Experimental Aflatoxicosis in Swine: Morphological and Clinical Pathological Results

D.M. Miller, B.P. Stuart and W.A. Crowell*

ABSTRACT

The morphological changes in livers of 30 feeder pigs fed diets containing corn contaminated by aflatoxins (0.0 μg aflatoxins/g feed, 0.4 μg aflatoxin/g feed, and 0.8 μg aflatoxin/g feed) were compared with changes in hematology, liver specific serum enzymes, serum proteins, and lymphocyte stimulation indices. Histologically, the livers were classified into five groups. Pigs fed the 0.8 μg/g diets had the most severe histological lesions of karyomegaly, bile ductule proliferation and hepatocellular degeneration plus elevated gamma-glutamyl transpeptidase, aspartate aminotransferase, and alkaline phosphatase. This group also had significantly lower total protein and albumin values compared to the control pigs. Variation in the severity of the histological lesions was seen in pigs fed 0.4 μg/g diets as well as variation in lymphocyte indices, liver specific serum enzymes, and electrophoretic results in the affected pigs in that group.

RÉSUMÉ

Cette expérience consistait à comparer les changements morphologiques du foie de 30 porcs à l'engraissement auxquels on servait de la moulée qui contenait du maïs sain ou contaminé avec les quantités suivantes d'aflatoxine: 0,4 et 0,8 μg/g, avec ceux du sang, des enzymes sériques spécifiques du foie, des protéines sériques et de l'indice de stimulation des lymphocytes. Les auteurs classifièrent les lésions hépatiques microscopiques en cinq catégories; elles s'avérèrent particulièrement marquées, chez les porcs dont la moulée contenait 0,8 μg d'aflatoxine/g et elles se caractérisaient par de la karyomégalie, de la prolifération des canalicules biliaires et de la dégénérescence des hépatocytes. Ces porcs affichèrent en plus une élévation de la transpeptidase gamma-glutamyle, de l'aspartate-transaminase et de la phosphatase alcaline. Par rapport aux témoins, ces porcs affichaient une baisse apréciable des protéines totales et de l'albumine. Les porcs dont la moulée ne contenait que 0,4 μg d'aflatoxine/g présentèrent par ailleurs des variations dans la gravité des lésions microscopiques du foie, ainsi que dans l'indice de stimulation des lymphocytes, les enzymes sériques spécifiques du foie et les résultats de l'électrophorèse.

INTRODUCTION

Aflatoxins are a group of mold metabolites which have varied toxic and carcinogenic properties depending on dose and duration of exposure. They cause serious disease in poultry, livestock and other animals (5, 23, 30, 35). Several reports have shown the association between aflatoxicosis and decreased humoral and cellular immunity in cattle, guinea pigs, dogs, rabbits, trout, laboratory animals (24, 25, 26, 28) and recently in swine (10, 18). Aflatoxin in the diet has been associated with increased incidences of infectious disease (25, 28).

Lesions and clinical signs of aflatoxicosis vary with the amount of toxin ingested, diet, species, feed, and age of the animal (3, 7, 22). Variation exists within the literature as to the signs and lesions present in feeder pigs fed 0.4 μg to 0.8 μg aflatoxin/g feed. Microscopically, hepatic lesions of karyomegaly, cytoplasmic degeneration, bile ductule proliferation, and increased fibrous tissue, have been observed in pigs fed 0.45 μg/g and higher levels of alfatoxin but were not always associated with significant depressions in feed conversion efficiency (12). Others have detected decreased feed conversion efficiency but no histological lesions in pigs fed diets containing up to 0.8 μg/g aflatoxin (1).

The relationship of aflatoxicosis and salmonellosis was discussed in our previous publication (18) in which it was reported that the pigs fed aflatoxin containing diets were clinically more severely affected by the induced salmonellosis. Also, increased humoral immunity and cellular immunity were present in the aflatoxin fed pigs compared to the controls. The present investigation was undertaken to assess the effect of feeding rations containing different concentrations of aflatoxin contaminated corn on

*Department of Veterinary Pathology, College of Veterinary Medicine, Athens, Georgia 30602 (Miller and Crowell) and Veterinary Diagnostic and Investigational Laboratory, Tifton, Georgia 31793 (Stuart).

Submitted November 13, 1980.

changes in the lymphocyte stimulation response using purified protein derivative and concanavalin A as stimulants and changes seen in hematology, clinical chemistry and electrophoretic patterns in the groups of swine. Histological lesions were described and placed into one of five defined classifications.

MATERIALS AND METHODS

Thirty mixed breed confinement raised pigs four weeks old weighing approximately 20 to 30 kg were obtained from a commercial herd. All pigs were given an anthelmintic, and were acclimated two weeks prior to the initiation of the feeding trial. Fecal samples were cultured for *Salmonella* spp.

A commercial, antibiotic free ration meeting NRC nutritional standards was formulated to contain either 0.0 μg/g, 0.4 μg/g, or 0.8 μg/g aflatoxin/g feed using naturally contaminated corn (20). The contaminated corn was analyzed to contain approximately 5000 ppb aflatoxin ($B_1+B_2+G_1+G_2$). The percent fungal contamination of the corn was *Aspergillus flavus* (100%), *Aspergillus niger* (20%), *Penicillum* spp. (5%) and Phycomycetes (3.3%). Aflatoxin free corn and supplement were mixed in an agar type feed mill in ratios to obtain 0.4 μg and 0.8 μg aflatoxin/g feed on the average. New feed was mixed and analyzed approximately every three weeks as needed. Samples from ten different areas of the feed were analyzed by two separate laboratories[1] using thin layer chromatography (15). Values ranged from 315 to 525 μg/g for the 0.4 μg/g diet and 733 to 943 μg/g for the 0.8 μg/g diet. Analysis for other mycotoxins was not available and therefore not performed at the time of the study.

The pigs were placed into a control and five experimental groups (five pigs each) as follows:

Control Ration
Control Ration/*Salmonella* inoculated
0.4 μg aflatoxin/g ration
0.4 μg aflatoxin/g ration/*Salmonella* inoculated
0.8 μg aflatoxin/g ration
0.8 μg aflatoxin/g ration/*Salmonella* inoculated

Heparinized blood (10 mL) and uncoagulated blood (20 mL) were obtained weekly from the cranial vena cava. The serum was harvested, frozen and stored at -65°C.

Hemoglobin (Hgb),[2] packed cell volume (PCV), white blood cell count (WBC)[2] and total protein[3] were determined using standard techniques (11). Bilirubin,[3] cholesterol,[4] albumin,[4] aspartate aminotransferase (AST),[4] alanine aminotransferase (ALT),[4] gamma-glutamyl transpeptidase (GGTP),[4] isocitric dehydrogenase (ICD),[5] blood urea nitrogen (BUN),[4] glucose,[4] and alkaline phosphatase (SAP)[4] were performed by a commercial laboratory.[6]

Blood was processed for recovery of lymphocytes within one hour of collection. The lymphocytes were separated by a modification of the Böyum method (2). Briefly, six mL heparinized blood was mixed with an equal volume of Hank's buffered salt solution and layered on top of 9 mL of Ficoll Paque[7] in a plastic conical centrifuge tube. After centrifugation at 400 × g for 30 minutes at 18-20°C, the lymphocyte-rich middle fraction was collected, diluted 1:3 with Hank's and centrifuged at 100 × g for ten minutes. This was repeated once with Hank's and once with RPMI 1640 media.[8] The cells were resuspended to a concentration of 2.0×10^6 cells/mL RPMI 1640 supplemented with 20% inactivated fetal calf serum, penicillin (100 units/mL) and streptomycin (100 μg/mL). Viability and concentrations of lymphocytes were determined by the trypan blue exclusion method.

Triplicate cultures, each containing 0.1 mL of cell suspension were placed in flat bottomed plastic microtiter plates with appropriate test antigen, mitogen, or control medium. Concanavalin A (Con A)[9] reconstituted according to manufacturer's instructions with RPMI 1640 was used at a concentration of 10 μL/mg of culture and purified protein derivative (PPD) of *Mycobacterium avium*[10] was used at a concentration of 2 μg/culture. All pigs had been sensitized with *M. avium* after consuming the rations for three weeks as reported in a separate publication (18).

All lymphocyte cultures were incubated at 37°C for three days in a humidified 5% CO_2 atmosphere. Eighteen hours before termination, cultures were pulsed with tritiated thymidine[11] (1 μCi/culture; specific activity, 6.7 CI/mmole). At the end of incubation, plates were placed in a refrigerator until processed by the method of Hartzman *et al* (14). Radioactivity was counted in a liquid scintillation spectrometer and results were expressed as counts per minute (cpm). The stimulation indices (SI) were determined by mean cpm of mitogen or antigen stimulated cultures divided by the mean cpm value of unstimulated control cultures.

[1]Plant Pathology, Coastal Plains Experiment Station and Tifton Diagnostic and Investigational Laboratory, Tifton, Georgia.
[2]Coulter-S, Coulter Electronics Inc., Hialeah, Florida.
[3]Jendrassik bilirubin, American Monitor Corporation, Indianapolis, Indiana.
[4]Hycel, Inc., Houston, Texas.
[5]Sigma Chemical Co., Saint Louis, Missouri.
[6]Phytochem Labs, Huntsville, Alabama.
[7]Pharmacia Fine Chemicals, Piscataway, New Jersey.
[8]Grand Island Biological Co., Grand Island, New York.
[9]Miles Laboratories, Inc., Elkhart, Indiana.
[10]National Veterinary Services Laboratories, Ames, Iowa.
[11]New England Nuclear Corporation, Boston, Massachusetts.

Electrophoresis of sera was performed using cellulose acetate membranes and commercial reagents.[12] After air drying, the strips were scanned using a 525 nm filter with albumin, alpha (α), beta (β) and gamma globulins (γ) peaks recorded as percentages and in grams per 100 mL (33).

Lyophilized *Salmonella choleraesuis* var. *kunzendorf* (10^9 organisms) obtained from and prepared according to the method of Troutt (34) were administered to the pigs after three weeks on the experimental ration. Feed was withheld 24 hours prior to oral inoculation.

The pigs were killed with intravenous pentobarbital after ten weeks on the rations. Multiple sections of liver, brain, lung, heart, kidney, spleen, mesenteric and cervical lymph nodes, small and large intestine and skeletal muscle were sampled for bacteriological and histological examination. Tissues for histological examination were fixed in 10% phosphate buffered neutral formalin. Five μm paraffin sections were prepared and stained with hematoxylin and eosin (H&E). Selected liver sections were also stained with Mason's trichrome, Gomori's reticulum, Oil Red O, and Periodic acid Schiff (with and without diatase).

Sections from multiple lobes of the liver were examined histologically. Karyomegaly was defined as nuclear diameters greater than approximately 11 microns (mean hepatocyte nuclear diameter 7.1 μ). Forty fields from five μm H&E liver sections were counted at 450× magnification and classified into one of five grades based on the following criteria:

O = No lesions present (Fig. 1)

I = Karyomegaly (less than ten cells with karyomegaly/40 fields, no other lesions (Fig. 2)

II = Karyomegaly (less than ten cells with karyomegaly/40 fields), hepatocellular degeneration characterized by swelling, increased eosinophilia, granularity and vacuolization of hepatocytes; equivocal bile ductule proliferation (Fig. 3)

III = Karyomegaly (greater than ten cells with karyomegaly/40 fields), hepatocellular degeneration, definite bile ductule proliferation, and mild fibrosis (Fig. 4)

IV = Karyomegaly (greater than ten cells with karyomegaly/40 fields) with hepato-

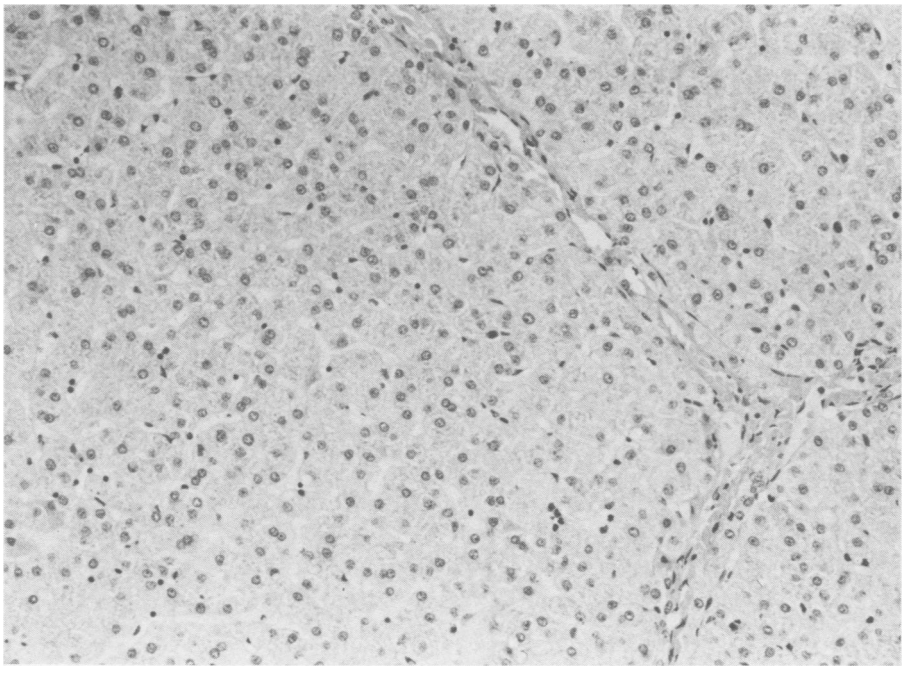

Fig. 1. Grade O. Normal liver from a control pig. H & E. X730.

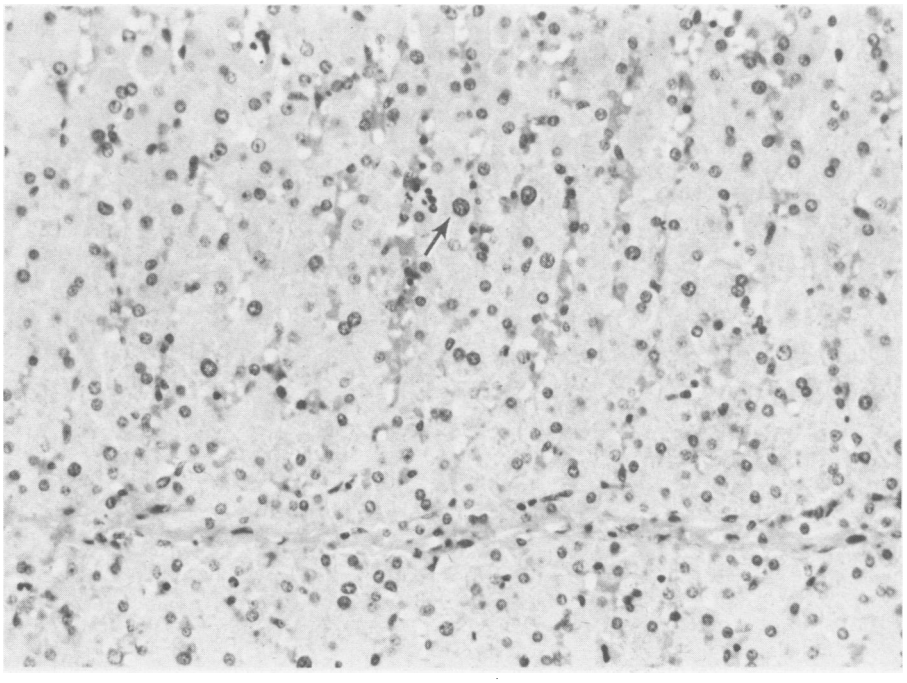

Fig. 2. Grade I. Mild karyomegaly (arrow) in the liver from a pig fed 0.4 μg aflatoxin/g feed. H & E. X730.

[12]Helena Laboratories, Beaumont, Texas.

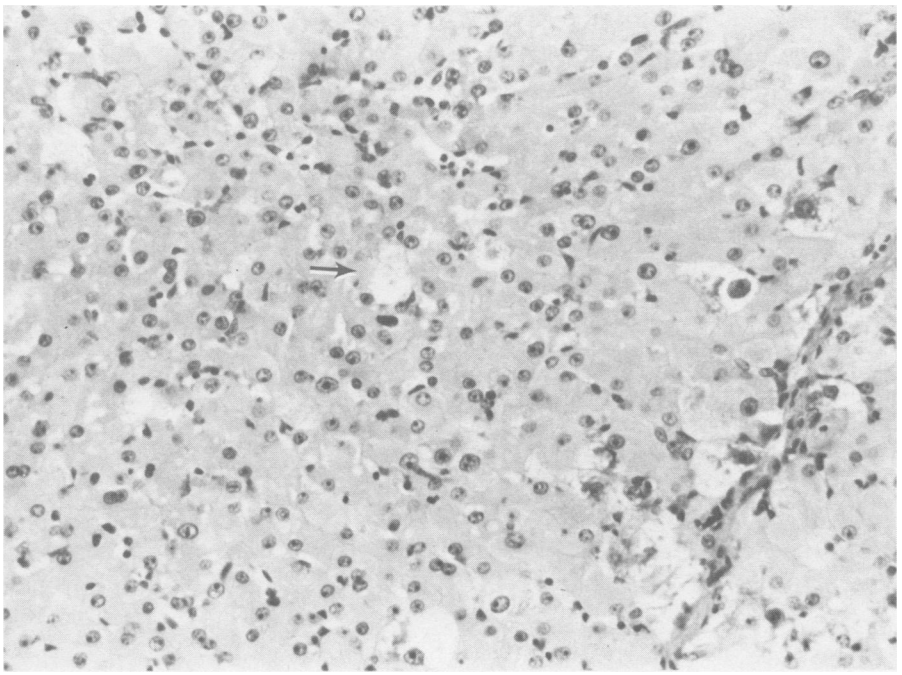

Fig. 3. Grade II. Karyomegaly, hepatocellular degeneration and vacuolization (arrow) in the liver from a pig fed 0.4 μg aflatoxin/g feed. H & E. X730.

cellular degeneration as in II and III, moderate to severe bile ductule proliferation, moderate to severe fibrosis and nodular hyperplasia (i.e. regeneration) (Fig. 5)

Duncan's multiple range tests (31) were used for evaluation of statistical significance of the data.

RESULTS

Table I gives the feed efficiency ratios of each group at seven and ten weeks on the rations. The best feed efficiency ratio was present in pigs consuming the 0.0 μg/g rations with least efficient feed conversion present in pigs consuming the 0.8 μg/g rations. This difference was significant ($p < 0.05$) between the groups fed the aflatoxin free (0.0 μg/g) rations and those consuming the aflatoxin containing rations (0.4 μg/g and 0.8 μg/g). Results of clinical signs of the *Salmonella* inoculated pigs have been reported (18). Although fever and diarrhea were seen, typical histological changes attributed to *Salmonella* cases were not observed due to the time elapsed from exposure to euthanasia. No *Salmonella* was isolated from any of the pigs after euthanasia.

Lymphocyte stimulation indices for Con A and PPD are tabulated (Table II). Stimulation ratios were not significantly different from control pigs.

Data analyzed one week post *Salmonella* and at termination of the project were considered representative of changes observed and are reported. In all pigs the hematocrit increased during the ten weeks of study. The hematocrit and hemoglobin of the controls were higher than these measures in the pigs consuming aflatoxin for ten weeks and was significantly higher ($p < 0.05$) at ten weeks but not at four (Table III). One week

TABLE I. Average Feed Efficiency Ratios (pounds feed consumed/pound weight gained) of Swine Fed Aflatoxin Containing Diets for Seven and Ten Weeks

Group[a]	7 Weeks	10 Weeks
0.0 μg/g[b]	2.17[f,g]	2.80[i,j]
0.0 μg/g/S[c]	3.02[k,f]	2.84[k,i]
0.4 μg/g	2.10[k,g]	3.28[k,j]
0.4 μg/g/S[c]	3.28[k,d,e]	3.00[k,h]
0.8 μg/g	3.86[d]	3.90
0.8 μg/g/S[c]	4.30[e]	3.11[h]

[c]S = Inoculated with *Salmonella choleraesuis* var. *kunzendorf* after consuming the rations for three weeks
[a] = Each group contained ten pigs
[b] = Indicates μg of mixed aflatoxins per gram of feed
[d,e,f,g,h,i,j] = Values followed by the same letter differ at $p < 0.05$. All other values differ from each other at $p < 0.01$
[k] = These values were not significantly different from each other

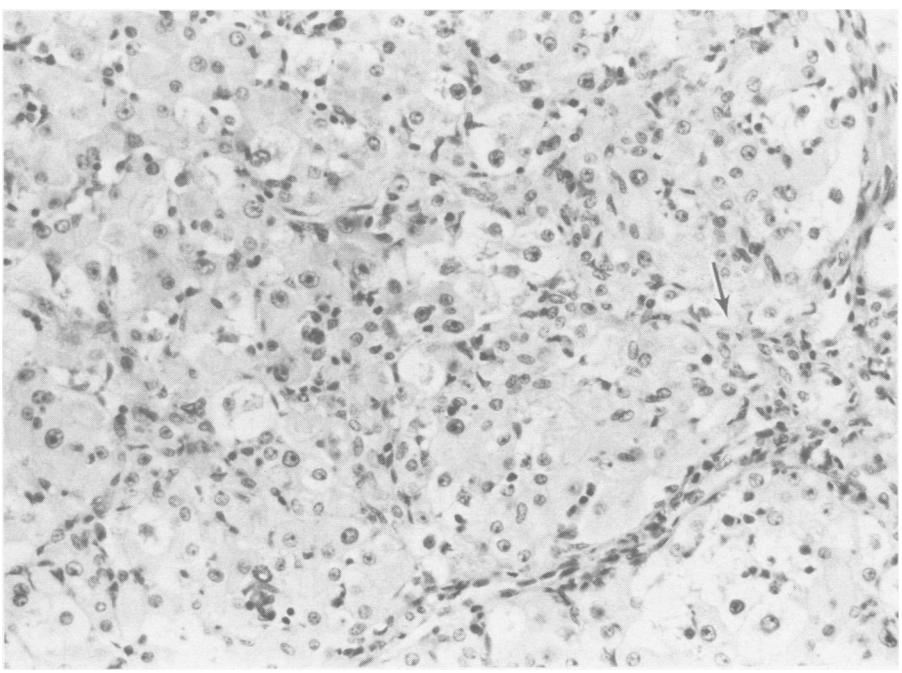

Fig. 4. Grade III. Karyomegaly, hepatocellular degeneration, vacuolization, and bile ductule proliferation (arrow) in the liver from a pig fed 0.8 μg aflatoxin/g feed. H & E. X730.

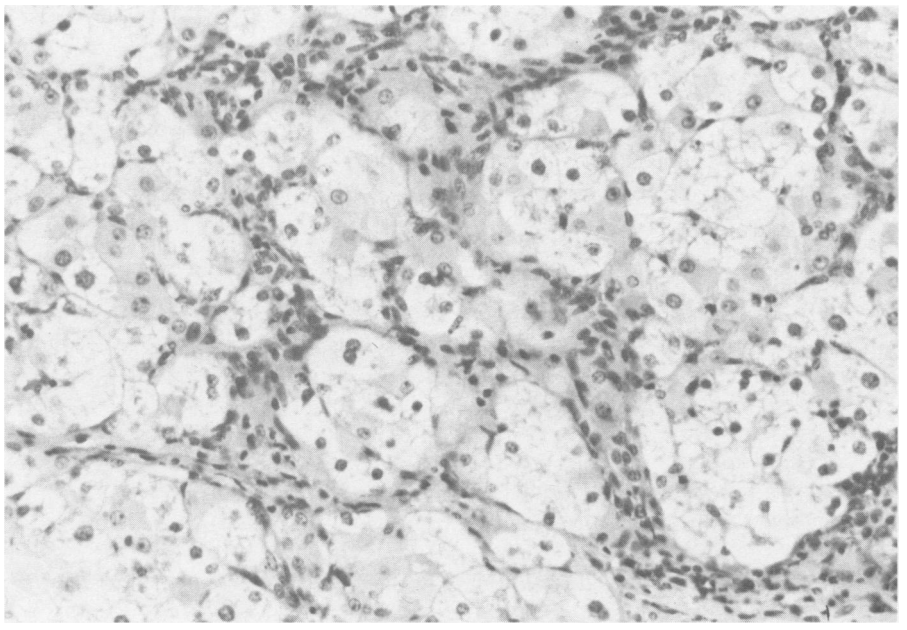

Fig. 5. Grade IV. Karyomegaly, vacuolization of hepatocytes, bile ductule proliferation and pseudolobulation in the liver from a pig fed 0.8 μg aflatoxin/g feed. H & E. X730.

TABLE II. Lymphocyte Stimulation Induced by Con A and PPD in Swine Fed Aflatoxin Containing Rations for Seven Weeks and Sensitized to *Mycobacterium avium*

Group[a]	Mitogen and Antigen	Lowest and Highest Δ CPM	Stimulation[d] Index
0.0 μg/g[b]	ConA	14,576 - 40,868	31
	PPD	2,525 - 5,513	27
0.0 μg/g/S[c]	ConA	20,705 - 58,233	67
	PPD	2,721 - 9,460	20
0.4 μg/g	ConA	21,789 - 45,951	29
	PPD	2,656 - 5,818	8.5
0.4 μg/g/S	ConA	7,382 - 70,564	64
	PPD	1,422 - 8,589	10
0.8 μg/g	ConA	14,226 - 37,185	54
	PPD	2,776 - 9,593	14
0.8 μg/g/S	ConA	13,101 - 95,989	26
	PPD	3,993 - 22,446	24

[a] = Each group was composed of ten pigs
[b] = μg/g indicates μg of mixed aflatoxins per gram of feed
[c] S = Indicates *per os* inoculation of pigs with *Salmonella choleraesuis* var. *kunzendorf* after the animals had been consuming the rations for three weeks
[d] = Stimulation Index = $\frac{\text{Stimulated - background}}{\text{Nonstimulated - background}}$

(four weeks on diet) postinoculation with *Salmonella choleraesuis* var. *kunzendorf*, white blood cell counts (Table IV) were significantly higher ($p < 0.05$) in inoculated pigs than in the noninoculated pigs, except among the control groups for which the white cell count was lower for inoculated pigs than for the noninoculated groups. No difference between *Salmonella* inoculated and noninoculated counterparts was seen after ten weeks on the rations.

Results of serum enzyme determinations are presented in Table V. After ten weeks, GGTP levels were higher (but not significantly) in pigs on the 0.8 μg/g and 0.4 μg/g diets than in the controls. Pigs fed 0.8 μg/g diets had significantly higher ($p < 0.05$) AST values than the controls and 0.4 μg/g groups on both test dates. The SAP values for pigs on the 0.8 μg/g diets were significantly higher ($p < 0.05$) than the controls. No significant differences were seen among the groups for ICD and BUN.

In all pigs fed aflatoxin containing diets for ten weeks, the total protein, albumin, alpha (α) and beta (β) globulins decreased and the gamma (γ) globulin levels increased (Table VI). The total protein and albumin values were significantly lower ($p < 0.05$) in the pigs fed aflatoxin diets when compared to the controls. No significant difference was present between groups when α and β globulin values were compared although β levels were lower in pigs fed aflatoxin. Gamma globulin levels were significantly higher in pigs consuming 0.8 μg/g aflatoxin diet and in the controls inoculated with *Salmonella*. Lower

TABLE III. Mean Hematological Values for Pigs Fed Aflatoxin Containing Rations

Test	Weeks on Diet	0.0 μg/g[a]	0.0 μg/g/S[b]	0.4 μg/g	0.4 μg/g/S	0.8 μg/g	0.8 μg/g/S
PCV (%)	4	38 ± 2.4[c]	38 ± 2.5	37.2 ± 1.8	37.8 ± 2.1	36.6 ± 2.1	39.6 ± 2.1
	10	42 ± 2.3[d]	44 ± 1.9[d]	37.7 ± 1.8	40 ± 3.0	35.7 ± 1.5	37.7 ± 2.0
Hgb (gm/dl)	4	12.6 ± .8	12.9 ± .8	10.4 ± .5	12.4 ± 1.3	12.1 ± 1	13 ± .5
	10	14.4 ± .9[d]	15.7 ± .6[d]	13.3 ± .8	13.5 ± 1.2	12.8 ± .6	12.7 ± .6

[a] = Indicates μg of mixed aflatoxins per gram feed
[b] = Indicates *per os* inoculation with *Salmonella choleraesuis* var. *kunzendorf* after consuming the rations for three weeks
[c] = All values are means (± SD) of duplicate samples of ten pigs
[d] = Indicates significant difference ($p < 0.05$) from others on same line but not each other

A/G ratios were present in pigs consuming the aflatoxin diets. This difference was significant from the controls without *Salmonella* but not those inoculated with *Salmonella*.

Tan to yellow, firm livers were observed in all pigs fed rations containing 0.8 μg/g aflatoxin. Two pigs from the 0.8 μg/g group died during the eighth and ninth weeks of the feeding trials. Both pigs had yellow, firm livers and serosanguinous ascitic fluid. One pig had marked subcutaneous edema and hemorrhage around venipuncture sites. Mild to moderate edema of the gall bladder wall was present in two pigs from the 0.8 mg/g group and one pig from the 0.4 μg/g group. The livers of three pigs fed the 0.4 μg/g ratios were light tan. The livers from the other 0.4 μg/g and control pigs were grossly normal.

The most severe histological lesions were present in pigs consuming the 0.8 μg/g level of aflatoxin. Hepatic changes in these pigs were compatible with criteria of grades III or IV (Figs. 4, 5) except one that was placed into grade II; the latter had moderate karyomegaly (17 cells/40 fields) without bile ductule proliferation or increased connective tissue. A spectrum of hepatic lesions was present in the ten pigs fed the low level of aflatoxin (0.4 μg/g). Two of these pigs had no histological lesions whereas eight had varying degrees of mild karyomegaly, hepatocellular degeneration, bile ductule proliferation and mild fibrosis (Fig. 6). Livers of pigs given the *Salmonella* inoculation had more histological changes of karyomegaly, bile ductule proliferation and increased fibrous connective tissue than their non-inoculated counterparts for the 0.4 and 0.8 μg/g groups.

The highest content of lipid in hepatocytes (as observed in frozen

TABLE IV. Mean White Blood Cell Counts (cells/dL) for Pigs Fed Aflatoxin Containing Rations and Inoculated with *Salmonella choleraesuis* var. *kunzendorf*

Weeks on Diet	0.0 μg/g[a]	0.0 μg/g/S[b]	0.4 μg/g	0.4 μg/g/S	0.8 μg/g	0.8 μg/g/S
4	27,000 (19,000 - 32,000)	23,700 (20,800 - 27,000)	26,700 (20,400 - 32,700)	40,400 (32,200 - 50,000)	25,500 (22,600 - 29,300)	40,500 (27,200 - 58,200)
5	20,800 (18,000 - 26,200)	33,600 (21,500 - 46,100)	22,900 (19,750 - 28,000)	31,600 (28,900 - 36,300)	24,300 (20,800 - 27,100)	28,900 (20,400 - 37,200)
10	21,700 (18,800 - 30,000	22,800 (18,900 - 26,200)	19,900 (17,000 - 23,400)	21,400 (18,000 - 24,300)	24,500 (17,100 - 31,200)	23,200 (16,700 - 31,100)

[a] = μg/g indicates μg of mixed aflatoxins per gram feed
[b] = Indicates inoculation with *Salmonella*. Each value represents the mean of five pigs. Numbers in parentheses are ranges

TABLE V. Mean Serum Enzyme Activities in Pigs Fed Aflatoxin Containing Diets

Enzyme	Weeks on Diet	0.0 μg/g[a]	0.0 μg/g/S[b]	0.4 μg/g	0.4 μg/g/S	0.8 μg/g	0.8 μg/g/S
GGTP	4	25.8 ± 11.2[c]	17.5 ± 4.3	31.6 ± 11.7	18.6 ± 16.3	23.2 ± 8.6	23.6 ± 4.8
	10	26 ± 9.7	20 ± 5.1	50.8 ± 23.1[d]	21.2 ± 12.9	37.5 ± 7.5[d]	26 ± 13.1
ICD	4	187.8 ± 87.5	224.2 ± 58.5	200 ± 60.9	148.2 ± 42.7	158.1 ± 47.1	253.4 ± 140.2
	10	228.2 ± 50.9	214.5 ± 74.7	241.4 ± 110.8	181.2 ± 61.3	153.7 ± 121.7	211 ± 121.1
SGOT	4	14.4 ± 8.1	21.7 ± 13	16 ± 4.6	9.2 ± 4.2	40 ± 32.7	29.8 ± 14.1
	10	20.5 ± 8.2	16.25 ± 7.9	11.6 ± 2.3	12 ± 3.1	21.4 ± 12.7[d]	24 ± 40.1[d]
ALP	4	40.6 ± 19	60.5 ± 25.4	73.2 ± 5.2	41.2 ± 17	107.8 ± 69.3	56.2 ± 45
	10	51.1 ± 25	35.5 ± 8.5	60.8 ± 15[d]	42.6 ± 11.1	109.8 ± 79.4[d]	64.2 ± 25[d]

[a] = μg/g indicates μg of mixed aflatoxin per gram feed. Each group contained ten pigs
[b] = Indicates inoculation with *Salmonella cholerasuis* var. *kunzendorf* after consuming the diets for three weeks
[c] = Indicates mean ± SD. GGTP and ICD data are expressed in Sigma units/mL. SGOT and SAP are expressed in I.U
[d] = Significantly different ($p < 0.05$) from others but not values with same letter

TABLE VI. Mean Levels of Plasma Proteins in Pigs Fed Aflatoxin Containing Rations for Ten Weeks

	0.0 μg/g[a]	0.0 μg/g/S[d]	0.4 μg/g	0.4 μg/g/S	0.8 μg/g	0.8 μg/g/S
Albumin[b]	3.1 ± .45[c]	2.9 ± .46[c]	2.28 ± .54	2.34 ± .48	2.32 ± .44	2.22 ± .24
α globulin[b]	1.14 ± .18	1.4 ± .33	1.3 ± .29	1.3 ± .25	1.14 ± .38	1.4 ± .37
β globulin[b]	1.02 ± .15	1.1 ± .17	.90 ± .14	.90 ± .17	.94 ± .18	.92 ± .43
γ globulin[b]	.82 ± .36	1.2 ± .15[e]	.90 ± .41	1.04 ± .24	1.5 ± .78[e]	1.14 ± .34[e]
A/G ratio	1.05 ± .07	.86 ± .07[e]	.87 ± .10[e]	.74 ± .21[e]	.65 ± .12[e]	.70 ± .21[e]
Total Protein[b]	7.42 ± .43[e]	7.52 ± .45[e]	6.88 ± .48[f]	6.88 ± .42[f]	6.12 ± .65	6.06 ± .61

[a] = μg/g indicates μg of mixed aflatoxins per gram feed
[b] = Expressed as g/dL
[c] = Indicates mean ± SD
[d]S = Indicates inoculation with *Salmonella cholerasuis* var. *kunzendorf* seven weeks previously
[e,f] = Indicates significant differences ($p < 0.05$) from other values but not each other

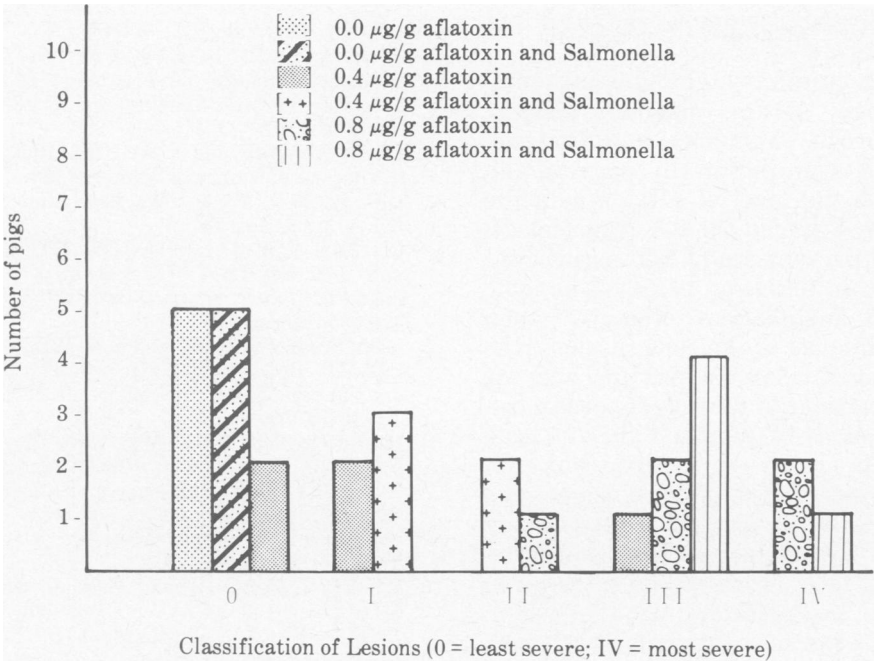

Fig. 6. Frequency and classification of histological liver lesions present in swine fed aflatoxin containing diets for ten weeks.

sections of liver stained with Oil Red O) was in livers from pigs fed the 0.8 μg/g rations. The highest content of glycogen (as observed in PAS stains) was present in the control groups (0.0 μg/g). Results for fat and glycogen content in hepatocytes was variable in pigs fed 0.4 μg/g rations.

Karyomegaly, hepatocellular vacuolization, hyaline degeneration, bile ductule proliferation and fibrosis were not seen histologically in the livers of pigs fed the control diets (0.0 μg/g).

DISCUSSION

In swine fed aflatoxin contaminated rations in south Georgia in 1977, an increased incidence of aflatoxicosis and salmonellosis was observed at the Veterinary Diagnostic and Investigational Laboratory at Tifton, Georgia (32). The dramatic increase in the number of cases of salmonellosis may have been due to the immunosuppressive effects of aflatoxin present in the animals' feed.

Aflatoxin contaminated rations result in decreased feed efficiency and growth rates in swine (12, 30). Generally levels below 0.3 ng/g aflatoxin B_1 do not affect growth rates (19). In our study, 0.4 μg/g and 0.8 μg aflatoxin/g feed did decrease feed efficiency. After seven weeks on the diets the feed efficiency ratios were poorest for those pigs on the diets. By ten weeks the difference in ratios was not as great, however, between the 0.4 and 0.8 μg/g groups, due to slower growth rates by the larger controls and recovery from the Salmonella and anorexia.

Aflatoxin has been studied for many years for its toxic , carcinogenic and immunosuppressive effects on various animal species (10, 25, 26). The only recent work involving the effect of aflatoxin on the immune system of swine demonstrated that there was an interference with the development of acquired immunity following erysipelas vaccination in swine fed aflatoxin containing rations (10).

Pigs consuming aflatoxin containing rations for three weeks prior to intravenous inoculation with sheep erythrocytes had less hemagglutinating antibody to the sheep erythrocytes nine days post-inoculation than pigs fed control rations (18). This suggested that aflatoxin had a depressant effect on the humoral immune system.

Aflatoxin has been shown to interfere with protein synthesis and RNA production in the liver, which is the primary site of aflatoxin metabolism (6, 9). Thus pigs consuming the aflatoxin diets (0.4 μg and 0.8 μg/g) in this study had significantly decreased total serum protein and albumin and increased gamma globulins. The increased gamma globulin seen in cases of hepatic disease may be due to failure of the liver to modify the gamma globulins (4) and explain the increase in our study.

It has been shown in several species that aflatoxin may impair *in vitro* tests of cellular immunity through inhibition of function of T lymphocytes (18, 24, 26, 29). In chickens and rabbits, there has been reported an impairment of the mononuclear phagocytic system in aflatoxicosis (17, 27). Previous studies have shown that delayed hypersensitivity skin test correlate with the *in vitro* migration inhibition factor test, demonstrating a possible T lymphocyte defect in swine consuming aflatoxin containing rations (18). The effect of aflatoxin on the cell mediated immune system in guinea pigs was characterized by a significant suppression of cutaneous hypersensitivity to *Nocardia asteroides* sensitization (28). There was no statistically significant difference noted in the SI when PPD or Con A were used as stimulants of lymphocytes from pigs fed aflatoxin contaminated diets which could partially be explained by the large variation present within group values. A statistical difference was reported when PHA was used in a previous publication (18). Clearly, more experimental work is needed before definite conclusions can be drawn for the effects of aflatoxin on swine lymphocyte stimulation indices.

Several investigators have reported hematological and serum enzyme alterations induced by aflatoxin consumption in acute and chronic studies in young and adult swine (12, 13, 30). In our study, total leukocyte counts were transiently but significantly elevated in pigs receiving the *Salmonella* inoculum but later returned

to normal ranges. Our results substantiate those reports of decreased PCV and Hgb with increased AST and SAP in pigs fed aflatoxin containing diets. However, we did not find significant alterations in BUN or ICD as reported by several investigators (12, 13). Elevated ICD values have been seen in pigs consuming aflatoxin rations for at least 112 days (13); individual values and sample variation were not reported. Significant differences in ICD values were not present in this study. No relationship between histological lesions and ICD levels were observed which is compatible with the absence of frank necrosis in which elevated ICD values are seen.

In man, an increase in both GGTP and SAP indicates hepatic disease (4). This may also be the case in swine, since in the present study elevated GGTP and SAP levels correlated with histological evidence of bile ductule proliferation. In the pig, this enzyme has been reported to be present within the luminal borders of the biliary ducts (8, 21). The biochemical significance of GGTP has not been clearly established, although it may play an important role in protein and amino acid synthesis (16, 21).

Gross and histopathological lesions of aflatoxicosis vary with the amount of toxin consumed (12, 24). In the present study, pigs consuming the highest levels of aflatoxin (0.8 μg/g) had the most pronounced lesions; pigs consuming the lower level (0.4 μg/g) had lesions varying in severity from normal to those similar to the 0.8 μg/g groups. This can be explained by individual variation in susceptibility to the toxin and amount ingested per pig. Histological lesions were more severe in the 0.4 μg/g groups inoculated with *Salmonella* but lesions compatible with salmonellosis (such as sinusoidal leukocytosis, multifocal necrosis or mononuclear cell infiltration) were not seen. Histological evidence (karyomegaly, cytoplasmic degeneration, bile ductule proliferation) of aflatoxicosis in swine receiving 0.4 μg/g (12) has been reported by some workers but not observed by others (1).

In summary, changes in hematology, serum enzymes, electrophoresis, histology and lymphocyte stimulation in pigs varied with the level of aflatoxin in the diet. Pigs fed the 0.8 μg/g diets in the present study had significant serum enzyme, electrophoretic and histological changes when compared to the control pigs. Histological lesions, serum enzyme elevations and electrophoretic differences of pigs fed the 0.4 μg/g diets were generally, but not always, different from the control pigs. While feeding of rations containing 0.8 μg/g and 0.4 μg aflatoxin/g feed depressed both the humoral and cellular immune system of swine in a previous study (18), lymphocyte stimulation results using PPD and Con A were not significantly different in the present study.

REFERENCES

1. **BODNAR, M., M. DORMAN, B. JUHASZ** and **B. STEGEDI.** Experiments with aflatoxin. Magy. Allatorv. Lap. 390-396. 1965.
2. **BÖYUM, A.** Separation of leucocytes from blood and bone marrow. Scand. J. clin. lab. Invest. 21: 1-109. 1968.
3. **BROWN, J.M.M.** Biochemical changes in the livers of domestic birds poisoned with aflatoxin. S. Afr. med. J. 39: 778. 1965.
4. **BURK, M.D.** Hepatic function testing. Postgrad. Med. 64: 177-185. 1978.
5. **BURNSIDE, J.E., W.L. SIPPEL, J. FORGACS, W.T. CARLL, M.A. ATWOOD** and **E.R. DOLL.** A disease of swine and cattle caused by eating moldy corn II. Experimental production with pure cultures of molds. Am. J. vet. Res. 18: 817-824. 1957.
6. **BUTLER, W.H.** Early hepatic parenchymal changes induced in the rat by aflatoxin B_1. Am. J. Path. 49: 113-128. 1966.
7. **CARNAGHAN, R.B.A., C.N. HERBERT, D.S.P. PATTERSON** and **D. SWEASEY.** Comparative biological and biochemical studies in hybrid chicks. II. Susceptibility to aflatoxin and effects on serum protein constituents. Br. Poult. Sci. 8: 279-284. 1967.
8. **CLAMPITT, R.B.** and **R.J. HART.** The tissue activities of some diagnostic enzymes in ten mammalian species. J. comp. Path. 88: 607-621. 1978.
9. **CLIFFORD, J.I.** and **K.R. REESE.** The action of aflatoxin B_1 on the rat liver. Biochem. J. 102: 65-75. 1967.
10. **CYSEWSKI, S.J., R.L. WOOD, A.C. PIER** and **A.L. BAETZ.** Effects of alfatoxin on the development of acquired immunity to swine erysipelas. Am. J. vet. Res. 39: 445-448. 1978.
11. **DUNCAN, J.R.** and **K.W. PRASSE.** (Editors). Veterinary Laboratory Medicine. pp. 30-51, 79-80. Iowa: Iowa State University Press. 1977.
12. **GAGNÉ, W.E., D.L. DUNGWORTH** and **J.E. MOULTON.** Pathologic effects of aflatoxin in pigs. Path. vet. 5: 370-384. 1968.
13. **GUMBMANN, M.R.,** and **S.N. WILLIAMS.** Biochemical effects of aflatoxin in pigs. Toxic. appl. Pharmac. 15: 393-404. 1969.
14. **HARTZMAN, R.L., M.L. BACH, F.H. BACH, G.B. THURMAN** and **K.W. SELL.** Precipitation of radioactively labeled samples: A semiautomatic multiple sample processor. Cell Immunol. 4: 182-186. 1972.
15. **HOWIWITZ, W.** Official Methods of Analysis of the AOAC. pp. 462-480. Twelfth Edition. Washington, D.C.: Association Official Analytical Chemists. 1975.
16. **LATNER, A.L.** Clinical Biochemistry. pp. 558-571. Philadelphia: W.B. Saunders Co. 1975.
17. **MICHAEL, G.Y., P. THAXTON** and **P.B. HAMILTON.** Impairment of the reticuloendothelial system of chickens during aflatoxicosis. Poult. Sci. 52: 1206. 1973.
18. **MILLER, D.M., B.P. STUART, W.A. CROWELL, J.R. COLE, A.J. GOVEN** and **J. BROWN.** Aflatoxicosis in swine: Its effect on immunity and relationship to salmonellosis. Am. Ass. vet. lab. Diag. 21: 135-146. 1978.
19. **MONEGUE, H.J.** Effects of Aflatoxin in Young Swine. pp. 1-78. M.S. Thesis, University of Florida, Gainesville. 1977.
20. **MORRISON, F.B.** Editor. Feeds and Feeding. pp. 136-147, 492-505. Iowa: Morrison Pub. Co. 1961.
21. **NAFTALIN, L., V.J. CHILD** and **D.A. MORLEY.** Observations on the site of origin of serum gammaglutamyltranspeptidase. Clinica chim. Acta 26: 297-300. 1969.
22. **NEWBERNE, P.M.** Chronic aflatoxicosis. J. Am. vet med. Ass. 163: 1262-1269. 1973.
23. **NEWBERNE, P.M.** and **W.H. BUTLER.** Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals. Cancer Res. 29: 236-250. 1969.
24. **PAUL, P.S., D.W. JOHNSON, C.J. MICROCHA, F.F. SOPER, C.O. THOEN, C.C. MUSCOPLAT** and **A.F. WEBBER.** *In vitro* stimulation of bovine peripheral blood lymphocytes: Suppression of phytomitogen and specific antigen lymphocyte responses by aflatoxin. Am. J. vet. Res. 38: 2033-2035. 1977.
25. **PIER, A.C.** Effects of aflatoxin on immunity. J. Am. vet. med. Ass. 163: 1268-1269. 1973.

26. **PIER, A.C., R.E. FICHTNER** and **S.J. CYSEWSKI.** Effects of aflatoxin on the cellular immune system. Ann. Nutr. Aliment. 31: 781-788. 1977.
27. **RICHARD, J.L.** and **J.R. THURSTON.** Effect of aflatoxin on phagocytosis of Aspergillus fumigatus spores by rabbit alveolar macrophages. Appl. Microbiol. 30: 44. 1975.
28. **RICHARD, J.L., J.R. THURSTON** and **A.C. PIER.** Effects of mycotoxins on immunity. In Toxins: Animal, Plant and Microbiol. P. ROSENBERG, editor. pp. 801-817. New York: Pergamon Press. 1978.
29. **SAVEL, H., F.B. SCHAEFFER** and **T. CARDELLA.** Effect of aflatoxin B_1 upon photohemagglutinin transformed human lymphocytes. Proc. Soc. exp. Biol. Med. 134: 1112-1115. 1970.
30. **SISK, D.B., W.W. CARLTON** and **T.M. CURTIN.** Experimental aflatoxicosis in young swine. Am. J. vet. Res. 29: 1591-1602. 1968.
31. **STEEL, R.G.** and **J.H. TORRIE.** Principles and Procedures of Statistics. New York: McGraw-Hill Book Co. 1960.
32. **STUART, B.P., J.R. COLE** and **L.T. SANGSTER.** Aflatoxicosis: Its association with salmonellosis in south Georgia swine. Abstract #108. Scient. Proc. a. Meet. Am. vet. med. Ass. Dallas, Texas. 1978.
33. **SUNDERMAN, F.W.** Studies of the serum proteins: VI. Recent advances in clinical interpretation of electrophoretic fractionations. Am. J. clin. Path. 42: 1-21. 1964.
34. **TROUTT, H.F.** The Effects of Sublethal, Whole Body, Gamma Irradiation and Salmonella choleraesuis Infection in Swine with Special Reference to Histochemical Alterations in the Distribution of Succinic Dehydrogenase and Acid Phosphatase in the Ileum and Colon. Ph.D. Thesis, University of Missouri, Columbia. 1970.
35. **WYLLIE, T.D.** and **L.G. MOREHOUSE** (Eds.). Mycotoxicosis in swine. *In* Mycotoxic Fungi, Mycotoxins, Mycotoxicosis. An Encyclopedic Handbook. pp. 217-277. New York: Dekker. 1978.