IMMUNOPATHOLOGICAL RESPONSE OF KIDS IN PNEUMONIA

By

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THESIS

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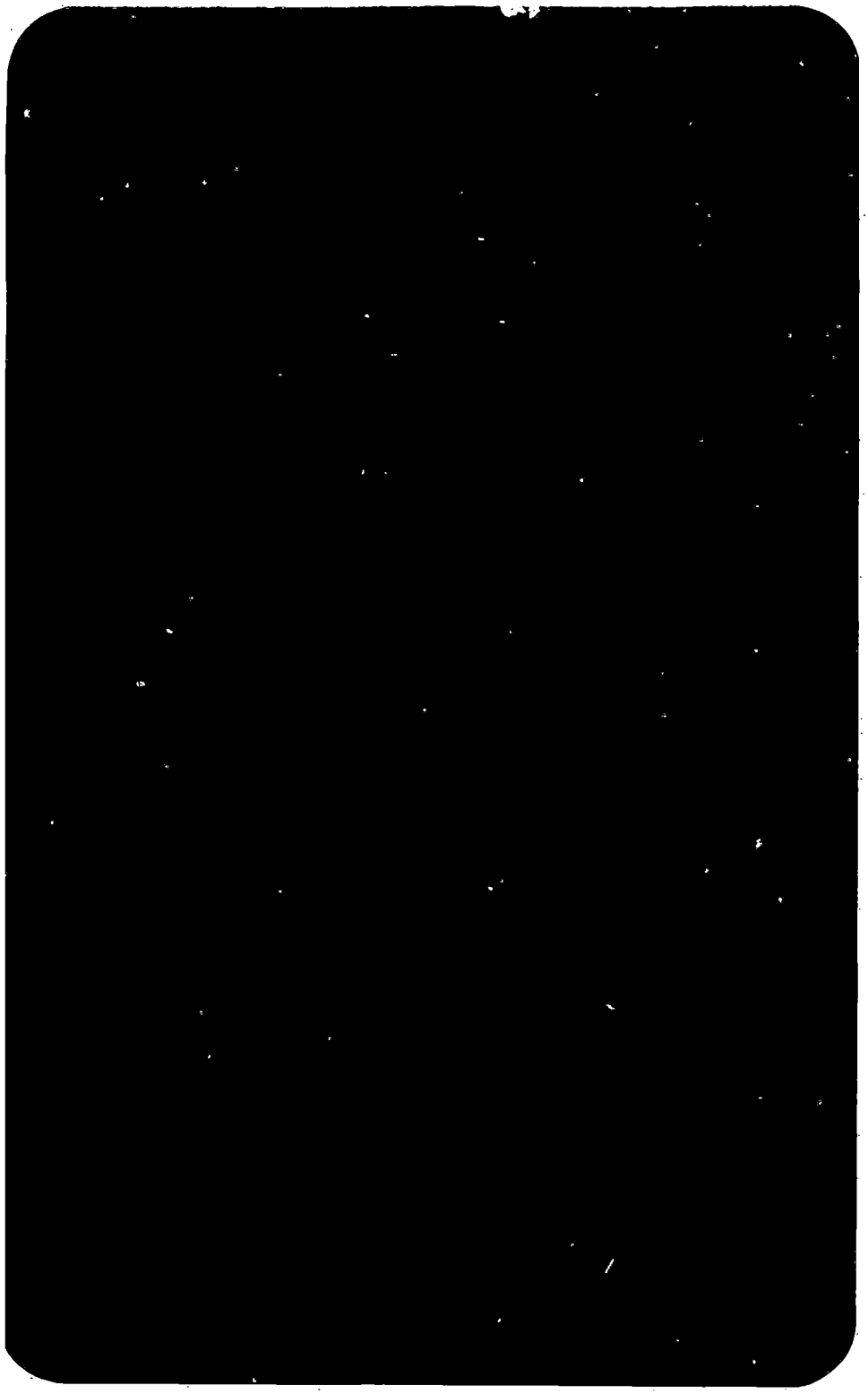

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I hereby declare that this thesis entitled IMMUNO-PATHOLOGICAL RESPONSE OF KIDS IN PNEUMONIA is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me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

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Certified that this thesis, entitled IMMUNOPATHOLOGICAL RESPONSE OF KIDS IN PNEUMONIA is a record of research work done independently by Sri. T.V. Anilkumar 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.

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T.V. Anilkumar

DEDICATED TO MY PARENTS

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CHAPTER 1

INTRODUCTION

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INTRODUCTION

The survival of an animal depends upon its ability to resist a wide variety of infections. This is achieved in the biological system by each species possessing certain innate immunological resistance which does not require previous contact with the specific infectious agent and by the acquired resistance which develops when the animal gets exposed to the infectious agents which possesses antigenic determinants. This specific active immunity is mediated through humoral and cell-mediated immune reactions. The importance of the immune mechanism in maintaining a disease free state is well illustrated by those diseases in which immune mechanism is deficient. Animals with deficiency in the humoral or cell-mediated immunity develop serious and sometimes fatal infections.

The immunological competency of the animal and resistance to infection is a relative state and depends upon a large number of complex variables. The dichotomy of the immune system is well known and these systems although have different developmental pat way, co-operate to a great extent in laying out an effective immune barrier in the host against the invading agent. As long as these two immune systems are functioning effectively the animal resists the infections contracted from the micro-environment and its survivability

is ensured. For the destruction of the invading agent and for the repair that has to follow, adequate participation of the immunocompetent cells is required. The defence and repair which are closely integrated phases of a defence reaction ensures protection of tissues against injuries induced by the invading agent.

It has now been recognised that it is the immunological competency that determines the fate of majority of the disease processes. The actiological agents are present everywhere and it is the deficiency of the immunological competency that leads to the occurrence of a disease. Once the disease is contracted, the outcome of the disease is largely determined by the immunological competency of the host. Against this background it has been now considered worthwhile to stimulate the immunological response which involves induction, suppression or amplification of the immune system as a part of an approach to treatment of diseases. Therapeutic stimulation of the immune system may be desirable in some cases with immunodeficiency while suppression may be necessitated in certain other cases. Immunomodulation may be specific or non-specific. Non-specific immunostimulation of late has been extensively used as an adjunct to therapy in many of the non-specific infections. Levamisole is one drug which has been used by many in animal and human medicines.

Although, much is not known about the immunopathological reactions taking place in the body, recently scientists are showing keen interest to understand the intricate and delicately balanced immunopathological reactions taking place in the body which plays a pivotal role in modulating the disease process within the host system.

It is an accepted fact that in most of the infectious diseases there is an immunological onslaught of the invading agent and the mechanism operates differently in different disease processes. However, its operational concept is not well understood. There has been reports of immunological breakdown in man and animals due to variety of internal and external factors. To understand the basic beneficial processes involved in these mechanisms there is need to understand the immunopathological reactions involved in the disease process.

Pneumonia is one of the common diseases encountered in kids and this causes considerable morbidity and mortality in the stock. Therefore, it was decided to take up an investigation to study the immunological background of the kids during the pneumonic process to assess the role of immune mechanism in pneumonia and to evaluate the scope of immunomodulation in the management of this disease.

Since aflatoxin is a mycotoxin which commonly contaminates the feed, taking this as a model of an immunosuppressive

agent its effect on the immune system of goat was also taken up for investigation in order to assess its role in precipitating the disease process as an immunosuppressive agent.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1. Incidence

2.1.1. Kerala State

Rajan et al. (1976) investigated into the causes of goat mortality in Kerala. Based on post-mortem observations, they reported that 16.64% of the deaths in goats were due to pneumonia. Fiftyone per cent of such cases were among kids below six months. In 1979, Manomohan et al. conducted a comprehensive study on the incidence and aetiology of kid mortality in the State. Out of 767 kids died during a period of five years (1974-1978), 170 kids (22.2%) were found to have died of pneumonia. They also found that pneumonia was the primary cause of death in kids below three months (92.96%). Nair (1982) found that mortality in goats due to pneumonia was 53.45%. Kids below six months of age were more prone to the disease (67.8%). Sreekumaran (1984) attributed pneumonia for 20.8% death in neonatal kids.

2.1.2. India

Minott (1950) reported that pneumonia was the important cause for kid mortality (39.3%). In Madhya Pradesh pneumonia was attributed as the cause of death for 13% of sheep and goats (Tiwari and Pandit, 1964). Gupta and Rajya (1969) made similar observations in Uttar Pradesh.

Bhagawan and Singh (1972) found pneumonic lesions in

170 goats out of 850 goats slaughtered in Tarai. Mittal (1976) stated that kid mortality was mostly due to pneumonia. He also observed highest mortality among kids below one month of age.

Banerjee and Gupta (1979) observed high incidence of goat mortality in Punjab, due to disease of the respiratory system. Sriramulu (1982) in Andhra Pradesh and Gupta and Verma (1984) in Bihar stated that pneumonia was the major cause of mortality among goats.

2.1.3. Other parts of the globe

Cottew and Lloyd (1965) described non-fatal respiratory disease in goats in Australia. Acute exudative pneumonia was a major cause of death in sheep and goats (Moro, 1970). Several authors reported the disease from different parts of the world, including Ranatunga, 1971 (Ceylon); Pearson et al. 1972 (Arizona); Rahman et al. 1975 (Bangladesh); Mandy et al. 1976 (Eastern Nigeria) and Ojo 1976 (River State of Nigeria).

Ojo (1977) stated that caprine pneumonia was reported as early as 1854. He reviewed, the then available literature on caprine pneumonia. His descriptions were mostly confined to pneumonia due to Mycoplasma spp.

Ramirez and Mijoan (1979) described the features of caprine pneumonia prevalent in Mexico. Caprine pneumonia was prevalent in many countries including Kenya (Masiga and

Rurangirwa, 1979); Belgium (Coussement et al. 1980); Sweden (Bolske et al. 1982) and Portugal (Concalaves, 1982).

2.2. Etiology and pathology

2.2.1. Pneumonia due to bacterial organisms

Cooper (1929); Pande (1943); Pillai (1965); Mugera and Kramer (1967); Ramachandran and Sharma (1969); Pegram (1974); Ojo (1976); Pillai et al. (1979); Sanjyal et al. (1980); Baker et al. (1980) Mordagode et al. (1981) and Vaushik and Kalra (1983) studied the etiological aspects of pneumonia. They attributed the following organisms as the etiological agents and pointed out that although many bacterial organisms were recorded as the etiological agents for caprine pneumonia, Pasteurella spp. and Streptococci spp. were the most frequent isolates. Other organisms included, Salmonella abortus ovis, Micrococcus spp., Fusiformis necrophorus, Citrobacter spp., Proteus spp., Enterobacter spp., Actinobacillus lignoresi, Escherichia coli, Moraxella bovis, Shigella spp., Haemophilus ovis, Erysipelothrix spp., Pseudomonas aeruginosa and Aruthia spp.

Pathology: Bacterial pneumonia in lambs was characterized histologically by the sequential occurrence of four distinct stages (Newsome et al. 1957). In stage I there was inflammatory oedema and proliferation of septal cells. It was followed by severe infiltration of leukocytes into the tissue (stage II). Inflammatory cells were replaced by fibrin in stage III. Gradual resolution occurred in stage IV.

Courlay and Barber (1960) observed severe oedema and congestion in pneumonic lung. Histologically inter-lobular connective tissue was prominent. In less acute cases the apical and cardiac lobes were consolidated and there was gangrenous pleuritis. Acute suppurative pneumonia, haemorrhagic pneumonia and interstitial pneumonia were also recorded in goats(Bhagavan and Singh, 1972). Sarkar and Bhattacharya (1975) observed multiple abscesses of varying size, adhesion of pleura and interlobular septa in Corynobacterium ovis infection. Manoraohan (1980) found consolidation of apical, cardiac and intermediate lobes of pneumonic lungs of goats. The cut surface was grey and coarsely granular and bronchial contents were mucopurulent.

2.2.2. Pneumonia due to viruses

Viruses were also reported as the cause for caprine pneumonia. Important viruses responsible for caprine pneumonia were Adenovirus, Respiratory syncytial virus, Reovirus type 1, 2 and 3, parainfluenza-3 virus and Retrovirus (Gupta, 1965). Chouhan and Singh (1969) and Mandy et al. (1976) isolated Psittacosis lymphogranuloma virus and stomatitis pneumoenteritis virus respectively from diseased lung.

Pathology: A few authors including Gupta and Rajya (1969) and Sims et al. (1983) described the pathology of viral pneumonia in goats. In most of the cases, apical and or cardiac lobes were only involved. Affected parts were consolidated, grey in colour and granular in consistency.

Histopathologically there was proliferation of epithelium and tendency to form syncytium. Peribronchial and perivascular cuffing of lymphocytes and accumulation of homogenous eosinophilic material in the alveolar space were also present.

2.2.3. Pneumonia due to Mycoplasma

According to Ojo (1977) Mycoplasma mycoides sub sp. Mycoides was the most common agent responsible for caprine pneumonia.

The pathological features varied in different cases of mycoplasmosis. However, unilateral lobar type of pneumonia (Longly, 1940) croupous and catarrhal pneumonia (Turner, 1959) and pleuritis and hepatisation of lobes (Filiai, 1965 and Coutew and Lloyd, 1965) were frequently encountered. The changes were similar in natural and experimental mycoplasma infections (Ojo, 1976). Observations made by Banerjee et al. (1979) from India agreed with the above findings. The lesions were bilateral and mediastinal lymph nodes were involved in some cases (Mashia and Nurangizwa, 1979) and fibrinous pleuritis was observed in others (Kasali and Ojo, 1980 and Cousserens, 1980). Narbi et al. (1981) and Bolske (1982) isolated the organisms from goats in Sweden and Goncalves (1982) in Portugal. Banasa et al.(1983) experimentally infected kids with Mycoplasma. They observed that the changes were similar in kids and adults. Gupta and Verma (1984) isolated and characterised different species of Mycoplasmal organisms from the respiratory tract of kids.

2.2.4. Pneumonia due to parasites

Ramachandran and Rajan (1967) recorded three cases of pneumonia associated with parasites in the lung of goats. According to Bon Durant (1978) goats of all ages were susceptible to Dictyocaulus filaria and this was the important agent responsible for verminous pneumonia in goats. Mirmo (1979) described six cases of verminous pneumonia in goats due to Muellerius spp. Upadhyaya et al. (1983) reported twelve cases of verminous pneumonia. The isolates included Dictyocaulus filaria and Protostrongylus rufescens.

2.3. Assessment of immunopathological response

2.3.1. 'T'-Lymphocytes in the peripheral blood

Jondal et al. (1972) demonstrated surface markers for identification of different cells in the peripheral blood. A large number of lymphocytes formed non-immune rosettes with sheep red blood cells. Such cells were thymus dependent cells. In the following year Wilson and Mitscherlichs (1973) employed the same technique and studied the distribution of T-lymphocytes and non-T lymphocytes in the peripheral blood of cattle. Moroin et al. (1979) used Holin peratia to demonstrate T-lymphocytes in blood and recommended the same for evaluating bovine lymphocytes. The number of E-rosette positive cells in the peripheral blood of Jakran goat was 9.59 ± 2.06 % (674.9 ± 185.6 cells/ml) (Wang and Chien, 1980). Donartini et al. (1983) observed an augmented T-lymphocyte response in

chronic diseases. They studied caprine arthritis-encephalitis due to retrovirus in goats.

T cell markers in blood: Antibodies, complement, bacterial products, yeasts, enzymes and haemagglutinating agents were used as 'T' cell markers in animals (Johnsson and Norein, 1983; Muller, 1984; McLonghlin, 1984 and Taymbal et al. 1984).

Acid alpha naphthyl acetate esterase activity as T cell marker

Acid alpha naphthyl acetate esterase (ANAE) activity was demonstrated in human lymphocytes by Li et al. (1973). The esterase activity was prominent in lymphocytes, especially 'T' cells and was used as a 'T' cell marker (Mueller et al. 1975). Later Osbaldiston et al. (1978) recognised that ANAE activity of lymphocytes could be used as a 'T' cell marker in different species of domestic animals. They successfully employed the technique in demonstrating the T cells in cat, dog, goat, guinea pig, hamster, pig, rabbit, rat and sheep. The esterase activity was recognised by the presence of magenta coloured granules or deposits in the cytoplasm. When the pH of the staining solution was altered, esterase positive reaction was seen in monocytes and neutrophils. Nemeth et al. (1979) evaluated ANAE positive cells of sheep suffering from lymphoma. Reddi et al. (1980) demonstrated ANAE activity in lymphocytes in the peripheral blood of cattle. They recommended the test for routine use in domestic animals. Esterase positive cells of sheep were recorded to decrease in Listeria monocytogenes

infection (Wachnik et al. 1981). Dhingra et al. (1982) observed that in T cells there was spherical or oval reddish brown granular reaction product adjacent to the cell membrane. But it was detected as diffuse red areas in the macrophages and monocytes. The number of NAE positive lymphocytes (26.84 ± 2.56) was the same as E-rosette forming cells (26.57 ± 2.05) in the peripheral blood of goats (Sulochana et al. 1982). According to Dixon and Moriarty (1983) the esterase activity was not a useful tool in evaluating ovine T cells.

2.3.2. Assessment of phagocytic activity of neutrophils

Bachner and Nathan (1967) observed that Nitroblue Tetrazolium (NBT) dye was reduced by neutrophils in vitro and reduction was enhanced during phagocytosis. In the following year, that property was used as an indicator to assess the phagocytic activity (Park et al. 1968). In man 8.5% neutrophils were NBT positive and was increased to 29-47% in bacterial illness. They pointed out that NBT could be used to differentiate pyogenic infection from others. Bacterial products like endotoxins enhanced the phagocytic property (Park and Good, 1970). The phagocytic property of neutrophils was attributed to NADH oxidase system (Anonymous, 1971).

Sara (1975) employed the NBT test to assess the function of neutrophils in the peripheral blood smear of normal and animals ailing from mastitis, and caprine pleuropneumonia. He observed that the number of NBT positive cells increased during infections. In rats the number of NBT positive cells

was found to be increased following stimulation by bacterial products (Khalifa, 1984).

2.3.3. Response to 2,4-Dinitrochlorobenzene

The hypersensitivity reaction induced by the chemical hapten, 2,4-Dinitrochlorobenzene (DNCB) was widely used as a cutaneous reaction to assess the cell-mediated immune response (CMI). It was used to assess the CMI in man (Brown et al. 1967; Dilber and Morton, 1970 and Alsabti, 1979). They used the test to evaluate CMI in patients suffering from different types of cancer.

In veterinary literature the reports on DNCB skin test were only few. Drummerstedt and Basse (1973) studied the reaction induced by DNCB in calves. They sensitised the skin for seven consecutive days and at different concentrations. Later they applied challenge dose and studied the response at 48 hours. Histologically they found perivascular cuffing of lymphocytes, macrophages and eosinophils. They recommended DNCB to assess the CMI in animals. Jennings (1979) evaluated the DNCB response of calves. He used a 2% solution of the chemical in acetone. He applied only one sensitising dose. Challenge dose was given after 16 days. Reddi et al. (1980) recommended the test for routine use for evaluating CMI in cattle. Rajan et al. (1981) standardised the technique for evaluating CMI in goats using DNCB. They used 0.4 ml of a 2% solution of DNCB in acetone for sensitisation. A challenge

dose of 0.25 ml was applied after 14 days. Skin thickness and histological appearance at 24 and 48 hours were taken as the criteria for evaluating CMI. By adopting the same procedure Paliwal et al. (1985) successfully evaluated CMI in goats in Johne's disease.

2.3.4. Response to phytohaemagglutinin

Phytohaemagglutinin (PHA), a non-specific mitogen was used as a haemagglutinating agent as early as 1949. Nowell (1960) found that PHA possessed remarkable ability to initiate mitosis of lymphocytes in cultures. The transformation of lymphocytes into blast cells was used as a marker for the assessment of CMI in man (Blease et al. 1973 and Zuckerman et al. 1977). They used the test to assess the CMI of normal individuals and cancer patients.

PHA was used to assess the immunological reactivity of normal and diseased animals. Both in vitro and in vivo tests with PHA were designed and employed in domestic animals to assess CMI. Powell (1980) described the in vitro use of PHA for evaluating the activity of chicken lymphocytes. Haggard et al. (1980) evaluated the CMI response of calves in experimental iodine toxicosis using PHA. Thein et al. (1981) employed both in vitro and in vivo PHA tests to assess the CMI in horses. They reported that response to PHA was an indicator of delayed type hypersensitivity reaction.

Rajan et al. (1982) designed and standardised the PHA

test in goats. They injected 10 μg of PHA intradermally. The degree of induration of the skin and the histological appearance after 24, 48 and 72 hours were the criteria for assessing the CMI activity. They recommended the use of PHA test as a convenient skin test for assaying the CMI response in goats.

PHA was used to assess the immunocompetence in horses (Khalil et al. 1982). It was also employed by Kelley (1982) to evaluate the effect of heat and cold stress on the immune system.

2.3.5. Immunoglobulins of goat

Feinstein (1969) demonstrated different classes of immunoglobulins (Ig) in goats. He found that goat Ig is similar to those in other species, with respect to structure and complement activity. Castro (1977) attributed antibody activity to all classes of Ig. There was significant correlation between Ig content in serum and humoral immune status. Further he estimated the Ig content in the serum as 31 mg/ml and 23.8 mg/ml in adult goats and kids. Variation in Ig concentration occurred in different breeds of goats (Nair and Balakrishnan, 1983).

2.3.6. Immunoglobulin in diseases of the Respiratory System

Williams et al. (1975) conducted quantitative studies using zinc sulphate turbidity test and radial immunodiffusion on bovine immunoglobulin. They observed that the mean

gammaglobulin content in the serum of normal calves was 25.27 ± 1.98 mg/ml while that of the pneumonic calves was only 16.72 ± 1.69 mg/ml. The survivability of calves with higher gammaglobulin content was more (Raja and Balakrishnan, 1979). Davidson et al. (1981) reported that the Ig concentration in normal calves (2698 mg/100 ml) was more than those suffering from pneumonia (1267 mg/100 ml). Olson (1982) employed gluteraldehyde coagulation test and evaluated the relation between serum immunoglobulin level and incidence of pneumonia and enteritis. The mortality rate due to these diseases was more in hypogammaglobulinaemic calves (31.3 %). Similar observations were made in kids (Nair and Balakrishnan, 1983). In kids with low serum Ig concentration the mortality ranged from 22.2-50%. Those kids with Ig concentration above 21 mg/ml had a greater chance of survival. Corbeil et al. (1984) investigated Ig content in serum and nasal secretion of calves at the onset of pneumonia. They found that pneumonia occurred at or just after the time when IgG_1, IgG_2 and IgA concentrations in serum and combined Ig concentration in serum and nasal secretion were lowest.

2.3.7. Determination of gammaglobulin in the serum

The conventional methods to determine gammaglobulin in the serum were Ammonium sulphate test, Specific method, Sodium sulphate test, Zinc sulphate turbidity test and Refractometer method (Waterson, 1967 and McBoath et al. 1971). They found high correlation between the values obtained by zinc-

sulphate turbidity test (ZST) and other methods to determine gammaglobulin in serum. There was direct relationship between the values obtained by ZST and paper electrophoresis in determining the concentration of gammaglobulin (Volvenko, 1975). Barbar (1976) compared the concentration of gammaglobulin obtained by various methods. He preferred ZST over other tests because of the accuracy, reliability and simplicity in procedure. Nandakumaran (1981) observed that ZST was useful in evaluating gammaglobulin in the serum of neonatal kids.

2.4. Immunopathological response of kids in aflatoxicosis

2.4.1. Introduction

Aflatoxins are a group of fungal metabolites produced by Aspergillus flavus, Aspergillus parasiticus and related fungi. Eventhough several fractions of the toxin have been reported B_1, B_2, G_1 and G_2 were recognised as the important ones.

Asplin and Carnaghan (1961) and Wogan (1966) reported that the susceptibility to the toxin varied with respect to species. Culvenor (1974) recorded the maximum safe level of toxin in the diet for different species of animals. He did not give the maximum safe level for goats. For sheep the level was 1.75 ppm in feed.

Animal feeds were often contaminated with aflatoxins (Izquierdocorser, 1976 and Brydon et al., 1980). In Kerala

State (India), Maryamma et al. (1982) conducted an analytical study on livestock feeds. They found that more than 50% of the animal feed samples contained sufficient concentration of aflatoxin to cause toxicity. They also observed that the prevalence of disease was more among those animals fed contaminated feeds.

2.4.2. Aflatoxicosis in young animals

Milk was the major source of aflatoxins for young animals. Aflatoxins appeared in the milk of cows fed aflatoxin contaminated feed (Masri et al. 1967). Toxicity was not reduced by spray drying and pasteurisation of milk (Allcroft et al. 1967). Adamastouanu et al. (1974) found that aflatoxins can reach the foetus through placental circulation.

2.4.3. Aflatoxicosis in goats

Maryamma et al. (1975) studied the clinicopathological features of caprine aflatoxicosis. They recorded anaemia, lethargy, weakness, delayed blood clotting time, icterus, weight loss, increase in serum enzyme activity and death. There was reduction in RBC count and decrease in haemoglobin concentration. Samrajeewa (1975) recorded spontaneous outbreaks of aflatoxicosis in goats in Sri Lanka. Out of 500 animals, 194 died after consuming contaminated feed. Toxicity was found to be increased by pretreatment with phenylbutazone and two beta flavones with or without methane sodium thiosulphate.

However phenylbarbital with glycerine and methane sodium thiosulphate had beneficial effects in reducing the toxicity (Hatch et al. 1982a). The same authors made an attempt to treat toxicity with antibacterials and steroids. Ampicillin, sulpha drugs and diethyl carbonate were not effective. However, goats responded favourably to treatment with activated charcoal, anabolic steroids and oxytetracycline (Hatch et al. 1982b and Hatch et al. 1982c). Clark et al. (1984) evaluated the clinicopathological features of caprine aflatoxicosis. Decreased food consumption, slight to moderate weight loss, mucopurulent nasal discharge, dyspnoea, coughing, lethargy, icterus, diarrhoea and subnormal body temperature were recorded before the death. There was increase in RBC count, PCV, haemoglobin concentration, serum bilirubin concentration and serum activity of aspartate aminotransferase, isocitric dehydrogenase and ornithine carbamyl transferase. Goats did not show consistent dose related changes, especially with respect to total protein and serum activities of alanine aminotransferase and alkaline phosphatase.

Miller (1984) recorded the pathological changes in aflatoxicosis in goats. In the blood there was an increase in gammaglobulin level. Eventhough there was reduction in betaglobulin levels, the changes were not dose related. At necropsy the carcase was found icteric. Other lesions were ascites, pallor of liver, petechial haemorrhages of mucus

membranes and nasal discharge. Microscopic changes included, bile duct proliferation, hepatocytic karyomegaly, hepatocellular degeneration, pneumonia, rhinitis and proximal renal tubular nephrosis. The pathological changes varied from animal to animal.

2.4.4. Aflatoxins and immunity

2.4.4.1. Effect on resistance of the body to diseases

The effect of aflatoxin on the resistance of the body varied. Smith et al. (1969) reported that it did not affect resistance in poultry. But Pier and Meddleston (1970) and Hamilton and Harris (1971) reported that aflatoxin reduced the resistance. Many reports pointed out that in aflatoxicosis, the resistance of poultry to diseases was poor and mortality rate was increased (Broun and Abrams, 1973, and Boonchuvitat and Hamilton, 1975). Miller et al. (1978) reported that pigs on aflatoxin contaminated diet were highly susceptible to salmonellosis.

2.4.4.2. Effect on antibody formation

Galikeev et al. (1968) studied the effect of aflatoxin on antibody formation. They found that in mice the agglutination titre in typhoid infection was depressed. There was reduction in the number of plasma cells in the lymphnode and spleen. Antibody titres were lower in birds in aflatoxicosis (Pier and Meddleston, 1970; Pier et al. 1972; Adinarayanaiah et al. 1973). However, in certain cases there was an increase

in gammaglobulin content in the serum (Richard et al. 1973 and Thurston, 1974). According to Pier et al. (1974) there was no change in antibody titre.

2.4.4.3. Effect on cell-mediated immune response

Aflatoxin B_1 inhibited the activity of human blood lymphocytes. The stimulation of lymphocytes by phytohaemoagglutinin was reduced (Savel et al. 1970). Pier et al. (1972) noticed involution of the thymus in turkey. They postulated that aflatoxins inhibited the activity of T lymphocytes. Aflatoxin reduced the production of migration inhibition factor in vitro and reduced the intensity of cutaneous hypersensitivity reactions in vivo (Pier et al. 1976). Aflatoxins significantly reduced the phagocytic property of macrophages (Miller et al. 1978). Bodine et al. (1984) made similar observations and stated that aflatoxins and their metabolites like aflatoxicol and aflatoxin B_2 inhibited the function of T lymphocytes.

2.5. Immunomodulatory effect of levamisole

Levamisole, an anthelmintic drug, also was demonstrated to have immunomodulatory properties when half the anthelmintic dose was administered.

Anthelmintic property of levamisole was reported as early as 1966 (Thienpont et al. 1966), but its immunomodulatory property was detected only in 1971 (Renoux and Renoux, 1971). Hadden et al. (1975) found that levamisole increased the

intracellular cyclic guanosine monophosphate which controlled the motility and secretions. They further found that levamisole enhanced the mitogen induced T-cell proliferation.

Avery (1975) postulated that levamisole behaved as an anti anergic drug by stimulating the maturation of blood borne host defence cells. According to Goldstein, 1978, levamisole mimicked the activity of thymic hormone thymopoietin and was brought about through receptor sites on the effector leukocytes.

Levamisole stimulated antibody production, enhanced graft-vs-host reaction, increased phagocytosis by macrophages and inhibited tumour growth. Levamisole was more effective in restoring effector function of T lymphocytes and macrophages by stimulation of precursor T-cells to differentiate into mature cells (Matsuura et al. 1979).

Jan (1981) studied the in vivo effect of levamisole in calves. He injected the drug (I/M.) at the rate of 7 mg/kg body weight. He found an enhanced non-specific immune response at 24 hours, which persisted for a period of not less than five days.

Olah et al. (1981) studied the effect of levamisole in chicken. At a dose rate of 0.2 mg/kg body weight, there was enhanced cutaneous response to PPD. In sections, heterophils and basophils were seen increased in number, eventhough there was no change in the number of mononuclear phagocytes.

Levamisole also increased the immune response to specific antigen like Brucella abortus (Kaneene et al. 1981).

Jayappa and Loken (1982) observed an enhanced activity of bovine polymorphonuclear leukocytes following levamisole administration.

Immunotherapy using levamisole

Tripodi et al. (1973) used levamisole for immunotherapy for cancer in man. It was found to restore cell-mediated immunity in man. Only a few workers have attempted immunomodulation in animals using levamisole. Onodera et al. (1980) treated cases of mastitis in bovines. They gave the drug at the rate of 7.5 mg/kg body weight with antibiotic as intramammary infusion. Using California mastitis reagent they evaluated the response of cattle. Many animals recovered and the authors stated that the beneficial effect was due to immunomodulation by levamisole.

Confer and Aldinger (1981) observed that levamisole enhanced the response to PHA in chicken affected with Marek's disease. But levamisole with antibiotics had no beneficial effect in the treatment of bronchopneumonia in calves (Pakhomov, 1982) and bovine herpes virus infection (Babiuk and Misra, 1982).

Ishikawa et al. (1982) and Anderson (1984) recommended levamisole for the treatment of mastitis in cattle. The drug

was more useful for protecting the animals from infections. However, it had little effect on treatment. He observed that levamisole was useful for controlling nematodis when used at the stage of drying off and parturition.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental design

The experiments were conducted on cross-bred male kids, obtained from the All India Co-ordinated Research Project on Goats, Mannuthy and Koneri Goat Farm, Tellichery. Kids of two to four months of age were used for the study. Animals were managed according to the standard recommendations, in four groups. Group I consisted of six healthy kids, Group II and III consisted of six pneumonic kids each. Pneumonic animals were randomly selected from the herd based on the clinical symptoms. The disease was confirmed by physical examination. Kids suffering from acute pneumonia were only included for the study. Group IV consisted of six kids. They were given crude aflatoxin, prepared in mice culture, at the daily dose rate of 0.25 mg/kg body weight. The toxin was blended with jaggery and administered orally to each kid. Blood samples were collected from the jugular vein once in three days for twenty four days.

3.2. Total and differential leukocyte count

Heparinised blood was used for the determination of total leukocyte count. Blood smears were prepared immediately after collection. Total leukocyte counts and differential leukocyte counts were determined according to the method

described by Schalm (1965). Absolute counts of lymphocytes and neutrophils were calculated from the values obtained.

3.3. Enumeration of acid alpha naphthyl acetate esterase positive cells in the peripheral blood

Number of acid alpha naphthyl acetate esterase (ANAE) positive cells in the peripheral blood was determined.

Blood smears were prepared from the peripheral blood. The smears were fixed immediately after preparation and before drying. The fixative contained six parts of acetone and four parts of 0.038 M sodium citrate (pH 5.4). Smears were immersed in the fixative for thirty seconds, rinsed in distilled water and air dried (Giorno and Beverly, 1980). Smears were labelled and stored at room temperature.

A reaction mixture was prepared for staining the smears. In 40 ml of 0.067 M phosphate buffer (pH 5.0) 2.4 ml of hexazotized pararosaniline and 10 mg of alpha naphthyl acetate (Loba) dissolved in 0.4 ml acetone were added and the final pH of the reaction mixture was adjusted to 5.0 with 2 N sodium hydroxide.

The hexazotized pararosaniline was prepared by mixing equal volumes of two solutions. (1) Freshly prepared 4% sodium nitrate 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. The hexazotized pararosaniline which formed was shaken and then allowed to

stand for one minute before adding it to the reaction mixture (Knowles et al. 1978).

The slides were incubated in the reaction mixture for eighteen to twenty-one hours at room temperature and then rinsed thoroughly with distilled water. The slides were counter stained with one per cent toludine blue for forty-five to sixty minutes. The slides were then washed with distilled water, dehydrated in ascending grades of ethylalcohol, cleared in xylol and mounted in DPX. The slides were observed 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 were counted and recorded.

3.4. Phagocytic activity of Neutrophils

Nitro-blue tetrazolium (NBT) dye reduction test was performed in vitro to assess the phagocytic activity of neutrophils. The procedure described by Peacock and Toner (1980) was followed.

Heparinised blood was used for the test and was performed immediately after collection. The dye solution was prepared by dissolving six milligrams of nitroblue tetrazolium (Sisco Research Laboratories, Bombay) in 2.5 millilitre of normal saline. Equal quantity of dye solution and blood were mixed and incubated at 37°C for thirty minutes. Smears were prepared in duplicate after incubation. Smears were then stained

with Wright's stain. Those neutrophils with brownish granules in the cytoplasm were considered as positive cells. The number of positive cells in every two hundred neutrophils were counted. Absolute number of NBT positive cells was calculated and recorded.

3.5. Response to phytohaemagglutinin

Cell-mediated immune response (CMI) of normal kids, kids ailing from acute pneumonia and kids fed aflatoxin for seven days was evaluated using phytohaemagglutinin-M (PHA). Each group contained six animals. Response to PHA was determined by employing the method described by Rajan et al. (1982).

Ten microgrammes of PHA-M (Difco Laboratories, USA) were dissolved in 0.1 ml of distilled water. The animals were prepared by closely clipping the hair on the neck. Two areas were marked for injecting the PHA. The thickness of the skin in the area was determined using a vernier callipers. Ten microgrammes of PHA was injected I/D at each site. The skin thickness was determined at 24 and 48 hours after inoculation. Biopsy specimens were collected from the site after 24 and 48 hours. The specimens were fixed in 10% formal saline. Paraffin sections were cut at 5-6 microns thickness and stained with Harri's Haematoxylin-eosin. The histological changes were studied.

3.6. Response to 2,4-Dinitrochlorobenzene

Cell-mediated immune response was also studied using the

hapten 2,4-Dinitrochlorobenzene (DNCB). The test was performed in six healthy kids and six kids having clinical symptoms of pneumonia. The method recommended by Rajan et al. (1981) was followed.

The skin test was done on the sides of the neck. Two areas were marked on either side of the neck and were closely clipped. A metallic ring of 3.5 cm diameter was used to mark the areas. A two per cent solution of 1-chloro-2,4-dinitrobenzene (Loba-Chemievienfischamend) was prepared in acetone. For primary sensitisation 0.4 ml of the solution was applied over one side. Solution was applied slowly and drop by drop and was made to evaporate quickly by blowing. The challenge dose was applied after 14 days. Before application of the challenge dose, the thickness of the skin over the two sites were measured using a vernier calliper. A challenge dose of 0.25 ml of the solution was applied at each site. After 24 and 48 hours the thickness of the skin was again measured. The diameter of the reaction zones was also measured using a scale. From selected animals, biopsy specimens were collected from the reaction site. The specimens were fixed in formal saline. Paraffin sections were cut at 5-6 microns thickness and stained with Harri's haematoxylin and eosin. The histological changes were recorded.

3.7. Determination of gammaglobulin in the serum

Zinc sulphate turbidity test (McEwan, et al. 1970) was

followed with suitable modifications. The modifications were similar to those followed by Nandakumar (1981) for evaluating the gammaglobulin in the serum of kids.

A working solution of zinc sulphate ($ZnSO_4$ 7 H_2O, Sarabhai Chemicals) was prepared by diluting 4.1 ml of 5% $ZnSO_4$ 7 H_2O to one litre of freshly boiled and cooled double distilled water to give a final concentration of 205 milligrams per litre.

Tubes were arranged in rows in the rack. For each serum sample three tubes were kept. Six millilitres of zinc sulphate solution was poured into the first two tubes and a similar amount of distilled water in the third row, which was taken as control. Diluted serum (one in six in distilled water) was poured into corresponding tubes in the first two rows, so that 0.1 ml of diluted serum was added to six ml of zinc sulphate solution. Tubes were gently shaken and allowed to stand at the room temperature for one hour. The turbidity developed in each tube was then read in a spectrophotometer (Baush and Lamb) at a wave length of 490 *mμ*-metre. The null adjustment was made against zinc sulphate solution. The reading of the control was substracted from the average readings of the test solutions to arrive at the optical density of the individual sample. The optical density was then converted into immunoglobulin content (mg/ml of serum) using a standard curve. An estimation of the total protein in the serum was also made (Inchiosa, 1964).

Preparation of the standard curve

Commercial bovine gammaglobulin (Sigma Chemicals, USA) was dissolved in pooled pre-colostral kid serum to get final concentrations ranging from 2 mg/ml to 80 mg/ml. They were diluted with distilled water at the ratio of one in six. The optical densities were determined in spectrophotometer. The optical density was plotted in a graph paper against the concentration of gammaglobulin and a standard curve was prepared (Fig.1).

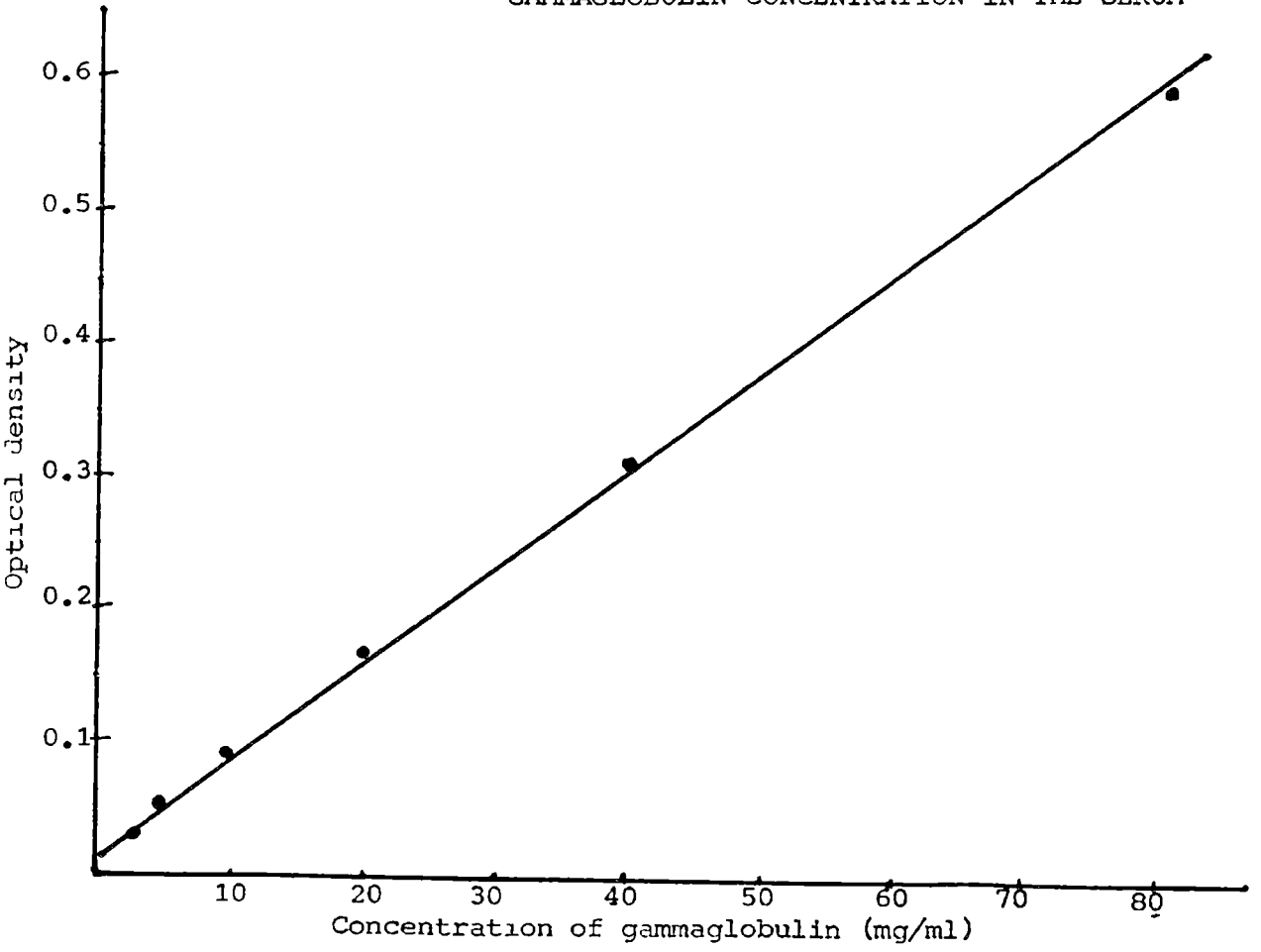
3.8. Immunological response of kids to immunomodulators

Immunomodulatory effect of levamisole was studied. A single injection of Levamisole (Helmonil, Alvel, Madras) was given sub-cutaneously at the rate of 3.75 mg/kg body weight. The injection was given to six healthy kids, six pneumonic kids (early acute stage) and six kids fed aflatoxin for ten days. Total leukocyte count, differential leukocyte count, number of NAE positive cells in the peripheral blood, number of NBT positive cells in the peripheral blood and gammaglobulin concentration in the serum were evaluated after 48 hours, employing the methods already described. The evaluations were repeated after five days.

3.9. Production and quantitation of Aflatoxin

A toxigenic strain of Aspergillus parasiticus was obtained from 'The Central Food Technology Research Institute', Mysore.

Fig.1. STANDARD CURVE FOR THE DETERMINATION OF GAMMAGLOBULIN CONCENTRATION IN THE SERUM



The fungus was grown in rice. Crude toxin was prepared according to the method described by Shotwell et al. (1966). Cultures containing the toxin was pooled together and thoroughly mixed. It was then powdered. Representative samples were taken from the powdered rice culture and the toxin content in the sample was assayed following the method of Jones and Goldblatt (1969).

Adequate quantity of toxin to feed six kids at a daily dose rate of 0.25 milligram per kilogram body weight was prepared.

3.10. Post-mortem examination

Post-mortem examinations was conducted when the animals died. Two pneumonic kids and all the six aflatoxin fed kids died. Gross and histological changes were recorded. For histopathological studies Carri's haematoxylin and eosin was used (Bancroft and Cook, 1984).

3.11. Statistical analysis

Statistical analyses were done wherever required according to the methods described by Steel and Torrie (1960).

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

The immunopathological response of kids spontaneously suffering from pneumonia and kids experimentally dosed with aflatoxin was studied. Besides this the response of the kids ailing from pneumonia and which were administered levamisole was also studied. For comparison, age matched control goats were used.

4.1. Immunopathological response of pneumonic kids

4.1.1. Pathology

Two kids out of twelve died during the course of the experiment. One kid died after twenty-one days. The other died after eighteen days in spite of the treatment with levamisole. Gross and histological lesions were similar in both the kids.

Gross lesions included fibrinous pleuritis, consolidation of apical and intermediate lobes of the right lung and pericarditis. Congestion of the liver and catarrhal enteritis were present.

Gram positive coccal organisms and organisms with bipolar staining characters were recovered from the diseased lung.

Histological changes were characterised by congestion, emphysema and presence of inflammatory exudate consisting of

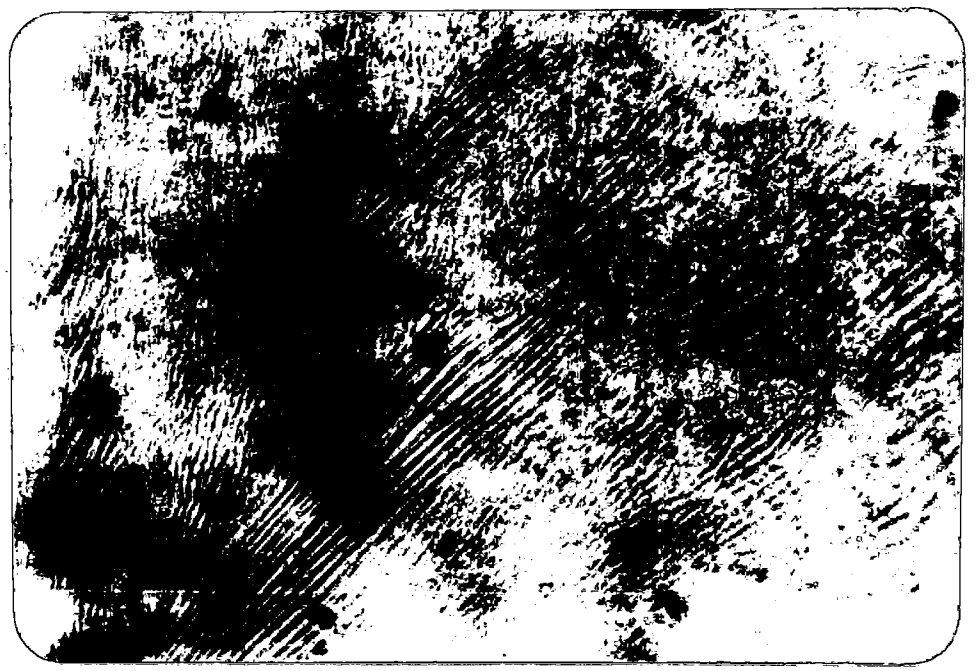
neutrophils, plasma cells and occasional macrophages in the alveoli and bronchi. Degeneration of bronchial epithelium and an exudate rich in neutrophils in the bronchial lumen were also observed (Fig.2).

4.1.2. Total leukocyte count

The leukocyte count of individual animal is detailed in appendix I. The peripheral blood leukocyte counts at different periods are shown in figure 4. The peripheral blood of control kids contained a mean number of 6615.74 ± 869.17 leukocytes per microlitre. Pneumonic kids had leukocytosis and the count was 13202.78 ± 1892.11 cells/μl. Pneumonic kids treated with levamisole also had leukocytosis (12795.93 ± 2759.36 cells/μl). There was no significant difference in the leukocyte count between the two groups of pneumonic kids irrespective of the treatment with levamisole. However, the values were significantly higher than that of the controls.

4.1.3. Number of lymphocytes and acid alpha naphthyl acetate esterase positive cells in the peripheral blood

Number of lymphocytes and alpha naphthyl acetate esterase (ANAE) positive cells in the peripheral blood was determined. The absolute number of these cells are given in appendix II and III. A few monocytes and neutrophils were also ANAE positive, but could be differentiated from the lymphocyte by diffuse reaction in the cytoplasm of the former. ANAE positive lymphocytes had one or two magenta coloured granules in



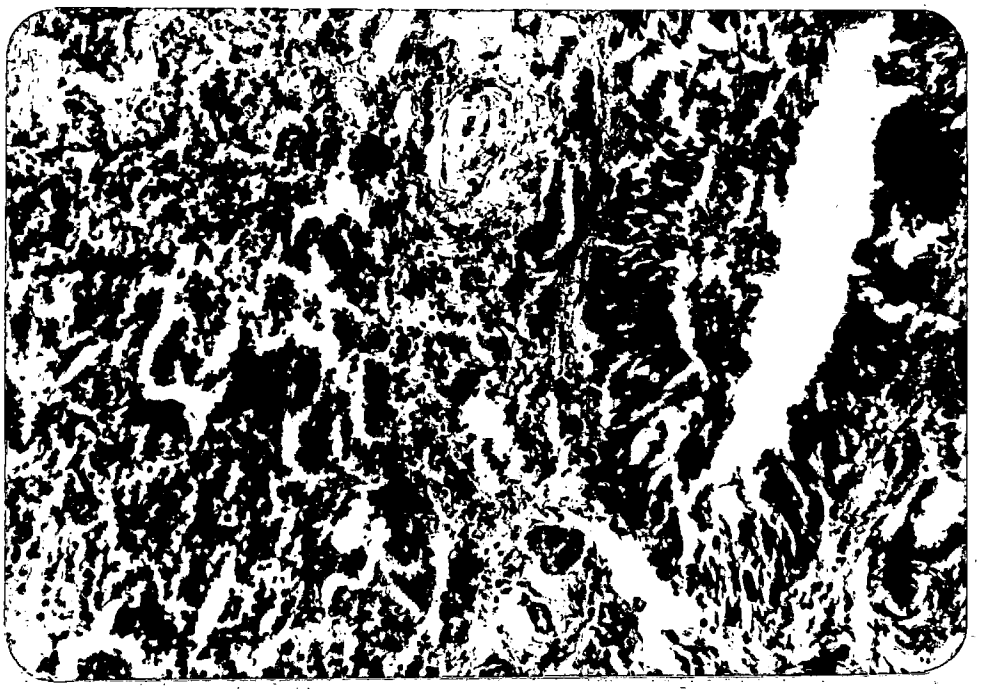
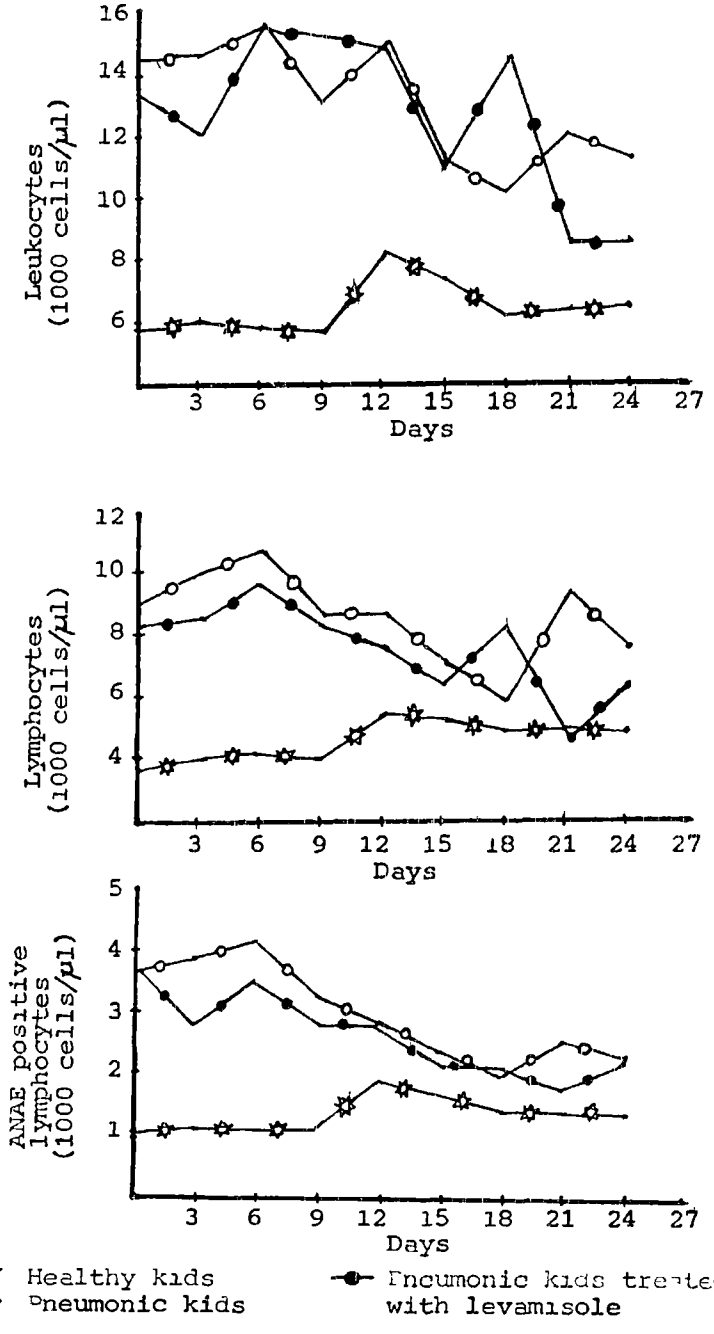


Fig.4. Leukocytic response of pneumonic kids-1



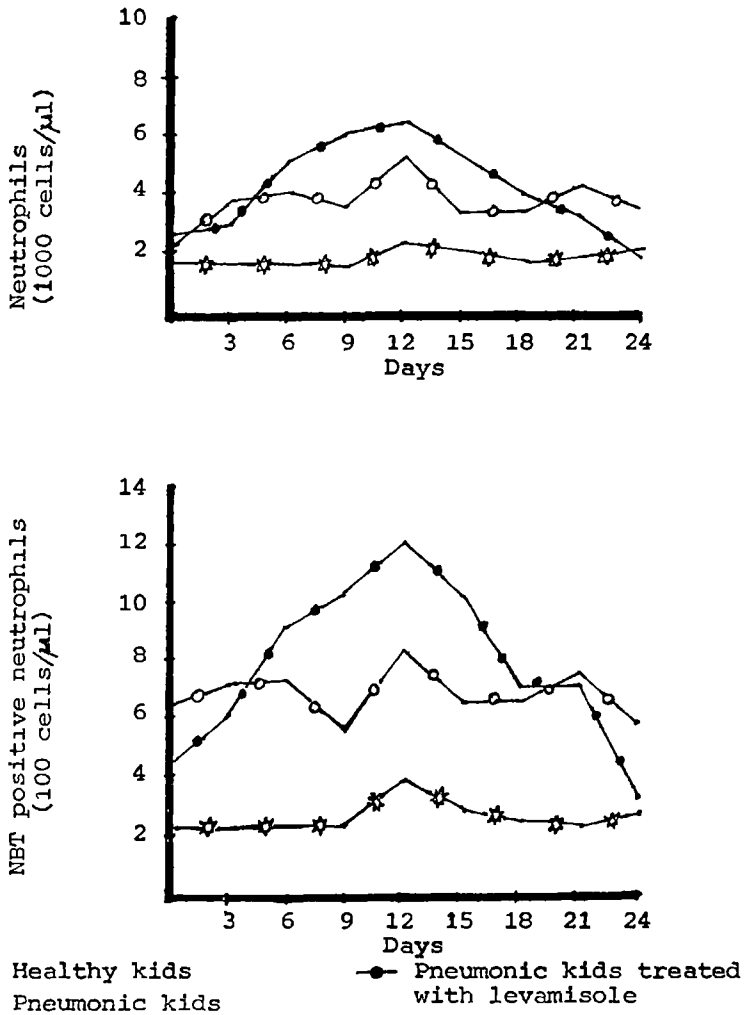
the cytoplasm (Fig.3). In the pneumonic kids, 3083.71 ± 743.17 cells/µl were ANAE positive out of 8664.66 ± 1517.8 lymphocytes/µl. Kids which were treated with levamisole had 2676.54 ± 685.61 cells/µl of ANAE positive lymphocytes out of 7659.81 ± 1486.07 lymphocytes/µl. Eventhough these values were not significantly different from each other, both of them were significantly higher than those of healthy kids. For healthy kids only 1422.30 ± 271.99 cells were ANAE positive out of 4666.69 ± 662.61 lymphocytes per microlitre of blood. The variation in the number of lymphocytes and ANAE positive cells in different periods are shown in figure 4.

4.1.4. Assessment of Neutrophil function

Unstimulated nitro-blue tetrazolium (NBT) test (in vitro) was performed in the peripheral blood to assess the neutrophil activity. The number of neutrophils and NBT positive cells was determined and are detailed in appendix IV and V.

Out of 1977.27 ± 213.31 neutrophils, only 274.33 ± 55.1[illegible] cells were NBT positive in one microlitre of the peripheral blood of healthy kids. However, in pneumonic kids 606.32 ± 84.18 NBT positive neutrophils were present out of 3037.53 ± 865.22 neutrophils in one microlitre of the peripheral blood. In kids treated with levamisole, 792.91 ± 306.68 neutrophils were NBT positive out of 4290.78 ± 1640.25 neutrophils. Irrespective of the treatment with levamisole, pneumonic kids had a higher number of NBT positive cells when compared to the

Fig. 5. Leukocytic response of pneumonic kids-2



healthy kids. The variation of the values in each period are shown in figure 5.

4.1.5. Cutaneous response to 2,4 Dinitrochlorobenzene (DNCB)

Cutaneous response to DNCB was studied in six normal kids and six pneumonic kids. Circumscribed oedematous reaction zone was observed after 24 hours after the application of DNCB. The reaction persisted even after 48 hours. The diameter and degree of induration at the reaction zone are detailed in table 1 and 2.

The diameter of the reaction zone at the end of twenty-four and fourty-eight hours was 4.916 ± 0.303 cm and 4.625 ± 0.372 cm for the normal kids. The corresponding values for the diseased kids were 4.896 ± 0.477 centimeters and 4.500 ± 0.385 centimeters. The increase in skin thickness after twenty-four hours for normal and diseased kids were 3.5 ± 0.9487 mm and 6.417 ± 0.6646 mm respectively. However, after forty-eight hours, the change was only 1.833 ± 0.6031 mm and 4.7917 ± 0.9542 mm respectively.

Histology

Moderate to severe oedema was observed in the dermis. Engorgement of the blood vessels and infiltration of inflammatory cells were present. The chief inflammatory cells involved were lymphocytes and macrophages and were seen in the upper part of the dermis and epidermis, particularly around

Table 1. DNCB Hypersensitivity reaction: Diametry of the reaction zone (Diameter in centimetre)

Animal No.	Normal animals						Animal No.	Pneumonic animals					
	24 hours			48 hours				24 hours			48 hours		
	Site I	Site II	Average	Site I	Site II	Average		Site I	Site II	Average	Site I	Site II	Average
514	4.75	5.00	4.875	4.50	4.00	4.25	406*	4.25	4.25	4.250	-	4.50	4.500
99*	5.25	5.00	5.125	-	4.50	4.50	427	4.75	4.75	4.750	4.00	4.50	4.250
520*	5.25	5.00	5.125	4.75	-	4.75	128	5.00	4.50	4.750	4.75	4.00	4.375
527	5.00	5.50	5.250	5.00	5.50	5.25	424*	4.75	4.75	4.750	4.25	-	4.250
162	4.25	4.75	4.500	4.50	5.00	4.75	28	5.50	5.00	5.250	5.25	4.75	5.000
513	4.25	5.00	4.625	4.25	4.25	4.25	111	6.00	5.25	5.625	5.25	4.50	4.625
		** SE=	4.916± 0.303		**SE =	4.625± 0.372			**SE =	4.896± 0.477		**SE =	4.500± 0.285

* Biopsy specimens were collected from those animals after 24 hours

** SE = Standard error

Table 2. DTH Hypersensitivity reaction: Data on skin thickness (mm)

Animal No.	Normal animals						Animal No.	Pneumonic animals					
	24 hours			48 hours				24 hours			48 hours		
	Site I	Site II	Average	Site I	Site II	Average		Site I	Site II	Average	Site I	Site II	Average
514	5.0	3.0	4.0	2.0	2.0	2.0	406*	5.0	7.0	6.0	-	5.0	5.0
99*	5.0	5.0	5.0	-	2.0	2.0	427	10.0	5.0	7.5	7.5	5.0	6.2
520	3.0	4.0	3.5	3.0	-	3.0	128	7.0	5.0	6.0	5.0	5.0	5.0
527	3.0	2.0	2.5	2.0	1.0	1.5	424*	7.0	7.0	7.0	5.0	-	5.0
162	3.0	3.0	3.0	1.0	2.0	1.5	28	5.0	7.0	6.0	3.0	4.0	3.5
513	3.0	2.0	2.5	1.0	1.0	1.0	111	5.0	7.0	6.0	3.0	5.0	4.0
			**SE = 3.5± 0.9487			**SE = 1.033± 0.6031				**SE = 6.4167± 0.6646			**SE = 4.791 0.954

* Biopsy specimens were collected from these animals after 24 hours

** SE = Standard error

blood vessels. Oedema and cellular reactions were prominent in those specimens collected from pneumonic kids.

4.1.6. Cutaneous response to phytohaemagglutinin (PHA)

Cutaneous reaction induced by PHA in six kids ailing from acute broncho pneumonia was compared with six healthy kids. The degree of induration of skin after 24 hours and 48 hours is given in the table 3. The degree of induration was 2.667 ± 0.816 mm and 1.667 ± 0.253 mm in normal kids and 4.00 ± 0.447 mm and 2.33 ± 0.606 mm in pneumonic kids. There was significant increase in the skin thickness of the pneumonic kids than the controls. However, it was lesser in the kid died due to pneumonia.

Histology

Oedema, congestion of capillaries, infiltration of lymphocytes and macrophages and perivascular cuffing of lymphocytes were the major histological changes. The changes were prominent in the tissues collected from pneumonic kids when compared to the control kids.

4.1.7. Humoral immune response

The concentration of total protein in the serum was also determined using the Biuret method. The concentration in the period is shown in figure 6. Gammaglobulin concentration in the serum was determined using the zinc sulphate turbidity test and the data are detailed in appendix VI. The concentration of the total protein as well as the gammaglobulin

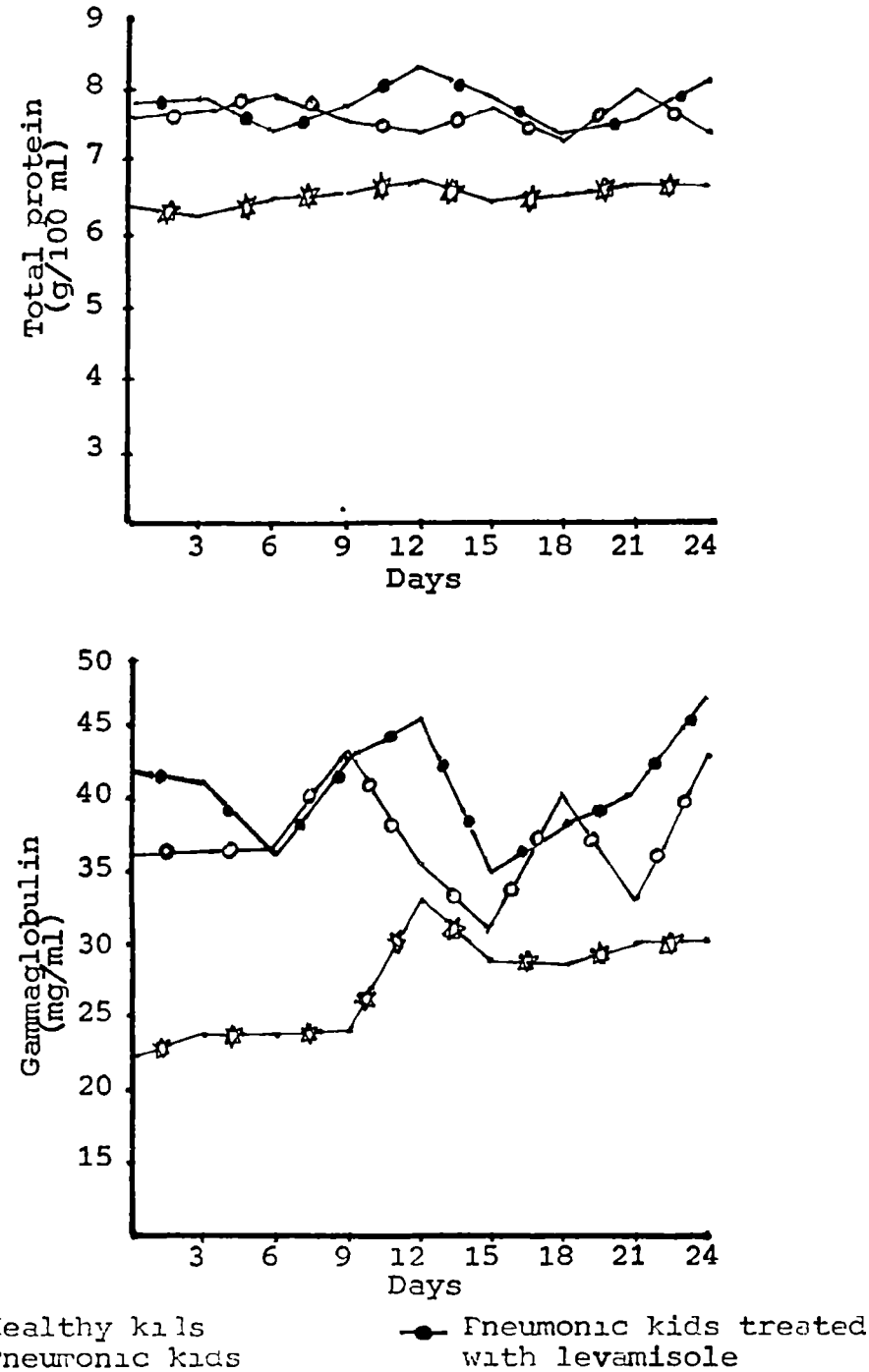
Table 3. Cutaneous response to phytohaemagglutinin: Measurement of skin thickness

Experimental group	Animal No.	Skin thickness 0 hrs (mm)			Standard error	Increase in skin thickness after 24 hrs(mm)			Standard error	Increase in skin thickness after 48 hrs (mm)			Standard error
		Site I	Site II	Average		Site I	Site II	Average		Site I	Site II	Average	
Normal animals	514	1.0	1.0	1.0	1.0±0	3.0	2.0	2.5	2.667±0.816	2.0	1.0	1.5	1.167±0.258
	99*	1.0	1.0	1.0		5.0	3.0	4.0		-	1.0	1.0	
	520	1.0	1.0	1.0		2.0	3.0	2.5		1.0	2.0	1.5	
	527*	1.0	1.0	1.0		3.0	2.0	2.5		-	1.0	1.0	
	162*	1.0	1.0	1.0		3.0	3.0	3.0		1.0	-	1.0	
	513	1.0	1.0	1.0		1.0	2.0	1.5		1.0	1.0	1.0	
Pneumonic animals	406*	2.0	2.0	2.0	1.333±0.516	4.0	3.0	3.5	4.00±0.447	-	2.0	2.0	2.333±0.606
	427*	1.0	1.0	1.0		4.0	5.0	4.5		3.0	-	3.0	
	424*	1.0	1.0	1.0		4.0	5.0	4.5		-	3.0	3.0	
	457**	2.0	2.0	2.0		4.0	4.0	4.0		1.0	2.0	1.5	
	111	1.0	1.0	1.0		4.0	3.0	3.5		2.0	3.0	2.5	
	28	1.0	1.0	1.0		4.0	4.0	4.0		2.0	2.0	2.0	

* Biopsy specimens were collected from these animals after 24 hours

** Died due to pneumonia

Fig. 6. Serum profile of pneumonic kids



concentrations were higher in pneumonic kids than the controls.

4.2. Immunopathology of experimental aflatoxicosis in kids

4.2.1. General findings

Aflatoxin B_1 produced by toxigenic strain of Aspergillus parasiticus, raised in crude rice cultures was fed to six kids. The quantity of the toxin in 1000 g of crude rice culture was 60 milligram. Toxin was fed at the rate of 0.25 mg/kg body weight daily. The quantity of the toxin consumed by each kid before they died is given in table 4. One kid died on the nineth day of the experiment. Others lived for a period of 30-35 days. The number of days survived by each kid is shown in the table 4.

Table 4. Quantity of toxin consumed by kids in experimental aflatoxicosis and duration of survival of kids

Kid No.	Number of days in experiment	Quantity of toxin consumed
101	8 days	9.30 mg
267	33 days	43.20 mg
3439	34 days	37.70 mg
154	34 days	38.95 mg
279	32 days	32.65 mg
163	29 days	23.60 mg
Total		190.56 mg

Reduction in feed intake, loss in body weight, unthriftiness, dysponea, nasal discharge and respiratory difficulties were shown by the animals. The changes in body weight are shown in the figure 7. After death of the kids autopsy was conducted. The cause of death of the kids was attributed to pneumonia.

4.2.2. Leukocytic response

The leukocyte count in the peripheral blood of individual animals is detailed in appendix I. The changes in the number of leukocytes in the peripheral blood over different periods of the experiment are shown in figure 8. The leukocyte count increased significantly on the sixth day. Maximum count was recorded on the 15th day. Later it gradually decreased.

4.2.3. Number of lymphocytes and alpha naphthyl acetate esterase (ANAE) positive lymphocytes in the peripheral blood

The absolute number of lymphocytes and ANAE positive cells in each period is shown in figures 8 and 9 and detailed data in appendix II and III. The mean cell count indicated that while 1422.30 ± 271.99 ANAE positive cells were present in 4666.69 ± 662.61 lymphocytes in one cu.cm.litre of peripheral blood of normal kids, only 1251.82 ± 219.07 ANAE positive cells were present in 6008.13 ± 1126.40 lymphocytes in one cu.cm.litre of the peripheral blood of kids fed with aflatoxin. There was significant reduction in the number of lymphocytes and ANAE positive cells in later stages of the experiment, among kids fed aflatoxin.

Fig.7. Experimental aflatoxicosis:
Mean body weight of kids

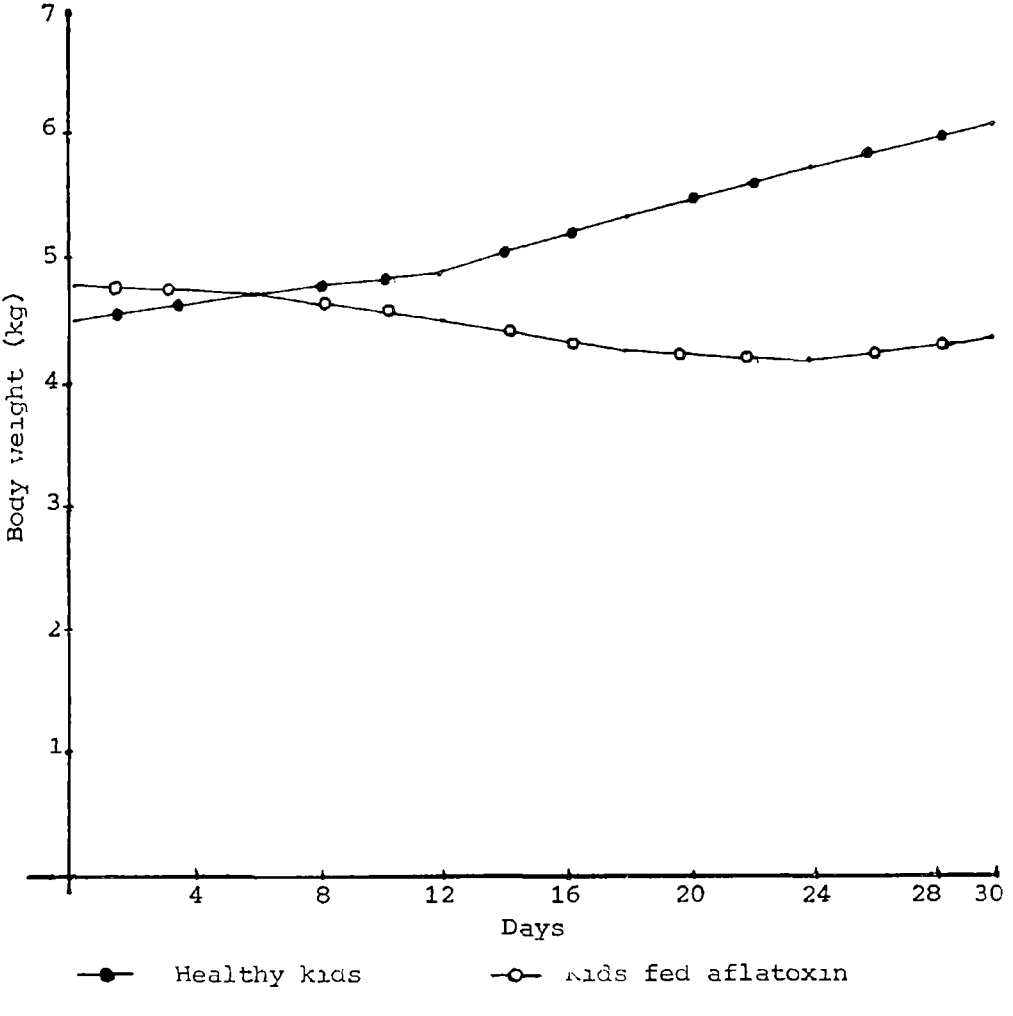


Fig.8. Experimental aflatoxicosis:
Leukocytic response-1

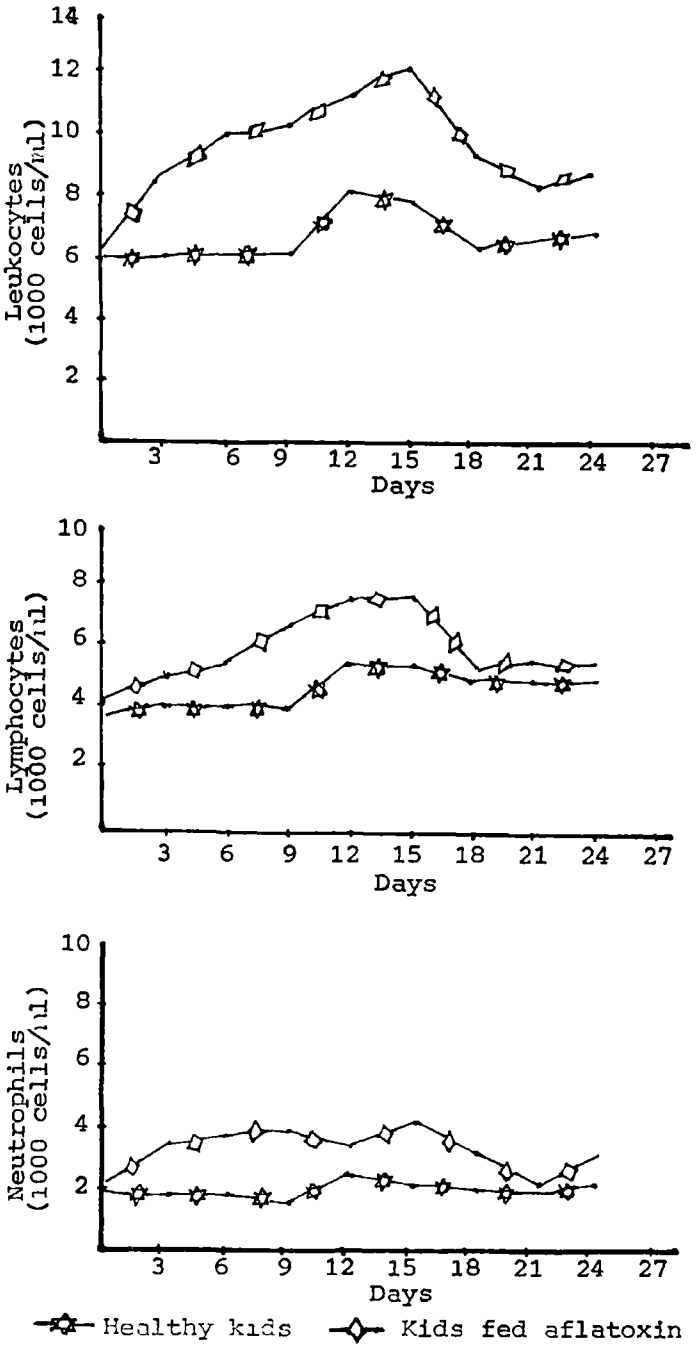
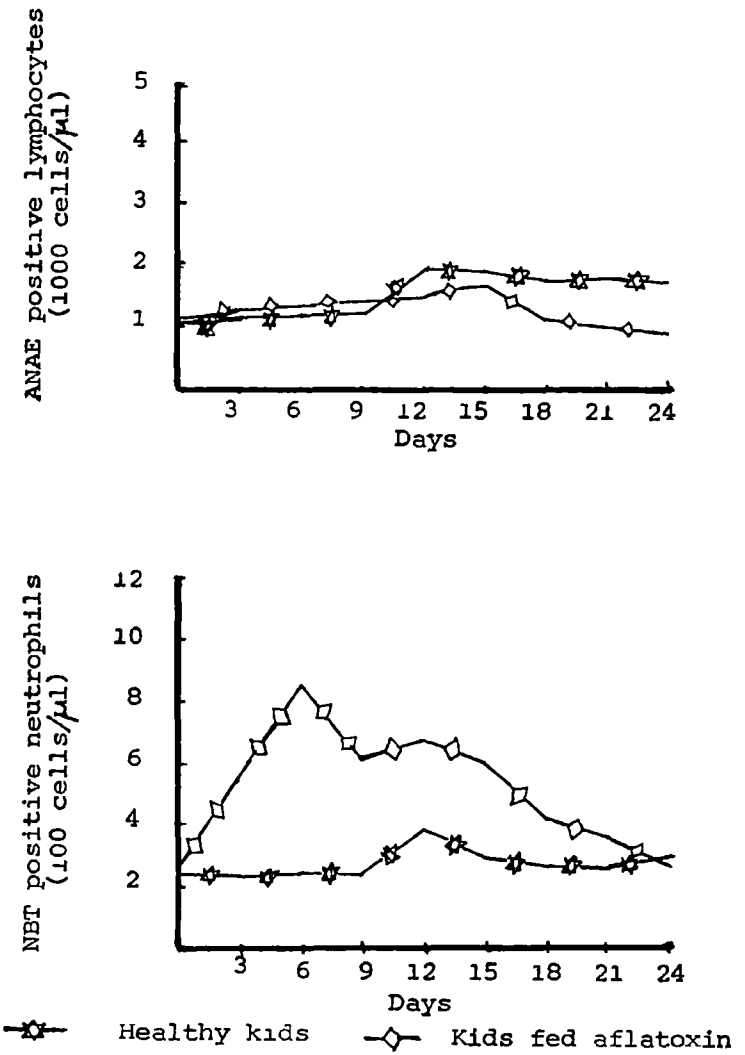


Fig. 9. Experimental aflatoxicosis:
Leukocytic response-2



4.2.4. Assessment of the neutrophil function

Nitro-blue tetrazolium (NBT) dye reduction test was performed to assess the activity of the neutrophils. The number of neutrophils and NBT positive cells in the peripheral blood of individual animal is detailed in appendix IV and V. The changes in each period is shown in figures 8 and 9. In the normal kids, 274.33 ± 55.10 cells out of 1977.27 ± 212.31 neutrophils were NBT positive. Neutrophilia was noticed upto the sixth day when it had 843.29 ± 461.33 NBT positive cells in unit volume of the peripheral blood of kids dosed with aflatoxin. Later it decreased progressively to 278.50 ± 94.26 NBT positive cells per microlitre of the blood, which was not significantly different from the normal.

2.5. Cutaneous response to phytohaemagglutinin (PHA)

Cutaneous response to PHA was evaluated in six kids fed aflatoxin for seven days. The induration in the skin developed after 24 and 48 hours was compared with the age matched controls. The biometry of the skin is detailed in table 5. The increase in skin thickness for healthy and diseased kids after 24 hours was 2.667 ± 0.816 mm and 1.033 ± 0.931 mm respectively. After 48 hours the corresponding values were 1.167 ± 0.258 mm and 0.00 ± 0.57 mm respectively. One kid died on the second day of the test and so the reactions could not be evaluated on the 48th hour. The values obtained from other kids indicated a reduced response for PHA in kids dosed with aflatoxin.

Table 5. Cutaneous response to phytohaemagglutinin: Measurement of skin thickness (mm)

Experimental group	Animal No.	Skin thickness 0 hrs			Standard error	Increase in skin thickness after 24 hours			Standard error	Increase in skin thickness after 48 hours			Standard error
		Site I	Site II	Average		Site I	Site II	Average		Site I	Site II	Average	
Healthy kids	514	1.0	1.0	1.0	1.00± 0	3.0	2.0	2.5	2.667± 0.816	2.0	1.0	1.5	1.167± 0.258
	99*	1.0	1.0	1.0		5.0	3.0	4.0		-	1.0	1.0	
	520	1.0	1.0	1.0		2.0	3.0	2.5		1.0	2.0	1.5	
	527*	1.0	1.0	1.0		3.0	2.0	2.5		-	1.0	1.0	
	162*	1.0	1.0	1.0		3.0	3.0	3.0		1.0	-	1.0	
	513	1.0	1.0	1.0		1.0	2.0	1.5		1.0	1.0	1.0	
Kids fed aflatoxin	101	1.0	1.0	1.0	1.167± 0.408	1.0	1.0	1.0	1.833± 0.931	Died			0.80± 0.57
	267	1.0	1.0	1.0		4.0	3.0	3.5		2.0	1.0	1.5	
	3439*	1.0	1.0	1.0		2.0	2.0	2.0		1.0	-	1.0	
	154	2.0	2.0	2.0		1.0	2.0	1.5		1.0	0	0.5	
	279*	1.0	1.0	1.0		1.0	1.0	1.0		0	-	0	
	163*	1.0	1.0	1.0		2.0	2.0	2.0		-	1.0	1.0	

*Biopsy specimens were collected from these animals after 24 hours

4.2.6. Humoral immune response

The concentration of gammaglobulin was determined to evaluate the humoral immune response. The concentration of gammaglobulin gradually increased from 23.08 ± 3.46 mg/ml to 44.4 ± 8.52 mg/ml in 12 days. Later the concentration remained at the same level without significant changes (Fig.10). A consistent increase in the concentration of total protein was also noticed during the period (Fig.10).

4.2.7. Pathology

Kids dosed with aflatoxin died at varying intervals of time. The survival period of each kid is shown in table 4. The lesions were similar in all the kids. The liver was slightly enlarged and brownish yellow in colour with rounded borders. Two animals showed petechial haemorrhages on the surface. Gall bladder was distended with yellowish green bile. Histologically hepatocytes showed granular degeneration, fatty change and necrosis. Hepatocytomegaly was also a feature. The sinusoids were engorged and showed focal areas of haemorrhage in the parenchyma (Fig.11 to 13). There was slight but perceptible interstitial fibrosis, pseudolobulation and haemosiderosis in two of the kids.

Lesions in the lung were characteristic of acute bronchopneumonia. Right apical and intermediate lobes of all animals were fleshy and consolidated. In two animals, the changes were also present in the diaphragmatic lobes, which

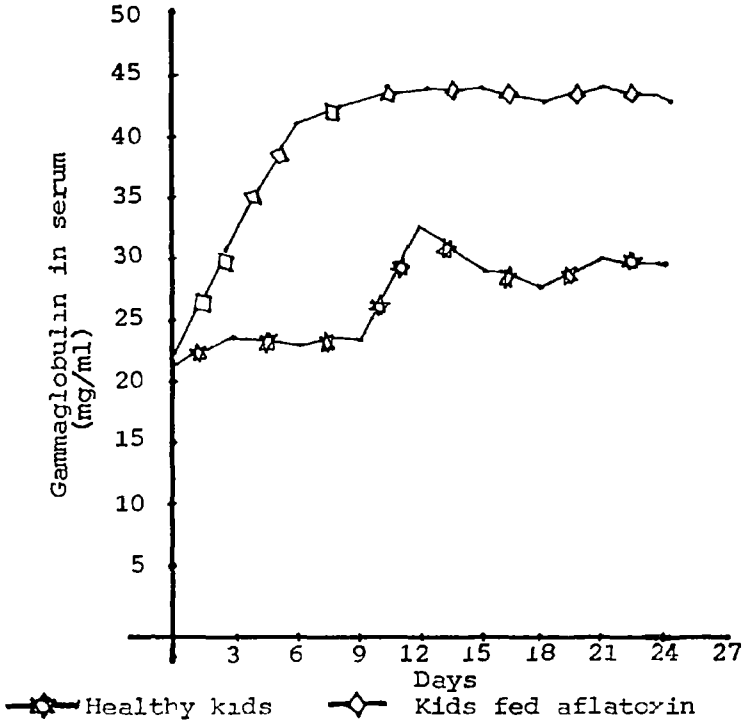
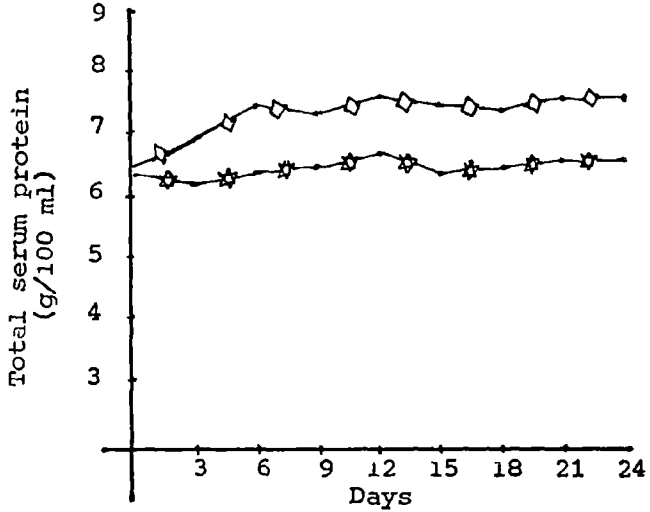
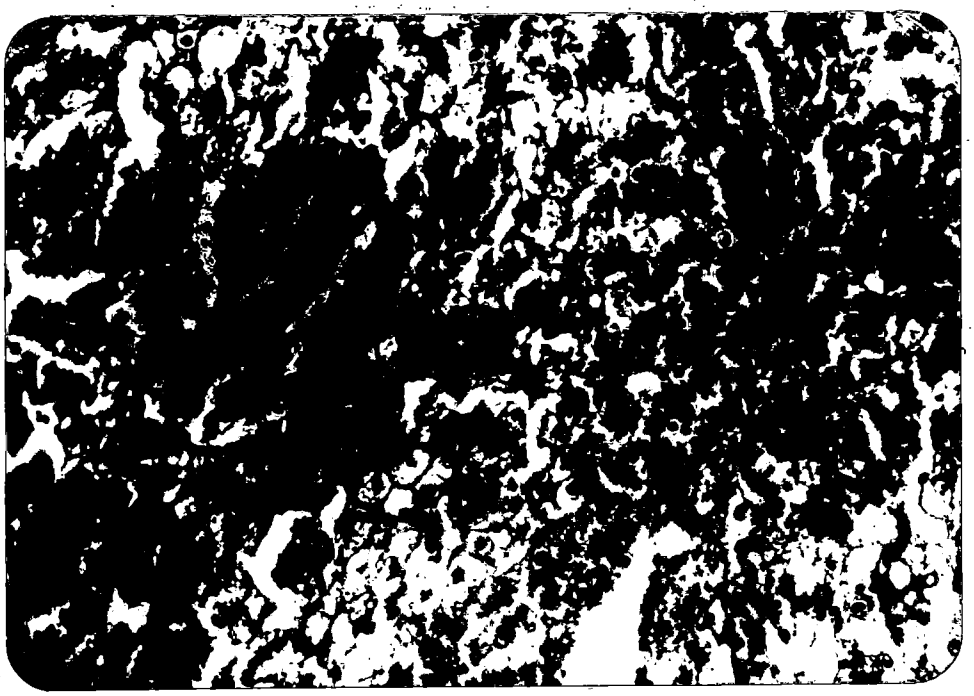

50
45
40
35
30
25
20
15
10
5
Gammaglobulin in serum (mg/ml)
3
6
9
12
15
18
21
24
27
Days
Healthy kids
Kids fed aflatoxin

Fig.10. Experimental aflatoxicosis:
Serum profile





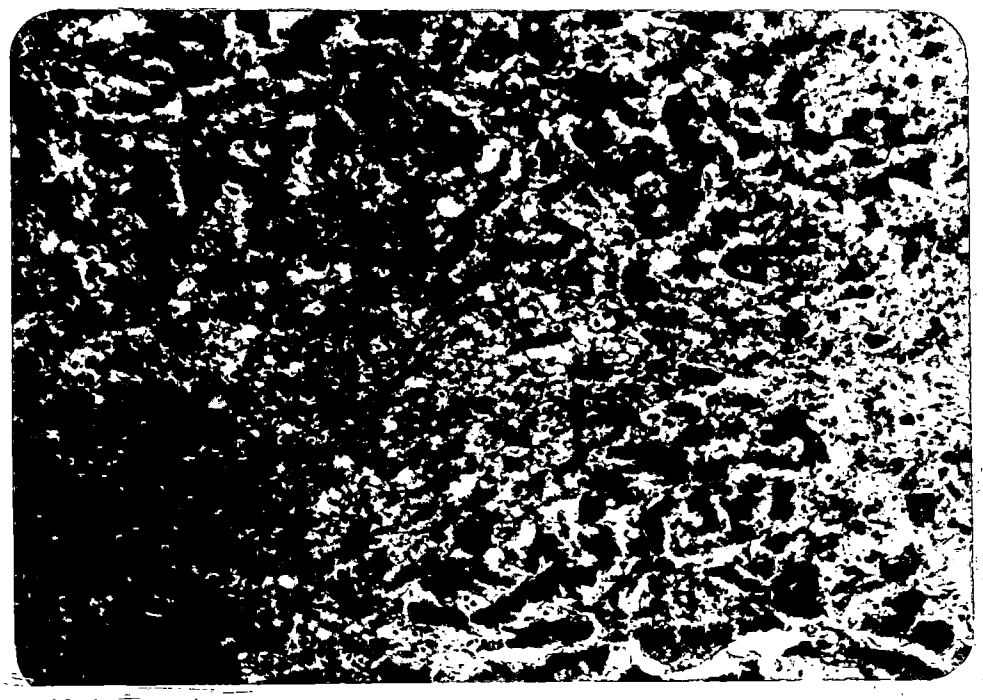


Fig.13. Liver - Aflatoxicosis - Granular degeneration, necrosis and haemorrhage. H and E x 400.

Fig.14. Bronchial lymph node - Aflatoxicosis - Oedema in the medulla and depletion of lymphoid cells in the para-cortex and cortex. H and E x 200.

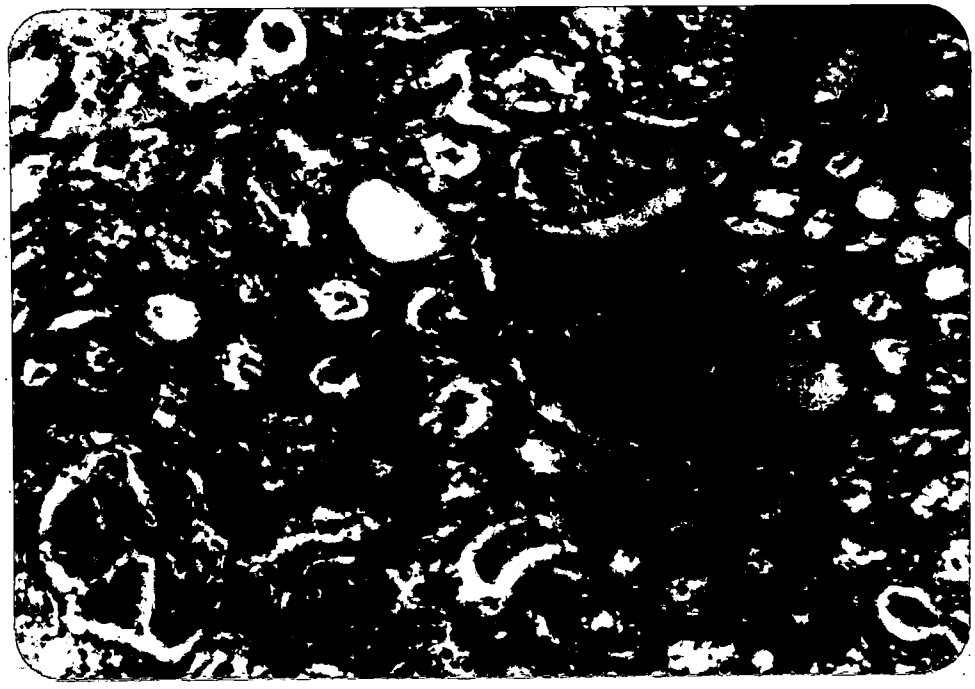
were partly consolidated. Congestion, haemorrhage and infiltration of neutrophils, lymphocytes, macrophages and plasma cells were present in the lung alveoli. There was degeneration of bronchial epithelium and occasional accumulations of lymphocytes in the peribronchial areas.

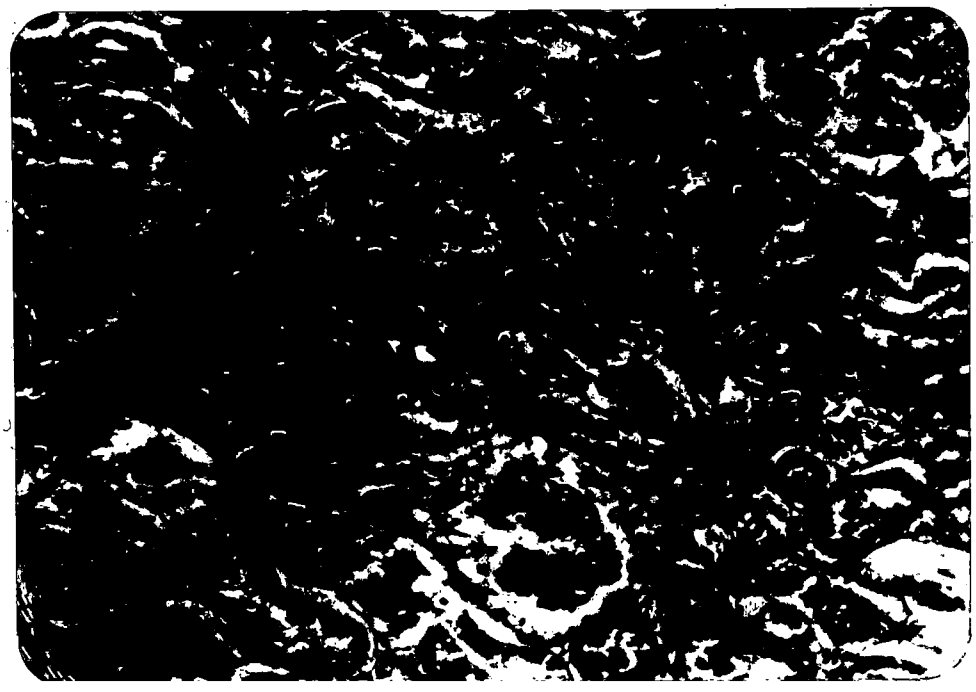
Grossly the bronchial and mediastinal lymph nodes were slightly enlarged, soft and fleshy. Cut surface was juicy. Histologically severe oedema was present in the medulla. Depletion of immunocompetent lymphoid cells was evident in the cortex and paracortex. The number of cortical lymphoid follicles was very few and poorly organised (Fig.14).

Focal greyish white areas were present uniformly distributed on the surface of the kidney. The cut surface revealed greyish white streaks in the cortex. Histological changes were characteristic of toxic nephrosis, with degeneration and desquamation of tubular epithelial cells, formation of hyalin casts, glomerular oedema, atrophy and presence of tubular epithelial reflux (Fig. 15 to 18).

4.3. Immunological response of the kids to immunomodulators

The <u>in vivo</u> immunomodulatory effect of levamisole was studied. Various immunological data of six kids suffering from acute pneumonia, and five kids fed aflatoxin for ten days were evaluated and were compared with the age matched controls. The number of leukocytes, lymphocytes, ANAE positive lymphocytes, neutrophils, NBT positive neutrophils and





concentration of gammaglobulin in the peripheral blood were evaluated. The blood samples were analysed before the administration of levamisole and forty-eight hours and five days after the administration of levamisole. The results obtained are shown in table 6 and figures 19 and 20.

There was significant increase in the leukocyte count (140.63%), lymphocyte count (137.03%), number of AINE positive cells (158.70%), neutrophils (145.20%), NBT positive neutrophils (168.20%) and concentration of gammaglobulin (135.76%) by 48 hours after the administration of levamisole in normal kids. The immunostimulation was persistent after five days.

The change in the leukocytic response and concentration of gammaglobulin of pneumonic kids was statistically insignificant. However, lymphocytic leukocytosis occurred in kids fed aflatoxin 5 days after administration of levamisole. The number of leukocytes, lymphocytes and AINE positive cells increased by 115.73%, 115.17% and 137.98% respectively (Table 6

Fig.19. Immunological response of kids to levamisole-1

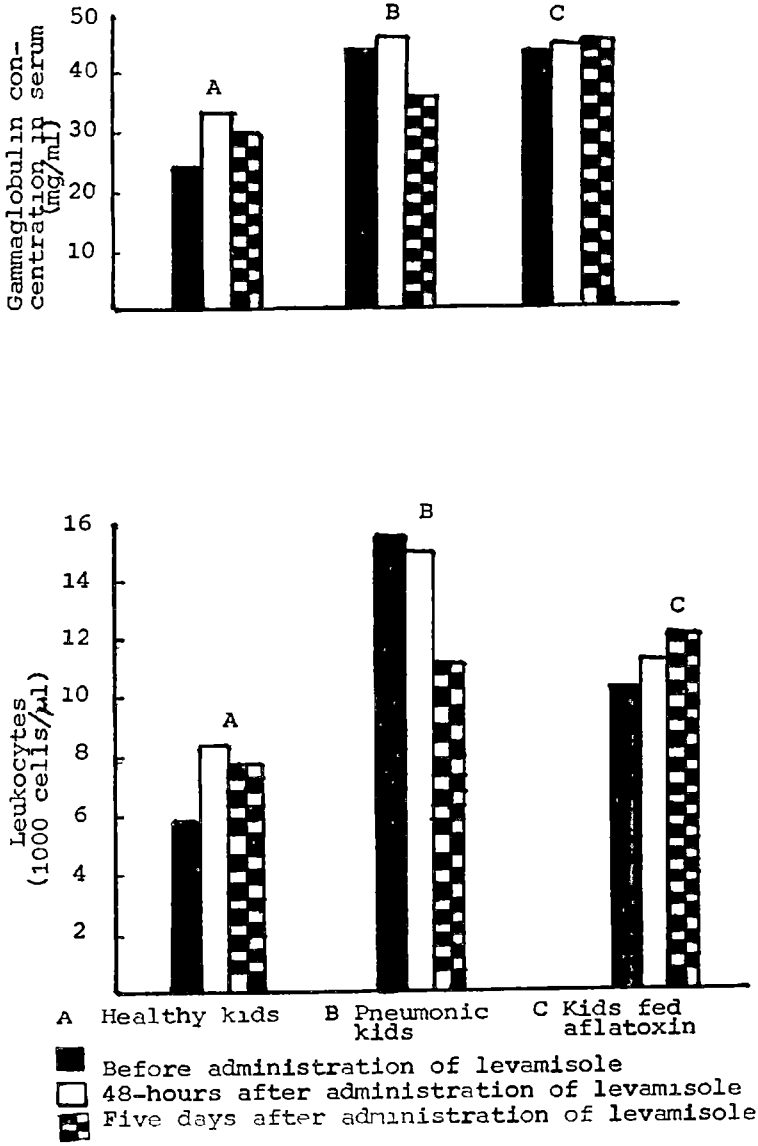


Fig. 20. Leukocytic response of kids to Levamisole-2

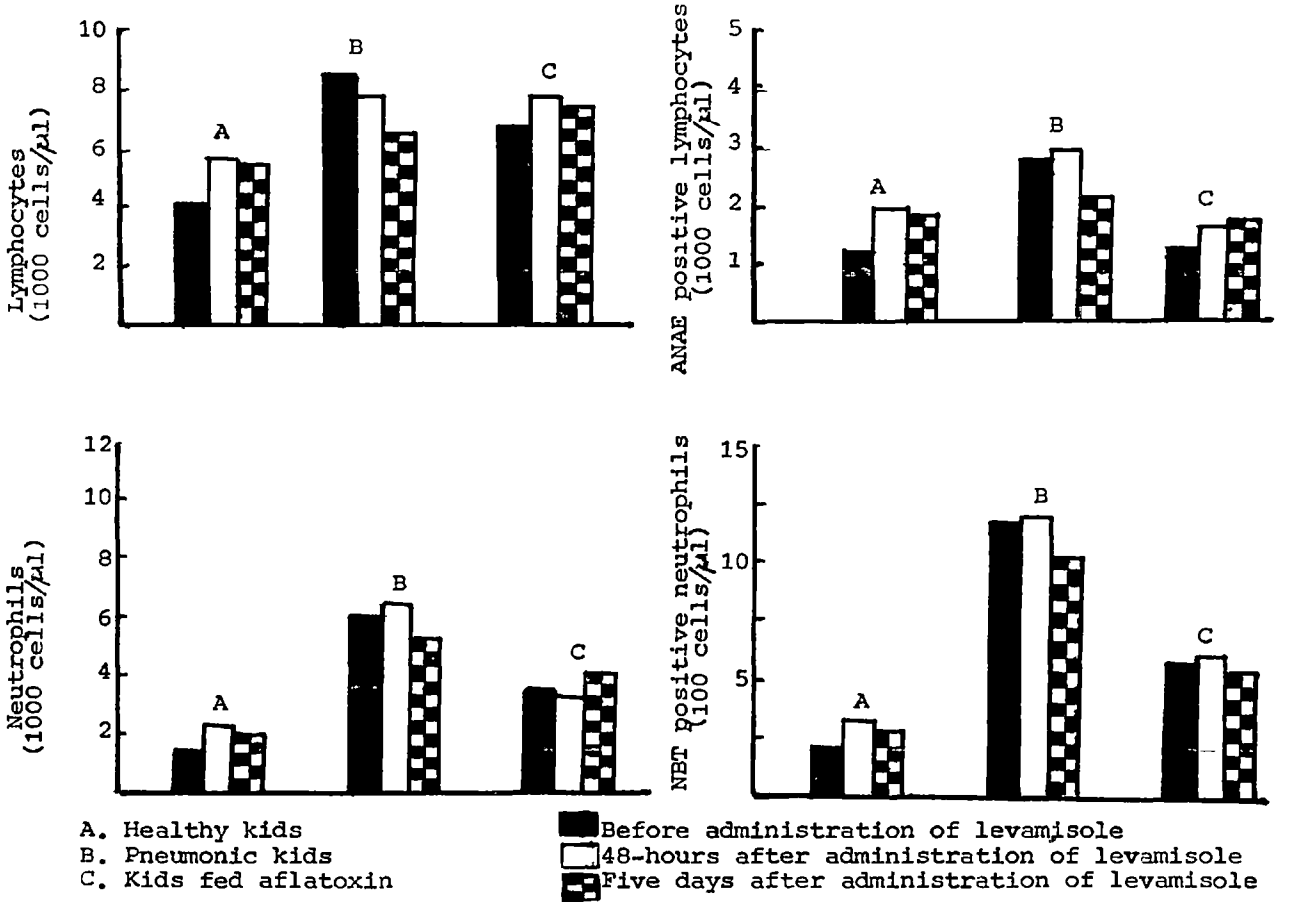


Table 6. Data on immunological parameters in kids dosed with levamisole

Parameter (in peripheral blood)	Experimental group	Before injection	48 hrs after injection		5 days after injection	
			Value	%	Value	%
Leukocyte number/µl	C	5866.67	8250	140.63	7858.33	133.95
	P	1557.5	15000	96.31	11041.67	70.90
	A	10370	11380	105.46	12120	115.73
Lymphocyte number/µl	C	4050.17	5549.92	137.03	5541.67	136.83
	P	8565.25	7859.67	91.76	6414.92	74.90
	A	6624.8	7822.6	118.08	7629.6	115.17
ANAE positive cells number/µl	C	1210.15	1020.45	150.7	1722.09	142.30
	P	2879.73	2939.80	102.09	2117.26	112.64
	A	1236.81	1555.33	125.73	1706.40	137.98
Neutrophils number/µl	C	1661.58	2417.83	145.51	2113.0	127.17
	P	6184.75	6543.92	105.81	5440.42	87.97
	A	3802.7	3588.2	94.36	4163.9	109.5
NBT positive cells number/µl	C	237.29	399.13	168.2	299.80	126.34
	P	1140.26	1207.88	105.93	1048.33	91.94
	A	612.31	652.60	106.58	597.93	97.65
Gammaglobulin in the serum mg/ml	C	23.92	33	137.96	29.3	122.62
	P	43.08	45.67	106.01	35.08	81.43
	A	43.4	44.22	102.32	44.4	102.30

C - Normal kids

P - Pneumonic kids

A - Kids fed aflatoxin

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

Protection and recovery from infections are the key biological features to survival of animals and the former is achieved through the co-operative interaction of specific antibody, proteins of the complement system, phagocytic leukocytes and T lymphocyte sub-populations (Perryman, 1982). Substantial evidence have accumulated to show that mortality from infectious diseases is largely due to the failure of the immune system. Against this background the immunological status of the kids ailing from pneumonia was documented. All the pneumonic kids showed significant leukocytosis compared to normal kids. This could be considered as an indication of the stimulation of the immune system. Leukocytosis can be expected during infections (Benjamin, 1978 and Sharma, 1978). There was significant lymphocytic and neutrophilic leukocytosis. Neutrophilia is considered as a hallmark of bacterial infections and an increase in the number of neutrophilic leukocytes goes to prove that there has been stimulation of the non-specific immune mechanism.

Neutrophils are the chief phagocytic cells in the peripheral blood and they are active even without sensitisation (Tizard, 1982). During bacterial infections neutrophilia occurs. In the present study significant neutrophilia was observed in the early and middle (acute phases) of pneumonia

and as evidenced by the Nitroblue-tetrazolium (NBT) dye reduction test, the phagocytic activity was also found to be enhanced during the disease. There was significant increase in the number of NBT positive neutrophils in the peripheral blood, throughout the disease process. The observations thus indicate that during pneumonia there is significant neutrophilia and an increase in their functional capability. It can be concluded that these cells also have an important role in the defence reactions in the host elucidated during the pneumonic process. Functional capacity of neutrophils and survival rate of kids appears to have significant correlation.

Lymphocytes are considered as the key cells which are responsible for effective defence within the host (Tizard, 1982). There was significant correlation between the lymphocytic response and survival of kids since, those kids having a relatively high lymphocyte count survived the disease. This observation is in agreement with those of Sharma et al. (1978) and support the observation that the lymphocytes are the key cells which are responsible for effective defence in the host (Tizard, 1982) and the conclusion that immunological interference is an important aspect of defence against infections.

In the present study estimation of alphanaphthyl acetate esterase (ANAE) activity was employed as a reliable 'T' cell marker and Sulochana et al. (1982) had observed that there was no significant difference in the number of E-rosette forming cells and ANAE positive cells in the goat. These

lymphocytes with one or two well defined circumscribed reddish spots in the cytoplasm were considered as positive cells. Fixation of the blood smear is an important step in the technique of demonstrating the ANAE activity. Traditionally the procedure described by Pinkus et al. (1979) is adopted, for the same. In this technique the technician has to necessarily keep the fixative and the staining solutions at a temperature of 4°C. For the present investigation to evaluate the ANAE activity in caprine peripheral blood smear, the method described by Giorno and Beverly (1980) was followed. The methodology was found useful in demonstrating the ANAE activity in the goat and is recommended for field use since the procedure is simple and can be done at room temperature.

In the present investigation the number of ANAE positive lymphocytes (T cells) in the peripheral blood of normal goats was found to be 1422.30 ± 271.99 cells/μl and was in the expected range (Chandra et al. 1980; Yang and Shein, 1980; Banks and Greenlee, 1982 and Sulochana et al. 1982).

There was appreciable increase in the number of ANAE positive lymphocytes in pneumonic kids. An increase in T lymphocyte sub-population has been considered as an important indication of the participation of the cell-mediated immune response (CMI) and has been reported in many human and animal diseases. However, there has not been many studies on the role of 'T' lymphocyte response in the defence against diseases in goat. Wachink et al. (1981) documented an increase

in 'T' lymphocytes in Listeriosis in goats. The cutaneous reactions induced by 2,4-dinitrochlorobenzene (DNCB) and phytohaemagglutinin (PHA) are further evidences to support the above conclusion. DNCB test and PHA test were useful in evaluating the CMI response in Johne's disease in goats and found an enhanced CMI in the pre-clinical stage (Paliwal et al. 1985). The present evidences support the conclusion that there has been stimulation of the CMI response following pneumonia.

The concentration of total protein and gammaglobulin in the serum was found to be high throughout the disease process. Benjamin (1978) suggested that there can be increase in the concentration of gammaglobulin due to B-cell response and total protein during infection.

In this investigation the causative agents associated with pneumonia were not identified. However, irrespective of the agent involved the data on leukocytic response and cutaneous response to PHA and DNCB goes to support the conclusion that there has been significant enhancement in the cell-mediated immune response of pneumonic kids. The concentration of gammaglobulin was also found to be increased during pneumonia, indicating that there has been stimulation of the humoral immune system. Although no treatment was attempted with antibiotics or any chemotherapeutic agents, ten kids out of twelve recovered from the infection. It may be pointed out that as per the data documented the cell-mediated immune system of

those kids died of the disease had not been enhanced to the same level as that of the kids that recovered from the disease. Thus the importance of immunological response in bringing about recovery from disease appears to be very significant. This observation further demonstrated that if attempts are made to stimulate the immune system it would be rewarding. The study has thus clarified that immunological response has an important role in causing recovery from infections and there is further scope for better management of pneumonic kids if procedures for immunomodulation are employed.

All the kids dosed with aflatoxin developed symptoms like dyspnea, cough, catarrhal nasal discharge which became mucopurulent, reduced food intake and unthriftiness and the condition progressed to pneumonia. Miller et al. (1984) also described the development of pneumonia in experimental caprine aflatoxicosis. The gross and microscopical changes in the liver gave further evidence of aflatoxicosis, and were similar to those observed by earlier workers in caprine aflatoxicosis (Maryamma and Sivadas, 1975; Miller et al. 1984 and Clark et al. 1984).

Examination of blood of kids receiving dietary aflatoxin revealed leukocytosis. The number of leukocytes increased to 12120 ± 4182 cells/µl of blood on the 15th day after oral administration of the toxin. The initial leukocytosis can be explained as a reflection of response to pneumonia the animals picked up. Later the number gradually reduced. Leukocytosis

was not a feature of caprine aflatoxicosis (Clark et al. 1984). However, Miller et al. (1978) observed leukocytosis in pigs following aflatoxicosis. The number of lymphocytes, neutrophils and NBT positive cells increased in the initial stages, however there was decline in these values in the later phases of the experiment. A similar pattern of changes was not observed in the number of ANAE positive lymphocytes. As the days advanced the number of ANAE positive lymphocytes in the peripheral blood came down significantly when compared to normal animals. No reports are available which describes the changes in the number of ANAE positive lymphocytes (T cells) in the peripheral blood of goats fed aflatoxins. Histological picture of the lymphnodes revealed severe depletion of cells in the paracortical area and the cutaneous response of aflatoxin fed kids to PHA was significantly lower than the control kids. These are further evidences which will support the conclusion that there has been suppression of cell-mediated immune response of goat affected with aflatoxicosis. Thus it would be reasonable to conclude that kids fed aflatoxin along with the diet picked up infection as a result of immunosuppression induced by the toxin.

Suppression of cellular immunity in aflatoxicosis has been reported in man (Seval et al. 1970), guinea pig (Pier et al. 1976), swine (McLoughlin et al. 1984) and bovines (Dedine et al. 1984). However, no report so far has appeared

describing the effect of aflatoxin on the cell-mediated immune response of goats.

All the kids dosed with aflatoxin developed pneumonia and there was increase in the total serum protein and gamma-globulin in them. Miller et al. (1984) also documented increase in the concentration of total plasma protein and gammaglobulin in the serum of goats dosed with aflatoxin. He supported the opinion made by Richards et al. (1978) who reasoned concomittant infections as the cause for hypergamma-globulinaemia. In this context it is worthy to note that eventhough the kids developed pneumonia and had leukocytosis, there was decrease in the number of ANAE positive lymphocytes (T cells) in the peripheral blood. This implies that there was increase in the number of non-T lymphocytes since there was leukocytosis. A majority of non-T lymphocytes in the peripheral blood is B-lymphocytes which are responsible for antibody production. This may be the reason for increase in the concentration of gammaglobulin and total protein in the serum.

The failure of the T-cell mediated immune response may be considered as the cause for the death due to pneumonia in aflatoxin fed kids. This has got significant practical relevance, since in field conditions the animals are exposed to varying levels of dietary aflatoxins. Certainly infectious agents are present every where. When there is break down in

the efficiency of the immune system the infectious agents gain an upper hand and cause the disease which may eventually lead to death of the animal. Therefore, in order to reduce the incidence of diseases in livestock there is need to minimise the aflatoxin content in the feed. Regular screening of feed samples for aflatoxins may be advocated as a mandatory measure to reduce the incidence of diseases. This will definitely aid in better management of diseases and to reduce mortality.

The efficiency of the immune system determines the fate of the invading organisms in the body of the host. Survival or death is again governed by the efficiency of the immune system. Increased susceptibility to infections can be due to the immune deficiency of the host caused by variety of factors like introduction of exotic genes by cross-breeding, the production stress on the animals as well as the modern intensive methods of husbandry practices. If sufficient immunostimulation occur following infection in the body there is better scope for destruction of the invading agent and survival from diseases. In the management of diseases, of late immunomodulation has been attempted to boost up the immunological efficiency and levamisole has been recommended as an efficient immunomodulator (Mathew and Mathew, 1986). So far no study has been undertaken to evaluate the immunomodulatory effect of levamisole in kids.

In the present study lymphocytic and neutrophilic leukocytosis were observed 48 hours after the administration of levamisole in kids, at the dose rate of 3.75 mg/kg body weight. The activity of neutrophil was also found to be augmented as evidenced by the Nitroblue tetrazolium dye reduction test. There was also enhancement in the number of SRBC positive lymphocytes (T cells) in the peripheral blood and concentration of gammaglobulin in the serum. The immunomodulatory effect was persistent even after five days of dosing. The present investigation showed that adequate immunomodulatory effect occurred when half the anthelmintic dose (3.75 mg/kg body weight) of levamisole was administered in goats. This observation has got significant practical relevance, since goats, like other livestock are likely to suffer from infectious diseases when their immune system is at a suppressed state. In such occasions levamisole will be helpful as a prophylactic in restoring and improving the efficiency of immune response.

Immunomodulation was found insignificant in uncurbed kids as evidenced by leukocytic response and serum gammaglobulin concentration in blood. Larsen et al. (1985) also did not find any desirable effect in modulating the immune response of calves suffering from respiratory diseases. According to Tizard (1982) and Keller (1982) levamisole restored the efficiency of the immune system only when it was deficient. Pathenove (1982) observed increase in the number of 'T cells'

and gammaglobulin, ten days after treatment with antibiotics and levamisole in calves suffering from acute bronchopneumonia.

No reports have so far appeared describing the effect of levamisole on the immune system of animals which were dosed with aflatoxin. Immunopathological response was evaluated in kids after feeding aflatoxin for seven days. In the present investigation no immunomodulation was observed, 48 hours after the administration of levamisole (3.75 mg/kg body weight) in kids dosed with aflatoxin at the rate of 0.25 mg/kg body weight. However, immunostimulation was observed 5 days after the administration of levamisole and this was characterised by neutrophilic leukocytosis. Eventhough there was no significant change in the total number of lymphocytes, there was increase in the number of ANAE positive lymphocytes (T cells). Goldstein et al. (1978) suggested that levamisole mimic the action of thymic hormones. This may be the reason for the increase in the number of T cells in the blood. It would be worth while to study the effect of administration of antibiotics and levamisole together and also the effect of levamisole at different dose levels.

Although there was neutrophilia, there was no change in the activity of neutrophils as indicated by NBT test in kids fed aflatoxin. Significant change was not observed in the concentration of gammaglobulin in the serum subsequent to levamisole therapy in aflatoxin fed kids. The dose regime

and role of levamisole in restoring the immunosuppressive effect of aflatoxin need further study and clarification.

CHAPTER 6

SUMMARY

SUMMARY

Immunopathological response of twelve kids (2-4 months of age) spontaneously affected with pneumonia and six kids experimentally dosed with aflatoxin was evaluated. The results were compared with those of six age matched healthy kids.

Immunopathological response in pneumonic kids was studied for the first time. The response of the kids was evaluated based on the leukocytic response, T-cell sub-population in blood, *in vitro* neutrophil activity, gamma-globulin concentration in the serum and cutaneous response to 2-4, Dinitrochlorobenzene (DNCB) and Phytohaemagglutinin (PHA).

The alpha naphthyl acetate esterase (ANAE) activity was used as a 'T' cell marker, to evaluate the T-cell sub-population in the peripheral blood. The method described by Giorno and Beverly (1980) for demonstrating the ANAE activity which has not been tried in animals before, was used and found to be a useful practical method, to evaluate the ANAE activity of lymphocytes in the peripheral blood of goats.

There was lymphocytic leukocytosis in the peripheral blood of pneumonic kids, throughout the disease process. There was also significant increase in the T-cell sub-population in pneumonic kids which indicated an enhancement of cell-mediated immune (CMI) response.

Cutaneous reaction induced by DNCB and PHA also revealed that the pneumonic kids had an enhanced C'MI.

The concentration of gammaglobulin in the serum was also higher in pneumonic kids.

Ten kids out of twelve kids recovered from the disease and it was concluded that there was significant immunostimulation following pneumonia in kids. However, immunomodulation was not observed in pneumonic kids 48 hours and five days after the administration (s/c) of levamisole at the dose rate of 3.75 mg/kg body weight.

Immunopathology of experimental aflatoxicosis was studied for the first time in goats. Immunopathological response of six kids dosed with 0.25 mg/kg body weight of crude aflatoxin was evaluated. Histopathological changes in the liver were pathognomonic of aflatoxicosis. All the kids developed pneumonia and died at varying intervals of time.

Leukocytic response and neutrophil activity was enhanced in the initial phase of the experiment (first 15 days) which gradually declined in the later stages (15-30 days). The number of ANAE positive lymphocytes was not increased during the course of the experiment. The cutaneous response of diseased kids to PHA was also significantly low after seven days of exposure to the toxin. It was therefore concluded that aflatoxin caused suppression of the C'MI response in goats.

Total protein and gammaglobulin concentration in the serum

were found higher in kids fed aflatoxin and this was attributed to concomittant infection.

It was pointed out that this observation has got practical significance since animals are often exposed to aflatoxin through contaminated feeds and are likely to pick up fatal infections due to immunosuppression. It was suggested that there should be regular screening of the feed for aflatoxin and the feed should not contain more than the permissible level of aflatoxin.

Immunomodulatory effect of levamisole in kids was studied for the first time. The leukocytic response, number of T lymphocytes in the peripheral blood, *in vitro* neutrophil activity and serum concentrations of gammaglobulin indicated significant immunostimulation, 48-hours after the administration of levamisole s/c at the rate of 3.75 mg/kg body weight. It was therefore, suggested that levamisole could be used to boost the efficiency of the immune system in apparently healthy kids when outbreaks of pneumonia occur in a herd.

Levamisole was not found to cause immunostimulation when the immune system was already stimulated due to pneumonia in kids. However, there was immunostimulation following levamisole administration in kids fed aflatoxin as evidenced by neutrophilic leukocytosis five days after the administration of levamisole.

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IMMUNOPATHOLOGICAL RESPONSE OF KIDS IN PNEUMONIA

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ABSTRACT OF A THESIS

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ABSTRACT

The immunopathological response of kids spontaneously affected with pneumonia and kids experimentally dosed with aflatoxin was evaluated, for the first time, employing various immunological markers. Attempts were also made to modulate the immune response of kids for the first time using levamisole.

There was enhancement of the immune response in pneumonic kids as evidenced by leukocytic response, number of T-lymphocyte sub-population in the peripheral blood, *in vitro* neutrophil activity, concentration of gammaglobulin in the serum and cutaneous response to 2,4-Dinitrochlorobenzene and phytohaemagglutinin.

Immunological background of kids fed arlatoxin (at the rate of 0.25 mg/kg body weight) was evaluated. Immunostimulation (in first 15 days) was followed by immunosuppression (from 15 days until death). The kids developed pneumonia and died. It was concluded that at a dose rate of 0.25 mg/kg body weight aflatoxin caused immunosuppression in goats.

At a dose rate of 3.75 mg/kg body weight (o/c) levamisole induced immunostimulation in kids. It did not cause immunomodulation in pneumonic kids.

APPENDICES

APPENDIX-I

Leukocyte count in the peripheral blood (numbers/µl) of healthy kids (A), pneumonic kids (B), pneumonic kids treated with levamisole (C) and kids fed aflatoxin (D)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
I	A	5800	5600	5500	6450	6000	5350	5933.33	609.64
	B	16000	15450	16550	7000	19800	11150	14325.0	4532.85
	C	16450	15500	7000	10200	14850	15900	13316.67	3826.84
	D	5350	5900	6250	6700	7100	6050	6225.0	615.43
II	A	6100	5750	5700	6400	6000	5450	6033.33	501.66
	B	8150	17200	16550	12450	17800	16550	15783.33	3758.4
	C	8750	8050	11950	8450	17350	17500	12008.33	4419.1
	D	8150	10050	8900	6700	10300	8100	8700.0	1347.1
III	A	5900	5550	5600	6450	6750	5650	5983.33	501.66
	B	15400	13250	15950	14550	18350	17400	15816.67	1860.56
	C	15100	15200	16000	9800	18500	19850	15741.67	3480.29
	D	14050	9350	5500	9150	11500	9450	9833.33	2834.9
IV	A	5850	5200	5950	6100	6350	5750	5866.67	388.16
	B	15000	10200	18350	7300	18600	9600	13175.0	4810.17
	C	15300	14400	16750	10300	17750	18950	15575.0	3058.88
	D	9850	15050	8750	9300	8900	-	10370.0	2650.61
V	A	8150	7450	7700	9100	9950	7150	8250.0	1074.71
	B	10950	17000	19450	10350	16650	8200	15100.0	4688.92
	C	15050	16000	16950	17350	14300	9550	15000.0	2871.93
	D	11550	8700	8400	13250	15000	-	11380.0	2858.89

(contd.)

Appendix-1 contd.

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
VI	A	7900	7350	7800	8100	8750	7250	7858.33	545.36
	B	13550	12700	16850	9000	9100	7550	11458.33	3517.59
	C	16700	7050	18750	5900	10050	7800	11041.67	5390.7
	D	18050	9000	7800	11250	14500	-	12120.0	4182.79
VII	A	6050	6950	6300	7350	5750	5900	6383.33	633.77
	B	9300	17050	10900	9850	7850	7400	10391.67	3507.77
	C	17700	12500	18550	8650	16750	-	14830.0	4164.82
	D	12500	8750	7250	8900	7950	-	9070.0	2028.73
VIII	A	6800	6000	5950	6250	7750	6550	6550.0	671.57
	B	9300	15400	10950	12500	-	-	12037.0	2594.67
	C	9300	8500	-	-	-	-	8900.0	565.67
	D	9550	7450	8250	6500	9250	-	8200.0	1262.93
IX	A	7000	6800	7100	5950	6350	6900	6683.33	443.47
	B	9250	17500	9750	10450	-	-	11737.5	3873.06
	C	7850	9650	-	-	-	-	8750.0	1272.79
	D	10050	8300	9700	8950	6250	-	8650.0	1502.91

R_1 ..... R_6 - Serial number of experimental animals in each group

I ..... IX - Period (interval of the period is three days)

SE - Standard error

APPENDIX-2

Absolute number of lymphocytes in the peripheral blood (numbers/μl) of healthy kids(A), pneumonic kids (B), pneumonic kids treated with levamisole (C) and kids fed aflatoxin (D)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
I	A	3488	3504	3685	3805.5	4761	3745	3844.75	462.99
	B	11680	9579	6289	5460	15444	8808.5	9043.42	3859.41
	C	10857	12710	5600	7446	11434.5	1287.9	8222.57	4311.94
	D	3969	3776	4702.5	3953	5183	5287.5	4478.5	667.87
II	A	4087	4025	3933	3840	4760	3651.5	4049.42	380.18
	B	5460.5	9632	11916	8839.5	12460	12247	10092.42	2713.46
	C	5075	4508	7289.5	6844.5	13706.5	12600	8337.25	3887.25
	D	3097	5427	5963	4221	7210	4617	5098.17	1433.35
III	A	3776	3996	3976	4192.5	4927.5	3898.5	4127.75	414.84
	B	8470	11395	10367.5	8584.5	12845	14094	10959.33	2269.74
	C	8154	10944	8320	7742	10915	13299.5	9895.75	2185.34
	D	7025	5903.5	3575	5581.5	7820	3874.5	5629.92	1680.77
IV	A	4036.5	3484	4165	4209	4381.5	4025	4050.17	306.45
	B	9030	7354	13120	4818	12834	5760	8897.67	3490.31
	C	5049	10656	6867.5	7828	9052.5	11938.5	8565.25	2521.01
	D	5122	10234	5075	8510	4183	-	6624.8	2607.82
V	A	5297.5	5215	5035	6552	6368	4862	5549.92	723.81
	B	10991	12580	7391	6210	9990	5822	8830.67	2759.03
	C	6498.5	9440	6949.5	11104	7436	5730	7859.67	2020.86
	D	6699	8264	5292	10600	8258	-	7822.6	1985.24

(contd.)

Appendix-2 contd.

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
VI	A	5293	5439	5226	6237	6125	4930	5541.67	523.44
	B	7859	8890	10110	5760	6279	3624	7087.0	2340.58
	C	6513	4864.5	12937.5	4189	5929.5	4056	6414.92	3338.03
	D	9386	6660	5338	8325	8439	-	7629.6	1613.63
VII	A	4174	4865	4624	6690	5115	4485	4990.50	887.94
	B	5766	9207	5668	5220.5	4788.5	5328	5996.33	1611.0
	C	6372	8875	15130	5536	5197.5	-	8222.1	4121.03
	D	6875	6037.5	5132.75	6052	4293	-	5678.05	969.63
VIII	A	4628	4920	4656.5	6187.5	5270	4519.5	4963.5	680.65
	B	14743	9856	5475	7250	-	-	9331.0	4031.74
	C	4557	5100	-	-	-	-	4825.5	388.20
	D	6398.5	4917	6048	4615	6290	-	5653.70	827.15
IX	A	5040	4556	5325	4121.5	5767.5	4485	4882.5	607.88
	B	6197.5	10500	7800	6479	-	-	7744.13	1965.58
	C	5181	8009.5	-	-	-	-	6595.25	200.05
	D	7236	5229	4947	6503.5	3312.5	-	5451.60	1519.42

R_1 ..... R_6 - Serial number of experimental animals in each group

I ..... IX - Period (Interval of the period is three days)

SE - Standard error

APPENDIX-3

Absolute number of ANAE positive cells in the peripheral blood (numbers/µl) of healthy kids (A), pneumonic kids (B), pneumonic kids treated with levamisole (C) and kids fed aflatoxin (D)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
I	A	939.8	931.84	1031.8	1179.71	1523.5	1235.85	1140.42	226.92
	B	4438.4	3352.65	3899.18	2293.2	5405.4	2466.28	3642.52	1191.24
	C	3582.81	3431.7	2576	2755.02	3659.04	3734.91	3734.91	497.38
	D	1071.63	944	1034.55	948.72	1192.09	1533.4	1120.73	221.83
II	A	1185.23	1328.25	1494.54	844.8	1142.4	912.80	1151.35	245.41
	B	2238.5	3178.56	6315.48	3800.98	4111.8	3919.04	3927.39	1353.77
	C	1877.75	1577.8	2998.7	2737.8	3937.92	4284	2885.66	1060.49
	D	774.25	1573.85	1132.97	1139.67	1802.5	1015.4	1239.77	379.62
III	A	1206.5	1358.64	1033.75	1131.98	1576.8	1013.61	1220.21	215.29
	B	3133.9	4671.95	4147	3262.11	4495.75	5496.66	4201.23	895.60
	C	2690.82	3720.96	3328	2554.86	4366	4308.84	3508.25	795.78
	D	1896.75	1425.88	715	1172.1	1798.6	891.14	1316.38	470.47
IV	A	1296.8	1289.08	1082.9	1136.43	1489.71	966	1210.15	186.08
	B	2970	3063.05	5152.6	1927.2	4748.5	2476.8	3389.61	1281.35
	C	1464.2	3942.72	2266.28	2974.64	3168.38	3462.17	2879.73	887.87
	D	1024.4	2149.4	913.5	1302	794.77	-	1236.81	543.63
V	A	1848.18	1825.25	1451.45	2227.68	2419.84	1750.32	1920.65	348.42
	B	3297.3	4025.6	2365.12	2049.3	3796.2	2387.02	2986.26	831.36
	C	1949.55	3304	2571.32	4805.76	2751.32	2177.4	2939.89	1063.25
	D	1540.77	1500	1050.4	2014	1567.5	-	1555.33	338.59

Appendix-3 contd.

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
VI	A	1693.76	1458.52	1724.58	1746.36	2082.5	1626.8	1722.09	204.84
	B	2593.47	3289.3	3134.1	2180.8	2323.23	1377.12	2484.34	695.89
	C	2019.03	1702.58	4784.88	1382.37	1719.56	1095.12	2112.26	1364.68
	D	2252.64	1790.2	1548.02	1498.5	1434.63	-	1706.40	335.03
VII	A	1544.56	1508.15	1387.2	1939.2	1381.05	1435.2	1532.56	209.60
	B	1672.14	3130.38	1983.8	2088.2	1532.32	1704.96	2018.63	582.58
	C	2166.48	3372.5	1898.1	1550.8	1507.28	-	2099.03	761.15
	D	1512.5	1287.5	1010.53	1149.08	858.6	-	1163.80	251.86
VIII	A	1689.8	1382.4	1350.39	1670.63	1581.	1310.65	1492.48	169.55
	B	3660.05	3085.36	1642.5	2392.5	-	-	2695.10	872.35
	C	1686.09	1734	-	-	-	-	1710.05	33.88
	D	1348.67	934.2	709.6	969.15	1009.6	-	993.24	227.81
IX	A	1012	1275.68	1204	2020.11	1497.97	1390.35	1400.02	346.02
	B	2169.13	3465	2418	1878.91	-	-	2482.76	690.90
	C	1865.16	2563.04	-	-	-	-	2214.10	493.48
	D	1030.12	1045.8	890.46	1006.7	695.62	-	933.74	146.41

R_1 ..... R_6 - Serial number of experimental animals in each group

I ..... IX - Period (Interval of the period is three days)

SE - Standard error

APPENDIX-4

Absolute number of neutrophils in the peripheral blood (numbers/µl) of healthy kids (A), pneumonic kids (B), pneumonic kids treated with levamisole (C) and kids fed aflatoxin (D)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
I	A	2030	1904	1705	2396.5	2139	1391	1925.91	347.5
	B	3360	4944	7252	1050	3564	2118.5	2118.5	2182.20
	C	3783.5	2480	700	2440	3118.5	2862	2565.33	1037.15
	D	3234	2065	2970	2479	1917	1621.5	2381.08	628.67
II	A	1891	1725	1596	2432	1904	1635	1863.83	306.12
	B	2282	6880	3972	2980	4094	3475	3948.5	1583.63
	C	3237.5	3300	3346	1436.5	3123	4375	3136.33	949.2
	D	5053	3919.5	2937	2010	2804	3248	3341.92	1040.95
III	A	1888	1498.5	1624	1612.5	2497.5	1751.5	1812.0	361.32
	B	6622	1855	4625.4	4947	4487.5	2784	4220.15	1684.60
	C	6040	3800	7680	1764	6475	5359.5	5186.42	2110.55
	D	6182	3179	1540	3568.5	3335	5103	3812.92	1620.67
IV	A	1696.5	1560	1487.5	1647	1968.5	1610	1661.58	166.68
	B	5850	2142	4954.5	1679	5022	3168	3802.58	1213.22
	C	9486	3168	8710	2060	7810	5974.5	6184.25	3037.34
	D	4334	4214	3237.5	2511	4717	-	3802.7	904.89
V	A	2445	2235	2156	2457	3283.5	1930.5	2417.83	467.15
	B	6822	3570	11281	3519	4995	2214	5400.17	3280.55
	C	9034.5	6080	9322.5	4058	6435	3533.5	6543.92	3280.55
	D	4851	2088	2352	2650	6000	-	3585.20	1757.13

(Cont'd.)

Appendix-4 contd.

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
VI	A	2449	1911	2340	1863	2012.5	2102.5	2113.0	235.77
	B	4742.5	3429	5223.5	2610	2821	3322	3691.33	1056.83
	C	9352	2185.5	12750	1416	3819	3120	5440.42	4550.48
	D	7761.5	2340	2198	2700	5820	-	4163.9	2300.99
VII	A	1633.5	2088	2040	1503	2402.5	2070	1956.17	330.47
	B	2046	7161	4469	4235.5	2276.5	1776	3660.67	2064.50
	C	9558	3250	3249	2941	1552.5	-	4110.10	3125.25
	D	5375	2368.5	2145	2848	3259.5	-	3199.2	1290.57
VIII	A	1836	1680	2224	1897.5	2325	2030.5	1998.83	263.53
	B	3534	4312	5037	4250	-	-	4283.25	614.12
	C	4371	2805	-	-	-	-	3588.0	1107.33
	D	2674	2011.5	1152	1885	2590	-	2062.5	615.49
IX	A	1960	2048	1420	1590	3256.5	2001	2045.92	646.13
	B	2590	5950	1852.5	3257.5	-	-	3412.5	1786.34
	C	2276.5	1447.5	-	-	-	-	1861.85	586.40
	D	2512.5	2905	4753	2416.5	2875	-	3092.4	953.03

R_1 ..... R_6 - Serial number of experimental animals in each group

I ..... IX - Period (Interval of the period is three days)

SE - Standard error

APPENDIX-5

Absolute number of NBT positive cells in the peripheral blood (numbers/µl) of healthy kids (A), pneumonic kids (B), pneumonic kids treated with levamisole(C) and kids fed aflatoxin (D)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
I	A	304.5	247.5	187.55	190.92	256.68	208.65	239.63	65.41
	B	672	791.04	1165.12	162	641.52	360.15	631.97	348.14
	C	643.2	496	154	465.12	592.5	400.68	458.58	172.96
	D	254.38	333.65	246.43	144.62	225.89	227.01	238.66	60.78
II	A	226.92	189.75	127.68	291.84	266.56	277.95	230.12	62.54
	B	337.94	1307.2	953.28	537.84	695.98	495.1	712.89	314.20
	C	777	660.1	602.3	301.7	593.4	700	605.75	163.57
	D	404.04	744.71	616.77	361.8	576.8	680.4	564.09	152.07
III	A	188.8	194.81	227.36	177.38	374.63	175.15	233.02	76.61
	B	1191.96	296.8	878.85	890.46	688.13	417.6	727.3	331.14
	C	845.6	608	1612.8	335.16	1359.75	857.52	936.47	437.40
	D	1050.94	667.59	261.8	713.7	733.7	1632.96	843.29	461.33
IV	A	186.62	202.8	208.25	296.46	255.9	273.7	237.29	44.21
	B	1111.5	449.8	792.72	269.64	303.52	538.56	577.46	322.42
	C	1992.06	506.88	1393.6	329.6	1562	1057.41	1140.26	637.62
	D	606.76	674.24	485.63	351.54	943.4	-	612.31	222.22
V	A	293.4	279.95	388	417.67	591.03	424.71	399.13	112.5
	B	1091.5	714	1353.72	738.99	749.25	420.66	844.69	327.78
	C	1897.25	1216	1398.38	777.28	1287	671.37	1207.88	445.22
	D	824.67	354.96	446.88	556.5	1080	-	652.60	296.83

(contd.)

Appendix-5 contd.

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
VI	A	440.82	326.87	274.4	223.56	301.88	231.28	299.80	79.72
	B	995.93	548.64	940.23	548.1	394.94	498.3	654.36	250.0
	C	1589.84	393.89	3060	212.4	534.66	499.2	1048.33	1098.40
	D	731.38	351	373.66	486	1047.6	-	597.93	293.18
VII	A	294.03	333.6	285.6	225.45	246.27	269.1	275.68	38.0
	B	429.66	1360.59	759.33	889.46	387.01	230.88	676.16	415.57
	C	1529.28	505	617.31	588.2	186.3	-	701.22	496.02
	D	506.25	378	340.3	426.56	488.92	-	412.78	71.62
VIII	A	293.75	252	244.64	265.65	372	243.66	278.62	49.4
	B	600.78	689.92	1057.77	680	-	-	757.11	204.37
	C	874.2	532.95	-	-	-	-	703.58	241.3
	D	427.84	261.49	463.68	226.2	414.4	-	350.72	107.13
IX	A	235.2	285.65	213	274.9	423.35	324.16	292.71	74.95
	B	492.1	952	314.93	621.76	-	-	595.20	269.05
	C	364.24	303.98	-	-	-	-	334.11	42.61
	D	301.5	190.5	227.77	241.65	431.25	-	278.53	94.20

R_1 ..... R_6 - Serial number of experimental animals in each group

I ..... IX - Period (Interval of the period is three days)

SE - Standard error

APPENDIX-6

Concentration of gammaglobulin in the serum (mg/ml) of healthy kids (A), pneumonic kids (B), pneumonic kids treated with levamisole (C) and kids fed aflatoxin (D)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SE
I	A	20	24	22.5	21.5	20	24	22.0	1.82
	B	36	16.5	36	37.5	43	48	36.17	10.73
	C	36	40	44	47	43	47	42.83	4.26
	D	21	25.5	21.5	29	20	21.5	23.08	3.46
II	A	22.5	27	25.5	21.5	24	22.5	23.83	2.09
	B	41.5	36	16.5	35	39	52	36.67	11.61
	C	31	49.5	35	48	43	43	41.08	6.68
	D	35	31	33.5	32	37.5	27	32.67	3.01
III	A	21.5	29.5	21.5	24	20	24	23.42	3.37
	B	47	24	37.5	27	45.5	37	36.33	9.37
	C	37.5	35	28	40	47	32	36.58	6.61
	D	53	40	41.5	44	40	28	41.08	8.05
IV	A	22.5	25.5	27	21.5	21.5	25.5	23.92	2.38
	B	62.5	29.5	51	41.5	37.5	36	43.0	11.9
	C	43	43	44	39	48	41.5	43.08	2.97
	D	47	37.5	47	53.5	32	-	43.4	8.55
V	A	37	28	25.5	35	35.5	37	33.0	4.97
	B	44	25.5	48	31	31	36	35.92	8.58
	C	53.5	43	47	37	44	49.5	45.67	5.71
	D	45.5	44	39	57.5	35	-	44.2	8.52

(contd.)

		R_1	R_2	R_3	R_4	R_5	R_6	Mean	SD
VI	A	29.5	29.5	31	25.5	29.5	31	29.33	2.02
	B	29.5	24	28	31	36	37.5	31.0	5.05
	C	32	29.5	35	47	32	35	35.08	6.2
	D	41.5	48	43	53.5	36	-	44.4	6.65
VII	A	31	27	32	29.5	21	29.5	28.33	3.97
	B	32	27	37.5	36	60	48	40.08	11.99
	C	29.5	41.5	32	41.5	43	-	37.5	6.25
	D	41.5	44	44	49.5	39.5	-	43.7	3.75
VIII	A	31	33.5	29.5	28.5	32	26.5	30.17	2.58
	B	31.5	24	40	41.5	-	-	34.25	8.13
	C	45.5	36	-	-	-	-	40.78	6.72
	D	40	44	44	51	43	-	44.4	4.04
IX	A	29.5	31	32	31	29.5	29.5	30.42	1.07
	B	41.5	22.5	45.5	43	-	-	43.0	10.55
	C	47	48	-	-	-	-	47.5	0.71
	D	40	36	45.5	48	48	-	43.5	5.32

R_1 ..... R_6 - Serial number of experimental animals in each group
I ..... IX - Period (Interval of the period is three days)
SE - Standard error