IMMUNOPATHOLOGICAL RESPONSE OF PIGS IN AFLATOXICOSIS

By

M. GOPALAKRISHNAN NAIR

THESIS

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DECLARATION

I hereby declare that this thesis entitled IMMUNO-PATHOLOGICAL RESPONSE OF PIGS IN AFLATOXICOSIS is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate-ship, fellowship, or other similar title, of any other University or Society.

Signature of the candidate : M Gopalakrishnan Nair

Name of the candidate : M. Gopalakrishnan Nair

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CERTIFICATE

Certified that this thesis entitled IMMUNOPATHOLOGICAL RESPONSE OF PIGS IN AFLATOXICOSIS is a record of research work done independently by Sri. M. Gopalakrishnan Nair under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

Name of the Guide : Dr. K.I. Maryamma
(Chairman, Advisory Board)

Designation : Professor,
Department of Pathology.

Mannuthy,
7-10-'86.

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DEDICATED TO MY BELOVED
PARENTS AND BROTHERS

CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

Fig. No.

Fig. No.

27 Liver - Experimental aflatoxicosis - Bile duct hyperplasia and focal lymphocytic accumulation
H & E x 250

28 Gall bladder - Experimental aflatoxicosis - Oedema and lymphocytic infiltration
H & E x 250

29 Spleen - Experimental aflatoxicosis - Haemorrhage
H & E x 250

30 Kidney - Experimental aflatoxicosis - Degeneration of the lining epithelium of the proximal convoluted tubules and formation of hyaline casts
H & E x 400

31 Hepatic lymphnode - Experimental aflatoxicosis - Haemorrhage and haemosiderosis
H & E x 250

32 Hepatic lymphnode - Experimental aflatoxicosis - Depletion of lymphocytes and dilatation of lymphatics
H & E x 250

33 Mesenteric lymphnode - Experimental aflatoxicosis - Reactive lymphnode - Diffuse hyperplasia of lymphoid tissue
H & E x 160

34 Thymus - Petechiae and echymotic patches, oedema

35 Thymus - Haemorrhage and lymphoid cell depletion
H & E x 250

36 Thymus - Degeneration of Hassal's corpuscles and haemorrhage
H & E x 250

Introduction

INTRODUCTION

Mycotoxins are fungal metabolites which have been known for many years but their significance in the causation of disease and their role as a major factor in causing disease was not known till recently. Epidemics of dry gangrene and nervous derangement which were popularly known as 'St. Anthony's Fire' caused by the ingestion of sclerotia of Claviceps purpurea swept Europe from the 11th through the 16th centuries. This important disease syndrome drew attention of the people all over the world and was a well recognised disease entity of mycotoxic origin. However, the recent interest in mycotoxicosis was initiated following the death of large number of turkeys in Great Britain in 1960 due to consumption of peanut meal contaminated with aflatoxin. Since then extensive work has been done on the various aspects of aflatoxin on different species of animals and voluminous literature has accumulated on aflatoxicosis during the last two decades. Now it has emerged out as a factor which accounts directly or indirectly for many of the diseases in domestic animals of hitherto ill defined actiology.

Although, various aspects of aflatoxicosis have been studied and vast amount of information has been gathered, the real significance of the problem has not been recognised by the personnel directly engaged in livestock production programmes. As in the case of an ice berg where we see only the small portion above the surface, only the acute toxicity induced

by this toxin has been appreciated but the lower massive part of the ice berg which as far as this toxin is concerned is the chronic toxicity due to continued ingestion of low levels of toxin remains obscure to a great extent. The adverse effects of chronic toxicity on the economic livestock production programmes appears to be tremendous. This aspect remains to be assessed and it perhaps awaits the development of better diagnostic criteria and wide recognition of the condition.

The toxigenic fungi are ubiquitous in distribution in the environment and their toxin production is determined by variety of factors. The unfavourable storage conditions of the feed particularly in an environment with high humidity as in Kerala which is coastal in geographical lay out and the heavy rainfall in this region favours mold growth and toxin production in feed commodities. This perforce leads to exposure of livestock to mycotoxins through the contaminated feed. However, their significance has not been precisely assessed and its impact on the livestock farming has not been accurately evaluated.

The limited studies undertaken in the Department of Pathology, Veterinary College, Mannuthy, during the last few years have shown that aflatoxicosis is an important problem, particularly in the case of pigs, which are relatively very susceptible to this toxicity when compared to other species of livestock and in most of the pig farms in Kerala it has choked the production potential of pigs. The mortality pattern of pigs recorded and the supportive evidence of aflatoxin induced

hepatopathy are proofs to this valid conclusion. In the investigations undertaken so far the effect of low doses of aflatoxin has not been taken into consideration. This aspect cannot be overlooked and warrants investigation.

The enquiries undertaken on the biological effects of aflatoxin in different species of animals all over the world have categorically shown that aflatoxin in higher doses causes acute hepatitis, haemorrhagic syndromes and death. The wide spectrum of changes in low doses have also demonstrated the teratogenic, mutagenic and carcinogenic effects. Besides this, other notable effects of continued consumption of low doses of aflatoxin are an alteration of serum protein profile and adverse effects on the immune system. It has been documented in certain species of animals that the immunosuppressive effect of the toxin would lead to acquired immunodeficiency and this perforce leads to disease outbreaks. It is well-known that it is the immunological background of the animal that determines the emergence of disease syndromes and if the problem is viewed against this background, it has tremendous impact on the disease prevalence in the animal population in general and pigs in particular since they are very susceptible to this toxin.

Considering these aspects a project to study the immune response of pigs in aflatoxicosis was designed and executed and the results obtained during the course of this investigation have been documented in the ensuing pages.

Review of Literature

REVIEW OF LITERATURE

2.1. Aflatoxicosis in pigs

Aflatoxins, a group of closely related metabolites of Aspergillus flavus, Aspergillus parasiticus and Penicillium rubrum, are well known potent hepatotoxins possessing carcinogenic, mutagenic and teratogenic activities. These toxins occur as natural contaminants in animal feeds.

2.1.1. Incidence of spontaneous cases of aflatoxicosis.

Several confirmed instances of aflatoxicosis in pigs were reported under field conditions.

A disease in pigs caused by consumption of moldy corn was reported by Sippel et al. as early as 1953. Loosmore and Harding (1961) described an episode of toxicity in which peanut meal was identified as the causative component in the diet of pigs. Following this report, Hornby et al. (1962) observed retarded growth in animals which consumed toxin contaminated feed. Gibbons reported moldy corn poisoning in pigs in 1965.

Incidence of toxicity in pigs due to aflatoxin was reported by Wilson et al. (1967). When fed toxic Penicillium rubrum culture, pigs showed severe haemorrhagic gastro-enteritis with death occurring more quicker than Aspergillus flavus cultures.

Smith et al. (1976) reported 94 cases of aflatoxicosis in pigs over 22 months period in North Carolina. The aflatoxin

B_1 concentration in the corn ingredient of the feed in these cases was 5.18 mg/kg.

Hayes et al. (1978) found aflatoxin B_1 in the plasma of three of the nine pigs examined, the values ranging from 5.1 to 36.7 ng/ml. Though it was not detected in the kidneys, 0.12 ng/g was found in the pale yellow liver of one pig. During the period 1965-1968 at the piggery farm attached to the Veterinary College, Mannuthy, out of the 815 pigs died, 85% of them showed varying degree of liver damage and aflatoxin was attributed as the cause (Rajan et al. 1981). The authors also indicated contamination of feed with aflatoxin to be a possible cause of ethmoid tumor in pigs.

Sriramamurthy et al. (1981) recorded an outbreak of acute aflatoxicosis in Large White Yorkshire pigs at Tirupathi. The suspected feed contained 2.4 ppm of aflatoxin B_1. The affected pigs recorded lowered feed consumption and weight gain. Discontinuance of the incriminated feed and supplementation of Liv. 52 controlled the outbreak. Aflatoxin B_1 at 500 µg/kg was found in the stomach contents of 80 pigs died of peracute toxicity on consumption of moldy bread (Ketterer et al. 1982). Anorexia and unthriftiness of growing animals were seen in chronic toxicity.

Dhanvantari et al. (1982) recorded a natural outbreak of aflatoxicosis in Large White Yorkshire pigs. The offending groundnut cake contained 16 ppm of aflatoxin and the grower

ration on fresh basis contained 2.4 ppm of aflatoxin. The morbidity and mortality rates were 18.13% and 10.36% respectively. The severity of the lesion was more in growers and among-growers it was particularly more severe in males.

Feed samples containing more than 360 ppb of aflatoxin were found to be the cause of mortality and unthriftiness in 54 herds during a three year period (Wilson et al. 1984).

Chauhan et al. (1984) reported twenty cases of hepatocellular carcinoma in pigs and attributed groundnut cake having an average contamination level of 1 mg/kg as the cause.

Dykes (1986) observed aflatoxin B_1 at high concentration (2000 ppb) in the feed of a pig herd. The affected animals had severe sarcoptic mange infection and did not respond to an aggressive treatment protocol for the same. Clinically the animals exhibited muscular weakness, depression, pneumonia, rectal bleeding and terminal convulsions. The study demonstrated the multitude of consequences and immuno-suppressive effects of aflatoxin.

2.1.2. Experimental studies.

2.1.2.1. LD_{50} of aflatoxin B_1 in pigs.

Jones and Jones (1969) reported the LD_{50} for pigs as 0.6 mg/kg body weight. According to several research sources, the acute LD_{50} of aflatoxin B_1 in young pigs was 0.52 mg/kg body weight and in older swine 1.0-2.0 mg/kg body weight (Hatch, 1982).

2.1.2.2. Susceptibility of pigs to aflatoxin.

Dietary concentrations of aflatoxins that caused toxicosis (liver damage and death) in swine were: growing pigs - 0.41 µg/g or more for 12-24 weeks, pregnant sows - 0.3-0.5 µg/g for four weeks (Edds, 1973; Ciegler, 1975; Buck et al. 1976).

Earlier research on porcine aflatoxicosis indicated that this species was more susceptible at an younger age of 1-4 weeks. Pregnant sows were more susceptible than mature cattle and sheep (Edds and Osuna, 1976; Edds, 1979).

2.1.2.3. Effect of aflatoxin on the growth rate and feed efficiency of pigs.

A number of reports and reviews describing the dietary effects of graded levels of aflatoxin on pigs indicated no acute toxic effects below 700 ppb. Consumption of rations containing upto 690 ppb aflatoxin did not seem to affect the performance of pigs during the finishing period when they gained weight from 120 to 200 lbs (Duthie et al. 1966), but substantial improvement occurred in the growth rate when they were placed on a toxin free diet. Hintz et al. (1967a) conducted feeding trials on 12-14 week old pigs with rations containing 10 to 1550 ppb aflatoxin and found that levels above 450 ppb adversely affected the weight gain and feed conversion. Similar observations were made by Gagne et al. (1968). However, toxic concentrations exceeding 280 ppb caused depression in growth rate and feed conversion efficiencies of growing pigs (Sisk et al. 1968; Allcroft, 1969; Armbrecht et al. 1971).

Studies of Keyl and Booth (1971) indicated that even higher levels (450 ppb and above) were required to depress the growth rate and feeding efficiencies of growing pigs.

Inclusion of aflatoxin B_1 at a level of 450 ppb in the ration of 5.5-7.7 kg pigs for a 28 day feeding period produced significantly lower daily gains than a control group on standard ration and returning the treated group to a normal ration resulted in their resuming a normal growth curve (Neufville, 1974).

Iwasaki et al. (1974) fed diet containing 0.55-0.6 ppm aflatoxin B_1 for 122 days to four miniature pigs and found transient inappetence and slight retardation of growth. Monegue (1977) compared average daily gain in two groups of seven pigs each of Duroc-Yorkshire x Hampshire barrows and gilts receiving either a control ration or a ration naturally contaminated with aflatoxin B_1 at 400 ppb level and found significant growth suppression in pigs which consumed aflatoxin. It was also observed that levels of 100, 200 and 300 ppb of aflatoxin B_1 in the ration did not significantly affect average daily gains, feed consumption or efficiency.

Cysewski et al. (1978) observed lesser weight gain in pigs fed aflatoxin B_1 at 1.3 mg in the feed for 25 days. Impaired growth rate and feed conversion were evident in pigs fed diet containing 0.5 mg/kg of aflatoxin B_1 (Armbrecht, 1978). Average daily gain was reduced linearly in pigs receiving

diets containing 385 ppb and higher levels of aflatoxin but the feed efficiency was significantly influenced only when 1,480 ppb aflatoxin was fed (Southern and Clawson, 1979).

Miller et al. (1981) observed decreased feed conversion efficiency in piglings fed diets containing 0.4 µg/g and 0.8 µg/g of aflatoxin respectively.

Osuna and Edds (1982) made similar observations in pigs fed 0.2 mg/kg body weight of aflatoxin B_1 for 10 days.

Ho (1982) reported loss of appetite and decreased weight gain in pigs fed crude aflatoxin at 90 µg/kg body weight for six weeks. Considerable reduction in weight gain occurred in pigs fed ration containing 400 ppb aflatoxin B_1 (Wu et al. 1982) and in pigs fed 0.15 mg/kg body weight of crude aflatoxin (Vishalakshan et al. 1984).

Tapia and Seawright (1985) observed significant decrease in the growth rate and feed consumption in young pigs fed dietary aflatoxin at 0.750 mg/kg feed.

2.1.2.4. Haematology and clinical chemistry.

Early work by Harding et al. (1963) showed that four to six week old pigs fed on a toxic groundnut meal, which presumably was contaminated with aflatoxin, responded with changes in the levels of serum enzymes and liver composition but the aflatoxin content of this meal stands unspecified. Besides, with dietary aflatoxin levels of 750 ppb or more, alterations in the electrophoretic patterns of pig serum

proteins indicated a relative decrease in the albumin, alpha$_1$, alpha$_2$ and beta globulin levels and considerable increase in the gamma globulin level (Annau et al. 1964). Cysewski et al. (1968) produced acute aflatoxicosis in young pigs having body weights ranging from 11.4-21.4 kg, by giving aflatoxin B_1 at 1.98 mg/kg body weight. The pigs showed depression and initial pyrexia followed by subnormal temperature. Shivering, muscular tremors and weakness were noticed from the sixth hour onwards at irregular intervals. Fresh blood appeared in the faeces after 24 hours. Altered liver function was detected after three hours and was marked at six hours. SGOT and SGPT activities were elevated markedly after 6-9 hours. The packed cell volume remained normal until 12 hours before death after which it declined. Leukocyte counts and prothrombin time were elevated after 12-24 hours. Differential leukocyte count indicated neutrophilia and lymphopenia. Significant decrease in all the components of protein with maximum decrease of β-globulins was observed.

Sisk et al. (1968) produced experimental aflatoxicosis in two week old pigs by the daily administration of aflatoxin B_1 for 23 days at dose levels of 83.4, 166.8 and 333.6 µg/kg body weight. The smallest dose did not produce clinical evidence of disease in the pigs, but mild hepatic changes were observed microscopically. The medial dose level (166.8 µg/kg) was obviously toxic to young pigs, and the lesions were similar to but less severe, than those observed in pigs administered

with the largest dose level. The largest dosage resulted in depression, anorexia, haemoconcentration and icterus.

Alterations in liver function, as represented by changes in blood and liver enzymes, occurred in pigs after consumption of feed containing as little as 51 ppb aflatoxin B_1. Along with this, plasma albumin, plasma-albumin-globulin ratio, non-protein nitrogen and urea nitrogen were decreased (Gumbmann and Williams, 1969).

Hauser et al. (1971) reported haemorrhagic syndrome in pigs which consumed feed containing 150 ppb of aflatoxin B_1. The routine handling of the pigs led to massive subcutaneous haemorrhage. Addition of Vitamin K at the rate of 22 mg/kg body weight reversed the prothrombin times to normal and haemorrhages ceased.

Young pigs exposed to aflatoxin at 450 ppb level in the ration for 28 days exhibited a significant increase in prothrombin time (Neufville, 1974).

A carcinogenic response in brood sows was observed by Shalkop and Armbrecht (1974) when the sows were fed aflatoxin B_1 and G_1 at the dose rates of 31 µg, 10 µg and 40 µg per kilogram body weight daily during growth, gestation and lactation periods respectively. Both four and ten week old pigs, when fed with rations containing high amounts of proteins (17-20.6%) vs low concentrations (11.4-14%), showed stunting, icterus and haemorrhage, when exposed to 95 and 99 µg/kg body weight of

aflatoxin (Edds and Osuna, 1976). In pigs fed with 1.3 mg of aflatoxin B_1 in the diet for 25 days, Cysewski et al. (1978) did not observe any change in the plasma activity of SGPT, SGOT levels and prothrombin times. The total plasma protein, alpha and beta globulin levels decreased while the gamma globulin level showed an increase. These changes were not statistically significant. Only the albumin value in aflatoxin fed pigs expressed a significant difference from that of the normal.

Drabek et al. (1979) observed that ingestion of 5.4-10.5 mg/kg live weight of aflatoxin B_1 caused drowsiness and apathy within three hours and death within 12-20 hours. A dosage of 1.4-3.1 mg/kg body weight of the toxin resulted in death of the animals within 3-26 days.

In experiments conducted by Southern and Clawson (1979), in pigs fed 750 ppb and above dietary levels of aflatoxin, no significant effect was noticed on the total serum proteins, albumin and IgG fraction but the IgM fraction was found to be increased.

Miller et al. (1981) observed a decrease in haematocrit value and haemoglobin level in pigs fed diet containing 0.4 µg/g and 0.8 µg/g aflatoxin respectively for ten weeks. Though Salmonella cholerae suis var kuzendorf was inoculated on the third week of the experiment, no significant effect was observed on the total leukocyte count. Serum levels of GGT, ALP and AST were higher in aflatoxin fed pigs. The total

protein, albumin, alpha and beta globulins and the albumin: globulin ratios decreased while the gammaglobulin level increased. The pigs inoculated with Salmonella had a higher gammaglobulin level.

Aflatoxin B_1 when fed daily to young pigs at a dose rate of 0.2 mg/kg body weight for ten days, significant increase in the haemoglobin levels and erythrocyte count was observed from the sixth day and this was followed by an abrupt drop in haemoglobin, PCV and RBC values by the tenth day of the experiment. Liver specific enzyme levels were increased after 48 hours. The total plasma protein and all the protein fractions were significantly reduced (Osuna and Edds, 1982). Ho (1982) fed pigs crude aflatoxin B_1 and G_1 daily at dosages of 23, 44 and 90 µg per kilogram body weight for six weeks and observed that in the higher dosage groups there was yellowish discolouration of urine within three weeks. Pigs that died manifested jaundice and rectal haemorrhage. The levels of SGPT, SGOT, LDH, ALP and bilirubin showed an increase in the group given the highest dosage whereas the serum cholesterol, triglycerides, total lipids, albumin, globulin and total plasma protein levels decreased during the latter part of the experiment.

Miller et al. (1982) recorded increase in isocitric dehydrogenase, alkaline phosphatase, sorbitoldehydrogenase and aspartoaminotransferase activity in six of the eight pigs orally dosed with aflatoxin at 1.2 mg/kg body weight. There

was significant increase in gamma glutamyl transpeptidase activity.

Vishalakshan et al. (1984) did not observe any significant alteration in the total erythrocyte count, haemoglobin, packed cell volume, erythrocyte sedimentation rate and total leukocytic values in adult pigs fed daily with crude aflatoxin at 0.15 mg/kg body weight for three months but a variation in the absolute count of lymphocytes was observed. Dietary aflatoxin at levels of 0.375 mg/kg and 0.750 mg/kg fed to young pigs did not alter the levels of serum protein, bilirubin, SGPT, alkaline phosphatase, gammaglutamyl transferase and urinary gammaglutamyl transferase (Tapia and Seawright, 1985).

2.1.3. An overview of aflatoxicosis in pigs in India.

2.1.3.1. Spontaneous cases.

Aflatoxicosis in pigs was encountered for the first time in Kerala during the period 1965-1968 in the piggery farm attached to the Veterinary College. The groundnut cake fed to the pigs which contained high level of aflatoxin was incriminated as the cause for liver damage (Rajan et al. 1981). They also attributed aflatoxin as one of the important etiological factor associated with sinus tumour in pigs. Outbreak of acute aflatoxicosis in pigs in Tirupathi was recorded by Sriramamurthy et al. (1981).

Dhanvantari et al. (1982) made clinical observations on a natural outbreak of aflatoxicosis in Large White Yorkshire pigs.

Chauhan et al. (1984) recorded twenty cases of hepatocellular carcinoma in pigs.

2.1.3.2. Experimental studies.

An assessment of the cell-mediated immuno response of adult pigs in subacute aflatoxicosis was made by Vishalakshan et al. (1984).

2.1.4. Pathology of aflatoxicosis in pigs.

In acute cases normal handling of the pigs led to massive subcutaneous haemorrhage (Hauser et al. 1971). Gross haemorrhage occurred in many parts of the body, especially in the ham areas. The increased pressure in the gluteal muscles led to ataxia, and animals presented a dog like sitting position with tachypnoea and panting (Edds, 1979).

2.1.4.1. Acute toxicity:

2.1.4.1.1. Liver.

Grossly, the liver was enlarged, icteric and contained petechiae. Liver lesions and increase in the liver weights indicated toxin stress. The gall bladder was in some cases atrophic (Burnside et al. 1957; Loosmore and Harding, 1961; Annau et al. 1964; Wilson et al. 1967; Keyl et al. 1968; Gagne et al. 1968; Keyl and Booth, 1971; Osuna et al. 1977; Edds, 1979; Lu and Ho, 1982; Osuna and Edds, 1982). Microscopically, early liver changes were seen in about three hours (Patterson, 1973). Disorganization of hepatocytes with fatty changes occurred. By six hours, the cells were swollen and

centrilobular congestion and necrosis were evident. These changes were accompanied by karyorrhexis and pyknosis. Infiltration by neutrophils and lymphocytes occurred by 12 hours in the necrotic areas. Congestion continued to increase and was accompanied by leukocyte infiltration. Bile duct hyperplasia and bile casts in canaliculi were also evident. Those changes were in accordance with the observation made by Sippel et al. (1953); Burnside et al. (1957); Loosmore and Harding (1961); Allcroft et al. (1961); Harding et al. (1963); Wilson et al. (1967); Armbrecht (1978); Miller et al. (1982).

Lower dosages extended over a few weeks produced many of the features described. However, reticulum and collagen fiber proliferation and bile duct hyperplasia were observed along with intra cellular glycogen depletion and lipid infiltration. These reactions required several days to take place (Shalkop et al. 1967; Sisk et al. 1968).

2.1.4.1.2. Kidney.

Increase in the weight, swelling and haemorrhage were reported by Burnside et al. (1957); Keyl et al. (1968); Lu and Ho (1982).

2.1.4.1.3. Spleen.

Engorgement of the spleen occurred in acute cases. Microscopical examination revealed lymphoid depletion (Burnside et al. 1957; Wilson et al. 1967).

2.1.4.1.4. Lymphnodes.

Congestion and oedema of the lymphnodes were reported by Burnside et al. (1957).

2.1.4.1.5. Gastro-intestinal tract.

Haemorrhagic gastroenteritis with occasional erosions and ulceration was reported by Burnside et al. (1957).

2.1.4.1.6. Peritoneal cavity.

Amber coloured fluid in moderate quantities was observed by Burnside et al. (1957); Loosmore and Harding (1961).

2.1.4.1.7. Heart.

Hydropericardium with accumulation of an amber coloured fluid associated with petechiae and icterus of the heart muscle were observed by Burnside et al. (1957); Loosmore and Harding (1961).

2.1.4.1.8. Thyroid.

Increased thyroid body weight ratio and histologically, evidence of thyroid hypoplasia were recorded (Loosmore and Harding, 1961; Wilson et al. 1967).

Petechial haemorrhages were found in the fat, muscle, and subcutaneous tissue (Armbrecht, 1978).

2.1.4.2. Chronic toxicity.

2.1.4.2.1. Liver.

Grossly, the liver developed a hard fibrous texture and the entire organ was dark yellow with scattered raised brown lumps and coarse nodularity. In earlier stages, the gall bladder of some animals was distended. In advanced cases, the gall bladder was atrophic and the wall was oedematous.

The bile was sometimes dark and had a 'thick' consistency (Wilson et al. 1967; Iwasaki et al. 1974; Armbrecht, 1978). Microscopically, the kind of liver lesion and its degree of extensiveness were dependent on the time and dosage rate relationship that preceeded the examination. There was pronounced centrilobular necrosis. The cytoplasm of the cells was granular and vacuolated or completely absent. There was pronounced karyomegaly of the surviving hepatic cells. Increased proliferation of reticular fibres, pseudolobulation with regenerating islands of hepatic cells, bile duct proliferation and progressive increase in collagen fibres were also evident. Most of the regenerating cells contained neutral fat globules. As the lesion progressed there was numerous foci of lymphocytes and few eosinophils and large nodules of regenerating liver cells with a collagen capsule extending throughout the parenchyma and above the liver surface (Gippel et al. 1953; Burnside et al. 1957; Loosmore and Harding, 1961; Harding et al. 1963; Shalkop et al. 1967; Sisk et al. 1968; Armbrecht et al. 1971; Miller et al. 1981). Hepatocellular carcinoma was observed by Shalkop and Armbrecht (1974) and Chauhan et al. (1984).

2.1.4.2.2. Kidney.

Grossly, petechiae or linear haemorrhages were evident in the cortex or at the cortico-medullary junction. Icteric fluid was sometimes found in the pelvis. Microscopically, karyomegaly and vacuolar degeneration of uriniferous tubules

were observed (Wilson et al. 1967; Armbrecht, 1978). In some cases metastatic nodules of hepatocellular carcinoma were seen (Chauhan et al. 1984).

2.1.4.2.3. Spleen.

Metastatic lesions of hepatocellular carcinoma were observed (Chauhan et al. 1984).

2.1.4.2.4. Lymphnodes.

In short term cases (less than one year), congestion and haemorrhages were found in the hepatic lymphnodes. In protracted cases (over two years) metastases of malignant cells from the liver were occasionally seen (Armbrecht, 1978; Chauhan et al. 1984).

2.1.4.2.5. Gastrointestinal tract.

Depending on prior dosage, haemorrhages were seen. Metastatic seeding of hepatocellular carcinoma occurred in the omentum (Armbrecht, 1978).

2.1.4.2.6. Peritoneal cavity.

Metastatic lesions of hepatocellular carcinoma were observed by Chauhan et al. (1984).

2.1.4.2.7. Thyroid.

Increased thyroid body weight ratio was observed by Loosmore and Harding (1961) and Wilson et al. (1967).

2.1.4.2.8. Adrenal gland.

A hyperplastic response was observed, depending, in part, on the prior exposure rate (Armbrecht, 1978).

2.2. Immune response of different species to aflatoxins

Aflatoxin exerts an effect on resistance to infection and development of acquired immunity. The fact that aflatoxin binds DNA, thus inhibiting protein formation, attracted a number of people to study its effect on immunity and resistance.

2.2.1. Effect of aflatoxin on the humoral immune response.

2.2.1.1. Laboratory animals.

The first demonstration of the effect of aflatoxin on antibody formation was in mice in which typhoid agglutinin titres were low after subcutaneous injection of aflatoxin (Galikeev et al. 1968). Lowered titres occurred when vaccination began before aflatoxin administration; however, no antibody formation or minimal titres occurred when aflatoxin administration preceeded vaccination or if aflatoxin and vaccine were administered simultaneously. In this study, the quantity of aflatoxin administered was not given. Thurston et al. (1974) observed that oral dosing of guinea pigs with 0.1 mg and 0.3 mg aflatoxin B_1 daily for four weeks had no effect on the antibody production in Brucella abortus vaccinated animals, however they had lesser weight gain than their controls. Splenic atrophy with nearly complete disappearance of Malpighian corpuscles was observed in rats which were fed aflatoxin (Franco et al. 1981). The primary and secondary antibody response were found to be suppressed in adult male mice given 0, 30, 145 or 700 µg/kg body weight of aflatoxin B_1 orally (Reddy et al. 1983).

2.2.1.2. Turkey.

In situations uncomplicated by disease some investigators have used different antigens to assess the effect of aflatoxins on antibody production. According to observations made by Pier and Heddleston (1970), there was no diminution in antibody response to Pasteurella multocida in turkeys fed 0.25-0.5 ppm of aflatoxin B_1. Pier et al. (1971) observed no change in antibody level in turkeys given New Castle Disease virus vaccine before, during or after 0.5 ppm aflatoxin consumption, but a lag in interferon production was noted during the first 24 hours in aflatoxin fed birds. Density gradient turkey serum fractions did not show qualitative or quantitative antibody differences but impaired resistance to Pasteurella multocida infection in turkeys vaccinated against fowl cholera was not necessarily associated with antibody, as the deficit could be overcome by giving vaccinated birds either normal or immune serum prior to challenge inoculation (Pier et al. 1972). Tests used to measure antibody production in these studies were indirect haemagglutination, agglutination, complement fixation and precipitation.

In a single study in which a mycotic agent, Aspergillus fumigatus, was used to infect turkeys fed either normal feed or feed containing 0.5 ppm aflatoxin, no impairment in precipitin production was noted; in fact, 4 of 12 birds on aflatoxin diet had precipitins to Aspergillus fumigatus, but none of the 13 birds fed normal feed had precipitins (Richard et al. 1973).

2.2.1.3. Chicken.

Aflatoxin caused a highly significant and dose dependent reduction in circulating antibodies in broiler chicken (Thaxton and Hamilton, 1971). Adinarayaniah et al. (1973) observed no measurable effect on antibody production against Salmonella pullorum antigen in chicken fed 15 µg aflatoxin B_1 daily for 73 days.

In chicken fed diets containing 0.625-10 ppm aflatoxin and given an intravenous injection of sheep red blood cells, there was a lag in antibody production during the first nine days after antigen administration (Thaxton et al. 1974). However, by the twelfth and fifteenth day after antigen injection, antibody titres of the birds fed the higher levels of aflatoxin were greater than those of the controls. The relative size of the bursa of Fabricius was reduced by 30% in birds fed 10 ppm aflatoxin. Similar results were obtained in chicken fed 10 ppm aflatoxin and infected with one of several species of Salmonella (Boonchuvit and Hamilton, 1975). In these studies aflatoxin fed throughout the experimental period, caused a dramatic increase in antisalmonella agglutinins in infected birds. Tung et al. (1975) recorded suppression of the IgG fraction chicken fed aflatoxin at dose levels of 2.5-10 µg/g of feed, but the IgM level was normal.

Significant decrease in the concentration of serum IgG and IgA but not IgM occurred in chicks fed dietary aflatoxin

at 2.5 µg/g from hatching to four weeks or between two and four weeks of age (Giambrone et al. 1978).

The formation of antibodies in chicks against New Castle Disease vaccine was adversely affected at 0.2 ppm dietary aflatoxin (Mohiuddin et al. 1981). The HI titre to New Castle Disease was decreased in layers fed aflatoxin (Boulton et al. 1982).

Rajasekhara Reddy et al. (1982) employed larger doses of aflatoxin (1-4 ppm) in chicks and bursal regression was observed at the dose rate of 1.25 ppm and above.

Aflatoxin (2.5 µg/g) along with ochratoxin (2 µg/g) caused a significant reduction in the relative weight of the bursa of Fabricius (Campbell et al. 1983).

2.2.1.4. Cattle.

Aflatoxin B_1 concentration ≥ 10 µg/ml significantly suppressed the lymphocyte responsiveness to poke weed mitogen in vitro, thereby inhibiting the humoral immunity (Paul et al. 1977) whereas aflatoxin was not found to exert any effect on the antibody production in steers fed naturally contaminated maize containing 800 ng aflatoxin per gram feed, for 17.5 weeks (Richard et al. 1983).

2.2.1.5. Goat.

The humoral immunity was lowered in goats fed aflatoxin B_1 and this rendered them more susceptible to rhinitis and

pneumonia (Miller et al. 1984). The gammaglobulin levels were found to be higher in the aflatoxin fed goats.

2.2.1.6. Pigs.

Miller et al. (1978) reported that the humoral immunity was lowered in pigs fed aflatoxin and they were more susceptible to salmonellosis.

2.2.2. Effect of aflatoxin on phagocytosis.

The role of the cells of the mononuclear phagocyte system is not completely understood (Van Furth, 1970). Since aflatoxin decreased complement activity and complement is involved in the opsonization process an effect of aflatoxin on phagocytosis by the mononuclear phagocyte system might be expected (Muller-Eberhard, 1970, 1971).

Michael et al. (1973) demonstrated an impairment of the mononuclear phagocyte system during aflatoxicosis in the chicken. Levels of 0, 0.625, 1.25, 2.5 and 5.0 µg of aflatoxin per gram of feed were given to chicken for three weeks. At the end of the experimental period, colloidal carbon was injected into the birds, and disappearance of the carbon was determined nephlometrically on blood samples obtained from the birds. The reduction in clearance was significant in those birds receiving 1.25 µg of aflatoxin per gram of feed whereas 5 µg of aflatoxin per gram of feed reduced the clearance to one-fourth of the control rate.

Richard and Thurston (1975) demonstrated a decrease in phagocytosis of Aspergillus fumigatus spores by alveolar macrophages from rabbits given aflatoxin. Rabbits were given daily doses of aflatoxin B_1 equivalents of 0, 0.03, 0.05, 0.07 and 0.09 mg for two weeks. Incorporation of serum from the rabbits given aflatoxin in the culture system resulted in less stimulation of macrophages from control rabbits when compared with the same system incorporating control serum. Stimulation of phagocytosis was least when both serum and macrophages from aflatoxin treated rabbits were used in the culture system. Chang et al. (1976) demonstrated an impairment of phagocytosis of Staphylococcus aureus by chicken heterophils in chicken given dietary aflatoxin at levels of 0.625 µg/g of feed and above. Chemotactic ability of leukocytes from chicken given 2.5 µg aflatoxin/g of feed was also inhibited.

Impairment of phagocytic activity of chicken monocytes in aflatoxicosis was also reported by Chang and Hamilton (1978).

2.2.3. Effect of aflatoxin on cellular immunity.

The thymus and thymus derived lymphocytes appear to be exceptionally sensitive to the effects of aflatoxins B_1 and M_1.

2.2.3.1. Mice.

Exposure of adult mice to aflatoxin B_1 at dose rates of 0, 30, 145 or 700 µg/kg body weight orally for 2-4 weeks resulted in suppression of lymphocyte blastogenesis at all levels tested (Reddy et al. 1983).

2.2.3.2. Guinea pig.

Chung et al. (1970) demonstrated delayed hypersensitivity reaction against aflatoxin B_1, B_2, G_1 and G_2 when administered intradermally. Significant suppression of cutaneous hypersensitivity to a Nocardia asteroides sensitin in guinea pigs fed 0.035 mg aflatoxin B_1 an in vitro reduction in migration inhibition factor with cultured peripheral blood leukocytes in aflatoxin recipients was reported by Pier (1981).

McLoughlin et al. (1984) observed reduction in the number of T lymphocytes in the peripheral blood of guinea pigs fed aflatoxin at 0.06 mg/kg body weight daily for three weeks.

2.2.3.3. Turkey.

Thymic involution occurred in turkeys fed a diet containing 0.5 ppm of aflatoxin B_1 for two weeks and this was likely to affect delayed hypersensitivity and graft versus host reactions (Pier et al. 1972).

2.2.3.4. Chicken.

Thymic involution to the extent of 55% occurred when birds were dosed with 0.625-10 ppm of aflatoxin (Thaxton et al. 1974).

The cell-mediated immunity was suppressed in chicken fed 2.5 µg/g aflatoxin in the diet from 2-4 weeks of age or from hatching to four weeks of age (Giambrone et al. 1978). The The authors also observed diminution in hypersensitivity skin

reaction of delayed type to tuberculin in chicken given aflatoxin from hatching to 7 weeks of age.

2.2.3.5. Cattle.

Aflatoxin concentration of ≥ 10 µg/ml significantly suppressed the lymphocyte response of cattle to phytomitogens *in vitro*. Lymphocyte response of *Mycobacterium bovis* infected animals to specific antigen PPD was significantly suppressed at aflatoxin concentration of 0.5 µg/ml. Fifty to 100 fold higher concentration of aflatoxins were required to produce 50% suppression of lymphocyte response to phytomitogens (PHA, Con A) as compared with that of PPD (Paul *et al.* 1977).

A waning of delayed cutaneous hypersensitivity reaction was observed by Richard *et al.* (1983), when steers were fed a ration containing 800 ng/g of aflatoxin.

Aflatoxin B_1 and Q_1 at concentration of 10 µg/ml strongly inhibited lymphoblastogenesis of bovine lymphocytes stimulated with PHA. Moderate to strong inhibition of blastogenesis by aflatoxin B_1 and its metabolites inhibited T lymphocyte function such as killer, helper, effector, or other immune processes and thus compromised the immunological surveillance mechanism (Bodine *et al.* 1984).

2.2.3.6. Horses.

Marked depletion of lymphocytes in the blood was observed in equine aflatoxicosis (Angsubhakorn *et al.* 1981).

2.2.3.7. Pigs.

Miller et al. (1978) studied the effect of aflatoxin on cellular immunity. It was demonstrated that pigs fed aflatoxin gave decreased response to mitogen by 3 H Thymidine uptake and macrophage inhibition. Decreased cellular response was shown to dermal challenge with Mycobacterium antigen.

Vishalakshan et al. (1984) assessed the cellular response of pigs fed aflatoxin at 0.15 mg/kg body weight for three months. It was observed that there was progressive reduction in the total lymphocyte count. The T lymphocyte count assessed by ANAE technique was found to be appreciably reduced by the 8th week.

2.2.3.8. Man.

Aflatoxin B_1 inhibited the activity of human blood lymphocyte cultures established with the addition of 0.1-200 µg of aflatoxin B_1/ml and stimulated with phytohaemagglutinin. Aflatoxin also decreased stimulation of lymphocytes by potent antigens such as tuberculin and mumps antigen (Savel et al. 1970).

2.2.4. Effect of aflatoxin on resistance to disease.

2.2.4.1. Chicken.

Brown and Abrahms (1965) consistently isolated Salmonella from cases of aflatoxicosis in poultry. Subsequent studies demonstrated that aflatoxin incorporated in the diet at 5 µg/g

had no significant effect on mortality in two different breeds of chickens given oral doses of Salmonella gallinarum (Smith et al. 1969).

The incorporation of aflatoxin at levels of 0.625 to 10 µg/g in the diet of chicken increased their susceptibility to Candida albicans infection (Hamilton and Harris, 1971). Enhanced infection was determined by measuring the relative crop weight of the birds. Tung et al. (1971) demonstrated that a smaller dose of 0.625 ppm of dietary aflatoxin could cause increased susceptibility to infections.

Increased susceptibility and mortality due to Eimeria tenella infection was found in chickens on diets containing 0.2 ppm of aflatoxin B_1 and 2.5 ppm aflatoxin (Edds et al. 1973; Wyatt et al. 1975; Edds and Simpson, 1976). Interaction between aflatoxicosis and natural infection with Salmonella worthington on weight gain was demonstrated (Wyatt and Hamilton, 1975). Increased mortality was reported in chicken fed aflatoxin at 10 µg/g of feed and infected with either one of several species of Salmonella which caused paratyphoid in chicken (Boonchuvit and Hamilton, 1975). Giambrone et al. (1978) reported increased incidence of Marek's disease in aflatoxin fed chicken.

2.2.4.2. Turkey.

Pier and Heddleston (1970) demonstrated impairment of acquired resistance to fowl cholera in turkeys fed dietary

aflatoxin at 0.25-0.5 ppm of B_1 equivalents either during or after the period of immunization to Pasteurella multocida. The birds did not have protection against challenge although birds on normal feed or fed aflatoxin before immunization were protected.

In studies on turkeys in which New Castle Disease Virus vaccine was used, there was no impairment of acquired immunity (Pier et al. 1971). Richard et al. (1973) observed no increase in susceptibility to aerosols of Aspergillus fumigatus in turkey poults fed a diet containing 0.5 ppm of aflatoxin B_1 equivalents. Although more birds on aflatoxin diet had visible airsac lesions, no differences were found between exposed birds on aflatoxin diet and those on normal diet upon histopathologic examination of lung lesions.

2.2.4.3. Hamster.

Aflatoxin added to the ration of hamsters did not increase their susceptibility to Mycobacterium paratuberculosis (Larsen et al. 1975).

2.2.4.4. Cattle.

In calves fed diet containing aflatoxin B_1 at 0.5-1.0 mg/kg body weight there was enhanced susceptibility to Fasciola hepatica infestation (Ddds and Osuna, 1976).

2.2.4.5. Goat.

The cell-mediated immune response was found to be suppressed in goats fed aflatoxin B_1 (Miller et al. 1984).

2.2.4.6. Pig.

Aflatoxin B_1 consumption (1.3 mg/pig/day) interfered with the development of immunity following vaccination with Erysipelothrix rhusiopathiae bacterin (Cysewski et al. 1978). Joens et al. (1981) observed that pigs fed aflatoxin at 0.07-0.14 mg/kg body weight had increased susceptibility to Treponema hyddysenteriae infection but aflatoxin did not affect the acquired immunity in pigs following recovery from swine dysentry.

Finishing swine had mange in spite of an aggressive treatment protocol for sarcoptic mange. The feed of these pigs was found to contain aflatoxin B_1 (2000 ppb), B_2 (225 ppb), G_1 (70 ppb) and G_2 (10 ppb). Many of them had subcutaneous abscesses and bronchopneumonia also (Dykes, 1986).

2.3. Assessment of immune response

2.3.1. Evaluation of humoral immune response.

2.3.1.1. Quantitation of gamma globulins in the serum.

The conventional methods to determine gammaglobulins in the serum were Ammonium sulphate test, specific method, sodium sulphate test, zinc sulphate turbidity test and Refractometer method (Patterson, 1967; McBeath et al. 1971). They found a high correlation between the values obtained by zinc sulphate turbidity test (ZST) and other methods to determine gammaglobulin in the serum.

A direct relationship between the values obtained by ZST and paper electrophoresis in determining the concentration of

gammaglobulin was recorded by Volvenko (1975). Barber (1976) compared the concentration of gammaglobulin obtained by various methods. He preferred ZST over other tests because of the accuracy, reliability, and simplicity in procedure.

Pfeiffer et al. (1977) compared single radial immunodiffusion, ZST, serum electrophoresis and Refractometer methods for quantitation of bovine immunoglobulins. Single radial immunodiffusion proved useful for quantification when either class or subclass information was needed. ZST measurements gave accurate results for total immunoglobulin and was found to be equally reliable as serum electrophoresis but quantitation of immunoglobulins from total proteins was not reliable. Nandakumar (1981) observed that ZST was useful in evaluating gammaglobulin in the serum of neonatal kids. Fivaz and Currel (1981) used ZST for quantifying total serum immunoglobulin in diagnosing hypogammaglobulinaemia in foals. A comparison of globulin levels measured by ZST method revealed a good correlation with single radial immunodiffusion readings.

2.3.2. Assessment of cell-mediated immune response.

2.3.2.1. Acid alphanaphthyl acetate esterase (ANAE) activity as T cell marker.

Li et al. (1973) demonstrated acid alphanaphthyl acetate esterase (ANAE) activity in human lymphocytes. The esterase activity prominent in lymphocytes, especially T^{+} cells was used as a T cell marker (Mueller et al. 1975). Later Osbaldiston et al. (1978) recognised that ANAE activity of

lymphocytes could be used as a T cell marker in different species of domestic animals. They successfully employed the technique in cat, dog, goat, guinea pig, hamster, rabbit, rat, sheep and pig. The esterase activity was recognized by the presence of reddish brown granules or deposits in the cytoplasm. Reddi et al. (1980) demonstrated ANAE activity in the peripheral blood lymphocytes of cattle and this test was recommended for routine use in domestic animals.

Dhingra et al. (1982) observed that in T cells there was spherical or oval reddish brown granular reaction product adjacent to the cell membrane.

Rajan et al. (1982) used ANAE as a T cell marker in the peripheral blood of pigs in evaluating the immunopotentiation response of pigs sensitized with 2,4-dinitrochlorobenzene (DNCB).

Sulochana et al. (1982) observed that the number of ANAE positive lymphocytes (26.84 ± 2.56) was the same as E-rosette forming cells (26.57 ± 2.05) in the peripheral blood of goats.

The cell-mediated immune response in experimental aflatoxicosis of pigs was assessed employing ANAE activity in the peripheral blood (Vishalakshan et al. 1984).

2.3.2.2. Response to mitogen - phytohaemagglutinin (PHA).

Many mitogens are lectins of plant origin. The first observations of lymphocyte transformation concerned the effects of phytohaemagglutinin (PHA), an extract of the red kidney bean Phaseolus vulgaris (Nowell, 1960). Janossy and Greaves

(1971) recognized PHA as a T-cell specific mitogen. Porcine T lymphocytes responded well to PHA (Binns et al. 1972b; Hardy and Ling, 1973; Crumpton et al. 1976).

Zuckerman and Lo Bugilo (1977) used PHA as a skin test for the evaluation of cellular immunocompetence in normal and cancer patients respectively.

PHA caused a direct reaction without prior sensitization and this test was advantageously employed in evaluating the cell-mediated immunity in man in recent years (Marchalonis, 1978).

Haggard et al. (1980) reported intra-dermal PHA response in experimental iodine toxicosis in young cattle.

Thein et al. (1981) employed both in vitro and in vivo PHA tests to assess the cell-mediated immunity in horses. They reported that the response to PHA was an indication of delayed type hypersensitivity reaction.

Rajan et al. (1982) used PHA for evaluating the cell-mediated immunity in goats and recommended this for routine use.

PHA was employed by Kelley et al. (1982) to evaluate the effect of heat and cold stress on the immune system. Reddy and Rajan (1984) employed PHA intradermally to assess the cell-mediated immunity in cattle bearing carcinoma of the Ethmoid mucosa.

2.3.2.3. Response to 2,4-dinitrochlorobenzene (DNCB).

The cutaneous sensitization test with DNCB has been considered as one of the reliable tests in measuring the cell-mediated immune status in man (Brown et al. 1967; Eliber and Morton, 1970).

Many workers (Malaviya et al. 1973; Chakravorthy et al. 1973) have reported positive responses in 85 per cent to 100 per cent of normal control subjects. Brummerstedt and Basse (1973) were the first to report the possibility of using the test to evaluate cell-mediated immunity in calves.

Jennings (1979) evaluated the DNCB response in calves. DNCB skin test was standardised in cattle by Reddy et al. (1981). Subsequently Rajan et al. (1982) evaluated the efficacy of this test in goats. DNCB was used for evaluating the cell-mediated immunity in pigs by Rajan et al. (1982). They standardised the technique in pigs. In the same year, Rajan et al. reported that DNCB induced a generalised stimulation of cell-mediated immune response as indicated by increased number of ANAE positive (T cells) in the peripheral blood of pigs.

2.3.3. Assessment of phagocytic activity.

2.3.3.1. Neutrophils.

Bachner and Nathan (1967) observed that Nitroblue tetrazolium (NBT) was reduced by neutrophils in vitro and reduction was enhanced during phagocytosis. This property was used as an indicator to assess the phagocytic activity of neutrophils

in man (Park et al. 1968). In man 8.5% of the neutrophils were positive and was increased to 29-47% in bacterial infections. Bacterial products like endotoxin enhanced the phagocytic property (Park and Good, 1970).

Sara (1975) employed the NBT test to assess the function of neutrophils in the peripheral blood smear of normal and animals ailing from mastitis and in caprine pleuro pneumonia cases. It was observed that the number of NBT positive cells increased during infections. Khalifa et al. (1984) used NBT test for detecting phagocytic activity in adult rats infected with bacteria.

2.3.3.2. Macrophages.

Hedley and Currie (1978) used NBT test to assess the function of the peripheral blood monocytes of cancer patients.

Reddy and Rajan (1984), for the first time employed NBT salt reduction test to assess the functional activity of activated macrophages in the impression smears of lymphnodes obtained from Dthmoid tumour bearing animals.

Materials and Methods

MATERIALS AND METHODS

3.1. Production and quantification of aflatoxin

Aspergillus parasiticus obtained from the Central Food and Technological Research Institute, Mysore, was used for preparing rice culture. The method described by Shotwell et al. (1966) was adopted for the production of aflatoxin in the laboratory. The toxin was extracted as per the method of Pons and Goldblatt (1969). The concentration of aflatoxin was determined by Thin layer chromatography employing minimum fluorescence extinction method (AOAC, 1975).

3.1.1. Biological assay for aflatoxin.

Day-old Khaki Campbell ducklings weighing 45-50 g procured from the Duck Farm, Niranam were used for the study. Five ducklings were fed crude aflatoxin extract in propylene glycol orally by means of a thin flexible polyethylene crop tube at the dose rate of 30 µg/duckling. Five other ducklings kept as controls were fed pure propylene glycol. The ducklings were maintained on aflatoxin free diet. The gross lesions and histopathology of liver of the ducklings that died were studied.

3.2. Experimental design

Twenty, clinically healthy Large White Yorkshire male piglings of 2-3 months of age belonging to the Pig Breeding Farm, Mannuthy were randomly selected for the experiment. The pigs were kept under observation for two weeks before

commencement of the experiment during which period they were screened for common parasitic diseases and other ailments.

The ration schedule followed in the farm was adopted for these animals. Every consignment of the feed was screened for aflatoxin before feeding it to the pigs.

The animals were assigned at random to three treatment groups: Group I and Group II consisting of seven animals each and Group III consisting of six animals. The pigs were housed in separate pens for convenience of individual feeding. Group I and Group II were given 0.1 and 0.2 milligrams of crude aflatoxin per kilogram body weight daily and Group III was kept as control. The aflatoxin was mixed with the ration of each animal and was fed orally for a period of three months.

The body weight of the animals was recorded before commencement of the experiment and subsequently at fortnightly intervals. The daily feed intake was also recorded.

3.3. Haematological studies

Blood samples from all the animals were collected from the anterior vena cava before commencement of the experiment, 48 hours after the administration of toxin, and thereafter at ten days interval for 90 days.

Procedures described by Schalm (1975) were followed for the determination of total erythrocyte count, erythrocyte sedimentation rate, packed cell volume, haemoglobin, total and differential leukocyte counts. Absolute counts of

neutrophils and lymphocytes were calculated from the values obtained.

3.4. Assessment of cell-mediated immune status

3.4.1. Enumeration of acid alpha naphthyl acetate esterase (ANAE) positive lymphocytes in the peripheral blood.

Wet smears prepared from the peripheral blood, were immediately fixed. The fixative contained six parts of acetone and four parts of 0.038 M sodium citrate (pH 5.4). The smears were kept in the fixative for 30 seconds, rinsed in distilled water and dried. The labelled smears were stored at room temperature (Giorno and Beverly, 1980). For staining the smears, a reaction mixture was prepared as follows:

In 40 ml of 0.067 M phosphate buffer (pH 5.0), 2.4 ml of hexazotized pararosaniline and 10 mg of alpha naphthylacetate (Loba) dissolved in 0.4 ml acetone was added and the final pH of the reaction mixture was adjusted to 5.8 with 2 N sodium hydroxide.

The hexazotized pararosaniline was prepared in the following manner:

Equal volumes of two solutions (1) freshly prepared 4% sodium nitrite in distilled water and (2) one gram of pararosaniline hydrochloride (Sigma Chemicals) dissolved in 20 ml of distilled water and 5 ml of 12 N hydrochloric acid were combined. The hexazotized pararosaniline which formed was shaken and then allowed to stand for one minute before adding it to the reaction mixture (Knowles et al. 1978).

The slides were incubated in the reaction mixture for 18-21 hours at room temperature and then rinsed thoroughly with distilled water and then counterstained with 1% toludine blue for 45-60 minutes. The slides were then rinsed thoroughly with distilled water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in DPX and examined under oil immersion objective of a microscope. Those lymphocytes with localised orange and nodular reaction product in the cytoplasm were considered as positive cells (T lymphocytes). The number of positive cells in every hundred cells was counted and recorded.

3.4.2. Cutaneous hypersensitivity reactions.

3.4.2.1. Response to phyto haemagglutinin-M (PHA-M).

At the end of two months of the experimental period the cellular response of both aflatoxin fed pigs and the controls was evaluated using PHA-M. The method described by Rajan et al. (1982) was employed.

Ten microgrammes of PHA-M (Difco Laboratories, USA) were dissolved in 0.1 ml distilled water. The site chosen was the back of the ear where the skin was comparatively loose. The hairs over the area were clipped. Two sites were marked for injecting PHA. The thickness of the skin at these two sites was measured using a vernier caliper. At each site 0.1 ml of distilled water containing 10 µg of PHA-M was administered I/D. The skin thickness at these sites were measured at 24, and 48 hours. Pieces of skin were taken for biopsy at the

same intervals and fixed in 10% formalin. The biopsy specimens were processed for histo-pathological studies by the routine method and sections were cut at 4-5 μ thickness and stained with Harris haematoxylin and eosin (H & E). The histological changes were studied.

3.4.2.2. Cutaneous response to 2,4-dinitrochlorobenzene (DNCB).

On the 70th day of the experiment, the cell-mediated immune response of the pigs to 2,4-dinitrochlorobenzene (DNCB) was evaluated. The method described by Rajan et al. (1982) was followed.

The site selected for sensitisation and challenge was near the base of the ear towards the head. All the animals were sensitised on the left side and challenged 14 days later on the right side. The area was shaved and a three centimeter diameter area was demarcated by holding a metal ring on to the skin at the site. The sensitation dose was a single application of 0.25 ml of 2% DNCB (Loba-Chemiewien Fischamend) in acetone to each of two 3 cm diameter sites. The DNCB solution was applied slowly drop by drop and allowed to evaporate quickly by blowing. The metal ring was kept at the site until the solution evaporated. The preparation of the site, the mode of application of DNCB solution and the dose for challenge were exactly the same as for sensitization.

The thickness and diameter of the skin at the two sites before challenging and at 24 and 48 hours after challenge were

measured. Skin biopsies at 24 and 48 hours were taken from four animals for histological studies. The tissues were fixed in 10% buffered formalin and paraffin sections cut at 4-5 μ were stained with haematoxylin and eosin.

3.5. Evaluation of humoral immune response

3.5.1. Determination of total protein in the serum.

Total serum proteins were estimated by the biuret assay method of Inchiosa (1964).

3.5.2. Determination of gammaglobulin in the serum.

Zinc sulphate turbidity test described by McEwan et al. (1970) was followed with suitable modifications. The modifications were similar to those followed by Nandakumar (1981) for evaluating the gammaglobulin in the serum of kids.

3.6. Assessment of phagocytic activity by Nitro blue tetrazolium salt (NBT) test

3.6.1. Neutrophils.

The procedure described by Peacock and Tomar (1980) was followed to assess the phagocytic activity of neutrophils in vitro, using Nitroblue tetrazolium (NBT) dye reduction test.

The dye solution was prepared by dissolving six milligrams of Nitroblue tetrazolium (Sisco Research Laboratories, Bombay) in 2.5 ml of normal saline. Heparinised blood was used for the test and the test was performed immediately after blood collection.

Equal volumes of the dye solution and blood were mixed in a polyethylene container and incubated at 37°C for 30 minutes. Smears were prepared in duplicate, air dried and stained with Wright's stain. Those neutrophils with blue-black (formazan) deposits in the cytoplasm were counted as positive. The number of positive cells in every hundred neutrophils were counted. The percentage of positive cells was calculated and recorded.

3.6.2. Macrophages.

The dye solution was prepared as above. The method of Reddy and Rajan (1984) was adopted and the NBT dye reduction test was performed on the lymph node impression smears in vitro.

Immediately after death or following slaughter, the prefemoral lymph node on one side was dissected out. The lymph node was cut across with a clean blade. The surface was blotted and impression smears prepared from the cut surface on clean glass slide. The glass slide was kept in a Petri-dish and the NBT salt solution was poured over the impression smears and incubated at 37°C for 30 minutes. During this period care was taken not to allow the solution to evaporate by keeping moist cotton in the petri-dish. After incubation the smears were kept at room temperature for 15 minutes and then stained with Wright's stain. The cells in the smears were examined for the NBT positive macrophages with formazan deposits.

Two hundred macrophages were identified based on their

morphological characters. The proportion of NBT positive and negative cells was counted. The NBT response was expressed as percentage of macrophages containing formazan deposits.

3.7. Morbid anatomy and histopathology

At the end of the experimental period all the experimental as well as the control animals were sacrificed and subjected to detailed post mortem examination. The method advocated by FAO/SIDA (1968) was followed for post mortem. Animals that died during the experimental period were also subjected to detailed post mortem. The gross lesions were recorded. The weights of liver, kidney, spleen and heart were recorded. Representative samples of tissues collected from various organs of all the animals were fixed in 10% formalin.

For histopathological studies, tissues were processed by the routine method. Paraffin sections cut at 4-5 μ thickness were stained with Harri's haematoxylin and eosin as described by Sheehan and Hrapchak (1980). Besides this, special staining techniques (Van Gieson's picrofuchsin, Gridley's modification of silver impregnation method of staining reticular fibres) were employed wherever necessary. The procedures described by Sheehan and Hrapchak (1980) were followed.

For the demonstration of fat in tissues, the frozen sections were stained with Sudan III (Lillie and Fullmer, 1976).

3.8. Statistical analysis

Statistical analysis was done using students 't' test according to the method described by Snedecor and Cochran (1967).

Results

RESULTS

The immunopathological response of piglings dosed with aflatoxin at two levels (0.1 mg/kg body weight and 0.2 mg/kg body weight) was studied for a period of three months and the results were compared with age matched control piglings.

4.1. Quantification of aflatoxin

The concentration of aflatoxin produced on rice culture and quantified by thin layer chromatography, on an average ranged from 95-105 µg per gram.

4.1.2. Biological assay for aflatoxin.

The ducklings dosed with crude aflatoxin died within 3-4 days with diffuse haemorrhages in all the visceral organs. The characteristic histologic findings were bile duct hyperplasia and haemorrhage in the liver.

4.2. General findings

The quantity of aflatoxin fed to each pigling of Group I and Group II and the period of survival of each animal in these two experimental groups are shown in table 1.

The piglings which were administered aflatoxin showed depression as compared to the healthy controls. During the experimental period of 90 days, one pig of group I died on the 72nd day and five pigs of group II died on the 21st, 23rd, 67th, 77th and 85th day of the experiment.

Table 1. Details of aflatoxin administered to the pigs

Sl. No.	Group I (0.1 mg/kg body weight)			Group II (0.2 mg/kg body weight)		
	Number of days	Total aflatoxin administered (mg)	Fate	Number of days	Total aflatoxin administered (mg)	Fate
1	72	142.37	Died	21	63	Died
2	90	108.70	Sacrificed	90	259.24	Sacrificed
3	90	122.2	Sacrificed	77	211.56	Died
4	90	138.18	Sacrificed	23	41.4	Died
5	90	144.6	Sacrificed	67	124.0	Died
6	90	179.32	Sacrificed	85	134.56	Died
7	90	220.8	Sacrificed	90	180.52	Sacrificed

All the aflatoxin fed pigs contracted sarcoptic mange infection towards the latter part of the experimental period while the control group of pigs maintained under identical management conditions remained healthy.

4.2.1. Body weight.

The mean body weights (kg) at fortnightly intervals of both the aflatoxin fed groups and control groups are given in Fig.1(a)

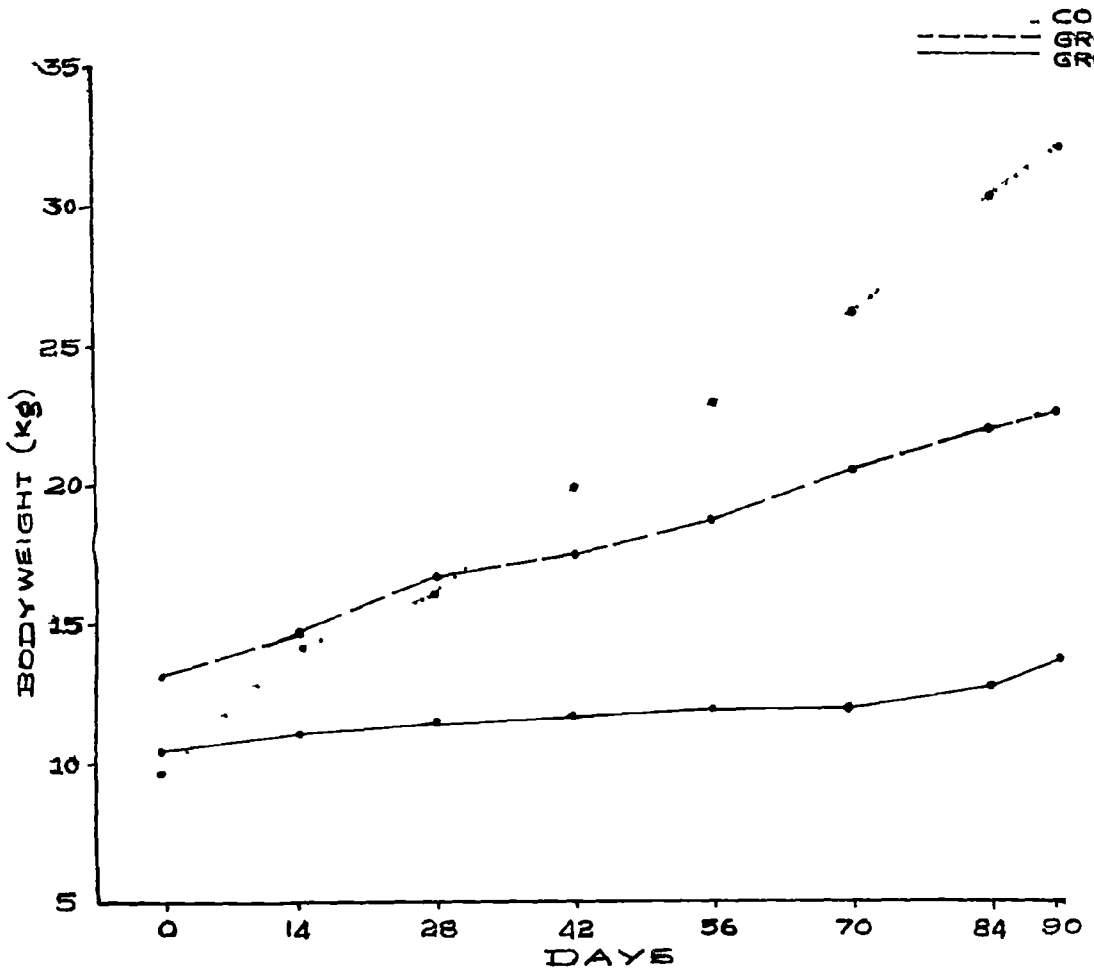
Animals in the control group showed a gradual increase in the body weight from an initial 9.71 ± 0.25 kg to 32.36 ± 2.41 kg by the 90th day.

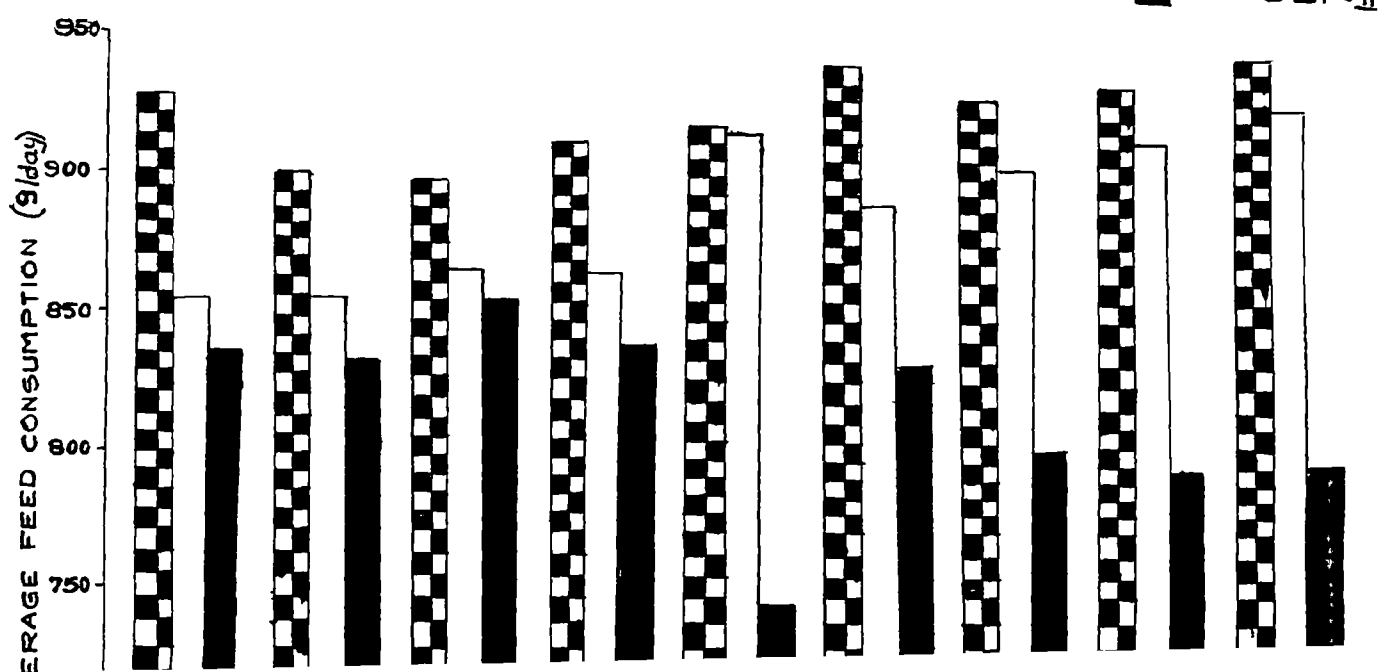
Group I animals recorded a gradual increase in body weight from 13.14 ± 0.94 kg to a maximum value of 22.33 ± 2.85 kg by the 90th day. The reduction was significant ($P < 0.05$) from that of the control animals on the sixth fortnight and on the 90th day.

The body weight of group II animals increased gradually from an initial value of 10.42 ± 1.21 kg to 13.6 ± 2.41 kg on the 90th day. There was a significant ($P < 0.05$) reduction in weight from that of the control group from the third fortnight onwards.

4.2.2. Feed consumption.

The average feed consumption (grams/pig/day) of the experimental and control animals at ten days interval is shown in Fig.1(b).


CONTROL
GROUP-I
GROUP-II
BODY WEIGHT (kg)
35
30
25
20
15
10
5
0
14
28
42
56
70
84
90
DAYS


CONTROL
GROUP-I
GROUP-II
AVERAGE FEED CONSUMPTION (g/day)
950
900
850
800
750

It was observed that the aflatoxin fed pigs consumed less feed compared to the control pigs. The decreased feed consumption was more marked in group II pigs given the higher dose.

4.3. Haematological studies

The haematological parameters recorded at different time intervals of both the experimental and control group of pigs are set out in table 2.

4.3.1. Erythrocyte sedimentation rate.

The data on ESR are shown in Fig.2. The ESR of the control animals ranged from 9.00 ± 1.00 mm/h to 13.16 ± 0.60 mm/h during the experimental period.

In Group I animals, a gradual increase was observed from the initial value of 5.86 ± 1.18 mm/h to 20.50 ± 1.36 mm/h by the 90th day. The values were significantly higher ($P < 0.05$) than that of the control animals on the 80th and 90th day.

In Group II animals, the ESR values showed a rapid increase from 4.29 ± 1.43 mm/h to 13.49 ± 0.92 mm/h on the 10th day. Following a transient decrease on the 20th day (11.86 ± 1.20 mm/h), the values increased to 29.00 ± 1.00 mm/h on the 90th day. The increase in ESR values was significant ($P < 0.05$) from the 10th day onwards.

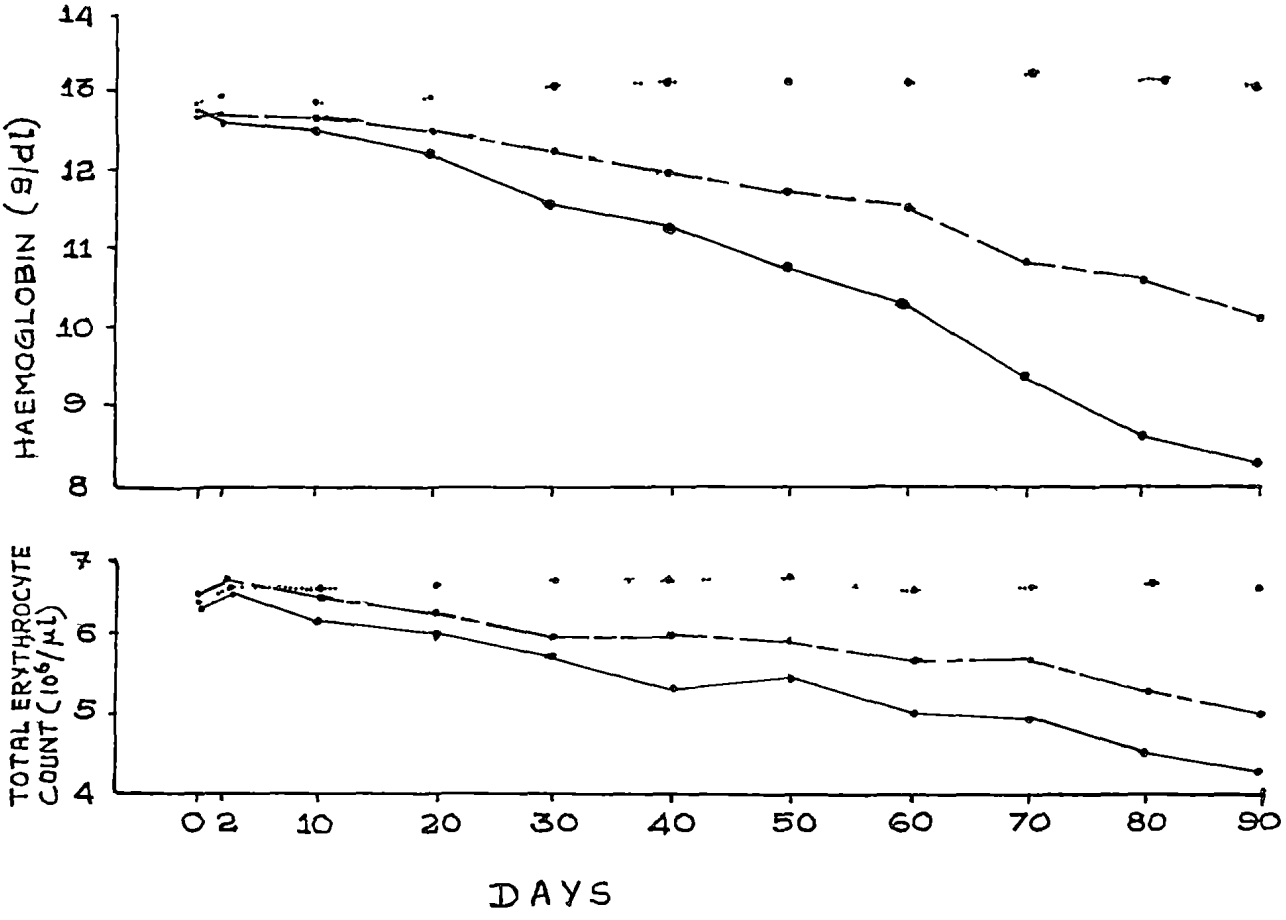
4.3.2. Packed cell volume.

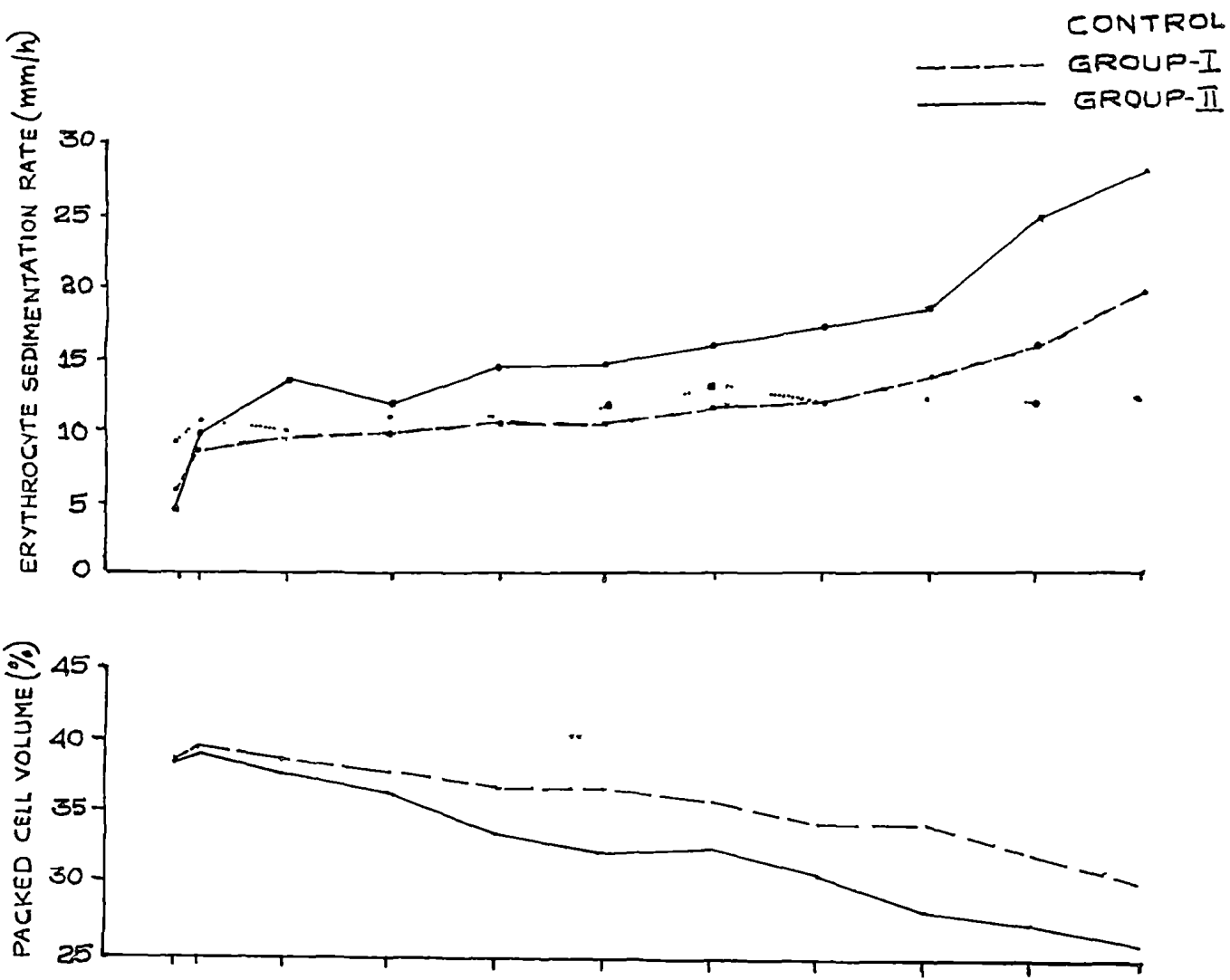
The PCV values are represented in Fig.2. In the control animals, the PCV values ranged from 38.00 ± 0.67% to 40.16 ± 0.48% during the experimental period.

Table 2. Haemogram of the control and experimental pigs (Mean ± S.E.)

Parameter	Group	Interval in days										
		0	2	10	20	30	40	50	60	70	80	90
ESR (mm/h)	C	9.00 ±1.00	10.16 ±1.01	9.83 ±0.47	10.66 ±0.61	10.66 ±1.02	11.83 ±1.07	13.16 ±0.60	11.83 ±0.87	12.16 ±0.65	11.83 ±0.54	12.16 ±0.65
	I	5.86 ±1.18	8.42 ±1.58	9.14 ±1.07	9.57 ±0.52	10.71 ±0.42	10.57 ±0.64	11.57 ±0.65	12.43 ±0.78	14.43 ±1.25	16.50* ±1.47	20.50* ±1.36
	II	4.29 ±1.43	9.86 ±1.60	13.49* ±0.92	11.86 ±1.20	14.40* ±0.50	14.80* ±0.37	16.20* ±0.58	17.60* ±0.75	19.25* ±0.48	25.67* ±1.20	29.00* ±1.00
PCV (%)	C	38.00 ±0.67	39.33 ±0.49	39.33 ±0.33	39.50 ±0.43	40.00 ±0.58	40.16 ±0.48	39.67 ±0.33	39.50 ±0.34	39.83 ±0.30	39.83 ±0.30	39.50 ±0.42
	I	39.00 ±0.53	39.57 ±0.69	38.71 ±0.60	37.71* ±0.52	36.43* ±0.43	36.43* ±0.43	35.71* ±0.52	34.14* ±0.59	34.14* ±0.59	32.16* ±0.40	30.83* ±0.48
	II	38.00 ±0.78	39.00 ±0.70	37.57* ±0.64	36.29* ±0.68	33.20* ±0.37	32.20* ±0.37	32.80* ±0.58	30.80* ±0.58	28.25* ±0.85	27.00* ±0.57	25.50* ±0.50
Hb (g/dl)	C	12.87 ±0.17	12.90 ±0.21	12.87 ±0.15	12.90 ±0.08	13.03 ±0.17	13.10 ±0.15	13.16 ±0.12	13.16 ±0.15	13.26 ±0.16	13.13 ±0.13	13.06 ±0.15
	I	12.69 ±0.20	12.71 ±0.20	12.66 ±0.17	12.51 ±0.19	12.24* ±0.17	11.97* ±0.11	11.71* ±0.13	11.53* ±0.15	10.89* ±0.16	10.67* ±0.18	10.10* ±0.15
	II	12.71 ±0.29	12.69 ±0.27	12.49 ±0.19	12.23* ±0.19	11.58* ±0.21	11.28* ±0.24	10.76* ±0.17	10.32* ±0.19	9.35 ±0.22	8.60* ±0.11	8.30* ±0.10
RBC count (10^6/µl)	C	6.47 ±0.11	6.61 ±0.08	6.61 ±0.07	6.63 ±0.08	6.70 ±0.09	6.70 ±0.07	6.73 ±0.06	6.61 ±0.05	6.65 ±0.05	6.71 ±0.07	6.65 ±0.05
	I	6.56 ±0.09	6.63 ±0.11	6.49 ±0.11	6.33 ±0.09	6.08* ±0.09	6.07* ±0.07	5.94* ±0.10	5.70* ±0.10	5.72* ±0.10	5.33* ±0.08	5.10* ±0.07
	II	6.43 ±0.13	6.53 ±0.11	6.29* ±0.10	6.05* ±0.11	5.72* ±0.25	5.34* ±0.07	5.48* ±0.11	5.14* ±0.07	4.90* ±0.09	4.57* ±0.09	4.30* ±0.01

C - Control, I - Group I, II - Group II * $P < 0.05$


HAEMOGLOBIN (g/dl)
14
13
12
11
10
9
8
TOTAL ERYTHROCYTE COUNT ($10^6/\mu l$)
7
6
5
4
0 2 10 20 30 40 50 60 70 80 90
DAYS


CONTROL
GROUP-I
GROUP-II
ERYTHROCYTE SEDIMENTATION RATE (mm/h)
0
5
10
15
20
25
30
PACKED CELL VOLUME (%)
25
30
35
40
45

In Group I animals, a gradual increase from the initial value of 39.00 ± 0.53% to 30.83 ± 0.48% was observed by the 90th day. The decrease was significant ($P < 0.05$) compared to the control animals from the 20th day onwards.

In Group II animals, from the initial value of 38.00 ± 0.66% a decrease to 25.50 ± 0.50% was observed by the 90th day. The variations from the control values were significant ($P < 0.05$) from the 10th day onwards.

4.3.3. Haemoglobin concentration.

The haemoglobin concentration of the experimental and control groups of pigs is shown in Fig.2.

The haemoglobin concentration in the control animals ranged from 12.87 ± 0.17 g/dl to 13.26 ± 0.16 g/dl during the experimental period.

In Group I animals, from the initial value of 12.69 ± 0.20 g/dl, the haemoglobin concentration decreased gradually to 10.10 ± 0.15 g/dl on the 90th day. The variations were significant ($P < 0.05$) from that of the control animals from the 30th day onwards.

Group II animals also recorded a lower value from an initial value of 12.71 ± 0.29 g/dl to 8.30 ± 0.10 g/dl on the 90th day. The variations were significant ($P < 0.05$) from the 20th day onwards.

4.3.4. Total erythrocyte count.

The total erythrocyte count of the experimental groups I and II and the control animals is shown in Fig.2.

The total erythrocyte count in the control animals ranged from 6.47 ± 0.11 million/µl to 6.73 ± 0.06 million/µl during the experimental period of 90 days.

In group I animals, from the initial value of 6.56 ± 0.09 million/µl, a gradual decrease to 5.10 ± 0.07 million/µl was observed by the 90th day. The values from the 20th day onwards showed a significant reduction ($P < 0.05$) from that of the control animals.

In group II animals, from the initial value of 6.43 ± 0.13 million/µl a decrease to 4.30 ± 0.01 million/µl was observed by the 90th day. The variations from the control values were significant ($P < 0.05$) from the 10th day onwards.

4.3.5. Total leukocyte count.

Figure 3 illustrates the leukocyte response. The Mean ± S.E. leukogram of the control and experimental pigs is represented in table 3.

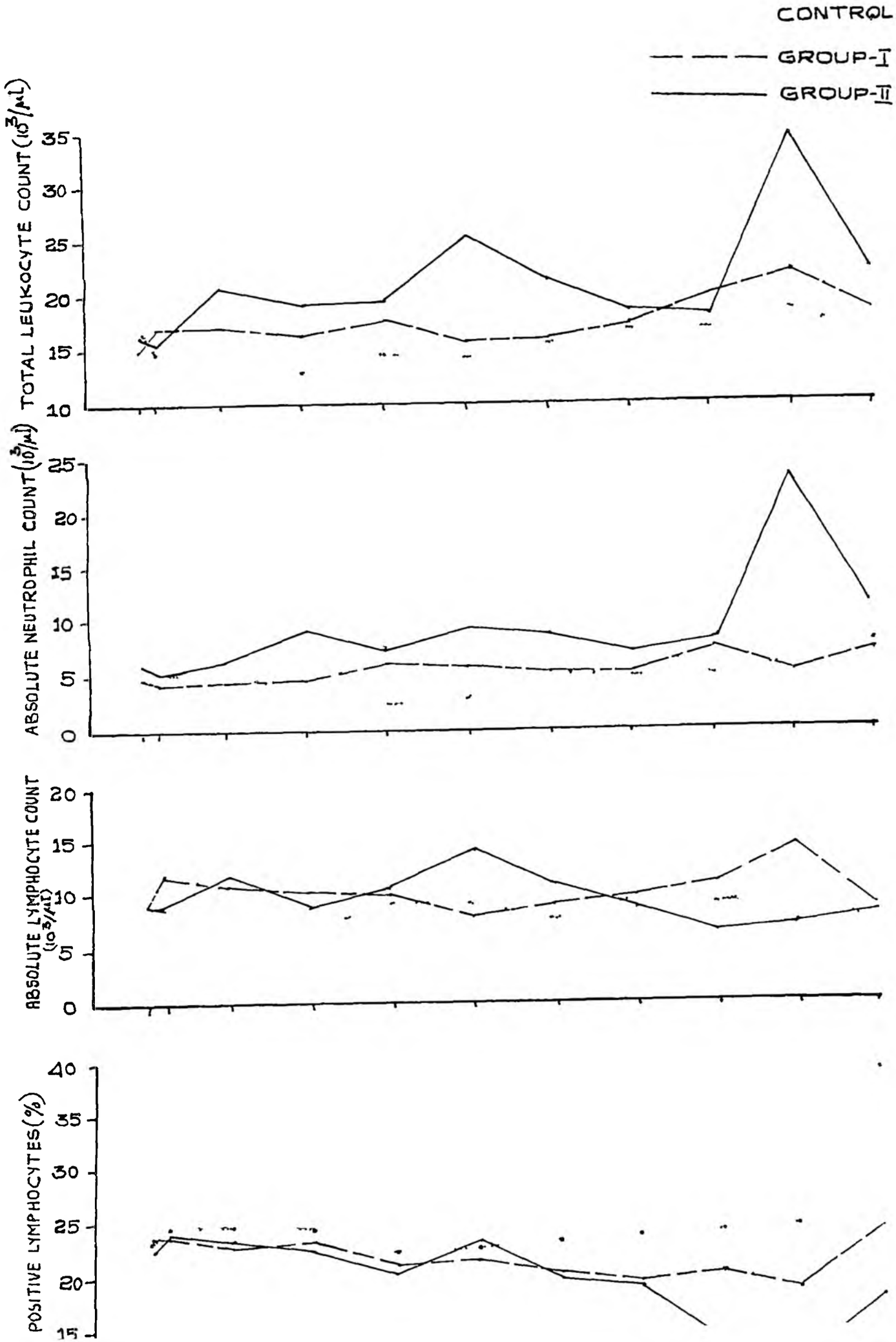
The total leukocyte count of the control animals ranged from 12.97 ± 1.37 (10^3/µl) to 18.67 ± 1.80 (10^3/µl) during the experimental period.

In group I animals, the values were at a higher level throughout the experimental period. From the initial value of 15.06 ± 3.03 (10^3/µl), a maximum of 22.37 ± 3.08 (10^3/µl) value was observed on the 80th day. This was followed by a

Table 3. Leukogram of the control and experimental pigs (Mean ± S.E.)

Parameter	Group	Interval in days										
		0	2	10	20	30	40	50	60	70	80	90
Total leukocyte count ($10^3/\mu l$)	C	16.39 ±1.95	14.46 ±1.69	13.38 ±1.55	12.97 ±1.37	14.46 ±1.63	14.38 ±1.65	15.27 ±1.76	16.75 ±1.46	16.87 ±1.80	18.67 ±1.01	15.28 ±1.86
	I	15.06 ±3.03	16.81 ±2.21	16.78 ±0.95	16.27 ±0.77	17.65 ±2.37	15.83 ±0.99	15.84 ±0.84	17.17 ±1.37	20.37 ±2.50	22.37 ±3.08	18.53 ±2.89
	II	16.02 ±1.76	14.92 ±1.50	20.54 ±1.92	18.74 ±2.20	19.39 ±1.86	25.29* ±4.56	21.34* ±1.62	18.35 ±1.27	17.90 ±2.84	34.38* ±9.20	22.20 ±0.49
Absolute neutrophil count ($10^3/\mu l$)	C	5.63 ±0.93	5.07 ±0.59	5.08 ±0.57	4.54 ±0.53	3.41 ±0.65	3.81 ±0.58	5.10 ±0.68	5.30 ±0.62	5.06 ±0.61	5.64 ±0.56	5.35 ±0.98
	I	4.57 ±0.97	4.33 ±0.55	4.48 ±0.71	4.57 ±0.62	6.25 ±2.25	5.88 ±0.78	5.31 ±0.55	5.39 ±0.75	7.52 ±2.86	5.24 ±1.01	7.43 ±1.85
	II	5.74 ±0.74	5.10 ±0.81	6.30 ±1.13	8.93 ±2.51	7.01* ±1.30	9.15* ±1.83	8.85* ±1.18	6.88 ±1.25	8.39 ±1.58	23.93* ±11.74	11.54* ±1.08
Absolute lymphocyte count ($10^3/\mu l$)	C	9.83 ±1.20	8.97 ±1.18	8.03 ±1.06	7.67 ±0.60	10.10 ±0.90	10.08 ±0.95	8.87 ±1.02	10.80 ±1.03	10.69 ±1.21	11.83 ±1.30	9.30 ±0.90
	I	9.16 ±2.59	12.08 ±2.18	11.76* ±0.88	11.34* ±0.53	10.99 ±1.24	9.33 ±0.83	10.42 ±0.52	11.15 ±0.90	12.54 ±1.69	16.01 ±2.29	10.84 ±2.65
	II	9.59 ±1.08	9.31 ±1.00	13.28* ±0.83	9.48 ±0.86	11.87 ±1.86	15.30 ±3.54	11.89* ±0.44	10.40 ±1.37	8.14 ±1.06	8.87 ±1.80	10.10 ±0.80
ANAE positive lymphocytes (%)	C	23.00 ±1.15	24.33 ±0.71	24.83 ±0.60	24.33 ±0.61	22.16 ±0.54	22.33 ±0.56	22.83 ±0.70	23.16 ±0.48	23.66 ±0.42	23.83 ±0.47	38.33 ±0.66
	I	23.28 ±1.34	23.00 ±0.90	22.42* ±0.65	23.00 ±0.81	21.00 ±0.69	20.71* ±0.42	20.42* ±0.57	20.28* ±0.42	21.28* ±0.52	19.66* ±0.56	25.83* ±0.60
	II	22.28 ±0.56	23.28 ±0.52	23.14 ±0.59	22.14* ±0.67	20.80 ±0.58	23.00 ±0.70	19.40* ±0.50	18.60* ±0.59	13.25* ±0.85	11.66* ±0.33	17.50* ±0.50

C - Control, I - Group I, II - Group II * $P < 0.05$


CONTROL
GROUP-I
GROUP-II
TOTAL LEUKOCYTE COUNT ($10^3/\mu L$)
35
30
25
20
15
10
ABSOLUTE NEUTROPHIL COUNT ($10^3/\mu l$)
25
20
15
10
5
0
ABSOLUTE LYMPHOCYTE COUNT ($10^3/\mu l$)
20
15
10
5
0
POSITIVE LYMPHOCYTES (%)
40
35
30
25
20
15

reduction to 18.53 ± 2.89 ($10^3/\mu l$) on the 90th day. The values were not statistically significant from that of the control values.

In group II animals, from the initial value of 16.02 ± 1.76 ($10^3/\mu l$), a higher level was maintained with the values reaching a maximum of 34.38 ± 9.20 ($10^3/\mu l$) on the 80th day followed by a reduction to 22.20 ± 0.49 ($10^3/\mu l$) on the 90th day. The variations were significant ($P < 0.05$) from that of the control values on the 40th, 50th and 80th day.

4.3.5.1. Absolute neutrophil count.

The absolute neutrophil count of all the groups of animals is shown in Fig.3. The absolute neutrophil count of the control animals decreased from the initial value of 5.63 ± 0.93 ($10^3/\mu l$) to 3.41 ± 0.65 ($10^3/\mu l$) on the 30th day followed by a transient increase on the 40th day to 3.81 ± 0.58 ($10^3/\mu l$). Thereafter, a higher level in the range 5.06 ± 0.61 ($10^3/\mu l$) to 5.64 ± 0.56 ($10^3/\mu l$) was maintained till the end of the experiment.

Group I animals, a higher level was maintained throughout the experimental period. The values ranged from 4.57 ± 0.97 ($10^3/\mu l$) to 7.52 ± 2.86 ($10^3/\mu l$). The variations from the control values were not significant.

In group II animals, the absolute neutrophil count maintained a higher level throughout the experimental period. From the initial value of 5.74 ± 0.74 ($10^3/\mu l$) the value

reached a maximum of 23.93 ± 11.74 ($10^3/\mu l$) on the 80th day and thereafter it decreased to 11.54 ± 1.08 ($10^3/\mu l$) on the 90th day. The variations from the control values were significant ($P < 0.05$) on the 30th, 40th, 50th, 80th and 90th day.

4.3.5.2. Absolute lymphocyte count.

The absolute lymphocyte count of all the groups of animals is set out in Fig.3. From an initial value of 9.83 ± 1.20 ($10^3/\mu l$) in the control group, the values decreased to a minimum of 7.67 ± 0.60 ($10^3/\mu l$) on the 20th day. This was followed by a gradual increase with the values reaching a maximum of 11.83 ± 1.30 ($10^3/\mu l$) on the 80th day. Thereafter, it showed a decrease towards the initial value.

In group I animals, a higher count was maintained. From the initial value of 9.16 ± 2.59 ($10^3/\mu l$) the values reached a maximum of 16.01 ± 2.29 ($10^3/\mu l$) by the 80th day. This was followed by a decrease to 10.84 ± 2.65 ($10^3/\mu l$) on the 90th day. The variations from the control values were significant ($P < 0.05$) only on the 10th and 20th day.

In group II animals, the absolute lymphocyte count increased from the initial value of 9.59 ± 1.08 ($10^3/\mu l$) to 13.28 ± 0.33 ($10^3/\mu l$) on the 10th day. The values then decreased to 9.48 ± 0.86 ($10^3/\mu l$) on the 20th day followed by an increase reaching a maximum of 15.30 ± 3.54 ($10^3/\mu l$) on the 40th day. Thereafter, the values gradually decreased reaching a minimum of 8.14 ± 1.06 ($10^3/\mu l$) on the 70th day and increased to 10.10 ± 0.80 ($10^3/\mu l$) on the 90th day. The variations from

the control values were significant ($P < 0.05$) only on the 10th and 50th day.

4.4. Assessment of cell-mediated immune response

4.4.1. Distribution of T lymphocytes in the peripheral blood.

Alphanaphthyl acetate esterase (ANAE) activity in the peripheral blood lymphocytes of all the animals was demonstrated to assess and evaluate the distribution of T lymphocytes.

Cells which revealed one or two well defined reddish-brown nodular reaction product at the periphery of the cytoplasm close to the cell membrane were counted as T lymphocytes and those which did not possess the reaction product were taken as other lymphocytes. The monocytes, neutrophils and eosinophils also showed slight esterase activity but the reaction was generally diffuse and lymphocytes could be distinguished from them by other morphological features (Fig.4).

The percentage of ANAE positive cells of the control and experimental animals at different time intervals is represented in table 3 and shown in Fig.3. In the control animals, the values ranged from $22.16 \pm 0.54\%$ to $24.83 \pm 0.60\%$ till the 80th day. On the 90th day the value increased to $38.33 \pm 0.66\%$.

In group I animals, the value decreased gradually from $23.28 \pm 1.34\%$ to $19.66 \pm 0.56\%$ by the 80th day and it increased to $25.83 \pm 0.60\%$ on the 90th day. The variations from the control values were significant ($P < 0.05$) on the 10th day and from the 40th day onwards.

In group II animals, from an initial count of 22.28 ± 0.56% the value decreased to 11.66 ± 0.33% on the 80th day. The values increased to 17.50 ± 0.50% on the 90th day. The variations from the control values were significant ($P < 0.05$) on the 20th day and from the 50th day onwards.

4.4.2. Cutaneous hypersensitivity reaction.

4.4.2.1. Response to phytonaemagglutinin-M (PHA-M).

Cutaneous response to PHA-M was evaluated on the 60th day in all the animals except in group II in which only five animals out of seven could be evaluated, the other two died within 60 days.

The skin induration developed after 24 and 48 hours of administration of PHA in the experimental groups I and II was compared with that of the controls. The biometry of the skin thickness is documented in table 4. The measurement of skin thickness at 24 and 48 hours revealed significantly lesser response ($P < 0.05$) in both the experimental groups when compared to the controls.

Histopathology of the skin

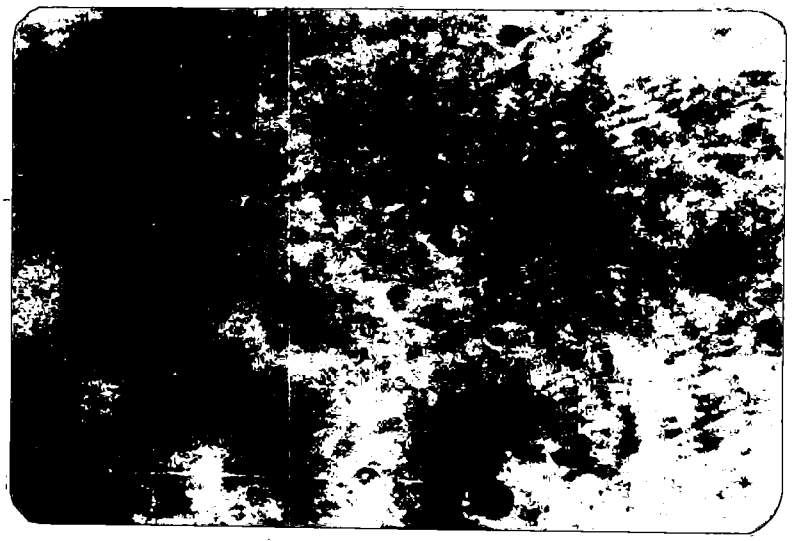
Twenty-four hours post-inoculation

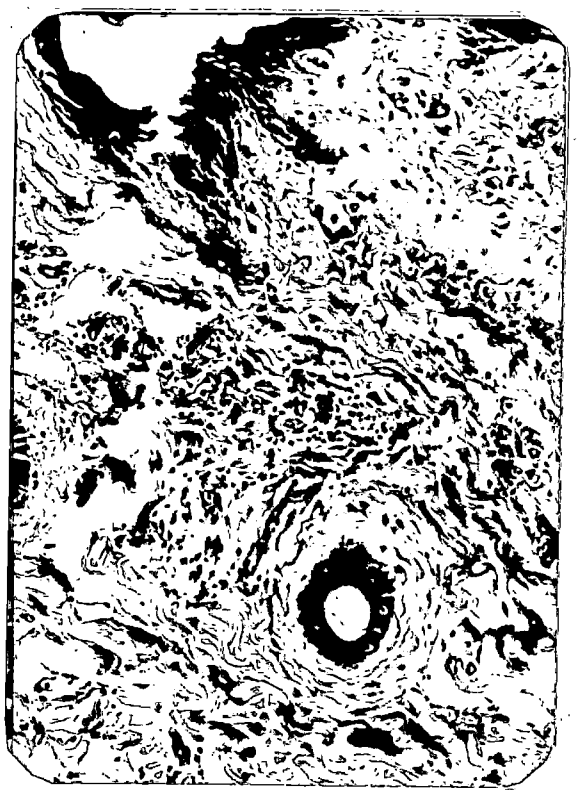
Slight to moderate focal infiltration of mononuclear cells was observed in the epidermis and in the dermal layer. The cells were mostly lymphocytes with a few macrophages intermingled with them. Occasionally neutrophils were also seen scattered among them. The infiltration of cells were mostly

Table 4. Biometry of the skin thickness before and after administration of PHA-M (Mean ± S.E.)

	Thickness before challenge (mm)	Increase in thickness after 24 hours (mm)	Increase in thickness after 48 hours (mm)
Control	2.00 ± 0.00	2.58 ± 0.15	1.87 ± 0.00
Group I	2.00 ± 0.00	1.71 ± 0.11*	1.14 ± 0.00*
Group II	2.00 ± 0.00	0.95 ± 0.05*	0.30 ± 0.00*

* $P < 0.05$





confined to the upper layer of the dermis. There was varying degree of oedema. In some of the sections the lymphocytic infiltration was more around the hair follicles. The severity of the reaction was less in aflatoxin fed groups. The least response was seen in group II animals (Fig.5-7).

Forty-eight hours post-inoculation

Cellular changes appeared more intense. Mononuclear infiltration was more diffuse and extended into the deeper layers of the dermis also. At places focal collection of these cells were seen. Capillary congestion and perivascular infiltration of lymphocytes and oedema were also evident. The cellular response was less intense in group I and least in group II animals as compared to the controls (Fig. 8-10).

4.4.2.2. Response to 2,4-dinitrochlorobenzene (DNCB).

The test was conducted on the 80th day of the experiment. Group I consisted of six animals and group II only two animals, since the other animals in both the groups had died earlier.

After application of the sensitizing dose there was slight reddening of the area indicating mild local reaction which subsided on the next day. The biometry of the skin thickness and the diameter following DNCB application are shown in table 5.

Twenty-four hours after administering the challenge dose all the animals showed reaction. The area appeared indurated. The skin thickness of the experimental groups I and II was

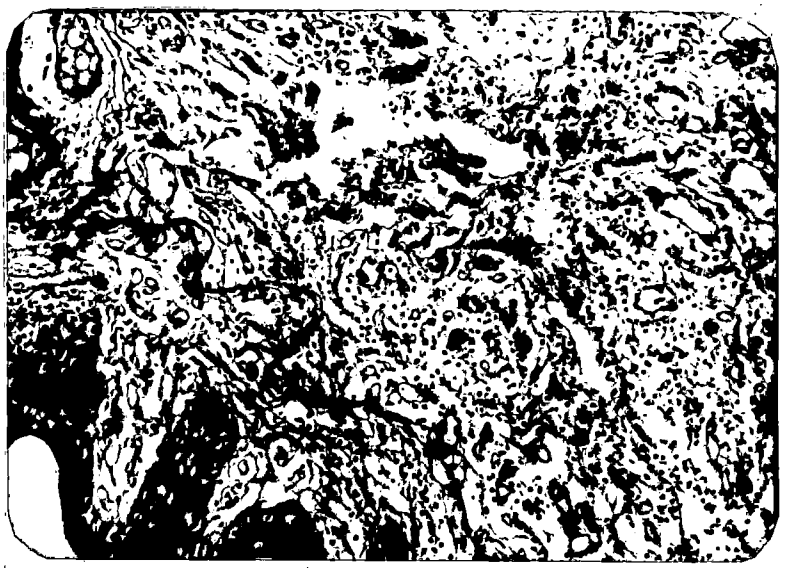


Table 5. Biometry of the skin thickness and diameter before and after challenge with DNCB (Mean ± S.E.)

	Thickness before challenge (mm)	Increase in thickness after challenge (mm)		Diameter before challenge (mm)	Increase in diameter after challenge (mm)	
		24 hours	48 hours		24 hours	48 hours
Control	4.83 ± 0.15	10.67 ± 0.43	8.70±0.50	3.00±0.00	2.92 ± 0.15	2.25 ± 0.21
Group I	4.78 ± 0.15	7.48 ± 0.18*	6.10±0.25*	3.00±0.00	2.36 ± 0.20	1.85 ± 0.19
Group II	4.52 ± 0.07	5.98 ± 0.57*	5.10±1.45*	3.00±0.00	1.52 ± 0.00*	1.00 ± 0.00*

* $P < 0.05$

significantly lower ($P<0.05$) both at 24 and 48 hours when compared to the reaction in the control group.

The increase in the diameter seen at 24 and 48 hours in group I animals was less than the control animals but was not statistically significant. The increase in diameter at 24 and 48 hours in the group II animals was significantly low ($P<0.05$) when compared to the control group.

Histopathology of DNCB reaction

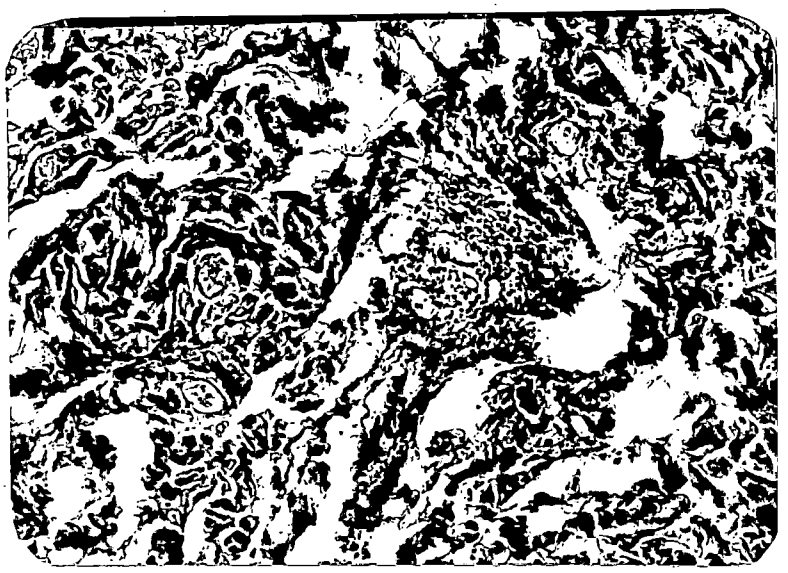
Twenty-four hour post-challenge

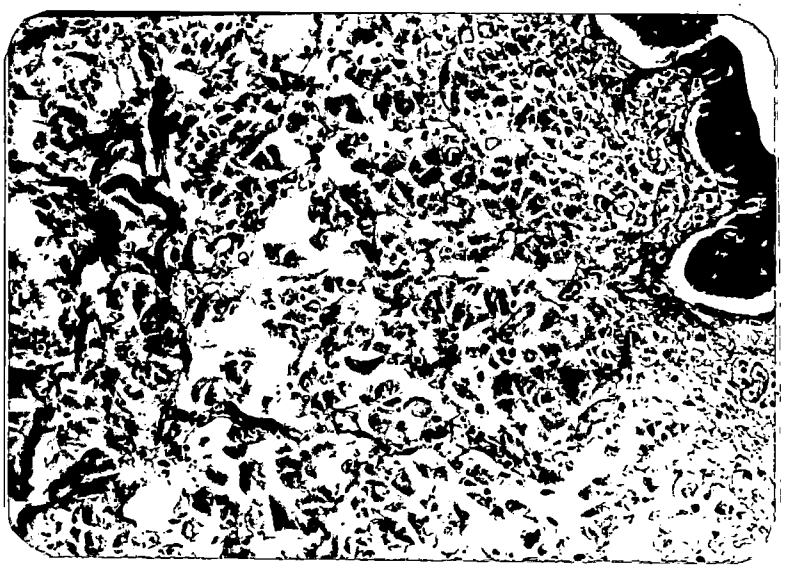
The focal and scattered infiltration of mononuclear cells mainly made up of lymphocytes were observed in the epidermis and upper part of the dermis. Moderate number of macrophages, a few neutrophils and eosinophils were also seen intermingled with them. Infiltration of these cells was mostly seen in the perivascular region. There was congestion of capillaries. The oedema separated the muscle bundles in the dermal layer.

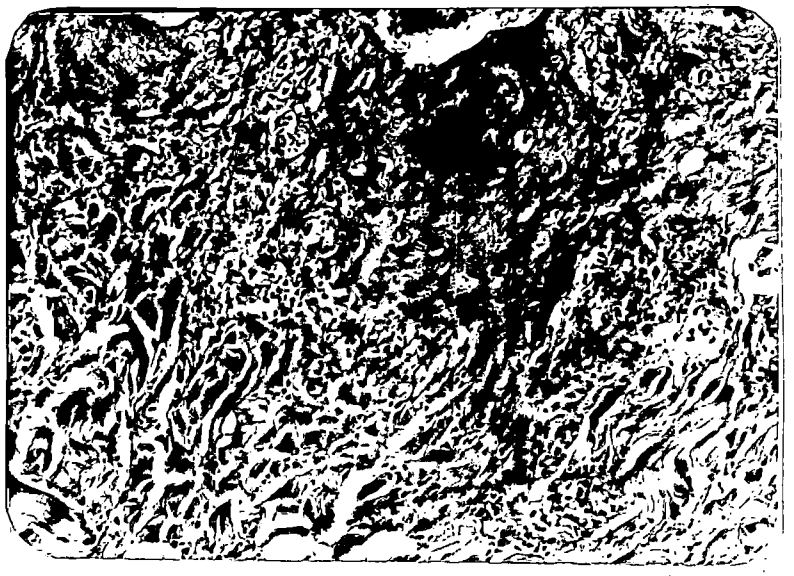
In the experimental groups the cellular response was of similar type but the reaction was less intense. The oedema and perivascular accumulation of cells were less pronounced (Fig. 11-13).

Forty-eight hour post-challenge

The cellular infiltration was more diffuse and intense. There was pronounced accumulation of lymphocytes and macrophages in the perivascular location but vascular changes were







loss. In a few sections capillary congestion and slight oedema were still discernible in the dermal layer.

Although the pattern of histological reaction was identical in the toxin fed groups, the degree of response in group II animals was relatively much less as evidenced by the less amount of cellular infiltrate and oedematous change (Fig.14-16).

4.5. Evaluation of humoral immune response

4.5.1. Determination of total protein in the serum.

The mean concentration of total serum proteins determined by the biuret method at different periods is set out in table 6 and is also shown in Fig.17. The total protein concentration in the control animals ranged from 6.60 ± 0.10 g/dl to 6.76 ± 0.09 g/dl during the experimental period of 90 days. The total protein concentration decreased from an initial value of 6.54 ± 0.13 g/dl to 5.31 ± 0.25 g/dl in group I animals and in group II animals from 6.61 ± 0.13 g/dl to 4.30 ± 0.00 g/dl, on the 90th day. The variations from the control values were significant ($P < 0.05$) from the 20th day onwards.

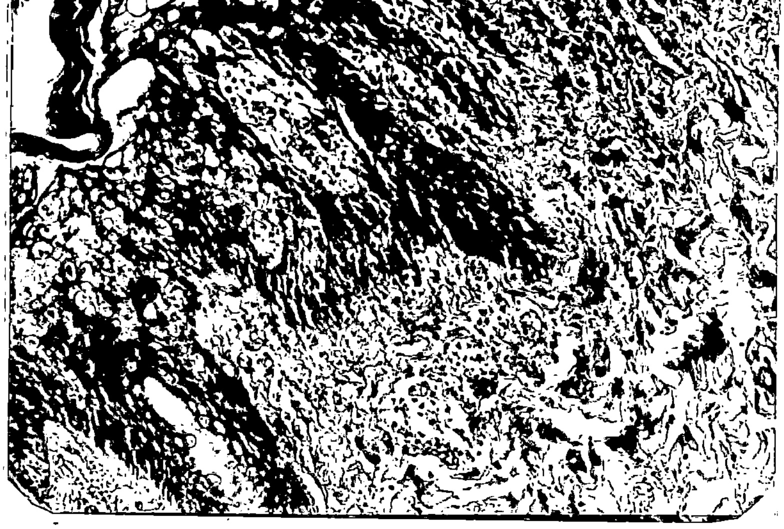
4.5.2. Determination of gammaglobulin in the serum.

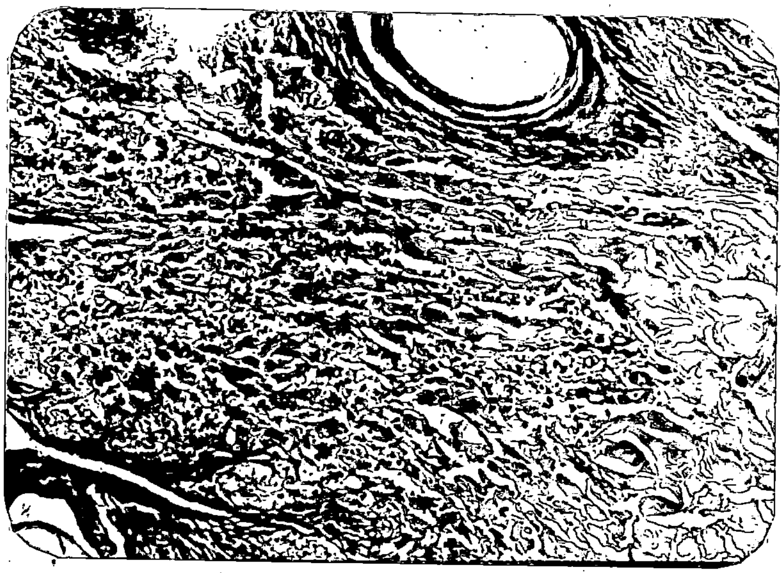
The data on mean gammaglobulin concentration of the control and experimental groups at different periods are set out in table 6 and are illustrated in fig. 17. In the control animals, the gammaglobulin concentration decreased from an initial value of 1.49 ± 0.00 g/dl to 1.40 ± 0.00 g/dl by the

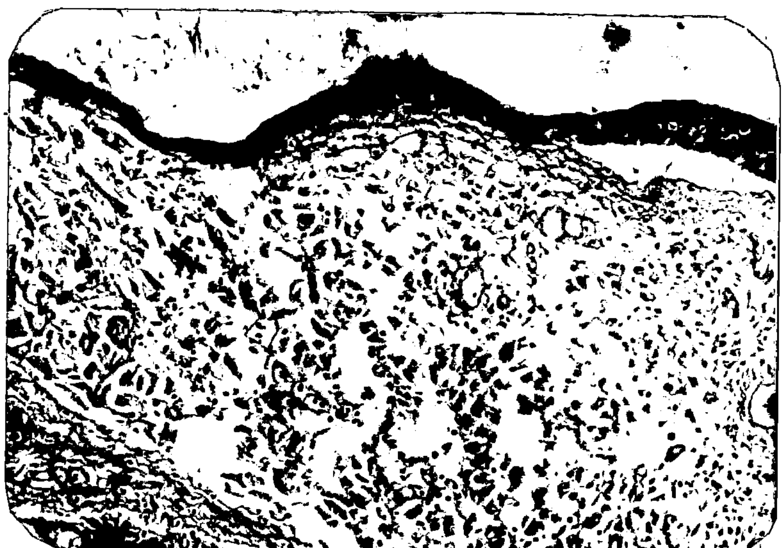
Table 6. Serum protein profile of control and experimental pigs (Mean ± S.E.)

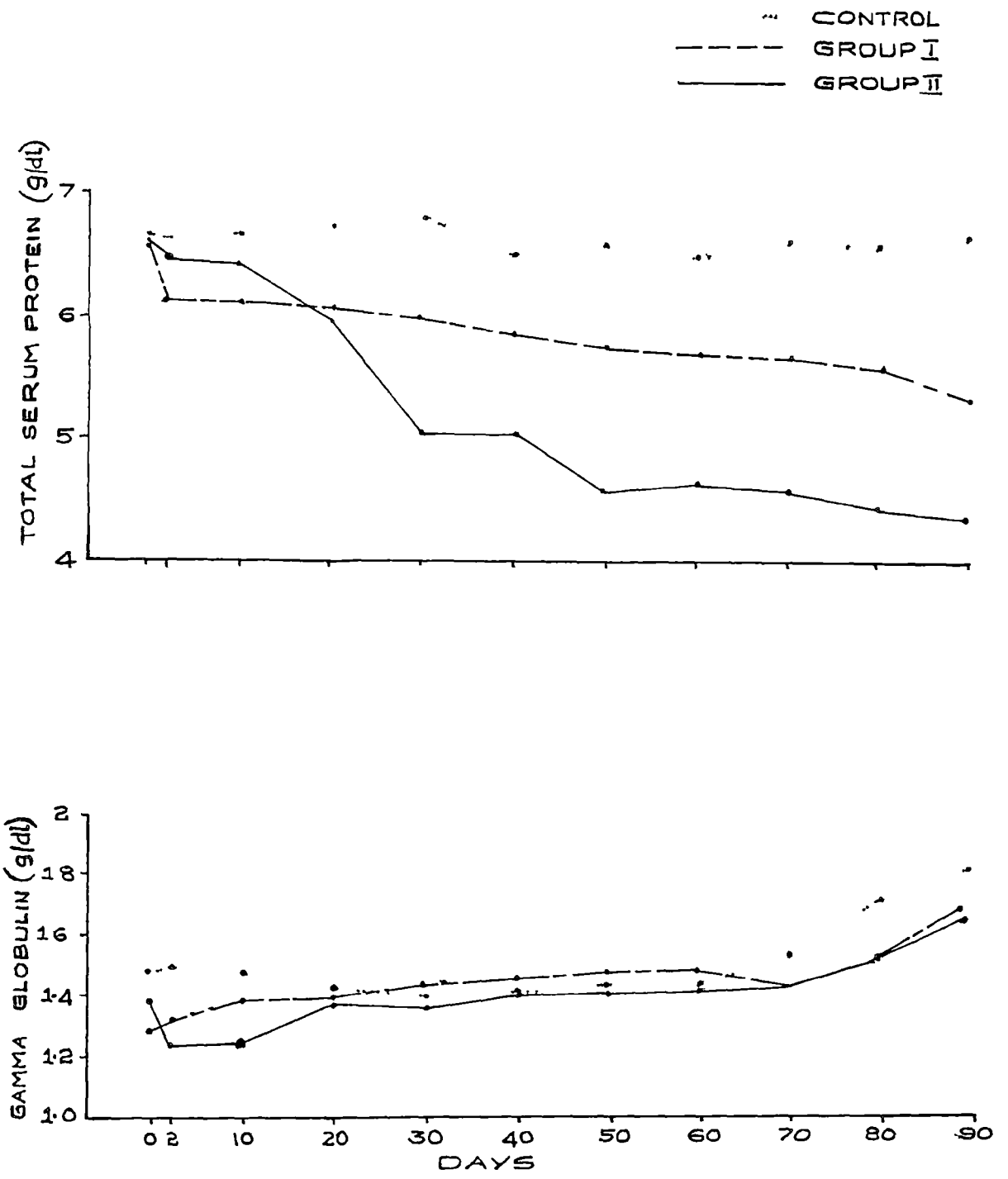
Interval in days	Total serum protein (g/dl)			Gammaglobulin (g/dl)		
	Control	Group I	Group II	Control	Group I	Group II
0	6.63 ±0.13	6.54 ±0.13	6.61 ±0.13	1.49 ±0.00	1.29 ±0.00	1.40 ±0.14
2	6.61 ±0.10	6.12 ±0.15	6.43 ±0.15	1.50 ±0.00	1.33 ±0.00	1.22* ±0.00
10	6.63 ±0.16	6.17 ±0.14	6.38 ±0.10	1.47 ±0.00	1.39 ±0.10	1.22* ±0.00
20	6.70 ±0.13	6.05* ±0.14	5.91* ±0.13	1.42 ±0.00	1.38 ±0.00	1.39 ±0.10
30	6.76 ±0.09	5.87* ±0.14	4.98* ±0.38	1.40 ±0.00	1.43 ±0.00	1.36 ±0.00
40	6.48 ±0.17	5.82* ±0.13	5.04* ±0.39	1.40 ±0.00	1.46 ±0.00	1.40 ±0.09
50	6.53 ±0.18	5.71* ±0.11	4.52* ±0.18	1.46 ±0.00	1.47 ±0.00	1.40 ±0.00
60	6.46 ±0.16	5.69* ±0.12	4.60* ±0.12	1.45 ±0.00	1.47 ±0.00	1.42 ±0.00
70	6.56 ±0.14	5.64* ±0.12	4.55* ±0.06	1.54 ±0.00	1.42 ±0.00	1.42 ±0.00
80	6.53 ±0.08	5.54* ±0.19	4.40* ±0.05	1.73 ±0.00	1.50* ±0.00	1.50 ±0.10
90	6.60 ±0.10	5.31* ±0.25	4.30* ±0.00	1.81 ±0.00	1.61* ± 0.00	1.62 ±0.02

* $P < 0.05$








CONTROL
GROUP I
GROUP II
TOTAL SERUM PROTEIN (g/dl)
7
6
5
4
GAMMA GLOBULIN (g/dl)
2
1·8
1·6
1·4
1·2
1·0
0 2
10
20
30
40
50
60
70
80
90
DAYS

40th day and then gradually increased reaching a maximum of 1.81 ± 0.00 g/dl on the 90th day.

In group I animals, the values gradually increased from the initial concentration of 1.29 ± 0.00 g/dl to 1.61 ±0.00 g/dl by the 90th day. The values were significantly lower ($P < 0.05$) than the control group on the 80th and 90th day. In group II animals, the gammaglobulin concentration reduced from an initial value of 1.40 ± 0.14 g/dl to a significantly lower ($P < 0.05$) value of 1.22 ± 0.00 g/dl compared to the control on the 2nd day but from the 20th day onwards the values increased gradually reaching a maximum of 1.62 ± 0.02 g/dl on the 90th day. The variations from the control values were significant ($P < 0.05$) on the 2nd and 10th day.

4.6. Assessment of the phagocytic activity of neutrophils and macrophages using Nitroblue tetrazolium salt (NBT) test

Cells with blue-black (formazan) deposits in the cytoplasm were counted as NBT positive (Fig.18).

4.6.1. Neutrophils.

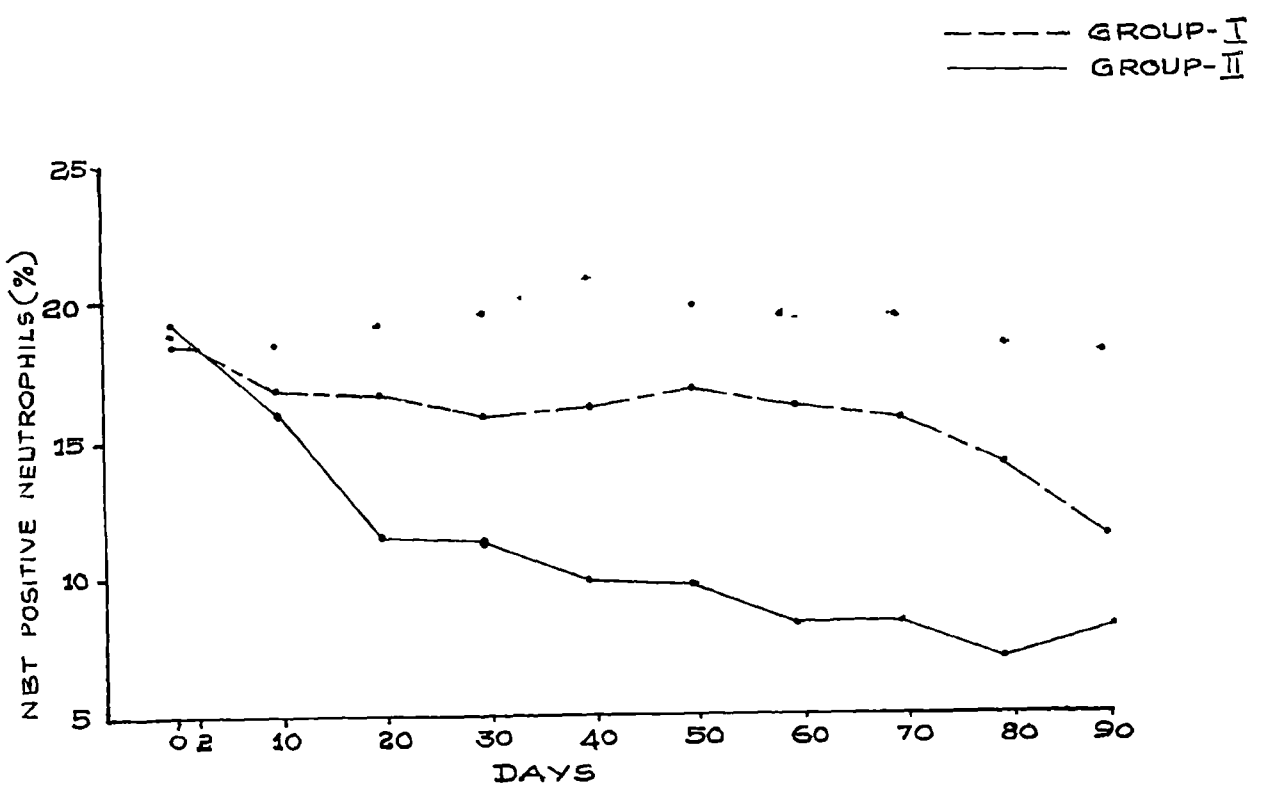
The data on the mean NBT positive neutrophils of the control and experimental animals at different periods are detailed in table 7. Figure 19(a) illustrates the change. In the control group, the number of positive cells ranged from 18.00 ± 1.66% to 20.83 ± 0.83%. In group I animals, the percentage of positive cells declined from an initial value of 18.43 ± 0.75% to 11.33 ± 0.84% by the 90th day. The

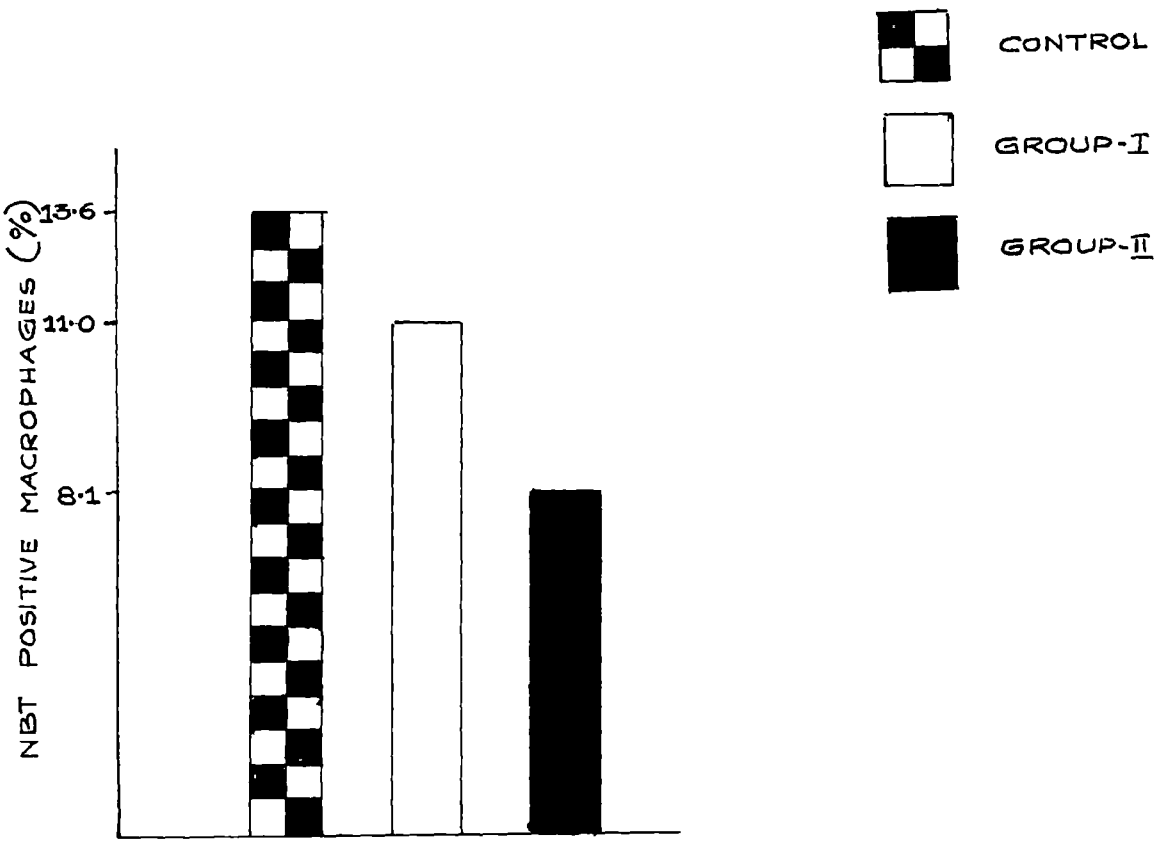
Table 7. Assessment of the phagocytic activity using NBT salt reduction test (Mean ± S.E.)

Interval in days	NBT positive neutrophils (%)		
	Control	Group I	Group II
0	18.83±0.75	18.48±0.75	19.00±1.00
2	18.33±0.66	18.29±1.26	18.43±0.87
10	18.83±0.60	16.71±1.43	16.00±1.80
20	19.16±0.83	16.86±0.93	11.71±0.52*
30	19.5 ±1.20	15.57±1.10*	11.40±0.75*
40	20.83±0.83	16.00±0.98*	9.80±0.66*
50	19.83±0.70	16.71±0.89*	9.60±0.40*
60	19.16±0.60	15.86±1.31*	8.40±0.50*
70	19.33±0.71	15.71±1.10*	8.25±0.63*
80	18.33±0.61	14.00±0.90*	7.00±1.00*
90	18.00±1.66	11.33±0.84*	8.00±0.00*

NBT positive macrophages (%)		
Control	Group I	Group II
13.66	11.00*	8.14*

* $P < 0.05$


GROUP-I
GROUP-II
NBT POSITIVE NEUTROPHILS (%)
25
20
15
10
5
0 2
10
20
30
40
50
60
70
80
90
DAYS


CONTROL
GROUP-I
GROUP-II
NBT POSITIVE MACROPHAGES (%)
13·6
11·0
8·1

variations being significant ($P < 0.05$) from the control animals from the 20th day onwards. In group II animals, from initial value of 19.00 ± 1.00%, the value declined to 8.00 ± 0.00% on the 90th day. The variations from the control values were significant ($P < 0.05$) from the 10th day onwards.

4.6.2. Macrophages.

The mean macrophage response to NBT salt performed on the prefemoral lymphnode impression smear is compared in table 7 and fig.19(b).

In the control group, the NBT positive macrophages were on an average 13.66%. In group I animals, the number of positive cells was 11.00% and a considerable decrease was observed in group II animals in which the number of positive cells was only 8.14%. The variations from the control values were significant ($P < 0.05$) in both group I and group II.

4.7. Morbid anatomy and histopathology

During the course of the experiment, one animal in group I died on the 72nd day and five animals in group II died on the 21st, 23rd, 67th, 77th and 85th day respectively. All the other animals in the experimental as well as control group were sacrificed on the 90th day. In the animals that died, there was subcutaneous petechial haemorrhage and haemorrhage in all the visceral organs. The carcase was moderately icteric. There was varying degree of ascitis and hydropericardium in all the dead as well as sacrificed animals in

group I and group II. The severity of the lesions was more in group II. The mean relative weights of the liver, kidney, heart and spleen expressed as percentage of body weight are given in table 8.

Table 8. Mean relative weight of liver, kidney, heart and spleen expressed as percentage of body weight

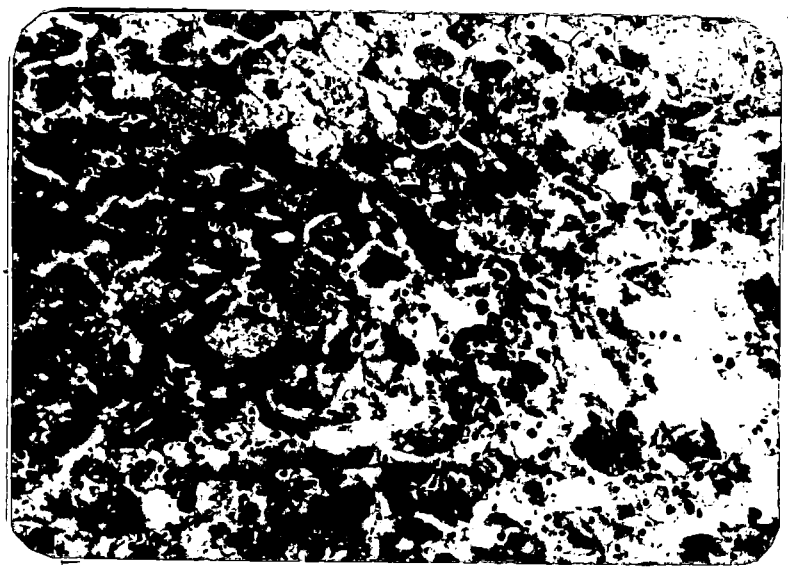
	Group I	Group II	Control
Liver	9.74 ±0.45	10.22* ±0.33	8.85 ±0.44
Kidney	3.76* ±0.17	4.45* ±0.13	3.19 ±0.08
Spleen	2.59 ±0.24	2.48 ±0.20	2.01 ±0.04
Heart	4.05* ±0.11	4.62* ±0.18	3.35 ±0.10

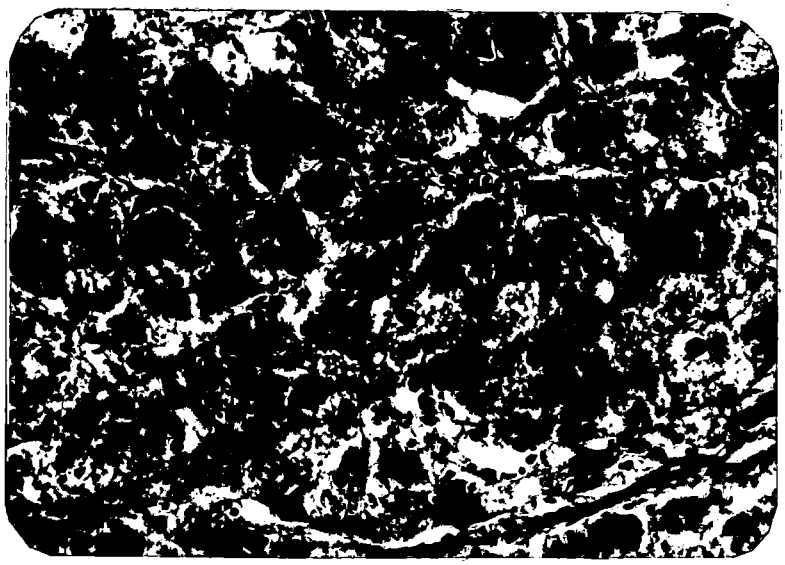
* Indicates significance at 5% level ($P < 0.05$)

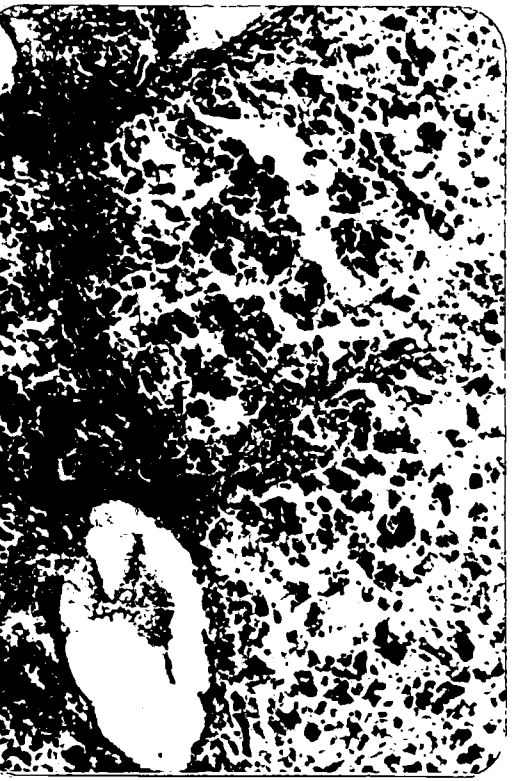
4.7.1. Liver.

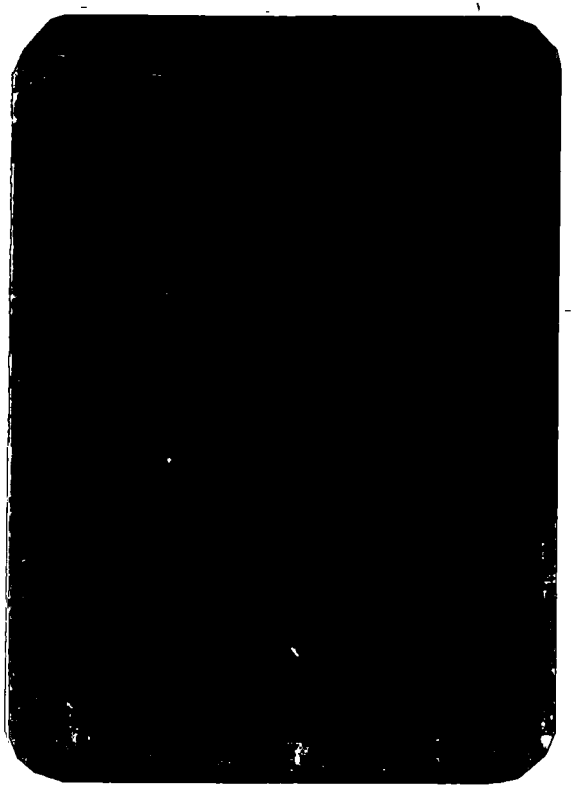
It is evident from the table 8 that the weight of the liver in group II was significantly higher ($P < 0.05$) when compared to that of the control animals. The liver was enlarged, yellowish and petechial haemorrhages were seen on all the lobes and in the parenchyma (Fig.20).

In one animal of group I, the liver had a hard fibrous texture and the organ contained scattered raised pale brown nodules (Fig.21). Histologically, pronounced fatty change and centilobular necrosis were seen (Fig.22). Staining with









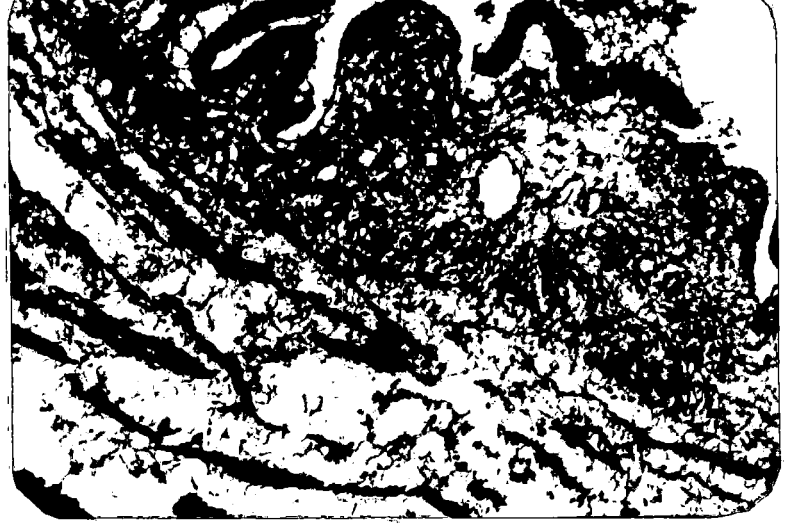
Sudan II and Sudan III revealed fatty cysts in certain foci. Hyaline bodies resembling councilman bodies were seen in the degenerating hepatic cells (Fig.23). The normal lobular appearance of the liver was accentuated by biliary proliferation and bile stasis. Staining by Van Gieson's method revealed large bands of fibrocollagenous connective tissue coursing through the lobules. This gave a picture of post necrotic cirrhosis (Fig. 24, 25).

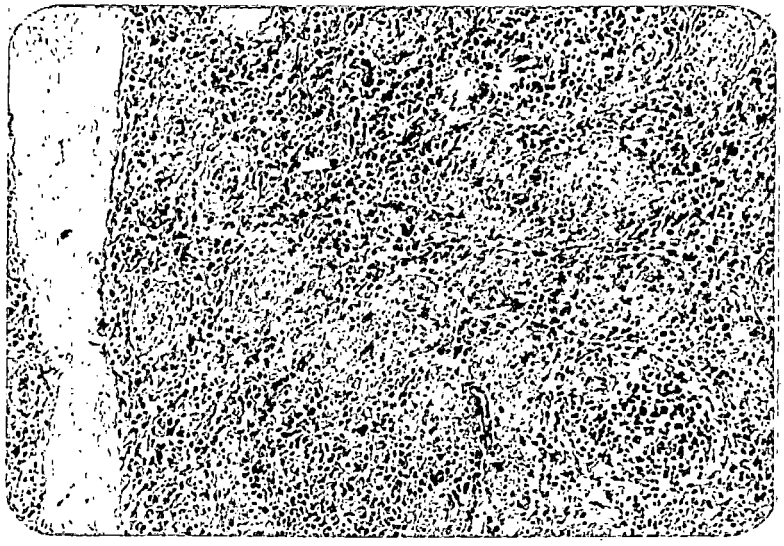
There was rupture of sinusoids and focal areas of haemorrhage. Reticulum staining revealed collapse of the lobular architecture characterised by collapse and reduplication of reticulum. Focal lymphocytic infiltration was also a common finding (Fig. 26, 27).

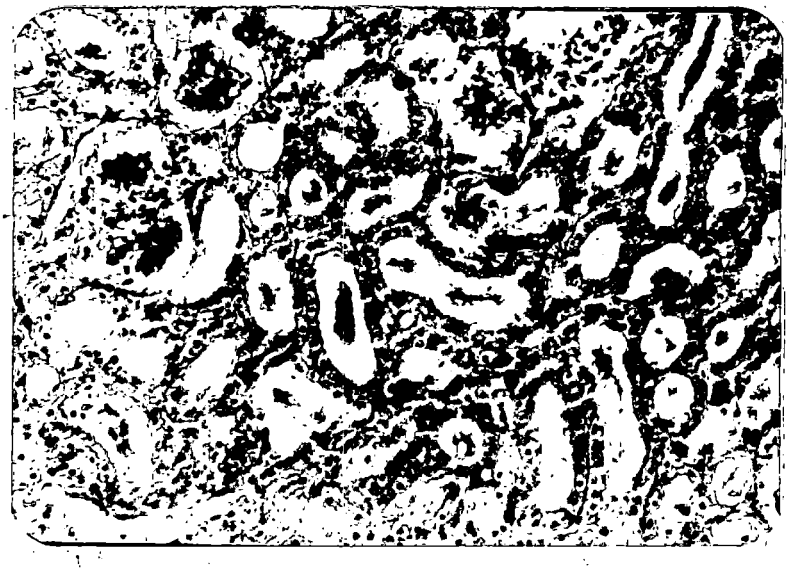
The gall bladder of all the animals in group I and group II was moderately distended with pale thin yellow bile and the wall was oedematous. Histologically, in the gall bladder there was moderate oedema of the mucosa and moderate lymphoid infiltration (Fig. 28).

4.7.2. Spleen.

The mean relative weights of the spleen did not indicate any statistically significant difference between the experimental and control groups. There was no appreciable gross lesion except in one animal belonging to group II in which the spleen had small nodules on the surface. Microscopically, in all the experimental animals there was haemorrhage and haemosiderosis (Fig. 29).







4.7.3. Kidney.

The mean relative weight of the kidney of the experimental group showed a significant increase ($P < 0.05$) compared to the control animals. Grossly, in both group I and group II, the kidneys showed yellowish grey discolouration of the cortical region and in some cases petechiae were also seen. Gelatinisation of the fat in the renal pelvis was a consistent finding. Histologically, the changes were confined to the tubules. Tubular degeneration and desquamation of the epithelium and formation of hyaline casts were seen (Fig.30). In few cases these changes were accompanied by scattered foci of haemorrhage.

4.7.4. Pathology of the lymph nodes.

Gross changes were evident only in the hepatic, mesentric, prescapular and prefemoral lymphnodes which showed moderate enlargement. In most of the hepatic lymphnodes there was cortical haemorrhage and few were oedematous. The microscopic lesions observed in different lymphnodes of group I and group II are set out in tables 9 and 10.

4.7.4.1. Hepatic lymphnode.

In many cases there was cortical haemorrhage and few were oedematous. Microscopically, there was diffuse haemorrhage and this was associated with deposition of haemosiderin pigment (Fig.31). In most of the cases, there was depletion of lymphoid cell population in the cortical and medullary region. The

Table 9. Reaction of the lymphnodes
Group I

	Reactive	Haemorrhage	Lymphoid depletion	Normal
Hepatic	++	++++	+++	-
Renal	+	-	-	+++
Mesenteric	+++	-	-	-
Bronchial	-	-	-	++++
Prescapular	++++	-	-	-
Prefemoral	++++	-	-	-

(+ Mild, ++ Moderate, +++ Severe)

Table 10. Reaction of the lymphnodes
Group II

	Reactive	Haemorrhage	Lymphoid depletion	Normal
Hepatic	++	++++	++++	-
Renal	+	-	-	++++
Mesenteric	++++	-	-	-
Bronchial	+++	-	-	+
Prescapular	++++	-	-	-
Prefemoral	++++	-	-	-

(+ Mild, ++ Moderate, +++ Severe)

proliferating stromal tissue was seen to replace most of the lymphoid tissue. In one animal of group II there was dilatation of lymphatics. Focal areas of congestion and haemorrhage were also seen (Fig.32). Sinus histiocytic reaction was sparse to minimal. A few lymphnodes were of reactive type. The consistent feature of this group was diffuse hyperplasia of lymphoid tissue in the cortical, paracortical and medullary region. The lymphoid cells in this group were large with eosinophilic cytoplasm and loosely arranged chromatin. There was also diffuse paracortical and focal histiocytic reaction characterized by islands of histiocytes in the medullary region.

4.7.4.2. Renal lymphnode.

In one animal in group I and in group II, the lymphnode was of reactive type.

4.7.4.3. Mesenteric lymphnode.

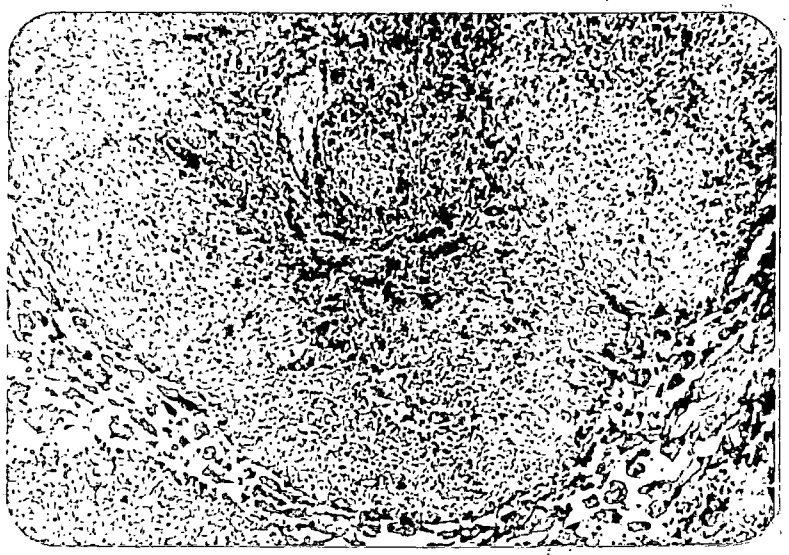
These were of reactive type (Fig.33).

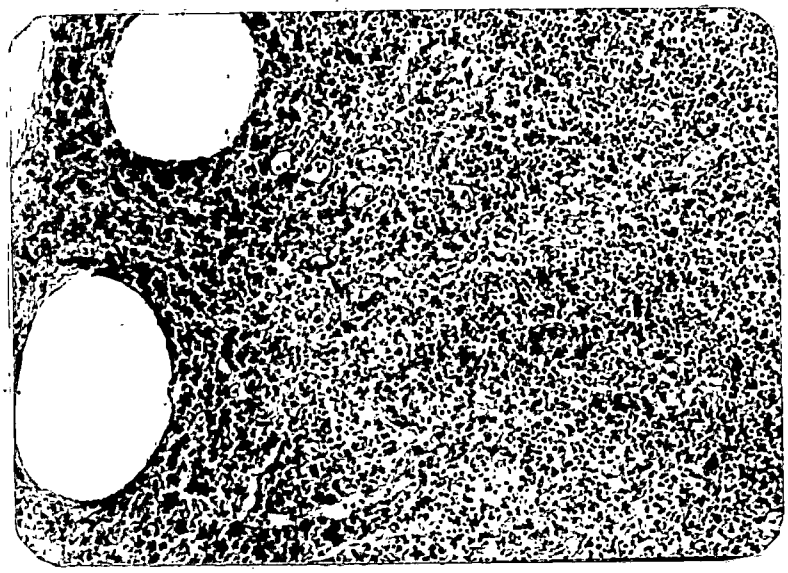
4.7.4.4. Bronchial lymphnode.

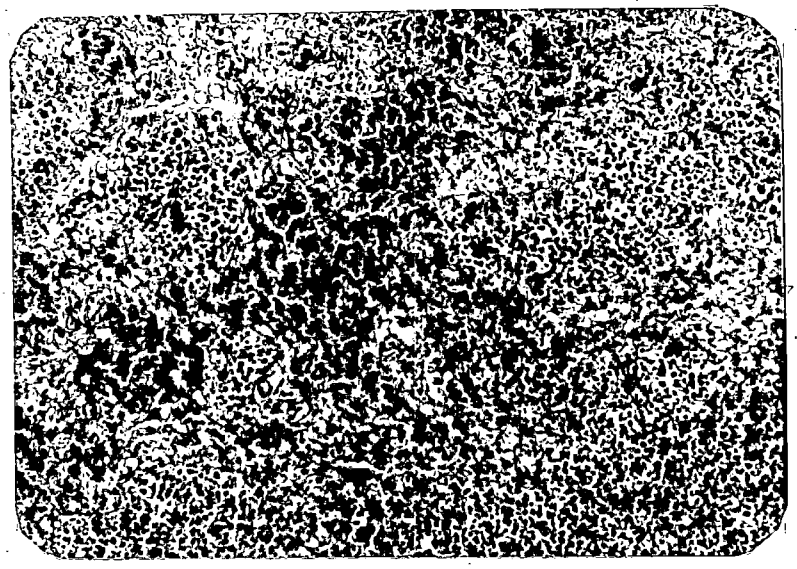
A reactive nature was evident in group II.

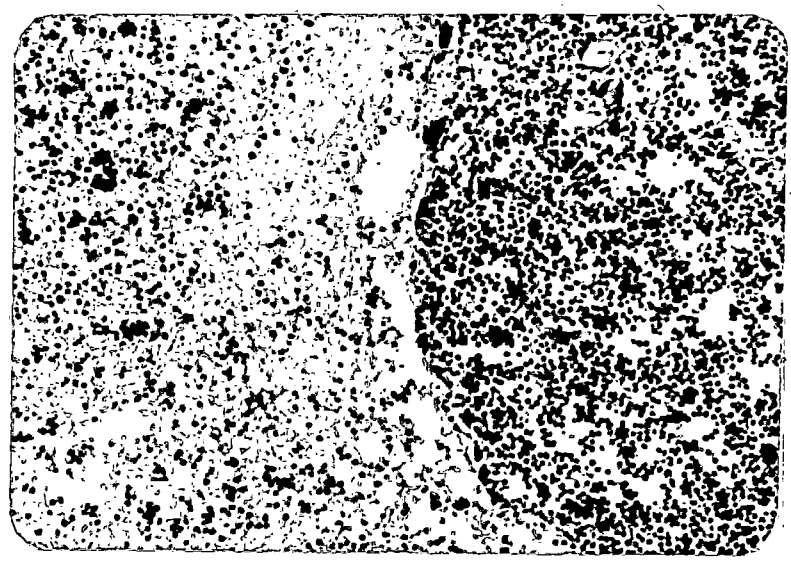
4.7.4.5. Prescapular and prefemoral lymphnodes.

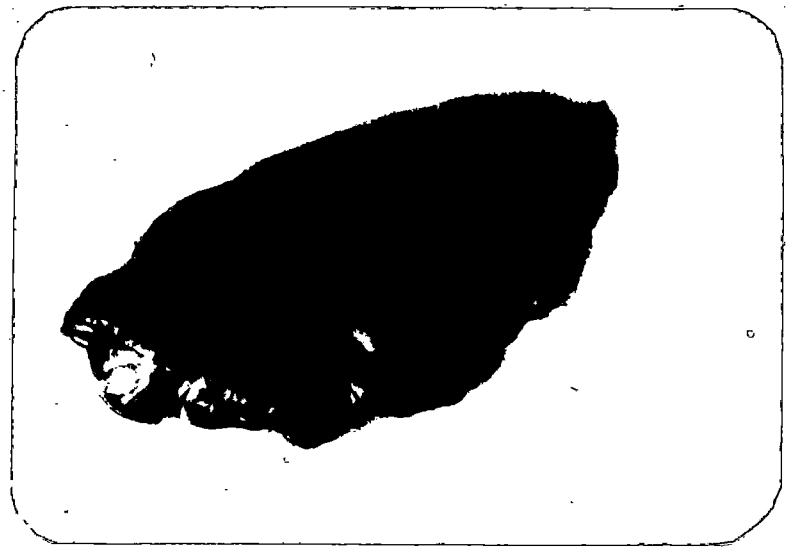
In both group I and group II the prescapular and prefemoral lymphnodes were of a reactive nature with sinus histiocytosis. The consistent feature of this group was diffuse hyperplasia of lymphoid tissue in the cortical, paracortical and medullary region. Few lymphoid follicles in the cortical











region exhibited germinal centre formation. This area appeared pale compared to other parts of the lymphnodes. Sinus histiocytosis was characterized by the presence of large oval cells with abundant slightly foamy granular eosinophilic cytoplasm in the medullary region.

4.7.5. Thymus.

Grossly, the thymus was slightly oedematous and contained petechiae and echymotic patches both on the surface as well as in the parenchyma (Fig.34). Histologically, there was multiple focal areas of haemorrhage in the parenchyma and in the interstitial tissue. The lymphoid tissue was widely separated by foci of haemorrhage. Lymphoid cells were few and loosely scattered (Fig.35). Islands of Hassal's corpuscles undergoing degeneration were seen amidst the area of haemorrhage (Fig.36).

Discussion

with aflatoxin. This could be attributed to the damaging effects of aflatoxin on the hepatic tissue, and this would perforce lead to variation in the total protein content and the albumin:globulin ratio. The mange infection observed in the pigs would again be a contributory factor for the increased ESR.

The reduction in PCV, haemoglobin and total erythrocyte count in the toxin fed pigs reflects anaemia and this again can be a factor which would contribute to the increase in ESR. Cysewski et al. (1968); Osuna and Edds (1982) made similar observations whereas this feature was not observed by Vishalakshan et al. (1984) in pigs fed aflatoxin.

It is reasonable to explain that the pigs contracted infection as a result of immunodeficiency and the leukocytosis observed is only a consequence of the disease process. Increase in the total leukocyte count in pigs fed aflatoxin was observed by Cysewski et al. (1968); (1978); Miller et al. (1978) and Dhanvantari et al. (1982). However, Osuna and Edds (1982) did not observe any significance in tho total leukocyte count in experimental aflatoxicosis of pigs.

There was no significant difference in the absolute lymphocyte count in pigs dosed with aflatoxin. This might be attributed to the fact that the experimental group of pigs had picked up mange infection. This inflammatory response may be responsible for masking the immunosuppressive effect of aflatoxin. It is pertinent to point out that in spite of

severe mange infection, the absolute lymphocyte count did not show a significant absolute lymphocytosis. This is an observation that would support the conclusion that in the pigs dosed with aflatoxin there was immunosuppression. This has also been clarified by other immunological markers employed for assessing the immunological response. In this context it may be pointed out that Cysewski et al. (1968) and Dhanvantari et al. (1982) have observed lymphopenia in pigs fed aflatoxin.

Assessment of ANAE positive cells (T cells) in the peripheral blood showed a significant decrease in the aflatoxin fed pigs indicating that in these animals the T-cell response was relatively suppressed. Similar observations were made by Vishalakshan et al. (1984) in pigs fed aflatoxin. McLoughlin et al. (1984) also observed a decrease in the number of T cells in aflatoxin fed guinea pigs. There was however a transient increase in the percentage of ANAE positive cells in later stages. This has to be considered as an after effect of DNCB application in these pigs to assess the CMI response. Rajan et al. (1982) recorded an increase in the percentage of ANAE positive lymphocytes in pigs following DNCB challenge. They suggested induction of esterase enzyme in T cells following DNCB challenge to be the probable reason for their increase. This increase in T cell population was not reflected on the absolute lymphocyte count suggesting that the immune system at this stage is suppressed. It may be pointed out that Ranki et al. (1976) has shown the possibility of some stimulated

B cells exhibiting ANAE activity. This is a point which has to be clarified by further studies.

The cell-mediated immune response (CMI) and indirectly the functioning of the macrophage-lymphoid system in pigs fed aflatoxin was assessed employing cutaneous hypersensitivity tests. PHA-M and 2,4-dinitrochlorobenzene (DNCB) were used for the first time for assessment of the cell-mediated immune response in aflatoxicosis in pigs. These tests clearly demonstrated a lowered cell-mediated immune response in aflatoxin fed pigs and this was reflected by significantly lower values in skin thickness and in DNCB, the diameter of the reactive area also, at 24 and 48 hours. It is significant to observe that the histological changes in the toxin fed pigs at the site of inoculation were less pronounced when compared to the control animals. These observations would lead to the conclusion that there has been significant reduction of CMI response in pigs dosed with aflatoxin. Suppression of CMI response of pigs in aflatoxicosis was also recorded by Miller et al. (1978). The assessment of the CMI response employing DNCB and PHA test as markers has shown that the response to these markers of CMI was dose dependent. Pier (1981) suggested that aflatoxin may impair the cellular immune response through inhibition of the function of T lymphocytes and possibly lymphokine production. Haemorrhage and lymphoid depletion observed in the thymus of aflatoxin fed pigs indicates that aflatoxin may exert a direct effect on the immune system of

pigs. Depletion of lymphocytes and involution of thymus in chicken fed aflatoxin were recorded by Pier et al. (1972) and Thaxton et al. (1974). Maryamma (1973) also observed capillary engorgement and granular degeneration of thymic corpuscles of goats fed aflatoxin. According to Cooper et al. (1966) thymic defect would be more likely to affect delayed hypersensitivity and graft-versus-host reactions than antibody production and bacterial clearance. These observations would support the conclusion that there is immunological suppression in aflatoxicosis.

The damaging effects of aflatoxin on the hepatic tissue was reflected by the lowered total serum protein concentration in aflatoxin fed pigs indicating that continued low-level consumption of aflatoxin had an inhibitory effect on the protein synthesis. Decreased protein synthesis could also be attributed to reduced feed consumption by the toxin fed animals. These findings correlate with that of Annau et al. (1964); Cysewski et al. (1968); 1978; Miller et al. (1981); Osuna and Edds (1982) and Ho (1982).

Earlier workers (Edds, 1973; Buck et al. 1976) suggested that aflatoxin impaired protein synthesis by inhibiting the activity of the DNA and RNA polymerases. Busby and Wogan (1981) opined that polysome disaggregation was consistently associated with, and may be the basis for the inhibition of protein synthesis by aflatoxin treatment. In the present study, the gammaglobulin levels of the toxin fed pigs was significantly

low indicating that aflatoxin might exert an effect on the humoral immune response. Decreased gammaglobulin levels in aflatoxicosis of pigs was also recorded by Southern and Clawson (1979); Ho (1982); Osuna and Edds (1982). Suppression of humoral response in pigs fed aflatoxin and their consequent enhanced susceptibility to Salmonella infection was pointed out by Miller et al. (1978).

It is very pertinent to point out that in the experimental studies carried out in this investigation, the pigs fed aflatoxin contracted mange. The control animals did not pick up infection although they were maintained under identical managemental conditions. This is an important point and need to be stressed. This is a practical problem emerging as a consequence of immunosuppression. Dykes (1986) also reported mange infection in pigs following consumption of aflatoxin contaminated feed and he also put forward the same hypothesis.

The apparently increasing trend in the gammaglobulin level despite a decrease in the total serum protein concentration observed in the aflatoxin fed pigs is an indication of the change following liver damage. This observation is true to the hypothesis made by Richard et al. (1978) according to which when the liver is damaged, it does not remove or eliminate the antigens that have been absorbed by the gastrointestinal tract and have made their way to the liver and thus allows the antigens to interact with antibody producing cells resulting in increased gammaglobulin production. Increased

gammaglobulin levels in aflatoxin fed pigs was also recorded by Annau et al. (1964); Gumbmann and Williams (1969); Cysewski et al. (1978) and Miller et al. (1981). The NBT reduction test employed to assess the phagocytic activity of neutrophils revealed a considerable reduction in the number of NBT positive cells in the aflatoxin fed pigs. It is relevant to refer here that NBT salt reduction test was first applied on the neutrophils as an indicator to assess their function by Park et al. (1968) in man and they considered it as an important test to assess the neutrophil function. They observed an increase in the number of NBT positive cells in bacterial infection. However, in the present investigation, a decrease in their number was noticed in aflatoxin fed pigs in spite of the neutrophilia. The functional activity of macrophages assessed by the NBT salt test in the lymphnode impression smear showed a decrease in the percentage of NBT positive macrophages. This observation is complementary to that observed in the case of neutrophilic leukocytes.

The studies undertaken therefore showed that aflatoxin impairs the function of at least more than one of the cell types of the phagocytic system, which was also dose related. This observation is akin to that Michael et al. (1973), Chang et al. (1976); Chang and Hamilton (1978) in aflatoxicosis of chicken. They recorded impairment of the phagocytic activity of heterophils and monocytes.

Richard and Thurston (1975) also documented decreased

phagocytic activity of macrophages in rabbits fed aflatoxin. The gross and histopathological changes observed in the present study were related to the dose of the toxin. Pigs given the lower dose were less severely affected. The observation that five of the seven animals which received higher dose (group II) of aflatoxin died while only one animal out of seven in (group I) animals given the lower dose died is an indication of the dose related response of animals to aflatoxin.

Subcutaneous haemorrhage and haemorrhage in almost all the visceral organs observed in the experimental animals were similar to the findings reported by Hauser (1971), Edds (1979) and Sriramamurthy et al. (1981).

According to Bababunmi and Bassir (1969), the haemorrhagic effect of aflatoxin B_1 like that of 4-hydroxy coumarin, may be due to specific inhibition of the synthesis of prothrombin due to the competition for the apoenzyme, and not to any generalized hepatocellular damage. This can also be due to the inhibitory effect on the synthesis of clotting protein. The icteric nature, increased liver weight, pronounced hepato-cellular necrosis, hepato-cytomegaly and hepatokaryomegaly, proliferation of reticular and collagen fibres, fatty infiltration, bile duct proliferation and infiltration with mononuclear cells are suggestive of chronic aflatoxicosis and these findings are in accordance with those of earlier workers (Sippel et al. 1953; Burnside et al. 1957; Loosmore and Harding, 1961; Harding et al. 1963; Annau et al. 1964; Shalkop et al. 1967; Sisk et al. 1968;

Armbrecht et al. 1971; Newberne, 1973 and Miller et al. 1981).

According to Newberne and Butler (1969), the liver is the primary target organ of aflatoxin action and becomes infiltrated with fatty deposits when sufficiently high levels of aflatoxins are administered. Since aflatoxin inhibits protein synthesis and prevents apoprotein formation required for lipoprotein formation, the triglycerides cannot be mobilised out of the cell without lipoprotein formation and fatty infiltration results (Osuna and Edds, 1982).

Hepatic necrosis is thought to result when glutathione reserves have been drastically depleted by conjugation with toxic intermediates so that the toxic intermediates are free to bind covalently to vital cellular macromolecules (Hatch, 1982).

There was varying degree of oedema of the gall bladder in the experimental group of pigs. This can be attributed to poor drainage of bile. This was also recorded by Wilson et al. (1967); Iwasaki et al. (1974) and Armbrecht (1978) in aflatoxicosis of pigs.

The degenerative pathobiological changes observed in the toxin fed animals indicated that aflatoxin had secondarily affected the kidneys also. This is in accordance with the findings of Wilson et al. (1967), Armbrecht (1978) and Dhanvantari et al. (1982).

Haemorrhage and haemosiderosis in the spleen of toxin fed animals are reflections of toxin induced injury. Haemorrhage, haemosiderosis and lymphoid cell depletion observed in the hepatic lymphnodes were suggestive of aflatoxin induced injury to the blood vessels of the lymphnodes and to the destructive effect of aflatoxin on the lymphoid cell population. The latter is a feature which would substantiate a reduction in cell-mediated immune response.

The reactive nature of the mesenteric lymphnode may be a part of the inflammatory reaction seen in the intestine. The reactive lymphadenopathy of the prescapsular and prefemoral lymphnode in the aflatoxin dosed mange infested pigs is to be considered as a consequence of the severe mange infection seen in these pigs. Maryamma (1973) also observed a reactive nature in the prescapular; parotid, hepatic and mesenteric lymphnodes of goats fed aflatoxin.

By the experimental studies undertaken it was clarified that aflatoxin has a significant suppressive effect on the immune system of pigs. The decreased response to cutaneous hypersensitivity agents PHA-M and DNCB along with reduction of ANAE positive cells is suggestive of suppression of CMI response. The decreased level of gammaglobulin indicates a decreased humoral response and the reduction of NBT positive neutrophils and macrophages points to lowered phagocytic response. These observations were supported by the gross and histopathological lesions in the liver, kidney, spleen, and

the lymphnodes. It was also observed that this immunosuppression would lead to enhanced susceptibility to infection. All the aflatoxin dosed pigs contracted mange infection. In the situation prevalent in the farm when the pigs are fed a diet contaminated with aflatoxin eventhough at a low level, the immunosuppression induced in the pigs predispose them to infections. Although the final postmortem diagnosis may be often pneumonia, enteritis or some other disease the basic cause may be an aflatoxin induced acquired immunosuppression. This fact will be generally overlooked. It may be pointed out that many of the biological agents responsible for causing the disease are prevalent in the environment and it is the competency of the immune system which determines the prevalence of the disease in an animal. The observations made in this study have therefore, great practical relevance and the results stress the need for screening the feed samples for aflatoxin contamination and the necessity to feed pigs a diet free of aflatoxin. This preposition may have limitations as in a State like Kerala with a hot and highly humid climate, heavy fungal contamination is likely to occur and the situation may warrant to adopt a policy of living with aflatoxin. This implies that efforts should be made to develop a suitable, simple and cheap technology to detoxify aflatoxin in the feed.

Summary

SUMMARY

An experimental study was undertaken to study the effect of aflatoxin on the immune system of pigs.

Aflatoxin obtained from the cultures of Aspergillus parasiticus and standardised in the laboratory was used for the experiment. Twenty Large White Yorkshire male pigs of 2-3 months of age were randomly grouped as controls, group I and group II. Aflatoxin was administered orally at the dose rate of 0.1 mg/kg body weight and 0.2 mg/kg body weight to group I and group II animals respectively for a period of 90 days.

Clinically, the toxin fed animals were dull and depressed. They consumed less feed and the body weight recorded at fortnightly intervals was significantly low when compared to the control animals.

There was increase in ESR and reduction in PCV, haemoglobin and total erythrocyte count in the aflatoxin fed pigs.

There was no significant variation in the neutrophil and lymphocyte count in the experimental animals. However, after the infection there was slight neutrophilia.

During the course of the experimental period the pigs fed aflatoxin contracted mange infection. This observation was considered as a clinical evidence of immunosuppressive effect of aflatoxin.

The T lymphocytes (ANAE positive cells) in the peripheral blood of the toxin fed pigs were significantly low than the control pigs.

The macrophage-lymphoid system in the experimental and control pigs was assessed by monitoring the cell-mediated immune response employing PHA and DNCB hypersensitivity tests. The cell-mediated immune response in the aflatoxin fed pigs was significantly low.

The serum protein and gammaglobulin concentration were significantly low in pigs fed aflatoxin. These observations indicated suppression of the humoral immune response.

The phagocytic activity of the neutrophils and macrophages was assessed by the Nitroblue tetrazolium (NBT) reduction test. A decrease in the phagocytic response was observed in the aflatoxin fed pigs.

Haemorrhages were seen in almost all the visceral organs. The liver of aflatoxin fed animals was enlarged and icteric. The gall bladder was distended and oedematous. Fatty change, centrilobular necrosis, haemorrhage, biliary hyperplasia, fibrous tissue proliferation, reticular collapse and focal lymphocytic infiltration were the characteristic histological features. Haemorrhage and haemosiderosis were evident in the spleen. Tubular degeneration and focal haemorrhages were seen in the kidney. The thymus and the lymphnodes revealed depletion of lymphoid cells and focal areas of haemorrhage. Some

of the lymphnodes revealed reactive proliferative response.

By employing two different dose levels of aflatoxin it was clarified that the biological effects of aflatoxin was dose dependent.

From the observations made, it was concluded that aflatoxin suppressed the cell-mediated and humoral immune response.

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DISCUSSION

A study was carried out to assess the effects of aflatoxin on the immune response of pigs at two different dose levels, 0.1 and 0.2 mg/kg body weight.

The general clinical manifestations in the pigs dosed with aflatoxin were depression, stunted growth and progressive weight loss. The data collected on these parameters indicated that the adverse effects of aflatoxin were more pronounced in pigs which were given 0.2 mg/kg body weight of aflatoxin. This observation showed that the adverse effect of aflatoxin was dose dependent. Newberne and Butler (1969) recorded that the toxic effects of aflatoxin B_1 varied depending on the dose and duration of exposure. The decrease in body weight observed in the aflatoxin fed pigs was associated with reduced feed consumption. Armbrecht et al. (1971) indicated that the effects of aflatoxin on weight gain and feed conversion were among the most sensitive indicators of aflatoxicosis. If these are considered as marker indicators of aflatoxicosis in pigs, in the present investigation there was evidence for these changes and it could be surmised that aflatoxin had induced the anticipated biological effects. Earlier workers (Sisk et al. 1968; Cysewski et al. 1978; Edds, 1979; Pier, 1981; Osuna and Edds, 1982; Ho, 1982 and Vishalakshan et al. 1984) had also made similar observations.

There was significant increase in the ESR of pigs dosed

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IMMUNOPATHOLOGICAL RESPONSE OF PIGS IN AFLATOXICOSIS

By

M. GOPALAKRISHNAN NAIR

ABSTRACT OF A THESIS

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Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

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ABSTRACT

The immune response of pigs fed aflatoxin at two different dose levels, 0.1 and 0.2 mg/kg body weight was studied.

The reduction in weight gain observed in the aflatoxin fed pigs was associated with decreased feed consumption and total serum protein concentration.

There was increase in ESR and decrease in PCV, haemoglobin and total erythrocyte count in the toxin fed groups.

The neutrophilic and lymphocytic count did not show any significant variation except when the animals contracted mange infection. Following infection there was slight neutrophilia.

The cell-mediated immune response was assessed employing ANAE, PHA and DNCB as markers. A decrease in the immune response was documented in the aflatoxin fed pigs.

The humoral immune response was found to be suppressed as evidenced by a decrease in the concentration of total serum protein and gammaglobulin in the toxin fed groups.

The phagocytic activity of neutrophils and macrophages was assessed using Nitroblue tetrazolium (NBT) test. The data revealed a decreased phagocytic activity.

Haemorrhages were seen in almost all the visceral organs. The liver of aflatoxin fed animals was enlarged and icteric. The gall bladder was distended and oedematous. Fatty change, centrilobular necrosis, haemorrhage, biliary hyperplasia,

fibrous tissue proliferation, reticular collapse and focal lymphocytic infiltration were the characteristic histological features. Haemorrhage and haemosiderosis evident in the spleen. Tubular degeneration and focal haemorrhages were seen in the kidney. The thymus and the lymphnodes revealed depletion of lymphoid cells and focal areas of haemorrhage. Some of the lymphnodes revealed reactive proliferative response.

Regular screening of feed for aflatoxin was advocated as a routine farm operation to prevent the deleterious effect of aflatoxin on the health and productivity of pigs.