EFFECTS OF ALBENDAZOLE THERAPY ON SERUM PROTEINS AND LEUCOCYTE COUNTS IN HYDATID PATIENTS.

BY

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DECLARATION

I, Charles Aono Omwandho hereby declare that this is my orginal work and has not been presented for award of a degree in any other University.

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I dedicate this thesis to my parents Mr. and Mrs. Michael and Petronela Omwandho Aono, my brothers Silvan, Maurice, Thomas, Francis and to my sisters Rose and Jacinta for the help and encouragement they have accorded me throughout the study.

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TABLE OF CONTENTS

ABBREVIATIONS

Alb	-	Albumin
DEAE-c	-	Diethylaminoethyl cellulose
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediamine tetra-acetic acid
IgG	-	Immunoglobulin G
Mr	-	Relative molecular weight
OD	-	Optical density
PAS	-	Periodic acid Schiff's reagent
RNA	-	Ribonucleic acid
SDS	-	Sodium dodecyl sulphate
SDS PAGE	-	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TEMED	-	N,N,N',N'-tetramethylethylene diamine
α	-	Alpha
β	-	Beta
γ	-	Gamma
(v/v)	-	Volume to volume ratio
(w/v)	-	Weight to volume ratio
λ	-	Wavelength

UNIT ABBREVIATIONS

°C	–	Degree centigrade
"	–	Inch
Kg	–	Kilogram
µ	–	Micron
mg	–	Milligram
ml	–	Millilitre
mm^3	–	Cubic millimetre
ng	–	Nanogram
nm	–	Nanometre

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

SUMMARY

Hydatidosis (hydatid disease) and echinococcosis refer to infections by the metacestode (larval) and strobilar (adult) stages of Echinococcus granulosus. The infection is a cyclozoonosis of major public health and economic importance in all the inhabited continents. Except in special cases, it is a dead end infection in man.

In Kenya, hydatid disease has been reported in man, domestic and wild animals. A report by Eugster (1978) indicated that in Kajiado District alone 44.7% of cattle, 9% of sheep and 6.3% of goats had hydatid cysts. Human hydatidosis in Kenya is mainly found among the Suk in West Pokot, the Rendille, the Shangilla, the Maasai and the Turkana. Sporadic cases are known to occur in other parts of Kenya among the Luo and Kikuyu (Okello and Kyobe 1981).

Turkana District of Kenya is reported to have the highest incidence of human hydatidosis in the world and the number of patients seeking treatment in the District is about 96 per 100,000 population annually (O'Leary 1976, French and Nelson 1983). As this figure represents mainly self diagnosed cases, it underestimates the real incidence which is presumably much higher (Okello 1986).

Although infection, prevalence and mortality rates due to hydatid disease may appear low in comparison with those for some other diseases, the morbidity associated with the disease is

considerable and hydatid patients often require multiple surgical interventions. Extensive secondary hydatid disease often becomes inoperable and the involvement of bones usually requires amputation.

Economic losses due to hydatidosis include the cost of hospitalization, the incapacity to do work especially after surgery and loss of life in some cases. Large amounts of livestock viscera are also condemned due to heavy infections with hydatid cysts.

A large number of serological tests and their attendant problems in hydatid disease of man and animals have been described (Rickard 1979). These tests have suffered from lack of sensitivity, specificty or both (Yong and Heath, 1979). The serological diagnosis and sero-epidemiological studies of human hydatid disease have been based to a large extent on "Arc 5" antigen, first described by Capron et al (1967) and later characterized by Bout et al (1974). The sensitivity and specificity of these tests have been very poor and an unacceptably high number of false negatives have been recorded (Okello and Chemtai 1981). Detection of hydatid disease in livestock before slaughter is usually clinically impossible since the infected animals show no clinical signs, hence collection of epidemiological data for hydatid disease particularly to establish prevalence have mainly depended on collection of data in slaughter houses and in most cases, accurate and reliable records are not available.

Both surgery and chemotherapy have been used in treatment of hydatid disease in Kenya. However, due to lack of adequate surgical facilities, late diagnosis and other complications such as the occurrence of multiple cysts in the abdomen and thorax, surgical treatment is possible in less than 50% of the cases in Turkana (Okello 1986). The recurrence rate is high among the surgically treated individuals (20% in Turkana) and many of the recurrent cases are inoperable (French 1984). Inoperable hydatidosis is thus a major problem and the need for medical treatment has long been realized. The benzimidazole carbamate group of drugs including flubendazole, mebendazole and albendazole have been used in experimental treatment of human hydatid disease.

In view of the economic and public health importance of hydatidosis, there is need to investigate and fully understand the course of the disease in individuals undergoing various types of treatment such as surgery and chemotherapy. This study was undertaken to determine the effects of albendazole therapy on serum protein levels and leucocyte counts in hydatid patients. Results indicated abnormal reduction in serum protein concentrations on admission of patients but this was found to recover over the treatment period with albendazole therapy. Electrophoretic studies using cellulose acetate membrane and SDS polyacrylamide gels showed a reduction in albumin and pre-albumin levels accompanied by elevated levels of IgG. In most cases (75%), albumin levels were as low as one third the amounts in normal subjects while pre-albumin was eliminated altogether

from circulation at the height of infection. Determination of IgG levels using Ion Exchange Chromatography showed 2 -3 fold elevation on admission of patients but this later underwent a gradual decrease towards normal values with treatment. It was suggested that the reduction in serum protein concentrations may have resulted from impaired synthesis and release of albumin by the liver due to hydatid infection, loss of appetite observed in patients during early days post-admission, low dietary protein uptake by the patients and probably the loss of proteins in urine due to glomerulonephritis consequent to deposition of immune complexes in the kidneys of these patients. The loss of protein in urine due to glomerulonephritis was also suggested by Okello (1988). The elevation in circulating IgG in patients was thought to have resulted from its increased production aimed at combating the infection and to replace the IgG that is lost from circulation after penetrating the hydatid cyst membranes.

The presence of hydatid cysts was shown to suppress leucocyte proliferation in the patients. However, the use of albendazole led to a temporary decrease in lymphocyte, monocyte and neutrophil counts. This was followed by a proliferative burst ending with a gradual decline to normal levels in 8 out of 12 (66.6%) cases. In 2 out of 12 (16.7%) cases, there was an immediate elevation in absolute and differential leucocyte counts followed by a gradual decline while in another 2 cases, initial administration of albendazole was followed by a progressive decline in lymphocyte and neutrophil counts, accompanied by a consistent increase in monocyte and eosinophil counts throughout therapy. It is likely that the latter were cases in

which the hydatid cysts were already undergoing regression at the beginning of therapy. Eosinophil counts were well above the normal range in 6 out of 12 (50%) cases and did not exhibit consistency in counts in response to albendazole administration. Total haemoglobin, packed cell volume and erythrocyte counts were normal in all the cases studied showing that hydatid disease is not associated with anaemia. The initial decline in leucocyte counts at the beginning of therapy is suspected to have resulted from the elimination of old leucocytes (probably defective) by albendazole. This hypothesis remains to be tested.

From this study, it was concluded that chemotherapeutic use of albendazole restores serum protein concentration accompanied by polyclonal leucocyte proliferation in hydatid patients. The detailed mechanisms by which albendazole contains hydatid disease remains unclear and needs to be investigated further.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1.0 Global distribution of human hydatid disease

The adaptability of Echinococcus granulosus the causative agent of hydatid disease, to a wide variety of host species and the repeated transportation of domestic animals between different parts of the world have made the present broad geographical distribution of the disease possible. The disease occurs from North of Arctic circle to as far south as Tierra del Fuego Argentina, Stewart Island and New Zealand (Schantz 1982).

In the higher latitudes of the Western hemisphere including parts of Canada and Alaska, the disease is confined to sylvatic animal intermediate hosts hence the risk of human infection is restricted largely to the indigenous Eskimos and Indian tribes that are involved in hunting and trapping (Cameron 1960, Rausch 1960). In America, the highest infection prevalence is observed in the intensive sheep raising areas of southern South America (Argentina, Uruguay, Central Peru, Bolivia and Southern Brazil), where the simultaneous presence of sheep and dogs combine to produce optimal conditions for the perpetuation of the cestode (Williams et al 1971).

In Europe, the highest prevalence in human infection is reported from countries adjacent to the Mediterranean Sea (i.e Spain, Italy, Yugoslavia, Greece and Cyprus). Little human morbidity is reported in parts of Western Europe, Great Britain and Ireland (Thompson and Smyth 1975).

Infections occur in most Asian countries including the People's Republic of China, Kampuchea, Viet-Nam, the

Phillipines, Taiwan and Indonesia with high prevalence in Iran, India, Nepal and Pakistan (Matossian et al 1977).

High prevalence is reported in Northern Africa. In East Africa, infection in domestic livestock is widespread but infection in humans is mainly limited to certain groups in North-Western Kenya and Uganda where a set of poorly understood environmental and cultural factors combine to produce one of the highest human morbidity rates ever reported (Mann 1974), Owor and Bitakaramire 1975, O'Leory 1976).

1.2.0 The hydatid worm (Echinococcus granulosus)

Echinococcus granulosus is a cosmopolitan parasitic tapeworm belonging to the cyclophiledian class of the taenid family. It requires both intermediate and definitive mammalian hosts for its complete development. The intermediate hosts include among others the domestic sheep (Ovis ammon f. aries) goats (Capra aegagrus f. hircus) and cattle (Bos primigenus f. taurus). The definitive hosts include domestic dogs (Canis familiaris), the dingo (Canis dingo Linnaeus) and the jackal (Canis aureus Linnaeus) and the wolf (Canis lupus Linnaeus) among others.

In Kenya, the camel and human beings are important intermediate hosts while the silver backed jackal (Canis aureus), Lions (Panthera leo) and spotted hyena (Crocuta crocuta) as well as the hunting dog are important definitive hosts (Eckert et al 1981).

It causes hydatid disease, sometimes called hydatidosis in the intermediate host and echinococcosis in the definitive hosts.

1.2.1 Structure of adult Echinococcus granulosus

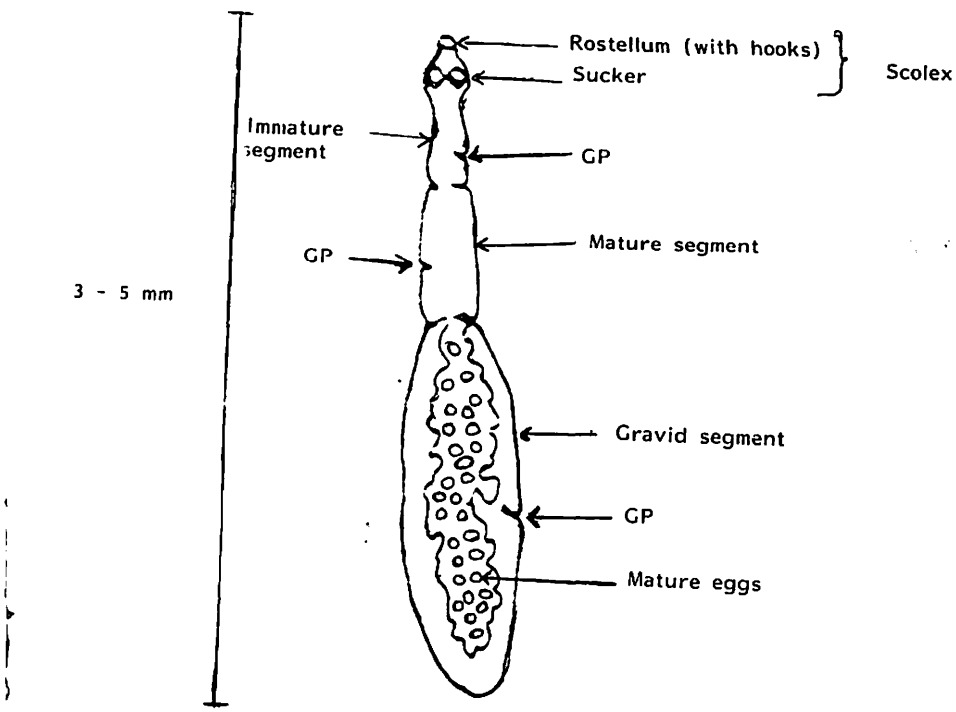
The fully developed strobilae of E. granulosus constitutes a scolex with four suckers, a double row of rostellar hooks (28 to 50 in number) plus three to four proglottids (Fig. 1).

The entire strobilae measures 3 to 5 millimetres. The cestode is hermaphrodite with 35 to 45 testes and a genital pore placed slightly behind the middle of each segment. The first proglottid adjacent to the scolex is sexually immature but is usually followed by two to three hermaphroditic proglottids in different stages of maturity. The final proglottid has a gravid uterus with lateral diverticulae, containing between 200 to 800 eggs (Arundel 1972) and is detached approximately every two weeks following which the penultimate proglottid becomes gravid.

Figure 1 : Schematic diagram of adult E. granulosus

Adapted from Rausch and Bernstein (1972).

GP - Genital pore


Rostellum (with hooks)
Sucker
Scolex
Immature segment
GP
GP
Mature segment
3 - 5 mm
Gravid segment
GP
Mature eggs

1.2.2. Life cycle of E. granulosus

Two types of life cycle exist for the complete development of E. granulosus. These are:

i) The sylvatic cycle between wild carnivores such as the wolf and wild ungulates such as the moose.

ii) The synanthropic cycle between domestic carnivores especially domestic dogs and domestic ungulates such as goats, cattle and sheep. In this cycle, man may occasionally be involved as the intermediate host.

The adult worm is found in the small intestines of the definitive canine hosts. Eggs are shed with faeces of definitive hosts into external environment and when ingested by a suitable intermediate host, the oncospheres (embryos) hatch and become activated leading to their complete liberation from the embryonic envelope. This results from the digestion of embryonic membranes from without by the host enzymes as well as from the mechanical activity and lytic processess of the activated oncospheres (Hatch and Smyth 1975). Lytic secretions may then facilitate the passage of motile oncospheres through the intestinal mucosa into the host's circulatory system via venous and lymphatic pathways (Heath 1971). The oncospheres are thereafter distributed via the host's circulatory system to various sites where the post-oncospheral development continues. The final localization of the taeniid is determined by anatomic (Capron et al 1968), and physiological factors of the host as well as the strain of the cestode among other factors (Wilson et al 1978).

Cystic development, a process involving degeneration of the oncospheral stage and emergence of metacestode stage begins a few days after the oncospheres reach their preferred sites (liver, lungs, heart, kidney and other organs). Successful *in vitro* cultures of *E. granulosus* oncospheres showed that within four to seven days, the larvae had changed into typical bladder forms with a germinative layer and within ten days, the latter had formed acellular laminations (Heath and Lawrence, 1976). In general, hydatid cysts increase in diameter from one to five centimetres each year while protoscolices (scolices with the rostellum and suckers deeply withdrawn into the post sucker region) are formed as early as four months but may require more than a year in some cases.

When metacestodes (hydatid cysts) are ingested by a suitable definitive host, the protoscolices, stimulated by bile, pH and presumably other host factors evaginate in the upper part of the duodenum (Smyth 1964). They then make their way between the villi and may enter the crypts of Lieberkuhn achieving intimate contact with the host intestinal mucosa (Smyth 1968). Proglottidation is thereafter initiated and strobilar development proceeds until the gravid adult stage is reached in approximately 48 days (Gemmell 1962).

Figure 2 : E. granulosus ; Schematic life cycle diagram showing sylvatic (dotted lines) and synanthropic cycles. Adapted from Anderson et al 1974).

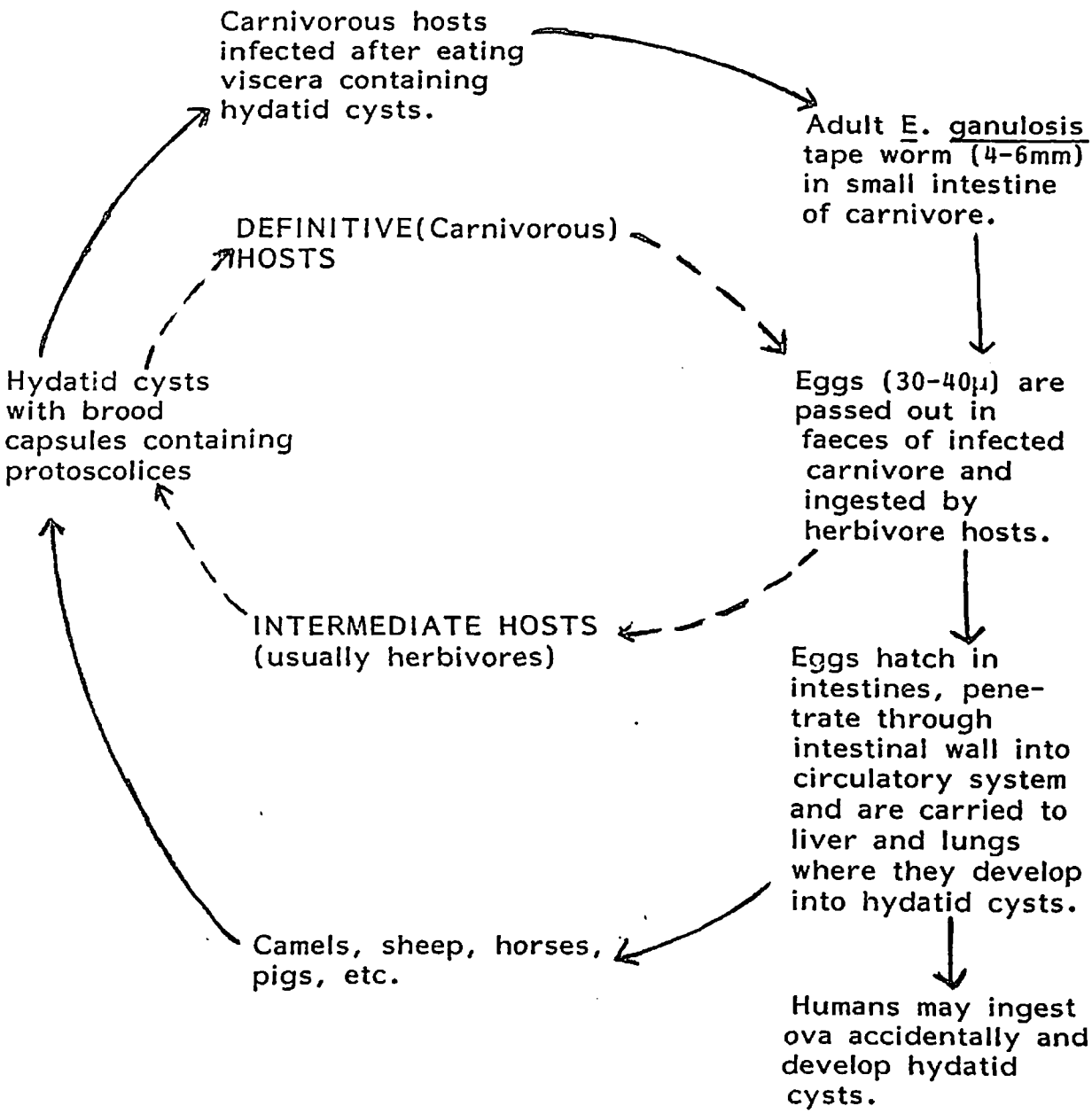
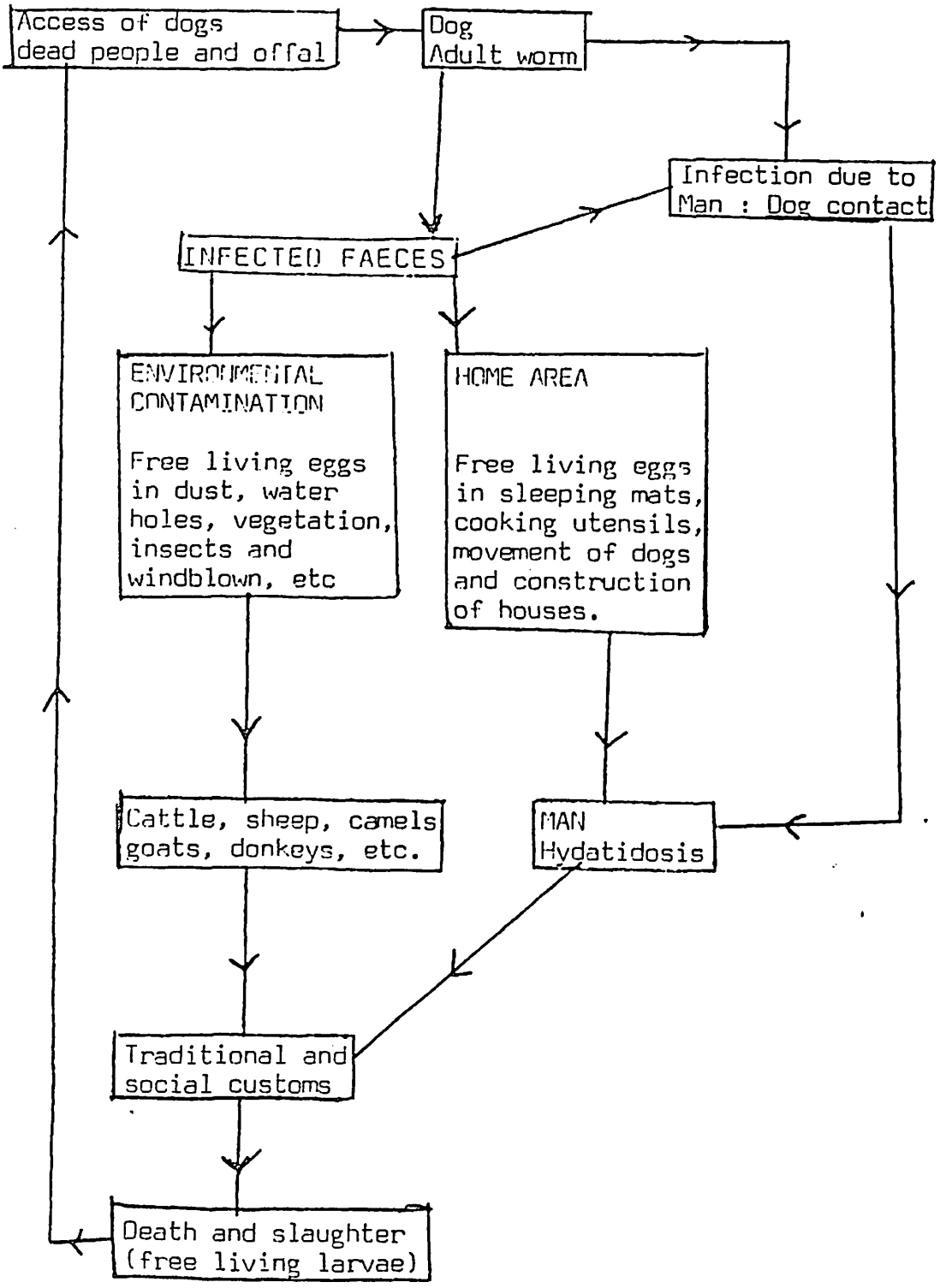

Carnivorous hosts infected after eating viscera containing hydatid cysts.
Adult E. ganulosis tape worm (4-6mm) in small intestine of carnivore.
DEFINITIVE(Carnivorous) HOSTS
Hydatid cysts with brood capsules containing protoscolices
Eggs (30-40μ) are passed out in faeces of infected carnivore and ingested by herbivore hosts.
INTERMEDIATE HOSTS (usually herbivores)
Eggs hatch in intestines, penetrate through intestinal wall into circulatory system and are carried to liver and lungs where they develop into hydatid cysts.
Camels, sheep, horses, pigs, etc.
Humans may ingest ova accidentally and develop hydatid cysts.

Figure 3 : Life cycle of Echinococcus granulosus with special relevance to the Turkana situation. Adapted from MacPherson 1986.

Life Cycle of E. granulosus with Special Relevance to Turkana District



1.2.3 Structure of hydatid cyst

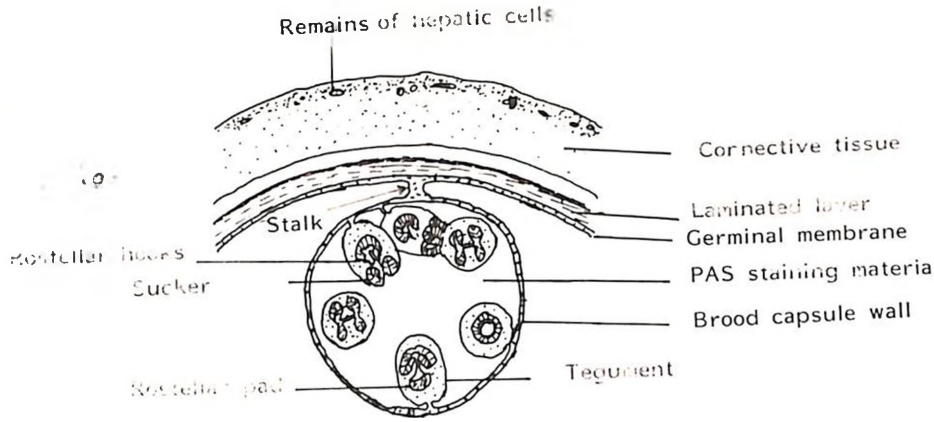
A fully developed cyst of E. granulosus is typically unilocular and fluid filled. The cyst structully consists of an inner germinative layer of cells supported by a characteristic acidophillic staining acellular laminated membrane of variable thickness. Cytoplasmic extensions of the germinative layer unite to form a syncitium which is differentiated into numerous microtriches that project peripherally towards the host tissues surrounding the cyst (Wilson et al, 1978).

The cyst is surrounded by a host produced granulomatous adventitial reaction of extremely variable intensity. Small secondary cysts called brood capsules bud internally from the germinative layer and by polyembryony produce multiple protoscolices.

In humans, the slowly growing hydatid cysts may attain a volume of many litres and may contain many thousands of protoscolices. This form, the hydatid cyst confers protective immunity to subsequent infection that is remarkable for the longevity and potentially unlimited growth with production of multiple protoscolices (Matossian and Malakian 1977), cited by Schantz (1982).

Figure 4 : A diagramatic presentation of the larval form (Hydatid cyst) of E. granulosus .

Adapted from Morseth D.J. (1967).


Remains of hepatic cells
Connective tissue
Laminated layer
Germinal membrane
Stalk
Flagellar hooks
Sucker
PAS staining material
Brood capsule wall
Tegument

1.3.0 Hydatid disease in man

There are numerous descriptions of the diverse clinical manifestations of hydatid disease in medical and surgical literature. It is apparent that many human infections remain asymptomatic and hydatid cysts are frequently observed as incidental findings at autopsy at rates much higher than the reported local morbidity (Schantz et al 1982). In other instances, the severity and nature of signs and symptoms produced by larval forms of the cestode are extremely variable and never pathogenic. The particular manifestations are determined by the site of localization and size of cysts (Schantz 1982).

The incubation period of human hydatid infection is highly variable and often prolonged for several years. It has been suggested that human E. granulosus infection is most often acquired in childhood (Schwabe and Abou-Daoud, 1961). Nevertheless, age-specific incidence and prevalence rates increase gradually with age, suggesting that new infections continue to occur throughout human lifespan (Schantz 1982). Moreover, surgical incidence rates among persons aged twenty five years or older was shown to decline significantly following successful interruption of parasite transmission in Australia and New Zealand (Beard 1978). These observations suggest that the latent period between infection and diagnosis in many cases may only be a few years.

Hydatid cysts of the liver may become relatively large before producing symptoms because of the large size of the organ and the distensible body area. Signs and symptoms

present may include hepatic enlargement with or without palpable mass in the right upper quadrat, right epigastric pain, nausea, loss of apetite and vomiting. Rupture or leakage of the cysts usually result in acute intermittent allergic manifestations. Urgent complications on initial presentation of patients with hepatic cysts may include traumatic or spontaneous rupture, thoracobilia, biliary fistula and secondary bacterial infection (Schantz, 1982).

Intact hydatid cysts in the lungs may cause no symptoms but leakage or rupture cause chest pain, coughing, dysphenia and hemoptysis (Yamashita et al 1957) cited by Schantz (1982). The first symptoms of cerebral cysts may include raised intracranial pressure or focal epilepsy, while kidney cysts may be manifested by loin pain or hematuria. Bone cysts are often asymptomatic until pathological fractures occur and because of a resemblance, they are often misdiagnosed as tuberculous lesions (Booz 1972). The prognosis for hydatidosis of the bone is poor and amputation of the affected limb is frequently necessary.

Cysts in the heart are especially dangerous because they may rupture and cause systemic dissemination of the prostoscolices, anaphylaxis or cardiac tamponade (Schantz 1982).

1.3.1 Cyst localization and pathology

In most published reports of surgical series, the most frequently reported site of localization of hydatid cysts is the liver (50 - 70%), followed by the lungs (20 - 30%) and less frequently the spleen, kidney, bones, central nervous system and elsewhere (Schantz 1982).

The relative frequency of specific sites of localization often differs from one country to another because of differences in the availability and use of diagnostic technology. Pulmonary cysts for example are diagnosed relatively more often in areas where mass miniature radiography is employed in tuberculosis control programmes, while cysts in multiple locations are detected more frequently where liverscans are performed routinely on patients with pulmonary cysts (Schantz et al 1976).

Larvae of the northern sylvatic strain for example localize predominantly in the lungs indicating that cestode strain differences may also affect sites of localization (Wilson et al 1968).

The slowly growing hydatid cyst is well tolerated by the human host until it becomes large enough to cause disfunction. Cyst rupture often resulting from trauma may cause a variety of immediate and delayed sequelae. Mild to severe anaphylactoid reactions and occasionally death may follow the sudden release of cyst fluid (reviewed by Schantz 1982). These shock reactions are largely a result of allergic reactions in the sensitized host.

1.3.2 Host response and survival of hydatid cysts in immunocompetent hosts

The prolonged survival of hydatid cysts in immunocompetent host suggests an interference with immune function of the host. Capron et al (1968) demonstrated that the parasite is able to synthesize and coat itself with host antigenic determinants. Based on this, Varela-Diaz et al (1974) suggested that the

incorporation or induction of host antigens in recipient animals must occur sufficiently rapidly to avoid immunological recognition by the host. The authors proposed a structural antigenic conformation of the metacestode wall which is favourable for cyst survival and further suggested production of low antigenic stimulus by healthy hydatid cysts which is insufficient to induce host response adverse to their survival. Studies by Kwa and Liew (1978) demonstrated that Taenia taeniaeformis larvae coated with normal or immune sera were able to shed surface bound antibodies at room temperature and survived better than the uncoated controls when transplanted into normal hosts. This suggested that the immunoglobulins on the surface may protect the parasites from attack by host antibodies in vivo. Such a situation can be envisaged to occur in E. granulosus infections.

Gershon and Kondo (1971) demonstrated that adoptive transfer of spleen cells from mice made specifically tolerant to sheep red blood cells (SRBC), prevents co-operation of normal T- and B- cells in syngeneic recipients leading to loss of response to subsequent immunization with sheep red blood cells. In a separate study, Allan et al (1981) demonstrated that adoptive transfer of mesenteric lymph node cells from E. granulosus equinus infected mice into syngeneic normal responder recipient mice leads to depletion of T-cell population and suppression of the antibody response of the recipient mice to sheep red blood cells. When the donor mice were made specifically tolerant to sheep red blood cells but were not infected with E. granulosus, the authors demonstrated non-specific tolerance sufficiently solid to interfere

substantially with the co-operation of T- and B- cells within the syngeneic recipient during their response to simultaneous immunization with sheep red blood cells (a T and B cell stimulator). In a separate study, Yusuf et al (1975) reported an inverse relationship between the indirect haemagglutination antibody titres and lymphocyte transformation activity in 10 seropositive hydatid infected humans. Ali-Khan (1978 a&b) demonstrated a reduction in T-cells in the cortex and peri-arterial areas of lymph nodes and spleens of swiss mice 13 months after infection with E. granulosus. Similar histochemical changes were described in chronic infections with E. granulosus (Ali-Khan 1978c). These results suggested that chronic infection with E. granulosus over a period of 13 months altered the composition of T-cell population in favour of non-specific T-cell suppressor activity. This resultant destruction of the parasite reactive T-cells may enable the parasite to subvert immune system of the host.

Dixon et al (1978) reported blastic stimulation of Balb/c mouse lymphocytes by living protoscolices after 72 hours incubation. This mitogenic response was found to be T-cell dependent and was suggested to be an in vitro representation of the immunosuppressive mechanisms favouring survival of the parasite in the host (Harrison and Parkhouse 1985). Low specific anti-hydatid antibody titres and negative intradermal and leucocyte migration tests were demonstrated in sheep with high hydatid antigen load (Judson et al 1985).

It has also been suggested that the larval cestodes probably

produce spontaneous agglutinins consisting of macromolecular polysulphated polysaccharide chains which are able to interact with the host's complement fixation activity leading to prolonged survival of the parasite (Orihara 1973, Perricone et al 1980).

1.4.0 A review of hydatid disease in Kenya

For many years, hydatidosis was thought to occur mainly among the Turkana, the Suk in West Pokot bordering Turkana, the Samburu, the Shangilla and Rendille near Lake Turkana. However, it has also been found in other areas of the country (Eugster, 1978, Macpherson 1981, Okello and Kyobe 1981).

The earliest reported systematic study of hydatidosis in Kenya was carried out by Wray (1958) who reviewed earlier reports concerning the disease. He reported that 117 cases were noticed during the period 1952 - 1955 of whom more than half were Turkana tribesmen. Another 64 cases were recorded at Kitale Hospital during the period 1957 - 1961.

Table 1 : Incidence of human hydatidosis in Maasailand (1952 - 1955) according to Wray 1958

Year	Kajiado	Narok
1952	1	0
1953	2	3
1954	3	3
1955	6	3
Total	12	9

Schwabe (1969) estimated the incidence of hydatid disease in Turkana District to be 40 per 100,000 population per year. Elsewhere in Kenya, hydatid cases were relatively few and 55 cases only were seen in the four year period between 1958 to 1961 on referral to Kenyatta National Hospital (Nelson and Rausch 1963).

Roettcher (1973) operated on 163 hydatid patients in 8 hospitals between January 1968 to 1972. He reported that the pastoral tribes of Kenya (Maasai and Turkana) were more affected by hydatid disease than the other tribes of Kenya that were mainly engaged in agricultural activities. Over a five year period of study in Turkana District (1971 - 1975), O'Leary (1976) reported that the incidence of human hydatid disease was 96 per 100,000 population per year. The results of this study are summarized on Table 2.

Table 2 : Cases of hydatid disease in Turkana District (1971 - 1975) according to O'Leary (1976).

Place	1971	1972	1973	1974	1975	Total
Lodwar Hospital	40	61	63	63	58	286
Kakuma Hospital	44	56	75	68	80	323
Lokari Hospital	9	4	12	12	25	62
Lokitaung Hospital	(All referred to Lodwar Hospital)	4	4	3	2	13
Lorogumu H/C	3	2	2	1	5	13
Lokichor H/C	-	-	-	-	2	2
Cases referred out of District through MOH (Not included above)	5	21	24	21	19	90
Grand Total	101	148	180	169	198	789

Key

H/C - Health Centre

MOH - Medical Officer of Health.

During this study, O'Leary reported different prevalence rates of human hydatidosis between various sexes and age groups. Of the 789 cases recorded (Table 2), 457 (58%) were aged over 15 years while 332 (42%) were under 15 years. 183 (55%) of the children were females while 149 (45%) were males. 293 (64%) of the adults were females and 164 (36%) were males. The site of localization of cysts were, (liver 66%), lungs (less than 10%) and 24% in other anatomic locations. French (1980) analysed the age and sex distribution of 355 cases in Turkana District and reported a marked increase in incidence in females between 20 and 35 years (the child bearing age) (Table 3).

French and Nelson 1982 reported the highest incidence of human hydatid disease in the North West and North Eastern parts of Turkana District. The central part had intermediate while the south had the lowest incidence. The range of incidence expressed as cases per 100,000 population per annum ranged over 10 fold from 17 in the South to 198 in the North West.

Table 3 : Number of patients with hydatid disease by age groups and the male to female ratio (French 1980)

Age groups	<5	5-14	15-24	24-34	35-45	45+	Total
Total No. of patients	7	99	65	116	45	23	355
Males	2	42	24	28	11	9	116
Females	5	57	41	83	34	14	239
Male to female ratio	1:2.50	1:1.36	1:1.71	1:3.14	1:3.09	1:1.56	1:2.06

A number of attempts have been made to explain the high incidence of human hydatidosis among the Turkana people. O'Leary (1976) suggested that the great intimacy between the Turkana and their working dogs is significant in the transmission of the disease. Nelson and Rausch (1963) noted that the Turkana dogs are allowed to lick eating utensils and to clean children after defaecating or vomiting. Mann (1974) suggested that the Turkana tradition of placing human corpses in open burial sites where they can be scavenged on by domestic dogs and wild carnivores creates a unique situation where man, other than domestic dog or wild animals is the major intermediate host of E. granulosus. He noted that wild animals including scavengers, birds of prey and coprophagal arthropods share waterholes with humans and that both soil and water are contaminated by faeces of man, animals and birds thus leading to enhanced transmission of the disease. Because of the higher incidence of hydatidosis among the Turkana as compared to other nomadic tribes, like the Maasai, Suk and Samburu, also living off animals with high hydatid infection rate and with similar attitude towards dogs and dead bodies, the author suggested that Turkana area is a high endemic spill over to the neighbouring Sudan, Ugandan and Ethiopian countries also inhabited by other nomadic tribes.

1.5.0 Diagnosis of human hydatid disease

Several techniques have been used in serological diagnosis of human hydatidosis. A review by Kagan (1968) gave a comprehensive summary of these methodologies. These include

indirect haemagglutination (IHA) which uses whole hydatid cyst fluid antigens, latex agglutination (LA) and complement fixation (CFT) both using whole sera from infected individuals, indirect immunofluorescence, (IIF) and counter-Immunoelectrophoresis (CIE) both using whole or fractionated particles of protoscolices, radioimmunoassay (RIA) based on partially purified hydatid antigens and immunoelectrophoresis (IEP) based on 'arc 5' antigen (the major antigen of hydatid cyst fluid). The enzyme linked Immunosorbent Assay (ELISA), first utilized by Faraj et al (1975) in the diagnosis of hydatid disease uses either purified 'arc 5' antigen or crude hydatid cyst fluid.

The IEP test was first described by Capron et al (1967) and was until recently the only immunodiagnostic technique for human hydatidosis with which no false positive results had been reported (Varela-Diaz et al 1975 a&b). However, later reports by Varela-Diaz et al (1978) indicated that human sera from one case of polycystic hydatidosis due to Echinococcus vogeli and from a case of multiple myeloma were positive in IEP test for hydatidosis based on 'arc 5' antigen. The occurrence of false positive results was further confirmed by Yong and Heath (1979), and later by Okello and Chemtai (1981).

Non serological diagnosis has been done using such techniques as radiography, radio-isotope scanning, ultrasonic echotopography and computerised axial topography.

1.6.0 Treatment of human hydatid disease

Both surgery and chemotherapy have been used in treatment of human hydatidosis in Kenya. However, because

of lack of adequate surgical facilities, late diagnosis and other complications such as occurence of multiple cysts in the thorax and abdomen, surgical treatment is possible in less than 50% of the cases in Turkana District. The recurrence rate is high (20%) and most recurrent cases are inoperable. (French 1984).

The benzimidazole carbamate group of drugs including flubendazole, mebendazole and albendazole, have been used in treatment of human hydatidosis (Schantz et al 1982, Roche et al 1982). Little success has been realized with use of mebendazole and flubendazole.

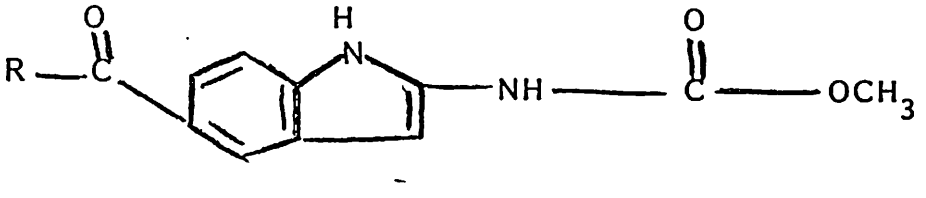
1.6.1 Mebendazole in treatment of human hydatid disease

Mebendazole (5-benzoyl-2-methoxycarbonyl amino benzimidazole) (fig. 4) has also been used in treatment of Echinococcosis in animals (Heath and Lawrence 1978). It irreversibly inhibits glucose uptake by the parasites leading to immobilization and subsequent expulsion of the parasites from the gut.

Earlier chemotherapeutic use of mebendazole against larval E. granulosus in humans was carried out by Bekhti et al (1974). Okello and Chemtai (1981) used mebendazole at 40mg/kg body weight daily for four weeks and reported no reduction in cyst size and numbers during and after chemotherapy. Reports by Okello (1984) indicated that mebendazole is effective against human hydatidosis if used in doses leading to serum drug levels of 100ng/ml in patients who eat margarine in their food routinely. It appears therefore that mebendazole would be of little use in cases where patients cannot afford to eat fatty foods.

Side effects of mebendazole include occasional diarrhoea and abdominal pains together with mild bone marrow depression. Okello (1984) postulated that use of mebendazole may lead to release of massive amounts of parasite antigens into circulation which may result in immune complex nephritis from depositions in the kidney.

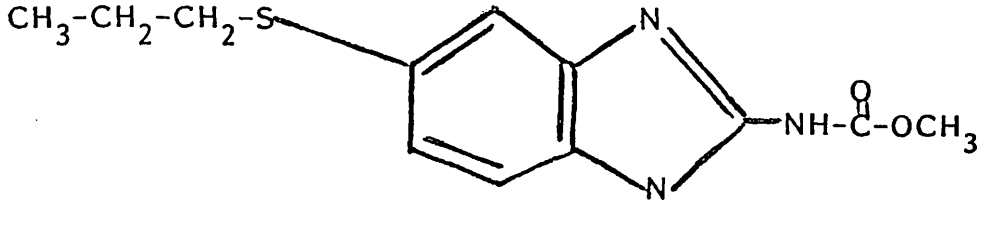
Figure 5 : Structure of mebendazole



1.6.2 Albendazole in treatment of human hydatid disease

Albendazole, $C_{12}H_{15}N_3O_2S$ (fig. 5) which has also been used in treatment of infections caused by Askaris lumbricoides, Ancylostoma duodenale, Necator americanus, Trichuris trichiura, Enterobius vermicularis, Strongyloides stercolaris, Taenia solium, Taenia saginata, Hyemenolepis nana and Hyemenolepis diminuta has shown promising results in experimental treatment of human hydatidosis (Saimot et al 1983, Okello 1984 & 1986, Pawlowski 1985). It is abenzimidazole derivative with relative molecular weight of 265 daltons.

Figure 6 : Structure of albendazole



1.6.3 Mechanism of action of albendazole

Albendazole interferes with normal metabolism of the parasites by selectively blocking glucose uptake by intestinal helminths and their tissue dwelling larvae (Smith Kline and French Laboratories 1982). This inhibition leads to depletion of glycogen storage within the parasite, resulting in reduced formation of adenosine triphosphate (ATP) which is essential for reproduction and survival of the parasites. This leads to death of the parasites.

1.6.4 Albendazole dosing in treatment of human hydatid disease

Patients with hydatid disease require 10 mg/kg weight divided in two equal doses daily for eight to twelve weeks. Larger doses such as 15 - 20 mg/kg body weight may occasionally be necessary especially for hepatic hydatids (Morris et al 1983, Okello 1984). Albendazole should also be administered to patients with hydatidosis requiring surgery both pre- and post operatively to avoid recurrence of infection. (Smith Kline and French laboratories 1982).

1.6.5 Clinical pharmacology of albendazole

Following an oral dose, albendazole is rapidly absorbed and metabolised in the liver to the active sulfoxide metabolite which then diffuses into the intestines and tissues where it acts on the parasites. Detectable plasma levels of the sulfoxide metabolite are found in patients within half an hour after the initial dosing. The maximum mean plasma level is reached in about three hours (Smith Kline and French Laboratories 1982).

The serum half life of the sulfoxide is eight to nine hours and serum levels are detectable upto seventy two hours after the initial dosing (Smith Kline and French Laboratories 1982). The concentration of the unchanged albendazole is low in both plasma and in the gastro-intestinal fluid.

The drug penetrates tissue well with high concentrations found in the liver and kidney and is excreted almost entirely via the kidneys (Smith Kline and French Laboratories 1982). Over 95% of the metabolite can be recovered in urine within twenty four hours.

1.6.6 Adverse effects of albendazole

Albendazole appears to be a safe drug with only minor infrequent side effects. These include dryness of the mouth, mild epigastric discomfort, nausea, weakness and diarrhoea. These side effects have not been proved to be related definitely to the drug since intestinal helminthiasis itself may be associated with these same symptoms.

Two cases of slight, transient neutropenia and two patients out of many hundreds of cases studied showed transient elevation of the serum enzymes SGOT and SGPT (Garcia 1981, Firth 1983).

1.7.0 AIMS AND OBJECTIVES OF THE STUDY

In view of the health and economic importance of hydatidosis, there is need to fully understand the course of the disease in individuals undergoing various types of treatment such as surgery and chemotherapy. Relatively little data is available on the *in vivo* effects of albendazole in patients undergoing treatment, apart from the effects on the parasite itself.

The objectives of this study were:

1. To assess total serum protein concentrations in hospitalized patients on albendazole therapy through tretment to discharge.

2. To separate and semiquantitate individual serum proteins by cellulose acetate membrane and SDS polyacrylamide gel electrophoresis and to determine changes in IgG levels throughout treatment by ion exchange chromatography in these patients.

These findings would assist in understanding some of the multiple effects of albendazole therapy.

CHAPTER 2

MATERIALS AND METHODS

2.1.0 Patients

Sixteen patients were included in the study, twelve of whom were from Turkana District of Kenya. Only clinically proven cases of human hydatidosis and who were at the time not suffering from any other parasitic infection as determined by stool, urine and bloodsmear examinations were included in the study. All patients had hepatic cysts and received albendazole therapy in Ward 28 of Kenyatta National Hospital, Nairobi, Kenya over a period of four to seven months.

2.2.0 The control group

The control group comprised six volunteer staff and post-graduate students in the Departments of Medicine and Biochemistry of the University of Nairobi. All were healthy individuals who had no history of any ailments for at least four months prior to initiation of the study.

2.3.0 Hydatid cyst fluid (HCF)

Both pulmonary and hepatic hydatid cyst fluids were donated by the Immunology Unit of Kenyatta National Hospital, Nairobi in lyophilized form.

2.4.0 Albendazole dosage used in this study

Patients were put on Albendazole, 20 mg per kilogram body weight divided in two equal doses daily.

2.5.0 Bleeding and preparation of blood samples for haematological analysis

Three drops of sodium EDTA (anticoagulant) were put in a universal sample bottle and air dried. A gauge (19x1½") needle fitted to a 10 ml syringe were used to obtain 2 mls of peripheral blood from both patients and controls by venipuncture between 8.15 - 9.30 a.m. The blood was transferred into sample bottles and gently agitated to ensure even mixing with the anticoagulant.

2.5.1 Haematological analysis

Routine haematological examinations were carried out once in one to three weeks. These included an estimation of haemoglobin, total red blood cell counts, packed cell volumes absolute and differential leucocyte counts. Other than the differential leucocyte counts, all the above haematological parameters were determined by feeding 1.5 mls of blood containing anticoagulant into a Coulter counter model S_5 and the results were automatically determined and printed out.

2.5.2 Differential leucocyte counting

A drop of blood was put on a microscope slide resting horizontally at one third the distance from one end of the slide. A second slide was rested on the first one at forty five degrees to the later and touching the blood drop on one side. Blood was allowed to spread along the contacting edge of the second slide by adhesion. The second slide was then sheared carefully towards the distal end of the horizontal one in order

to make a blood smear (a thin film of blood cells). The slide containing the blood smear was placed on a slide rack, to air dry and thereafter fixed in methanol for five minutes. The slide was removed and flooded with Jenner stain for cytoplasmic (RNA) staining made by mixing 1:4 (v/v) stock Jenner solution to 0.1 M phosphate buffer pH 6.8. Staining was done for five minutes after which the stain was poured off and replaced with Giemsa solution for nuclear (DNA) staining made by mixing 1:9 (v/v) stock Giemsa solution to 0.1 M phosphate buffer pH 6.8 and further stained for ten minutes. The slide was washed with phosphate buffer (0.1 M pH 6.8) and air dried. Differential leucocyte counts were performed using oil immersion lens at x 1000 magnification in an ordinary light microscope (Leitz).

Five different slides were made and counted for each test sample to obtain mean leucocyte counts.

2.5.3 Preparation of serum

Between 6 and 10 mls of peripheral blood were drawn from patients and controls between 8.15 and 9.30 a.m. The blood was allowed to stand at room temperature for three hours in Universal sample bottles (without anticoagulant) to allow for clot formation.

A spatula was used to separate the clot from glass wall of sample bottle. The clot was kept at 4° overnight. The clear serum was aspirated and centrifuges for twenty five minutes at x 1000 g in the cold (at 4°C) using a bench centrifuge. The supernant (clear serum) was aspirated using a sterilized pasteur pipette and was immediately decomplemented

by heating at 56°C for thirty minutes in water bath.

Serum samples were taken for various tests while unused serum was kept at -20°C with a drop of 0.1% (w/v) sodium azide as a preservative for every ml of serum. Serum was prepared from patients once in every four weeks of treatment.

2.5.4 Determination of serum protein concentrations

Serum protein concentrations were determined using the method described by Lowry et al (1951). A standard protein curve was constructed using BSA fraction V (0 - 100 mg) obtained from Sigma Chemical Company, U.S.A. Optical densities were read at 750 nm using PYE Unicam SP 1800 Spectrophotometer. All experiments were performed in triplicates to obtain average optical density values.

2.6.0 SDS Polyacrylamide gel electrophoresis (SDS PAGE)

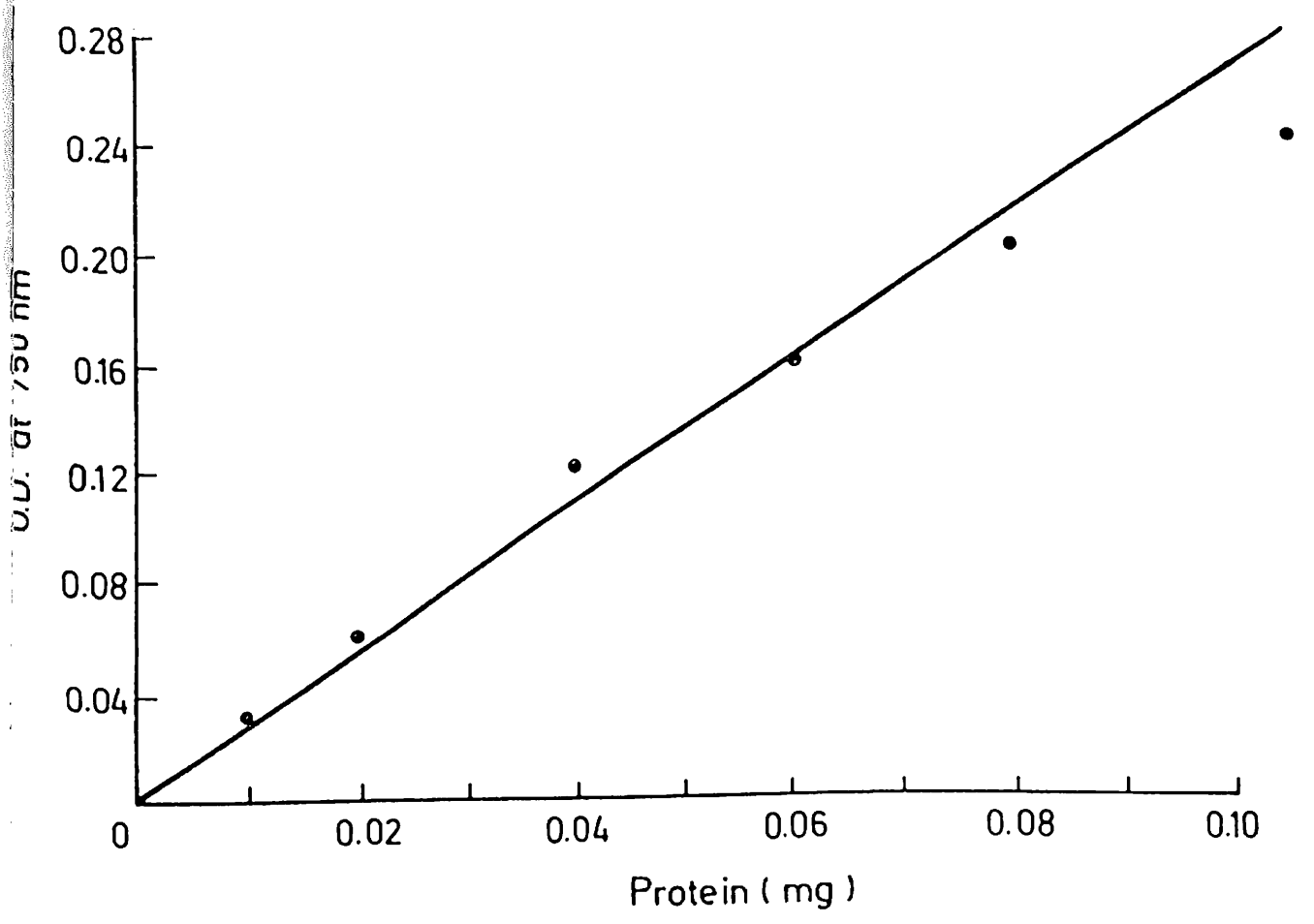
Electrophoresis under reducing conditions was performed on a vertical electrophoresis unit using Tris-buffered discontinuous system described by Laemmli (1970). Gradient running gels of 1.5 mm thickness containing 5 - 15% (w/v) acrylamide and 0.13 - 0.4% (w/v) bis-acrylamide were cast followed by a stacking gel of 3% acrylamide and 0.08% bis-acrylamide. 50 µls each of ammonium persulphate (APS) were separately added to initiate the formation of free radicals and was followed in each case by 10 µls of TEMED as a catalyst to enhance polymerization in preparing 5 and 15% gels. 20 µls and 9 µls respectively of APS and TEMED were added in preparing stacking gels. 1.5 molar Tris/Hcl buffer pH 8.3 was used as resolving buffer.

Samples for electrophoresis were boiled for three minutes in Laemmli sample buffer containing 0.0625 M Tris-HCl pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol and 0.01% (w/v) bromophenol blue. A total of five microlitres of 50% (v/v) serum sample : sample buffer were loaded into each sample well. A stacking voltage was set at 50 volts for the first thirty minutes of the run. Electrophoresis was then performed at 150 volts for three and a half hours to four hours until the tracking dye [0.01% (w/v)] bromophenol blue had reached the bottom end of the slab gel. After electrophoresis, gels were stained for polypeptides with 0.05% (w/v) Coomassie brilliant blue (6 grams in 92:400:508 mls of glacial acetic acid:methanol:distilled water). Staining was done overnight following which the gel slabs were destained in five changes of the destainer (92:500:408 mls of glacial acetic acid: methanol:distilled water). The gels were then stored in 7% (v/v) glacial acetic acid. The same procedure of electrophoresis, staining, destaining and storage was used for hydatid cyst fluid except that twenty microlitres of 50% (v/v) of sample: sample buffer was loaded into each sample well due to its low protein content.

Table 4 : Standard proteins used and their respective relative molecular weight (Mr)

Marker	Mr.
Phosphorylase b	94,000
Bovine Serum Albumin	67,000
Ovalbumin	43,000
Carbonic Anhydrase	30,000
Soya bean trypsin ihibitor	20,000
β-Lactalbumin	14,000

Figure 7 : The Lowry protein standard curve


0.28
0.24
0.20
0.16
0.12
0.08
0.04
0
0.02
0.04
0.06
0.08
0.10
Protein (mg)

2.6.1 Cellulose acetate membrane electrophoresis

This technique was used to determine the relative amounts of the five major serum proteins i.e. albumin, α_1, α_2, β- and γ-globulins.

Five microlitres of serum sample was applied in thin streaks, one centimetre long on cellulose acetate membrane and allowed to air dry. Upto four membranes were put on the same electrophoresis apparatus with filter paper bridges to complete the circuit.

Electrophoresis was performed for two and a half hours at eight volts per centimetre length of the membrane using veronal buffer pH 8.6, prepared by dissolving 10.3 grams of sodium diethyl barbiturate in a total volume of one litre using glass distilled water. The membrane strips were removed with forceps and dipped in 0.2% (w/v) Ponceau S stain in 3% (w/v) trichloro-acetic acid (TCA) and allowed to stain for ten minutes. Excess stain was removed by shaking the membrane strips in five changes of 5% (v/v) glacial acetic acid. The strips were then dried in the oven at 90°C for ten minutes.

2.7.0 Ion exchange chromatography

Anion exchange chromatography was performed using DEAE-cellulose matrix No.8382, of capacity 0.92 milli-equivalents per gram.

2.7.1 Preparation of adsorbent (DEAE- cellulose)

Ten grams of DEAE-cellulose powder was suspended in

excess amount of distilled water. The larger particles were allowed to settle by gravity and fine particles were thereafter removed by repeatedly decanting the supernatant. The resin was allowed to remain in 0.3 M potassium dihydrogen phosphate (KH_2PO_4) overnight then transferred to a funnel and most of the KH_2PO_4 aspirated through a filter using a suction pump. The cake was resuspended in 0.3 M KH_2PO_4 and aspirated again then washed five times with distilled water. It was then transferred into a beaker and suspended in 500 mls of 0.5 N sodium hydroxide (NaOH) and allowed to stand for four hours. Sodium hydroxide was aspirated through a funnel and the resin was further washed with 500 mls of 0.5 N sodium hydroxide. The resin was thereafter allowed to stand in a beaker with 500 mls of 95% (v/v) ethanol for two hours, then washed once again with 500 mls of 95% (v/v) ethanol, once with 800 mls of 0.05 N sodium hydroxide and eight times with 800 mls of distilled water. The pH was set using equilibration buffer (0.01 M phosphate buffer pH 8.0).

2.7.2 Chromatographic separation of serum proteins on DEAE-c column

Elution was performed as outlined by Fahey and Terry (1967). The cellulose matrix (prepared as indicated in section 2.7.1) was washed and equilibrated with starting buffer (0.01 M phosphate buffer pH 8.0). The equilibrated resin was packed in a 1 x 25 cm Pharmacia column and further washed with five hundred millilitres of starting buffer.

To 0.6 mls of serum sample, 0.2 mls each of starting buffer and 50% (v/v) glycerol were added, mixed, then carefully loaded onto the column using a sterile pasteur pipette. 80 mls of starting and limiting buffer (0.01 and 0.3 M phosphate buffers of pH 8.0 respectively) were added into the first and second chambers of a gradient mixer GM1 (Pharmacia). A thin hollow plastic tube was used to connect the first chamber to the top of the column and a fraction collector to the lower end of the column. The flow rate was set at 30 mls per hour and the adsorbed protein was eluted off the column in batches of 1.2 mls into each test tube using a gradient of 0.01 to 0.3 M phosphate buffer pH 8.0 with constant stirring in the first chamber of the mixer, directly connected to the top of the column.

Elution was carried out in the cold (at $4^{o}C$) and optical densities of the eluate were read at room temperature using PYE Unicam SP 1800 spectrophotometer at λ 280 nm.

2.7.3 Determination of cyst size and locations

Anatomical location of cysts were determined by ultrasonic scanning. Ultrasonography was also used periodically throughout the study to determine the changes in cyst size as an index of recovery in response to Albendazole therapy.

CHAPTER 3

RESULTS

3.1.0 Ultrasonic scanning and Urinalysis

Repeated determination of cyst size by ultrasoniography indicated that Albendazole gradually reduces cyst size during treatment. All the 12 patients had normal urine with no proteins, sugars or casts.

3.1.1 Serum protein concentrations

Using methods of Lowry et al (1951), it was demonstrated that total serum proteins were abnormally low on admission of hydatid patients. Results however indicated that Albendazole restores total serum protein concentrations through progressive increase during therapy. These results are provided on Table 5. Patient 1 (800740), serum protein concentration 48 mg/ml was discharged soon after the first blood sample was drawn hence no further follow up was made. It was however shown that her total serum protein concentration at discharge (48.0mg/ml) was nearly as high as those observed in controls. Patient 3 (815137) had serum protein concentration amounting to 34.5 and 37.0 mg/ml respectively at the first and second bleeding but thereafter died before completion of therapy. Patients 15 and 16 (776969) and 828760 respectively) were transferred to Optical Ward for retro-orbital operation and were therefore not available for further study. Their serum protein concentrations were 39.8 and 43.2 mg/ml respectively at first and only bleeding. It was noted that in patient 6 (772254) the serum protein concentration did not follow the pattern shown by all the other eleven patients. It was not established why this was so but it is possible that the patient

was suffering from another infection(s) which was not detected at the beginning of therapy. This patient was however treated successfully and discharged with no cysts in the liver. The leucocyte response in this patient was similar to those observed in the other patients treated (see Figure 17 page 060).

Table 5 : Total serum concentration at four weeks intervals over treatment period.

Patient No	Serum protein concentrations mg/ml				
	I	II	III	IV	V
2 (800791)	36.0	41.0	39.0	51.0	53.0
4 (805815)	39.0	36.0	43.0	44.0	46.0
5 (865703)	31.0	32.0	38.0	45.0	49.0
6 (772254)	36.0	34.0	31.0	35.0	36.0
7 (826521)	41.0	43.0	39.6	43.4	47.6
8 (797552)	38.0	41.0	42.6	49.0	58.0
9 (808087)	37.0	34.0	43.0	46.0	51.3
10 (861184)	35.2	38.4	41.0	47.0	49.0
11 (849829)	40.0	39.0	40.2	42.0	43.0
12 (808085	36.0	40.0	42.0	46.4	50.6
13 (811537)	36.9	37.8	40.4	45.4	48.8
14 (808092)	32.7	34.6	36.8	42.7	47.6
Mean	36.6	37.6	39.7	44.7	48.3
SD	2.7	3.3	3.2	3.8	5.1

The study also revealed that the average serum protein concentration in control individuals ranged between 49.4 and 56.1 mg/ml with a standard deviation ranging from 0.25 to 3.14 (Table 5).

Table 6 : Total serum protein concentration of control individuals taken at four week intervals

SUBJECT	Protein concentrations (mg/ml)			Mean	SD
	I	II	III		
A	56.0	56.4	55.8	56.07	0.31
B	54.8	57.0	55.2	55.67	1.17
C	51.2	49.6	52.4	51.07	1.40
D	53.8	54.0	55.6	54.47	0.98
E	48.2	49.2	52.2	49.86	1.69
F	54.8	56.0	55.4	55.40	0.6

3.2.1 SDS Polyacrylamide gel electrophoresis

Electrophoresis of patients sera at different stages of treatment using 5 - 15% gradient running gels showed marked reduction in serum albumin and pre-albumin accompanied by a distinct high density protein in the gamma globulin region of the electropherogram. In most cases, the amount of circulating albumin was nearly as low as one third the normal amount while pre-albumin was eliminated altogether from circulation. Results indicated progressive changes in serum protein electropherograms leading to achievement of normal amounts at the end of therapy (plates 1 & 2).

Electrophoresis of hydatid cyst fluid under the same conditions gave similar electropherograms to those of human sera

but showed an additional protein band between albumin and globulin regions of the electropherogram. Attempts to identify the serum equivalent of this protein band were fruitless and it was suspected to be one of the hydatid antigens (Plate 3).

Plate 1 : 5 - 15% gradient SDS PAGE of sera.

CA - Control A

4P - Patient 4 (805815)

5P - Patient 5 (865703)

6P - Patient 6 (808748)

5D - Patient 5 (865703) after successful treatment

6D - Patient 6 (772254) after successful treatment

8D - Patient 8 (797552) after successful treatment

Alb - Albumin

Pr Pre-albumin

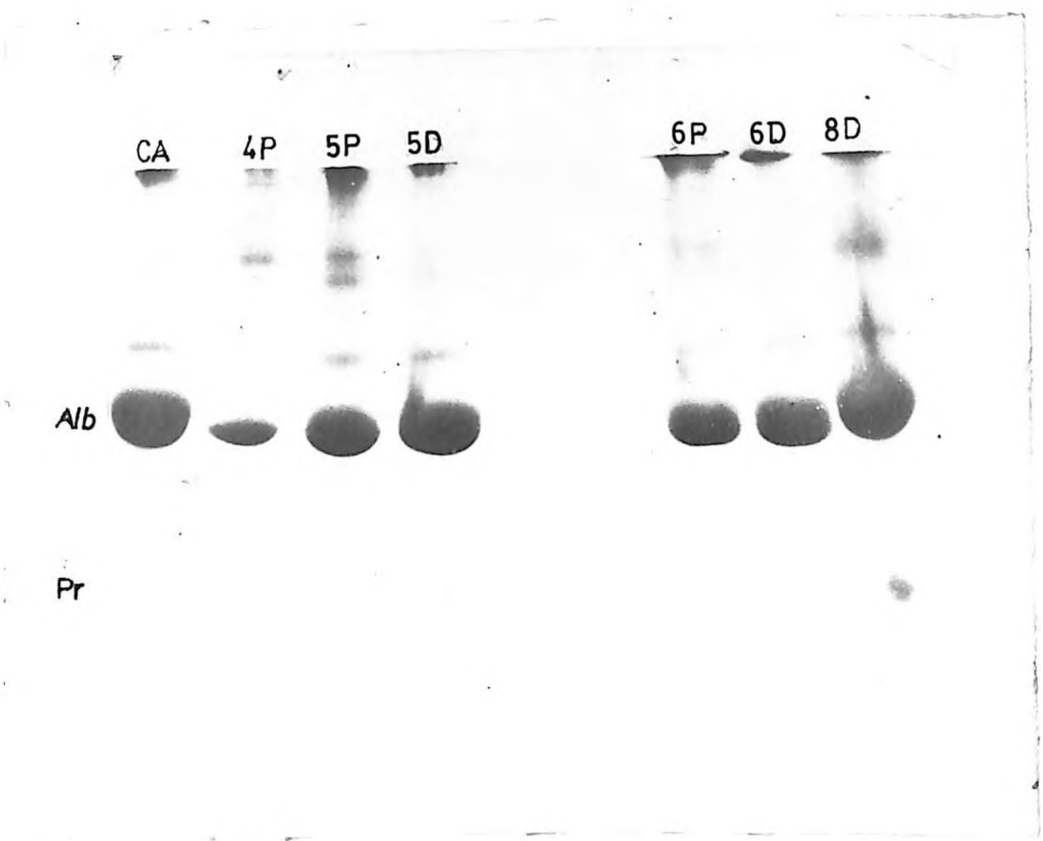

CA
4P
5P
5D
6P
6D
8D
Alb
Pr

Plate 2 : 5 - 15% gradient SDS PAGE of sera

CD - Control

7P - Patient 7 (826521)

9P - Patient 9 (808087)

12P - Patient 12 (808085)

7D' - Patient 7 (826521) after surgery

7D'' - Patient 7 (826521) after successful treatment

12D - Patient 12 (808085) after successful treatment

Alb - Albumin

Pr - Pre-albumin

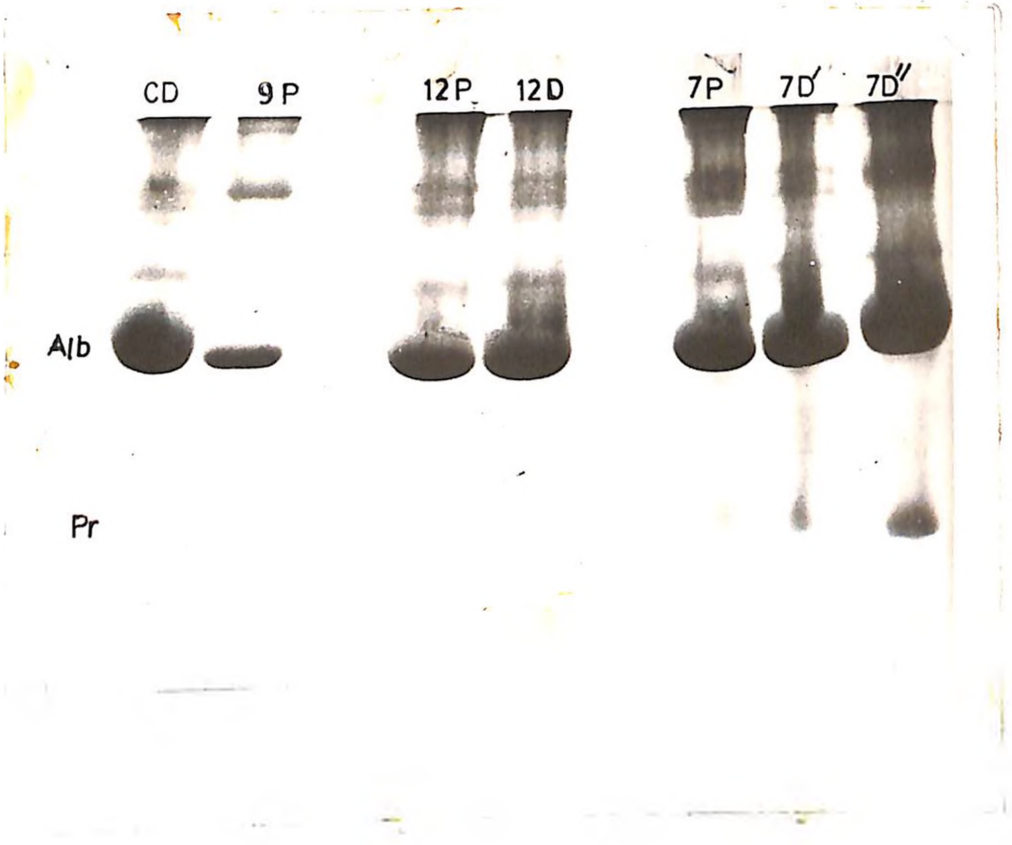

CD
9P
12P
12D
7P
7D'
7D''
Alb
Pr

Plate 3 : 5 - 15% gradient SDS PAGE of sera and hydatid cyst fluid.

CE - Control E

2P - Patient 2 (800791)

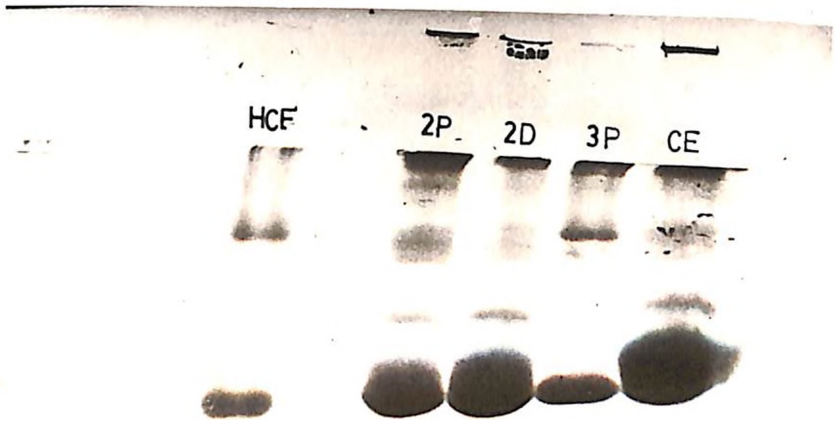
3P - Patient 3 (815137)

2D - Patient 2 (800791) after successful treatment

Alb - Albumin

Pr - Pre-albumin

HCF - Hydatid Cyst Fluid


HCF
2P
2D
3P
CE
Alb
Pr

3.2.2 Cellulose acetate membrane electrophoresis

Electrophoresis on cellulose acetate membranes showed a reduction in serum albumin accompanied with increased amounts of gamma globulins in patients receiving albendazole treatment. The electropherograms appeared normal or nearly so at the end of therapy. Patient 1 who was discharged soon after initiation of the study had more albumin than patient 2 who was at the height of infection then (Plate 4). Similar electropherograms were obtained when serum from patient 1 was run against that from control A (Plate 5). However, patient 2 was shown to have much higher amounts of gamma globulins than control A (Plate 6).

Plate 4 : Cellulose acetate membrane electropherogram of sera obtained from patients 1 and 2.

1 - Patient 1 (800740)

2 - Patient 2 (800791)

Alb - Albumin

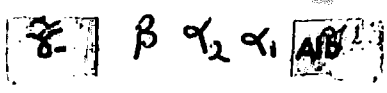
α_1 - Alpha 1 globulin

α_2 - Alpha 2 globulin

β - Beta globulin

γ - Gamma globulin

1



2

Plate 5 : Cellulose acetate membrane electropherogram of of sera obtained from control A and patient.

1 - Patient 1 (800740)

CA - Control A

Alb - Albumin

α_1 - Alpha 1 globulin

α_2 - Alpha 2 globulin

β - Beta globulin

γ - Gamma globulin

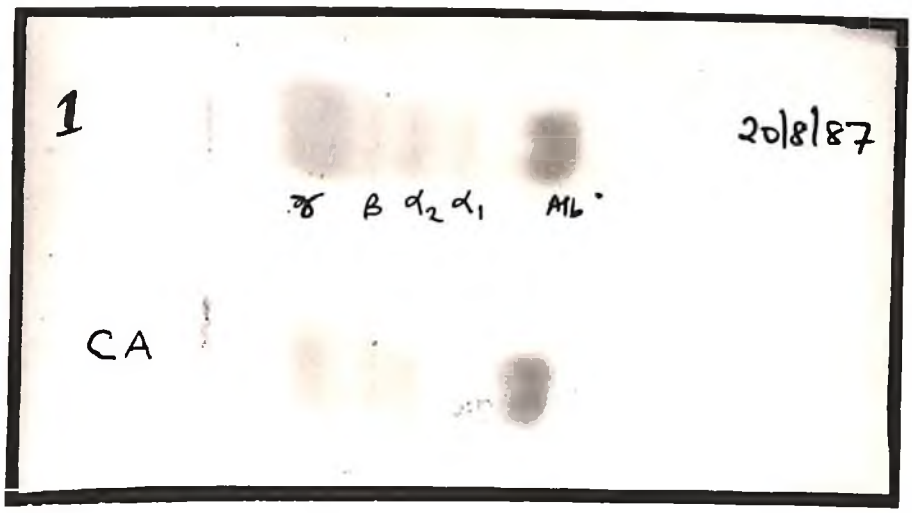

1
20/8/87
γ β α2 α1 ML
CA

Plate 6 : Cellulose acetate membrane electropherogram of sera obtained from Control A and patient 2.

2P - Patient 2 (800791)

CA - Control A

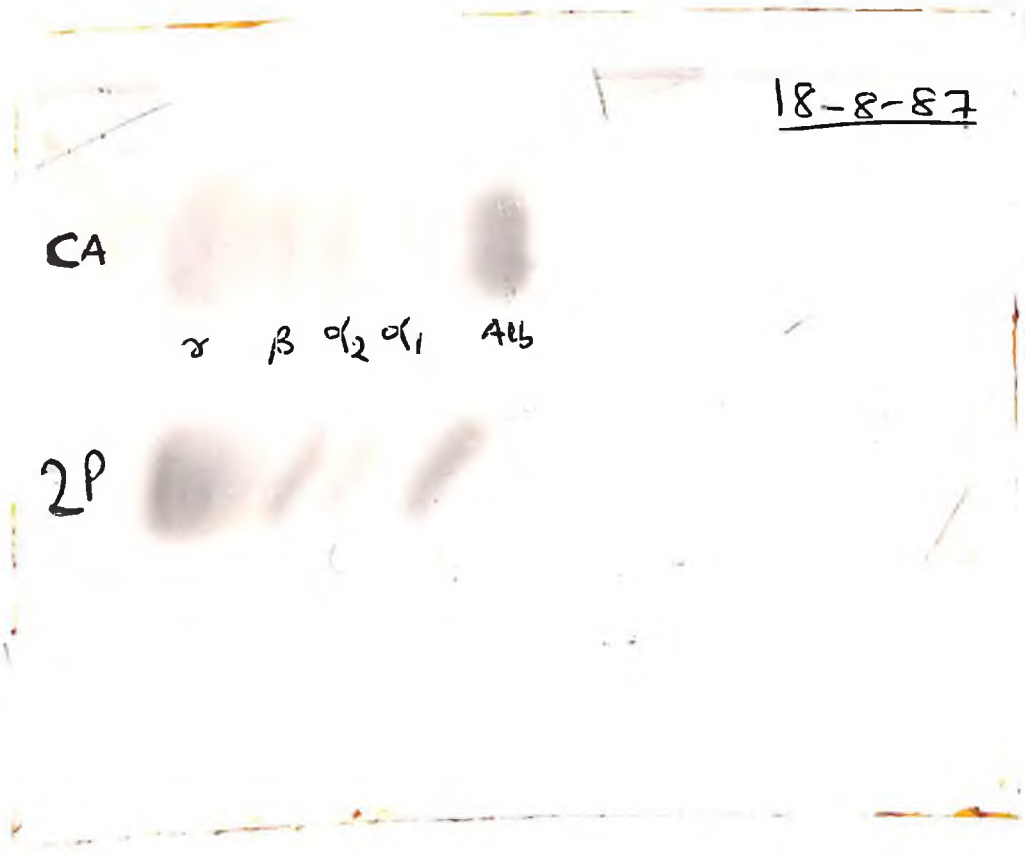
Alb - Albumin

α_1 - Alpha 1 globulin

α_2 - Alpha 2 globulin

β - Beta globulin

γ - Gamma globulin


18-8-87
CA
γ β α2 α1 Alb
2P

3.3.0 Ion exchange chromatography

Chromatographic separation of sera on DEAE-cellulose matrix (anion exchanger) showed 2 to 3 times as much IgG in patients as in controls at the beginning of therapy. This was followed by a progressive decrease which was observed as a progressive reduction in optical density peaks accompanied with lesser areas covered by the IgG portion of sera eluted from the column.

Hydatid cyst fluid was shown to have similar elution profile as human sera but with lower protein concentrations, having mean absorption peaks of 0.87 and 0.23 for IgG and albumin as compared to 4 and 1.6 respectively for normal human serum. This together with the portion of elution area covered by the two proteins demonstrated that normal human serum contains approximately ten times as much IgG and albumin as hydatid cyst fluid. there was no direct relationship between albumin peaks and continued albendazole therapy. These results are on figures (6 to 7). In all cases, the IgG portion of the eluate were diluted between 4 to 16 times to obtain protein concentrations detectable within the optical density range (limits) used in each experiment (0-2.0 OD units).

Table 7 : Changes in IgG peaks observed at four week intervals during Albendazole therapy in 12 patients

Patient No	IgG Peaks in OD units				
	I	II	III	IV	V
2(800791)	10.2	10.1	8.8	7.4	7.0
4(805815)	12.8	11.6	11.0	6.8	6.6
5(865703)	9.8	9.6	9.6	8.0	7.6
6(772254)	10.2	8.8	7.6	7.5	7.5
7(826521)	9.8	9.2	8.6	8.7	7.2
8(797552)	12.0	11.8	11.7	9.2	6.8
9(808087)	11.0	11.2	11.2	10.0	7.0
10(861184)	11.6	11.0	10.2	9.9	6.6
11(849829)	12.6	12.6	12.2	9.6	6.9
12(808085)	9.6	9.5	9.1	7.7	7.2
13(811537)	10.0	9.7	9.1	8.0	7.2
14(808092)	9.9	9.1	9.1	8.1	7.0
Mean	10.79	10.35	9.85	8.41	7.05
SD	1.16	1.24	1.40	1.05	1.31

Figure 8 : Changes in mean IgG peaks (OD units) observed at 4 week intervals during Albendazole therapy in 12 hydatid patients.

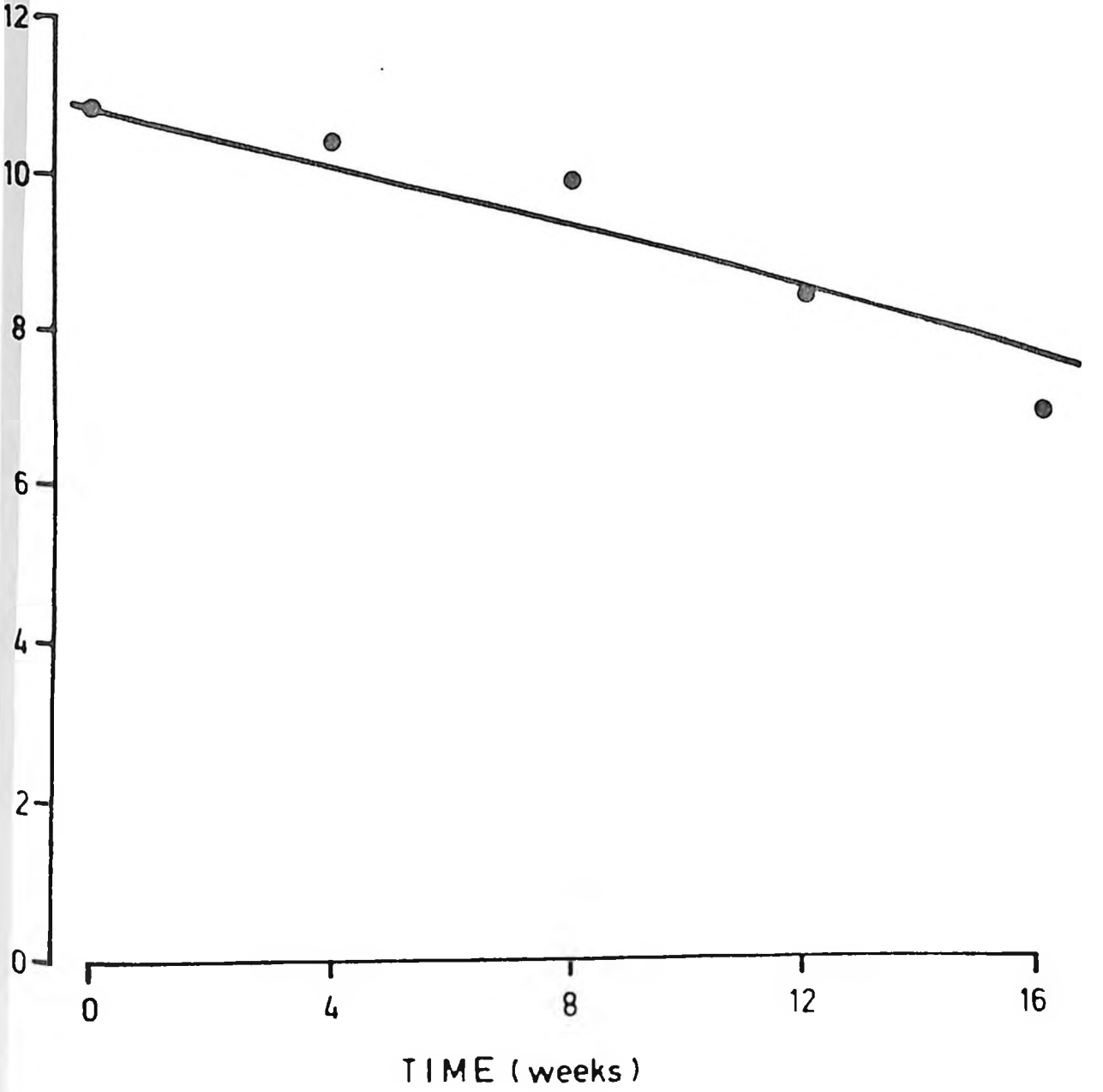

12
10
8
6
4
2
0
0
4
8
12
16
TIME (weeks)

Figure 9 : DAEE-C chromatograms from the mean of Optical densities at λ 280 nm observed in 12 patients at the beginning of therapy.

A - IgG peak

B - Albumin peak

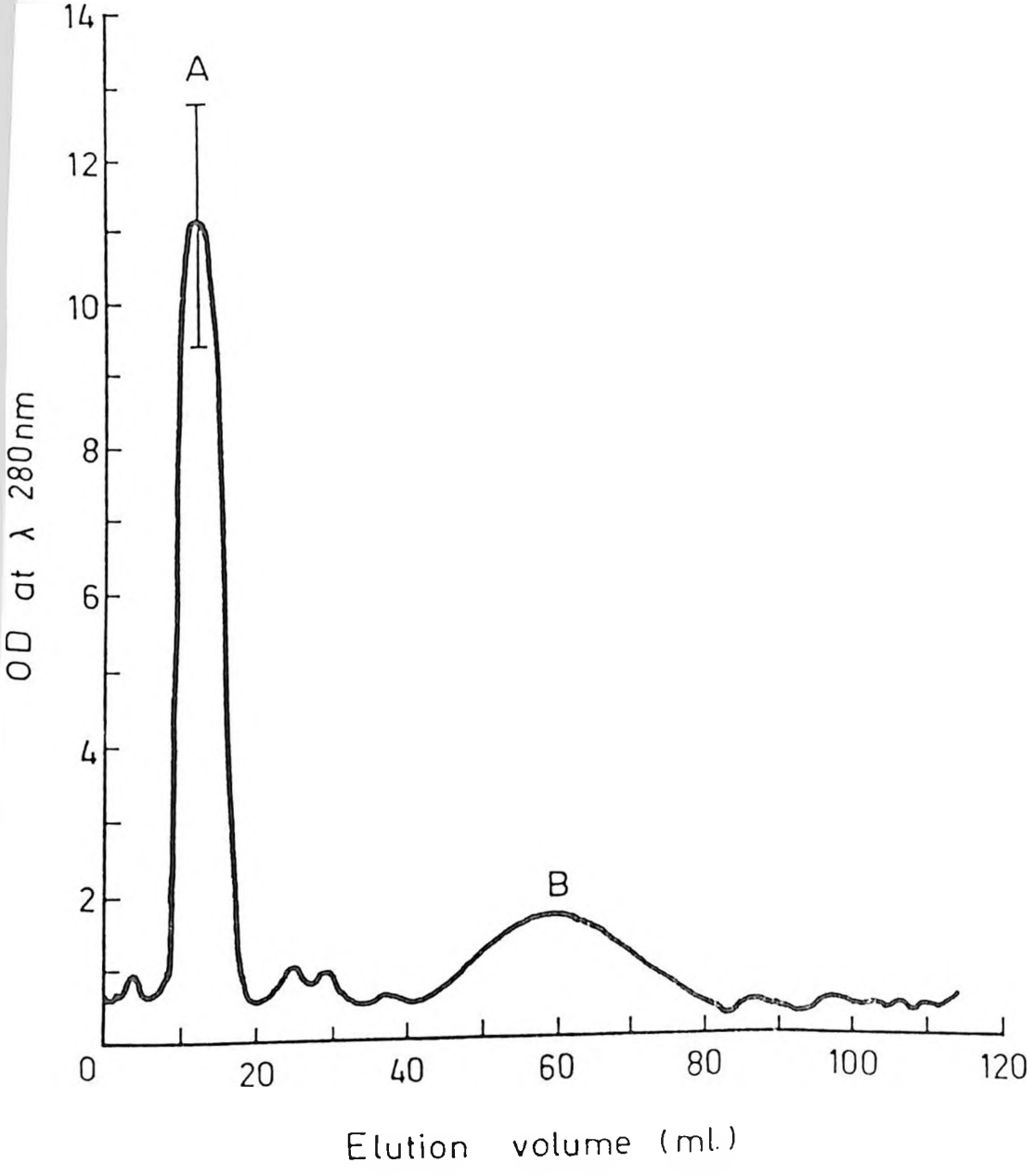
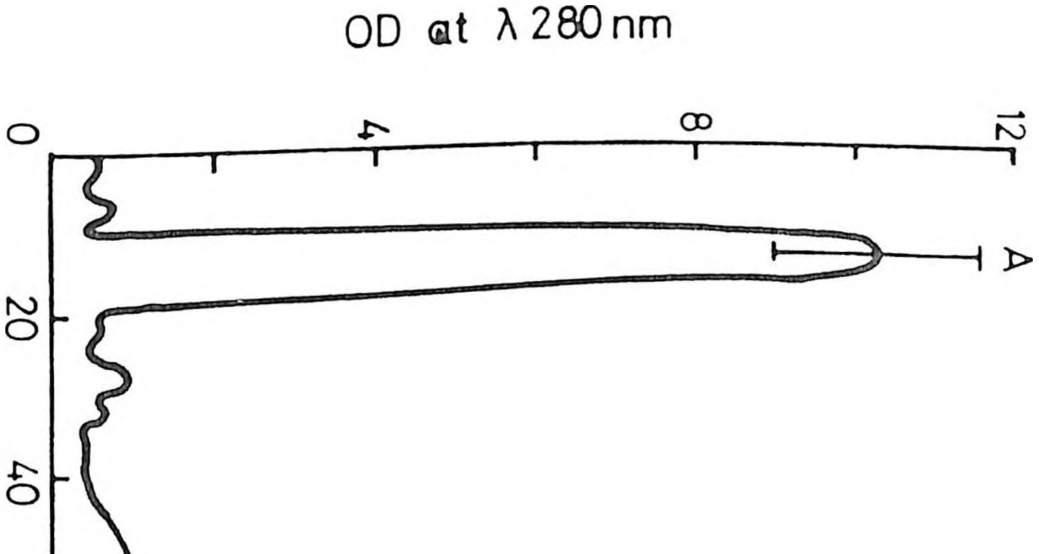

14
12
10
8
6
4
2
0
20
40
60
80
100
120
A
B
OD at λ 280nm
Elution volume (ml.)

Figure 10 : DEAE-c chromatograms from the mean of optical densities at λ 280nm observed in 12 patients at the end of 4 weeks post-admission.

A - IgG peak

B - Albumin peak


OD at λ 280 nm
0
4
8
12
A
0
20
40
Elution

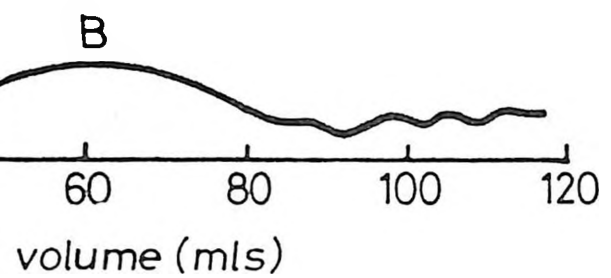

B
60
80
100
120
volume (mls)

Figure 11 : DEAE-c chromatograms from the mean of optical densities at λ 280nm observed in 12 patients at the end of 8 weeks post-admission.

A - Igh peak

B - Albumin peak

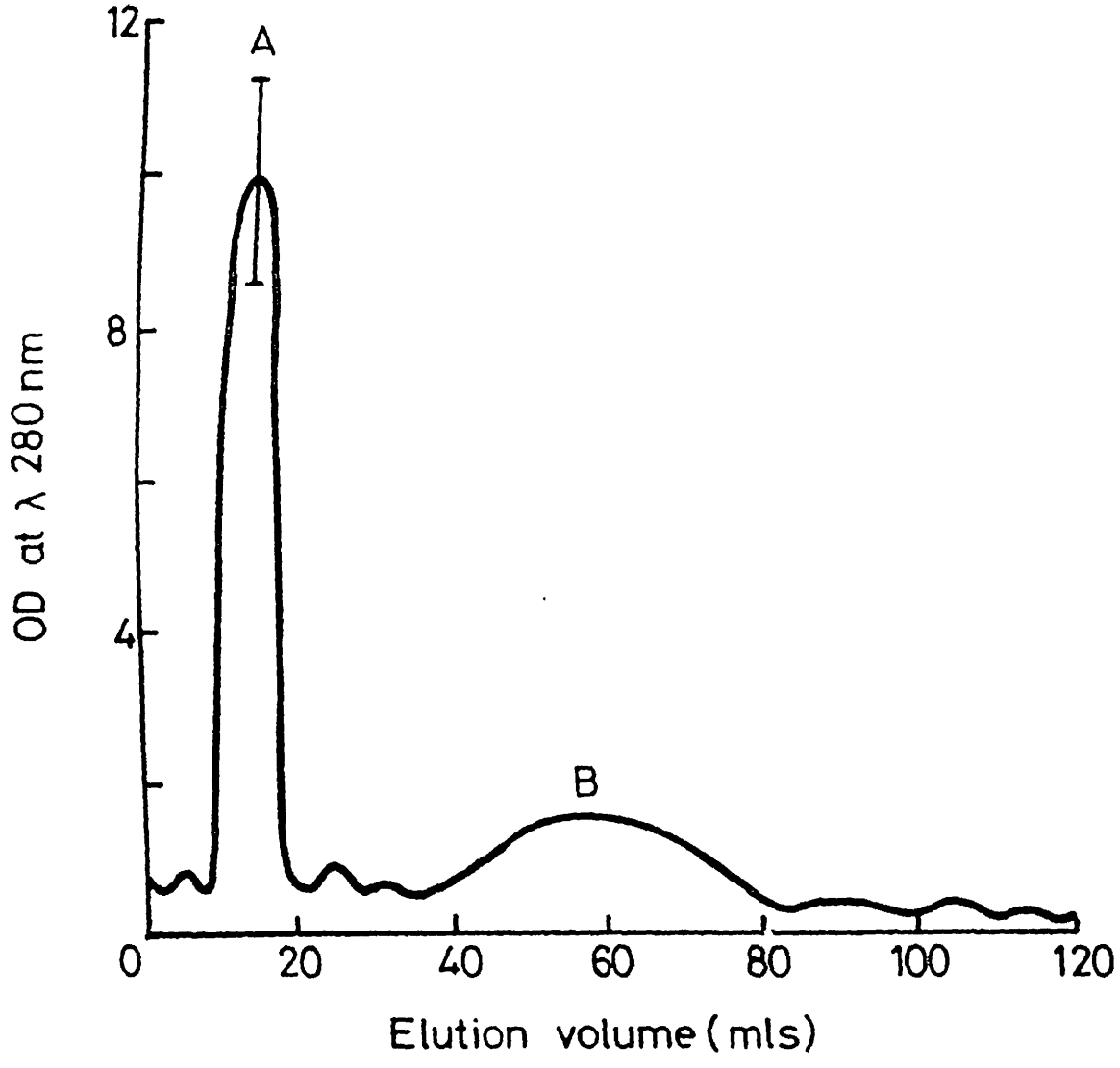

12
8
4
0
A
B
OD at λ 280 nm
20
40
60
80
100
120
Elution volume (mls)

Figure 12 : DEAE-c chromatogram from the mean of optical densities at λ 280nm observed in 12 patients at the end of 12 weeks post-admission.

A - · IgG peak

B - Albumin peak

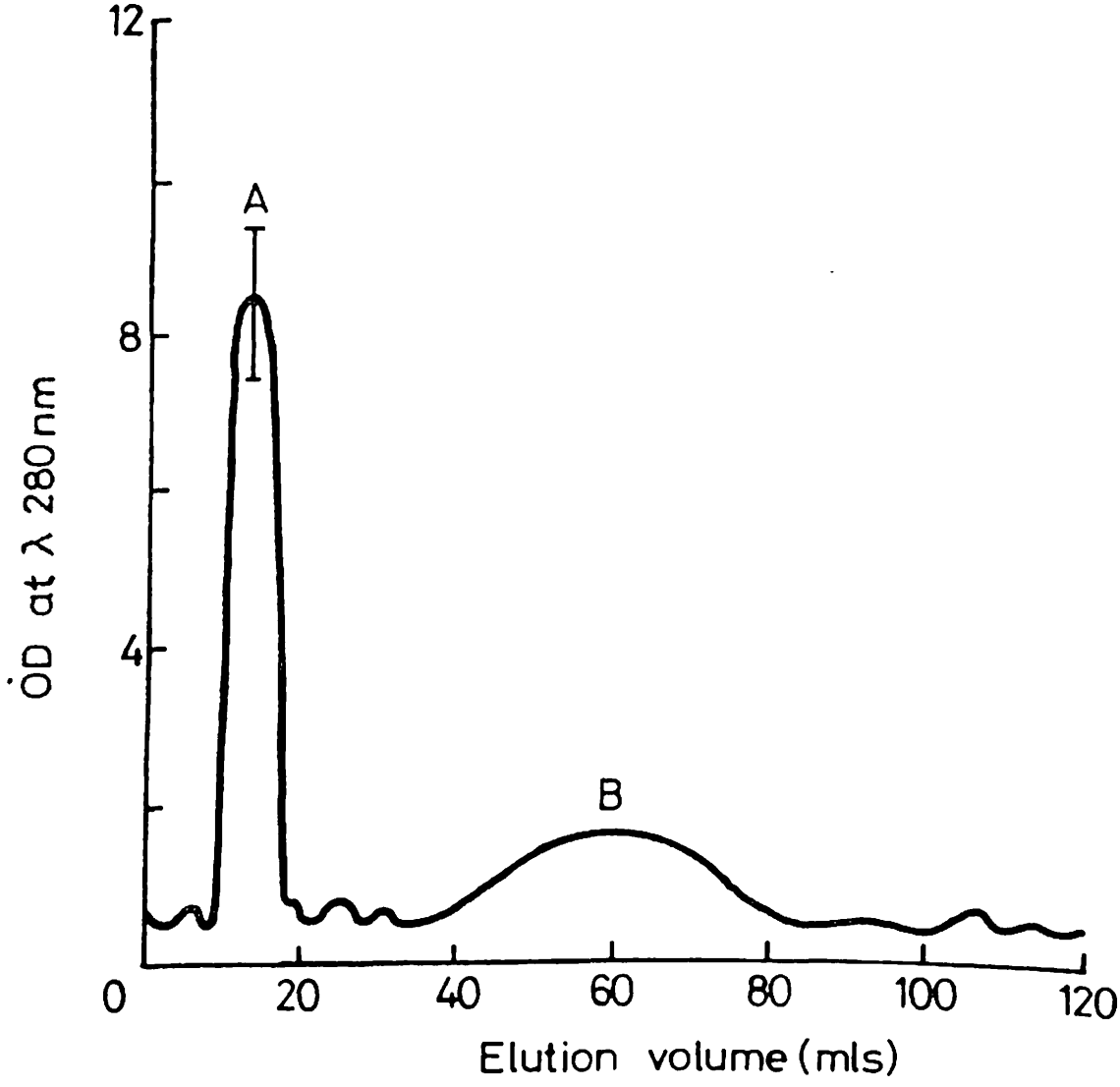

12
8
4
0
A
B
OD at λ 280nm
20
40
60
80
100
120
Elution volume (mls)

Figure 13 : DEAE-c chromatograms from the mean of optical densities at λ 280nm observed in 12 patients at the end of 16 weeks post-admission.

A - IgG peak

B - Albumin peak

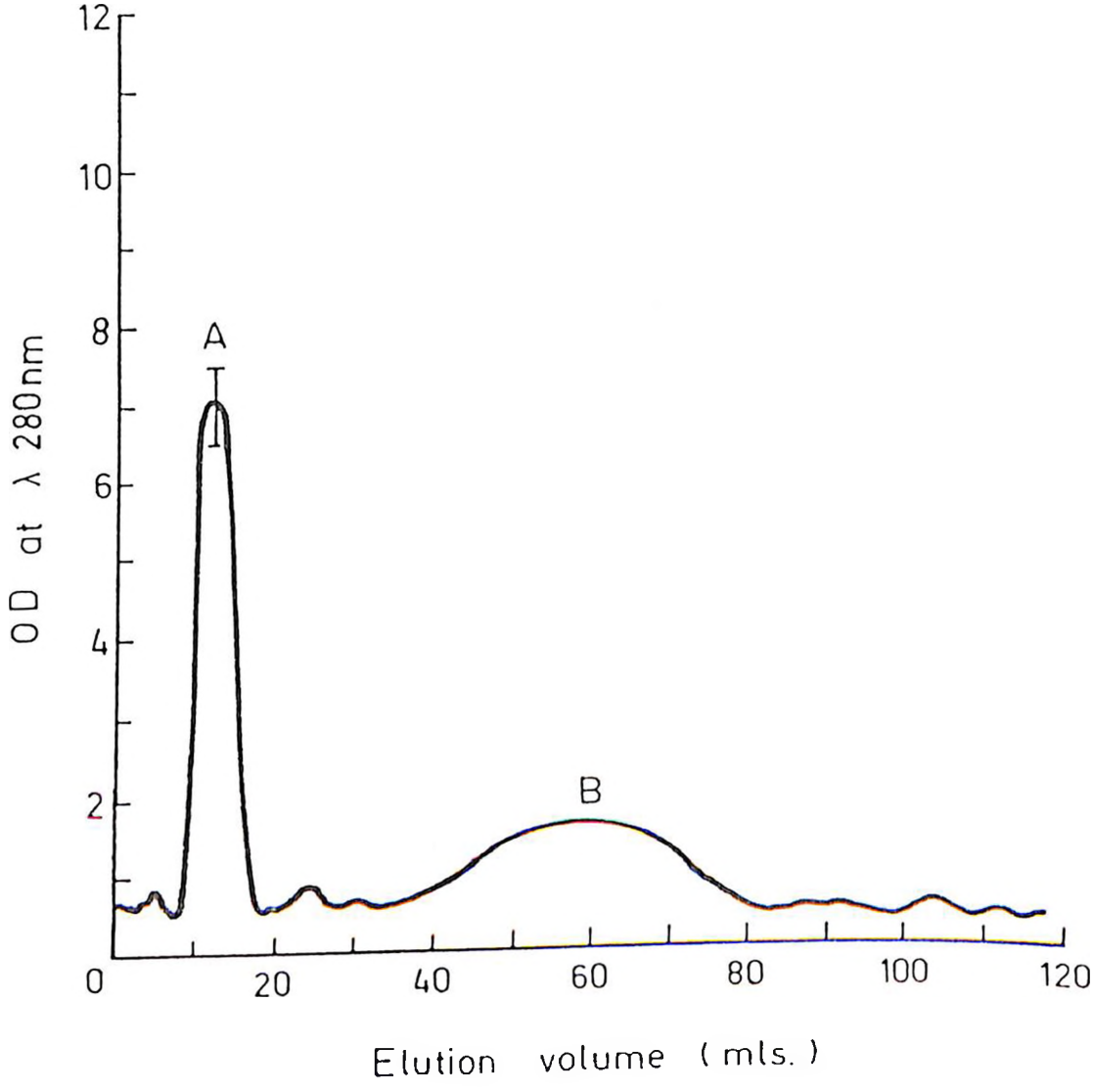

12
10
8
6
4
2
0
OD at λ 280nm
A
B
20
40
60
80
100
120
Elution volume (mls.)

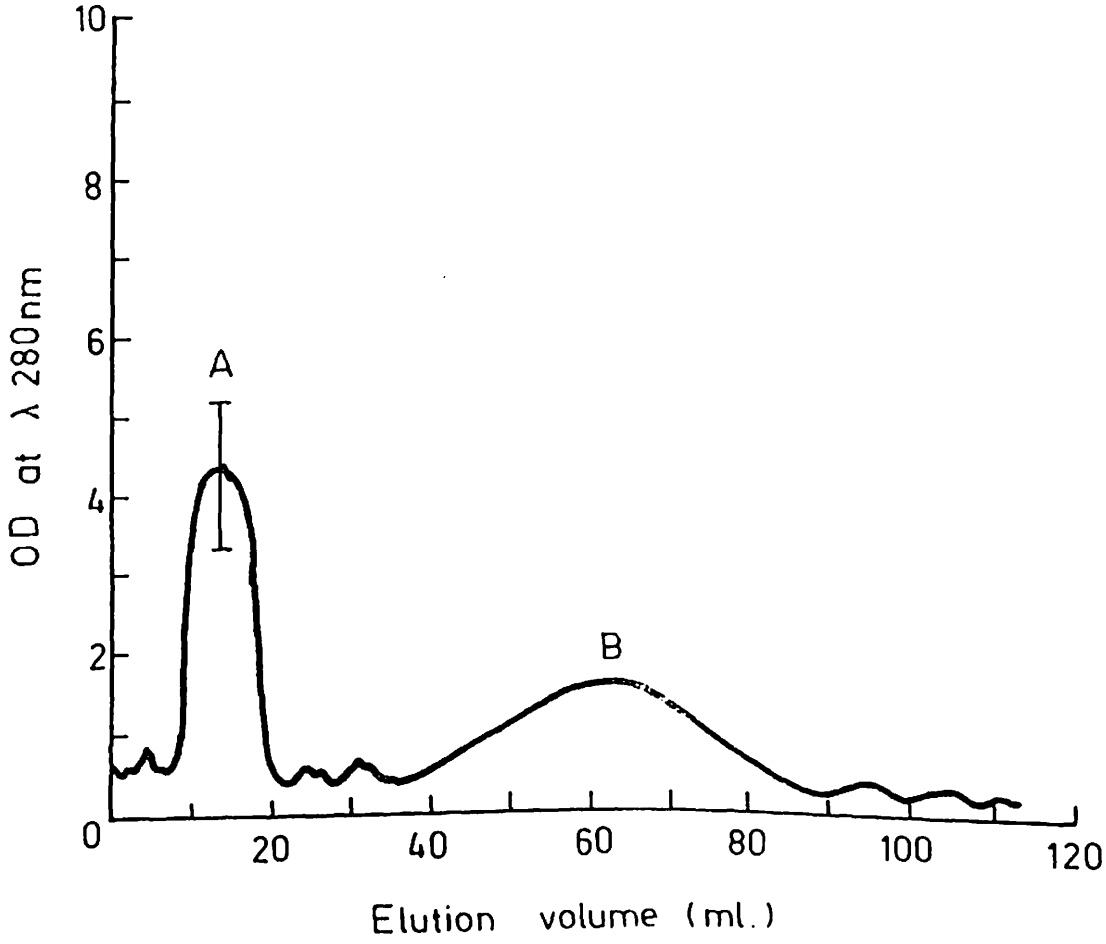
Figure 14(i) : DEAE-C chromatogram from the mean of optical densities at λ 280 nm observed in 6 control individuals.

A - IgG peak

B - Albumin peak

Figure 14(ii) : DEAE-C chromatogram from the mean of optical densities at λ 280 nm observed in hepatic hydatid cyst fluid.

i)


OD at λ 280nm
10
8
6
4
2
0
A
B
20
40
60
80
100
120
Elution volume (ml.)

)

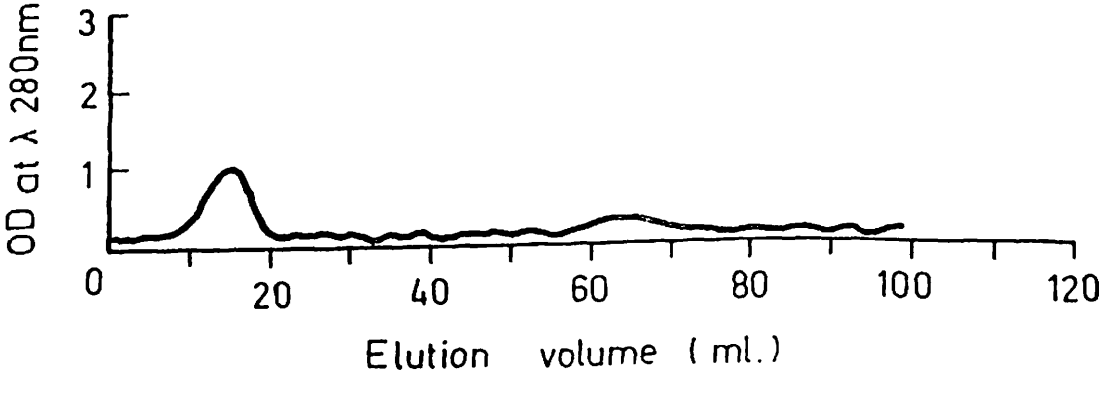

OD at λ 280nm
3
2
1
0
20
40
60
80
100
120
Elution volume (ml.)

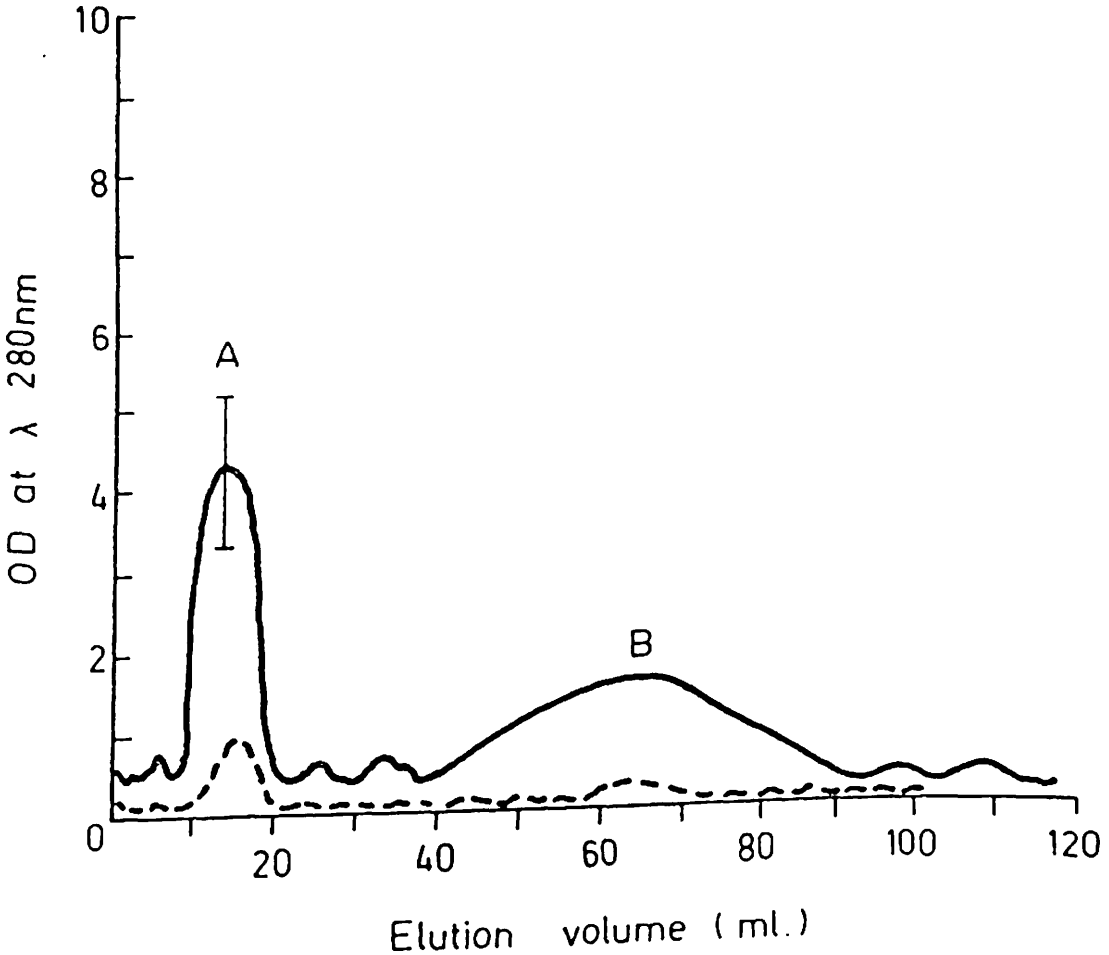
Figure 15 : Combined DEAE-C chromatograms from the mean of optical densities at λ 280 nm observed in control sera and hepatic hydatid cyst fluid.

—— Elution profile of control sera

---- Elution profile of hydatid cyst fluid

A - IgG peak

B - Albumin peak


10
8
6
4
2
0
OD at λ 280nm
A
B
20
40
60
80
100
120
Elution volume (ml.)

3.4.0 Haematological analysis

Total leucocyte counts were on the higher side of the normal range at admission of patients but underwent a rapid increase following albendazole administration. This was followed by a gradual decline to normal levels at the completion of therapy (Figs. 12 to 14).

In 8 out of 12 set 1 (66.6%) cases, initial lymphocyte counts were high but exhibited a brief and temporary decline followed by a rapid proliferative phase ending with a gradual decline. Similar results were obtained for monocytes. Leucocyte counts of two patients Nos 12(808085) and 6(772254) were taken to represent this observation (Figs 12 and 13).

In two out of 12 (16.7%) cases set 2, there was an immediate elevation in total and differential leucocyte counts followed by a gradual decline. In another two cases set 3 initial administration of albendazole was followed by a progressive decline in numbers of lymphocytes, and neutrophils accompanied by a consistent increase in monocyte and eosinophil counts throughout therapy. It is likely that the latter were cases in which the hydatid cysts were already undergoing regression at the beginning of therapy. Leucocyte counts of two patients No. 8(797552) and 9(808087) respectively were taken to represent the response in sets 2 & 3 respectively (Figs 14 & 15).

Eosinophil counts were well above the normal range in 6 out of 12 (50%) cases studied and did not exhibit consistency in numbers in response to albendazole administration. Total haemoglobin, packed cell volume and erythrocyte counts were normal.

These observations clearly show that albendazole administration in most cases leads to temporary decline followed by a rapid proliferation of lymphocytes, neutrophils and monocytes in hydatid patients. This is followed by a gradual decline leading to achievement of normal levels at the end of therapy.

Figure 16 : Leucocyte counts of patient No. 12(808085) during albendazole therapy.

T - Total (absolute) leucocyte counts

L - Lymphocyte counts

N - Neutrophil counts

M - Monocyte counts

E - Eosinophil counts.

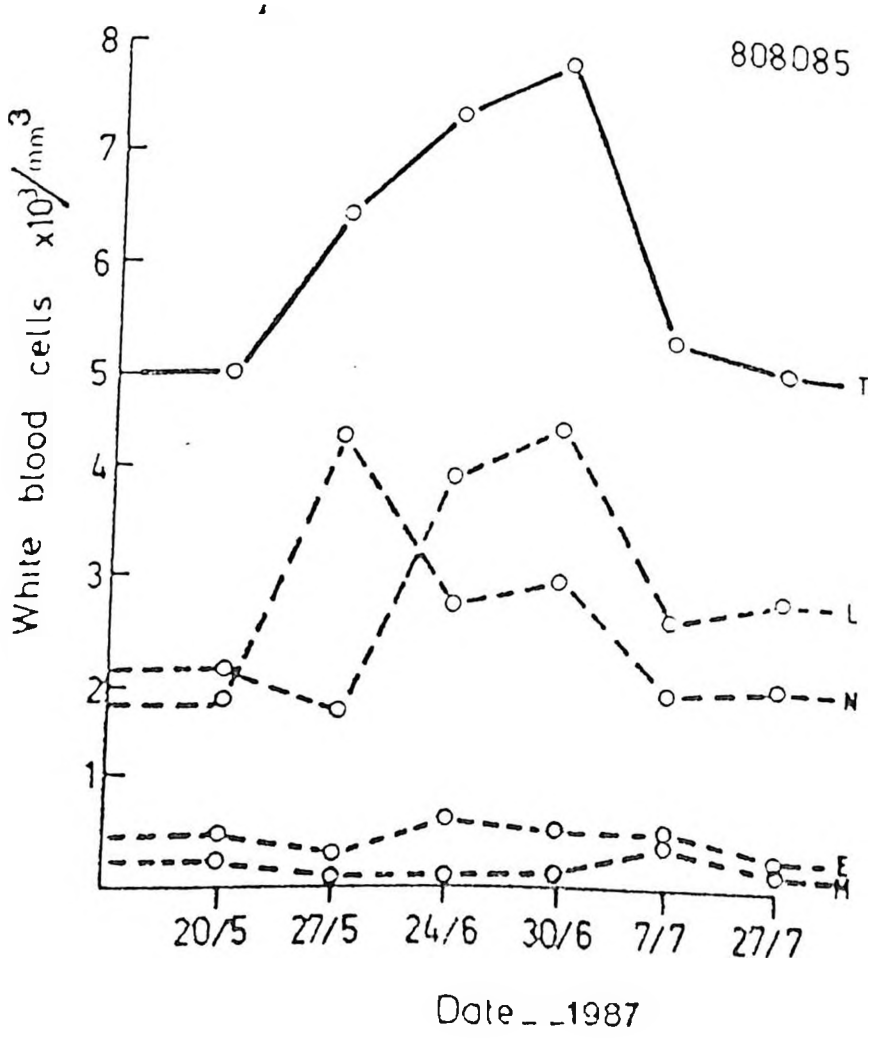

808085
White blood cells x10³/mm³
8
7
6
5
4
3
2
1
T
L
N
E
M
20/5
27/5
24/6
30/6
7/7
27/7
Date _ _1987

Figure 17 : Leucocyte counts of patient No. 6(772254) during albendazole therapy.

T - Total (absolute) leucocyte counts

L - Lymphocyte counts

N - Neutrophil counts

M - Monocyte counts

E - Eosinophil counts.

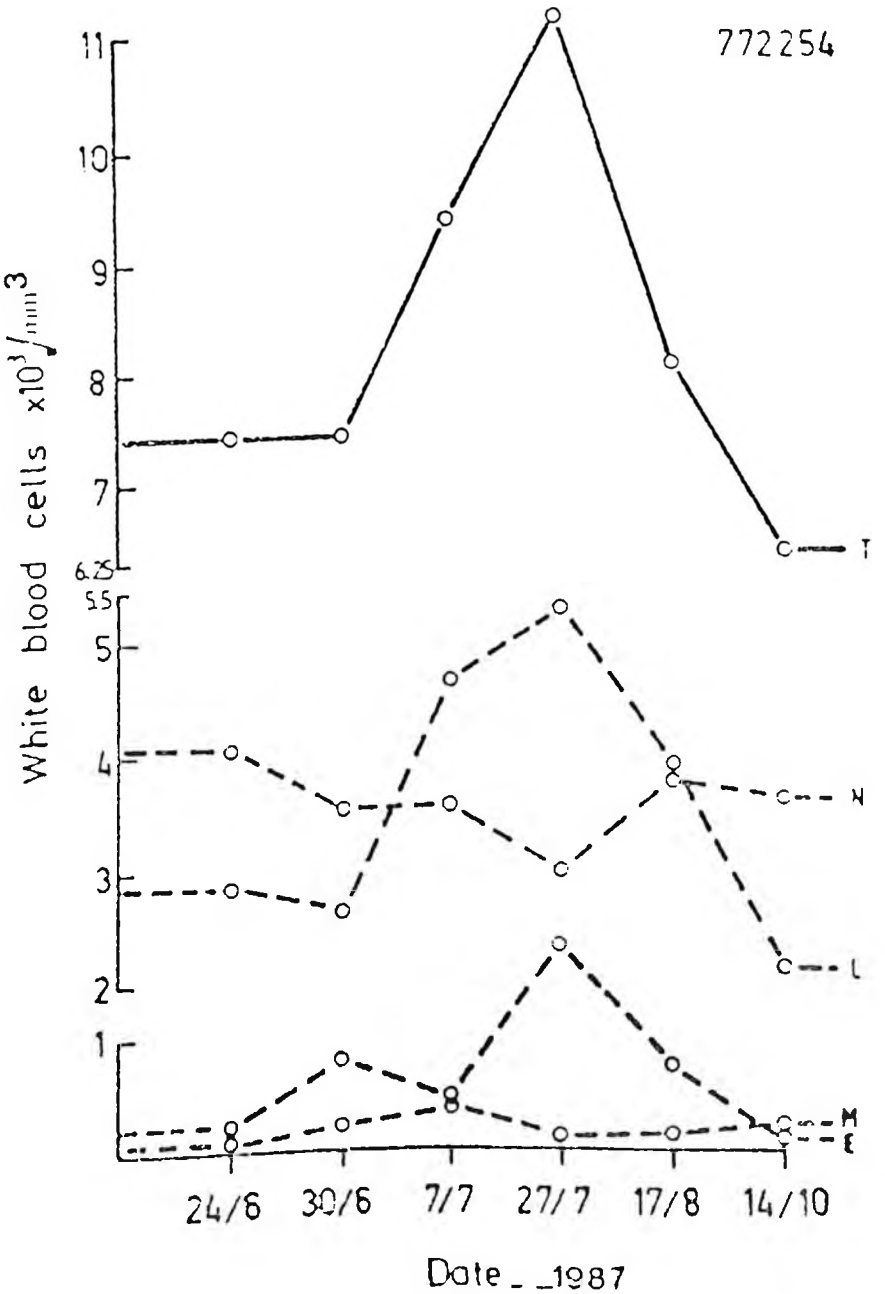

772254
White blood cells x10³/mm³
11
10
9
8
7
6.5
5.5
5
4
3
2
1
T
N
L
M
E
24/6
30/6
7/7
27/7
17/8
14/10
Date _ _1987

Figure 1A : Leucocyte counts of patient No. 8(797552) during albendazole therapy.

T - Total (absolute) leucocyte counts

L - Lymphocyte counts

N - Neutrophil counts

M - Monocyte counts

E - Eosinophil counts.

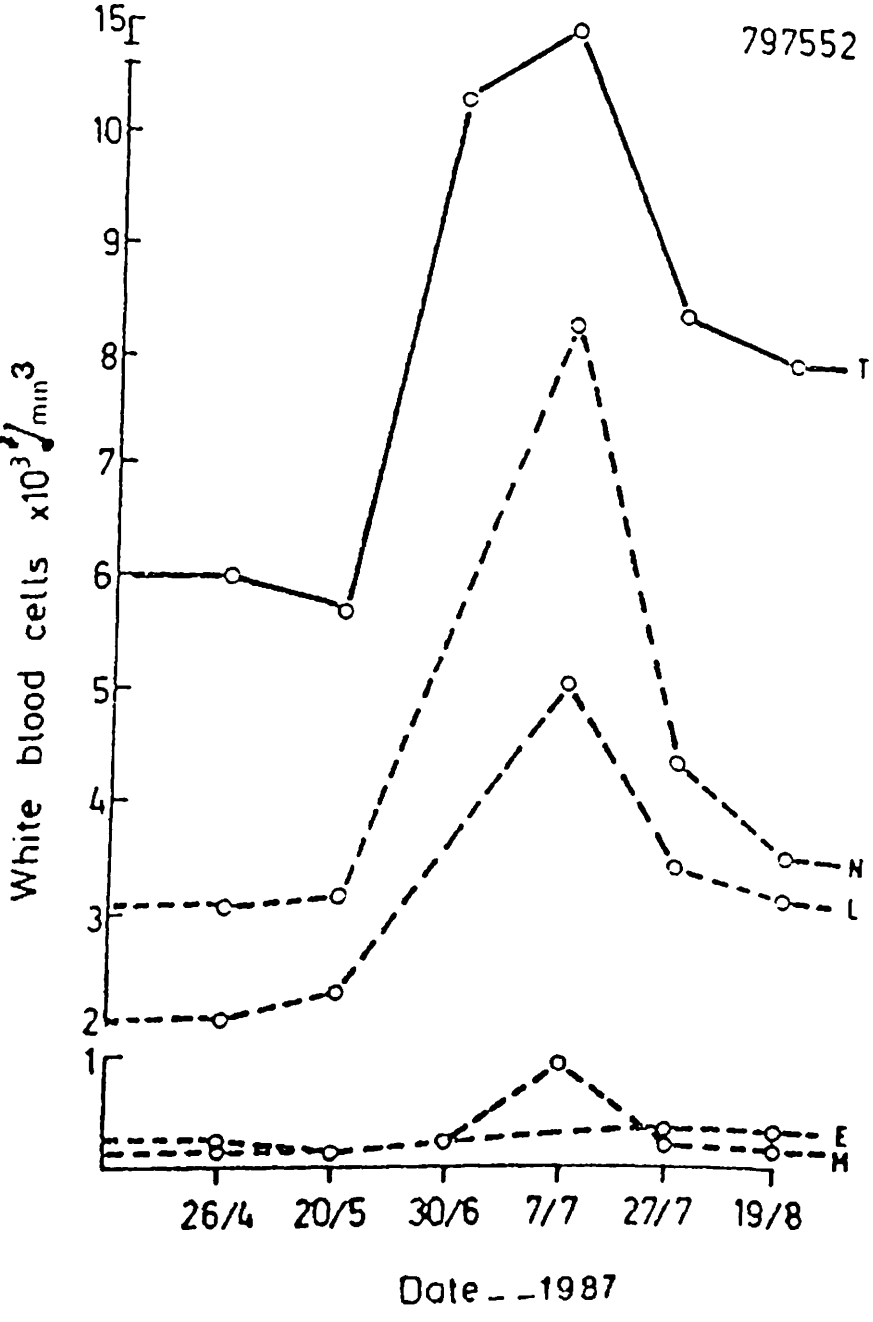

797552
White blood cells x10³/mm³
15
10
9
8
7
6
5
4
3
2
1
T
N
L
E
H
26/4
20/5
30/6
7/7
27/7
19/8
Date - -1987

Figure 19 : Leucocyte counts of patient No. 9(808087) during albendazole therapy.

T - Total (absolute) leucocyte counts

L - Lymphocyte counts

N - Neutrophil counts

M - Monocyte counts

E - Eosinophil counts.

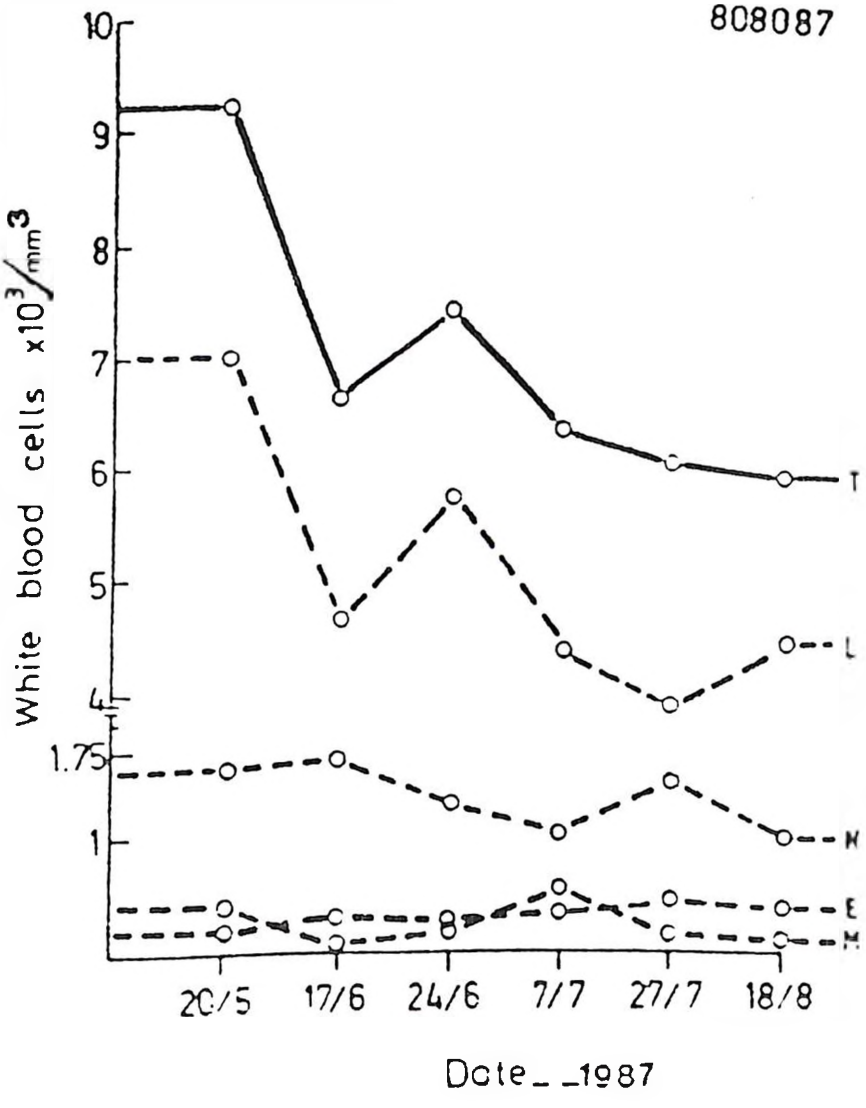

808087
White blood cells x10³/mm³
10
9
8
7
6
5
4
1.75
1
T
L
M
E
M
20/5
17/6
24/6
7/7
27/7
18/8
Date_ _1987

Figure 20 : Mean leucocyte counts of 12 patients taken at quarterly intervals over treatment period.

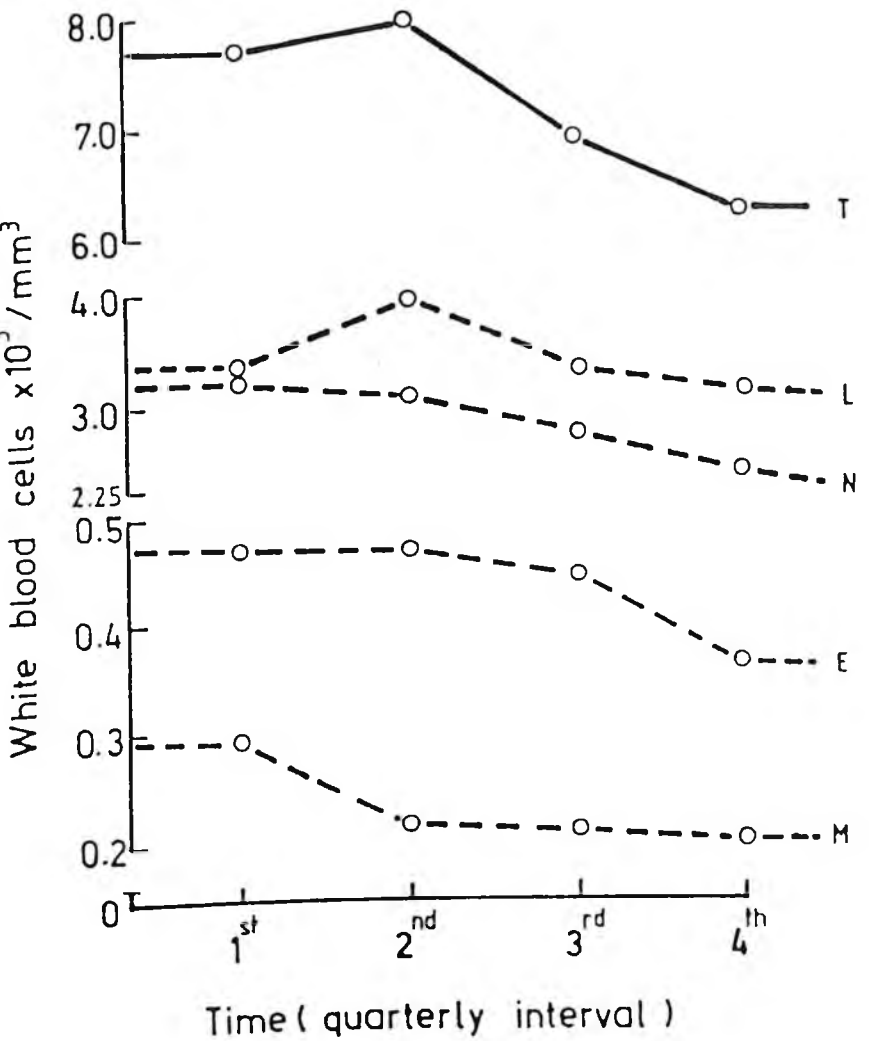
T - Total (absolute) leucocyte counts

L - Lymphocytes counts

N - Neutrophil counts

M - Monocyte counts

E - Eosinophil counts.


8.0
7.0
6.0
4.0
3.0
2.25
0.5
0.4
0.3
0.2
0
T
L
N
E
M
1st
2nd
3rd
4th
White blood cells x10³/mm³
Time (quarterly interval)

CHAPTER 4

DISCUSSION

Due to public health and economic importance of hydatid disease, there is need to fully understand the course of the disease in hospitalized patients receiving various types of treatment such as surgery and chemotherapy. This study was undertaken to determine the effects of albendazole therapy on serum proteins and leucocyte counts in patients admitted with cystic hydatid disease at Kenyatta National Hospital, Nairobi.

Results indicated abnormal reduction in total serum protein concentrations on admission of the patients as determined by methods of Lowry et al (1951). This suggests an anomaly in metabolism of these proteins. Electrophoretic studies using cellulose acetate membrane (CAM) and SDS polyacrylamide gel electrophoresis showed a reduction in albumin and pre-albumin accompanied with elevated levels of IgG. Quantitative determination of IgG levels by anion exchange chromatography showed 2 - 3 fold elevation at admission of patients. It is suggested that the reduction in serum protein concentrations observed at admission of patients may have been due to impaired synthesis and release of albumin by the liver due to hydatid cyst infection, loss of appetite observed in patients during the early days post admission, low dietary protein uptake by patients and the loss of proteins in urine due to glomerulonephritis consequent to deposition of immune complexes in the kidneys of patients. Okello (1988) reported mesangial proliferative glomerulonephritis in a 27 year old Turkana male admiteed at Kenyatta National Hospital with hepatic hydatid disease. The author suggested that the nephropathy was likely to have resulted from immune complex depositions

in the kidneys. Although little work has been published on the problem of nephropathy in human hydatidosis, it is well known that parasitic infections such as malaria (Wing et al 1972) and schistosomiasis (Sabbour et al 1979) can cause glomerulonephritis. The elevation of IgG at admission is likely to have resulted from its increased production in attempt to contain the infection and to replace IgG that penetrated the cysts and are thereafter lost from circulation. This would be consistent with the findings of Mazzaco (1923), who demonstrated that hydatid cyst fluid contained all the components of host serum but in much lower concentrations. Similar observations were reported by Coltorti and Varela-Diaz (1974) that host IgG, albumin and peroxidase penetrate the germinal membranes of hydatid cysts. It has also been shown that both α- and β-serum components are found in hydatid cyst fluid (Chordi and Kagan 1965). Studies by Coltorti and Varela-Diaz (1975b) demonstrated that the entry of macromolecules into hyaline cysts (cysts containing transparent fluid and with unaltered membranes) is irregular and on this basis, they proposed that the passage of macromolecules from the laminated layer into the hydatid fluid resulted from occassional formation of microfissures in the germinal membrane tegument. To account for the release of macromolecules from hydatid cysts with unaltered membranes, Lascano (1975) suggested that the tegument of hydatid cyst germinal membrane constituted only a physical barrier to penetration of micromolecules.

The flow of macromolecules into and out of the hydatid cyst fluid was further demonstrated by Coltorti and Varela-Diaz (1976) who showed that hydatid cysts could survive in vitro when pierced with fine gauge (22-27G) needles.

The results of this study also agree with the findings of Sweatman et al (1963), who reported a distinct decrease in albumin accompanied with an increase in the globulins particularly the gamma and beta fractions thereby giving an overall decrease in serum albumin/globulin ratios in sheep with short term infections superimposed on chronic infections. Matossian et al (1976) reported a significant increase in mean serum IgG levels in 83 Lebanese patients with either hepatic or pulmonary hydatidosis. Increased serum IgG levels have also been reported in patients with amoebiasis, schistosomiasis and other illnesses (reviewed by Matossian et al 1976). In trypanosomiasis, a non-specific hyper gamma globulinaemia in the mouse was associated with polyclonal B- activation and loss of B-cell antigen and mitogen response (reviewed by Cox et al, 1984). Terry et al (1982) suggested that these elevations in total immunoglobulin levels may occur as a consequence of polyclonal lymphocyte activation in vivo leading to secretion of immunoglobulins of many specificities.

The results of this study also revealed that albendazole gradually restores serum protein levels particularly IgG and albumin over the treatment period. This is in agreement with the results of Mousa et al (1976), who demonstrated a significant lowering of albumin and beta globulins accompanied with an increase in serum alpha and gamma globulins in patients suffering from schistosomiasis. In the same study, the authors also demonstrated that use of niridazole as a chemotherapeutic agent in these patients led to a gradual change in gamma globulin and albumin levels towards normal values with treatment.

The study also demonstrated that leucocyte counts were on the higher side of the normal range at admission but underwent a rapid increase following albendazole administration. This was followed by a gradual decline to normal levels at the end of therapy. In 8 out of 12 (66.6%) cases, initial lymphocyte and monocyte counts were high but exhibited a brief and temporary decline followed by a rapid proliferative phase which ended with a gradual decline. In 2 out of 12 (16.7%) cases, there was an immediate elevation in absolute and differential leucocyte counts followed by a gradual decline. In another 2 cases, initial administration of albendazole was followed by a progressive decline in numbers of lymphocytes, and neutrophils accompanied by a consistent increase in monocyte and eosinophil counts throughout therapy. It is likely that the latter were cases in which the hydatid cysts were already undergoing regression at the beginning of therapy. Eosinophil counts were well above the normal range in 6 out of 12 (50%) cases studied and did not exhibit consistency in numbers in response to albendazole administration. These results confirmed active participation of the various leucocyte populations in E. granulosis infections, which is suppressed by presence of hydatid cysts and that albendazole administration leads to enhancement of the host's immunological activity. It has been shown that hydatid cysts suppress non-specific immunity to sheep red blood cells in mice (Allan et al 1981). Similar suppression was demonstrated by Yusuf et al (1975) who reported an inverse relationship between indirect haemagglutination antibody titres and lymphocyte

transformation activity in ten seropositive hydatid infected humans. The participation of cell-mediated immune response to hydatid antigens was also demonstrated by Siracusano et al (1988).

Specific suppression of cell mediated immune response to hydatid antigens was demonstrated after prolonged Echinococcus multilocularis infection in mice (Ali-Khan 1978a). The specific loss of reactivity was further confirmed by Ali-Khan (1978c) when he demonstrated T-lymphocyte depletion in the T-dependent areas of lymphoid organs of E. granulosus infected Swiss mice 13 months after infection. Jenkins et al (1984) performed cell culture experiments and demonstrated that the functional composition of lymphocyte population is altered in favour of non-specific T-cell suppressive activity during prolonged E. granulosus infection. These findings confirm that the presence of hydatid cysts in vivo suppress cell mediated immunity in the hosts.

Although it is known that albendazole acts by selectively blocking glucose uptake into the parasite, the results of this study suggest that in addition, the drug probably creates fissures on the cyst membranes thereby leading to loss of membrane integrity and subsequent leakage of antigens which in turn cause a temporary decline in leucocyte proliferation followed thereafter by an amplified proliferative activity. This is consistent with the hypothesis of Yarzabal et al (1974) that the detection of serological activity in E. granulosus infected individuals is dependent on cysts membrane alterations which allow antigen release. It is therefore thought that these two mechanisms probably work together in containing the infection.

The initial decline in leucocyte counts on admission of patients may as well have resulted from elimination of old (probably defective) leucocyte by albendazole.

The ability of albendazole to provoke a temporary depression in leucocyte followed by a rapid proliferation was particularly pronounced for lymphocytes and monocytes which are important in mounting effective immune response to parasitic infections. The central role of eosinophils, macrophages and neutrophils, observed by Capron et al (1982) in schistosomiasis probably also applies to E. granulosus infections. This has yet to be confirmed.

It would appear that there is need to fully understand the detailed mechanisms by which albendazole contains hydatidosis. Furthermore, a closer look at the response by various sub-populations of T- and B-lymphocytes and a determination of the role played by monocytes during treatment with albendazole would be necessary. Further work should also be carried out to determine the response by various subclasses of IgG (i.e. IgG 1,2,3 and 4) during treatment with albendazole. This information would be useful in diagnosis, assessment of prognosis and immunostaging hydatid patients during treatment.

CONCLUSIONS

This study clearly shows that:-

(a) Albendazole restores serum protein concentrations to normal levels in hydatid patients. This is accompanied

by a progressive increase in albumin and pre-albumin together with a consistent decline in IgG levels throughout treatment period

(b) Initial administration of albendazole leads to a temporary decline in leucocyte counts followed thereafter by polyclonal leucocyte proliferation which ends with a progressive decline in counts to normal levels at the end of therapy.

(c) The detailed mechanisms by which albendazole contains hydatid disease are far from clear and need to be investigated further.

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