects on the growth and viability of embryos at this stage. The apparently delayed development of the liver may be linked with the failures in yolk sac circulation, since the liver develops from the proximal anterior wall of the yolk sac and is also a site of haemopoiesis. None of these effects, however, parallels the abnormalities that have so far been observed in embryos of streptozotocin-diabetic rats (i.e. malformations of the nervous system and heart, skeletal deficiencies and exomphalos[7]). So it seems likely that these latter abnormalities were attributable to the maternal diabetes rather than to any direct effects of streptozotocin. Moreover, since the female rats were injected with streptozotocin either before mating or on day 0, and it is eliminated from the body within 4–6 h[3], only early cleavage stages of embryos could be at much risk of exposure to the drug. The present work shows, however, that administration of streptozotocin to rats at post-implantation stages of pregnancy could have severe effects on the further development of the embryos.

Modification of hepatotoxic effects of aflatoxin B_1 in rabbits by immunization[1]

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Summary. Reduction of acute toxic effect of aflatoxin B_1 was achieved by immunizing the rabbits with small amounts of bovine serum albumin-aflatoxin B_1 conjugate. Rabbits after immunization showed lower mortality, near normal serum isocitric dehydrogenase activity, no abnormality in livers when challenged with a single dose of aflatoxin B_1. The results suggest that immunization might be used prophylactically against aflatoxicosis.

Aflatoxin B_1 (afla B_1) is one of the most potent environmental carcinogens and hepatotoxins produced by *Aspergillus parasiticus* and *A. flavus*. Because of the potential hazard of this toxin to human and animal health, the chemistry and the biochemical and pathological effects of afla B_1 have been studied extensively in the last decade[4,5]. Unlike most bacterial toxins, afla B_1 and other mycotoxins are small molecular weight fungal metabolites with diverse chemical structures. While these toxins are devoid of any antigenicity, an afla B_1-1-(0-carboxymethyl)-oxime can be prepared through derivation[6,7]. The new derivative has a carboxyl group which is readily coupled to a protein for immunization. Using this approach, investigators in this and other laboratories[7,8] have produced antibody in rabbits showing high affinity to afla B_1 after the animals were immunized with either bovine serum albumin (BSA)-alfa B_1 conjugate or with polylysine-afla B_1 conjugate. The antibody was also found to be useful in the radioimmunoassay for afla B_1. The present study was carried out in order to find whether or not immunization might be used prophylactically against aflatoxicosis.

Material and methods. Since rabbits are among the most sensitive animals with regard to afla B_1 toxicity, this species was selected for study. Albino female rabbits weighing 3.5 kg were divided into 6 groups of 3–7 rabbits each. 3 groups of rabbits were immunized with BSA-afla B_1 conjugate (210 µg per rabbit) which contained 13 moles of afla B_1/mole of BSA, according to the method previously described[8]. Rabbits in the other 3 groups were raised under the same conditions but were not immunized. Antibody titers of the immunized rabbits were determined by a binding method[9] every week starting from the 4th week after immunization. 6 weeks after immunization, all the animals were challenged with a single dose of pure alfa B_1 by i.p. injection. Mortality and serum isocitric dehydrogenase activity (ICDH, Sigma method[10]) were monitored. Limited histological examinations on

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Effect of immunization on the susceptibility of rabbits to aflatoxin B_1*

Afla B_1 dose (mg/rabbit)	Mortality (No. of deaths/surviving) Control	Immunized
0.75	3/7	0/7
1.0	2/4	0/4
0.75 + 1.50**	2/3	2/3

* Rabbit size was 3.5 kg. Mortality was recorded in 2–7 days after a single dose of i.p. injection with pure afla B_1.
** Challenged with 0.75 mg first and then again with 1.5 mg afla B_1 17 days after the first injection.

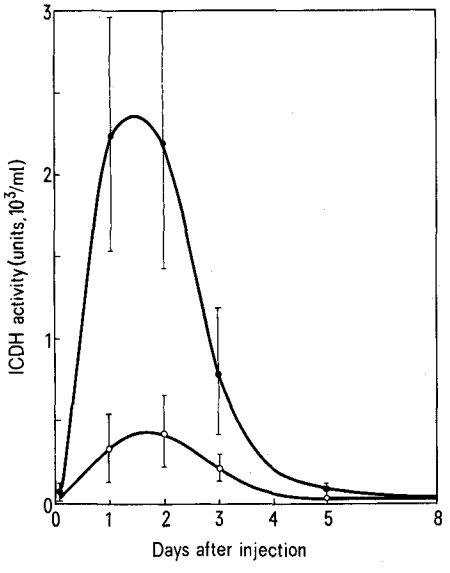

Fig. 1. Serum isocitric dehydrogenase activity of the immunized (-○-○-) and nonimmunized (-●-●-) rabbits after receiving a single dose (0.75 mg/rabbit) of aflatoxin B_1.

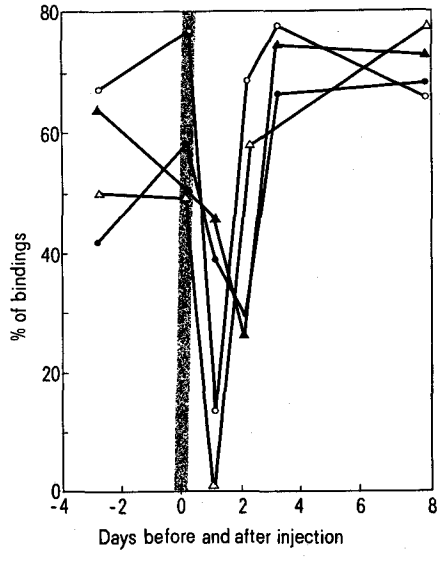

Fig. 2. Binding capacity of rabbit antiserum with ^{3}H-afla B_1 before and after rabbits received a single dose (0.75 mg/rabbit) of aflatoxin B_1. All serum was precipitated with $(NH_4)_2SO_4$ at 33% saturation and a final dilution of 1:500 was made prior to assay. Representation of experimental results from 4 rabbits is presented.

the livers of the dead and surviving rabbits were kindly performed by Prof. J. R. Allen of the Pathology Department, University of Wisconsin-Madison.

Results and discussion. The effects of immunization on the mortality of challenged rabbits are summarized in the table. When rabbits were challenged with afla B_1 at LD_{50} or slightly higher dosages (LD_{50} of afla B_1 = 0.3 mg/kg), all the immunized rabbits were protected from the acute toxic effect. In contrast, the expected mortality rate was observed in the unimmunized control rabbits. Protection is not absolute, however, since immunized rabbits challenged with roughly a 2 LD_{50} dose 17 days after the first challenge showed a higher than 50% mortality.

The protective effect of immunization was also evident from the serum ICDH tests and histological examinations. Figure 1 shows that the serum ICDH activity in the control rabbits increased significantly after they received afla B_1 (0.75 mg/rabbit), whereas the enzyme activity of immunized rabbit serum increased only slightly above normal range. Histological examinations of the rabbit livers revealed that the control rabbits died from a typical aflatoxicosis, including a centrilobular necrosis, and fatty infiltration in the liver. The livers of immunized rabbits which survived the challenge showed no abnormality.

In order to determine whether the protective effect was due to the interaction of afla B_1 with anti-afla B_1 antibody, the binding capacity of rabbit serum with ^{3}H-afla B_1 was monitored throughout the experiments. Figure 2 shows that the binding capacity decreased rapidly after the rabbits received the challenge with unlabelled afla B_1 but returned to near normal levels 3–4 days afterwards. The results indicate that immediately after the animals received the mycotoxin, the binding sites of the serum antibody were occupied by the unlabelled afla B_1; as soon as the toxin left the bloodstream, perhaps through excretion, the new binding sites which are probably located in the newly synthesized antibody, became available for the *in vitro* binding with ^{3}H-afla B_1. Rabbits in the 3 group, which showed little protection when challenged a second time with afla B_1 (2 LD_{50} dose), may have had insufficient antibody to bind and neutralize the toxin. It is also interesting to speculate that since metabolism plays an important role for afla B_1 toxicity, interaction of afla B_1 with homologous antibody could hinder the activation of afla B_1 to an active molecule, thus preventing the manifestation of the toxic effects.

The present study indicates that it is possible to increase the resistance in animals to aflatoxicosis by immunization, but it is not known whether immunization might inhibit the afla B_1 induced hepatocarcinogenic effect in animals. Peck and Peck[11] suggested that immunization might be a practical means to prevent cancer in humans. In their study of tumor induction in rats with 2-anthrylamine, they demonstrated that a 50% inhibition of tumor formation was achieved by immunizing Sprague-Dawley female rats with 2-anthrylaminohuman serum albumin-41 conjugate. Therefore, it is possible that the carcinogenic effect of afla B_1 might also be inhibited after immunization. Studies in our laboratory are currently directed to verify such a possibility. Since we have used only a one-step multiple sites injection method for immunization, continuous boosting of the animals with BSA-afla B_1 conjugate would likely produce higher antibody titers and thus the greater protective effect. Improved methods for immunization warrant further investigation.

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