Effect of Aflatoxin on Complement Activity in Guinea Pigs (36130)

JOHN R. THURSTON, JOHN L. RICHARD, SIGMUND J. CYSEWSKI, ALLAN C. PIER, AND CHARLES K. GRAHAM

National Animal Disease Laboratory, Veterinary Sciences Research Division and Biometrical Services Staff, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Aflatoxins are collectively hepatotoxic substances produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*. They have been reported to inhibit some enzyme systems and to alter *in vivo* protein synthesis, including those serum globulins produced by the liver (1–5).

Many species of domestic and laboratory animals are susceptible to aflatoxin (1). Recent investigations of aflatoxicosis have been directed toward determining the effect of aflatoxin on enzyme systems (2–4) and immunity (5, 6). Choice of animal species used in these investigations was determined by the nature of the study. Interest in the effect of aflatoxin on serum complement activity directed attention to use of guinea pigs which are suceptible to aflatoxin (1) and have depressed complement levels when treated with some other hepatotoxins (7).

In preliminary experiments, guinea pigs given measured amounts of aflatoxin apparently had suppressed serum complement activity. The present investigation was done to examine in greater detail the relationship of complement activity to intake of aflatoxin. Also considered in the study was the effect of aflatoxin on condition of the guinea pigs as reflected in weight changes, and histopathologic changes of their livers.

Materials and Methods. Guinea pigs. Female guinea pigs were distributed randomly into 12 groups of 5 animals. The guinea pigs were fed a commercially prepared diet[1] and water *ad libitum* throughout the experiment. Chloroform extracts of a 200-g sample of the guinea pig feed were assayed for aflatoxin by thin-layer chromatography (8) and no aflatoxin was detected.

Aflatoxin. Partially purified aflatoxin (PPA) produced by *Aspergillus flavus* was from the same lot used in a previous study (8); and dose levels were determined on the basis of toxic equivalents of fraction B_1 in the PPA (8).

Administration of aflatoxin to guinea pigs. Doses of aflatoxin were prepared and administered to guinea pigs by the following technique: The desired quantity of PPA was dissolved in chloroform and the solution was added to 11 g of lactose. After evaporation of chloroform, the dry PPA and lactose were thoroughly mixed and used to fill 100 No. 5 gelatin capsules.[2] Filled capsules were stored in the dark at 4°. A single capsule was given daily per os to each guinea pig. Capsules were prepared to provide a daily dose of fraction B_1 of from 0.05 to 0.005 mg. The daily dose of B_1 given to guinea pigs in each group is shown in Figs. 1 and 2. Capsules prepared with solvent and lactose, but no PPA were given to guinea pigs in group 11. Guinea pigs in group 12 served as untreated controls. Treatment was for 20 consecutive days.

Complement determinations. Blood samples were obtained from each guinea pig by cardiac puncture before administration of PPA was initiated, and 1 day after the conclusion of the experimental period, *i.e.*, on day 21. Serum samples were stored at —70°, and complement titrations, using a 50% end point (9), were done within 48 hr after collection.

Postmortem and histopathologic examinations. All guinea pigs were killed after blood samples were collected on day 21. Postmortem examinations were made and sections of liver were placed in 10% formalin. Paraffin-

[1] Rockland Guinea Pig Diet, TEKLAD, Inc., Monmouth, IL 61462.

[2] Eli Lilly & Co.

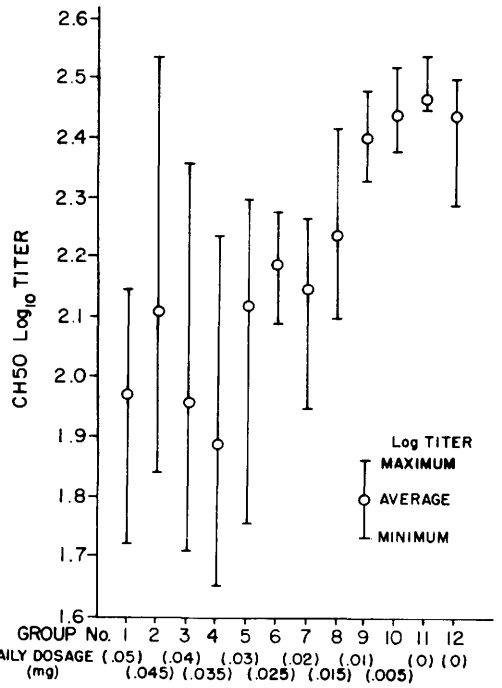

FIG. 1. Maximum, minimum, and average CH_{50} $\log_{10}$ titers of guinea pigs after daily doses of aflatoxin (PPA) for 20 days. The milligrams of B_1 equivalents given daily to guinea pigs in each group are shown in parentheses.

embedded sections were stained with hematoxylin and eosin stain.

Results. Analysis of variance (10) indicated there were no significant[3] differences among the pretreatment average titers[4] or weights of the 12 groups of guinea pigs. Subsequently titers and weights of guinea pigs in each treatment group were compared with control group 11 by means of Dunnett's procedure [cited in Ref. (10)].

At the end of PPA administration, the average complement titers of guinea pigs in groups 1 through 5 were significantly less that that of guinea pigs in the treatment control group (Fig. 1). Average titers of groups 6, 7 and 8 approached significant reduction in titer. The average weights of guinea pigs in groups 1 through 8 were significantly less than the average weight of the control group (Fig. 2).

Wide differences in complement titers occurred among guinea pigs in groups 1 through 8, ranging from elevated titers to titers depressed below the lower confidence limit (log titer 2.12) established by complement titers of control group 11. The differences within the groups are shown by the range of titers and weights depicted in Figs. 1 and 2. Seven combinations of reactions occurred, relating depressed and normal complement activity to weight gain or loss and the presence or absence of histopathologic changes in the liver (Table I). Microscopic alterations occurring in livers of guinea pigs in this study were typical of aflatoxicosis as previously reported (11).

Discussion. Suppression of complement activity, retardation of weight gain, and histopathologic changes were observed in guinea pigs given daily doses of PPA for 20 days. The average effects were dose related, but individual guinea pigs varied widely in their response to PPA and in the relationship of complement depression to weight loss and liver change. The individual variation in response of guinea pigs given accurate daily doses of PPA was consistent with variations in re-

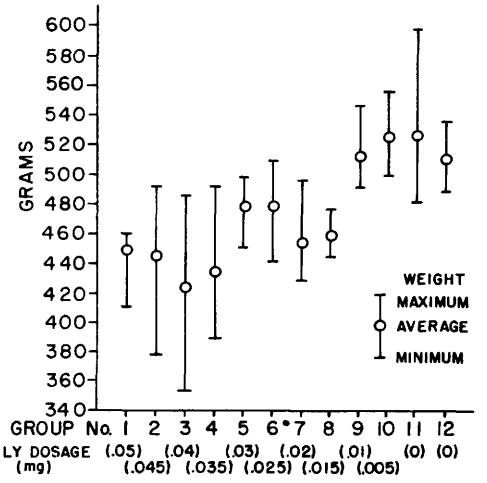

FIG. 2. Maximum, minimum, and average weights of guinea pigs after daily doses of aflatoxin (PPA) for 20 days: The amounts (mg) of B_1 equivalents given daily to guinea pigs in each group are shown in parentheses.

[3] Significance is used in all instances to mean $p < .05$ compared to the control.

[4] Titers refer in all instances to the $\log_{10}$ titer of the CH_{50} end point.

TABLE I. Relationship of Complement Depression of Guinea Pigs, Groups 1 Through 8, Relative to Gain or Loss in Weight, and Presence of Hepatic Lesions.

Guinea pigs from group no.[a]	Complement[b]	Weight[c]	Hepatic lesions present[d]
1,2,2,3,4,5,5,7	Depressed	Gain	Yes
1,3,3,3,4,4	Depressed	Loss	Yes
1,1,6,6,7,8	Depressed	Gain	No
2	Depressed	Loss	No
1,2,8	Normal	Gain	Yes
3,4,4,5,5,6,6, 6,6,7,7,8,8	Normal	Gain	No
2,7,8	Normal	Loss	No

[a] Five animals/group; see Figs. 1 and 2 for explanation of groups.

[b] A log titer of less than 2.12 was considered depressed.

[c] Gain or loss relative to pretreatment weight.

[d] Yes indicates presence of microscopic alterations in the liver compatible with a diagnosis of aflatoxicosis.

sponse observed in studies of other animal species fed aflatoxin (5, 6). In the present case, where PPA was administered, the observed variations in response were less likely to be related to feeding or drinking habits than if PPA had been incorporated in a ration (11).

In general, a daily intake equivalent to 0.03 mg or greater of fraction B_1 resulted in depressed complement activity associated with changes in the liver. A daily intake equivalent to 0.015 mg or greater of B_1 caused a significant reduction of weight, showing the general condition of guinea pigs was more responsive to aflatoxin, on the average, than either complement activity or liver change. The latter factor was studied by conventional techniques, and it is possible that special procedures would show changes in the liver more closely associated with individual variations in complement titers and weight.

Log titer 2.12 was used as the critical titer for determining that a particular guinea pig had depressed complement activity (Table I). Log titer 2.12 (serum dilution 1:130) was equivalent to the average log titer of guinea pigs in group 5, which was significantly less than the average log titer of the control guinea pigs. No guinea pig in any group had a pretreatment titer of less than 2.21 (1:162).

The possibility that complement depression resulted from a relative increase in the dose of PPA because of weight loss was considered. Fourteen of the 21 guinea pigs (Table I) that had a depressed complement titer following treatment had a net gain in weight over their pretreatment weights. The gain was, in almost every case, much less than occurred in guinea pigs in groups 9 and 10 or in control guinea pigs.

The responses observed were related to a 3-week treatment period. The selection of 3 weeks was somewhat arbitrary, but was of sufficient duration that the effect of small increments in dose levels could be observed. The administration of aflatoxin for longer periods of time could result in less individual variation among guinea pigs or the demonstration of changes in titer, weight, and tissues at dose levels that caused no detectable changes in the present investigation.

Summary. Guinea pigs were dosed once daily with partially purifed aflatoxin in measured amounts per os for 20 days. A significant average depression of complement titers occurred in guinea pigs given a daily dose of aflatoxin equivalent to 0.03 mg or greater of fraction B_1. Guinea pigs given a daily dose of aflatoxin equivalent to 0.015 mg or greater of fraction B_1 had average weight significantly below that of control guinea pigs. Microscopic changes in the liver were detected mostly in guinea pigs given 0.03 mg or more of B_1 equivalents. Considerable individual variation in complement titers, weights, and liver changes were noted among the guinea pigs.

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