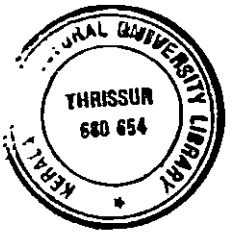
IMMUNOPATHOLOGICAL RESPONSE OF DUCKS IN AFLATOXICOSIS

By

P. BALAKRISHNAN



THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Thrissur

1992

TO

MY MOTHER

AND

EVER LOVING MEMORY OF MY FATHER

DECLARATION

I hereby declare that this thesis entitled **"IMMUNOPATHOLOGICAL RESPONSE OF DUCKS 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, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy,

P. BALAKRISHNAN 20/11/92

CERTIFICATE

Certified that this thesis entitled "**IMMUNOPATHOLOGICAL RESPONSE OF DUCKS IN AFLATOXICOSIS**" is a record of research work done independently by **Dr. P. Balakrishnan** 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.

Mannuthy,
20-11-1992

20.11.92
Dr. K.I. Maryamma
Professor
Centre of Excellence
in Pathology
(Major Advisor)

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. P. Balakrishnan**, a candidate for the degree of **Master of Veterinary Science** in Pathology, agree that the thesis entitled **"IMMUNOPATHOLOGICAL RESPONSE OF DUCKS IN AFLATOXICOSIS"** may be submitted by **Dr. P. Balakrishnan** in partial fulfillment of the requirement for the degree.

30.11.92

Dr. K.I. Maryamma
Professor
Centre of Excellence in Pathology
(Chairman, Advisory Committee)

20/11/92

Dr. A. Rajan
Director
Centre of Excellence
in Pathology
(Member)

Kabala. 20/11/92.

Dr. K.V. Valsala
Associate Professor
Centre of Excellence
in Pathology
(Member)

20/11/92

Dr. K. Chandrasekharan Panicker
Professor
Department of Parasitology
(Member)

External Examiner
12/4/93.

ACKNOWLEDGEMENT

I wish to express my sincere thanks and gratitude to Dr. K.I. Maryamma, Ph.D., Professor, Centre of Excellence Pathology and chairperson of the Advisory committee for her inspiring guidance, stimulating discussions, constructive criticism, valuable advice and constant encouragement throughout this work.

I am deeply indebted to Dr. A. Rajan, Ph.D., Director, Centre of Excellence in Pathology, member of advisory committee for his expert guidance, support, interest, generous help and co-operation during the course of the study.

I am extremely grateful to Dr. K.V. Valsala, Ph.D., F.R.V.C.S., Associate Professor, Centre of Excellence in Pathology and Dr. K. Chandrasekharan Panicker, Ph.D., Professor Department of Parasitology, members of the Advisory committee for their valuable help and suggestions rendered during the study.

I wish to express my sincere gratitude to Dr. M. Krishnan Nair, Ph.D., F.R.V.C.S., Director, Research Co-ordination for his valuable advice co-operation and personal interest throughout this work.

I wish to express my appreciation and sincere gratitude to Dr. K.M. Ramachandran, M.V.Sc., F.R.V.C.S., Professor, Dr.T. Sreekumaran, Ph.D and Dr. C.B.Manomohan, Ph.D., Associate

Professors; Dr. N.D. Nair, M.V.Sc, Assistant Professor; Dr. N. Vijayan, M.V.Sc and Dr. M. Gopalakrishnan Nair, M.V.Sc Junior Assistant Professors, Centre of Excellence in Pathology for their invaluable help, co-operation and constant encouragement.

My thanks are due to Dr. B.Gangadharan, Dr.T.Leena Devi Junior Assisstant Professors and Dr. S.P.Deshpande and Dr. Sreekrishnan, my colleagues, for their warm friendship and co-operation during the entire period of the study.

I am extremely grateful to Dr. A. Sundararaj, Professor and Head of Pathology, Dr. M.Thiagarajan, Professor and Head, Dr.T. Sivakumar, Assisstant Professor, Department of Livestock Production and Management and Dr. Musthaq, Department of Biochemistry, Madras Veterinary college for placing the facilities of their departments at my disposal during the course of the study.

My sincere thanks are due to Dr. Amir Abbas Farshid, Ph.D., for his warm friendship, patience and valuable collaborations.

The help rendered by Dr. J. Radhakrishnan in the analysis of data is gratefully acknowledged.

I wish to express my sincere gratitude to the Vice Chancellor, Kerala Agricultural University, Dr. G. Nirmalan, Dean-incharge, Dr. Zachariah Cherian, Professor, Academic cell college of Veterinary and Animal Sciences for their invaluable support and encouragement.

My sincere thanks are due to Dr. D.D.Kulkarni, Dr. Karpe, Dr.C.R. Predhan and Dr. Latif, Ph.D. Scholars and Dr. S. Sivakumar for their warm friendship and continuous interest in my well being.

I am thankful to Ms. M & P Computers, Trichur for the neat and prompt data processing.

P. BALAKRISHNAN

CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

Introduction

INTRODUCTION

Mycotoxins are fungal metabolites which have been known for many years but their significance in the causation of disease and their role 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 ingestion of sclerotia of Claviceps purpurea swept Europe from the 11th through the 16th centuries. The problem of mycotoxicosis in the health of man and livestock assumed considerable significance only after the outbreak of Turkey 'X' disease during 1960 involving the death of large number of turkeys in England. It was due to the consumption of peanut meal contaminated with aflatoxin.

Aspergillus flavus and Aspergillus parasiticus are the two most important species which produce aflatoxins. Aflatoxins are hepatotoxic, nephrotoxic, carcinogenic, mutogenic and teratogenic in livestock and poultry. The degree of susceptibility to aflatoxin varies among poultry. Ducklings are highly susceptible and turkey poults, gooslings, chicken and quails follow in the decreasing order of susceptibility. The effects of aflatoxins vary with dose, length of exposure, species, breed and nutritional status. These toxins may be lethal when consumed in large doses; sub-lethal doses produce chronic toxicity and chronic exposure at low level results in neoplastic changes,

primarily in the liver in a number of animal species. In general, young animals of any species are more susceptible to the acute toxic effects of aflatoxins than are older animals of the same species. Susceptibility also varies between species of animals and birds. Extensive work has been done on the effects of aflatoxin in different species of animals and it has emerged out as a hazardous element which accounts directly or indirectly for many of the diseases in domestic animals of hitherto ill-defined aetiology.

The chronic toxicity due to continued ingestion of low levels of this toxin is responsible for heavy economic loses encountered in livestock and poultry production. The toxigenic fungi are ubiquitous in distribution in the environment and the Indian agro-climatic conditions with high humidity and heavy rainfall favour mould growth and toxin production in feed commodities. This 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.

In recent years mycosis and mycotoxicosis are assuming considerable importance in the aetiology of many unknown diseases in poultry. Aflatoxins in the diet of poultry cause reduction in production, reduced feed intake, increased susceptibility to infectious diseases and even mortality. Severity of aflatoxicosis is related to its dosage and duration of intake of aflatoxins within a flock. It is characterised by haemorrhages in many

tissues, reduced growth rate, anorexia, and decreased egg production in laying flock.

The biological effects of mycotoxins are manifold but their effect on the immune system of the host is perhaps most important. The adverse effect of aflatoxins on the immune system causes diminished resistance to infections. It has been observed that vaccines fail to confer immunity in the birds consuming aflatoxin contaminated feed which was due to the immunosuppressive effect of the toxin. Thus, the inadvertent use of aflatoxin contaminated feed may defeat the aim of controlling various poultry diseases by vaccination.

The effect of aflatoxin on the cell-mediated as well as humeral immune system has been well documented in chicken. However, only very limited work has been carried out to assess the immune response of ducks in aflatoxicosis. Therefore, a project was designed to study the effect of aflatoxins on the immune response of ducks and the results obtained during the course of this investigation have been documented.

Review of Literature

REVIEW OF LITERATURE

2.1 Aflatoxins:

Aflatoxins are polycyclic, unsaturated compounds consisting of a coumarin nucleus flanked by a highly reactive bifuran system on one side and a pentanone or a six membered lactone on the other. Although 17 aflatoxins were isolated (WHO,1979), only four of them were studied extensively from the toxicological point of view. Because of their intense fluorescence in ultraviolet light the four were designated by letters B_1, B_2, G_1 and G_2 representing their blue and green fluorescence in ultra violet light. Two other familiar aflatoxins, M_1 and M_2 which were infact metabolites of B_1 and B_2 were labelled so because of their presence in milk of animals (previously exposed to B_1 and B_2).

Christensen and Nelson (1976) observed that the minimum, optimum and maximum temperatures for aflatoxin production were 12 °C, 27 °C and 40 °C to 42 °C, respectively.

Among the food-producing animals, gooslings, ducklings and turkey poults were reported to be most susceptible. Female rats were most resistant to aflatoxicosis (Cavalheiro, 1981 and Malkinson et al. 1982).

2.2 General Toxicity:

2.2.1.Duck:

Asplin and Carnaghan (1961) reported that the signs of acute

aflatoxicosis in ducklings were anorexia, poor growth rate, ataxia and death in opisthotonus following convulsions. In birds over three weeks of age subcutaneous haemorrhages of legs and feet were very characteristic. The duckling was recommended by Carnaghan (1965), as a convenient species for aflatoxin bioassay because of its rapid response to aflatoxin and manifested by marked bile duct hyperplasia 48 to 72 hours after exposure.

Ostrowski Meissner(1983) reported experimental aflatoxicosis in duck. There was reduced body weight gain when 50 ug/kg aflatoxin B_1 was given and the intensity of damage and toxicity were dose dependent.

The aflatoxin B_1 at the dose level of 50 to 400 µg/kg feed caused, in addition to reduction in growth and utilisation of protein, liver damage and also affected most of the blood constituents (Ostrowski Meissner, 1984).

Uchida et al. (1988) studied the effect of aflatoxin B_1 on duck livers with duck hepatitis B virus infection and stated that short term administration resulted in increased number of incomplete viral particles in dilated cisternae of the endoplasmic reticulum.

2.2.2. Chicken:

Hamilton (1971) reported a severe spontaneous outbreak of aflatoxicosis in North Carolina in which 50% of a flock of laying hens died within 48 h, after feeding on a highly toxic maize containing 100 ppm aflatoxin.

Edds (1973) stated that susceptibility of chickens to toxic effects of aflatoxin B_1 varied with several factors such as breed, strain, age, nutritional status, amount of toxin intake and also the capacity of liver microsomal enzymes to detoxify aflatoxin B_1.

Newberne (1973) reported that chickens exposed to as low as 0.2 to 1 ppm of aflatoxin in diet, showed poor growth rate, reduced feed efficiency, marked drop in egg production, liver damage, bile duct proliferation and most importantly, decreased resistance to common infectious diseases including coccidiosis.

At low levels of feed contamination, exposed chickens showed general weakness, failure to gain weight with concomitant decline in feed efficiency and egg production (Doerr et al., 1983).

Dalvi and Mcgowan (1984) observed substantial decrease in weight gain, feed efficiency and hepatic microsomal drug metabolising enzymes in Rock type broiler chicken when they were administered high dietary levels of aflatoxin B_1 (0 to 10 ppm).

2.3 Effect of aflatoxin B_1 (AFB_1) on the immune system:

2.3.1 Humoral immune system:

2.3.1.1 Duck:

Brown and Abram (1965) isolated Salmonella from ducklings with typical aflatoxicosis. They concluded that aflatoxins caused hypoproteinemia which included low levels of globulins and thus made the birds susceptible to Salmonella infection.

2.3.1.2 Chicken:

Thaxton and Hamilton (1971) reported the dose related decrease in ability of chicken to form haemagglutinins during aflatoxicosis. They attributed the cause of immunosuppression to impaired reticulo-endothelial system.

Michael et al. (1973) assessed the functioning of the reticulo-endothelial system and it was seen that the rate of colloidal carbon clearance was significantly reduced.

Edds et al. (1973) reported that aflatoxins reduced the resistance against infection by Candida albicans, Eimeria tenella and Marek's disease virus.

Thaxton et al. (1974) reported that feeding graded levels of aflatoxin from hatching, significantly depressed haemagglutination. Response at 3, 6 and 9 days after sheep red blood cell injection were studied. They opined that the degree of immunosuppression was directly related to the concentration of the dietary aflatoxin.

Decreased serum IgG of chicken fed with aflatoxin at the rate of 2.5 µg/g of diet was reported by Tung et al. (1975). They stated that aflatoxin or its metabolites bound randomly to template of DNA and inhibited the larger transcribing units.

Giambrone et al. (1978) reported decreased serum immuno-globulin fractions of IgG and IgA but not IgM in chicks fed aflatoxins at the rate of 2.5 µg/g of feed from hatching to four weeks of age. They opined that the reduction in the IgA in chicken could increase the susceptibility of chicken to local

infections and the reduction in IgM concentration to a marked level in chicken could be explained by the ontogeny of Ig production during later stages of embryonic life and therefore, aflatoxin fed to newly hatched chicks will be too late to impair IgM producing cells.

Mohiuddin et al. (1981) reported that chickens fed with 0.2 ppm of aflatoxin over a period of 21 days had depressed antibody titre against Ranikhet disease vaccines.

Campbell et al. (1983) reported that aflatoxin in Broiler chicks caused hypoproteinemia, lymphocytopenia, decreased relative weight of the bursa of Fabricius and depression of complement activity which indicated the immunosuppression.

Stewart et al. (1985) reported decreased complement activity in chicks given dietary aflatoxins. Marshaly et al. (1986) reporteddecreased RNA synthesis and protein synthesis in chicks given dietary aflatoxin.

Pier (1986) summarised the effects of aflatoxin on acquired resistance and immunity. The data showed that variety of infections in different species of animals are affected by exposure to aflatoxin. The immunosuppressive effect of aflatoxin appeared to be on both the units of dichotomous immune system. The studies revealed that it has more pronounced effect on the CMI and the complement system. The antibody response was normal to diminished and also dose dependent. IgA and IgG concentrations diminished whereas the IgM concentration was unaffected. Dose

related reduction in size of the bursa of Fabricius was observed.

Sharma (1988) observed that when purified aflatoxin at the rate of 0.25 µg fed to chicks from the second day of age, lowered the protection level against fowl pox virus.

Kadian et al. (1988) observed that continuous presence of aflatoxin B1 in the diet had an immunosuppressive effect in chickens and even its withdrawal had residual effect on phagocytic activity and delayed type hypersensitivity.

Rao et al. (1988) evaluated the effect of dietary aflatoxin on immune responses against Ranikhet disease virus in chicks. The serum protein decreased with each increase in aflatoxin level from 0.5 ppm onwards.

Padmanabhan (1989) studied the immunosuppressive effect of aflatoxin and observed that the immunosuppressive effect appeared to be antigen specific. He explained that inhibition of RNA polymerase, increased lysosomal activity, inhibition of reticulo-endothelial system and inhibition of specific immunological system as the possible ways in which aflatoxin acted.

Decreased antibody response to infected sheep red blood cells in aflatoxin fed chicks were observed (Virdi et al. 1989)

2.3.1.3 Turkey:

Pier and Heddleston (1970) studied the effect of aflatoxin on immunity in turkeys. They observed that aflatoxin B1 consumed at the rate of 0.25-0.5 ppm during or after the period of immunization against Pasturella multocida interfered with the

development of acquired resistance in 20-67% of turkey poults.

Pier et al. (1971) observed no change in antibody level in turkeys given New Castle 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 Pasturella multocida infection in turkeys vaccinated against Fowl cholera was not necessarily associated with antibody, as the deficit was overcome by giving vaccinated birds either normal or immune serum prior to challenge inoculation (Pier et al. 1972).

2.3.1.4 Laboratory animals:

In rats, aflatoxin appears to exert their primary effects on RNA polymerase as a result of binding to DNA. (Clifford and Rees, 1967). Galikeev et al. (1968) reported a marked depression in antibody formation in mice, when aflatoxin was given before or during the vaccination period and a moderate depression when aflatoxin was given after vaccination.

Lafarge and Frayssinet (1970) observed that aflatoxin had been shown to inhibit RNA polymerase. They concluded that the immunosuppression resulted due to inhibition of the synthesis of specific immunoglobulins.

Reddy et al. (1983) studied the dose and time related responses of immunological functions in mice fed 0, 30, 145 and 700 ug/kg body weight of aflatoxin B_1. A decrease in humoral

immunity was observed.

2.3.1.5. Other animal species:

Paul et al. (1977) reported that aflatoxin B1 significantly suppressed the bovine lymphocyte responsiveness to poke weed mitogen in vitro, thereby inhibiting the humoral immunity.

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

Humoral immunity was lowered in goats fed aflatoxin B1 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.

Balaraman and Arora (1987) reported that aflatoxin at 50 to 250 ppb levels in colostrum significantly affected the passive immunity status of the neonatal calves.

In pigs, Panangala et al. (1986) reported significant reduction in complement titres with dietary aflatoxin at the rate of 560 ug/kg.

Gopalakrishnan Nair (1986) reported reduction in the humoral immune response to experimental aflatoxicosis in pigs.

2.3.2. Cell-mediated immune system:

2.3.2.1. Chicken:

Michael et al. (1973) observed impaired reticulo-endothelial response in experimental aflatoxicosis of chicken. There was decreased ability to process the antigenic components.

The chemotactic activity of heterophils to phagocytose was markedly reduced in chicken fed a diet containing 2.5 ug of aflatoxin/g of feed, reflecting upon the impaired cellular immunity (Chang et al).

Giambrone et al. (1978) reported that chicken fed 2.5 ug of aflatoxin /g diet from hatching to 84 weeks was deficient in cell-mediated immunity as measured by graft versus host reaction.

Chang and Hamilton (1979) observed reduced phagocytic activity of chicken monocytes during aflatoxicosis.

Giambrone et al. (1985) studied the effect of purified aflatoxins on broiler chicken and stated that aflatoxins affected the cell-mediated immune response significantly as measured by delayed type of hypersensitivity reaction.

Mohiuddin et al. (1986) studied the phagocytic activity in aflatoxin fed chicken. They reported that the phagocytic activity of heterophils and Kupffer cells were markedly depressed.

Kadian et al. (1988) reported reduced phagocytic activity due to the immunosuppressive effect of aflatoxin in chicken.

Rao et al. (1988) also observed reduced cell-mediated immune response in chicks in experimental aflatoxicosis as measured by T lymphocyte count, cutaneous reaction to 2,4,dinitro chlorobenzene and graft-versus host reaction.

2.3.2.2 Turkey:

Pier et al. (1972) revealed thymic involution in turkeys fed a diet containing 0.5 ppm aflatoxin B_1. This had affected the

delayed hypersensitivity and graft-versus host reactions.

Giambrone et al. (1985) observed significant effects of purified aflatoxins on cell-mediated immune response in birds receiving 400 ppb of pure aflatoxin B_1 and B_2.

2.3.2.3 Laboratory animals:

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

Mc Loughlin 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.

Reddy et al. (1987) studied the immune function of CD-1 mice exposed to aflatoxin B_1 and observed that the synthesis of DNA was decreased in lymphocyte cultures and primary antibody production by splenic cells in animals challenged with T dependent antigen was affected by aflatoxin B_1.

2.3.2.4 Other animal species:

Richard et al. (1983) observed in steers delayed cutaneous hypersensitivity when corn naturally contaminated with aflatoxin was given at the rate of 800 ug/g feed.

Bodine et al. (1984) Observed moderate to strong inhibition of blastogenesis resulting in inhibition of T-lymphocyte function such as killer, helper, effector or other immunological

processess when bovine lymphocytes were treated with aflatoxin.

Decreased cell-mediated immune response was observed in kids fed aflatoxin (Sinha and Arora, 1984).

Visalakshan et al. (1984) studied the effects of alpha naphthyl acetate activity in lymphocytes in pigs and found that the total lymphocytic count was reduced while erythropoietic activity was not affected.

Aflatoxin suppressed in vitro bovine and caprine lymphocytic response to phytomitogens (Sinha and Arora, 1985).

Gopalakrishnan Nair (1986) studied the effects of aflatoxin given in single or multiple doses in cattle. In single dose, complement activity and bacteriostatic activities decreased but returned to normal by 168 h. However, in multiple dosed animals only the bacteriostatic activity decreased and persisted for two weeks. They concluded that aflatoxin affected both complement dependent and independent serum bacteriostatic activity.

2.4 Pathology:

2.4.1 Duck:

Yadgiri (1970) observed diffuse liver cell necrosis, shrunken cells with pyknotic nuclei and fatty vacuolation in the cytoplasm of liver cells in ducks. The liver cell damage had always been more severe in periportal zones than in the centrilobular zones and there was mild bile duct hyperplasia, during aflatoxicosis.

Peckham (1975) reviewed the aflatoxin induced. lesions in

ducklings. There were hydropericardium, petechae on the pancreas, diffuse haemorrhages on the kidney, enlarged gall bladder and duodenal inflammation.

Vacuolar changes and ductular proliferation were the prominent changes in the liver of ducks fed aflatoxin-appended turkey feed for 14 days. Many hepatocytes had clear cytoplasm and were swollen making sinusoids difficult to distinguish. Bile duct proliferation was confined mainly to the portal areas. Karyomegaly and mitotic figures were common within hepatocytes. Disseminated individual hepatic cell necrosis was minimal (Hoerr et al. 1986).

2.4.2. Chicken:

Archibald et al. (1962) observed pale enlarged kidneys which had fine network of urate deposit in chickens.

Carnaghan et al. (1966) observed enlarged and grey coloured livers in chicken which received 1.5ppm of aflatoxin in the experimental diet. The liver was enlarged and soft with petechial haemorrhages. Thereafter, there was reduction in the size of the liver with increasing firmness and texture until the seventh week, with well defined, raised nodular lesions on the liver surface. Diffuse white pinhead sized foci were found from the sixth week of age.

Muller et al. (1970) observed no gross lesions in birds that received diet containing 0.5, 1.0 ppm aflatoxin while at 2.0 ppm to 4ppm levels, liver appeared pale and lighter compared to the

normal limits.

Vanzytveld et al. (1970) observed in the dead broiler chicks which were given 256.6 ug to 513 ug of aflatoxin, by daily intra-crop incubation for 6 weeks, mottled liver with small reddish spots of 1 to 2mm size, which tended to be firm, and the edges somewhat rounded. No evidence of nodule formation was observed. Liver colour varied from slightly pale to intense pale yellow, Kidney and spleen were usually pale in affected birds.

Tung et al. (1971) reported that small amount of aflatoxin in the diet increased capillary fragility and susceptibility to bruising.

Thaxton et al. (1974) reported atrophy of the bursa and thymus in chicken fed high doses aflatoxin.

Tapia et al.(1980) reported enlarged and pale liver, with severe degenerative lesions and proliferation of hepatocytes in the peripheral region in chicken in aflatoxicosis.

Mohiuddin (1982) reported that feeding of aflatoxin 100 and 200 ug /day /diet for 35 days resulted in congestion of the liver and atrophy of testes and absence of spermatogenesis.

A hypertrophy and hepatic hyperlipaemia were seen during chronic aflatoxicosis in broiler chicks (Doerr et al. 1983).

Moorthy et al. (1985) observed hepatic lesions in chickens given 6.25 ppm and 3.12 ppm aflatoxin in feed. Important gross lesions included yellow liver with haemorrhages, haematomas and

nodules. The important histological findings were lipidosis, sinusoid congestion, perivascular haemorrhages, bile duct hyperplasia, phlebitis and veno-occlusive lesions.

Hoerr et al. (1986) reported that experimental broiler chicken given 100 and 200 ppb aflatoxin B1 for 35 days had liver changes with pleomorphic hepatocytes, disruption of the hepatocellular plates and necrosis of individual hepatocytes. Chicken fed 400 and 800 ppb aflatoxin B1 had, in addition, marked inflammation of portal areas, and ductular proliferation. The disorganization of the hepatocellular plates was marked due to pleomorphism of the hepatocytes and ductular proliferation. Hepatocyte with cytoplasmic, PAS-positive granules were confined to the central lobules. Vacuolar change in the hepatocyte cytoplasm was found only in the portal area. Cellular exudate in the portal area consisted of either heterophils or mononuclear cells and these cells extended to all portions of the lobule. Large intrahepatic bile ducts had hyperplastic mucosa. Fibrosis and karyomegaly were minimal.

Mohiuddin et al.(1986)reported periportal fatty change, loss of normal hepatic architecture, necrosis of hepatic cells with prominent kupffer cells in chicken fed aflatoxin at the rate of 20ppm per day for three months.

2.4.3. Turkey:

Stevens et al. (1960) reported enlargement and congestion of the kidneys as a constant feature in turkeys.

Silver and Ostler (1961) observed diffuse necrosis of liver parenchyma, proliferation of the bile duct epithelial cells and frequent haemorrhages during the outbreak of aflatoxicosis in turkey poults in 1960.

The prominent cellular changes in the liver included swelling and vacuolation of the parenchymal cells, enlargement of the nucleus and in some cases dissolution of nucleolus (Edds, 1973).

Turkeys given aflatoxin 100, 200, 400 and 800 ppb in feed had shown liver lesions. Ductular proliferation in the trabecular and less commonly tubular form was prevalent in the portal areas of all of the lobules. Centrilobular hepatocytes had increased cytoplasmic eosinophilia. Vacuolar change was minimal, mild fibroplasia occured only if ductular proliferation was extensive (Hoerr et al. 1986).

2.5 Assessment of immune response:

2.5.1 Evaluation of cell-mediated immune response:

2.5.1.1 Acid alphanaphthyl acetate esterase (ANAE) activity as T cell marker:

Li et al. (1973) demonstrated acid alphanaphthyl 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).

Osbaldiston et al.(1978) successfully employed ANAE as T cell marker in cat, dog, goat, guinea pig, hamster, rabbit,rat,

sheep and pig.

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.

Valsala et al. (1981) demonstrated the ANAE activity as a lymphocyte marker in peripheral blood leukocytes of ducks.

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

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

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

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

2.5.1.2. Response to mitogen-phytohaemagglutinin-M. (PHA-M):

Nowell (1960) observed the effects of phytohaemagglutinin (PHA), an extract of the red kidney bean Phaseolus vulgaris, for inducing lymphocyte transformation.

Janossy and Greaves (1971) recognized PHA as a T-cell specific mitogen. Zuckerman and lo Bugilo (1973) used PHA as a skin test for the evaluation of cellular immunocompetence in

normal and cancer patients respectively.

Marchalonis (1978) employed PHA in evaluating the cell mediated immunity in man scince PHA caused a direct reaction without prior sensitization.

Heggard 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 of hypersensitivity reaction.

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

Kelly et al. (1982) employed PHA 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.

2.5.1.3. 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; Bliber and Morton, 1970).

Chakravorthy et al. (1973) 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.

DNCB skin test was standardised in cattle by Reddy et al. (1981). Valsala et al. (1981) described the DNCB test for assessing cell-mediated immune response of ducks.

Rajan et al. (1981) evaluated the efficacy of this test in goats. Rajan et al. (1982) used DNCB skin test for evaluating the cell-mediated immunity in pigs and also 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.

Gopalakrishnan Nair (1986) evaluated the cell-mediated immunity in experimental aflatoxicosis in pigs using DNCB test.

Ashturkar et al. (1989) assessed the cellular immunity in experimental diaphragmatic hernioplasty by using DNCB test.

2.5.2 Evaluation of humoral immune response:

2.5.2.1 Serum protein fractions:

Brandt et al. (1951) carried out gel electrophoresis of chicken sera at different age groups. The low gamma globulin exhibited by the young birds is in keeping with the fact that these birds may not have developed the antibodies which would be produced by later exposure to various organisms. However, the possibility could not be excluded that a large portion of the gamma globulin formed in older birds might be a normal development as the birds matured.

Glick (1968) studied the serum protein electrophoresis patterns in acrylamide gel electrophoresis of the chicken sera. All the gels contained a heavy stained band of transferrin, approximately midway between the origin and albumin, the origin was the separation point between the stacking and separating gel. The first two bands beyond the origin, and a stained area 4-8 mm below the origin represented immunoglobulins. The absence of the stained area in the electrophorograms of chemically bursectomised birds indicated reduced immunoglobulin levels.

Morgan and Glick (1972) employed polyacrylamide gel electrophoresis to sera of chickens. At 2-3 weeks of age birds attained ability to produce IgG in appreciable quantities. IgM which was generally absent or very low at hatching increased rapidly during the first week of post-embryonic life. Both surgical and hormonal bursectomy resulted in delay in IgG production where as IgM and transferrin were higher. They also observed that surgical bursectomy plus 700 r of the whole gamma-irradiation resulted in decrease in both IgG and IgM levels.

In experimental ochratoxicosis in Japanese quails, Firshid (1992) observed significant reduction in IgM and IgG factors of immunoglobulins.

Materials and Methods

MATERIALS AND METHODS

Aflatoxin B$_1$ (AFB$_1$) obtained from the Sigma Chemical Company, St. Louis, USA was used in this experiment.

3.1 Dose and route of administration:

The AFB$_1$, dissolved in rectified spirit, was administered by oesophageal intubation to the experimental ducks at the dose rate of 0.075 mg/kg body weight on every alternate day till the end of the experiment.

3.2 Experimental design:

One hundred and ten day-old, White Pekin ducklings were procured from the Govt. Duck Farm, Niranam and were maintained on aflatoxin free feed up to four weeks of age. Water was provided ad libitum.

These ducklings were divided at random into four groups. Fifty six ducklings were allotted to group I. These ducklings were again divided into two sub-groups A and B consisting of twentyeight ducklings each. Sub-group A was used as the experimental group and sub-group B served as the control. Seven ducklings from each subgroup were sacrificed at fortnightly intervals up to two months.

Twentyfour ducklings were allotted to Group II which formed four sub-groups, A, B, C, and D of equal numbers (six). Sub-group

A was used to assess the cutaneous hypersensitivity reaction to 2,4-dinitrochlorobenzene (DNCB) after the fourth fortnight and sub-group B served as control. Sub-group C was used to evaluate the cell-mediated immune response of the ducks to phyto-haemagglutinin-M (PHA-M) after the fourth fortnight. Sub-group D was used as control.

The Group III comprised of eighteen ducklings with two sub-groups A and B. Twelve ducklings were maintained as experimental in sub-group A and six ducklings were maintained in sub-group B which served as control. These ducklings of sub-groups A and B were vaccinated with Ranikhet Disease F_1 vaccine (BAIF, INDIA) to assess the humoral immune response on the first and fourth fortnight post-vaccination. The antibody titre was estimated by employing Haemagglutination Inhibition test.

The Group IV consisted of twelve ducklings with two sub-groups A and B. Sub-group A served as experimental group with six ducklings and sub-group B consisting of six ducklings served as control. This group was used at the end of the fourth fortnight to monitor the phagocytic efficiency of the reticuloendothelial cells employing Carbon clearance assay.

All the experimental ducks in the above groups (Group I, II, III and IV) were given pure aflatoxin B_1 (Sigma, USA.) at the rate of 0.075 mg/kg body weight on alternate days for eight weeks.

3.3 Haematological studies:

Blood samples from all the ducks were collected from the

jugular vein at the end of each fortnight for the determination of haemoglobin, total and differential leukocyte counts and for ANAE positive lymphocytes. The procedure described by Valsala (1968) was followed for the determination of total leukocyte count (TLC) and the differential leukocyte count (DLC).

3.4 Assessment of cell-mediated immune status:

3.4.1 Enumeration of alpha naphthyl acetate esterase (ANAE) activity in the peripheral blood lymphocytes:

Wet smears, prepared from the peripheral blood at the end of each fortnight 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 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 2N 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 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 to 21 hours at room temperature and then rinsed thoroughly with distilled water and then counterstained with 1% toludine blue for 45 to 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:

Response to phyto-haemagglutinin-M (PHA-M):

Six White Pekin ducks each from experimental and control groups were employed for the study.

100 ug of PHA -M (Difco laboratories, USA) was dissolved in 0.1 ml of distilled water and was given intradermally in the web region. The thickness of the skin at the site of injection was measured using a vernier caliper at 24, 48 and 72 hours. Pieces of skin from the web region were taken for biopsy during the same intervals and fixed in 10% formalin. The biopsy specimens were processed for histopathological studies by the routine method and

sections cut at 5 u thickness were stained with Harris haematoxylin and eosin (Shuhan and Hrapchak, 1980).

3.4.3 Cutaneous response to 2,4-dinitrochlorobenzene (DNCB):

At the end of the fourth fortnight, cell mediated immune response of the ducks to DNCB was evaluated. The method described by Valsala et al. (1981) was followed.

Six white pekin ducks each from experimental as well as control groups were employed for the study. The site of application of DNCB was on the back in the thoraco-lumbar region 1cm lateral to vertebral column. The sensitising dose was applied at one site on the left side and the challenge dose was applied at two separate sites behind the area of sensitisation. The area was defeathered and cleaned two days prior to the test to avoid any traumatic factors interfering with the test.

One percent solution of 1 chloro-2-4dinitrobenzene (Loba) in acetone was employed for sensitising and challenge. The test area was demarcated by a metallic ring 2cm in diameter and having a raised border. For sensitising and challenge the diameter of the area of exposure was the same. The metallic ring was placed on the prepared are and the DNCB solution was dropped slowly 2 to 3 drops at a time using a tuberculin syringe with needle. This was allowed to dry immediately by blowing so as to avoid the solution running down the sides. The challenge dose was applied 14 days after application of the sensitising dose. In the control group the same procedure was repeated with acetone alone. The thickness

of the skin at the site of the test and the area of the reaction zone were measured before sensitisation and 24, 48 and 72 hours after challenge. From one of the sites of reaction, tissues for biopsy were taken during the same intervals. Tissues were fixed in 15% formalin and paraffin sections cut at 5u thickness were stained with haematoxylin and eosin.

3.4.4 Assessment of phagocytic activity:

Carbon clearance Assay:

The phagocytic efficiency of the reticulo-endothelial cells was monitored by carbon clearance assay described by Glick et al. (1964).

At the end of the fourth fortnight six White Pekin ducks each from the experimental and control groups were employed for the study.

The supernatant fraction of india ink, centrifuged at 3000 g for 30 minutes, was injected into the brachial vein of ducks at the rate of 1 ml/kg body weight. Blood (100 ul) was collected from the opposite wing before and at 5 minutes and 15 minutes after carbon injection and transferred into 2ml of 1% sodium citrate. The samples were centrifuged at 50 g for 4 minutes. The relative amount of carbon remaining in the supernatent of the sample was estimated by spectrophotometric determination of the absorbance of the samples at 675 nm wavelength, using samples collected before carbon injection as zero values.- Density readings were converted into logarithmic scale (In). The

phagocytic index (PI) was calculated as negative of the slope of the line determined by absorbance time.

3.5 Evaluation of humoral immune responses:

3.5.1 Biochemical estimation:

The serum was separated and total protein and albumin were estimated using commercial kits in chematric analyser. Miles. India, USA. The total serum globulin level was estimated by deducting total serum albumin from the total protein.

At the end of the fourth fortnight the serum samples from both experimental and control groups were subjected to Polyacrylamide Gel Electrophoresis (PAGE) to estimate the different serum protein fractions.

3.5.2 PAGE:

The serum samples were subjected to PAGE as per the method described by Davis (1964).

3.5.2.1 Reagents:

3.5.2.1.1 Preparation of electrode buffer (Solution A):

Tris (36.6 g) and 0.23 ml of TEMED with 48 ml of 1.0 N hydrochloric acid were taken in a 100 ml volumetric flask. The contents were dissolved and the volume was made up to the mark with distilled water. The pH of the solution was adjusted to 8.9 with distilled water. (TEMED-NNN 'N' Tetramethyl ethylene diamine).

3.5.2.1.2 Gel solution (Solution B):

Acrylamide (28 g) and 0.735 g of bisacrylamide were dissolved in 100 ml of distilled water.

3.5.2.1.3 Ammonium persulphate solution (0.14%) (Solution C):

Ammonium persulphate (0.14 g) was dissolved in 100 ml of distilled water.

3.5.2.1.4 Electrolyte buffer (Solution D):

Glycine (6 g) and Tris (28.3 g) were dissolved in one litre of distilled water. The pH of the solution was adjusted to 8.3.

3.5.2.1.5 Staining solution (Solution E):

One gram Amido Black (10 B) was dissolved in a minimum amount of methanol and the volume of 100 ml was made up with 7% acetic acid solution.

3.5.2.1.6 Destaining solution (Solution F):

Seven per cent sucrose solution.

3.5.2.1.7 Sucrose solution (Solution G):

Twenty per cent sucrose solution.

3.5.2.1.8 Preparation of gel solution:

One part of solution A, two parts of solution B, one part of water and four parts of solution C were mixed well and immediately used for polymerization.

3.5.2.2 Procedure:

One end of the gel tube was closed and it was filled 3/4 th

with the prepared gel mixture. A drop of distilled water was added above the gel to prevent contact with oxygen of the atmosphere. The tubes were then kept in an upright position for about 30 minutes for polymerisation.

After polymerisation the tubes were fixed in the upper compartment of the electrophoretic apparatus. The water layer on the top of the gel was removed with a strip of filter paper. The lower compartment was filled with electrode buffer.

10 ul of a one per cent serum solution in 20 per cent sucrose was applied to the tube. As a marker to one of the tubes, a little bromophenol blue was added to indicate the movement of the run. The tubes were then filled up with the electrode buffer and also the upper tank. The upper tank was closed with the lid and then electrical connections were given.

Initially a current of 2 mA/tube was given until the sample entered the separation gel in a concentrated band and then it was raised to 4 mA/tube till the end of the run. It was run for one hour after which the tubes were removed.

The gel was removed from the tube by immersing the tubes in distilled water and inserting a five gauge needle between the gel and the inner wall of the tube. By five rotation of the needle along the inner wall from both sides the gel was removed and placed in the staining solution (Solution F) for one hour. Then it was destained with seven per cent acetic acid repeatedly till the interspaces between the fractions were clear.

The scanning of the gel tubes was performed by soft Laser Scanning Densitometer (Zeinch, Model SLR-TREF, Biomed Instruments Inc., USA) using Laser mode as the light source.

3.5.3 Response to Ranikhet Disease Vaccine:

Twelve experimental and six control ducks were vaccinated against RDF vaccine (BAIF, India) and antibody response was measured by employing haemagglutination inhibition test.[1]

3.6 Gross anatomy and histopathology:

At each experimental period all the experimental as well as the control birds were sacrificed and subjected to detailed autopsy as described by FAO/ SIDA (1968). The gross lesions were recorded. Representative samples of tissues collected from various organs of all the birds were fixed in 10% formalin. The tissues were processed by the routine method for histopathological studies. Paraffin sections cut at 5 u thickness were stained with Harris haematoxylin and eosin as described by Sheehan and Hrapchak (1980).

3.7 Statistical Analysis:

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

RESULTS

4.1 Haematology:

4.1.1 Hb: The average values for the Hb concentration of experimental and control groups are furnished in Table-1.During the first fortnight there was slight decrease in the Hb concentration of the AFB_1 fed ducks. Similarly same pattern of lower Hb values were seen in the second, third and fourth fortnights. The fall in the Hb concentration of the AFB_1 fed ducks was not, however, statistically significant.

4.1.2 TLC:

The TLC values for the experimental and control groups during the course of the experiment are given in Table-1 and (Fig.1). The AFB_1 fed ducks showed a dose and time dependent decrease in the TLC values when compared to the controls. This decrease was significant ($P < 0.05$) during the first fortnight and subsequently was found to be statistically highly significant ($P < 0.01$) during second, third and fourth fortnights.

4.1.3 DLC:

The average percentage values of heterophils, eosinophils, monocytes and lymphocytes are furnished in Table-1. During the first fortnight there was highly significant ($P < 0.01$) increase in heterophils with significant ($P < 0.05$) reduction in the

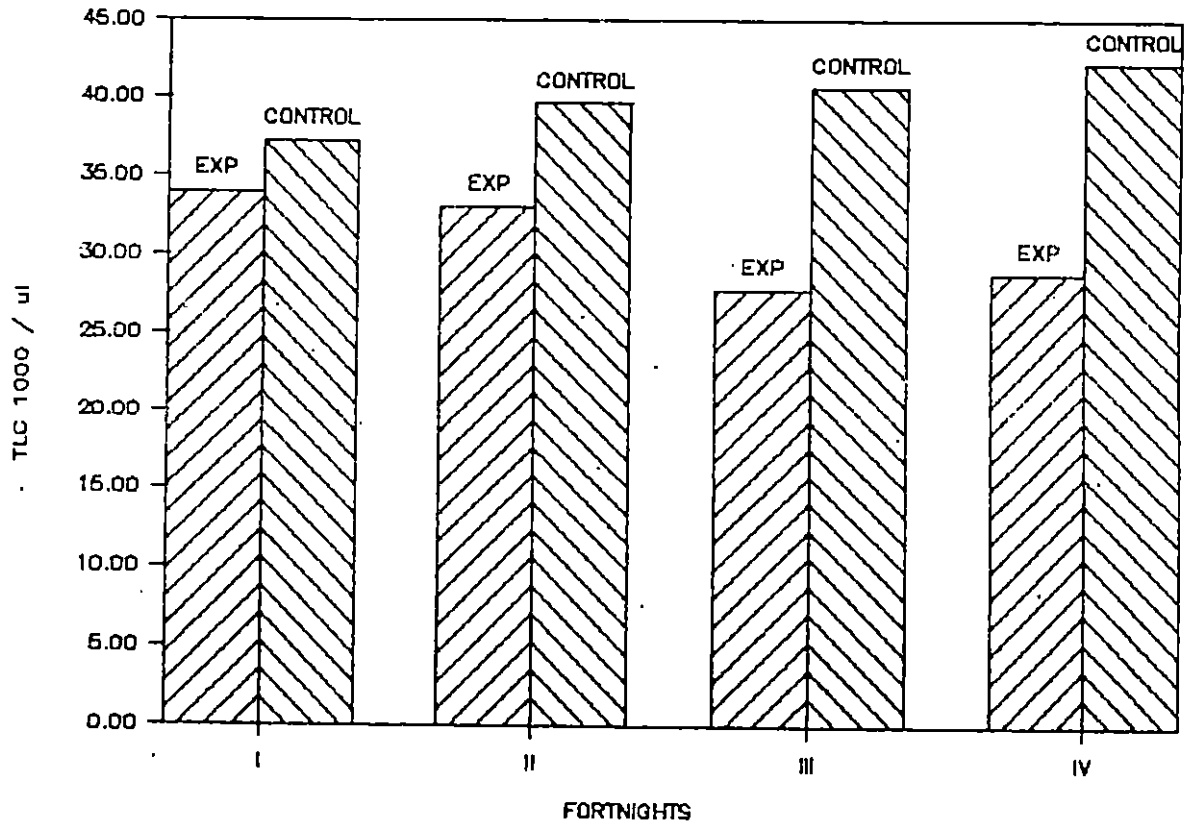


Fig.1 Average TLC (Experimental and Control)

lymphocyte percentage of AFB_1 fed chicks compared to the control(Fig. 2). There was also reduction in eosinophil and monocyte percentage of AFB_1 fed ducks which was found to be significant ($P< 0.05$) and highly significant ($P< 0.01$) respectively. During the second fortnight, though there was reduction in the lymphocyte percentage, and increase in the heterophil percentage in the AFB_1 fed individuals this was not statistically significant. The changes in eosinophil and monocyte percentage were insignificant. In the third fortnight the increase in the heterophil percentage and decrease in the lymphocyte percentage were found to be highly significant ($P< 0.01$), whereas the changes in the percentage of eosinophils and monocytes were insignificant. This continued to be so in the fourth fortnight. The increase in heterophils during the fourth fortnight was significant ($P< 0.05$) while there was highly significant($P< 0.01$) reduction of lymphocytes (Fig.2).

4.2 Enumeration of ANAE positive cells:

The percentage mean values of ANAE positive cells in the peripheral blood of the experimental and control groups are furnished in Table 1 and (Fig. 3). During the first fortnight the AFB_1 fed ducks showed a mild reduction in the ANAE positive cells which was not significant statistically whereas the reduction was found to be significant ($P< 0.05$) in the second fortnight and subsequently the reduction was found to be highly significant ($P< 0.01$) during the third and fourth fortnights.

TABLE 1

AVERAGE (MEAN ± S.E.) OF HAEMOGLOBIN, TLC, DLC AND T LYMPHOCYTES [EXPERIMENTAL AND CONTROL]

FORT-NIGHTS	GROUP	HAEMO-GLOBIN [g/dl]	TOTAL LEUCOCYTE COUNT [10 ul]		HETERO-PHILS [%]		EOSINO-PHILS [%]		BASO-PHILS [%]	LYMPHO-CYTES [%]		MONO-CYTES [%]		ANAE POSITIVE LYMPHO-CYTES [%]	
FIRST	EXPERI-MENTAL	8.7142 ± 0.2582	34.0000 ± 1.1428	*	47.0000 ± 0.7284	**	2.1428 ± 0.3740	*	0	40.3571 ± 0.3740	*	1.0000 ± 0.2857	**	12.2857 ± 1.1012	
	CONTROL	8.7714 ± 0.3062	37.2142 ± 0.3478		40.7142 ± 0.3892		3.2857 ± 0.2645		0	51.8571 ± 0.5506		4.1428 ± 0.3148		12.7142 ± 0.8259	
SECOND	EXPERI-MENTAL	8.8857 ± 0.2675	33.0714 ± 1.5825	**	41.5714 ± 0.9658		2.2857 ± 0.2645		0	55.0000 ± 1.3093		1.1428 ± 0.3740		10.7142 ± 0.9195	*
	CONTROL	9.6857 ± 0.4744	39.6714 ± 0.4893		39.1428 ± 1.9527		2.4285 ± 0.3967		0	57.0000 ± 2.0303		1.4285 ± 0.3967		15.2857 ± 1.1377	
THIRD	EXPERI-MENTAL	8.8285 ± 0.2811	27.7857 ± 1.7492	**	39.1428 ± 1.7492	**	2.1428 ± 0.3148		0	58.0000 ± 2.0800	**	0.8577 ± 0.4251		12.0000 ± 0.6060	**
	CONTROL	9.5142 ± 0.2453	40.6714 ± 0.8563		29.8571 ± 1.3913		1.5714 ± 0.2753		0	71.7142 ± 2.8407		1.1428 ± 0.4251		17.0000 ± 0.9897	
FOURTH	EXPERI-MENTAL	10.8000 ± 0.3023	28.8571 ± 0.8613	**	38.1428 ± 1.9423		2.1428 ± 0.3740		0	58.8571 ± 1.5574	**	0.8571 ± 0.3740		9.8571 ± 0.5865	**
	CONTROL	10.8857 ± 0.6192	42.2428 ± 0.8128		30.0000 ± 1.8182		1.7142 ± 0.2645		0	67.1428 ± 1.7891		1.1428 ± 0.2414		19.7142 ± 1.1196	

* [p< 0.05] ** [p< 0.01]

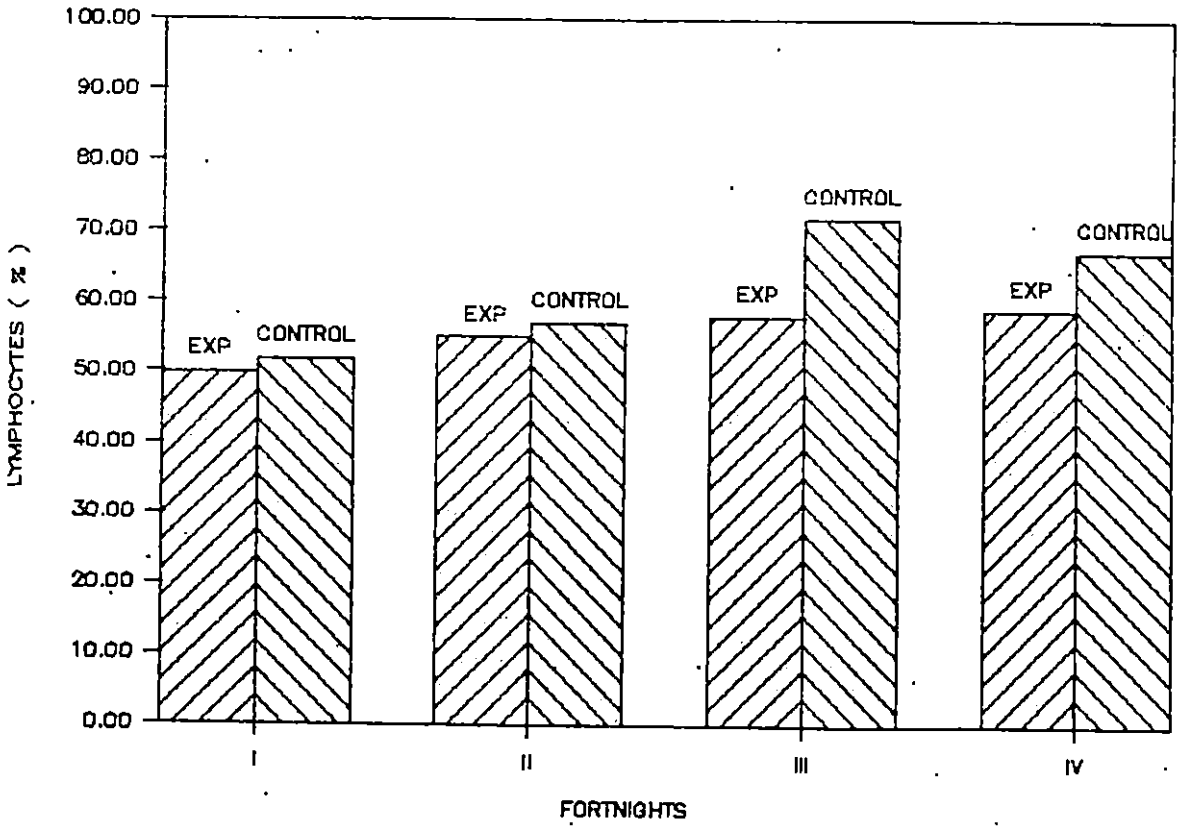


Fig.2 Average lymphocytes (Experimental and Control)

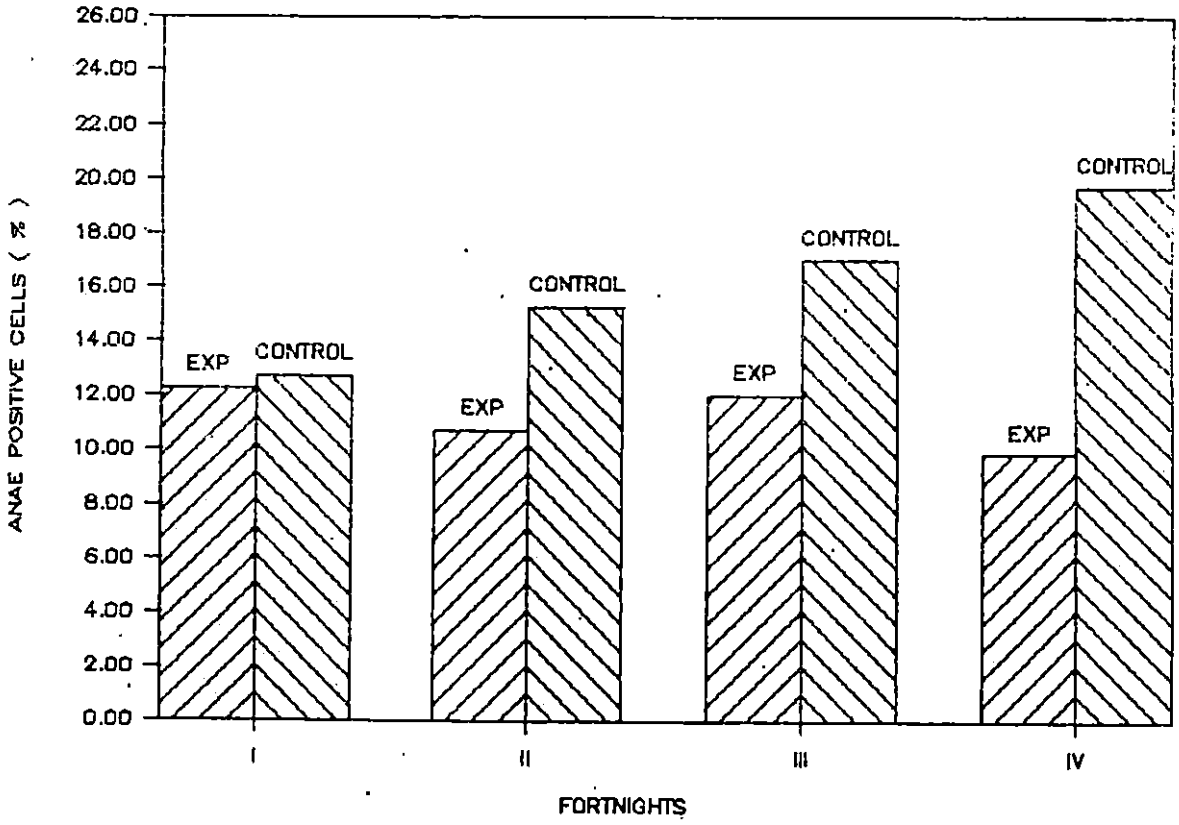


Fig.3 Average ANAE positive cells (Experimental and Control)

4.3 Total serum protein, serum albumin and serum globulin:

The average total serum protein, serum albumin and serum globulin of the experimental and control groups are given in Table 2. The AFB_1 fed ducks showed a reduction in the total serum protein during the course of the experiment (Fig. 4). Similarly there was reduction in serum globulin in the AFB_1 fed birds which was found to be initially insignificant statistically in the first fortnight and during the second, third and fourth fortnights it was found to be highly significant ($P< 0.01$) (Fig. 5). Serum albumin pattern during the first and second fortnights did not show significant change in the experimental groups whereas during the third and fourth fortnight there was a rise in serum albumin level of the experimental groups which was found to be highly significant ($P< 0.01$).

4.4 Serum protein fractions:

The results of the PAGE analysis of serum samples of experimental and control groups are furnished in (Fig. 6 to Fig. 17)and shown in (Fig. 20 and 21). In the AFB_1 fed ducks there was reduction in IgM and IgG fractions of immunoglobulins.

4.5 Response to Ranikhet disease vaccine:

The haemagglutination inhibition titre values are furnished in Table 3. The data indicated reduction in the titre of AFB_1 fed ducks.

4.6 Cutaneous response to DNCB:

The average values of skin thickness are furnished in Table

TABLE 2

AVERAGE (MEAN ± S.E.) OF TSP, S.Al, S.Gl [EXPERIMENTAL AND CONTROL]

FORTNIGHTS	1ST FORTNIGHT		2ND FORTNIGHT		3RD FORTNIGHT		4TH FORTNIGHT	
GROUPS	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL
TOTAL SERUM PROTEIN [g/dl]	3.1028 ± 0.2929	3.6128 ± 0.2709	2.5928 ± 0.1715 **	4.1642 ± 0.0774	2.4957 ± 0.2726 **	4.1000 ± 0.0782	2.6157 ± 0.2784 **	4.4428 ± 0.1588
SERUM ALBUMIN [g/dl]	1.1285 ± 0.0561	1.1857 ± 0.0241	1.1285 ± 0.0389	1.3285 ± 0.0661	1.3157 ± 0.0508 **	1.1976 ± 0.0274	1.3428 ± 0.0445 **	1.2857 ± 0.0425
SERUM GLOBULIN [g/dl]	1.9742 ± 0.2489	2.2710 ± 0.2571	1.4642 ± 0.1655 **	2.8357 ± 0.0628	1.1800 ± 0.2387 **	2.9023 ± 0.0804	1.2728 ± 0.2558 **	3.1571 ± 0.1226

* [P< 0.05]

** [P< 0.01]

TABLE 3

HAEMAGGLUTINATION INHIBITION TITRES OF EXPERIMENTAL AND CONTROL DUCKS INOCULATED WITH R.D.F VACCINE [1]

FORTNIGHTS	HAEMAGGLUTINATION INHIBITION TITRE	
	EXPERIMENTAL	CONTROL
FIRST	320	640
	640	640
	640	640
	1280	640
	640	1280
	640	640
FOURTH	1280	2560
	160	320
	320	640
	1280	2560
	160	320
	320	640

TABLE 4

BIOMETRY OF THE SKIN THICKNESS [MEAN ± S.E.] BEFORE AND AFTER CHALLENGE WITH 2,4-DINITROCHLOROBENZENE [EXPERIMENTAL AND CONTROL]

GROUP	SKIN THICKNESS [mm] BEFORE CHALLENGE	SKIN THICKNESS [mm] AFTER CHALLENGE		
		24 h	48 h	72 h
CONTROL	1.0	5.5833 ± 0.5702	5.0000 ± 0.5621	4.0833 ± 0.4025
EXPERIMENTAL	1.0	2.7167 ** ± 0.1566	1.7500 ** ± 0.2886	1.5833 ** ± 0.2805

** [P< 0.01]

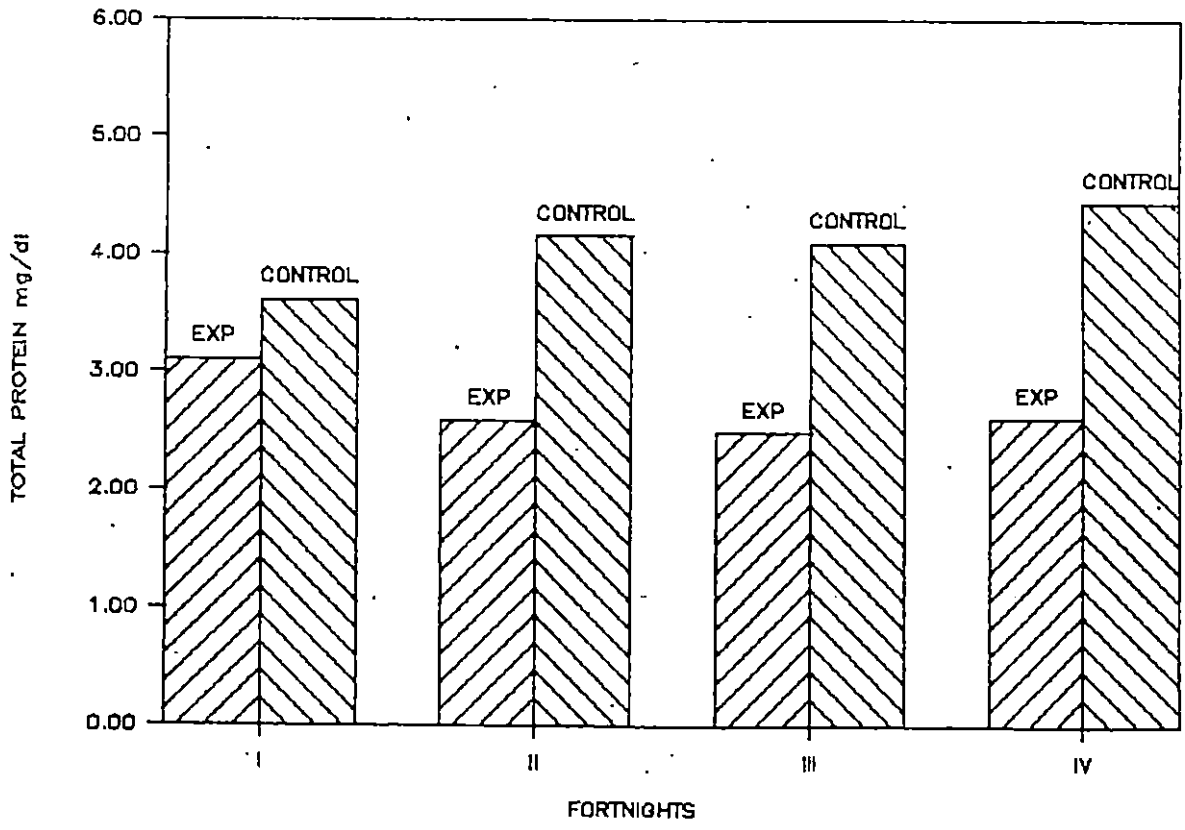


Fig.4 Average total serum protein (Experimental and Control)

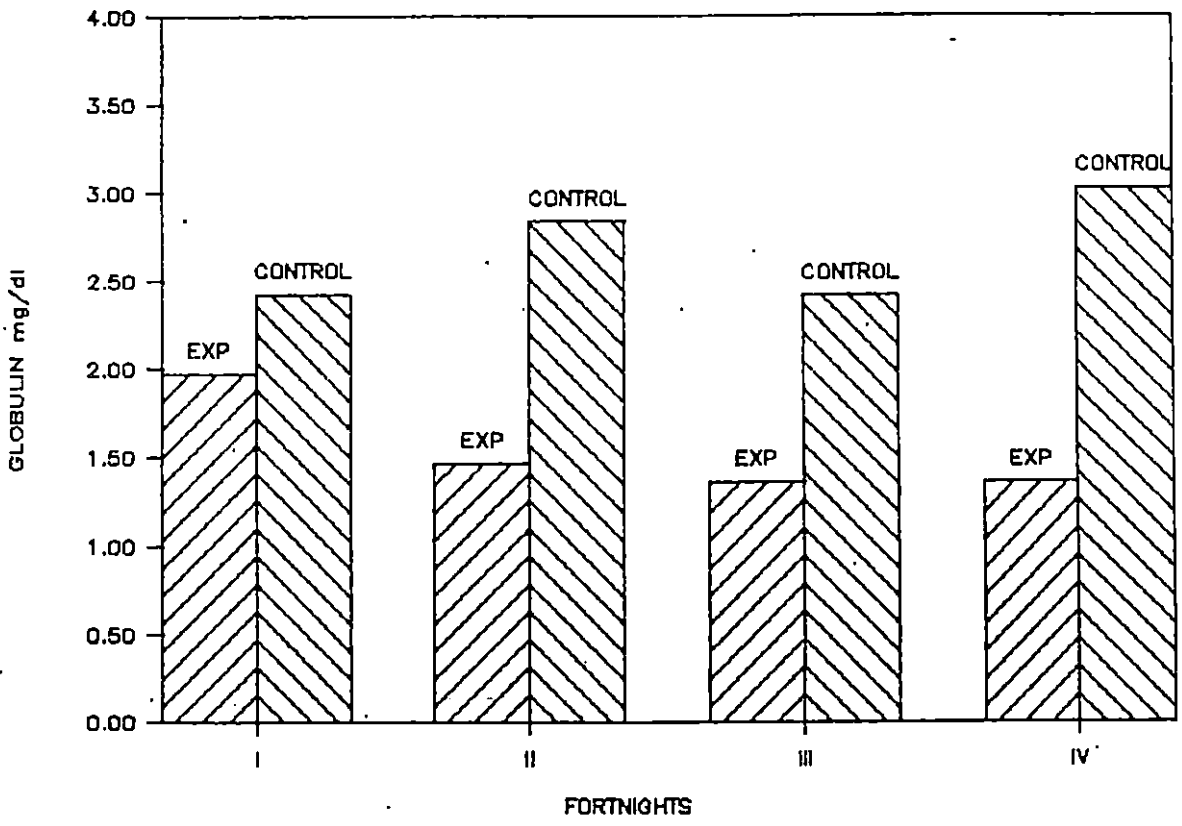
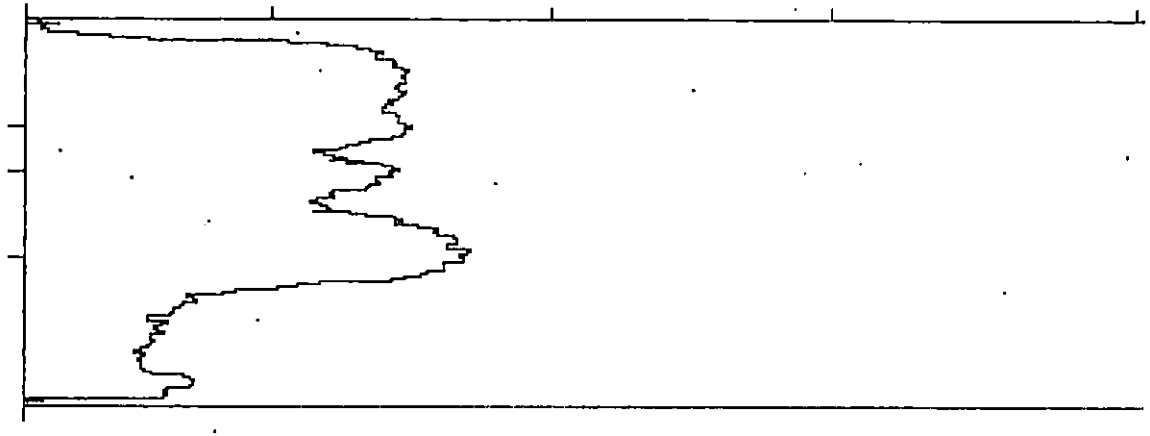


Fig.5 Average serum globulin (Experimental and Control)

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



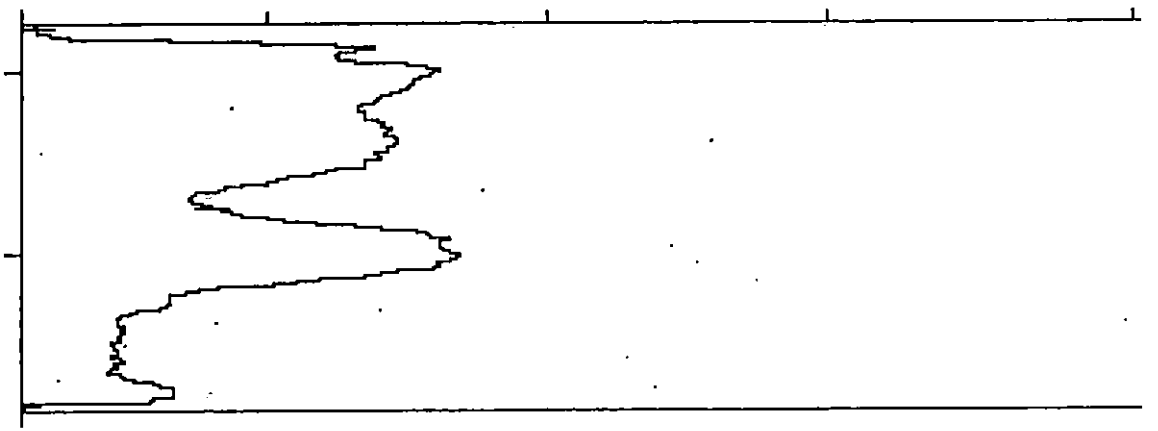
T-19

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	1.787	111648	91	1226.9	41.81	41.8071
2)	2.521	41859	86	486.7	15.67	15.6743
3)	4.007	113548	103	1102.4	42.52	42.5186
TOTAL:		267055			100.00	100.0000

Fig.6 PAGE Analysis - Duck serum sample No.1 - Experimental

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



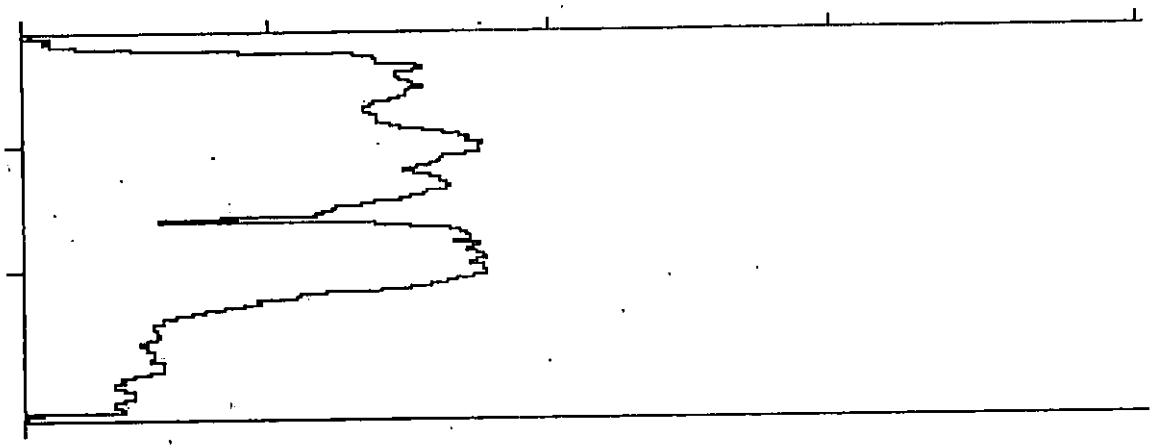
T-55

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	0.742	134676	96	1402.9	55.02	55.0183
2)	3.883	110108	101	1090.2	44.98	44.9817
TOTAL:		244784			100.00	100.0000

Fig.7 PAGE Analysis - Duck serum sample No.2 - Experimental

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



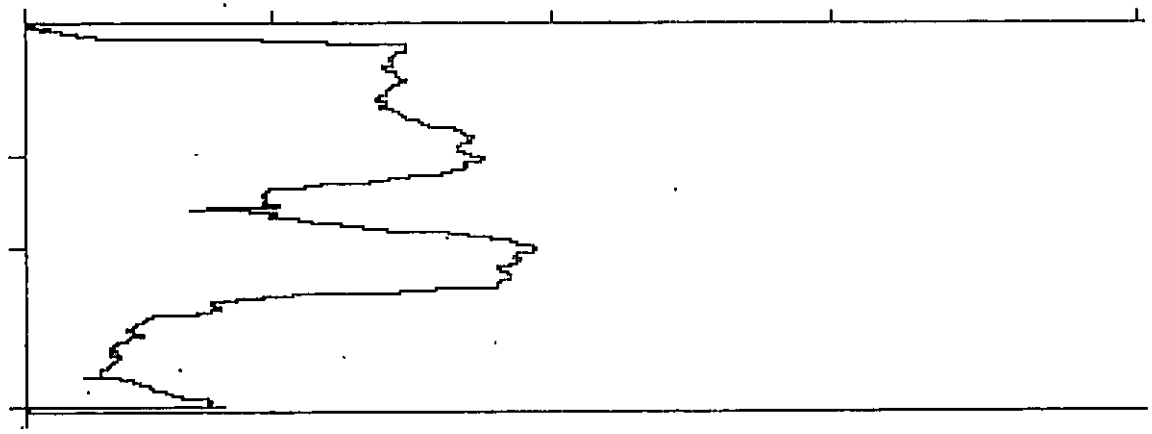
T-18

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	1.867	167307	107	1563.6	55.84	55.8413
2)	3.990	132304	107	1236.5	44.16	44.1587
TOTAL:		299611			= 100.00	100.0000

Fig.8 PAGE Analysis - Duck serum sample No.3 - Experimental

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



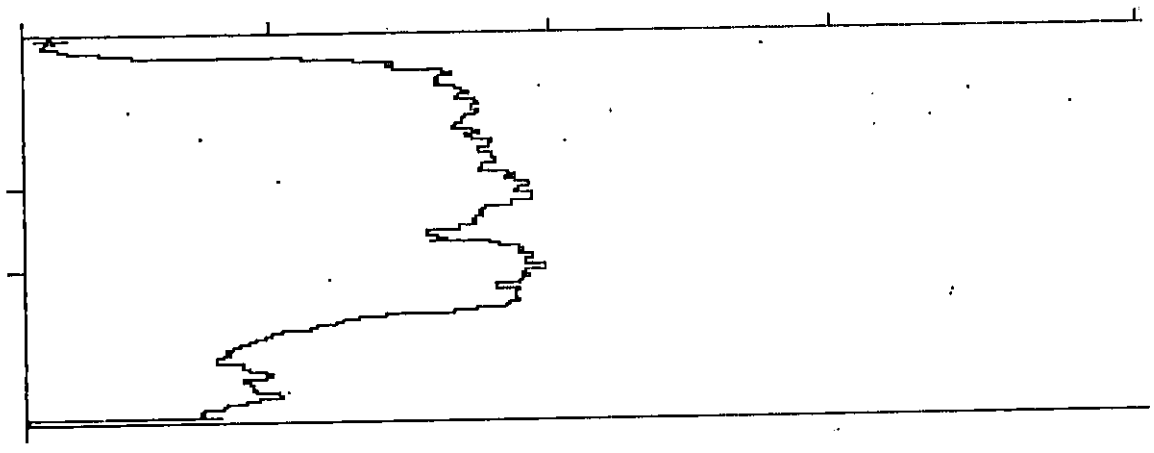
T-17

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	2.243	152342	106	1437.2	57.54	57.5397
2)	3.780	109067	117	932.2	41.19	41.1948
3)	6.492	3351	43	77.9	1.27	1.2655
TOTAL:		264759			100.00	100.0000

Fig.9 PAGE Analysis - Duck serum sample No.4 - Experimental

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



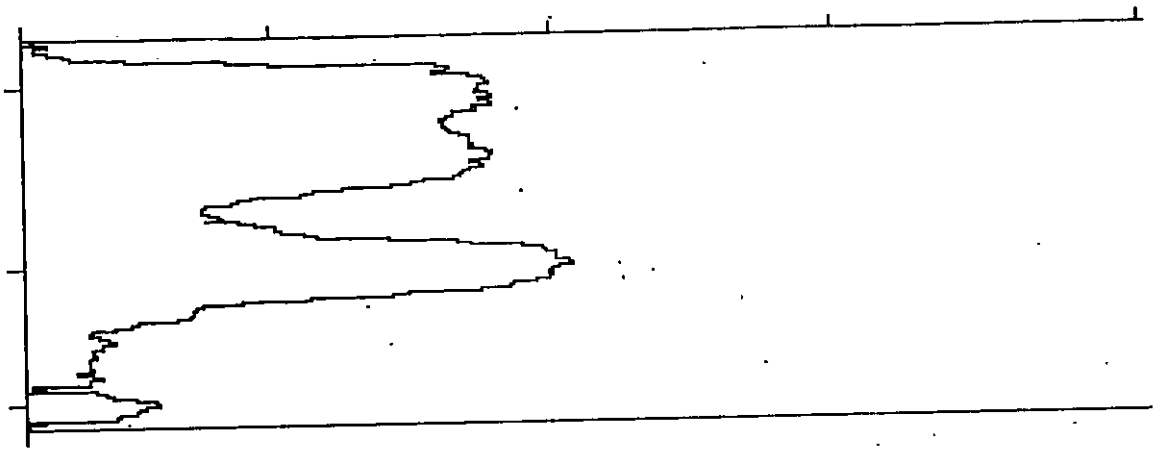
T-16

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	2.526	212078	117	1812.6	59.02	59.0218
2)	3.945	147243	119	1237.3	40.98	40.9782
TOTAL:		359321			100.00	100.0000

Fig.10 PAGE Analysis - Duck serum sample No.5 - Experimental

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



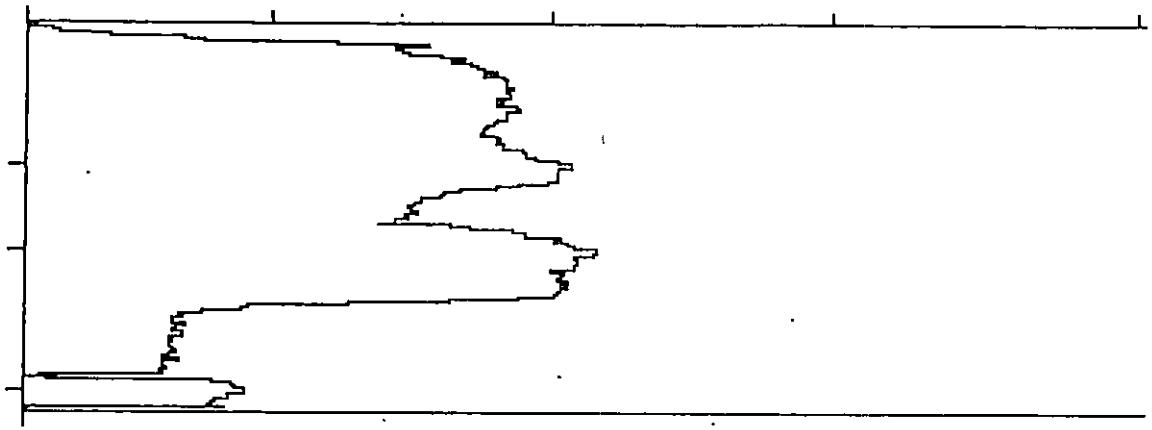
T-14

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	0.750	152893	108	1415.7	58.18	58.1833
2)	3.876	104672	126	830.7	39.83	39.8329
3)	6.160	5213	30	173.8	1.98	1.9838
TOTAL:		262778			100.00	100.0000

Fig.11 PAGE Analysis - Duck serum sample No.6 - Experimental

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



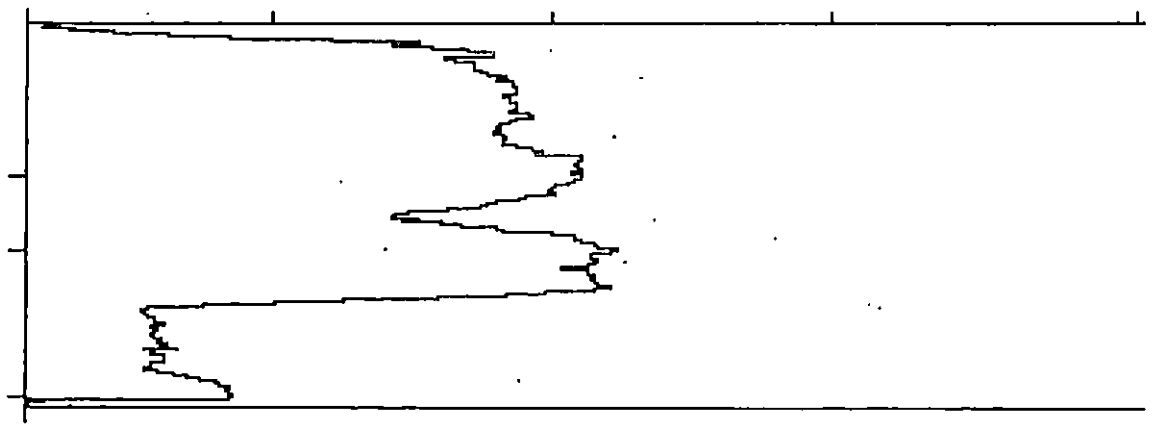
C10

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	2.374	200925	126	1594.6	59.27	59.2713
2)	3.839	130999	131	1000.0	38.64	38.6437
3)	6.205	7068	51	138.6	2.09	2.0850
TOTAL:		338992			100.00	100.0000

Fig.12 PAGE Analysis - Duck serum sample No.1 - Control

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



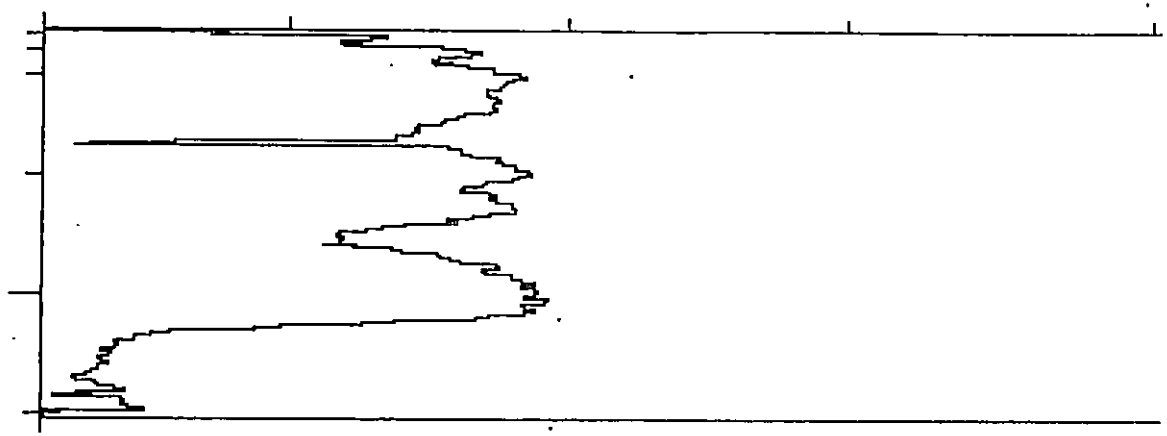
c-3

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	2.587	216265	131	1650.9	62.80	62.7976
2)	3.877	120231	137	877.6	34.91	34.9119
3)	6.376	7888	49	161.0	2.29	2.2905
TOTAL:		344384			100.00	100.0000

Fig.13 PAGE Analysis - Duck serum sample No.2 - Control

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



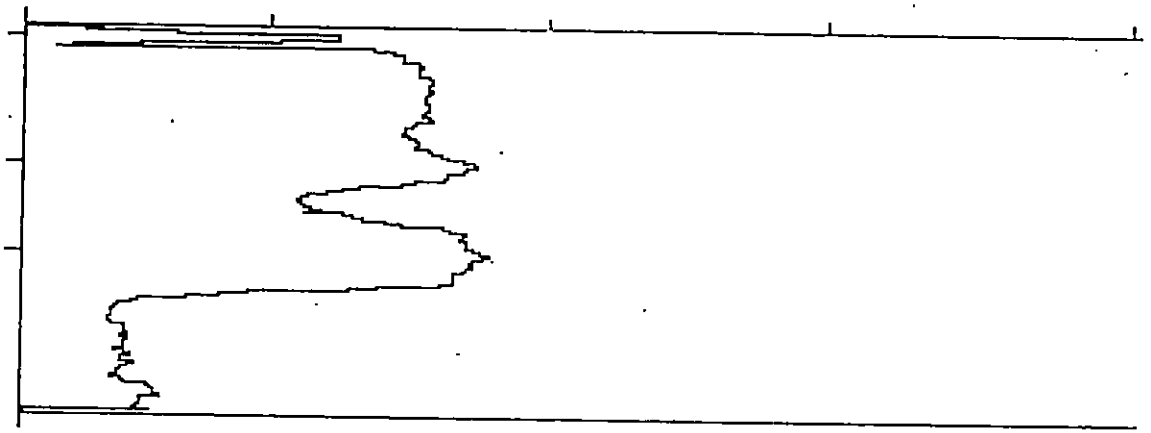
C-2

PEAK	POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	0.022	4184	96	43.6	1.42	1.4196
2)	0.320	17467	106	164.8	5.93	5.9262
3)	0.725	77169	110	701.5	26.18	26.1820
4)	2.397	97532	115	848.1	33.09	33.0907
5)	4.453	96007	116	827.6	32.57	32.5733
6)	6.495	2382	29	82.1	0.81	0.8082
TOTAL:		294741			100.00	100.0000

Fig.14 PAGE Analysis - Duck serum sample No.3 - Control

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



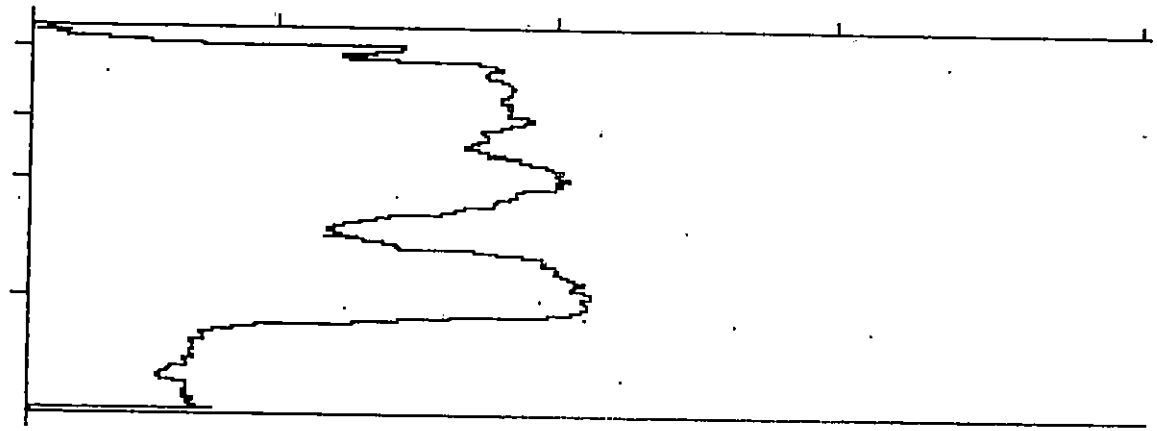
C-4

	PEAK POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	0.128	7657	76	100.8	2.77	2.7740
2)	2.261	152061	104	1462.1	55.09	55.0893
3)	3.788	116309	107	1087.0	42.14	42.1367
TOTAL:		276027			100.00	100.0000

Fig.15 PAGE Analysis - Duck serum sample No.4 - Control

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



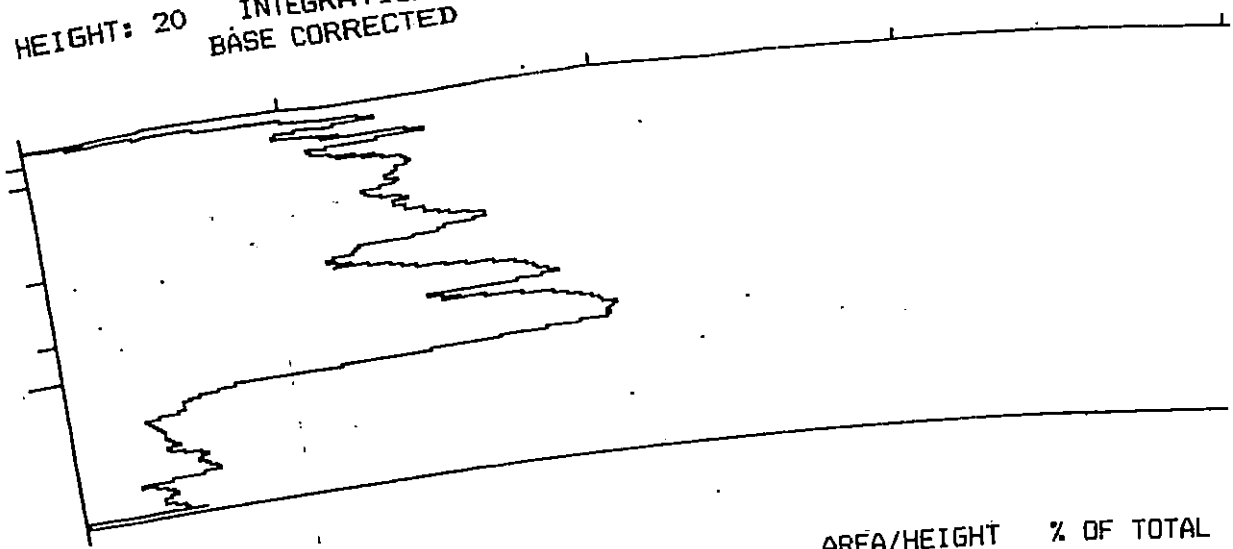
C-5

	PEAK POSITION	AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
1)	0.314	12065	91	132.6	3.53	3.5340
2)	1.511	107820	116	929.5	31.58	31.5818
3)	2.545	87336	126	693.1	25.58	25.5818
4)	4.542	134178	131	1024.3	39.30	39.3024
TOTAL:		341399			100.00	100.0000

Fig.16 PAGE Analysis - Duck serum sample No.5 - Control

BIOMED INSTRUMENTS

PEAK HEIGHT: 20 INTEGRATION RESOLUTION: 100% PEAK WIDTH: 10 GRAPH LENGTH: 5
BASE CORRECTED



C-1

PEAK POSITION		AREA	HEIGHT	AREA/HEIGHT	% OF TOTAL	WEIGHT
	0.227	14557	82	177.5	5.03	5.0266
1)	0.578	16644	88	189.1	5.75	5.7474
2)	2.214	101687	97	1048.3	35.11	35.1139
3)	3.350	50492	111	454.9	17.44	17.4356
4)	4.005	106213	125	849.7	36.68	36.6766
5)						
TOTAL:		289592			100.00	100.0000

Fig.17 PAGE Analysis - Duck serum sample No.6 - Control

4 and (Fig. 18). The AFB$_1$ fed ducks showed highly significant ($P < 0.01$) reduction in the skin thickness compared to the control groups at 24 h, 48 h and 72 h post-challenge.

4.7 Cutaneous response to PHA-M:

The average skin thickness values of experimental and control groups are furnished in Table 5 and (Fig. 19). There was reduction in skin thickness in the experimental group when compared to the controls at 24h, 48h and 72h post-administration, which however was not statistically significant.

4.8 Phagocytic response:

The optical density of serum samples collected during the carbon clearance assay from AFB$_1$ fed and control groups are furnished in Table 6. There was significant increase in the optical density of serum of AFB$_1$ birds compared to those from the controls which was indicative of reduction in phagocytic activity of AFB$_1$ ducks.

4.9 Pathology:

4.9.1 Gross pathology:

4.9.1.1 AFB$_1$ fed group:

4.9.1.1.1 Liver:

The liver of the AFB$_1$ ducks was enlarged , pale yellow and friable. The intensity of these changes were more towards the end of the experiment. Some of the livers showed streaks of haemorrhages at the end of fourth fortnight.

TABLE 5

BIOMETRY OF SKIN THICKNESS [MEAN ± S.E.] IN RESPONSE TO PHYTOHAEMAGGLUTININ-M [EXPERIMENTAL AND CONTROL]

GROUPS	SKIN THICKNESS CHALLENGE [0 h]	SKIN THICKNESS [mm] AFTER CHALLENGE		
		24 h	48 h	72 h
EXPERIMENTAL	1.0	3.3333 ± 0.2405	2.6250 ± 0.0778	1.5833 ± 0.1402
CONTROL	1.0	3.8330 ± 0.2545	3.1250 ± 0.1932	1.8750 ± 0.1640

TABLE 6
CARBON CLEARANCE ASSAY- OPTICAL DENSITY AND PHAGOCYTIC INDEX
[EXPERIMENTAL AND CONTROL]

EXPERIMENTAL				CONTROL			
SERUM NO.	CLEARANCE TIME [mts}	OPTICAL DENSITY [OD]	PHAGOCYTIC INDEX [PI]	SERUM NO.	CLEARANCE TIME [mts}	OPTICAL DENSITY [OD]	PHAGOCYTIC INDEX [PI]
1	0	0.271	0.0916	1	0	0.101	0.0087
	5	0.131			5	0.104	
	15	0.065			15	0.090	
2	0	0.051	0.0241	2	0	0.132	-0.0142
	5	0.042			5	0.244	
	15	0.035			15	0.182	
3	0	0.063	-0.0229	3	0	0.083	0.1615
	5	0.148			5	0.171	
	15	0.103			15	0.010	
4	0	0.202	0.0296	4	0	0.039	-0.0419
	5	0.263			5	0.108	
	15	0.140			15	0.086	
5	0	0.025	-0.0783	5	0	0.086	0.0262
	5	0.156			5	0.123	
	15	0.108			15	0.064	
6	0	0.023	-0.1123	6	0	0.019	-0.0844
	5	0.261			5	0.130	
	15	0.180			15	0.091	
AVERAGE	0	0.110	0.0054	AVERAGE	0	0.080	-0.0071
	5	0.210			5	0.164	
	15	0.116			15	0.102	

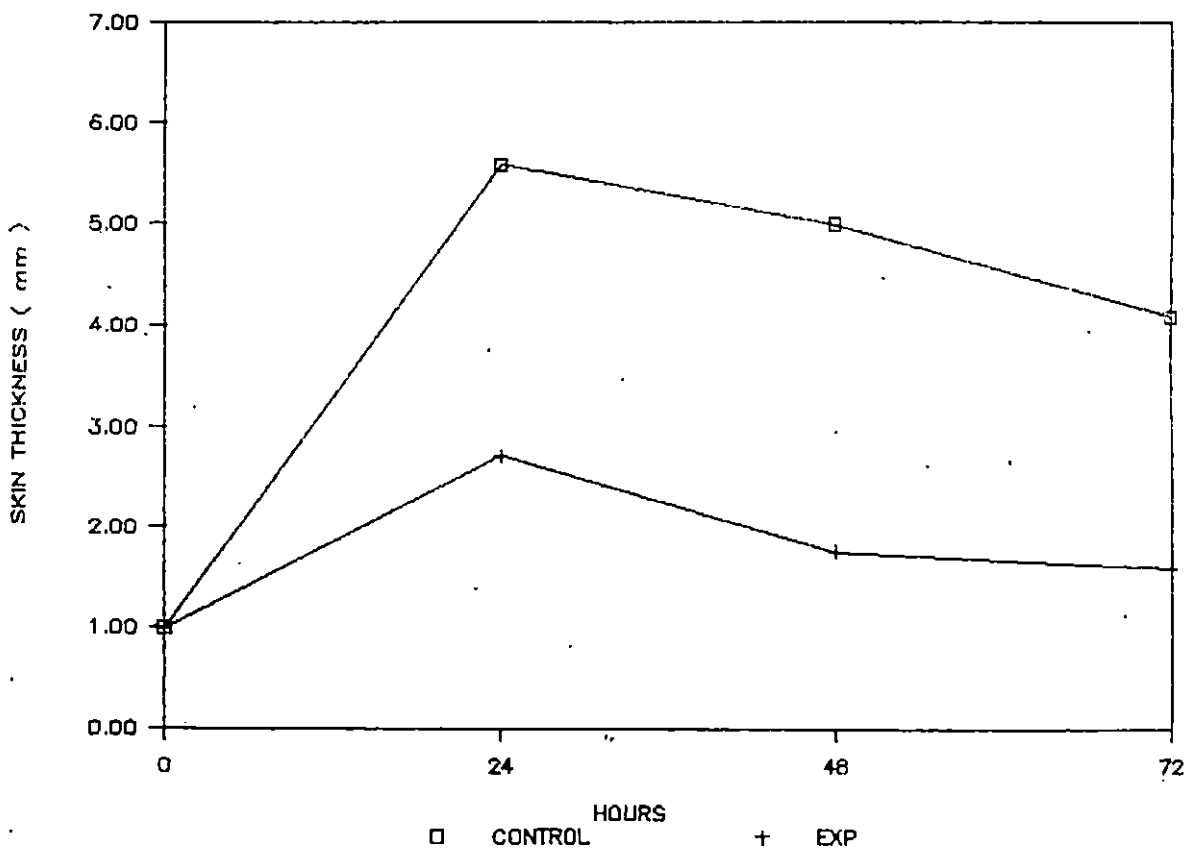


Fig.18 Average skin thickness in response to DNCB (Experimental and Control)

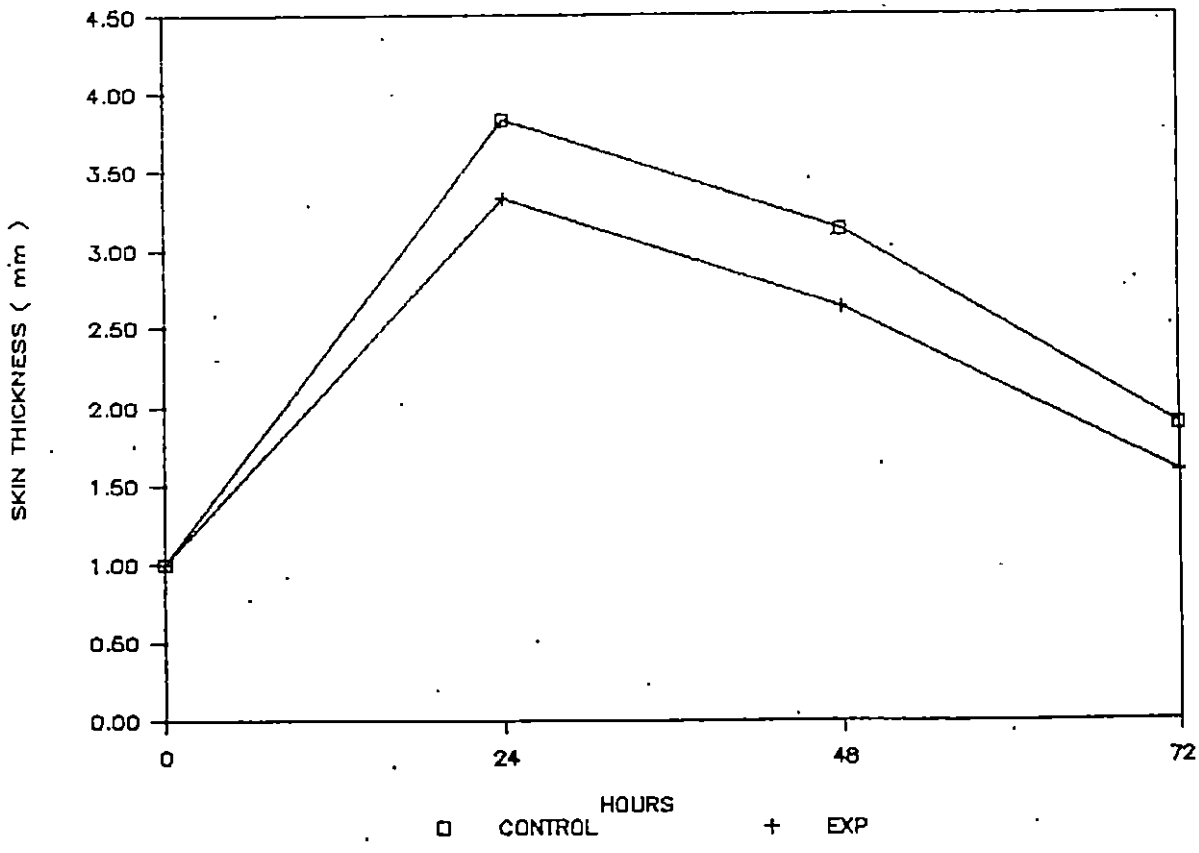


Fig.19 Average skin thickness in response to PHA-M (Experimental and Control)

4.9.1.1.2 Kidney:

Some of the AFB$_1$ fed individuals showed congestion and petecheal haemorrages.

4.9.1.1.3 Lymphoid organs:

In general, the gross changes in the bursa of Fabricius, spleen and thymus were restricted to the size of the organs. The lymphoid organs of the AFB$_1$ fed birds appeared atrophied. The caecal tonsils also appeared to be atrophied and in few cases the surface revealed focal haemorrhagic spots.

4.9.1.2 Control group:

No gross abnormalities were observed in the control group at autopsy.

4.9.2 Histopathology:

4.9.2.1 AFB$_1$ fed groups:

4.9.2.1.1 Liver:

The histopathological changes during the course of the experiment revealed the dose and time dependent changes. The hepatic changes varied from fatty change during the first fortnight of the experiment (Fig. 22 & 23) to severe degenerative changes during the end of the experiment(Fig. 24, 25 & 26). Some ducks showed fatty change in the peripheral areas during the first fortnight. These changes were more intense in the subsequent second, third and fourth fortnights. Some of the hepatic cells showed pyknotic nuclei. There was dissociation of

hepatocytes leading to disruption of structural integrity of the hepatic architecture(Fig. 26). Necrosis was evident. In some cells the cytoplasm was granular in nature with few fat globules. During the fourth fortnight the above said changes were more intense with paracentral and centrilobular necrosis. There was marked proliferation of the epithelium of bile ducts with occasional ductule formation(Fig. 27, 28 & 29). There was severe disorganisation of hepatic chords and grouping of hepatocytes and acinilike formation in few areas(Fig. 30 & 31).

4.9.2.1.2 Kidney:

Granular degeneration, focal necrosis and desquamation of the epithelial cells lining the proximal convoluted tubules were seen(Fig. 32). Vacuolation of a few epithelial cells in the glomerular capillaries was observed in few places.

4.9.2.1.3 Bursa of Fabricius:

During the first fortnight distribution of lymphoid cells in the germinal centre of bursa of Fabricius were sparse (Fig. 33). Degenerative and necrotic changes were also seen in the follicles (Fig. 34). The changes in the epithelial layer were focal necrosis and desquamation of lining cells in some follicles. During the second fortnight, the necrotic process was more extensive both in the germinal centre and at the periphery of the follicles (Fig. 35 & 36). Necrosis of epithelial cells was also evident. There was reduction in the size of the follicles(Fig. 37). This was more pronounced than that was observed in the first

fortnight. Desquamation of lining epithelial cells of follicles was also noted. Marked reduction in the size of the follicles was seen during the third and fourth fortnights with loss of lymphoid elements. The regular follicular pattern was lost with interstitial fibrosis (Fig. 38). Collagen fibres were seen interspersing the follicular structure. Majority of the lymphoid elements had undergone necrobiotic changes.

4.9.2.1.4 Spleen:

The histological changes in the spleen were dose dependent and the intensity of the changes were more during the fourth fortnight. The spleen showed lymphoid depletion and most of the cells had undergone necrotic changes (Fig. 39). Due to the severe loss of lymphoid elements some of the spleen sections had a washed-out appearance (Fig. 40).

4.9.2.1.5 Thymus:

The thymic changes included atrophy and depletion of lymphoid elements from the parenchyma due to necrobiotic processes (Fig. 41). Interstitial edema was also evident in some cases. More intense lympholytic changes were seen during the fourth fortnight.

4.9.2.1.6 Caecal tonsil:

The changes in the caecal tonsils were haemorrhages and degeneration of lymphoid cells. Focal areas of necrosis were seen during the course of the experiment (Fig. 42).

4.9.2.2 Control group:

No significant histological changes were observed in the liver, kidney, bursa of Fabricius, spleen thymus and caecal tonsils of control ducks.

4.9.2.3 Cutaneous response:

4.9.2.3.1 Gross and histopathology of skin:

4.9.2.3.1.1 DNCB:

At 24 h the skin of AFB_1 fed individuals was warm and diffusely oedematous. The reaction was more severe in the control group compared to AFB_1 fed birds. Histologically at 24 h there was diffuse congestion and haemorrhages with infiltration of mononuclear cells along with lymphocytes and macrophages 9Fig. 43 & 44). Oedema was evident. The intensity of these reactions was subsequently reduced during 48 h and 72 h.

4.9.2.3.1.2 PHA-M:

There was hyperaemia and oedema of the skin during the first 24 h in both AFB_1 fed and control birds. The cutaneous reaction in the control birds showed severe response.

Histological picture during the first 24 h included congestion and oedema of capillaries, infiltration of monocytes macrophages and lymphocytes (Fig. 45 & 46). During this period the control group showed more cellular response than the AFB_1 fed birds.

During the subsequent 48 h and 72 h the cellular reaction

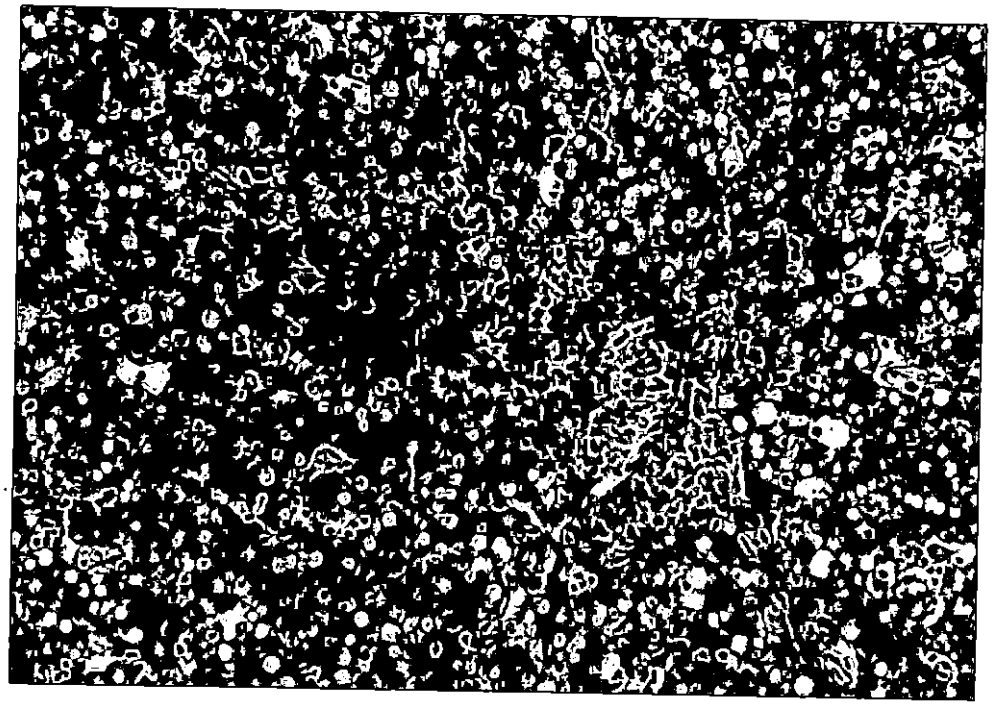
had subsided and the intensity did not vary between the control and AFB_1 fed birds (Fig. 47 & 48).

Fig.20 Polyacrylamide gel electrophoretic patterns - Duck serum samples 1, 2, 3, 4, 5 & 6 - Experimental

Fig.21 Polyacrylamide gel electrophoretic patterns - Duck serum samples 1, 2, 3, 4, 5 & 6 - Control

Fig.22 Liver - 1st fortnight - experimental - diffuse fatty changes - H&E x 250

Fig.23 Liver - 1st fortnight - experimental - diffuse fatty changes - H&E x 250



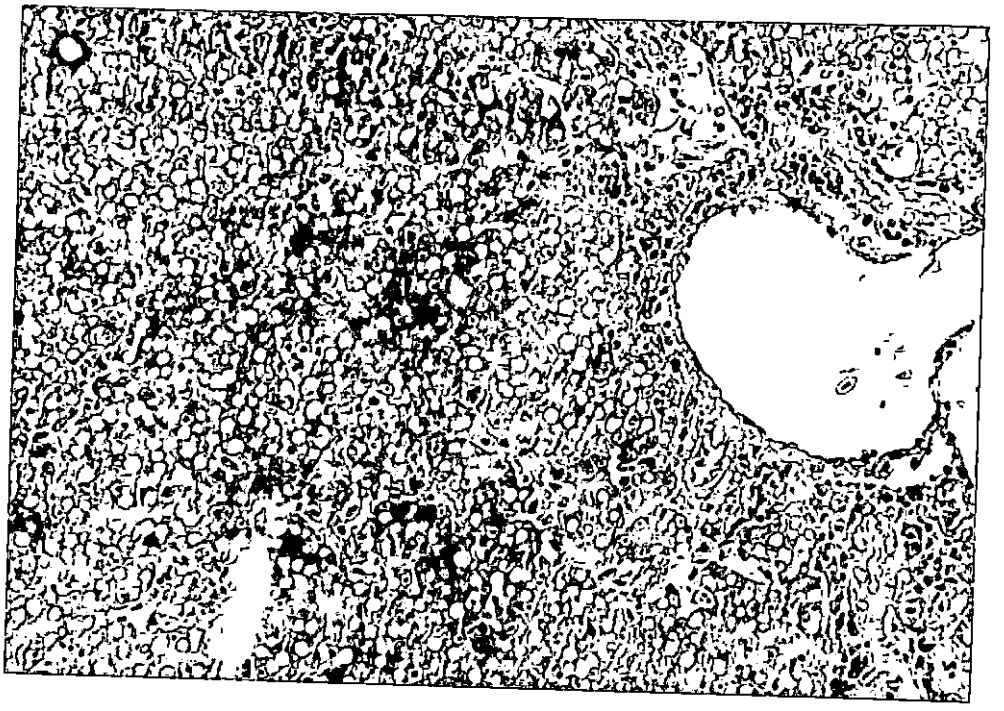
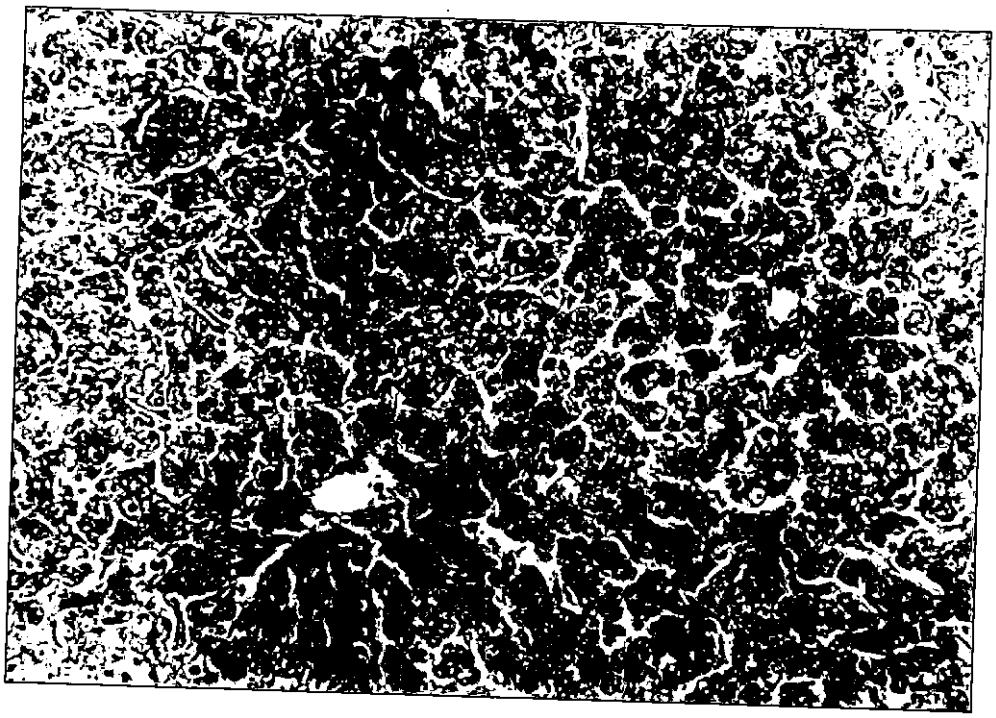


Fig.24 Liver - 3rd fortnight - experimental - degenerative changes - H&E x 250

Fig.25 Liver - 4th fortnight - experimental - severe degenerative changes - disassociated hepatic cords and loss of structural integrity - H&E x 250



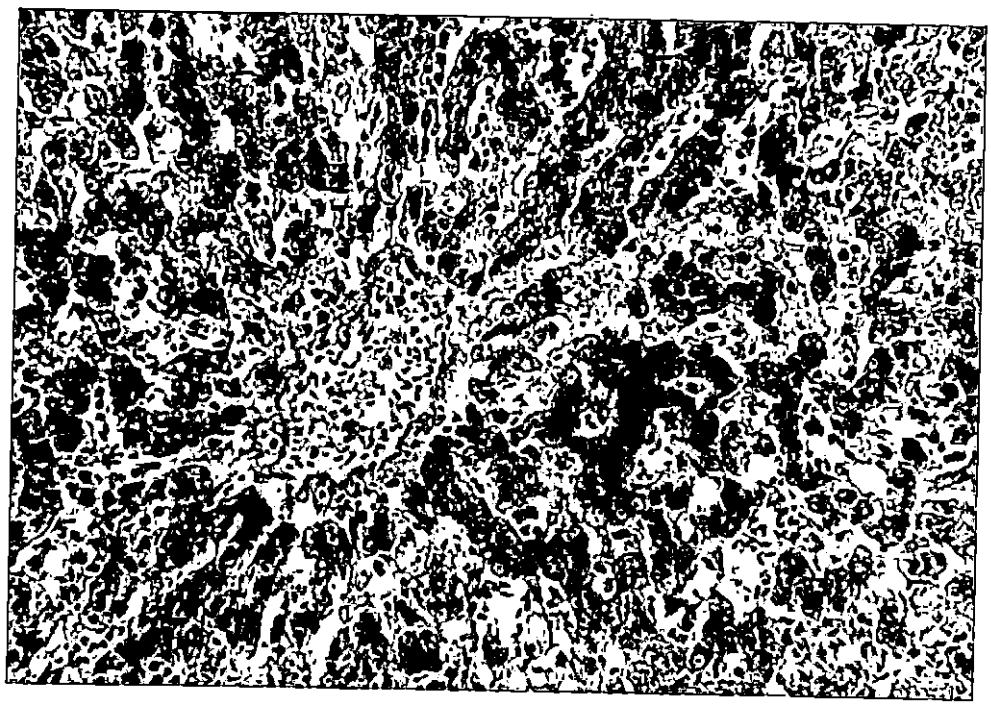
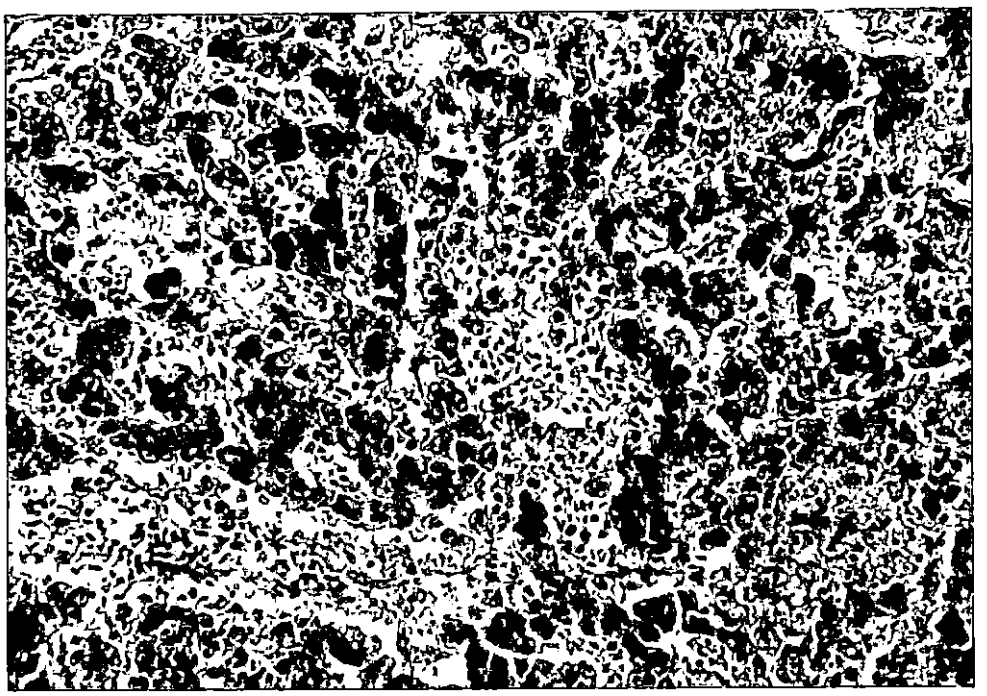


Fig.26 Liver - 4th fortnight - experimental - degenerative and necrotic changes - haemorrhage and total loss of architecture - H&E x 250

Fig.27 Liver - 4th fortnight - experimental - pseudolobulation and bile duct proliferation - H&E x 250



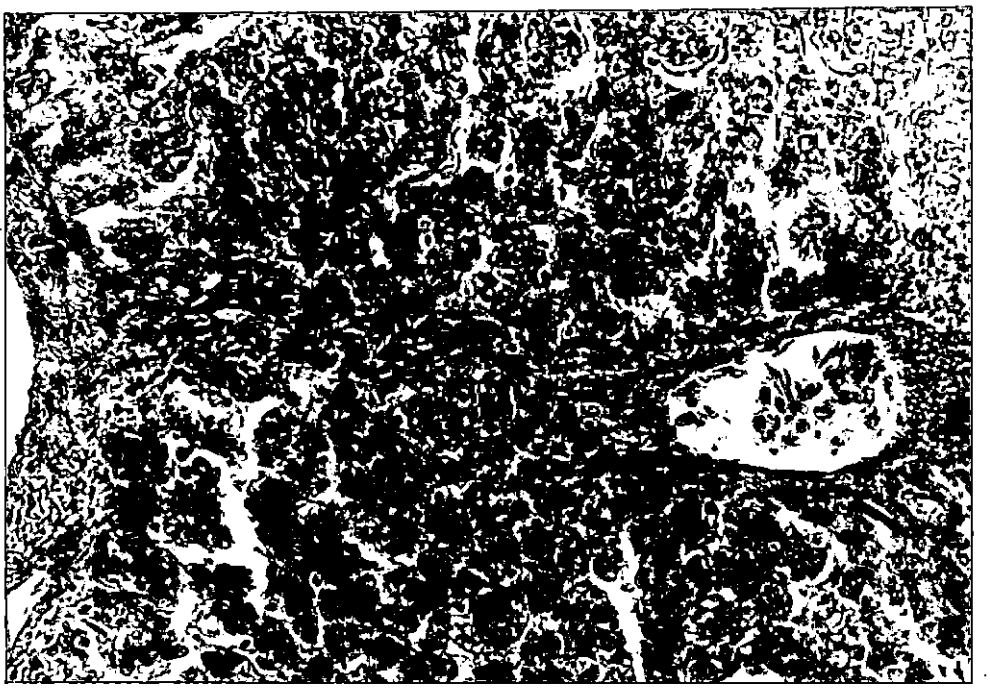
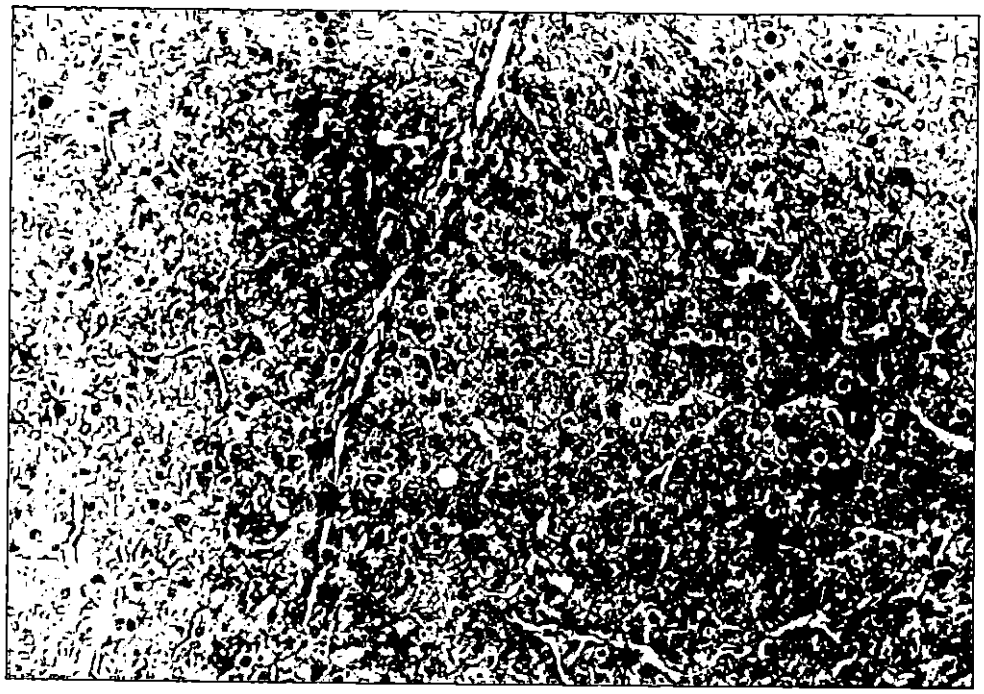


Fig.28 Liver - 4th fortnight - experimental - bile duct proliferation - H&E x 250

Fig.29 Liver - 4th fortnight - experimental - necrotic changes and loss of hepatic architecture - H&E x 250



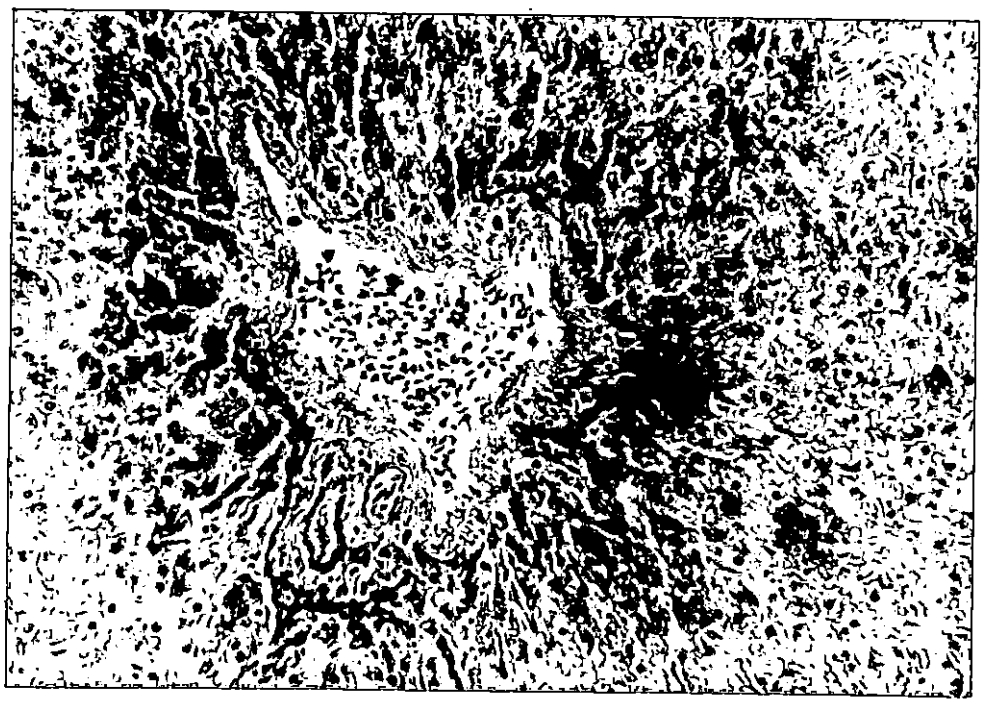
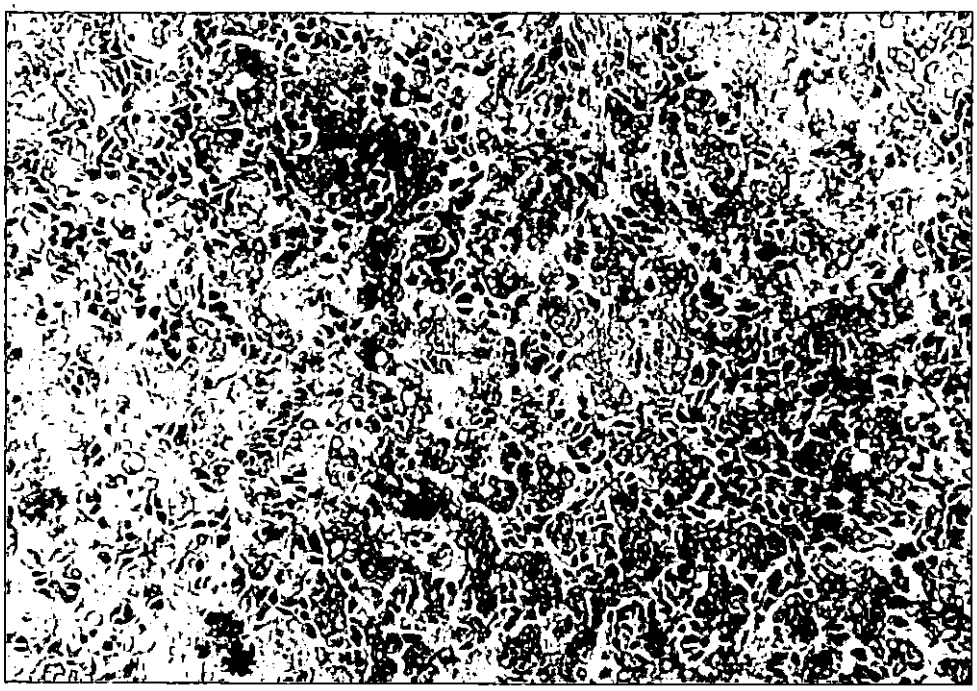


Fig.30 Liver - 4th fortnight - experimental - congestion - severe degenerative and necrotic changes - H&E x 250

Fig.31 Liver - 4th fortnight - experimental - complete loss of hepatic architecture - severe haemorrhage - H&E x 250



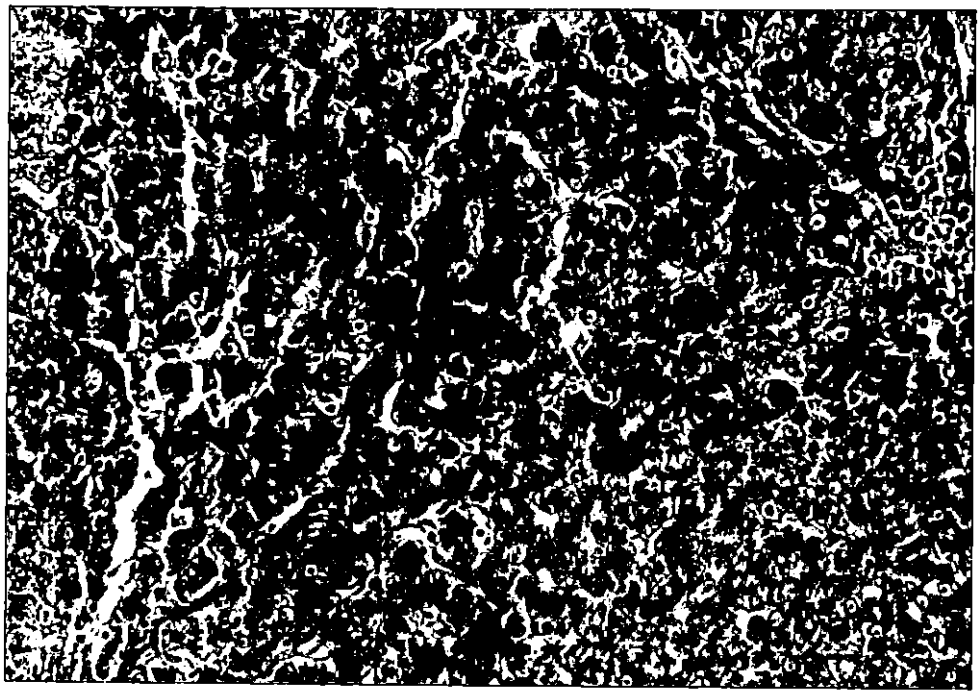
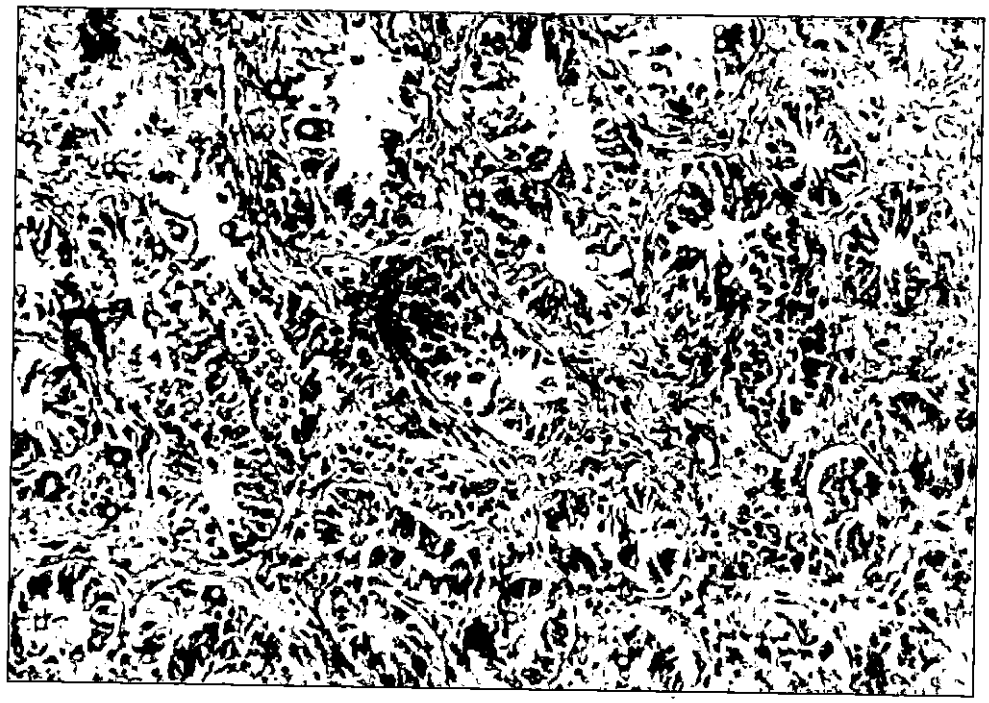


Fig.32 Kidney - 4th fortnight - experimental - severe tubular degeneration, necrosis and fibrosis - H&E x 250

Fig.33 Bursa of Fabricius - 1st fortnight - experimental - atrophy of follicles and depletion of lymphoid cells - H&E x 250



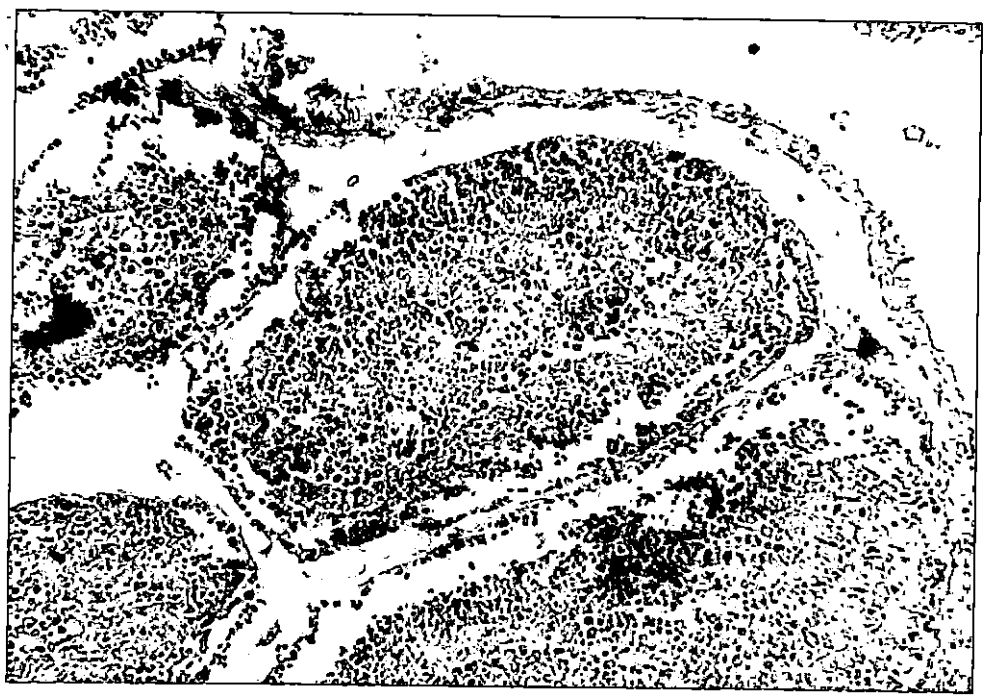


Fig.34 Bursa of Fabricius - 1st fortnight - experimental - necrotic changes with atrophy of follicles - H&E x 250

Fig.35 Bursa of Fabricius - 2nd fortnight - experimental - sparse lymphoid cells - fibrosis - H&E x 400

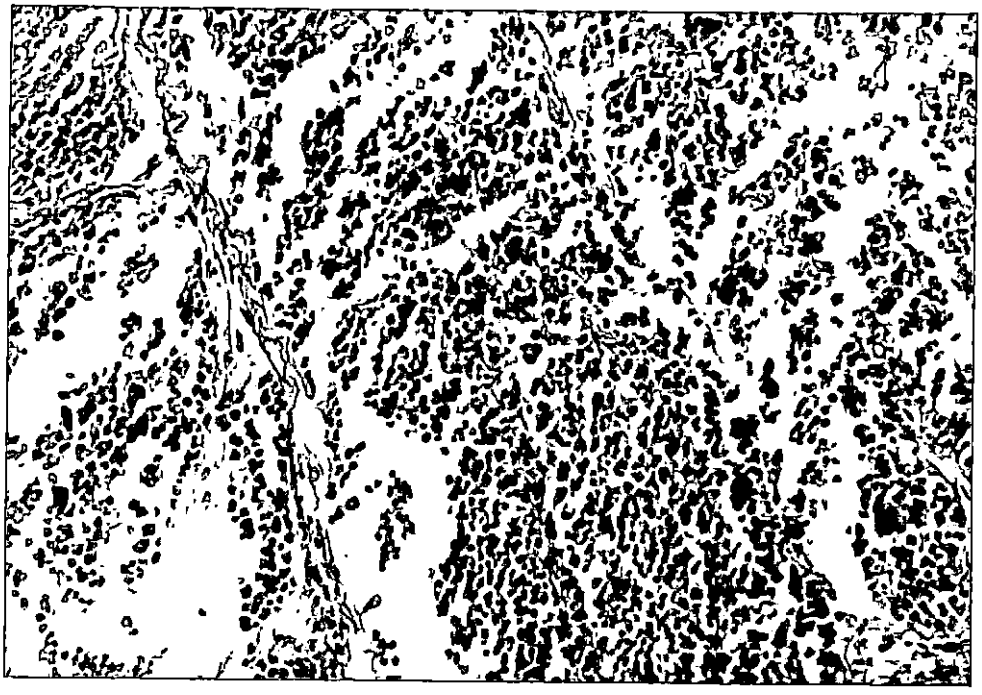
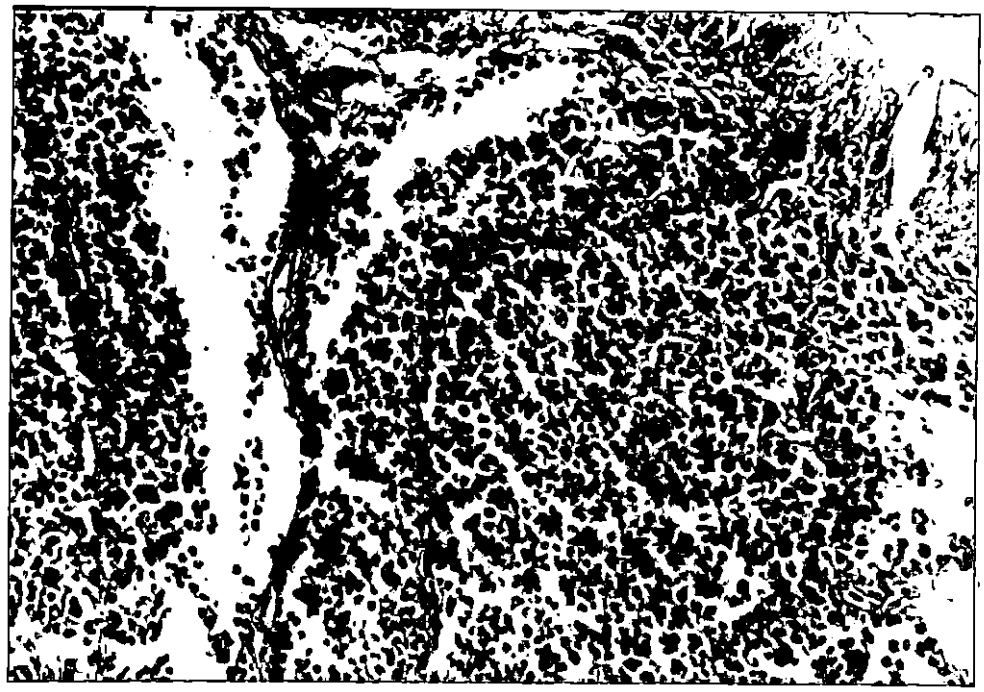
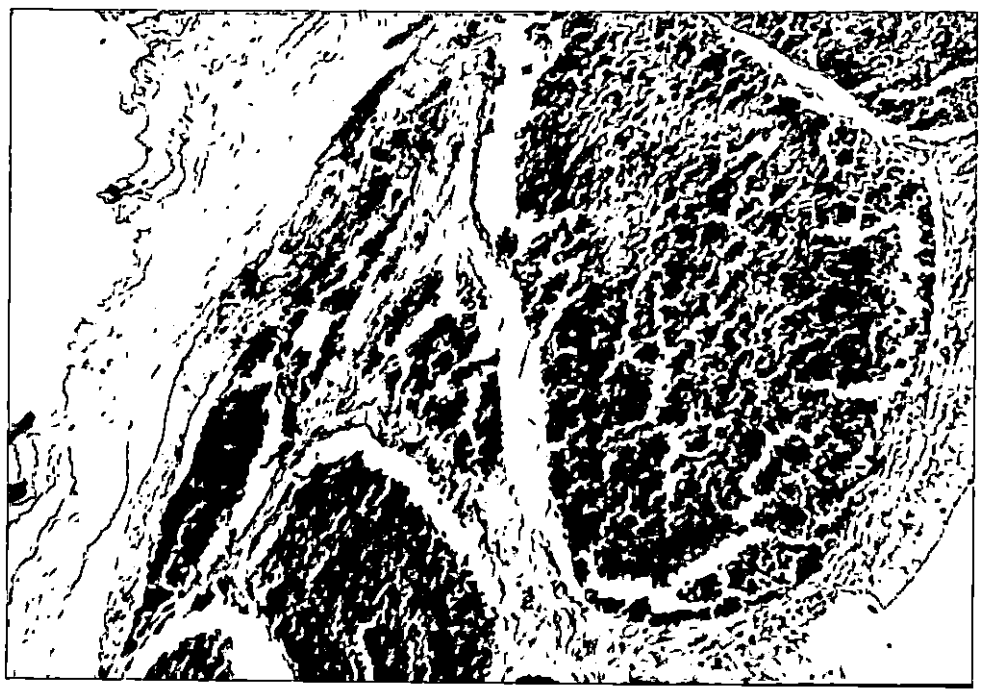
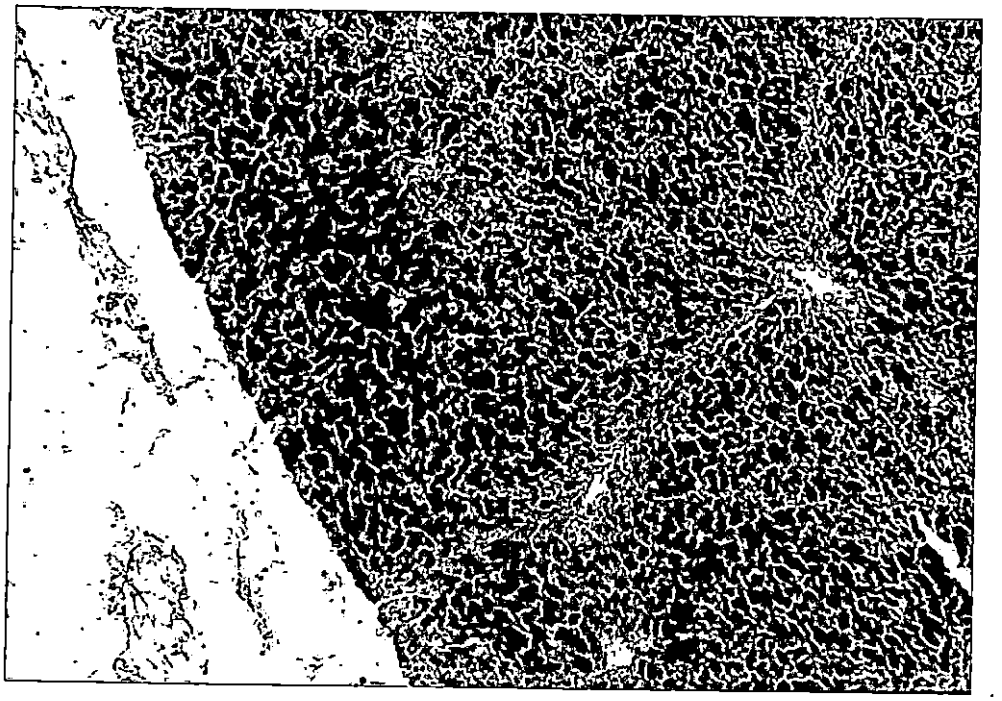


Fig.36 Bursa of Fabricius - 2nd fortnight - experimental - loosely packed lymphoid cells - oedema - H&E x 400

Fig.37 Bursa of Fabricius - 3rd fortnight - experimental - follicular atrophy - interstitial fibrosis - H&E x 250







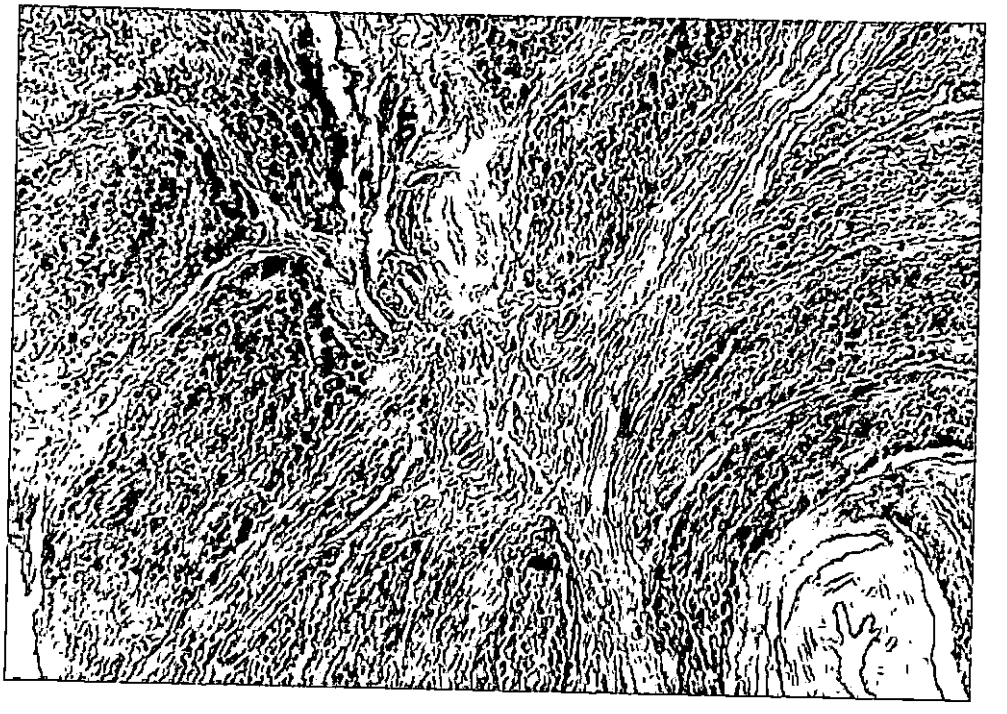


Fig.38 Bursa of Fabricius - 4th fortnight - experimental - loss of follicular patterns - severe interstitial fibrosis - H&E x 250

Fig.39 Spleen - 1st fortnight - experimental - loss of lymphoid cells - necrotic changes - H&E x 250

Fig.40 Spleen - 4th fortnight - experimental - loss of lymphoid cells - degeneration - haemorrhage - H&E x 250

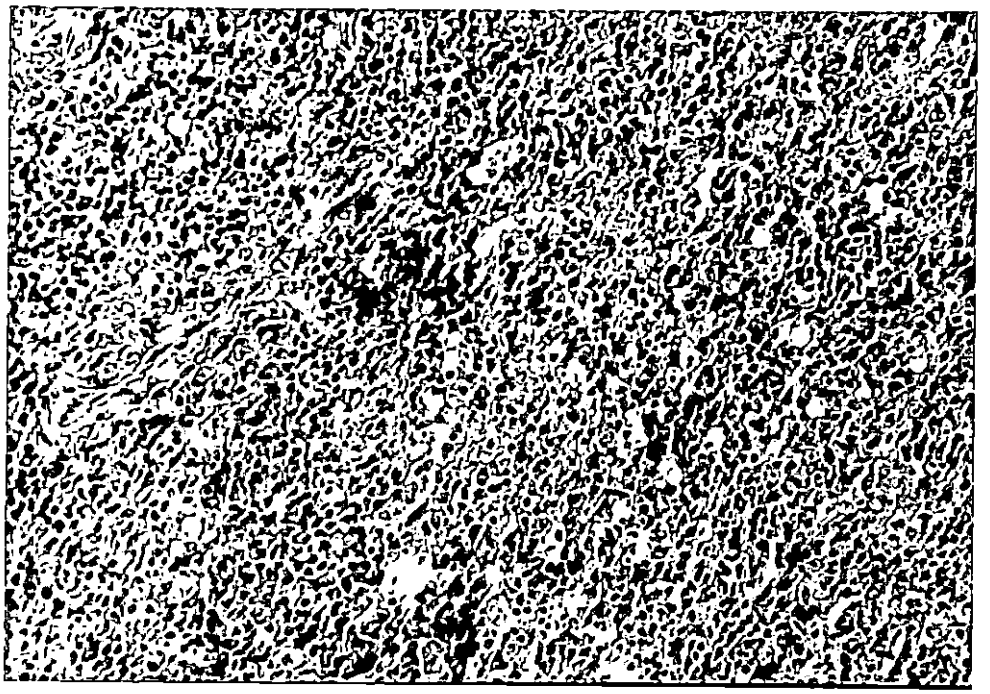
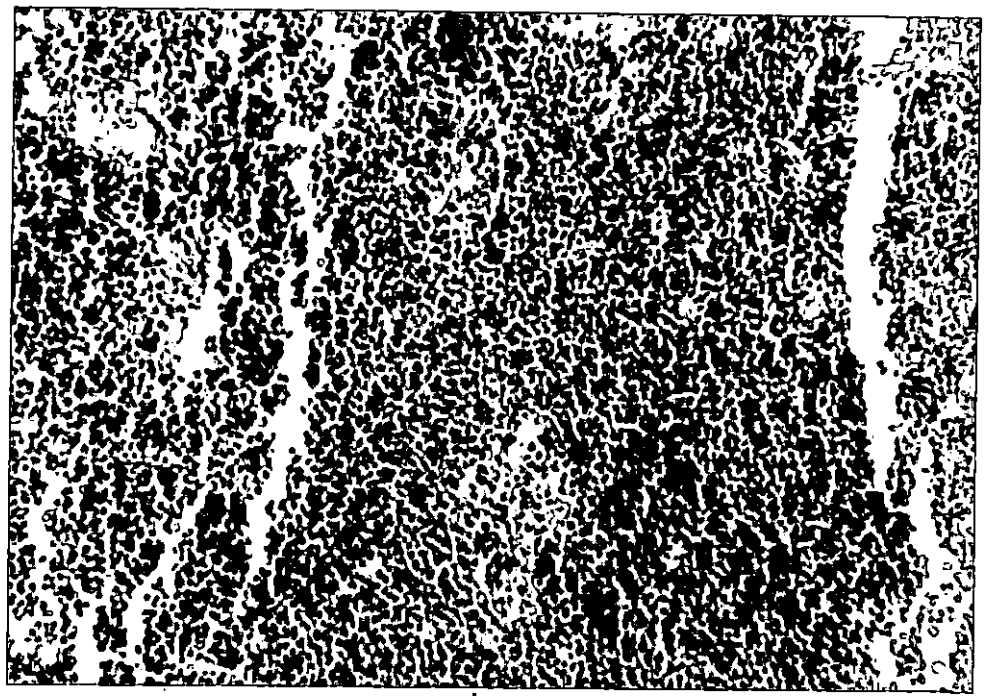


Fig. 41 Thymus - 4th fortnight - experimental - loosely packed lymphoid cells - oedema - H&E x 250

Fig.42 Caecal tonsil - 4th fortnight - experimental - loss of lymphoid cells - oedema - H&E x 250



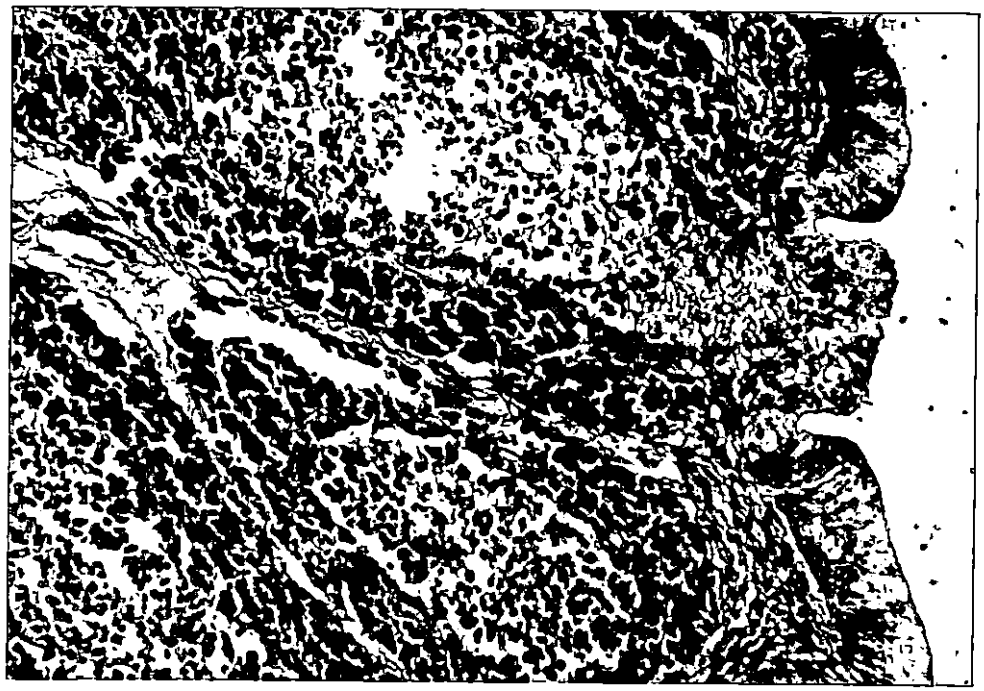
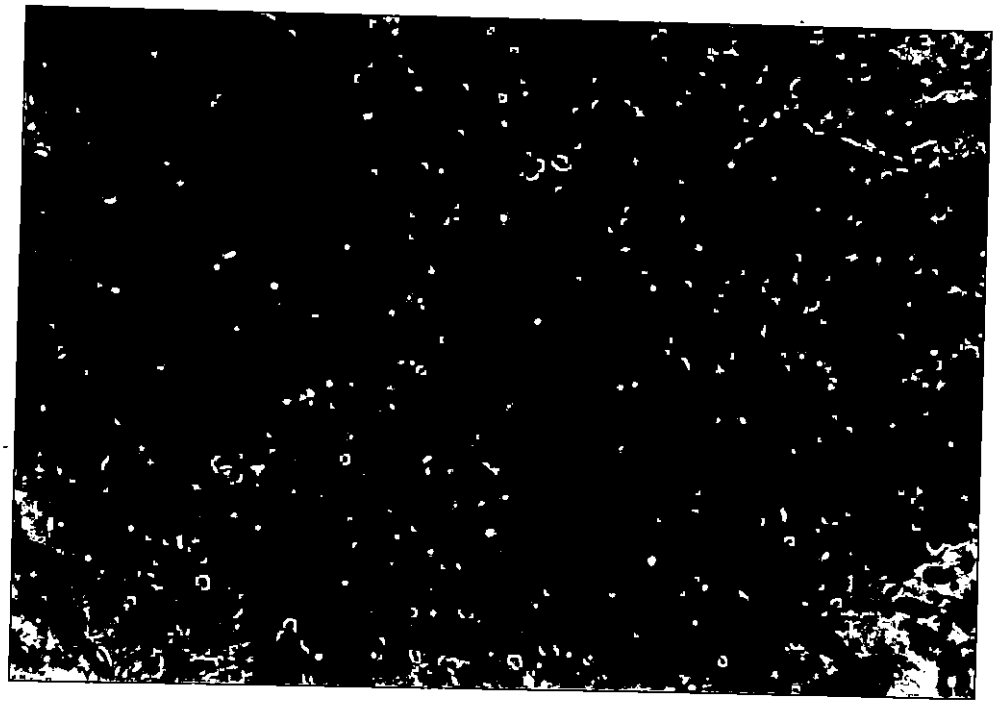


Fig.43 Skin - DNCB - 24 h - experimental - mild cellular infiltration and oedema - H&E x 250

Fig.44 Skin - DNCB - 24 h - control - severe haemorrhage - cellular infiltration and oedema - H&E x 250



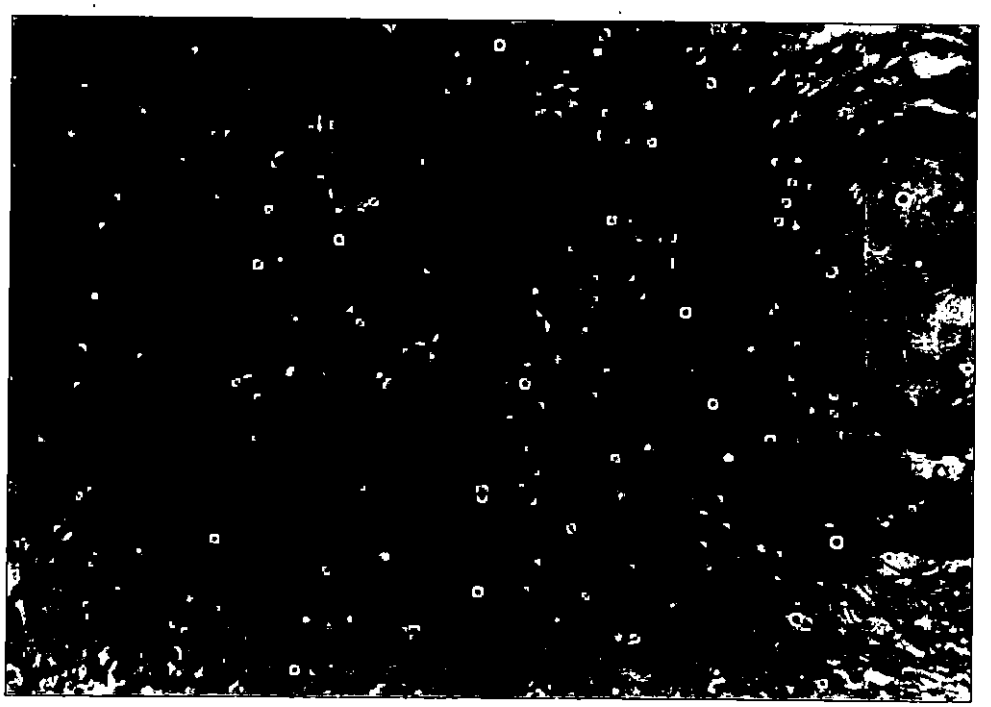


Fig.45 Skin - PHA-M - 24 h - experimental - cellular infiltration and oedema - H&E x 250

Fig.46 Skin - PHA-M - 24 h - control - severe cellular infiltration and oedema - H&E x 250

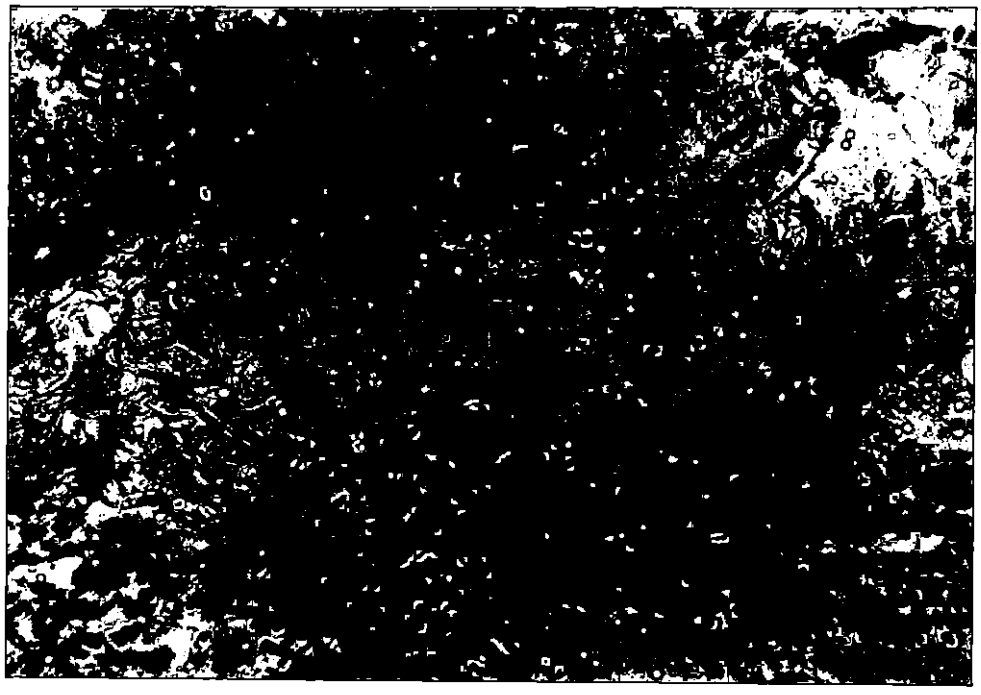
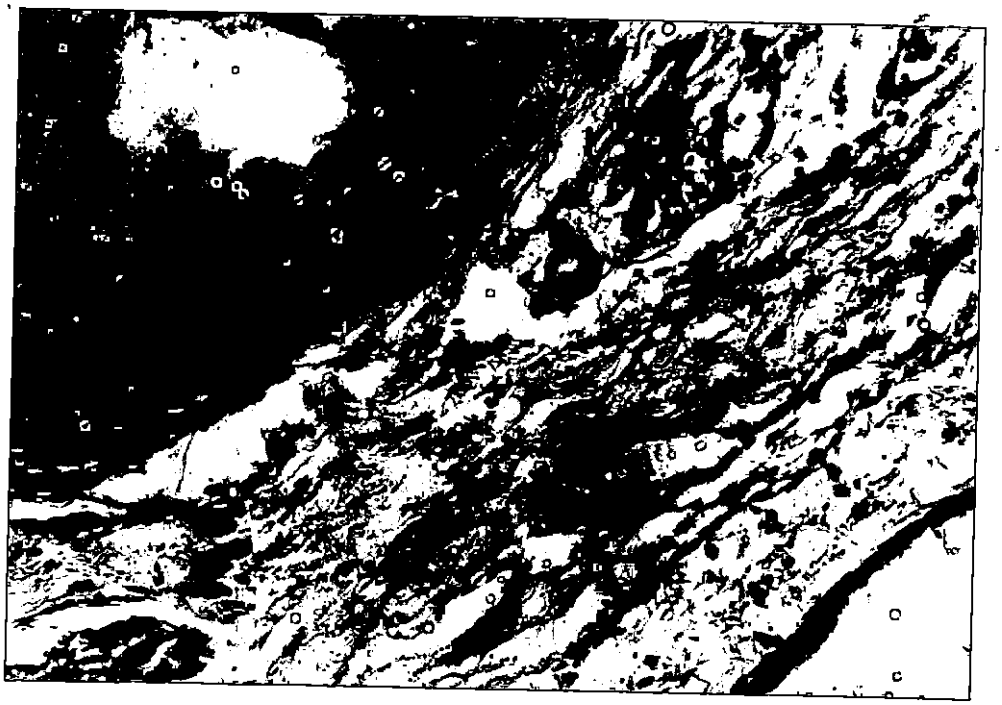


Fig.47 Skin - PHA-M - 48 h - experimental - cellular infiltration and oedema - H&E x 250

Fig.48 Skin - PHA-M - 48 h - Control - cellular infiltration and oedema - H&E x 250



Discussion

DISCUSSION

This investigation was designed to study the effects of dietary AFB_1 on the immune system of ducks. The humoral and cell-mediated immune responses were assessed using suitable markers. The results of the haematological parameters established the pathological effect of AFB_1, on the haemopoietic system of ducks.

During the course of this experiment there was moderate decrease in the haemoglobin concentration of the AFB_1 fed ducks. This observation clearly indicated the adverse biological effect of AFB_1 on the haemopoietic system at the given dose level. The reduction in the haemoglobin concentration could be correlated with the hepatic damage and to the direct effect of the toxin on the protein synthesis. Gopalakrishnan Nair (1986) also observed decreased haemoglobin concentration in aflatoxin fed pigs. Mohiuddin *et al*. (1986) also observed reduction in haemoglobin concentration during experimental aflatoxicosis in chicken.

The mean values of TLC during the course of the experiment revealed dose dependent reduction of the immunocompetent cells. This is a reflection of the adverse effects of AFB_1 on the immune system leading to immunosuppression. The results of the DLC indicated lymphopenia and relative heterophilia with a total leucopenia. Similar observations were made by Campbell *et al*. (1983) and Mohiuddin *et al*. (1986).They also reported lymphopenia

with relative heterophilia in experimental aflatoxicosis in chicken.

During the course of this experiment the ANAE positive cells showed a dose dependent decrease from the first to the fourth fortnights. This in turn shows the adverse biological effect of aflatoxin B_1 on the cells responsible for the cell-mediated immunity in particular and the immune competency of aflatoxin fed ducks in general. Similar observations were also made by Gopalakrishnan Nair (1986) in pigs. In general, the adverse affect of AFB_1 is mainly due to inhibition of protein synthesis (Edds, 1973) and Buck et al. (1976). The cells involved in the immune system as well as the haemopoietic system mainly go through sequential steps namely activation, differentiation and proliferation. Hence the inhibition of the protein synthesis at any of these steps could have adverse effect on the normal physiological ability of the cells. The participation of these immunocompetent cells in the defence mechanism of the body is well established. From this investigation the adverse effect of the AFB_1 on the immune system was demonstrated.

From the results of this experiment it is clearly evident that dietary AFB_1 caused significant reduction in the total serum protein and serum globulin. This clarifies the inhibitory action of AFB_1 on the protein synthesis. This reduction would in turn bring about the defective immunoglobulin synthesis leading to an inefficient humoral immune response. Similar observations were also reported by Rao et al. (1988) and Reddy et al. (1982). The

reduction of serum globulin concentration brings about the defective immunoglobulin synthesis. The immunoglobulin fractions in the serum of the AFB_1 fed ducks and their control were assessed. This assessment clearly brought to light the reduction in the immunoglobulin fractions mainly IgM and IgG in AFB_1 fed groups. This observation is in agreement with Giambrone *et al.* (1978). The defective protein synthesis and subsequent adverse effect on the immunoglobulin production during aflatoxicosis in ducks demonstrated the adverse biological effect on the antibody mediated immune response.

In order to assess the effect of AFB_1 on the humoral immune response to R.D. vaccine, AFB_1 fed ducks were vaccinated against Ranikhet disease and the HI titre was estimated. It was clarified that there was significant reduction of HI titre in the AFB_1 fed ducks. This showed that there is a definite interference in the process of antibody synthesis. This fact is supported by the low serum protein profile and also the lack of normal lymphoid components in the histological sections of bursa of Fabricius. In the field situations when ducks consume aflatoxin contaminated diet over a period of time in small doses that is bound to have damaging effect on the immune system and consequent immunosuppression. This can lead to breakdown of immunity after vaccination or poor response to vaccination. Thus the observations made in this study has great relevance and practical application. The cellular immunity was assessed with various

suitable immunological markers. The cutaneous reaction to DNCB and PHA-M clearly demonstrated that the cellular reaction was significantly lowered in AFB$_1$ fed ducks due to adverse effect on the cell-mediated immune response which was supported by reduction in the ANAE positive T lymphocytes in the peripheral blood of these ducks and the histological appearance of the T cell dependent lymphoid organ namely thymus which showed lympholytic changes. The reduction in the cell-mediated immunity in aflatoxicosis was also observed by Gopalakrishnan Nair (1986) in pigs and Anil Kumar (1986) in goats.

The earlier studies showed that aflatoxin impairs the function of at least more than one of the cell types of phagocytic system which was also dose related.

Richard and Thurston (1975) also documented decreased phagocytic activity of alveolar macrophages in rabbits fed aflatoxin.

Michael et al. (1973) showed dose dependent action of aflatoxin on the ability of reticulo-endothelial system to clear colloidal carbon. The dietary aflatoxin at 1.25 ug/g and 5 ug/g of feed significantly decreased the clearance of colloidal carbon from circulation by the reticulo-endothelial system of the chicks. A decreased phagocytic activity of reticulo-endothelial system and circulating monocytes was noted in aflatoxin fed birds at a dose level of 20 ppm over a period of 12 weeks (Chang and

Hamilton 1979a, 1979b).

The assessment of phagocytic response of reticulo-endothelial cells also indicated a reduced activity in the aflatoxin fed ducks. This observation is in agreement with Kadian et al. (1988). From the results of these investigations it was clearly evident that there was an adverse effect of AFB_1 on the humoral and cellular immunity which resulted in immunodeficiency.

THe histological observations on the vital organs like liver, clearly showed that in ducks liver is the primary target organ. The fatty change observed in the liver is mainly due to inhibition of protein synthesis and aberration in the lipo-protein formation. The triglycerides , hence cannot be mobilised without lipoprotein formation , and fatty infiltration resulted (Osuna and Edds, 1982). Similar observations were made by Gopalakrishnan Nair (1986), Mohiuddin (1982), Moorthy (1985) and Mohiuddin et al. (1986).

Hatch (1982) stated that the hepatic necrosis resulted when glutathione reserves were drastically depleted by conjugation with toxic intermediates and the toxic intermediates were free to bind covalently to vital cellular macromolecules. This mechanism could be the possible way in which the aflatoxin B_1 causes hepatic necrosis. The histological observation of the kidney in this experiment clearly demonstrated the degenerative pathobiological effect of AFB_1 on this organ. Similar

observations Dhanvantari et al. (1962), Wilson et al. (1967), Armbrecht (1978) and Gopalakrishnan Nair (1986).

The main observations in the lymphoid organs namely bursa of Fabricius, spleen, thymus and caecal tonsil were of lympholysis, atrophy and extensive loss of immunocompetent cells. Similar observations were also made by Giambrone et al. (1978), Reddy et al. (1982) and Rao et al. (1988) in chicken. The histological changes of bursa of Fabricius clearly demonstrated the necrobiotic action of AFB_1 on the bursa dependent immunocompetent lymphocytes. This was supported by reduction in the total protein and immunoglobulin fractions and reduced antibody formation in response to the Ranikhet disease vaccine. These observations demonstrated the defective functioning of the humoral immune system due to dietary aflatoxin. Similarly the histological observations in the spleen, thymus and caecal tonsils were of lymphoid depletion. The thymus dependent T cells showed a significant reduction as was clearly demonstrated by enumeration of these cells in the peripheral blood and reduction in cutaneous response to DCNB and PHA. This was well correlated with the histological appearance of the thymus.

From the experimental data there is clear evidence that atleast part of the immunosuppressive action of AFB_1 is attributable to its direct cytotoxic effect on the lymphoid cells. It is also possible that the non-lethal doses of AFB_1 exert an inhibitory effect on those lymphocyte functions requiring increased protein synthesis like synthesis of

antibodies and production of lymphokines. The lymphocytic action of the toxin may adversely influence the phagocytic function of the macrophages where T cell differentiation and co-operation are required for this function. Direct cytotoxic action of the toxin on the macrophages may also cause reduced rate of phagocytosis and lowered cell-mediated immune response.

The result of this investigation has clearly indicated that aflatoxin B_1 although primarily a hepatotoxin, had adverse biological effect on immune system. The pathobiological effects of these toxins on the reticulo-endothelial system were found to be an adverse effect. Apart from these effects AFB_1 also caused degenerative and necrotic changes in the liver and kidney of ducks.

Hence from the result of this investigation the need for quantitative assessment of aflatoxin in the diet of ducks was brought to light. On many occasions under the field conditions, in spite of vaccinations the outbreak of contagious diseases is a problem to the duck farmers, since they lead to heavy economic loss. This study clearly showed that aflatoxin can be a major cause of breakdown of immunity in ducks. Therefore, the regular analysis and assessment of duck feed for aflatoxin contamination becomes mandatory.

Summary

SUMMARY

An experimental study was undertaken to elucidate the immunopathological response of ducks in aflatoxicosis. Cell-mediated as well as humoral immune response were assessed using suitable immunological markers.

One hundred and ten day old, White Pekin ducklings were reared up to four weeks of age on aflatoxin free feed. Aflatoxin B_1 (AFB_1) was administered orally to the experimental ducks at the rate of 0.075 mg/kg body weight on every alternate day till the end of the experiment.

The results of various haematological parameters during the experimental period could clearly establish the pathological effect of AFB_1 on the haemopoietic system of ducks at the given dose level. There was moderate decrease in the haemoglobin concentration of the AFB_1 fed ducklings.

The immunosuppressive effect of AFB_1 was demonstrated by the dose dependent reduction in total leukocyte count (TLC), T cell dependent lymphopenia, a relative heterophilia and a total leucopenia. The reduction in ANAE positive T cells and reduction in the cutaneous response to 2,4-dinitrochlorobenzene (DNCB) and Phytohaemagglutinin-m (PHA-M) confirmed the immunotoxicity of AFB_1 on the cell-mediated immune system.

The assessment of phagocytic response of reticulo-

endothelial cells also indicated a reduced activity in the AFB_1 fed ducks.

The humoral immune system was evaluated by estimating the total serum protein (TSP), serum globulin (SGI) and various serum immunoglobulin fractions. The AFB_1 at the above dose level had significant biological effect on the humoral immune system. The breakdown of humoral immunity was further confirmed by reduction in the antibody titre in the AFB_1 fed ducks vaccinated with RDF vaccine.

The gross and histological features of AFB_1 induced toxicity consisted of enlarged , pale and friable liver with streaks of haemorrhages, congested kidney with petechial haemorrhages, atrophied spleen, thymus, bursa of Fabricius and caecal tonsils.

The histopathological changes clearly revealed that liver is the primary target organ in case of aflatoxicosis in ducks. Fatty change, paracentral and centrilobular necrosis, proliferation of bile ducts and ductules, severe disorganisation of hepatic cords and grouping of hepatocytes with acini-like formations in few areas were observed in the liver. Tubular degeneration and focal necrosis of lining epithelium of proximal convoluted tubules were seen in the kidney.

Histologically the lymphoid organs showed degenerative changes and loss of lymphoid elements. In the bursa of Fabricius apart from loss of regular follicular pattern with interstitial fibrosis, necrobiotic changes occurred in the majority of lymphoid elements. Severe loss of lymphoid elements in the spleen

was observed. The thymus revealed atrophy, necrosis, interstitial edema and intense lympholytic changes. Caecal tonsils revealed haemorrhages, degeneration and necrosis of lymphoid cells.

During the course of this investigation, it was demonstrated that AFB_1, apart from having hepatotoxic effect, had significant adverse biological effect on both humoral as well as cell-mediated immune system.

References

REFERENCES

Anil Kumar, T.V. (1986). Immunopathological response of goats in pneumonia. M.V.Sc. Thesis. Kerala Agricultural University.

Archibald, R.M., Smith, S.J. and Smith, J.D. (1962). Brazilian groundnut Toxicosis in Canadian Broiler Chickens. Can. Vet. J., 3: 322-325.

Armbrecht, B.H. (1978). Mycotoxicosis in swine. In Mycotoxic fungi, Mycotoxins and Mycotoxicosis. An encyclopedic hand book. Eds, Wyllie, T.D. and Morehouse, L.G., New York, Vl.2, pp. 227-236.

Ashturkar, R.W., Gujar, M.B. and Bhokre, A.P. (1989). Assessment of cell-mediated immunity in allografted diaphragmatic hernioplasty. Indian J Anim. Sci., 59: 763-765.

Asplin, F.D. and Carnaghan, R.B.A. (1961). The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings. Vet. Rec., 73: 1215-1219.

Balaraman, N. and Arora, S.P. (1987). Effects of aflatoxin on immunoglobulin status in blood serum of neonatal calves. Indian J. Anim. Sci. 57: 61-63.

Bodine, A.B., Fisher, S.F. and Gangjee, S. (1984). Effect of aflatoxin B and major metabolites on

phytohaemagglutinin stimulated lymphoblastogenesis of bovine lymphocytes. J. Dairy Sci., 67: 110-114.

Brandt, L.W., Clegg, R.E. and Andrews, A.C. (1951). The effect of age and degree of maturity on the serum proteins of the chicken. J. Biol. Chem. 191: 105-111.

Brown, J.M. and Abrams, L. (1965). Biochemical studies on aflatoxicosis. Onderstepoort j. Vet. Res., 32: 119-146.

Brummerstedt, E. and Basse, A. (1973). Cutaneous hypersensitivity to 2,4-dinitrochlorobenzene in calves. Nord. Vet. Med., 61: 232-236.

Buck, W.B., Osweiler, G.D. and Van Gelder, G.A. (1976). Clinical and Diagnostic veterinary toxicology. Kendall/Hunt Publishing Co. Dubuque, Iowa, pp. 153-256, 263-268.

Campbell Jr., M.L., May, J.D., Huff, W.E. and Doerr, J.A. (1983). Evaluation of Immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. Poultry Sci., 62: 2138-2144.

Carnaghan, R.B.A. (1965). Hepatic tumours in ducks fed a low level of toxic groundnut meal. Nature., 208: 308.

Carnaghan, R.B.A., Lewis, G., Patterson, D.S.P. and Allcroft, R. (1966). Biochemical and pathological aspects of groundnut poisoning in chickens. Pathol. Vet., 3: 601-615.

Cavalheiro, A.C.L. (1981). Aflatoxins and aflatoxicosis, a review. World's Poult Sci. J., 37: 34-38.

Chakravorthy, B.C., Curutchet, H.P., Coppolla, F.S., Park, C.M.,

Blaylock, W.K. and Lawrence, W. (1973). The delayed hypersensitivity reaction in cancer patients: Observations made on sensitization by DNCB. Surgery. **73**: 730-735.

Chang, C.F and Hamilton, P.B. (1979 a). Impaired phagocytosis by heterophils from chickens during aflatoxicosis. Toxicol. Appl. Pharmacol. **48**: 459-466.

Chang, C.F. and Hamilton, P.B. (1979 b). Impairment of phagocytosis in chicken monocytes during Aflatoxicosis. Poult. Sci., **58**; 562-566.

Christensen, C.M. and Nelsen, G.H. (1976). Mycotoxins and mycotoxicosis. Mod. Vet. Pract., **57**: 367-371.

Clifford, J.I. and Rees, K.R. (1967). The action of aflatoxin B_1 on the rat liver. Biochem. J., **102**: 65-75.

Dalvi, R.R. and McGowan, C. (1984). Experimental induction of chronic aflatoxicosis in chickens by purified aflatoxin B_1 and its reversal by activated charcoal, phenobarbital and reduced glutathione. Poult. Sci., **63**: 485-491.

Davis, B.J. (1964). Disc electrophoresis- II, Methods and application to human serum proteins. Am. new York Acad. Sci., **121**: 404-428.

Dhanvantari, D., Subba Rao, N., Reddy, J.S.N., Alahasingari, N. and Rao, D.S.T. (1982). Clinical observations on a natural outbreak of aflatoxicosis in large white

Yorhshire pigs. Indian J. Vet. Med., 2: 41-48.

Dhingra, V.K., Gupta, R.K.P. and Sadana, J.R. (1982). Demonstration of acid alpha naphthyl acetate esterase activity in bovine lymphocyte and monocytes or macrophages. res. Vet. Sci., 33: 26-30.

Doerr, J.A., Huff, W.E., Wabeck, C.J. Chaloupka, G.W., May, J.D. and Merkley, J.W. (1983). Effects of low chronic aflatoxicosis in broiler chickens. Poult. Sci., 62: 1971-1977.

Edds, G.T. (1973). Acute aflatoxicosis. A review. J. Am. Vet. Med. Assoc., 162: 304-309.

Edds, G.T, Nair, K.P.C & Simpson, C.F. (1973). Effect of aflatoxin B on resistance in poultry against cecal coccidiosis and Marek's disease. Am. J. Vet. Res. 34: 819-826.

Eliber, F.R. and Morton, D.L. (1970). Impaired immunologic reactivity and recurrence following cancer surgery. Cancer., 25: 362-367.

FAO/ SIDA (1968). A guide to Autopsy Techniques. Stockholm, Vol. IV. pp. 1-10.

Farshid, A.A (1992). Ochratoxicosis in quails (Coturnix coturnix Japonica) with special reference to immunopathological response. Ph.D. thesis. Kerala Agricultural University.

Galikeev, K.L., Raipov, O.R. and Manyasheva, R.A. (1968). Effect of aflatoxin on dynamics of antibody formation.

(Trans.) Byul. Eksper. Biol. Medic. (U.S.S.R)., 65: 88-90.

Giambrone, J.J., Ewert, D.L., Wyatt, R.D. and Edison, C.S. (1978). Effect of aflatoxin on the humoral and cell-mediated immune system of the chicken. Am. J. Vet. Res., 39: 305-308.

Giambrone, J.J., Diener, U.L., Panangala, V.S. and Hoerr, F. (1985). Effects of aflatoxin on young broiler chicken. Poult. Sci., 64: 1678-1680.

Giorno, R and Beverly, S. (1980). Acid alpha naphthyl acetate esterase activity in stored buffy coat smears. J.Histochem. Cytochem., 28: 181-182.

Glick, B., Sato, K and Cohenour, F. (1964). Comparison of the phagocytic activity of normal and bursectomized birds. J. Reticuloendothel. Soc. 1: 442-449.

Glick, B. (1968). Serum protein electrophoresis pattern in acrylamide gel: Patterns from normal and buraless birds. Poult. Sci., 47: 807-814.

Gopalakrishnan Nair, M. (1986). Immunopathological response of pigs in aflatoxicosis. M.V.Sc. Thesis. Kerala Agricultural University, Trichur.

Haggard, D.L., Stowe, D.H., Conner, G.H. and Johnson, D.W. (1980). Immunologic effect of experimental iodine toxicosis in young cattle. Am. J. Vet. Res., 41: 539-543.

Hamilton, P.B. (1971) A natural and extremely severe occurrence of aflatoxicosis in laying hens. Poult. Sci., 50: 1880-1882.

Hatch, R.C. (1982). Poisons causing abdominal distress or liver or kidney damage. In Veterinary Pharmacology and Therapeutics. Eds. Booth, N.M. and Mcdonald, L.E., Iowa State University Press, 5th Edn., pp. 1034-1039.

Hoerr, F.J., D'Andrea, G.H., Giambrone, J.J. and Panangala, V.S. (1986). Comparative histopathologic changes in aflatoxicosis. inDiagnosis of Mycotoxicosis. Eds. Richard, J.L. and Thurston, J.R:, Martinus Nyhoff Publishers, Dordnecht. pp. 179-189.

Janossy, G. and Greaves, M.F. (1971). Lymphocyte activation. 1. Response of T and B lymphocyte to phytomitogen. Cl.Exp. Imm., 9: 483-498.

Kadian, S.K., Monga, D.P. and Goel, M.C. (1988). Effect of aflatoxin B_1 on the delayed type hypersensitivity and phagocytic activity of reticulendothelial system in chickens. Mycopathologia., 104: 33-36.

Kelley, K.W., Greenfield, R.E., Evermann, J.F., Parish, S.M. and Perryman, I.E. (1982). Delayed type hypersensitivity,

contact sensitivity and phytohaeagglutinin skin test response of heat and cold stressed calves. Am. J. Vet. Res., 43: 775-779.

Knowles, D.M., Hoffman, T., Ferrarini, M. and Kunkel, H.G. (1978). The demonstration of acid alphanaphthyl acetate activity in human lymphocytes: Usefulness as a tcell marker. Cellular Immunol. 35: 112-123.

Lafarge, G. and Frayssinet, C. (1970). The reversibility of inhibition of RNA and DNA synthesis induced by aflatoxin in rat liver. A tentative explanation for carcinogenic metabolism. Int. J. Cancer., 6: 74-83.

Li, C.Y., and Yam, L.T. (1973). Esterases in human leukocytes. J. Histochem. Cytochem., 21: 1-12.

Malkinson, M., Shlosberg, A., Egyed, M.N., Avidar, Y., Perl, S., Weisman, Y., Nobel, T. and Bogin, E. (1982). Hepatic cirrhosis suggesting aflatoxicosis in a flock of geese. Vet. Rec., 110: 101-103.

Marchalonis, J.J. (1978). Cell cooperation in immune responses. cited by Rajan et al. (1982).

Mashaly, R.I., Salem, M.H., Mahmoud, Z.H., El-Deeb, S.A., El-Sharawi, G. and Ismail, A.A. (1986). Effect of aflatoxins on body weight gains and on protein and RNA synthesis in chickens. Indian J. Anim. Sci., 56: 698-702.

McLoughlin, M.E., Pier, A.C. and Thurston, J.R. (1984). Use of bacteria and yeasts to identify T lymphocytes in

peripheral blood and lymphoid tissues of healthy guinea pigs and guinea pigs fed aflatoxin. Am.J. Vet. Res., 45: 1136-1141.

Michael, G.Y., Thaxton, P. and Hamilton, P.B. (1973). Impairment of the Reticuloendothelial system of chickens during aflatoxicisis. Poult. Sci., 52: 1206-1207.

Miller, D.M., Stuart, B.P., Crowell, W.A., Cole, J.R., Goven, A.J. and Brown, J. (1978). Aflatoxicosis in swine: its effect on immunity and relationship to salmonellosis. Am. ass. of Vet. Lab. Diagnosticians., 21: 135-146. Cited in Vet. Bull. (1981). Abstr. 146.

Miller, D.M., Clark, J.D., Hatch, R.C. and Jain, A.V. (1984). Caprine aflatoxicosis: serum electrophoresis and pathological changes. Am. J. Vet. Res., 45: 1136-1141.

Mohiuddin, S.M., Mahendranath, D., Yadagiri, B. and Ahmed, S.R. (1981). Studies on the effect of a aflatoxin on antibody synthesis against Ranikhet disease vaccine in chicks. Indian J. Anim. Sci., 51: 77-82.

Mohiuddin, S.M. (1982). Note on the effect of aflatoxin on testes in poultry. Indian J. Anim. sci., 52: 481-482.

Mohiuddin, S.M., Vikram Reddy, M., Madava Reddy, M. and Ramakrishna, K. (1986). Studies on phagocytic activity and haematological changes in aflatoxicosis in poultry. Indian Vet. J., 63: 442-445.

Moorthy, A.S., Mahendar, M. and Ramarao, P. (1985).

Hepatopathology in experimental aflatoxicosis in chicken. Indian J. Anim. Sci., 55: 629-632.

Morgan, G.W. Jr. and Glick, B. (1972). A quantitative study of serum proteins in bursectomised and irradiated chickens. Poult. Sci., 51: 771-778.

Mueller, J., Brundere, G., Buerki, A., Keller, K.V., Wera, M.W. and Cottier, H. (1975). Non-specific acid esterase activity. A criterion for differentiation of T and B lymphocytes in mouse lymph nodes. Eur. J. Immunol., 5: 270-274.

Muller, R.D., Carlson, C.W., Scnenimick, G. and Marshfield, G.S. (1970). The response of chicks, ducklings, gooslings, pheasants and poults to graded levels of aflatoxicosis. Poult. Sci, 49: 1346-1350.

Newberne, P.M. (1973). Chronic aflatoxicosis. J. Am. Vet. Med. Assoc., 163: 1262-1267.

Nowell, P.C. (1960). Phytohaemagglutinin. An initiator of mitosis in cultures of normal human lymphocytes. Cancer. Res., 20: 426-431.

Osbaldiston, G., Sullivan, P.J. and Fox, A. (1978). Cytochemical demarkation of esterases in the peripheral blood leukocytes. Am. J. Vet Res., 39: 683-685.

Ostrowski-Meissner, H.T. (1983). Effect of contamination of diets with aflatoxins on growing ducks and chicken. Trop. Anim. Hlth. Production., 15: 161-168.

Ostrowski-Meissner, H.T. (1984). Biochemical and physiological

responses of growing chickens and ducklings to dietary aflatoxins. Comp. Biochem. Physiol., 79: 193-204.

Osuna, o. and Edds, G.T. (1982). Toxicology of aflatoxin B 1 Warfarin and Cadmium in young pigs: Clinical Chemistry and blood coagulation. Am. J. Vet. Res., 43: 1387-1394.

Padmanaban, V.D. (1989). Role of Aflatoxins as immunomodulations. J. Toxicol. Toxin. Rev., 8: 239-245.

Panangala, V.S., Giambrone, J.J., Dier, V.L. Davis, N.D., Hoerr, E.J., Miller, A., Scuacty. and Wilt, G.R. (1986). Am. J. Vet. Res., 47: 2062-2067.

Paul, P.S., Johnson, D.W., Mirocha, C.J., Soper, F.F., Thoen, C.O., Muscoplat, C.C. and Weber, A.G. (1977). In Vitro stimulation of bovine peripheral blood lymphocytes. Suppression of phytomitogen and specific antigen lymphocytic responses by aflatoxin. Am. J. Vet. Res., 38: 2033-2035.

Pier, A.C. and Heddleston, K.L. (1970). The effect of aflatoxin on immunity in Turkeys. I. Improvement of actively acquired resistance to bacterial challenge. Avian. Dis., 14: 797-809.

Pier, A.C, Heddleston, K.L., Boney, W.A. and Lukert, P.D. (1971). The effect of aflatoxin on immunity. Proc. xix Cong. Mundial de Med. Vet. Zootech. 1: 216-219.

Pier, A.C., Heddleston, K.L., Cysewski, S.J. and Patterson, J.M. (1972). Effect of aflatoxin on immunity in turkeys. II.

Rreversal of impaired resistance to bacterial infection by passive transfer of plasma. Avian. Dis. **16**: 381-387.

Pier, A.C. (1986). Immunomodulation in aflatoxicosis. In: Diagnosis of Mycotoxicosis. Eds. Richard, J.L. and Thurston, J.R., Martinus Nyhoff Publishers, Dordrecht, pp. 143-148.

Peckham, M.C. (1975). Poisons and Toxins. In: Diseases of Poultry . Eds, Hofsted, M.S., Calnek, B.W. Helmoldt, C.F., Reid, W.W. and Yadev. Jr. H.W., 6th Edn. Oxford and IBH publishing Co., India. pp. 1134-1140.

Rajan, A., Reddi, M.V. and Sreekumaran, T. (1981). Evaluation of the cell-mediated immune response in goats employing 2,4-dinitrochlorobenzene. Kerala J. Vet. Sci, **12**: 266-270.

Rajan, A., Reddi, M.V. and Sreekumaran, T. (1982). Evaluation of the cell-mediated immune response in goats by phytohaemagglutinin. Indian J. Pathol. Microbiol., **25**: 78-80.

Rao, A.N., Reddy, V.R., Rao, P.V., Sharma, B.J. and Mohiuddin, S.M. (1988). Effect of dietary aflatoxins on the development of immunity against Newcastle disease virus in chicken. Indian. J.Anim. Sci. **58**: 77-80.

Rajan, A., Reddi, M.V., Sreekumaran, T., Vijayan, N. and Valsala, K.V. (1982). Evaluation of the non-specific immunopotentiation induced by 2,4-dinitrochlorobenzene in pigs based on T lymphocyte enumeration in peripheral

blood. Kerala J. Vet. Sci., 13: 49-54.

Rajan, A., Reddi, M.V., Sreekumaran, T., Valsala, K.V. and Vijayan, N. (1982). Evaluation of the cell-mediated immune response in pigs using 2,4-dinitrochlorobenzene. Vet. Rec., 110: 173-174.

Rao, A.N., Reddy, V.R., Rao, P.V., Sharma, B.J and Mohiuddin, S.M(1988). Effect of dietary aflatoxin on the development of immunity against Newcastle disease virus in chicken. indian. J. Anim. Sci., 58: 77-80.

Reddi, M.V., Rajan, A., Sulochana, S. and Nair, M.K. (1980). Demonstration of acid alpha naphthyl acetate esterase activity in bovine leukocytes in the peripheral blood smear. Curr. Sci., 49: 954.

Reddi, M.V., Rajan, A. and Sulochana, S. (1981). Evaluation of cell-mediated immune response in cattle employing 2,4-dinitrochlorobenzene. Vet. Immunol. Immunopathol., 2: 483-489.

Reddy, M.V. and Rajan, A. (1984). An assessment of the cell-mediated immunity in cattle bearing carcinoma of the ethmoid employing 2,4-dinitrochlorobenzene and phytohaemagglutinin. Indian Vet. J., 61: 549-554.

Reddy, R.V., Sharma, R.P., Taylor, M.J. (1983). Dose and time related response of immunologic functions to aflatoxins in mice. Develop. Toxicol. Environ. Sci., 11: 431-434. Cited in Rev. Med. Vet. Mycol. (19850. 20: abstr. 1928.

Reddy, R.A., Reddy, V.R., Rao, P.V. and Yadgiri, B. (1982). Studies on the effect of dietary aflatoxin on the haematology and performance of commercial broiler chicks. Indian J. Anim. Sci., 52: 405-410.

Reddy, R.V., Taylor, M.J. and Sharma, R.P. (1987). Studies on immune function of Col. mice exposed to aflatoxin B_1. J. Toxicol., 43: 123-132.

Richard, J.L. and Thurston, J.R. (1975). Effect of aflatoxin on phagocytosis of Aspergillus fumigatus spores by rabbit alveolar macrophages. Appl. Microbiol. 30: 44-47.

Richard, J.L., Pier, A.C. Stubblefield, R.D., Shotwell, O.L., Lyan, R.L. and Cutlip, A.C (1983). Effect of feeding corn naturally contaminated with aflatoxin on feed efficiency, physiologic, immunologic and pathologic changes and on tissue residues in steers. Am. J. Vet. Res., 44: 1294-1299.

Sharma, D.K. (1988). Immunosuppressive effect of aflatoxin in Fowl pox virus vaccine. Poultry., V: 181.

Sheehan, D.C. and Hrapchak, B.B. (1980). Theory and Practice of Histotechnology. The C.V. Mosby Company, London, 2nd Ed. pp. 142-143, 184-189.

Siller, W.G. and Ostler, D.C. and. Ostler, D.C. (1961). The histopathology of an entrohepatic syndrome of turkey poults. Vet. Rec., 75: 134-138.

Sinha, R.R.P. and Aroraa, S.P. (1985). Indian J. Animal. Nutr., 1: 19-23.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods The Iowa State University Press, USA., 6th Edn., pp. 91-296.

Stevens, A.J., Saunders, C.W., Spence, J.B. and Newnham, A.G. (1960). Investigations into "disease" of turkey poults. Vet. Rec., 72: 627-628.

Stewart, R.G., Skeelee, J.K., Wyatt, R.D., Brown, J., Page, P.K., Russel, I.D. and Lukert, P.D. (1985). The effect of aflatoxin on compliment activity in broiler chickens. Poultry Sci., 64: 616-619.

Sulochana, S., Jayaprakasan, V., Pillai, R.M. and Abdulla, P.K. (1982). T and B lymphocytes in the peripheral blood of goat. Kerala J. Vet. Sci., 13: 71-78.

Tapia, M.O., Lopez, J.A. and Casaro, A.P. (1980). Aflatoxicosis in broiler fowl. Poult. Abst. 7: No. 1903.

Thaxton, J.P. and Hamilton, P.B. (1971). Immunosuppression in chicks by aflatoxicosis. Poult. Sci., 50: 1636.

Thaxton, P., Tung, H.T. and Hamilton, P.B. (1974). Immunosuppression in chickens by aflatoxin. Poult. Sci, 53: 721.

Thein, P., Hechler, H. and Mayr, A. (1981). Comparative studies on the activity of paraimmunity induced PIND-AVI, mitogen PHA-P and rhinopneumoniatis virus on the peripheral lymphocytes of the horse. Zentral Blatt Fur Veterinarmedizin Cited in Vet. Bull. (1982), 52: Abstr.

2648.

Tung, H.T., Smith, J.W., Hamilton, P.B. (1971). Aflatoxicosis and bruising in the chicken. Poult. Sci., **50**: 795.

Tung, H.T., Wyatt, R.D., Thaxton, P. and Hamilton, P.B. (1975). Concentration of serum proteins during aflatoxicosis. Toxicol. Appl. Pharm., **34**: 320-326.

Uchida, T., Suzuki, K., Esumi, M. and Shikata, T. (1988). Influence of aflatoxin B_1 intoxication on duck livers with Duck hepatitis B virus infection. Cancer Research., **48**: 1559-1565.

Valsala, K.V. (1968). Reproductive Pathology in the hen. M.Sc. Thesis. University of Kerala.

Valsala, K.V., Krishnan Nair, M. and Rajan, A. (1981). Demonstration of acid alpha naphthyl acetate esterase activity in the peripheral blood leukocytes of ducks. Kerala J. Vet. Sci., **12**: 262-265.

Valsala, K.V., Rajan,A. and Krishnan Nair, M. (1981). Evaluation of cutaneous hypersensitivity to 2,4-dinitrochlorobenzene in ducks. Kerala J. Vet. Sci., **12**: 332-336.

Vanzytveld, W.A., Kelly, D.C. and Dennis, S. M. (1970). Aflatoxicosis. The presence of aflatoxins or their metabolites in livers and skeletal muscles of chickens. Poult. Sci., **49**: 1350-1356.

Virdi, J.S., Tiwari, R.P., Saxena, M., Khanna, V., Singh, G., Saini, S.S. and Vadehra, D.V. (1989). Effects of

aflatoxin on the immune system of the chick. J. Appl. Toxicol., 9: 271-275.

Vishalakshan, M.M., Maryamma, K.I., Valsala, K.V., Sreekumaran, T. and Rajan, A. (1984). Clinicopathological features of subacute aflatoxicosis in pigs with special reference to lymphocyte population. Kerala J. vet. Sci., 15: 146-152.

Wilson, B.J., Teer, P.A., Barney, G.H. and Blood, F.R. (1967). Relationship of aflatoxin to epizootics of toxic hepatitis among animals in southern United states. Am. J. Vet. Res., 28: 1217-1230.

World Health Organisation., 1979. Environmental health criteria II. Mycotoxins. WHO Publications, Geneva, Switzerland, pp. 28-50.

Yadagiri, B. (1970). Aflatoxins toxicity in different species of animals (Cited from A.R. Reddy, M.V.Sc. Thesis, APAU. (1980).

Zuckerman,K.S. and LoBugilo, A.F. (1977). Phytohaemagglutinin skin tests in cancer patients. Cancer., 39: 2355-2361.

IMMUNOPATHOLOGICAL RESPONSE OF DUCKS IN AFLATOXICOSIS

By

P. BALAKRISHNAN

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Thrissur

1992

ABSTRACT

THe immunopathological effects of AFB_1 in ducks were studied in this investigation. Both cell-mediated as well as humoral immune system were assessed using suitable immunological markers.

White Pekin ducklings were maintained on aflatoxin free feed. AFB_1 was administered orally to the 4 week old experimental ducklings at a dose level of 0.075 mg/kg body weight on every alternate day till the end of the experiment.

There was reduction in ANAE positive T cells and reduction in the cutaneous response to 2,4-dinitrochlorobenzene (DNCB) and phytohaemagglutinin-M (PHA-M) confirming the immunotoxicity of AFB_1 on the cell-mediated immune system.

The assessment of phagocytic response of reticulo-endothelial cells also indicated a reduced activity in the AFB_1 fed ducks.

The humoral immune system was evaluated by estimating the total serum protein (TSP), serum globulin (SGI) and various serum immunoglobulin fractions. The AFB_1 at the above dose level had significant biological effect on the humoral immune system. It was further confirmed by reduction in the antibody titre in the AFB_1 fed ducks vaccinated with RDF vaccine.

The gross and histopathological features of AFB_1 induced toxicity consisted of enlarged, pale and friable liver with

haemorrhages, congested kidney with petechial haemorrhages, atrophied spleen, thymus, bursa of Fabricius and caecal tonsils.

There was marked histopathological changes in the liver, it being the primary target organ in case of toxicity induced by AFB_1. Fatty changes, paracentral and centrilobular necrosis, proliferation of bile ducts and ductules, severe disorganisation of hepatic cords and grouping of hepatocytes with acini-like formations, were seen in the liver. Tubular degeneration and focal necrosis were seen in the kidney. THe lymphoid organs like bursa of Fabricius, spleen, thymus and caecal tonsils showed degenerative changes and loss of lymphoid elements. There were loss of follicular pattern with interstitial fibrosis and necrobiotic changes in lymphoid elements in the bursa of Fabricius. The thymus revealed atrophy, necrosis, interstitial edema and intense lympholytic changes. Caecal tonsils revealed haemorrhages, degeneration of lymphoid cells and necrosis.

In this investigation the immunosuppressive effects of AFB_1 on the humoral and cell-mediated immune system of ducks were evaluated. Regular screening of feed for AFB_1 was advocated as a routine farm practice to prevent the deleterious effect of AFB_1 on the health and productivity of ducks.