creases in total activity, with no significant differences in free cathepsin activity. Adrenalectomized animals maintained with saline had greater free cathepsin activity in liver and muscle than their pair-fed and *ad libitum*-fed controls, suggesting that the increased free activity was not due to electrolyte imbalance or starvation.

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Aflatoxin Toxicity in Beef Cattle (32652)

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Loosmore and Markson in 1961 first reported the adverse effects of aflatoxin in cattle (1). Subsequently, Clegg and Bryson described an outbreak of poisoning in cattle which was attributed to Brazilian groundnut (peanut) meal (2). In 1963 Allcroft and Lewis reported their findings on the effects of toxic Brazilian peanut meal for calves, cows, and heifers (3). In calves the first symptomatic effect of continuous ingestion of toxic peanut meal was a reduced growth rate followed by severe tenesmus a few days before death. Postmortem examination revealed fibrosis of the liver, ascites, and visceral edema.

Since aflatoxin is toxic for cattle, this raises the question as to whether a "no effect" or harmless level of intake of aflatoxin for cattle can be established. The primary purpose of this work was to relate the level of intake of aflatoxin B_1 to the degree of toxic effects produced in a beef cattle feeding study under practical conditions.

Methods and Materials. Twenty tons of prime quality California grown cottonseed meal, containing not less than 41% protein and not more than 0.04% free gossypol, was purchased locally for the feeding trial. Three tons of this meal was shipped to the Southern Regional Research Laboratory for fortifying with rice which had been cultured with mold at the Northern Regional Research Labora-

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TABLE I. Weight Gains and Feed Utilization of Cattle Fed Cottonseed Meal Containing Graded Levels of Aflatoxin B_1 for 133 Days.

Item	Aflatoxin B_1 in the ration (ppb)				
	Control	100	300	700	1000
No. of animals	10	10	10	10	9[e]
Initial weight (lb)	401	427	417	406	433
Daily gain (lb)	2.51[a]	2.63[a]	2.40[a]	1.90[b]	1.76[b]
Daily feed consumption[d] (lb/100 lb body wt.)	2.61[a]	2.66[a]	2.59[a]	2.27[b]	2.07[b]
Gain/feed (lb)	0.175	0.164	0.159	0.155[a]	0.152[a]

[a,b] Comparable means with a different letter superscript are significantly different at the 1% level.

[c] One steer died on the 59th day.

[d] Dry matter.

[e] Eight steers from each of these groups were confined in metabolism cages for a 1-week period.

tory.[3] The aflatoxin content of the fortified meal averaged 10,670 parts per billion (ppb) of aflatoxin B_1 and 1070 ppb of G_1. By proper blending of the fortified and unfortified cottonseed meal, the levels of aflatoxin B_1 indicated in Table I were formulated. The composition of the basal ration was as follows (in %): alfalfa hay 14, oat hay 6, beet pulp 8, milo 20, barley 24, cottonseed meal 15, fat 2, molasses 10 and salt 1. Vitamin A and a small quantity of oyster shell flour were also added.

Fifty cross-bred beef steers purchased on the open market at age 6–8 months and in a body weight range of 400–500 lb were used. The animals were randomly divided and housed in groups of 5, two groups of 5 steers being assigned to each level of aflatoxin fed. Animal weights were recorded initially and at 4-week intervals, each weighing being preceded by withholding of feed and water overnight. Feed consumption records were maintained for each group of 5 streers.

Blood samples (50 ml) were taken by jugular vein from 10 steers per week during the feeding trial for hematology and biochemical measurements. Complete autopsies at the rate of 5 steers per week were initiated when the feeding trial had progressed for 133 days. The last group was slaughtered at 196 days. Organ weights were recorded for liver, kidneys, spleen, heart, and adrenals. Tissues were also preserved in formaldehyde for microscopic examination. The pathological and biochemical findings will be the subjects of separate reports. Blood, liver, kidneys, muscle, and fat samples were also saved for aflatoxin residue studies which will also be the subject of a separate report.

Results. Mean weight gains, feed consumption, and feed efficiency ratios at the end of 133 days are summarized in Table I. Weight gains were significantly lower when the level of aflatoxin in the ration was 700 ppb or higher. The feed efficiency values decreased as the level of aflatoxin fed increased.

Organ weights expressed in grams per 100 pounds body weight are tabulated in Table II. Both liver and kidney weights were significantly increased at the 700 and 1000 ppb levels of aflatoxin intake. Spleen, heart, and adrenal weights appear to be within normal limits and not influenced by the aflatoxin intake. The livers from 3 of 8 steers fed 1000 ppb and 2 of 10 steers fed the 700 ppb level of aflatoxin B_1 were grossly abnormal being greyish in color, enlarged, and having a fibrous or rubbery texture. Below 700 ppb no gross liver abnormalities were observed. Two steers fed the 1000 ppb level died; one

[3] The aflatoxin was produced on a rice substrate at the Northern Regional Research Laboratory, Peoria, Ill., as described by O. L. Shotwell, *et al.* (4). The procedure used for fortification of the cottonseed meal at the Southern Regional Research Laboratory, New Orleans, La., was described previously (5).

TABLE II. Effect of Aflatoxin B_1 on Organ Weights of Beef Cattle.

Ration[a] aflatoxin B_1 (ppb)	Mean organ weights (gm/100 lb body wt.)				
	Liver ± SE	Kidneys ± SE	Spleen	Heart	Adrenals
Control	605 ± 12	90 ± 3	98	193	1.5
+ 100	665 ± 18[b]	99 ±12	103	194	1.9
+ 300	610 ± 10	97 ± 3	83	189	1.8
+ 700	739 ± 54[b]	107 ± 3[c]	92	208	2.1
+1000	720 ± 51[b]	107 ± 3[c]	89	193	1.6

[a] Ten steers per group; 2 steers died on 1000 ppb group.
[b] $p = <.05$
[c] $p = <.01$

on the 59th day and one on the 137th day of the feeding trial.

Conclusions. Under simulated practical conditions, graded levels of aflatoxin fortified cottonseed meal were fed to young beef cattle. The levels of aflatoxin B_1 fed in the ration to groups of 10 steers for 133–196 days were 0, 100, 300, 700, and 1000 parts per billion (ppb). Significant growth inhibition, decreased feed efficiencies, and increased liver and kidney weights were observed in the steers fed 700 and 1000 ppb. Gross evidence of liver damage in these groups was also detected. No apparent abnormalities were seen in the groups fed 100 and 300 ppb aflatoxin.

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Specificity of Inhibition by Antiserum of the Development of Immediate and Delayed Hypersensitivities in Mice* (32653)

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Antiserum taken from hyperimmunized mice can impair development by other mice of delayed hypersensitivity to protein antigens (1). We have suggested that this effect is specific because inhibitor appeared in the sera of donor mice only upon their being heavily re-exposed to the antigen to which they were already hypersensitive. Nevertheless, such massive exposure to one antigen conceivably might elicit formation of a broadly effective inhibitor, like the α-globulin of Kamrin (2) and Mowbray (3–5), or of excess immunoglobulin which could suppress formation of the same kind of product (i.e., antibody) by feedback mechanisms specific for that class of immunoglobulin rather than for antigen (6). To examine these possibilities we set up the experiments reported here, seeking direct evidence for the antigenic specificity of this inhibition.

Materials and Methods. We used CF-1 female mice and purified protein antigens according to previously described techniques (1). Crystalline human serum albumin (Pentex, Inc.), bovine serum albumin (Pentex,

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