IMMUNOLOGICAL STUDIES IN PATIENTS WITH HOOKWORM INFECTION IN ZARIA

BY

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D E C L A R A T I O N

The author hereby declares that the work presented here for the Master of Science Degree in Microbiology was conducted solely by herself under the supervision of Dr. A.A. Ahmad (of the Microbiology Department, Faculty of Science) and Dr. G.C. Onyemelukwe (of the Department of Medicine, Faculty of Medicine), Ahmadu Bello University, Zaria.

No part of it has been presented for any award or degree and reference to published literature is adequately acknowledged.

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This thesis meets the regulation governing the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to Scientific knowledge and literary presentation.

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D E D I C A T I O N

This work is dedicated to the Nigerian Hookworm patients without whom this work could not have been possible and to the hope of the eradication of this disease from Africa and indeed the world in the nearest future.

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ABSTRACT

Peripheral blood mononuclear cells from 57 Nigerian patients with (Necator americanus and Ancylostoma duodenale) hookworm infection were examined for lymphocyte subpopulations, leucocyte response to P.P.D., for immunoglobulins (IgG, IgM, IgA and slgA) and complements (C and C,) and for the effect of antihelminthic treatment with mebendazole and lavamisole on the lymphocyte populations. Anaemia was assesse.i in terms of hypochroraia and level of haemoglobin. The patients were divided into five groups comprising those with (a) marked anaemia (b) mild to moderate anaemia (c) no anaemia (d) hookworm iniection alone and (e) hookworm and other parasites.

In comparison with a group of 2/ healthy subjects as controls, VBC counts were aigniiicantly elevated in ail the subgroups ($P < 0.05$). A significant eosinophilia was also observed in all the subgroups ($P < 0..5$). Differential leucocyte and neutrophil counts were not significantly difierent fron the controls before and after treatment. From the T-cell compartment, anti-Leu U positive (total) T-cell3 and anti-Leu-3a positive (Helper/Inducer) T-cells were significantly depressed in all the subgroups compared to controls ($p < 0.001$). The difference in anti-Leu-2a positive (suppressor/cytotoxic) T-cells between patients and controls was not significant ($P < 0.1$). Leucocyte migration responses to purified protein derivative (F.P.D.) was also significantly recuced in all tne subgroups ($P < 0.005$).

IgA was significantly elevated in patients with anaemia of mild to moderate severity and with hookworm only (P <0.05) while slgA was significantly elevated in all the subgroups as compared to the controls. IgM was significantly elevated in patients with marked anaemia, in patients without anaemia and in those with hookworm and otner parasites (P <0.05). However, the difierences in igG levels between patients and controls were not significant (P >0.l). C4 was significantly elevated in patients with marked anaemia, mild to moderate anaemia and in those with hookworms only while C3 levels were not significantly different in the subgroups when compared to the controls.

In 2o patients eff'ectively treated with mebendazole and levamisolo, a significant partial reversibility of the lymphocyte alterations was observed after 4 weeks of treatment (P < 0.05).

WBC counts were reduced, while anti Leu-4 and anti Leu-3a cells were significantly elevated compared to pre-treatment values in all the subgroups (P <0.05).

These results show that hookworm infection in Nigerian patients is characterized by depressed total and helper T-cell and leucocyte responses which are partially restorable by treatment with levamisole and mebendazole and which might be responsible for the prolonged life of hookworms in man.

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CHAPTER ONE

INTRODUCTION

The duration of infections with hookworms in man has remained a controversial subject with reports varying in their estimates from 2 to 4 years under the re-infection conditions encountered in the field (Anderson and May, 1985) to 15 years in the case of an experimental self-infection (Palmer, 1955; WHO, 1981). Nevertheless, acquired immunity to hookworms has been difficult to demonstrate and humans living in endemic areas, may often expect to carry worms throughout life (Anderson and May, 1982). However, Josimovic-Alasevic et al.(1988), insist that since hookworm infections often lead to chronic disease, they can serve as models for the study of the long term effects of continuous challenge of the immune system by defined antigens.

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The hallmark of hookworm infection, is iron deficiency anaemia. However, the development of anaemia is very much dependent on the ability of the host to resist infection (Gilman, 1982). This resistance to infectious diseases has been related with the immune system (Ernesto-Mendes, 1981). There are several ways by which an immune response can influence invading organisms and these can be divided into three categories. The first category mediated by cells include effector mechanisms which involve cells exclusively. For example, the direct cytotoxic action of lymphocytes and macrophage participation (Roitt, 1985). The second mechanism comprises the effects that can be attributable to antibodies and or other serum components. These include essentially, lysis and antigenic modulation (Chapel and Haeney, 1984). The third way involves those situations where both types

of factors cooperate, as in the enhanced phagocytosis and perhaps digestibility of opsonized particles (Ernesto-Mendes, 1981).

Research on the immunology of hookworm and other parasitic infections has three outstanding characteristics (Rowe, 1980):

a). Complexity, due to the fact that successful parasitism involves the interweaving of intricate mechanisms of host and parasite,

b). Fascination, in view of the many insights into fundamental immunologic, genetic and biochemical processes involved and

c). Importance, since parasitic infections constitute an enormous burden of disease on mankind with social and economic consequences which are difficult to exaggerate.

Of the available epidemiological evidence on hookworm infections, it is not known whether multiple infections under natural conditions confer protective immunity in humans. Under laboratory conditions, repeated infection with small doses of *N. americanus* larvae produced infection each time. There was however no decrease in worm load (Ogilvie *et al.*, 1978). Rises in IgE and IgG antibody levels have been recorded and antibody to hookworm acetylcholinesterase has also been found. The epidemiologic curve of hookworm infection suggests the presence of some degree of immunity, although this is of short duration (Gilman, 1982). High worm burdens were found in children and the elderly but reductions

in worm burdens were seen in young and middle aged adults (Miller, 1979). Functional protective immunity has not been demonstrated in humans under experimental conditions and there is the possibility that both multiple and chronic hookworm infections, as occur in nature, may be needed for protective immunity in immunocompetent hosts (Gilman, 1982).

In rats, it has been demonstrated that Nippostrongylus brasiliensis is expelled two to three weeks after infection. However, rats with iron and protein deficiencies do not efficiently expel the worms (Pritchard, 1988). Expulsion is said to be a combined effort of antibodies, T-lymphocytes and B-cells (Gilman, 1982). Expulsion may also be avoided or delayed by giving multiple small infections or by infecting young rats (Schad and Page, 1982). The possibility of the occurrence of immune evasion has been suggested, with the observation of the so-called "adapted" worms that develop in immune hosts. By comparison with normal adult worms, adapted worms are less antigenic, less quickly expelled, stunted and have a typical acetylcholinesterase isoenzyme pattern (Pritchard, 1988). Local anaphylaxis has also been suggested as one of the mechanisms responsible for expulsion of N. brasiliensis (Bloch and Malveaux, 1985).

AIM AND OBJECTIVES OF THE STUDY

Although parasitic infections are now attracting increasing attention from immunologists, the knowledge available is patchy and often inadequate, especially as a basis for further research needed for disease control (Rowe, 1980). The above information in rats and man of hookworm infection,

provide a point of departure for more defined studies on the immunological response to hookworm infection in man. However, the rodent-nematode model does not closely mirror the human reaction to hookworm. Thus there is the need to discover and develop an animal model that closely resembles the human situation. There is also the need to study closely hookworm infection in man in order to:

a). increase our knowledge of the state of human immunity to hookworm infection.

b). help in the planning of effective therapy for hookworm infection and

c). aid in the development of a vaccine which can be used to eliminate hookworm infection.

The aim of this study was:

i). to determine immunoglobulin and Complement levels in hookworm patients as a means of estimating the humoral immune response to the infection.

ii). to monitor absolute and relative numbers of T-lymphocytes and their subsets (T-suppressor 'Leu-2a' and T-helper 'Leu-3a' cells) in these patients with a view to observing any impairment in cellular immunity.

iii). to assess leucocyte function in hookworm infection, by detecting in vitro leucocyte migration to purified protein derivative (P.P.D)

iv). to study the alterations in T-lymphocyte and their subsets in these patients after treatment with levamisole (KETRAX I.C.I) and mebendazole (VERMOX JANSSEN) in order to assess their contributions to the immune response.

v). to determine re-infectivity rates after treatment.

CHAPTER TWO

LITERATURE REVIEW

2. Definition

In man, the consequences of hookworm infection may be conveniently separated into primary and secondary effects. Those associated mainly with the migration of infective larvae and the presence of adult worms are designated 'primary' signs and symptoms (Miller, 1979). These usually constitute the term hookworm 'infection'. The signs and symptoms which are consequences of physiological, biochemical and haematological disturbances, following persistent intestinal infection are designated 'secondary' effects (Oyerinde, 1982). They represent the common term, hookworm 'disease' (Miller 1979). However this latter term refers to a variety of disorders often associated with so many concomitant factors, which are not necessarily a direct consequence of the presence of intestinal hookworms.

2.1. Hookworm Infection and Disease

Hookworm infection and its association with anaemia, has been recognized since the middle 1800's (Carr and Pritchard, 1986). The two major hookworm species in man are *Ancylostoma duodenale* and *Necator americanus* (Keymer and Bundy, 1986). These worms are responsible for chronic gastrointestinal tract infections in 800 million people throughout the world (Schad and Anderson, 1985). The effects of the disease are not dramatic, since mortality is

relatively uncommon. Anaemia and the resultant decrease in worker productivity are both common and chronic and lead to a prolonged loss of economic productivity (Pasta et al.,1979). Evidence of slow learning and apathy in children with significant hookworm infections has also been demonstrated. Due to these chronic effects, hookworm disease may produce substantially more damage to the health of a nation, than would a more acute disease with higher mortality rates (Gilman, 1982).

Despite these facts, interest in hookworm infection, has been declining as evidenced by its omission from the World Health Organization (W.H.O.'s) list of the six major tropical diseases of the world (Quartely Bibliography of major Tropical Diseases, UNDP/WHO, 1982). There has also been no recorded attempts to identify immunomodulatory factors produced by these worms or to stimulate protective immunity using either attenuated larvae, worm homogenates or purified antigen (Carr and Pritchard, 1986).

The established adult worms of hookworms in man, appear to be resistant to the immune defence mechanisms and to persist for very long periods of time. However, available evidence suggests that the worms are immunogenic but survive despite the induction of an immune response (Ogilvie and Worms, 1976).

2.2. History

Hookworm infection was described in Italy, Arabia and Brazil even before the discovery of Ancylostoma duodenale (the Old World hookworm) in 1838. Necator americanus (the New World hookworm) was not described until 1902 and was reportedly brought to the United States of America with the importation of slaves from West Africa (Brown and Neva, 1975). Between 1877 and 1900 metamorphosis of the free-living rhabditiform larvae and the etiopathology, symptomatology, diagnosis and therapy of hookworm infection were established. In Nigeria, both Ancylostoma duodenale and Necator americanus have been reported (Oyerinde, 1978; Oyerinde, 1982). Necator americanus is however more common, appearing as 83% of the total hookworm infection in Northern Nigeria (Ramsay, 1934; 1935), 69% of the infections in Lagos and 100% of infections in Akufo, Ibadan (Stoll and Hsieh, 1962).

2.3. Prevalence and Significance

The geographical distributions of human hookworms is limited because the larvae require temperatures above 10°C and plenty of moisture to survive. Ancylostoma duodenale is subtropical and does not survive in the northern hemisphere above 52° latitude (Brown and Neva, 1975). The optimum temperatures suitable for Necator americanus are higher, in the range of 25-28°C (Miller, 1979; Hominick, Dean and Schad, 1987). N.americanus

has a wider geographical distribution compared to A. duodenale, but the distribution of both species overlap. Mixed infections are common although, there appears to be little competition between the two worms when they are both present in the same host (Gilman, 1982).

Hoagland and Schad (1978), comparing the life history parameters of A. duodenale and N. americanus found the former to be more of an opportunist owing to its ability to infect by both the percutaneous and oral route. N. americanus infects only percutaneosly (Edington and Gilles, 1961). Of the adult worms, A. duodenale has a shorter life span, a greater ability to arrest its development and a higher production of eggs (Gilman, 1982). N. americanus on the other hand, causes five to ten times less blood loss and seems better equipped to parasitize humans due to its longer life span (Keymer and Bundy, 1988). Black people are less susceptible to N. americanus than are whites living under the same unsanitary conditions. Nutrition may play some part since black people appear to be better nourished than their poor white neighbours (Schad and Anderson, 1985). Also, since this parasite appeared early in Africa, there may have been some natural selection (Brown and Neva, 1975).

The world prevalence of hookworm infection in man has been shown by Anderson and May (1982), to exceed the prevalence of almost all other helminthic infections and other individual diseases with exception of the common cold. This situation has been improved in developed countries where hookworm disease but not necessarily hookworm infection has been almost eradicated (Miller, 1979). However, improvement in developing

countries, are almost nullified by other problems such as population expansion, which by analogy with the well established association in the veterinary context, between epizootic parasitism and population density, often lead to a resurgence of parasitic infection and disease. Views on the significance of hookworm infection in man generally vary. Ansari (1971), omitted it from the list of the considered five most important parasitic diseases of man. Ettling (1981), concluded that hookworm infection may be the one link to explain the retardation of backward people. However, Howe (1980), is of the opinion that hookworm infection, as one of the global parasitic infections is important as it constitutes a largely unappreciated but enormous bulk of disease on man and domestic animals.

2.4. Morphology

Adult hookworms are small, cylindrical, fusiform, grayish white nematodes (Edington and Gilles, 1981). The females 9 to 13 by 0.35 to 0.6mm) are larger than the males (5 to 11.0 by 0.3 to 0.45mm). A. duodenale is larger than N. americanus (Hsieh, 1971). Both possess the relatively thick cuticle which is characteristic of the species, however N. americanus has a more prominently striated cuticle (Hsieh et al., 1972). There are single male and paired female reproductive organs. The posterior end of the male has a broad, translucent, membranous caudal bursa, with rays which are used for attachement to the female during copulation (Brown and Neva, 1975). The major morphologic difference in the species are

usually detected in the shape of the worms, the buccal capsule and the male bursa. Hence the bursa is very important in the morphology of the hookworms. The vulva is located anterior to the middle of the body in Necator and posterior in Ancylostoma.

The eggs of the species are almost indistinguishable, differing only slightly in size: N. americanus 64 to 76 by 36 to 40 u and A. duodenale 56 to 60 by 36 to 40 u (Brown and Neva, 1975). Of the infective larvae, N. americanus larvae have a more developed boring spine which is useful for penetrating the skin than do A. duodenale larvae. This morphologic difference may be partly responsible for the higher percentage of N. americanus that successfully complete the migratory life cycle to become adults (Gilman, 1982).

Bonner (1979a), in studying Nippostrongylus brasilensis larvae in rats, gives an insight into the ultrastructure of the rhabditiform larvae of hookworms. The intestinal tract has an absorptive structure with a well developed layer of microvilli. This is very suitable for energy synthesis and growth (Banwell and Schad, 1978). The intestinal cells contain abundant rough endoplasmic reticulum and ribosomes (Bonner, 1979b). When these rhabditiform larvae moult, the old cuticle is kept as a detached sheath over the new, third stage cuticle and the larvae become filariform or L_3. Ultrastructural examination of the L3 intestine reveals narrow cells that have few microvilli and lack synthetic structures. The lumen of the intestine comprises membranous whorls and lipid droplets. These features explain the failure of the filariform larvae to incorporate

significant levels of radioactive uridine into RNA due to a lack of synthetic activity. When the larvae infect their host, synthetic activity is commenced and the intestinal cells are seen to contain ribosomes and endoplasmic reticulum (Bonner, 1979b).

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2.5 Life Cycle of Hookworms

The life cycles of all hookworm species are direct (Anderson and May, 1982). The ova hatch in one or two days in aerated moist soil at optimal temperatures of 22 to 33°C. They produce rhabditiform larvae which feed on bacteria and faecal matter (Brown and Neva, 1975). The rhabditiform larvae pass through a 7 to 10-day free-living cycle in the soil, moulting twice to become the sheathed 'filariform' larvae which are infective to man (Ellington and Gilles, 1981). Hookworm filariform larvae are restricted in their lateral movements and usually remain close to the area where the stool is deposited. They move vertically upwards through the soil to the surface upon attraction by contact (thigmotaxis), temperature, oxygen and carbon dioxide. In a suitable environment, the larvae remain infective for several months (Banwell and Schad, 1978). However, under tropical conditions, 90% of the larvae die in the first 3 weeks (Gilman, 1982). A duodenale worms, during unfavourable seasonal conditions, produce dormant larvae. In Bengal, larvae acquired during the rainy season of one year appear to remain dormant until just before the monsoon of the following year when they resume development and mature (Schad and Page, 1982). In patients given A. duodenale infection, the phenomenon of dormant larvae was further corroborated by unusually long prepatent periods

(Nawalinski, Schad and Chowdhury, 1978). Consequently, there is observed, a decrease in total output of eggs by the worms (Nawalinski, Schad and Chowdhury, 1978). There has been no report on production of dormant larvae in N. americanus infections.

Man is infected by larvae penetrating his skin. The usual site of infection is the dorsum of the feet or between the toes. Miners and farmers may acquire the infection on the hand and fishermen have been infected by sitting on infested stream banks (Brown and Neva, 1975). A. duodenale infects orally when present on vegetables grown in faecally contaminated areas (Odugbemi et al.,1982). There has been reports of auto-reinfection in a number of patients due to the occurrence of adult worms in submucosal closed cysts in the human intestine (Schad and Anderson, 1985). Hookworm infection has also been recorded in children under 12 months old (Odugbemi et al.,1982). Views put forward by workers to explain the presence of the parasite include prenatal or transmamary infection (Nwosu, 1982), mechnical transfer by flies (Oyerinde, 1976) and contamination of the infants feed with hookworm eggs (Odugbemi et al.,1982).

The larvae migrate by entering the lymphatics or venules and are then carried in the blood through the heart to the lungs where, they are unable to pass through the capillary barrier. They break out of the capillaries into the alveoli, and ascend the bronchi and trachea, where they are swallowed and passed down into the intestines. This takes about one week (WHO, 1987). During this period the larvae undergo a third moult and

acquire a temporary buccal capsule, which enable the adolescent worms to feed. After a fourth moult, mature egg laying females are produced in 5 to 6 weeks after infection. The adult worms live in the upper part of the small intestine, mainly in the jejunum (Faust, Beaver and Jung, 1975). One female Ancylostoma produces about 30,000 eggs and one female Necator about 9,000 eggs per day (Edington and Gilles, 1981).

The length of time from infection to first appearance of hookworm eggs in the faces of the host vary within the species but are relatively constant for each species (WHO, 1981). N. americanus in man has one of the longest prepatent periods (about 44-100 days) (Miller, 1979). Various host influences (for example, age resistance) have been shown to delay worm maturation. Species of host, has been demonstrated to influence hookworm development by either complete rejection of the parasite, reduced success in establishment or restriction of worm growth (Miller, 1979).

2.6 Parasite Antigens

There is no doubt that parasite surface antigens generate an immune response that is capable of acting against parasites in - vitro and in-vivo (Philipp, Parkhouse and Ogilvie, 1980). For example, monoclonal antibodies have been directed against surface determinants of S. mansoni schistosomula and shown to mediate in-vitro killing of larvae (Tavares et al.,1983). Also, using the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), Pritchard et al.(1985) were able to obtain significant quantities of relatively clean

surface antigens of Nematospiroides dubius. The material released by CTAB was able to retain its antigenicity as shown by immunoprecipitation with post infection sera (Pritchard et al., 1985). The latter report was in response to the quest for purifiable material for bioassay of parasite surface antigens. In 1986, Pritchard et al, studied the surface antigens of adult Necator americanus and found them to be recognizeable by post infection hamster sera. The antigens were also recognized by a range of human sera, and this added support to the relevance of the hamster model to the human situation. The major cuticular antigens resolved at molecular weights 93000, 67000, 56000, 43000, 32000 and 25000. Utilizing immunoblotting experiments, long term infection (117 days) hamster serum recognized several somatic and ES antigens of adult N. americanus (Carr and Pritchard, 1986). Many of these were strongly immunogenic but of minor protein species. They were also consistently predominant in the Excretory-Secretory (ES) products of the hookworms (Carr and Pritchard, 1986). These characteristics of the resolved antigens could be explained on the basis of the hypothesis by Ogilvie, Maclaren and Worms (1975), that nematode antigens are actually enzymes produced by the excretory, secretory, amphidial and eosophageal glands of hookworms. Examples of these antigens include an acetylcholinesterase by N. americanus and a protease by Ancylostoma caninum (Ogilvie, Maclaren and Worms, 1975) which cleaves IgA. N. americanus haemoglobinase, a 'feeding enzyme' is another antigen being studied (Pritchard et al., 1988).

2.6.1 Hookworm Acetylcholinesterase

This enzyme is found in the oesophageal and amphidial glands of the worms from where it is actively synthesized and released by the worms in vitro (Ogilvie, Maclaren and Worms, 1975). The Necator acetylcholinesterase enzyme is membrane-bound within the worm and is secreted as a rather large molecule; while that of Ancylostoma is present in a soluble form. All animals infected with acetylcholinesterase producing worms produce specific antibodies against the enzyme. For example, IgG class antibodies to N. americanus acetylcholinesterase have been found in a man infected for nine years with this hookworm (Ogilvie, Maclaren and Worms, 1975). Antibodies to Necator acetylcholinesterase were also detected about 12 weeks after the second infection in a self-infected person over a 27- month period. The level of this antibody was however, observed to fall following worm removal (Ogilvie et al., 1978).

There is a sharp increase in the level of acetylcholinesterase produced during the period between infection and gut invasion by the worms. In Nippostrongylus brasiliensis, acetylcholinesterase activity per unit weight of worm increases almost four fold during the first three days of infection in rats (Sanderson and Ogilvie, 1971). For those species which adopt the secretion of acetylcholinesterase, it has been speculated that the enzyme secretion has survival value for the parasite (Philipp, 1984). For example, Ogilvie and Jones (1971), put forward the hypothesis that,

acetylcholinesterase (AChE) may act as a "biochemical holdfast" of the worms, by inhibiting locally the peristaltic movements of the gut during the expulsion process. Ogilvie, Maclaren and Worms (1975), also argue that in the advent of infection with more than one acetylcholinesterase producing worm, there might be an impairment of the specificity of the antibody production, rendering it ineffective. However Philipp (1984), suggests that in addition to the above mentioned effects, the enzyme may delay worm expulsion by interfering with the process of mucus excretion by intestinal goblet cells. This interference is achieved by the exertion of an effect on the acetylcholine of the gut lumen which is responsible for the induction of mucus secretion (Philipp, 1984).

Since the fourth - stage larvae, immature fifth stage and adult worms but not infective third stage larvae of nematodes secrete acetylcholinesterase, the antibodies to this enzyme are a measure of the response to the adult worms but not the infective larva (Ogilvie *et al.*, 1978). Hence, any immune response induced by the migration of the infective larvae, cannot be effectively assessed by measurement of antibodies to acetylcholinesterase.

The antibodies to acetylcholinesterase are of the IgG_1 subclass and they affect production of the enzyme by the worms (Rhoads, 1984).

2.7 PATHOLOGY - AT THE INVASIVE STAGE

2.7.1 Signs and Symptoms due to Infective Larvae

The first symptom after cutaneous penetration is a dermatitis known as 'ground', 'Coolie' or 'dew' itch (Adetuyibi, 1980). There is intense itching, oedema and erythema and later a papulo-vesicular eruption which may last for up to two weeks. A similar reaction is also produced in the form of a bronchitis or pneumonitis in the lungs if considerable numbers of larvae migrate (Edington and Gilles, 1981). This cutaneous reaction of the host has however not been well defined immunologically. The fact that bacteria too can intensify and extend inflammatory reactions has been confirmed by the production of little or no reaction by axenic larvae of N. americanus when they penetrate the skin (Miller, 1979). In endemic areas, these early symptoms either pass unnoticed or are rare. In contrast to the consequences of infection with some abnormal species of hookworm eg. Ancylostoma brasiliensis, infections with human hookworm rarely produce the serpentine tracks known as cutaneous 'larva migrans' (Gaillard, 1974).

2.7.2 Signs and Symptoms due to Migratory Larvae

The pulmonary migratory phase of human hookworm infection has not been associated with significant clinical symptoms but the following stage which entails the movement of larvae through the upper respiratory tract shows a lot of primary signs and symptoms. Many of which include coryza, pharyngitis, laryngitis, and

discomfort and pain in the throat when speaking and swallowing (Faust, Beaver and Jung, 1975). At this time, larvae may be recovered from the sputum in infected patients (Faust, Beaver and Jung, 1975). These upper respiratory signs may start as early as three days and continue for two weeks after infection, for example Loeffler's syndrome and asthmatic symptoms.

2.7.3 Signs and Symptoms due to the adult worms

The mature worms usually inhabit the small intestine, but may be found as low as the ileum when crowding results due to heavy infections (Kalkofen, 1974). These adult worms secrete an anticoagulant and an acetylcholinesterase, but it is not known whether cytolytic enzymes are secreted into the tissues (Croll, 1976). Usually symptoms due to the adult worms do not develop until the onset of anaemia (WHO, 1981) and they are usually abdominal. Of the abdominal symptoms recorded, epigastric pain has been a uniform occurrence (Miller, 1979). Other symptoms which follow this abdominal pain include depressed appetite, indigestion, colicky cramping, nausea, vomiting, flatulence, persistent aching of the stomach when it is empty and diarrhoea (Miller, 1979). Other less frequent symptoms are indigestion, dysentary and constipation (Gilman, 1982; Oyerinde 1982). Pain in hookworm infection has often been reported as extremely severe and has been confused with that which accompanies duodenal ulcers, cholecystitis and gastric carcinomas. This is not too unexpected as radiological finding in hookworm infection and ulceration reveal many close affinities (Miller, 1979). However, the pathology of hookworm infection in

human is less clear because of the inclusion of cases from the tropics where tropical enteropathy is common or endemic. In areas where tropical enteropathy is not endemic, intestinal biopsies of individuals with heavy hookworm infestation do not show any significant mucosal changes (Banwell and Schad, 1978).

The above mentioned symptoms such as the effects due to the migration of larvae through the lungs and the settlement of adult worms in the upper intestine are consequences of the direct damage to the host by the larvae and adult worms. Rarely has any major fatal consequence due to these activities been reported (Miller, 1979; Gilman, 1982). However the most important pathological features of hookworm infection are those due to:-

i). anaemia and iron loss,

ii). hypoalbuminaemia and

iii). the cardiac insufficiency and congestive failure due to the former two features (Miller, 1979).

2.8 PATHOLOGICAL CHANGES FOLLOWING THE INVASIVE PHASE

2.8.1 Hookworm Anaemia

Anaemia in hookworm infection can be described as a primary symptom considering the fact that it is directly a consequence of the blood-sucking activities of the worms. However, it can

also be a secondary sign as it can be affected by the treatment of the anaemic status of the patient, (that is, iron therapy) without removal of the hookworms (Miller, 1979 ; Michaelsen, 1985). Anaemia as a primary sign is acute and haemorrhagic but rarely occurs in humans. (Garcia Salas et al.,1971). While iron reserves last it is normochromic and hyperplastic. Anaemia in hookworm infection is much more common as a chronic iron-deficiency anaemia. The occurrence and extent of this anaemia depending on a complex interplay between host and parasite. The anaemia unlike the acute anaemia described above is progressive, microcytic and hypochromic (WHO, 1981). Chronic iron - deficiency anaemia develops as a result of exposure to hookworm infection or re-infection and manifestation of the anaemia may take months or years (Roche and Layrisse, 1966). In cases where the infection is low (as observed by worm load) and dietary intake and iron load are adequate, hookworm infection, may remain for long periods of time without any evidence of significant anaemia (Banwell and Schad, 1978). Hence, hookworm infection is present in many areas of the world where anaemia may be rare as is the case in the more advanced nations of the world (Martin, 1972; Miller, 1979). The pathogenesis of chronic iron - deficiency anaemia is dependent on the iron content of the patients diet, the state of his iron reserves and the intensity and duration of the infection (Miller, 1979). Hence, these factors comprise hookworm mediated iron loss and other losses of iron from the patients body. These factors are not the same in every country and must therefore always be taken into account for a proper evaluation of the infection. For instance, in the Kweneng District of Botswana, anaemia was not prevalent even though hookworm

infection is endemic (Michaelsen, 1985). This was said to be because malaria is not endemic in the area and the children have a high iron intake from the diet of sorghum as a staple food. Sorghum has a high iron content and is also cooked in iron pots (Michaelsen, 1985). In Nigeria, iron intake is also high; about 21-30mg daily. Pathologically people whose only source of chronic blood loss is hookworm infection show no evidence of iron depletion, as shown by iron - deficiency anaemia, unless they harbour more than 800 worms (Gilles, Watson-Williams and Ball, 1964). On the other hand, in Mauritius, with a total food iron content of 5-10mg daily, even moderate hookworm loads of less than 800 worms were sufficient to cause blood loss leading to anaemia (Stott, 1961). Evidence from these studies indicate that anaemia will not develop if stores of iron can be maintained (Gilman, 1982). While the development of iron - deficiency anaemia is rather slow and silent its reversal and cure can be achieved very rapidly in the absence of any complicating intercurrent conditions. The anaemia and resulting iron loss will occur faster and even at lower worm burdens in growing children who have increased iron requirements and menstruating women who have increased loss of blood than in adult males (Roche and Layrisse, 1966). Pregnant women with an increased demand for iron are also more vulnerable to hookworm anaemia (Gilman, 1982).

The pathological changes due to the anaemia are many. Depending on the degree of anaemia which may be as low as 2gm of haemoglobin per 100ml of blood, the patient experiences dyspnea on exertion, and weakness and dizziness (Gilles, 1985). It is surprising however that with

haematological measures close to these values, the patient is still up and about. This indicates that since blood loss is minimal although chronic, physiological compensation by the circulatory system may permit active participation in exercise and even work by severely anaemic patient having hemoglobin values as low as 4% (Cook, 1980). Severe and potentially fatal circulatory failure, sudden death associated with stress factors such as second stage labour and overload of a hyperkinetic circulation after blood transfusions have been associated with hookworm anaemia patients (Gilles, 1985). The appetite may be enormous or poor and associated with pica. Heavily infected children may be physically, mentally and sexually retarded. Eosinophilia and leukocytosis are marked. The stools may contain gross blood and occult blood is readily found (Layrisse, Linares and Roche, 1965). Early interruptions of pregnancy and neonatal deaths have been associated with the anaemia and it has been found to be the most important cause of repeated abortions and miscarriage in hyperendemic areas (Keymer and Bundy 1988).

In some tropical countries eg India and Colombia, there have been reports of a superadded folic-acid megaloblastic anaemia. This has often been masked by the severe iron - deficiency anaemia and it only becomes overt after partial haematological response to iron therapy (Cook, 1986). Speculations as to its pathogenesis have been left to a variety of factors which include defective folic acid absorption, deficient folic acid in the diet and increased demand on folic acid reserves (Cook, 1986).

A mechanism which may likely contribute to anaemia secondary to hookworm infection is the shortened life of the red cell in infected patients. Interest in haemolysis as a pathogenic mechanism in hookworm anaemia has been renewed since the discovery of shortened survival of ^{51}Cr - labelled erythrocytes in infected and anaemic patients (Megahed et al.,1972). However, the severity of the reduction in half - life of the red cells has been shown to be related to the chronicity of the anaemia rather than to its immediate severity. It persists after vermifuge and so is probably secondary to the anaemia or is associated with malnutrition (Roche and Layrisse, 1966).

2.8.2 Hypoalbuminaemia and Oedema

In addition to anaemia, loss of protein is another important manifestation of hookworm infection. The mechanism responsible for protein loss into the intestine are similar to those responsible for loss of iron and the protein loss is well in excess of the red blood cell loss (Gilman, 1982). Plasma is directly ingested by the worm and the ensuring breach in epithelial continuity, with the resulting leak of plasma from the injured tissue results in loss of both protein and iron (Gilman, 1982). Blackman et al.(1965), were able to establish a relationship between faecal protein loss and hookworm load. They found a daily loss of 0.1g of albumin per 100 N. americanus hookworms. However, although iron - deficiency anaemia is a hall mark of heavy hookworm infection, malnutrition has not

been found to be so (Crompton , 1986). Villarejos et al. (1970), in their studies in South America found no association between hookworm egg counts and level of serum albumin. This finding was not surprising considering the multitude of variables that affect the levels of serum albumin. For example, anaemia, which is known to affect liver function, coincidental disease of the liver, loss of appetite and failure to reabsorb aminoacids from the albumin passing into the gut (Edington and Gilles, 1981).

The aetiology of oedema is not primarily anaemia but is a direct consequence of the hypoproteinaemia and hypoalbuminaemia of chronic hookworm infections. Brown et al.(1981), found that the serum albumin concentration was often low in hookworm anaemia patients but attributed severe oedema to hypoalbuminaemia rather than to other conditions such as heart failure which may also be present. Brumpt and Hothi Sang (1955), found that oedema did not respond well to mercurial diuretics in their patients even after their anaemia had been corrected but subsided rapidly after they were dewormed. They suggested that oedema might be due to hypoproteinaemia following loss of plasma proteins in the stools and that hookworm disease could be added to the list of possible causes of protein - losing enteropathy. Oedema in chronic hookworm infection has been recorded in dependent limbs and as generalized anaesarca (Gilles, Watson - Williams and Ball, 1964), but it is relatively uncommon among young ones (Brown et al., 1981).

2.8.3 Cardiac Insufficiency and Congestive Failure

Other signs and symptoms accompany chronic hookworm infection and are associated with cardiac insufficiency and congestive failure. They can be attributed to the anaemia and hypo-albuminaema. The list includes breathlessness, palpitation, tachycardia, cardiac dilation, venous distension, systolic murmurs, angina pectoris, vertigo, weakness and fainting (Miller, 1979). Upon examination of the electrocardiograms of chronic hookworm cases, they have been found to be abnormal. Cardiac dilation has also been found as a common occurrence in profoundly anaemic chronic hookworm cases and Fowler, Knight and Patel (1968), found that reduction in anaemia correlated well with reduction in heart size.

Other changes which occur in chronic and anaemic hookworm cases include, the impairment of mental processes and aptitude. Children are often tired and unhealthy. There is dullness, confusion, poor memory and an appearance of stupidity. Thus in areas where hookworm infection and anaemia are endemic, there is much loss of educational potential (Miller, 1979). In pregnant women, complications of pregnancy such as toxaemia, pre-eclampsia and eclampsia have also been reported.

2.9 The Immune Response in Hookworm Infection

There have been comparatively few studies on the immune response to *Necator americanus* and *Ancylostoma duodenale*. The general

complaint has been on the issue of antigenic material with which to conduct the tests. In most of the tests or studies done, a wide variety of antigenic material, instead of a specific one has been obtained from the cuticle of the larvae or adult worms (Carr and Pritchard, 1986). The antigen most frequently tried out has been the infective third stage larva (Schad et al.,1975). This stage of the parasite is very easily available. However in another study, carried out by Ogilvie et al.(1978), antibody responses were measured in a volunteer infected four times with N. americanus over a 27 month period. The main source of antigen in this case was culture fluid in which adult hookworm had been maintained for several days. Antibodies were detected by the enzyme linked immunosorbent assay (ELISA) (Voller, Bartlett and Bidwell, 1976) to worm acetylcholinesterase and by the radio allergosorbent test (RAST) for IgE antibodies (Wide, Bennich and Johanson, 1967. Lewis, Salimonu and Osunkoya (1978), were able to detect using the fluorescent antibody technique, antibodies to the surface antigens of hookworm larvae. Basically, all the studies require standardization of the hookworm antigens and improvement in the specificity of the technique (Lewis, Salimonu and Osunkoya, 1978; Carr and Pritchard, 1986).

2.9.1 Production of Specific antibody in Hookworm Infection

T-helper cells are involved in the production of specific antibody during parasite infections (Cohen and Warren, 1982). For example, apart from the usual display of antibodies normally present in

hookworm infections, precipitates (immune) have been clearly demonstrated around the oral and excretory orifices of Nippostrongylus brasiliensis incubated in immune serum from rats. Ogilvie (1963), believes that these immune precipitates are no guide to immunity since they are not detected after one or two normal infections, although the rats at this stage are resistant to challenge infection. On the other hand, immune precipitates have been developed in the serum from rats infected with extracts of adult worms in adjuvants, even though, rats treated in this way are not resistant to challenge (Ogilvie, 1963).

2.9.2 NON-SPECIFIC IMMUNOGLOBULIN SYNTHESIS

2.9.2.1 (a) Secretory Immunoglobulin A (SIgA)

Intestinal and faecal IgA responses against different developmental stages of Nippostrongylus brasiliensis were studied in hooded lister rats (Wedrychowicz, Maclean and Holmes, 1984). They were not able to find any correlation between secretory IgA levels and degree of protection. However, analysis of intestinal mucosa, intestinal contents and faecal IgA specific to larval or adult hookworm antigens suggests that the IgA is probably induced by antigens common to all intestinal stages. Also secretory IgA responses against surface antigens of the intestinal L_4 (4th stage larvae) did not show any stage specificity, although this was detected in sera (Wedrychowicz, Maclean and Holmes, 1984).

In another study carried out on 30 cases of hookworm infestation in New Delhi, Oswal et al.(1979), found that secretory IgA showed significant alteration in hookworm patients as compared to normal controls. The levels of secretory IgA were significantly lower than those of control subjects. This selective lack of secretory IgA in the gut of these patients was thought to predispose them to colonization by hookworms (Oswal et al.,1979).

(b) Serum IgA

Serum IgA is the predominant immunoglobulin in seromucous secretions. Oswal et al.(1979), found a significant inverse correlation between the ova load and serum IgA levels in hookworm patients. They advocate the need for further studies to investigate whether IgA as an antibody could have acted as an immunologic barrier against hookworm larvae immediately after they had migrated from the skin to the venous circulation.

2.9.2.2 Immunoglobulin G (IgG)

IgG is the major immunoglobulin in normal human serum, accounting for 70-75% of the total immunoglobulin pool (Roitt, Brostoff and Male, 1985). Oswal et al.(1979), found that in hookworm patients, levels of serum IgG were significantly higher than those of normal controls . Similar higher values of IgG have been reported by Higashi and Chowdhury (1971) although the rise in IgG was not found to be significant. In an East African population higher

IgG levels were observed in patients with helminthiasis (Banwell et al.,1967, Higashi and Chowdury, 1971). Poulain, Luffan and Pery (1976) in their study also came to the conclusion that specific antibody activity in the serum could be found in the fraction containing IgG in rats infested with N. brasiliensis.

2.9.2.3 Immunoglobulin M. (IgM)

Raised immunoglobulin levels in parasitic diseases are common and IgM levels are especially raised in trypanosomal and malarial infections (Molineaux and Gramiccia, 1980). Very high IgM levels are reached early in the infection and it has been suggested that frequent antigenic variation of the malarial and trypanosomal parasite may contribute to the raised IgM levels (Brown and Brown, 1965).

Of the evidence so far, IgM levels are not significantly altered in hookworm infection cases (Oswal et al., 1979). However it is known that in the course of immunization to a variety of antigens, IgM serum antibodies are preferentially represented early in the response (World Health Organization, 1987). The absence of a specific rise in IgM in hookworm infection has been attributed to the disease being a chronic infestation.

2.9.2.4 Immunoglobulin E (IgE)

IgE antibodies are regularly found in large quantities in sera of humans or animals infected with helminths (Bloch and Malveaux, 1985). Some of these include infection with Ascaris lumbricoides

Toxocara Spp, Trichinella spiralis, Schistosoma japonicum, Wuchereria bancrofti and hookworm (Ito, Sawada and Sato, 1972), and that IgE might play a role in immunity was suggested by the results of Amman et al. (1969), who showed that 9 out of 11 patients with ataxia telangiectasia and recurrent sinopulmonary infections were deficient in IgE and IgA. Basically the normal range of IgE from analysis of serum from normal subjects varied over a 130 - fold range from 6 to 780mg per milliliter (Gleich, Averbeck and Swedlund, 1974). Other values with a mean of 24.8 and a range of 61 to 1000mg per milliliter have been reported (Jarrett and Miller, 1982).

The mechanisms involved in potentiating IgE production in hookworm infection are still under study, but the following have been observed:-

i). Rats infected with N. brasiliensis produce a substantial increase in the number of IgE - B cells in the mesenteric lymph nodes (Mayrhofer, Bazin and Gowers, 1976; Ishizaka, Urban and Ishizaka, 1976).

ii). T-lymphocytes in the mesenteric lymph nodes of rats infected with N. brasiliensis produce a soluble factor that stimulates IgE - B cells (Suemura, Yodoi et al, 1980);

iii). IgE-B cells produce lymphokines which recruit new IgE - B cells (Urban, Ishizaka and Ishizaka, 1977);

iv). Soluble products produced by parasites are very potent mitogens for IgE - B cells (Ogilvie and Jones, 1973).

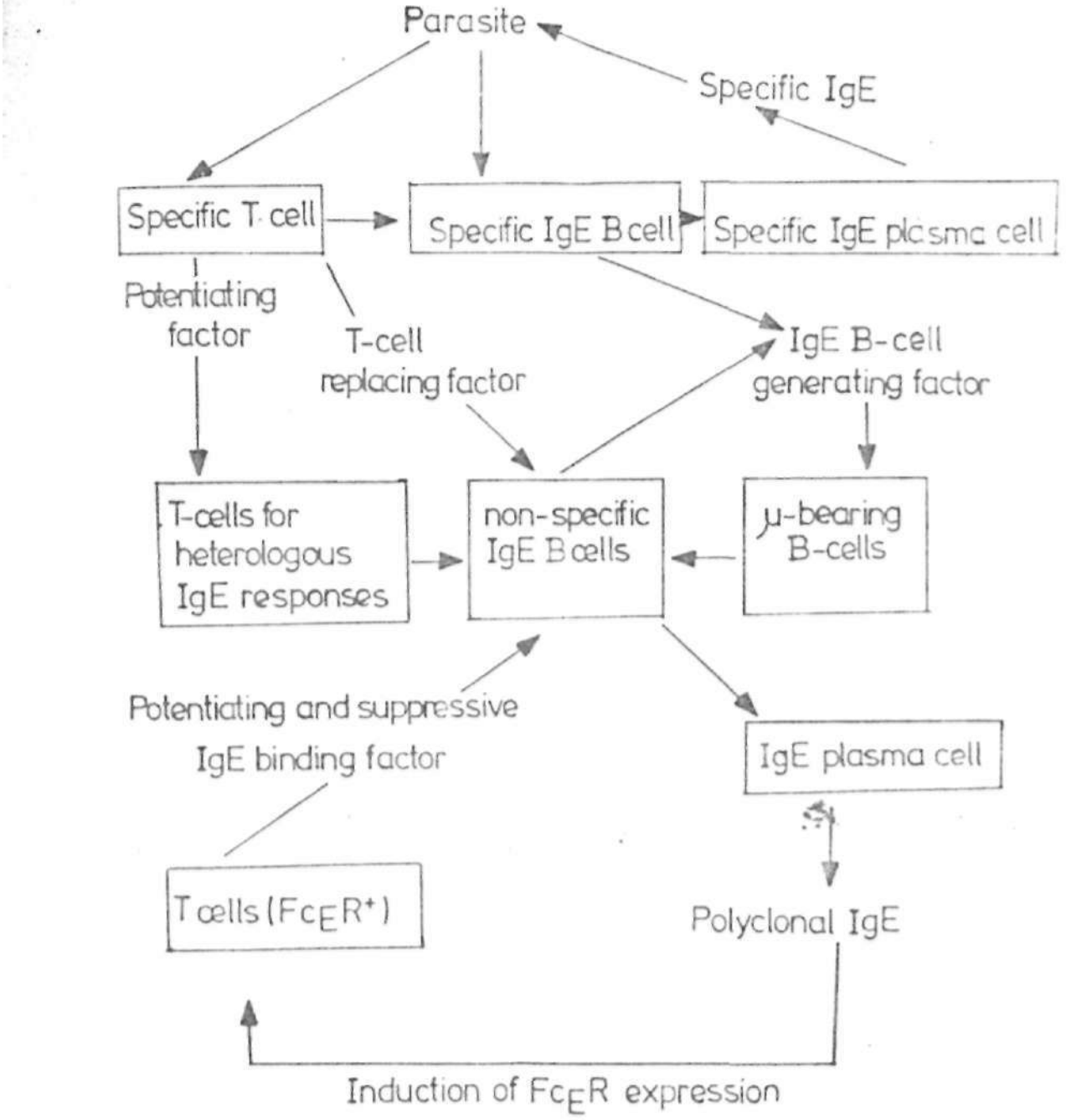


Fig. 1. Scheme for the regulation of parasite-specific and non-specific IgE in helminth infection.

The increase of IgE levels during childhood is rather slow. Adult values also are not stabilized until 15-20 years of age (Berg and Johansson, 1969). This may be due to the behaviour characteristics of the worms. For example, worms restricted to the lumen of the digestive tract do not appear to have increased specific IgE levels (Radermecker et al., 1974). In a study of a self - hookworm infected individual, total IgE levels fell after the first infection and then increased gradually after the third and fourth infections (Ogilvie et al., 1978). Specific IgE antibodies were not detected until after the third and fourth infections. This contrasts with the total IgE levels of individuals harbouring hookworm infections in endemic areas (Kojima, Yokagawa and Toda, 1972, Biroum-Noerjasin, 1973, Grove, Burston and Forbes, 1974). The results however correlate well with those of Hall et al. (1985), who found that a relationship in time exists between the elevation of total serum IgE, parasite specific IgE and the potentiated IgE response to unrelated antigen. During primary infection, the potentiated IgE and total response rise synchronously to a peak. Parasite specific IgE rises to a peak two to three weeks later (Hall et al., 1985). Obviously from the above study, even though IgE is known to be selectively elevated in hookworm and other parasitic infections (Jarrett and Miller, 1982), only a small proportion of this IgE is antiparasite antibody. In most cases, the titre of pre-existing antibody is increased but much of the IgE has no recognizable antigen specificity (Orr, Riley and Doe, 1972). The mechanisms of non-specific IgE increase may involve depletion of suppressor T-cells (Watanabe et al.,1977). It has been shown that non-specific IgE production does not occur in T-lymphocyte - deprived animals (Jarrett and Ferguson, 1974).

Grove and Forbes (1974), have reported an increased resistance to hookworm infestation in an atopic population as assessed by faecal egg counts. In animals, Jarrett and Stewart (1972), found that in addition to inducing an IgE response to helminth antigens, helminth infestation potentiates antibody responses to non-helminth antigens. These observations have led to the suggestion that high levels of serum IgE, produced as a result of parasitic infection, protect against the development of atopic disease (Jarrett and Bazin, 1974). Experimental evidence to support this has shown that rats producing a high titre of IgE antibody to one allergen show diminished passive sensitization to another (Jarrett et al., 1971). Also subjects with a high serum IgE level following hookworm infection showed a diminished Prausnitz - Küstner (P-K) reaction to guinea pig dander (Barzaal et al., 1973).

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2.9.3 THE CELLULAR IMMUNE RESPONSE

2.9.3.1 Effector Function of Macrophages

Activated macrophages are important in the control of infections caused by Tryparosoma Cruzi, Leishmania and Plasmodium spp (Mitchell, 1979). Macrophages activated by lymphokines to become tumoricidal, inhibit the multiplication of Leishmania donovani (Mitchell, 1979). During phagocytosis, perturbation of the membrane of macrophages, can induce a respiratory burst and production of oxygen metabolites which are cytotoxic for

many parasites (Nathan, 1983). These macrophages are also able to release more superoxide and hydrogen peroxide than resident macrophages (Capron, et al., 1983, Nathan, 1983). There is now increasing evidence that macrophages can exert an effector function in hookworm infection. They have been found to kill infective larvae of N. brasillensis in vitro, in the presence of complement and to enhance IgG - dependent eosinophil - mediated killing of Trichinella spiralis larvae (Mackenzie et al.,1980). Stage specific macrophage cytotoxicity for Dipetalonema vitae was also elicited by Immunoglobulin E (Ouassi et al., 1981). IgE dependent macrophage killing of schistosomula of Schistosoma mansoni has also been demonstrated (Capron et al., 1975).

2.9.3.2 The role of the Eosinophil

Eosinophilia has been observed for many years in allergies and parasitic infections (Grove, Burston and Forbes, 1974). Larvae of hookworms and other helminths such as Ascaris and Schistosoma pass through the lungs on their way to the habitat of the adult worm in the intestine or bladder venous plexus. A pneumonitis and bronchitis result with eosinophilia during this passage of the larvae through the lungs.

This syndrome has been described by various names which include, 'tropical eosinophilia' and 'Loefflers' syndrome' (Archer, 1963). It has been suggested that mast cells when activated by IgE -

specific antibodies release eosinophil chemotactic factors. Eosinophils attracted to the parasite by worm derived factors are thus stimulated to proliferate by eosinophil stimulation promotor (ESP a lymphokine) released by antigen stimulated T-cells. These stimulated eosinophils are said to act in two main ways:

i). in association with specific antibody, they kill the worm by antibody dependent cytotoxicity (ADCC) (Leading Article Lancet, 1971); and

ii). Enzymes released from the eosinophils exert a controlling influence on the substances released from mast cells (Leading Article, Lancet, 1971). Eosinophils have also been shown to phagocytose antigen - antibody complexes (Taylor and Muller, 1980). The eosinophil thus appears to be a major effector cell in helminth infections even though it is not known whether they are a direct response to antigenic stimulus or whether they are a second order phenomenon such as a consequence of the presence of free amines, antigen - antibody complexes or the release of lymphokines (Grove, Burston and Forbes, 1974). The role of the eosinophil remains obscure despite its continuous association with hookworm and other nematode infection. Eosinophilia was also notably decreased in long standing cases of strongyloidiasis (Sato et al., 1985).

2.9.3.3 Immunological Responses of the Intestinal Mucosa

Infection with helminth parasites of mucous surfaces stimulates a hyperplasia of mucosal mast cells (Hall et al., 1985). Lymphocytes of hookworm - infected rats on in-vitro stimulation with specific antigen release factors causing a pronounced mucosal mastocytosis in normal rat bone marrow (Haig et al., 1982). These T-lymphocytes induce an increase in the number of inflammatory cells and goblet cells and enhance secretion of mediators and mucus. In the presence of antibody, expulsion of worms enveloped in mucus is effected by the muscular activity that accompanies the increased secretions. Infective larvae of Trichinella spiralis can be trapped by intestinal mucus from immune rats both in-vivo and in-vitro (Lee and Ogilvie, 1980). An immunologically mediated intestinal goblet cell hyperplasia develops during primary infection of rats with Nippostrongylus brasiliensis (Miller and Nawa, 1979), and an increased turnover of mucin precedes the expulsion of these worms from the gut (Miller et al., 1981). Pre-treatment of immune rat intestines with mustard oil, which strips off the mucus layer or with cysteine or papain (mucolytic agents) permits the establishment of a challenge worm population (Miller and Huntley, 1982). Comparable hyperplastic changes have been reported in Necator americanus infection (Sheehy et al., 1962), and are said to contribute to impaired absorption of vitamin A, xylose and fat (Barriga, 1981).

2.9.3.4 T-Lymphocyte Responsiveness

Decreased T-lymphocyte responsiveness has been recorded in a variety of conditions (Edwards, Rowland and Lee, 1973), which include parasitic diseases (Weiler, Weinbaum and Herrod, 1979). There are at least two helminth infections where the lack of responsiveness has been shown to be specific for the parasite antigens and suppressor serum factors demonstrated. These are schistosomiasis (Ottesen, Weller and Heck, 1977) and filariasis (Piessens et al., 1980; Genta, Ottesen and Poindexter, 1983).

Genta et al. (1983), found that lymphocyte responsiveness to strongyloides antigen was enhanced when these cells were cultured in 10% normal human serum. Lymphoproliferative responses to other antigens and mitogens were unaffected, suggesting that serum suppressor factors may develop in chronic strongyloidiasis. In addition, they found a decreased responsiveness of lymphocytes to the T-cell mitogen phytohaemagglutinin (PHA). This was not affected by the substitution of autologous serum with normal human serum in the cultures.

In the rat, cell-mediated responses to hookworm, Nippostrongylus brasiliensis were recorded by Blumdell-Hassell (1974), and Dobson and Soulsby (1974) reported a similar response to Trichostrongylus colubriformis in the guinea pig. Taylor and Turton (1976) using a blastogenic technique , also demonstrated that an antigen prepared from the infective larvae of Necator americanus possesses the capacity to act as a specific antigen

stimulus for peripheral blood lymphocytes during a hookworm infection. These correlate well with the study of Tschudi et al., (1972), where lymphocyte transformation in Chagas disease was studied.

Further studies on cell-mediated immunity in hookworm infection, has shown that infection with N. brasiliensis in the rat influences the growth of tumours. The growth of the Walker Sarcoma tumour in rats infected with N. brasiliensis was either enhanced or suppressed according to the timing of the infection in relation to tumour growth (Schloen, 1980). Rats infected five days before tumour implantation showed complete inhibition of the tumour growth.

2.9.3.5 B-Lymphocyte Responsiveness

B-cell responsiveness has either been decreased or increased in different helminthic infections. In the investigation of the cellular basis of immunosuppression in C57B1/6 Ascaris suum infected mice, in vitro studies of the primary antibody response were conducted in mice splenocytes. Cultures from infected mice repeatedly gave reduced antibody responses to sheep erythrocytes (Crandall, Crandall and Jones, 1978). Antibody responses to a T-dependent antigen, DNP-Ficoll were also demonstrated but these were not increased. The observed serum reactivity with diverse antigens and the high IgM response to the infection seemed to indicated the presence of non-specific B-lymphocyte stimulation during this period of infection

(Crandall, Crandall and Jones, 1978). The anti-DNP response suggests polyclonal B-cell activation (Varga, Konigsberg and Richards, 1973; Coutinho and Moller, 1975).

In Nematosprioides dubius infected CBA mice, FACs analysis of lymphoid tissues indicated a substantial increase in both frequency and number of lymph node B-cells, although their proportion returned to normal the fourteenth day after infection (Parker and Inchley, 1985). The presence of these B-cell activators in A. suum and N. dubius infections were not investigated but A. suum infection is known to induce an IgM antibody response to phosphorylcholine (PC) which parallels the antibody response to DNP (Brown and Crandall, 1976). Polyclonal B-cell activation (PBA) prior to an antigenic stimulation has been reported to inhibit specific antibody responses due to a temporary loss of antigen sensitivity of B-cells (Diamantstein et al., 1976). Polyclonal B-cell activation has also been suggested as a cause of immunosuppression in other infections (Greenwood, 1974). In experimental trypanosomiasis, in which elevated IgM and immunosuppression are associated, the presence of polyclonal B-cell activation and depressed immune responses have been correlated (Hudson et al., 1976).

2.9.3.6 Cellular Changes in Hookworm Infection

Cellular changes were observed in CBA mice infected with Nematospiroides dubius. Increased cell division in spleens and mesenteric lymph nodes was evident three days after infection, reaching a peak at day 7, as adult worms started to emerge

(Parker and Inchley, 1985). These levels however returned to normal by day 14 (for spleen cells) and by day 28 (for lymph node cells). The proportion of T-cells remained unaltered in the spleen but decreased by day 7 in the lymph nodes and was still below normal at day 21 (Parker and Inchley, 1985). The ratio of mouse suppressor (Ly 2^+) to helper (L_3 T_4^+) cells showed a decrease in the lymphodes at day 7, suggesting an alteration in the balance of T-cells in favour of T-helper lymphocytes (Parker and Inchley, 1985).

Similarlly, Crandall, Crandall and Jones (1978), investigated quantitative changes in splenocyte populations during early A. suum infection. They found a decrease in cell numbers by the eight days, followed by an increase to very high numbers by the end of the second week of infection. Differential leucocyte counts of spleen cells gave an increase in blastoid cells, large lymphocytes, plasma and eosinophils, with a relative decrease in small lymphocytes (Crandall, Crandall and Jones, 1978). The thymus size was also greatly reduced on the 8th day.

2.9.4 Possibilities of a Functional Acquired Immunity

The detection of specific antibody in hookworm infection has been useful only in clinical diagnosis and in epidemiological studies. In general it correlates very poorly with the clinical immune status, indicating or suggesting that much of the specific antibody formed has no protective effect. However, some patterns in

infection such as the increase in worm burdens in the first 10 years of life, its reduction in the next 20-to-30 years and its rise again in old age support the hypothesis that age resistance and acquisition of functional immunity play a protective role (Miller, 1979). Another supporting evidence for the hypothesis occurs in the results of treatment. The rate of re-infection decreased as numbers of worms increased so that after 2-3 years the intensity of infection amounted to only 50-60% of pre-treatment levels (Miller, 1979). In the absence of any immunity, the rate of increase in hookworm burdens should be the reverse case (Miller, 1979).

Also, under conditions favourable for larvae growth and infection, the controlling factor for the hookworm disease in the Ancylostoma caninum - dog system is immunity. It is therefore also reasonable to infer such a protective mechanism in the Ancylostoma duodenale infection of man (Miller, 1979).

Arguments against the theory of functional immunity are based on the facts that incidence of hookworm infection in man is high (Michaelsen, 1965) and that it is relatively easy to re-establish infections in volunteers. However, high incidence and low intensity with no acute disease in members of a population are certaintly not an indicator of susceptibility (Miller, 1979).

Most of the information on the immune mechanisms which affects hookworms comes form the study of the response of laboratory rodents to hookworms. The model system has been Nippostrongylus brasiliensis infection in rats

(Ogilvie and Jones, 1971; 1973). The immunological control of N. brasiliensis in rats involves several effector aims of the immune response. Both antibodies and sensitized lymphocytes must affect the worms before they are expelled (Ogilvie and Worms, 1976). No essential role for IgE antibodies, mast cells or eosinophils has been defined in this system. The protective antibodies are most often IgG_1 antibodies (Ogilvie and Worms, 1976). This antibody mediated step is characterized by four properties which include the ability to:-

1. act on the worms in the absence of complement
2. effect a reduction in the metabolism of the worms
3. affect secretion of enzymes from the glands of the worms and
4. damage the worms (Ogilvie and Jones, 1973).

2.10 The Haematological response in Hookworm Infection

The haematological response of rats was studied 10 days after infection with Nippostrongylus brasiliensis larvae. Erythrocyte numbers were significantly lower than controls. Haemoglobin (Hb) concentration also dropped significantly (Cheema and Scofield, 1982). Mean corpuscular haemoglobin and mean corpuscular volume increased significantly and this was also the trend observed in the reticulocyte counts (Cheema and Scofield, 1982). This result was comparable with the survey of Martin (1972), among rural school children in Georgia, United States. He found a proportion of children with heavy hookworm infestation to be associated with low haemoglobin and haematocrit values. However, few of these

children could be categorized as anaemic. Some very heavy infections in the children even had normal haematocrit and haemoglobin values (Martins, 1972); Davis, 1973). A differential study of blood films showed leucocytes had significant differences compared with controls. Lymphocytes were increased in number while percentages and absolute counts for neutrophils were lower. The percentage of monocytes dropped with increasing levels of infection and absolute numbers were significantly lower only after infection with 5,000 larvae (Cheema and Scofield, 1982). Basophils were not seen in infected rats and only one or two showed up in the controls. On the other hand, eosinophil percentage and absolute counts were greater at all levels of infection (Cheema and Scofield, 1982).

2.11. The Diagnosis of Hookworm Infection

This is based on the demonstration of the hookworm ova in the patients stool (Adetuyibi, 1980). The ovum is roughly spherical in shape and contains eight nuclei. These eggs may be found in direct faecal films but in light infections concentration methods may be required. Such methods include the zinc sulfate centrifugal flotation and the formalinether concentration methods (Knight et al., 1976). With these techniques, cases with fewer than 400 hookworm eggs may be found. As compared to the direct coverglass mount method which measures only samples of more than 1,200 eggs per gram of faeces, the concentration techniques, were able to increase the number of positive findings several fold (Faust and Russell, 1964). The essence of the diagnostic examination of the eggs of the worms is

to permit differentiation of the hookworm anaemia and disease from the nutritional deficiency anaemia and oedemas and from the other helminthic infections. The eggs of hookworm found in fresh faeces are observed to be in the early stages of cleavage and the patients may harbour more than one specie of the parasite (Azikiwe, 1984).

Egg counting methods such as the Stoll dilution egg count or Kato's thick smear method, are useful as indicators of infection before and after therapy (Faust, Beaver and Jung, 1975).

2.11.1 Coproculture Identification

Faecal cultivation for the routine detection of human hookworm infection has been in use for more than 60 years. The technique was however pre-empted by the use of the salt - flotation technique. With the advent of Harada and Mori's simple test-tube culture method in 1951, the benefits of faecal coproculture were again ascertained (Harada and Mori, 1955). This method has been used extensively in surveys to detect hookworm infection in human populations and been reported to give a high percentage of positive findings (Hsieh et al., 1972). An application of the coproculture technique is the modified test-tube filter paper (MTFC) cultivation technique by Hsieh (1971). This gives balanced generic yields as it is adapted to be applied with the Stoll dilution egg counting to furnish a relationship between the relative Necator and Ancylostoma egg burdens and prevalence in the same stool specimen (Hsieh, 1971). The greater consequence this new type of information gives is the bearing it

makes available in the study of problems of clinical and non clinical hookworm; in pre- and post treatment phases, the efficacy of drugs and other related problems where both species of hookworm are endemic (Hsieh, 1971).

2.11.2 Immunological Diagnosis

This has been hampered by the relative ease of diagnosis by faecal examinations for hookworm ova. Of the immunological tests performed on hookworm subjects many include intradermal tests with various crude antigenic preparations. Positive immediate hypersensitivity reactions were obtained from antigens prepared from A. duodenale, N. americanus and A. caninum (Yamanaka, 1960). The antigens were prepared by tryptic digestion of larvae or adult hookworms and by aqueous extraction. These yielded protein and polysaccharide antigens respectively (Pritchard et al., 1985; Pritchard et al., 1986). The relevance of the intradermal tests as a useful screening test for hookworm was found in endemic areases. In non-endemic areas false positive readings of about 20% were obtained (Miller, 1979). Intensity of reaction in skin tests have been correlated with intensity of the infection, but the relationship has been described as imprecise (Miller, 1979). However, comparing intradermal tests with coprological methods it has been found that intradermal tests were more sensitive for detecting light infections. The presence of these false positive tests has been blamed on the reduction of antigen specificity due to a various collection of reasons which

include:-

- contamination of the antigen (which may be adult worms or larvae) with enteric and faecal microorganisms.
- exposure of the human population to larvae of canine and feline hookworms (Miller, 1979).

2.11.2.1 Immunoglobulin E as a Diagnostic Tool

The study of immunoglobulin E is particularly useful in non-endemic areas where clinicans have less experience with parasitic infections and patients can be misdiagnosed. Many parasitic infections will cause substantially raised total IgE levels (Greenwood and Whittle 1981). Infact, levels of up to 10-20,000KU/L have been obtained. Specific antibodies also have been found in connection with infections due to Echinococcus, Ascaris and Schistosoma (Radermecker et al., 1974). Specific diagnosis of any offending parasite can be achieved using specific allergens for the parasite IgE antibodies. However, this has not been too easy in hookworm patients due to a lack of suitable specific antigenic material (Hall et al., 1985). On the whole, measurement of IgE antibodies has been shown to be more specific for parasite diagnosis than measurement of IgG and other antibodies.

2.11.2.2 Serological Diagnosis

Other immunological reactions elicited in-vitro by hookworms and their antigens include a wide range of serological reactions. Ball and Bartlett (1969), have shown that several antibodies are

produced in response to infection with Necator americanus. These include precipitin (Ball and Bartlett, 1969; Sood et al., 1972), haemagglutination (Ball and Bartlett, 1969), complement fixation (Ball and Bartlett, 1969), gel - diffusion (Singh, 1965), latex floculation (Rombert et al., 1967) and specific immunofluoresence reactions (de Azevedo et al., 1971; Lewis, Salimonu and Osunkoya, 1978). Delayed antibody responses to continuing hookworm infection or re-infection had been earlier reported by Soulsby and Gilles (1965). Sood et al. (1972) used haemagglutination and precipitation techniques for the detection of antibodies in hookworm patients and healthy individuals. Failure of these workers to detect haemagglutination antibodies in most (42 out of 50) patients was attributed to short duration of the infection. They were not able to detect hookworm antibodies by agar gel precipitation also. Ball and Bartlett (1969), believe that the haemagglutination reaction is unhelpful because it was commonly found to be negative in light infections and because of its delay in appearance.

Yeates and Ogilvie (1976), knowing that hookworms secrete acetylcholinesterase, worked on the production of a stable radioactive label to be used in the measurement of anti-enzyme antibodies to hookworm acetylcholinesterase. The possibility of using this as a diagnostic tool was however reduced due to cross - reactions between conjugates derived from Necator americanus and Nippostrongylus brasiliensis. N. americanus is the only human parasite known to secrete acetylcholinesterase (Yeates and Ogilvie, 1976).

2.12 Treatment of Hookworm Infection

By 1962, over 50 drugs had been tested for activity against the hookworms and since then, there has been no shortage of new candidates as chemotherapeutic agents (Davis, 1973). Drugs available for the treatment of hockworm then included thymoland carbon tetrachloride which are now considered of historical interest only (Gerwel and Pawlowski, 1970). Hexyl resorcinol, a phenol derivative, has moderate efficacy in hookworm infection but its employment has diminished markedly because of its topical irritant properties in children (Davis, 1973; Wyatt, 1982). Among the older compounds, both tetrachloroethylene (TCE) first introduced 1925 (Rowland, 1966) and bephenium hydroxynaphthoate (Alcopar) remain in use. TCE is thought to be more effective against N. americanus, than A. duodenale (Wyatt 1982). In fact, Rowland (1966) using worm counts estimated that 3 daily doses of 4ml of tetrachloroethylene removed only 56% of the worms which were mainly N. americanus. However, TCE has been found to be very toxic and is best avoided in very anaemia patients or young children (Wyatt, 1982). Also, in the presence of concurrent Ascaris infection, piperazine must always be used first to get rid of the ascariasis, in order to prevent TCE-mediated Ascaris migration (Davis, 1973). Other side effects include epigastric burning, vomiting, abdominal pain, dizziness and a feeling of drunkenness.

Alcopar is safer than TCE although it is more expensive. It is also more useful against A. duodenale than N. americanus (Wyatt, 1982). The drug is given as dispersible granules, 5 grams for adults and

children over 2 years and 2.5 grams for children weighing less than 10kg. It may be used during pregnancy (Wyatt, 1982). Side effects are mainly mild gastrointestinal discomforts (Davis, 1973; Biroum-Noerjasin, 1973).

Pyrantel embonate or pyrantel pamoate (Combantrin) is one of the antihelminthies effective against both species of hookworm. The drug is given in a dose of 10mg per kg up to a maximum of 1 gram which may be repeated after 1 or 2 days. Side effects include dizziness and gastrointestinal disburbances (Kale et al., 1982).

Bitoscanate (Jonit) is also effective against hookworms irrespective of the species. It is however more toxic than pyrantel and no advantages are offered (Gill, 1986).

2.12.1 Mebendazole in the Treatment of Hookworm Infection

Mebendazole as used for trichuris infections, i.e. one 100mg tablet twice a day for three days for adults and children over the age of 2 years is effective against Necator and Ancylostoma species of hookworm (Wyatt, 1982; Gill, 1986). It should however not be given to pregnant women (Mims, 1988). It is virtually free of other side effects although further experience is needed (Gill, 1986). Using mebendazole as a follow-up therapy 14 days after treatment with levamisole Ogilvie et al. (1978), found that a 100% reduction in egg count to zero was achieved on all occasions. Chongsuphajaisiddhi et al. (1978), also reported a

93.9% reduction rate in 137 children aged 6-15 years using mebendazole. Mebendazole is also the best drug for trichuriasis (Chongsuphajaisiddhi et al., 1978; Wyatt, 1982; Gill; 1986).

2.12.2 Levamisole in the Treatment of Hookworm Infection

Levamisole is a levo-isomer of dl-tetramisole. It has a formula of 1-2,3,5,6-tetrahydro-6-phenyl imazole (2,1-5) thiazole (Symoens and Rosenthal, 1977). It was developed as an anti-helminthc agent but its ability to stimulate the immune response the subject of further investigations. Its mechanism of stimulating the immune response is basically undnown, but it has been observed to stimulate both helper and supressor T-cells (DeCree and Symoens, 1979). In general, it also has a net effect of being able to restore to normal, the cellular response to antigens. It seems to influence host defence mechanisms in a way similar to that of other immune modulators (DeCree and Symoens, 1979). It has inducing properties on precursor T-lymphocytes. It also mimics the effects of cyclic GMP-inducing agents on effector leucocytes. In T-cell enriched mouse spleen cells (Wright et al., 1977), it increases cyclic GMP but lowers cyclic AMP concentrations.

The same has been reported in polymorphonuclear cells in the early phase of chemotaxis (Wright et al., 1977). This suggests that levamisole promotes the expression of receptors on T-lymphocytes, which seems to be mediated through changes in intracelluar nucleotides. In vivo, the activity in the sulfa radical stimulates

bone marrow activity, while in vitro, from the halide radical, it stimulates blast transformation (Symoens and Rosenthal, 1977). Levamisole is able to act on macrophages and polymorphs to increase random migration, chemotaxis, antibody, and complement receptor activity, adherence, phagocytosis, nitroblue tetrazolium (NBT) reduction, peroxidase activity and intracellular killing (DeCree and Symoens, 1979). It also increases spontaneous and mitogen - or antigen - induced proliferation of T-cells, active and total E-rosette formation, cytotoxicity, lymphokine production, suppressor activity and plaque cell formation.

Levamisole does not directly stimulate B-cells (Symoens and Rosenthal, 1977). It does not have any effect on their proliferative response to mitogens and it has no antibody production. Instead, if B-cell activity is pathologically increased, it is returned to normal levels as shown by reduced members of Ig-bearing or EAC-rosette forming cells and by the normalization of antibody levels (Symoens and Rosenthal, 1977). Levamisole however has the ability to enhance the immune response in compromised and immunosuppressed hosts (De Cree and Symoens, 1979).

As concerns trials of levamisole in infections, it has been claimed to control herpes virus infection of the cornea, mouth and genitalia (Racz, 1977; Holborow and Lessof, 1981). Mouth ulcers have improved with this treatment and persistent warts have healed (De Cree et al., 1978). Patients with Hodgkins disease have shown a conversion from negative to postitive skin tests (Holborow and Lessof, 1981). In a controlled trial in rheumatoid athritis patients, levamisole was claimed to be as

effective as d-penicillamine and more effective than placebo. Levamisole favourably influences the course of a considerable number of diseases which are usually chronic or recurrent (Symcens and Rosenthal, 1977). Some of these diseases include immunodeficiency diseases like the Wiskott-Aldrich syndrome (Spitler et al., 1977) and chronic granulomatous disease (Griscelli, 1976); allergic diseases such as atopic dermatitis (Bruninx, 1973), infections such as chronic rhinitis (De Loore, 1977), pyogenic infections (Zilko, 1975) and so on. In cancer cases, animal studies have shown that levamisole may prolong the remission induced classical anti-cancer treatment but has little effect when used alone in the treatment of advanced cancer (Symoens and Rosenthal, 1977). The immunological effects of levamisole on cancer patients include a consistent effect of restoration of cutaneous delayed hypersensitivity to various antigens (Klefstrom, 1977).

A single intake of a therapeutic dose of levamisole (2.5mg/kg) is practically devoid of side effects as shown in studies where levamisole was used as an anthelmintic (Moens et al., 1978). Repeated intake which is needed for immunotherapy may however cause severe adverse reactions in a susceptible sub-population (De Cree and Symoens, 1979). These reactions which are idiosyncratic may necessitate discontinuation of therapy. They include skin rashes, febrile illness, mouth ulceration and agranulocytosis. Neutropenia is also a common side effect (Symoens et al., 1978). However, levamisole is basically well tolerated by 80-95 percent of patients. The highest well tolerated dose in man (2.5mg/kg) happens to be the therapeutic dose in antihelminthic and immunotherapeutic indications (De Cree and Symoens, 1979). A single dose is effective against [illegible] and restores immune function for at least 5 days (Mowat, 1978).

2.12.3 Iron Supplementation in Therapy of Hookworm Infection

Supplements of iron for infected individuals in endemic hookworm areas have been effective in controlling anaemia (Basta et al., 1979). Replenishment of iron is currently the most rational and achievable intervention available for conteracting the effects of the anaemia caused by hookworm. It's advantage is that of being a cheap and effective method for treating iron-deficiency anaemia irrespective of it aetiology (Gilman, 1982). Supplementation may be provided in available food staples such as sugar or flour, depending on local preferences (Michaelsen, 1986). Oral supplementation of iron in malnourished patients may however have drastic effects. For example, in poorly nourished Somali nomads (a milk dependent people) supplementation of iron was associated with an increased risk of bacterial and protozoal infection (Murray et al., 1978). These results are puzzling since in the iron-deficient host who is receiving oral supplements, the ingested iron would be bound to transferrin and little free iron would then be available for use by bacteria and protozoa (Payne and Finkelstein, 1978). However, invasion of microorganisms is only possible if iron is available for growth of the organisms (Van Asbeck and Verhoef, 1986) and the hypothesis that an iron-rich host environment predisposes to infection has been established by an overwhelming number of studies dealing with the subject (Van Asbeck and Verhoef, 1983).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Patients and Controls

Patients studied numbered fifty-seven (57), and they were taken from patients attending out-patients clinic or in the wards of the Ahmadu Bello University Teaching Hospital. The controls, numbering twenty-six (26) were volunteers age and sex matched with the patients who were either civil servants or students of the Ahmadu Bello University, Zaria. The age of the patients ranged between 12 and 70 years with a mean of 31.0 years. The controls had an age range of 16 years to 45 years, with a mean of 28.0 years. There were 44 males and 13 female patients. Amongst the controls, there were 20 males and six females. The patients were subjects with heavy or mild hoodworm load depending on their egg counts and they were grouped into those having marked, moderate, mild or no anaemia based on their blood results.

3.2 METHODS

3.2.1 Specimen Collection

(a) Blood:- Peripherial blood samples were taken by venepuncture. About 25ml of blood was drawn from each patient and controls. Ten ml of blood was put into a tube containing heparin (250 i.u/10ml) for T-lymphocyte assay, 8ml into another heparinised tube for leucocyte migration inhibition test and 5ml was allowed to clot in a tube for serum. The serum was separated out and stored at - 20°C until further use. The remaining 2ml of blood was put into an EDTA bottle for haematological assessment.

(b) Stool:- Stool specimens were collected from all subjects in screw capped containers. The samples were examined immediately for parasite ova and protozoal cysts. Egg. counts were then made by the Stoll method while a small portion of the sample was smeared onto filter paper for hatching (STOLL, 1963).

3.2.2 White Blood Cell Count

A 1:20 dilution of the heparinized blood (20ul in 400ul) was made in WBC dilution fluid (3% glacial acetic acid with a tinge of gentian violet). It was used to charge a Neubauer counting chamber and the total WBC was counted in the WBC areas under a microscope at a magnification of x160. The WBC was expressed as count per litre.

3.2.3 Differential Lymphocyte Count

A small drop of the blood in EDTA was placed on a microscope slide and spread out to give a thin film. This was air dried, stained with Leishman stain and the percentage of lymphocytes counted under a microscope at a magnification of x1000.

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3.2.4 T-CELL ENUMERATION

3.2.4.1 Separation of Lymphocytes

The method of Gupta and Good (1979) was employed. The 10ml of heparinised blood was diluted 1:2 with Eagle's minimum essential medium (MEM) and gently layered on 3ml of Ficoll-Histopaque density gradient in a 10ml tissue culture tube (i.e about 7ml of the diluted blood). This was centrifuged at 1,800 rpm, for 30 minutes at room temperature. The lymphocyte layer at the Ficoll - MEM interphase was gently removed with a Pasteur pipette and transferred to another 10ml tissue culture tube. The cells were washed three times with MEM by re-suspending them in the medium and then centrifuging at 1,800 rpm for 10 minutes at room temperature. After the final wash, the cells were resuspended in 1ml of MEM containing 5% inactivated absorbed foetal calf serum (FCS) and the cell concentration adjusted to 4×10^6/ml. More than 95% of the cells were viable as tested by trypan blue dye exclusion test.

3.2.4.2 Preparation of 0.5% Sheep Red Blood Cells (SRBC)

About 3ml of sheep red blood cell in Alsever's solution was centrifuged at 2,000 rpm for 15 minutes at $4^{\circ}C$. The packed cells were washed 3 times with MEM. Then, 50ul of the packed red cells were removed and resuspended in 10ml of MEM solution to give a 0.5% suspension.

3.2.4.3 Rosette Technique

The method outlined by Spiegelberg and Gonzalez-Molina (1980) was use Of the lymphocyte suspension separated from the heparinised blood, 200ul was taken and mixed with 200ul of the 0.5% sheep red blood cell (SRBC) suspension and incubated at 37°C for 5 minutes. This was centrifuged at 1,000 rpm, for 5 minute at 4°C and incubated at 4°C overnight. The cell pellet obtained was gently resuspended and 100 lymphocytes were counted for rosette formation in a drop on a microscope slide. A rosette is defined as a lymphocyte with 3 or more SRBC attached to it. The result was quoted as percentage positive of peripheral blood lymphocytes.

Absolute numbers of the lymphocytes were calculated from the following formula

$$\frac{a}{100} \quad x \quad \frac{b}{100} \quad xX = Y \times 10^9/L$$

Where a = % of rosette positive cells.

b = % peripheral blood lymphocytes

X = White blood cell count ($x\ 10^9/L$).

Y = Absolute no. of rosette positive lymphocytes $x\ 10^9/L$.

3.2.4.4 Immunofluorescence Microscopy Technique

The method described in the Becton-Dickinson Source book (1981) was adopted. Fifty microlitres of the lymphocyte suspension was placed in a small plastic tube and 20ul of the monoclonal antibody

(Leu 2a, Leu 3a or Leu 4) added. This was incubated for 30 minutes on ice at 4°C, after which it was centrifuged and washed twice at 1,000 rpm, for 5 minutes at 4°C. After the final wash, the cells were resuspended in a drop of minimum essential medium. A drop of the cell suspension was placed on a glass slide, covered with a coverslip and sealed with nail varnish. This was kept at 4 C for 15 minutes for the cells to settle down and the flourescence read by screening random fields. A field was selected and viewed under normal light and the total number of cells in a visible area recorded. The UV light was then switched on and the number of cells in the same area that stained bright peripherally with fluorescence dye were recorded separately as positive. Several fields were scanned until a total of 100 lymphocytes (that is, both positive and negative were counted. The number of positive cells was expressed as a percentage of the total count. The absolute number was calculated using the above formula for positive rosetting cells.

3.2.5 Leucocyte Migration Inhibition Test (LMIT)

The method described by Rosenberg and David (1970) was employed. The 8ml of heparinised blood separated above, was allowed to settle at 37 C for 1 hour. The plasma was removed and transferred to another plastic tube and washed three times with RPMI containing 15% FCS, by centrifuging at 1,800 rpm for 10 minutes at 37°C. At the end of the final wash, the cell pellet was resuspended in 1 ml of the medium and the cell concentration adjusted to 1 x 10^7 cells/ml. This cell suspension was then aspirated into capillary tubes leaving about one-third of the tubes empty. The empty end of the capillary

tubes were then sealed with plasticine. The sealed capillary tubes were then placed in tissue culture tubes and centrifuged at 1,800 rpm for 10 minutes at 37°C. They were cut about one millimeter below the cell liquid interface and placed.in a migration chambers and anchored firmly with a small dab of sili cone grease. The chambers were filled with either RPMI medium with 15% FCS alone (control) or with 100 iu/ml PPD (test). Coverslips were then placed on the chambers and sealed in place with sili cone grease. The test was performed in duplicates. The chamber plate was incubated perfectly flat at 37 C for 24 hours, after which it was placed on a microscope and the area of migration was projected onto a piece of graph paper. This was drawn and the area was measured by counting the number of squares encircled. The percentage migration inhibition was calculated from the following formula:

$$\% \text{ inhibition} = 1.0 - \frac{\text{test area}}{\text{control area}} \times 100$$

3.2.6 Quantitation of Serum Immunoglobulins and Complement Levels

The single radial immunodiffusion technique described by Mancini et al.(1965) was employed. Various dilutions (Neat, 1:2, 1:4, and 1:8) of standard serum which had been calibrated against a WHO immunoglobulin standard serum were assayed along with the samples.

A 1% noble agar in Tris Sodium (pH 8.2) barbitone buffer was prepared by heating until the agar was completely dissolved. This was allowed to cool down to 56°C. The antiserum was diluted 1:15 in this sodium

barbitone buffer and warmed up to 56°C in a water bath. Equal volumes of the agar and diluted antiserum were then mixed. About 15ml of the mixture was layered on a clean 17.5cm by 13.0cm glass plate. The final concentration of the agar then became 1:5 while the final dilution of the antiserum became 1:30. The agar was allowed to set at room temperature before wells of 2mm in diameter were made using a gel punch. Using a 5ul capillary tube, a constant volume (5ul) of the specimens and the standard serum were added to the wells. After all specimens were added, the plates were incubated in a moisture chamber at room temperature for 24 hours. The diameter of the formed precipitin rings were measured with an eye micrometer. The values obtained for the standard serum (the serial dilutions) were used to plot a reference curve on a line-log graph paper with concentration on the logarithmic scale and the diameter on the linear scale. Values corresponding to the sample diameters were then read off the reference curve.

3.2.7 STOOL EXAMINATION

3.2.7.1 Direct Smear Method

Under this technique, a pin head size of faeces were taken from each specimen and emulsified in a drop of normal physiological saline. The smear was made so that newsprints could still be visible through it. A weak solution of Lugols iodine, in place of saline was also used to make the smear for the purpose of examining protozoal cysts. Glass coverslips (44mm x 22mm) were

then applied and the slide examined for hookworm ova (Faust, Beaver and Jung, 1975).

3.2.7.2 Stoll EGG Counting Technique

The method described by Stoll and Hausheer (1926), was adopted. A 3g quantity of faeces was weighed out into a screw capped glass bottle with 42ml of distilled water (N/10 sodium hydroxide NaOH) was used in place of water in case faeces was hard. Some glass beads were then added to the faeces in water and thoroughly comminuted. A 0.15ml of the suspension was then pipetted onto a glass slide and covered with a coverslip. The total number of eggs in the sample including any in the fluid exuded from the sides of the coverslip were then counted.

Eggs counted were present in $\frac{3 \times 0.15g}{45}$ of faeces.

This gave a 0.01g of faeces. Therefore eggs counted (x 100) would give the number per gram of faeces (EPG).

3.2.7.3 Identification of Species of Hookworm Larva: The modified test-tube filter paper cultivation method of Hsieh (1971) was employed. A lengt wise crease was made in a filter paper strip (30 by 150mm) to act as a wick for the larvae to travel up and using a wooden applicator 0.1gm of faeces was smeared on the paper crease. This was done in such a wa as to leave about 4cm at the end of the filter paper unsmeared. The smeared strip was inserted into a test tube with 10ml sterilized water, unsmeared portion downwards, making sure that the faecal smear does not touch the water. The test-tube was

covered with a piece of cellophane, fixed tightly in place with a rubber band. Several tiny holes were punctured in the cellophane sheet with a dissecting needle. The tubes were then kept at room temperature (24°-29°C) for 6-8 days. In order to examine the infective larvae, the cellophane sheet was removed from the top of the tube and discarded, then distilled water was added to the tube until the water level covered the top of the filter paper strip. This was left standing for about 5 hours, after which the filter strip was removed with forceps and discarded. The supernatant water was carefully drawn off by means of a transfer pipette until the water level was just about 25mm from the bottom of the test tube. The suspension was shaken and rapidly transferred to a conical centrifuge tube where the larvae were concentrated by centrifuging at 1,500 rpm for 3 minutes. The supernatant water was carefully pipetted off the concentrated larvae leaving about 3ml of water at the conical bottom. A drop of Dobell and O'Connor's solution was added and more water (about 2.5ml) removed as soon as the iodine tint covered the bottom of the centrifuge tube.

The whole sediment was agitated and transferred as a drop to a microslide and covered with a cover glass (22 x 50mm). The slide was examined under the microscope at a magnification of x 160 for the presence of distinguishing features on the larvae present such as the conspicuously striated cuticle of Necator, the length of the body, the mouth spear and the blunt anterior end.

3.2.8 Treatment and follow-up

The patients were treated using both Ketrax and Vermox alternatively. Lavamisole was given 2.5mg per kg start, while Vermox was given in a dose of 100mg b.d. for 3 days. Whenever possible the tablets were taken in the presence of the researching candidate. The patients were followed up for 4 months in order to record any changes in the cellular response after treatment and to observe any evidence of i.e. infection. At this point, amny (29) of the patients defaulted after the initial treatment. Of the 28 patients who returned for follow-up, 25% were treated on Ketrax, 28.57% on Vermox and 42.85% had a serial combination of vermox and ketrax.

3.2.8 Statistical analysis

Analysis of the results was done using the students t-test.

CHAPTER 4

RESULTS

4.1 Clinical Findings

The patients were diagnosed to be suffering from hookworm infection based on the recovery of hookworm eggs in their stools. There were 44 males and 13 females (male: female ratio of 3:1). The age range of the patient varied from 13 to 70 years, the mean being 30 ± 14 years. Controls were well screened, healthy volunteer subjects. There were 21 males to 7 females (male: female ratio of 3:1). The age range was from 16 to 45 years, with the mean being 28 ± 7 years.

4.1.1. Parasitological findings

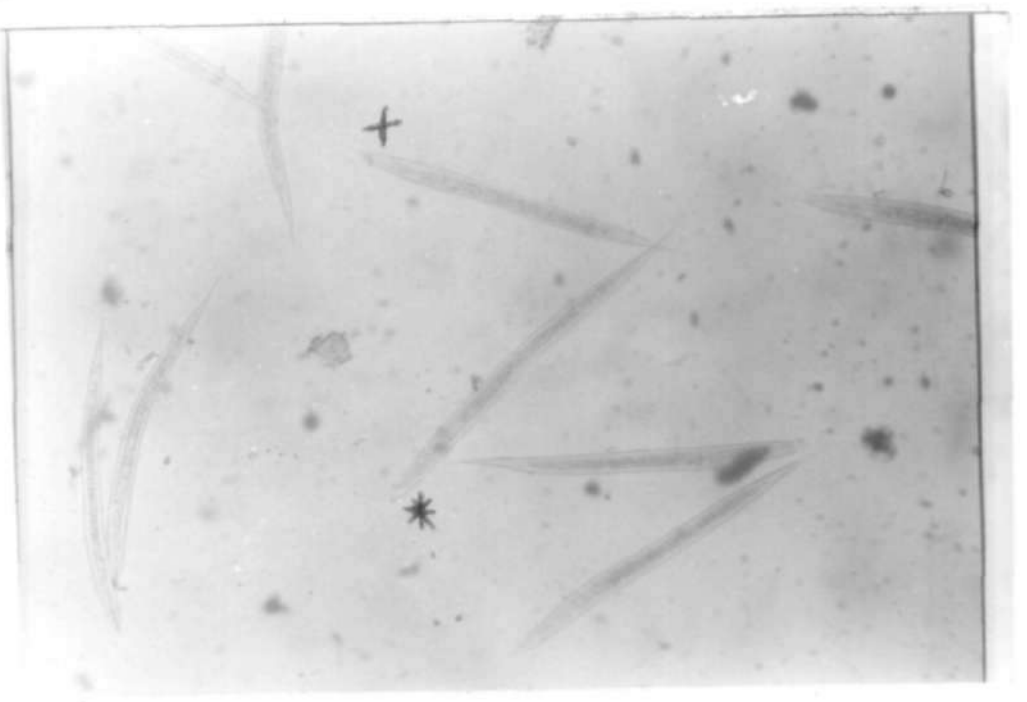
The intensity of infection as indicated by the average egg excretion in stool was 800 ova per gram of stool (range 100 - 2900 eggs per gram of stool). However, four (7%) cases of superinfection ranging from 15,200 to 111,200 eggs per gram of stool were recorded (Tables 1 & 2). From the test - tube filter paper culture results, Necator americanus was the predominant hookworm species occurring alone in 67% of the 57 samples (Page 65). Ancylostoma duodenale was always found together with N. americanus and occurred in 5% of the samples. Other intestinal

PLATE 1



Hookworm Ova x 400

PLATE 2



Filariform larvae of Necator (+) and Ancylostoma (*) x 160

TABLE 1

T-LYMPHOCYTE RESULTS IN PATIENTS WITH SUPERINFECTION

NAME	Age	Sex	Stoll count	Hb	WBC	Differential lymphocyte + ecsin		Anti Leu 4+	Rosettes at 4°C	T-help.	T-supp.	MLF
K. Duniya	65	M	30600	11	3.6	*2.16 60	0.43 22		0.66 31			
D. Mijintaba	45	M	15200	4	3.0	1.80 60	0.18 10		0.72 40			
B. Mohammadu	35	M	82500	7	3.9	1.24 32	0.05 4	0.60 48	0.53 43	0.32 26	0.22 18	
A. Abdullahi	25	M	111200	6	5.1	1.22 24	0.01 1	0.49 40	0.45 37	0.23 19	0.26 22	24

TABLE 2

IMMUNOGLOBULIN AND COMPLEMENT RESULTS IN PATIENTS WITH SUPERINFECTION

NAME	I.U. IgM	I.U IgG	I.U. IgA	mg/L $SIgA$	% of std C_3	% of std C_4
K. Duniya	298	376	760	41.6	145	46
D. Mijintaba	119	125	95	6.24	112	46
B. Mohammadu	205	158	190	10.66	145	104
A. Abdullahi	167	139	190	10.66	73	46

* Absolute numbers $x10^9/L$.

Parameters not statistically analysed because the sample size was too small.

parasites that were observed along with hookworm infection include Ascaris lumbricoides (4), Trichuris trichiura (1), Taenia spp (1), Strongyloides spp (3), Schistosoma mansoni (2), Giardia lamblia (4) and Entamoeba histolytica (1). Malaria parasites were detected in two of the thin blood films and patient no 2 (appendix I) had microfilaria in his skin snip (Table 2). The patients were then subgrouped into those with hookworm only and those with hookworm and other parasites for comparison of the immune parameters.

4.1.2 Haematological Findings:-

The thin blood films consistently showed hypochromic anaemia in 49% of the cases. The mean haemoglobin (Hb) level was 8.3g/dl. (± 2.96), while the mean packed cell, volume was 33±11% (Table 3). A significant relationship ($P \leq 0.05$) was found between level of haemoglobin and ova load (Figure 2). On the basis of haemoglobin levels and degree of hypochromia, the hookworm patients were again divided into those with marked anaemia (Hb 1-8g/dl, hypochromasia +++), moderate to mild anaemia (Hb 9-11g/dl, hypochromasia ++) and patients with no signs of anaemia. A mild degree of eosinophilia (1-6%) was observed in 77% of the cases. Only in 16% of the patients was marked eosinophilia (> 6%) demonstrated. Mean percent eosinophilia was however elevated in all the hookworm subgroups as compared to the controls (Table 4).

TABLE 2

PARASITOLOGICAL PROFILE IN 57 NIGERIAN HOOKWORM PATIENTS

Parasite Specie and Combination		No of Sample	Percent of Sample
Hookworm alone	Necator americanus	38	66.66%
	Ancylostoma duodenale	3	5.26%
		41	71.93%
Hookworm + Giardia lamblia		4	7.02%
Hookworm + Ascaris lumbricoides		3	5.26%
Hookworm + Strongyloides stercoralis		2	3.51%
Hookworm + A lumbricoides + Trichuris trichuria + P. falciparum		1	1.75%
Hookworm + S. stercoralis + S. mansoni		1	1.75%
Hookworm + Schistosoma mansoni		1	1.75%
Hookworm + Plasmodium falciparum		1	1.75%
Hookworm + Taenia saginata		1	1.75%
Hookworm + Entamoeba histolytica		1	1.75%
Hookworm + Onchocerca volvulus		1	1.75%
Total		57	100%

TABLE 3

PACKED CELL VOLUME (PCV) LEVELS IN HOOKWORM PATIENTS BEFORE AND AFTER TREATMENT

	Before Treatment	4/52 after treatment	P. Value
Patients	33 ± 11	38 ± 9	
n	55	25	P < 0.001
Range	11 - 48	15 - 48	
Controls	42 ± 4	43 ± 5	
n	28	27	P > 0.1
Range	34 - 48	39 - 52	
P. value	P < 0.001	P < 0.01	

TABLE 4

Differential analysis of peripheral blood mononuclear cells in Hookworm patient subgroups and controls

Cell Type	Marked anaemia $\bar{X}$ SD	Moderate anaemia $\bar{X}$ SD	No anaemia $\bar{X}$ SD	Hookworm only $\bar{X}$ SD	Hookworm + other parasites $\bar{X}$ SD	Total Hookworm patients $\bar{X}$ SD	Controls $\bar{X}$ SD	P. value groups I, II, III, IV V & VI vs group VII resp.
WBC $x10^9/L$	5.12 ± 3.22	4.95 ± 2.31	4.40 ± 1.82	4.9 ± 3.0	4.1 ± 1.5	4.72 ± 2.3	3.76 ± 1.5	*P $\angle$0.01 for all group resp.
n	15	15	27	41	16	57	28	
range	1.9 - 12.3	2.7 - 9.3	1.4 - 12.3	1.5 - 12.3	1.4 - 8.0	1.4 - 12.3	1.2 - 8.3	
Lymphocytes ($x10^9/L$)	2.20 ± 1.64	2.23 ± 1.31	2.09 ± 0.75	2.27 ± 1.2	1.84 ± 0.66	2.16 ± 1.15	1.80 ± 0.87	N.S. P$\geq$0.1 for groups vs group VII resp.
n	15	15	27	41	16	57	28	
range	0.79 - 7.25	1.07 - 6.51	0.15 - 3.33	0.15 - 7.25	0.72 - 3.06	0.15 - 7.25	0.57 - 4.31	
Neutrophils ($x10^9/L$)	2.08 ± 1.25	1.99 ± 1.01	1.73 ± 1.17	1.91 ± 1.18	1.84 ± 1.05	1.88 ± 1.14	1.77 ± 0.75	N.S. P$\geq$0.1 for all groups vs group VII resp.
n	15	15	27	41	16	57	28	
range	0.42 - 4.52	0.70 - 4.64	0.26 - 5.12	0.26 - 4.65	0.53 - 5.12	0.26 - 5.12	0.9 - 3.99	
Eosinophils ($x10^9/L$)	0.25 ± 0.14	0.43 ± 0.30	0.38 ± 0.35	0.35 ± 0.31	0.40 ± 0.29	0.36 ± 0.30	0.06 ± 0.04	* P $\angle$0.001 for all the groups vs group VII resp.
n	15	15	27	41	16	57	28	
range	0.05 - 0.49	0.03 - 1.08	0.05 - 1.35	0.05 - 1.35	0.08 - 1.09	0.03 - 1.32	0.0 - 0.18	
Monocytes (%)	3.0 ± 1.33	1.5 ± 0.85	1.9 ± 0.51	2.2 ± 0.6	1.7 ± 0.52	2.0 ± 0.58	1.5 ± 0.6	P $\geq$0.5 for all the groups vs group VII resp.
n	15	15	27	41	16	57	28	
range	0.0 - 6.0	0.0 - 3.2	1.0 - 2.9	1.0 - 3.5	0.0 - 2.8	0.0 - 3.5	0.0 - 32	

N.S. = not statistically significant * = statistically significant n = number of samples

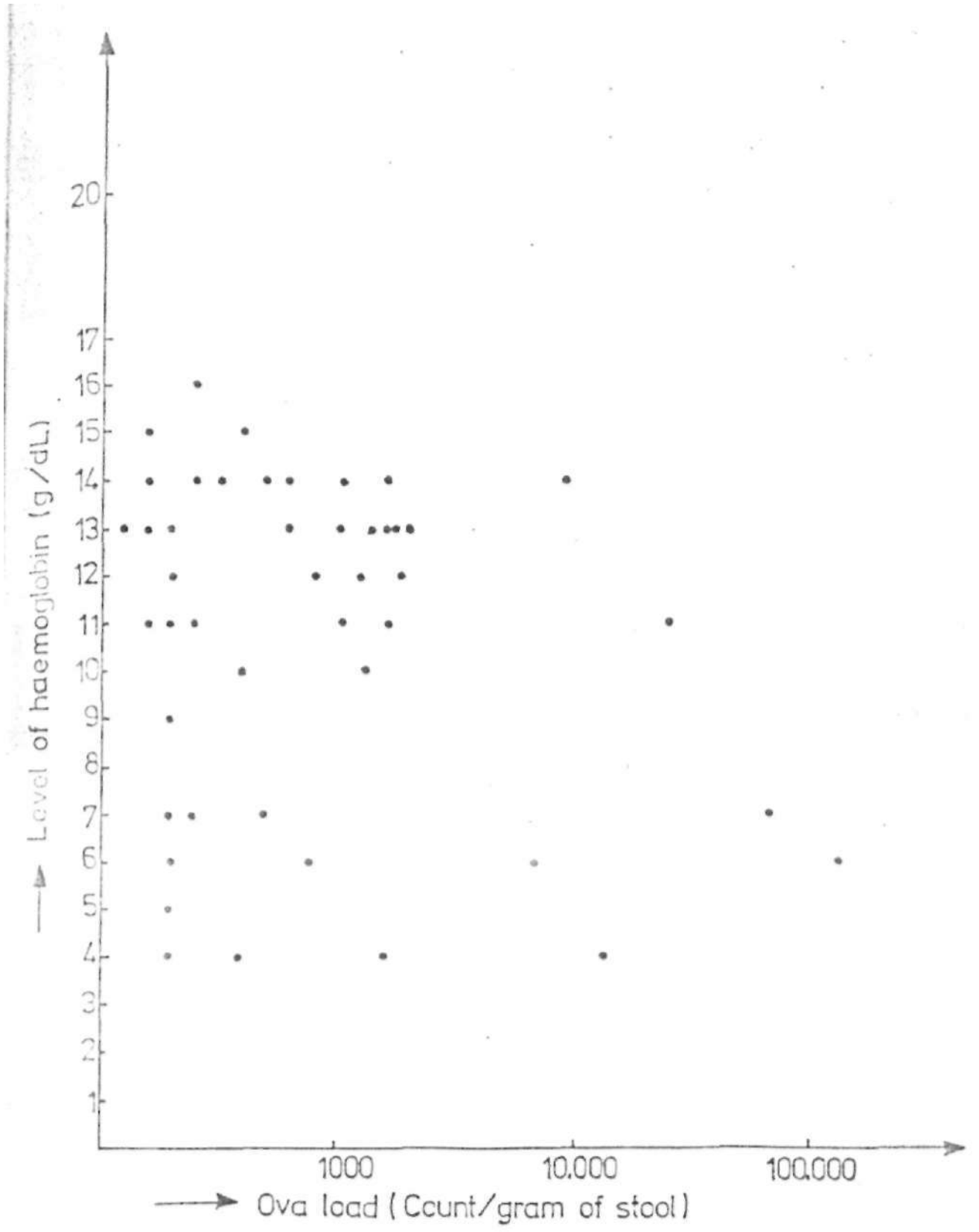


Fig. 2. Relationship between ova load and level of haemoglobin

4.2 White Blood Cell (WBC) Count

The white blood cell count in the various subclasses of hookworm patients studied and in controls are indicated in Figure 3. Patients with marked anaemia had a mean ± S.D of 5.12± 3.22 x 10^9/L with a range of 1.9 to 12.3x10^9/L; those with mild to moderate anaemia had a mean of 4.95±2.31x10^9/L with a range of 2.7 to 9.3x10^9/L, while those who showed no anaemia had a mean of 4.40± 1.82x10^9/L with a range of 1.4 to 8.3x10^9/L. The controls had a mean of 3.76±1.5x10^9/L with a range of 1.2 to 8.3x10^9/L. The mean of each group compared with mean of the controls were not found to be statistically significant using the F-test ($P > 0.05$) even though values were higher than those of the controls.

However, patients with hookworm and other parasites as a group had a value of white blood cells (4.1±1.5x10^9/L compared to patients with hookworm infection alone (4.9±3.0) x10^9/L. Comparing both subgroups with the controls patients with hookworm infection alone had a significantly higher ($P \leq 0.05$) value of white blood cells (Table 4).

Post-treatment white blood cell readings of all the hookworm patients gave a mean of 3.7±0.8x10^9/L. This was significantly lower than the w.b.c. readings before treatment of 4.72±2.3x10^9/L ($P \leq 0.05$). However there was no statistical difference between the post treatment value and that of controls ($P > 0.1$) (Table 9-).

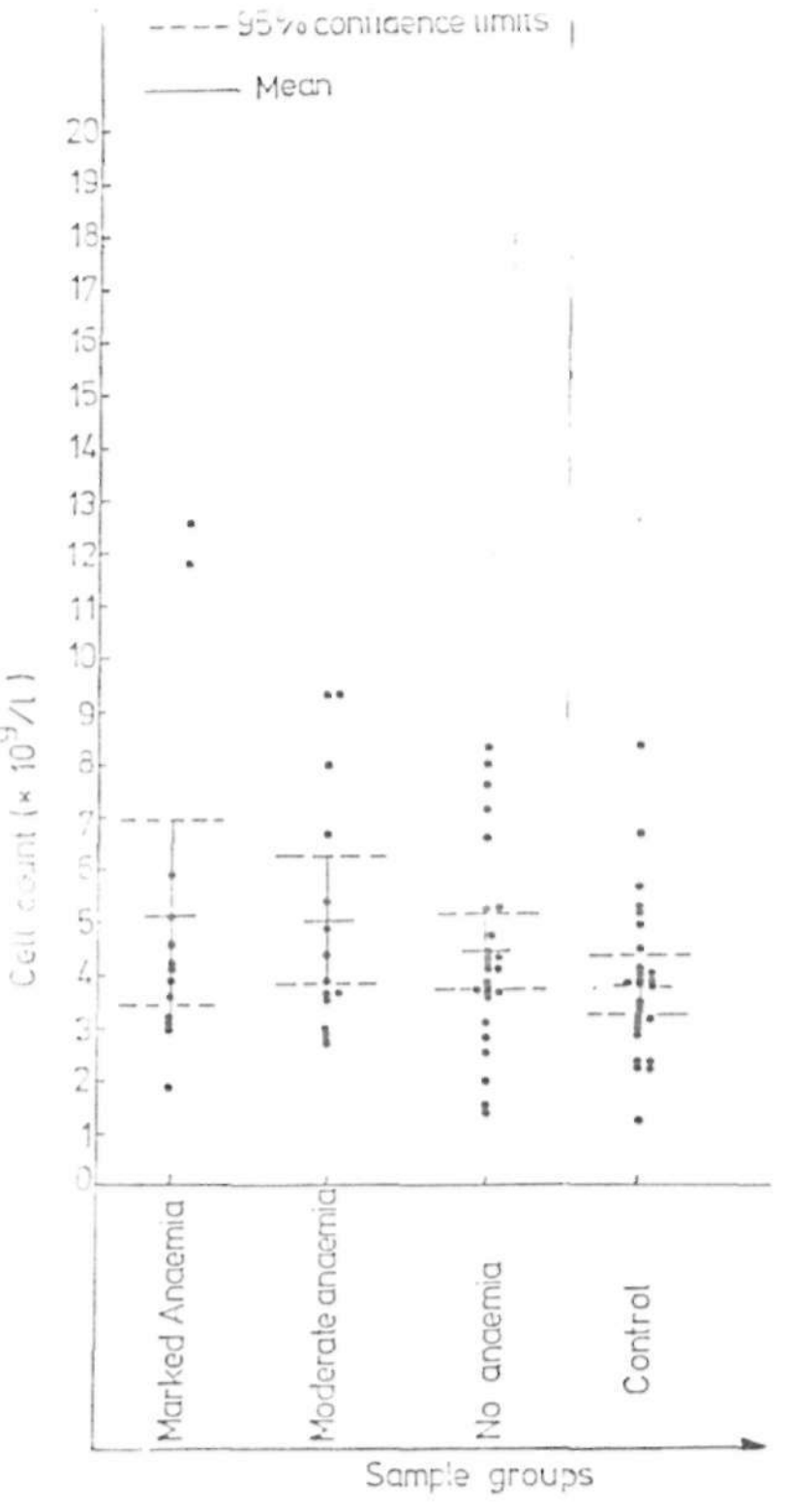


Fig 3 Mean Distribution of white blood cell counts in hookworm patients and controls.

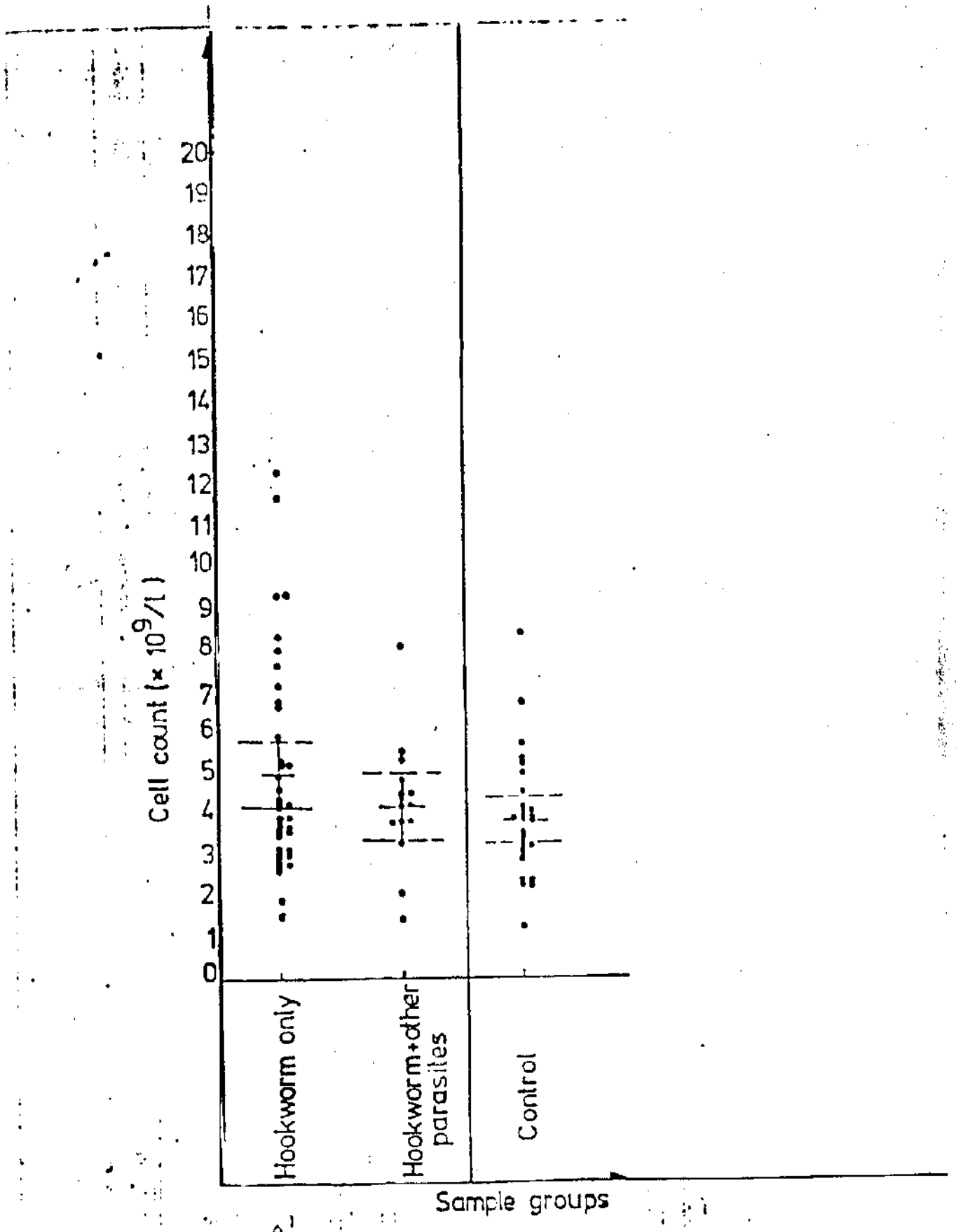


Fig. 3. Mean Distribution of white blood cell counts in hookworm patients and controls

4.3 Differential Lymphocytes Count

The mean percent lymphocyte count in the peripheral blood of hookworm patients and controls are indicated in Table 4. Marked anaemia patients had a mean of 52±24% with a range of 18% to 97%, moderate to mildly anaemic patients had a mean of 47±15% and a range of 26% to 72%, patients without anaemia had a mean of 51±15% and a range of 4% to 70%. Patients with hookworm only had a mean of 51±19% and a range of 18% to 97%, while patients with hookworm and other parasites had a mean of 46±12% and a range of 25% to 60%. Compared with the controls who had a mean of 48± 13% (range of 29% to 84%), none of the groups was found to be significantly different ($P > 0.3$, $P > 0.4$, $P > 0.2$, $P > 0.2$ and $P > 0.3$ for each of the groups respectively). All the hookworm patients together gave a mean of 50±17% and a range of 4% to 97%. Again, this was not significantly different from the control reading ($P > 0.1$).

Figure 4 shows the mean absolute number of the peripheral blood lymphocytes in the same patients and controls. Expressed as count $x10^9/L$, markedly anaemic patients had a range of 0.79 to $7.25x10^9/L$ and a mean of 2.20±1.64, moderate to mildly anaemic patients had a range of 1.07 to $6.51x10^9/L$ and a mean of 2.23±1.31, patients with no indications of anaemia had a range of 0.15 to 3.33 and a mean of $2.09±0.75x10^9/L$. Patients with hookworm only had a range of 0.15 to $7.25x10^9/L$ with a mean of $2.27+1.28x10^9/L$, while patients with hookworm other parasites had a mean of 1.84±0.66 and a range of 0.72 to $3.06x10^9/L$. Control subjects had a mean of 1.80±0.87 and

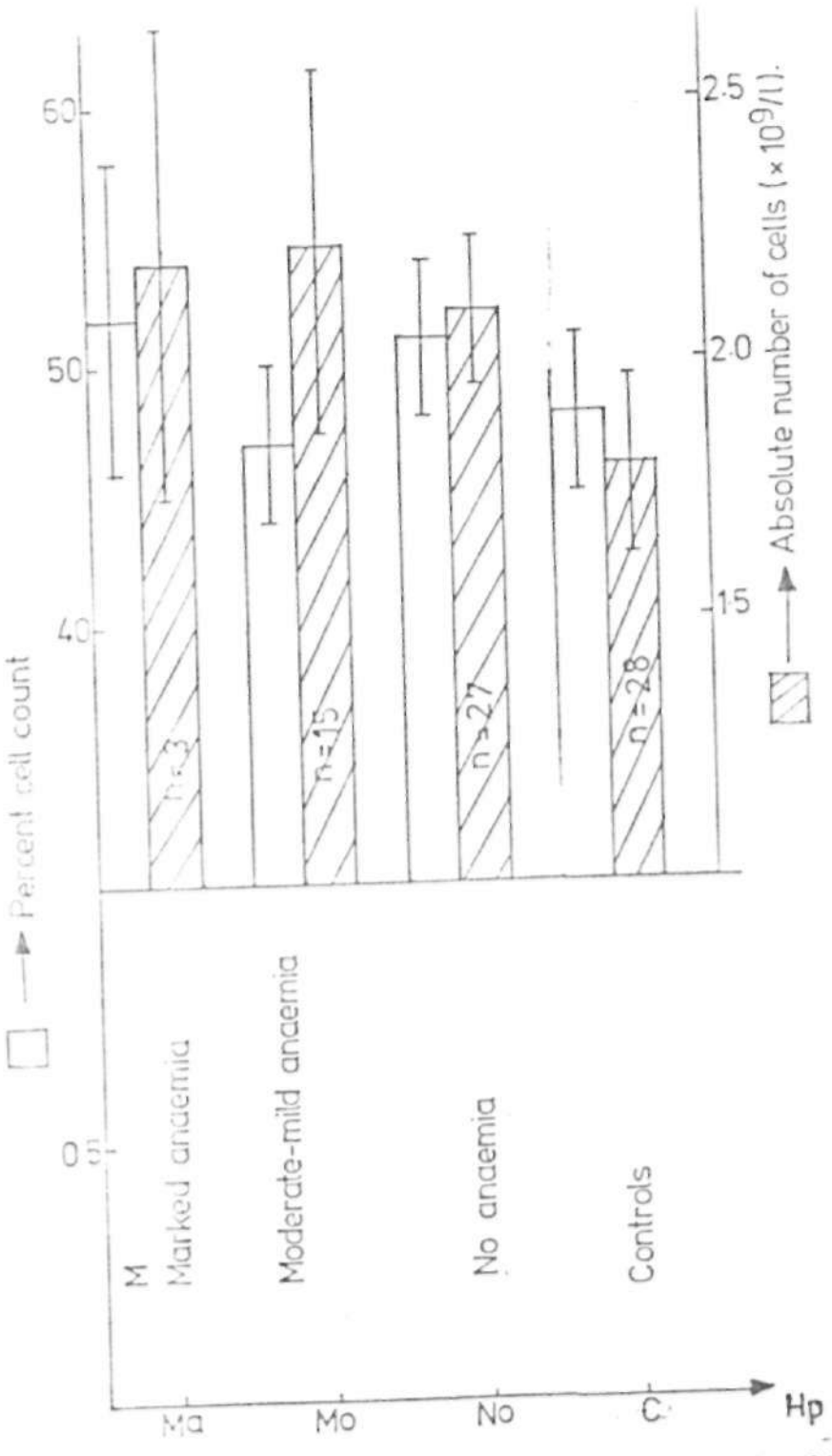


Fig 4. Mean (±1 S.E.M) absolute no & percents of lymphocytes (differential) in the peripheral blood of Hookworm patients & controls

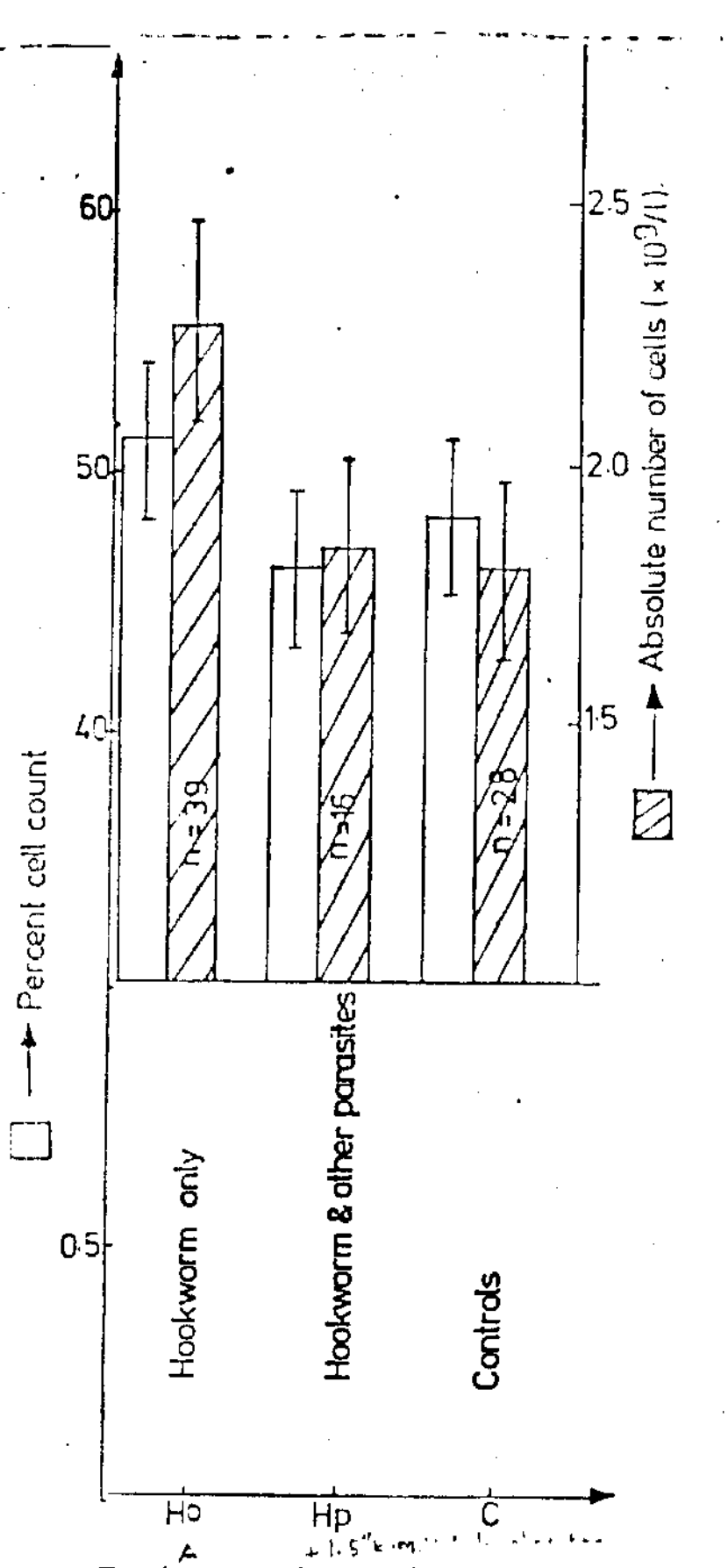


Fig. 4. Mean (±1S.E.M) absolute no & percents of lymphocytes (differential) in the peripheral blood of Hookworm patients & controls

a range of 0.57 to $4.31x10^9/L$. None of the subgroups was significantly different from the controls ($P > 0.1$ for al of the groups). For all the hookworm patients together, the mean absolute lymphocyte count was $2.16 \pm 1.15x10^9/L$ with a range of 0.15 to $7.25x10^9/L$. Again, this was not not significantly different from that of the controls ($P>0.1$).

TABLE 5

LEVELS OF SERUM IMMUNOGLOBULINS IN NORMAL CONTROLS AND HOOKWORM PATIENTS SUBGROUPS

Ig CLASS	I Marked Anaemia (15)	II Mild-moderate anaemia (15)	III No anaemia (27)	IV Hookworm only (41)	V Hookworm & Other parasites (16)	VI Controls (28)	P. value groups I,II,III, IV & V. vs. group VI, resp.
IgA (1.U/ml)	156 ± 112	244 ± 212	198 ± 133	201 ± 174	128 ± 74	125 ± 84	NS; < 0.05; NS; < 0.05; NS
R	n = 15 (24 - 380)	n = 15 (36 - 760)	n = 27 (31 - 760)	n = 41 (24 - 760)	n = 16 (48 - 285)	n = 27 (35 - 361)	
IgG (1.U/ml)	206 ± 92	284 ± 247	216 ± 93	270 ± 262	213 ± 133	232 ± 206	NS; NS; NS; NS; NS.
R	n = 14 (125-455)	n = 14 (112-957)	n = 27 (125-554)	n = 40 (125-957)	n = 16 (112-693)	n = 25 (92 - 957)	
IgM (1.U/ml)	331 ± 319	160 ± 80	211 ± 173	167 ± 65	202 ± 94	130 ± 47	< 0.005; NS, < 0.001; NS; < 0.005
R	n = 15 (80 -1148)	n = 13 (65-321)	n = 27 (88-1023)	n = 37 (65-802)	n = 14 (100-405)	n = 28 (69-321)	
SIgA(g/l) (I.U/ml)	8.9 ± 7.1	17.2 ± 15.7	10.2±8.06	12.25±11.62	10.34±7.19	2.79±2.07	< 0.001; < 0.001; < 0.001; < 0.001; < 0.001.
R	n = 15 (0.73-26.0	n = 15 (1.77-41.6)	n = 27 (1.76-41.6)	n = 41 (0.73-41.6)	n = 16 (3.3-26.0)	n = 28 (0.73-10.66)	

The values are given as mean ± S.D.

n = No. of samples

Values in parenthesis indicate the range - R

NS = Not statistically significant

TABLE 6

MEAN VALUES (X) AND STANDARD DEVIATIONS (S.D) OF SERUM IMMUNOGLOBULINS AND COMPLEMENT LEVELS IN ALL HOOKWORM PATIENTS TOGETHER AND CONTROLS

Humoral Component	Hookworm Patients n = 57 $\bar{X} \pm$ SD (Range)	Controls n = 28 $\bar{X} \pm$ SD (Range)	P.Value
IgA (I.U)	180±155 (24-760)	125±84 (35-361	P/ 0.05*
SIgA (I.S)	11.7±10.5 (0.728-41.6)	2.8±2.1 (0.728-10.7)	P/ 0.001*
IgG I.U./ml	254±233 (112-1584)	282±206 (92-957)	P > 0.20
IgM I.U./ml.	232±216 (65-1148)	129±47 (69-321)	P/ 0.0005*
C_3 I.U./ml.	109±51 (21-290	112±47 (49-185	P [illegible].10
C_4 I.U./ml.	61±36 (11-144)	43±25 (11-98)	P/ 0.005*

* Statistically significant.

n = number of samples.

4.4 Serum Immunoglobulin Levels

IgA

Serum levels of IgA in hookworm patients and controls are indicated in Table 5.and Figure 5. Patients with marked anaemia had a mean ± SD of 156±112 I.U per ml (range 24-380 i.u), moderate to mild anaemia 244±212 (range 36-760i.u), no anaemia 158±133i.u/ml(range 31-760 i.u). Patient with hookworm alone had a mean of 201±174 i.u/ml (range 24-760i.u), while patients with other parasites in addition to hookworm had a mean of 128±74 i.u/ml (range 48-285). The controls had a mean ± SD of 125±84 (range 35-361 i.u/ml). Patients with hookworm only and those in the category 'moderate to mild' anaemia had significantly higher results than those of the controls ($P \leq 0.05$ for both of them). The other categories were not statistically different from the controls ($P > 0.5$ for all of them). Analysing all the hookworm patients gave a higher level of IgA 180±155 i.u (range 24 to 760 i.u/ml compared to the controls ($P \leq 0.05$) (Table 6).

Secretory IgA

The distribution of serum secretory IgA (IIS) levels is shown in figure 6 and Table 5. Patients with marked anaemia had a range of 0.7g/L to 26.0g/L with a mean of 8.9±7.1g/L, those with moderate to mild anaemia had a range of 1.77g/L to 41.6g/L with a mean of 17.2±15.1g/L, no anaemia had a range of 1.76g/L to 41.6g/l and a mean of 10.2±8.06g/L. Patients with hookworm only had a range of 0.73g/L to 41.6g/L and a mean of 12.25±11.62g/L, while patients with hookworm and other parasites had a range of 3.3g/L to 26.0g/L and a mean of

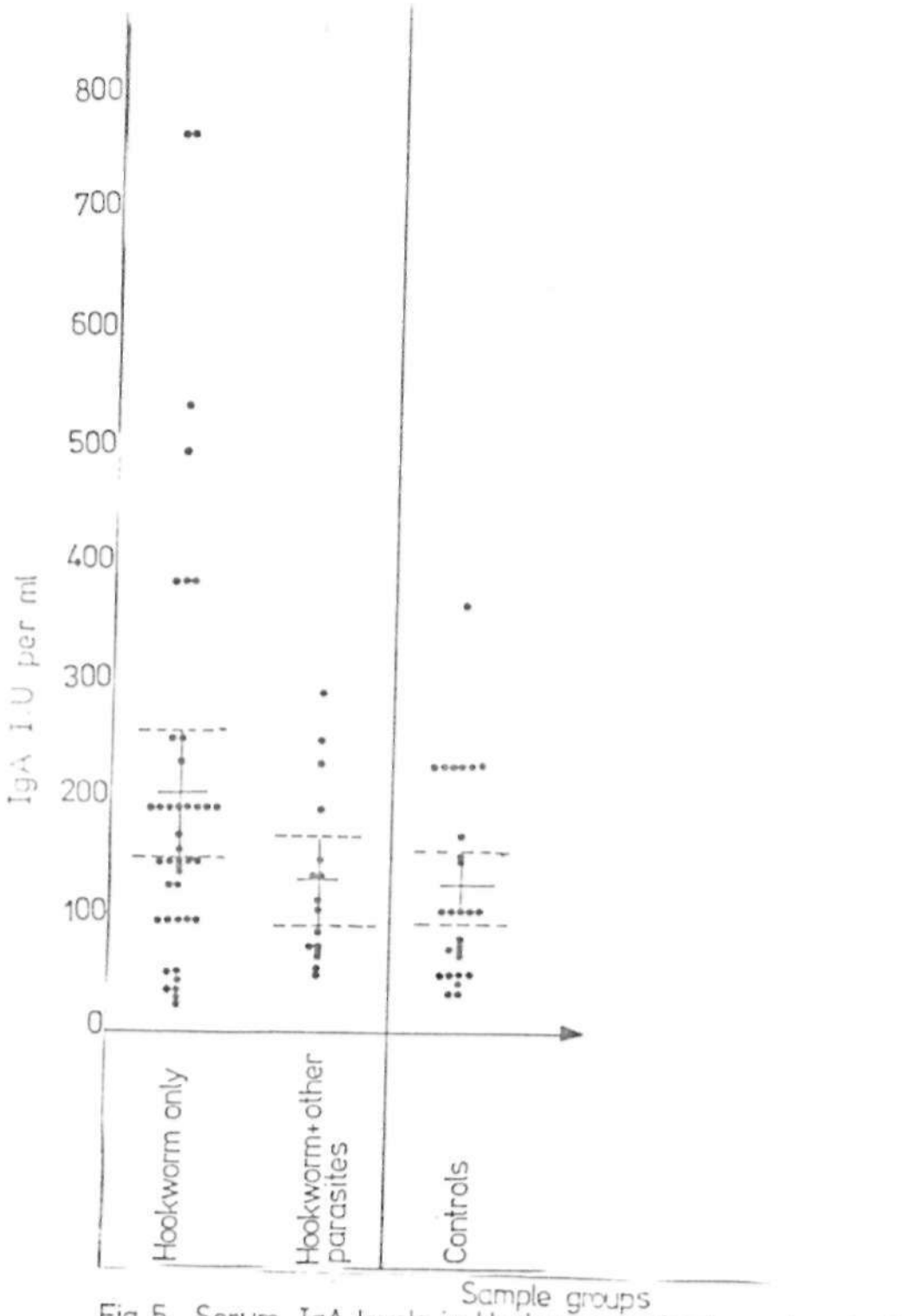


Fig. 5. Serum IgA levels in Hookworm patients and controls

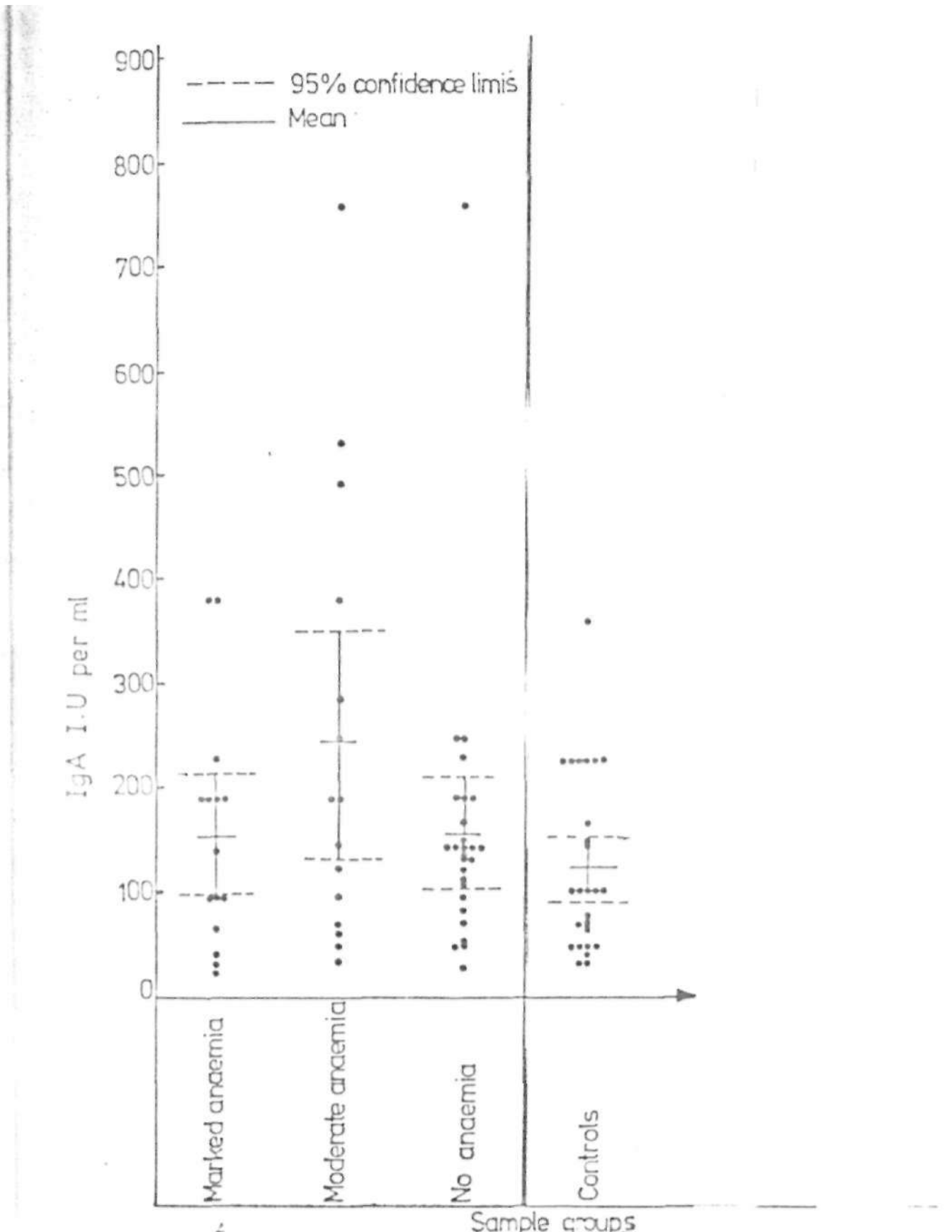


Fig. 5 Serum IgA levels in Hookworm patients and controls

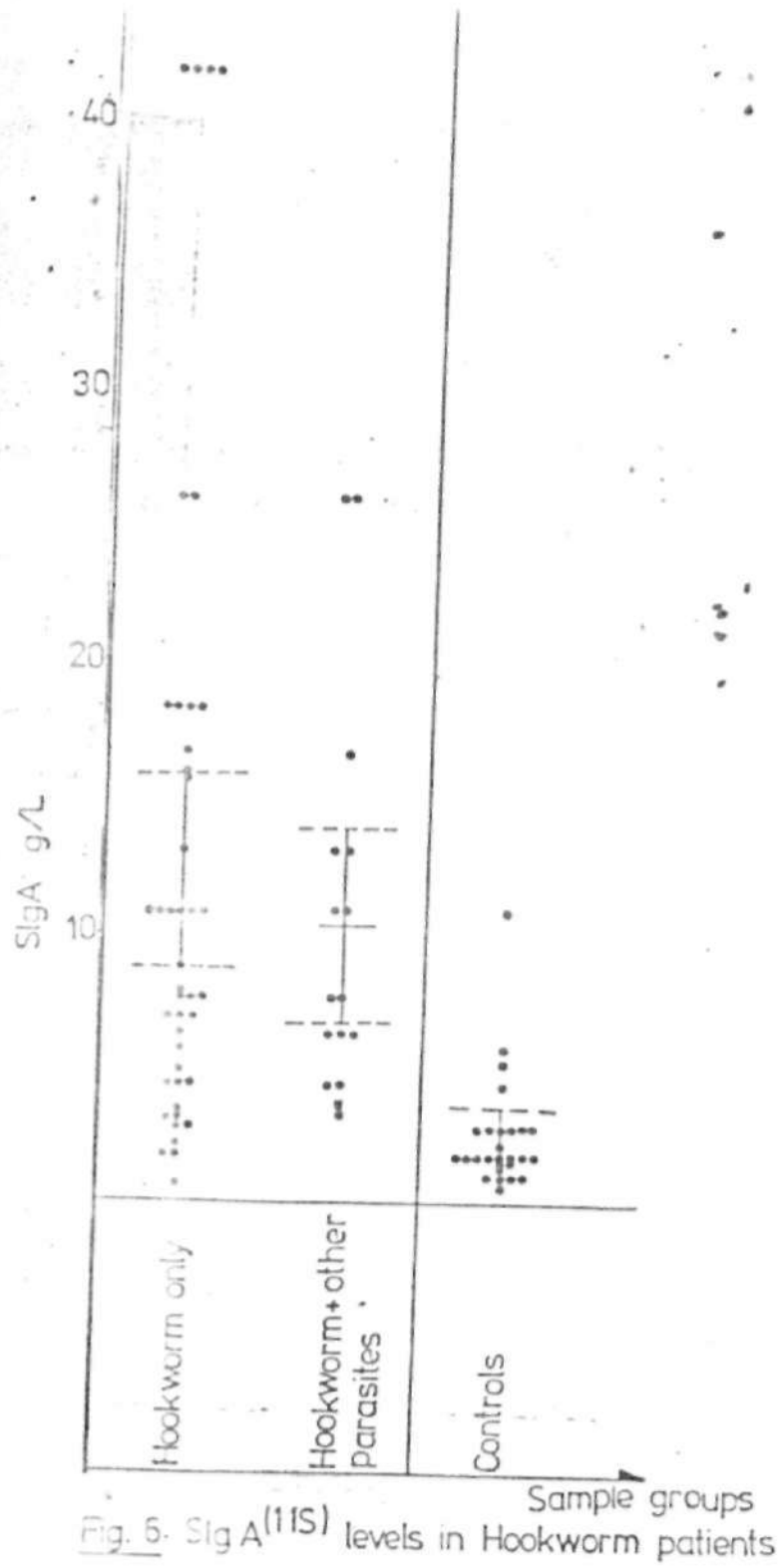


Fig. 6. Sig A(11S) levels in Hookworm patients and controls

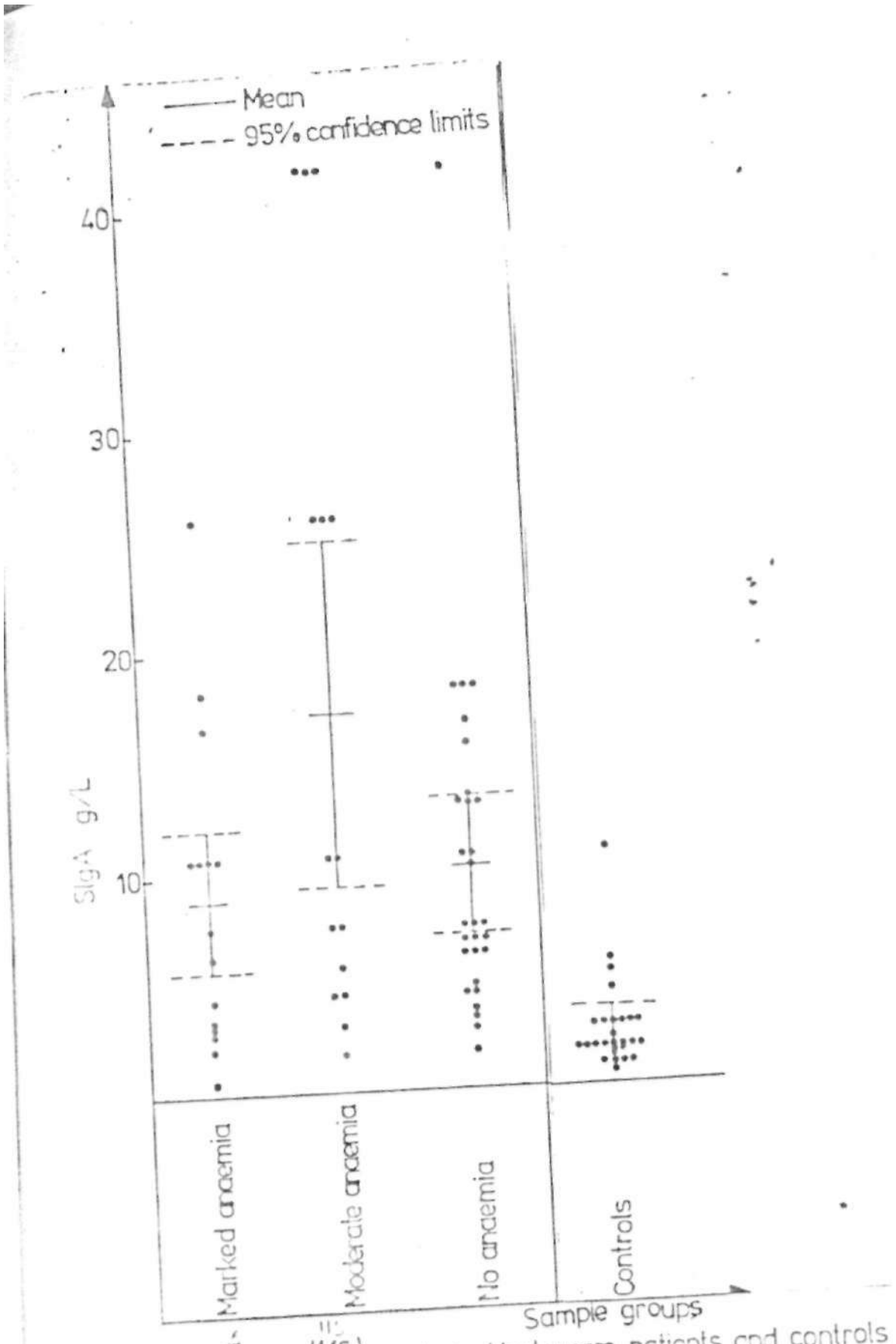


Fig. 6. Sig A(11S) levels in Hookworm patients and controls

10.3±7.19g/L. The results from each group were significantly higher than those of the controls with a range of 0.73g/L to 10.7g/L and a mean of 2.79g/L ($P < 0.001$ for each groups respectively). The mean SIgA level for all the hookworm patients was also significantly higher than those of the controls (11.7±10.5g/L with a range of 0.7g/L to 41.6g/L; $P < 0.001$) (Table 6).

Serum IgG

Figure 7 and Table 5 shows the mean serum levels of IgG in the various groups of hookworm patients and controls studied. Marked anaemia patients had a range of 125 i.u/ml to 4551.u/ml and a mean of 206±92 i.u/ml, moderate to mild anaemia patients a range of 1121.u/ml to 9571.u/ml and a mean of 284±247i.u/ml, no anaemia patients, a range of 125 i.u/ml to 5541.u/ml and a mean of 214±931.u/ml. Patients with hookworm alone had a range of 125 i.u/ml to 957 i.u/ml and a mean of 270±2621.u/ml, while patients with hookworm and other parasites had a range of 1121.u/ml to 693 i.u/ml and a mean of 213±133 i.u/ml. None of these values was significantly different from those of the controls with a range of 921.u/ml to 9571.u./ml and a mean of 282±2061.u./ml ($P > 0.2$, $P > 0.8$, $P > 0.2$, $P > 0.5$ and $P > 0.2$ for the different groups respectively. The mean serum level of 254±233 (range 112 to 1584) i.u/ml of all the hookworm patients together was also not statistically different from those of the controls ($P > 0.2$) (Table 6).

Serum IgM

The mean ± S.D values for the levels of serum IgM in the different hookworm subgroups are indicated in Table 5. and Figure 8. The various readings are as follows; marked anaemia; a range of 881.u/ml to 1148 i.u/ml and a mean

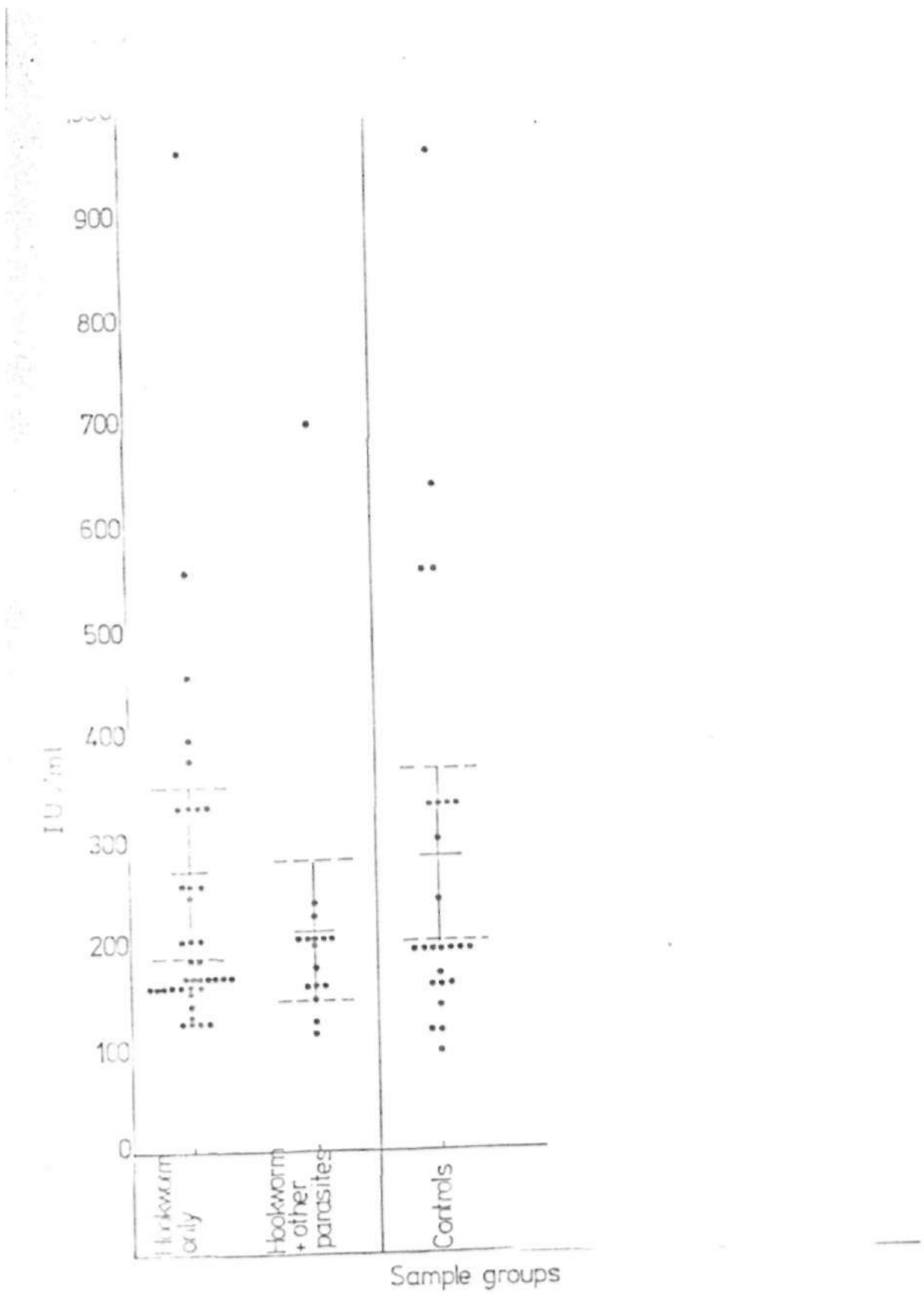


Fig. 7 Serum IgG levels in Hookworm patients and controls

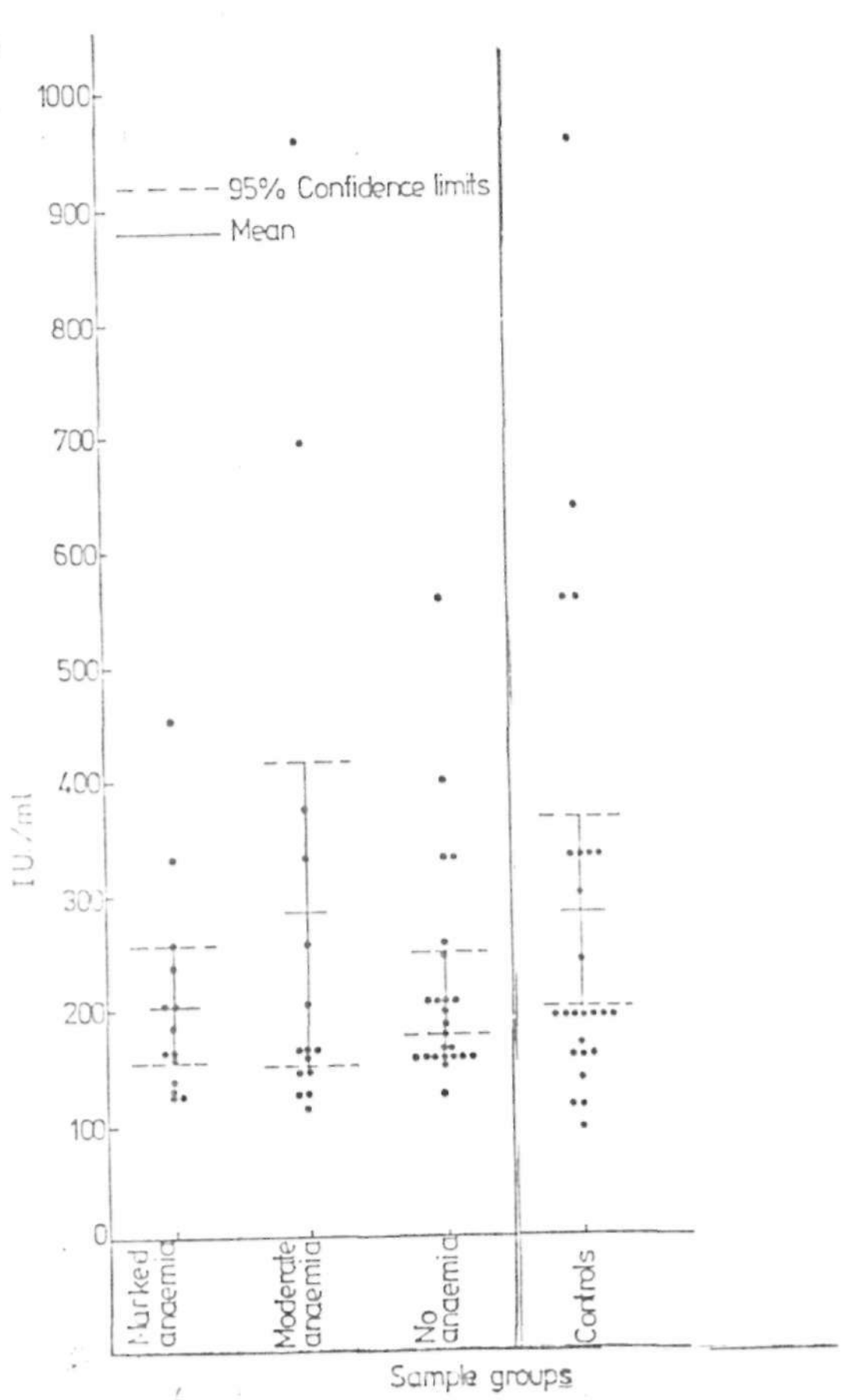


Fig. 7. Serum IgG levels in Hookworm patients and controls

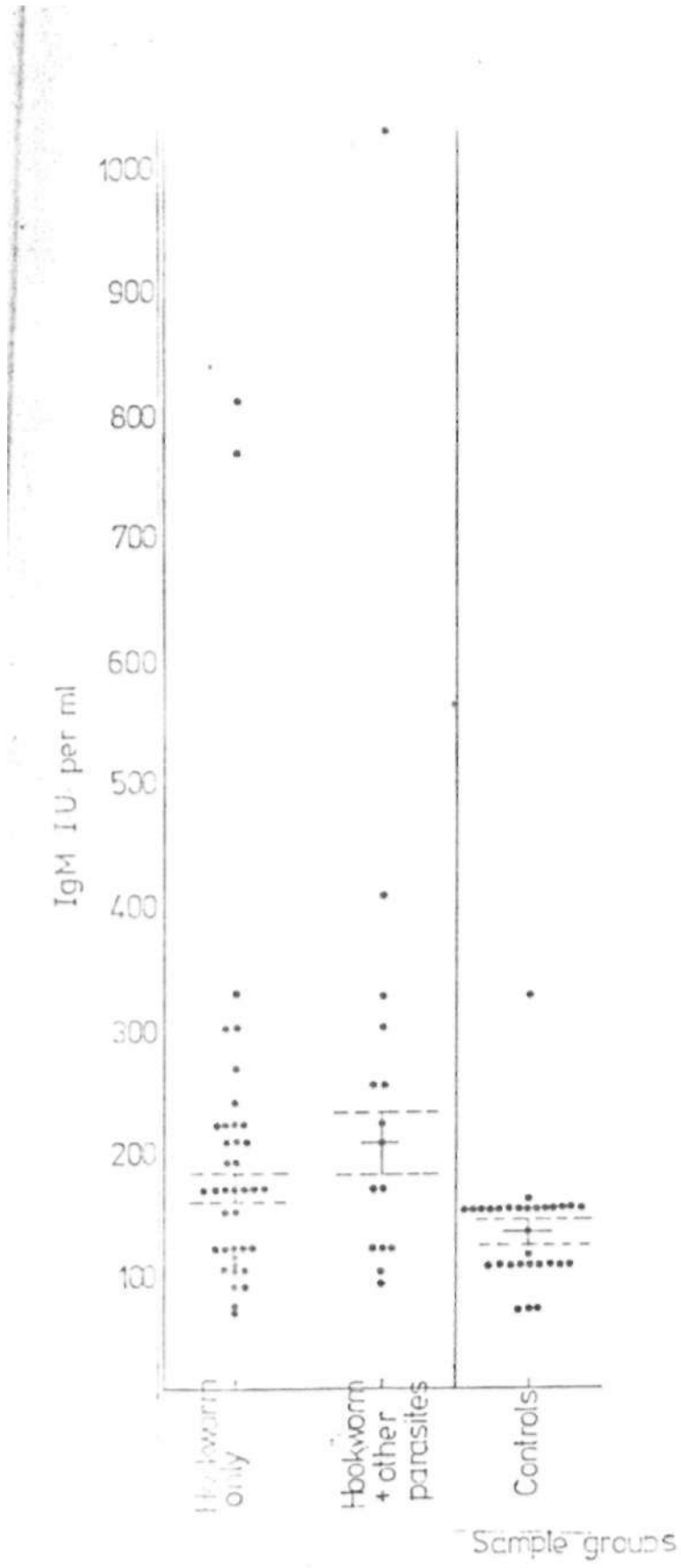


Fig. 8. IgM levels in Hookworm patients and controls.

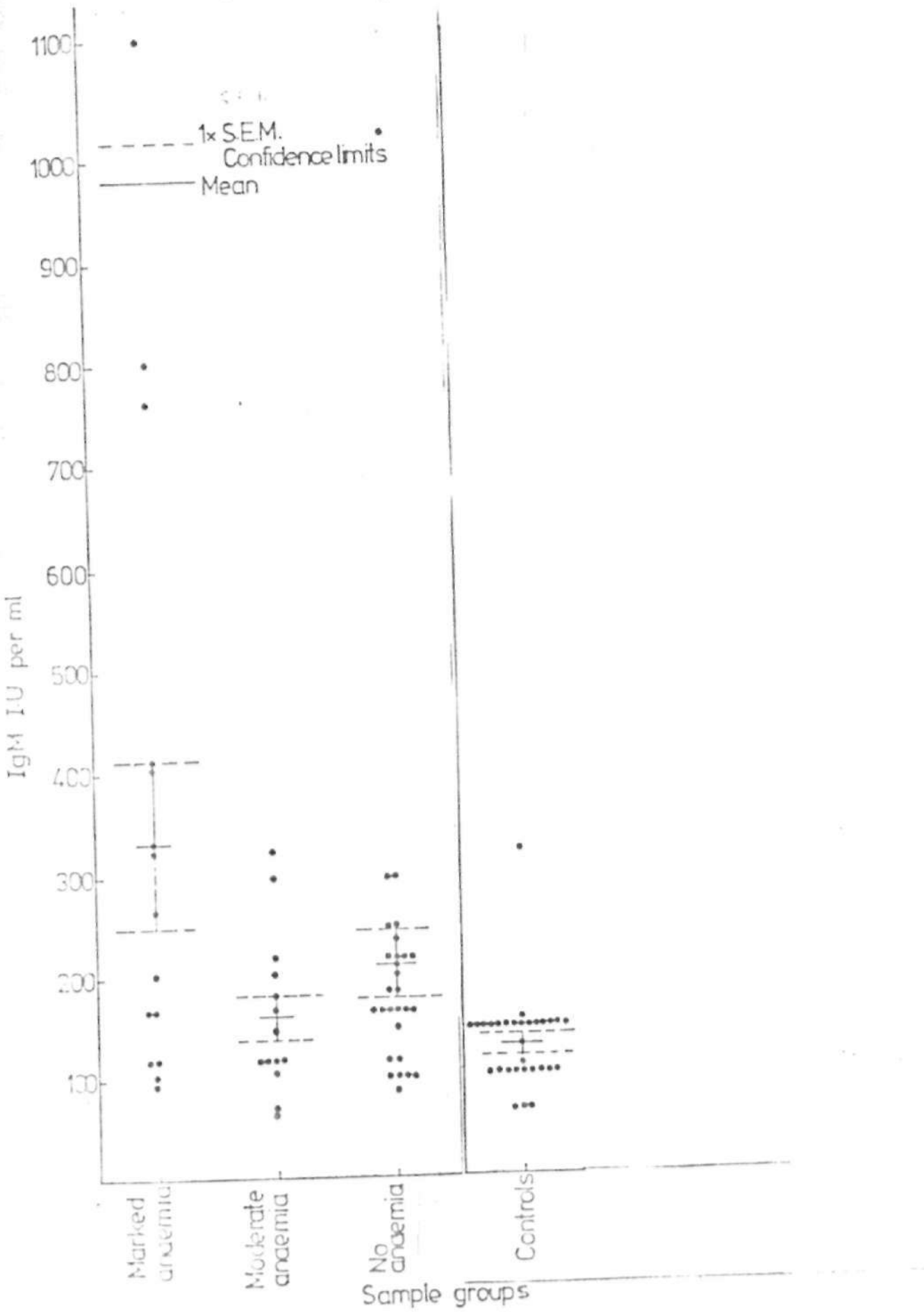


Fig 8. IgM levels in Hookworm patients and controls

of 331±319i.u/ml, moderate to mild anaemia: a range of 65i.u/ml to 321i.u/ml and a mean of 160±80i.u/ml, no anaemia, a range of 88i.u/ml to 1023iu./ml and a mean of 211±173 i.u/ml. Patients with hookworm alone had a range of 65 i.u/ml to 8.02 i.u/ml and a mean of 167±65i.u/ml while patients with hookworm and other parasites had a range of 100i.u/ml to 405 i.u/ml and a mean of 202±94 i.u/ml. The controls had a range of 69 i.u/ml to 321 i.u/ml and a mean of 130±47i.u/ml. Compared with the controls, patients with marked anaemia, no anaemia and hookworm and other parasites had significantly higher results ($P \leq 0.005$) while those with moderate to mild anaemia and those with hookworm alone, were not significantly different ($P > 0.2$ and $P > 0.05$ respectively) even through they also recorded higher values. All hookworm patients together gave a range of 65 i.u/ml to 1148i.u/ml and a mean of 232±216 i.u/ml. They were also significantly higher than those of the controls ($P \leq 0.005$) (Table 6).

TABLE 7

LEVELS OF SERUM COMPLEMENT IN NORMAL CONTROLS AND HOOKWORM PATIENTS SUBGROUPS

Complement Component / H/W sub group	Marked Anaemia (I)	Mild moderate anaemia (II)	No anaemia (III)	Hookworm only (IV)	Hookworm & Other parasites (V)	Controls (VI)	P. value; groups I,II,III IV, V vs. group VI, resp.
C_3 % std.	92 ± 64 n = 15 (21-290)	110 ± 59 n = 15 (55-290)	119 ± 37 n = 27 (70-231)	114 ± 58 n = 41 (21-290)	98 ± 28 n = 16 (55-145)	112 ± 36 n = 28 (49-185)	NS; NS; NS; NS; NS;
C_4 % std	68 ± 41 n = 15 (11-144)	68 ± 39 n = 15 (26-144)	53 ± 32 n = 27 (20-144)	68 ± 38 n = 41 (26-144)	43 ± 24 n = 16 (26-80)	43 ± 25 n = 28 (11-98)	< 0.05; <0.05; NS; <0.001; NS

The values are given as mean ± S.D.

n = No. of Samples

Values in parenthesis indicate the range

NS = not statistically significant.

4.5 Serum Complement Levels

C_3 Complement

Serum levels of C_3 expressed as percentage of standard in hookworm patients and controls are indicated in Figure 9 and Table 7.

In the first group, patients with marked anaemia had a mean ± SD of 92±64% and a range of 21 to 290% of standard, moderate anaemia (Mn) patients, had 110±59% and a range of 55 to 290% of standard and no anaemia (Na) patients had 119±37% and a range of 70-231% of standard. In the second group, patients with hookworm alone had a mean ± SD of 114± 58% and a range of 21 to 21 to 290% of standard as opposed to patients with hookworm infection and other parasites who had 98±28% and a range of 55 to 145% of standard. The controls had a mean ± SD of 112±36% and a range of 49 to 185% of standard. There was no statistically significant difference within the groups and compared with the controls ($P > 0.05$) each time. Serum C_3 levels in all the hookworm patients gave a mean of 109±51% of standard and a range of 21 to 290%. This was also not statistically different from that of the controls ($P > 0.1$) (Table 6).

C_4 Complement

Figure 10 and Table 7 show the mean ±S.D levels of C_4 expressed as a percentage of standard in hookworm patients and controls. Marked anaemia patients had a range of 11 to 144% and a mean of 68±41% of standard, moderate to mild anaemia had a range of 26 to 144% and

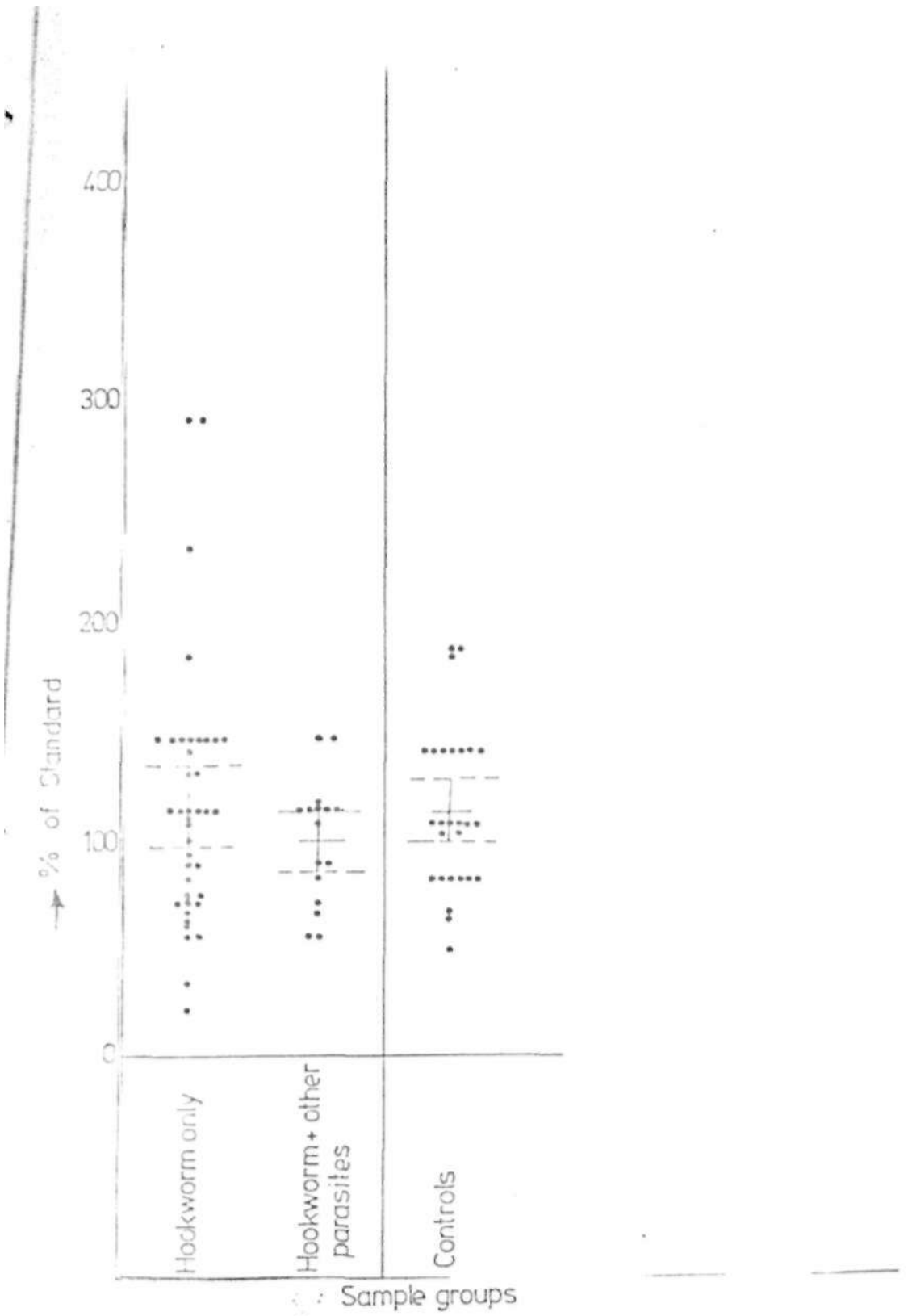


Fig 9 Serum C_3 level in Hookworm patients and controls

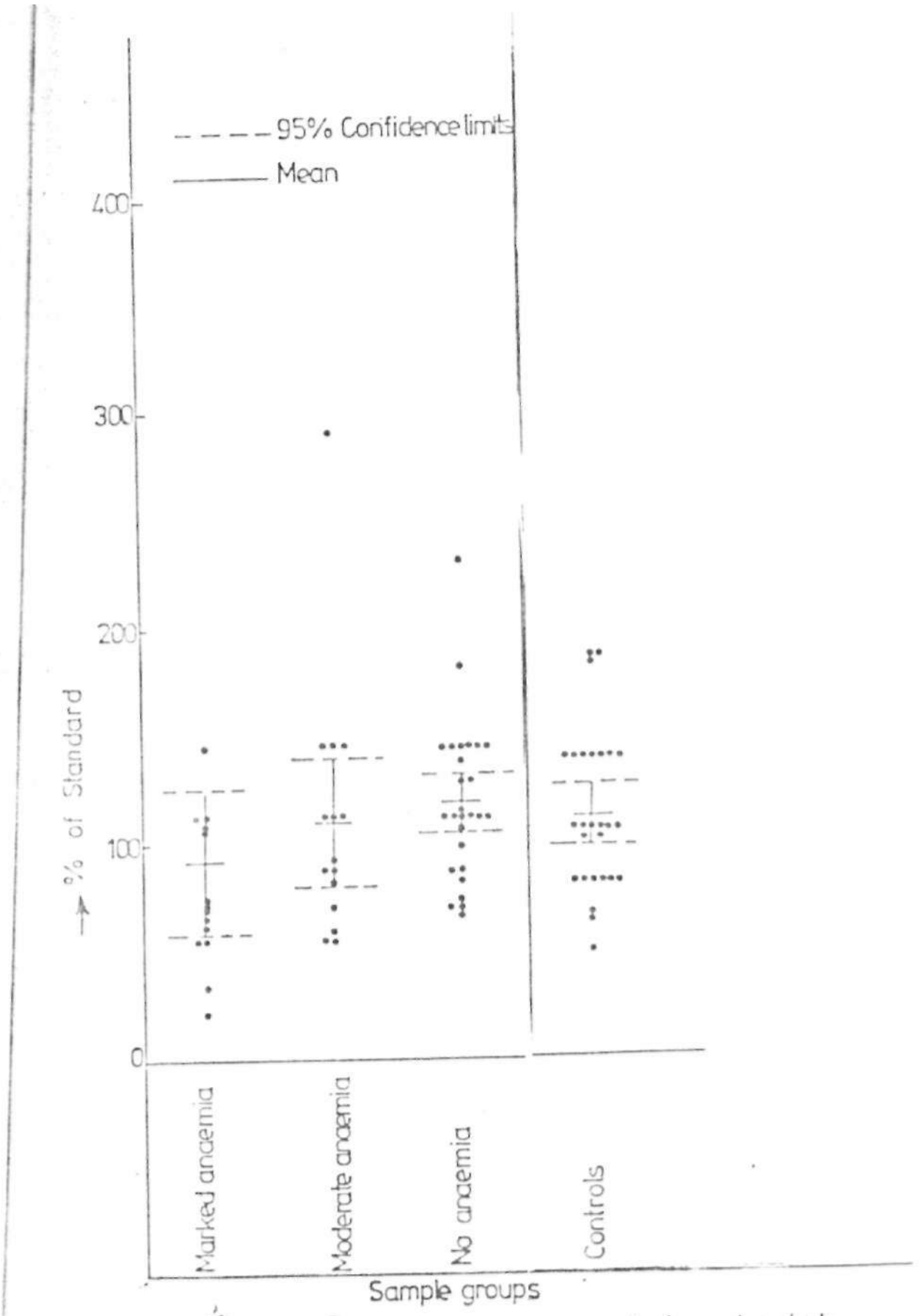


Fig. 9. Serum C_3 level in Hookworm patients and controls

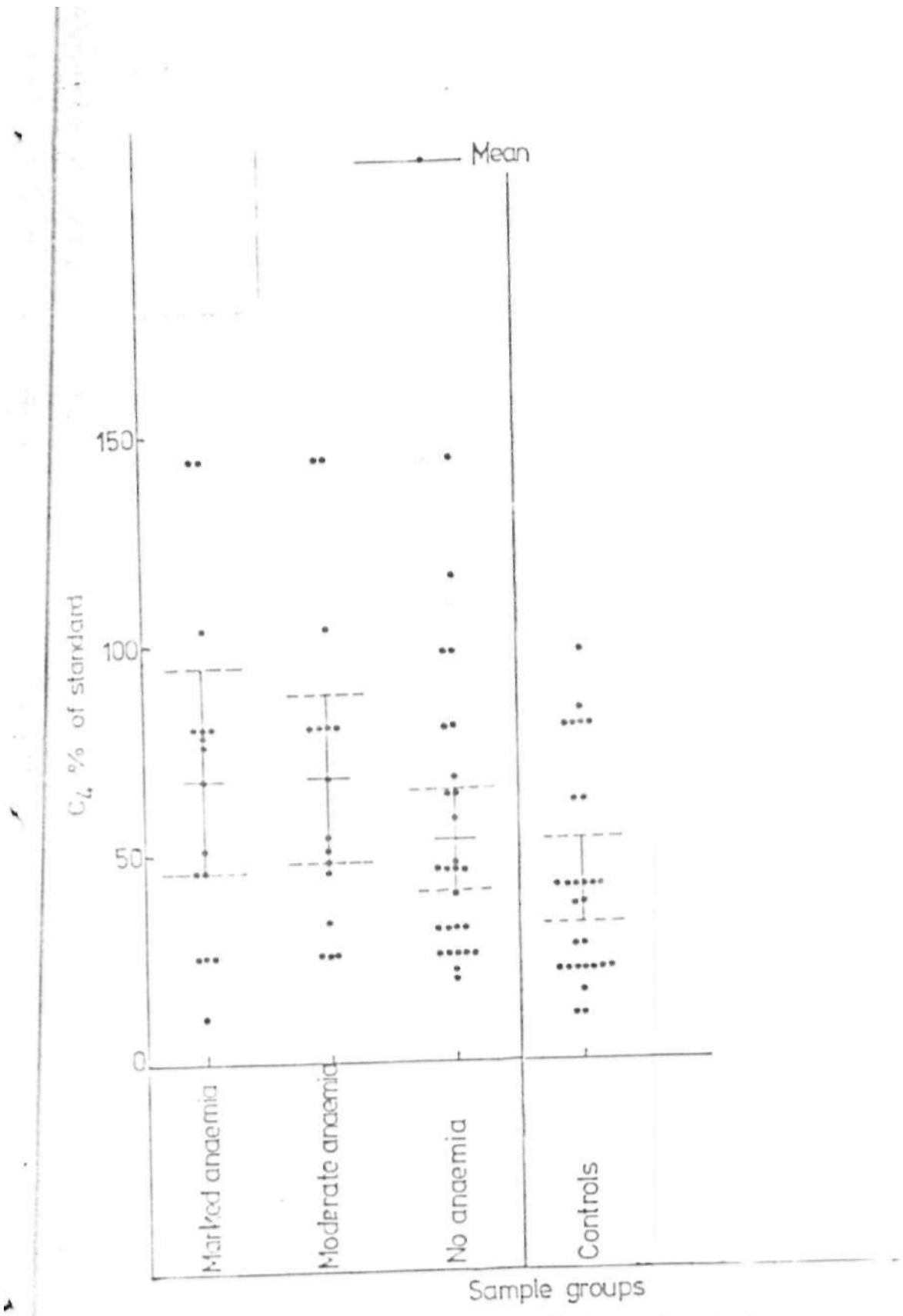


Fig 10 C_4 levels in Hookworm patients and controls

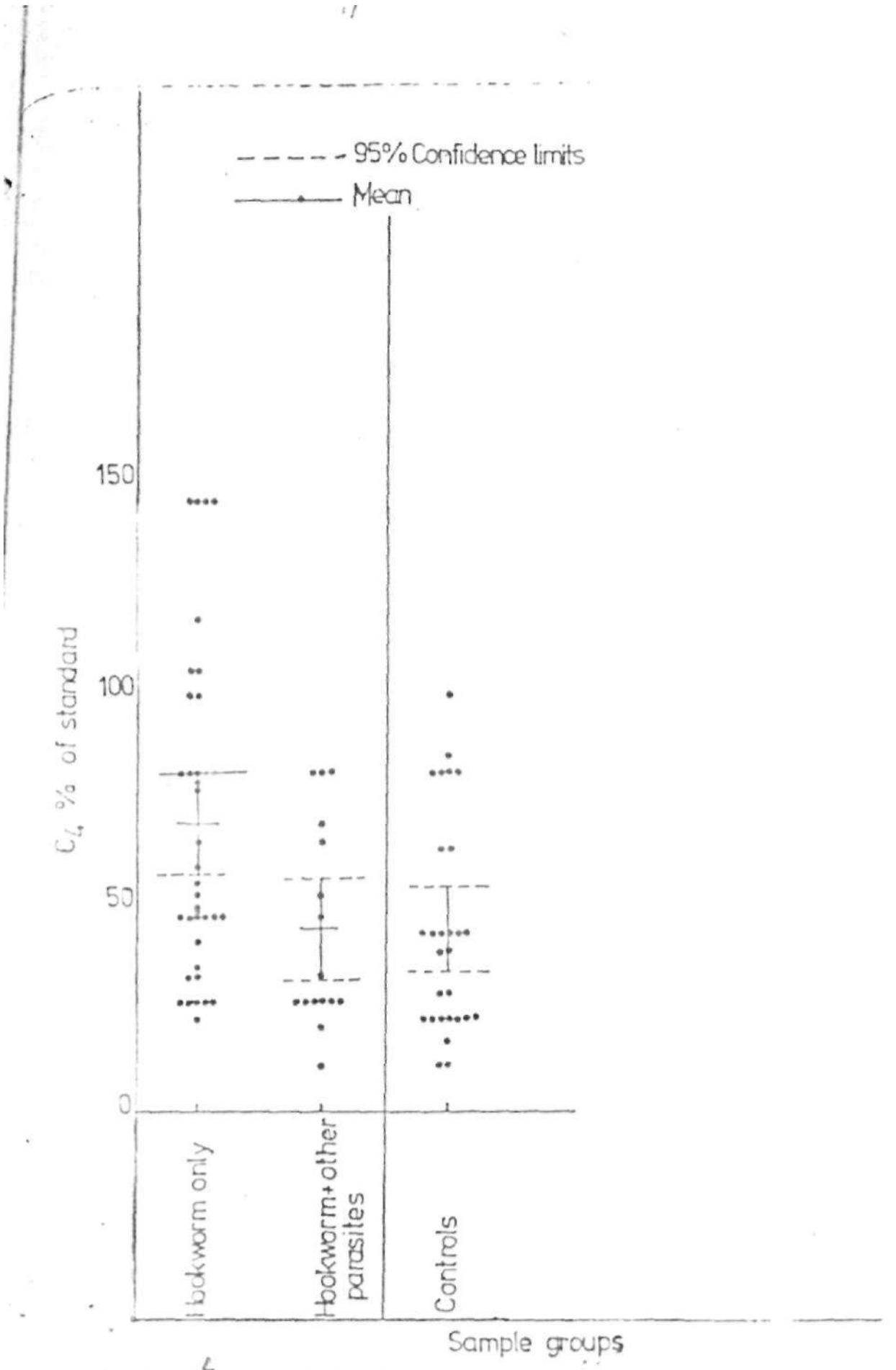


Fig. 10. C_4 levels in Hookworm patients and controls

a mean of 53±32% of standard. Patients with hookworm only had a range of 26 to 144% and a mean of 68±38% of standard, while patients with other parasites as well as hookworm had a range of 26 to 80% and a mean of 43±24% of standard. The controls had a range of 11 to 98% and a mean of 43±25% of standard. Compared with the controls, patients with marked anaemia, moderate to mild anaemia and hookworm only had values significantly higher than the controls ($P \leq 0.05$). Patients with no anaemia and hookworm along with other parasites were not significantly different ($P > 0.2$ and $P > 0.8$ respectively). Analysis of all the hookworm groups gave a range of 11 to 144% and a mean of 61±36% of standard. This was also significantly higher than that of the controls ($P \leq 0.05$) (Table 6).

TABLE 8

MEAN VALUES (X) AND STANDARD DEVIATIONS (S.D) OF IN-VITRO CELLULAR TESTS IN ALL HOOKWORM PATIENTS TOGETHER AND CONTROLS

	Hookworm Patients n x S.D.	Controls n x S.D	P.Value
Late Rosettes (Total T-cells) at 4°C	+ 1.06 ± 0.7	1.04±0.5	P>0.10
(%)	57 45 ± 9.0	28 58 ±6.0	P/ 0.05
Anti-Leu 4 (Total T-cells) positive cells	+ 1.08 ± 0.8	0.91±0.1	P>0.10
(%)	34 48 ± 9.0	17 59±7.0	P/ 0.05*
Anti-Leu 3a (Helper) positive cell	+ 0.60 ± 0.4	0.50±0.2	P>0.10
(%)	34 25 ± 6.0	17 32± 4.0	P/ 0.05*
Anti-Leu 2a (Suppressor) positive cells	+ 0.41 ± 0.2	0.34±0.2	P>0.10
(%)	34 21 ± 5.0	17 23 ±4.0	P>0.10
Percent migration inhibition to purified protein derivative (P.P.D) (%)	28 35 ± 16.0	16 56 ± 6.0	P/ 0.001*

* Statistically significant

n = No of Samples

+ = absolute numbers as opposed to relative percentage.

4.6 T-CELLS

4.6.1 Anti-Leu 4 Positive or Pan T Cells:-

The distribution of percent anti-Leu 4 positive cells in the subgroups of hookworm patients and controls is indicated in Figure II. Patients with marked anaemia had a range of 27% to 61% and a mean of 45.3± 9.07%, those with moderate to mild anaemia had a range of 31% to 65% and a mean of 50.2±9.86% while no anaemia patients had a range of 31% to 60% and a mean of 47.9±6.68%. Patients with hookworm only had a range of 31% to 65% and a mean of 49.2±8.0.3%, while those with hookworm and other parasites had a range of 27% of 59% and a mean of 44.5±9.38%. All of the groups had significantly lower levels of total T-cells as compared to the controls with a range of 49% to 69% and a mean of 59±6.64%. ($P \leq 0.005$ for all of them). All the hookworm patients, range 27% to 65% also had a significantly lower mean value (48±9.0%) compared to the controls ($P < 0.05$) (Table 8).

Absolute numbers for Pan T cells are shown in Figure 12. Marked anaemia patients had a range of 0.31 to 3.48×10^9/L and a mean of $1.12 \pm 0.91 \times 10^9$/L, mild to moderate anaemia had a range of 0.33 to 4.23×10^9/L and a mean of $1.27 \pm 1.09 \times 10^9$/L while no anaemia patients had a range of 0.05 to 1.70×10^9/L and a mean of $0.97 \pm 0.43 \times 10^9$/L. Patients with hookworm only had a range of 0.05 to 4.23×10^9/L and a mean of $1.21 \pm 0.85 \times 10^9$/L, while patients with hookworm and other parasites had a range of 0.31 to 1.50×10^9/L and a mean of

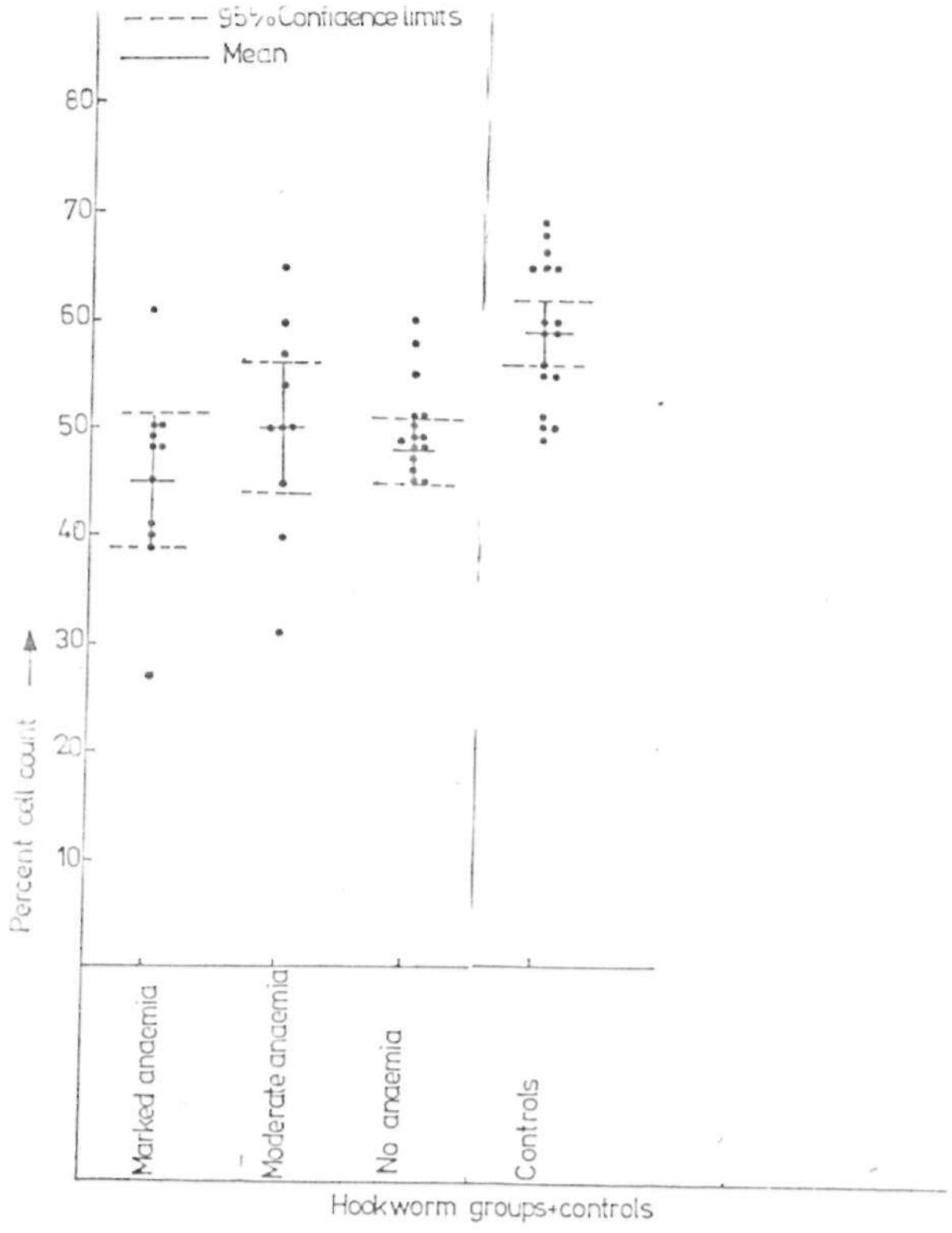


Fig 11. Mean percent anti Leu-4 positive cells in the peripheral blood of Hookworm patients and controls.

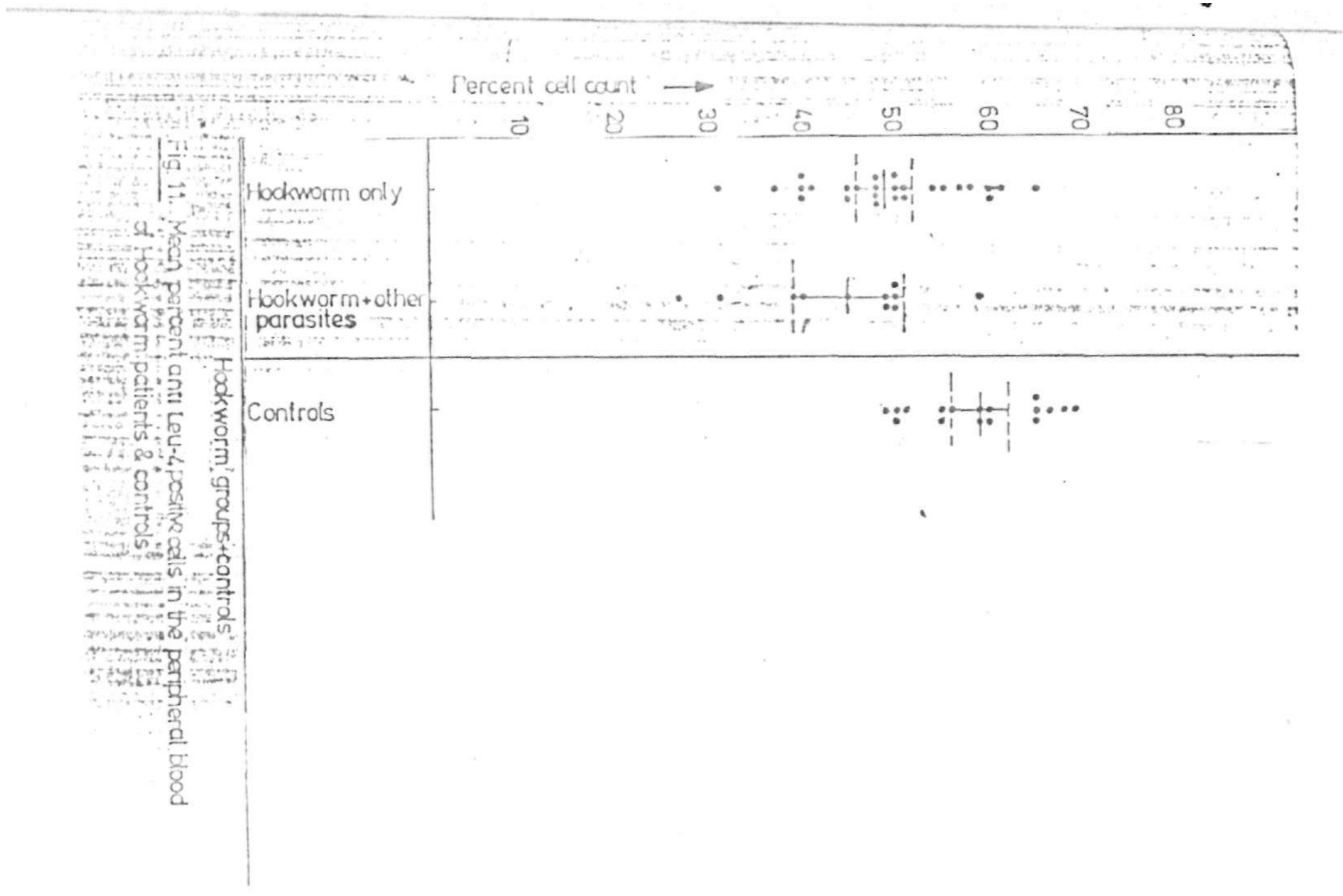


Fig. 11. Mean percent anti Leu-4 positive cells in the peripheral blood of Hookworm patients & controls. Hookworm groups+controls

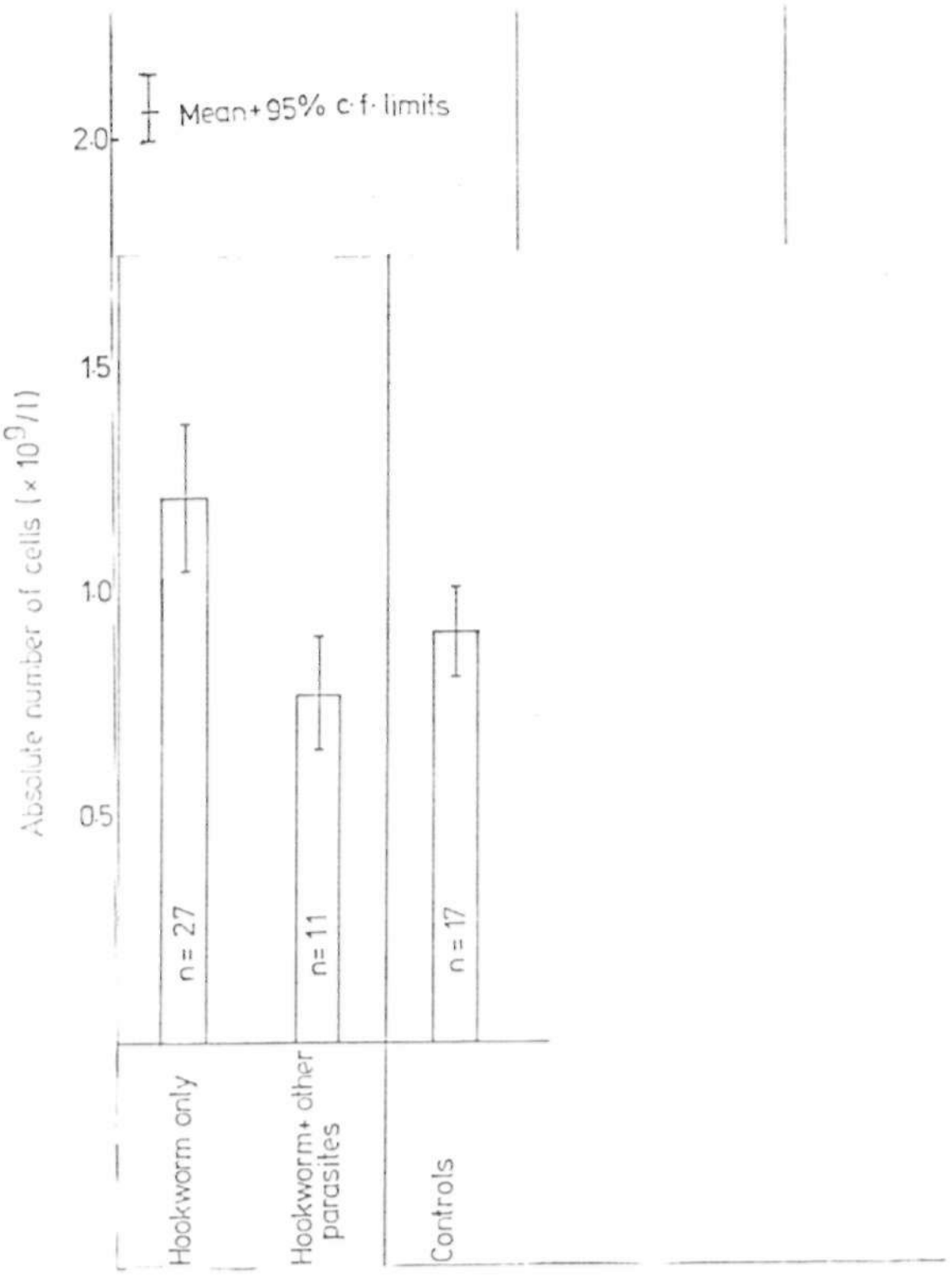


Fig 12. Mean (±I.S.E.M.) absolute number of anti Leu 4positive cells in Hookworm patients and controls.

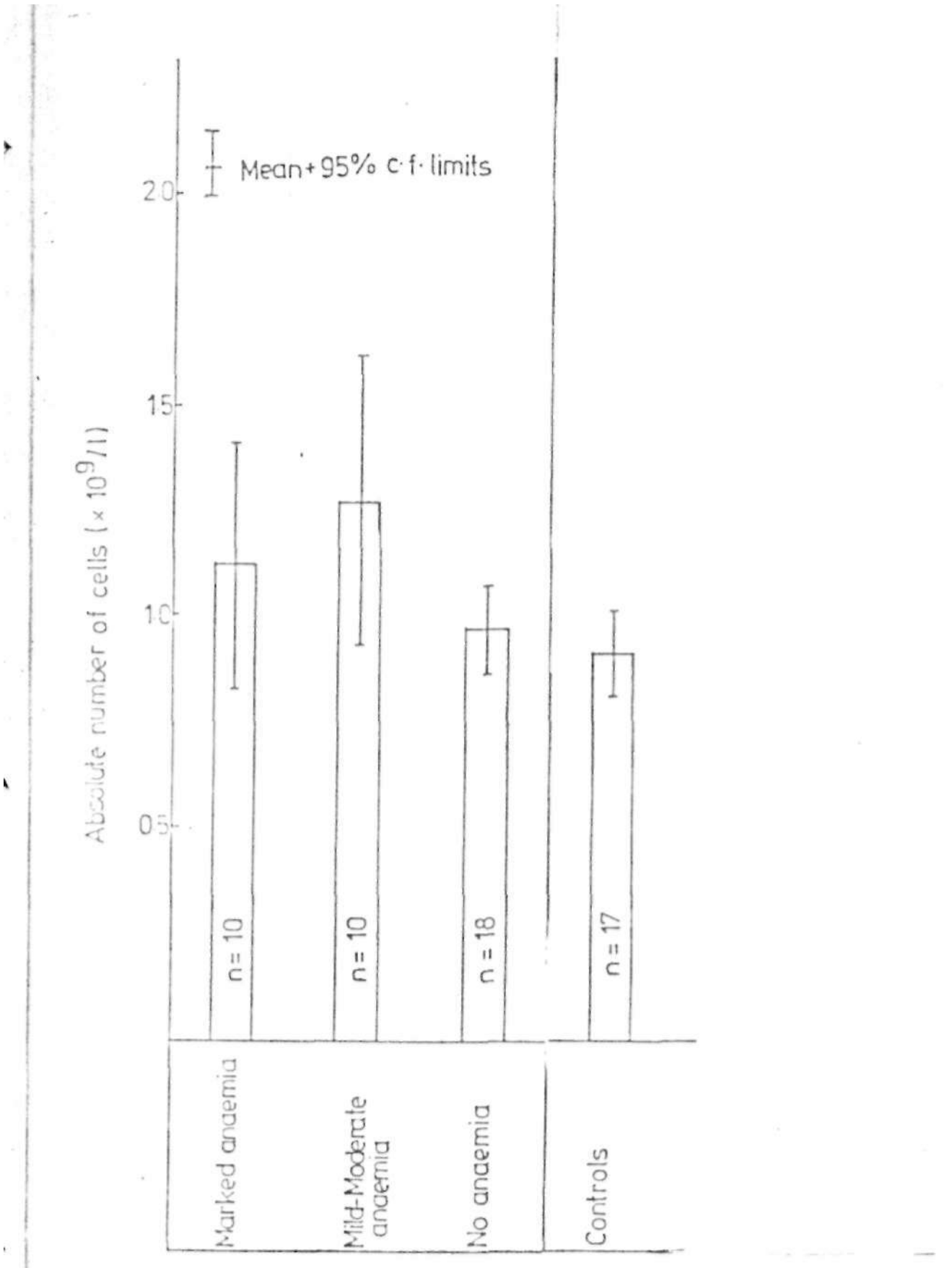


Fig. 12. Mean (+ I.S.E.M.) absolute number of anti-Leu 4 positive cells in the peripheral blood of hookworm patients + controls

0.77±0.4x10^9/L. The controls had a range of 0.48 to 1.89x10^9/L and a mean of 0.91±0.42x10^9/L. None of the groups had results significantly different from those of the control group ($P > 0.2$ for all respectively). All the hookworm patients together gave a mean of 1.08±0.8x10^9/L and again the result was not significantly higher than that of the control group ($P > 0.1$) (Table 8).

4.6.2 Late Sheep Rosette Forming or Total T Cells

Percent Total T-cells in the peripheral blood of hookworm patients and controls as determined by the formation of late rosettes with sheep red blood cells are shown in Figure 13. The results are as follows for the subgroups:- marked anaemia; a range of 24% to 60% and a mean of 42±8%, moderate to mild anaemia; a range of 28% to 65% and a mean of 45±11% and no anaemia; a range of 24% to 58% and a mean of 47±6%. Patients with hookworm only; a range of 24% to 65% a mean of 46±8% and hookworm and other parasites; a range of 24% to 55% and a mean of 43±9%. The controls had a range of 50% to 70% and a mean of 58±13%. All the groups were significantly lower than the control group ($P \leq 0.001$ for all the groups). The values for all the hookworm patients put together; range 24% to 65% and a mean of 45±9% was also very significantly lower than the control value ($P \leq 0.05$) (Table 8).

The absolute numbers of each of the hookworm subgroups are indicated in Figure 14. None of the groups were significantly different from

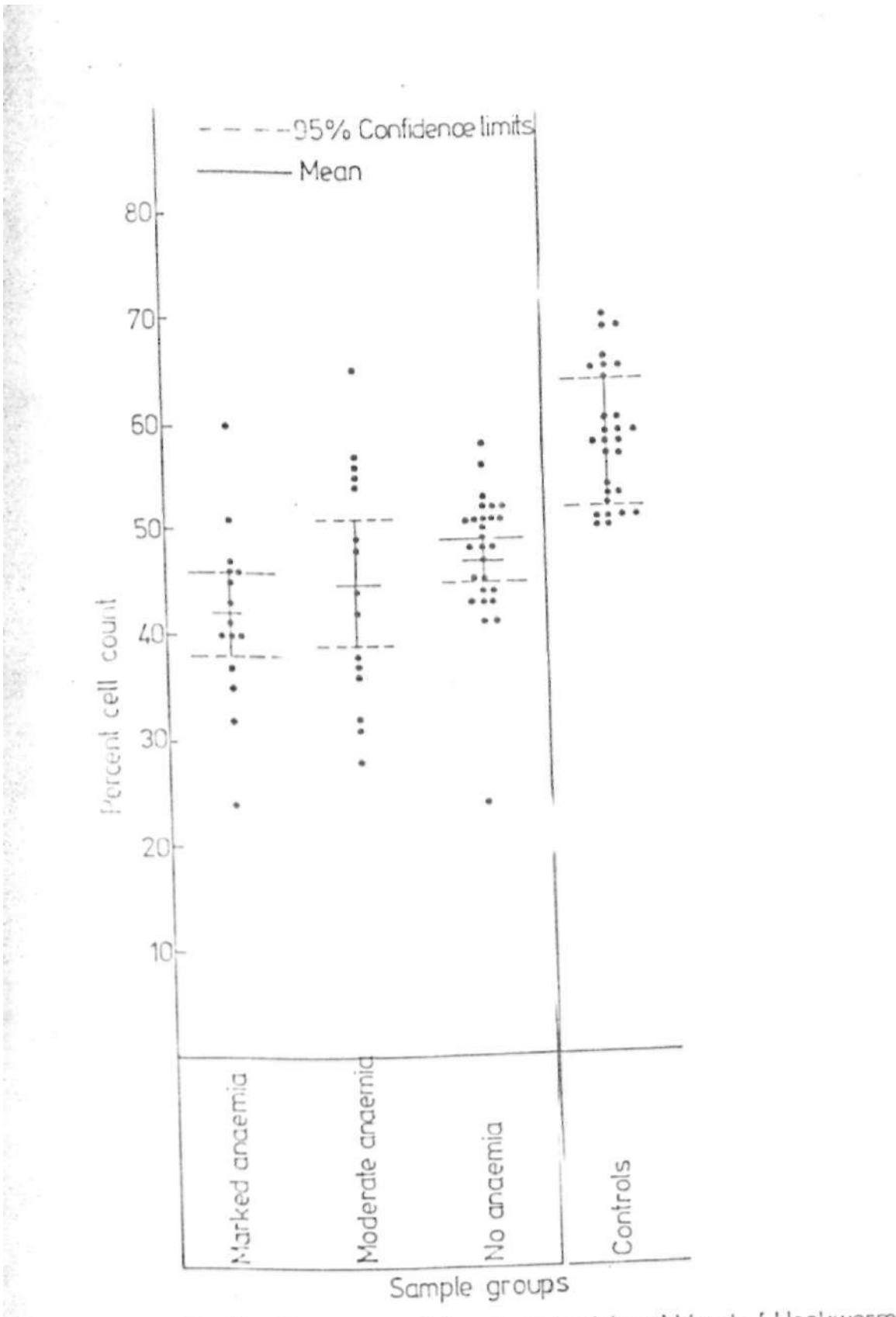


Fig. 13. Mean percent T. cells in peripheral blood of Hookworm patients & controls (late rosettes)

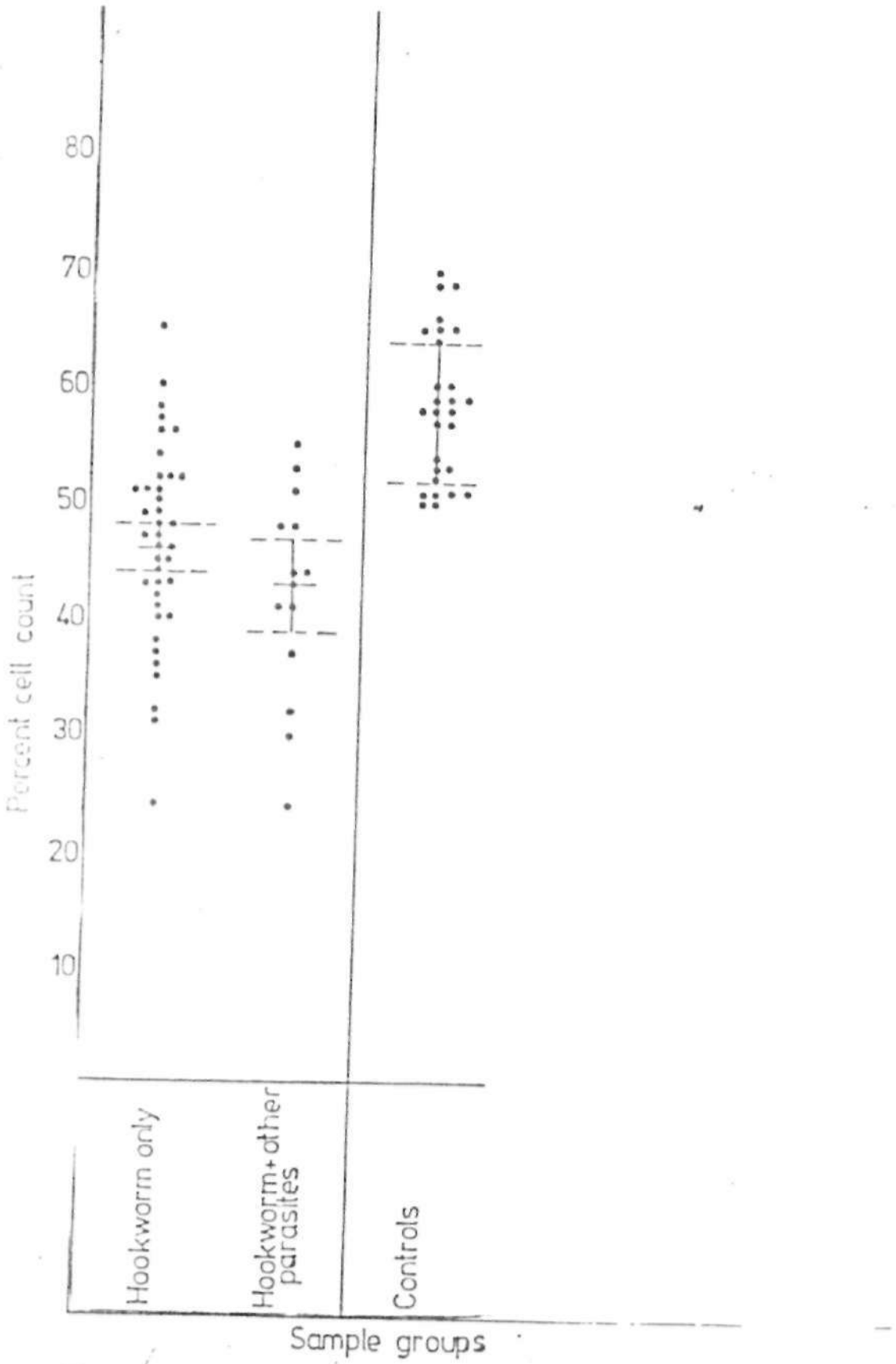


Fig. 13. Mean percent T. cells in peripheral blood of Hookworm patients & controls (Iaterosettes)

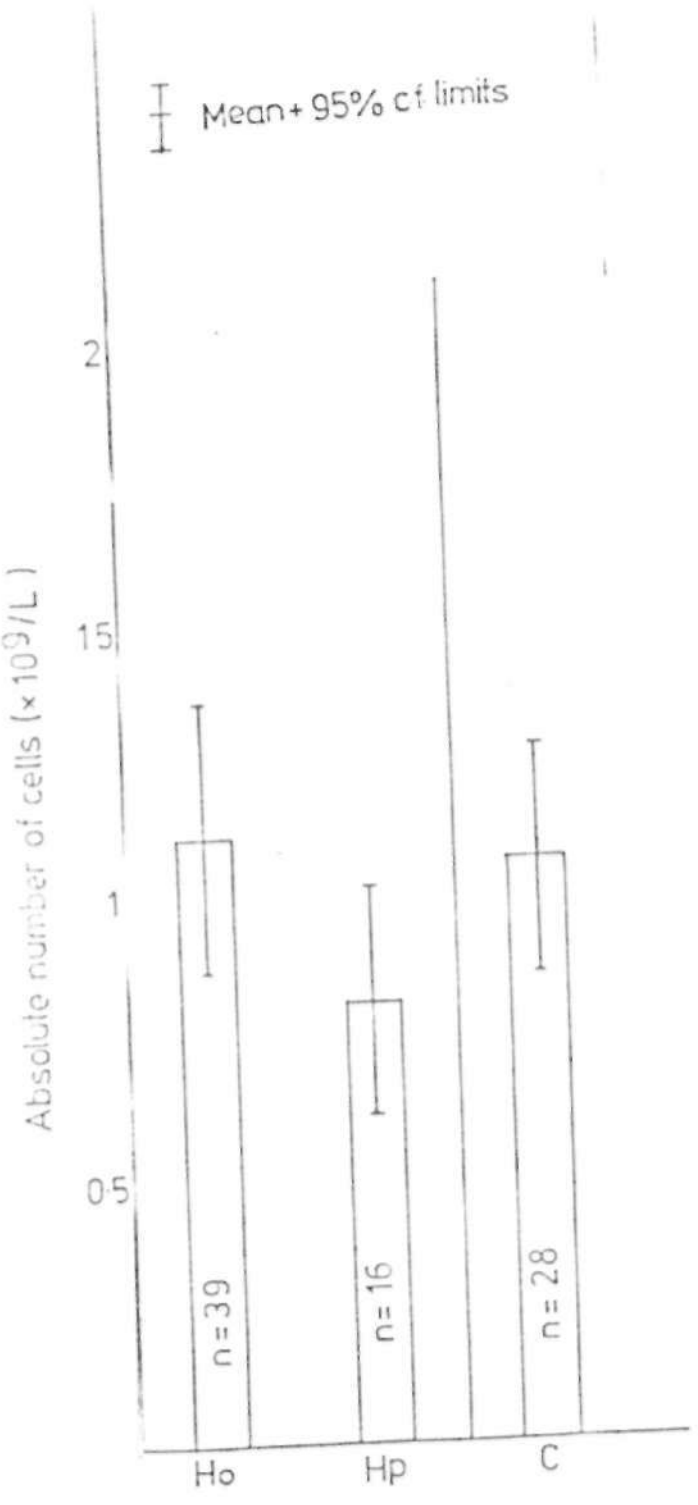


Fig 14. Absolute numbers of late Rosettes (total T-cells) in Hookworm patients & controls

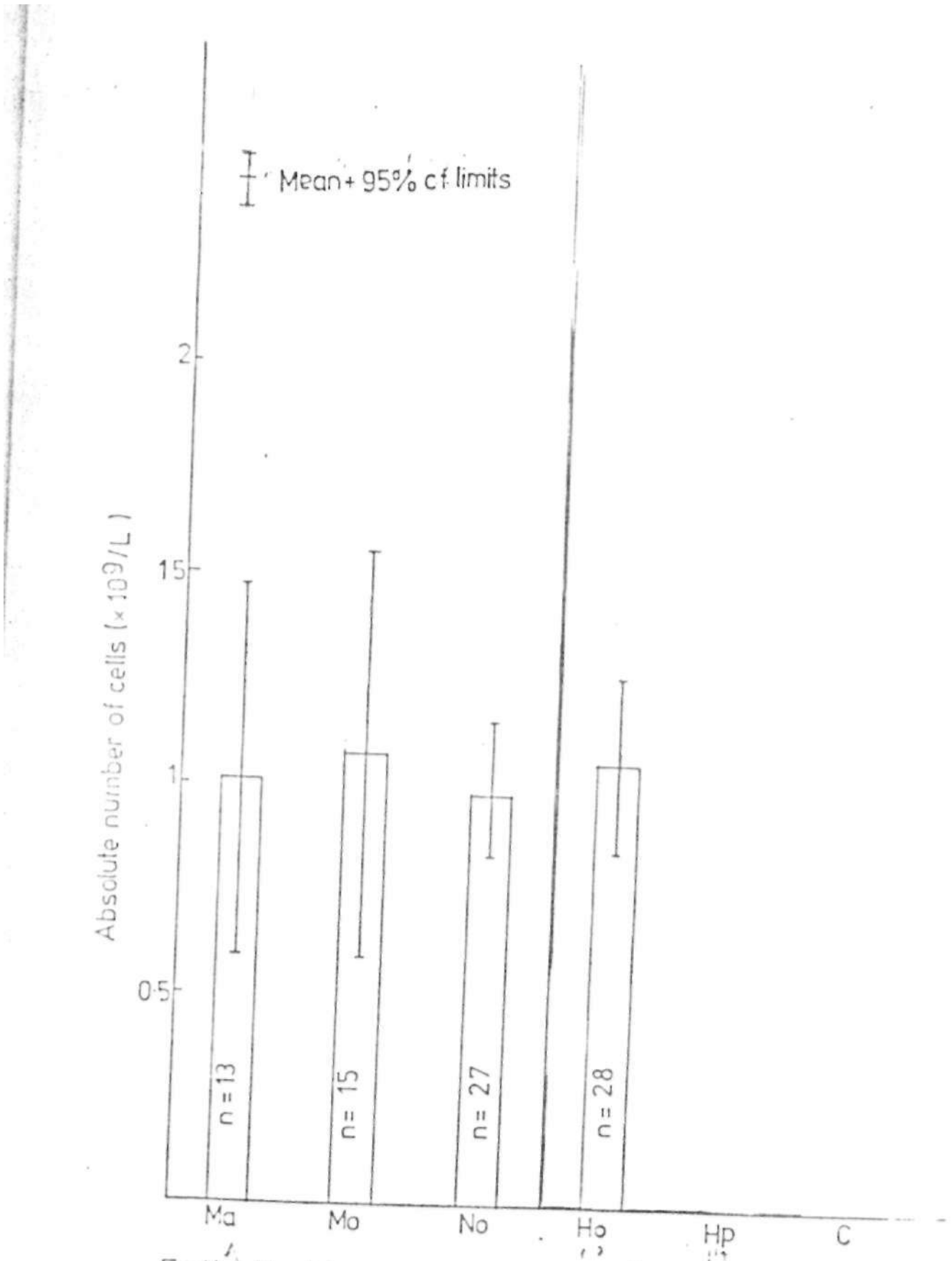


Fig. 14. Absolute numbers of late Rosettes (total T-cells) in Hookworm patients & controls

those of the controls ($P>0.1$ for all the groups). Marked anaemia patients had a range of 0.25 to $1.36x10^9$/L and a mean of $1.01\pm 0.75x10^9$/L, moderate to mild anaemia patients had a range of 0.30 to $4.23x10^9$/L and a mean of $1.07\pm0.94x10^9$/L no anaemia patients had a range of 0.02 to $1.47x10^9$/L and a mean of $0.98\pm0.44x10^9$/L patients with hookworm only had a range of 0.02 to $4.23x10^9$/L and a mean of $1.10\pm0.73x10^9$/L, while the hookworm and other parasites group had a range of 0.20 to $1.47x10^9$/L and a mean of $0.80\pm41x10^9$/L. The controls had a range of 034 to $2.46x10^9$/L and a mean of $1.05\pm 0.54x10^9$/L. All the hookworm patients together gave a range of 0.02 to $4.23x10^9$/L and a mean of $1.06\pm0.7x10^9$/L. This was also not significantly different from the controls ($P > 0.01$) (Table 8).

4.6.3 Anti Leu 3a Positive or Helper T Cells

The mean percent helper T cells are shown in Figure 15. Patients with marked anaemia, had a range of 10% to 29% and a mean of $22\pm$ 6%, moderate to mild anaemia, a range of 19% to 37% and a mean of $28\pm$ 6%, no anaemia had a range of 15% to 34% and a mean of $26\pm$ 5%. The hookworm only subgroup had a range of 15% to 37% and a mean of 25 ± 6%, and those with hookworm and other parasites had a range of 10% to 28% and a mean of 22 ± 6%. The control group gave a mean of 32 ± 4% and a range of 25% to 37%. All the groups were found to be significantly lower than the control group ($P/_ 0.001$ for all the groups). All the hookworm patients gave a mean of 25 ± 6% and a range of 10% to 37% and was significantly lower than the control group ($P/_ 0.005$) (Table 8).

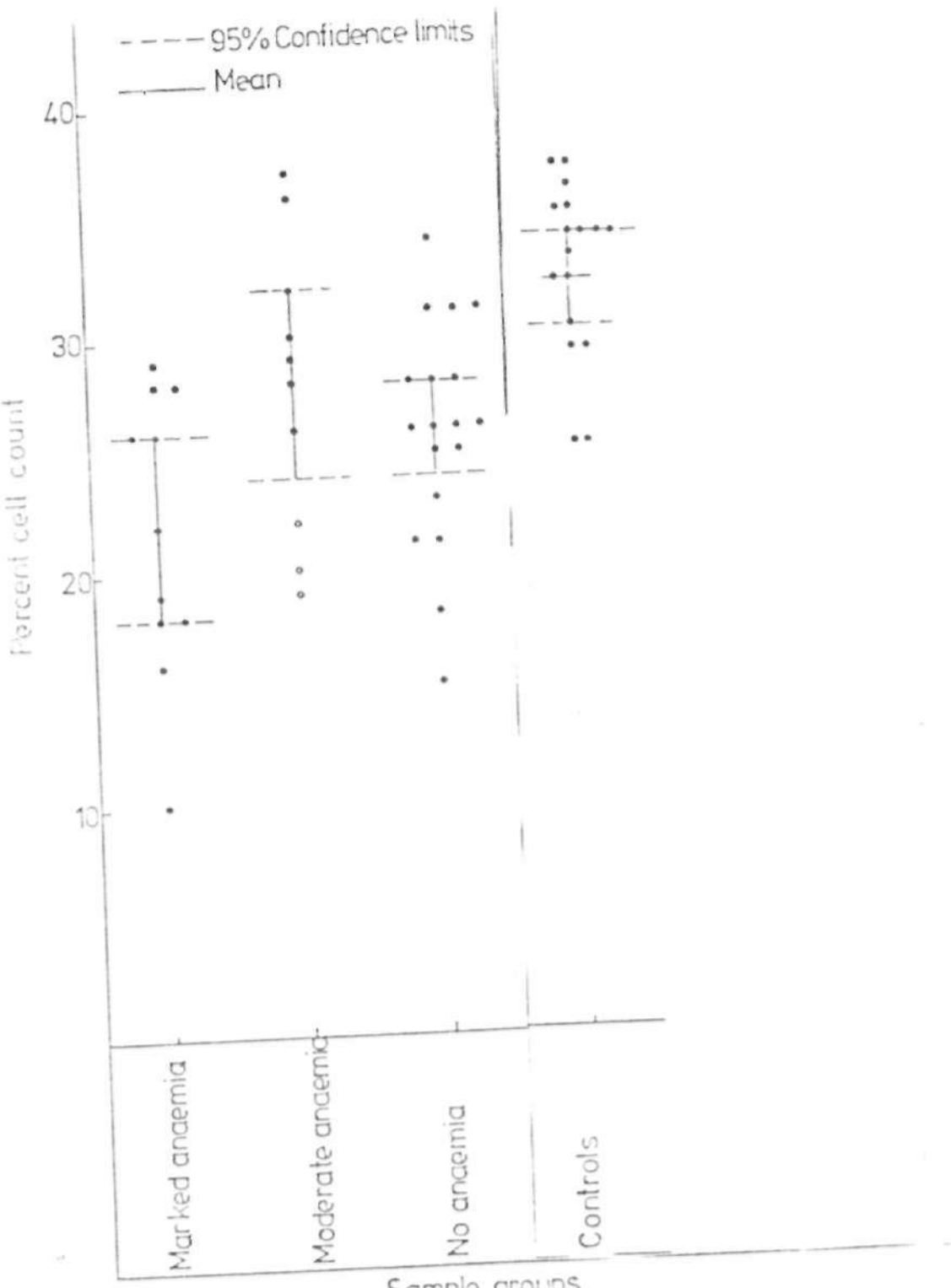


Fig. 15. Mean percent anti Leu-3a positive cells in the peripheral blood of Hookworm patients & controls

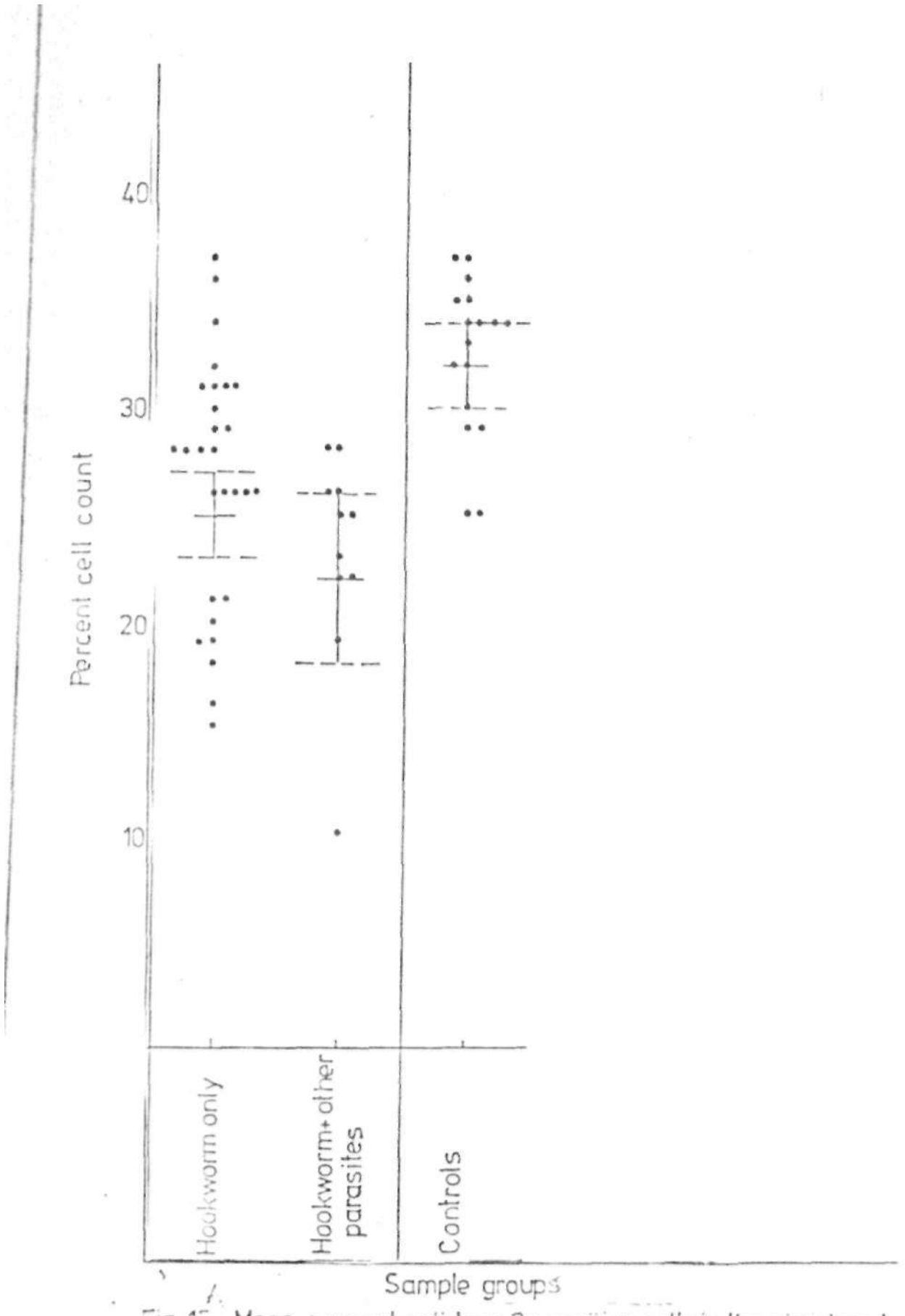


Fig. 15. Mean percent anti Leu-3a positive cells in the peripheral blood of Hookworm patients & controls

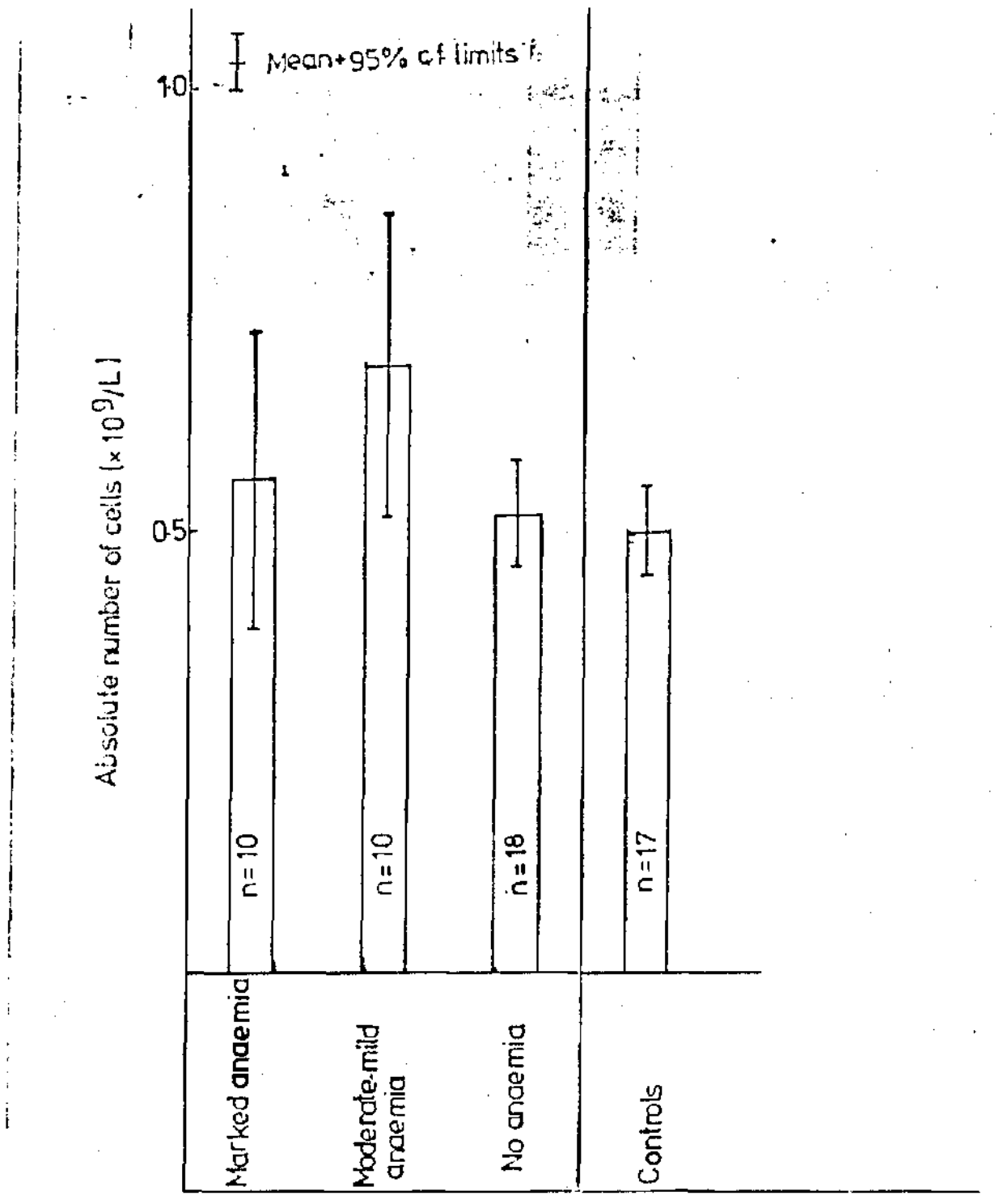


Fig. 15. Mean (+I.S.E.M) absolute number of anti Leu 3a postive cells in the peripheral blood of Hookworm patients & controls

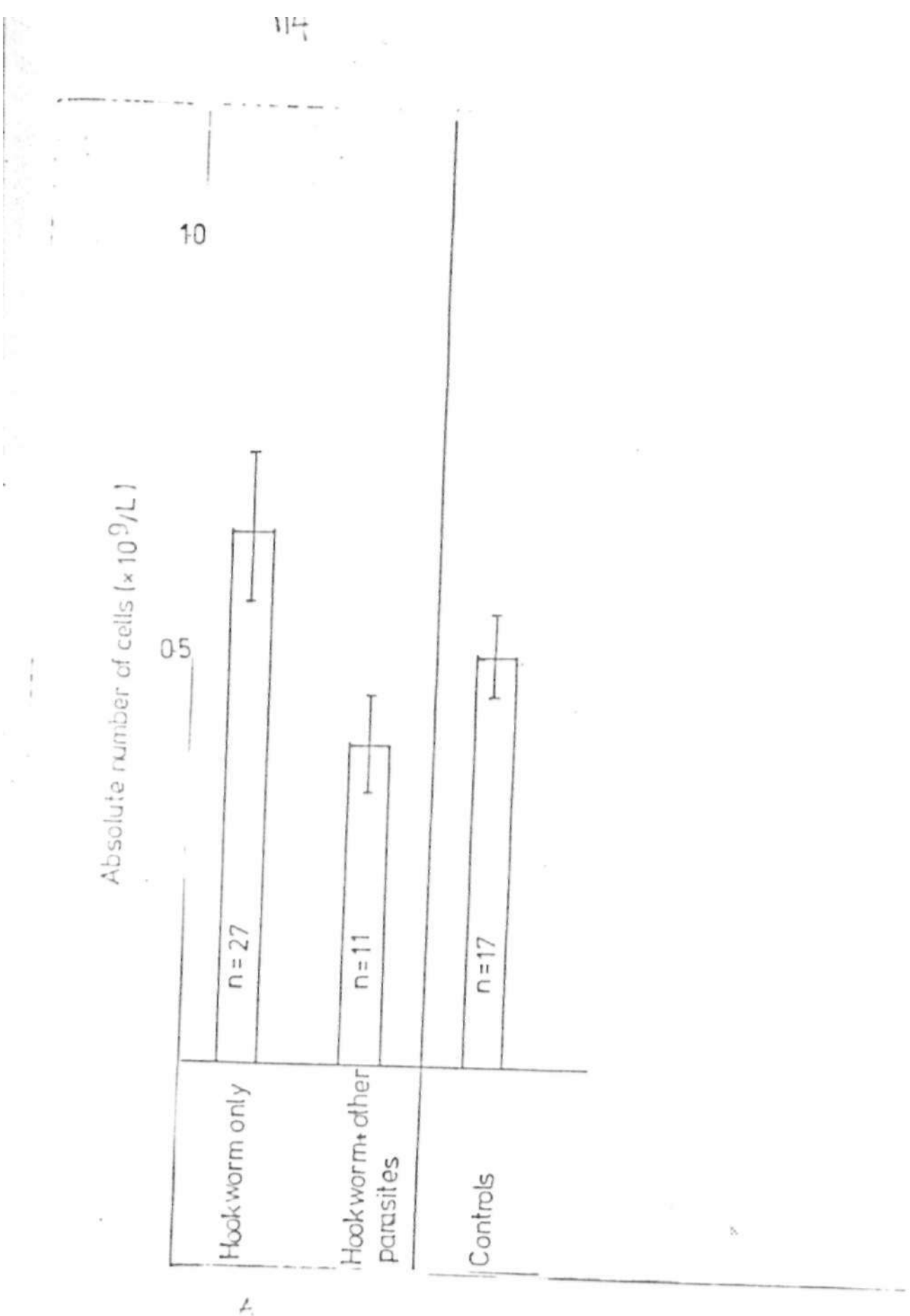


Fig. 15. Mean (+I.S.E.M) absolute number of anti Leu 3a postive cells in the peripheral blood of Hookworm patients & controls

Absolute numbers of helper T-cells in the peripheral blood of hookworm patients are indicated in Figure 16. Patients with marked anaemia had a range of 0.17 to 2.03 and a mean of 0.56±0.55, moderate to mild anaemia, had a range of 0.20 to 2.10 and a mean of 0.69±0.54, no anaemia had a range of 0.02 to 1.02 and a mean of 0.52±0.27. The hookworm only, subgroup had a range of 0.02 to 2.10 and a mean of 0.05±0.47, and those with hookworm and other parasites had a range of 0.17 to 0.76 and a mean of 0.39±0.21. The controls had a range of 0.23 to 0.98 and a mean + SD. of 0.50±0.23. None of the groups was significantly different from the controls ($P \geq 0.1$ for all groups). All the hookworm patients together also had an insignificantly different reading of 0.60±0.4 (range 0.02 to 2.10) ($P \geq 0.1$).

4.6.4 Anti-Leu 2a Positive or Suppressor T Cells

Percent distributions of suppressor T cells can be seen in Figure 17. Patients with marked anaemia had a range of 11% to 29% and a mean of 21±5%, those with moderate to mild anaemia, a range of 12% to 29% and a mean of 21±5%, no anaemia patients, a range of 14% to 37% and a mean of 21± 6%. Patients with hookworm only had, a range of 16% to 29% and a mean of 22±4% and those with other parasitic infections coupled with hookworm infection, had a range of 11% to 37% and a mean of 19±8%. The controls had a range of 14% to 29% and a mean of 23±4%. None of the groups was significantly different from the controls ($P \geq 0.1$ for all groups). Analysis of all the hookworm patients together gave a mean of 21±5% and was not significantly different from controls (Table 8).

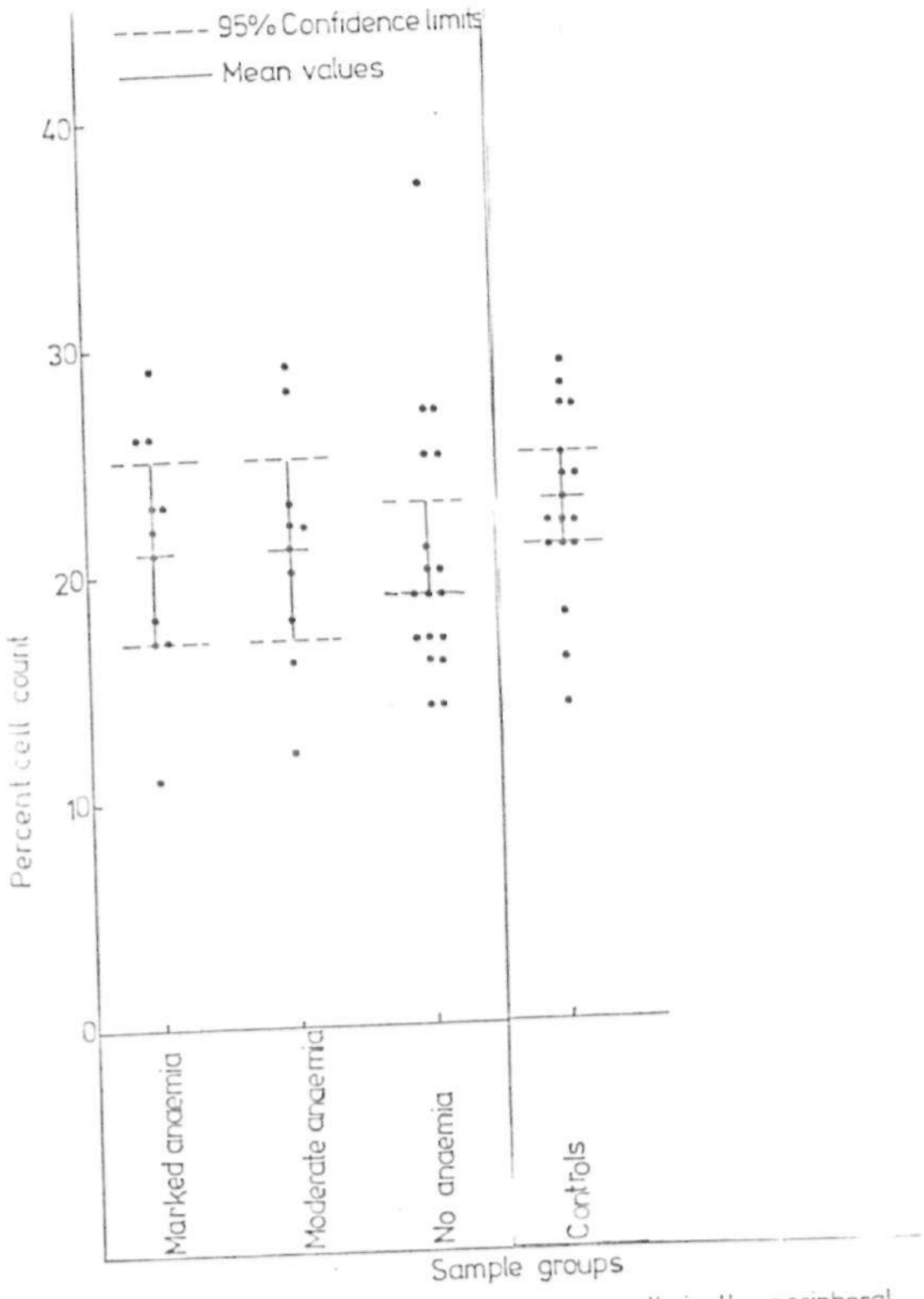


Fig 17. Mean percent anti Leu-2a positive cells in the peripheral blood of Hookworm patients and controls.

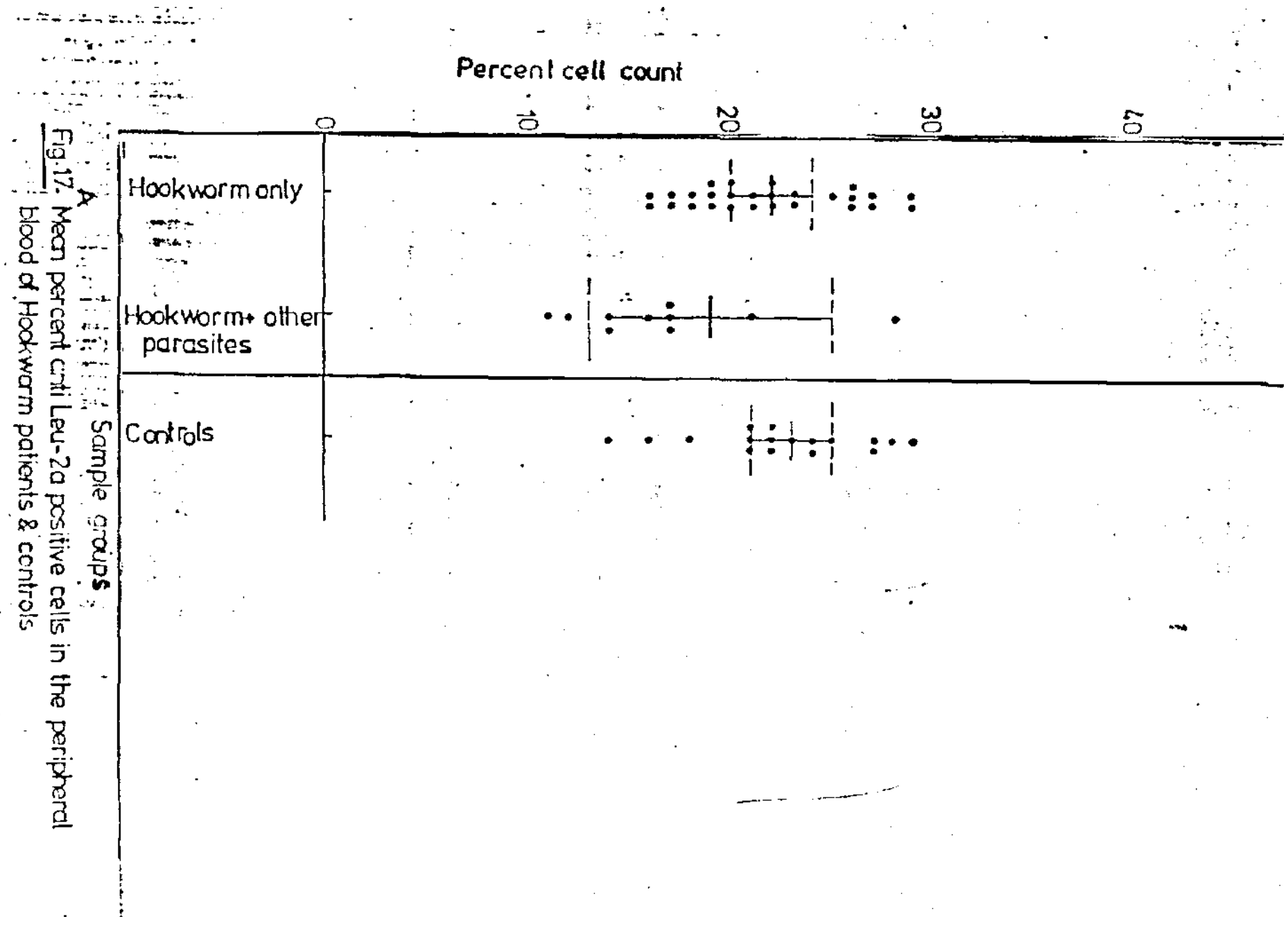


Fig.17. Mean percent cell Leu-2a positive cells in the peripheral blood of Hookworm patients & controls

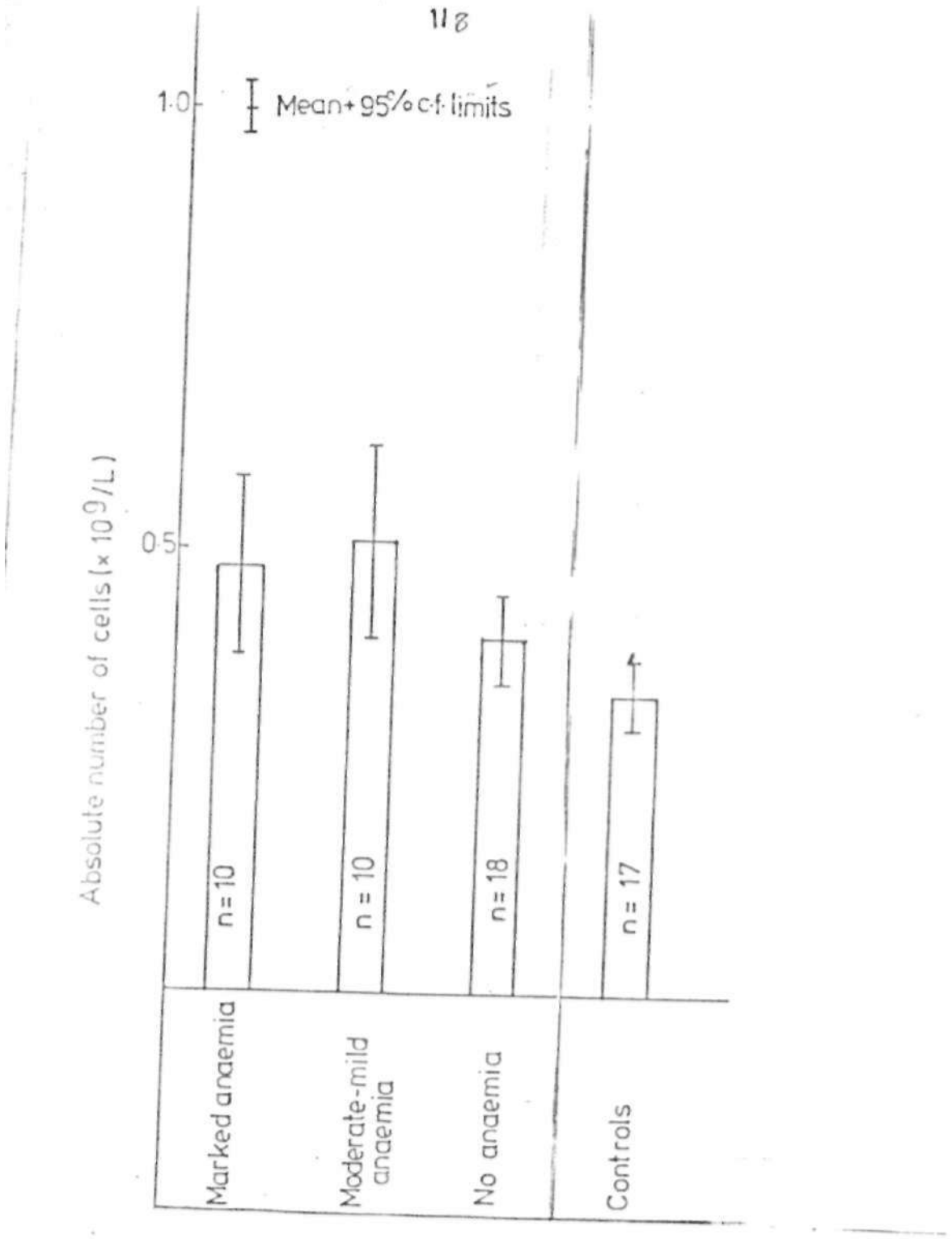


Fig. 18. Mean (+ I S E M) absolute number of anti Leu 2a positive cells in the peripheral blood of Hookworm patients and controls.

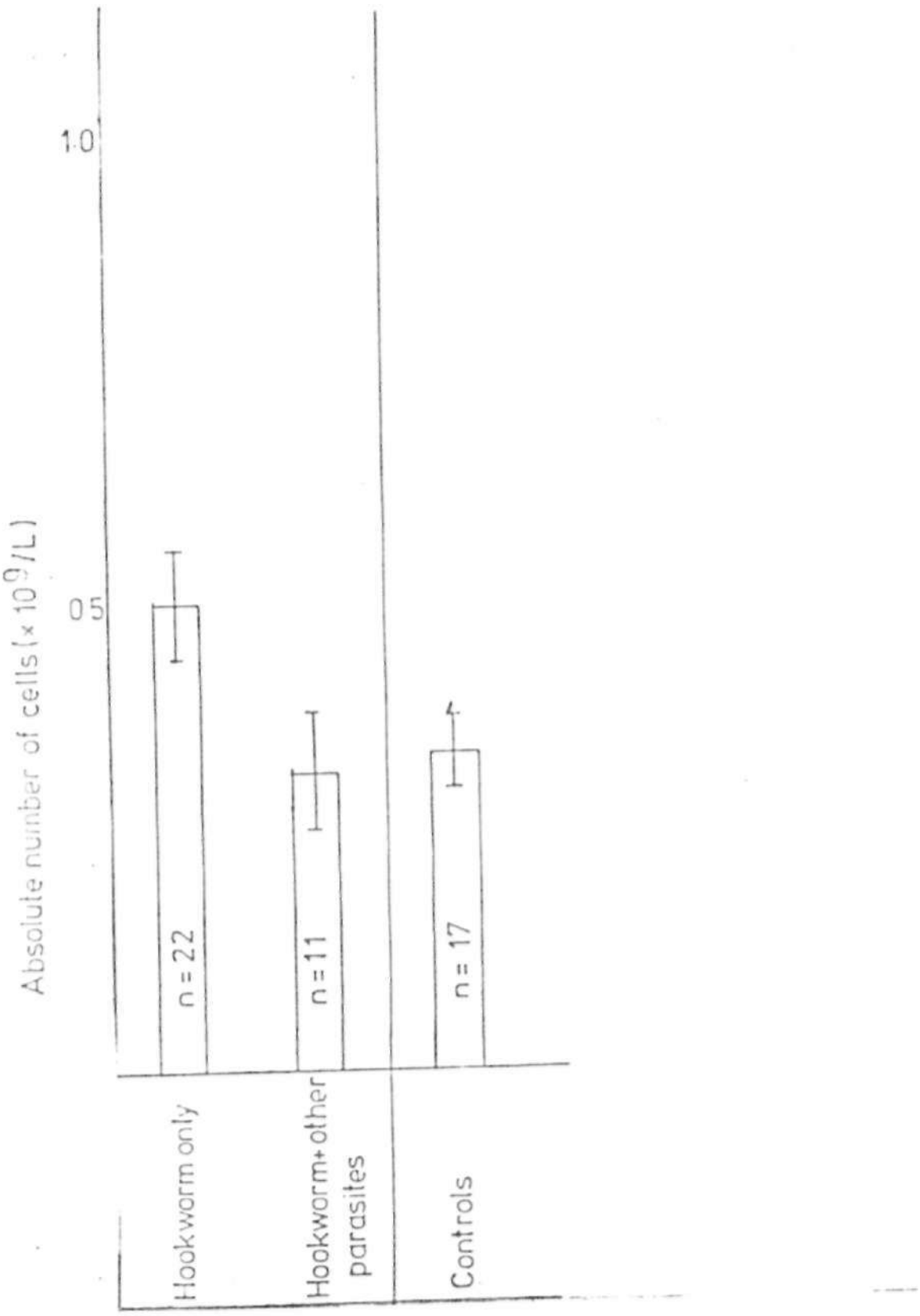


Fig. 18. Mean (± I.S.E.M.) absolute number of anti-Leu 2a positive cells in the peripheral blood of Hookworm patients & controls

Figure 18 shows the distribution of absolute numbers of suppressor T cells in hookworm patients and controls. Marked anaemia patients gave a mean of 0.48±0.32 (range 0.13 to 1.23), moderate to mild anaemia, a mean of 0.51±0.36 (range 0.13 to 1.43), no anaemia, a mean of 0.40 ±0.19 (range 0.02 to 0.79), 'hookworm only' a mean of 0.50±0.29 (range 0.02 to 1.43) and patients with hookworm and other parasites had a mean of 0.32±0.20 (range 0.12 to 0.79). None of the subgroups was significantly different from the controls with a range of 0.15 to 0.78 and a mean of 0.34±0.18 ($P > 0.1$). All the hookworm patients together gave a mean of 0.41±0.2 and was also not significantly different from the controls ($P > 0.1$) (Table 8).

4.7 Leucocyte Migration Inhibition Tests

Figure 19. shows the percent migration inhibition against P.P.D in hookworm patients and controls. Patients with marked anaemia had a mean of 30±20% and a range of 3% to 57%, those with moderate to mild anaemia had a mean of 32±14% and a range of 13% to 59%, those with no signs of anaemia had a mean of 41±16% and a range of 11% to 60%. Those patients with hookworm as the only parasite had a mean of 35±18% and a range of 3% to 59%, while those with hookworm and other parasites had a mean of 37±13% and a range of 22% to 57%. The controls had a range of 48% to 69% and a mean of 56±6%. All the groups were significantly lower than the controls ($P \leq 0.005$ for each group). The hookworm patients all together gave a range of 3% to 60% and a mean of 35±16%. They were also significantly lower than the control ($P \leq 0.001$) (Table 8).

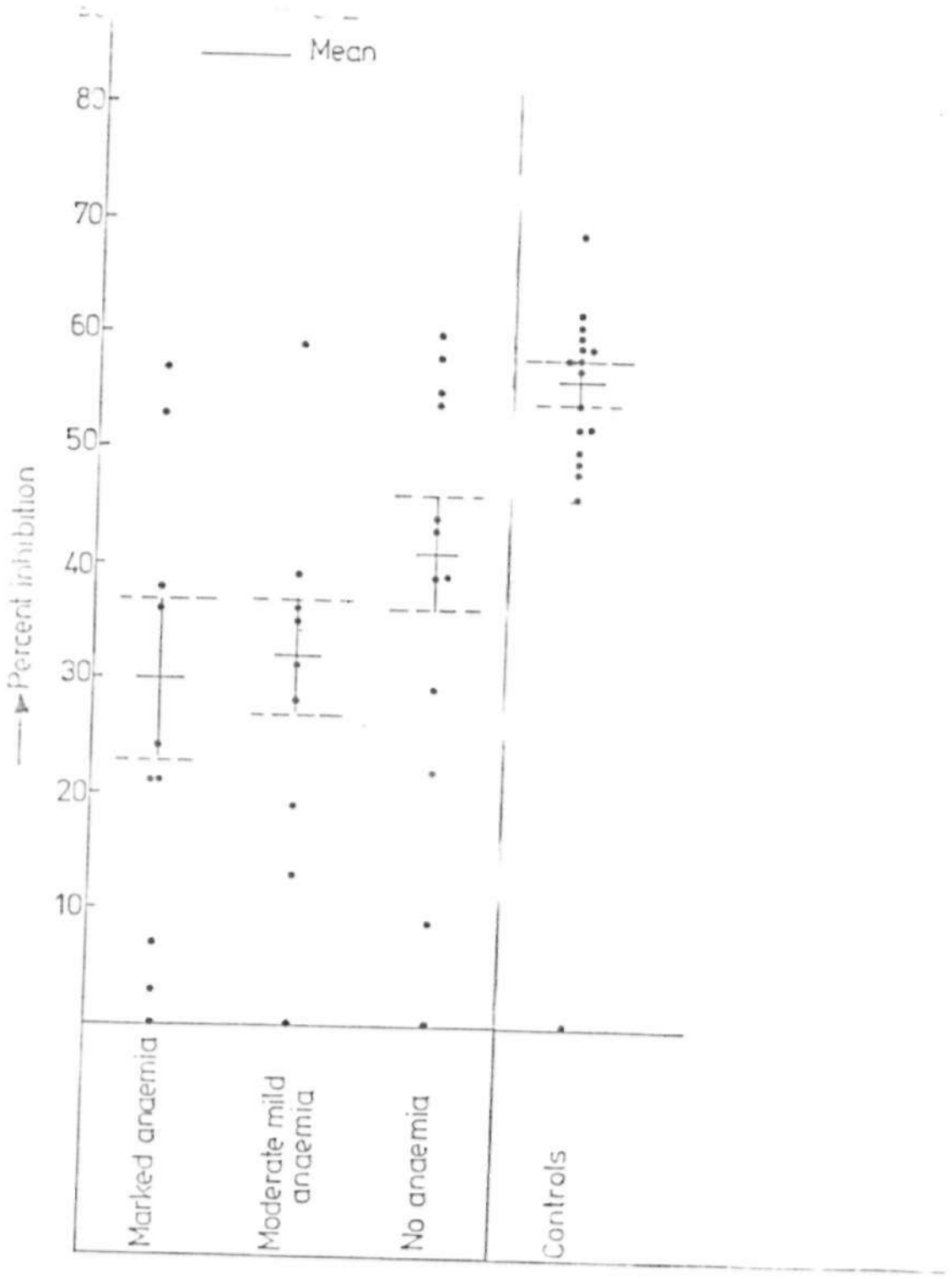


Fig 19. Mean percent (+1 S.E.M) migration inhibition to P.P.D in hookworm patients & controls.

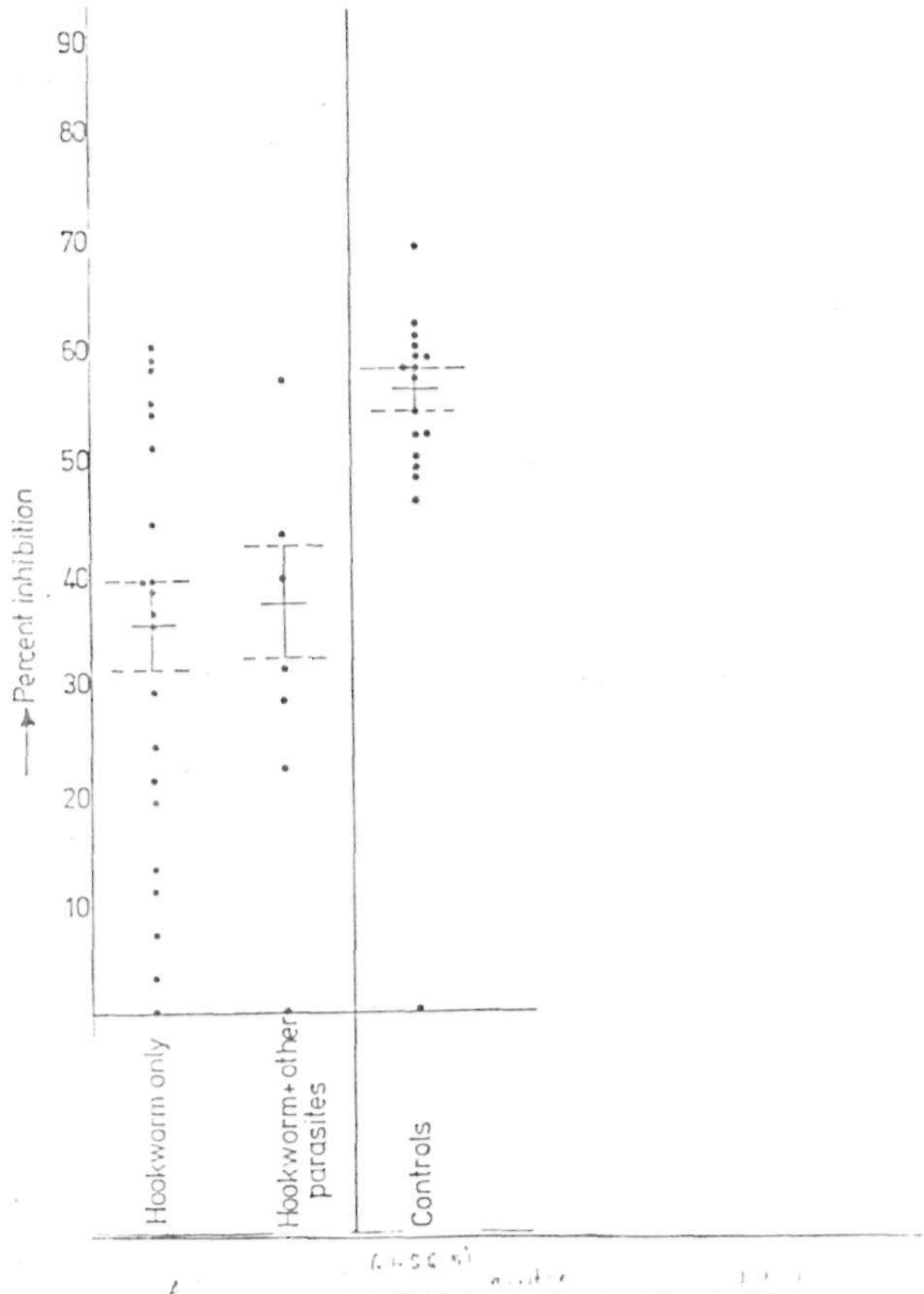


Fig. 19. Mean percent (+1 S.E.M) migration inhibition to P.P.D. in hookworm patients & controls

TABLE 9

EFFECT OF ANTIHELMINTHIC, MEBENDAZOLE (VERMOX) AND LEVAMISOLE (KETRAX) ON NUMERICAL AND FUNCTIONAL LYMPHOCYTE PARAMETERS IN HOOKWORM INFECTION.

TESTS	Before treatment n $\bar{x}$ SD	Hookworm patients patients 4/52 treatment n $\bar{x}$ SD	Controls n $\bar{x}$ SD
White blood cells (WBC)($x10^9$/L)	55 4.7±2.3	28 3.8±1.8^{+}	28 3.8 ± 1.5
Differential lymphocytes (%)	** 2.16±1.15 55 50 ± 17	1.76±0.89$_{NS}$ 28 46 ± 8	1.80 ± 0.87 28 48 ± 13
Late rosettes at 4°C (Total T cells) (%)	** 1.06±0.17 55 45 ± 9	0.89±0.44 28 51 ± 5.5^{+*}	1.05 ± 0.54 28 58 ± 6
Anti-Leu 4 positive (Total T cells) (%)	** 0.08±0.80 34 48 ± 9	0.91±0.50 19 52 ± 3.2^{+*}	0.91 ± 0.42 17 59 ± 7
Anti-Leu 3a positive (Helper T cells) (%)	** 0.60±0.41 34 25 ± 6	0.50±0.25 19 28 ± 6.7*	0.50 ± 0.23 17 32 ± 4
Anti-Leu 2a positive (suppressor T cells) (%)	** 0.41±0.2 34 21 ± 5	0.38±0.26$_{NS}$ 19 21 ± 3.0	0.34 ± 0.18 17 23 ± 4
Percent migration inhibition to P.P.D. (%)	28 35 ± 16	10 49 ± 8 +*	16 56 ± 6

** Indicate absolute numbers for the relative percentages
+ Significantly different from parallel group values of patients before treatment ($P < 0.001$)
* Significantly different from parallel group values of control subjects ($P < 0.05$)
NS No statistically significant difference between group values of patients before treatment and controls ($P > 0.1$

4.8 Cumulative effect of antihelmintic treatment with mebendazole (vermox) and Levamisole (Ketrax) on numerical and functional lymphocyte parameters in human hookworm infection.

4.8.1 White Blood Cell Counts

The distribution of peripneral blood white blood cells in all the hookworm patients and controls are indicated in Table 9 and Figure 20. Before treatment values ($4.7 \pm 3 \times 10^9/L$) were much more elevated compared to the values four weeks after treatment ($3.8 \pm 8 \times 10^9/L$). The results after treatment were not significantly different from those of the controls ($P \geq 0.1$).

4.8.1 Differential Lymphocyte Counts

Mean percent differential lymphocyte counts were read as 46 ± 8% four weeks after treatment. None of these were significantly different compared to the results before treatment, with a mean of 50 ± 17% or with controls, 48 ± 13% ($P > 0.1$). Figure 21, gives the mean percent and absolute differential counts of lymphocytes in the peripneral blood of hookworm patients before and after treatment. The mean absolute counts of differential lymphocytes, recorded some significant differences four weeks after treatment ($1.76 \pm 0.89 \times 10^9/L$), compared with the absolute counts before treatment of $2.16 \pm 1.15 \times 10^9/L$ ($P < 0.05$). The results after treatment were not significantly different from the controls.

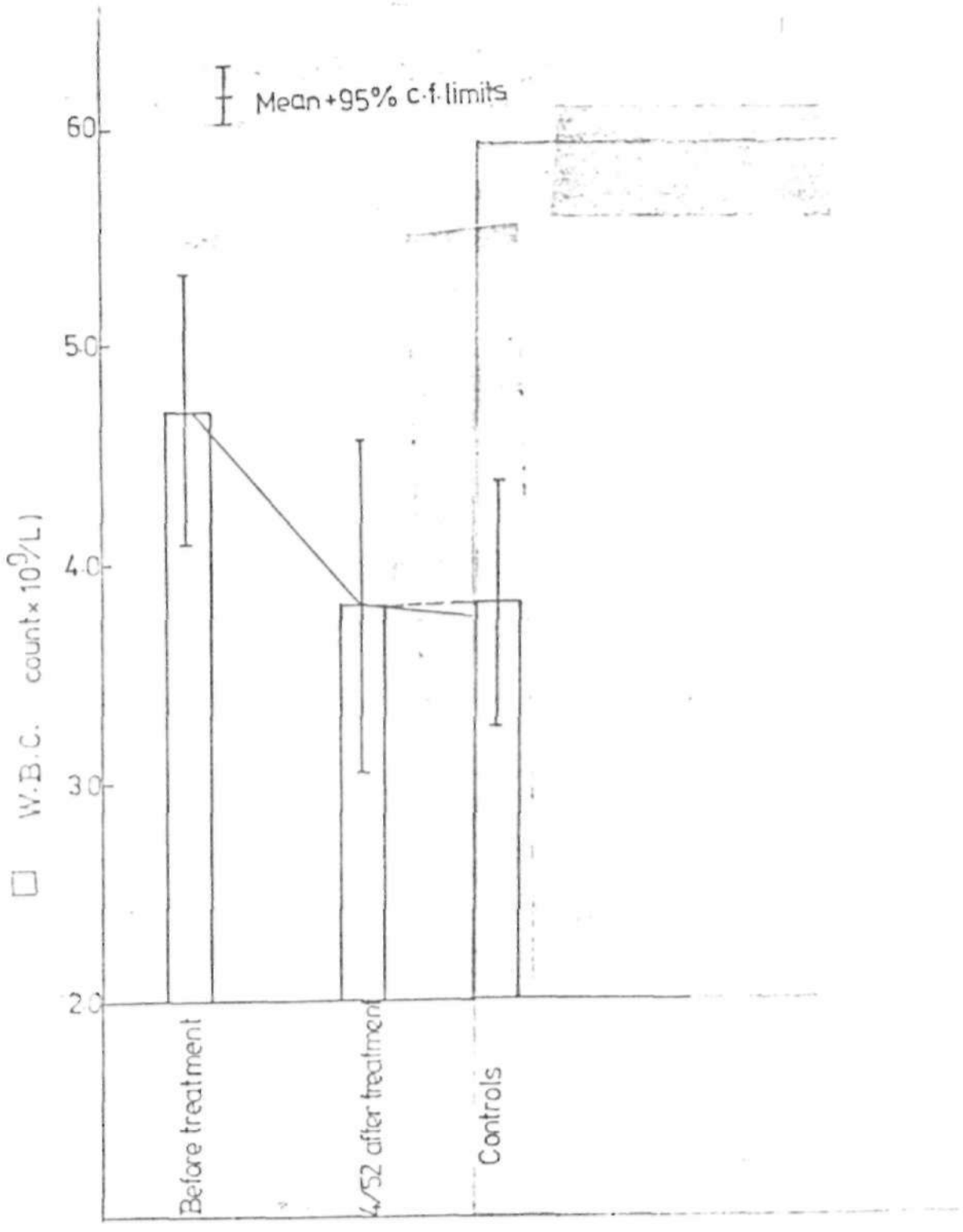


Fig. 20. Mean ± 2 S.E.M. white blood cell count in the peripheral blood of Hookworm patients before and after treatment.

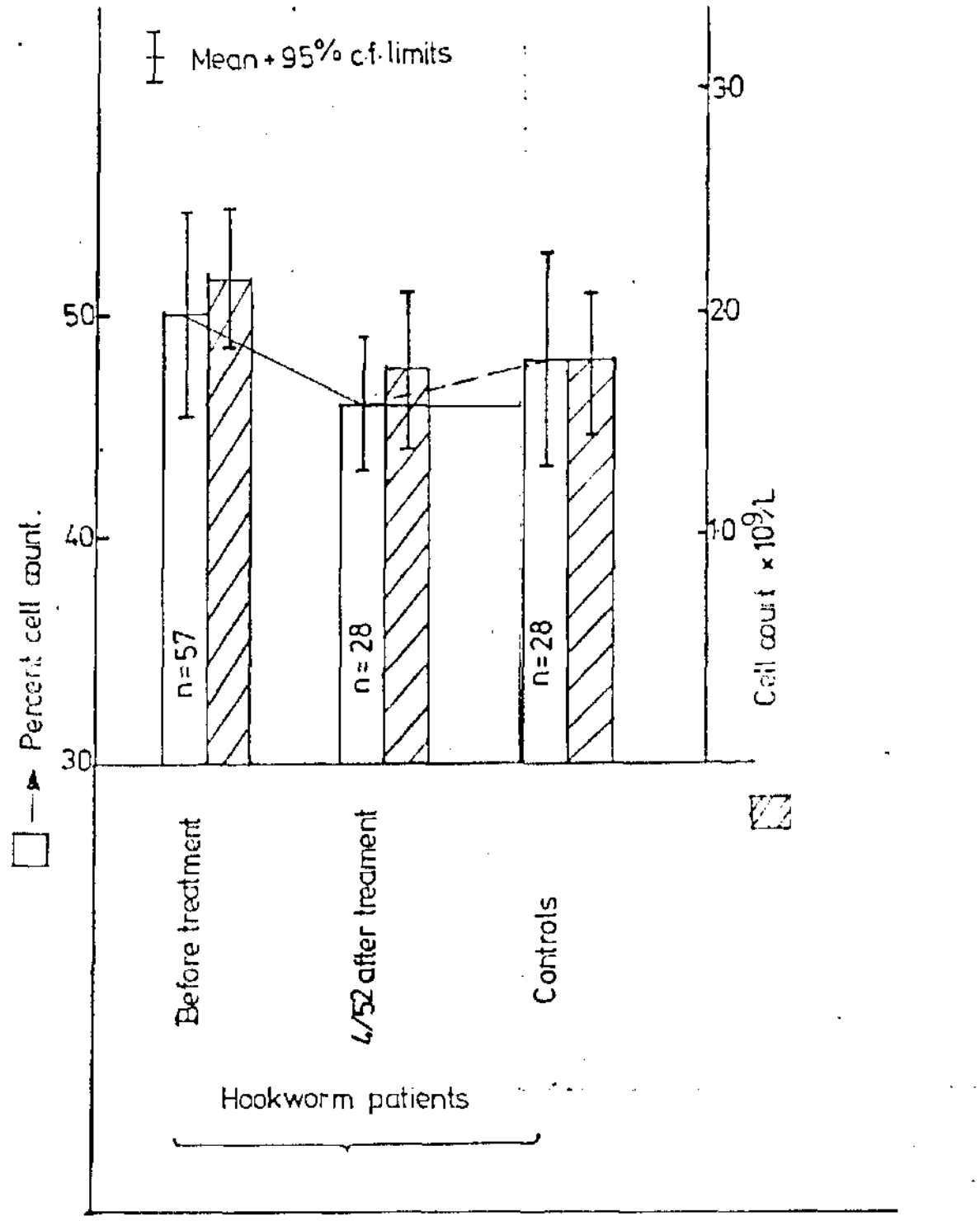


Fig. 21. Mean ± 95% c.f. limits of differential lymphocytes in the peripheral blood of hookworm patients before and after treatment.

4.8.3 Anti Leu 4 Positive (Total T-) Cells

The mean percent and absolute counts of anti Leu 4 positive cells are shown in Figure 22. The mean percent T-cell reading four weeks after treatment was 52 ± 3.2%. This was significantly higher than the mean of 48 ± 9% obtained before treatment ($P < 0.001$), but significantly lower than the mean obtained for the control subjects of 59 ± 7.0% ($P < 0.05$). Mean absolute count four weeks after treatment was 0.91 ± 0.50 $x10^9/L$. There was no significant difference compared with values before treatment of 1.08 ± 0.8 $x10^9/L$ or with controls of 0.91 ± 0.42 $x10^9/L$ ($P > 0.1$). There was also no significant difference between values four weeks after treatment.

4.8.4 Percent late Rosetting (Total T-) Cells

Figure 23, shows the mean percent and absolute counts of late rosetting lymphocytes in the blood in hookworm patients before and after treatment. The mean percent lymphocyte values four weeks after treatment. This was significantly higher than pre-treatment values of 45 ± 9.0% and significantly lower than values from control subjects of 58 ± 6.0% ($P < 0.01$ and $P < 0.05$ respectively). The mean absolute counts four weeks after treatment was 0.89 ± 0.44 $x10^9/L$. However there was no significant difference between these reults and pretreatment values of 1.06 ± 0.7 $x10^9/L$ or with control results of 1.05 ± 0.54 $x10^9/L$ ($P > 0.1$).

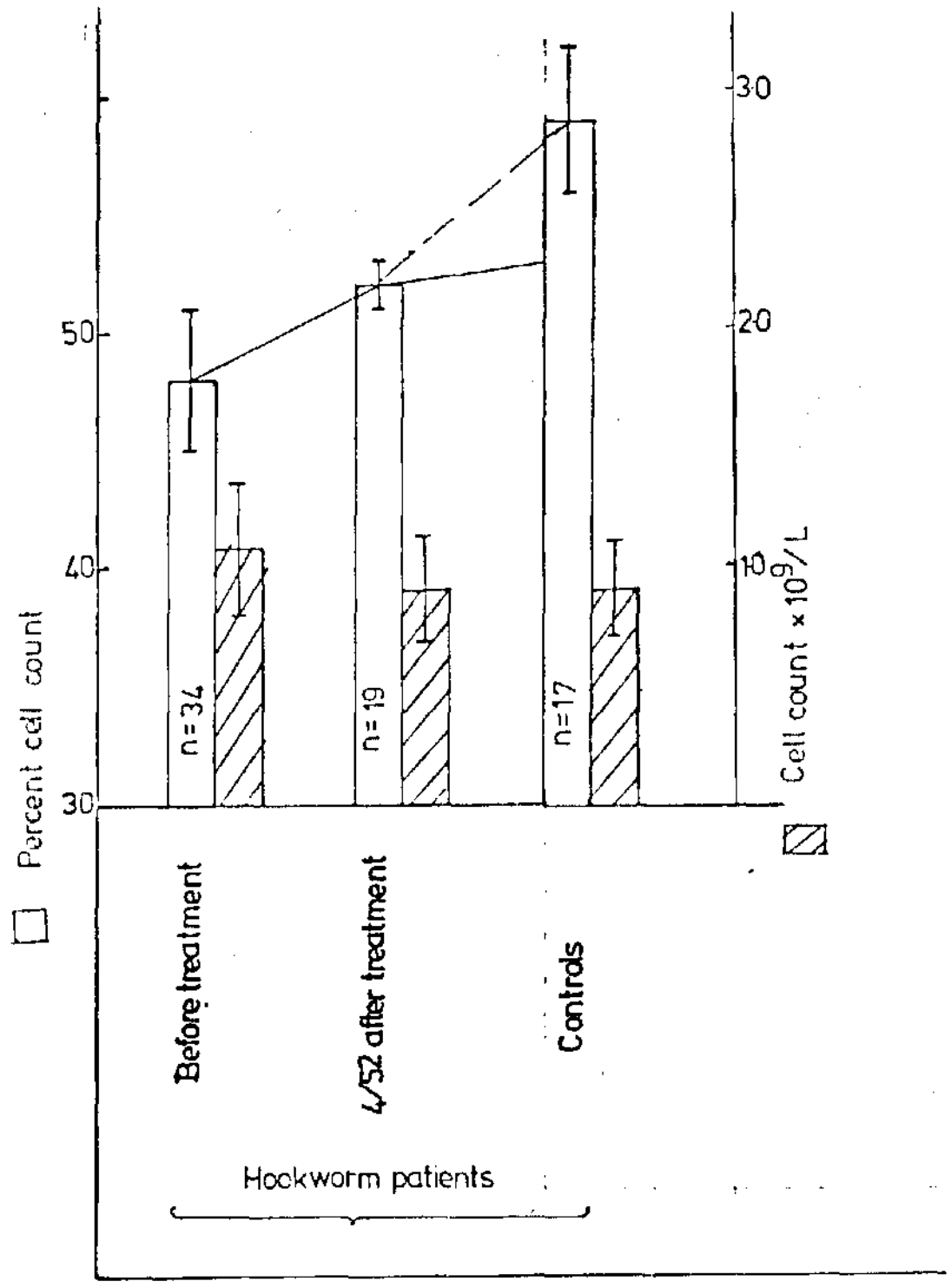


Fig. 22. Mean and 95% of limits of anti-Leu4 positive cells in the peripheral blood of hookworm patients before and after treatment.

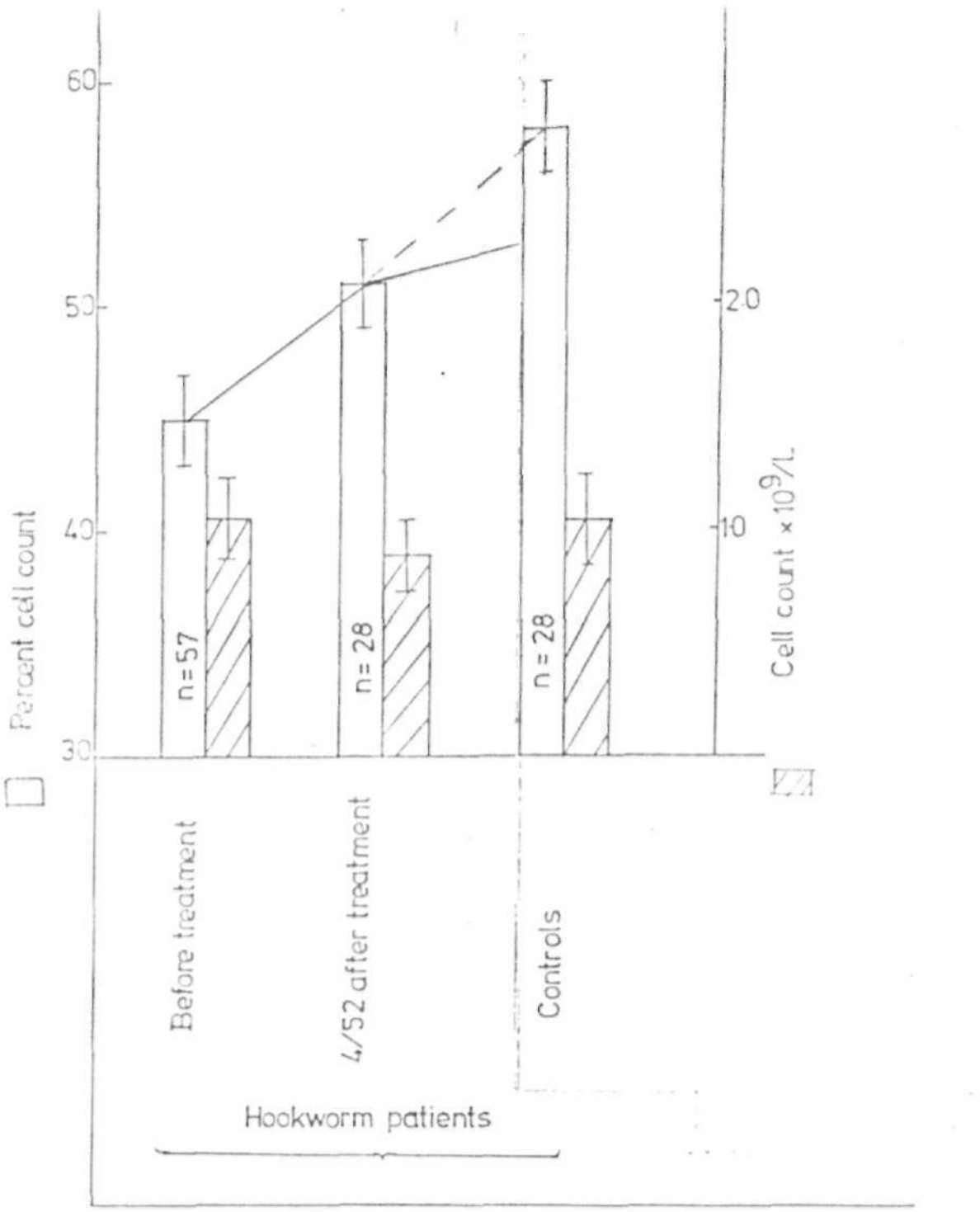


Fig. 23. Mean (+2 S.E.M.) late rosettes in the peripheral blood of hookworm patients before treatment, after treatment.

4.8.5 Anti Leu-3a (Helper T) Positive Cells

The mean percent and mean absolute counts as per positive anti Leu-3a cells in hookworm patients before and after treatment are indicated in Figure 24. The mean value four weeks after treatment was 28 ± 6.7%. The mean post treatment absolute counts of four weeks ($0.50 \pm 0.25 \times 10^9/L$) was not significantly different from control and pretreatment values of $0.50 \pm 0.23 \times 10^9/L$ and $0.60 \pm 0.4 \times 10^9/L$ respectively ($P \geq 0.1$).

4.8.6 Anti Leu 2a Positive (Suppressor T-) Cells

Figure 25 indicates the mean percent and mean absolute counts of Anti leu 2a positive cells in the peripheral blood of hookworm patients and controls. The mean + S.D. in the controls was 23 ± 4%. Pretreatment values gave a mean of 21 ± 5%, while those four weeks after treatment gave a mean of 21 ± 3%. There was no significant difference between values before and after treatment or with controls ($P \geq 0.1$). There was also no significant difference between the mean absolute counts before ($0.41 \pm 0.2 \times 10^9/L$ and after ($0.38 \pm 0.26 \times 10^9/L$; $0.35 \pm 0.15 \times 10^9/L$) treatment or with controls ($0.34 \pm 10 \times 10^9/L$) ($P \geq 0.1$).

4.8.7 L.M.I.P. After treatment

The mean percent leucocyte migration inhibition to purified protein derivative (P.P.D), in hookworm patients before and after treatment and in controls is recorded in Table 9 and Figure 26. Percent values four weeks after treatment gave a mean of 49 ± 8%. The results were significantly higher than the pretreatment values of 35 ± 16% ($P < 0.01$), but considerably lower than control values of 56 ± 6% ($P < 0.05$).

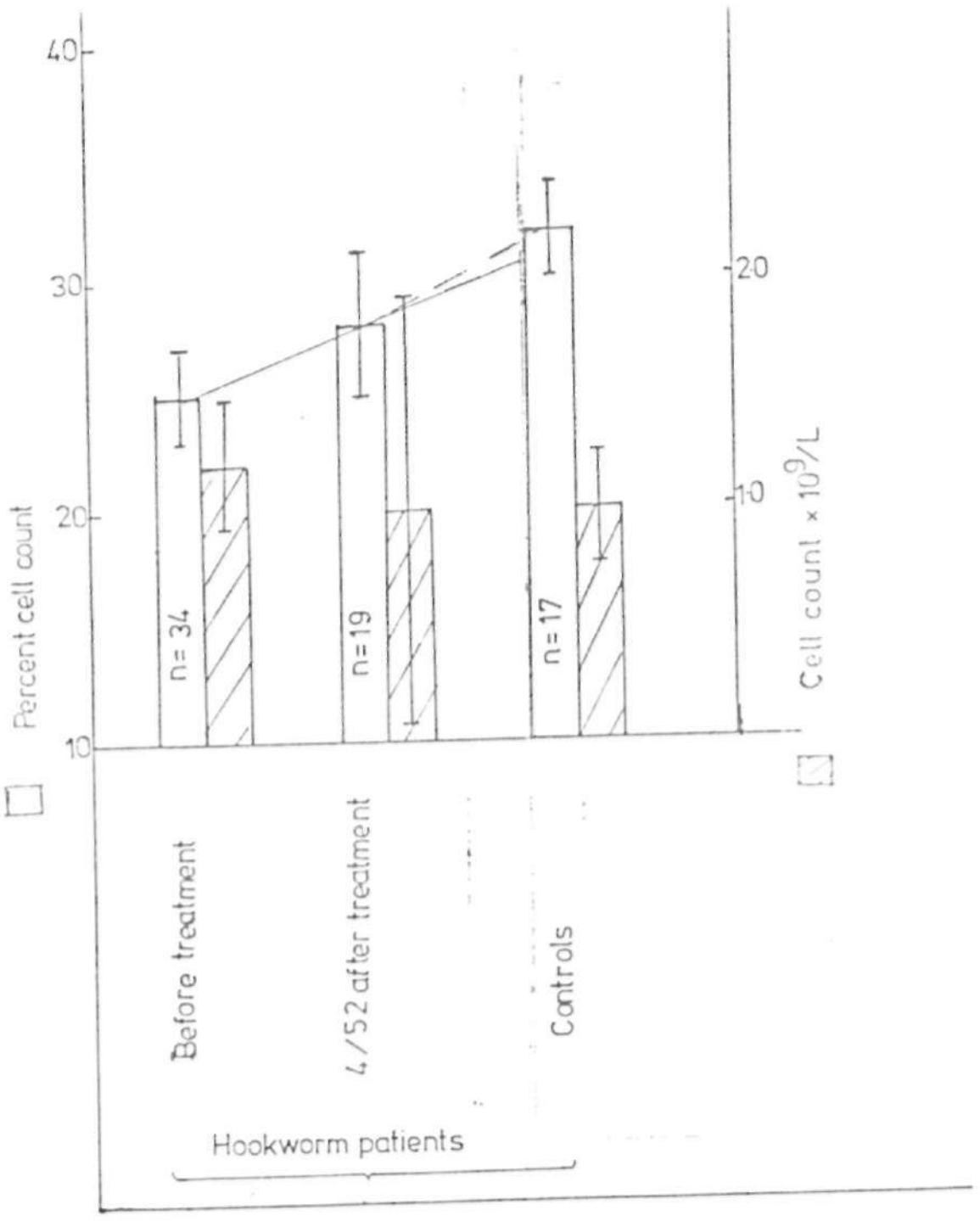


Fig. 24. Mean +95% of limits of anti Leu-3a positive cells in the peripheral blood of hookworm patients before and after treatment.

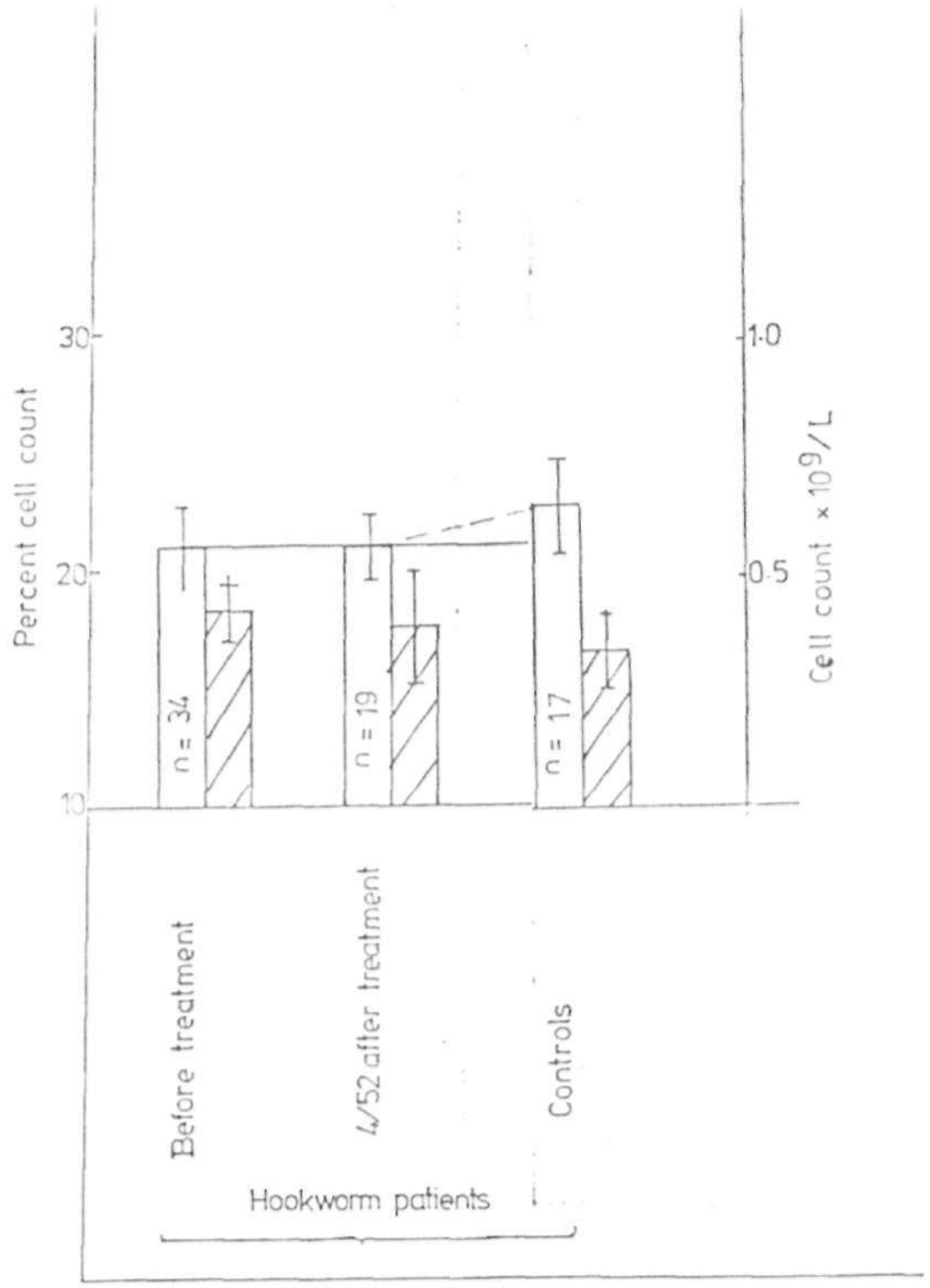


Fig. 25. Mean+95% Confidence limits of anti Leu 2a positive cells in hookworm patients before & after treatment

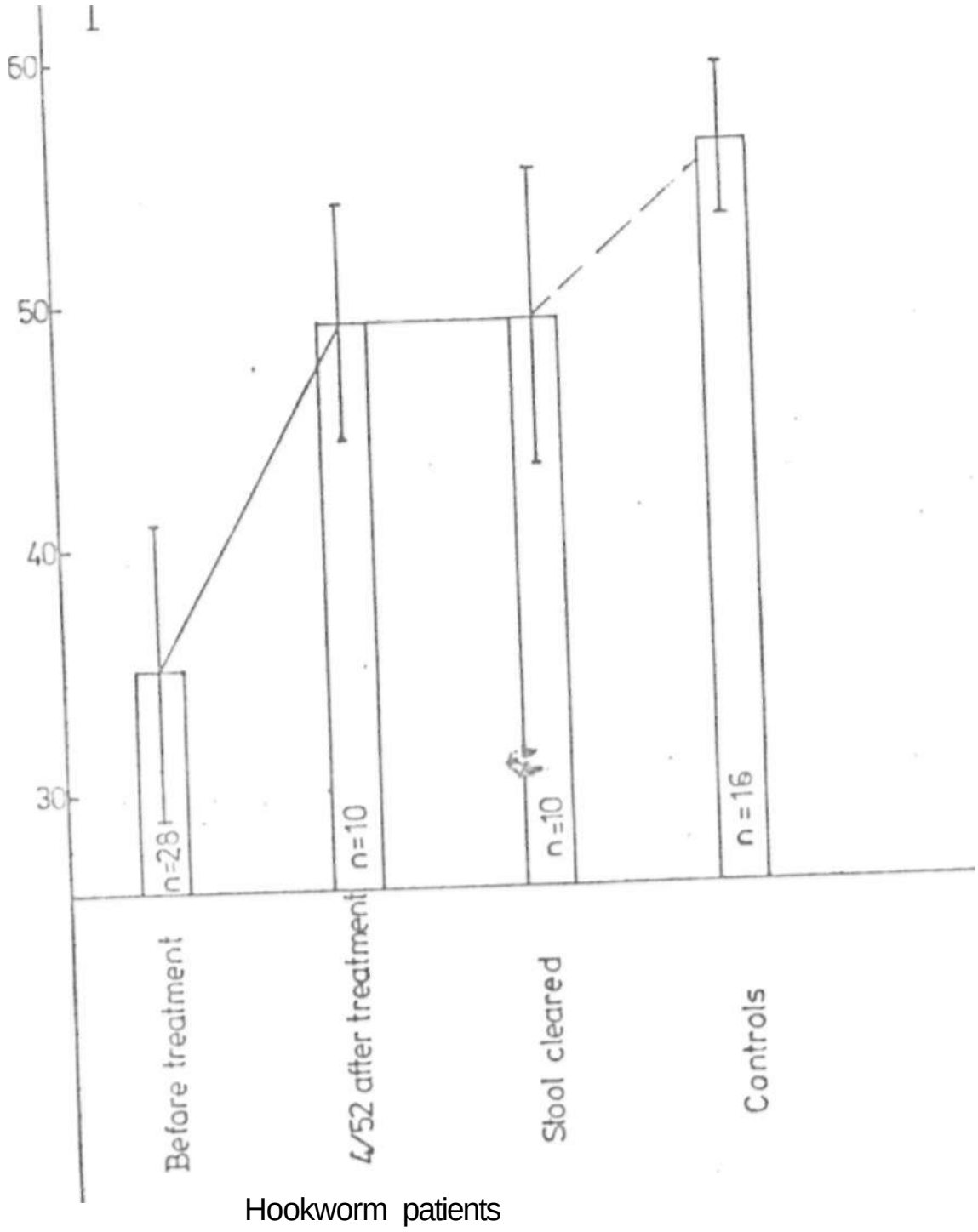


Fig 26. Meant 95V.ctWsmigration inhibition to PPD in hookworm ————patients before and alter taetment and after stool was ckxxed

Even though there were some increases in the parameters 4 weeks after treatment, the differences were not significant as is shown in table 10.

The patients who were treated on Ketrax, recorded no significant difference in their differential lymphocytes (44.28% ± 11.07% -- 44.14% ± 7.43% -- 53.25 ± 2.63%), late rosetting T-cells (47.71 ± 8.26% -- 48.42 ± 8.16%), T-helper cells (26.6 ± 7.09% -- 25.25 ± 6.13%) and T-suppressor cells (22.4 ± 4.16% -- 23.25 ± 4.35%) after four weeks. There was a significant decrease ($P < 0.05$) in the white blood cell count from 5.16 ± 3.33 $x10^9/L$ to 4.11 ± 2.69 $x10^9/L$ and while the MIF indices were increased from 31.67 ± 6.66% to 43.00 ± 6.08%, it was not statistically analysed because the samples were too few.

The patients who were treated on Vermox, after four weeks, recorded no significant difference in their differential lymphocyte counts (57.70 ± 10.74% -- 45.13 ± 8.29%), anti leu 4 positive T-cells (48.33 ± 7.55% -- 51.33 ± 2.16%), late rosetting T-cells (48.38 ± 4.95% -- 52.25 ± 2.92%), T-helper cells (27.66 ± 4.32% -- 32.00 ± 5.87%) and T-suppressor cells (18.50 ± 5.28% -- 21.16 ± 2.40%). There was however a significant decrease ($P < 0.05$) in the white blood cell counts from 4.89 ± 1.59 $x10^9/L$ to 3.24 ± 1.28 $x10^9/L$

After four weeks, the patients who were treated with a serial combination of Ketrax and Vermox had no significant difference in their differential lymphocyte counts (51.85 ± 14.93% -- 46.38 ± 8.16%), anti Leu 4 positive T-cells (48.33 ± 9.08% -- 50.88 ± 3.89%), late rosetting T-cells (46.85 ± 8.91% -- 51.23 ± 5.12%), T-helper cells (25.22 ± 8.01% -- 27.44 ± 6.98%) and T-suppressor cells (22.33 ± 7.76% -- 20.22 ± 2.49%). There was however,

Pre- and Post-treatment Parameters in the follow-up patients treated with Ketrax and Vermox

	WBC	Differential	Anti leu 4 cells	Late Rosetting	T-helper cells	T-supp. cells	MIF	Cure rate after 1 month
Pretreatment Parameters (n=7)	5.16 ± 3.33	44.28 ± 11.07	50.20 ± 7.43	47.71 ± 8.26	26.60 ± 7.09	22.40 ± 4.16	31.67 ± 6.66	
Post treatment (Levamisole) Parameters (n=7	4.11 ± 2.69	44.14 ± 7.27	58.25 ± 2.63	48.42 ± 8.16	25.25 ± 6.13	23.25 ± 4.35	43.00 ± 6.08	57.14%
	<0.05	NS	NS	NS	NS	NS	NA	
Pretreatment Parameters (n=8)	4.89 ± 1.50	51.70 ± 10.74	48.33 ± 7.55	48.38 ± 4.95	27.66 ± 4.32	18.50 ± 5.28	38.50 ± 7.33	
Post treatment (Mebendazole) Parameters (n=8)	3.24 ± 1.28	45.13 ± 8.29	51.33 ± 2.16	52.25 ± 2.92	32.00 ± 5.87	21.16 ± 2.40	52.25 ± 6.95	87.5%
	<0.05	NS	NS	NS	NS	NS	NA	
Pretreatment Parameters (n=12)	4.54 ± 2.70	57.85 ± 14.93	48.33 ± 9.08	46.85 ± 8.91	25.22 ± 8.01	22.33 ± 7.76	40.33 ± 19.03	
Post treatment (Levamisole + Mebendazole) Parameters (n=12)	3.96 ± 1.36	46.38 ± 8.16	50.88 ± 3.89	51.23 ± 5.12	27.44 ± 6.98	20.22 ± 2.49	50.33 ± 7.02	58.33%
	<0.05	NS	NS	NS	NS	NS	NA	

a significant ($P < 0.05$) decrease in the white blood cell counts from $4.54 \pm 2.70 \times 10^9/L$ to $3.96 \pm 1.36 \times 10^9/L$.

There was also no significant difference in the parameters after the use of Vermox or Ketrax alone or the combined use of Ketrax and Vermox together. There was also no significant difference between the absolute numbers of the parameters.

Re-occurence of Hookworm ova in stool

Of the 28 patients who returned for the follow-up study, four were later observed to be re-producing eggs (hookworm) in their stool. Their STOLL counts had dropped to 100 eggs per gram of stool and two of them had stopped releasing hookworm eggs in their stools altogether. The new reading of STOLL counts for the from patients 200, 400, 400, 600 eggs per gram of stool respectively. The white blood cell count reading were 3.1, 3.3, 4.1 and $4.2 \times 10^9/L$ respectively. The percent differential lymphocyte counts were 38%, 40%, 42% and 43% respectively. The absolute counts for differential lymphocytes were 1.24, 1.56, 1.68 and $1.76 \times 10^9/L$. The total T-cell counts as determined by percent late rosetting cells were 49%, 49%, 51% and 54%. The absolute counts for Total T-cells were 0.67, 0.76, 0.82 and $0.90 \times 10^9/L$. No statistical comparison was made with any of these parameters because of the smallness of the size of the sample.

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CHAPTER FIVE

DISCUSSION

Peripheral blood white cell responses

A marked increase in the level of peripheral blood eosinophils was observed in all the subgroups in this study when compared with the controls ($P < 0.001$). The study however did not investigate the degree of local accumulation of eosinophils in the gut, but this has been observed by Moqbel et al. (1986) in rat models. The highest increases were observed in patients with anaemia of mild to moderate severity, in those without anaemia and in those with hookworms and other parasites. This is not surprising however as eosinophilia is a major hallmark of hookworm and other helminth infections (Sefus and Bienenstock, 1982) and eosinophils are active in protective immunity against helminths (Yong and Lawson, 1984). The mechanisms for eosinophilic leucocytoses have been reviewed by Walls et al.(1971) and may involve proliferation in association with T-cells or activation by either antibody - antigen or lymphocyte-antigen reactions. In mice infected with Schistosoma mansoni (another helminth), it was observed that increases in the levels of blood eosinophils were not significant if the mice had been depleted of their T-lymphocytes by irradiation (Phillips et al., 1977). Thus it appears that eosinophilic responses require a functioning T-lymphocyte population (Wardle, 1988). This may well explain the reduced eosinophilia in the patient subgroups with marked anaemia in this study, in which the lowest total and helper T-cell responses were recorded. It is speculative whether the lymphocyte - antigen reactions are impaired in this subgroup.

The mechanisms through which eosinophils act to produce protective immunity are varied. Eosinophils are attracted towards the worms by parasite derived chemotactic factors (eosinophil chemotactic factor of anaphylaxis ECF-A) and stimulated to proliferate by eosinophil stimulation promoter (ESP), lymphokines derived from antigen stimulated helper T-cells (Butterworth, 1984). In association with specific antibody (IgG) eosinophils kill newborn larvae of Trichinella spiralis in rats and Schistosoma mansoni schistosomula in humans by antibody dependent cellular cytotoxicity (Butterworth, 1984). They have also been shown to degranulate and release their stored mediators following contact with adult worms or their larvae (Helmers, 1980) and to undergo transmembrane perturbation via IgG Fc receptors, thus triggering the preferential generation of slow reactive substance of anaphylaxis (SRS-A) leucotrienes (Shaw et al., 1985). These leucotrienes are shown to cause smooth muscle contraction, increase vascular permeability and enhance the production of mucus from bronchial tissues (Lewis and Austen, 1984). The SRS-A leucotrienes (LTC_4, LTD_4) released in the gut also have smooth muscle contractile (Moqbel, 1986) and mucus hypersecretive properties (Miller et al., 1983) for the gut. Leucotriene B_4 (LTB_4) also has the ability to enhance eosinophil mediated complement dependent killing as demonstrated in studies on schistosomula of S. mansoni by human cells in vitro (Moqbel et al., 1983). Smooth muscle contraction in the gut may contribute to cases of spontaneous excretion of intestinal helminths.

The possible involvement of leucotrienes in the pathogenesis of helminth-induced inflammatory reactions in the gut has been previously studied by Moqbel et al. (1986). Rats immunized by infecting twice with rat hookworm N. brasiliensis were challenged intravenously with the worm antigen two weeks after immunization. The rats developed a generalized anaphylactic response with the small intestine being the major shock organ. The major pathological changes which included oedema, increased mucosal permeability, epithelial shedding and mucus hypersecretion were associated with local eosinophil infiltration and increased numbers of mucosal mast cells. Also significant amounts of LTB_4 and LTC_4 were released into the gut lumen following antigen challenge of the immune rats (Moqbel et al., 1986). In another study using rapid expulsion (RE) of N. brasiliensis from the guts of immune rats as a measure of the concetration of LTB_4 and LTC_4, Moqbel et al. (1986) demonstrated high concentrations of both LTB_4 and LTC_4 in the rats. The release of these leucotrienes during gut anaphylaxis and rapid expulsion of nematode worms from rats is suggestive of a role for the leucotrienes in inflammation associated with eosinophilia and changes leading to the expulsion of the worms from the intestine. Furthermore, Kay (1979) speculated on the role of the eosinophil in helminth parasitic death (in schistosomiasis). The immunological 'link up' consists of mast cells, IgE, complement and eosinophils interacting to provide an effective mechanism against invasion by helminth parasites. Following their migration through the skin, schistosomula trigger IgE-sensitized mast cells with the release of pharmacologic mediators including ECF-A and histamine. These mast cells adhere to complement-coated schistosomula by their membrane

receptors for complement. Mast cell mediators promote eosinophil recruitment and 'arming' for parasite death. Thus ECF-A tetrapeptides (Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu) and histamine mobilize human eosinophils and enhance eosinophil mediated, complement dependent parasite death (Kay, 1979). In a similar manner, migration of hookworm larvae through the heart to the lungs and through the lungs, result occasionally in a pulmonary reaction (with bronchaspasm) where minute heamorrhages are accompanied by eosinophilic and leucocytic infiltrations; the pulmonary infiltration with eosinophils (PIE) syndrome (Cook, 1986). In this way, the increases in peripheral blood eosinophilia in this study may be of great importance in limiting the establishment of infection or the magnitude of worm load. This is corroborated by the fact that eosinophilia was greatest in the patient subgroups without anaemia and with moderate anaemia where the worm load was reduced as compared to those with marked anaemia. Also the presence of a greater number of eosinophils in patients without anaemia and those with moderate anaemia as compared to those with marked anaemia where the worm load was higher, could possibly indicate a 'sequestration' of eosinophils at the site of worm attachment in the small intestine. This 'drain' on the blood eosinophils would indicate that the eosinophils are activated to attack the worms and would highlight their importance in the response to infection.

Eosinophils have receptors for C3, C4, IgA and IgE (Kay, 1974). Even though IgE was not studied in this case due to the inability to secure RAST reagents at the time of study, it is well established that IgE is raised in hookworm infection (Biroum-Noerjasin, 1973; Ogilive et al.,

1V70J. 'From t.is study, the raised IgA, SlgA and CU levels in j&tienta with moderate anaemia correlate weii with the trend ir. soaino.hiiia.

• i.:A has boon reported to have the highest binding capacity Tor eosinophils and consequently to induce eosinophil deL'ranulaticn (.ou-j:".a~2.1er. et **al.,** I^c-j. **Thus** the increased, eosinophils in the sub.jroups in this study in c.).-.^ir.ation with increased CU complement and IgA may cor.tribute to the inflammatory response in the gut to infection by hookworms.

The effects of eosinophils on helminths have been most effectively studied in huaana and mice, where it is interesting to note that t..e -cult worms ! enolved many different mechanisms to evade the effects of the host protective function. 3o~e cf the parasites defence aecr.anisrr.s include t:.e acquisition of host antigens like blood group antijor.s ir.c the presence of circulating immune complexes which inhibit the action of cytotoxic cells • Lve a pair.at the worms. Others include the suppression of the hoat3 :tivatin/j macroph ve-supprosnor or T-sup?rv;:7or colls and the •avage o£ a peptice fron lgG **t..at** inhibits cellular r?3;onsea (Lutterworth, i). In t.is study, T-supprcsor cells were normal in ill the subgroups, but r-helper celi3 were decreased and this could have seen mode of depressing host responses by T-suppressor cells. In the hookworm infection of rats, the -eat example of immune evasion by hookworms so far, infection with £, :•:• -.sili- -:3is suppresses the ability of draining mesenteric lymph nodes (*'X«) ceils to respond to trinitrophenylbovinea$_0$-tma.:iobulin ^TIl-HOG) (7ritthar:d, 1?ci6). '

Other reports from the peripheral white blood cell compartment in this study, include an increase in differential lymphocytes as opposed to a decrease in neutrophil leucocytes. The difference after comparing absolute numbers and relative percentages was however not significant ($P \geq 0.1$). This correlates well with the result of Cheema and Scofield (1982) who studying haematological changes in hookworm infection also found a non-significant increase in lymphocytes while neutrophils were decreased. Total white cell counts were also increased in this study. The increase in total white blood cell counts may have been largely due to the increase in lymphocytes and this could represent an increase in B-cells. B-cells were however not studied in this survey, but the increases in IgA and IgM may reflect indirectly, expansion specifically and non-specifically of B-cell clones.

Cell-mediated immunity and MIF reactions

From this arm of the immune response, results indicate that in all the patient subgroups the relative percentages of total T- and helper T-cells were increased while suppressor T-cell values remained normal. Absolute numbers of the different T-cell subpopulations remained unaffected and were not significantly different from those of the controls, probably due to the higher total white blood cell values observed in the patients. Very significant ($P < 0.001$) reductions in total T- and helper T-cells were observed in patients with marked anaemia as compared to those with mild to moderate anaemia. This finding is consistent with the result of Grantham et al. (1982) who found that in children with iron-deficiency anaemia, secondary to hookworm infections, of a mild to moderate severity,

the major finding was a subtle T-cell deficiency. In this study, the degree of T-cell depression was lesser in patients with no anaemia as compared to those with moderate anaemia and this in turn was less compared to those with marked anaemia. This is corroborated by the fact that the effects of superinfection were most observed in the cellular arm of the immune response where the total T-cells were most reduced with a range of 31 to 43% (0.45 to 0.66 $x10^9$/L - absolute range). Haemoglobin readings as low as 4.0g/dl were also obtained. This indicates that heavy worm burdens are associated with a decrease in the level of haemoglobin and the quantity of total T-cells but not a depression of the humoral immune response, as the immunoglobulin results were normal. It could then be implied that the lymphocytes, are reduced partly due to 'sequestration' and or partly due to lymphocytotoxic effects of the worms.

Many parasitic infections, of both protozoan and metazoan origin, are now associated with some degree of immunosuppression in their hosts. For example, Allison and Eugui (1983) have reported a decrease in the

number of circulating total T-cells in human peripheral blood in malaria, while Genta et al. (1983) and Gastl et al. (1984) have also reported a decrease in circulating total and helper T-cells in humans with strongyloidiasis and schistosomiasis (both nematode infection) respectively. Patients in this study who had other concurring parasites (some of which include malaria, strongyloides, and schistosome parasites) had a greater depression of their total and helper T-cell values as compared to those with hookworms only.

Production of leucocyte migration inhibition factor to purified protein derivative (P.P.D) was also found to be significantly depressed in this study ($P < 0.05$) for all the subgroups. Krantman et al. (1982) and Chandra and Saraya (1975) also found similar results while studying iron-deficiency anaemia in hookworm patients. In this study, variations in MIF levels correlated with the degree of anaemia (marked anaemia < moderate anaemia < no anaemia) and the decrease in total and helper T-cells.

The exact mechanism for the depression of the cellular responses is not clear but Pritchard (1986) suggests the release of immunomodulatory factors in the form of parasite excretory-secretory products and enzymes which may

initiate non-specific suppressor cell activity. This idea is further corroborated by a study in the model hookworm-mice system of N. dubius in mice, where an impairment in the cellular response to sheep red blood cells was observed. This was attributed to parasite induced physical damage to the intestine with impaired food absorption and consequent malnutrition and the release of excretory-secretory products (Behnke and Hannah, 1982). The hookworm excretory-secretory products include a histolytic anticoagulant metalloprotease, and acetylcholinesterase as well as the newly isolated N. americanus haemoglobinase (Pritchard et al., 1986). However, the full contribution of these products to the host-parasite relationship and model-systems is being intensely investigated. Hookworm acetylcholinesterase has been implicated in a 'biochemical hold-fast' mechanism which aids in the retention of hookworms in the intestine by inhibiting locally the peristaltic movements of the gut during the expulsion process (Sullivie and Jones, 1971). Pritchard (1986) is also of the opinion that metabolism of essential fatty acids (EFAs) and the consequent local eicosanoid release at the site of larval penetration may cause local immunosuppression in the skin. Even though the patients in this study were at a stable state at which the parasites were evidently producing eggs in the gut, this speculation may be extended to the local site of attachment in the gut, thus enhancing the non-expulsion of the worms.

Another suggestion to consider would be that those cellular interactions needed for manifestation of positive in vivo and in vitro responses to parasite antigens may be impaired by the extent and severity of the disease. Thus it would be expected that patients with other parasites as well as

those with marked anaemia should be the most immunosuppressed. Both subgroups are representative of infections with excess antigen and consequently an overload of the immune system. In such cases, there is stimulation of suppressor T-cells and limitation of antigen-induced lymphocyte proliferative responses by the production of antigen-specific suppressor factors. This interpretation is supported by the observation of this type of suppression in human schistosomiasis (Butterworth et al., 1982). In this study, suppressor cells were normal for all the subgroups, but at the expense of a reduced helper cell subpopulation, indicating the probable suppressive effect of suppressor T-cells on helper T-cells.

Jesimovic - Alsevic et al. (1988), reporting IL-2R (interleukin - 2 receptor) levels in patients with parasitic disease found only mildly elevated levels of IL-2R in hookworm patients as compared to the very significantly elevated levels in patients with systemic infections like strongyloidiasis, schistosomiasis, malaria and filariasis. Helper T-cells in the presence of accessory cells and repeated stimulation by antigen, produce IL-2 which binds to its receptor IL-2R to result in total and helper T-cell proliferation (Wardle, 1988). The possibility of a defect in the expression of IL-2R on T-cell surfaces or the binding of IL-2 to its receptor might be responsible for the mild helper T-cell responses in hookworm infection, as found by Wardle (1988) (Figure 27). However in this study, T-helper populations were reduced in quantity and in function as shown by the defective MIF responses in all the subgroups, especially the patient subgroup with marked anaemia. Metabolic blockage or the effects of suppressor factors on helper cells may also have affected the production of IL-2. In a similar study,

Bastl et al. (1984) studying numerical and functional alterations of T-lymphocytes in human schistosomiasis, attributed the decrease in CD_3^+ (total T) and CD_4^+ (helper T) cells in patients with schistosomiasis with a depressed capacity of mononuclear cells to respond to mitogens and antigens in vitro and an insufficient release of IL-2 into culture supernatant.

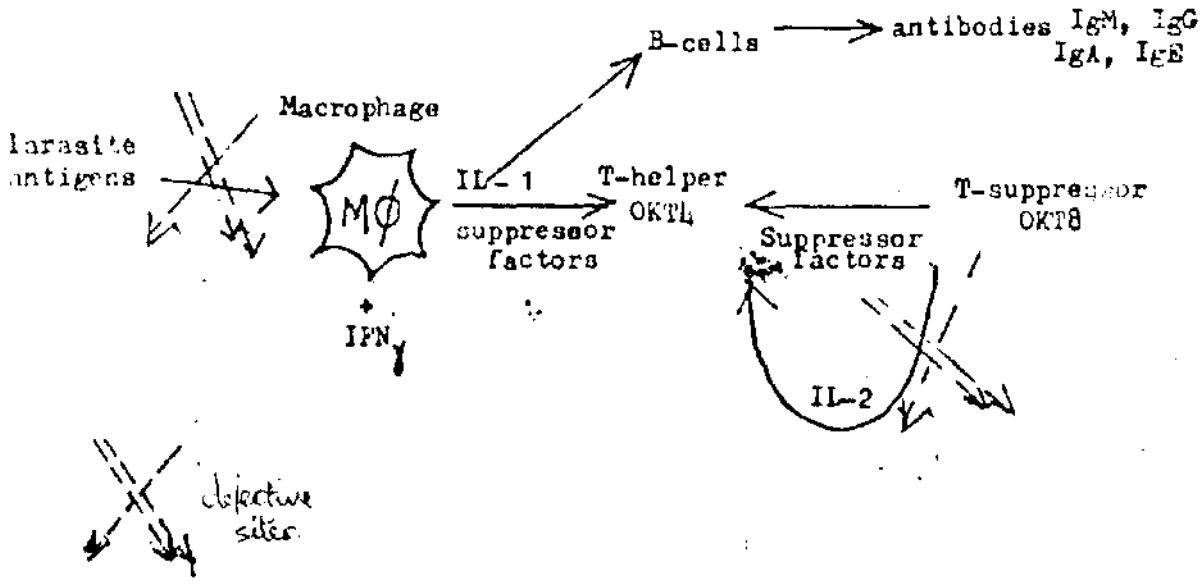


Fig. 37.: Probable defective sites in the Immune Response to parasite infection.

Immuno Jobulin Synthesis

Significantly elevated levels of IgM, IgA and secretory IgA (SIgA) ($p < 0.05$) were recorded in patients in the different subgroups of this study. In a similar study by Higashi and Chowdhury (1971) in ascariasis, trichuriasis and hookworm infections, high values of immunoglobulin especially IgG were found in both patients and controls. Oswal et al. (1979) also found that the levels of IgG and IgA were elevated in the serum of hookworm patients

in India. In this study, both patients and controls had normal levels of IgG while IgA levels were elevated. Since IgM antibodies appear early in an infection and IgG antibodies later (WHO, 1987) the elevated IgM levels have been reported to be indicative of an acute as opposed to chronic infestation (Oswal et al., 1979). However in this study, it is difficult to speculate as such for the observed IgM elevation because the determination of the period of the patients infection was difficult due to inconsistencies in the medical reports. However, in medicine, an acute infection would refer to less than 2 weeks but the length of time from infection to production of hookworm eggs in the faeces for N. americanus is approximately 14 weeks. Therefore, since our patients were all excreting eggs, the infections could not be described as acute.

An alternative explanation for the high IgM response in this study could be an intense antigenic stimulation by the worms under conditions of depressed T-lymphocyte function which could predispose to prominent T-independent antibody responses. This is supported by the fact that the most significant elevations in IgM levels were observed in patients with marked anaemia and those with hookworms and other parasites in whom the T-cells (total and helper) were most depressed.

High levels of serum IgA were observed in patients with mild to moderate anaemia in this study. Oswal et al. (1979) also observed such values in his study in hookworm infested subjects as IgA is the predominant immunoglobulin in the mucosal defence system. They also suggested that IgA could have acted as an immunologic barrier against the hookworm larvae immediately

after they had migrated from the skin to the portal venous circulation. Serum IgA levels and secretory IgA levels were also elevated in Northern Nigeria patients with amoebic liver abscess due to the intestinal parasite Entamoeba histolytica (Oyeyinka and Onyemelukwe, 1983). They suggested the elevated IgA levels to be due to unexplained host or parasite factors and to play a role in the activation of the alternative pathway of the complement system. This does not seem to be the case in this study as normal levels of C3 were reported while C4 (a major component of the classical pathway) levels were increased. Although, in the absence of complement turnover studies or detection of breakdown products of complement, the conclusion that the alternative complement pathway is not being activated cannot be made. IgA and other immunoglobulins like IgM and IgG may couple parasite antigens to form immune complexes.

Secretory IgA levels have been found in serum and other body fluids in intestinal parasitic infections in man (Befus and Bienenstock, 1984). For example, Oswal et al. (1979) found very low SIgA levels in the jejunal secretions of hookworm patients. They suggested that low levels of SIgA might predispose the patients to colonization by the worms. Contrary to this report serum SIgA levels in this study were significantly higher in the serum of the patients from all the subgroups compared with controls ($P < 0.001$), although jejunal secretions were not studied in this survey. It has been suggested that SIgA may act indirectly upon the parasite through helper and suppressor T-cells which bear isotype specific receptors for IgA (Befus and Bienestock, 1984) in an IgA dependent cellular cytotocity

reaction. Also as reported previously, Abu-Ghazaleh et al. (1989), have speculated a link between SIgA and the eosinophilic response in helminth infections. Secretory IgA is secreted into the gut lumen by exocytosis and from there it passes into the mucous surface of the epithelial layer. The mucosa associated lymphoid tissue has a wide distribution in the Peyer's patches of the gut (Holborow and Lessof, 1981) and SIgA is more efficient at agglutinating than IgG as it has four combining sites. It is therefore tempting to speculate that the rise in IgA observed in this study has a protective role to play in inflammation in the gut against hookworm infection. However, since these hookworm patients were not deficient in SIgA it could be that these reactions are not efficient in expelling the worms, hence their persistence in the gut. SIgA in this study was highest in patients with mild to moderate anaemia but lowest in those with marked anaemia (the highest worm load), thus indicating that increased levels of SIgA are necessary to reduce worm survival and the risk of severe disease.

The increased production of immunoglobulin (IgA, SIgA and IgM) in this study is similar to that observed by Keller and Keist (1972) in rats infected with the rat hookworm N. brasiliensis and might suggest an increase in SIg^+ or B-lymphocytes at the expense of a reduced T-cell compartment (Gastl et al., 1984). Keller and Keist (1972) found out that antibody production was not impaired in hookworm infected rats but that the cell-

mediated step involving T-lymphocytes was readily suppressed. Yong and Dobson (1984) also reported that Angiotrongylus cantonensis infection of rats was characterized by a prominent antibody mediated-response but not a cellular response to PHA or the worm antigen. Genta et al. (1983) also found that in human strongyloidiasis, T-cell responses to PHA were reduced and this was attributed to a decrease in circulating T-cells and an increase in suppressor T-cells. They also found the presence of suppressor factors in serum. Gastl et al. (1984) reported a decrease in total and helper T-cells in contrast with the high concentrations of IgG, IgM and IgE antibodies usually present in schistosomiasis. They suggested that the alteration in these T-cell subpopulations in schistosomiasis might precede the development of the hepatosplenic form of the disease. Keller and Keist (1972) furthering their studies on hookworm infected rats, found that worms were only expelled from the rats, when the antibody damaging step was followed by the action of sensitized lymphocytes. In this study, the depressed MIF indices indicate that the lymphocytes were not sensitized to heterologous antigens. However, it has been reported that in mice, N. dubius infection depresses the hosts lymphocyte responses to homologous antigen (Wakelin, 1986). Sensitized lymphocytes are required to produce lymphokines which stimulate B-cell blast formation, eosinophil chemotaxis, mast cell proliferation, polymorph chemotaxi and lymphocyte proliferation. In the gut, normal T-lymphoblasts obtained from peripheral lymph nodes do not localize in the intestine until there is inflammation, as occurs in nematode infections in mice (Bienenstock, 1982). However the lymphocytes of the intestinal leucocyte compartment are reported to be 25% T-helper/inducer cells and 85% suppressor/cytotoxic cells in man and mice. The intestinal lamina of mice has approximately equal numbers of helper and suppressorcells (10-15%) (Beius, 1986). There is therefore a large proportion of suppressor cells in the intestine and hookworms might stimulate non-specific

suppressor cell activity to their own benefit in the intestine. These suppressor cells acting on helper cells would inhibit antigen induced lymphocyte proliferation, as observed in this study. On this basis then, it could be suggested that the reduced capacity to respond immunologically to hookworms and the prolonged life of hookworms in man might be due to the lack of an effective cell-mediated response to hookworm antigens.

Complement levels

IgM levels were significantly elevated in hookworm patients with marked anaemia, moderate anaemia and hookworm only ($P < 0.05$). C3 levels on the other hand were not significantly different in patients and controls. These results are similar to the results obtained by Ahmed and Choudhury (1971) in patients with ascariasis, trichuriasis and hookworm infection. With respect to IgG values, they found no significant difference between patients and controls but values of total haemolytic complement CH50 were significantly elevated. They attributed the elevated complement levels to a response to tissue injury. In patients with inflammatory bowel disease, Ward and Eastwood (1974) reported rises in C4 levels in the inactive disease and this they considered as evidence of continuing immunopathology. Hodgson, Potter and Elliott (1977) working on the same disease however found elevated C4 levels in the active form of the disease. Hospital controls as opposed to normal blood donor controls also had a mean higher C4 level. They suggested that the increase in serum C4 levels are non-specific and common in ill-health. The presence of inflammatory processes in intestinal parasitic infections including hookworm infection has been documented (Bruce et al., 1965; Ward et al., 1975). The cells involved in the pathogenic mechanisms

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following injection, a phenomenon known as spontaneous cure' (Wakelin, 1978). This has been observed in the rat-hookworm (N. brasiliensis - rat) system (Wakelin, 1978). This effect could have contributed to the effectiveness of the chemotherapeutic drugs (levamisole and mebendazole) in eliminating the worms. Also while the increase in C_4 levels could reflect a response to an inflammatory state, it could also be a means by which the immune system eliminates or controls the worm load. This interpretation is supported by the fact that 27 (47%) of the patients in this study had low egg loads of below 500 eggs per gram of stool before treatment.

The effect of chemotherapy and re-occurrence of hookworm ova in stools

There was an increase in the numbers of circulating total and helper T-cells and MIF indices and a reduction in egg counts to near zero levels after four weeks of effective treatment with levamisole and mebendazole in this study. This was to be expected even though levamisole and mebendazole have different pharmacokinetics as antihelmintics. While levamisole is rapidly absorbed from the gut and acts by stimulating ganglion like structures in the worms, thereby causing neuromuscular inhibitions of a depolarizing type which results in paralysis and elimination of the worms (Moens et al., 1978); mebendazole is not readily absorbed in the gut and acts by blocking the glucose uptake of the worms (Chavarria et al., 1973). Both drugs have been reported to give more than 86% cure rates in patients with hookworm infection, ascariasis and trichuriasis (Chavarria et al., 1973; Moens et al., 1978). In this study, 49% of the patients were cured after an average of 4 weeks after treatment and some of these after the use of a

combination of both Vermox and Ketrax.

The observation that the drugs took at least a month to effect a cure could be due to the heaviness of the worm load or the persistence of the worms despite the induction of an anti-parasitic state by the drugs. N. brasiliensis for example has the ability to change its antigens within a single instar (Maizels et al., 1983) and Nematodiroides dubius and Necator americanus have been shown to, directly shed their surface antigens (Pritchard et al., 1985). These examples of antigen turnover coupled with the physical attachment of adult N. americanus and A. duodenale often make expulsion difficult even in the face of a very active inflammatory process or drug effect.

Levamisole has been reported to repair basic immunological impairments such as antigen induced proliferation of T-cells, lymphokine (MIF) production,

total E-rossette formation and antibody and complement receptor activity (De Cree and Symoens, 1979). Hence the changes observed in the cellular immune response after four weeks of treatment, such as the significant increases in circulating total and helper T-cells and MIF indices ($P < 0.05$), could have been due to a boost to the immune system of the patients by levamisole and the removal of the worm load by both mebendazole and levamisole. Even though the immunoglobulins and complement levels after treatment were not measured due to some difficulties in the securing of antisera at the time of the study, it is known that levamisole does not directly stimulate B-cells or antibody production (De Cree and Symoens, 1979). Even when B-cell activity is pathologically increased, it is returned to normal levels as shown by reduced numbers of Ig-bearing cells and by the normalization of antibody levels (De Cree and Symoens, 1979).

The results from Table 10 of the breakdown of the immune parameters before and after treatment with each drug and a combination of both drugs indicate that there is a non significant response from the cellular immune response. This proves that while the total, helper and suppressor T-cells are favourably affected both in function and quantity, an adequate immune response had not yet been mounted. With the use of vermox alone, there was a decrease in the white blood cell count but an increase in total T-cells and the helper and suppressor T-cells. This increase in the cells of the immune system is reflected functionally in the increase in the secretion of the leucocyte migration inhibition lymphokine. Though none of these results were statistically significant, vermox achieved an 87.5% cure rate on the patients on whom it was used after 4 weeks. This result was different from that of obtained from the use of Ketrax and the combination of Ketrax and Vermox

where, the cellular immune parameters were also increased but cure rates of 57.14% and 58.33% respectively were achieved. The lack of correlation between restoration of immunological function by these drugs and clinical responsiveness or parasite load would imply that the underlying defect responsible for the disease, mainly the hookworms, had not been effected. Thus it would appear that the ideal drugs for treatment of hookworm infection would be one that can influence the cellular immune response as well as achieve a very effective means of eliminating the worm. It would also appear that elimination of the worms is necessary for restoration of the immune response and consequently an insight into the possibility that hookworms might on their own effect a suppressive action on major immunocompetent cells would be very necessary. However, these results due to the smallness of the sample size and the non-statistically significant results are very inconclusive and would require further studies on hookworm infection and its effect on the immune response.

The issue of reinfection poses a problem in the areas of hookworm endemicity (Nawalinski et al., 1978). In this study, four months after treatment, four of the patients followed-up had hookworm ova in their stools. In all the four cases, the patients were again observed to have infection with the same worm that had previously infected them (that is Necator americanus), thus eliminating any suggestions of cross-immunity. In mice infected with the mouse hookworm Nematospiroides dubius, there is the development in some of a strong cellular and humoral immunity which results in loss of worms and prevents further establishment (Wakelin, 1986). Hence immunity acquired from previous hookworm infection can protect against subsequent infections. However, immunity to gastrointestial nematodes (such as Ascaris and hookworms) has now been shown

to be a heritable characteristic, in that individuals carrying heavy or light burdens in an infected population are liable to reacquire heavy or light burdens respectively after chemotherapy (Anderson, 1986). In a study of the epidemiology of intestinal helminths in India, Elkins et al., (1986) found that there was a predisposition for infection with heavy or light worm loads of Ascaris, Trichuris or hookworms. Those with low egg counts at the beginning remained low while those who were high at the beginning remained high, after chemotherapeutic control. In the past, predisposition and development of immunity to gastrointestinalnematodes, was dependent on behavioural and social factors (Schad et al., 1983) but laboratory studies on rodent nematode models are now showing that genetic and immunological factors are involved (Wakelin, 1986). For example immunity to Trichinella spiralis infection, as studied by duration of worm survival in the intestine is dependent on the development of an immunological (T-lymphocyte mediated) and inflammatory (myeloid cell-mediated) response (Wassom et al., 1984) and this in turn is dependent on the interaction with MHC-linked genes Ts-1 and Ts-2 which influence the activity of T-cell subsets. On the other hand, in parasitic diseases of both protozoan and metazoan origin like malaria or schistosomiasis, continued stimulation of the immune system by small numbers of organisms surviving the initial immune attack (*premunition*) is an important factor in maintaining prolonged immunity (Anderson, 1986). Immunity to clinical Plasmodium falciparum malaria depends upon continued subclinical infection (Deans and Cohen, 1983) and in schistosomiasis, as long as adult worms are present in the portal vein, re-infection with cercariae is partially controlled (Butterworth et al., 1984). Evidence of premunition has not been reported in hookworm infection

before but the evidence of re-infection or reoccurence of the hookworm ova in the stools in this study as well as in other epidemiological and control studies indicate that there may be a basic immunological or genetic defect which exposes the patients to infection again and again. This implies frequent check-ups and treatment if worm burdens are to be suppressed to low levels on a long term basis.

CONCLUSION

1. This study has shown that Nigerian patients infected with hookworms as opposed to normal healthy controls have a significant reduction of relative percentages of circulating total and helper T-cells and of leucocyte proliferative responses to P.P.D. indicating that hookwormscould cause abnormal lymphocyte distributions and functions in humans.

2. Serum levels of IgA, IgM, SIgA and C4 are elevated in these patients while IgG and C3 levels are normal, suggesting the possibility of polyclonal B-cell activation by T-independent antigens such as the polysaccharide cuticular antigens of the hookworms.

3. There is a partial reversibility of the depressed lymphocyte responses four weeks after treatment which may be attributed to the effects of the drugs on the immune system and the removal of the worm load.

4. Re-occurrence of hookworm ova was observed in four of the subjects four months after treatment, stressing the need for constant epidemiological assessment of the worm burdens in the community and the establishment of effective control measures.

The above also bring about the recognition of the need for new strategies in the study of hookworm and other gastrointestinal infections in man. These include a need to use the peripheral blood as well as concurrent studies of the gastric mucosal responses as a means of studying immunological and inflammatory events in the intestine. There is also the need to use specific parasite antigens to study antibody and molecular responses during the course of infection, for example, interleukin, complement, IgG subclasses and tumour necrosis factor assays.

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APPENDIX I

PROFILE FOR THE PROPOSED RESEARCH IN HOOKWORM INFECTION

Patients Name:.....

Address:-.....

.....

Age:.....

Infection: Hookworm Infection

Stage of Infection

Other Parasites

Hookworm asthma

Hookworm anaemia Hb———

Hookworm no symptom Albumin———

Symptoms:

Stoll Egg Count:-

Diff. Counts:-

WBC:-

Humoral (antibody) levels:-

IgM: IgA:-

IgG: IgE:

Anti acetylcholinesterase Ab:-

Cellular Immunity:-

Total T:-

T :-

Tr:-

LMIT:-

Blast formation (?):-

Skin Test (?):-

Repeat after treatment (Vermox):

No.	NAME	AGE	SEX	PARASITE STATUS	STOOL CONSIS-TENCY	STOLL EGG COUNT	SPECIE	
1.	Felicia Baten	22	F		F	600	N.a	Abd. pains, cramps
2.	Adamu Bankaro	40	M	Giardia lamblia(+)	M	6200	Na+	Anaemia,
3.	Igwe Fidelis	19	M	Entamoeba histolitica^{+++}	D	300	Na+ Ad	Bloody stool, abd. pain.
4.	Mike Musa	23	M		F	100	N.a	Med. checkup
5.	Daluwai Dumiya	65	M	Peptic ulceration	M	38600	N.a	Epigstric pain, tender abd.
6.	Yusuf'u Zubairu	45	M	A. lumbricoides (400)	M	1600	N.a	Abd. pains
7.	Saka Ismaila	13	M	I. trichiura (2,000) malaria A. lumbricoides (2100)	M	200	Na	Skin sepsis, malaria
8.	Onowumi Ajiboye	25	F	A. lumbricoides (800)	M	200	Na	Loss of weight
9.	Mba Medline	20	F		M	2000	Na	Pregnant, fever
10.	Danjuma Mijintaba	45	M		M	15200	N.a	Hypochrom. anaemia (+++) wasting
11.	Mohammed Umaru	31	M		MD	200	N.a	Abd pain, fever, diarrhoea.
12.	Barnabas Paul	23	M		F	1700	N.a	Abd. pain, fever
13.	Chollom Iyop	55	F		F	700	N.a	Headache, passing loose stool.
14.	Bagson Theresa	29	F		F	200	N.a	Diff. in breathing, headache.
15.	Sule Yau	25	M		M	1500	N.a	Abd. pain, fever
16.	Attin James	28	M	S. mansoni (400), Strongyloides larvae (+)	M	600	N.a	Med. checkup, head-aches
17.	Elisha Sunday	13	M	Taenia ova (300)	D	300	N.a	Pyrexia, loss of appetite
18.	Umaru Alhassan	13	M		M	400	N.a	Fever, headache
19.	Abbas Ibrahim	22	M		M	800	N.a	Med. check-up
20.	Akao Ezekiel	45	M		F	2000	N.a	Febrile, jaundiced
21.	Abdullahi Haliru	19	M	Malaria rings (++)	F	2500	N.a	Loss of apetite, pale.
22.	Salka Daniel	70	M		MD	300	N.a	Body weakness gener dizziness, abd. pai
23.	Buhari Mohammadu	35	M		MD	82500	N.a	Vomiting, headache
24.	Auchan Abdullahi	70	M		MD	2900	Na+ Ad	Paraplegic
25.	Garba Akilu	30	M	Giardia lamblia (+)	M	800	N.a	Epigastric pain
26.	Jumare Sule	37	M		F	9400	N.a	Paraplegic

No.	NAME	AGE	SEX	PARASITE STATUS	STOOL CONSIS-TENCY	STOOL EGG COUNT	SPECIE	
27.	Adamu Simon	27	M	Microfilaria (++)	MD	2100	Na+ Ad.	Pallor, epigastric pain
28.	Ketah Felix	33	M	Strongyloides larvae(+)	F	400	N.a	Bloody stool, abdominal pain
29.	Mohammed Mohammadu	42	M		MF	200	Na	Dizziness, Headache, wt. los
30.	Mohammed Aishatu	50	F		M	700	N.a	Lymphadenopathy, C.L.L.
31.	Mohammed Shuaibu	23	M		F	200	N.a	Abdominal pain, fever
32.	Junaidu Lawal	23	M		F	200	N.a	Abdominal pain, cramps
33.	Muntari Ibrahim	24	M	A. lumbricoides (8,600)	MF	500	N.a	Medical check-up.
34.	Ogheibei Maryam	54	F		F	1200	Na	Hypertensive
35.	Gaiyas Talatu	38	F		F	200	Na	Abdominal pain, headach
36.	Maazu Samaila	20	M		F	200	Na	Jaundiced, afebrile
37.	Ahmed Abdullahi	25	M		F	111200	N.a	Anaemic, feverish
38.	Jatau Hakiya	20	F		MD	300	N.a	Pregnant, anaemic
39.	Taru Zakaria	42	M		M	600	N.a	Anaemic, wasting
40.	Umaru Zainab	14	F	Hypochronasia (++)	MD	300	N.a	Loose bloody stool
41.	Nwuaan Mary	33	F	Strongyloides larvae (++)	MF	300	N.a	Epigastric pain, headache, neck pain.
42.	Yahaya Hadiza	18	F		MF	300	N.a	Anorexia, constipation, preg
43.	Tsar Atsen	20	M	Giardia lamblia (+)	MF	900	N.a	Lower abdominal pain
44.	Adamu Lawal	35	M		MF	900	N.a	General oedema, renal failur
45.	Bala Nicodemus	13	M		F	300	N.a	Hypochron (++), Abd. swellir
46.	Maleka Mohammed	13	M	S. mansoni (+)	D	300	N.a	Septicaemia, fever
47.	Abdullahi Abubakar	55	M		MF	200	N.a	Bilateral nephrolithiasis re
48.	Odang Moses	13	M		F	2600	N.a	Hypochr(++), bloody stool
49.	Tungwang Marcus	30	M		F	1300	N.a	Vomiting, passing watery sto
50.	Adamu Yau	33	M		F	1600	N.a	Renal failure, anaemic
51.	Audu Ladidi	22	F		M	300	N.a	General body weakness, Megal blastic anaemia
52.	Abdullahi Ibrahim	24	M		M	400	N.a	Hepatomegaly fever, splenom lly, abdominal pain
53.	Miu Ibrahim	27	M	Giardia lamblia (+)	F	300	N.a	VDRL-ve, feverish
54.	Aliyu Sani	25	M		F	400	N.a	Epigastric pains
55.	Umaru Haruna	20	M		F	1100	N.a	Abdominal pain
56.	Abdu Haliru	21	M		F	200	N.a	Headache, abdominal pains
57.	Dalhatu Hussaini	23	M		F	2100	N.a	Fever, abdominal pains

APPENDIX

LIST OF CONTROLS

S/No.	NAME	AGE	SEX	STATUS	CONSIS-TENCY	STOOL	SPECIE
1.	Kolawole Olatunde	16	M	Student	F	-	-
2.	Ronke IgE	19	F	"	F	-	-
3.	Bola Olatunte	23	F	Staff	F	-	-
4.	Victor Amurawaiye	36	M	"	F	-	-
5.	Joseph Egbe	28	M	"	F	-	-
6.	Thomas Oyeniyi	30	M	"	F	-	-
7.	Betty Lawal	33	F	Staff(Preg.)	F	-	-
8.	V.K. Clorunshola	24	M	Staff	F	-	-
9.	Joseph Gubel	32	M	Staff	F	-	-
10.	Mikhael Wandzu	24	M	Student	F	-	-
11.	Abubakar Kwande	30	M	Med. checkup	F	-	-
12.	Kunle Salami	30	M	Staff	F	-	-
13.	Commeh Emmanuel	38	M	Staff	F	-	-
14.	Musa Ibrahim	45	M	Staff	F	-	-
15.	Kabir Umar	23	M	Staff	F	-	-
16.	Mohammadu L. Umar	25	M	Staff	F	-	-
17.	Gupta Rachana	20	F	Student	F	-	-
18.	Modupe Olatunde	19	F	Student	F	-	-
19.	Dr. Iwuchukwu	38	M	Staff	MF	-	-
20.	Mathias Ideh	40	M	Staff	F	-	-
21.	Kenneth Ihenetu	27	M	Staff	F	-	-
22.	Mohammed Ali	29	M	Staff	MF	-	-
23.	Idris Ibrahim	22	M	Staff	MF	-	-
24.	Mohammed Abdullahi	25	M	Blood donor	F	-	-
25.	Abubakar Abdullahi	30	M	" "	F	-	-
26.	Cecilia Sule	25	F	Student	F	-	-
27.	Nada Usman	30	M	Student	F	-	-
28.	Alice Adeoye	34	F	Trader	F	-	-

Ketrax I.C.I.

Levamisole HCl 40mg

Vermox Janssen

Mebendazole 100mg

APPENDIX

White blood cell, differential lymphocyte and T-cell counts and migration inhibition in Hookworm Patients after stool was clear

	PCV	WBC ($x10^9$/L)	Differential lymphocytes ($x10^9$/L)	Absol. No ($x10^9$/L)	Resette at 4°C (%)	Absol. No ($x10^9$/L)	Anti-Leu 4+ (%)	Absol No. ($x10^9$/L)	Anti leu 3a(%)	Absol No. ($x10^9$/L)	Anti leu 2a(%)	Absol No. ($x10^9$/L)	MI (%)
N	28	28	28	28	28	28	20	20	20	20	20	20	10
Range	31-48	2.2-5.2	30-78	0.93-3.04	42-48	0.47-1.73	48-59	0.50-1.62	25-39	0.26-0.90	17-19	0.17-0.66	37-67

APPENDIX

TOTAL TIME OF STOOL CLEARANCE AFTER TREATMENT

Serial No.	Stoll count other 4 weeks	Drug Used	Total time of stool clearance
1.	100	Ketrax+ Vermox	8 weeks
2.	100	Ketrax + Vermox	8 weeks
4.	100	Ketrax	8 weeks
7.	NIL	Vermox + Ketrax	4 weeks
9.	900	Ketrax	8 weeks
11.	100	Ketrax + Vermox	8 weeks
12.	100	Vermox + Ketrax	4 weeks
13	NIL	Vermox + Ketrax	4 "
14	100	Vermox	8 "
16	NIL	Ketrax + Vermox	4 "
17	NIL	Vermox	4 "
18	NIL	Ketrax	4 "
19	NIL	Vermox	4 "
20	NIL	Vermox + Ketrax	4 "
22	NIL	Ketrax	4 "
24	NIL	Vermox + Ketrax	4 "
25	NIL	Vermox	4 "
27	200	Ketrax + Vermox	8 "
28	NIL	Vermox	4 "
31	NIL	Ketrax	4 "
32	NIL	Vermox	2 "
33	400	Ketrax + Vermox	8 "
36	NIL	Ketrax + Vermox	4 "
37	NIL	Ketrax	4 "
41	NIL	Vermox	4 "
48	NIL	Ketrax	4 "
49	NIL	Vermox	4 "
54	NIL	Ketrax + Alcopar	2 "

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