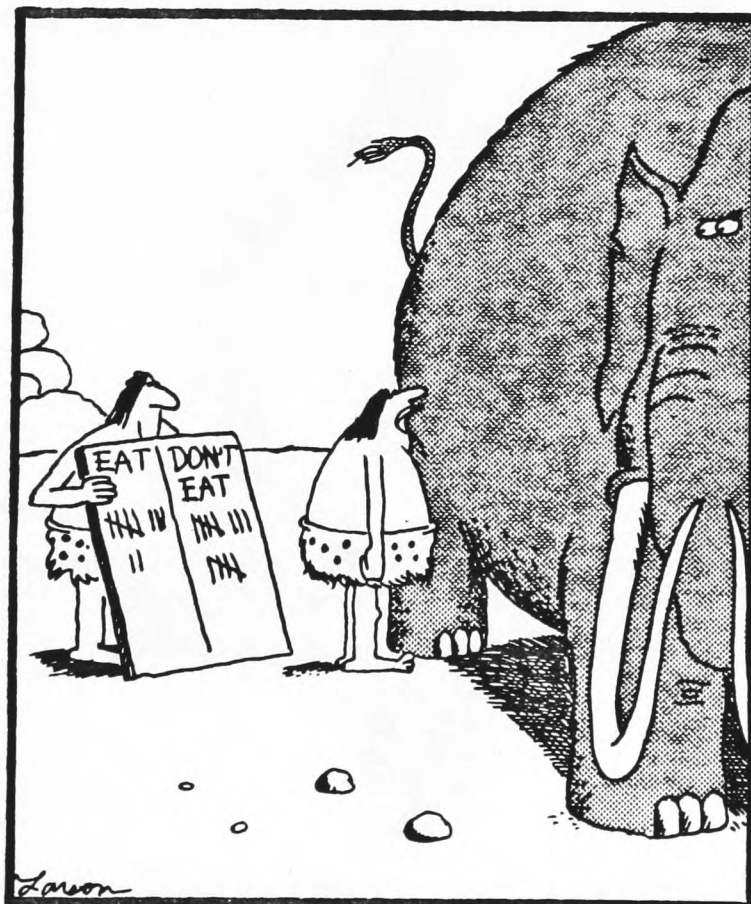
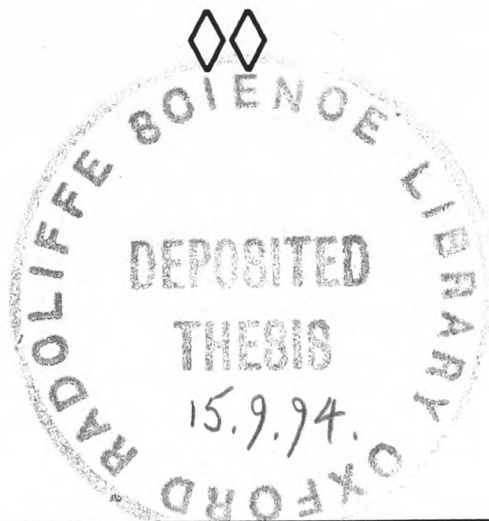


“Feed a Cold,
Starve a Fever”.....?



PARASITES & HOST NUTRITION

Denver Dudley Stanton Dale



THESIS SUBMITTED FOR THE DEGREE
DOCTOR OF PHILOSOPHY



UNIVERSITY OF OXFORD
ENGLAND

1993

ST JOHN'S COLLEGE

TRINITY TERM

.....for my dearest Mum & Dad.....

Abstract: Doctor of Philosophy Thesis
University of Oxford
Trinity Term
1993

“Parasites & Host Nutrition”

Denver Dudley Stanton Dale
St. John's College



This Thesis examines nutritional aspects of the host/parasite association. Although it is well recognized that parasites greatly influence the nutritional status of their hosts, the effects of variations in host nutritional status on the relationship between host and parasite remain largely unexplored. Preliminary assimilation of scattered evidence led to the generation of the present thesis that changes in host nutritional status significantly influence both host and parasite success. This thesis was investigated with respect to three host/parasite systems:

I. Experiments examining the influence of vertebrate host nutrition on *Schistosoma mansoni* infection and disease sequelae, employing a murine model, focused on three areas:

1. Externally imposed host protein deficiency during single and multiple infections showed that schistosomes invading deficient hosts were less successful than those invading well-fed animals. Parasite survival, growth and fecundity were significantly reduced. Parasite antigenicity fell as a result, and in the presence of protein deficiency-induced immunosuppression, host pathogenesis was largely suppressed.

2. The influence of externally imposed fluctuations in host protein status on mature infection showed that providing increased protein diets to mice harboring schistosomes which had matured under deficiency largely reversed the deleterious effects of protein undernutrition on the parasite. The resultant rise in antigenicity, in the presence of host immune activity restored by refeeding, produced a marked increase in disease expression. Conversely, a period of acute protein deficiency in hosts harboring schistosomes which had matured under conditions of protein repletion significantly reduced parasite reproductive success and led to marked amelioration of chronic immune-mediated pathology.

3. Externally imposed host protein deficiency during vaccination and drug treatment showed that deficient hosts were less able to capitalize on the protection against schistosome infection which these interventions provided to well-fed animals. Immunosuppression known to accompany protein deficiency was thought to have compromised immune-mediated elimination of invading parasites in the vaccinated host, and immune-mediated elimination of damaged adult parasites in hosts receiving praziquantel treatment.

II. Experiments examining the interactions between invertebrate host nutrition and *S. mansoni* infection showed that schistosome growth and replication are significantly reduced in snails maintained on qualitatively complete, but quantitatively inadequate diets. In addition to decreased cercarial output, time-dependent cercarial infectivity post-shedding was reduced when development took place in nutritionally deficient snails.

III. A review of published evidence which examines the influence of host nutritional status on malaria showed that the majority of the various forms of nutritional deficiency tend to suppress malarial replication and disease in both the vertebrate and invertebrate host. This review provides an accessible basis for future work.

Experimental results presented in this document support the thesis presented above. Furthermore, they suggest that the protection which host nutritional deficiency provides against some parasites may consist of changes within the host rendering conditions suboptimal for fulminant parasite exploitation. As infection is well documented to induce largely host-mediated changes in the ambient conditions within the host by way of the metabolic response, this Thesis suggests that the ability of the host to respond to infection in this way may provide a form of defence against parasites susceptible to fluctuations in ambient conditions.

The implications which follow this suggestion are serious: refeeding nutritionally deficient humans in the presence of active infection can remove the protection which ambient defences may provide against some diseases by restoring conditions suitable for parasite survival, reproduction and exploitation.

This Thesis concludes that the formulation and implementation of successful public health policy must include informed consideration of the complex nutritional interactions between parasite, host and disease.

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And finally, I reserve my deepest appreciation and love for Dr. Tricia Ellis Markusen M.D., for bestowing upon me the ultimate gift, and for being my best friend.

Despite invaluable input from the above named and others during the preparation of this document, the contents and opinions expressed herein are entirely my own, and I accept sole responsibility for them. No part of this thesis has been previously submitted for any degree of the University of Oxford or elsewhere. All work contained herein is entirely my own, except where otherwise indicated.

Denver D.S. Dale
Los Angeles, California, USA. 01 June, 1993.

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CHAPTER ONE

PARASITES & HOST NUTRITION



*“De duobus malis minus est semper eligendum”*¹

Thomas À Kempis (circa 1380-1471)



Chapter Summary

This chapter examines nutritional aspects of the host/parasite relationship. Although parasites have a well recognized impact on host nutrition, the majority of the nutritional costs associated with infection are host-mediated. It appears that host undernutrition, whether endogenously induced or exogenously imposed, can produce physical and chemical conditions within the host which are suboptimal for parasites success. It is suggested that host nutritional fluctuations can lead to changes in infection and disease independent of the other, and shown that nutritional fluctuations can lead to both increases and decreases in parasitic infection and disease. The implications of counterintuitive changes in disease in response to host nutritional fluctuations are discussed in terms of the field situation, where schistosomiasis and malaria are implicated as responding antagonistically to host undernutrition.

1.1: INTRODUCTION

All organisms require nourishment in order to develop and propagate. Primary producers and consumers gather nutrients from their environment and assemble them into living tissues. Predators exploit their efforts at consolidation, and ascending the food chain, successively higher animals represent increasingly rich islands of organic material in a relatively depauperate environment (Ricklefs, 1980).

Although providing inherent nutritional advantages, predation can be difficult to sustain; consumed quarry represent spent resources which may be difficult and costly to replace. As a result, prey availability is commonly a major determinant of predator success (Ricklefs, 1980). Some organisms have largely escaped this constraint, however, by spending an extended period of time feeding from a single prey individual. Parasites employ a highly specialized form of predation in which exploitation of the prey resource (the host) by the predator (the parasite) is continuous and on a sustainable level relative to the predator's lifespan.

Parasitism is a lucrative strategy - parasites enjoy a rich supply of nutrients provided under the highly regulated conditions of the host interna. By reducing endoregulatory requirements, however, such conditions lead to increasing dependence of the parasite on its host (Bryant & Behm, 1989).

Parasite reliance upon its host for food intensifies along the continuum of intimacy exhibited by host/parasite associations, culminating in obligate endoparasitism where the parasite's entire nutritional requirements must be met from within the host (Bryant & Behm, 1989;

FOOTNOTE

¹ “Of two evils, the lesser is always to be chosen”.

Hall, 1985; Meshnick & Cerami, 1985). In addition to food, parasites rely to some extent upon their hosts for shelter. In intimate associations, where the parasite exists within the stable environment of the host interna, selection may largely abandon parasite robustness in favour of reproductive output. Parasites thus unable to independently maintain homeostasis rely upon their host to maintain the stable physical and chemical conditions pivotal to their success (Hall, 1985; Meshnick & Cerami, 1985). The difficulties experienced in culturing parasites *in vitro* attest to their precise nutritional and physico-chemical requirements, and suggest that fluctuations in *in vivo* conditions significantly influence parasite success.

Parasites & Host Nutrition: Mutually Inclusive

Physical and chemical conditions within hosts fluctuate as a result of exogenous and endogenous factors. Among the latter, parasites influence the host interna both by their nutritional costs and by inducing disease. As a result, parasites commonly have an appreciable impact on the environment upon which their own success depends. Thus host and parasite forge an intimate, bidirectional relationship which is fundamentally nutritional in nature (Hall, 1985). Their complex interaction suggests that to view either parasitic infection or host nutrition in isolation is highly artificial.

The ultimate outcome of the nutritional relationship between host and parasite assumes crucial significance when viewed in the light of the global prevalence and coincidence of nutritional stress and parasitic diseases in impoverished human communities (Bundy & Golden, 1987). For this reason, the following general introduction focuses almost exclusively on infection and human nutrition, although fundamental similarities exist between humans and other animals with respect to nutrition/parasite interactions. In attempting to understand the complex relationship between parasites and host nutrition, it is useful to begin by examining the effects each has upon the other.

1.2: THE EFFECTS OF PARASITES ON HOST NUTRITIONAL STATUS

By definition, parasites impose nutritional costs on their hosts. Not only do they compete with the host for available nutrients and metabolites, but pathophysiological changes within the host in response to parasitic infection commonly have serious nutritional consequences.

Competition for Nutrients

Parasites may compete with their hosts for nutrients at almost any stage in the complex process of nutritional repletion. Gastrointestinal parasites, such as the human roundworm *Ascaris lumbricoides*, have immediate access to ingested food, and any uptake by such parasites directly reduces nutrient availability to the host (Schultz, 1982; Stephenson & Holland, 1987). However, the majority of parasites deprive the host of nutrients which have been metabolized and/or incorporated into host tissues at some prior expense to the host. For

example the hookworms, *Necator americanus* and *Ancylostoma duodenale*, suck blood and tissue fluids from feeding lesions in the intestinal mucosa of their human hosts, resulting in the loss of various essential nutrients, such as protein and iron, and metabolites, such as haemoglobin and transferrin (McDowell, 1981; Schad & Banwell, 1985; Stephenson & Holland, 1987).

Parasites are commonly much smaller in terms of biomass than their hosts, and thus the nutrients taken to meet their metabolic requirements generally represent a relatively small proportion of host food intake (Crompton & Nesheim, 1982; Hall, 1985). There are, however, occasions when parasitic competition for nutrients can have a significant impact on the host.

Hosts existing in or near nutritional deficit are sensitive to small changes in nutrient availability (Torún & Viteri, 1985; Wood & Calloway, 1985); any nutrients lost to parasites can only exacerbate nutritional stress, and may precipitate disease in hosts which are pushed across clinical deficiency thresholds. This is particularly likely when parasites have a higher affinity for essential nutrients than their host. For example, the pseudophyllidean tapeworm *Diphyllobothrium latum*, which absorbs high levels of vitamin B₁₂ from food in the intestinal lumen of its human host, can induce clinical deficiency of this vitamin even when dietary intake would otherwise be adequate (von Bonsdorff, 1956).

Whilst nutrient losses attributable to parasite uptake are frequently insignificant with respect to individual host intakes, it should be noted that, on a population level, the collective nutritional burden imposed by highly prevalent parasite infections in impoverished human communities may be considerable. For example, it has been calculated that individual *Ascaris lumbricoides* can consume 0.14 grams of carbohydrate daily (Cabrera, 1984). In the Philippines, some 20 million Filipinos each harbor approximately 20 *Ascaris*. Therefore estimates of the food lost directly to *Ascaris* per day in that country suggest the equivalent of 1000 fifty kilogram sacks of rice, or 50 tonnes (Cabrera, 1984).

Although nutrient losses to parasite uptake and the resultant impact on host nutritional status increase in proportion to the number and nutritional demands of individual parasites, higher parasite burdens are more likely to be nutritionally important with regard to their pathophysiological effects on host structure and function than to their direct cost in nutrients.

Parasitic Disease

Parasites induce a plethora of changes (collectively known as parasitic disease) in the host which result from both direct damage to host tissues by, and, more significantly, host responses to the physical and chemical presence of parasites (Holmes, 1986; 1987). Parasitic diseases are responsible for the most serious nutritional consequences of infection and may disrupt the complex process of host repletion at any stage, from acquiring food to its

successful intracellular use (Keusch, 1985a; Keusch & Scrimshaw, 1986; Rosenberg & Bowman, 1984; Stephenson, 1980; Stephenson & Holland, 1987; Tomkins & Watson, 1989).

Food Acquisition

Mental aptitude and physical ability are required to gather, grow and/or earn food. Lethargy resulting from general malaise or nervous damage (Hajduk, Englund, Mahmoud & Warren, 1985) arising from host responses to infection can reduce the motivation to secure food (Crompton, 1986; Hall, 1981). For example African Sleeping Sickness, a disease caused by host responses to several species of the flagellate protozoan, *Trypanosoma*, induces chronic lassitude, resulting in a marked loss of productivity (Hajduk *et al.*, 1985).

In addition, parasitic infection commonly results in tissue damage which can reduce the host's physiological capacity required to secure food (Bengtsson, Pehrson, Bjorkman, Brohult, Jorfeldt, Lundbergh, Rombo, Willcox & Hanson, 1988; Crompton, 1986; Hall, 1981). For example, anaemia secondary to hookworm disease reduces the blood's capacity to transport oxygen, resulting in decreased aerobic, and therefore work performance (Latham, Stephenson, Kurz & Kinoti, 1990; Stephenson & Holland, 1987).

Moreover, when chronic disease leads to on-going nutritional stress, mental development can be significantly impaired. School children suffering from persistent anaemia display decreased mental performance; it follows that anaemia secondary to some parasitic infections, such as hookworm disease, if unchecked, may impair the mental development of chronically infected individuals (Brozek & Church, 1984; Calloway, 1982; Crompton, 1986). Recent evidence, in which geohelminth infection has been clearly related to reduced academic performance (Nokes, Cooper, Robinson & Bundy, 1991), confirms this notion. Accordingly, increasing attention is being paid to the role of infection in impairing effective learning (Halloran, Bundy & Pollitt, 1989; Pollitt, 1990).

It appears that regardless of food acquisition opportunities, parasitic disease can significantly influence the amount and type of food acquired by the host by inducing host responses which deter and/or impede its acquisition.

Ingestion

Once food is acquired, intake is under neural and hormonal control (Morgensen & Calaresu, 1978). Parasitic disease frequently reduces host appetite by inducing pain and/or vomiting in response to ingestion, whilst the expectation of recurrent symptoms can deter further intake (Crompton, 1984). In addition cytokines, such as Interleukin 1 (IL-1), released from macrophages activated by infection, may induce fever and endotoxin release which combine to reinforce inappetance (Dinarello, 1990; Keusch & Farthing, 1986). Anorexia is a response common to many parasitized hosts (Hall, 1985; Stephenson & Holland, 1987), and has been

the subject of previous attention (Crompton, 1984; Crompton, Arnold, Walters & Whitfield, 1985; Crompton, Walters & Arnold, 1981; Hart, 1990; Latham *et al.*, 1990; Rosenberg & Bowman, 1984).

Other work has shown that host food preference can change in response to parasitic disease. That rats heavily infected with the nematode *Nippostrongylus* avoid those food flavors which they associate with morbidity demonstrates that parasitic disease can qualitatively influence food intake (Keymer, Crompton & Sahakian, 1983a). Humans too may display perversions of normal food intake during parasitic disease; for example, hookworm disease has been associated with the development of pica and geophagia in children (Schad & Banwell, 1985).

In rare instances, parasitic disease can physically obstruct ingestion. The severe oesophageal swelling which can occur in response to acute infection with the protozoan *Trypanosoma cruzi* (the causative agent of Chagas Disease) blocks food intake, thus rapidly leading to host undernutrition (Nogueira, Coura & Chen, 1985).

In summary, regardless of food availability, parasitic disease can significantly influence the amount and type of food eaten by the host by inducing host responses which deter, alter and/or obstruct intake.

Digestion

Parasites frequently impair host digestive functions, and several mechanisms have been identified (see Rosenberg & Bowman, 1984). Whilst some trematode flukes disrupt bile flow from the liver of their human hosts, reducing digestive function (Bunnag & Harinasuta, 1985), other parasites, such as *Ascaris*, damage the brush borders of their host's intestinal epithelial cells active in enzyme secretion essential to the digestion of specific compounds. Such damage can produce host intolerance to important nutrients, such as lactose (Carrera, Nesheim & Crompton, 1984; Hall, 1985). In addition, *Ascaris* has been found to secrete an antiproteolytic substance *in vitro* which is thought to be active in inhibiting digestive attack on the parasite *in vivo* by blocking the action of pepsin, cathepsin E, trypsin and chymotrypsin. Despite local effects, it is thought these antienzymes do not grossly impair host digestion (Hall, 1985; Pawlowski & Arfaa, 1985).

In addition to disrupting food degradation, parasites can stimulate intestinal activity which alters the flow of food through areas of the alimentary tract active in digestion and absorption. Among numerous parasitic infections which alter gastric flow, ascariasis, amoebiasis (Mata, 1985) and giardiasis (Roberts-Thomson, 1985) can significantly reduce the amount of time food takes to pass through the host, reducing food digestion and absorption, and thus increasing nutrient excretion. Whilst many other parasites similarly increase transit rates (Rosenberg & Bowman, 1984), heavy infection with *Ascaris* can

physically obstruct gastric flow, inducing vomiting and other nutritionally expensive responses (Blumenthal & Schultz, 1975).

Absorption

Once food is decomposed by digestion, nutrients become available for active uptake into host tissues. Malabsorption is a feature common to many parasitic diseases, as the sensitive intestinal surfaces active in uptake present parasites with a site which is rich in nutrients yet relatively free of immune defences (Hall, 1985). Parasites can injure the delicate absorptive surfaces of the host gut by their physical and chemical presence eliciting pathogenic responses. For example host responses to *Ascaris* infection produce marked changes in the jejunal mucosa, including height and width reductions of villi, elongation of crypts, a decrease in the crypt:villus ratio and round cell infiltration of the *lamina propria* (Beaver, Jung & Cupp, 1984).

In addition, many protozoan parasites seriously damage host mucosal surfaces, leading to ulceration and contributing to malabsorption (Banwell, 1985). In severe cases, necrosis of intestinal surfaces in response to parasite activity can produce lesions through which nutrients are lost into the excretory passages. For example, enteropathies secondary to amoebic infection can lead to severe intestinal haemorrhage (Sepulveda & Martinez-Palomo, 1985). Although some reabsorption may occur downstream in the intestine, such tissue losses are serious and can rapidly lead to severe nutritional deficits (Stephenson & Holland, 1987).

Once actively absorbed through the intestinal wall, nutrients are transported by circulating cells to sites of metabolic activity. Disturbance of cell dynamics by parasitism can therefore impair nutrient transport by disrupting concentration gradients and reducing cell availability and carrying capacity (Keusch & Farthing, 1986). For example filarial worms can obstruct the lymphatic flow of their human hosts, causing malabsorption (Banwell, 1985), whilst the increased rate of erythrocyte destruction in the enlarged spleen during schistosomiasis may reduce the nutrient carrying capacity of the blood (Stephenson & Holland, 1987).

Metabolism

By far the greatest nutritional impact of parasitic disease occurs when infection triggers numerous responses within the host which drastically alter metabolism. These are largely mediated by various bioactive peptides (collectively known as lymphokines) released by activated macrophages in response to infection. Among the many factors released (see Klein, 1990), IL-1 induces marked systemic changes in nutrient intake (described above), metabolic dynamics and nutrient excretion which combine to rapidly and significantly reduce nutritional status in the infected host (Dinarello, 1990; Keusch & Farthing, 1986) (Table 1, overleaf).

<i>Protein metabolism</i>	Increased nitrogen loss (negative balance) Catabolism of muscle protein Conversion of amino acids to glucose Decreased synthesis of albumin, transferrin Increased synthesis of acute-phase proteins by liver Proliferation of phagocytes & lymphoid cells
<i>Carbohydrate metabolism</i>	"Pseudodiabetes" Increased glucose oxidation Peripheral (muscle) insulin resistance Augmented gluconeogenesis
<i>Mineral metabolism</i>	Removal of plasma iron to the liver Reticuloendothelial system uptake of zinc Increased plasma ceruloplasmin copper Urine, stool and sweat losses of Mg, P, K & S
TABLE 1. Metabolic responses to infection which alter host nutritional status (adapted from Keusch & Farthing, 1986).	

Lymphokine release leads to the production of pyrogens and acute phase proteins (Klein, 1990). Pyrogens induce fever which increases the host's basal metabolic rate (BMR; 13-15% increase in BMR.degree celcius⁻¹), which in turn metabolizes nutrients at an abnormally high rate, wasting energy and increasing the demand for other essential nutrients (Hall, 1985). The principal metabolic response to infection is, however, the induction of catabolic dominance. In severe and/or chronic infection, anorexia and increased metabolic requirements may lead to gluconeogenesis (the production of glucose by the liver from amino acid precursors released from contractile proteins of muscle), resulting in negative nitrogen balance and wasting of lean body mass (Keusch & Farthing, 1986; Keusch & Scrimshaw, 1986).

Micronutrient dynamics are also markedly altered in response to infection. Acute phase proteins (such as transferrin and lactoferrin) sequester micronutrients (such as iron and zinc) from host circulation into non-utilizable storage compounds, thereby reducing their bioavailability (Keusch & Farthing, 1986; Kochan, 1973; Peto & Hershko, 1989).

Nutrient Excretion

The predominantly catabolic response of hosts to infection leads to a net increase in the wastage of nutrients to excretion (Keusch & Scrimshaw, 1986). Clearly, the host response to infection, largely mediated by IL-1, produces a rapid and marked reduction in host nutritional status, whilst simultaneously potentiating immune activity (Figure 1, overleaf).

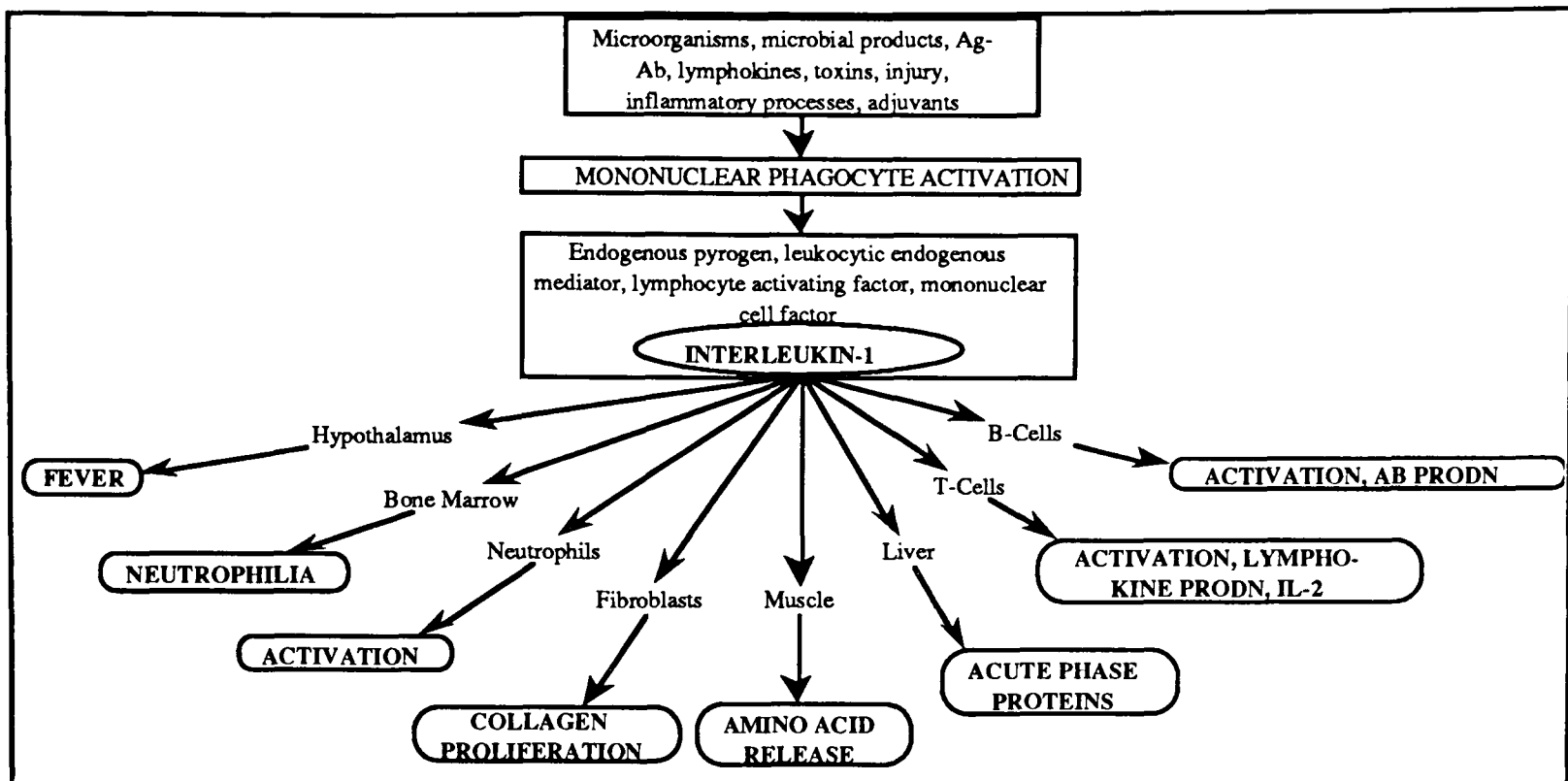


FIGURE 1. Infection-induced IL-1 release potentiates immune activity whilst simultaneously altering host metabolism, resulting in significant reductions in nutritional status (Ag=antigen; Ab=antibody; AB=antibody) (adapted from Keusch, Cerami & Takaku, 1990 and Keusch & Farthing, 1986).

Nutrient Diversion

Nutrients otherwise required by the host may be diverted to fuel expensive biosynthetic functions in response to parasitic infection and disease; examples include immunity (Hall, 1985; Keusch & Scrimshaw, 1986) and tissue repair (Dreizen, 1979).

Secondary Infections

Parasitic disease can change the host in such a way as to reduce its active defences to, and/or increase its suitability for secondary infections (Coovadia, 1988; Klein, 1990). In serious cases of disease, induced nutritional stress can lead to a reduction in host immunity (Coovadia, 1988), which is nutrient-dependent (see, for example, Chandra, 1988). A fall in immune defences reduces the host's ability to actively resist subsequent infections as well as control existing, subclinical infections. For example, some protozoan parasites can rapidly multiply and become pathogenic in the immunocompromised host (Ellner, 1985).

Other parasites actively immunosuppress their hosts by various mechanisms (Ellner, 1981; Ellner, 1985). Among numerous examples, acute *T. cruzi* infection can non-specifically suppress both the humoral and cell-mediated immune responses (Nogueira *et al.*, 1985), with obvious implications for active host resistance to secondary infections.

As noted previously, parasites have precise requirements in terms of nutritional, physical and chemical conditions. It has been shown that nutritional stress secondary to parasitic disease may render conditions within the host more suitable for extant organisms, with a resultant increase in their numbers and the evolution of disease. For example, the intestinal damage caused by some gastrointestinal parasites can increase gut acidity, leading to bacterial

overgrowth and accompanying nutritional stress (Mata, 1985). Clearly, secondary infection can only add to the host's nutritional burden by increasing the amount of nutrients lost to direct uptake by parasites and by compounding the nutritional costs of the diseases they induce.

Host Responses to Infection: A Paradox

In summary, pathophysiological host responses to parasites can radically reduce host nutritional status by impairing food intake, altering metabolic dynamics, and increasing nutrient waste (Figure 2). This is a well studied area, and indeed parasites have been clearly implicated as major contributors to nutritional problems in developing countries (McGregor, 1988; Stephenson & Holland, 1987).

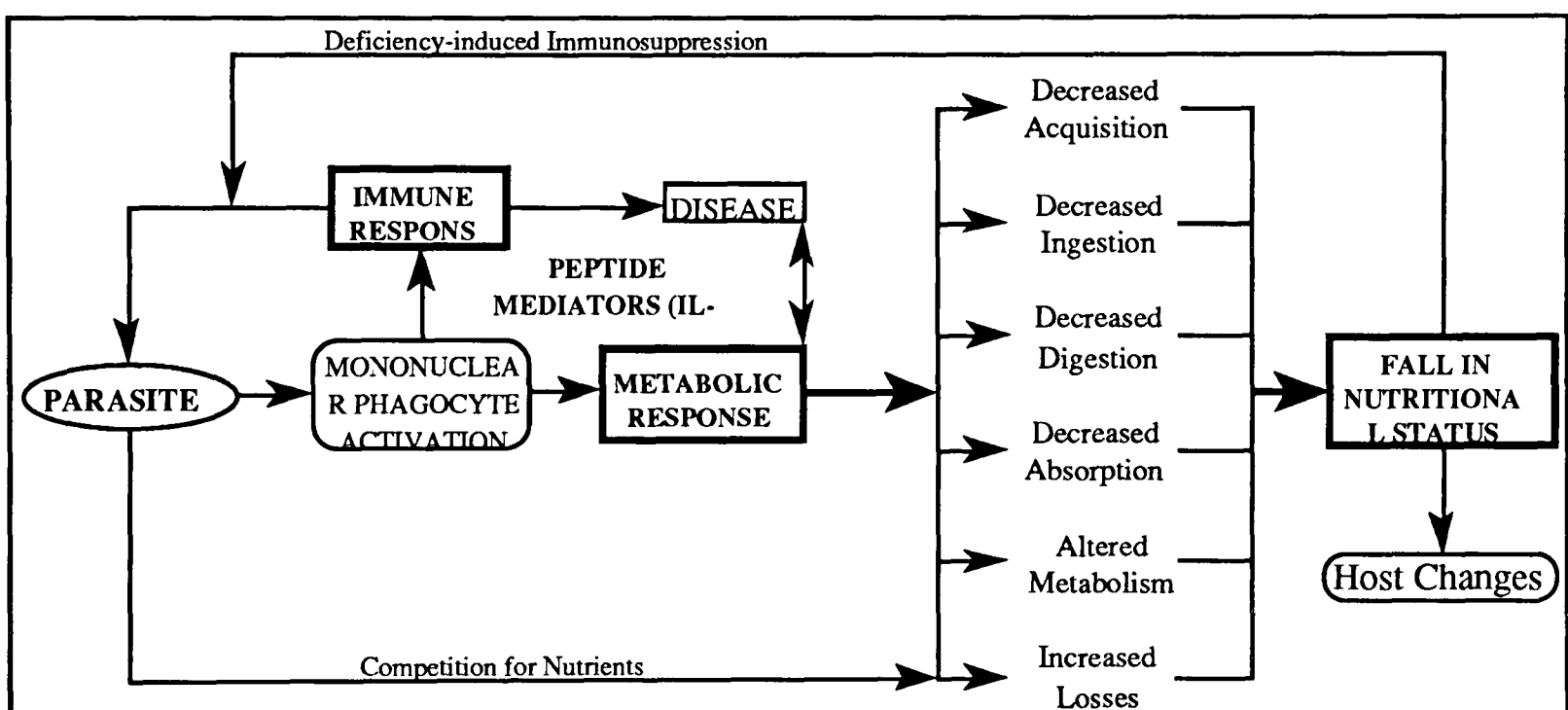


FIGURE 2. Summary of the effects of parasitic infection and disease on host nutritional status; when infection leads to undernutrition-induced immune suppression, what controls parasite exploitation? Perhaps host changes...

However, the impact of parasitic infection on the host contains an intriguing, yet unexplored paradox. Having detected infection, the immune recognition system responds simultaneously in two ways: the first, and well described response is to potentiate the immune system by releasing amplifiers which set in motion the complex immune actions designed to attack invading parasites (Klein, 1990).

The second, and virtually unexplored, effect of immune recognition of non-self is the metabolic response to infection (see Keusch & Farthing, 1986). Lymphokines (and perhaps some of the many other soluble factors) released by activated macrophages initiate a complex series of metabolic events within the infected host which combine to produce an acute and marked fall in host nutritional status by reducing nutrient intake, increasing metabolic requirements, decreasing metabolic efficiency and precision, and increasing excretion (Keusch & Farthing, 1986; Klein, 1990). This host-mediated reaction appears largely responsible for the nutritional costs of infection.

Thus, precisely when they require maximum nutritional resources to fuel expensive immune defences, it appears that hosts respond to infection by mobilizing immunity, whilst simultaneously producing a marked *reduction* in host nutritional status. That such an apparently inappropriate response to infection occurs, yet nutritionally disadvantaged, and therefore immunosuppressed hosts are not overcome by their parasites, suggests there exists a mechanism in addition to immunity which limits parasite exploitation.

As parasites depend upon their hosts to provide stable food and shelter, the answer may lie in the effects which deteriorating host nutritional status has on their environment and thus the parasites themselves (Figure 2). Undernutrition can result from either internally induced responses, such as those described above, or from qualitatively and/or quantitatively insufficient food intake. As the majority of work in this area addresses the effect of externally imposed nutritional changes on the host/parasite association, the following discussions focus exclusively on this aspect.

Whilst hosts, and particularly humans, are of parasitological interest primarily because of the diseases they suffer, it must be remembered that hosts are simultaneously reservoirs of infection. The impact of host nutrition on parasites is thus important in two respects: (1) host nutrition potentially influences parasite survival and reproductive success, which shapes parasite transmission dynamics, and (2) host responses shaped by nutritional status combine with infection dynamics to determine disease expression, our ultimate concern. It is therefore instructive to examine the effects of host nutrition on both parasite success and the expression of disease.

1.3: THE EFFECTS OF HOST NUTRITIONAL STATUS ON PARASITES

As described above, parasites inevitably influence host nutrition. However being a bidirectional association, the nature of the host resource is, in turn, of pivotal importance to parasite survival and reproductive success. This aspect of the host/parasite relationship remains virtually unexplored since it is tacitly assumed that malnourished hosts suffer greater parasitic disease severity than well-fed hosts simply because their compromised immune defences are less able to defeat infection (Bundy & Golden, 1987).

However the dependence of parasites on their hosts to provide a stable environment (Bryant & Behm, 1989; Hall, 1985), and the fundamental changes in that environment which result from the fall in nutritional status and the induction of disease (Holmes, 1987; Torún & Viteri, 1985), suggest that such an assumption may be overly simplistic.

Once host contact is made, the survival and reproductive success of parasites depends upon the ability of the host to marshal effective defences. The influence of changes in host nutritional status on parasite success is therefore likely to reflect the effects of host nutritional status on the host defences.

Host Defence: I. Immunity

The mammalian immune system comprises a complex array of host defences which obstruct foreign material from entering the body, or recognize and remove it once inside (Klein, 1990). It is highly biosynthetic in nature, and as such is nutritionally expensive to assemble, maintain and deploy (Hall, 1985). Various forms of malnutrition differentially impair components of the immune system (Chandra, 1988; Dreizen, 1979; Keusch, Wilson & Waksal, 1983; Suskind, 1977; 1980), which is considered to be the major determinant of parasite success (David, 1985). It is therefore generally recognized that nutritional changes, by modifying immune efficacy, affect the host's ability to actively influence parasite survival and reproductive success.

Although maintaining the complex array of immune responses requires a wide range of nutrients, the following summary of the effects of undernutrition on immune activity focuses primarily on protein-energy malnutrition (PEM), as it is the most widespread nutritional deficiency among human populations exposed to parasitic infections (Solomons & Keusch, 1981).

Antigen Non-Specific Immunity

Also known as Innate or Natural Immunity, antigen non-specific immunity comprises physical barriers and non-specific cellular and non-cellular responses to extraneous material entering the body which are not qualitatively modified by prior antigenic experience (Klein, 1990).

Physical Defences

The skin and mucosal surfaces constitute the host's first line of defence against infection, presenting a physical barrier to invading parasites. Within these barriers, secretions of active substances, such as IgA, mucous and mucosal lysozymes, attack foreign bodies (Klein, 1990). Protein malnutrition limits the biosynthesis required for cellular renewal and maintenance, thus compromising the integrity and secretory abilities of these surfaces and therefore the host's capacity to obstruct and attack invading parasites, and to contain spreading infection (Dreizen, 1979).

Cellular Components

Once internal, parasites are assailed by mobile phagocytic defence cells which ingest or attack identified foreign material. General polymorphonuclear leukocyte (PMNL) function, including motility (chemotaxis), phagocytic capacity, activation of the oxidative metabolic burst and intracellular microbicidal activity and monocyte-macrophage activities, is generally

depressed by malnutrition (Table 2a) (Chandra, 1989; Dreizen, 1979; Keusch, 1985b; Keusch *et al.*, 1983).

Non-specific inflammatory responses dependent upon antigen presentation by T lymphocytes, such as Delayed Type Hypersensitivity (DTH) responses and the killing of intracellular pathogens, may be impaired secondary to T-cell defects resulting from malnutrition (see below). As a result, granuloma and tuberculocidal mechanisms are depressed, and parasite elimination compromised (Keusch, 1985b).

(a) Phagocytes		(b) Complement	
Parameter	Observation	Parameter	Observation
Chemotaxis.....Delayed	Classical pathway activation..... Impaired
Mobilization of marrow reserves.....Depressed	Alternative pathway activation..... Impaired
Particle ingestion.....Normal	Component levels.....Decreased
Lysosomal fusion.....Normal	Regulatory proteins (C1 inhibitor & β 1H)..... Relatively normal
Bactericidal activity.....Decreased (variable)	Opsonic function.....Diminished
Degranulation.....Normal	C3 conversion products.....Present <i>in vivo</i>
Oxidative burst.....Blunted	Immunoconglutinin.....Increased
Iodination.....Impaired		

TABLE 2. Summary of the effects of human protein-energy malnutrition on components of the antigen non-specific immune system (adapted from Keusch, 1985b).

Non-Cellular Components

Complement

PEM simultaneously produces an increase in complement activation and a decrease in its production by limiting protein-dependent biosynthesis, thus leading to rapid fall in complement levels (Table 2b); the result is a deficiency in complement activity necessary for normal phagocyte function, including chemotactic and opsonic functions, and for execution of the classic and alternative pathways to lytic cell killing. The role of complement in amplifying inflammation, by acting as a vascular permeability enhancer promoting the egress of PMNLs from the circulation to sites of infection, is similarly depressed (Keusch, 1985b).

Lymphokines

Lymphokines are non-immunoglobulin secreted products of sensitized lymphocytes and monocytes which amplify and regulate non-specific defences by influencing the behavior of target cells involved in immunity and inflammation (Klein, 1990). As such, a malnutrition-induced fall in lymphocyte and monocyte production and secretion is thought to induce an appreciable reduction in lymphokine activity (Hoffman-Goetz, 1988).

Others

The activity of lysozymes (small protein enzymes with microbicidal activity contained in serum, tears, saliva, sweat, respiratory and intestinal mucous and monocytes), properdin (a

serum euglobulin integral in the alternate complement pathway involved in resistance to infections of bacterial, viral and protozoal origins) and interferon (comprised of various factors active in amplification of effector cells and effective in impairing adenosine triphosphate formation in microbial cells) is decreased during PEM due to the reduction in protein biosynthesis required for their manufacture (Dreizen, 1979; Hoffman-Goetz, 1988).

Transferrin (produced in the liver) and lactoferrin (present in milk, tears, saliva, bile and leukocytes) are protein molecules endowed with inherent antimicrobial properties by virtue of their actively binding free iron required by these organisms into unusable compounds (Keusch & Farthing, 1986). As the production of these components is largely protein-dependent, it is likely that malnutrition which impairs protein synthesis suppresses their production and activity (Dreizen, 1979).

Antigen Non-Specific Immunity Falls with Malnutrition

Malnutrition significantly reduces the integrity of the host's physical defences and the production of active cellular and non-cellular defence components within the tissues by impairing their biosynthesis. It can be stated, therefore, that malnutrition leads to a general depression of non-specific immunity.

Antigen Specific Immunity

The second, and more potent components of the host's immune defence are the antigen specific systems. The humoral and cellular immune systems are qualitatively and quantitatively modified by previous experience with antigens, and provide rapid and targeted responses to infection by memory of antigens previously encountered, and their recognition upon re-exposure (qualitative), followed by subsequent clonal proliferation of specific defence products (quantitative).

Cellular Immunity

Cell mediated immunity (CMI) owes its effectiveness largely to the host's ability to proliferate and concentrate various cells active in destroying invaders. Indicators of cellular immunity activity include the number and proportion of circulating T lymphocytes, lymphocyte transformation and proliferation induced by mitogens and antigens, the production of soluble mediators with immunological properties following exposure to mitogens and antigens, and dermal DTH to a variety of ubiquitous recall antigens or following prior sensitization to specific agents (Chandra, 1983).

Various components of the cellular immune response are compromised during nutritional deficiency, such as PEM (Table 3a, overleaf). The most pronounced influence of PEM on CMI is a marked reduction in the number and proportion of T lymphocyte populations in the

peripheral circulation (Keusch, 1985b). It is thought these changes are associated with involution and abnormalities of areas of the central (thymic) and peripheral (spleen, tonsil, lymph node) lymphoid organs active in T-cell growth, resulting in a marked reduction in thymocyte synthesis, particularly in the organ cortex. This reduction is exacerbated by impaired terminal differentiation and maturation of T-cell precursors (Keusch *et al.*, 1983).

In vitro proliferation of lymphocytes activated by antigens and mitogens during PEM has been reported as either impaired or normal. The most common defect is a depression of the proliferative mitotic response as measured by responses to mitogenic lectins, such as phytohaemagglutinin (Keusch, 1985b).

Information on the production of soluble mediators by sensitized lymphocytes in PEM is limited; although a decrease in production has been implicated, the predominant effect appears to be a reduction in the responsiveness of target cells (Hoffman-Goetz, 1988).

PEM depresses both afferent (sensitization) and particularly efferent (response) limbs of the cutaneous DTH response following injection of recall antigens such as *Candida albicans* or streptokinase-streptodornase, or following neosensitization with Bacille Calmette-Guérin vaccine or the chemical hapten, 2-4-dinitrochlorobenzene (see Keusch, 1985b). As the DTH response is the result of a number of sequential steps (sensitization via antigen presentation by macrophages {activation}, cellular recognition of antigenic determinants {memory}, release of lymphokines from T-cells, and changes in vascular walls enabling migration of inflammatory cells to the distressed site), observed dermal anergy to both primary and secondary challenges suggests that one or more of these processes are impaired during PEM (as outlined above).

(a) T Lymphocytes		(b) B Lymphocytes	
Parameter	Observation	Parameter	Observation
Thymus gland.....Atrophy & lymphocyte depletion. Decreased thymic serum hormone	Peripheral lymphoid organs.....Partial preservation of cellularity of B-lymphocyte regions
Peripheral lymphoid organs.....Cell depletion from T-dependent areas	Circulating lymphocytes.....Normal number
Circulating lymphocytes.....	...Relatively normal numbers but decreased proportion of mature T lymphocytes	Serum immunoglobulins..... Normal to elevated levels
Mitogen responses <i>in vitro</i>Depressed	Secretory immunoglobulins..... Decreased levels
Mediator production.....Inadequate data, but appears to be decreased	Secretory antibody responses..... Impaired
Delayed type skin hypersensitivity.....Impaired, particularly efferent limb	Serum antibody responses..... Variable; may fall secondary to T-cell defects

TABLE 3. Summary of the effects of human protein-energy malnutrition on components of the antigen-specific immune system (adapted from Keusch, 1985b).

Humoral Immunity

Host protection provided by the humoral immune response consists largely of the ability of circulating B lymphocytes to synthesize specifically reactive immunoglobulins in response to

antigenic challenge. These attach to foreign cells, facilitating their attack by macrophages, or through complement fixation (Klein, 1990). Malnourished hosts may exhibit a polyclonal hyperimmunoglobulinaemia due to increased exposure to parasitic antigens (Chandra, 1984), however specific responses are largely unaffected, or depressed secondary to T-cell defects (see above and Table 3b, previous page).

Unlike the general reduction of T-cell producing areas (see above), regions of peripheral lymphoid organs active in B-cell synthesis are largely preserved during PEM; the number of circulating B lymphocytes therefore remains relatively normal. Despite this, serum antibody responses to vaccine probes have been variously reported to be normal or depressed (Chandra, 1984). In such cases, it appears that the largely intact B-cell responses require triggering via the antigen presenting cell, which often requires T-cell help. Therefore the antibody immune response in PEM is often blunted secondary to acquired T-cell defects (see above and Keusch, 1985b).

Secretory IgA and other antibodies in secretions are generally depressed by malnutrition, leading to defective mucosal immune responses. This is thought to result from changes in lymphocyte populations during nutritional stress, although little work has studied these effects (Keusch, 1985b).

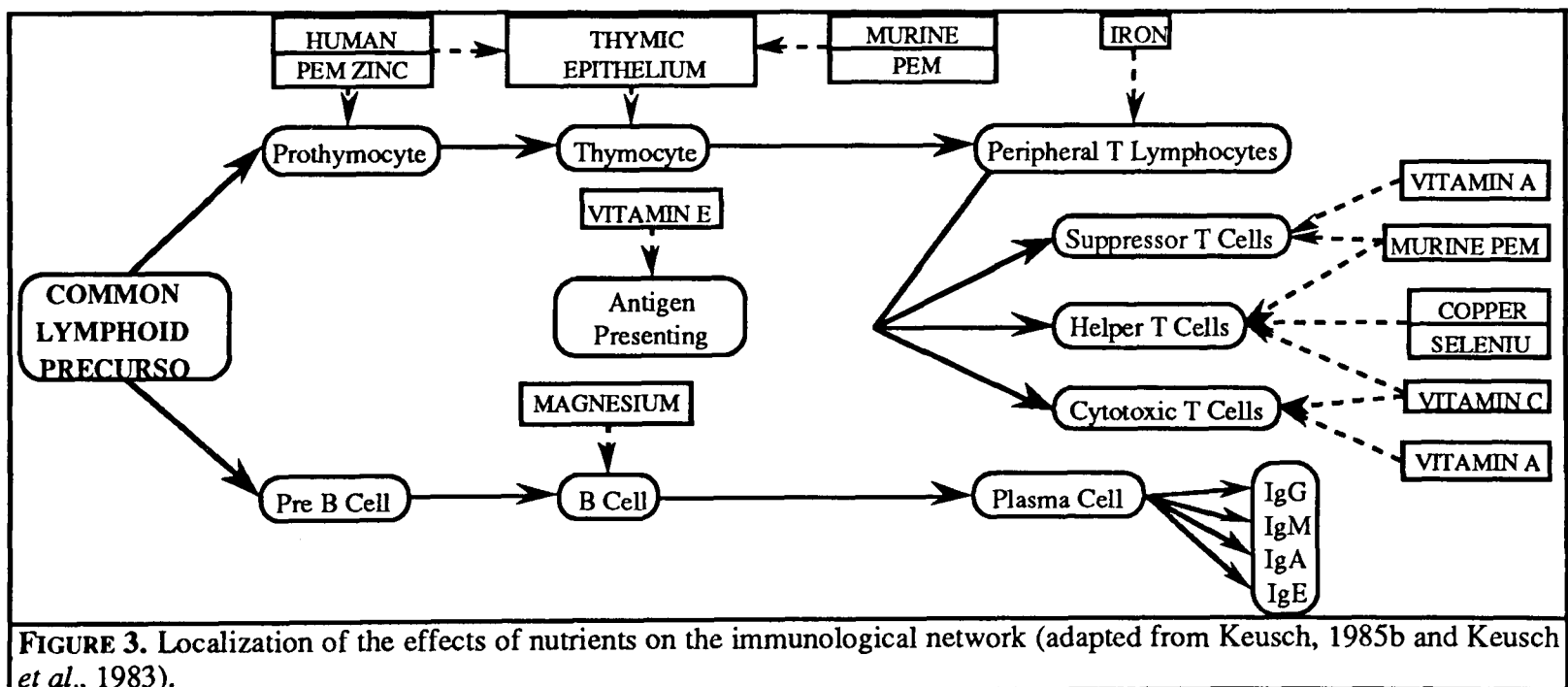


FIGURE 3. Localization of the effects of nutrients on the immunological network (adapted from Keusch, 1985b and Keusch *et al.*, 1983).

Antigen Specific Immunity Falls with Malnutrition

It appears that the most significant changes in immunocompetence induced by PEM are in cell-mediated immunity, the bactericidal activity of neutrophils, the complement system, and the secretory IgA response. Other components of the specific immune responses, such as the induction of immunoglobulin proliferation, may be blunted secondary to these defects. A summary of these effects appears as Table 3 (above). In addition to PEM, previous work has shown that deficiencies of certain other nutrients impair various components of the vertebrate

immune system (Figure 3, previous page) (see reviews by Bendich & Chandra, 1990; Chandra, 1988; 1989; Keusch, 1985b and Keusch *et al.*, 1983).

Host Nutrition & Immunity

Clearly various forms of malnutrition reduce the host's qualitative and quantitative ability to block and actively eliminate parasitic infection by disrupting mucosal and cutaneous integrity, diminishing non-specific resistance factors, reducing phagocytic activity, retarding wound healing, depressing cell-mediated immunity and impairing humoral antibody activation. However deficiency-induced immune suppression is rapidly restored by refeeding (Chandra, 1979).

It follows that the infected host would benefit from maximizing nutritional status in order to optimize immune defences. However observations that the paradoxical metabolic response to infection, which leads to a rapid and marked decrease in host nutritional status (see above), is well preserved across vertebrate taxa (Keusch & Farthing, 1986) suggests that such seemingly deleterious changes may provide some advantage in the face of parasitic infection.

Although it is widely believed to provide the sole reactive defence against infection, host immunity is not always predictive of host resistance (Murray & Murray, 1989). The following section suggests that changes in nutritional and physico-chemical conditions within the host can profoundly influence parasite success.

Host Defence: II. Suitability

Changes in the individual host in response to infection are not limited to the immune system. As described earlier, infection can lead to an acute and rapid decrease in host nutritional status, with resultant changes in internal conditions, by stimulating the release of bioactive peptide mediators from activated macrophages which alter nutrient intake, use and excretion (see above and Keusch & Farthing, 1986). The result of such a response is to produce physico-chemical changes within the host which are identical to those produced by externally imposed undernutrition. It is suggested that such changes in the characteristics of the food and shelter which the host represents to the parasite can compromise the invader's survival and reproductive success.

Nutritional Environment

As parasites inhabit nutrient-rich sites, their metabolic requirements are commonly small in relation to those of the host (see above). A reduction in nutrients and metabolites which continues to sustain the host is thus unlikely to starve the parasite (Rogers, 1962). However as parasites rarely store nutrients, in those cases where the parasite has a greater requirement for a specific nutrient than the host, a low level or absence of this nutrient from the host diet

(or the absence of its metabolites from host tissues) can rapidly lead to parasite malnutrition (Bryant & Behm, 1989; Meshnick & Cerami, 1985). Although parasites may attempt to compensate for deficiencies in their food supply by increasing uptake (Rogers, 1962) or altering body composition (Keymer, Crompton & Walters, 1983b), an inability to meet essential requirements can limit parasite survival and reproduction (Scrimshaw, Taylor & Gordon, 1968).

The plasmodia, for instance, must synthesize essential folates *de novo* because, unlike their human hosts, they are unable to utilize intact dietary folates (McGregor, 1988). If other essential nutrients are available, yet the precursors essential to folate synthesis are not present in sufficient quantities, parasite replication is limited and failing parasite numbers are overcome by host immunity (see Chapter 6 and Ferone, 1977 and Krungkrai, Webster & Yuthavong, 1990).

Indeed hosts can actively deprive parasites of nutrients. Among the numerous metabolic changes induced by lymphokines (such as IL-1) released from stimulated macrophages in response to infection, anorexia reduces food intake (see above and Dinarello, 1990 and Hart, 1988), lowering both nutrient and metabolite availability. Furthermore, in some human communities, traditional treatments include withholding food from ill individuals (Latham, 1975; Lusty & Diskett, 1984; Tomkins & Watson, 1989). In addition to reducing nutrient ingestion, IL-1 stimulates the removal of micronutrients essential to parasite metabolism from general circulation by triggering the release of acute-phase proteins which bind them into unusable storage compounds (see above and Peto & Hershko, 1989).

In addition to the amount of food ingested, changes in food types available to the host can affect parasites by changing their nutritional environment - food may be toxic to the parasite or alter the host in some way which influences parasite success. Human milk presents an important example. Not only does breastfeeding confer protective antibodies to suckling infants, but it contains substances with antiparasitic properties, such as the enzyme, Bile salt stimulating lipase (which can kill the vegetative forms of *Giardia lamblia* and *Entamoeba histolytica in vitro* {Gillin, Reiner & Wang, 1983}).

Hosts can also influence parasite success by altering their dietary intakes in response to infection. In the same way that hosts can avoid foods they associate with illness (Keymer *et al.*, 1983a) thus limiting further infection by the oral route, so too hosts may favour those foods which make them feel better (Johns, 1990). Natural vermifuges and natural anti-protozoans (Bryant & Behm, 1989; Phillipson & Wright, 1991) are obvious examples. The effects of host diet on parasites has been the subject of recent reviews (Crompton, 1987; 1989).

Physical Environment

The complex cellular structure of living organisms is maintained by constant biosynthesis and repair fuelled by continuous cellular nourishment. Reductions in host nutritional status fundamentally alter tissue conditions by disrupting these functions (Dreizen, 1979). Where tissue alterations affect physical attributes of the host on which parasites depend, or to which they are adapted, nutritional deficiency can influence parasite success (Mettrick, 1980).

Malarial parasites rely on host erythrocytes for food and shelter during schizogony and gametogony. It has been demonstrated that host riboflavin deficiency disrupts normal synthesis of the erythrocytic membrane (Vial & Ancelin, 1989). In contrast to normal cells, the high oxidant stress produced by malarial replication lyses malformed, fragile cells before parasite maturation has been completed; expelled, immature plasmodia are overcome by immune defences and the infection is suppressed (see Chapter 6 and Thurnham, 1985a).

In addition to relying on host structures, many parasites are sensitive to fluctuations in physical conditions because in their stable environment, endoregulatory abilities are often lost (Bryant & Behm, 1989). Fluctuations in such factors as temperature, tissue pH, and fluid and electrolyte balances resulting from protein-energy malnutrition (Torún & Viteri, 1985) may impair parasite survival and reproduction (Dixon, 1989; Kwiatkowski & Greenwood, 1989; Williams & Nesse, 1991). Indeed hosts can actively produce such changes regardless of food availability by the activities of peptide mediators secreted in response to infection, producing anorexia, fever and accompanying sequelae (see above and Hart, 1988; Keusch *et al.*, 1990 and Keusch & Farthing, 1986).

In addition to the direct effects of their environment, parasites may suffer from hyperparasitism promoted by host malnutrition. For instance, the growth of bacteria during malnutrition-induced pH changes within the host (Jackson & Golden, 1978) has been shown to seriously compromise parasite survival and reproduction (Krakower, Hoffman & Axtmayer, 1940).

Biochemical Environment

Malnutrition fundamentally alters biochemical activities within host tissues by limiting the nutrients available to fuel them and by changing the conditions under which they occur (Torún & Viteri, 1985). Many parasites have lost certain essential biochemical processes (termed “streamlining”) because their needs are normally met by the host, and little or no selective pressure exists to retain them (Bryant & Behm, 1989). Where malnutrition changes such activities on which parasites depend, it may influence parasite success.

Malaria provides an example; intra-erythrocytic replication by the plasmodia generates free oxygen radicals. The stress these produce in the erythrocyte is normally reduced by intracellular antioxidants, such as the tocopherols (Vitamin E). If effective antioxidants are in poor supply, unchecked stress lyses the cell before parasite replication has been completed, resulting in the expulsion and immune elimination of immature merozoites. Thus dietary antioxidant deficiencies have been shown to limit malarial replication (see Chapter 6 and Eckman, Eaton & Jacob, 1976).

Nutrition & Host Suitability

Clearly, malnutrition can decrease the host's susceptibility to parasitic exploitation by altering the nutritional, physical and biochemical characteristics of the resource it represents to the parasite. Hosts can actively reinforce these deficiencies or create them independent of food availability by the metabolic response to infection (see above); however a fall in host nutrition, whether imposed or induced, may carry the cost of increased deficiency disease.

Parasite Success & Host Nutrition

For those parasites which multiply within the vertebrate host (microparasites), success consists of initial survival, and more importantly, subsequent replication. For those parasites which do not multiply within the vertebrate host (macroparasites), success consists of initial survival and subsequent sexual reproduction. The success of parasites encountering a host depends upon the ability of the host to maximize immune defences and minimize susceptibility. As described above, the impact of reductions in nutritional status have conflicting effects on these defences: malnutrition may increase host susceptibility to infection by compromising the immune defences, but may simultaneously decrease the suitability of hosts to parasites by making ambient conditions suboptimal for parasite survival and/or reproduction.

Immune Defences

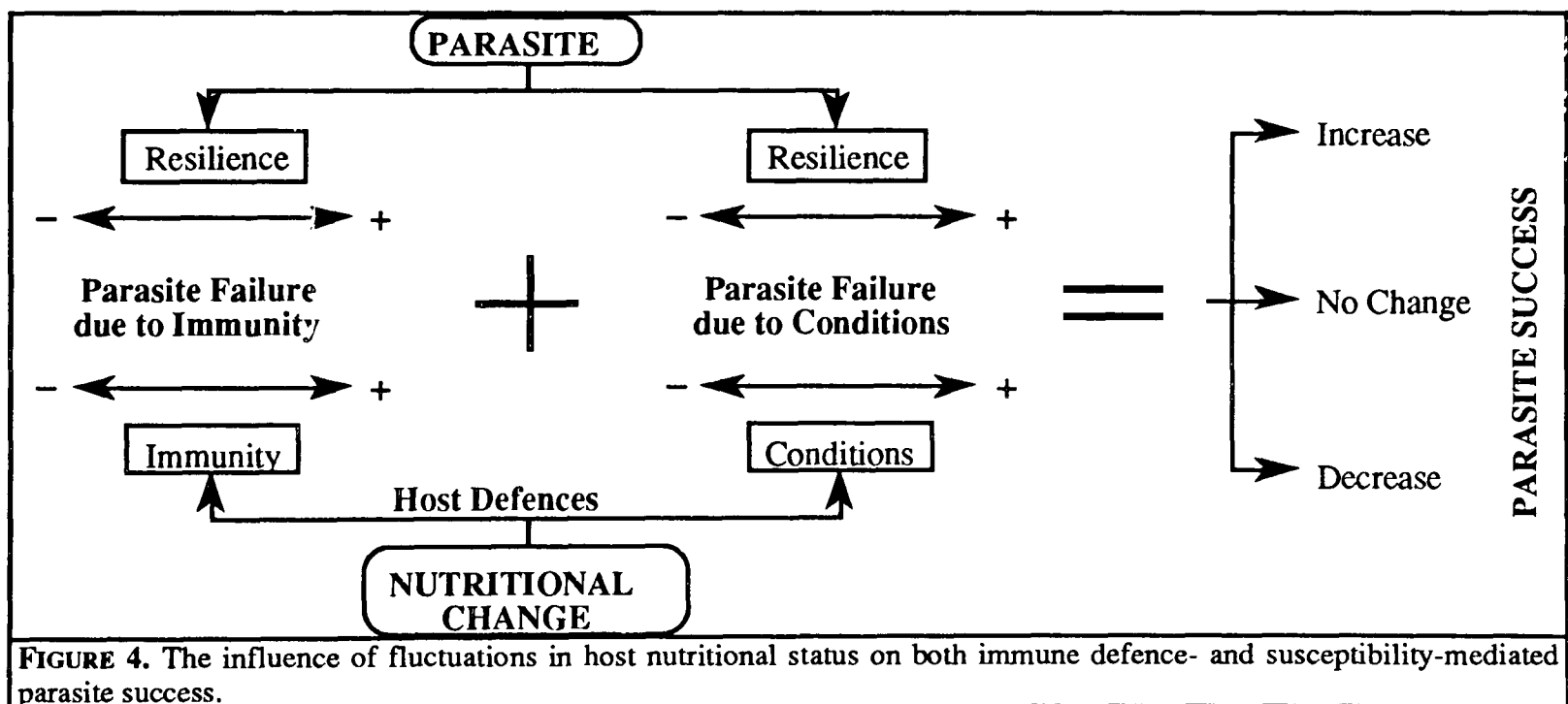
Isolating the effect of the immune defences on parasites, it is clear that a fall in host nutritional status leads to a decrease in immunity and thus an increase in parasite success (see above). This intuitive effect has been clearly demonstrated by the work of Slater (1987; 1988) and Slater and Keymer (1986; 1988) in which malnutrition-induced immunosuppression led to an increase in helminth success within vertebrate hosts. Reversing such a decrease by refeeding can lead to an immune-mediated decrease in parasite success; numerous examples of the benefits of refeeding have been presented elsewhere (Tomkins & Watson, 1989). Although intuitive, focusing solely on the immune component of host defence neglects the influence of ambient conditions on parasite success outlined above.

Host Suitability

Isolating the defensive effects of a change in host conditions on parasitic infection, it appears that a fall in host nutritional status may lead to a decrease in host suitability and thus a decrease in parasite success, as described above. If refeeding alters conditions existing under the prevailing nutritional status, this may lead to an increase in host suitability, and thus parasite success.

Parasite Success & Host Nutrition: The Dichotomy

There appears, in summary, conflicting effects of host malnutrition on host defences: a reduction in host nutritional status has the simultaneous, yet opposing effects of increasing host susceptibility to infection (by reducing immune defences) whilst simultaneously impairing parasite survival and reproductive success (by altering the food and shelter which the host provides for the parasite). Depending on the way host nutritional status affects them, and the parasites resilience to each, malnutrition can therefore lead to an increase, no change, or a decrease in parasite success (Figure 4). It might be expected that refeeding can reverse these effects by restoring immune responses and normal conditions.



Implications

Increases in parasite success with a reduction in host nutritional status, and their reversal during refeeding, are well documented (Scrimshaw *et al.*, 1968). However the thesis that host malnutrition can lead to decreases in parasite success is counterintuitive, and thus worthy of further attention. Although central to parasite population dynamics, parasitic success within the host does not, however, equate in a simple fashion to disease intensity.

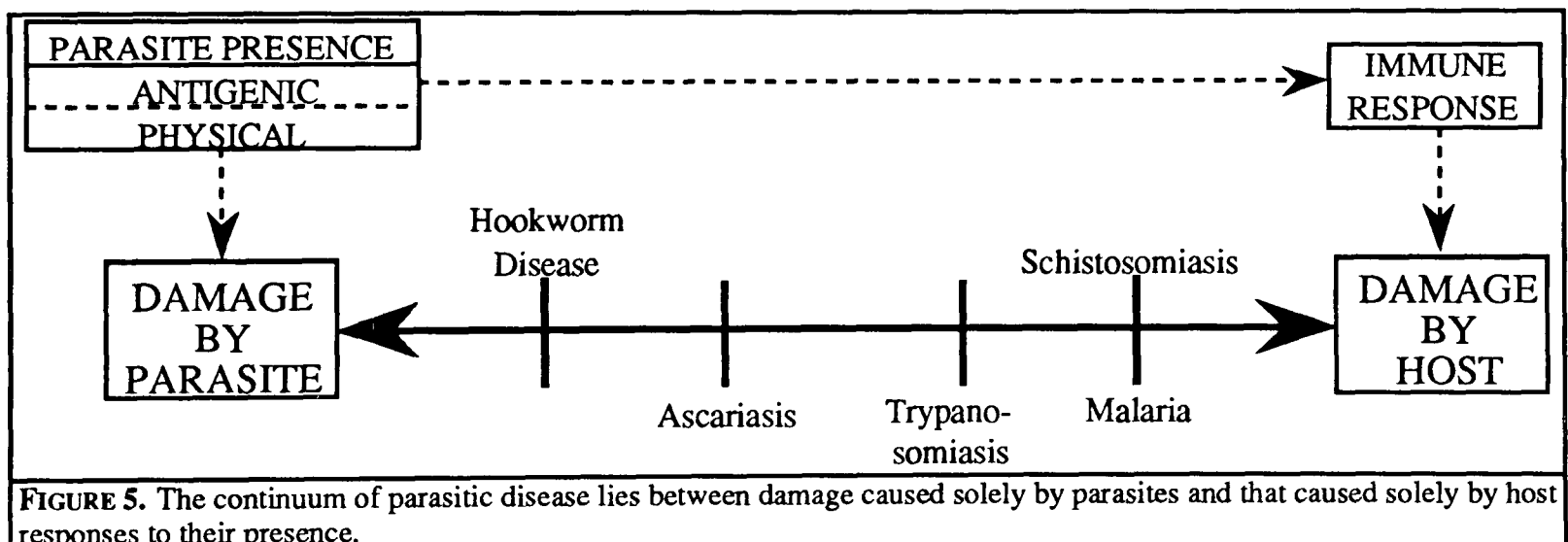
1.4: THE EFFECTS OF HOST NUTRITIONAL STATUS ON DISEASE

Infection & Disease Intensity are Non-Linear

It is an unfortunate fact that the terms “infection” and “disease” are commonly used interchangeably. Infection is a term describing the act of parasitism on a host, whilst disease refers to those changes in host structure and function which accompany infection. By using the terms indiscriminately, it has become implicit that they are simple cause and effect: a change in infection intensity will produce a corresponding (in size & direction) change in disease, whilst any change in disease simply reflects a like change in infection intensity. Such simplistic dogma ignores the complex interaction between host, parasite and disease.

Parasitic Disease

Parasitic disease represents some combination of the damage caused by parasites and that produced by hosts in response to the physical and antigenic presence of parasites. Given the improbability of either damage occurring in isolation (parasites invariably exact some toll from their hosts & hosts invariably respond to infection in some way), it follows that all parasitic disease lies on the continuum delimited by these two extremes (Figure 5).



Any nutritional change in the host produces a new total disease state reflecting the positive, neutral or negative effects of the nutritional change on the parasite- and host-induced components of parasitic disease. In order to elucidate the individual contributions of each disease type to total parasitic disease, it is instructive to look at the likely effects of host nutrition on each.

Damage Done by the Parasite

If we isolate the effects of parasites directly on their hosts, it is likely that the pathologic results of infection are directly proportional to parasite numbers. Changes in host nutritional status will therefore influence direct parasite damage in a fashion approximating its influence on parasite numbers (see above). Although intuitively a major component, direct

damage by parasites, which are generally small in biomass in relation to their hosts, is localized and therefore represents a correspondingly small proportion of parasitic disease. Of far greater importance is both localized and systemic damage incurred by host responses to parasite presence.

Damage Done by the Host

In the majority of infections, host responses to infection are responsible for the greater part of pathology. All other things being equal, if the host did not respond to parasite presence, little damage would ensue, other than the assimilation of host tissue into parasite tissue. Disease severity is thus largely the product of parasite antigenicity and host responses to it. Antigenic challenge being equal (although it may rise or fall - see above), a change in nutrition will thus reflect the change in host immunity which mediates both immunological (immunopathological) and non-immunological (metabolic) changes in host structure and function. The impact of malnutrition on such indirect disease thus depends upon the balance of the effects of malnutrition on these two factors.

If nutritional deficiency reduces immunopathology to a greater extent than it increases deficiency disease, a net fall in disease results. If deficiency disease is increased to a greater extent than immunopathology is reduced, malnutrition produces a net increase in disease.

Although consideration given to this aspect is commonly small or non-existent, their systemic effects and marked ability to alter host structure and function make immune-mediated responses to infection of major importance.

In summary, by altering parasite antigenicity and host responses to it, changes in host nutritional status can produce either an increase or a decrease in host-mediated disease.

Total Parasitic Disease

Total disease therefore rises and falls with changes in nutritional status depending upon parasite susceptibility to changing immunity and host conditions, and the influence of host nutritional status on these factors (see previous section), in combination with the extent of the immune response, not simply immunity as current dogma dictates.

Parasitic Disease & Host Nutrition: The Dichotomy

Therefore depending on the contribution of each form of damage, nutritional changes in either direction can produce increases or decreases in disease. Nutritional changes can therefore produce a dichotomy of disease expression. That disease can increase in response to a fall in host nutrition, and decrease with an increase in host nutrition is well documented (Scrimshaw *et al.*, 1968; Tomkins & Watson, 1989), however the notion that disease can

decrease in response to a fall in host nutrition and rise in response to refeeding remains counterintuitive and largely unexplored.

The ideas presented above suggest that host malnutrition does not inevitably lead to an increase in parasitic disease, and may indeed reduce parasitic disease, regardless of whether parasite success increases or decreases. It is interesting to note that the demonstration of a dichotomy of disease effects following nutritional change in the presence of different parasites may shed some light on the possible derivation of the proverb, "Feed a cold, starve a fever".

1.5: SYNERGY & ANTAGONISM

In their now classic monograph, Scrimshaw and colleagues (1968) examined the interactions of host nutrition and parasitic infection. Although identifying both increases and decreases in disease due to host undernutrition of various nutrients, their failure to recognize changes in infection independent of disease intensity accompanying nutritional fluctuations reinforced the erroneous assumption that these changes are simple cause and effect. Their definitions of disease sequelae thus dealt solely with those infections in which disease severity altered, regardless of parasite success, as described below.

Undernutrition & Parasitic Disease Synergy

It is generally assumed that the influence of host undernutrition on parasitic disease is synergistic: by compromising host resistance and thus facilitating the development of parasites and parasite-induced morbidity, acute undernutrition is thought to produce a total disease state of greater severity than that which might be predicted by the combined effects of parasitism and undernutrition working independently (Beisel, 1982; Chandra, 1979; 1984; Mata, 1985; Scrimshaw, Taylor & Gordon, 1959; Scrimshaw *et al.*, 1968). In synergistic associations, a period of undernutrition exacerbates parasitic disease. As examples, the experimental model for human hookworm infection, *Heligmosomoides polygyrus* in laboratory mice (Slater, 1987; 1988; Slater & Keymer, 1986; 1988) and the lung symbiont of man, *Pneumocystis carinii* (see (Solomons & Keusch, 1981) both display the increased parasite success and host pathology characteristic of disease synergy in undernourished hosts.

In such cases, it is likely that the predominant effect on the parasite is a fall in immunity. The increase in parasite success provided by the fall in immunity outweighs the negative effects on the parasite resulting from changes in host conditions. In synergy, undernutrition exacerbates total disease. This situation is reversed by refeeding.

If a period of undernutrition produces no change in total disease, either immunity and host suitability are unaffected, or they are both equally and oppositely affected; in both cases no net change in disease results.

Undernutrition & Parasitic Disease Antagonism

If a period of host undernutrition leads to a total disease state less than that predicted by the addition of undernutrition and parasitic disease working independently, undernutrition is said to be antagonistic to total disease (Scrimshaw *et al.*, 1968). In such cases, it is likely that the predominant effect on the parasite is a fall in the suitability of host conditions. The decrease in parasite success imposed by the imperfect conditions outweighs the benefits for the parasite provided by reductions in host immunity. In antagonism, undernutrition alleviates parasitic disease; this is reversed by refeeding.

A Definition Revisited

By divorcing parasite success and disease severity (see previous sections), we need to address the impact of changes in host nutrition individually with respect to parasite success and parasitic disease.

Parasite Success

Changes in parasite success are not easy to quantify, however a useful measure is the Intrinsic Reproductive Rate (R_0) which has been defined elsewhere (Anderson, 1986; Anderson & May, 1985; 1991). When parasite success rises as a result of a change in host nutrition, it is suggested that the term “ R_0 -Synergy” be used, and when it falls, “ R_0 -Antagonism”. Reversing the nutritional change will reverse these effects (Figure 6).

		NUTRITIONAL STATUS	
		-	+
PARASITE SUCCESS	+	R_0 Synergy	Reversed R_0 Antagonism
	-	R_0 Antagonism	Reversed R_0 Synergy

FIGURE 6. Terms suggested for use when describing increases and decreases in parasite success which result from changes in host nutritional status.

Disease Severity

When net disease rises as a result of host nutritional change, it is suggested that the term “Patho-Synergy” be used, and when it falls, “Patho-Antagonism”. Reversing the nutritional change will reverse these effects (Figure 7, overleaf).

		NUTRITIONAL STATUS	
		-	+
PARASITIC DISEASE	+	Patho-Synergy	Reversed Patho-Antagonism
	-	Patho-Antagonism	Reversed Patho-Synergy

FIGURE 7: Terms suggested for use when describing increases and decreases in host disease which result from changes in host nutritional status.

Implications

The implications of host nutritional fluctuations producing independent changes in parasite success and host disease are twofold: observing a fall in host disease can mask increases in parasite success (pivotal to parasite transmission dynamics), whilst observing a fall in parasite numbers does not necessarily result in a reduction in disease.

1.6: IN THE FIELD: PARASITES, DISEASE & HUMAN NUTRITION

Coincidence

Poverty is the overriding factor imposing both parasitic infection and undernutrition on the inhabitants of Developing Countries (Bundy & Golden, 1987; Chandra, 1979; 1984; Dunn, 1985; Keusch & Farthing, 1986; Solomons & Keusch, 1981). Poverty largely removes the ability of human communities to dampen fluctuations in food availability resulting from seasonal, catastrophic and man-made factors. Undernutrition is a global problem of distressing proportions, affecting over 800 million people worldwide, and associated with more than 10 million human deaths each year (Stephenson & Holland, 1987).

Poverty also largely compromises effective sanitation, health education and on-going treatment and control of parasitic infections; as a result, the parasites of man enjoy enormous global success in areas exposed to nutritional problems. In addition, undernutrition and parasitic diseases are both chronic conditions, are most common in growing children and tend to occur together in the same individuals (Bundy & Golden, 1987).

Whilst each factor is important in its own right, their frequent coexistence and metabolic intimacy suggest that to view either human nutrition or parasitic infection in isolation is highly artificial (Keusch & Farthing, 1986; Scrimshaw *et al.*, 1968).

Interventions Must Focus on Disease Management

The coincidence of infection and undernutrition in human populations of Developing Countries creates an imperative on more developed countries to lend assistance. The aim of this assistance must be the reduction of net *disease*. This chapter shows that this may not always be achieved by intuitive means. By fully understanding host defence mechanisms, we can design interventions which compliment, not counter, the protection they provide.

Nutritional Interventions

Undernutrition remains the greatest problem in developing countries, and nutritional support is frequently prescribed. The coincidence of undernutrition and parasitic infection suggests that such interventions commonly reach infected people; although the primary aim is to reduce deficiency disease (Kennedy & Knudsen, 1985), an accompanying assumption is that food similarly reduces parasitic disease. This assumption may be fundamentally flawed, because as shown above, disease can increase as a result of refeeding.

Observing an increase in disease with increasing nutritional status entices the simplistic reaction that starving people is unethical; indeed it is, however ignoring the full implications of refeeding is even more so. It must be understood that increasing host nutrition to levels optimal for other human populations (such as those existing in the absence of endemic parasitic infections) may see the displacement of deficiency disease by more severe parasitic diseases if control measures do not accompany food. This aspect of the host parasite relationship forms the basis of Chapter 3.

Treatment & Control Interventions

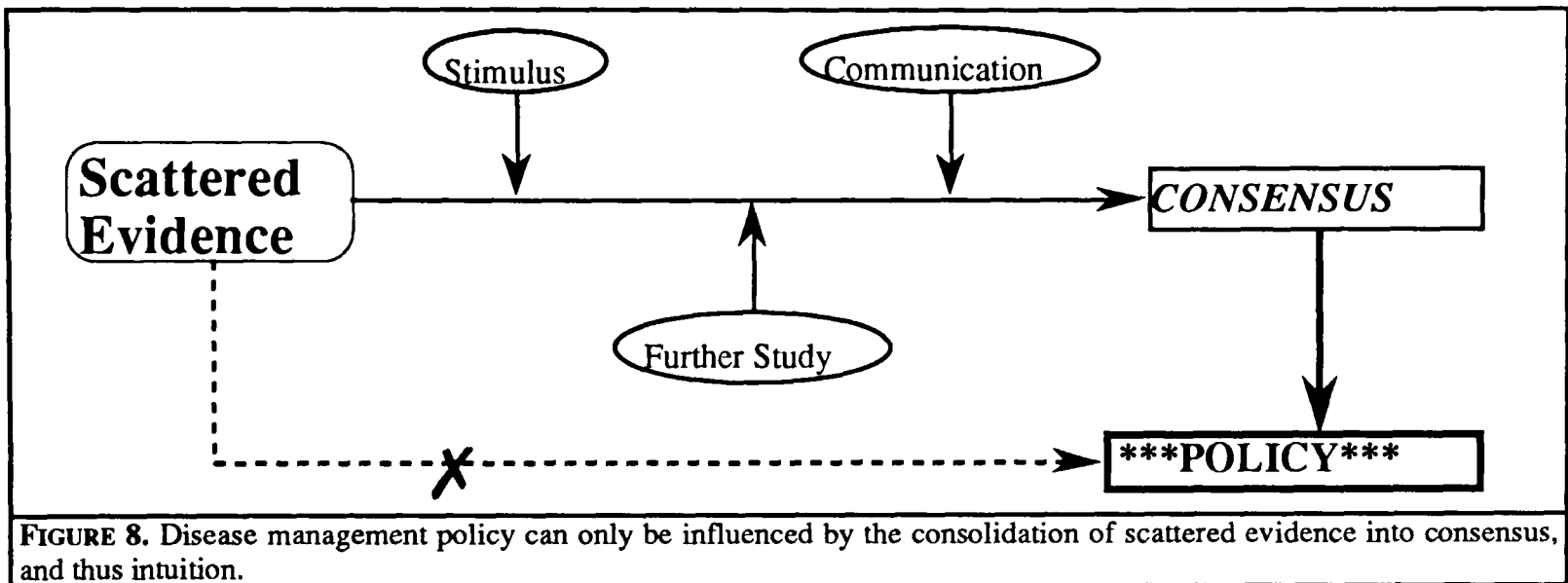
As technical advances continue to facilitate cost-effective interventions, increasing numbers of people receive help in the form of drugs and vaccines. The coincidence of infection and undernutrition in developing regions means that these interventions commonly reach human populations which are immunosuppressed. As the success of patient-based interventions generally rely on host immunity, and as the immune system is greatly depressed by undernutrition, it follows that these interventions may suffer reduced efficacy in undernourished populations. Other drugs effectively starve parasites, and thus host nutritional status will be an important determinant of their activity. This aspect of disease control forms the basis of Chapter 4.

Ineffective Disease Management

It appears that in some cases we perceive protective changes in the host as disease. By treating that disease, it may be that we are removing host protection. If we do not

compensate by replacing natural defences with effective artificial ones, such as drugs and vaccines, parasite exploitation may increase.

We must therefore reconcile the treatment of short term nutritional distress with longer term reductions of total disease. This presents a difficult task, as interventions are judged on their short-term, intuitive effects. However longer term monitoring will be required to reveal the net disease results of specific interventions.



Scattered evidence will not influence health policy; only further study which precipitates consensus will have an impact on the design and execution of health policy in Developing Countries (Figure 8). This thesis is designed to stimulate further work on the effects of deficiency and refeeding, and the efficacy of treatment and control, by examining two diseases implicated as responding in counterintuitive ways to nutritional change. Schistosomiasis and malaria are not mere infections, but the most damaging helminth and protozoan parasites of humans, respectively.

1.7: THESIS

That externally-imposed undernutrition sufficient to produce changes within the host may compromise parasite success and reduce disease expression during some infections. And that the occurrence of these effects has implications for the effective interventional control of certain parasitic diseases.

Aims

The aim of the present dissertation was to gather evidence for the thesis, to carry out experiments which examine certain aspects of it, and to report the findings in an attempt to stimulate discussion, future work and ultimately informed consensus in a relatively unexplored area of Medical Zoology.

Structure

As concerted examination of nutritional effects is almost entirely lacking from the field of parasitology, it was decided to touch upon a number of different areas in order to stimulate discussion on a wide range of topics. Addressing a number of areas has led to the generation of disparate chapters, and thus each is necessarily self-contained. Due to similar underlying themes, this construction has meant that some repetition was unavoidable.

This document consists of three main sections:

Schistosome infection and disease in the vertebrate host are examined in Chapters 2, 3 and 4 which study the nutritional aspects of simple infection dynamics, dietary interventions, and treatment and control interventions, respectively.

Chapter 5 investigates the nutritional relationship between schistosomes and their molluscan intermediate hosts.

Chapter 6 reviews the literature on the nutritional interactions between malarial parasites and their hosts, and is included specifically to allow others access to this collection of literature, perhaps providing a basis for further work in this area.

Chapter 7 concludes the thesis by recapping experimental indications, introducing a novel theorem of host defence, and exploring the implications of the present thesis for optimizing the results of disease control interventions in human communities endemic for both parasitic infection and undernutrition.

CHAPTER TWO

SCHISTOSOMES & VERTEBRATE HOST NUTRITION

SINGLE & MULTIPLE INFECTIONS



“Indeed there is no goodness in the worm”

Shakespeare, *Antony & Cleopatra*



Chapter Summary

This chapter examines the influence of externally imposed host protein deficiency on both parasite and host during the early stages of *Schistosoma mansoni* infection. Previously published results indicate that schistosome development and host pathogenesis are variably reduced in undernourished vertebrate hosts. The present chapter consolidates this evidence and explores the influence of host nutritional status on parasite success and disease development. Experimental results confirm that suboptimal conditions within the undernourished host significantly reduce the numbers of parasites which establish, their sex ratios, size, the proportion of females which are fecund, the proportion of eggs which are normal and their size, and the number of eggs which successfully undergo tissue migration. Hosts which become infected with schistosomes benefit from previously established undernutrition as a result of decreased granulomata formation and organ inflammation producing lower morbidity. These results have implications for human populations exposed to endemic undernutrition and schistosome infection.

2.1: INTRODUCTION

Members of the family Schistosomatidae are dioecious Digenea which inhabit specific blood vessels of their avian and mammalian hosts, and which require a phase of asexual replication within a molluscan intermediate host to complete their lifecycle (Beaver, Jung & Cupp, 1984; Rollinson & Southgate, 1987). The present chapter examines the nutritional interaction between schistosomes and their vertebrate hosts in terms of the criteria described in Chapter 1. Although this, and the following three chapters refer largely to work on mansonian schistosomes (which induce the hepato-splenic form of schistosomiasis), the broad similarities in parasite development and disease aetiology observed between *S. mansoni* and other schistosome species permit general extrapolation of many of the following observations.

The schistosomes are well studied due to their medical and economic importance; by virtue of the chronic and debilitating diseases they induce, schistosomes are widely believed to be the most damaging helminths of man (Mahmoud, 1984), and second only to malaria in their public health importance in tropical and sub-tropical areas (Iarotski & Davis, 1981; Maddison, 1986).

Schistosomes & Host Nutrition: Mutually Inclusive

It is well established that schistosomes have a significant impact on the nutritional status of their hosts, and excellent reviews have appeared elsewhere (Stephenson, 1989; Stephenson &

Holland, 1987; Stephenson, Latham & Mlingi, 1986). However as obligate endoparasites, schistosomes are also entirely dependent upon their hosts to provide suitable food and shelter. Thus schistosome infection and disease (von Lichtenberg, 1987; Warren, 1987), and the nutritional stress which results (Stephenson & Holland, 1987), commonly affect the characteristics of the resource upon which the parasites themselves depend. The intimate, and fundamentally nutritional nature of their relationship suggests that examination of schistosome infection or host nutrition must include consideration of the other. In attempting to understand the complex relationship between schistosomes and host nutrition, it is useful to begin by examining the effects each has upon the other.

2.2: THE EFFECTS OF SCHISTOSOMES ON VERTEBRATE HOST NUTRITION

By their definition as parasites, schistosomes impose nutritional burdens on their hosts; in addition to competing with the host for nutrients, schistosomes frequently induce disease which may impair the host's ability to satisfy nutritional requirements (Akpom, 1982; DeWitt, 1962; Kinoti, Latham & Oduori, 1986; Stephenson, 1989; Stephenson & Holland, 1987; Stephenson *et al.*, 1986).

Competition for Resources

Mansonian schistosomes inhabit the mesenteric-portal vasculature of their vertebrate hosts (Beaver *et al.*, 1984). Despite a voracious appetite for erythrocytes and other nutrients, their small biomass in relation to that of the human host (even in relatively heavy infections), and the nutrient-rich site of infection, suggest that parasite uptake is of minor importance in terms of host nutrition.

Schistosomal Disease

By far the greatest nutritional costs of schistosome infection occur when the antigenic and physical presence of schistosomes elicit host responses which produce structural and functional changes within the host. These changes, manifest as disease, commonly impair the host's ability to acquire, utilize and retain nutrients.

The initial nutritional impact of schistosome infection occurs when macrophages activated by schistosome antigens release peptide mediators (Colley, 1987), such as the Interleukins, which induce acute metabolic changes (Keusch, Cerami & Takaku, 1990; Keusch & Farthing, 1986). As described in Chapter 1, this systemic response leads to a rapid and marked reduction in host nutritional status by altering the intake, use and excretion of nutrients (see also Table 1, overleaf). Subsequent and chronic immunopathology reinforces nutritional reductions when localized tissue damage impairs the host's physiological ability to meet essential nutritional requirements (Stephenson, 1989; Stephenson & Holland, 1987; Stephenson *et al.*, 1986) (Table 1, overleaf).

Phase	Event	Clinical Features	Nutritional Outcome
Invasion.....	Cercarial penetration & dermatitis.....	<p>Papular rash, intense itching.....</p> <p>Fever</p> <p>Weakness</p> <p>Cough</p>	<p>?Decrease food intake</p> <p>Increase nitrogen loss</p> <p>?Decrease food intake</p>
Maturation, early oviposition..... (Acute Phase)	Egg production & allergic reaction..... & early granuloma formation	<p>Eosinophilia.....</p> <p>Fever</p> <p>Weakness</p> <p>Lassitude</p> <p>Muscle pain</p> <p>Anorexia, weight loss</p> <p>Nausea</p> <p>Vomiting</p> <p>Diarrhoea</p> <p>Dysentery</p> <p>Abdominal pain</p> <p>Splenomegaly</p> <p>Hepatomegaly</p> <p>Ulceration of GI mucosa, blood loss in stool</p> <p>Pulmonary or cerebral manifestations</p>	<p>Increase nitrogen loss</p> <p>?Decrease food intake</p> <p>Decrease nutrient intake</p> <p>Decrease food intake</p> <p>Increase nutrient loss & decrease food intake</p> <p>Increase nutrient loss</p> <p>Increase nutrient loss</p> <p>Decrease food intake</p> <p>Increase blood loss</p> <p>Decrease food intake</p>
Established & late infections..... & complications (Chronic & Late Chronic Phases)	Egg production & granuloma formation.... & fibrosis & calcification	<p>Fatigue.....</p> <p>Abdominal pain</p> <p>Intermittent diarrhoea or dysentery</p> <p>Blood loss in stool</p> <p>Bleeding from polyps</p> <p>Bleeding ex oesophageal varices, haematemesis</p> <p>Anaemia (relieved by splenectomy)</p> <p>Severe growth retardation(hormone imbalance)</p> <p>Hypoproteinaemia</p> <p>Oedema</p> <p>Ascites</p> <p>Jaundice</p> <p>Hepatic coma</p> <p>Pulmonary hypertension</p> <p>CNS involvement, epilepsy</p>	<p>?Decrease food intake</p> <p>Decrease food intake</p> <p>Increase nutrient loss & increase blood loss</p> <p>Increase blood loss</p> <p>Increase blood loss</p> <p>Increase blood loss & decrease food intake</p> <p>?Decrease food intake</p> <p>}} - Potential to produce clinical illness which decreases food intake & increases nutrient losses</p>

TABLE 1: Stages of *S. mansoni* & *S. japonicum* infections, their clinical features, and potential nutritional outcomes (adapted from Stephenson & Holland (1987) and Stephenson, Latham & Mlingi (1986)).

Host Responses to Schistosomes: A Paradox

The host response to schistosome infection is largely mediated by lymphokines which set in motion two distinct reactions: the immune system is potentiated, whilst the metabolic response to infection simultaneously produces a marked fall in nutritional status. As outlined in Chapter 1, the occurrence of these two responses appears paradoxical, for it might be expected that hosts would tend to conserve nutritional resources in order to fuel expensive immunity. That such an apparently inappropriate host response to infection occurs suggests there exists a host-mediated mechanism, in addition to immunity, which limits schistosome success.

As schistosomes are entirely dependent upon their hosts to provide stable food and shelter, the answer may lie in the effects which deteriorating host nutritional status have on their environment and thus the parasites themselves. Undernutrition can result from either internally induced responses, such as those described above, or from qualitatively and/or quantitatively insufficient food intake. As previous work in this area addresses the effects of externally imposed nutritional changes on the host/schistosome association, the following discussion focuses exclusively on this aspect.

Although disease remains the primary focus of schistosome research, it must be remembered that hosts are also reservoirs of infection. The impact of host nutrition on the schistosome/host relationship is thus important in two respects: (1) host nutrition potentially influences schistosome survival and reproductive success, which together shape parasite transmission dynamics; and (2) immune responses mediated by host nutritional status combine with infection dynamics to determine disease expression in man. It is therefore instructive to examine the effects of host nutrition on both schistosome success and the expression of consequent disease.

2.3: THE EFFECTS OF VERTEBRATE HOST NUTRITION ON SCHISTOSOMES

The impact of schistosomes on host nutrition has been the subject of enthusiastic investigation (see above), however the converse effect, that of host nutrition on schistosomes themselves, remains largely unexplored.

Current dogma holds that undernourished, and therefore immunocompromised (Chandra, 1984; 1988) hosts are less able to resist schistosome infection (see Chapter 1 and Scrimshaw, 1988; Scrimshaw, Taylor & Gordon, 1959; 1968). However if we consider that undernutrition alters the internal conditions of the host upon which schistosomes are entirely dependent, such an assumption appears overly simplistic.

Most of the evidence reviewed here relates to the use of laboratory animals, and its extrapolation to human infections must therefore remain open to question (Keusch, Wilson & Waksal, 1983). However, as ethical constraints preclude human studies, and given that the development of schistosomes and schistosomiasis in permissive animal hosts closely parallels that seen in man (DeWitt, Oliver-Gonzalez & Medina, 1964; Warren, 1961), consideration of experimental evidence appears to be justified.

Schistosome Survival & Reproductive Success

Schistosomes do not multiply within the vertebrate host, and therefore the number of adults reaching maturity in the host is a simple product of the number of cercarial challenges and the proportion of these which are successful in surviving to adulthood. Having made contact with a potential host, parasite success is determined by host defences. The impact of host nutritional status on schistosome success is therefore likely to reflect the impact of nutrition on host immunity and on the suitability of host internal conditions. A summary of past work on host nutritional effects on schistosomes appears as Table 2 (overleaves).

Cercarial Penetration

Host acquisition by cercariae is to some extent dependent upon snail nutritional status (this aspect of the schistosome lifecycle is dealt with in Chapter 5), however once contact is achieved, vertebrate host defences determine larval survival. Following increased parasite establishment in mice fed diets deficient in antioxidants (DeWitt, 1957b) and low in vitamin A (Parent, Rousseaux-Prevost, Carlier & Capron, 1984), it was suggested that nutrient deficiency may impair antibody-mediated elimination of invading cercariae. In addition, cutaneous inflammation during cercarial invasion is reduced in naive mice fed diets low in protein, and both delayed and reduced upon subsequent reinfection (Coutinho-Abath, 1962). Collectively, undernutrition appears to lower the immune elimination of invading cercariae.

However both protein and vitamin A deficiency also result in a degree of corneal thickening (Keusch *et al.*, 1983) which is thought to render local conditions unsuitable for cercarial survival (Coutinho-Abath, 1962; Turchetti-Maia, Bambirra, Coelho, Moraes e Santos & Vieira, 1983). This may have the compensatory effect of increasing the attrition of invading cercariae.

Schistosomular Development

Several hours after successfully entering the host's skin, cercariae metamorphose to the schistosomular stage, becoming wholly dependent upon the vertebrate host for food and shelter (Lawson & Wilson, 1980a; Wilson, 1987). The rate and degree of schistosomulum development will reflect nutrient availability to the parasite (Oyerinde, 1975; Rumjanek, 1987), suitability of the internal *milieu* and the impact of immune attack.

Year	Host	Nutrient Level	Challenge	Phase	Effect on Host	Effect on Parasite	Author
Single Deficiencies							
Choline							
1965	albino mice	deficient	150 cercs	acute, chronic	no effect	not done	Bhattacharyya, 1965
Folic Acid							
1959	unknown	unknown	unknown	unknown	unknown	decreased development	Maldonado, 1959
Niacin (Factor PP)							
1950	albino mice	deficient ^a	unknown	acute	no effect	not done	Pifano & Marcuzzi, 1950
Pantothenic Acid							
1975	swiss albino mice	zero level	1000 eggs ^b	acute	no effect	not done	Akpom & Warren, 1975b
Protein-Energy							
1949	albino rats	low protein	unknown	acute	decreased conjunctive lesions	not done	Menezes, Coelho & Magalhaes, 1949
1958	white mice	12.8, 20.2% protein	50 cercs i.p.	acute	increased mortality	decreased worm size decreased reproductive development decreased faecal egg output increased egg encapsulation	de Meillon & Paterson, 1958
1959	laboratory mice	low quality protein	unknown	unknown	increased mortality	decreased burden decreased reproductive development decreased oviposition	Maldonado, 1959
1962	swiss albino mice	8, 25, 60% protein	100 cercs	acute	delayed skin reaction decreased skin reaction increased duration of skin reaction decreased acquired responses	decreased cercarial establishment	Coutinho-Abath, 1962
1962	swiss albino mice	8, 25, 60% protein	150 cercs	acute	decreased spleen weight decreased liver weight decreased inflammation decreased liver regeneration decreased granuloma size	decreased faecal egg output	Coutinho-Abath, Magalhaes & Barbosa, 1962
1964	laboratory mice	high carbohydrate	unknown	unknown	decreased fibrogenesis	adversely affected	Zuckerman & Macdonald, 1964

Year	Host	Nutrient Level	Challenge	Phase	Effect on Host	Effect on Parasite	Author
1965	laboratory mice, rats	4% protein	150 cercs per mouse, 450 cercs per rat	acute	no effect	not done	Bhattacharyya, 1965
1967	laboratory mice	low protein	unknown	acute	splenic iron deposits	not done	Ashry, 1967
1969	swiss albino mice	25, 50% calorie-deficient; 4, 8, 12% protein	40 cercs	acute	increased deficiency symptoms decreased weight loss post-patency decreased pathology decreased splenomegaly decreased granuloma sizes decreased liver cell involvement	decreased worm size decreased tissue egg burdens altered worm burdens increased proportion males	Knauff & Warren, 1969
1975	swiss albino mice	50% calorie-deficient; 4, 8, 20% protein ^d	22 cercs	chronic	increased deficiency symptoms decreased organ damage & size decreased pathology decreased mortality increased fibrosis decreased residual disease ^f	decreased faecal egg output increased tissue egg burden ^e decreased egg viability increased tissue egg age	Akpom & Warren, 1975a
1975	swiss albino mice	25, 50% calorie-deficient; 4, 8, 12, 20% protein	1000 eggs ^b	acute	decreased granuloma size decreased host response decreased pathology	not done	Akpom & Warren, 1975b
1975	golden hamsters	protein (zero level)	200 cercs	acute	increased weight loss	decreased worm growth decreased tegumental height increased tegumental irregularities increased tegumental disintegration ^g	Oyerinde, 1975
1976	mice	low protein	unknown	unknown	impaired hepatic regeneration decreased liver inflammation	not done	Coutinho, 1976
1978	swiss albino mice	50% calorie-deficient; 4, 8, 20% protein ^d	22 cercs	chronic	not done	decreased faecal egg output decreased egg viability increased egg mortality decreased egg maturation residual decrease in egg viability ^h	Akpom, 1978
1981	swiss albino mice	4% protein	1000 eggs ⁱ	acute	decreased granuloma size	decreased egg antigenicity	Akpom, 1981
1986	swiss mice	4-5.6% protein	100 cercs	acute, chronic	decreased granuloma numbers decreased granuloma size decreased immunoactivity increased mortality	decreased proportion males decreased worm size decreased faecal egg count decreased burden	Magalhaes, Guaraldo, Zanotti-Magalhaes, de Carvalho, Sgarbieri & de Alcantara, 1986

Year	Host	Nutrient Level	Challenge	Phase	Effect on Host	Effect on Parasite	Author
1987	laboratory mice	4, 12% protein	trickle of 10 cercs.	acute	decreased acquired immunity	increased maturation time decreased growth decreased development	Sithithaworn, 1987
1987	swiss mice	high fat; high carbo-hydrate ^j	100 cercs	acute	decreased gluco-genesis decreased glyco-genesis	not done	Thompson, 1987
Vitamin A							
1940	white rats	zero level	3700-4100 cercs	acute, chronic	decreased parasite destruction altered cellular proliferation decreased lesion resolution decreased hepatopathology decreased immunoactivity vascular changes	increased burden altered site of infection increased size variation decreased size decreased sexual maturity increased bacterial presence decreased fecundity increased mortality	Krakower, Hoffman & Axtmayer, 1940
1983	swiss albino mice	deficient	60-70 cercs	acute	decreased weight gains	decreased development rate decreased egg viability decreased burden ^k	Turchetti-Maia, Bambilra, Coelho, Moraes e Santos & Vieira, 1983
1984	swiss albino mice	zero level	60 cercs	acute	decreased immunoactivity decreased serum IgG decreased inflammation decreased hepatopathology	not done	Aziz, El Raziky, Khalil, El Harrizi & Wassef, 1984
1984	Fisher rats	deficient	1000 cercs	acute	increased mortality decreased and delayed IgE antibody titres decreased humoral immunity	increased burden increased tissue egg load	Parent, Rousseaux-Prevost, Carlier & Capron, 1984
1986	swiss albino mice	zero level	60 cercs	acute	decrease immunoactivity decreased hepatopathology	not done	Aziz, Zaki, Tawadrous, El Harizi, Khalil, Sakla & Shoeb, 1986
Vitamin B1 (Thiamine)							
1950	white mice	deficient	unknown	acute	no effect	not done	Pifano & Marcuzzi, 1950
1975	swiss albino mice	zero level; vitamin analog	1000 eggs ^b	acute	decreased granuloma size ^l	not done	Akpom & Warren, 1975 ^b

Year	Host	Nutrient Level	Challenge	Phase	Effect on Host	Effect on Parasite	Author
Vitamin B₂ (Riboflavin)							
1975	swiss albino mice	zero level	1000 eggs ^b	acute	decreased granuloma size	not done	Akpom & Warren, 1975b
Vitamin B₆ (Pyridoxine)							
1959	unknown	unknown	unknown	unknown	unknown	decreased development	Maldonado, 1959
1975	swiss albino mice	zero level ^m ; vitamin analog	1000 eggs ^b	acute	decreased granuloma size	not done	Akpom & Warren, 1975b
Vitamin C (Ascorbic Acid)							
1944	guinea pigs	various levels	3500-4700 cercs ⁿ	acute	scorbutic symptoms decreased haemorrhage decreased eosinophilia	defective egg shells bacterial damage to caecae decreased fecundity increased egg destruction	Krakower, Hoffman & Axtmayer, 1944
1975	guinea pigs	deficient	10000 eggs i.v.	acute	decreased granuloma formation	not done	Akpom & Warren, 1975b
Zinc							
1983	golden hamsters	injected supplement	200 cercs	acute	reduced hepatomegaly increased splenomegaly	reduced burden	Mansour, Mikhail & Guirgis, 1983
1985	swiss albino mice	zero level	60 cercs	acute	decreased immunoactivity decreased hepatopathology	not done	Aziz, Fahmy, Tharwat, El Harrizi, Salama & Ibrahim, 1985
1988	golden hamsters	normal, x2, x3, x5	unknown	acute	decreased fibrosis up to x3 increased fibrosis over x3	not done	Aziz, Tawadrous, El-Harrizi, Atta, El-Raziky, Khalil & Darwish, 1988
Multiple Deficiencies							
1957	laboratory mice	vitamin E, factor 3 ^o & cystine	150 cercs	acute	decreased host reaction decreased hepatopathology	decreased sexual maturity decreased size decreased fecundity	DeWitt, 1957a
1957	laboratory mice	vitamin E, factor 3 ^o & cystine	150 cercs	acute	see DeWitt (1957a) (above)	increased burden altered sex ratios decreased sexual maturity decreased size decreased reproductive organs decreased fecundity decreased <i>in utero</i> egg viability	DeWitt, 1957b

Year	Host	Nutrient Level	Challenge	Phase	Effect on Host	Effect on Parasite	Author
1961	swiss albino mice	low protein, low fat ^p	70-150 cercs	acute	decreased drug efficacy	decreased size decreased sexual maturity decreased fecundity increased burden	Luttermoser & DeWitt, 1961
1961	swiss albino mice	deficient in protein & lipotrophic substances ^q	140 cercs	acute	no effect ^r		Warren, 1961
1976	mice	regional diets ^s	unknown	unknown	altered inflammation increased portal fibrogenesis	not done	Coutinho, 1976
1980	mice	regional diets ^s	unknown	unknown	altered inflammation increased portal fibrogenesis	not done	Coutinho, 1980
Fasting							
1983	swiss mice, golden hamsters	cyclic & absolute fasting	100 cercs. mouse ⁻¹ , 250 cercs. hamster ⁻¹	acute	not done	decreased glycogen content decreased protein content	Cornford, Diep & Rowley, 1983
1987	swiss mice	absolute fasting	100 cercs	acute	decreased liver glucogenic capacity	not done	Thompson, 1987

Notes

aDiet thought to be deficient in niacin.

bInoculated challenge of 1000 eggs intravenously (i.v.); or 1000 eggs intraperitoneally (i.p.=sensitization) followed by 1000 eggs i.v. Eggs recovered from well-fed donors.

cLacked dietary control comparisons.

d4 weeks of experimental diets, 20 weeks post-infection.

eThought to be due to reduced egg loss from tissues.

fFollowing host dietary depletion.

gResults were site dependent, and reversed upon host dietary repletion.

hResults reversed upon host dietary repletion; residual reductions greatest in most deficient diets.

iInoculated egg challenge of 1000 i.v., and 1000 i.p., followed by 1000 i.v. Eggs recovered from deficient and well-fed donors.

jExperimental feeding of 2 weeks duration, 4 or 7 weeks post-infection.

kReduction not significant.

lAnorexia may have compounded deficiency.

mAuthors unsure of effective deficiency.

nInoculated egg (from scorbatic and well-fed hosts) or percutaneous cercarial challenge.

o "Factor 3" = selenium (Bhattacharyya, 1965).

pBoth diets also lacked choline, inositol, folic acid and vitamin B₁₂.

qDeficient diets begun 5 weeks post-infection.

rHigh host mortality precluded definitive results.

sRegional diets were based on nutrient deficient diets consumed by people from areas of Brazil endemic for *Schistosoma mansoni* infection.

TABLE 2: Chronological summary, by nutrient, of experimental work on *Schistosoma mansoni* infection and host nutrition.

Increases in the time required for schistosomula to reach sexual maturity have been observed in hosts deficient in vitamin A (Turchetti-Maia *et al.*, 1983) and protein (Sithithaworn, 1987). Furthermore, the proportion of worms reaching sexual maturity and/or its extent have been reportedly reduced in hosts fed diets deficient in vitamin A (Krakower, Hoffman & Axtmayer, 1940), antioxidants (DeWitt, 1957a; 1957b), and protein (de Meillon & Paterson, 1958). In contrast, Krakower, Hoffman and Axtmayer (1944) found parasite growth and development to be normal in scorbutic guinea pigs, although the worms produced abnormal eggs. As the various humoral and cellular immune effector mechanisms responsible for schistosomule elimination in the intact host (such as eosinophil-mediated antibody-dependent cellular cytotoxicity - see Colley, 1987 and Hagan, 1987) are likely to be reduced by nutritional deficiency (see Chapter 1), it is suggested that the observed results are associated with changes in the parasite's physico-chemical environment.

Schistosomular Migration

Host nutritional status also appears to affect parasite migration. Infecting hosts deficient in vitamin A (Krakower *et al.*, 1940) or protein (Oyerinde, 1975) reportedly influences the final sites of worm establishment. Once established at different sites, it has been suggested that variations in the environment (Oyerinde, 1975) and host defences (Krakower *et al.*, 1940) at these sites induce differential survival of worms. As such effects were not observed in mature parasites (Oyerinde, 1975), it appears that nutrient deficiencies influence the sites of worm establishment and subsequent survival only when present during parasite development, suggesting the cues for successful larval migration are distorted.

Survival to Sexual Maturity

Evidence indicates that host nutritional status may influence schistosome sex ratios. Both increases (Knauff & Warren, 1969) and decreases (Magalhaes, Guaraldo, Zanotti-Magalhaes, de Carvalho, Sgarbieri & de Alcantara, 1986) in the proportion of males establishing in protein and/or energy deficient hosts have been reported. It has been demonstrated that both copulatory status and worm sex influences the ability of schistosomes to withstand the acute nutrient stress (Cornford, Diep & Rowley, 1983) which can cause serious parasite damage (Oyerinde, 1975). DeWitt (1957b) noted that more male worms established when antioxidant deficiencies were imposed subsequent to infection, but not when infections were established in already deficient animals. In addition, de Meillon and Paterson (1958) found male schistosomes to suffer the effects of host protein deficiency to a greater extent than females.

If we assume that host immune efficacy and the suitability of tissue conditions vary inversely with increasing nutritional deficiency, these results support the findings of Cornford *et al.* (1983), which indicate that male schistosomes are more vulnerable to nutritional stress than

are females. Due to the inherent preponderance of male schistosomes in well-fed hosts (Mitchell, Garcia, Wood, Diasanta, Almonte, Calica, Davern & Tiu, 1990), a higher proportion of females than males exist *in copula*. It is suggested that the differences in size and physiology between paired and unpaired worms (Cornford *et al.*, 1983; Rumjanek, 1987) may be the cause of the differential mortality of the sexes during nutrient stress, resulting in the death of a disproportionately high number of males. A change in sex ratios will alter mating probabilities of schistosomes (Macdonald, 1965).

Adult Schistosome Survival

Reductions in worm longevity have been reported in hosts fed diets deficient in vitamin A (Turchetti-Maia *et al.*, 1983) and protein (Magalhaes *et al.*, 1986). Other workers have described increases in parasite survival in relation to deficiencies in vitamin A (Krakower *et al.*, 1940; Parent *et al.*, 1984), antioxidants (DeWitt, 1957b), protein (Knauft & Warren, 1969), protein and lipotropic substances (Warren, 1961) and zinc (Mansour, Mikhail & Guirgis, 1983). As host immunity to adult parasites will be reduced during undernutrition, and given that schistosomes may suffer serious structural damage as a result of nutritional deficiencies (Cornford *et al.*, 1983; Oyerinde, 1975), it appears that observed reductions in survival reflect parasite undernutrition.

In addition, it may be that host undernutrition renders preferred sites of infection suboptimal for parasite survival. For example, schistosomes inhabiting hosts deficient in vitamin A (Krakower *et al.*, 1940) and vitamin C (Krakower *et al.*, 1944) have been reported to succumb more readily to bacterial infections than worms from well-fed hosts. Such infections markedly reduce parasite survival and fecundity. Therefore, even though worm burdens were initially higher in hosts fed diets deficient in vitamin A (Krakower *et al.*, 1940) and vitamin C (Krakower *et al.*, 1944), rates of parasite mortality increased rapidly as deficiency worsened. The time of sampling post-infection can therefore influence observed worm survival in deficient hosts.

On the other hand, it is likely that reported increases in parasite survival in undernourished hosts reflect reduced immune elimination of adult worms in the absence of nutritional deficiency severe enough to produce telling changes in the parasite's physiochemical environment. If the nutritional deficiency is less than that required to influence parasite survival to the extent that they numerically benefit from decreased host immunity, a net increase in adult worm burden may result. Thus the degree of nutritional insult may be an important determinant of parasite success.

Adult Schistosome Size

All studies which have reported schistosome size show marked reductions associated with nutrient deficiency, except for vitamin C (Krakower *et al.*, 1944) where no effect was

apparent. Size reductions have been noted in relation to deficiencies in vitamin A (Krakower *et al.*, 1940), antioxidants (DeWitt, 1957b), protein plus various other nutrients (Luttermoser & DeWitt, 1961), as well as during protein and energy undernutrition (de Meillon & Paterson, 1958; Knauft & Warren, 1969; Magalhaes *et al.*, 1986). As host immunity might be expected to exert less constraint on parasite growth when reduced by undernutrition, it appears that these size reductions may be the result of deficiency producing conditions suboptimal for schistosome growth. In addition to body size, nutrient deficiencies suppress the development of schistosome reproductive organs (DeWitt, 1957b; Magalhaes *et al.*, 1986), an effect likely to have a marked impact on fecundity.

Schistosome Egg Output

Female worm fecundity, egg survival and tissue migration combine to determine the number of schistosome eggs reaching the external environment.

Female Schistosome Fecundity

Female worm fecundity is reportedly reduced by feeding hosts a diet deficient in antioxidants (DeWitt, 1957b). Fewer than 1% of female worms recovered from deficient hosts had eggs *in utero*, all of which were abnormal. Conversely, virtually all female worms from well-fed hosts contained normal eggs. As adult schistosomes clearly suffer during host deficiency (see above), it is likely that both the organs and the biosynthesis involved in egg production are similarly affected.

Egg Survival

Decreases in egg viability have been reported in parasites inhabiting hosts fed diets deficient in vitamin C (Krakower *et al.*, 1944), antioxidants (DeWitt, 1957b), protein-energy (Akpom, 1978; de Meillon & Paterson, 1958; DeWitt, 1957b) and protein plus various other nutrients (Luttermoser & DeWitt, 1961). Once laid, developing eggs are known to use exogenous metabolites in their nucleic acid, protein and carbohydrate cycles, particularly during maturation and growth (Stenger, Warren & Johnson, 1967; Stjernholm & Warren, 1974), and may require essential nutrients to maintain integrity during immune attack (Krakower *et al.*, 1944). It is therefore likely that host undernutrition decreases egg survival by altering the concentrations of nutrients and biosynthesis required to maintain egg integrity.

Tissue Migration

It has been postulated that antigen produced by the developing miracidium, and exuded through the egg shell, facilitates the passage of eggs through the host tissues and into the faeces by eliciting immune responses which disrupt differentiated host tissues (Doenhoff, Musallam, Bain & McGregor, 1978; Doenhoff, Hassounah, Murare, Bain & Lucas, 1986).

By injecting eggs recovered from deficient hosts into well-fed mice and observing reduced lung granulomata, it was concluded that egg antigenicity may be reduced as a result of undernutrition (Akpom, 1981). Combined with undernutrition-induced immunosuppression (Chapter 1), this could result in a reduction in tissue passage rates, and an increased chance of egg elimination. Reported reductions in the number of eggs in faeces despite increases in tissue egg burdens during host undernutrition (de Meillon & Paterson, 1958; Parent *et al.*, 1984) are thus unsurprising.

Support for the notion that nutritional deficiency retards tissue migration and potentiates encapsulation was provided by the observation that eggs in the tissues of protein-deficient animals are more commonly found in granulomata than those from well-nourished control hosts (de Meillon & Paterson, 1958). Similar findings were reported in relation to vitamin C (Krakower *et al.*, 1944) and vitamin A deficiency (Parent *et al.*, 1984).

Egg Excretion from the Host

All studies which recorded faecal egg production in deficient hosts have reported reductions relative to well-fed controls. Hosts fed diets deficient in vitamin A (Krakower *et al.*, 1940), vitamin C (Krakower *et al.*, 1944), antioxidants (DeWitt, 1957a; 1957b), and protein-energy (Akpom, 1978; Akpom & Warren, 1975a; Coutinho-Abath, 1962; Coutinho-Abath, Magalhaes & Barbosa, 1962; de Meillon & Paterson, 1958; Knauft & Warren, 1969; Magalhaes *et al.*, 1986) produced fewer faecal eggs in total than did well-fed controls. These reductions could reflect decreased fecundity in undernourished schistosomes (see above) and/or increases in egg elimination and/or retention by the host (see above).

Miracidial Development within the Egg

Hatching soon after mature eggs contact fresh water, the free-living miracidium does not actively feed, as it lacks a functional gut (Sturrock, 1987). All the nutrients required for snail location and penetration must therefore be obtained during migration of the egg through the vertebrate host tissues. That eggs use exogenous metabolites (Stenger *et al.*, 1967; Stjernholm & Warren, 1974), and suffer from nutritional deficiency (Akpom, 1981; Krakower *et al.*, 1944), suggests that vertebrate host nutrition might influence the infectivity of the miracidium.

Clearly, host nutrition can influence schistosome egg production, survival, development, excretion, and therefore parasite reproductive success. In light of these observations, it may be that worm intensity estimates based on faecal egg counts are as much a function of host nutrition as of schistosome intensity.

Schistosome Success & Host Nutritional Status: Counterintuitive

In summary, schistosome survival and reproductive success appear to be reduced by host undernutrition. Schistosomes inhabit a nutritionally rich environment, and thus despite requiring a wide range of nutrients (Halton, 1967; McManus, 1986), it is unlikely that these effects are due to parasite starvation. In the presence of diminished immune responses, it is therefore suggested that the reductions in survival and reproductive success generally observed in schistosomes inhabiting undernourished hosts result from the deleterious effects of suboptimal physico-chemical conditions induced by undernutrition affecting parasite development, survival and reproduction.

Regardless of parasite success, however, disease must remain the primary focus. As described in Chapter 1, disease severity may not be linearly related to parasite success. Therefore changes in host nutrition do not inevitably influence the expression of schistosomiasis in an identical fashion to their influence on schistosome success.

2.4: THE EFFECTS OF VERTEBRATE HOST NUTRITION ON SCHISTOSOMIASIS

Schistosomes & Schistosomiasis are not Synonymous

There exists an almost universal assumption that disease severity in schistosomiasis is dependent upon parasite numbers (Warren, 1987). As the primary stimulus of chronic schistosomiasis is the parasite egg (Warren, 1961; 1987), numbers of which are likely to be some function of parasite numbers, it is not surprising that disease intensity can commonly be related to infection intensity. However both egg antigen *and* an appreciable immune response are required to produce disease. It follows that a change in one of these factors may alter disease severity independently of the other. For example, immune suppression, either by the administration of immunosuppressive drugs (Domingo, Cowan & Warren, 1967) or by thymectomy (Domingo & Warren, 1967) can suppress schistosomiasis regardless of parasite intensity.

Schistosomiasis

The various forms of schistosomiasis share a common aetiology: as host responses to parasite antigens induce pathology, schistosomiasis is known as an immunologic disease (Warren, 1961; 1987). The effects of host nutritional changes on schistosomiasis are therefore likely to reflect their impact on both parasite antigenicity and host immunity. The results of previous work on the effects of host nutritional deficiencies on schistosomiasis are presented in Table 2.

Acute Schistosomiasis

Cercarial dermatitis

Cercarial dermatitis is a sensitization phenomenon consisting of a transient and relatively harmless focal cutaneous inflammation in response to invading cercariae (von Lichtenberg, 1987; Warren, 1987) which rarely occurs in man following exposure to human-adapted schistosomes (Abdel-Wahab & Mahmoud, 1987). The alleviation of these cell-mediated symptoms by undernutrition (see Chapter 1), which has been shown to delay and reduce cutaneous responses to cercarial challenge (Coutinho-Abath, 1962), will therefore relieve, at best, only minor discomfort.

Toxaemic Phase

The toxaemic stage of schistosomiasis consists of an acute hypersensitivity reaction seen in a small proportion of individuals in response to a high level of antigen exposure resulting from the maturation of first infection, from the initial onset of oviposition, or following massive reinfection. Exposure incites localized and systemic immune responses which can produce severe morbidity (von Lichtenberg, 1987; Warren, 1987). Given that undernutrition suppresses the T and B-cell activation (see Chapter 1) responsible for its onset (von Lichtenberg, 1987), and that schistosome development, fecundity and egg antigenicity (see above) vary with host nutrition, it appears that the clinical extent of toxaemic schistosomiasis is likely to be a function of host nutritional status.

After some 5 or more weeks of heightened responses following the onset of egg production by mature worm pairs, immune activity rapidly falls as a result of modulation (endogenous desensitization; see Hang, Boros & Warren, 1974), and schistosomiasis is said to have entered the chronic phase.

Chronic Schistosomiasis

The extent of pathology in chronic schistosomiasis is a function of the quantity and antigenic quality of tissue eggs, and the degree of the host's ongoing inflammatory response to their presence (Akpom, 1981; Warren, 1975).

Schistosome Egg Antigenicity

As outlined above, the antigenic potential of schistosome infection falls during host undernutrition as the number of eggs and their persistence within the host is reduced. In addition, the observation that schistosome eggs recovered from deficient hosts lead to reduced granuloma sizes when inoculated into well-fed hosts (Akpom, 1981), demonstrates that a fall in their antigenicity may accompany host undernutrition.

However regardless of egg number or antigenicity, an appreciable immune response must occur if disease is to develop. Undernutrition is a potent immunosuppressor (see Chapter 1 and Chandra, 1988; Dreizen, 1979 and Keusch *et al.*, 1983) and much experimental evidence exists to suggest that undernutrition retards and reduces the antigen/immune aetiology of chronic schistosomiasis.

Pathological Host Responses

Host Immune Response to Egg Antigenicity

The immune response to schistosome eggs consists largely of a type IV cell-mediated, DTH reaction to soluble antigen secreted by the developing miracidium (Doenhoff *et al.*, 1986; Warren, Domingo & Cowan, 1967). Immune responses to egg antigens are reportedly reduced in mice fed diets deficient in vitamin A (Aziz, El Raziky, Khalil, El Harrizi & Wassef, 1984; Aziz, Zaki, Tawadrous, El Harizi, Khalil, Sakla & Shoeb, 1986; Krakower *et al.*, 1940; Parent *et al.*, 1984), protein (Akpom & Warren, 1975b; Coutinho-Abath *et al.*, 1962; Magalhaes *et al.*, 1986), vitamin C (Krakower *et al.*, 1944), zinc (Aziz, Fahmy, Tharwat, El Harrizi, Salama & Ibrahim, 1985) and diets based on foods eaten by undernourished human populations in Brazil (Coutinho, 1976; 1980). Mice fed diets deficient in zinc display suppressed immune activity (Aziz *et al.*, 1985; Aziz, Tawadrous, El-Harrizi, Atta, El-Raziky, Khalil & Darwish, 1988), which may explain the observations of Mansour and colleagues (1983) who recorded increased organ sizes in infected mice receiving zinc supplements. Extreme zinc excess during infection can also increase fibrosis (Aziz *et al.*, 1988).

Number & Size of Granulomata in Host Tissues

Prolonged immune stimulation in response to tissue eggs leads to the formation of T cell-mediated granulomatous tissue (Chandra, 1984). The number of granulomata in host tissues is a function of both the number of eggs produced and the proportion which are encapsulated. As discussed above, female worms inhabiting undernourished hosts produce fewer eggs, many of which are abnormal. Further, as abnormal eggs may not produce sufficient antigen (Akpom, 1981) known to facilitate successful tissue migration (Doenhoff *et al.*, 1978; 1986), it is likely that a larger proportion of these eggs will be retained in host tissues.

In contrast to this expectation, the number of granulomata in host tissues has been reportedly reduced by feeding infected animals diets deficient in vitamin A (Krakower *et al.*, 1940) and protein (Magalhaes *et al.*, 1986). It appears that despite the retention of a greater proportion of eggs in undernourished hosts, the combination of reduced worm fecundity, decreased egg survival and impaired immune activity produce fewer granulomata in deficient hosts.

Granuloma size is a critical determinant of clinical hepatosplenic schistosomiasis. Despite similar cellular composition (Knauff & Warren, 1969), granuloma size was reduced in infected hosts fed diets deficient in vitamin A (Aziz *et al.*, 1984; 1986; Krakower *et al.*, 1940), vitamin C (Akpom & Warren, 1975b; Krakower *et al.*, 1944), antioxidants (DeWitt, 1957a), protein-energy (Akpom, 1981; Akpom & Warren, 1975b; Coutinho-Abath *et al.*, 1962; Knauff & Warren, 1969; Magalhaes *et al.*, 1986; Menezes, Coelho & Magalhaes, 1949), thiamine, pyridoxine (Akpom & Warren, 1975b) and zinc (Aziz *et al.*, 1985; 1988).

This effect may reflect reduced antigenicity of individual eggs (see above) and/or reductions of the cell-mediated immunity (Chapter 1) responsible for granuloma formation (see above). By using eggs isolated from well-fed donor animals, Akpom and Warren (1975b) demonstrated reductions in granuloma formation following their inoculation into hosts deficient in various nutrients, thereby implicating the suppressive effects of nutrient deficiency on immune-mediated pathogenesis. It appears likely then that the decreased size of granulomata in undernourished hosts is a result of nutrient deficiency reducing both egg antigenicity and host immunity.

Tissue Disruption

The intestinal pathology, including haemorrhage and polyp formation (von Lichtenberg, 1987), accompanying schistosome infection results from mucosal disruption by, and host responses to parasite eggs during tissue migration. As schistosomes inhabiting undernourished hosts produce fewer, less antigenic eggs, and host immunity is reduced by undernutrition (see above), tissue disruption might be expected to fall with host nutritional status.

Fibrogenesis & Organ Dysfunction

Tissue disruption resulting from granuloma formation is reflected in organ damage, dysfunction (Cornford *et al.*, 1983; Pifano & Marcuzzi, 1950; Thompson, 1987) and the eventual induction of late-stage chronic disease. It has been suggested that the suppression of granuloma formation might avert the development of hepatosplenic disease (Domingo *et al.*, 1967; Domingo & Warren, 1967). In support, it appears that undernutrition not only decreases pathogenesis (see above) but also retards and reduces the fibroplasia and collagen maturation (Dreizen, 1979) central to liver fibrosis.

Organomegalies indicate the extent of organ inflammation (Thompson, 1987). Reduced organomegalies have been recorded in hosts fed diets deficient in vitamin A (Aziz *et al.*, 1986; Krakower *et al.*, 1940) and protein-energy (Akpom & Warren, 1975a; 1975b; Coutinho-Abath *et al.*, 1962; Knauff & Warren, 1969). The observation that a diet high in carbohydrate resulted in reduced fibrosis in deficient hosts was attributed to dietary effects on

the schistosomes (Zuckerman & Macdonald, 1964). Clearly, the rate and extent of chronic disease development is reduced by host undernutrition.

Schistosomiasis & Host Nutritional Status: Counterintuitive

In summary, schistosomes inhabiting undernourished hosts develop more slowly, to a lesser extent, and present less antigenic challenge than those inhabiting well-fed hosts. As a result, they stimulate less immune activity, and in the added presence of undernutrition-induced immunosuppression, induce less disease.

It appears that poor food availability and/or quality can reduce schistosome success and disease expression by rendering ambient conditions suboptimal for the parasite and by suppressing immune-mediated damage, respectively.

2.5: REVERSIBLE ANTAGONISM

Just as a reduction in host nutritional status can protect the host from fulminant schistosomiasis (antagonism between undernutrition and disease), it might be expected that refeeding reverses these effects - restoring conditions within the host and immune responses is likely to produce an increase in parasite success and disease. The effects of both undernutrition and its reversal assume crucial significance in human populations experiencing nutritional fluctuations in the presence of endemic schistosomiasis.

2.6: IN THE FIELD: SCHISTOSOMES, DISEASE & HUMAN NUTRITION

Human schistosomiasis is now endemic in 74 countries (World Health Organization, 1985), and present in 79 countries with a total population of 3 billion. As a result, almost 75% of the world's population lives in areas where schistosomiasis in one form or another is endemic (Mahmoud, 1984), and are thus at risk of infection (Sturrock, 1987).

Conservative estimates have placed the number of people exposed to infection at 500 to 600 million, of which between 200 and 300 million are reportedly infected. Some 20 million cases of clinical disease, and more than 1 million human deaths, are attributed to schistosome infection each year (Akpom, 1982; Crompton & Nesheim, 1982; Stephenson & Holland, 1987; World Health Organization, 1985).

Coincidence

As previously noted (Chapter 1), poverty is the overriding factor predisposing the inhabitants of Developing Countries to both parasitic infection and undernutrition, and thus those most affected are those least able to help themselves. It therefore falls to outside agencies to offer assistance, which commonly takes the form of discrete interventions.

Nutritional Interventions

Food and supplementary nutrients are frequently offered in response to nutritional deficiencies in developing countries (Berg, 1987). Because poverty underlies both undernutrition and infection, nutritional support commonly reaches populations suffering endemic parasitic infection (Bundy & Golden, 1987; Oppenheimer, 1989).

Whilst intuition suggests that interventions designed to reduce deficiency disease will also have the desirable corollary of increasing host resistance to parasitic infection and disease, the evidence presented in Chapter 1 opens this assumption to question. Indeed the evidence reviewed above suggests that, just as deficiency suppresses, so too refeeding may exacerbate schistosomiasis by increasing parasite antigenicity whilst enhancing immunopathologic responses to their presence. Clearly undernutrition requires correction, however it must be understood that in so doing, we potentially displace deficiency disease with increased schistosomiasis. Nutritional interventions and schistosomiasis are examined in more detail in Chapter 3.

Treatment & Control Interventions

Drug and vaccine interventions are increasingly available options for the control of parasitic disease in developing countries. Because poverty underlies both infection and undernutrition (see above), such treatment and control efforts commonly reach undernourished populations. As the drug and vaccine interventions planned for, or currently in use against schistosomiasis rely to some extent on the immune system for their activity (see Chapter 4), it is suggested that undernutrition-induced immunosuppression compromises their efficacy. Treatment and control interventions and schistosomiasis are examined in more detail in Chapter 4.

Intervention = Disease Management

Since Keusch (1982) reached the conclusion that “nutritional interventions can be planned, regardless of the presence of schistosomiasis”, little attention has focused on the nutritional relationship of schistosome and human hosts, other than to repeatedly demonstrate the already well documented deleterious effects of schistosome infection and disease on the host (Stephenson & Holland, 1987; Stephenson *et al.*, 1986). Clearly the most desirable option is to eliminate infection, however schistosome infection persists in many areas of the developing world, and indeed may be on the increase due to man’s development activities (Birley, 1985). Given that we remain unable to avoid this parasite, the major focus of interventions must be on minimizing disease. It is suggested that consideration of nutritional factors, such as those examined in this thesis, will contribute to this goal.

2.7: HYPOTHESIS

That externally imposed host undernutrition reduces schistosome success and disease development during the acute stages of infection by reducing host suitability to parasite exploitation and by suppressing immunopathogenesis, respectively.

Aims of Chapter Two

As protein deficiency is the most prevalent nutritional problem in developing areas (Keller & Fillmore, 1983) endemic for human schistosomiasis (Iarotski & Davis, 1981), the hypothesis was tested by manipulating the protein content of diets fed to mice exposed to single and multiple schistosome infections. The effects of dietary protein on schistosome infection and disease are reviewed with respect to their implications for the field situation.

2.8: MATERIALS & METHODS: THE VERTEBRATE HOST

This section describes materials and methods used in the experiments contained in Chapters 2, 3 and 4. Details of individual studies are contained in the Experimental Design sections within each experiment.

The Organisms

Schistosome

The Puerto Rican (PR-1) strain of *Schistosoma mansoni* Sambon, 1907 (see Beaver *et al.*, 1984 for synonymy) was established in the Department of Zoology, University of Oxford from patent albino *Biomphalaria glabrata* snails (Family Planorbidae, Subfamily Planorbinae) obtained from Dr. S. Ron Smithers at the National Institute of Medical Research (NIMR) at Mill Hill, London, where this highly permissive system has been maintained for a number of years.

Mouse

All mice used in experiments and in schistosome maintenance were of the inbred Agouti strain (CBA/Ca) obtained from Harlan Olac Limited (Bicester, Oxfordshire, UK). Mice were kept in large plastic rodent cages (North Kent Plastic Cages, Dartford, Kent, UK) in groups of 5 or 10. Standard animal house conditions of temperature ($22 \pm 0.2^\circ$ Celsius) and photoperiod (12:12 hours dark:light) were maintained throughout all experiments.

Mice used to passage the parasite for maintenance purposes were fed on standard laboratory chow (SLC) (Modified Porton Combined Diet; Special Diets Services Limited, Witham, Essex, UK), the theoretical composition of which is given in Appendix I. Water and food

were available *ad libitum* at all times. Animals used in experiments, which were 4-6 weeks of age at the start of procedures, were fed synthetic diets as described below.

Parasite Maintenance

Infected snails were maintained in the Department of Zoology at the University of Oxford; all snails were fed *ad libitum* unless otherwise indicated. Snail maintenance is described in Chapter 5.

The semi-natural paddle method (Crombie & Anderson, 1985; Meier & Meier-Brook, 1981) was used to infect mice both for parasite maintenance and for experimental purposes. Individual mice were placed in ventilated, enclosed chambers (diameter 100 mm, height 100 mm) in which 40 ml of preconditioned water¹ had been placed, to a depth of approximately 5 mm. The required number of cercariae were pipetted from a pool of more than 40 infected snails (held under direct bench lighting) and added to the water in the chamber. This was always done in mid-12 hour light phase in order to compensate for any diurnal fluctuations in cercarial output or infectivity. All cercariae were used within 2 hours of emerging from patent snails. After 0.5 hour, mice were removed from the paddling chambers and returned to their cages. Accuracy in the actual number of cercariae used in infections was verified by pipetting several replicates of the required number into petri dishes containing 20 ml water, adding 10 ml 70% alcohol and counting individual cercariae under a dissecting microscope.

Experimental Diets

In all experiments, mice were fed synthetic diets which differed only in respect of the percentage protein (weight for weight). All other ingredients were identical, and the proportions of protein were manipulated by the substitution of starch for casein, thereby keeping the diets theoretically isoenergetic. As mice are able to compensate for energy deficits by altering intakes (Crompton, Walters & Arnold, 1981), this was essential in order to achieve similar dietary, and thus total nutrient, intake. Two diets were used throughout Chapters 2, 3 and 4: the 16% ("high") protein diet was considered to be adequate for normal mouse growth (Slater, 1987), whilst the 3% ("low") protein diet was chosen as it was known to be inadequate for normal mouse growth (see Experiment 2.I). The composition of experimental diets is described in Appendix I.

Mice in Experiment 2.II were fed *ad libitum* from powdered food hoppers (Stainless Steel Powder Feed Hopper, North Kent Plastic Cages Limited, Dartford, Kent, UK) in order to measure food intakes. The hoppers in each cage were refilled each day from a 1 kg container of diet. At the end of each week, all remaining food was weighed and subtracted from the

FOOTNOTE

¹ Prior to use, water was conditioned by 2 weeks of aeration, followed by a further 2 weeks of aeration during which time the water contained a number of guppies. This provided the water with organic material and is thought to remove potentially toxic compounds.

initial stock, and the total group intake calculated; the mean of this figure provided an estimate of weekly intake of food for each mouse. Mice in all other experiments were fed synthetic experimental diets *ad libitum* from four 100 ml glass pots (in each cage) which were cleaned and refilled with fresh diet daily.

Radio-Attenuation of Cercariae

In Experiment 4.I, cercariae were attenuated by exposure to a radioactive cobalt source (^{60}Co) in the Sir William Dunn School of Pathology, University of Oxford. The source emitted gamma (γ) radiation at the rate of 1204 rad/minute at the source-to-cercariae distance used. Less than 30 minutes after their emergence from a pool of 40 infected snails, cercariae were exposed to the source for 40 minutes, thus receiving a total γ -radiation dose of 48.16 kRad. The cercariae were then immediately used to challenge mice during a 30 minute paddle infection described above.

Anthelmintic

In Experiment 4.II, pure praziquantel (2-cyclohexylcarbonyl- $\{1,2,3,6,7,11b\}$ -hexahydro-4h-pyrazin $\{2,1-a\}$ isoquinolin-4-one; marketed as Biltricide[®] by Bayer A.G., Leverkusen, Germany) was administered to mice at the recommended rate (3 doses of 100 mg praziquantel.kg⁻¹ live weight given orally, 4 hours apart). To emulsify the water-insoluble powder, pure praziquantel was homogenized in a 2% Tween-distilled H₂O solution. Praziquantel and dosage advice was provided by Dr. Peter Andrews of The Institute for Parasitology, Bayer A.G., Leverkusen, Germany.

Collection of Results

The following sequence of procedures was performed on each individual mouse in each experiment at the time of autopsy. Relevant results are reported in each experiment.

Faecal Egg Counts

Individual mice were placed in single rodent cages (North Kent Plastic Cages, Dartford, Kent, UK) over collecting grids (false floor) and provided with SLC and water *ad libitum*. After 24 hours, the mice were removed and the faecal pellets weighed on an electronic balance (1404 MP8-1; Sartorius, Göttingen, Germany; accuracy ± 0.01 g) and stored in universal tubes containing 25 ml formal-saline solution¹. In some experiments, individual mice were weighed immediately following the 24 hour collection period. To estimate their egg content, faeces were homogenized in a mortar and pestle and egg counts performed using a previously described concentration method (Knight, Hiatt, Cline & Ritchie, 1976).

FOOTNOTE

¹ Formal-saline solution consists of 10% formaldehyde and 0.85% NaCl in distilled H₂O.

Haematology

Plasma Collection

After warming the animals for several minutes under an 100 W lamp, each mouse was restrained in a bleeding cradle and the end 10 mm of the tail removed with surgical scissors. Blood from each mouse was carefully massaged from the tail-snip into two heparinized capillary tubes (Hawksley, Lancing, Sussex, UK) and one end sealed with Cristaseal® (Hawksley, Lancing, Sussex, UK). The tubes were then spun at 4000 G for 10 minutes in a microhaematocrit centrifuge (Hawksley, Lancing, Sussex, UK). The plasma fraction was stored in a sealed plastic microcentrifuge cup (Alpha Laboratories, Eastleigh, Hampshire, UK) at -20° C until required for antibody analysis.

Antibody Analysis

The mouse humoral immune response was quantified using a conventional Enzyme-Linked ImmunoSorbent Assay (ELISA) (Voller & Savigny, 1981) to detect circulating antibodies specific for prepared *S. mansoni* schistosomulum surface antigen (SSA). The method employed for SSA preparation and antibody estimation appears as Appendix II. As plasma samples within groups were pooled for analysis, the single result obtained for each group at each plasma dilution precluded statistical comparison between experimental groups.

Autopsy

Following faecal collection and bleeding, individual mice were sacrificed by a lethal intraperitoneal injection of 0.2 ml pentobarbitone sodium (200 mg/ml) solution (Euthatal®; May & Baker, Dagenham, Berkshire, UK). This compound is thought to interrupt schistosome adhesion to vascular walls by inducing muscular tenesmus within the parasite, thus facilitating maximal perfusion (Smithers & Terry, 1965). The following autopsy procedure was performed identically on all mice, regardless of infection status.

Worm Recovery

Worms were recovered from each mouse using a modified vascular hepatic-portal perfusion technique (Smithers & Terry, 1965). Following the loss of involuntary reflex after fatal injection (approximately 60 seconds), mice were placed in a large petri dish, opened dorsally and the heart exposed; care was taken not to disturb major blood vessels. A small incision was made in the hepatic-portal vein slightly proximal to the point of mesenteric venule radiation, and 50 ml of citrated saline solution¹ pumped under even and constant pressure

FOOTNOTE

¹ Citrated Saline solution consists of 3% Sodium Citrate and 0.85% NaCl in distilled H₂O.

(100 ml.minute⁻¹) into the left cardiac ventricle using a 27 gauge needle and a 50 ml syringe (Terumo Corporation, Tokyo, Japan).

The mouse was then thoroughly washed in a large beaker of clean tap water, the perfusate in the petri dish irrigated into the beaker, and the mixture allowed to stand for 10 minutes. The entire intestine was immediately removed from the carcass and the mesenteric venules examined for worms under a dissecting microscope. The beaker containing the sedimented perfusate was carefully decanted and all worms removed with a fine squirrel-hair brush (No.6, Pro Arte, UK). The perfusate was thoroughly checked for worms using a dissecting microscope. Worms were immediately preserved in a standard fixative¹ for later counting, sexing and measuring under a dissecting microscope using an ocular micrometer.

Eggs within female worms were measured using an ocular micrometer and, assuming they approximate ellipsoids, egg sizes were estimated by measuring the transverse radius (t) and the longitudinal radius (l) of the egg and calculating the volume of the ellipsoid using the formula $\frac{4}{3} \pi (t)^2 l$.

Organ Weights

After removing extraneous tissue, the liver, spleen and intestine were blot dried on absorbent paper and weighed on an electronic balance (Sartorius). Weights are expressed as a percentage of intact mouse weight. After weighing, the entire anterior liver lobe was fixed and stored in formal saline for later histological examination. The remaining organs were frozen in universal tubes at -20° C for estimation of schistosome egg content.

Tissue Egg Counts

The tissue digestion technique (Cheever, 1968) was used to estimate the number of schistosome eggs present in host tissues. Stored livers² and intestines were thawed, blot-dried, weighed, sliced in several pieces, and each organ placed in separate glass vials containing 25 ml of 5% Potassium Hydroxide in saline solution³. The vials were then placed in an incubator at 37° C for 16 hours, after which digestion was immediately arrested by replacing the supernatant with cold saline. After homogenation of the resuspended sediment, several aliquots were transferred to a petri dish where eggs were measured, counted and total numbers estimated.

FOOTNOTE

¹ Fixitive consisted of 10% Acetic acid and 10% formalin in distilled H₂O.

² All livers lacked anterior lobes which had been removed for histological examination; no compensatory allowances were made for this when calculating the egg content of livers.

³ Saline solution consists of 0.85% NaCl in distilled H₂O.

Histology

Circumoval granulomata in the livers of experimental mice were examined using the criteria of von Lichtenberg (1962), as described in Appendix III. Stained and mounted slides of serial liver sections were viewed under a dissecting microscope. The number of granulomata was counted in each of six serial liver sections and mean scores calculated, whilst granulomata size was similarly estimated using an ocular micrometer. Assuming eggs, and therefore granulomata, approximate ellipsoids, granuloma volume was estimated by measuring the transverse radius (t) and the longitudinal radius (l) of the granuloma and calculating the volume of the ellipsoid (see above).

Statistical Analyses

Data in Chapters 2, 3 and 4, and in Experiment 5.II, were square-root (real values) or \arcsin (\sin^{-1} ; percentage values) transformed to normalize variances (personal communication, Professor R. Hiorns, Department of Statistics, University of Oxford) and allow comparison between multiple groups by Analysis of Variance (ANOVA); results are presented as total degrees of freedom (df) and Scheffe F-Test values (F; * indicates significance at or above the 95% level). The one-tailed Student's T-test was used in some instances to statistically differentiate between groups which appeared to differ when graphed but did not prove so when examined by ANOVA; results are presented as total degrees of freedom (df), T-test values (t) and p values (p ; * indicates significance at or above the 95% level). Proportions were analyzed using the one-tailed Z Test, and results presented as Z values (* indicates a Z value > 1.64 , significant at or above the 95% level).

The range of untransformed data values and the sample size for each experimental group, together with the results of the above statistical tests, are provided in tables. Graphs (based on untransformed data, unless otherwise indicated) are provided for illustrative purposes, and provide estimates of error (confidence intervals) calculated from transformed data: the upper limit of the confidence interval represents the detransformed sum of the mean and standard errors of the transformed data used in the statistical comparisons. The lower extent of the confidence interval represents the detransformed difference between the mean and standard error of transformed data. Estimates of sample optical density (O.D.) recorded during antibody analysis in Experiments 2.III and 4.II were transformed for graphic presentation using the formula $\log(1+O.D.)$.

The two group comparisons contained in Experiment 5.I employed the one-tailed Student's T-test on untransformed data; results are presented as total degrees of freedom (df), T-test values (t) and p values (p ; * indicates significance at or above the 95% level). Graphs in this experiment plot the means of untransformed data, and confidence intervals represent estimates of error (standard error) calculated from untransformed data.

2.9: EXPERIMENTS

The hypothesis that externally imposed undernutrition reduces schistosome success and disease development during the acute stages of infection was tested by manipulating the protein content of diets fed to mice exposed to single or multiple infections. Protein was manipulated, as it is the most prevalent nutritional problem in Developing Countries where schistosomiasis occurs (see above).

The single infections used $100 \text{ cercariae.mouse}^{-1}$ as it had been found during previous studies to provide parasite establishment sufficient to permit statistical comparison between host dietary groups (D. Dale, unpublished data). Multiple infections used lower challenges in an attempt to avoid host death, whilst still providing appreciable schistosome infection.

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: I. ACUTE PHASE PRIMARY INFECTION

Abstract

This experiment examines the influence of externally imposed host protein deficiency on both schistosome success and host pathogenesis following exposure of naive mice to a single cercarial challenge. Results show that host protein deficiency reduces the survival of invading parasites only when established at the time of infection, whilst schistosome growth and reproductive success fell regardless of when deficiency developed. Protein deficient hosts suffered less disease, as measured by reduced liver damage and organ inflammation.

INTRODUCTION

Under natural conditions, schistosomes are thought to infect their vertebrate hosts continually at a low level (Crombie & Anderson, 1985). As they do not multiply within the vertebrate host, the number of schistosomes establishing as adults equates to the number of cercarial challenges and the proportion of these which are successful. Given that each adult schistosome results from a single infection event, it is useful to examine the outcome of a single cercarial challenge of an undernourished host. This experiment was designed to investigate the effects of host protein deficiency on the relationship between schistosomes and their vertebrate hosts following a single exposure.

Hypothesis

Previous experimental evidence led to the generation of the hypothesis that host protein deficiency significantly reduces parasite success and disease in the naive host during the acute stages of schistosome infection.

The influence of dietary protein on both parasite and host was examined at mouse autopsy six weeks after a single exposure to 100 cercariae.mouse⁻¹. Mice belonged to one of two dietary groups: those receiving low (3%) or those receiving high (16%) protein diets. The timing of experimental feeding with low protein diets relative to host infection determined whether host protein deficiency was established at the time of infection or developed during worm maturation.

EXPERIMENTAL DESIGN

One hundred and twenty mice were randomly assigned to two groups of 10, two groups of 20 and two groups of 30 mice housed in large cages each containing 10 animals. All mice were fed SLC for two weeks, after which one group of 20 mice received the 3% protein synthetic diet (D3) whilst the second group of 20 received the 16% protein diet (D16). Two weeks later, animals in the groups of 20 and 30 from both dietary régimes were individually

exposed to 100 cercariae.mouse⁻¹ (I.100; range 96-103¹) for 30 minutes, whilst mice in the two groups of 10 were handled identically in the absence of cercariae. All cercariae were gathered simultaneously from the same group of 40 patent snails.

Immediately following exposure, one of the two groups of 10, and one of the two groups of 30 mice still receiving SLC had that diet replaced by the 3% protein diet, whilst the remaining group of 10 and group of 30 mice had the SLC replaced by the 16% protein diet. Six weeks after infection, a 24 hr faecal specimen was taken from each mouse before autopsy (At) was performed as described in the Materials and Methods section of this chapter. The design of this experiment appears in Table 1.

<i>Week</i>		-4	-3	-2	-1	0	1	2	3	4	5	6
A	(10)	SLC	→	-	→	D3	→	-	-	-	→	At
B	(10)	SLC	→	-	→	D16	→	-	-	-	→	At
C	(20)	SLC	→	D3	→	I.100	→	-	-	-	→	At
D	(30)	SLC	→	-	→	I.100 D3	→	-	-	-	→	At
E	(20)	SLC	→	D16	→	I.100	→	-	-	-	→	At
F	(30)	SLC	→	-	→	I.100 D16	→	-	-	-	→	At

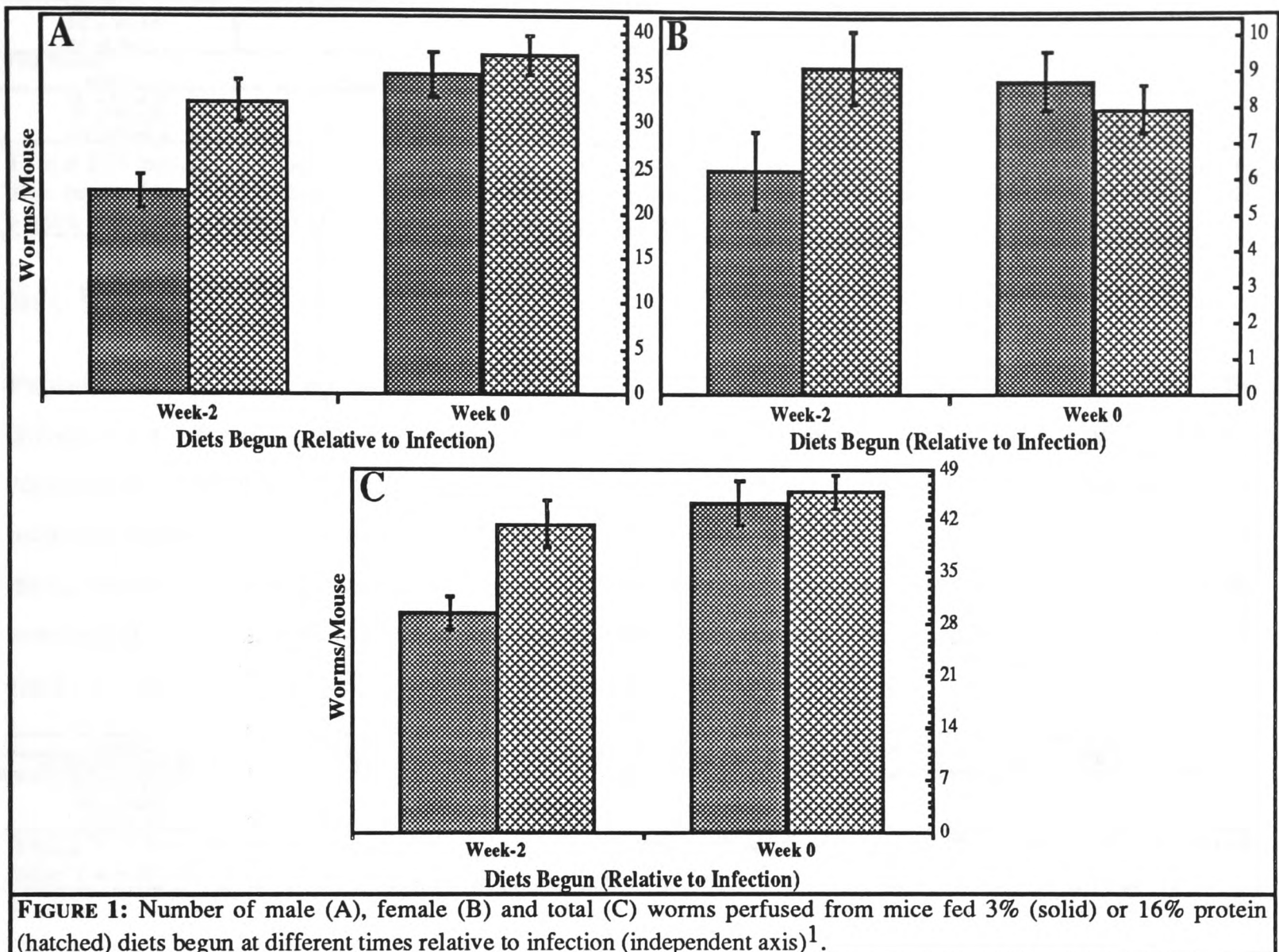
TABLE 1: Design of Primary Infection experiment (n=number of mice in group).

FOOTNOTE

¹ Numbers revealed by replicates of cercarial collection described in the Materials & Methods section of this chapter.

RESULTS: PARASITE

Number



Significantly fewer males established in hosts fed the low protein diet than in well-fed animals, although this only occurred when protein deficiency was established at the time of infection (Figure 1; Table 2, overleaf). Male numbers did not differ between mice fed the high protein diet and those which began the low protein diet at the time of infection. This was also the case for female worm establishment and total worm burdens.

FOOTNOTE

¹ Graphs depict detransformed means of transformed data. Upper and lower limits of confidence intervals depict the detransformed sum and difference, respectively, of the mean and standard error of the transformed data used during statistical comparison of results.

Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
Male Worms				
range ¹ (n)	5-33 (20)	15-77 (30)	19-62 (20)	20-65 (30)
3% at Week -2	-	0.0001*(-4.10;48)	0.001*(-3.51;38)	-
3% at Week 0	-	-	-	0.23(-0.73;58)
16% at Week -2	-	-	-	0.06(-1.60;48)
Female Worms				
range (n)	0-17 (20)	2-17 (30)	3-23 (20)	2-19 (30)
3% at Week -2	-	0.04*(-1.82;48)	0.03*(-1.90;38)	-
3% at Week 0	-	-	-	0.23(0.75;58)
16% at Week -2	-	-	-	0.15(1.06;48)
Total Worms				
range (n)	7-49 (20)	21-91 (30)	22-85 (20)	24-74 (30)
3% at Week -2	-	0.0002*(-3.81;48)	0.002*(-3.06;38)	-
3% at Week 0	-	-	-	0.34(-0.41;58)
16% at Week -2	-	-	-	0.14(-1.12;48)

TABLE 2: T-test comparisons² of mean male, female and total worm numbers perfused from mice fed 3% or 16% protein diets begun either 2 weeks prior to (Week -2), or at the time of infection (Week 0) (number above diagonal represents T-test p value followed by t value and degrees of freedom; n=number of mice perfused for worms).

Sex Ratio

Protein deficiency, by altering worm establishment (see above), produced sex ratio differences between groups. Males comprised a significantly lower proportion of worms recovered from mice begun on both experimental diets 2 weeks prior to, compared with animals begun at, the time of infection. Protein status did not affect sex ratios in mice fed diets begun 2 weeks prior to infection, but males comprised a lower proportion of worms recovered from protein-deficient compared with well-fed mice when diets were begun at the time of infection (Table 3).

Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
males/total worms (%)	76.36	79.74	77.69	82.34
3% at Week -2	-	1.65*	0.58	-
3% at Week 0	-	-	-	1.75*
16% at Week -2	-	-	-	2.69*

TABLE 3: Z Test comparison of sex ratios (male/total) of worms perfused from mice fed 3% or 16% protein diets begun either 2 weeks prior to (Week -2), or at the time of infection (Week 0).

FOOTNOTE

¹ Refers to the range of real values.

² Statistical comparisons between groups were carried out on transformed data sets, as described in the Materials & Methods section of this chapter.

Size: Mean Length

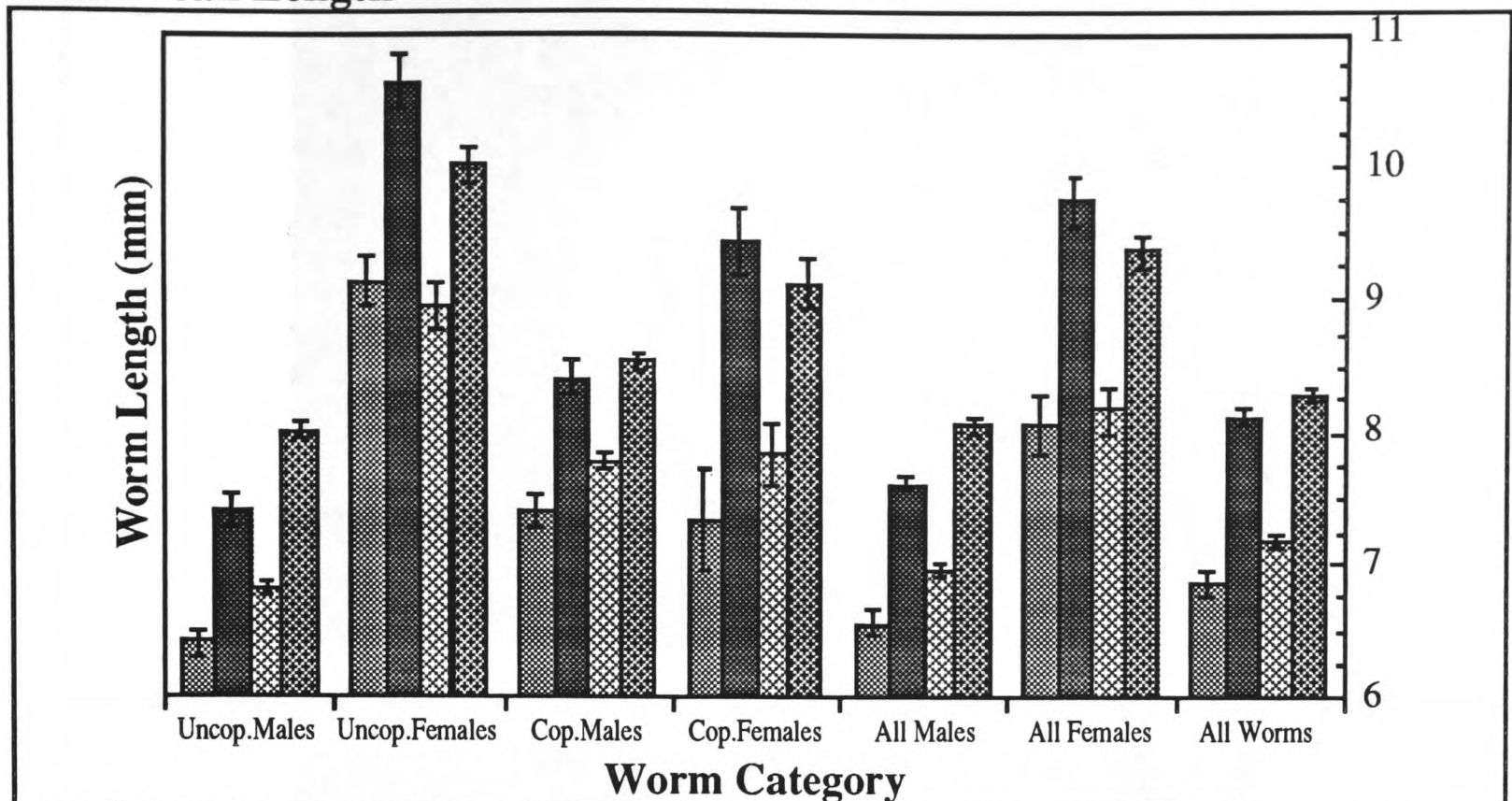


FIGURE 2: Mean length (mm) of all worm categories (independent axis) perfused from mice fed 3% (light) or 16% protein (dark) diets begun either 2 weeks prior to (solid) or at the time of infection (hatched).

Regardless of sex or copulatory status, worms establishing in mice fed low protein diets were significantly shorter than those inhabiting well-fed hosts (Figure 2). Females infecting mice which were well-fed at the time of infection but subsequently developed protein deficiency were not significantly different in size to those infecting mice which were already deficient at the time of infection. On the other hand, established deficiency saw the establishment of smaller males than those infecting hosts which developed deficiency after infection (Table 4). In addition to greater lengths, worms from well-fed hosts were generally observed to be thicker than those from protein deficient hosts (Plate 1, overleaf).

Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
Uncopulated Male Worms range (mm) (n)	0.8-10.8 (420)	1.1-11.9 (933)	1.0-11.5 (424)	0.9-11.9 (1021)
3% at Week -2	-	0.001*(-4.21;1351)	0.0001*(-7.83;842)	-
3% at Week 0	-	-	-	0.0001*(-16.95;1952)
16% at Week -2	-	-	-	0.0001*(-6.33;1443)
Uncopulated Female Worms range (mm) (n)	6.3-11.3 (59)	4.8-12.2 (77)	7.9-13.5 (43)	7.5-13.2 (71)
3% at Week -2	-	0.27(0.62;134)	0.0001*(-5.75;100)	-
3% at Week 0	-	-	-	0.0001*(-4.23;146)
16% at Week -2	-	-	-	0.007*(2.49;112)
Copulated Male Worms range (mm) (n)	5.5-9.4 (82)	5.5-10.9 (201)	6.3-12.5 (135)	6.5-12.1 (179)
3% at Week -2	-	0.001*(-3.11;281)	0.0001*(-7.45;215)	-
3% at Week 0	-	-	-	0.0001*(-7.66;378)
16% at Week -2	-	-	-	0.14(-1.07;312)
Copulated Female Worms range (mm) (n)	0.7-11.2 (82)	0.9-13.4 (201)	1.1-13.2 (135)	0.8-12.8 (179)
3% at Week -2	-	0.107(-1.25;281)	0.0001*(-4.80;215)	-
3% at Week 0	-	-	-	0.0001*(-4.43;378)
16% at Week -2	-	-	-	0.18(0.91;312)
All Male Worms range (mm) (n)	0.8-10.8 (502)	1.1-11.9 (1134)	1-12.4 (559)	0.9-11.9 (1203)
3% at Week -2	-	0.0001*(-4.86;1634)	0.0001*(-9.88;1059)	-
3% at Week 0	-	-	-	0.0001*(-17.68;2335)
16% at Week -2	-	-	-	0.0001*(-5.50;1760)
All Female Worms range (mm) (n)	0.7-11.3 (141)	0.9-13.4 (278)	1.2-13.5 (181)	0.8-13.2 (253)
3% at Week -2	-	0.32(-0.48;417)	0.0001*(-5.62;320)	-
3% at Week 0	-	-	-	0.0001*(-5.61;529)
16% at Week -2	-	-	-	0.045*(1.70;432)
All Worms range (mm) (n)	0.7-11.3 (643)	0.9-13.4 (1412)	1-13.5 (740)	0.8-13.2 (1456)
3% at Week -2	-	0.0002*(-3.61;2053)	0.0001*(-10.95;1381)	-
3% at Week 0	-	-	-	0.0001*(-16.58;2866)
16% at Week -2	-	-	-	0.017*(-2.11;2194)

TABLE 4: T-test comparison of mean worm sizes in experimental mice fed 3% or 16% protein diets begun either 2 weeks prior to (Week -2), or at the time of infection (Week 0) (number above diagonal represents T-test p value followed by t value and degrees of freedom; n=number of worms measured).

PLATE 1: Schistosome Size: Dietary Comparisons

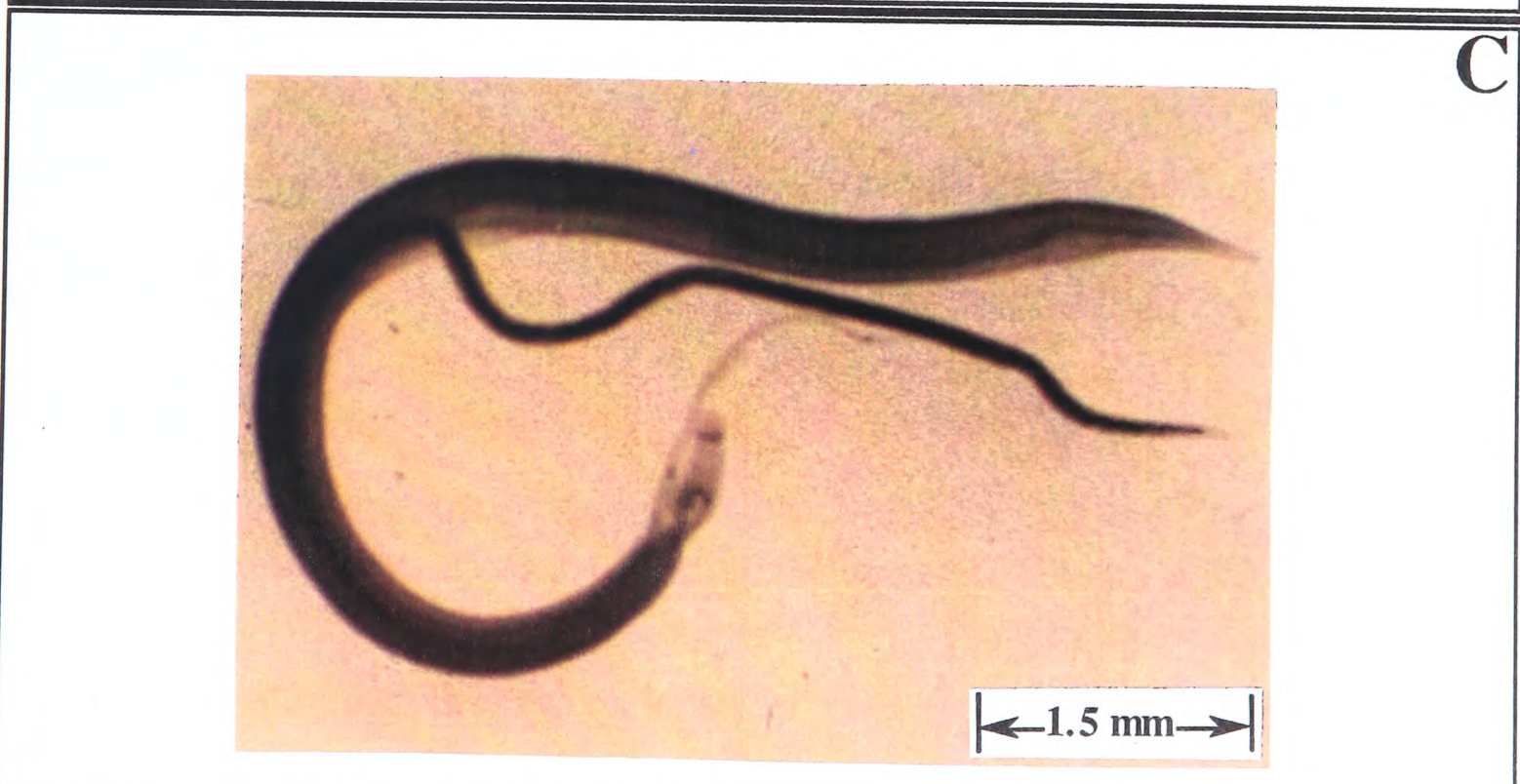


PLATE 1: Fragile female schistosome (A) perfused from a protein-deficient host and robust female (B) and pair *in copula* (C) recovered from a well-fed host (scales as shown).

Size: Frequency Distributions

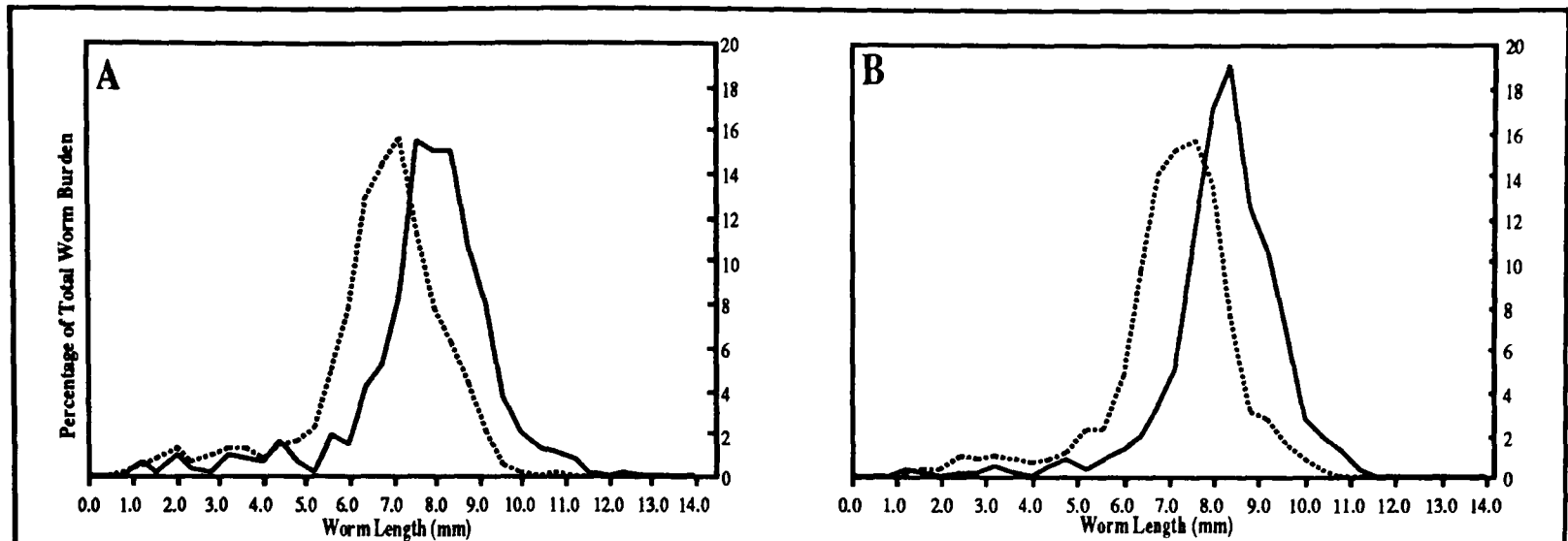


FIGURE 3: Frequency distributions (as % of total male burden) of the sizes of male worms (independent axis) perfused from mice fed 3% protein (dotted) or 16% protein (solid) diets begun either 2 weeks prior to (A) or at the time of infection (B).

Male size distributions (Figure 3) support the prior observation that worms perfused from protein deficient hosts are generally smaller than those recovered from well-fed hosts, regardless of when deficiency developed with respect to infection (see above). Note should be made of the wide range of male sizes arising from a single infection 6 weeks previously.

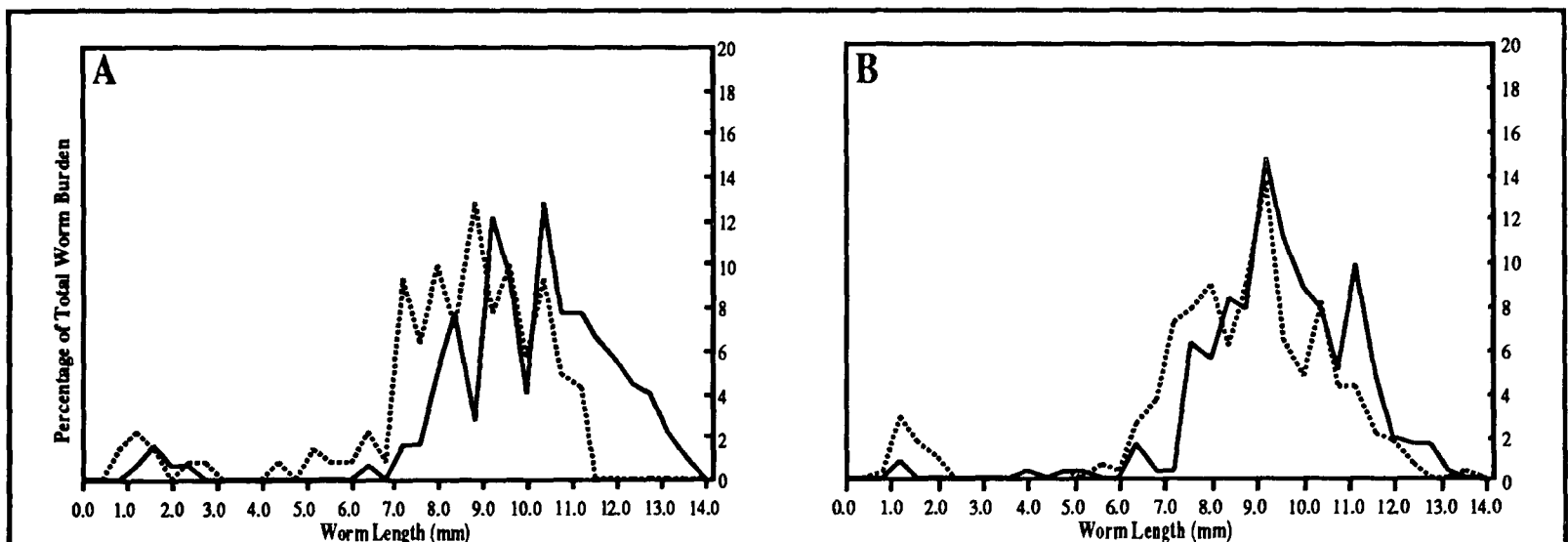


FIGURE 4: Frequency distributions (% of total female burden) of the sizes of female worms (independent axis) perfused from mice fed 3% protein (dotted) or 16% protein (solid) diets begun either 2 weeks prior to (A) or at the time of infection (B).

Female size distributions (Figure 4) verify that females inhabiting protein deficient hosts were generally smaller than those inhabiting well-fed hosts. Note should be made of the wide, bimodal distribution of female sizes arising from a single infection 6 weeks previously.

In Copula Worms

Figure 5 (overleaf) shows the relationships between the lengths of paired schistosomes recovered from hosts receiving differing dietary treatments. As described above, copulated worms in mice fed low protein diets were smaller than those found in well-fed hosts. All plots show male and female (combining both fecund and non-fecund females) lengths to be positively correlated (A. $df=81$, $r^2=0.48$, $p=0.0001^*$; B. $df=134$, $r^2=0.37$, $p=0.0001^*$; C. $df=200$, $r^2=0.42$, $p=0.0001^*$; D. $df=178$, $r^2=0.25$, $p=0.0001^*$). From this plot, it appears that

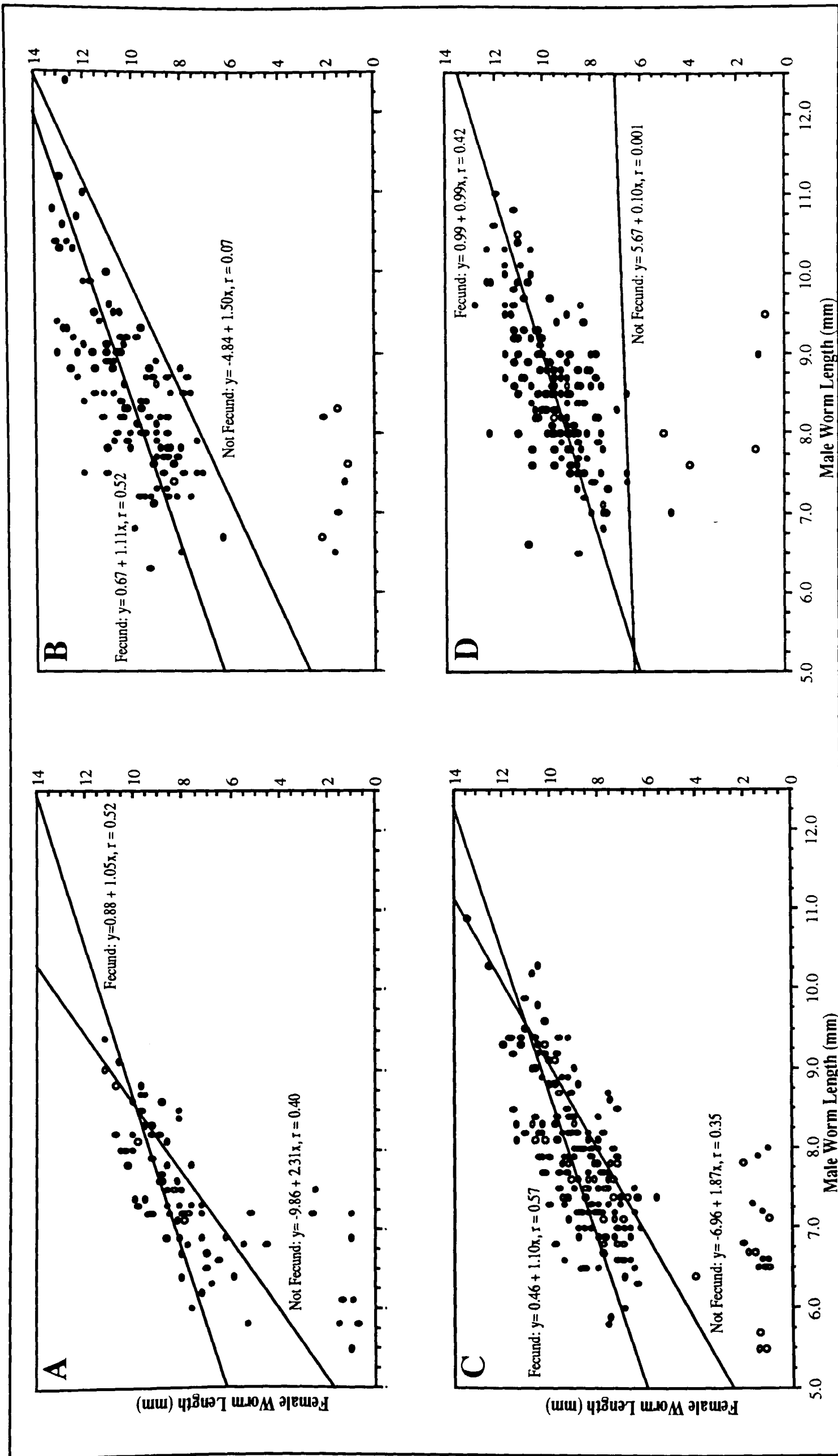
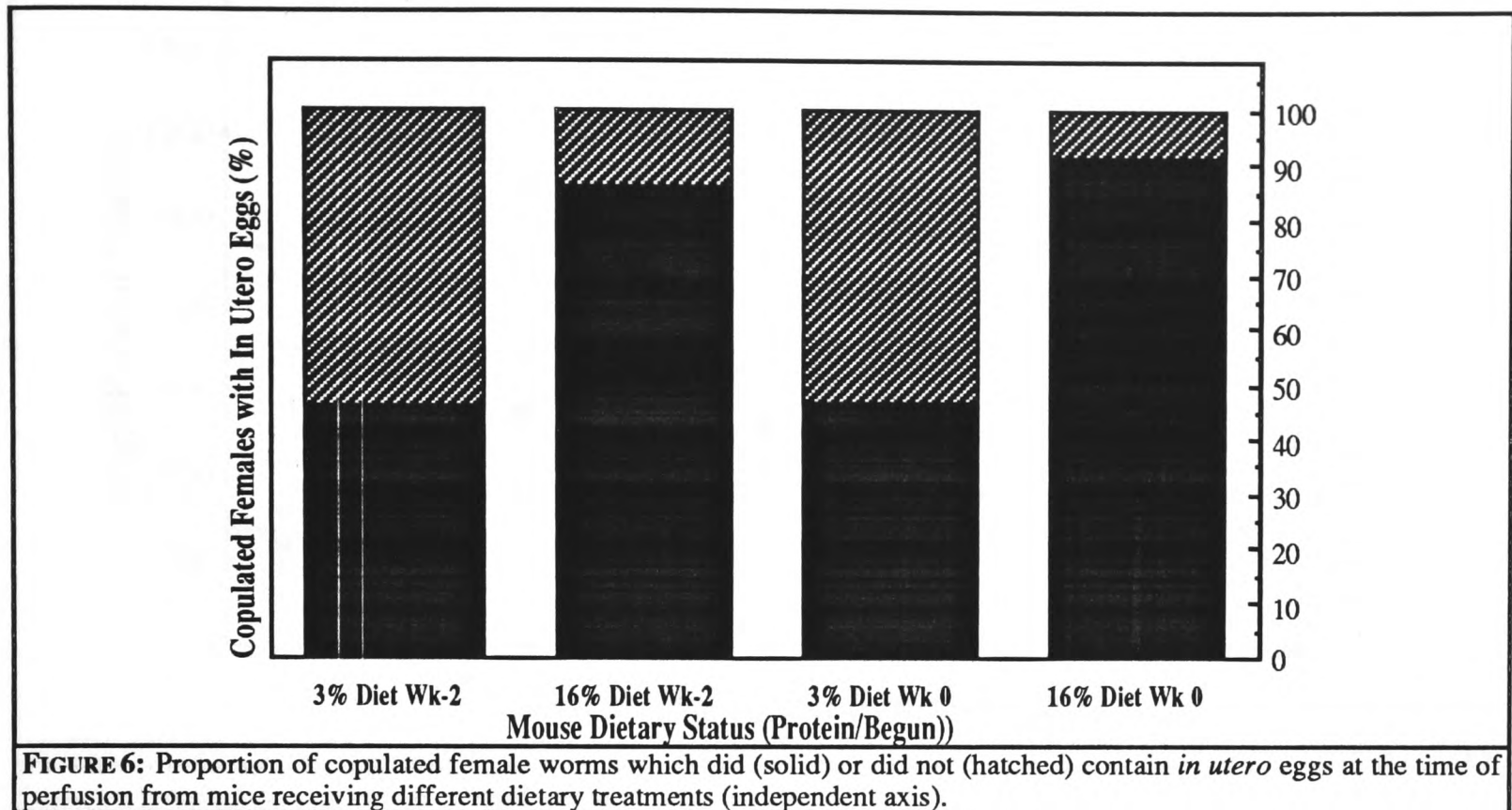


FIGURE 5: Worm sizes in experimental mice: Sizes of mated male and female (fecund J; not fecund E) worm pairs perfused from mice fed 3% protein (A & C) and 16% protein (B & D) diets begun either 2 weeks prior to (A & B) or at time of infection (C & D).

fewer paired females have *in utero* eggs when inhabiting mice fed low protein diets, regardless of when experimental feeding was begun.

Fecundity: Proportion of Females Gravid



A significantly lower proportion of female worms inhabiting protein-deficient hosts contained *in utero* eggs, regardless of whether deficiency was established at, or developed after infection (Figure 6; Table 5). The start of experimental feeding relative to infection did not influence the proportion of female worms which were fecund within either dietary group.

Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
females fecund (%)	46.34	46.77	85.93	91.62
3% at Week -2	-	0.08	6.23*	-
3% at Week 0	-	-	-	9.34*
16% at Week -2	-	-	-	1.61

TABLE 5: Z Test comparison of the proportions of copulated females which contain *in utero* eggs (gravid/total) when perfused from mice fed 3% or 16% protein diets begun either 2 weeks prior to (Week-2), or at the time of infection (Week0).

Fecundity: Faecal Eggs/Female

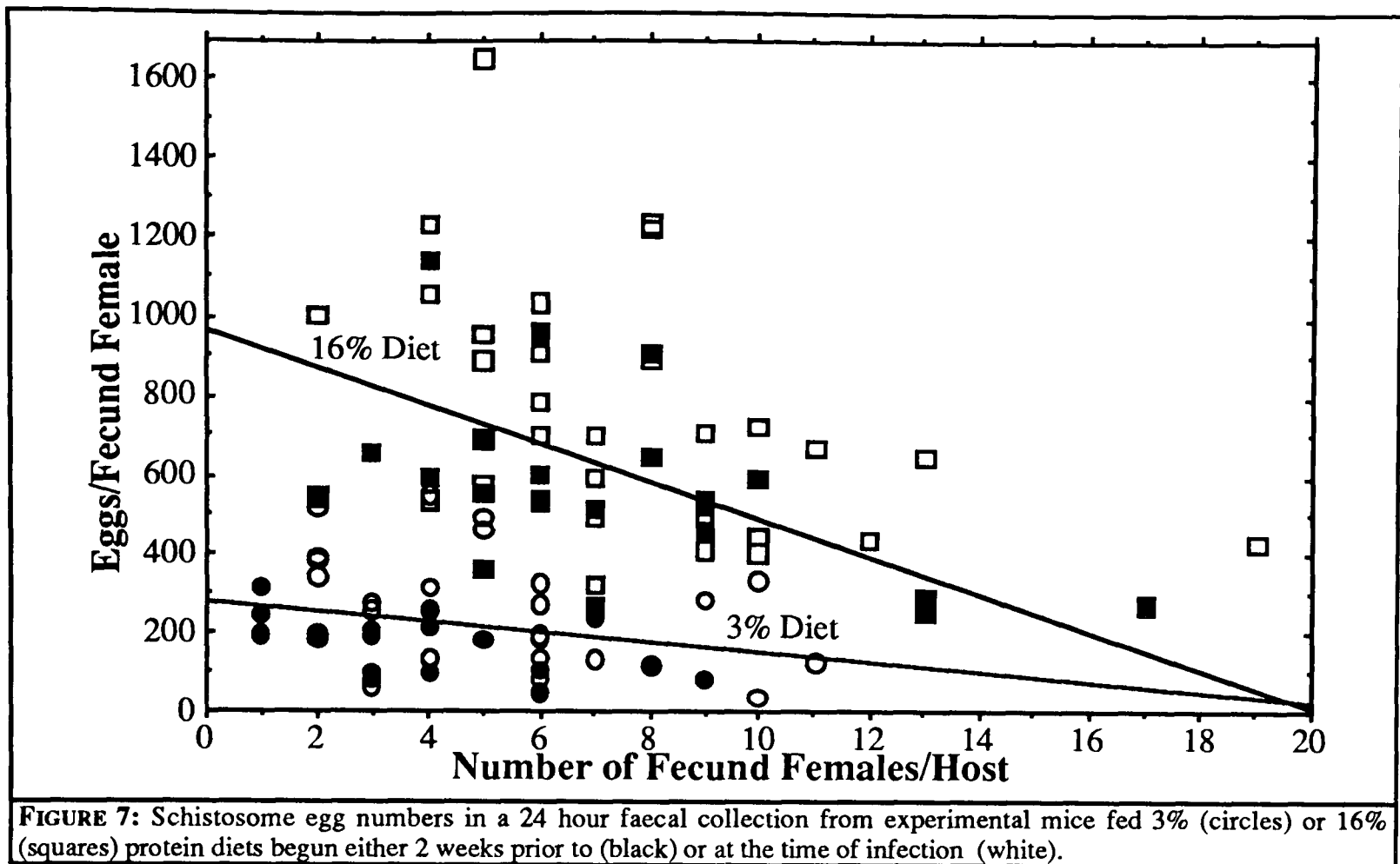


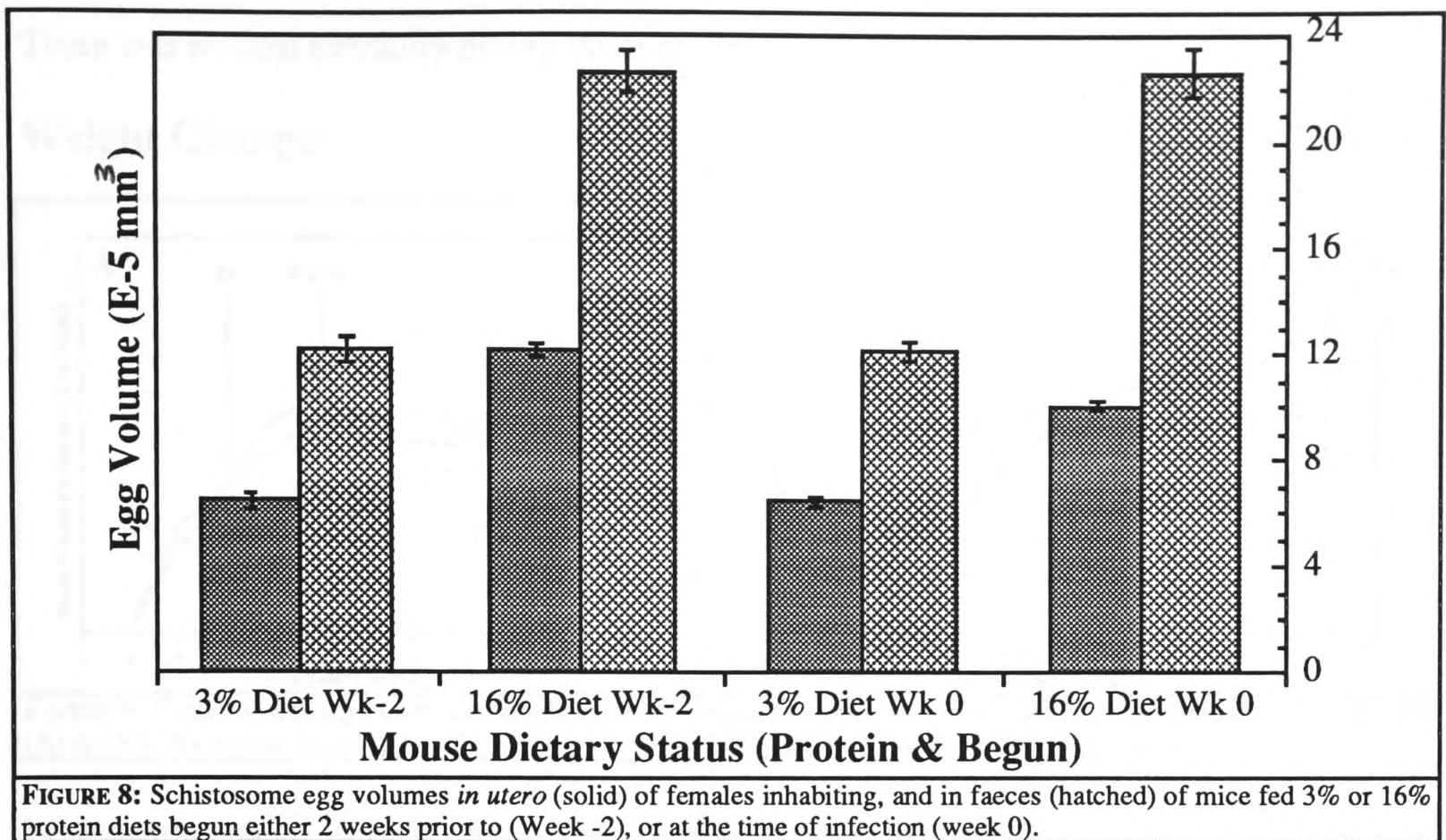
FIGURE 7: Schistosome egg numbers in a 24 hour faecal collection from experimental mice fed 3% (circles) or 16% (squares) protein diets begun either 2 weeks prior to (black) or at the time of infection (white).

Mice fed low protein diets produced significantly fewer faecal eggs than did well-fed animals (Table 6), regardless of whether deficiency was established at the time of, or developed after infection. Mice begun on the low protein diet excreted significantly fewer eggs than did animals begun on that diet at the time of infection. This also was the case in animals fed the high protein diet. Figure 7 shows the significant, negative correlation between faecal egg output.female⁻¹ and the number of gravid females in well-fed hosts ($df=49$, $y=967.11 + -38.03x$, $r^2=0.2$, $p=0.001^*$); no such association existed in protein-deficient hosts ($df=45$, $y=282.0 + -12.72x$, $r^2=0.07$, $p=0.076$).

Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
range (n)	47.5-315 (18)	34.5-547.5 (28)	257.3-1140 (20)	321.4-1653 (30)
3% at Week -2	-	0.01*(-2.38;44)	0.0001*(-8.05;36)	-
3% at Week 0	-	-	-	0.0001*(-8.59;56)
16% at Week -2	-	-	-	0.01*(-2.35;48)

TABLE 6: T-test comparison of faecal egg production/female worm by mice fed 3% or 16% protein diets (begun either 2 weeks prior to {Week -2}, or at the time of infection {Week 0}) during the 24 hour period before autopsy (number above diagonal represents T-test p value followed by t value and degrees of freedom; n=number of faecal specimens examined).

Fecundity: Egg Sizes



Eggs measured *in utero* of worms inhabiting mice fed low protein diets were significantly smaller than those found in worms recovered from well-fed animals (Table 7), regardless of when deficiency developed. A similar situation was observed with respect to eggs recovered from the faeces. Egg sizes increased markedly during tissue migration (uterus to faeces) in all groups, although these increases tended to be greater in eggs migrating through the tissues of well-fed hosts (Figure 8).

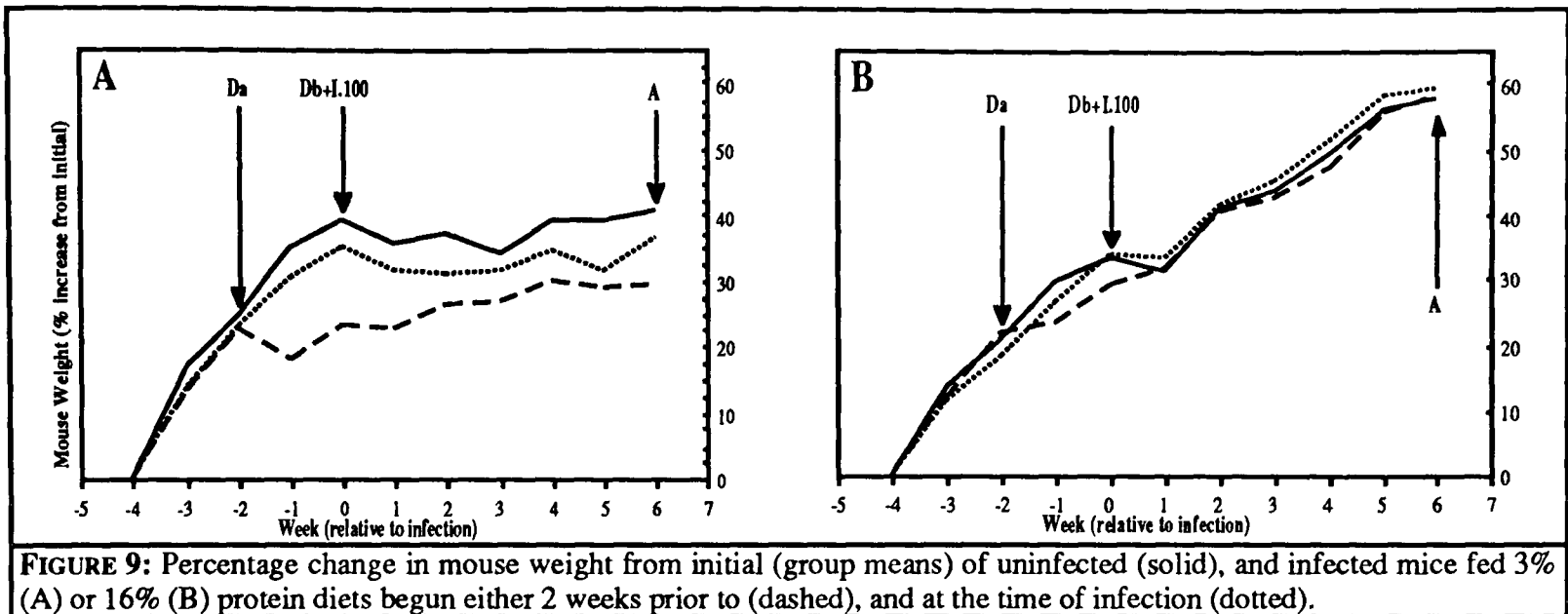
Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
In Utero Eggs				
range (E-5 mm ³) (n)	2.47-10.9 (38)	2.03-12.8 (94)	5.56-20.4 (116)	2.83-21.3 (165)
3% at Week -2	-	0.25(-0.69;130)	0.0001*(-10.26;152)	-
3% at Week 0	-	-	-	0.0001*(-11.90;257)
16% at Week -2	-	-	-	0.004*(2.64;279)
Eggs in Faeces df=95				
range (E-5 mm ³) (n)	7.19-14.4 (18)	8.55-16.0 (28)	15.4-29.2 (20)	11.2-32.2 (30)
3% at Week -2	-	0.29	38.9*	-
3% at Week 0	-	-	-	45.93*
16% at Week -2	-	-	-	0.08

TABLE 7: Statistical comparison of mean volumes of eggs *in utero* in copulated female worms (T-test p value followed by t value and degrees of freedom), and eggs recovered from the faeces (ANOVA F value) of mice fed 3% or 16% protein diets begun either 2 weeks prior to (Week -2), or at the time of infection (week 0) (n=number of eggs measured).

RESULTS: HOST

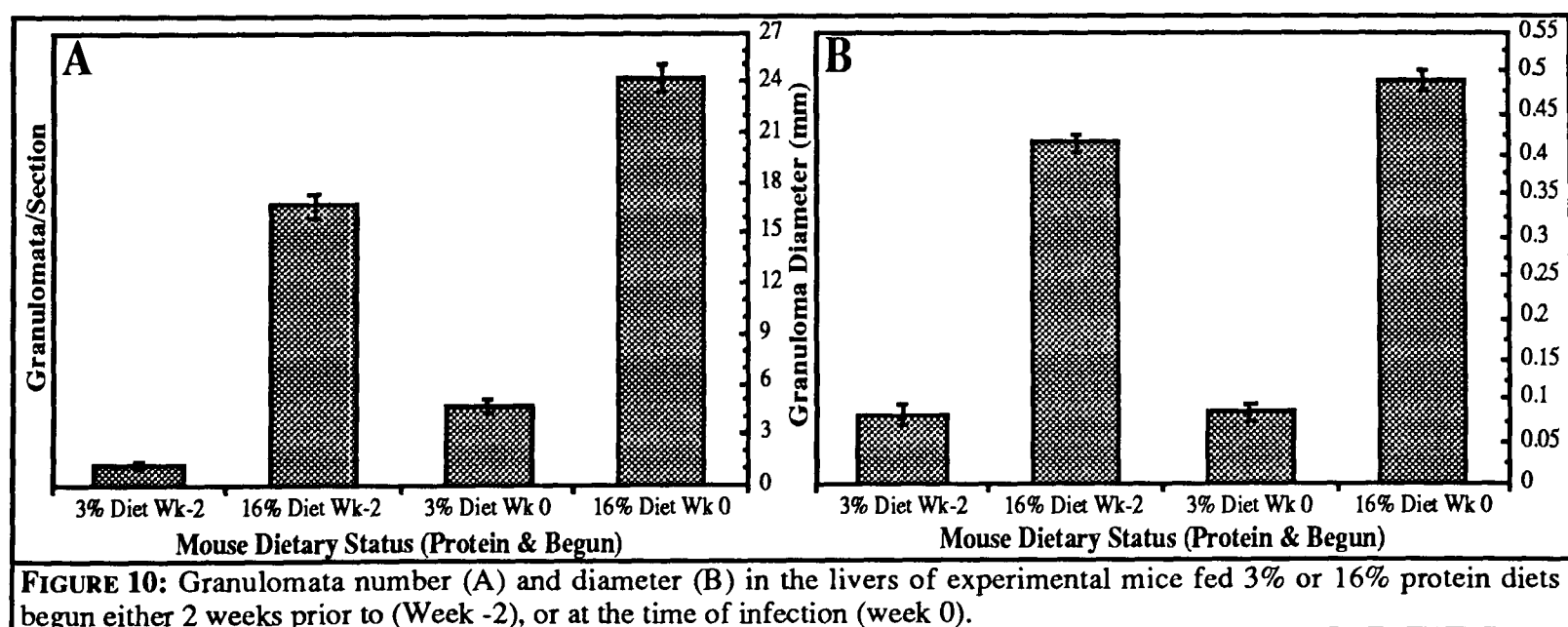
There was no host mortality during this experiment.

Weight Change



Low protein diets were inadequate for normal mouse growth (Figure 9). At the time of infection, mice begun on low protein diets 2 weeks previously were significantly lighter than those begun at that time ($df=59$, $F=7.72^*$). The weights of mice begun on a low protein diet at the time of infection fell significantly behind those of infected, well-fed animals at week 4 ($df=79$, $F=3.94^*$) and remained so for the duration of the experiment.

Liver Granulomata Formation: Number & Size



Mice fed low protein diets developed significantly fewer liver granulomata than did well-fed animals (Table 8, overleaf). Animals which were begun on experimental diets 2 weeks prior to infection had significantly fewer granulomata than did those animals receiving the same diet begun at the time of infection (Figure 10).

Liver granulomata in mice fed low protein diets were significantly smaller than those seen in well-fed hosts (Table 8). The timing of experimental feeding had no effect on the size of granulomata in mice fed low protein diets, however animals begun on the high protein experimental diet 2 weeks prior to infection had smaller granulomata than well-fed hosts begun on that diet at the time of infection (Figure 10). Liver granulomata prepared from hosts of differing protein status are depicted in Plate 2 (overleaf). Note should be made of the relatively weak cellular response surrounding schistosome eggs observed in the livers of protein deficient hosts, and the extensive tissue disruption associated with eggs in the tissues of well-fed animals.

Groups	3% at Week -2	3% at Week 0	16% at Week -2	16% at Week 0
Granuloma Number/Section df=575 range (n)	0-9 (118)	0-19 (170)	4-38 (120)	8-53 (168)
3% at Week -2	-	24.57*	169.6*	-
3% at Week 0	-	-	-	207.13*
16% at Week -2	-	-	-	15.55*
Granuloma Diameter range (mm) (n)	0-0.38 (64)	0-0.44 (118)	0.14-0.88 (100)	0.24-0.92 (140)
3% at Week -2	-	0.43(-0.18;180)	0.0001*(-14.64;162)	0.0001*(-19.43;256)
3% at Week 0	-	-	-	0.0001*(-4.41;238)
16% at Week -2	-	-	-	-

TABLE 8: Statistical comparison of mean number (granulomata/liver section) (ANOVA F value) and diameter of liver granulomata (T-test p value followed by t value and degrees of freedom) in experimental mice fed 3% or 16% protein diets begun either 2 weeks prior to (Week -2), or at the time of infection (week 0) (n=number of granulomata measured).

Organ Size

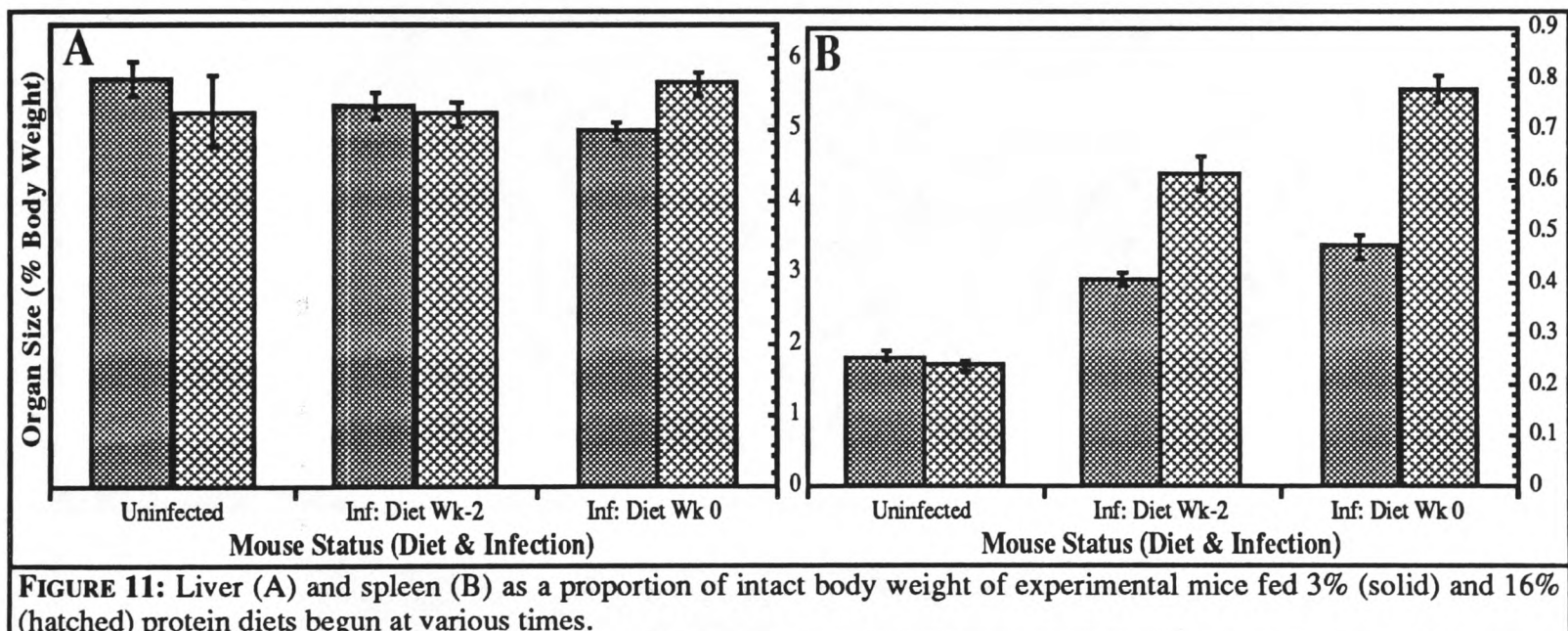


FIGURE 11: Liver (A) and spleen (B) as a proportion of intact body weight of experimental mice fed 3% (solid) and 16% (hatched) protein diets begun at various times.

Dietary protein did not affect liver sizes in uninfected mice, or those infected animals which began experimental feeding 2 weeks prior to infection (Table 9, overleaves). However the livers of infected mice which began to receive the low protein diet at the time of infection were significantly smaller than those of well-fed infected controls (Figure 11A). The timing of experimental feeding with respect to infection did not significantly influence liver sizes within dietary groups.

Dietary protein did not affect spleen size in uninfected mice. Infection produced a significant increase in spleen size in animals fed diets low in protein, whilst animals receiving high protein diets had significantly larger spleens than both uninfected animals and infected animals fed the low protein diet, regardless of when it was begun (Figure 11B).

PLATE 2: Granulomata Formation: Livers of Uninfected & Infected Mice

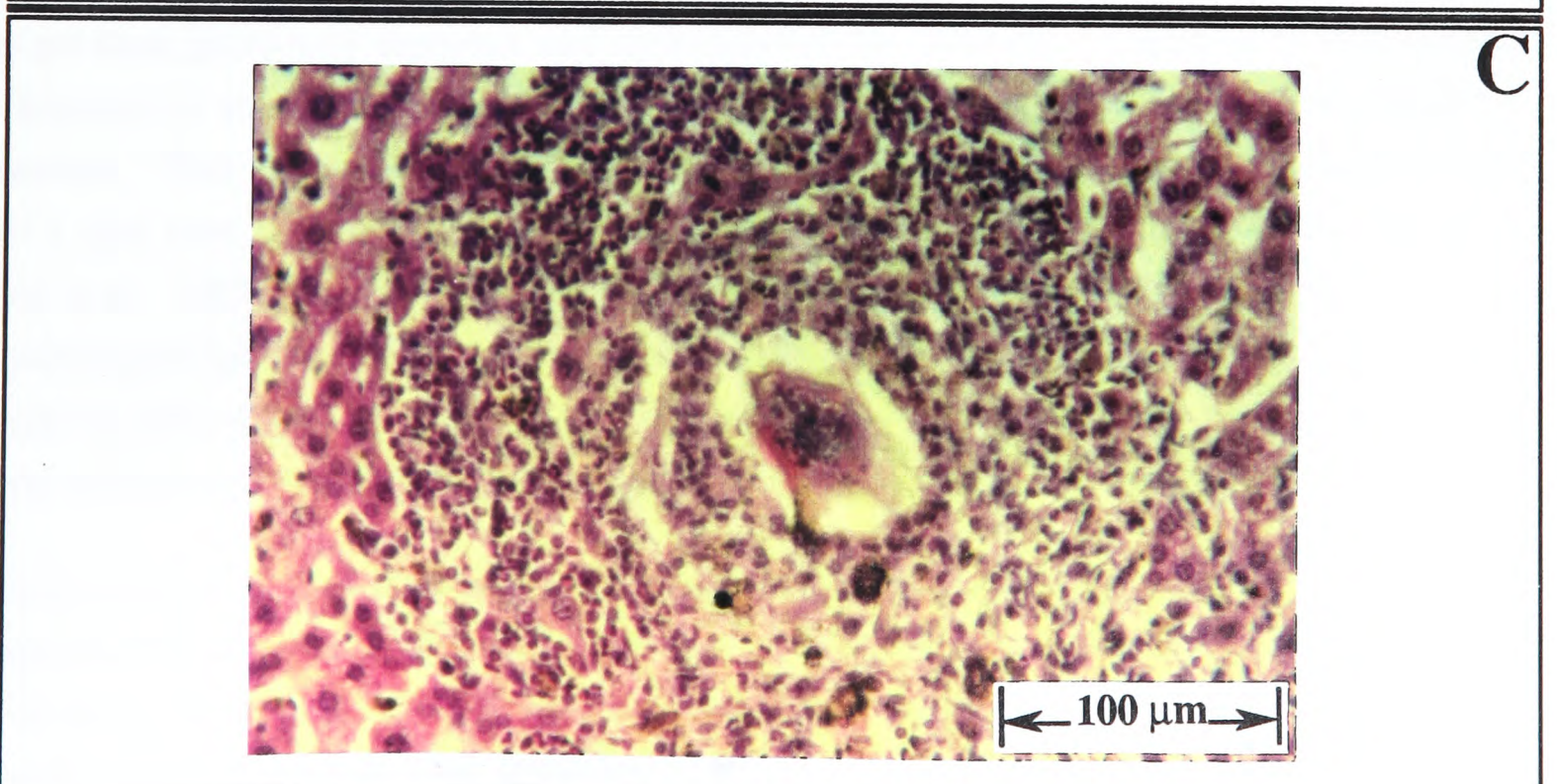
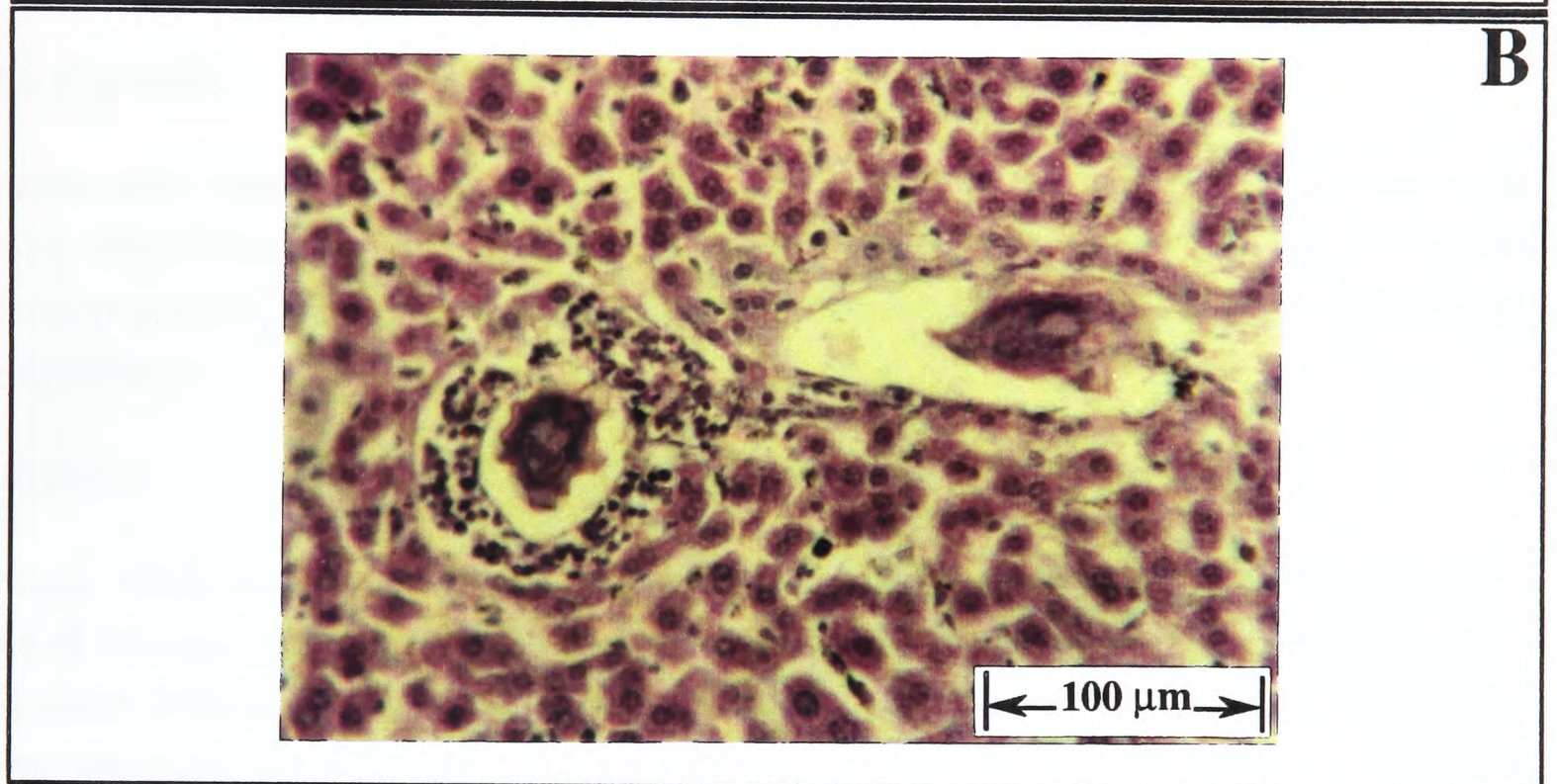
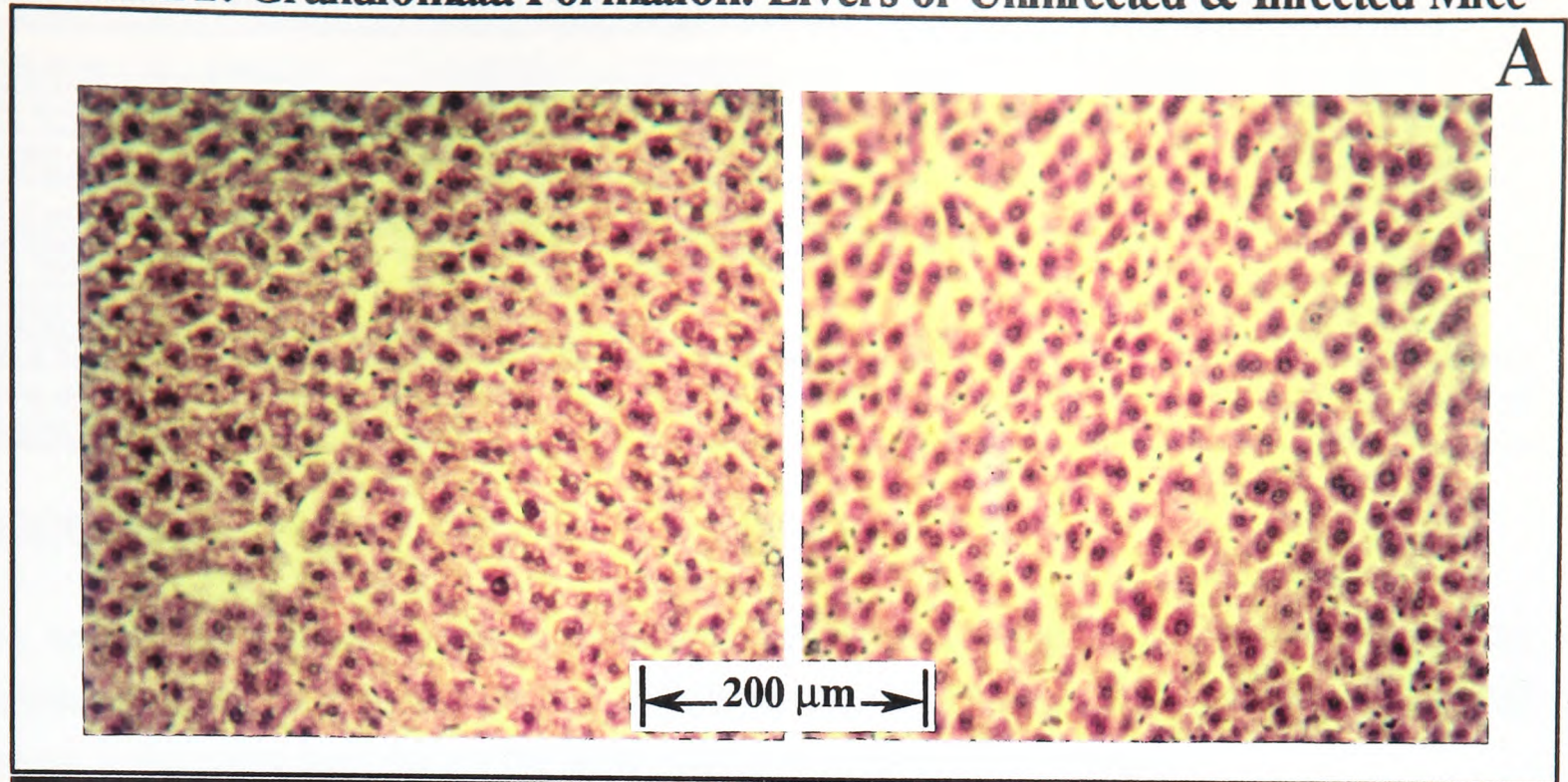


PLATE 2: Liver sections prepared from uninfected (A) mice fed 3% (left) and 16% (right) protein diets, and infected mice fed 3% (B) and 16% (C) protein diets begun 2 weeks prior to infection (livers collected at autopsy 6 weeks post-infection) (stain=haematoxylin & eosin; scales are as shown).

Groups	Uninfected 3%	3% at Week-2	3% at Week 0	Uninfected 16%	16% at Week-2	16% at Week 0
Liver						
range (%) (n)	4.61-6.87 (10)	3.82-6.98 (20)	3.61-6.84 (30)	3.54-6.88 (10)	4.32-6.18 (20)	4.66-7.15 (30)
Uninfected 3%	-	0.13(1.13;28)	0.004*(2.79;38)	0.15(1.07;18)	-	-
3%, Week -2	-	-	0.067(1.57;48)	-	0.32(0.49;38)	-
3%, Week 0	-	-	-	-	-	0.0002*(-3.83;58)
Uninfected 16%	-	-	-	-	0.45(0.14;28)	0.09(-1.38;38)
16%, Week -2	-	-	-	-	-	0.009*(-2.45;48)
Spleen						
range (%) (n)	0.21-0.31 (10)	0.31-0.5 (20)	0.29-0.72 (30)	0.19-0.29 (10)	0.34-0.97 (20)	0.54-1.11 (30)
Uninfected 3%	-	0.0001*(-7.13;28)	0.0001*(-6.01;38)	0.19(0.92;18)	-	-
3%, Week -2	-	-	0.012*(-2.34;48)	-	0.0001*(-5.90;38)	-
3%, Week 0	-	-	-	-	-	0.0001*(-8.54;58)
Uninfected 16%	-	-	-	-	0.0001*(-8.04;28)	0.0001*(-10.22;38)
16%, Week -2	-	-	-	-	-	0.0002*(-3.71;48)

TABLE 9: T-test comparisons of livers and spleen sizes (as mean % of intact body weight) in mice fed fed 3% or 16% protein diets begun at various times (number above diagonal represents T-test p value followed by t value and degrees of freedom; n=number of organs weighed).

DISCUSSION

The results presented in this experiment confirm the hypothesis, generated in the Introduction above, that host protein deficiency significantly reduces parasite success and disease in the naive host during the acute stages of schistosome infection.

The Parasite

Schistosomes which challenge naive, protein deficient mice are less successful than those which infect well-nourished animals. In addition to suffering greater attrition during the infection process, the size and reproductive success of those which survive is markedly diminished.

Numbers

Results which show decreased worm survival in mice which were protein deficient at the time of infection agree with previous studies which have examined the influence of protein and other deficiencies on schistosome establishment (see Introduction). However the observation that this effect did not occur when deficiency developed subsequent to infection has not been previously reported, and indicates that the deleterious effects of host nutritional deficiency on schistosome survival appear to be most severe during the early stages of infection. This supports previous suggestions that conditions within the undernourished host's skin may be suboptimal for invading cercariae (Coutinho-Abath, 1962; Turchetti-Maia *et al.*, 1983). As cercarial metamorphosis to the schistosomular stage involves marked physiological and biochemical changes within the parasite (Rumjanek, 1987; Wilson, 1987), it may be that this transition is impaired in some way which leads to increased attrition of larval schistosomes in undernourished hosts.

Previous work has shown that adult female schistosomes are more resilient to changes in host nutrition than are adult males (Cornford *et al.*, 1983), putatively due to a greater proportion of females as compared with male worms being *in copula* and benefiting from the physiological resilience this provides against nutrient stress (see Introduction). The observation that both male and female worms suffered greater attrition when protein

deficiency was established at the time of infection than when it developed subsequent to infection indicates that both sexes are vulnerable to suboptimal conditions during the larval stages of infection (see above). The differences observed in sex ratios between groups shows that statistically insignificant differences in survival of the sexes can none-the-less influence worm sex ratios within individual hosts; this is likely to have implications for worm mating probabilities (see above), particularly at low levels of infection (Macdonald, 1965).

Size

The significantly reduced sizes of schistosomes recovered from protein deficient hosts confirms previous studies which have shown impaired physical and reproductive development in schistosomes developing within hosts deficient in various nutrients (see Introduction). That males developing in mice which had established deficiencies at the time of infection were smaller than those infecting mice which developed deficiency subsequent to infection indicates that stunting takes place early in infection, and that deteriorating conditions during the later stages of worm development do not significantly impede growth. Females did not exhibit size differences when deficient diets were begun at different times, which supports the suggestion (above) that females are more resilient to nutritional fluctuations than males. Females from deficient hosts were, however, much smaller than those recovered from well-fed hosts (see Plate 1).

It should also be noted that copulated males were smaller than uncopulated males in protein deficient hosts. That this effect was not observed in well-fed mice lends support to the theory that male schistosomes provide physiological support to females (Cornford *et al.*, 1983), and thus are called upon to do so more in the suboptimal conditions experienced in undernourished hosts.

It is interesting to note the wide range of worm sizes arising from a single cercarial exposure in all host groups, indicating that schistosome growth and development continues for some time after a proportion of worms first reach patency.

Fecundity

Although males in worm pairs establishing in mice which were begun on low protein diets at the time of infection were larger than those in mice which were deficient at the time of infection, the subsequent development of host deficiency reduced the fecundity of their mates to a comparable level. It is thus likely that worm fecundity fluctuates with host nutritional status, regardless of the size of paired worms; this indicates that the process of egg production is impaired by host deficiency, independent of the state of worm reproductive development. Further, the observation that a significantly smaller proportion of females in deficient hosts had *in utero* eggs suggests that production is not simply slowed during times

of stress, but that egg production actually ceases in the majority of females. It is likely that these females tend to be those paired with smaller males (see Figure 5).

That density dependent egg excretion in well-fed hosts was not apparent in protein deficient animals suggests that resources limit worm fecundity in normal infections, whilst factors other than resources limit the fecundity of worms inhabiting undernourished hosts. This suggests that poor conditions impair egg production, regardless of nutrient availability. An additional factor may be that the immune responses thought to facilitate egg excretion (see above) are most pronounced in well-fed hosts which have had antigenic exposure to a larger number of worms.

Results showing that eggs *in utero* of female worms recovered from protein deficient hosts are smaller than those in females inhabiting well-fed hosts is likely to reflect the influence of suboptimal conditions within the host, as it is difficult to imagine female schistosomes being unable to absorb sufficient nutrients from the nutrient-rich hepatic-portal system to fuel normal egg production.

Results also show that eggs in the faeces of protein deficient hosts are markedly smaller than those voided by well-fed hosts. As miracidial development and growth occurs during tissue migration, this observation suggests that miracidial maturation is impaired in deficient hosts. Given that schistosome eggs pass through nutrient-rich tissues, it is suggested that reduced miracidial maturation reflects suboptimal physico-chemical conditions within undernourished host tissues rather than a lack of ambient nutrients.

As miracidia do not feed once outside the vertebrate host, any reductions in miracidial size and development are likely to impair the longevity, and thus infectivity, of miracidia originating from female worms inhabiting deficient hosts (see Chapter 5). In addition, impaired miracidial development within tissue eggs could lead to a reduction in soluble antigen secretion from the egg, thus lowering the stimulus central to the induction of pathology. This notion is consistent with previous work which has shown that eggs in the tissues of undernourished hosts are less antigenic than those isolated from well-fed hosts, and thus induce less pathology (Akpom, 1981).

The Host

The low protein diets used in the present experiment, and throughout this thesis, were inadequate for normal mouse growth, as shown by the significantly lower weights of uninfected animals maintained on these diets. As lean body weight is a primary indicator of low nutritional status (see Slater, 1987), it was considered that feeding with the low protein diet produced significant protein deficiency in experimental animals. The present experiment shows that the harmful effects of schistosome infection are markedly suppressed in mice fed low protein diets when compared with well-fed controls.

Weight

Mice fed the low protein diet begun two weeks prior to infection were significantly lighter than other animals at the time of infection, and thus it can be concluded that hosts fed the low protein diet were deficient at the time of exposure to cercariae. The provision of a low protein diet at the time of infection rapidly led to a significant loss of weight, and so it can be concluded that these hosts became deficient during the experimental period.

Granulomata Number

Results clearly show that protein deficient hosts develop less liver pathology during the initial stages of schistosome infection than do well-fed animals. That significantly fewer granulomata occurred in the livers of protein deficient hosts compared with well-fed animals most likely reflects a combination of reduced egg production by female worms, reduced egg antigenicity (see above) and diminished DTH responses central to their formation (see Introduction & Chapter 1).

Granulomata Size

The large and diffuse granulomata in livers of well-fed mice contrast sharply with the smaller, discrete lesions observed in the livers of protein deficient hosts. Indeed, eggs in the livers of protein deficient hosts were frequently seen to be free of any cellular infiltration (see Plate 2). As the aetiology of granulomata formation around schistosome eggs involves primarily type IV DTH to secreted antigen, it is likely that suppression of these responses secondary to protein undernutrition (see Introduction), combined with reductions in egg antigenicity (see above), produce a marked decline in granulomata size.

Although it has been shown that a drug-induced reduction in granuloma formation can increase host exposure to egg hepatotoxin and result in severe morbidity (Domingo *et al.*, 1967), it is likely that the simultaneous decrease in worm fecundity and egg toxicity and persistence in the tissues, together with an impaired immune response to antigen stimulation (see above), will produce a net decrease in the damage associated with patent schistosome infection in the undernourished host. Furthermore, it is suggested that reductions in the initial onset of liver damage may impair the subsequent development of chronic pathology.

Organ Size

Protein deficient hosts show reduced spleen and liver sizes during infection when compared with well-fed animals. As the increase in spleen size during infection is largely the result of elevated lymphoid activity, it appears that protein deficiency impairs thymocyte production in infected animals. The resultant fall in T cell activity is likely to reduce the egg-induced type IV DTH response which results in granulomata formation and liver inflammation. This

is consistent with the observation that liver sizes are reduced in protein deficient infected hosts when compared with well-fed animals. It is suggested that reduced organ inflammation during the acute stages of infection will lower the rate of development and extent of the more extensive damage characteristic of chronic disease.

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: II. ACUTE PHASE REPEATED INFECTIONS

Abstract

This experiment examines the influence of externally imposed host protein deficiency on the parasite and host at a single time point following repeated cercarial challenge. Results indicate that the recruitment of juvenile parasites is reduced in protein deficient hosts, whilst greater parasite reproductive success combined with increased immunopathology combine to reduce the survival of well-fed hosts exposed to repeated infection.

INTRODUCTION

The previous experiment has shown that host protein deficiency can significantly reduce the development of schistosomes and pathology in the vertebrate host following a single infection. Natural exposure to schistosomes is, however, thought to occur continually at relatively low levels. This has been recently modelled in the laboratory (Anderson & Crombie, 1985; Crombie & Anderson, 1985) employing the trickle method of repeated host exposure to cercarial challenge in an attempt to mimic the natural exposure of host to parasite.

Anderson and Crombie (1985) and Crombie and Anderson (1985) have demonstrated dose- and time-dependent acquired immunity to *S. mansoni* in well-fed laboratory mice. Although it is well documented that the acquisition of immunity is nutrient-dependent (see Chapter 1), no previous study has examined the influence of host nutritional status on the development of immunity during repeated schistosome infection. As schistosomiasis has an immune aetiology (see above), it was hypothesized that heightened responses accompanying repeated infection in intact hosts may be ameliorated by the immunosuppression accompanying imposed protein deficiency. The present experiment was designed to investigate the influence of protein deficiency on the ability of mice to acquire immunity to repeated *S. mansoni* challenge.

Hypothesis

That protein deficiency influences parasite success and reduces host disease in the acute stages of schistosome infection in hosts repeatedly exposed to infection.

The influence of dietary protein on both parasite success and host pathogenesis was examined at mouse autopsy 4 weeks after the last of 6 weekly exposures to either 5 or 20 cercariae.mouse⁻¹. Mice belonged to two dietary groups: those receiving 3% or 16% protein diets, all begun 2 weeks prior to cercarial exposure.

EXPERIMENTAL DESIGN

Sixty mice were randomly assigned to groups of 10, housed in large cages, and fed SLC. After two weeks, animals in three of the groups had the SLC replaced by powered food hoppers containing the 3% protein diet (D3), whilst those in the remaining three cages received hoppers containing 16% protein diet (D16).

After two weeks of feeding on experimental diets, mice from one group in each of the dietary régimes were individually exposed to 5 cercariae.mouse⁻¹ for 30 min (I.05). Mice from the second group of each dietary regime were similarly exposed to 20 cercariae.mouse⁻¹ (I.20; range 19-20), whilst those in the remaining two groups were similarly handled in the absence of cercariae. This procedure was repeated once each week for six weeks. All cercariae were gathered weekly from the same group of 40 patent snails for the duration of the experiment. Four weeks after final exposure, mouse weights were determined and autopsies (At) performed as described in the Materials and Methods section of this chapter. The design of this experiment is presented in Table 1.

Week Group (n)	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9
A (10)	SLC	→	D3	→	-	-	-	-	-	-	-	-	→	At
B (10)	SLC	→	D16	→	-	-	-	-	-	-	-	-	→	At
C (10)	SLC	→	D3	→	I.05	I.05	I.05	I.05	I.05	I.05	→	-	→	At
D (10)	SLC	→	D3	→	I.20	I.20	I.20	I.20	I.20	I.20	→	-	→	At
E (10)	SLC	→	D16	→	I.05	I.05	I.05	I.05	I.05	I.05	→	-	→	At
F (10)	SLC	→	D16	→	I.20	I.20	I.20	I.20	I.20	I.20	→	-	→	At

TABLE 1: Design of Repeated Infection Experiment (n=number of mice in group).

RESULTS: PARASITE

Number

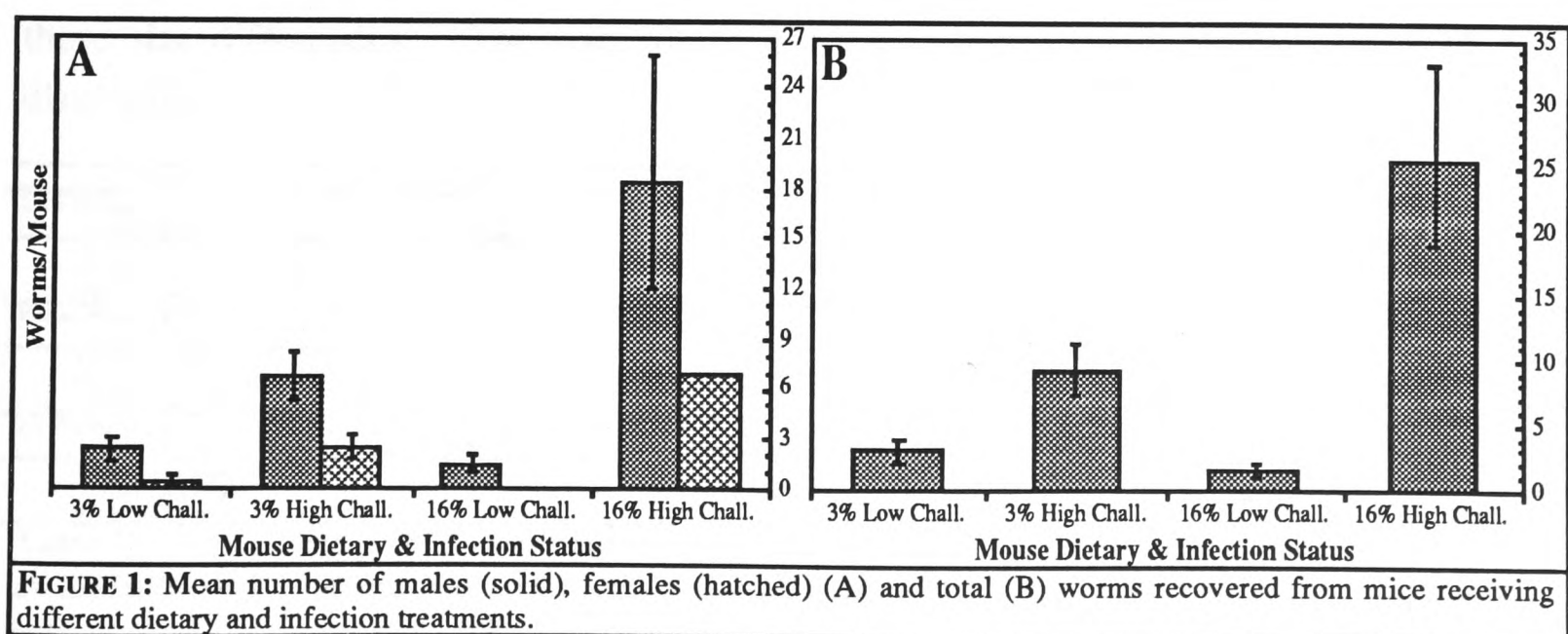


FIGURE 1: Mean number of males (solid), females (hatched) (A) and total (B) worms recovered from mice receiving different dietary and infection treatments.

There were no significant differences noted in the number of worms (males, females or total worm numbers) perfused from mice challenged with low levels of infection and fed differing

dietary protein levels. Significantly more worms (males and/or females) established in well-fed animals exposed to high levels of infection than similarly challenged protein deficient animals (Figure 1, previous page; Table 2).

Groups	3% Low Challenge	3% High Challenge	16% Low Challenge	16% High Challenge
Male Worms				
range (n)	0-5 (8)	2-13 (7)	1-2 (2)	12-26 (2)
3% Low Challenge	-	0.008*(-2.79;13)	0.30(0.55;8)	-
3% High Challenge	-	-	-	0.02*(-2.58;7)
16% Low Challenge	-	-	-	0.03*(-3.65;2)
Female Worms				
range (n)	0-2 (8)	1-7 (7)	0 (2)	7-7 (2)
3% Low Challenge	-	0.008*(-2.80;13)	0.13(1.23;8)	-
3% High Challenge	-	-	-	0.025*(-2.37;7)
16% Low Challenge	-	-	-	-
All Worms				
range (n)	0-7 (8)	3-16 (7)	1-2 (2)	19-33 (2)
3% Low Challenge	-	0.005*(-3.01;13)	0.20(0.87;8)	-
3% High Challenge	-	-	-	0.014*(-2.78;7)
16% Low Challenge	-	-	-	0.017*(-5.32;2)

TABLE 2: T-test comparisons of mean worm burdens in experimental mice (number above diagonal represents T-test p value followed by t value and degrees of freedom; n=number of mice perfused).

Size: Means

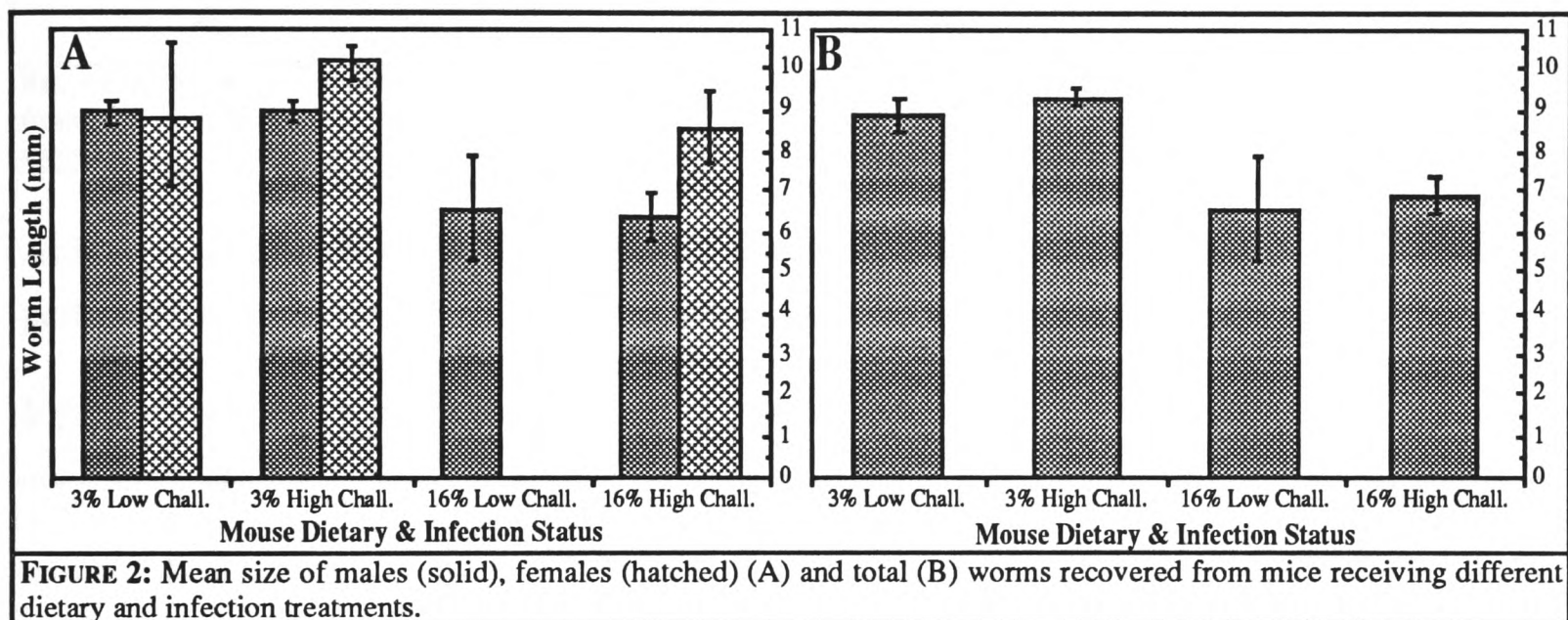


FIGURE 2: Mean size of males (solid), females (hatched) (A) and total (B) worms recovered from mice receiving different dietary and infection treatments.

Comparing mean sizes, male worms recovered from mice fed low protein diets and exposed to either low or high cercarial challenge were significantly longer than those recovered from similarly challenged mice fed high protein diets (Figure 2; Table 3). Females did not exhibit these size differences. The wide range of worm sizes prompted examination of size distributions.

Groups	3% Low Challenge	3% High Challenge	16% Low Challenge	16% High Challenge
Male Worms				
range (mm) (n)	6.9-12.15 (22)	6.08-12.98 (50)	4.43-8.76 (3)	0.62-11.85 (38)
3% Low Challenge	-	0.44(-0.15;70)	0.004*(2.89;23)	-
3% High Challenge	-	-	-	0.0001*(4.53;86)
16% Low Challenge	-	-	-	0.47(0.08;39)
Female Worms df=38				
range (mm) (n)	4.12-12.46 (6)	5.77-14.32 (19)	0 (0)	4.33-15.04 (14)
3% Low Challenge	-	0.47	-	-
3% High Challenge	-	-	-	1.21
16% Low Challenge	-	-	-	-
All Worms df=151				
range (mm) (n)	4.12-12.46 (28)	5.77-14.32 (69)	4.43-8.76 (3)	0.62-15.04 (52)
3% Low Challenge	-	0.13	0.76	-
3% High Challenge	-	-	-	7.96*
16% Low Challenge	-	-	-	0.02

TABLE 3: Statistical comparisons of mean worm sizes (males: number above diagonal represents T-test p value; females and all worms comparisons: number above diagonal represents ANOVA F value; n=number of mice perfused).

Size: Frequency Distributions

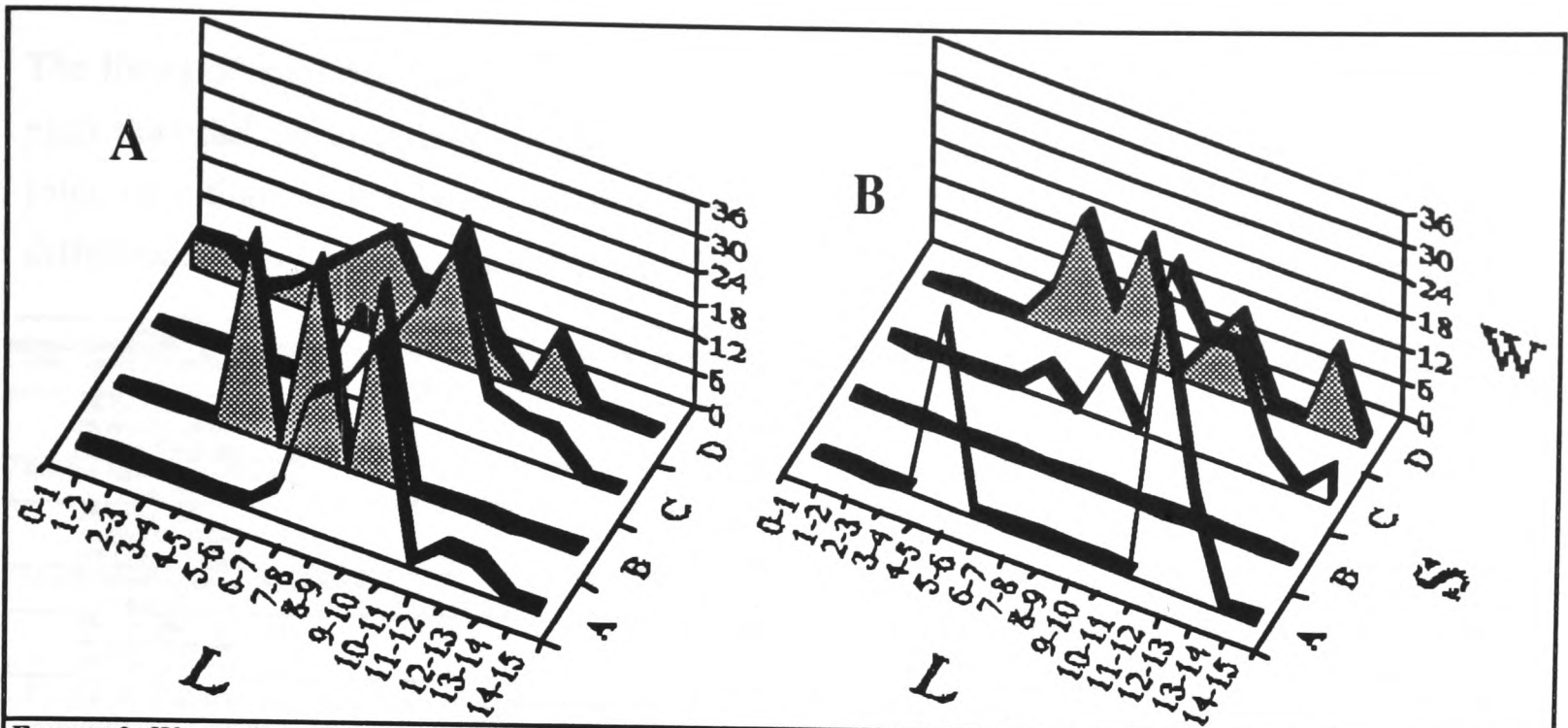


FIGURE 3: Worm sizes in experimental mice: Frequency distribution of sizes of male (A) and female (B) worms perfused from mice fed 3% protein (S:A & B) and 16% protein (S:C & D) diets and exposed to either 5 (S:A & C) or 20 (S:B & D) cercariae/week/mouse (L=worm length in mm, S=mouse dietary and infection status, W=% of worm burden).

Although both dietary treatments produce worms in the larger size classes (Figure 3), worms perfused from well-fed hosts contain a larger proportion of smaller worms.

Fecundity: Eggs/Female in Host Tissues

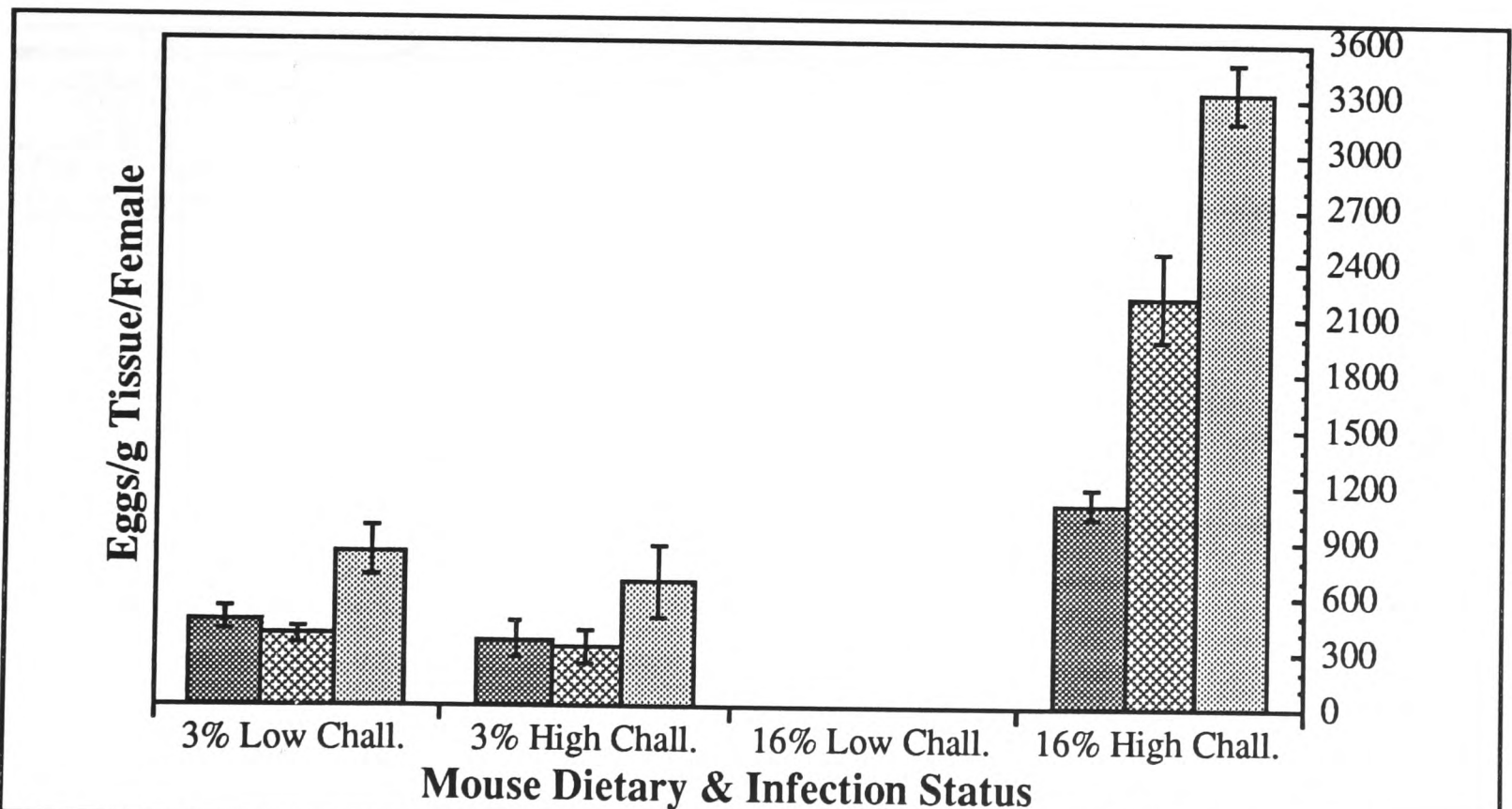


FIGURE 4: Schistosome egg numbers/female in the livers (solid), intestines (hatched) and both livers and intestines (dotted) of experimental mice receiving different dietary and infection treatments.

Both liver and intestinal tissue removed from well-fed hosts contained significantly more eggs/female than did tissues from protein deficient mice (Table 4, overleaf), regardless of infection treatment. Total tissue egg content.female⁻¹ was greater in well-fed animals than in protein-deficient animals. There were no significant differences in total tissue egg

content.female⁻¹ in mice fed low protein diets and exposed to differing infection rates (Figure 4, previous page).

The livers of mice fed low protein diets tended to contain a greater proportion of total tissue eggs than did the intestines. A greater proportion of total tissue eggs tended to occur in the intestines than in the livers of mice fed high protein diets. There were no significant differences between proportions of eggs in the livers or intestines across challenge groups.

Groups	3% Low Challenge	3% High Challenge	16% Low Challenge	16% High Challenge
Liver Eggs/g Tissue/Female range (n)	180-780 (10)	0-690 (9)	0 (0)	1020-1185 (2)
3% Low Challenge	-	0.19(0.89;17)	-	-
3% High Challenge	-	-	-	0.019*(-2.43;9)
16% Low Challenge	-	-	-	-
Intestine Eggs/g Tissue/Female range (n)	120-630 (10)	0-630 (9)	0 (0)	1980-2460 (2)
3% Low Challenge	-	0.14	-	-
3% High Challenge	-	-	-	12.97*
16% Low Challenge	-	-	-	-
Liver & Intestine Eggs/g Tissue/Female range (n)	300-1410 (10)	0-1320 (9)	0 (0)	3165-3480 (2)
3% Low Challenge	-	0.22	-	-
3% High Challenge	-	-	-	7.33*
16% Low Challenge	-	-	-	-

TABLE 4: Statistical comparisons of eggs/female in host tissues (liver eggs: number above diagonal represents T-test p value followed by t value and degrees of freedom; intestine and liver and intestine eggs: number above diagonal represents ANOVA F value (df=22); n=number of organs examined).

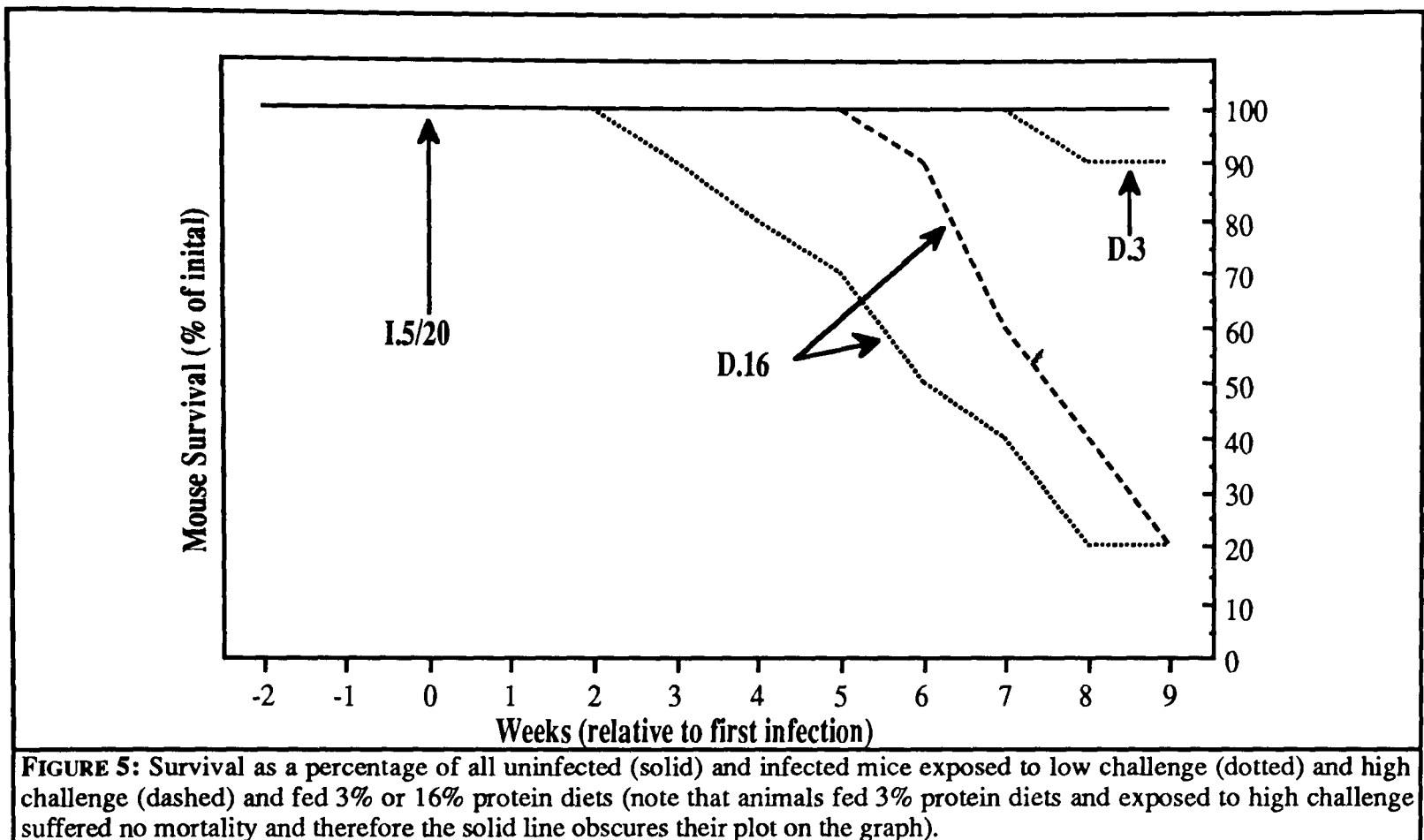
Fecundity: Egg Size

Eggs recovered from the livers of mice fed low protein diets were significantly smaller than those recovered from mice fed high protein diets (Table 5). Challenge intensity did not significantly influence egg volumes in mice fed low diets.

Groups	3% Low Challenge	3% High Challenge	16% Low Challenge	16% High Challenge
range (E-4 mm ³) (n)	1.59-4.91 (20)	1.67-4.21 (20)	- (0)	2.77-5.28 (20)
3% Low Challenge	-	0.003	-	-
3% High Challenge	-	-	-	4.72*
16% Low Challenge	-	-	-	-

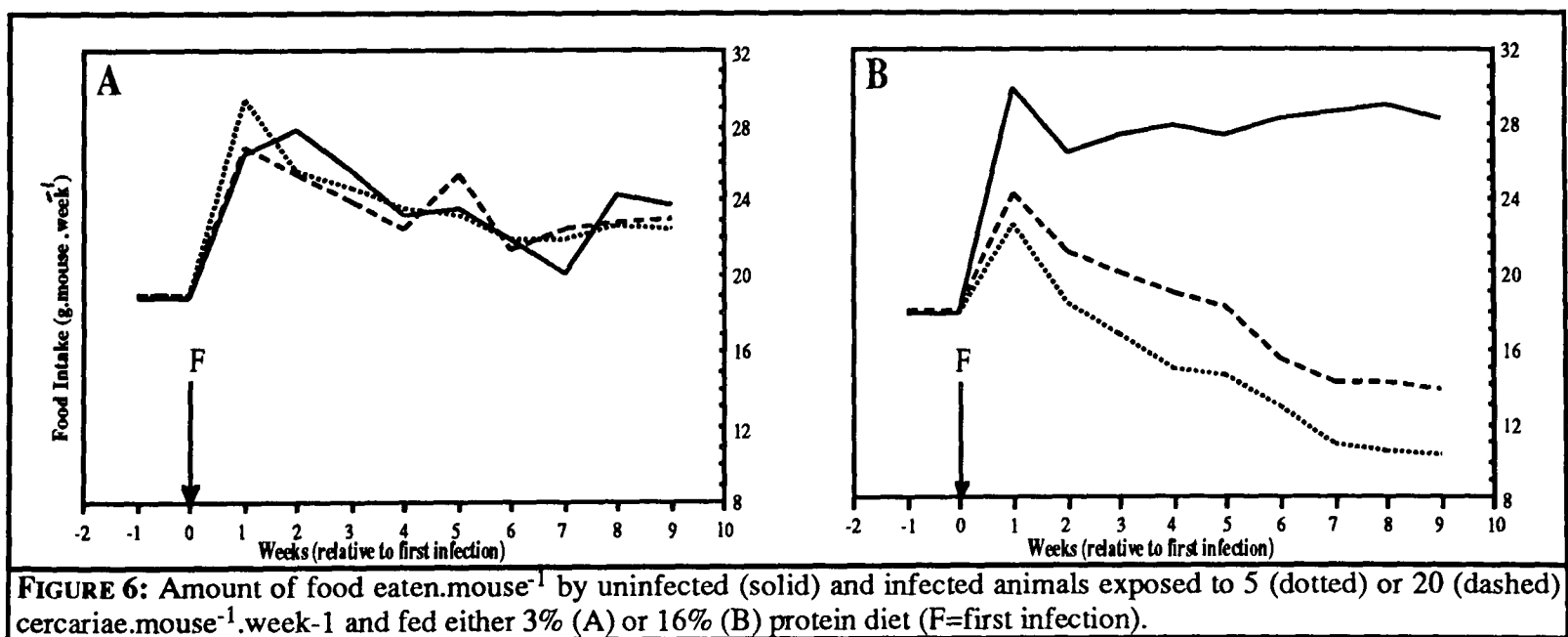
TABLE 5: ANOVA comparisons of schistosome egg volumes recovered from the livers of experimental mice receiving different dietary and infection treatments (number above diagonal represents ANOVA F value; n=number of eggs measured).

RESULTS: HOST



Uninfected mice did not suffer any parasite-induced mortality regardless of dietary treatment. Infection was associated with reduced survival of mice fed high protein diets at both challenge rates (Figure 5). Conversely, 95% of mice fed low protein diets survived infection.

Food Intake



Infected mice fed low protein diets did not differ in their intakes from similarly fed uninfected mice (Figure 6). Conversely, infected mice fed high protein diets showed a decline in food intake, when compared to similarly fed uninfected animals, which began soon after first infection and continued throughout the experiment.

Weight Change

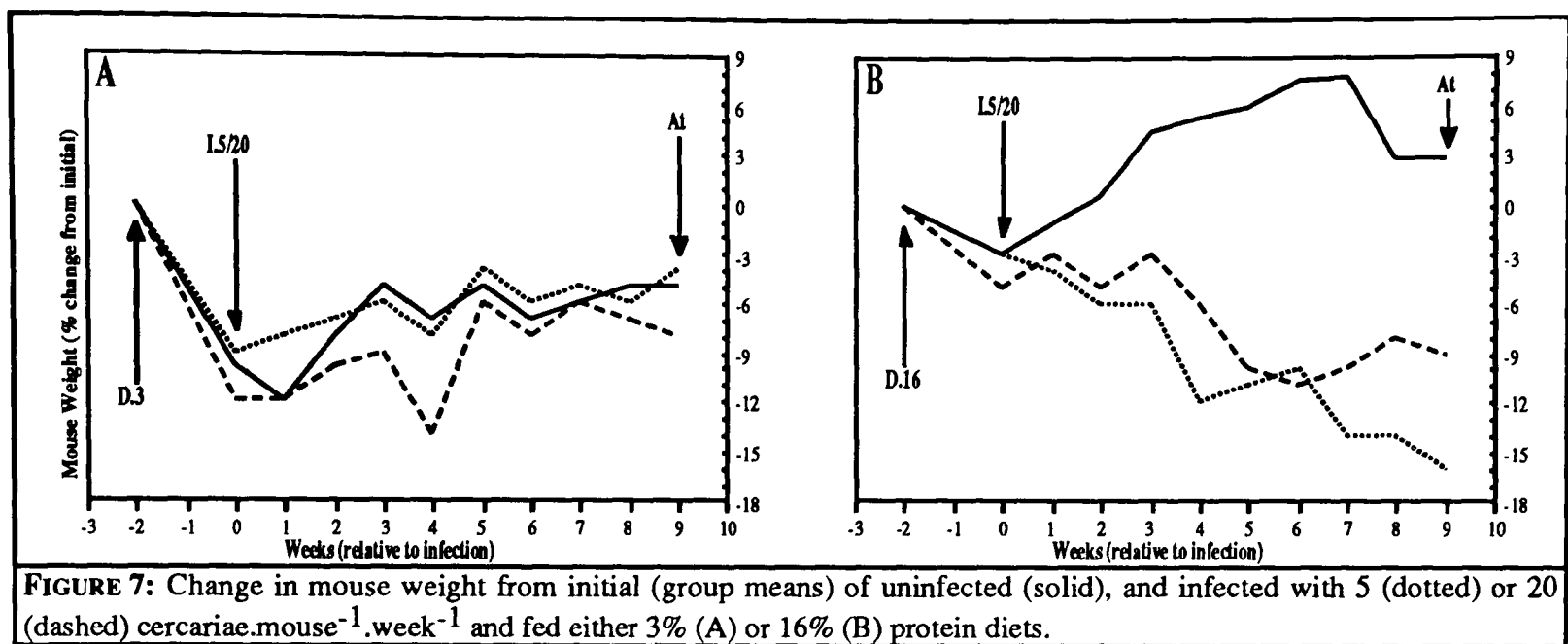


FIGURE 7: Change in mouse weight from initial (group means) of uninfected (solid), and infected with 5 (dotted) or 20 (dashed) cercariae.mouse⁻¹.week⁻¹ and fed either 3% (A) or 16% (B) protein diets.

Uninfected mice fed high protein diets initially lost a small degree of weight, recovering quickly to gain weight during the remainder of the experiment. After 11 weeks, weights of uninfected mice fed high protein diets were significantly greater than uninfected mice fed low protein diets ($t=1.76$, $p<0.05^*$). This observation shows the low protein diets to be inadequate for normal host growth.

As shown in Figure 7, the weights of infected mice fed low protein diets differed little from their uninfected controls. In contrast, infected animals fed high protein diets initially lost less weight. Some 3 weeks after first infection however, losses began to increase, until final weights of infected mice fed high protein diets were lower than infected mice fed low protein diets (low challenge: $t=2.75$, $p<0.01^*$; high challenge: $t=4.05$, $p<0.01^*$), and significantly lower than uninfected controls fed 16% protein (low challenge: $t=4.72$, $p<0.01^*$; high challenge: $t=2.15$, $p<0.05^*$).

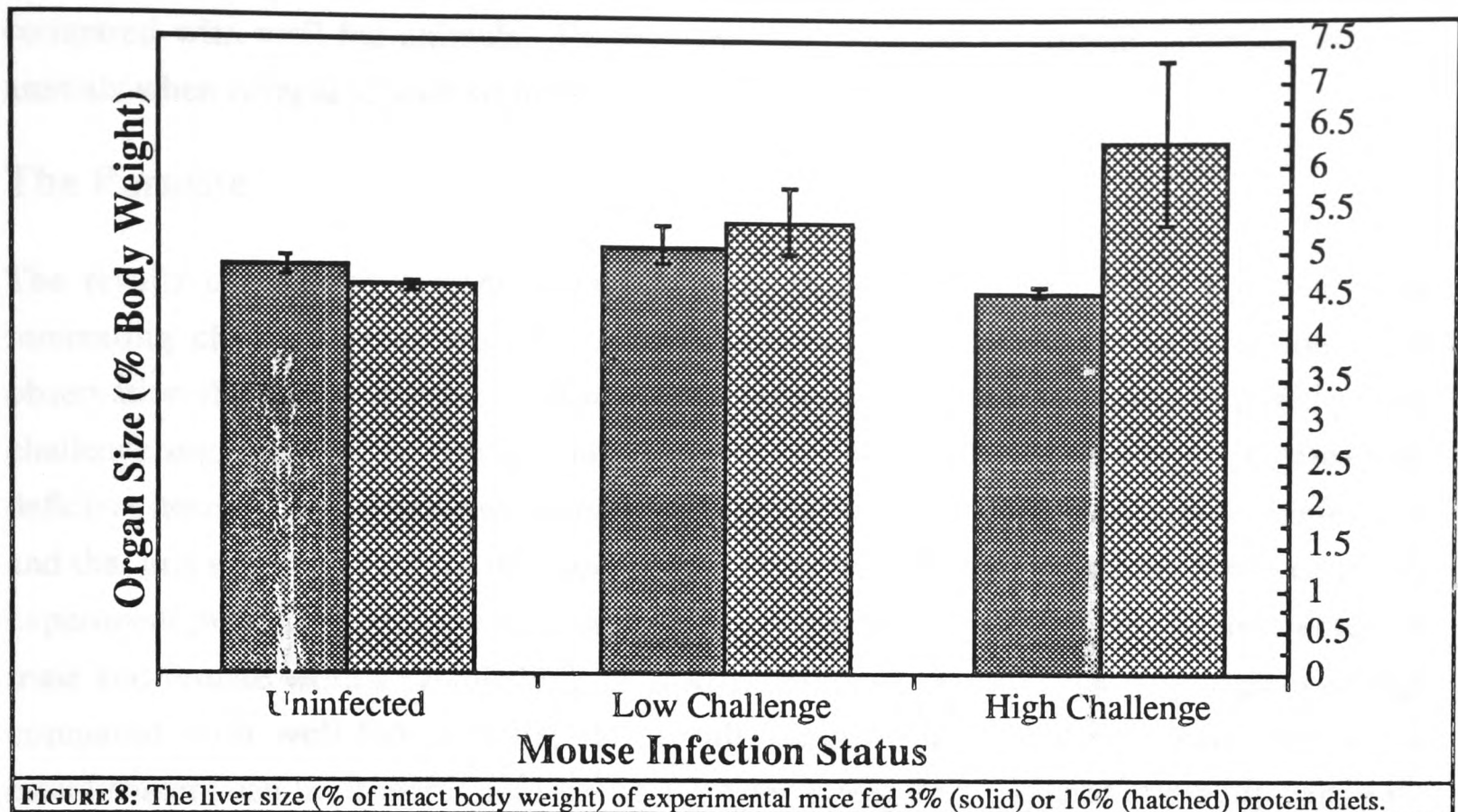
Liver Granulomata: Size

Mice fed high protein diets had significantly larger granulomata than mice fed low protein diets and exposed to either low or high challenge (Table 6). Animals fed low protein diets had liver granulomata of similar size, regardless of challenge intensity.

Groups	3% Low Challenge	3% High Challenge	16% Low Challenge	16% High Challenge
range (E-4 mm ³) (n)	0.58-4.05 (20)	3.17-4.33 (10)	- (0)	13.32-41.62 (10)
3% Low Challenge	-	0.35	-	-
3% High Challenge	-	-	-	20.34*
16% Low Challenge	-	-	-	-

TABLE 6: ANOVA comparisons of granulomata volumes in the livers of experimental mice (number above diagonal represents ANOVA F value; n=number of granulomata examined; df=39).

Liver Size



Differences in dietary protein levels did not influence liver sizes in uninfected mice (Figure 8; Table 7). Low levels of infection in protein deficient animals did not cause a significant increase in liver size over uninfected controls, however high levels of infection caused liver shrinkage in deficient animals. In contrast, both low and high levels of infection produced significant liver enlargement in well-fed animals. There was no difference in liver size between deficient and well-fed animals exposed to low levels of infection, however well-fed animals exposed to high levels of infection had significantly larger livers than similarly infected, deficient animals.

Groups	Uninfected 3%	3% Low Challenge	3% High Challenge	Uninfected 16%	16% Low Challenge	16% High Challenge
range (%) (n)	4.11-5.4 (10)	4.38-6.12 (8)	4.38-4.86 (7)	4.49-4.72 (5)	4.96-5.74 (2)	5.35-7.24 (2)
Uninfected 3%	-	0.16(-1.02;16)	0.026*(2.12;15)	0.075(1.53;13)	-	-
3% Low Challenge	-	-	0.03*(2.06;13)	-	0.30(-0.55;8)	-
3% High Challenge	-	-	-	-	-	0.002*(-4.15;7)
Uninfected 16%	-	-	-	-	0.009*(-3.45;5)	0.01*(-3.36;5)
16% Low Challenge	-	-	-	-	-	0.23(-0.92;2)

TABLE 7: T-test comparisons of livers as mean percentage of intact body weight (number above diagonal represents T-test p value followed by t value and degrees of freedom; n=number of organs weighed).

DISCUSSION

No previous study has examined the influence of host nutritional status on the dynamics of repeated schistosome infections. It is therefore useful to view the present results in relation to those reported in the previous experiment, whilst noting any modifications in the parasite and host which accompany repeated exposure to challenge.

The results presented in this experiment are broadly similar to those described in Experiment 2.I, and confirm the hypothesis generated above that host protein deficiency significantly reduces parasite success and disease development in hosts exposed to repeated infection. In

addition to confirming previously reported reductions in egg production and size, results indicate that the recruitment of larval schistosomes is reduced in protein deficient hosts when compared with well-fed animals. Pathogenesis was reduced in protein deficient, infected animals when compared with well-fed controls.

The Parasite

The results of this experiment largely parallel those of Experiment 2.I, however some interesting changes occur with the accumulation of host experience to infection. The observation that worm numbers did not differ between host dietary groups exposed to low challenge suggests that the greater worm survival seen in well-fed hosts when compared with deficient hosts in the previous experiment is reduced by an acquired immunity to reinfection, and that this effect was less in deficient hosts. However the light infections achieved in this experiment preclude definitive comment. This can be contrasted with the smaller number of male and female worms establishing in protein deficient animals receiving high challenge compared with well-fed animals; this result suggests that poor conditions for worm establishment can limit worm numbers to a greater extent than acquired immunity can in the well-fed, theoretically immunointact host.

The observation that male worms are on average larger in protein deficient hosts runs contrary to the evidence presented in Experiment 2.I. Although this result suggests that males are generally larger, size distributions show that a greater proportion of male worms in well-fed hosts appear in the smaller size classes. This indicates that well-fed hosts continue to accumulate schistosomes during repeated infection, despite a theoretical increase in acquired immunity. That undernourished hosts harbored fewer males in these size classes at autopsy suggests that deteriorating conditions during repeated infections reduce the survival of larval schistosomes.

From these indications, it appears that acquired immunity in well-fed hosts may lower total worm numbers by reducing the survival of worms of all ages, whereas the acquired resistance developed during protein deficiency does so by reducing the survival of larvae during later challenges. This suggests that invading schistosomes may be more susceptible to suboptimal conditions than to acquired immunity. Thus it may be that poor host nutrition may contribute to the generation of worm loads comprised mainly of larger worms. This may implicate suboptimal host conditions as contributing to the concomitant immunity commonly observed in natural schistosome infections.

In accordance with the results obtained in Experiment 2.I, which showed schistosome fecundity was reduced in protein deficient hosts when compared with well-fed controls, significantly higher egg counts.female⁻¹ worm were recorded in the tissues of well-fed hosts. That a greater proportion of tissue eggs occurred in the intestines of well-fed animals

suggests that in addition to greater egg production by females, egg migration from liver to intestine is more efficient in well-fed hosts. This lends support to the theory that immune responses facilitate egg migration through differentiated host tissues (Doenhoff *et al.*, 1978; 1986). It is suggested that the increased immune activity seen in well-fed animals facilitates egg migration, and thus increases parasite reproductive success over that seen in poorly fed hosts.

The observation that schistosome eggs lodged in the livers of protein deficient hosts are smaller than those in well-fed animals supports the results described in Experiment 2.I. It appears that despite greater egg retention in deficient hosts (see above), and thus increased egg age when compared with well-fed hosts, suboptimal conditions markedly suppress egg development (see Experiment 2.I).

The Host

Mice fed the low protein diet begun two weeks prior to first infection were significantly lighter than well-fed animals at the time of first infection, and thus it can be concluded that cercariae experienced deficient hosts.

Mice which were maintained on the high protein diet clearly suffered greater pathology than those receiving the low protein diet, and this led to a high death rate in the former. The increased time of active infection in this experiment (9 weeks) over Experiment 2.I (6 weeks) includes the period when schistosome egg production and resultant pathology became fulminant. Despite this crisis period, undernourished animals suffered negligible mortality; it is suggested that this was a result of reduced parasite antigenic stimulus (see above) combined with diminished immunopathology (see below).

Food intake fell in animals offered the high protein diet to a much greater extent than intakes of those receiving low protein diets. That this effect only occurred in infected animals, and was associated with infection, rather than disease (onset was immediately after first exposure to cercariae) suggests that schistosome infection was associated with anorexia. As anorexia has a largely immune aetiology (see Chapter 1), it is suggested that the various immune mechanisms responsible for anorexia were suppressed in undernourished hosts.

Results showing significantly smaller granulomata forming in the livers of protein deficient hosts when compared with those in well-fed animals agree with results reported in Experiment 2.I. It should be noted that high cercarial challenge in mice fed the low protein diet did not produce any significant increase in granulomata sizes. This suggests that repeated infection does not exacerbate granulomata sizes in deficient hosts, an idea consistent with the immune suppression theory of reduced pathology.

That liver sizes in animals fed the low protein diet and exposed to low infection did not differ from those in similarly challenged, well-fed animals, yet there was high mortality, may indicate that the more diseased mice died first, which might be expected. Under high challenge, mice fed low protein diets had smaller livers than did well-fed animals, an observation consistent with results in Experiment 2.I.

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: III. ACUTE PHASE TRICKLE INFECTIONS

Abstract

This experiment examines the progressive effects of externally imposed host protein deficiency on the parasite and host during multiple cercarial challenges. Results show that parasite recruitment is reduced to a greater extent during protein deficiency than during infection in well-fed hosts, despite a decreased immune response in the former. Worms inhabiting deficient hosts are generally less successful than those in well-fed controls.

INTRODUCTION

The previous experiment has shown that host protein deficiency can significantly reduce the development of schistosomes and pathology in the vertebrate host during repeated infections, whilst suggesting that host protein deficiency reduces parasite recruitment. However the single autopsy point used in that experiment precluded examination of the progression of parasite and disease development during multiple infections.

Under natural conditions, it is thought that the acquisition of specific immunity contributes to the convex relationship commonly observed between schistosome intensity and host age (Woolhouse, Taylor, Matanhire & Chandiwana, 1991). This suggests that a protective immune response exists in the human host, a notion which in turn fuels the search for an effective vaccine.

However infection also changes the host by inducing metabolic responses which produce nutritional stress and resultant pathology (see Chapter 1). As nutritional stress (see above) and pathology (McHugh, Coulson & Wilson, 1987) can compromise schistosome survival, it may be that the observed convexity may not simply reflect the acquisition of immunity and/or age-related changes in exposure, but also the acquisition of innate resistance resulting from deteriorating nutritional status rendering conditions suboptimal for invading schistosomes.

As no previous study has examined these issues, this experiment was designed to test the contribution of each form of host defence by employing the contrasting effects of externally imposed protein deficiency on innate resistance (increased due to suboptimal conditions) and acquired immunity (reduced; see Chapter 1). The ultimate measure of host defences however, is protection from disease, not infection. Therefore the undernourished host's resistance to parasites was also viewed in the light of the host's resistance to disease.

Hypothesis

That protein deficiency alters parasite success and host disease in the acute stages of schistosome infection in hosts exposed to multiple infection.

The influence of dietary protein on both parasite success and host pathogenesis was examined at mouse autopsy four weeks after the last of between 1 and 6 weekly exposures to either 15 or 60 cercariae.mouse⁻¹. Mice belonged to two dietary groups: those receiving 3% or 16% protein diets begun 2 weeks prior to first cercarial exposure.

EXPERIMENTAL DESIGN

Two hundred and sixty mice were randomly assigned to groups of 10, housed in 26 large cages, and fed SLC. After 2 weeks, 13 groups were begun on the 3% protein (D3) and 13 groups begun on the 16% protein diet (D16). After a further three weeks, mice from 12 groups (six from each of the two dietary régimes) were individually exposed to 15 cercariae.mouse⁻¹ (I.15; range 13-15) for 30 min, whilst those from a further 12 groups were exposed to 60 cercariae.mouse⁻¹ (I.60; range 57-62). All cercariae used in this experiment were gathered each week from the same group of 40 patent snails (no mortality). Mice from two control groups of 10 (one from each dietary régime) were handled identically to exposed animals, but in the absence of cercariae.

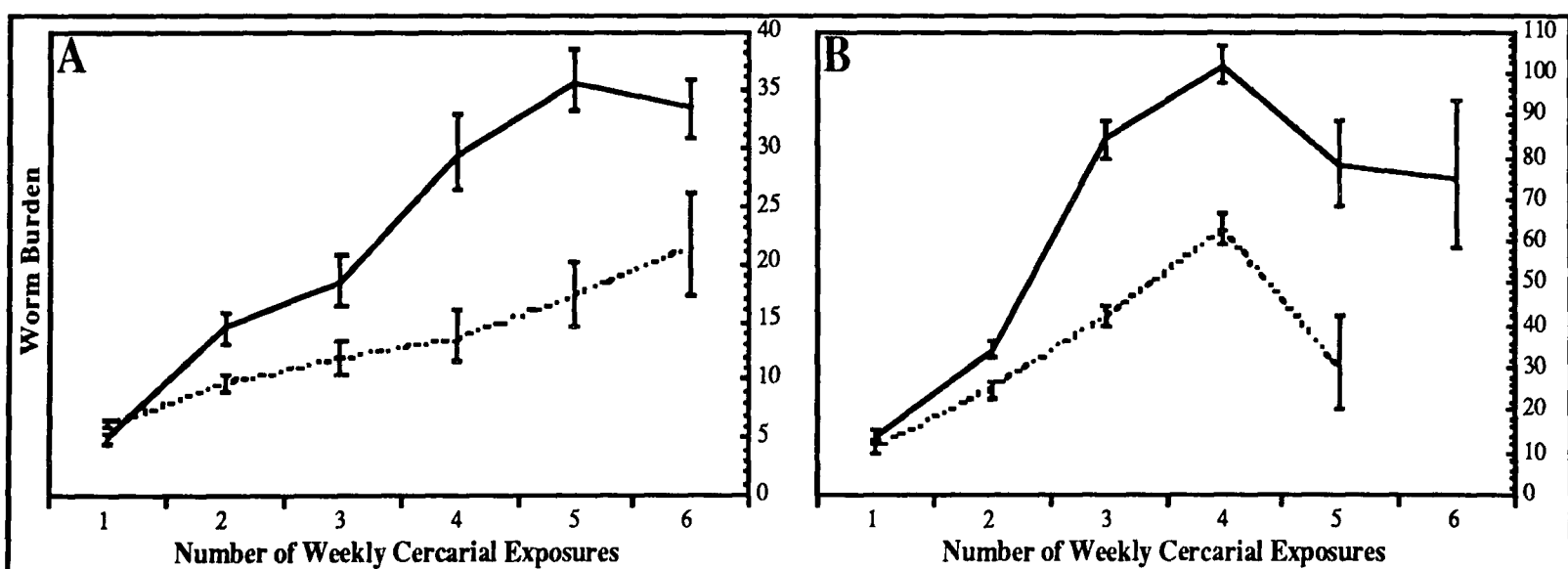
At the end of Week 0, only the two control groups remained unexposed to cercariae. In each of the subsequent five weeks, two more groups (one previously exposed to 15 and one to 60 cercariae) from each of the dietary régimes were handled in the absence of cercariae. In this way, each group of mice from the two dietary régimes received 1, 2, 3, 4, 5 or 6 weekly exposures to either 15 or 60 cercariae.mouse⁻¹, yet were handled identically. Four weeks after their final exposure, individual mice contributed a 24 h faecal sample and were bled and autopsied (At) as described in the Materials and Methods section of this chapter. The design of this experiment is presented in Table 1 (overleaf).

Week Group (n)	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9
A (10)	SLC	→	D3	→	-	-	-	-	→	-	-	-	-	→	At
B (10)	SLC	→	D16	→	-	-	-	-	→	-	-	-	-	→	At
C (10)	SLC	→	D3	→	→	I.15	→	-	→	At					
D (10)	SLC	→	D3	→	→	I.15	I.15	→	-	→	At				
E (10)	SLC	→	D3	→	→	I.15	I.15	I.15	→	-	→	At			
F (10)	SLC	→	D3	→	→	I.15	I.15	I.15	I.15	→	-	→	At		
G (10)	SLC	→	D3	→	→	I.15	I.15	I.15	I.15	I.15	→	-	→	At	
H (10)	SLC	→	D3	→	→	I.15	I.15	I.15	I.15	I.15	I.15	→	-	→	At
I (10)	SLC	→	D3	→	→	I.60	→	-	→	At					
J (10)	SLC	→	D3	→	→	I.60	I.60	→	-	→	At				
K (10)	SLC	→	D3	→	→	I.60	I.60	I.60	→	-	→	At			
L (10)	SLC	→	D3	→	→	I.60	I.60	I.60	I.60	→	-	→	At		
M (10)	SLC	→	D3	→	→	I.60	I.60	I.60	I.60	I.60	→	-	→	At	
N (10)	SLC	→	D3	→	→	I.60	I.60	I.60	I.60	I.60	I.60	→	-	→	At
O (10)	SLC	→	D16	→	→	I.15	→	-	→	At					
P (10)	SLC	→	D16	→	→	I.15	I.15	→	-	→	At				
Q (10)	SLC	→	D16	→	→	I.15	I.15	I.15	→	-	→	At			
R (10)	SLC	→	D16	→	→	I.15	I.15	I.15	I.15	→	-	→	At		
S (10)	SLC	→	D16	→	→	I.15	I.15	I.15	I.15	I.15	→	-	→	At	
T (10)	SLC	→	D16	→	→	I.15	I.15	I.15	I.15	I.15	I.15	→	-	→	At
U (10)	SLC	→	D16	→	→	I.60	→	-	→	At					
V (10)	SLC	→	D16	→	→	I.60	I.60	→	-	→	At				
W (10)	SLC	→	D16	→	→	I.60	I.60	I.60	→	-	→	At			
X (10)	SLC	→	D16	→	→	I.60	I.60	I.60	I.60	→	-	→	At		
Y (10)	SLC	→	D16	→	→	I.60	I.60	I.60	I.60	I.60	→	-	→	At	
Z (10)	SLC	→	D16	→	→	I.60	I.60	I.60	I.60	I.60	I.60	→	-	→	At

TABLE 1: Design of Trickle Infections experiment (n=number of mice in group).

RESULTS: PARASITE

Number: Males

FIGURE 1: Numbers of male worms recovered from mice fed 3% protein (dotted) and 16% protein (solid) diets following 1 to 6 weekly exposures to (A) 15 and (B) 60 cercariae.mouse⁻¹.

Significantly fewer male worms established in well-fed animals exposed to a single low level infection than established in similarly challenged, protein deficient animals. This result was reversed following 2 challenges and thereafter significantly fewer males established in poorly fed hosts than in well-fed animals (Figure 1; Table 2, overleaf). In animals exposed to high

levels of infection, there was no difference between dietary groups following a single challenge, however from Week 2 onwards, well-fed hosts became infected with significantly more male schistosomes than did similarly challenged, protein deficient hosts. Weeks 5 and 6 saw reductions in male worm numbers in mice exposed to high challenge intensity, regardless of dietary treatment.

3% Protein		Exposures	1	2	3	4	5	6
range (15cerc.wk-1) (n)			5-7 (10)	5-13 (10)	7-20 (10)	4-25 (10)	4-26 (10)	7-48 (10)
range (60cerc.wk-1) (n)			2-17 (10)	16-35 (10)	29-60 (10)	40-80 (10)	12-42 (4)	-
Comparison (15 chall.)	T-test p value (t;df)		0.048*(1.76;18)	0.003*	0.009*	0.0003*	0.0002*	0.018*
Comparison (60 chall.)	T-test p value (t;df)		0.15(-1.09;17)	(-3.09;18)	(-2.63;18)	(-4.11;18)	(-4.57;16)	(-2.27;17)
range (15cerc.wk-1) (n)			3-8 (10)	8-19 (10)	10-28 (10)	10-45 (10)	23-49 (10)	21-44 (10)
range (60cerc.wk-1) (n)			5-20 (10)	29-51 (10)	70-110 (10)	80-120 (10)	52-111 (10)	47-112 (10)
16% Protein		Exposures	1	2	3	4	5	6

TABLE 2: T-test comparisons of numbers of male worms establishing in mice fed 3% (upper) or 16% (lower) receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (central numbers represent T-test p values followed by t values and degrees of freedom).

Number: Females

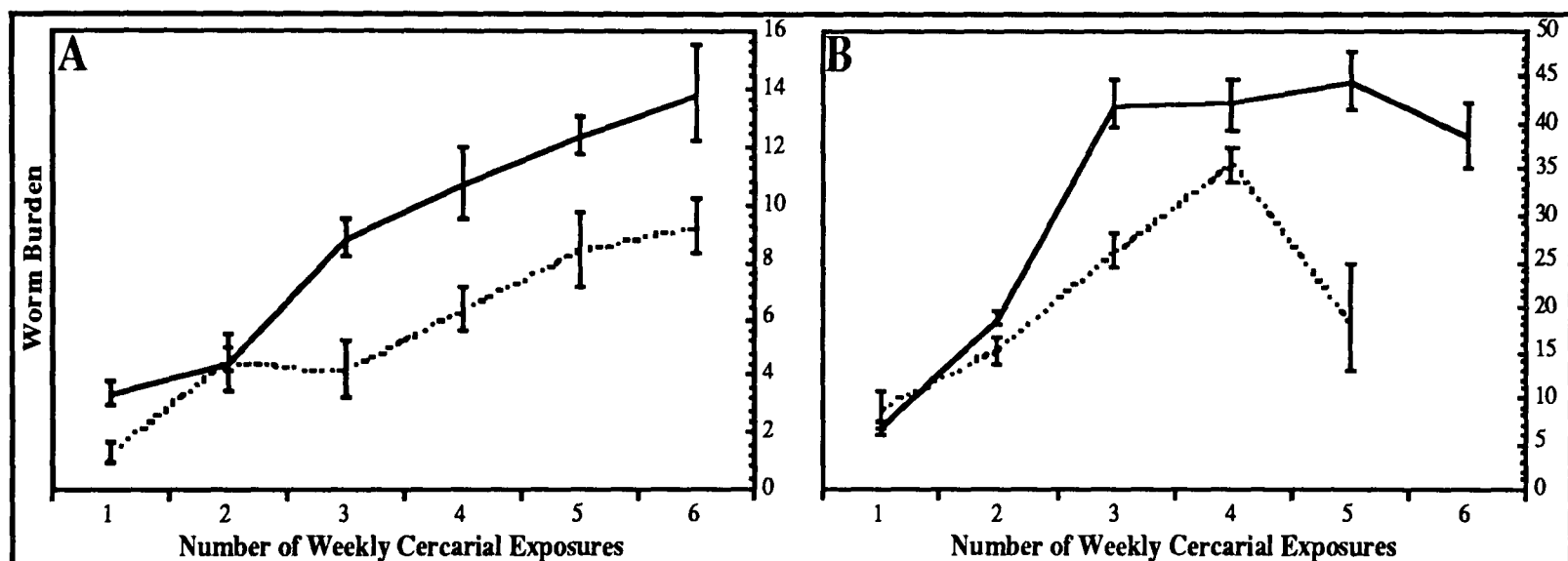


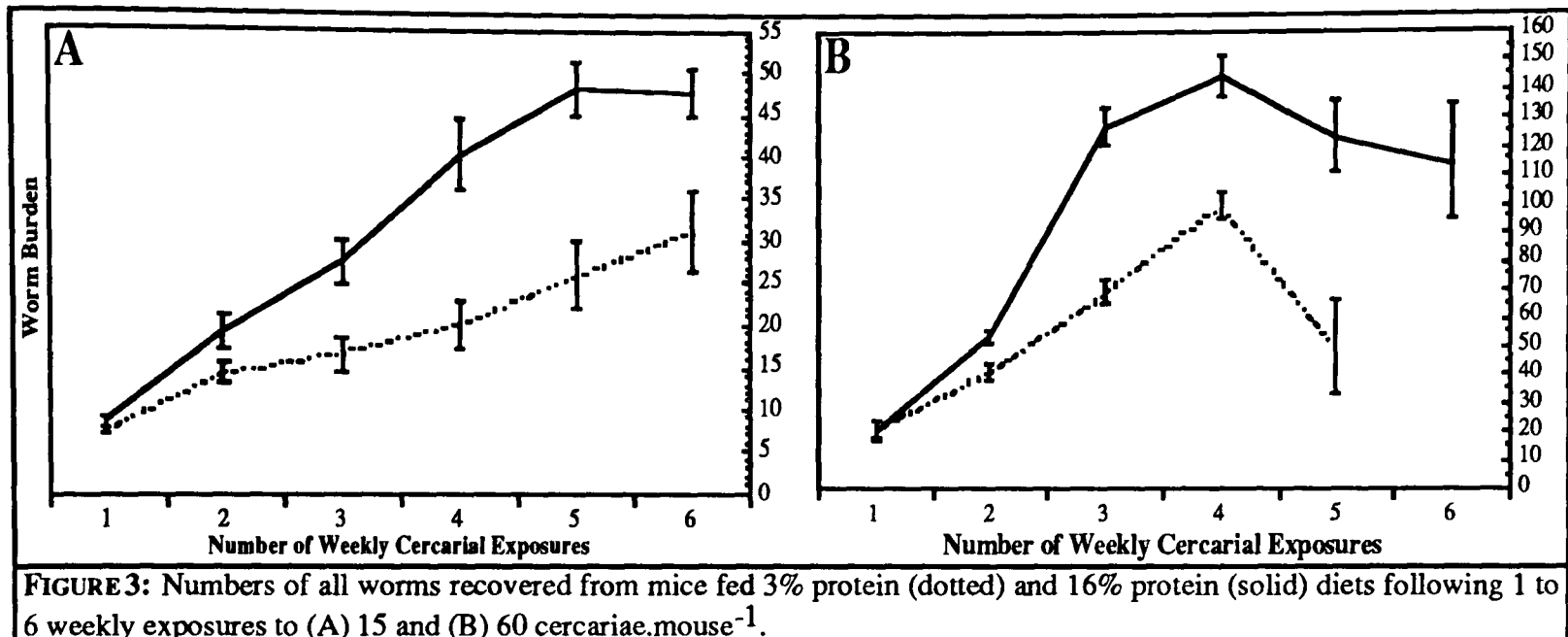
FIGURE 2: Numbers of female worms recovered from mice fed 3% protein (dotted) and 16% protein (solid) diets following 1 to 6 weekly exposures to (A) 15 and (B) 60 cercariae.mouse⁻¹.

Significantly more female worms established in well-fed animals exposed to a single low level infection than established in similarly challenged, protein deficient animals. There was no significant difference between these groups after 2 challenges, however thereafter significantly fewer females established in poorly fed hosts than in well-fed animals (Figure 2; Table 3). In animals exposed to high levels of infection, there was no difference between dietary groups following a single challenge, however from Week 2 onwards, well-fed hosts became infected with significantly more female schistosomes than did similarly challenged, protein deficient hosts. Weeks 5 and 6 saw reductions in female worm numbers in mice exposed to high challenge intensity, regardless of dietary treatment.

3% Protein		Exposures	1	2	3	4	5	6
range (15cerc.wk-1) (n)			0-2 (10)	3-7 (10)	0-8 (10)	2-10 (10)	2-13 (10)	5-14 (10)
range (60cerc.wk-1) (n)			0-16 (10)	9-22 (10)	19-38 (10)	24-44 (10)	9-28 (4)	-
Comparison (15 chall.)	T-test p value (t;df)		0.001*	0.47(0.09;18)	0.002*	0.003*	0.009*	0.012*
Comparison (60 chall.)	T-test p value (t;df)		(-3.60;18)	0.21(0.84;17)	(-3.39;18)	(-3.08;18)	(-2.65;16)	(-2.47;17)
range (15cerc.wk-1) (n)			2-7 (10)	1-10 (10)	7-13 (10)	5-17 (10)	9-16 (10)	6-21 (10)
range (60cerc.wk-1) (n)			4-12 (10)	16-24 (10)	28-51 (10)	31-63 (10)	33-55 (10)	34-49 (10)
16% Protein		Exposures	1	2	3	4	5	6

TABLE 3: T-test comparisons of numbers of female worms establishing in mice fed 3% (upper) or 16% (lower) receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (central numbers represent T-test p values followed by t values and degrees of freedom).

Number: Total Worms

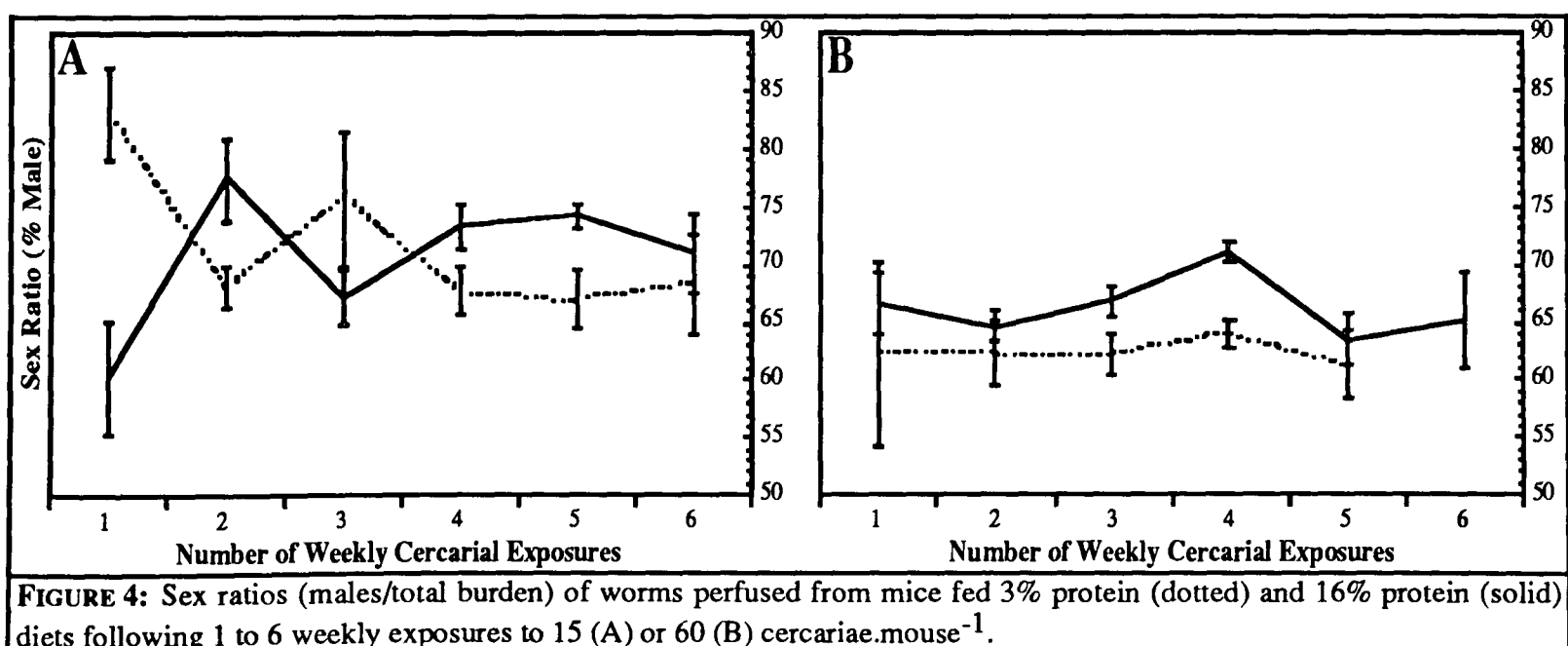


Although they did not differ after a single challenge, significantly more worms established in well-fed animals exposed to multiple low level infections than established in similarly challenged, protein deficient animals (Figure 3; Table 4). Results were the same for animals exposed to higher infection levels. Weeks 5 and 6 saw reductions in total worm numbers in mice exposed to high challenge intensity, regardless of dietary treatment.

3% Protein		Exposures					
range (15cerc.wk-1) (n)		1	2	3	4	5	6
range (60cerc.wk-1) (n)		5-9 (10)	8-20 (10)	7-27 (10)	6-33 (10)	6-38 (10)	12-59 (10)
Comparison (15 chall.)	T-test p value (t;df)	2-33 (10)	33-57 (10)	50-94 (10)	64-112 (10)	21-70 (4)	-
Comparison (60 chall.)	T-test p value (t;df)	0.08(-1.44;8)	0.022*	0.002*	0.0003*	0.0003*	0.005*
			(-2.18;18)	(-3.44;18)	(-4.09;18)	(-4.29;16)	(-2.89;17)
		0.46(-0.10;17)	0.0007*	0.0001*	0.0001*	0.004*(-3.65;7)	-
			(-3.76;18)	(-8.05;17)	(-5.56;18)		
16% Protein		Exposures					
range (15cerc.wk-1) (n)		1	2	3	4	5	6
range (60cerc.wk-1) (n)		6-12 (10)	11-28 (10)	17-37 (10)	15-57 (10)	34-64 (10)	37-62 (10)
		9-32 (10)	47-73 (10)	101-151 (10)	115-183 (10)	94-166 (10)	81-152 (10)

TABLE 4: T-test comparisons of total worm numbers establishing in mice fed 3% (upper) or 16% (lower) receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (central numbers represent T-test p values followed by t values and degrees of freedom).

Sex Ratio



Differential survival of the sexes (see above) produced sex ratio fluctuations (Figure 4; Table 5, overleaf), showing a tendency for males to comprise a high proportion of worm burdens in well-fed compared with undernourished hosts.

3% Protein		Exposures					
range (15cerc.wk ⁻¹)(n)	% male	1	2	3	4	5	6
range (60cerc.wk ⁻¹)(n)	% male	71.43-100(10)	61.11-78.57(10)	57.14-100(10)	57.14-75.76(10)	53.57-75(10)	48.15-84.44(10)
Comparison (15 chall.)	T-test p value (t;df)	0.001*(3.56;18)	0.022* (-2.18;18)	0.10(1.33;18)	0.04*(-1.89;18)	0.007* (-2.77;16)	0.30(-0.52;17)
Comparison (60 chall.)	T-test p value (t;df)	0.31(-0.49;17)	0.24(-0.73;18)	0.023* (-2.16;17)	0.0001* (-4.65;18)	0.29(-0.59;7)	-
16% Protein		Exposures					
range (15cerc.wk ⁻¹)(n)	% male	1	2	3	4	5	6
range (60cerc.wk ⁻¹)(n)	% male	30-75(10)	64.29-94.44(10)	55.56-75.68(10)	65.31-82.05(10)	67.65-77.78(10)	56.1-85.37(10)
Comparison (15 chall.)	T-test p value (t;df)	0.001*(3.56;18)	0.022* (-2.18;18)	0.10(1.33;18)	0.04*(-1.89;18)	0.007* (-2.77;16)	0.30(-0.52;17)
Comparison (60 chall.)	T-test p value (t;df)	0.31(-0.49;17)	0.24(-0.73;18)	0.023* (-2.16;17)	0.0001* (-4.65;18)	0.29(-0.59;7)	-

TABLE 5: T-test comparisons of percentage "maleness" of worm burdens (number of males/total burden) in mice fed 3% (upper) or 16% (lower) receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (central numbers represent T-test p values followed by t values and degrees of freedom).

Size: Means

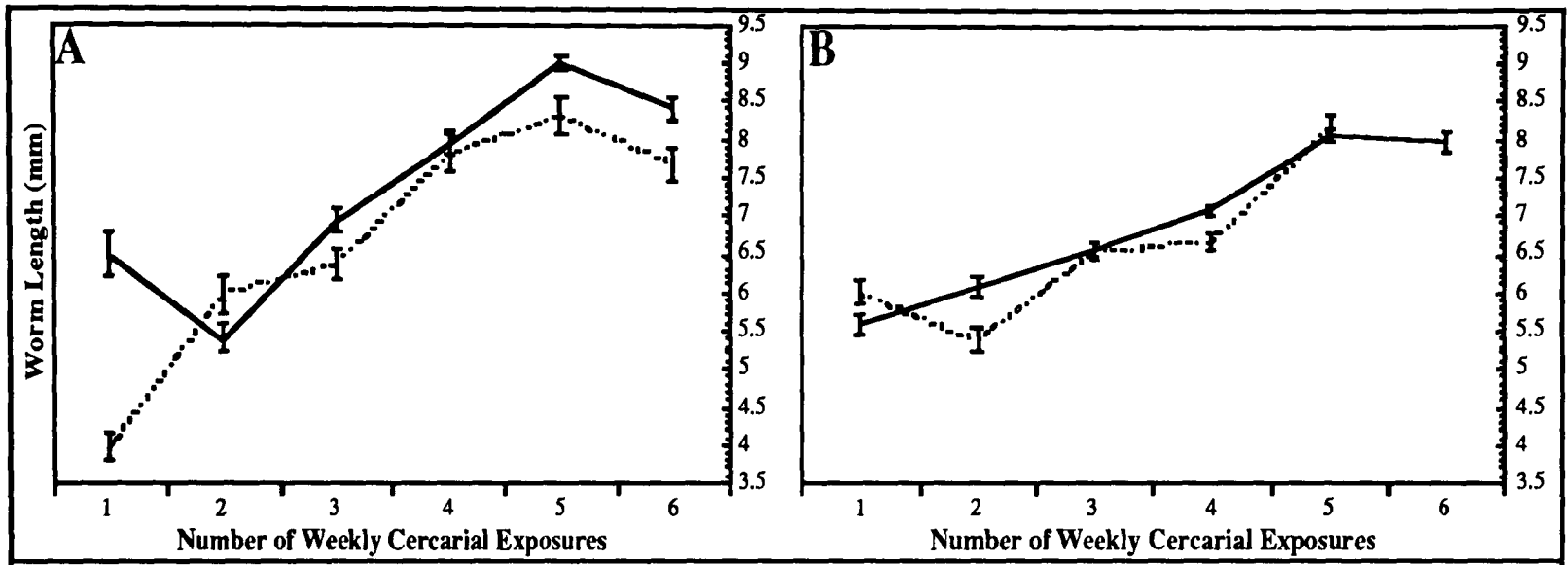


FIGURE 5: Mean length of male worms perfused from mice fed 3% protein (dotted) and 16% protein (solid) diets following 1 to 6 weekly exposures to 15 (A) or 60 (B) cercariae.mouse⁻¹.

Male worms recovered from mice fed low protein diets were generally shorter than those recovered from well-fed hosts exposed to multiple challenges of both lower and higher intensity (Figure 5; Table 6).

3% Protein		Exposures					
range (mm) (15cerc.wk ⁻¹)(n)		1	2	3	4	5	6
range (mm) (60cerc.wk ⁻¹)(n)		1.7-6.4 (61)	1.6-11 (98)	0.7-10.6 (122)	1.0-11.8 (143)	1.6-12.6 (143)	1.5-13.1 (208)
Comparison (15 chall.)	T-test p value (t;df)	0.001* (-7.93;110)	0.034* (-1.84;242)	0.006* (-2.52;310)	0.31(-0.50;443)	0.0004* (-3.35;501)	0.003* (-2.83;544)
Comparison (60 chall.)	T-test p value (t;df)	0.024* (1.98;243)	0.0004* (-3.33;600)	0.34 (-0.40;1191)	0.0001* (-3.70;1656)	0.33(0.44;572)	-
16% Protein		Exposures					
range (mm) (15cerc.wk ⁻¹)(n)		1	2	3	4	5	6
range (mm) (60cerc.wk ⁻¹)(n)		1.7-9 (51)	1.6-11.6 (146)	1.9-13.1 (190)	1.4-13.4 (302)	4.1-16.1 (360)	1-13.1 (338)
Comparison (15 chall.)	T-test p value (t;df)	0.001*(3.56;18)	0.022* (-2.18;18)	0.10(1.33;18)	0.04*(-1.89;18)	0.007* (-2.77;16)	0.30(-0.52;17)
Comparison (60 chall.)	T-test p value (t;df)	0.31(-0.49;17)	0.24(-0.73;18)	0.023* (-2.16;17)	0.0001* (-4.65;18)	0.29(-0.59;7)	-

TABLE 6: T-test comparison of male worm lengths in mice receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (central numbers represent T-test p values followed by t values and degrees of freedom).

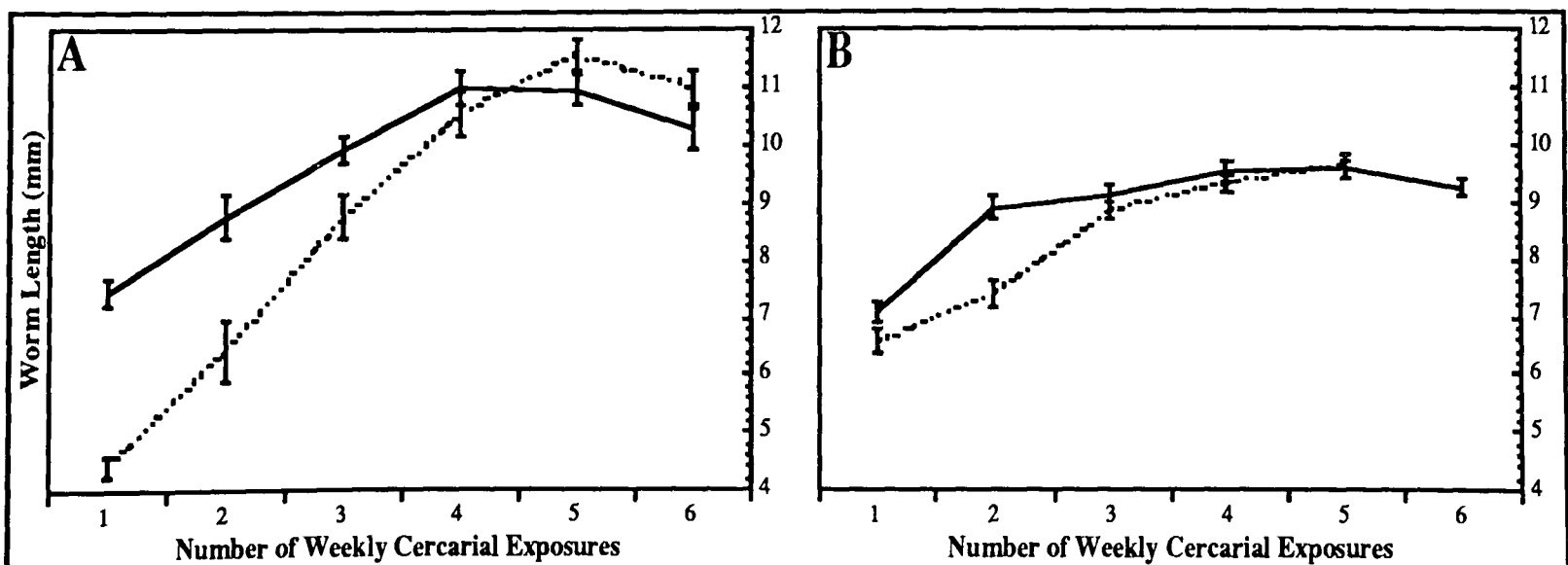


FIGURE 6: Mean length of female worms perfused from mice fed 3% protein (dotted) and 16% protein (solid) diets following 1 to 6 weekly exposures to 15 (A) or 60 (B) cercariae.mouse⁻¹.

Female worms establishing in poorly fed hosts were smaller than those infecting well-fed animals after 1, 2 and 3 low intensity challenges and 1 and 2 higher intensity challenges (Figure 6, previous page; Table 7). It appears that this early stunting subsequently disappeared, leaving no differences in female sizes across dietary groups.

3% Protein		Exposures					
		1	2	3	4	5	6
range (mm) (15cerc.wk ⁻¹)(n)		2.8-6.1 (15)	0.6-11.6 (46)	1.9-12.7 (47)	2.6-13.7 (65)	3.3-14.7 (70)	3.7-14.6 (84)
range (mm) (60cerc.wk ⁻¹)(n)		3.3-11.2 (98)	2.7-12.6 (156)	2.7-13.6 (265)	2.3-15.0 (357)	5.3-13.9 (58)	-
Comparison (15 chall.)	T-test p value (t;df)	0.0001*	0.0004*	0.005*	0.19(-0.89;173)	0.09(1.36;192)	0.086(1.37;224)
Comparison (60 chall.)	T-test p value (t;df)	(-8.98;48)	(-3.45;93)	(-2.61;134)	0.18(-0.93;780)	0.39(0.27;324)	-
		(-1.93;159)	(-4.66;341)				
16% Protein		Exposures					
		1	2	3	4	5	6
range (mm) (15cerc.wk ⁻¹)(n)		4.7-10.4 (35)	4.3-14.3 (49)	1.9-14.8 (89)	1.6-15.8 (110)	0.9-15.3 (124)	0.8-14.8 (142)
range (mm) (60cerc.wk ⁻¹)(n)		4.3-9.3 (63)	2.1-13.2 (187)	0.7-14.3 (380)	1.0-15.2 (425)	3.7-13.6 (268)	0.9-13.1 (155)

TABLE 7: T-test comparison of female worm lengths in mice receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (central numbers represent T-test p values followed by t values and degrees of freedom).

Size: Frequency Distributions

Frequency distributions were included to visualize the change in worm size distributions during repeated exposure.

Male

Males tended to be smaller in mice fed low protein, as opposed to high protein diets (Figure 7, overleaf). Higher challenge intensity saw reductions of male sizes within dietary groups.

Female

Females tended to be smaller in mice fed low protein diets (Figure 8, overleaves). Higher challenge intensity saw reductions of female sizes within dietary groups. It should be noted that a larger number of smaller females was found after several challenges in well-fed hosts than in protein-deficient hosts, regardless of challenge intensity.

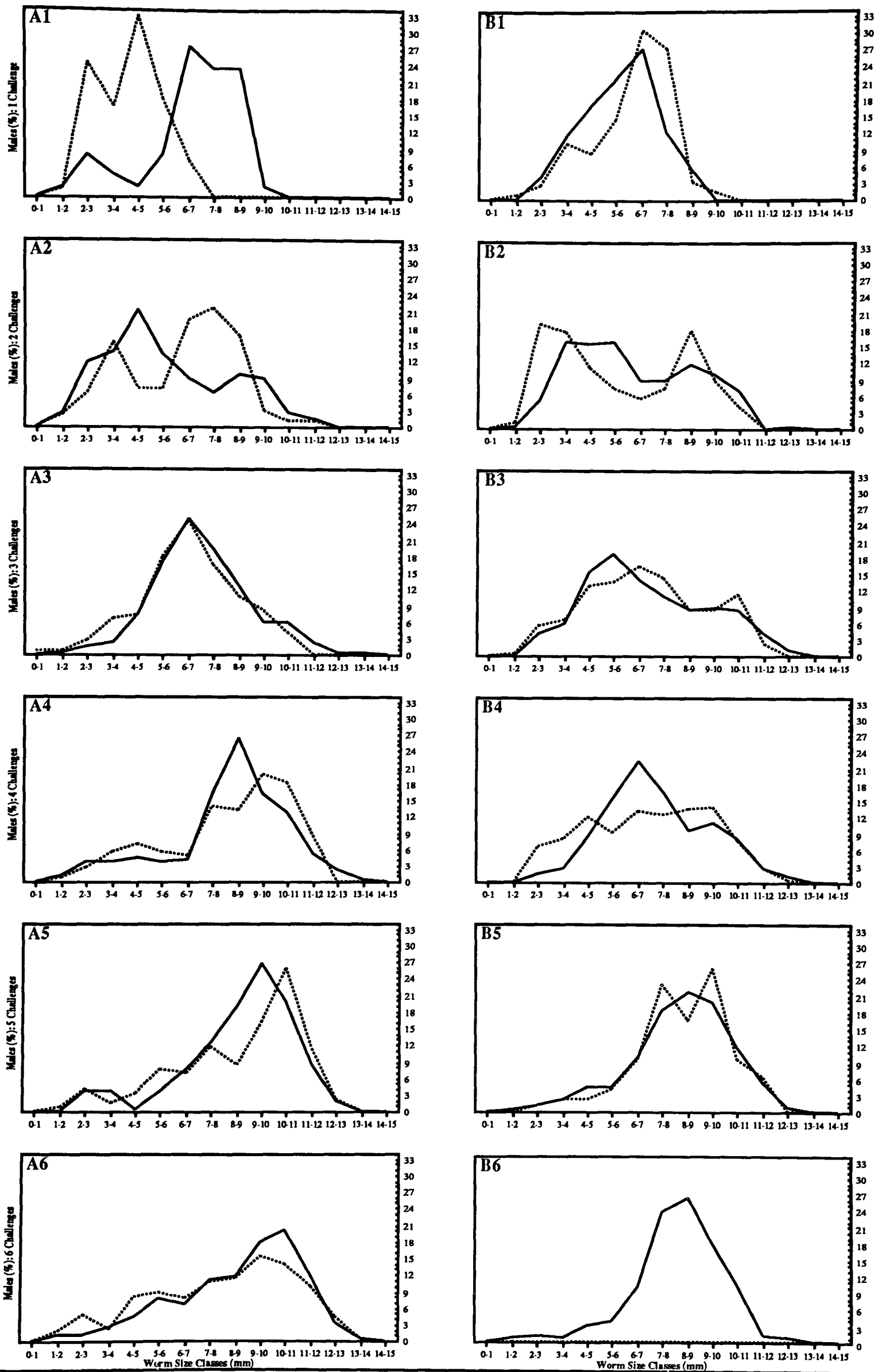


FIGURE 7: Frequency distributions (% of total males) of sizes of male schistosomes perfused from mice fed 3% (dotted) or 16% (solid) protein diets and receiving between 1 (top) and 6 (bottom) exposures to 15 (A) or 60 (B) cercariae.mouse⁻¹.week⁻¹.

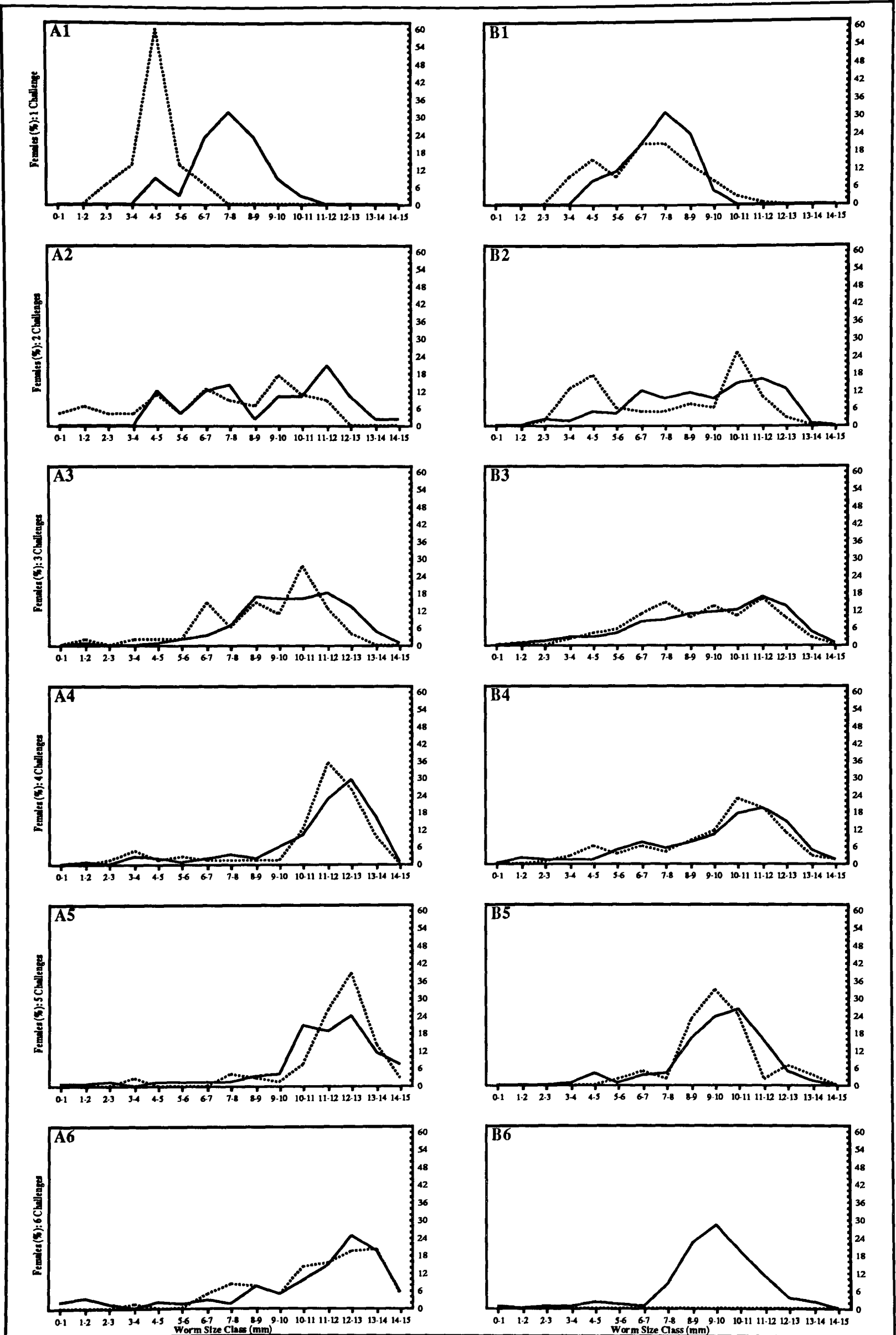
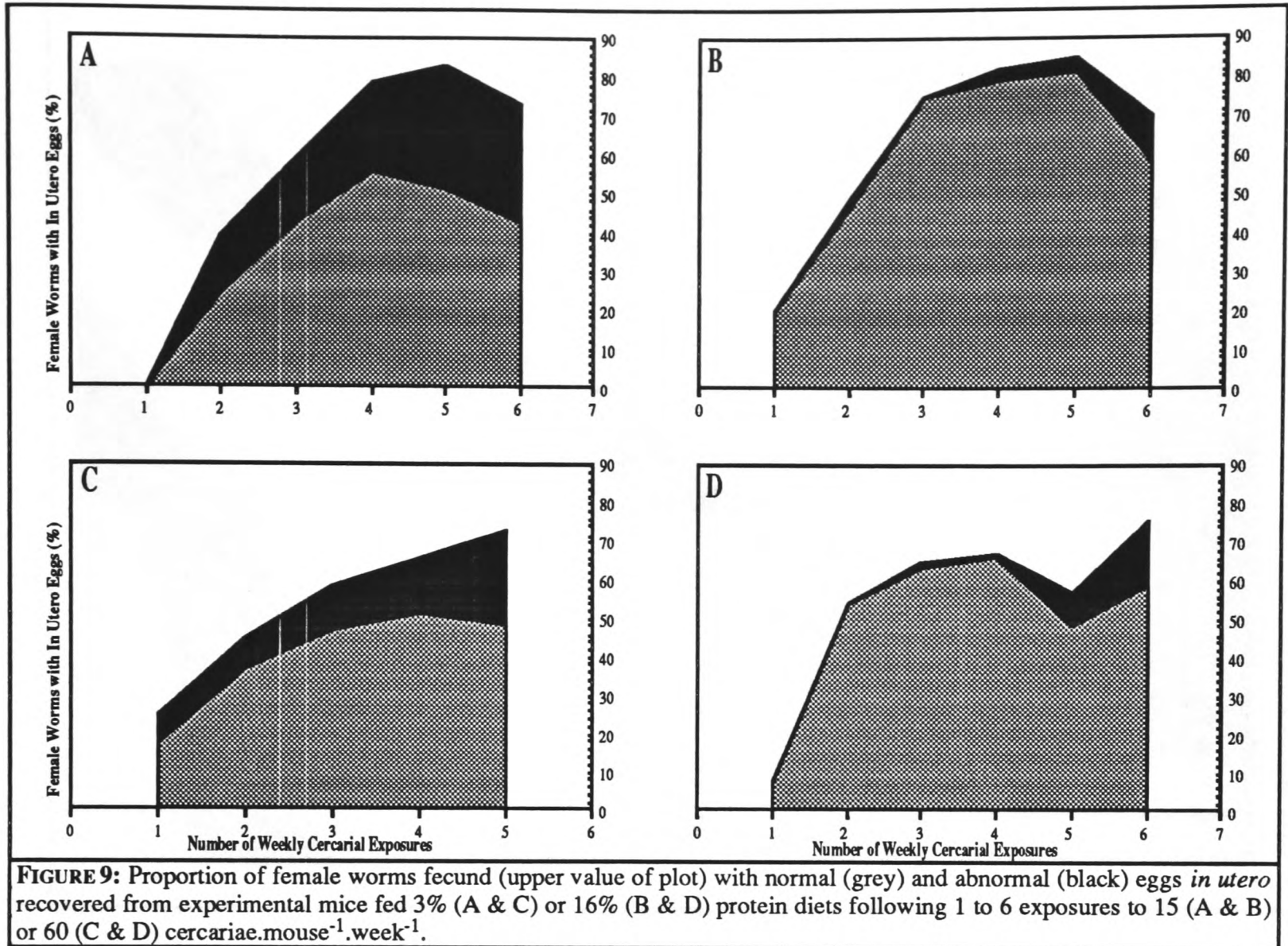


FIGURE 8: Frequency distributions (% of total females) of sizes of female schistosomes perfused from mice fed 3% (dotted) or 16% (solid) protein diets and receiving between 1 (top) and 6 (bottom) exposures to 15 (A) or 60 (B) cercariae.mouse⁻¹.week⁻¹.

Fecundity



Although a similar proportion of females are fecund in protein-deficient and well-fed hosts (Figure 9), those inhabiting mice fed the low protein diet produced an appreciably higher proportion of abnormal eggs. Females perfused from mice fed low protein and exposed to one low challenge did not contain *in utero* eggs. *In utero* egg development is depicted in Plate 1 (overleaf).

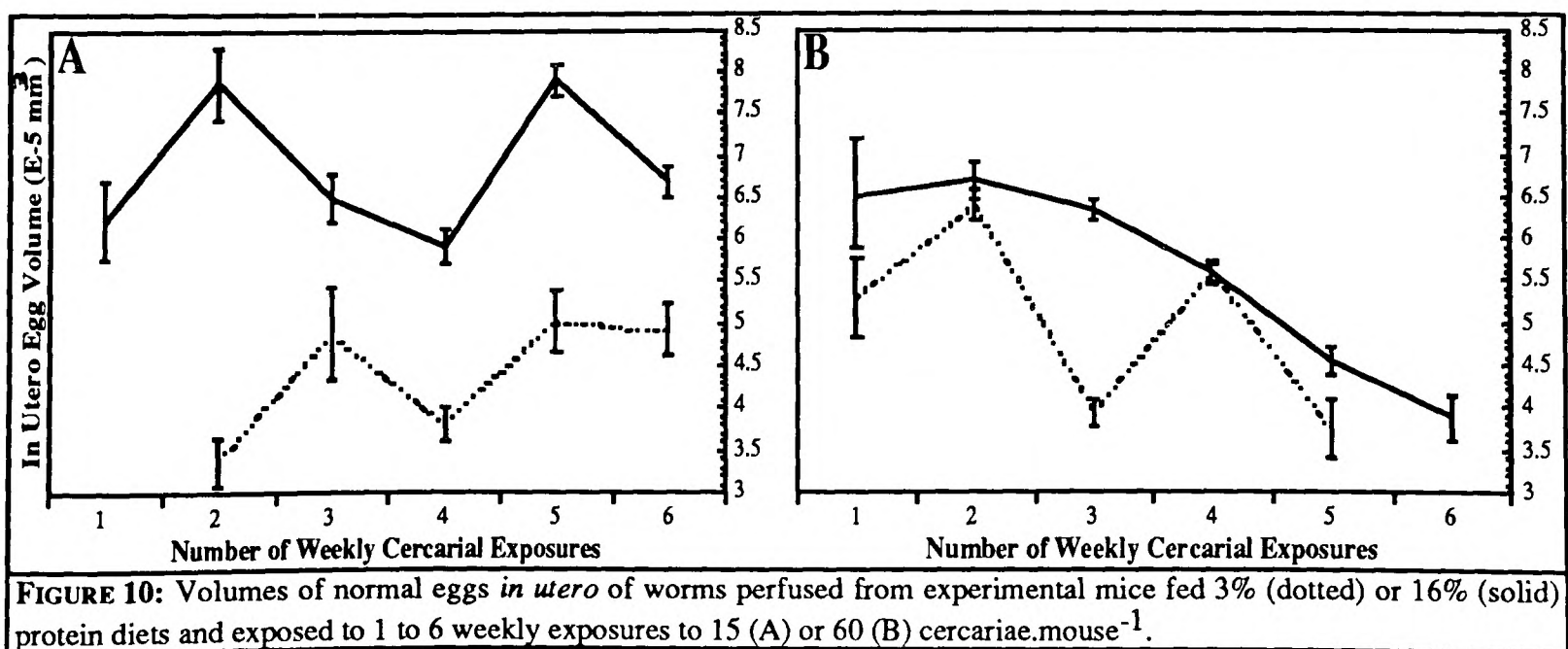
Fecundity: *In Utero* Egg Size

PLATE 1: *In Utero* Schistosome Egg Development

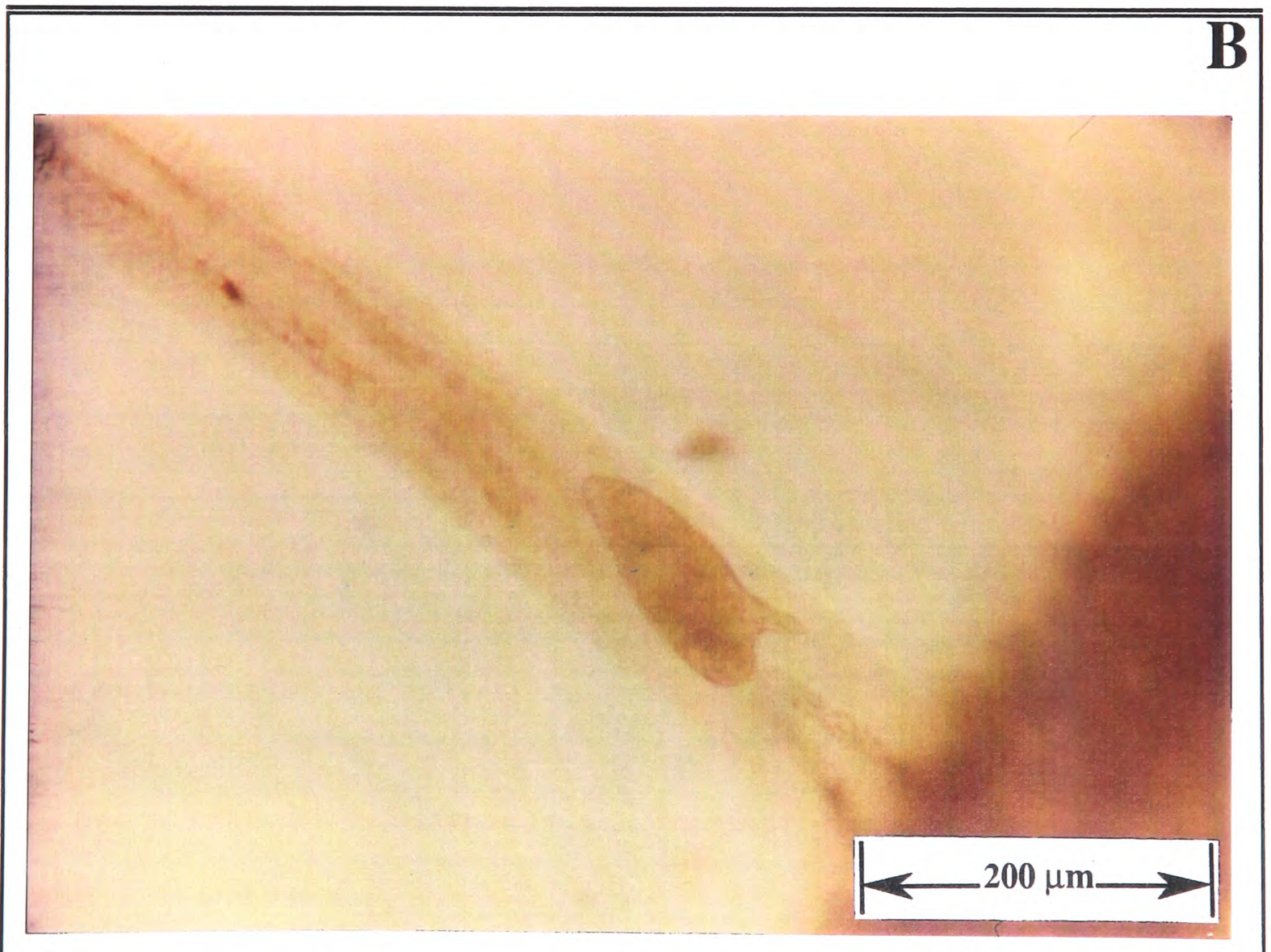
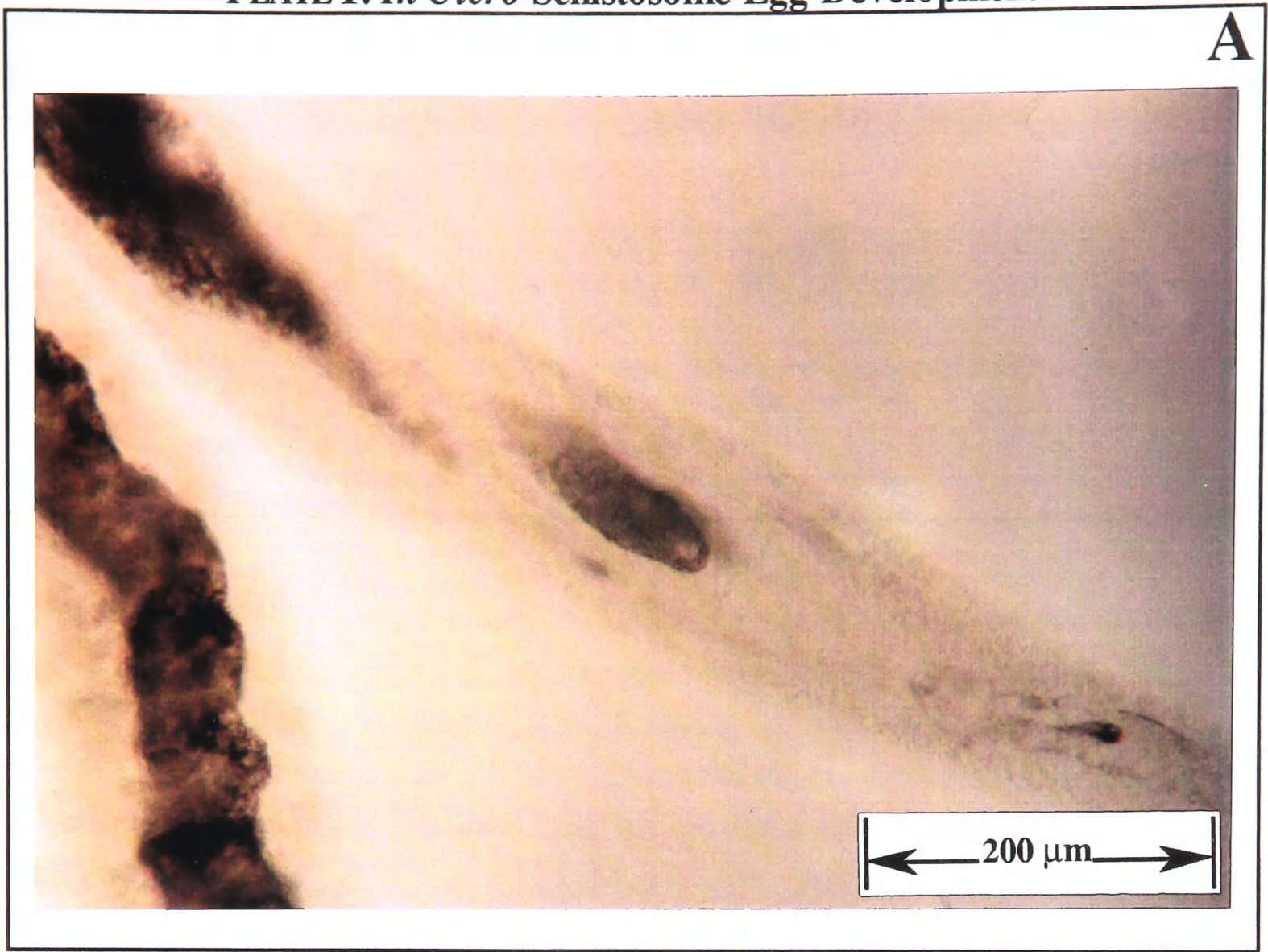


PLATE 1: Abnormally (A) and normally (B) developed eggs photographed *in utero* in female worms perfused from mice exposed to 5 high cercarial challenges and fed 3% and 16% protein diets, respectively (scales are as shown).

Worms inhabiting protein-deficient hosts exposed to low levels of infection have significantly smaller normal eggs *in utero* than do those recovered from well-fed hosts (Figure 10, previous pages; Table 8). This is generally true also for worms recovered from animals exposed to higher levels of infection.

3% Protein range (E-5 mm ³)	Exposures (15cerc.wk ⁻¹) (n)	1	2	3	4	5	6
range (E-5 mm ³)	(60cerc.wk ⁻¹) (n)	- (0)	2.08-5.04 (11)	1.13-9.61 (20)	2.48-9.42 (36)	1.56-9.52 (36)	1.72-8.87 (36)
Comparison (15 chall.)	T-test p value (t;df)	-	0.0001* (-7.97;32)	0.003* (-2.80;85)	0.0001* (-6.15;121)	0.0001* (-7.65;135)	0.0001* (-5.06;117)
Comparison (60 chall.)	T-test p value (t;df)	0.12(-1.23;20)	0.18(-0.93;156)	0.0001* (-11.82;364)	0.50 (-0.011;463)	0.03* (-1.89;156)	-
range (E-5 mm ³)	(15cerc.wk ⁻¹) (n)	4.5-7.56 (7)	3.78-11.08 (23)	3.26-11.27 (67)	2.9-13.3 (87)	2.83-13.5 (101)	2.96-14.24 (83)
range (E-5 mm ³)	(60cerc.wk ⁻¹) (n)	4.5-8.38 (5)	3.1-11.96 (101)	1.63-11.7 (241)	2.01-27.8(282)	0.47-15.1 (130)	0.49-17.56 (92)
16% Protein	Exposures	1	2	3	4	5	6

TABLE 8: T-test comparison of normal *in utero* egg volumes within female worms which are fecund in mice receiving 1-6 exposures to either 15 or 60 cercariae.mouse⁻¹ (n = number of eggs measured; central numbers represent T-test p values followed by t values and degrees of freedom).

RESULTS: HOST

Mortality occurred in 2 mouse groups: all 10 mice in the group receiving low protein diet and 6 high cercarial challenges died during week 8, having completed the maximum number of high exposures 3 weeks previously. Similarly, 6 mice in the group receiving low protein diet and 5 high challenges died during week 7, having completed their quota of 5 high exposures 2 weeks previously.

Weight Change

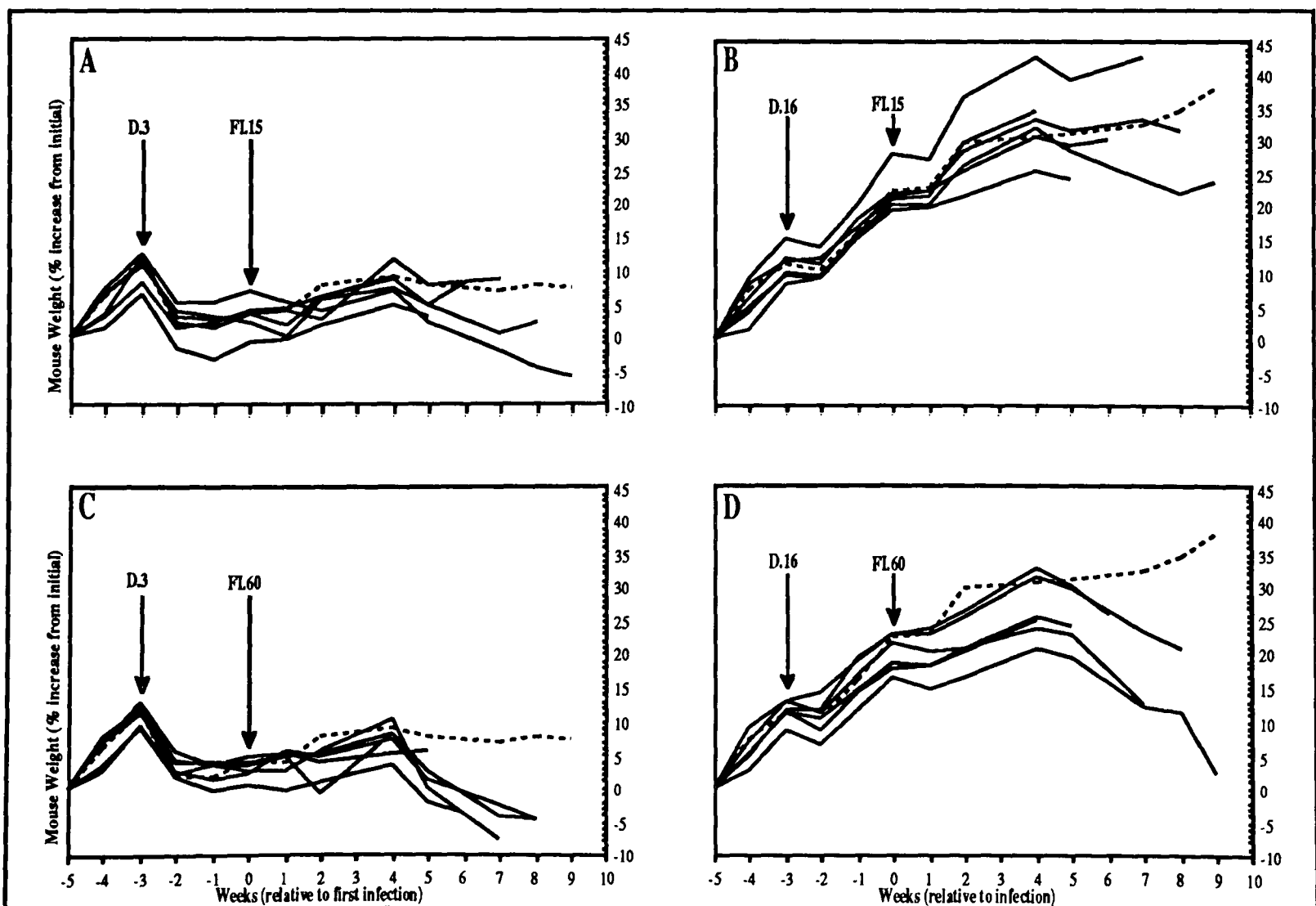


FIGURE 11: Change in weights of uninfected (dotted) and infected (solid) mice fed either 3% (A & C) or 16% (B & D) protein diets following 1 to 6 weekly to 15 (A & B) or 60 (C & D) cercariae.mouse⁻¹.week⁻¹ (number of exposures = {week of final weights} - 3).

Low protein diets were clearly deficient, as weights were lower in these mice from the time of first infection ($df=259, F=319.37^*$) and thereafter (Figure 11, previous page).

Antibody Response

Cercarial challenge produced an increase in IgG activity which was appreciably higher in well-fed mice and animals which received high challenges. Figure 12 (overleaf) is provided in order to visualize increases in antibody activity. Note should be made of the rapid and marked rise in antibody activity following multiple challenges in well-fed animals, contrasted with the noticeably smaller increase in antibody activity observed in protein deficient hosts. Pooling of plasma samples within groups for antibody analysis precluded statistical comparison of immune responses between groups.

Spleen Size

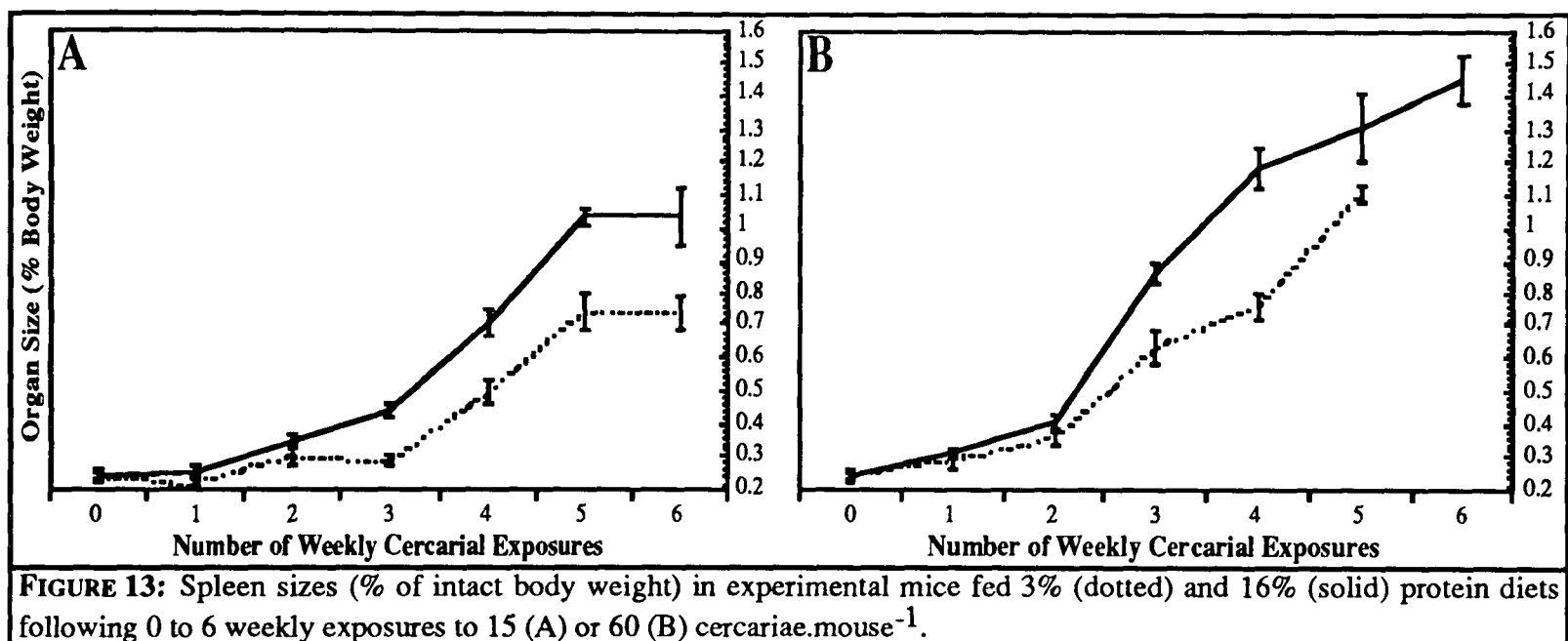


FIGURE 13: Spleen sizes (% of intact body weight) in experimental mice fed 3% (dotted) and 16% (solid) protein diets following 0 to 6 weekly exposures to 15 (A) or 60 (B) cercariae.mouse⁻¹.

Dietary protein had no influence on spleen sizes in uninfected animals (Table 9). Infection led to a significant increase in spleen sizes, regardless of dietary treatment (Figure 13). Mice fed low protein and exposed to low challenge generally had significantly smaller spleens than did similarly challenged mice receiving high protein diets, whilst poorly fed mice receiving high challenge had significantly smaller spleens than did similarly challenged mice receiving high protein diets.

3% Protein range(E-1 %)	Exposures (15cerc.wk ⁻¹)(n)	0	1	2	3	4	5	6
		1.55-2.77 (10)	1.71-2.93 (10)	1.46-4.36 (10)	2.16-3.43 (10)	3.43-6.45 (10)	5.31-9.32 (10)	5.74-10.34 (10)
range(E-1 %)	(60cerc.wk ⁻¹)(n)	1.55-2.77 (10)	1.77-4.06 (10)	2.57-4.47 (10)	4.12-9.22 (10)	5.79-9.8 (10)	8.43-13.62 (4)	- (0)
		0.438(0.159;18)	0.017*(-2.29;18)	0.06(-1.65;18)	0.0001* (-5.84;18)	0.0006* (-3.86;18)	0.0001* (-5.09;16)	0.009*(- 2.65;17)
range(E-1 %)	(15cerc.wk ⁻¹)(n)	0.438(0.159;18)	0.19(-0.90;18)	0.02*(-2.22;18)	0.002*(- 3.37;18)	0.0001* (-5.72;18)	0.20(-0.90;6)	-
		1.7-3.68 (10)	1.77-3.16 (10)	2.68-4.53 (10)	3.56-5.27 (10)	5.06-9.62 (10)	8.39-11.13 (10)	7.69-15.85 (10)
range(E-1 %)	(15cerc.wk ⁻¹)(n)	1.7-3.68 (10)	2.39-3.96 (10)	3.41-4.86 (10)	7.31-10.39 (10)	8.26-15.06 (10)	10.8-17.96 (10)	12.6-16.11(10)
		16% Protein	Exposures	0	1	2	3	4

TABLE 9: T-test comparisons of spleen sizes in mice receiving between 0 (uninfected) and 6 exposures to either 15 or 60 cercariae.mouse⁻¹ (n = number of organs measured; central numbers represent T-test p values followed by t values and degrees of freedom).

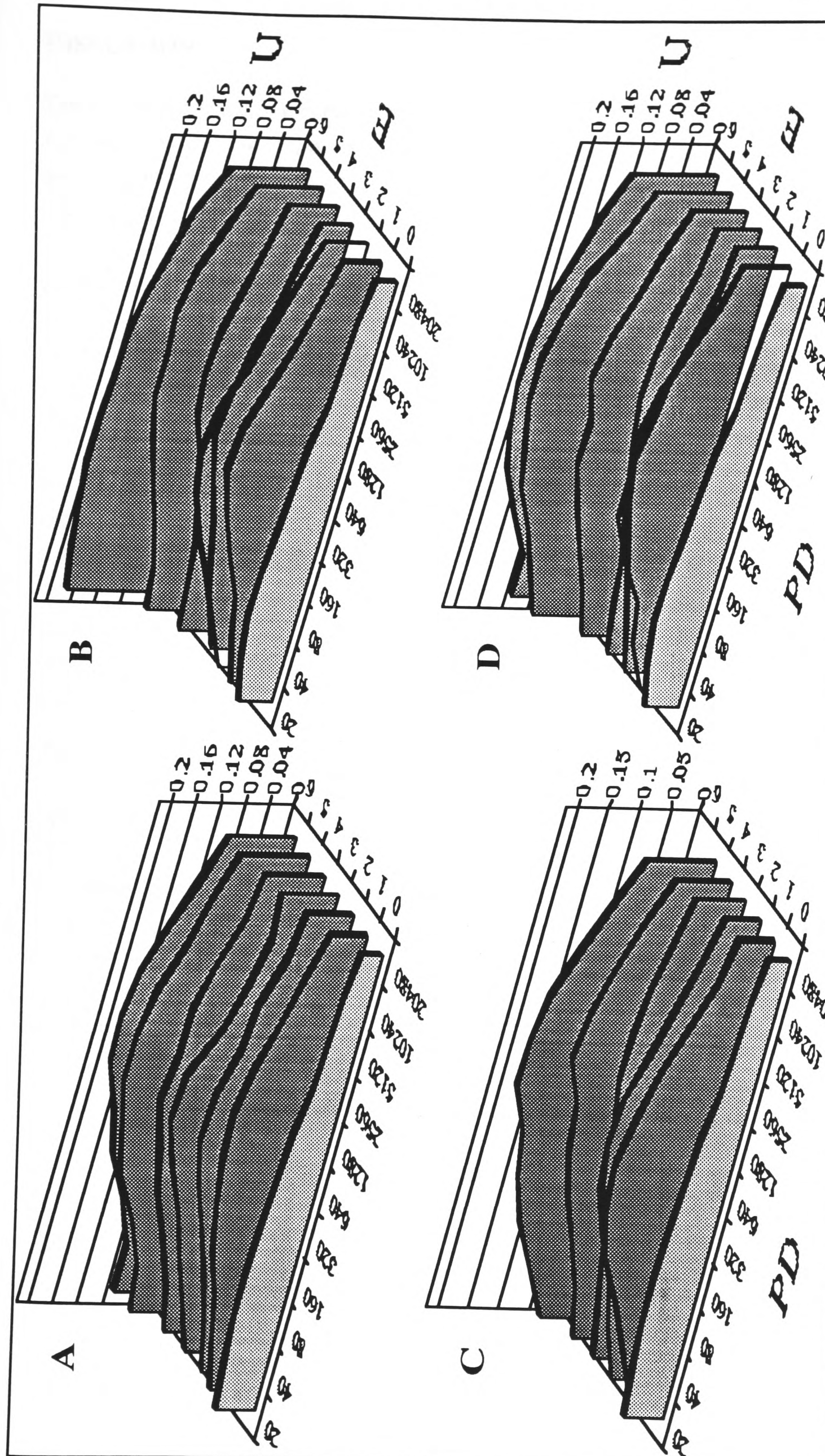


FIGURE 12: Plasma antibody responses (as measured by ELISA) to SSA in experimental mice fed 3% (A & C) or 16% (B & D) protein diets following 0 (front row of each plot) to 6 weekly exposures to 15 (A & B) or 60 (C & D) cercariae.mouse⁻¹ (solid) (PD=plasma dilution, E=number of exposures to cercariae, U=units {log(1+O.D.)}).

DISCUSSION

The results presented in this experiment are broadly similar to those described in Experiment 2.II, and confirm the hypothesis generated above that protein deficiency alters parasite success and host disease in the acute stages of schistosome infection in hosts exposed to multiple challenges. Although Experiment 2.II examined the influence on host nutritional status on the dynamics of repeated schistosome infections, the use of a single kill point precluded examination of the progression in parasite success and host disease. As no previous study has examined the effect of host protein status on the progression of the host/schistosome relationship during multiple exposures, it is useful to view the present results in relation to those reported in the previous experiment, whilst noting any modifications in the parasite and host which accompany increasing exposure to challenge.

Results in the present experiment show that multiple challenges saw the acquisition of resistance in both mouse groups; well-fed animals appeared to acquire an immunity to reinfection that was both time- and dose-dependent, whilst poorly fed hosts maintained, and increased, levels of resistance to reinfection greater than well-fed hosts in the absence of greater immune activity relative to well-fed animals. Morbidity rose with increasing challenge, and was more severe in well-fed hosts than in protein deficient animals, although high mortality occurred in the most heavily infected, undernourished animals.

The Parasite

In accordance with previous results (Experiments 2.I & 2.II), fewer male worms established within protein deficient hosts than in well-fed animals. The observation that male survival decreased during continued challenge in protein deficient hosts relative to well-fed controls, which are more immunoactive (see below), suggests that ongoing protein deficiency and parasite challenge progressively reduces the suitability of host conditions to schistosome larvae, which are thought to be susceptible to elimination during this period (see Experiments 2.I & 2.II). Male survival in well-fed hosts exposed to low challenge did fall after 5 previous challenges, which suggests that immunity to infection is acquired, however parasite survival remained lower in deficient animals, despite a weaker immune response by their hosts.

Support for the acquisition of non-immunological "resistance" in undernourished hosts exposed to multiple infections is provided by the observation that this trend is more marked in animals exposed to high challenge. It is suggested that the increase in resistance to male reinfection in those hosts receiving the low protein diet relative to well-fed animals, in the presence of decreased immune activity, demonstrates that acquired resistance may take the form of increasingly suboptimal conditions to parasite infection.

This notion is supported by the observation that female worms (which are more resilient to changing conditions within the host - see Chapters 2.I & 2.II) suffer decreased survival in protein deficient hosts when compared with well-fed animals. This shows that female worms also suffer reduced survival following deterioration in the host following multiple high challenges.

Results showing that male and female worms establishing in protein deficient hosts were generally smaller than with those from well-fed animals is consistent with observations in Experiments 2.I and 2.II. Results which show female worms recovered from protein deficient hosts were smaller than those recovered from well-fed controls after several challenges, yet this effect disappears in later infections suggests this effect is due to retarded growth, and that catch-up growth followed. This indicates that the time post-infection of autopsies might affect worm size comparisons between dietary groups.

It is interesting to note that frequency distributions of male worm sizes show that in protein deficient hosts exposed to high cercarial challenge, fewer worms in the smaller size classes appear after several exposures than worms in mice fed the high protein diet and similarly challenged, or mice fed either diet and exposed to low challenge. This further supports the theory that suboptimal conditions contribute to the acquisition of resistance in deficient hosts. A similar, and more marked, observation can be made in female worms (Figure 8).

Results indicating that larval recruitment continues in well-fed animals which are more immunoactive (see below), yet declines in deficient hosts following a number of challenges, suggests that rapidly deteriorating conditions may comprise a major component of resistance to reinfection in undernourished animals.

Evidence that conditions worsen more in poorly fed hosts and that this affects parasites is provided by the increasing proportion of female worms which produce abnormal eggs. That high challenge produces a fall in egg sizes in well-fed animals yet does not in poorly fed animals suggests that immunity may have an impact on their size, although not to any greater extent than protein deficiency alone.

The Host

Mice fed the low protein diet begun three weeks prior to first infection were significantly lighter than other animals during the infection period, and thus it can be concluded that cercariae experienced deficient hosts.

The high mortality observed in protein deficient animals receiving a number of high cercarial challenges reflects the damage inflicted by unnaturally high worm burdens in animals weakened by deficiency. Under milder challenges, well-fed animals appeared to suffer more

severe morbidity than protein deficient animals, as shown by greater weight losses during patent infection and increased spleen sizes.

Weight losses in all infected groups began approximately 4 weeks after first infection, and likely reflect morbidity accompanying the onset of schistosome egg production. Under low challenge conditions, weight losses between well-fed hosts and protein deficient animals were comparable, however high challenge produced increased losses which were more pronounced in well-fed animals.

Antibody (IgG) activity rose in response to multiple exposure in all groups, however well-fed animals appeared to produce a higher level of immune activity, especially those exposed to high challenge. This is consistent with the observations that spleen sizes rose to a greater extent in infected, well-fed hosts than in protein deficient animals, indicating increased T cell activity, especially in animals receiving high challenge. Given the apparently higher levels of immune activity in well-fed infected hosts, it is likely that immune-mediated damage characteristic of schistosomiasis was greater in well-fed hosts when compared with protein deficient animals, and this would explain their more severe weight losses.

2.10: CHAPTER DISCUSSION

This chapter examined the hypothesis, generated from the evidence presented in the Introduction (above), that host undernutrition reduces schistosome success and disease development during the acute stages of infection by reducing host suitability to parasite exploitation and by suppressing immunopathogenesis, respectively.

Schistosomes, Schistosomiasis & Vertebrate Host Nutrition

Results confirm the hypothesis, and show that the evolution of suboptimal conditions within protein-deficient hosts during continued exposure to schistosomes provides increasing protection from schistosome infection at a level greater than that observed in well-fed animals. The relatively low immune activity observed in deficient hosts did not impair this protection, and indeed led to lower immune-mediated pathology.

R₀-Antagonism & Host Undernutrition

Parasites which encounter poorly fed hosts are likely to be less successful than those which contact well-fed hosts, both in their ability to survive to maturity and their subsequent reproductive success. It appears, therefore, that schistosomes suffer R₀-antagonism when host protein deficiency is present during the acute stages of infection.

As the mechanisms involved in the observed R₀-antagonism appear to consist of the evolution of conditions suboptimal for parasite survival and reproduction, it may be that previous work reporting similar effects in hosts deficient in various other nutrients (see Introduction) involve similar mechanisms. The pathophysiological effects of protein and other deficiencies are well known, and may contribute to host resistance to schistosome infection.

Patho-Antagonism & Host Undernutrition

Although nutritional deficiencies involve the cost of pathophysiology, the protection provided against schistosome infection is secondary to the protection it provides against disease. In addition to reducing the agents of pathology (schistosome eggs), deficiency also reduces the immune responses to them which induce pathogenesis. Therefore the early stages of schistosomiasis in the vertebrate host displays patho-antagonism to protein deficiency, where disease expression is reduced as a result of host protein undernutrition. Again, other deficiencies which have been shown to ameliorate schistosomal disease (see Introduction) are likely to produce this effect in a similar fashion.

From these observations, it can be seen that various forms of undernutrition in the vertebrate host, despite involving the cost of nutritional deficiency, may protect the host from fulminant schistosomal infection and disease.

In the Field

Implications for human populations exposed to schistosome infection are clear: individuals which are undernourished are likely to harbor fewer, less fecund schistosomes, and will most likely suffer less from schistosomal disease. However it is probable that this will only occur when this individual is deficient relative to the prevailing nutritional status of that area.

As outlined above, there remains difficulty in extrapolating results obtained in animal models to the human situation. The severity of deficiency which occurs in experimental animals maintained on inadequate diets, the rate of parasite infection and the parasite load in relation to host physiology, as well as many other factors are likely to influence the absolute outcome of experimental infection in relation to those seen in humans. In addition, given the frequency of polyparasitism in developing countries, antagonism for schistosomiasis may be replaced by a synergistic association with another species of parasite or parasites.

However, despite the limitations of experimental work, it is suggested that the general mechanisms responsible for the generation of R_0 - and patho-antagonism in acute schistosome infection in experimental animals may be important determinants of the success of schistosomes and the development of the acute forms of schistosomiasis in the human host.

Conclusions

The development, within the undernourished host, of conditions suboptimal for invading and developing schistosomes may contribute to host resistance to infection and disease by reducing worm survival, development and egg production, and by reducing immunopathology.

Although the acute stage of schistosome infection is an important determinant of parasite success and the initiation of pathogenesis, the chronic stages of schistosomiasis are by far the most significant with respect to both parasite transmission and host disease. This led to the investigations presented in the following chapter.

CHAPTER THREE

SCHISTOSOMES & VERTEBRATE HOST NUTRITION

DIETARY INTERVENTIONS



“... our current state of knowledge offers little indication as to whether the food supplementation programmes currently in operation throughout the developing world will reduce or actually increase the transmission of some of the most prevalent ... infections.”

Bundy & Golden (1987)



Chapter Summary

This chapter examines the influence of externally imposed changes in host protein status on the parasite and host during the chronic stages of schistosome infection. Experiments explore the effects of both increases and decreases in the host protein status at which infection had matured. Results show that a period of protein deficiency reduces parasite reproductive success and disease expression in the previously well-fed host. Conversely, dietary repletion reverses the deleterious effects of prior host protein deficiency on schistosome success, whilst simultaneously boosting disease expression. These results have implications for human populations exposed to poor nutritional security in the presence of endemic schistosomiasis.

3.1: INTRODUCTION

Within developing countries, poverty largely removes the ability of human populations to store resources, and thus dampen natural, catastrophic and man-made fluctuations in food quality and availability (Harpham, 1986); as a result, these populations are frequently exposed to nutritional stress (Payne, 1985; Rohde & Chen, 1985). In response to such circumstances, benevolent organizations advocate nutritional support (Kennedy & Knudsen, 1985; Lusty & Diskett, 1984).

Due to the similarity in socio-economic conditions conducive to their persistence, recurrent undernutrition and parasitic infection commonly coexist in impoverished human communities (Bundy & Golden, 1987; Chandra, 1979; 1984; Dunn, 1985; Keusch & Scrimshaw, 1986; Solomons & Keusch, 1981). In addition, undernutrition and parasitic diseases are both chronic conditions, are most common in growing children and tend to occur together in the same individuals (Bundy & Golden, 1987). This coincidence suggests that those individuals most likely to suffer from parasitic diseases are also those most likely to selectively receive dietary supplementation.

3.2: DIETARY INTERVENTIONS

Nutritional supplementation and food fortification are widely practiced in developing countries where acute and chronic nutrient deficiencies occur (Kennedy & Knudsen, 1985; Lusty & Diskett, 1984). An intuitive, and widely extolled corollary to such interventions is that refeeding reverses the immune suppression which accompanies undernutrition (Chandra,

1984), thus restoring the individual's ability to resist infection (Lamprey & Sai, 1985). Although supplementation is clearly desirable in instances where synergy exists between undernutrition and disease, the thesis presented in Chapter 1 suggests that refeeding does not inevitably benefit the parasitized recipient.

In cases exhibiting R_0 - and patho-antagonism between undernutrition and parasitosis, undernourished hosts harbor less successful parasites and suffer less disease (see Chapter 1). Supporting evidence appears in Chapter 2, which demonstrates antagonism between host protein deficiency and early schistosome infection and disease. From these observations, it might be expected that refeeding undernourished hosts reverses antagonism, and potentially increases the transmission and severity of some parasitoses.

Although the acute stages of infection initiate pathogenesis in the human host, the most serious manifestations of schistosomiasis occur following a lengthy period of active infection (von Lichtenberg, 1987). As schistosomes are long lived in comparison to their maturation period (Wilkins & Gilles, 1987), and given that infection under natural circumstances is thought to occur continually at a low level (Crombie & Anderson, 1985), hosts will typically harbor a schistosome burden comprised predominantly of adult worms. It follows that the majority of schistosomes inhabiting hosts experiencing nutritional fluctuations are adults.

Although it has been shown that nutritional deficiency compromises larval schistosome survival and development to maturity (Chapter 2), little is known of the effects of host nutritional changes on schistosomes once their development is complete. Still less is known about the effects of nutritional fluctuations on the expression of chronic schistosomiasis. Given the probability of nutritional change in areas endemic for schistosomiasis, and the central role played by the fecund adult schistosome in both parasite transmission and disease development, it is clear these interactions are of potentially major importance. The present chapter examines the influence of both increases and decreases in host nutritional status on the success of adult schistosomes and on the expression of chronic disease.

Parasite Success

The establishment of schistosomes is reduced in undernourished hosts (Chapter 2). Once established however, the success of individual schistosome pairs in the vertebrate host pivots upon the number of their offspring which successfully contact a snail host; this depends upon adult worm longevity and fecundity, viable egg excretion from the host (Anderson, 1987) and miracidial infectivity (Anderson, Mercer, Wilson & Carter, 1982). The influence of host nutritional changes will therefore reflect the impact of nutritional changes on these components of schistosome success.

Adult Schistosome Survival

Previous work has shown that brief periods of host undernutrition appear to have little effect on the number, size (Akpom & Warren, 1975a; Warren, 1961) and tegumental integrity (Oyerinde, 1975) of worms which have reached maturity in well-fed hosts. Furthermore, refeeding deficient hosts following schistosome maturity reportedly reverses the deleterious effects of deficiency on the worms during their development (see Chapter 2), such as tegumental necrosis and failure (Oyerinde, 1975), reduced worm size and impaired somatic development (DeWitt, 1957b).

In essence, the deleterious effects of schistosome maturation in undernourished hosts appear to be reversed by host refeeding, whilst parasites which mature in well-fed hosts do not suffer greatly from short periods of host nutritional stress. Although this aspect requires further scrutiny, it may be that the greater rate of tegumental biosynthesis observed in more mature schistosomes (Rumjanek, 1987) permits more rapid repair of local necrosis which might lead to the elimination of less developed worms.

Schistosome Fecundity

Although short periods of undernutrition appear not to greatly affect adult worms (see above), it has been shown that the number (Akpom & Warren, 1975a) and viability (Akpom & Warren, 1975a; Krakower, Hoffman & Axtmayer, 1944) of eggs produced by schistosomes which reach maturity in well-fed hosts are reduced by a period of host undernutrition.

Conversely, refeeding deficient hosts following schistosome maturation reverses many of the detrimental effects of undernutrition on schistosome fecundity and egg viability (Akpom, 1978; Akpom & Warren, 1975a; DeWitt, 1957b; Krakower *et al.*, 1944) described in Chapter 2, although reductions may persist in proportion to the extent of previous deficiency (Akpom & Warren, 1975a).

The observation that increases in host dietary protein above levels regarded as adequate can actually boost worm fecundity above previous levels (Coutinho-Abath, Magalhaes & Barbosa, 1962) suggests that host nutrition is a normal constraint on schistosome fecundity and that feeding hosts diets of higher than usual nutritional value can boost parasite reproduction.

Egg Excretion from the Host

Once voided by the female worm, schistosome eggs must migrate through differentiated host tissues. As eggs must remain intact in order to withstand immune attack (Krakower *et al.*,

1944), observed reductions in egg integrity (see above) suggest that fewer will survive this migration.

It has been suggested that the immune response facilitates egg passage through host tissues by creating circumoval necrosis and tissue disruption in response to hepatotoxins released by the developing miracidium (see Chapter 2 and Doenhoff, Musallam, Bain & McGregor, 1978 and Doenhoff, Hassounah, Murare, Bain & Lucas, 1986). Thus, it might be expected that an undernutrition-induced fall in egg antigenicity and/or host immunity will reduce egg migration and excretion. A decrease in the proportion of live eggs (see above), and loss of egg antigenicity (Akpom, 1981) in undernourished hosts, combined with undernutrition-induced anergy (see Chapter 1) suggests this may be the case. That de Meillon and Paterson (1958) observed a fall in egg excretion during a period of reduced host nutrition supports this notion. Such a fall might be expected to be reversed during refeeding, as more eggs are produced, of which a greater proportion survive to successfully undergo toxin- and immune-facilitated migration.

Miracidial Infectivity

As pointed out in Chapter 2, miracidia do not feed once hatched; thus all nutritional requirements essential to fuel acquisition of the snail host must be accumulated during the tissue phase of egg development. A fall in host nutrition is unlikely to deprive the egg of sufficient nutrients on which to develop (they live in a rich environment and their needs are small), however a change in conditions can reportedly influence their development (see Chapter 2 and Akpom, 1981). If such effects occur, one might expect they are reversed by refeeding which restores suitable ambient conditions. Although a critical determinant of schistosome transmission dynamics (Anderson *et al.*, 1982), the effects of changing host conditions on miracidial infectivity remain unknown.

Evidence from Human Studies

It is clearly difficult, in both ethical and practical terms, to observe the effects of human nutrition on schistosome survival and fecundity, and thus no data currently exist. A single study by DeWitt and colleagues (1964) has, however, examined egg excretion by undernourished humans during refeeding. Although *S. mansoni* egg output was monitored throughout the experimental period in both the refed and control groups, the fluctuation in egg count counts obtained from the two groups was such that "interpretation was difficult or impossible" (DeWitt, Oliver-Gonzalez & Medina, 1964). Since these authors did not offer their results, and from the experimental evidence related above, it is suggested that the difficulty involved in interpretation may have consisted of counterintuitive results.

Although providing no indication of the relative contributions of female fecundity, egg survival or tissue migration (see Chapter 2), the observation of Murray and Murray (1977)

that the prevalence of schistosome egg excretion by humans rose during refeeding, suggests that their combination leads to a net increase in parasite success during refeeding.

Chronic Disease

As described in Chapter 2, the majority of disease in schistosomiasis results from host responses to parasite antigens, principally egg components (Kelly, 1987). Chronic disease differs from acute stages in that the host component is reduced by immune modulation (see below and Warren, Domingo & Cowan, 1967). Fluctuations in host nutrition will therefore affect disease expression as a function of their impact on antigenic quantity and quality and the changes in modulated immunopathology.

Schistosome Antigenicity

The extent of the pathologic stimulus provided by schistosome eggs will depend upon the numbers present and their individual antigenic quality. The number of viable eggs falls during host undernutrition and rises during refeeding, perhaps to levels above normal (see above).

As egg antigenicity is largely a function of the production of soluble toxins by the developing miracidium (Kelly, 1987), the reductions in egg antigenicity accompanying host undernutrition identified by Akpom (1981) are thought to reflect the effect of suboptimal conditions on miracidial survival and development (see Chapter 2). Zuckerman and Macdonald (1964), noting the change to a high carbohydrate diet at time of infection after 6 weeks of normal diet led to less fibrosis, attributed the effect to parasite undernutrition producing lower antigenic stimulus, most likely egg numbers and/or quality, as described above. It is likely that refeeding restores favorable conditions and thus egg antigenicity, although this has not been shown.

It should be noted that, as egg excretion is thought to be facilitated by toxin-induced, circumoval immunopathology (Doenhoff *et al.*, 1978; 1986), selection may favour an increase in egg antigenicity in host populations which are chronically undernourished, and therefore immunosuppressed. It is suggested that correcting such deficiencies by nutritional supplementation will restore immunity in the presence of selectively enhanced egg antigenicity, with potentially damaging results.

Host Immune Response

In the acute phase of schistosomiasis, delayed-type hypersensitivity (DTH) is responsible for the formation of tissue granulomata, which initiate pathogenesis (Warren *et al.*, 1967). The chronic form of the disease is characterized by the modulation of these cell-mediated immune (CMI) responses by an endogenous desensitization process (Hang, Boros & Warren,

1974) involving primarily suppressor T cells (Colley, 1987). Modulation, the onset of which commonly occurs after some 10 weeks of active infection in both humans and in experimental models of schistosomiasis (McLaren & Smithers, 1987), is thought to be advantageous to the host by lowering the immunopathology associated with tissue egg presence (Colley, 1987; Hagan, 1987).

It is unknown whether undernutrition, which produces general CMI suppression (see Chapter 1), will lead to a greater reduction in those T-cells which invoke DTH-mediated pathology or those which suppress it, and thus result in a decrease or increase in tissue damage, respectively. Although the results of refeeding, which might be expected to reverse these effects, are similarly unknown, the observation that, upon refeeding, reductions in granuloma size persisted in proportion to the extent of previous host dietary deficiencies, yet did enlarge (Akpom & Warren, 1975a), suggest that the effector mechanisms may be most affected by undernutrition and that refeeding may lead to an increase in chronic disease.

Further to the possible reduction in immunopathology accompanying host undernutrition, secondary cellular functions central to the consolidation of chronic schistosomiasis, chiefly collagen synthesis and fibrogenesis (von Lichtenberg, 1987), are suppressed by undernutrition (Dreizen, 1979), indicating the progression of chronic schistosomiasis may also be retarded.

Evidence from Human Studies

Support for the refeeding theory of increased egg antigenicity appears in the observation that egg excretion increased in chronically undernourished humans following refeeding (Murray & Murray, 1977), although this may also reflect increases in worm fecundity and/or increased immune-mediated excretion (see above).

In a purpose-designed study, DeWitt and colleagues (1964), working in Puerto Rico, used prisoner volunteers to study the clinical results of refeeding humans volunteers naturally infected with *S. mansoni*. The reported improvement in the nutritional status and general health of the patients following the provision of an enriched diet is to be expected, as the detrimental effects of deficiency were corrected. It is both unfortunate and surprising, however, that the effects of refeeding on pathology specifically due to schistosome infection were not reported.

The Unknown Effects of Dietary Interventions

Despite the frequency of natural and man-made nutritional fluctuations in those areas endemic for schistosome infection and disease, no study has been specifically designed to address the effects of changes in host nutrition on host and adult parasite. The evidence presented above suggests that deficiency can impair, and refeeding improve, adult

schistosome success. In accordance with the suppressive effects of undernutrition on the acute stages of disease (see Chapter 2), evidence suggests that chronic disease falls during reductions in host nutrition and resurges during refeeding. It is therefore a matter of some interest that Akpom (1982) has stated that "nutritional rehabilitation by mass feeding programs is unlikely to aggravate {schistosomiasis} in human populations".

3.3: HYPOTHESIS

That an increase in chronically deficient host nutrition increases adult schistosome success and exacerbates disease expression during the chronic stages. That a decrease in adequate nutrition has the converse effect of reducing schistosome success and ameliorating disease.

3.4: EXPERIMENTS

The hypothesis was tested by manipulating the protein content of diets (between inadequate and adequate levels; see Chapter 2) fed to mice harboring light, chronic schistosome infections. Diets were changed at 10 weeks post-infection in order to investigate the effects of dietary protein changes on both parasites which had matured, and disease which had reached the chronic stage, at the prior protein level. Light infections (20 cercariae.mouse⁻¹) were used in order to minimize host mortality during prolonged infection.

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: I. CHRONIC PHASE DIETARY PROTEIN INCREASE

Abstract

This experiment examines the influence of an externally imposed increase in host protein status on the parasite and protein-deficient host during chronic schistosomiasis mansoni. Results show that while correcting deficiency does not significantly influence adult schistosome survival, the limits on reproduction imposed by host deficiency are largely reversed during refeeding. In the presence of restored host immunity, this led to the resurgence of chronic pathology. These results suggest that refeeding chronically undernourished humans in the presence of active schistosome infection may increase parasite transmission and exacerbate the expression of chronic schistosomiasis.

INTRODUCTION

Chapter 2 has shown that the early stages of schistosome infection and disease are suppressed by host nutritional deficiency. However it is the chronic form of schistosomiasis which accounts for the majority of its public health significance.

Previous evidence has suggested that an increase in chronically poor nutritional status influences both parasite and host during chronic infections (see above). As no previous study has reported a comprehensive examination of this relationship, this experiment was designed to investigate the effects of host protein refeeding on the relationship between schistosomes and their vertebrate hosts during chronic infection which had developed under deficient conditions.

Hypothesis

Previous experimental evidence led to the generation of the hypothesis that reversing extant host protein deficiency increases parasite success and disease expression in the chronic stage of schistosome infection.

The influence of host protein repletion on schistosomes and pathology which had developed in protein-deficient mice was examined by autopsy at the time of diet change and 2 and 4 weeks later. Mice belonged to one of two dietary groups: those receiving 3% protein diet for the duration of the study or those which had their 3% protein diet replaced by a 16% protein ration 10 weeks after infection with 20 cercariae.mouse⁻¹.

EXPERIMENTAL DESIGN

Eighty mice were randomly assigned to groups of 10 housed in large cages and fed the 3% protein synthetic diet (D3). After two weeks, mice from six of the cages were individually exposed to 20 cercariae.mouse⁻¹ for 30 min (I.20; range 19-21). All cercariae were gathered simultaneously from the same group of 40 patent snails. The remaining two groups were handled identically in the absence of cercariae.

Ten weeks after cercarial exposure, three mice from each cage were autopsied (Atx3) as described in the Materials and Methods section of Chapter 2. At this time, one cage of uninfected mice and three cages of infected animals had their 3% protein diet replaced by the 16% protein diet (D16). The remaining cages continued to receive the 3% diet.

Two weeks later, a further three mice from each cage were autopsied (Atx3). After an additional two weeks, the remaining mice in each group were also autopsied (Atx4 & Atx12). The design of this experiment is presented in Table 1.

Week Group (n)	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A (10)	D3	→	-	-	-	-	-	-	-	-	-	→	At3	→	At3	→	Atx4
B (10)	D3	→	-	-	-	-	-	-	-	-	-	→	At3 D16	→	At3	→	Atx4
C (30)	D3	→	I.20	→	-	-	-	-	-	-	-	→	At9	→	At9	→	Atx12
D (30)	D3	→	I.20	→	-	-	-	-	-	-	-	→	At9 D16	→	At9	→	Atx12

TABLE 1: Design of Dietary Increase experiment (n=number of mice in group).

RESULTS: PARASITE

Number

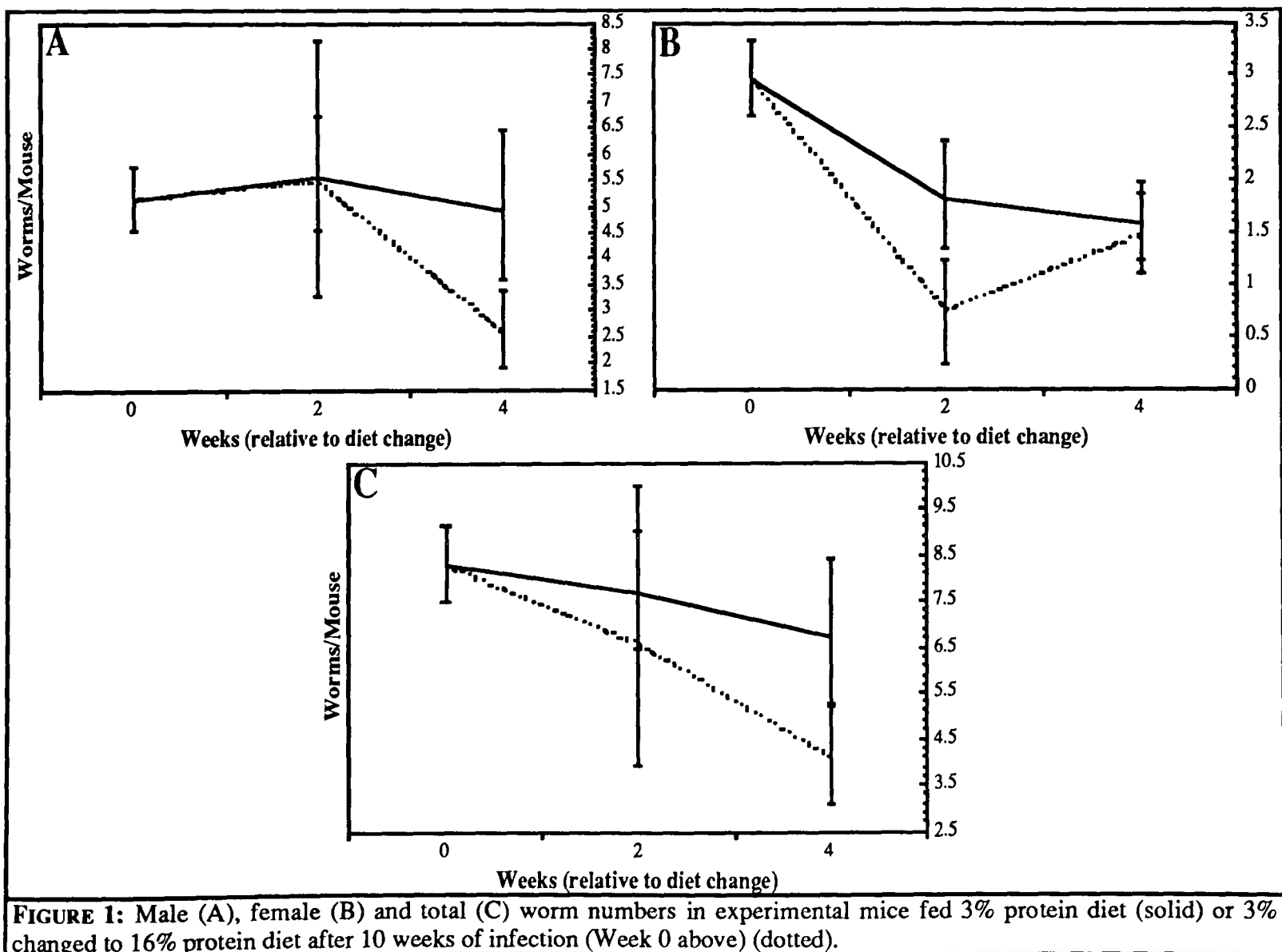


FIGURE 1: Male (A), female (B) and total (C) worm numbers in experimental mice fed 3% protein diet (solid) or 3% changed to 16% protein diet after 10 weeks of infection (Week 0 above) (dotted).

Although worm numbers tended to fall slightly in hosts which received an increase in dietary protein (Figure 1, previous page), changes were not statistically significant when compared with animals remaining on the low protein diet (Table 2).

Groups	3% Protein at Change	3% Protein + 2 weeks	3% Protein + 4 weeks	16% Protein + 2 weeks	16% Protein + 4 weeks
Male Worms					
range (n)	2-10 (20)	1-13 (9)	1-9 (7)	0-13 (6)	0-5 (9)
3% Protein at Change	-	0.36(-0.37;27)	0.44(0.16;25)	0.42(-0.19;24)	0.007*(2.65;27)
3% Protein + 2 weeks	-	-	-	0.48(0.04;13)	-
3% Protein + 4 weeks	-	-	-	-	0.07(1.61;14)
Female Worms					
range (n)	1-6 (20)	0-4 (9)	1-4 (7)	0-4 (6)	0-2 (9)
3% Protein at Change	-	0.04*(1.82;27)	0.015*(2.30;25)	0.003*(3.05;24)	0.006*(3.17;22)
3% Protein + 2 weeks	-	-	-	0.12(1.24;13)	-
3% Protein + 4 weeks	-	-	-	-	0.41(0.228;140)
All Worms					
range (n)	3-13 (20)	2-15 (9)	2-11 (7)	0-17 (6)	0-7 (9)
3% Protein at Change	-	0.34(0.415;27)	0.167(0.99;25)	0.22(0.78;24)	0.002*(3.17;22)
3% Protein + 2 weeks	-	-	-	0.36(0.37;13)	-
3% Protein + 4 weeks	-	-	-	-	0.09(1.39;14)

TABLE 2: T-test comparisons of mean worm numbers in experimental mice fed 3% protein for the duration of the experiment (3% at Change, 3%+2 weeks, 3%+4 weeks) or 3% changed to 16% protein 10 weeks post-infection (3% at Change, 16%+2 weeks, 16%+4 weeks) (number above diagonal represents t-test p value followed by t value and degrees of freedom; n=number of mice perfused for worms).

Size: Means

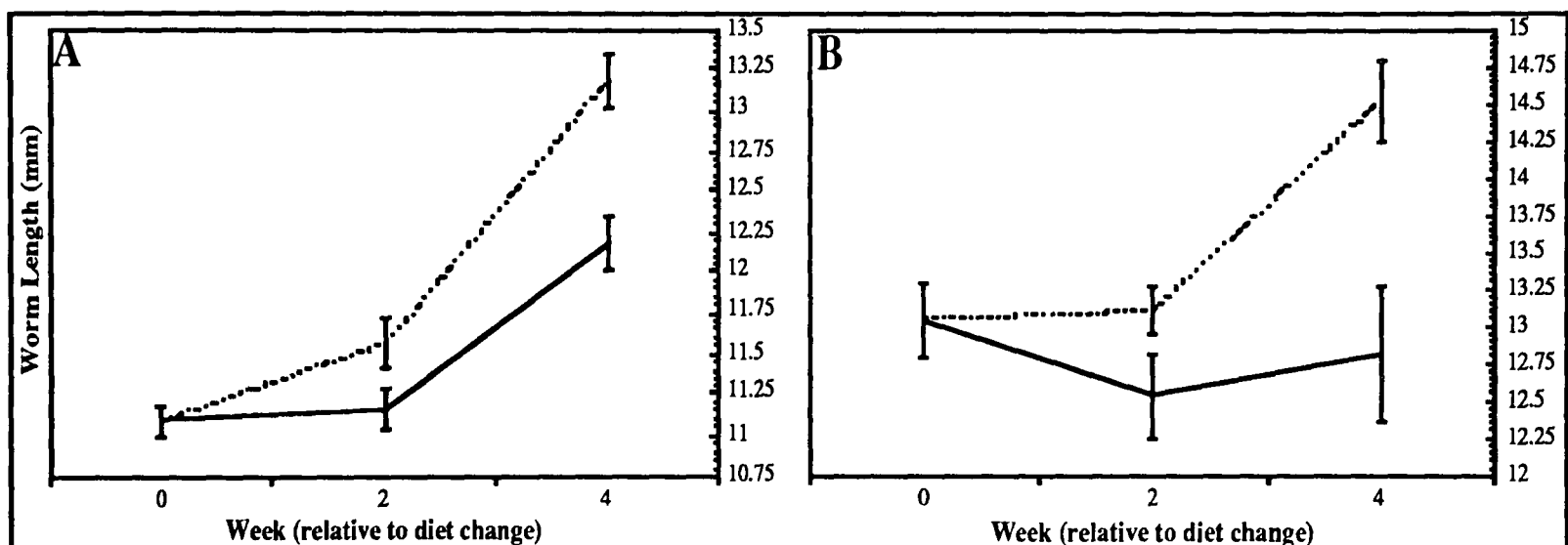


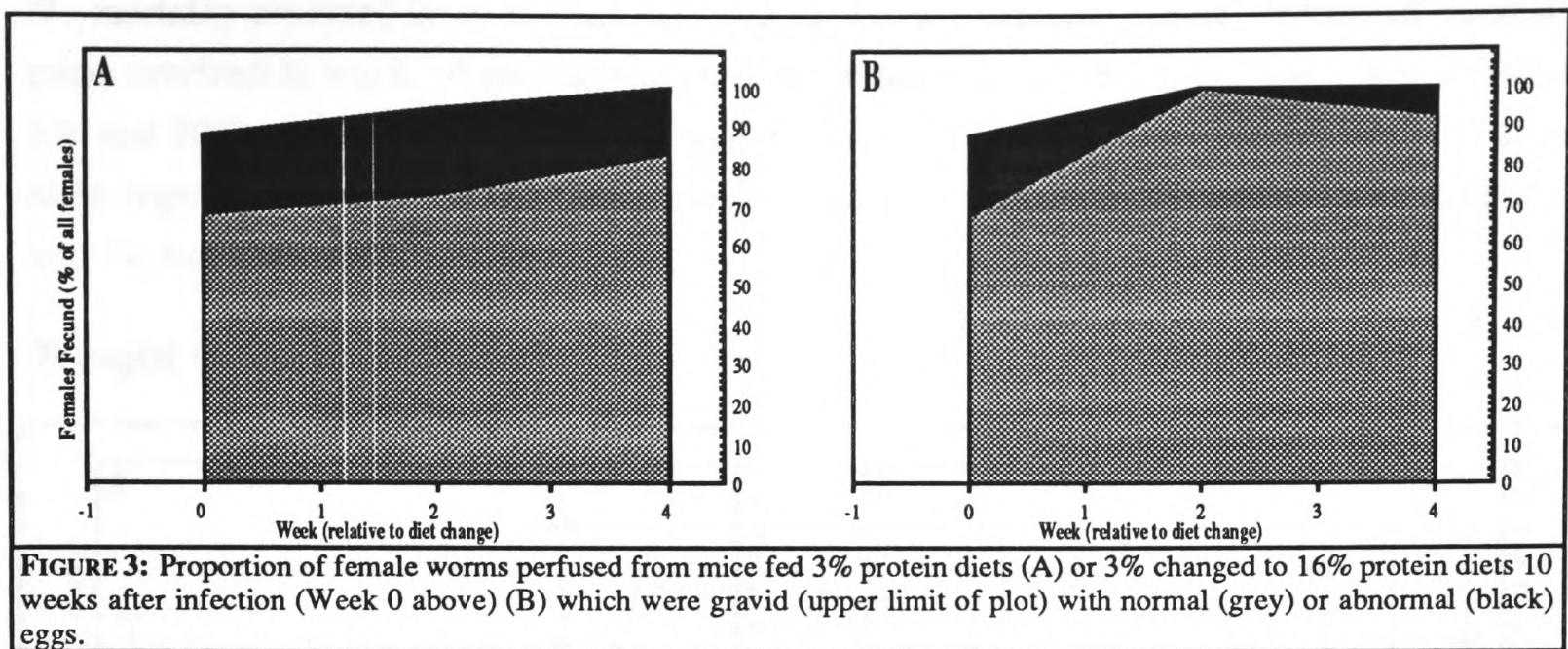
FIGURE 2: Length of male (A) and female (B) worms perfused from mice fed 3% protein (solid) or 3% changed to 16% protein 10 weeks after infection (Week 0 above) (dotted).

Male worms from both dietary treatments continued to grow during the experimental period, however those inhabiting hosts receiving an increase in dietary protein grew to be significantly larger than those infecting mice which continued to receive the low protein diet (Figure 2). Female worms inhabiting mice remaining on the low protein diet did not grow during the period studied after dietary change, however females inhabiting mice which began to receive the high protein diet grew to be significantly larger 4 weeks after dietary change than worms inhabiting mice which remained poorly fed (Table 3).

Groups	3% Protein at Change	3% Protein + 2 weeks	3% Protein + 4 weeks	16% Protein + 2 weeks	16% Protein + 4 weeks
Male Worms					
range (mm) (n)	7.5-14.6 (112)	8.3-13.3 (54)	10.2-15 (39)	8.2-13.6 (41)	11.3-15 (27)
3% Protein at Change	-	0.26(-0.66;164)	0.0001*(-5.27;149)	0.005*(-2.61;151)	0.0001*(-8.54;137)
3% Protein + 2 weeks	-	-	-	0.017*(-2.16;93)	-
3% Protein + 4 weeks	-	-	-	-	0.0001*(-3.92;64)
Female Worms					
range (mm) (n)	4.3-6.6 (63)	10.6-14.8 (19)	9.3-15.6 (12)	12.5-13.9 (9)	12.9-16.3 (15)
3% Protein at Change	-	0.13(1.14;80)	0.36(0.35;73)	0.48(-0.06;70)	0.005*(-2.64;76)
3% Protein + 2 weeks	-	-	-	0.09(-1.38;26)	-
3% Protein + 4 weeks	-	-	-	-	0.002*(-3.25;25)

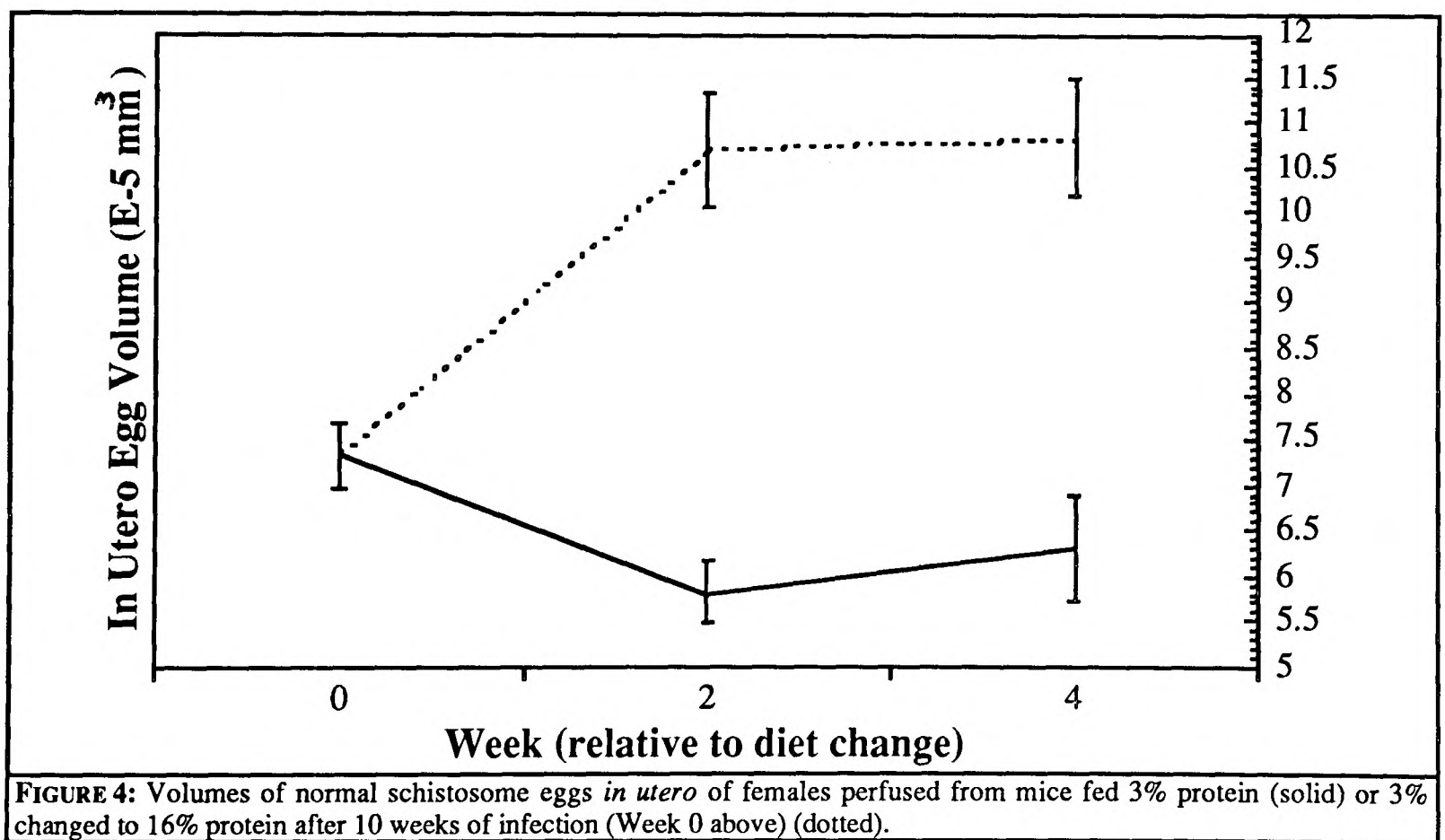
TABLE 3: T-test comparisons of mean worm lengths in experimental mice (number above diagonal represents t-test p value followed by t value and degrees of freedom; n=number of worms measured).

Fecundity: Proportion of Females Gravid



Refeeding mice fed low protein diets had little effect on the proportion of female worms which contained eggs (Figure 3, upper limit of plot), however it noticeably decreased the proportion of these eggs which appeared abnormal (Figure 3, black area).

Fecundity: *In Utero* Egg Size



Of those *in utero* eggs which appeared normal (see above), host protein repletion saw a rapid and sustained increase in *in utero* egg size (Figure 4; Table 4).

Groups	3% Protein at Change	3% Protein + 2 weeks	3% Protein + 4 weeks	16% Protein + 2weeks	16% Protein + 4 weeks
range (E-5 mm ³) (n)	3.19-14.14 (43)	3.75-9.24 (14)	3.26-10.34 (10)	9.05-15.97 (9)	7.86-18.32 (14)
3% Protein at Change	-	1.55	0.53	3.92*	6.03*
3% Protein + 2 weeks	-	-	-	6.73*	-
3% Protein + 4 weeks	-	-	-	-	5.97*

TABLE 4: *In utero* egg volumes (number above diagonal represents ANOVA F value; n=number of eggs measured; df=89).

RESULTS: HOST

No mortality occurred in uninfected mice during the experimental period. Whilst all infected mice survived to week 10 post-infection, those maintained on the low protein diet suffered 5% and 20% cumulative mortality by weeks 12 and 14 post-infection, respectively. Those mice begun on the high protein diet at week 10 suffered 20% and 25% mortality at weeks 12 and 14, respectively.

Weight Change

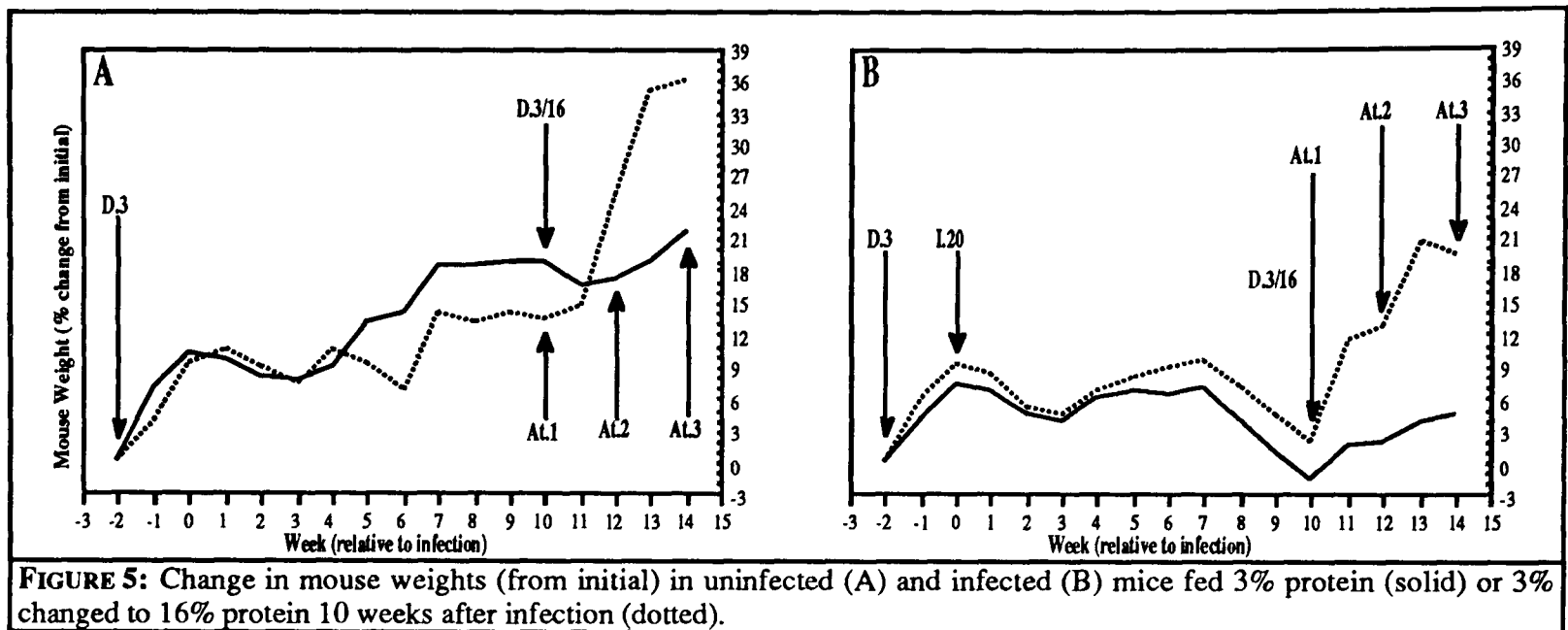


FIGURE 5: Change in mouse weights (from initial) in uninfected (A) and infected (B) mice fed 3% protein (solid) or 3% changed to 16% protein 10 weeks after infection (dotted).

Protein repletion of mice previously maintained on a low protein diet saw an immediate and significant increase in the body weight of infected animals one week after dietary change ($df=59$, $F=3.76^*$) which persisted for the duration of the experiment (Figure 5).

Liver Granulomata: Number & Size

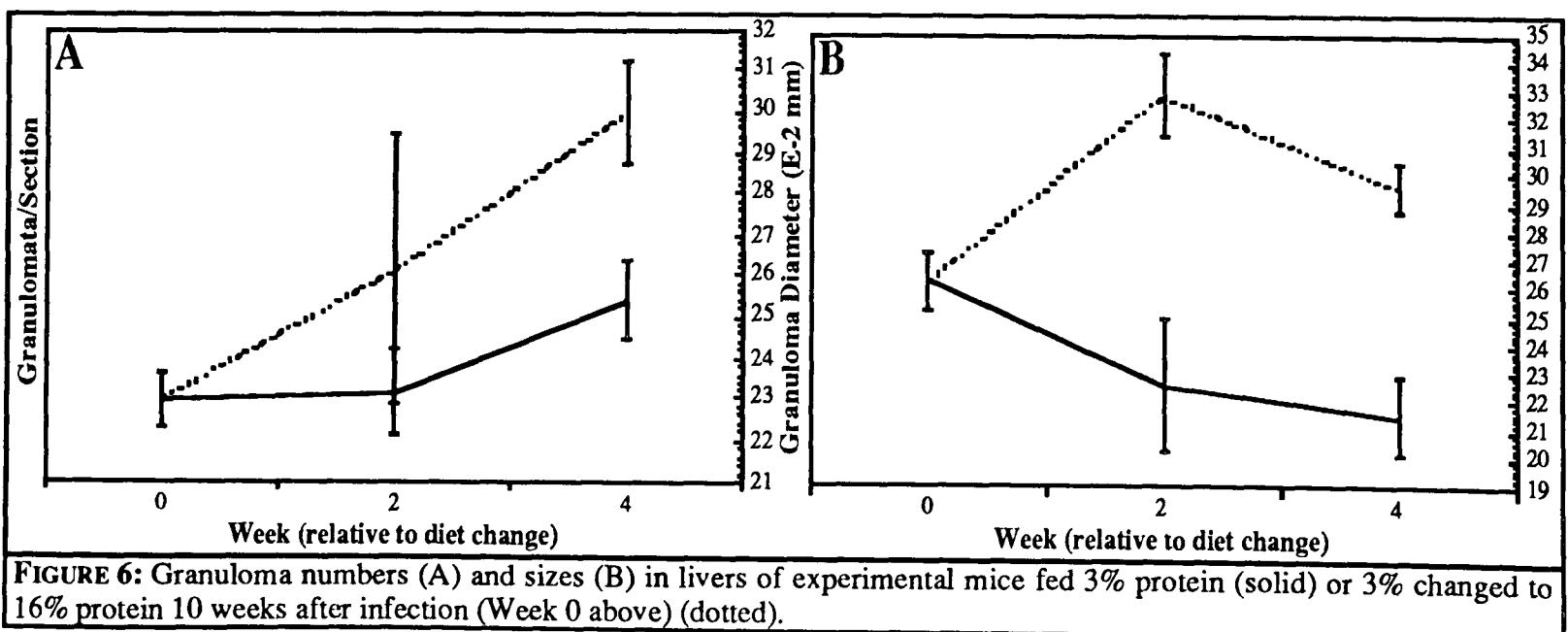


FIGURE 6: Granuloma numbers (A) and sizes (B) in livers of experimental mice fed 3% protein (solid) or 3% changed to 16% protein 10 weeks after infection (Week 0 above) (dotted).

The number of liver granulomata rose during the experimental period in all animals, regardless of dietary protein status, however animals which began to receive high protein diets had significantly more granulomata 4 weeks after dietary change than did animals which continued to receive the low protein diet (Table 5, overleaf).

After an initially rapid rise, granulomata sizes in mice receiving the high protein diet fell, but remained significantly above those observed in mice continuing to receive the low protein diet (Figure 6, previous page). A comparison of granulomata from mice maintained on the low protein diet and from mice changed to the high protein diet 4 weeks previously is shown in Plate 1 (overleaf). Note should be made of the resurgence of peripheral cellular infiltration around lesions in the refeed mouse liver when compared with the smaller lesion found in the liver of an undernourished mouse.

Groups	3% Protein at Change	3% Protein + 2 weeks	3% Protein + 4 weeks	16% Protein + 2 weeks	16% Protein + 4 weeks
Granuloma Number range (n)	12-42 (119)	9-41 (48)	16-38 (42)	7-46 (24)	13-44 (54)
3% Protein at Change	-	0.45(-0.12;165)	0.03*(-1.87;159)	0.066(-1.52;141)	0.0001*(-5.30;171)
3% Protein + 2 weeks	-	-	-	0.13(-1.11;70)	-
3% Protein + 4 weeks	-	-	-	-	0.003*(-2.83;94)
Granuloma Diameter range (mm) (n)	0-0.48 (100)	0-0.46(40)	0-0.32(35)	0.22-0.46 (20)	0.18-0.42 (45)
3% Protein at Change	-	0.93	1.42	1.48	0.77
3% Protein + 2 weeks	-	-	-	3.04*	-
3% Protein + 4 weeks	-	-	-	-	3.01*

TABLE 5: Statistical comparison of numbers (t-test) and diameter (ANOVA) of granulomata in the livers of experimental mice (number above diagonal represents p value followed by t value and degrees of freedom (t-test) or F value (ANOVA); n=number of granulomata measured).

Organ Size

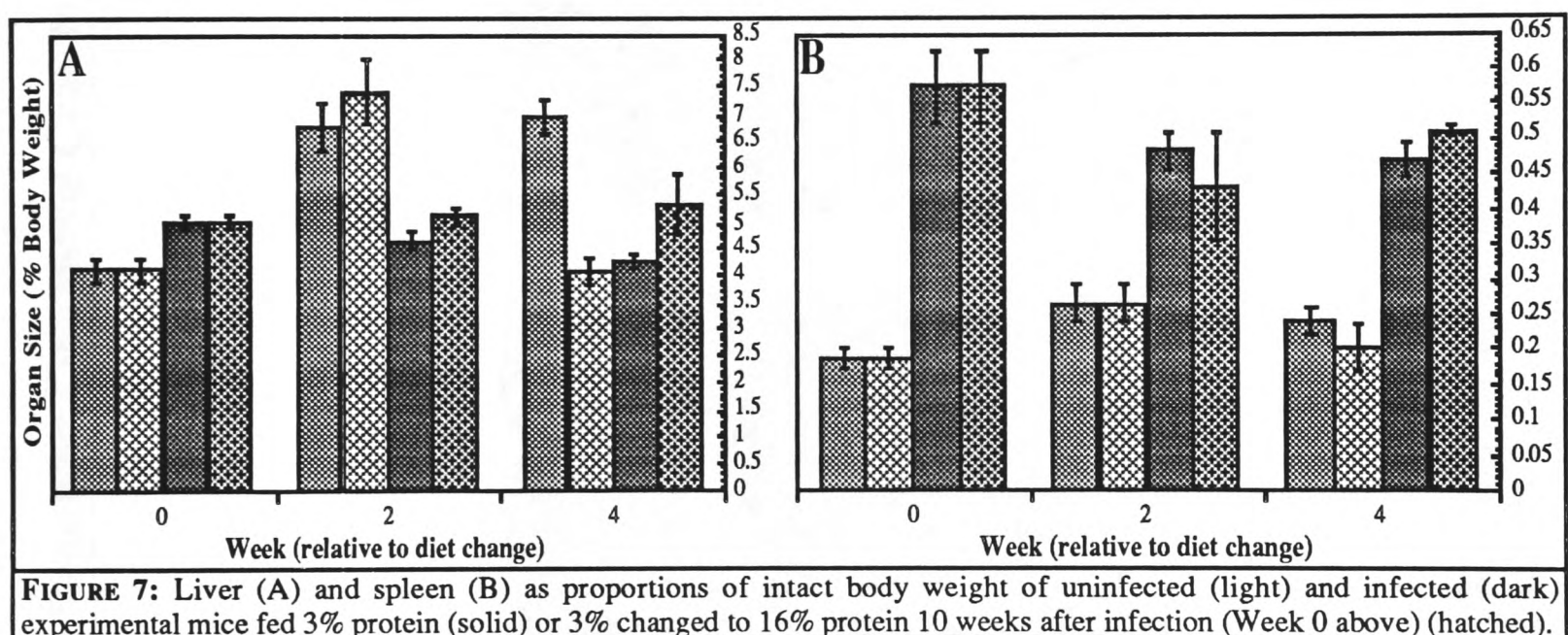


FIGURE 7: Liver (A) and spleen (B) as proportions of intact body weight of uninfected (light) and infected (dark) experimental mice fed 3% protein (solid) or 3% changed to 16% protein 10 weeks after infection (Week 0 above) (hatched).

Infection produced an artifactual decrease in apparent liver size (Figure 7), as infected livers did not readily absorb and retain fluid during perfusion, and thus weighed less than livers recovered from uninfected animals. Infected animals which had their diets changed to high protein had significantly larger livers at both 2 and 4 week post-dietary change than did infected animals which remained on the low protein diet (Table 6, overleaves).

Whilst infection produced a marked increase in spleen size (Figure 7), provision of a high protein diet to infected animals did not significantly influence their spleen size (Table 6, overleaves).

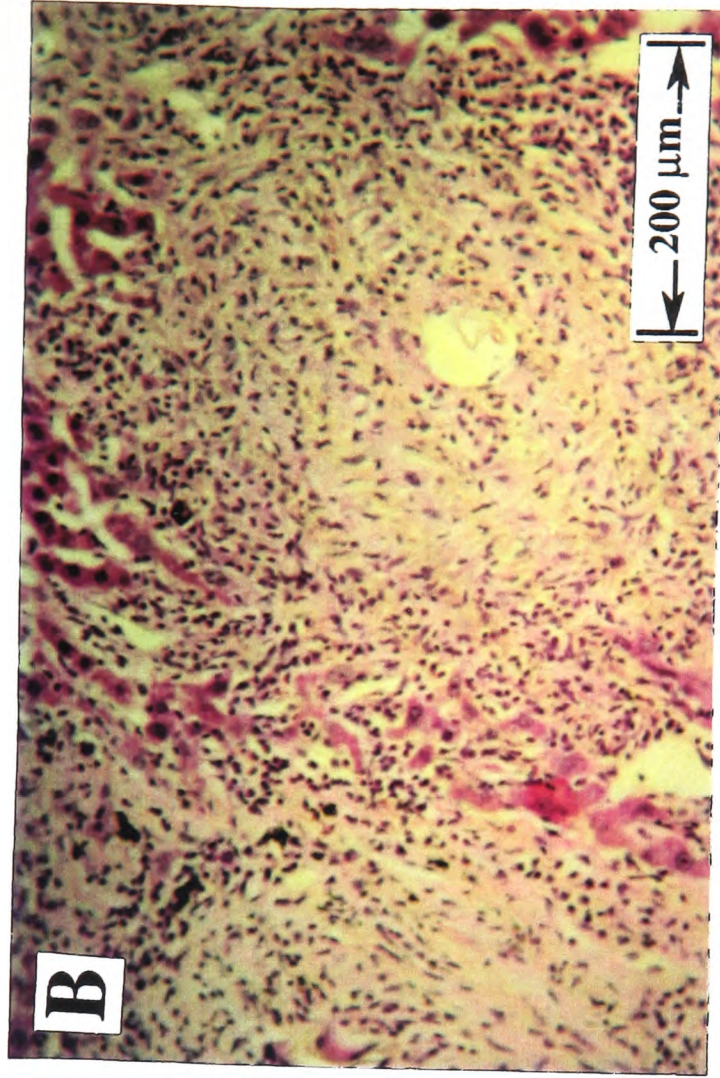
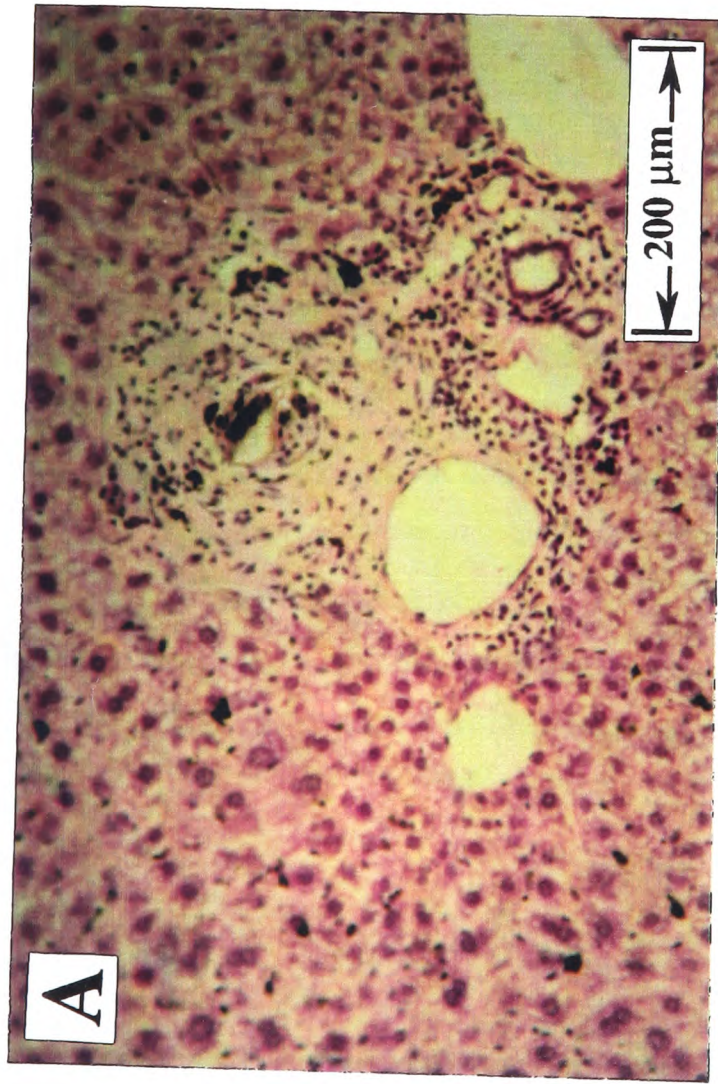
PLATE 1: Tissue Responses: Circumoval Granulomata in Mouse Liver

PLATE 1: Granulomata sizes in experimental mice: Liver sections prepared from mice after 14 weeks of schistosome infection and fed the 3% protein diet (A) or the 3% increased to 16% protein diet at week 10 (B) (stain=haematoxylin & eosin; scales are as shown).

Group	Un3-3A	Un3-3B	Un3-3C	Un3-16B	Un3-16C	Inf3-3A	Inf3-3B	Inf3-3C	Inf3-16B	Inf3-16C
Liver (n) range (%)	3.49-5.03 (6)	6.31-7.62 (3)	6-7.53 (4)	6.29-8.2 (3)	3.48-4.55 (4)	4.1-5.91 (19)	4.03-5.38 (9)	3.78-4.9 (7)	4.66-5.79 (6)	4.77-5.89 (2)
Spleen (n) range(B-1%)	1.36-2.49 (6)	2.32-3.19 (3)	2.06-2.86 (4)	2.26-3.17 (3)	1.25-2.87 (4)	2.93-12 (19)	3.33-6.09 (9)	3.79-5.58 (7)	1.78-6.52 (6)	5.02-5.17 (2)
Un3-3A	-	0.0002* (-6.48;7)	0.0001* (-7.54;9)	0.0002* (-6.72;7)	0.40(0.27;8)	0.001* (-3.44;23)	0.02* (-2.28;13)	0.33 (-0.45;11)	0.002* (-3.62;10)	0.02* (-2.55;6)
Un3-3B	0.014* (-2.75;7)	-	0.38(-0.34;5)	0.21 (-0.89;4)	0.0008* (6.18;5)	0.0001* (4.89;20)	0.0001* (6.70;10)	0.0001* (7.95;8)	0.002* (4.46;7)	0.06(2.16;3)
Un3-3C	0.029* (-2.21;8)	0.23(0.79;5)	-	0.25 (-0.72;5)	0.0002* (7.07;6)	0.0001* (6.07;21)	0.0001* (7.84;11)	0.0001* (9.03;9)	0.0003* (5.31;8)	0.03* (2.67;4)
Un3-16B	0.014* (-2.77;7)	0.49 (-0.02;4)	0.23(-0.81;5)	-	0.0009* (6.03;5)	0.0001* (6.21;20)	0.0001* (7.24;10)	0.0001* (7.90;8)	0.0008* (5.01;7)	0.046* (2.44;3)
Un3-16C	0.35(-0.40;8)	0.10(1.47;5)	0.16(1.08;6)	0.10(1.48;5)	-	0.002* (-3.23;21)	0.015* (-2.49;11)	0.22 (-0.81;9)	0.003* (-3.76;8)	0.03* (-2.60;4)
Inf3-3A	0.0002* (-4.12;23)	0.016* (-2.30;20)	0.004* (-2.88;21)	0.016* (-2.30;20)	0.002* (-3.22;21)	-	0.049* (1.72;26)	0.001* (3.43;24)	0.35 (-0.39;23)	0.25 (-0.69;19)
Inf3-3B	0.0001* (-8.16;13)	0.001* (-4.25;10)	0.0001* (-5.52;11)	0.0009* (-4.24;10)	0.0001* (-6.01;11)	0.13 (1.18;26)	-	0.02* (2.27;14)	0.03* (-2.14;13)	0.049* (-1.84;9)
Inf3-3C	0.0001* (-9.09;11)	0.0007* (-4.72;8)	0.0001* (-6.19;9)	0.0007* (-4.71;8)	0.0001* (-6.44;9)	0.13 (1.18;24)	0.39 (0.29;14)	-	0.0008* (-4.14;11)	0.009* (-3.10;7)
Inf3-16B	0.005* (-3.20;10)	0.09 (-1.49;7)	0.04* (-2.01;8)	0.09 (-1.49;7)	0.02* (-2.38;8)	0.09 (1.38;23)	0.25 (0.70;13)	0.31 (0.50;11)	-	0.33 (-0.47;6)
Inf3-16C	0.0001* (-11.09;6)	0.003* (-6.98;3)	0.0003* (-9.40;4)	0.003* (-6.99;3)	0.002* (-6.18;4)	0.35 (0.39;19)	0.33(-0.46;9)	0.23 (-0.78;7)	0.30(-0.55;6)	-

TABLE 6: T-test comparisons of organ sizes in experimental mice (number above diagonal represents p value followed by t value and degrees of freedom for liver comparison, below diagonal represents p value followed by t value and degrees of freedom for spleen comparison; n=number of organs measured) (Un=uninfected, Inf=infected, A=time of diet change, B=time of diet change +2 weeks, C=time of diet change +4 weeks).

DISCUSSION

The results presented in this experiment are in general agreement with previously published evidence (see Introduction), and confirm the hypothesis generated above that reversing extant host protein deficiency increases parasite success and disease expression in the chronic stage of schistosome infection.

The Parasite

Number

The present results indicate that having reached maturity in protein deficient hosts, worm survival is not significantly influenced by protein repletion. This observation is consistent with the results presented in Chapter 2 which indicate that the larval stages of *S. mansoni* are most vulnerable to suboptimal conditions within deficient vertebrate hosts. There is, however, a slight tendency for worm numbers to fall following host protein repletion, which suggests that the increased immune activity likely to accompany refeeding may have some impact on schistosomes which have developed under suboptimal conditions. This may indicate that, unlike adult worms which have matured under normal host conditions, schistosomes which have reached maturity in chronically deficient hosts may be less resistant to immune elimination. It is likely that further work could clarify this suggestion.

Size

A period of refeeding produces a significant increase in the growth of both male and female schistosomes when compared with those inhabiting hosts which remain protein deficient. This indicates that the stunting which occurs in schistosomes establishing within deficient hosts (see Chapter 2) can be reversed by host protein repletion in the later stages of development. The observation that male growth continues in deficient hosts after 10 weeks

suggests that the suboptimal conditions found within these hosts may retard worm growth, and perhaps maturation. Refeeding clearly rectifies these effects.

Proportion Fecund

A period of host protein repletion produced a marked increase in schistosome fecundity. Whilst the proportion of females producing eggs in hosts receiving an increase in dietary protein was similar to those remaining on the deficient diet, an increase in host dietary protein produced a marked rise in the proportion of these eggs which appeared normal. Such a rapid reversal of impaired egg production suggests that poor ambient conditions within deficient hosts (which are corrected by repletion), and not damaged or stunted worm reproductive organs, are the likely causes of abnormal egg production.

Whilst fewer females were fecund in deficient hosts after 6 weeks of infection than in well-fed hosts (see Experiment 2.I), the proportion of females which were fecund in deficient hosts 10 weeks after infection was similar to that seen in well-fed hosts after 6 weeks. These results suggest that host deficiency retards the onset of female schistosome fecundity, but that it does eventually reach similar levels to infections maturing in well-fed hosts, albeit with a higher proportion of abnormal eggs (see above).

***In Utero* Egg Size**

In addition to increasing the proportion of female worms producing normal eggs, a period of host repletion appears to reverse the deleterious impact of host deficiency on the size of normal eggs *in utero*. This may reflect the tendency for female sizes to increase following host repletion (see above), and/or the restoration of conditions more suitable for egg production. The increase in *in utero* egg sizes following repletion indicate that the reduced antigenicity associated with reduced egg sizes in deficient hosts (see Chapter 2) may be restored by host refeeding. In the presence of increased immune activity following repletion, increased antigenicity could boost immune-mediated egg excretion, thus enhancing parasite reproductive success. Increased egg antigenicity also has implications for the development of pathology in the host.

The Host

The provision of a high protein diet to chronically deficient mice led to an immediate and significant increase in body weight in all animals, regardless of infection status. This indicates that protein deficiency was established in animals prior to refeeding and that providing diets of higher protein content produced an appreciable increase in host nutritional status. The correction of protein deficiency in chronically infected mice led to a marked increase in the disease manifestations of schistosome infection. This was largely due to a significant increase in liver pathology.

Granulomata Number

The rapid and significant rise in the number of granulomata found in mouse livers following the provision of the high protein diet is likely to reflect increased egg production and antigenicity (see above), combined with the restoration of DTH (known to accompany host protein repletion) responsible for granulomata formation. It appears that an increased number of more antigenic eggs, in the presence of increased immune activity, produces a larger number of granulomata.

Granulomata Size

In addition to a significant increase in number, the size of lesions in the liver was observed to increase during protein repletion. This is likely to reflect an increase in egg antigenicity (see above) and/or an increase in the DTH reaction responsible for granulomata formation (see Chapter 2). The increase in granulomata size suggests that protein repletion restores the production of cell-mediated mechanisms active in granulomata promotion to a greater extent than those mechanisms active in their modulation. Therefore, the modulation which reduces organ inflammation during chronic schistosomiasis appears to be reversed by a period of host repletion. Therefore new eggs will elicit a larger immune response and produce larger granulomata, whilst eggs already existing in the tissues may attract renewed cellular activity (see Plate 1). The increase in granulomata number and size brought about by protein refeeding is likely to exacerbate chronic schistosomiasis.

Organ Size

The decrease in liver size accompanying infection, regardless of dietary treatment, is thought to be the result of decreased circulation in the diseased liver leading to less perfusate permeating through and being retained in the liver tissues. It is therefore suggested that future work on liver size be undertaken using organ dry weight comparisons. None-the-less, the significantly larger livers seen in well-fed, infected animals relative to animals remaining on the low protein diet supports the evidence above of greater inflammation and pathology accompanying protein repletion.

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: II. CHRONIC PHASE DIETARY PROTEIN DECREASE

Abstract

This experiment examines the influence of an externally imposed decrease in host protein status on the parasite and well-fed host during chronic schistosomiasis mansoni. Results show that while imposed deficiency does not significantly influence adult schistosome survival, parasite reproduction is significantly reduced. Combined with a fall in host immunity, this led to the amelioration of chronic pathology. These results suggest that an acute fall in human nutritional status in the presence of active schistosome infection may reduce parasite transmission and ameliorate the expression of chronic schistosomiasis.

INTRODUCTION

The previous experiment demonstrates that the suppressive effects of host protein deficiency on parasite and disease development are largely reversed by protein repletion. However, in areas endemic for schistosome infections, the prevailing nutritional level is frequently reduced by natural, catastrophic or man-made factors.

Although previous evidence has suggested that an acute decrease in host nutritional status influences both parasite and host during the early stages of infection (Chapter 2), no previous study has reported a comprehensive examination of this relationship in the chronic stages of infection. This experiment was designed to investigate the effects of a period of host protein deficiency on the relationship between schistosomes and their vertebrate hosts during chronic infection which had developed under normal nutritional conditions.

Hypothesis

That creating a period of acute protein deficiency in well-fed hosts decreases parasite success and disease development in the chronic stage of schistosome infection.

The influence of acute host protein-deficiency on schistosomes and pathology which had developed in well-fed mice was examined by autopsy at the time of diet change and 2 and 4 weeks later. Mice belonged to one of two dietary groups: those receiving 16% protein diet for the duration of the study or those which had their 16% protein diet replaced by a 3% protein ration 10 weeks after infection with 20 cercariae.mouse⁻¹.

EXPERIMENTAL DESIGN

Eighty mice were randomly assigned to groups of 10 housed in large cages and fed the 16% protein synthetic diet (D16). After two weeks, mice from six of the cages were individually exposed to 20 cercariae.mouse⁻¹ for 30 min (I.20; range 19-21). All cercariae were gathered simultaneously from the same group of 40 patent snails. The remaining two groups were handled identically in the absence of cercariae.

Ten weeks after cercarial exposure, three mice from each cage were autopsied (Atx3) as described in the Materials and Methods section of Chapter 2. At this time, one cage of uninfected mice and three cages of infected animals had their 16% protein diet replaced by the 3% protein diet (D3). The remaining cages continued to receive the 16% diet.

Two weeks later, a further three mice from each cage were autopsied (Atx3). After an additional two weeks, the remaining mice in each group were also autopsied (Atx4 & Atx12). The design of this experiment is presented in Table 1.

Week Group (n)	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A (10)	D16	→	-	-	-	-	-	-	-	-	-	→	Atx3	→	Atx3	→	Atx4
B (10)	D16	→	-	-	-	-	-	-	-	-	-	→	Atx3 D3	→	Atx3	→	Atx4
C (30)	D16	→	I.20	→	-	-	-	-	-	-	-	→	Atx9	→	Atx9	→	Atx12
D (30)	D16	→	I.20	→	-	-	-	-	-	-	-	→	Atx9 D3	→	Atx9	→	Atx12

TABLE 1: Design of Dietary Decrease experiment (n=number of mice in group).

RESULTS: PARASITE

Number

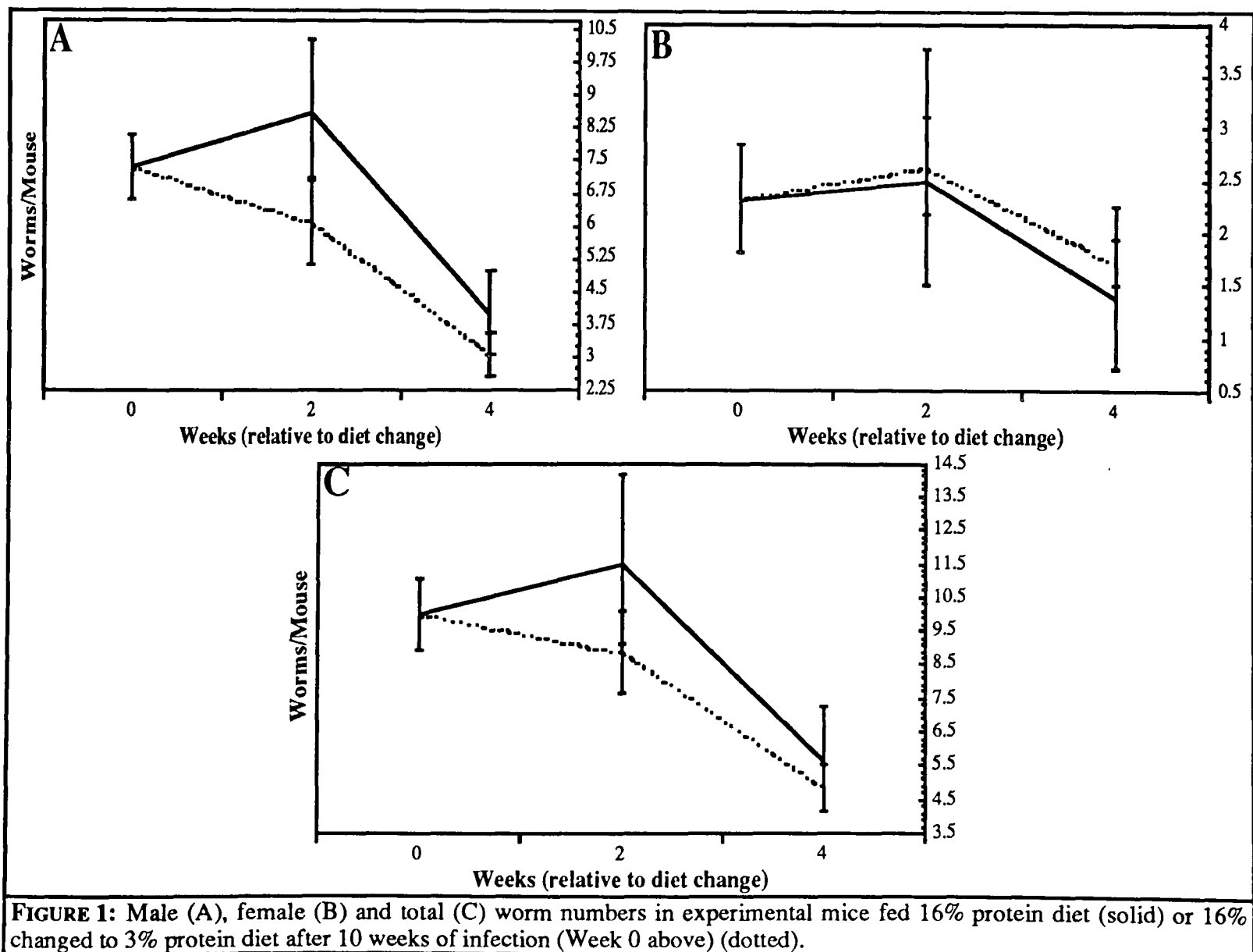


FIGURE 1: Male (A), female (B) and total (C) worm numbers in experimental mice fed 16% protein diet (solid) or 16% changed to 3% protein diet after 10 weeks of infection (Week 0 above) (dotted).

Although worm numbers tended to fall during the experimental period (Figure 1, previous page), there were no significant differences at any time point between the number of worms recovered from mice suffering a protein reduction and those which continued to receive the high protein diet (Table 2).

Groups	16% Protein at Change	16% Protein + 2 weeks	16% Protein + 4 weeks	3% Protein + 2 weeks	3% Protein + 4 weeks
Male Worms					
range (n)	3-14 (17)	2-12 (8)	2-7 (5)	3-10 (10)	1-5 (9)
16% Protein at Change	-	0.19	1.38	0.28	3.74*
16% Protein + 2 weeks	-	-	-	0.69	-
16% Protein + 4 weeks	-	-	-	-	0.13
Female Worms					
range (n)	0-6 (17)	0-6 (8)	0-4 (5)	1-6 (10)	1-3 (9)
16% Protein at Change	-	0.01	0.27	0.03	0.14
16% Protein + 2 weeks	-	-	-	0.004	-
16% Protein + 4 weeks	-	-	-	-	0.04
All Worms					
range (n)	4-16 (17)	2-17 (8)	2-11 (5)	5-16 (10)	2-7 (9)
16% Protein at Change	-	0.14	1.22	0.12	2.73*
16% Protein + 2 weeks	-	-	-	0.4	-
16% Protein + 4 weeks	-	-	-	-	0.05

TABLE 2: ANOVA comparisons of mean worm numbers in experimental mice fed 16% protein for the duration of the experiment (16% at Change, 16%+2 weeks, 16%+4 weeks) or 16% changed to 3% protein 10 weeks post-infection (16% at Change, 3%+2 weeks, 3%+4 weeks) (number above diagonal represents ANOVA F value; n=number of mice perfused for worms; df=48).

Size: Means

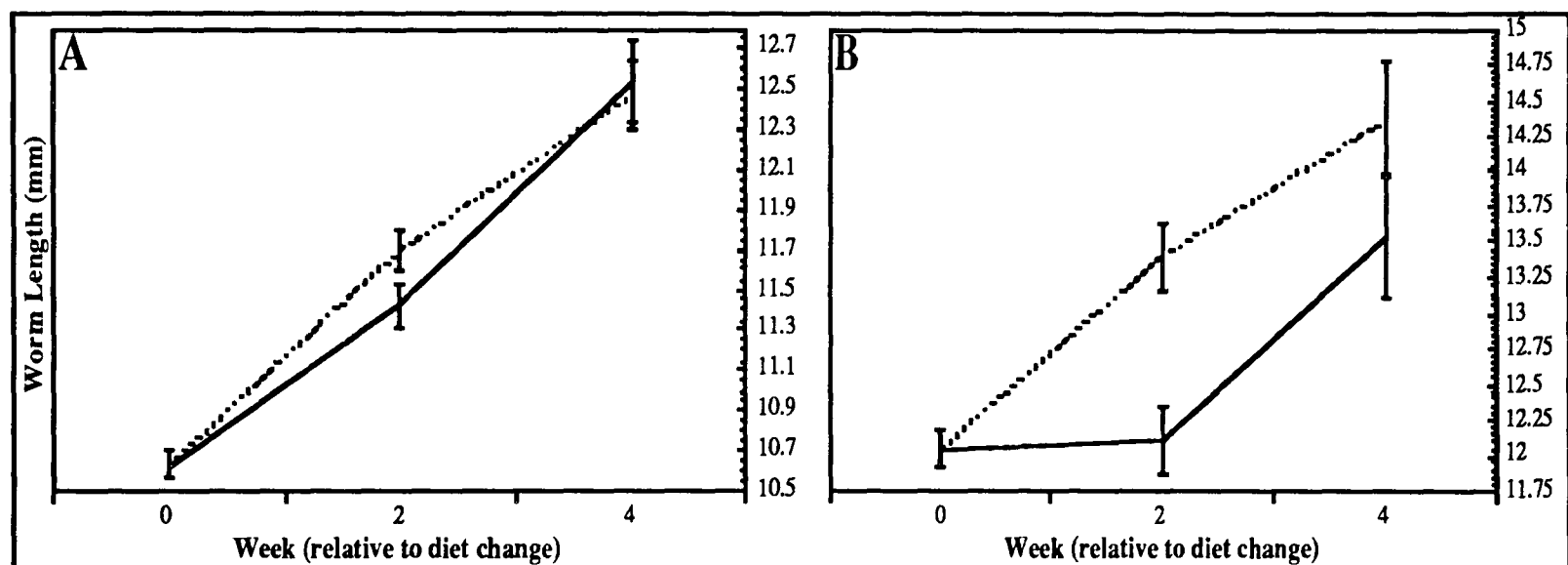


FIGURE 2: Length of male (A) and female (B) worms perfused from mice fed 16% protein (solid) or 16% protein changed to 3% protein 10 weeks after infection (Week 0 above) (dotted).

Males recovered from both well-fed mice and mice which suffered a decrease in protein intake continued to grow, and there were no apparent differences in length between the two groups of worms (Table 3). On the other hand, a decrease in host protein intake was associated with a significant increase in female schistosome length 2 weeks after repletion, which became statistically insignificant after a subsequent increase in female length in those hosts continuing to receive the high protein diet (Figure 2).

Groups	16% Protein at Change	16% Protein + 2 weeks	16% Protein + 4 weeks	3% Protein + 2 weeks	3% Protein + 4 weeks
Male Worms df=315					
range (mm) (n)	8.9-13.1 (129)	9.4-13.2 (73)	10.6-14.0 (21)	9.9-14.3 (64)	11.0-14.7 (29)
16% Protein at Change	-	11.03*	21.46*	16.45*	25.53*
16% Protein + 2 weeks	-	-	-	0.61	-
16% Protein + 4 weeks	-	-	-	-	0.03
Female Worms df=126					
range (mm) (n)	9.0-14.1 (47)	9.7-14.2 (27)	12.2-15.6 (9)	11.1-16.4 (28)	12.3-17.3 (16)
16% Protein at Change	-	0.009	3.22*	5.76*	11.11*
16% Protein + 2 weeks	-	-	-	4.15*	-
16% Protein + 4 weeks	-	-	-	-	0.56

TABLE 3: ANOVA comparisons of mean worm lengths in experimental mice (number above diagonal represents ANOVA F value; n=number of worms measured).

Fecundity: Proportion of Females Gravid

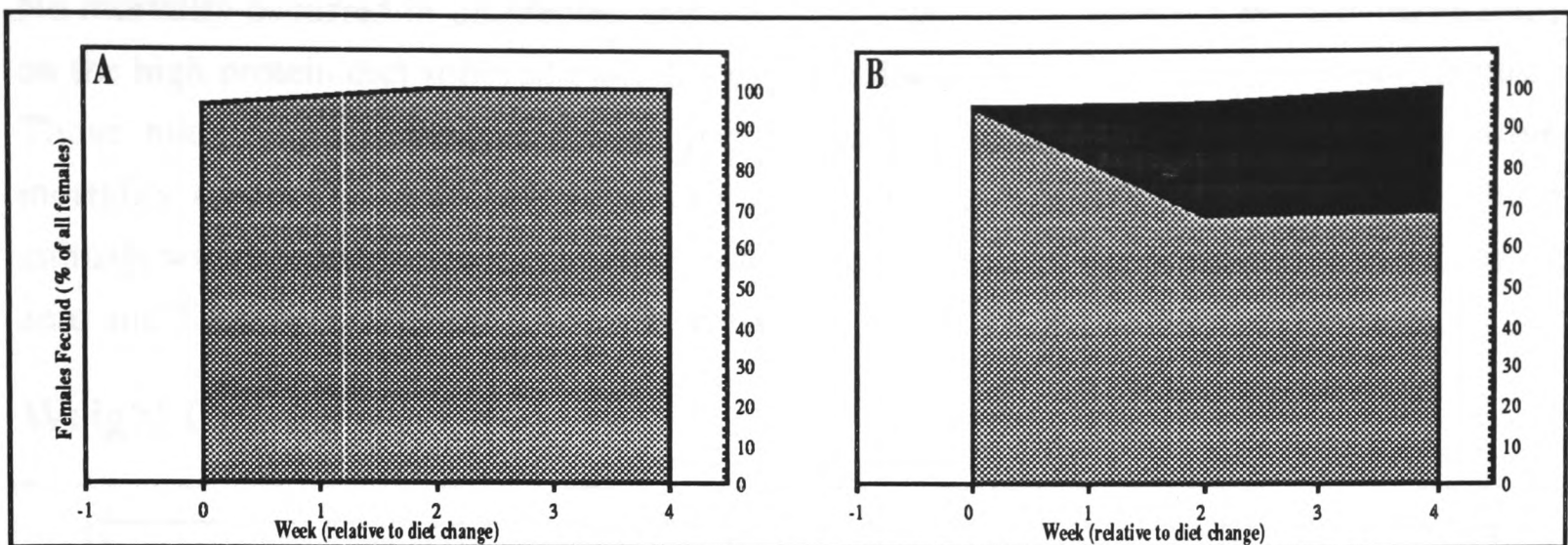


FIGURE 3: Proportion of female worms perfused from mice fed 16% protein diets (A) or 16% changed to 3% protein diets 10 weeks after infection (Week 0 above) (B) which were gravid (upper limit of plot) with normal (grey) or abnormal (black) eggs.

Although having no noticeable effect on the proportion of female worms which had eggs *in utero* (Figure 3, upper limit of plot), a reduction in host protein intake produced a marked increase in the proportion of these eggs which were abnormal (Figure 3, black area).

Fecundity: *In Utero* Egg Size

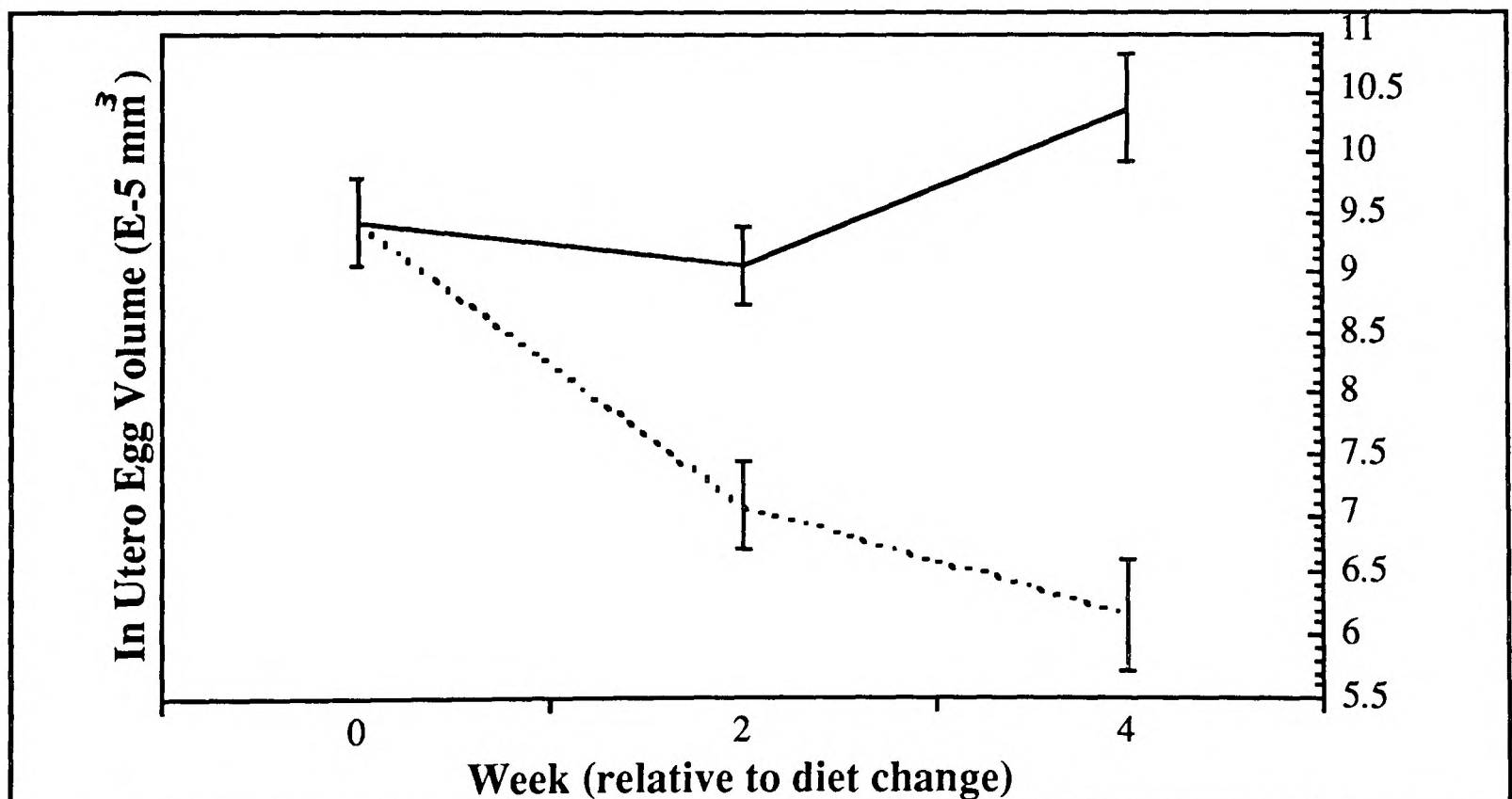


FIGURE 4: Volumes of normal schistosome eggs *in utero* of females perfused from mice fed 16% protein (solid) or 16% changed to 3% protein after 10 weeks of infection (Week 0 above) (dotted).

A reduction in host protein intake produced a rapid and sustained fall in the size of *in utero* eggs (Figure 4) which became significant after 2 weeks of deficiency (Table 4).

Groups	16% Protein at Change	16% Protein + 2 weeks	16% Protein + 4 weeks	3% Protein + 2 weeks	3% Protein + 4 weeks
range (E-5 mm ³) (n)	3.18-15.86 (45)	5.25-12.16 (27)	7.41-11.76 (9)	3.96-10.34 (19)	4.61-9.22 (11)
16% Protein at Change	-	0.12	0.38	4.91*	6.61*
16% Protein + 2 weeks	-	-	-	3.02*	-
16% Protein + 4 weeks	-	-	-	-	5.87*

TABLE 4: *In utero* egg volumes (number above diagonal represents ANOVA F value; n=number of eggs measured; df=110).

RESULTS: HOST

No mortality occurred in uninfected animals during the study. Infected animals maintained on the high protein diet suffered mortality of 7.5% during the 10 week period post-infection. Those mice which continued to receive the high protein ration displayed cumulative mortality of 16.67% and 25% at weeks 12 and 14 post-infection, respectively. Those animals which were placed on the low protein diet at week 10 saw cumulative mortality of zero and 5% at weeks 12 and 14, respectively.

Weight Change

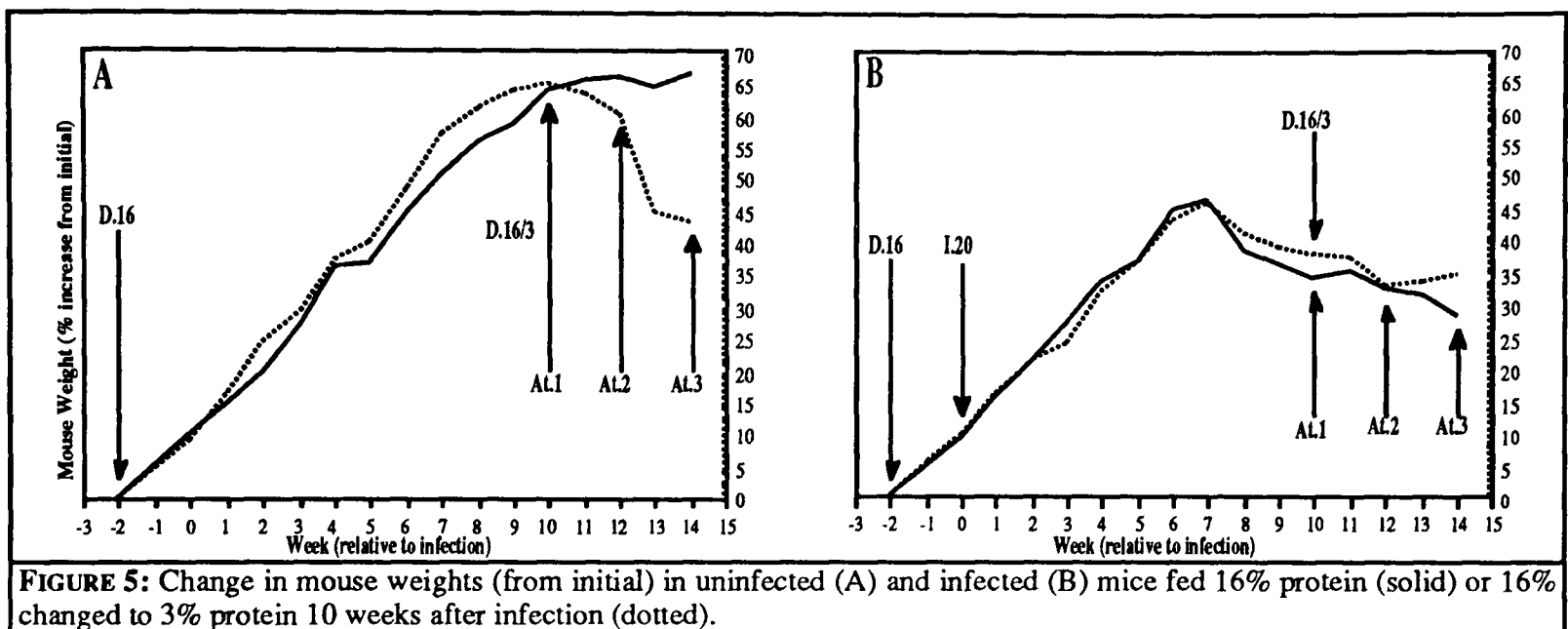


FIGURE 5: Change in mouse weights (from initial) in uninfected (A) and infected (B) mice fed 16% protein (solid) or 16% changed to 3% protein 10 weeks after infection (dotted).

Providing mice maintained on a 16% protein diet with a low protein diet saw a significant decrease in body weight in uninfected animals by week 12 ($df=53$, $F=2.93^*$). Infected animals which were begun on the low protein diet were not significantly different in weight to those which continued to receive the high protein diet (Figure 5).

Liver Granulomata: Number & Size

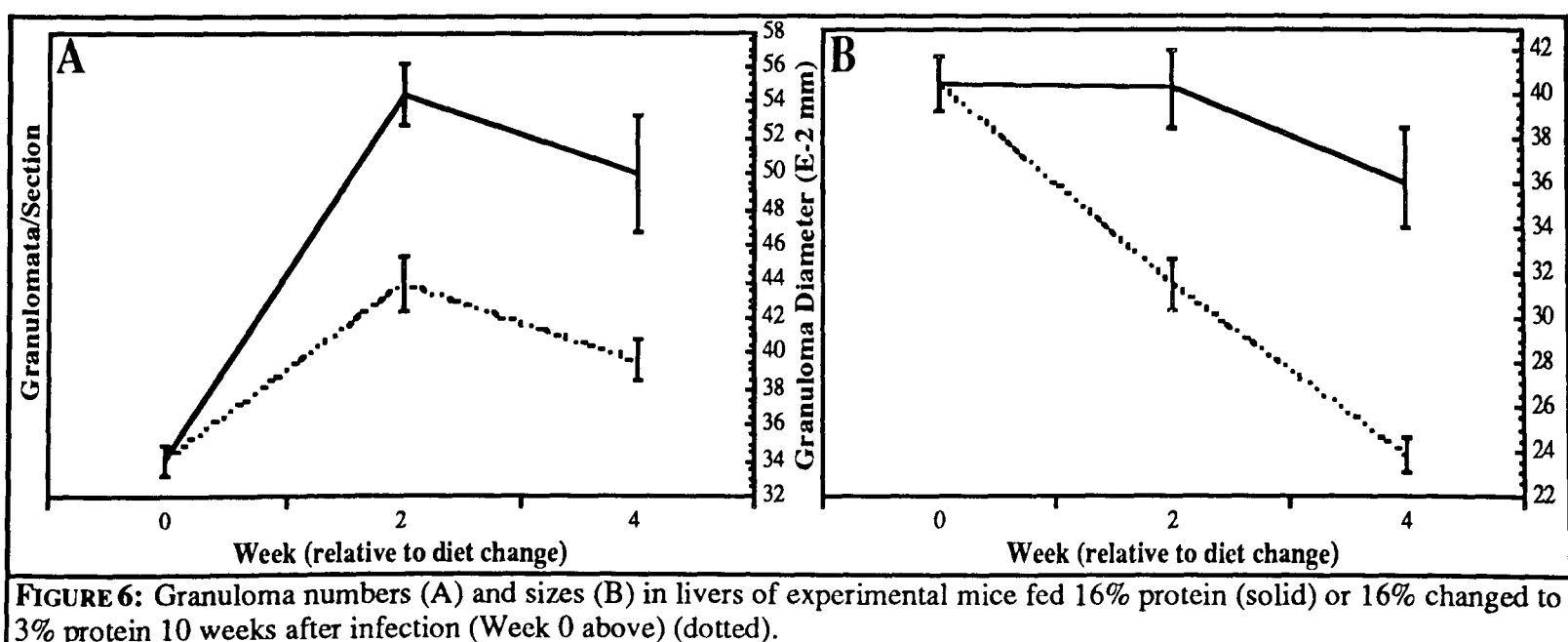


FIGURE 6: Granuloma numbers (A) and sizes (B) in livers of experimental mice fed 16% protein (solid) or 16% changed to 3% protein 10 weeks after infection (Week 0 above) (dotted).

Granuloma numbers rose in all animals during the experimental period (Figure 6), however those mice which suffered a reduction in dietary protein had significantly fewer liver

granulomata at both 2 and 4 weeks post-dietary change than animals which continued to receive the high protein diet (Table 5).

Whilst granulomata sizes remained similar throughout the experiment in mice maintained on the high protein diet (Figure 6), a reduction in dietary protein produced an immediate and significant reduction in granuloma size (Table 5). Granulomata from mice maintained on different dietary treatments appear in Plate 1 (overleaf). Note should be made of the reduced immune activity surrounding the new egg in the tissues of the deficient animal, and the extensive lesion surrounding the egg in the well-fed animal.

Groups	16% Protein at Change	16% Protein + 2 weeks	16% Protein + 4 weeks	3% Protein + 2 weeks	3% Protein + 4 weeks
Granuloma Number range (n)	df=260 16-53 (90)	37-73 (35)	33-86 (22)	24-69 (60)	23-58 (54)
16% Protein at Change	-	23.37*	10.3*	8.61*	2.78*
16% Protein + 2 weeks	-	-	-	4.97*	-
16% Protein + 4 weeks	-	-	-	-	3.54*
Granuloma Diameter range (mm) (n)	df=219 0.18-0.64 (75)	0.26-0.68 (30)	0.24-0.58 (20)	0.16-0.52 (50)	0.16-0.34 (45)
16% Protein at Change	-	0.006	0.81	6.99*	25.16*
16% Protein + 2 weeks	-	-	-	4.08*	-
16% Protein + 4 weeks	-	-	-	-	7.16*

TABLE 5: ANOVA comparison of numbers and diameter of granulomata in the livers of experimental mice (number above diagonal represents ANOVA F value; n=number of granulomata measured).

Organ Size

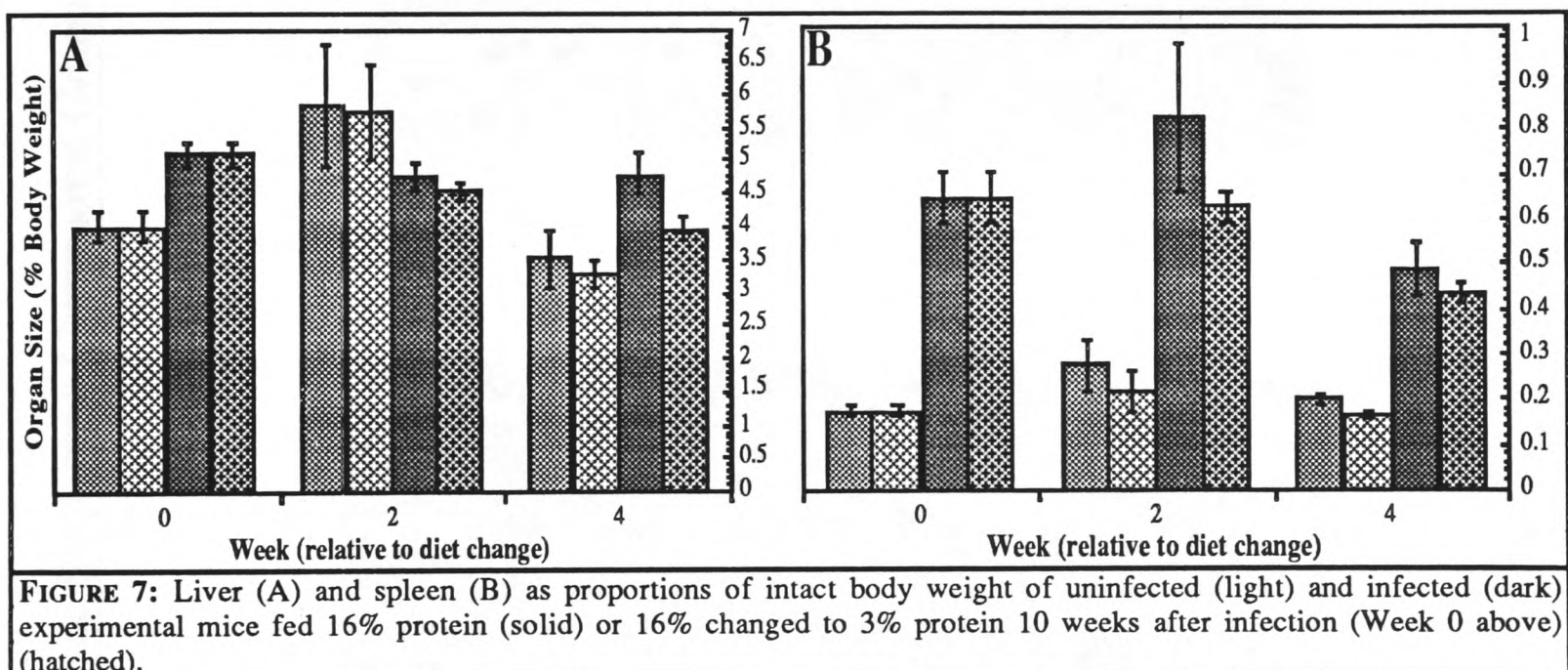


FIGURE 7: Liver (A) and spleen (B) as proportions of intact body weight of uninfected (light) and infected (dark) experimental mice fed 16% protein (solid) or 16% changed to 3% protein 10 weeks after infection (Week 0 above) (hatched).

Infection produced a significant increase in the size of both mouse livers and spleens, regardless of dietary treatment (Figure 7; Table 6, overleaves). Reduction of dietary protein produced an apparent decrease in liver and spleen sizes in infected animals.

As seen in the prior experiment, infection produced an artifactual decrease in apparent liver size (Figure 7A, Week 2), as infected livers did not readily absorb and retain fluid during perfusion, and thus weighed less than livers recovered from uninfected animals. Infected animals which had their diets changed to low protein had significantly smaller livers 4 weeks after dietary change than did infected animals which remained on the high protein diet (Table 6).

PLATE 1: Tissue Responses: Circumoval Granulomata in Mouse Liver

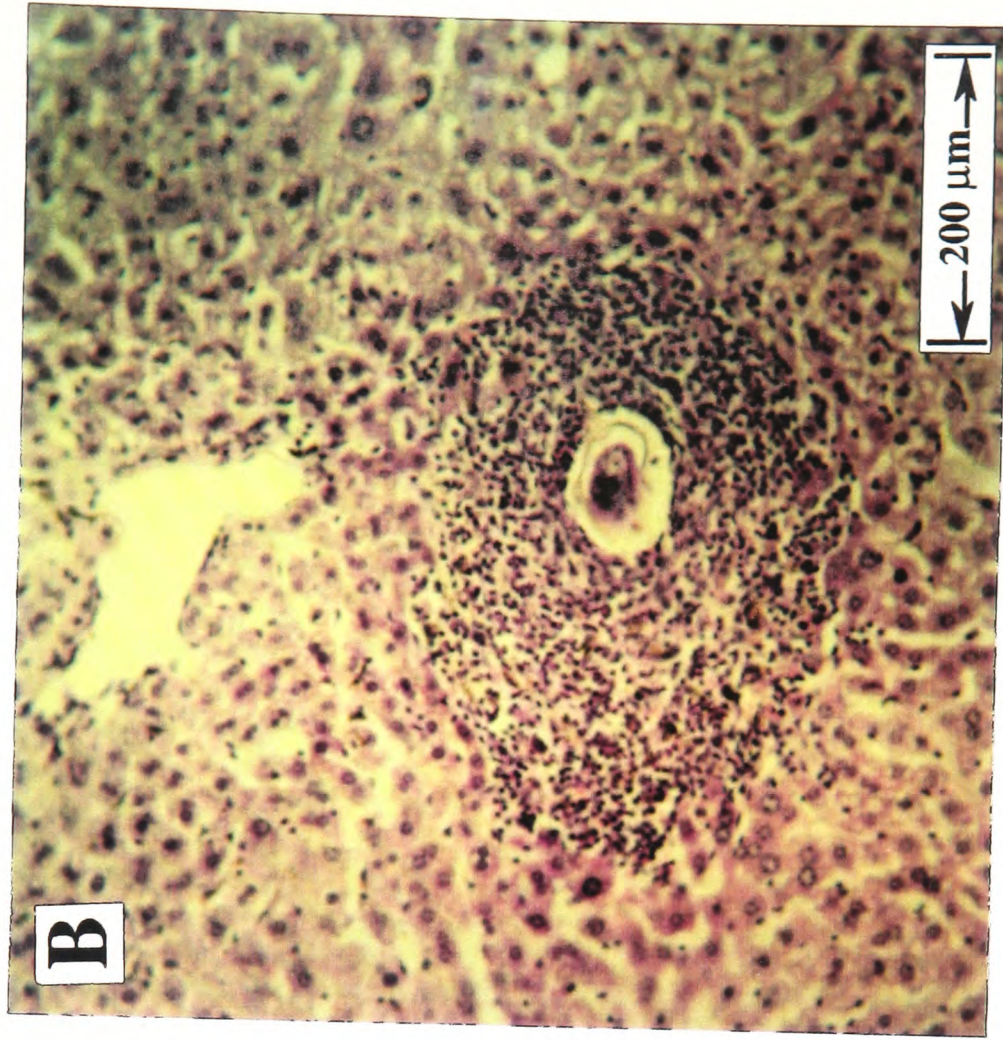
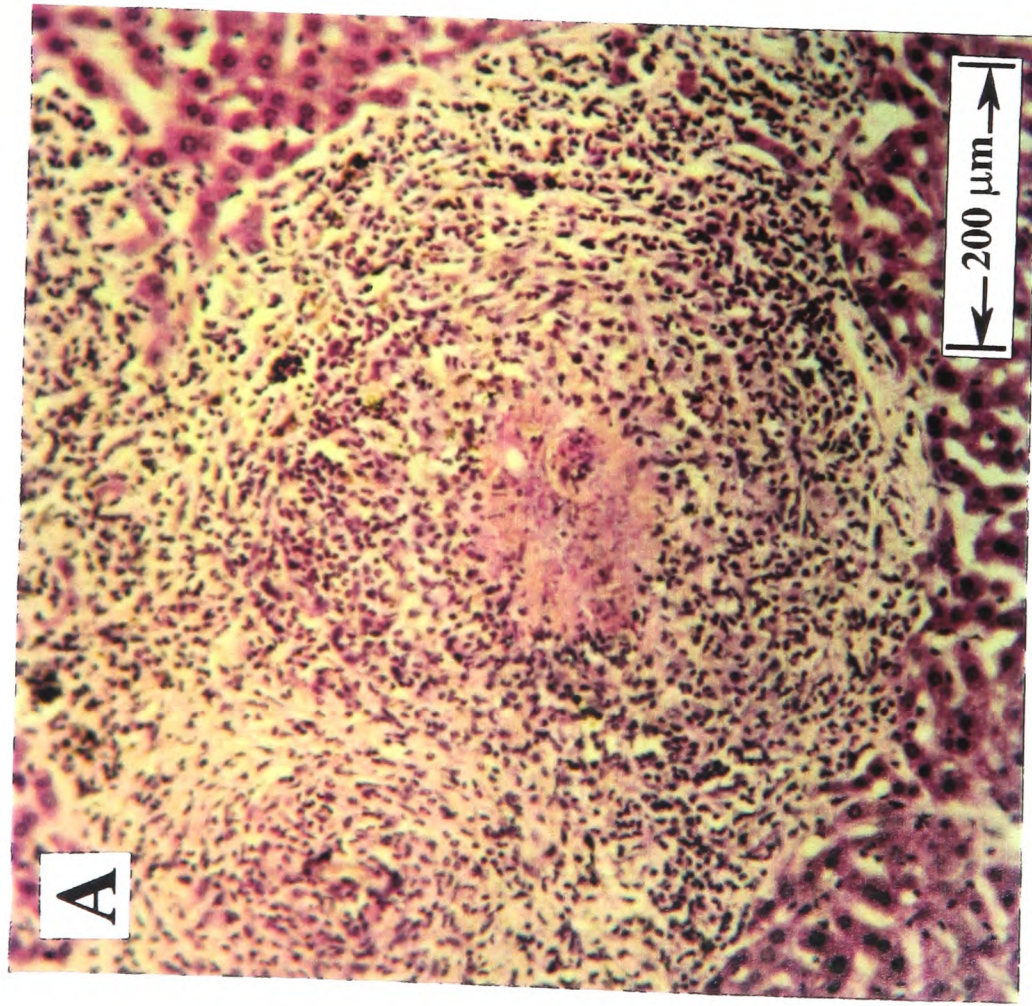


PLATE 1: Granulomata sizes in experimental mice: Liver sections prepared from mice after 14 weeks of schistosoma infection and fed the 16% protein diet (A) or the 16% decreased to 3% protein diet at week 10 (B) (stain=haematoxylin & eosin; scales are as shown).

Whilst infection produced a marked increase in spleen size (Figure 7), provision of a low protein diet to infected animals tended to reduce their spleen size. Although this reduction was not statistically significant when compared with similarly infected animals which remained on the high protein diet, spleens of infected mice which began to receive the low protein diet 4 weeks previously were significantly smaller than host spleens measured at the time of dietary change (Table 6).

Group	Un16-16A	Un16-16B	Un16-16C	Un16-3B	Un16-3C	Inf16-16A	Inf16-16B	Inf16-16C	Inf16-3B	Inf16-3C
Liver (n range (%))	3.29-4.63 (6)	4.17-7.38 (3)	3.03-4.87 (4)	4.55-7.05 (3)	2.99-3.86 (4)	3.75-6.31 (17)	4.07-5.92 (8)	3.71-5.52 (5)	3.68-5.06 (10)	3.2-4.7 (9)
Spleen (n range(B-1%))	1.33-2.04 (6)	1.78-3.75 (3)	1.65-2.2 (4)	1.55-3.08 (3)	1.48-1.87 (4)	3.57-13.0 (17)	1.69-14.6 (8)	2.54-5.87 (5)	4.59-8.24 (10)	2.79-5.04 (9)
Un16-16A	-	0.016* (-2.66;7)	0.15(1.10;8)	0.01* (-2.98;7)	0.03* (2.31;8)	0.002* (-3.27;21)	0.02* (-2.46;12)	0.03* (-2.14;9)	0.017* (-2.35;14)	0.44 (0.17;13)
Un16-16B	0.019* (-2.57;7)	-	0.03* (2.47;5)	0.46(0.10;4)	0.013* (3.16;5)	0.10 (1.33;18)	0.057 (1.75;9)	0.11(1.35;6)	0.013* (2.55;11)	0.003* (3.40;10)
Un16-16C	0.08(-1.57;8)	0.09(1.58;5)	-	0.02* (-2.74;5)	0.32(0.50;6)	0.0007* (-3.70;19)	0.007* (-2.95;10)	0.02* (-2.46;7)	0.004* (-3.14;12)	0.12 (-1.23;11)
Un16-3B	0.013* (-1.25;7)	0.23(0.82;4)	0.34(-0.43;5)	-	0.006* (3.78;5)	0.12 (1.21;18)	0.05* (1.83;9)	0.10 (1.42;6)	0.009* (2.81;11)	0.002* (3.80;10)
Un16-3C	0.33(0.46;8)	0.037* (2.26;5)	0.04* (2.16;6)	0.14(1.23;5)	-	0.0001* (-4.69;19)	0.0005* (-4.59;10)	0.003* (-4.01;7)	0.0001* (-5.61;12)	0.01* (-2.61;11)
Inf16-16A	0.0001* (-5.12;21)	0.006* (-2.78;18)	0.0004* (-3.94;19)	0.002* (-3.25;18)	0.0002* (-4.23;19)	-	0.13 (1.15;23)	0.20 (0.86;20)	0.02* (2.17;25)	0.0002* (4.15;24)
Inf16-16B	0.003* (-3.40;12)	0.04* (-1.96;9)	0.013* (-2.63;10)	0.028* (-2.18;9)	0.01* (-2.77;10)	0.11 (-1.29;23)	-	0.47 (-0.08;11)	0.19 (0.92;16)	0.003* (3.16;15)
Inf16-16C	0.0002* (-5.58;9)	0.03* (-2.30;6)	0.002* (-4.12;7)	0.01* (-3.06;6)	0.001* (-4.61;7)	0.07 (1.52;20)	0.07 (1.56;11)	-	0.20 (0.89;13)	0.009* (2.75;12)
Inf16-3B	0.0001* (-10.95;14)	0.0001* (-5.41;11)	0.0001* (-8.43;12)	0.0001* (-6.54;11)	0.0001* (-9.10;12)	0.39 (0.29;25)	0.10 (1.32;16)	0.02* (-2.30;13)	-	0.004* (3.04;17)
Inf16-3C	0.0001* (-8.95;13)	0.005* (-3.15;10)	0.0001* (-6.66;11)	0.0004* (-4.62;10)	0.0001* (-7.64;11)	0.005* (2.80;24)	0.012* (2.50;15)	0.17 (-0.99;12)	0.0001* (4.94;17)	-

TABLE 6: T-test comparisons of organ sizes in experimental mice (number above diagonal represents p value followed by t value and degrees of freedom for liver comparison, below diagonal represents p value followed by t value and degrees of freedom for spleen comparison; n=number of organs measured) (Un=uninfected, Inf=infected, A=time of diet change, B=time of diet change +2 weeks, C=time of diet change +4 weeks).

DISCUSSION

The results presented in this experiment confirm the hypothesis generated above that a period of acute protein deficiency in previously well-fed hosts reduces parasite success and disease expression in the chronic stage of schistosome infection.

The Parasite

Number

A period of host protein deficiency does not significantly reduce adult schistosome survival when compared with that seen in well-fed controls. This is consistent with the suggestion (see Chapter 2) that the majority of the deleterious effects of protein deficiency on schistosome survival occur during the larval stages.

Size

Acute protein deficiency did not affect male growth, however female lengths were significantly greater in deficient hosts 2 weeks after protein reduction. From observations made during worm examination, this appears to be the result of weakened longitudinal musculature allowing females recovered from deficient hosts to extend more during the

measuring process. Thus it appears that, despite an increase in length, mature females suffer from a period of host nutritional deficiency.

Proportion Fecund

In accordance with the observation that females are affected by a reduction in host nutrition, results show that, despite having little influence on the proportion of females producing eggs, a period of host protein deficiency leads to an immediate and sustained rise in the proportion of these eggs which are abnormal. This agrees with previous reports (see Introduction).

***In Utero* Egg Size**

Furthermore, those *in utero* eggs which remain normal during a period of protein deficiency are significantly reduced in size. This suggests that miracidial development will be reduced (see above), and as such, these eggs may be expected to be less antigenic than those produced by females in animals which continue to receive adequate rations. In addition to potentially compromising immune-mediated migration (see Chapter 2), the loss of antigenicity has implications for the stimulus central to disease development.

The Host

Uninfected animals placed on the low protein ration immediately lost significant body weight when compared with those which continued to receive the high protein diet. This indicates that the provision of the low protein diet was sufficient to produce deficiency. Infected animals began to lose weight prior to dietary protein reduction, indicating parasite-induced morbidity. Protein reduction did not alter the course of continuing weight loss.

Granulomata Number

A period of protein deficiency produced an immediate and significant reduction in both the number of liver granulomata and their size. It is likely that the fall in granulomata number reflects reduced egg presence, reduced antigenicity and a diminished immune response (see Chapter 2). As female worms inhabiting mice experiencing protein deficiency produce similar numbers of eggs to females in well-fed hosts (see above), yet a higher proportion of these are abnormal, it appears that abnormal eggs do not survive in the liver, or do not elicit a response. This agrees with previous work which suggests that egg integrity is essential for survival in the tissues (see Chapter 2).

Granulomata Size

In addition to reduced numbers, granulomata size falls in response to a decrease in host protein status. This is likely to reflect the immune suppression known to accompany protein deficiency (see Chapter 1). In the presence of modulation, it appears that protein deficiency

produces a greater decrease in those immune mechanisms responsible for lesion formation than those responsible for response modulation.

Organ Size

The significantly smaller livers seen in poorly-fed, infected animals relative to animals remaining on the high protein diet supports the evidence above of reduced inflammation and pathology accompanying protein undernutrition. It is also likely that spleen sizes in infected mice will eventually fall as a result of reduced DTH activity secondary to protein deficiency.

3.5: CHAPTER DISCUSSION

This chapter examined the hypothesis generated in the Introduction that an increase in previously deficient host nutrition increases adult schistosome success and exacerbates disease expression, whilst a decrease in previously adequate nutrition has the converse effect of reducing schistosome success and ameliorating disease expression.

Experimental results confirm that, whilst adult worms are largely resilient to changes in their environment, their reproductive success is rapidly compromised by a period of host deficiency. Conversely, the reduction in reproductive success observed in schistosomes which develop in deficient hosts is restored by host repletion. In concert with alterations in immune activity, deficiency and refeeding produce decreases and increases in disease expression, respectively.

Reversible R_0 -Antagonism & Host Undernutrition

Deficiency

The results presented above indicate that decreasing host protein status can reduce the reproductive success of schistosomes which have developed at a higher nutritional level. It follows that a period of protein deficiency in hosts infected with schistosomes may contribute to a decrease in schistosome transmission. Thus, it might be expected that the vertebrate host's contribution to schistosome transmission falls during times of nutritional stress, be it a result of natural, catastrophic or man-made factors.

Refeeding

Nutritionally deficient hosts harbor fewer, smaller and less fecund schistosomes than do well-fed animals (Chapter 2). The results presented above indicate, however, that increasing host protein status can largely restore parasite size and reproductive success. In addition to potentially increasing the host's susceptibility to new schistosome infections (Chapter 2), an increase in host protein status leads to increases in worm sizes, normal egg production and egg sizes. It follows that refeeding deficient hosts infected with schistosomes may increase schistosome reproductive success and therefore transmission.

Reversible Patho-Antagonism & Host Undernutrition

Deficiency

In much the same way as low protein diets protect hosts from fulminant schistosomiasis in the acute stages (Chapter 2), decreases in host protein status can markedly decrease the number and size of tissue responses to parasite eggs during chronic infection, and in the theoretical presence of reduced immunity, lead to an amelioration of chronic disease.

This is not to suggest starvation as a form of prophylaxis against schistosomiasis - deficiency disease is clearly to be avoided. However it does point to the fact that changes in the host interna which reduce viable egg production (see above) may provide relief from chronic disease. Simply reducing the immune effector component of schistosomiasis is impractical, as egg toxins remain intact and removing the protection provided by the granuloma formation has been shown to increase morbidity (Domingo, Cowan & Warren, 1967; Domingo & Warren, 1967).

Compounds which change the host interna in such a way as to reduce viable egg production may be effective in suppressing both disease and transmission, however these may have undesirable side-effects in the host, such as those seen in the use of antimonial drugs.

Refeeding

Whereas low protein diets protect hosts from fulminant schistosomiasis in the acute stages (Chapter 2), increases in host protein status can markedly increase the number and size of tissue responses to parasite eggs, and in the theoretical presence of restored immunity, lead to an increase in chronic disease. It is therefore suggested that deficient hosts infected with schistosomes should not be re-fed in the absence of curative chemotherapy. Furthermore, treatment must be sustained, as reinfection will occur in more suitable, replete hosts and may produce increases in parasite and disease development following reinfection to levels above those which existed prior to supplementation.

In the Field

Clearly, results showing a change in schistosome infection and disease with changing host nutritional status have implications for human populations exposed to poor nutritional security. Although the present results should be interpreted with appropriate caution accorded to studies involving experimental animals, indications of reversible R_0 - and patho-antagonism demand further study.

Little attention has been paid to the influence of host nutrition since the statement by Keusch (1982) that "schistosomiasis is a severe, sometimes fatal disease in certain individuals, and this fact must be considered independently of nutritional concerns". The observations contained in this chapter contradict this statement, and it is suggested that this aspect be looked at more closely.

Conclusions

Altering nutritional status in the presence of active parasitic infection and disease may change the expression of disease. It is therefore imperative that the disease implications, both

nutritional and parasitic, be considered during the formulation of policy concerning nutritional support in developing countries where schistosomiasis is endemic.

CHAPTER FOUR

SCHISTOSOMES & VERTEBRATE HOST NUTRITION

TREATMENT & CONTROL INTERVENTIONS



Chapter Summary

This chapter examines the influence of externally imposed protein deficiency on the vertebrate host's ability to capitalize on vaccination and chemotherapy against schistosome infection. Results of experiments which explore the influence of undernutrition on vaccination and praziquantel treatment show that their therapeutic values are diminished in protein deficient hosts. Vaccination produced significantly less protection against reinfection in deficient hosts when compared with well-fed animals. Unlike drug treatment in well-fed hosts, praziquantel administered to protein deficient hosts failed to eliminate all schistosomes. These results have implications for undernourished human populations receiving treatment and/or control interventions aimed at reducing schistosome infection and disease.

4.1: INTRODUCTION

It is widely accepted that the concomitants of poverty, chiefly poor sanitation and inadequate education, conspire to impose persistent infection and disease on human communities within developing countries (see Chapter 1). These populations are continually exposed to enormous medical and economic burdens as a consequence. As those populations most affected are similarly those least able to help themselves, it falls to benevolent organizations to provide what assistance they can.

The previous chapter describes how the policy of dietary supplementation, primarily aimed at preventing deficiency diseases but generally considered to impart some increased resistance to infection, requires closer scrutiny if inadvertent damage is to be avoided during refeeding. On the individual patient level, however, interventions specifically targeted at the treatment and control of parasitoses chiefly comprise chemotherapy and vaccination.

4.2: TREATMENT & CONTROL INTERVENTIONS

Treatment and control measures are generally offered in response to high prevalences of infection and disease. It is widely held that the majority of such interventions rely upon one of two mechanisms: vaccines potentiate immune-mediated elimination of invading parasites (Butterworth, 1987; Cox, 1990), whilst drugs generally inhibit some aspect of parasite metabolism or damage parasites in some way which increases their vulnerability to immune attack (Bryant & Behm, 1989).

The frequent coincidence of infection and poor food security in impoverished human communities suggests that those individuals most likely to selectively receive vaccination and/or chemotherapy are also those most likely to suffer from undernutrition. This chapter

describes how the success of such interventions, regardless of their precise mode of action, depends to some extent upon host nutritional status.

Nutritional Dependence of Vaccination

Vaccines potentiate extant immune defences by increasing the host's ability to recognize parasitic invasion and to generate appropriate defences (Klein, 1990). As undernutrition invariably reduces antigen presentation and the subsequent proliferation of effector mechanisms, it is generally recognized that vaccine immunity is largely suppressed secondary to undernutrition (Butterworth, Fulford, Dunne, Ouma & Sturrock, 1988; Chandra, 1972; 1979; Keusch & Scrimshaw, 1986; Keusch, Wilson & Waksal, 1983).

A great deal of effort has been channelled towards the search for an effective vaccine against schistosome infection. The observations that experimental hosts can acquire an effective immunity following antigenic challenge (Crombie & Anderson, 1985; McLaren & Smithers, 1987), and that schistosome numbers appear to decline in humans after a peak in the early teen years (Anderson, 1987; Wilkins, 1987; Woolhouse, Taylor, Matanhire & Chandiwana, 1991) fuel the search for a protective immune response which might be successfully harnessed through vaccination. Attention has largely focussed on the schistosomular stage.

The significant changes in ambient conditions which accompany the transition from free-living cercaria to endoparasitic schistosomulum trigger marked metabolic and physiological changes within the parasite (Rumjanek, 1987; Wilson, 1987). It is at this point which schistosomes are generally considered to be most vulnerable to immune elimination (Butterworth, 1987). Cell-mediated effector mechanisms which are thought to contribute to acquired resistance to infection consist chiefly of antibody-dependent, cell-mediated cytotoxicity (ADCC) responses during the skin phase of invasion, in concert with macrophage activity during this and later phases (Butterworth, 1987). It is likely that any successful vaccine will exploit these responses.

Undernutrition produces well documented immune suppression, particularly among the T-dependent responses central to CMI (see Chapter 1). The ability of the undernourished host to acquire, retain and exploit antigenic potentiation is thus clearly reduced (Dreizen, 1979). Although no evidence of this effect currently exists for vaccines effective against schistosome infection, it is suggested that undernutrition, which blunts the potentiating effect of vaccination by reducing antigen presentation and limiting the proliferative response which fuels the production of effector cells (Butterworth *et al.*, 1988; Chandra, 1972; 1979; Keusch & Scrimshaw, 1986; Keusch *et al.*, 1983), will similarly reduce their protection.

Given that the efficacy of any vaccine is immune-dependent, and given that a large proportion of potential recipients are likely to be undernourished (see above), it is suggested that vaccination against schistosome infection will be compromised in undernourished

humans. The first experiment in this chapter was designed to stimulate some discussion on an aspect of schistosome control which may warrant consideration if a vaccine is eventually forthcoming.

Nutritional Dependence of Chemotherapy

Antiparasitic drugs which act by inhibiting some aspect of parasite metabolism often do so by competing with nutrients for inclusion in essential metabolic processes (Bryant & Behm, 1989; Meshnick & Cerami, 1985). As varying nutrient concentrations can alter the probability of drugs or their metabolites successfully competing for metabolic inclusion (Maier & Riley, 1942), it is suggested that ambient nutrient levels will influence the efficacy of drugs which operate in this way. Furthermore, although their modes of action vary considerably, the effectiveness of antiparasitic compounds ultimately relies upon the host immune system consummating the removal of disabled parasites.

Schistosomicides & Host Nutrition

Despite obvious drawbacks, such as the lack of residual protection and the resultant cost of ongoing treatment, chemotherapy remains as the most effective form of schistosome control currently available (Marshall, 1987).

Antimonials

Evidence first arose of the effect of nutritional deficiency on the activity of antimonial drugs when Luttermoser and DeWitt (1961) examined the schistosomicidal efficacy of the drug stibophen in mice fed a diet deficient in protein, choline, inositol, folic acid, and vitamin B₁₂ ("fatty liver" diet) and a diet deficient in fat, pyridoxine HCl, D-biotin, vitamin A acetate, vitamin D₃, vitamin E, vitamin K, and inositol ("pellet" diet). It was found that drug efficacy was greatest in the optimal synthetic diet, and lowest in the most deficient, pellet diet.

Supporting evidence soon followed from human studies; Bell (1964) reported the results of treatment of 36 asiatic boys, aged 13-20 years, with intramuscular injection of sodium antimony dimercaptosuccinic acid (Astiban[®]). Eleven of the boys were vegetarians and 25 were not. A significantly higher proportion of non-vegetarians than of vegetarians was cured. The author, though unable to identify the factors responsible for these results, suggested that dietary habits influence the outcome of treatment.

The mechanisms responsible for the observed influence of host nutrition on antimonial treatment remained unknown until the demonstration by Doenhoff and Bain (1978) that stibophen activity was immune-dependent. This finding suggests that the action of stibophen pivots not simply on disabling the parasite, but also on the host's ability to eliminate them once damaged. In addition to showing immune-dependence, these results suggest that, if the

undernourished host is unable to eliminate damaged parasites, the worms may survive drug treatment.

Refeeding

Conversely, refeeding undernourished human hosts appears to restore drug activity. DeWitt and colleagues (1964) showed that treatment with stibophen (Faudin[®]) nine months after infected volunteers began receiving an enriched diet produced similar cure rates in both refeed and well-fed controls. It is likely this reflects the restoration of immunity following refeeding.

Where the prevailing nutritional status is a limiting factor on immune activity, it may be that refeeding which increases immunity above normal levels actually boosts drug efficacy. This might explain the observation that 2-methyl-1, 4-naphthoquinone, a compound with vitamin K (menadione) activity (may be an important physiological factor {Fitch, 1985}), can enhance the schistosomicidal effect of subcurative doses of stibophen (Beuding, Peters & Waite, 1947).

More Recent Drugs

Despite continuing widespread use, antimonals can induce toxic side-effects which, combined with other factors, make them suboptimal for long term use (Marshall, 1987). This has led to the production of a number of less toxic compounds which have proven highly effective against schistosomes (see Marshall, 1987 and Webster, 1987). However host immunity has again proven central to their activity, as demonstrated by observations that hycanthone, oxamniquine (Sabah, Fletcher, Webbe & Doenhoff, 1985) and praziquantel (Doenhoff, Sabah, Fletcher, Webbe & Bain, 1987; Sabah *et al.*, 1985) display reduced schistosomicidal activity in T cell-deprived animals, suggesting that these drugs rely upon the immune system to eliminate damaged parasites, and that disabled parasites can survive treatment if immune clearance is impaired.

Although these results clearly implicate immunity as central to drug efficacy, the effects of nutritional deficiency-induced immunosuppression of the activity of schistosomicides currently in use remains unexplored. Given that a large proportion of potential recipients are likely to be undernourished (see above), it is suggested that host nutritional status influences the activity of schistosomicidal drugs. As the current drug of choice is praziquantel, the second experiment in this chapter was designed to test its activity in deficient hosts in order to stimulate some discussion on this aspect of schistosome treatment.

The Unknown Effects of Nutritional Status

From the evidence reviewed above, it appears that fluctuations in host nutritional status may influence the host's ability to capitalize on treatment and control interventions by reducing the immune response required to carry out parasite elimination. Where estimations of vaccine and drug cure rates calculated from experimental observations and clinical trials form pivotal roles in the design of intervention strategies (Anderson & May, 1991), it may be that suboptimal nutrition in the field can compromise their projected therapeutic value.

4.3: HYPOTHESIS

That host nutritional status influences the effectiveness of vaccination and chemotherapy against schistosome infection, and that this has implications for the design of effective treatment and control strategies.

4.4: EXPERIMENTS

The hypothesis was tested by manipulating the protein content of diets fed to mice before vaccination or chemotherapy and observing reinfection, and cure rates and reinfection, respectively. Irradiated challenges and normal infections in this chapter made use of similar cercarial numbers to the single infection experiment presented in Chapter 2 (100.mouse⁻¹).

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: I. NORMAL INFECTION SUCCESS FOLLOWING IRRADIATED CERCARIAL CHALLENGE

Abstract

This experiment examines the influence of externally imposed host protein deficiency on the vertebrate host's ability to capitalize on vaccination against schistosome infection. Results show that vaccination of protein deficient mice provides significantly less protection against reinfection than that provided to replete hosts. These results have implications for the planned use of vaccines against schistosome infections in human populations experiencing periods of undernutrition.

INTRODUCTION

The observation that schistosome infection intensity in humans falls after reaching a peak early in the teen years suggests that humans may acquire an effective immunity to schistosome infection (Wilkins, 1987). The apparent occurrence of effective acquired immunity fuels one of the greatest goals of contemporary schistosome research - to discover a vaccine which provides protection from infection sufficient to reduce parasite success below that required to maintain transmission at a rate supporting chronic disease development.

Vaccines have inherent advantages over chemotherapy: vaccination provides residual protection which most drugs do not, thereby removing the need for costly repeated applications. Indeed it has been suggested that chemotherapy may even facilitate schistosome reinfection by removing concomitant immunity thought to be provided by the presence of active infection (Moloney, Hinchcliffe & Webbe, 1987).

Vaccines which potentiate and boost immune-mediated killing of larval schistosomes work by alerting the immune system to their antigenic presence, thus rapidly identifying and disabling invading schistosomes before they establish effective defences against immune attack (Butterworth, 1987; Colley & Colley, 1989; Sher, James, Correa-Oliveira, Hieny & Pearce, 1989; Simpson & Cioloi, 1987). Thus for vaccines to work effectively, the host must firstly recognize the protective target antigens, remember them and then be able to recognize them during normal infection in order to proliferate the relevant clonal lines in great enough numbers to capitalize on vaccine potentiation.

Protein undernutrition is commonly co-incident with schistosome infection (see above), and therefore attempts to control the disease using vaccines will commonly be administered in undernourished populations. As undernutrition impairs the recognition, memory and proliferative phases of the antigen-specific immune system (see Chapter 1), it is suggested that vaccine efficacy may be reduced in undernourished hosts.

As no previous study has examined the effects of host nutritional status on the activity of vaccination against schistosome infection, this experiment was designed to examine this interaction.

Hypothesis

That host protein deficiency reduces the therapeutic value of vaccination against schistosome infection.

The effectiveness of vaccination with irradiated cercariae was compared between well-fed and protein deficient mice examined at autopsy 4 weeks after a second, normal challenge. Mice belonged to one of two dietary groups: those receiving the 16% and those receiving the 3% protein ration. Dietary protein content provided hosts of differing nutritional status at the time of vaccination.

EXPERIMENTAL DESIGN

One hundred mice were randomly assigned to two groups of 10 and four groups of 20 mice housed in large cages each containing 10 animals. All mice were fed SLC for two weeks after which one group of 10 and two groups of 20 animals received the 3% protein synthetic diet (D3), whilst the remaining group of 10 and the two remaining groups of 20 received the 16% protein diet (D16).

After two weeks, both groups of 10 and one group of 20 from each dietary régime were exposed to 100 radiation-attenuated cercariae.mouse⁻¹ (RA.100; range 96-105) for 30 min less than 2 h after their emergence from a group of 40 snails. The two remaining groups of 20 animals (one from each dietary régime) were handled identically to the first, but in the absence of cercariae.

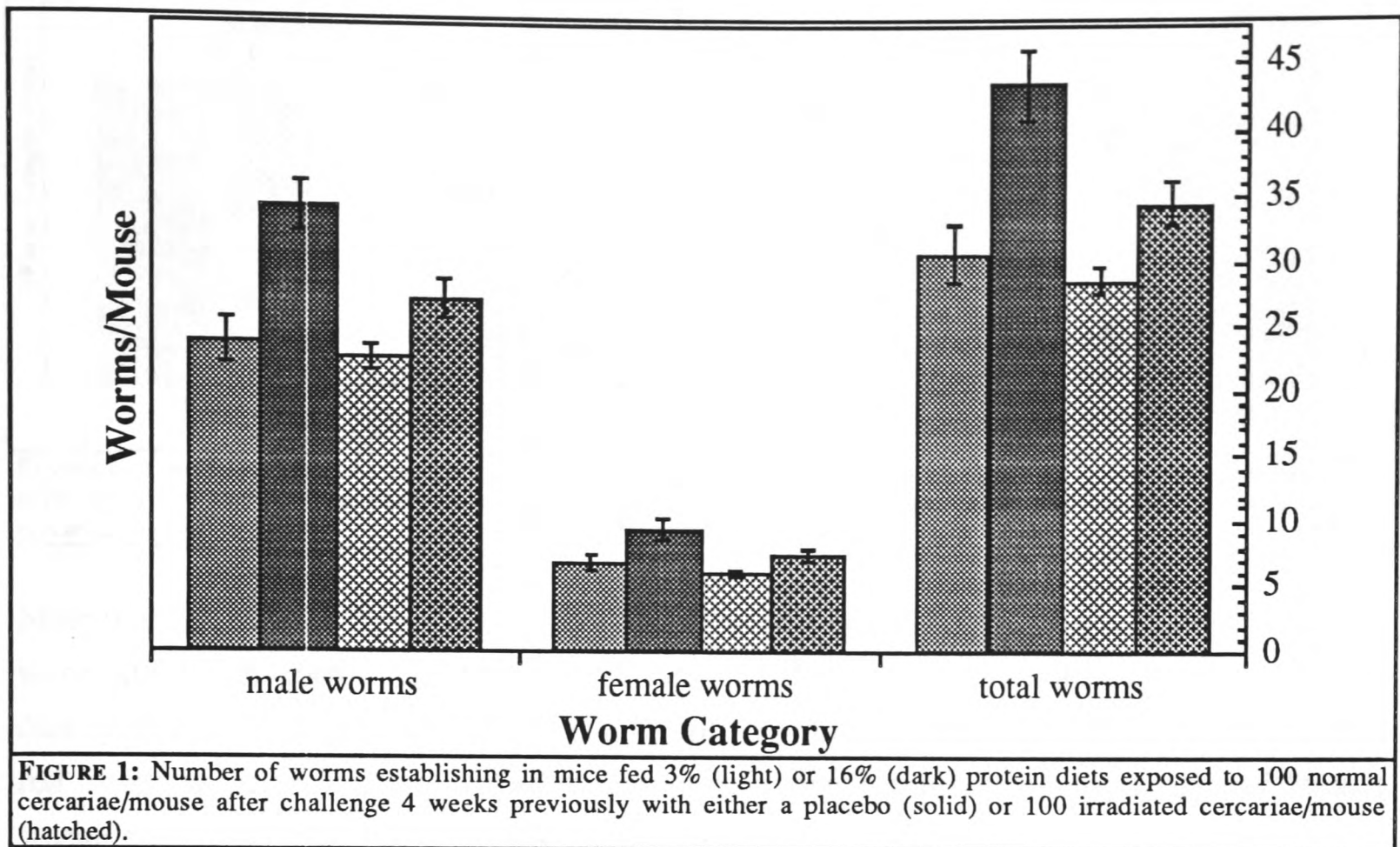
After four weeks, the two groups of 10 were autopsied (At) as described in the Materials and Methods section of Chapter 2. A blood sample was collected for antibody analysis before the remaining mice were exposed to 100 normal cercariae.mouse⁻¹ (I.100; range 96-103) for 30 min. All cercariae were gathered simultaneously from a group of 40 patent snails. Four weeks after exposure to normal cercariae, all animals were autopsied as described. The design of this experiment is presented in Table 1.

Week Groups (n)	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8
A (10)	SLC	D3	→	-	→	RA100	→	-	→	At				
B (10)	SLC	D16	→	-	→	RA100	→	-	→	At				
C (20)	SLC	D3	→	-	-	-	-	-	→	I.100	→	-	→	At
D (20)	SLC	D3	→	-	→	RA100	→	-	→	I.100	→	-	→	At
E (20)	SLC	D16	→	-	-	-	-	-	→	I.100	→	-	→	At
F (20)	SLC	D16	→	-	→	RA100	→	-	→	I.100	→	-	→	At

TABLE 1: Design of Irradiated Challenge & Normal Reinfection experiment (n=number of mice in group).

RESULTS: PARASITE

Number



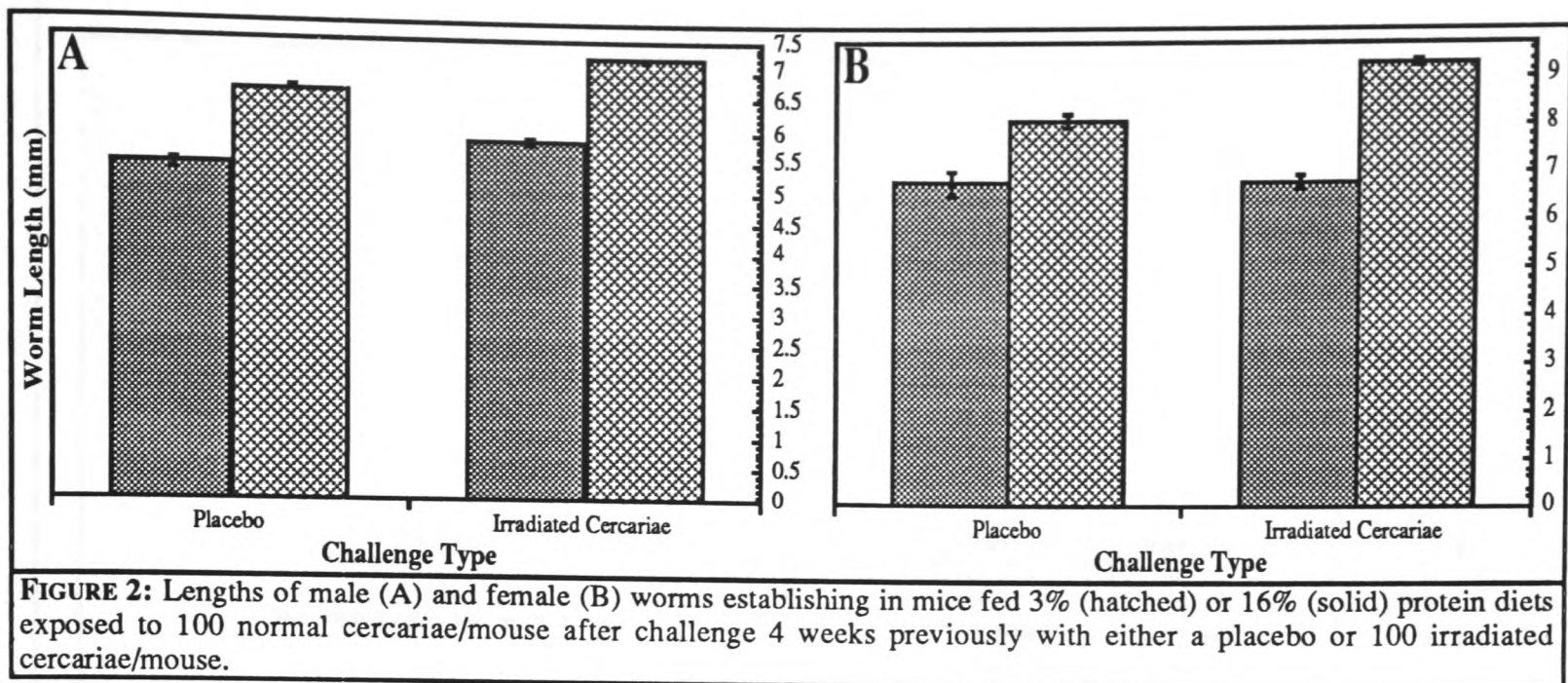
Autopsies of mice fed both low and high protein diets performed 4 weeks after exposure to 100 irradiated cercariae did not reveal any schistosomes. Prior exposure to irradiated cercariae produced a significant reduction in male, female and total worm numbers following normal infection in well-fed animals; this result was not observed in animals receiving the low protein diet (Figure 1; Table 2).

A prior irradiated challenge in mice fed the low protein diet saw 92.01% normal challenge reinfection when compared with animals which had not received the prior challenge; this compares with an 83.35% reinfection in well-fed mice. Therefore vaccination provided significantly more protection against reinfection in mice fed high protein diets than in protein deficient animals ($Z=483.9^*$).

Groups	3% Nil+100	3% Irrad.100+100	16% Nil+100	16% Irrad.100+100
Male Worms				
range (n)	9-35 (20)	16-30 (20)	23-50 (18)	15-33 (19)
3% Nil+100	-	0.28(0.59;38)	0.0002*(-3.92;36)	-
3% Irrad.100+100	-	-	-	0.005*(-2.75;37)
16% Nil+100	-	-	-	0.002*(3.05;35)
Female Worms				
range (n)	4-11 (20)	4-10 (20)	5-16 (18)	3-11 (19)
3% Nil+100	-	0.08(1.43;38)	0.005*(-2.75;36)	-
3% Irrad.100+100	-	-	-	0.013*(-2.33;37)
16% Nil+100	-	-	-	0.022*(2.10;35)
Total Worms				
range (n)	13-45 (20)	20-38 (20)	30-66 (18)	20-43 (19)
3% Nil+100	-	0.20(0.84;38)	0.0003*(-3.78;36)	-
3% Irrad.100+100	-	-	-	0.003*(-2.93;37)
16% Nil+100	-	-	-	0.003*(2.94;35)

TABLE 2: T-test comparisons of mean worm numbers in experimental mice exposed to 100 normal cercariae (+100) 4 weeks after exposure to either a placebo (Nil) or 100 irradiated cercariae.mouse⁻¹ (Irrad. 100) (number above diagonal represents p value followed by t value and degrees of freedom; n=number of mice perfused for worms).

Size: Means



Male worms perfused from hosts exposed to an irradiated challenge prior to normal infection were larger than those recovered from mice receiving a placebo challenge, regardless of dietary treatment (Figure 2). This was also only true of female worms recovered from well-fed hosts, as prior irradiated challenge had no noticeable effect on the length of female worms recovered from mice fed low protein diets (Table 3).

Groups	3% Nil+100	3% Irrad.100+100	16% Nil+100	16% Irrad.100+100
Male Worms df=2044				
range (mm) (n)	1.9-8.7 (454)	1.9-10.0 (453)	2.2-10.2 (621)	2.1-11.1 (517)
3% Nil+100	-	13.18*	108.18*	-
3% Irrad.100+100	-	-	-	194.43*
16% Nil+100	-	-	-	26.41*
Female Worms df=565				
range (mm) (n)	0.9-12.1 (127)	0.8-10.5 (123)	0.9-12.6 (172)	3.8-12.8 (144)
3% Nil+100	-	0.0002	11.53*	-
3% Irrad.100+100	-	-	-	66.89*
16% Nil+100	-	-	-	14.76*

TABLE 3: ANOVA comparisons of mean worm lengths in experimental mice (number above diagonal represents ANOVA F value; n=number of worms measured).

Size: Density-Dependence

Male sizes were significantly and negatively associated with total worm burdens both in well-fed mice which had received previous exposure to the placebo ($r^2=0.01$, $p=0.023$) and in well-fed mice which had received previous exposure to the irradiated challenge ($r^2=3.44E-3$, $p=0.0378$). There was no such relationship in mice fed the low protein diet (placebo: $r^2=4.2E-5$, $p=0.8898$; irradiated: $r^2=1.34E-3$, $p=0.3121$) (see Figure 3, overleaf).

Female sizes were significantly and negatively associated with total worm burdens in well-fed mice which had received previous exposure to the placebo ($r^2=0.04$, $p=0.0032$) but not in those which had received the irradiated challenge ($r^2=6.61E-4$, $p=0.6318$). There was no relationship between female sizes and total worm numbers in mice fed the low protein diet (placebo: $r^2=3.11E-3$, $p=0.5337$; irradiated: $r^2=1.43E-3$, $p=0.582$) (see Figure 4, overleaves).

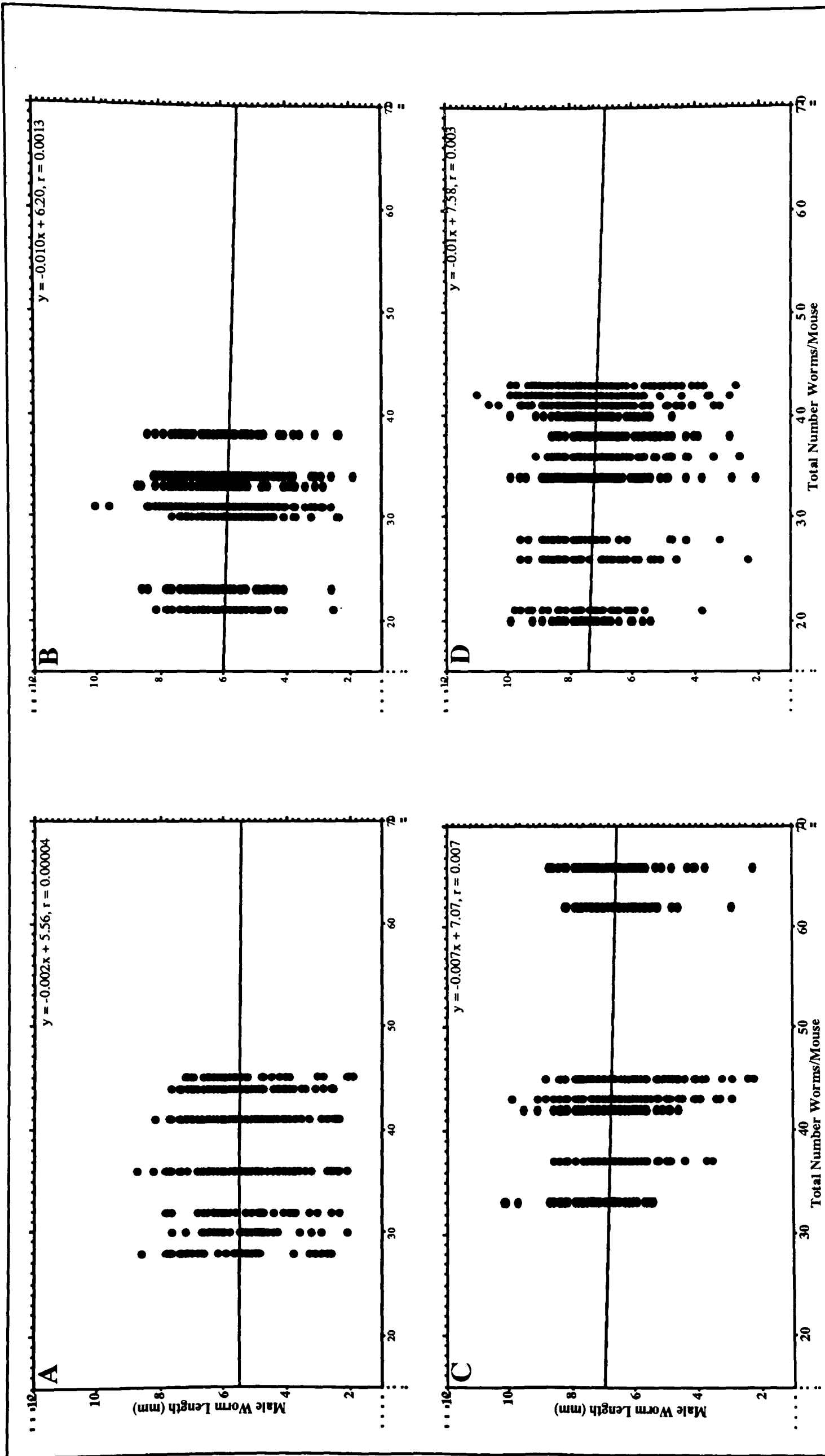


FIGURE 3: Length of male worms perfused from mice fed 3% protein (A & B) and 16% protein (C & D) diets challenged 4 weeks prior to infection with either a placebo (A & C) or an irradiated challenge (B & D) regressed against total worm burden/mouse.

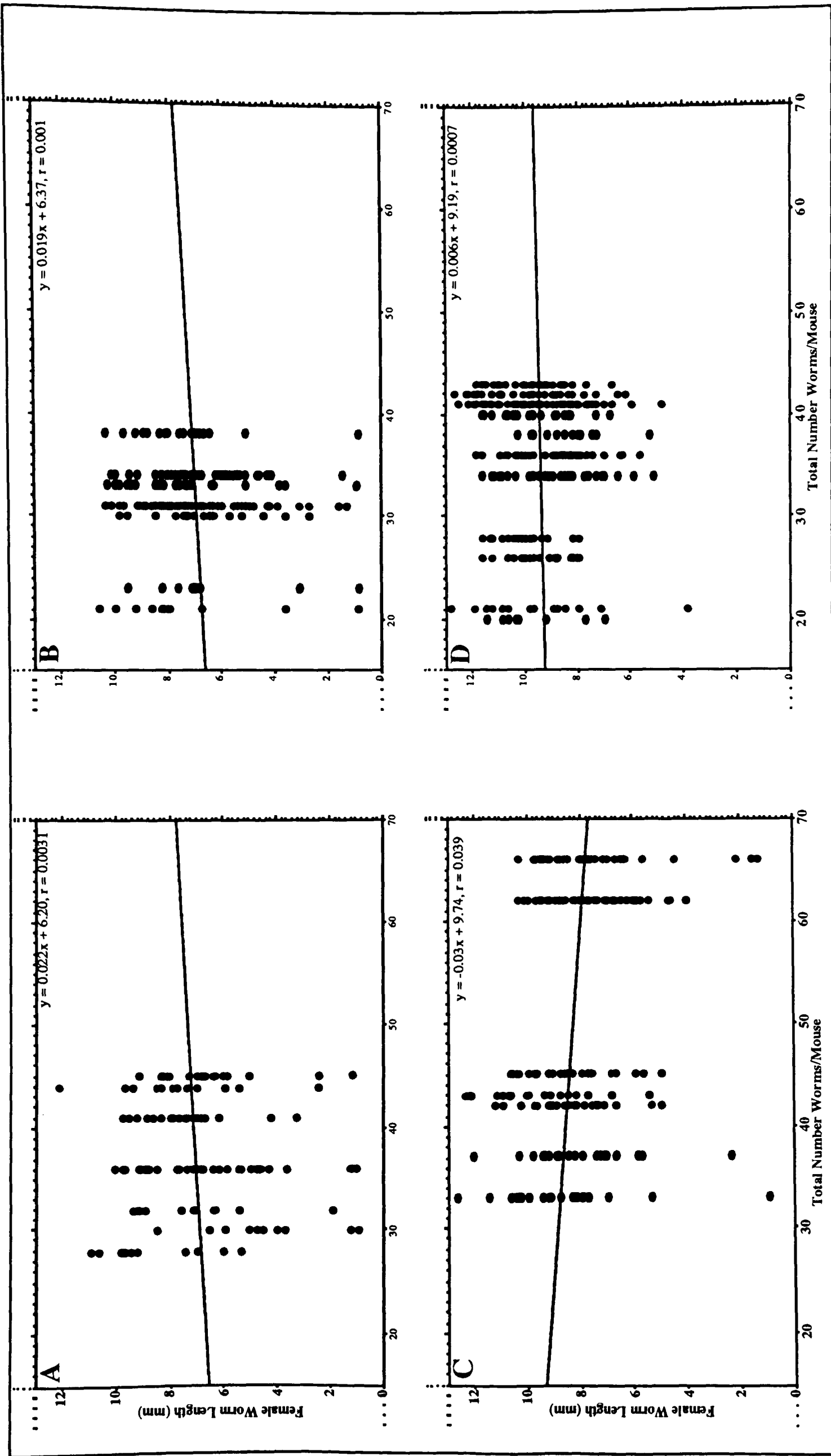


FIGURE 4: Length of female worms perfused from mice fed 3% protein (A & B) and 16% protein (C & D) diets challenged 4 weeks prior to infection with either a placebo (A & C) or an irradiated challenge (B & D) regressed against total worm burden/mouse.

Fecundity

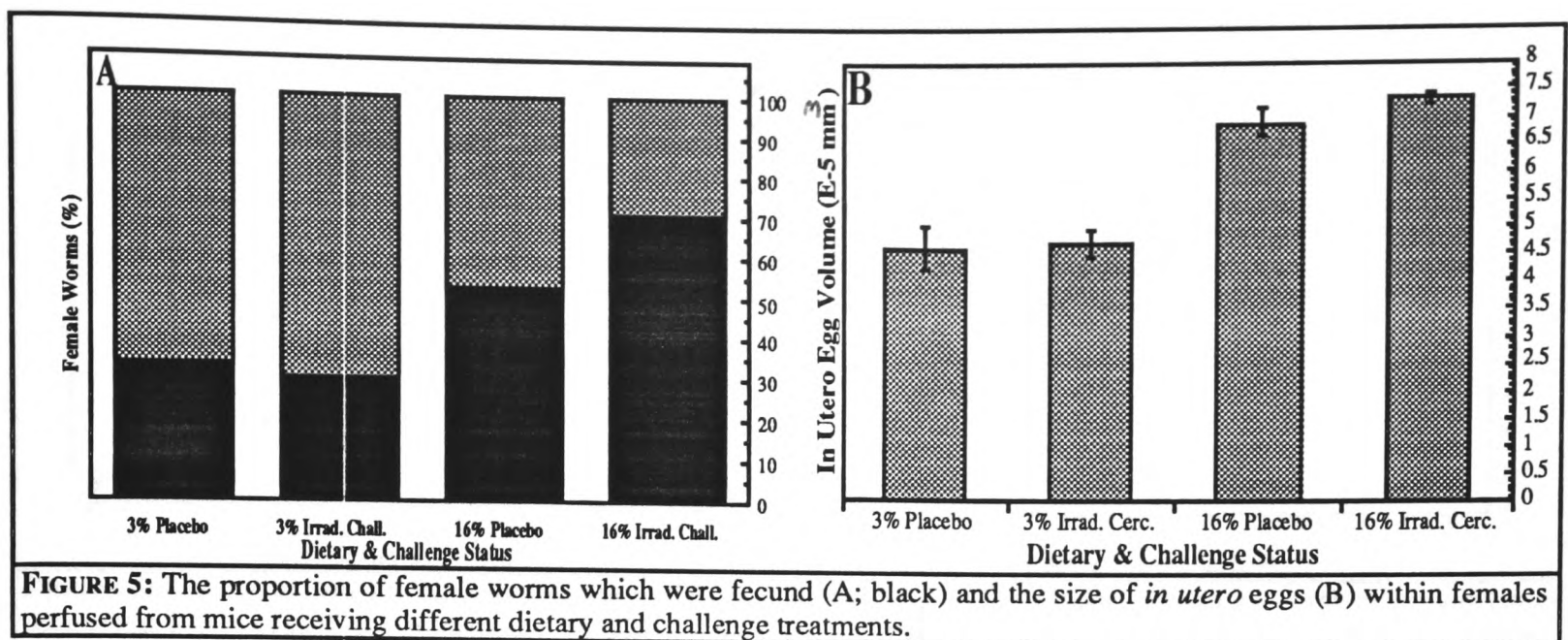


FIGURE 5: The proportion of female worms which were fecund (A; black) and the size of *in utero* eggs (B) within females perfused from mice receiving different dietary and challenge treatments.

Appreciably fewer female worms recovered from mice fed low protein diets were fecund than those from well-fed animals (Figure 5). Whilst prior challenge with irradiated cercariae had no effect on the proportion of females fecund in deficient hosts, irradiated challenge produced an apparent increase in the proportion of fecund females in well-fed hosts (Figure 5).

Females perfused from mice fed low protein diets contained smaller eggs *in utero* than did females perfused from well-fed hosts (Table 4). Prior exposure to irradiated cercariae had no significant effect on *in utero* egg size in either dietary group (Figure 5).

Groups	3% Nil+100	3% Irrad.100+100	16% Nil+100	16% Irrad.100+100
Egg Volumes df=275 range (E-5 mm ³) (n)	0.4-9.58 (47)	0.86-8.87 (37)	1.57-18.58 (90)	3.11-15.71 (102)
3% Nil+100	-	0.02	12.97*	-
3% Irrad.100+100	-	-	-	26.89*
16% Nil+100	-	-	-	0.89

TABLE 4: Statistical comparisons of the volumes of *in utero* eggs (number above diagonal represents ANOVA F value; n=number of eggs measured).

RESULTS: HOST

Mice exposed only to the irradiated challenge did not suffer any mortality, regardless of dietary treatment. No mortality was observed in mice fed low protein diets, regardless of challenge and infection status. Mice fed high protein diets and exposed to a placebo followed by normal infection suffered 10% mortality, whilst infected animals exposed to the irradiated challenge and normal infection suffered 5% mortality during the experiment.

Weight Change

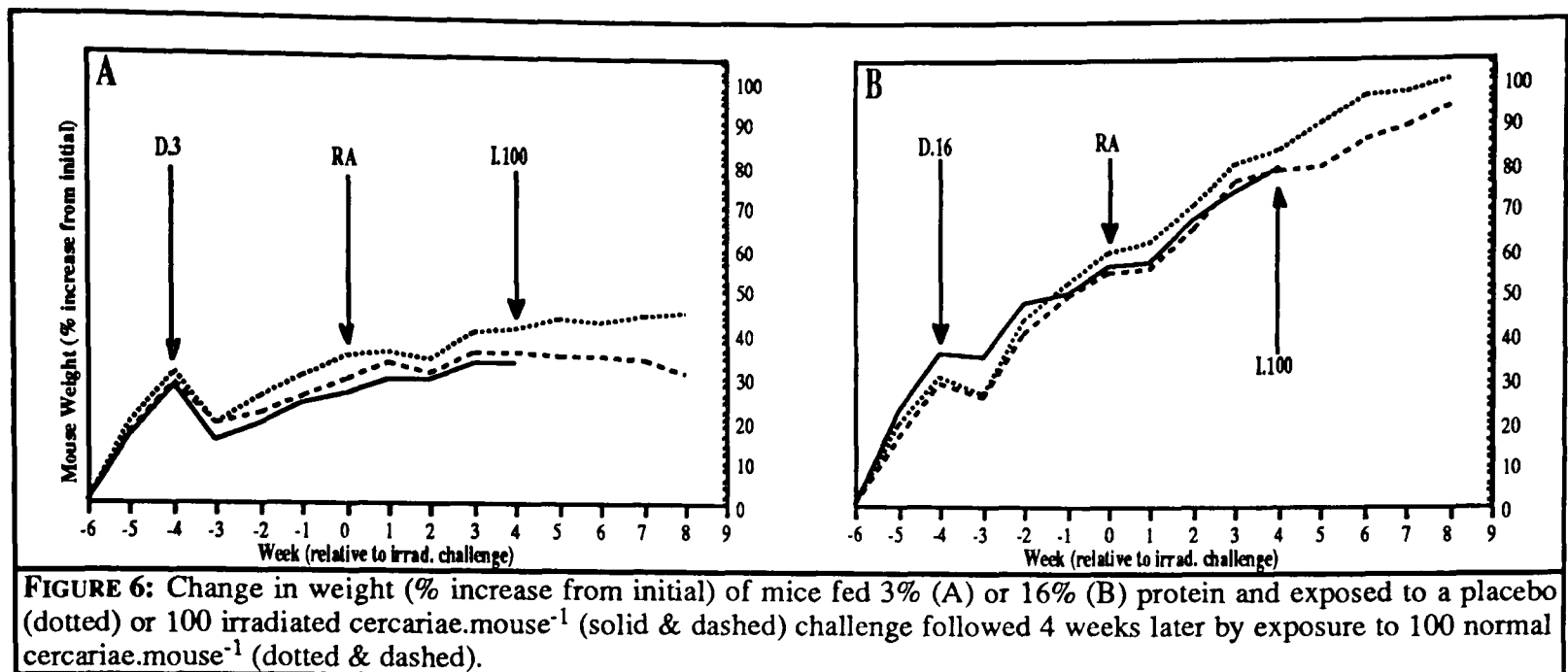


FIGURE 6: Change in weight (% increase from initial) of mice fed 3% (A) or 16% (B) protein and exposed to a placebo (dotted) or 100 irradiated cercariae.mouse⁻¹ (solid & dashed) challenge followed 4 weeks later by exposure to 100 normal cercariae.mouse⁻¹ (dotted & dashed).

Mice fed the low protein diet weighed significantly less than well-fed animals at the time of irradiated cercarial exposure (df=99, $F=88.47^*$) and at the time of normal challenge (df=99, $F=216.53^*$). There were no significant differences at either time between the weights of mice within dietary groups (Figure 6).

Antibody Response

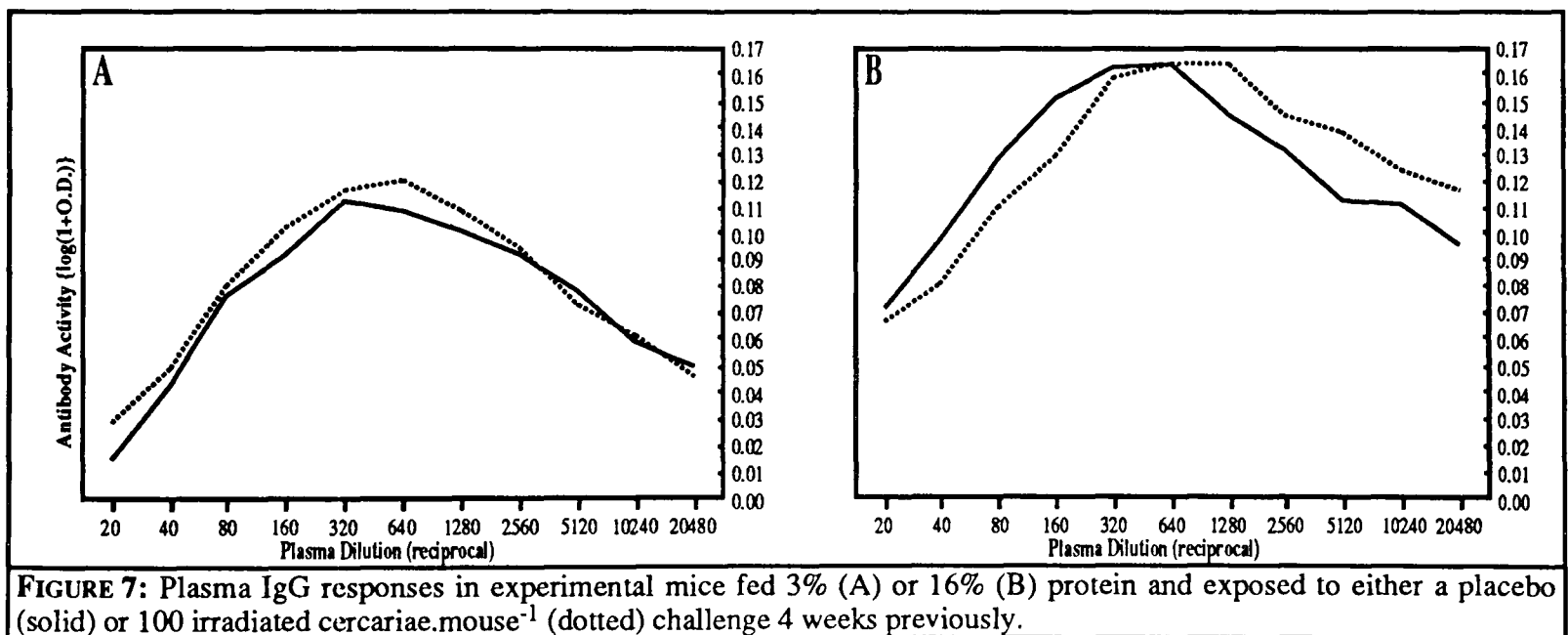


FIGURE 7: Plasma IgG responses in experimental mice fed 3% (A) or 16% (B) protein and exposed to either a placebo (solid) or 100 irradiated cercariae.mouse⁻¹ (dotted) challenge 4 weeks previously.

Well-fed mice tended to produce stronger antibody responses to SSA than did protein deficient hosts (Figure 7). Whilst previous exposure to irradiated cercariae had little effect on antibody levels in mice fed low protein rations, well-fed animals showed a marked increase in antibody activity in response to irradiated challenge, particularly at higher dilutions.

Organ Size

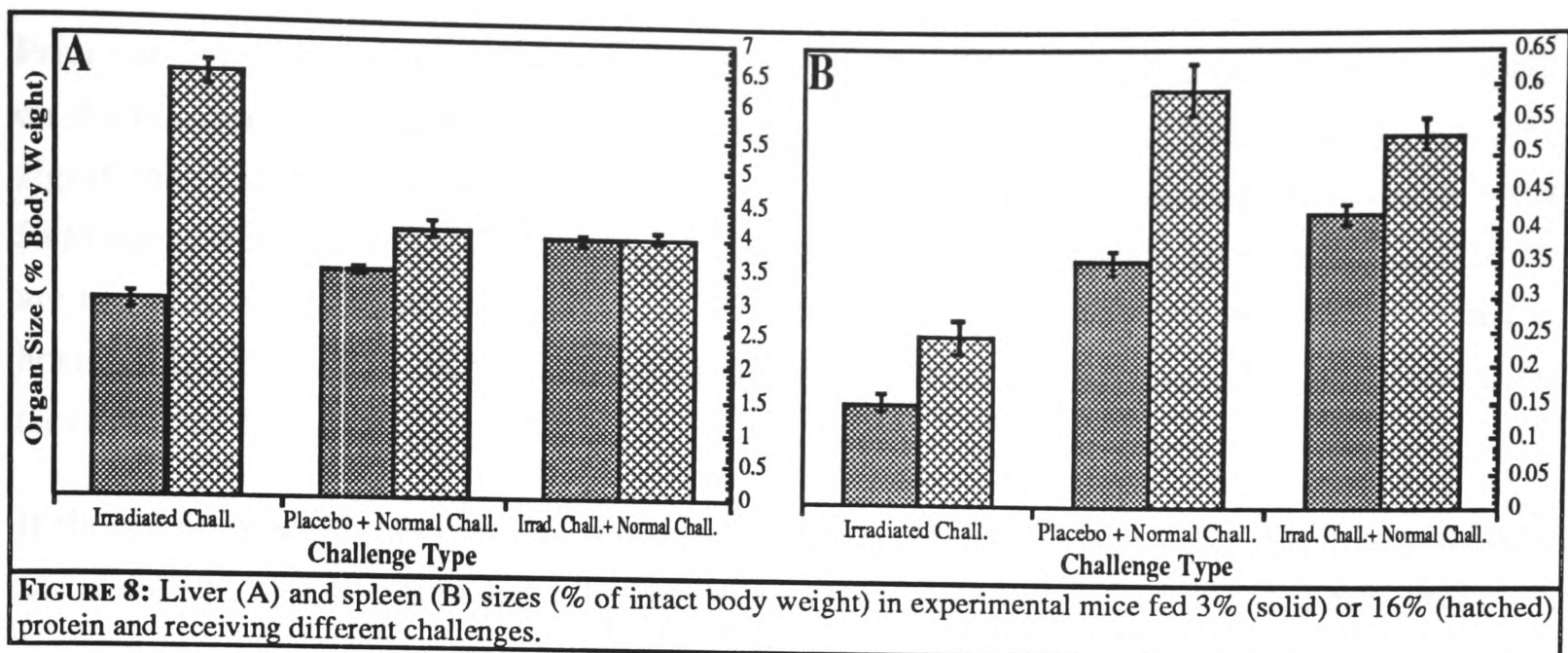


FIGURE 8: Liver (A) and spleen (B) sizes (% of intact body weight) in experimental mice fed 3% (solid) or 16% (hatched) protein and receiving different challenges.

Well-fed mice exposed to irradiated challenge had larger livers than similarly exposed, protein deficient hosts (Figure 8). Livers were larger in well-fed animals following a placebo challenge and normal infection, but not when preceded by the irradiated challenge (Table 5).

Well-fed mice had significantly larger spleens than mice fed the low protein diets, regardless of parasite exposure (Table 5). Spleens of animals from both dietary groups were larger following normal infection than those in mice exposed only to the irradiated challenge (Figure 8)

Group	3% Irrad	16% Irrad	3% Pl+Norm	3% Irrad+Norm	16% Pl+Norm	16% Irrad+Norm
Liver range (%) (n)	2.59-3.71 (10)	5.77-7.38 (7)	3.02-3.85 (20)	3.3-4.6 (19)	3.39-5.26 (17)	3.29-4.65 (19)
Spleen range (E-1%)(n)	0.97-1.98 (10)	1.83-3.69 (7)	2.06-4.56 (20)	2.99-5.62 (19)	3.82-9.14 (17)	3.94-6.54 (19)
3% Irrad	-	0.0001*(-16.02;15)	0.0001*(-4.33;28)	0.0001*(-6.88;27)	0.0001*(-5.70;25)	0.0001*(-7.41;27)
16% Irrad	0.001*(-3.74;15)	-	0.0001*(20.76;25)	0.0001*(13.68;24)	0.0001*(9.41;22)	0.0001*(13.91;24)
3% Pl+Norm	0.0001*(-8.38;28)	0.0009*(-3.47;25)	-	0.0001*(-5.22;37)	0.0001*(-4.82;35)	0.0001*(-5.84;37)
3% Irrad+Norm	0.0001*(-11.52;27)	0.0001*(-5.75;24)	0.003*(-2.95;37)	-	0.17(-0.99;34)	0.37(-0.33;36)
16% Pl+Norm	0.0001*(-9.25;25)	0.0001*(-5.92;22)	0.0001*(-6.43;35)	0.0001*(-4.60;34)	-	0.23(0.76;34)
16% Irrad+Norm	0.0001*(-13.64;27)	0.0001*(-8.07;24)	0.0001*(-7.15;37)	0.0001*(-4.50;36)	0.066(1.55;34)	-

TABLE 5: T-test comparisons of organ sizes in experimental mice (number above diagonal represents p value followed by t value and degrees of freedom for the liver; number below diagonal represents p value followed by t value and degrees of freedom for the spleen; n=number of organs measured).

DISCUSSION

The results presented in this experiment confirm the hypothesis generated above that host protein deficiency reduces the therapeutic value of vaccination against schistosome infection and disease.

The Parasite

Autopsy of mice from both dietary groups 4 weeks after the irradiated challenge did not reveal any schistosomes, and so it can be assumed that the number of worms present at autopsy result from the subsequent normal infection.

Number

Prior vaccination of protein deficient hosts with irradiated cercariae had no significant impact on the number of worms arising from a subsequent normal infection. This contrasts with the significant reductions in worm number resulting from normal challenge in well-fed hosts following prior challenge with irradiated cercariae. This suggests that protein deficient hosts are less able to capitalize on the immune potentiation which vaccination provided in well-fed hosts. It is likely this is due to impaired immune responses accompanying protein deficiency (see Chapter 1).

It should be noted, however, that whether vaccinated or not, protein deficient mice were still as "resistant" to reinfection as well-fed animals receiving appreciable vaccine protection.

Size

Worms recovered from mice receiving vaccination were larger than those perfused from mice receiving the placebo prior to normal infection. This suggests that vaccination, by reducing worm numbers establishing following reinfection, lowered density-dependent constraints on worm size.

Proportion Fecund

It is likely that the reported increase in the proportion of females fecund in vaccinated, well-fed hosts compared with those receiving the placebo reflects the reduction in density-dependent constraints on worm fecundity observed in well-fed animals (see Chapter 2) by reducing worm numbers, as reported above.

Size of *In Utero* Eggs

Vaccination had no discernable effect on egg sizes in either dietary group.

The Host

Mice fed the low protein diet begun four weeks prior to challenge with irradiated cercariae were significantly lighter than other animals at the time of vaccination, and thus it can be concluded that animals fed the low protein diet were deficient relative to well-fed animals at the time of the irradiated cercarial challenge. Vaccination had no effect on mouse weights, and thus hosts within each dietary group were of similar weight, and therefore nutritional status, at the time of reinfection.

Antibody Response

Vaccination produced an appreciable increase in the IgG responses of mice in both dietary groups, however the rise was greater in well-fed animals. This suggests well-fed animals were able to respond to the stimulus it provided in a more pronounced manner than deficient animals. This result is consistent with previous reports of reduced immune response to antigen stimulus in deficient animals (see Chapter 1 and Introduction).

Organ Size

The enlarged spleens noted in well-fed animals is likely to reflect greater T cell activity in these animals when compared to protein deficient hosts. Prior vaccination reduced spleen sizes in well-fed animals harboring normal reinfection but not in deficient animals, although the latter were still smaller than the former. It is likely this reflects the decreased immune activity accompanying the lower worm burdens observed in vaccinated, well-fed mice.

THE INFLUENCE OF DIETARY PROTEIN ON SCHISTOSOMIASIS MANSONI IN LABORATORY MICE: II. PRAZIQUANTEL ACTIVITY & PARASITE REINFECTION

Abstract

This experiment examines the influence of host protein deficiency on the therapeutic value of chemotherapy against schistosomiasis mansoni. Various nutritional deficiencies have produced well documented reductions in the efficacy of drugs previously used to treat schistosome infection. Results show that the therapeutic value of praziquantel is impaired in protein deficient mice when compared with that observed in replete hosts. These results have implications for the use of praziquantel against schistosome infection in human populations experiencing periods of undernutrition.

INTRODUCTION

Drugs have long been used to treat schistosome infections (Marshall, 1987), and presently constitute the single most effective and practical strategy to combat human schistosomiasis (Webster, 1987). Although their mode of action has not always been understood, early workers realized that host diet could influence the effectiveness of drug treatment (see above).

The activity of antischistosomal drugs in relation to host nutritional status is of interest because those socio-economic factors which predispose the inhabitants of developing countries to parasitic infections are also those which predispose these populations to undernutrition (see Chapter 1). Therefore schistosome infection is commonly endemic in human populations suffering chronic conditions of undernutrition.

Because nutritional deficiencies and parasitic disease are chronic conditions, are most common in growing children and tend to affect the same individuals (Chapter 1), those selectively most likely to receive drug treatment for schistosome infection are also those most likely to be undernourished. Clearly, in order to accurately predict the results of chemotherapeutic interventions, it is essential to characterize the effects of host nutrition on drug activity.

Praziquantel

Early antischistosomal drugs have since given way to more effective, less toxic compounds. The current drug of choice in schistosomiasis is praziquantel, a pyrazinoisoquinoline compound developed by Bayer which is active against all schistosomes. Praziquantel's mode of action is thought to involve rapid vacuolization of schistosome tegument produced by ion flux, followed by the immune elimination of disabled parasites (Andrews, 1985; Andrews, Thomas, Pohlke & Seubert, 1983; Marshall, 1987).

Mode of Action

Ambient Conditions

As the initial damage done to schistosomes by praziquantel consists of changes in the flow of calcium ions across the tegument (Andrews, 1985; Andrews *et al.*, 1983), it is not surprising that the concentrations of such trace elements at the schistosome surface influences the activity of praziquantel in vacuole formation (Xiao, Friedman, Catto & Webster, 1984). It follows that the ambient nutritional conditions within the host can influence the activity of this drug, although no data currently exist.

Immune Clearance

It has been shown that the efficacy of praziquantel is dependent upon host immunocompetence (Brindley & Sher, 1987; Doenhoff *et al.*, 1987; Sabah *et al.*, 1985), which appears to operate largely by antibody-mediated elimination of disabled parasites (Brindley & Sher, 1987). As host nutritional status influences immune function (see Chapter 1), it might be expected that the activity of praziquantel is influenced by the host's nutritional status.

Indirect evidence was recently reported by Mott, Dixon, Osei-Tutu, England and Davis (1985), who found persons with proteinuria and clinical signs of haematuria (likely to be associated with parasite-induced nutritional deficiency - see Chapter 1) had substantially lower parasitological cure rates when treated with praziquantel for *S. haematobium* than infected individuals without such signs. It is suggested that this reflects the immunosuppressive effects of protein deficiency on immune-mediated clearance of parasites following praziquantel treatment.

As praziquantel will be most commonly administered to undernourished individuals, and given that undernutrition reduces both the concentration of ambient nutrients required for drug activity and the host immunity responsible for schistosome elimination following treatment, it is suggested that host nutritional status affects the activity of praziquantel. As no previous study has examined this aspect of schistosome treatment, the present experiment was designed to do so.

Hypothesis

That protein deficiency decreases the success of praziquantel treatment.

The effectiveness of praziquantel treatment was compared at autopsy between well-fed and protein deficient mice treated 4 weeks after a primary challenge. Reinfection was studied to examine the influence of protein status on resistance following abrogated infection. Mice

belonged to one of two dietary groups: those receiving 16% and those receiving 3% protein ration. Dietary protein content provided hosts of differing nutritional status to drug treatment and during reinfection.

EXPERIMENTAL DESIGN

Eighty mice were randomly assigned to two groups of 5 and two groups of 35 mice housed in large cages each containing a maximum of 10 animals. All mice were fed SLC for two weeks, after which one group of 5 and one group of 35 mice were placed on the 3% protein synthetic diet (D3) whilst the second group of 5 and the second group of 35 mice received the 16% protein diet (D16). Three weeks later, animals in the groups of 35 from both dietary regimes were individually exposed to 100 cercariae.mouse⁻¹ (I.100; range 96-103) for 30 min. All cercariae were collected simultaneously from a group of 40 patent snails. Throughout the experiment, mice in the groups of 5 were handled identically in the absence of cercariae.

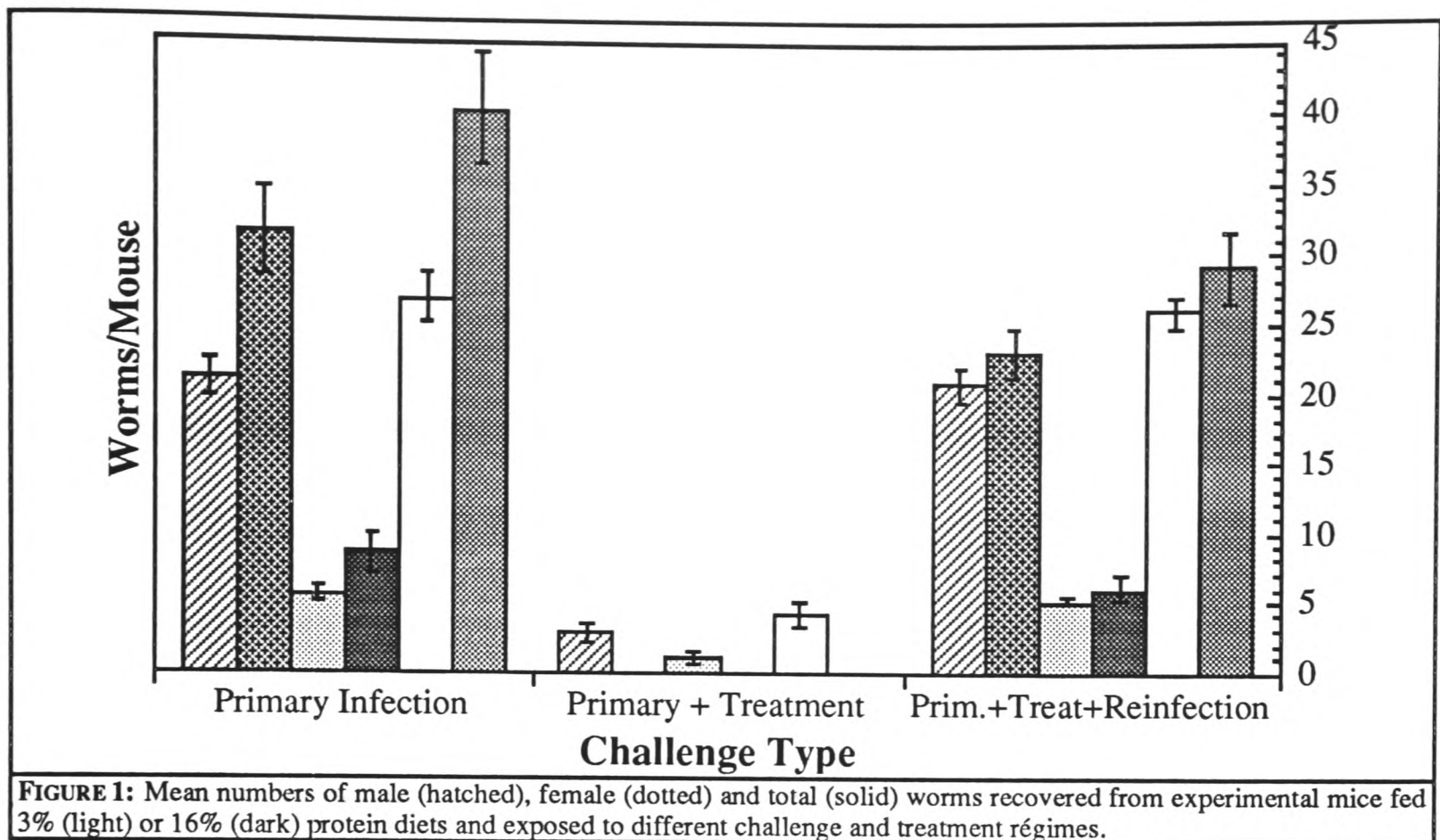
After 4 weeks, 10 mice from each infected group were autopsied (At) as described in the Materials and Methods section of Chapter 2. All remaining mice, including uninfected controls, received praziquantel treatment (T) as previously described (Chapter 2). One week later, 10 mice from each dietary régime were autopsied, whilst the remaining 15 animals in each dietary group were re-exposed to 100 cercariae.mouse⁻¹ (R.100; range 95-103) for 30 min. All cercariae were collected simultaneously from the same group of 40 patent snails. Four weeks after re-exposure, all remaining animals were autopsied as described. The design of this experiment is presented in Table 1.

Week Groups (n)	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9
A (5)	SLC	→	D3	→	-	-	-	-	-	-	-	-	-	^	At
B (5)	SLC	→	D16	→	-	-	-	-	-	-	-	-	-	^	At
C (10)	SLC	→	D3	→	→	I.100	→	-	→	At					
D (10)	SLC	→	D3	→	→	I.100	→	-	→	T	At				
E (15)	SLC	→	D3	→	→	I.100	→	-	→	T	R.100	→	-	→	At
F (10)	SLC	→	D16	→	→	I.100	→	-	→	At					
G (10)	SLC	→	D16	→	→	I.100	→	-	→	T	At				
H (15)	SLC	→	D16	→	→	I.100	→	-	→	T	R.100	→	-	→	At

TABLE 1: Design of Praziquantel and Reinfection experiment (n=number of mice in group).

RESULTS: PARASITE

Number



Praziquantel treatment eliminated all worms in mice fed high protein diets, whilst effecting an 86%, 79% and 84% reduction in male, female and total worms, respectively, in mice fed low protein diets when compared with mean pretreatment burdens (Figure 1; Table 2, overleaf). Praziquantel thus showed a higher efficacy in well-fed animals compared with protein deficient animals.

There was a tendency for fewer parasites to establish during reinfection than had established following primary infections in naive animals; these reductions were only significant, however, in well-fed animals, and for male worm numbers and total worm numbers only (Table 2). When reinfection was compared as a percentage of previous parasite success (and corrected for the proportion of worms in deficient mice which were likely the result of the initial infection), there was 81.04% reinfection in protein deficient hosts, and 72.83% reinfection in well-fed animals. Therefore praziquantel treatment provided significantly more protection against reinfection in the well-fed host when compared with the protein deficient host ($Z=50.83^*$).

Group	3% Primary	3% Pri+Treat	3% Pri+Treat+Reinfn	16% Primary	16% Pri+Treat	16% Pri+Treat+Reinfn
Males						
range (n)	16-27 (10)	2-6 (5)	16-25 (9)	22-39 (5)	0 (10)	16-31 (8)
3% Primary	-	0.0001*(12.13;13)	0.41(0.22;17)	-	0.0001*(12.35;13)	-
3% Pri+Treat	-	-	0.0001*(-13.21;12)	-	-	0.14(-1.13;15)
3% Pri+Treat+Reinfn	-	-	-	-	0.0001*(29.86;13)	0.01*(2.57;11)
16% Primary	-	-	-	-	-	0.0001*(-29.79;16)
16% Pri+Treat	-	-	-	-	-	-
Females						
range (n)	3-8 (10)	0-2 (5)	4-7 (9)	5-13 (5)	0 (10)	3-11 (8)
3% Primary	-	0.0001*(5.84;13)	0.27(0.63;17)	-	0.0001*(5.50;13)	-
3% Pri+Treat	-	-	0.0001*(-6.26;12)	-	-	0.16(-1.03;15)
3% Pri+Treat+Reinfn	-	-	-	-	0.0001*(17.04;13)	0.07(1.55;11)
16% Primary	-	-	-	-	-	0.0001*(-16.25;16)
16% Pri+Treat	-	-	-	-	-	-
All Worms						
range (n)	19-33 (10)	2-7 (5)	21-32 (9)	27-50 (5)	0 (10)	19-42 (8)
3% Primary	-	0.0001*(11.54;13)	0.36(0.36;17)	-	0.0001*(15.20;13)	-
3% Pri+Treat	-	-	0.0001*(-14.40;12)	-	-	0.13(-1.18;15)
3% Pri+Treat+Reinfn	-	-	-	-	0.0001*(29.82;13)	0.016*(2.44;11)
16% Primary	-	-	-	-	-	0.0001*(-26.10;16)
16% Pri+Treat	-	-	-	-	-	-

TABLE 2: T-test comparisons of mean worm numbers in experimental mice (number above diagonal represents p value followed by t value and degrees of freedom; n=number of mice perfused).

RESULTS: HOST

Uninfected mice suffered no mortality, regardless of dietary treatment. Infection produced no mortality in mice fed low protein diets up to the time of treatment, whilst 14.3% of mice fed high protein diets died during this period. Following praziquantel treatment and before autopsy, 20% of low protein mice died, whilst no mortality occurred in well-fed animals. Twenty-four percent and 28% of mice fed low and high protein diets respectively died during the period between reinfection and autopsy.

Weight Change

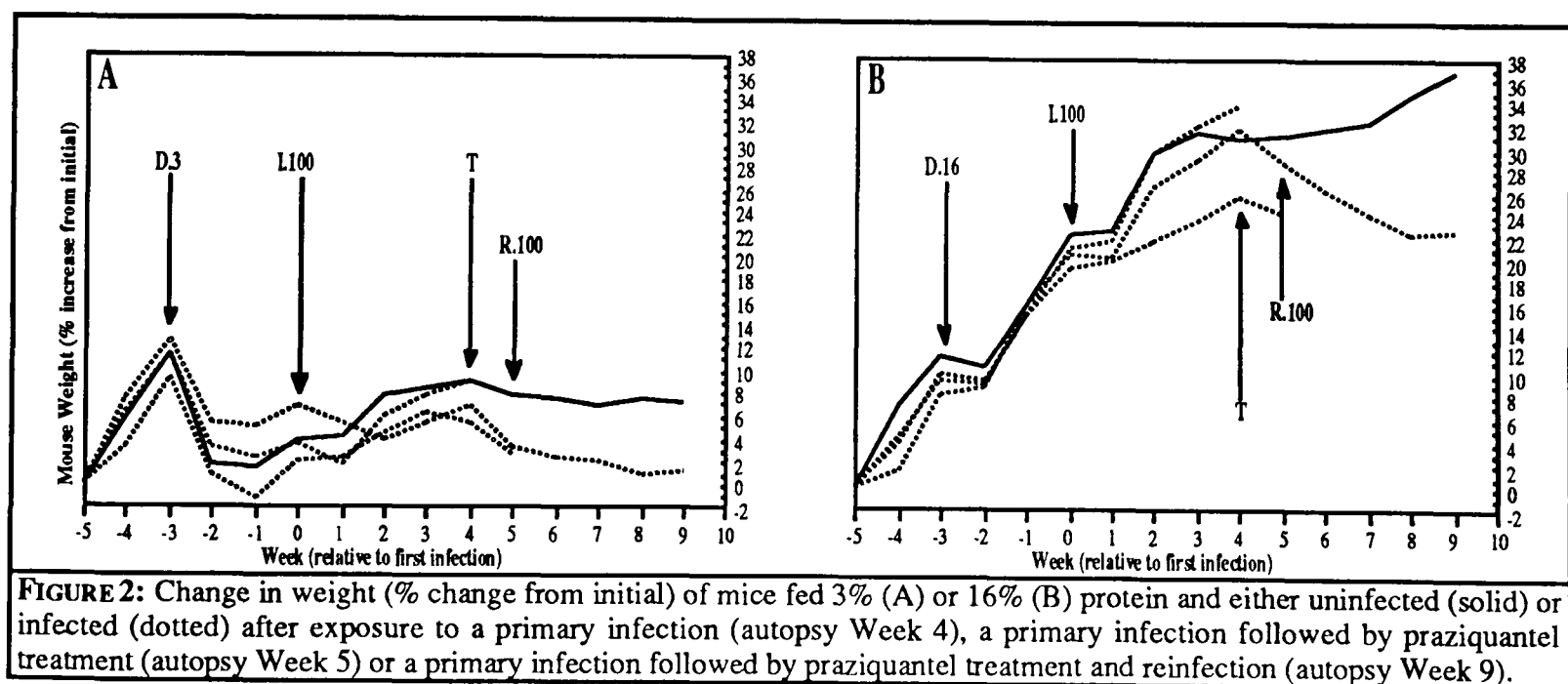


FIGURE 2: Change in weight (% change from initial) of mice fed 3% (A) or 16% (B) protein and either uninfected (solid) or infected (dotted) after exposure to a primary infection (autopsy Week 4), a primary infection followed by praziquantel treatment (autopsy Week 5) or a primary infection followed by praziquantel treatment and reinfection (autopsy Week 9).

Mice fed the low protein diet clearly weighed less than well-fed animals at the time of primary infection (week 0), at the time of drug treatment (week 4) and at the time of reinfection (week 5) (Figure 2). There were no significant differences at these times between the weights of mice within dietary groups.

Organ Size

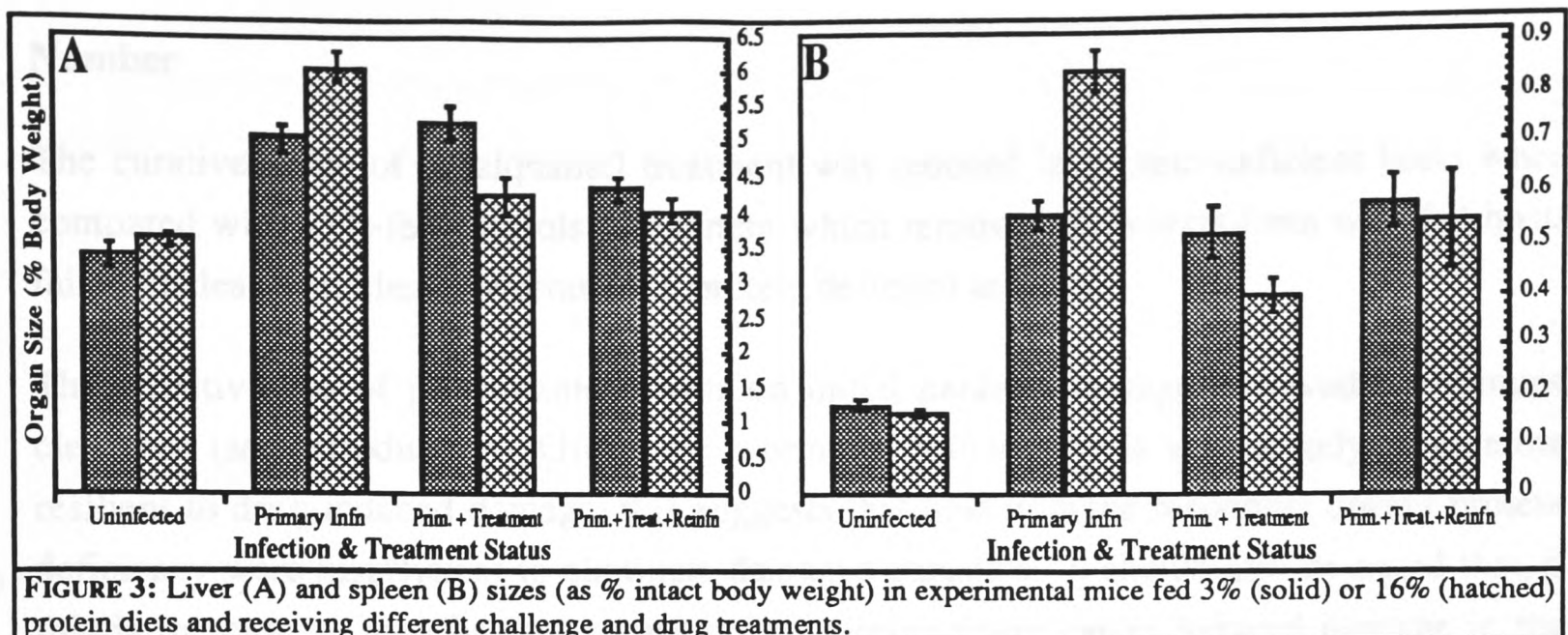


FIGURE 3: Liver (A) and spleen (B) sizes (as % intact body weight) in experimental mice fed 3% (solid) or 16% (hatched) protein diets and receiving different challenge and drug treatments.

Primary infection produced a large increase in both liver and spleen sizes, regardless of dietary treatment, although this increase was significantly greater in well-fed animals (Figure 3). Drug treatment produced a significant reduction in liver and spleen size in well-fed animals when compared with protein deficient animals, but had no significant effect on liver or spleen sizes in animals fed low protein diets (Table 3). Reinfection led to a significant reduction in the liver size of protein deficient hosts, but did not significantly alter post-treatment liver sizes in well-fed animals. While reinfection did not significantly alter spleen sizes in undernourished hosts, reinfection led to a significant increase in the size of spleens in well-fed animals.

Groups	Uninfected 3%	Uninfected 16%	3% Primary	3% Pri+Treat	3% Pri+Treat+Reinfn	16% Primary	16% Pri+Treat	16% Pri+Treat+Reinfn
Liver range (%) (n)	2.88-3.79 (5)	3.44-4.05 (5)	3.91-5.91 (10)	4.65-6.08 (5)	3.74-5.51 (9)	5.66-6.81 (5)	3.26-5.4 (10)	3.74-4.33 (8)
Spleen range (E-1%) (n)	1.54-2.15 (5)	1.48-1.79 (5)	4.5-6.6 (10)	4.1-6.75 (5)	3.83-8.09 (9)	7.3-9.73 (5)	2.87-6.22 (10)	4.46-7.32 (8)
Uninfected 3%	-	0.13(-1.19;8)	0.0001* (-5.59;13)	0.0001* (-6.87;8)	0.002*(-3.58;12)	0.0001* (-10.70;8)	0.02*(-2.27;13)	0.016*(-2.77;6)
Uninfected 16%	0.12(1.25;8)	-	0.0001* (-4.98;13)	0.0001* (6.52;8)	0.007*(-2.85;12)	0.0001* (-10.72;8)	0.056(-1.71;13)	0.03*(-2.23;6)
3% Primary	0.0001* (-10.48;13)	0.0001* (-11.00;13)	-	0.25(-0.70;13)	0.008*(2.69;17)	0.004* (-3.20;13)	0.009* (2.61;18)	0.01*(2.71;11)
3% Pri+Treat	0.0001* (-6.60;8)	0.0001* (-6.96;8)	0.24(0.74;13)	-	0.004*(3.18;12)	0.02*(-2.53;8)	0.009* (2.68;13)	0.005*(3.73;6)
3% Pri+Treat+Reinfn	0.0001* (-6.00;12)	0.0001* (-6.24;12)	0.35(-0.40;17)	0.23(-0.76;12)	-	0.0001* (-6.05;12)	0.37(0.35;17)	0.19(0.91;10)
16% Primary	0.0001* (-16.21;8)	0.0001* (-16.90;8)	0.0001* (-6.34;13)	0.0005* (-4.96;8)	0.002*(-3.63;12)	-	0.0002* (4.76;13)	0.0002*(6.86;6)
16% Pri+Treat	0.0002* (-4.58;13)	0.0002* (-4.91;13)	0.0005* (3.89;18)	0.03*(2.10;13)	0.003*(3.14;17)	0.0001* (8.12;13)	-	0.35(0.41;11)
16% Pri+Treat+Reinfn	0.0009* (-5.32;6)	0.0007* (-5.60;6)	0.41(0.23;11)	0.41(-0.23;6)	0.36(0.36;10)	0.007(3.48;6)	0.04*(-1.90;11)	-

TABLE 3: T-test comparisons of organ sizes in experimental mice (number above diagonal represents p value followed by t value and degrees of freedom of liver comparisons; number below diagonal represents p value followed by t value and degrees of freedom of spleen comparisons; n=number of organs measured).

DISCUSSION

The results presented in this experiment confirm the hypothesis generated above that host protein deficiency decreases the success of praziquantel treatment.

The Parasite

Number

The curative value of praziquantel treatment was reduced in protein deficient hosts when compared with well-fed controls. Treatment which removed all worms from well-fed hosts failed to clear a number of worms from protein deficient animals.

The effectiveness of praziquantel pivots on initial parasite damage followed by immune clearance (see Introduction). Given that worms in deficient hosts are unlikely to be more resilient to drug-induced damage, this suggests that host immune responses during protein deficiency were insufficient to eliminate damaged parasites. It should also be noted that, if this is the case, it may be that parasites can survive praziquantel-induced damage in the absence of immune elimination, even when the parasites were weakened secondary to host deficiency (see Chapter 2). The observed survival of both male and female worms suggests that both sexes may be capable of reversing drug-induced damage.

The Host

Mice fed the low protein diet were significantly lighter at the time of treatment than well-fed animals, and therefore it appears that praziquantel was administered to deficient hosts. Hosts fed the low protein diet were also lighter than animals fed the high protein diet at the time of reinfection.

Mortality was greater in deficient animals following treatment than in well-fed hosts. This suggests that this drug causes stress in the host. It has been suggested that nutritional deficiencies can potentiate the toxic effects of normally safe drugs (Torún & Viteri, 1985), and the present results support this notion.

Despite curative chemotherapy, weights of well-fed animals continued to decline steeply, suggesting that the damage is residual, or that the deleterious effects of reinfection are exacerbated by prior infection.

Organs

The persistence of liver and spleen enlargement in deficient animals following drug treatment is likely to reflect the incomplete clearance of infection. This is supported by the observation that complete worm elimination in well-fed hosts produced a significant fall in both liver and spleen sizes.

4.5: CHAPTER DISCUSSION

This chapter examined the hypothesis that host nutritional status influences the effectiveness of vaccination and chemotherapy against schistosome infection. Results supported the hypothesis, and provided some additional points of interest.

Parasite Control & Vertebrate Host Nutrition

The observation that protein deficient hosts are less able to capitalize on the protection which vaccination provides in the well-fed host reflects their impaired immune response to the stimulation vaccination provides. This result was not surprising, as it is well documented that a wide variety of vaccination régimes suffer from host undernutrition (Chandra, 1983).

However the protection afforded to well-fed animals by the regime used in the above experiment, although significant, fell far short of complete. Unless some future vaccine is 100% effective, which is unlikely, humans exposed to subsequent infection will acquire some worm burden. Results presented above which indicate that the reduction of density-dependent constraints on schistosome size and fecundity in well-fed hosts following reduced worm establishment in vaccinated hosts suggests that even though vaccinated hosts may harbor fewer worms, these are larger and more fecund. As egg production is central to both parasite transmission and host pathogenesis, it may be that the protection which vaccines provide against worm numbers overestimates the protection it provides from parasite transmission and pathology.

It is interesting to note that the number, size and fecundity of schistosomes found at reinfection in protein deficient hosts, regardless of prior exposure to irradiated challenge, remained lower than those in well-fed hosts, whether vaccinated or not.

Disease Control & Vertebrate Host Nutrition

Results indicate that praziquantel administration in protein deficient hosts produces incomplete cure, in contrast to full clearance in well-fed animals. This result supports the hypothesis generated from previous evidence which showed praziquantel to be dependent upon the host immune system and the ambient concentrations of certain nutrients. These results suggest that projections of drug efficacy based on cure rates achieved in well-fed subjects may overestimate drug activity. Where these projections form part of dosage strategies which seek to maximize the benefit of finite resources, a fall in activity may compromise the value of the scheme.

In the Field

It is clear that the host's nutritional status can influence not only the effectiveness of some drugs and vaccines, but also the residual impact of these interventions.

Several comments arise from these findings. First, given the coincidence of such interventions in impoverished communities exposed to chronic undernutrition, and as host undernutrition influences the properties of treatment or control interventions, testing should be done in hosts of similar nutritional status to those for which the intervention is intended (Luttermoser & DeWitt, 1961).

Second, as vaccine and drug treatment may pivot on host nutritional status, in the absence of nutritional support during intervention, it has been suggested that treatment and control interventions be planned to coincide with seasonal peaks in nutritional status (Keusch & Scrimshaw, 1986; Suskind, Sirishinha, Vithayasai, Edelman, Damronsak, Charupatana & Olsen, 1976).

Finally, the observation of altered reinfection following treatment or partial vaccine protection is clearly of importance. It suggests that an important component of interventions will be the ongoing ability to reinforce initial reductions in infection and disease.

Conclusions

Host nutritional status was shown to have a significant impact on the therapeutic value of vaccination and drug treatment against schistosome infection.

CHAPTER FIVE

SCHISTOSOMES & INVERTEBRATE HOST NUTRITION



“Worm nor snail, do no offence”
Shakespeare, *Midsummer Night's Dream*



Chapter Summary

This chapter examines the influence of host nutritional status on the snail-schistosome relationship. Previous experimental evidence has indicated that cercarial production is reduced in undernourished snails. The present chapter consolidates this evidence and explores the influence of snail undernutrition on parasite success and host survival. Results indicate that miracidia invading poorly fed snails initiate the production of fewer, smaller cercariae when compared with those infecting well-fed snails. As a result, deficient hosts suffer less parasite-induced mortality. In addition, the infectivity of emergent cercariae appears to be influenced by snail nutritional status during cercariogenesis. These results have implications for snail population dynamics, and schistosome persistence and transmission, in areas where snail nutrition fluctuates naturally, or as a result of human activities.

5.1: INTRODUCTION

Continued schistosome transmission, prerequisite for the development of chronic schistosomiasis (Warren, 1987), pivots on the ability of adult schistosomes to achieve an intrinsic rate of reproduction greater than unity (Anderson & May, 1991). Under natural conditions, the chances of individual cercariae encountering a suitable host are slim. As a result, the majority of larval schistosomes succumb to environmental stress before a host is acquired (Anderson, 1987). This high level of larval attrition is counteracted by massive asexual replication within the snail host (Jourdane & Théron, 1987).

It has been well documented that single *S. mansoni* miracidia may initiate an intramolluscan phase of replication which eventually produces thousands of cercariae, each of which is capable of developing into an adult schistosome (Sturrock, 1987). The larval amplification provided by the intramolluscan stage of the schistosome lifecycle is thus of clear epidemiological significance.

Because the success of individual miracidia rests upon massive replication within the snail (Sturrock, 1987), small variations in the proportion of miracidia which establish, and/or in the subsequent proliferation of each, are likely to produce large fluctuations in cercarial output. It is therefore clear that the ability of schistosomes to optimize intramolluscan replication is pivotal to their transmission dynamics.

Snail Nutrition & Schistosomiasis: Mutually Inclusive

One of the major factors determining the outcome of a chance encounter between a miracidium and a susceptible snail is likely to be host defences. In view of their highly

biosynthetic nature (Loker & Bayne, 1986; Meuleman, Bayne & van der Knaap, 1987), it might be expected that these defences are influenced by the snail's nutritional status.

Once a suitable host is acquired by the miracidium, schistosome challenge produces a marked impact on the snail (Bayne & Loker, 1987). Parasite competition for nutrients (Thompson & Mejia-Scales, 1989), diversion of nutrients to defence and tissue repair, and metabolic and physiological host responses to infection (Shaw & Erasmus, 1987) combine to markedly alter the survival and reproductive strategies of individual snails (Bayne & Loker, 1987; Minchella, 1985). Given their profound influence on snail hosts, and the fact that parasite transmission depends critically upon both individual snail survival and continued host availability, it is clear that larval schistosomes have an appreciable impact on the resources upon which their own success depends. This suggests that to view either schistosome transmission or snail nutrition in isolation is artificial. In order to explore the complex relationship between snail and schistosome, it is useful to begin by examining the effects each has upon the other.

5.2: THE EFFECTS OF SCHISTOSOMES ON INVERTEBRATE HOST NUTRITION

Schistosomes have a significant impact on those snails with which they come into contact, and this has been the subject of recent attention (Bayne & Loker, 1987).

The Individual Host

Fecundity

Miracidial challenge elicits a nutritionally expensive array of internal defences (Meuleman *et al.*, 1987; van der Knaap & Loker, 1990). This may divert nutrients which might otherwise be needed by the snail for growth and reproduction (Bayne & Loker, 1987). It is at this stage that many infections are eliminated, however regardless of infection success, exposure to parasites can radically alter snail metabolism (Becker, 1980a; 1980b; Shaw & Erasmus, 1987) and physiology (Liebsch & Becker, 1990), including feeding (Thompson & Mejia-Scales, 1989) and reproductive (Minchella, 1985; Minchella & Loverde, 1981) strategies.

Growth

Although growth appears largely unaffected during the early stages of schistosome infection, the observed negative correlation between snail growth and periods of high cercarial production (Théron & Moné, 1984) suggests that nutrients are used by the parasite and/or nutrients are diverted to tissue repair following migration-induced damage. Bayne and Loker (1987) have considered conflicting evidence on this subject, however it is clear that schistosome infection can markedly influence snail growth.

Survival

Infection culminates in cercariogenesis where the great majority of the host biomass is assimilated into parasite tissue. At this stage nutrient losses directly to parasite uptake, and those lost to tissue repair, combine with the damage resulting from cercarial escape to endanger snail survival (Bayne & Loker, 1987; Christie, Foster & Stauber, 1974; Pan, 1965; Smith, 1986; Thompson & Mejia-Scales, 1989).

Clearly schistosomes have a marked impact on the survival and reproductive success of individual snails. As a result, it is likely that infection influences snail population dynamics, which in turn influences the availability of hosts to subsequent miracidia (Anderson, 1978; Carter, Anderson & Wilson, 1982).

5.3: THE EFFECTS OF INVERTEBRATE HOST NUTRITION ON SCHISTOSOMES

The characteristics of schistosome transmission depend critically upon the temporal and spatial distribution of cercariae in the environment. This distribution is largely determined by the density of infected snails and the productivity of the snail-schistosome relationship (Anderson & May, 1979; Carter *et al.*, 1982; Wilkins, 1987; Wilson, 1987).

Having acquired a suitable host, the primary determinants of schistosome success during the snail phase of the lifecycle are the establishment of miracidia, the number of cercariae which result, and their individual infectivities (Jourdane & Théron, 1987).

Miracidial Establishment

As snail defences are pivotal to miracidial survival (Bayne & Yoshino, 1989; Meuleman *et al.*, 1987; van der Knaap & Loker, 1990) and energetically expensive to mount (Eveland & Ritchie, 1972; Minchella, 1985; Minchella & Loverde, 1981; 1983), nutritional changes sufficient to alter host defences are likely to be important determinants of miracidial survival in the snail host.

Cercariogenesis

Once established, miracidia make almost total use of snail biomass to fuel asexual replication, at the expense of host reproduction and ultimately survival (Bayne & Loker, 1987; Minchella, 1985; Minchella & Loverde, 1981; 1983; Théron & Moné, 1984; Whitfield, 1981). That parasite establishment and replication within the snail are commonly density-dependent (Jourdane & Théron, 1987; Kastenholtz, 1984; Meier & Meier-Brook, 1981) and result in host starvation (Becker, 1980a; 1980b; Christie *et al.*, 1974; Shaw & Erasmus, 1987) suggests nutrient availability is a limiting factor on parasite replication. In addition, nutritional stress drastically alters the physiochemical conditions under which the parasites

develop (Thompson & Mejia-Scales, 1989). Cercariogenesis is therefore also likely to be influenced by changes in snail nutrition which alter the nutritional and physical characteristics of the resource which the host represents to the parasite.

Experimental evidence from a variety of digenean species confirms that snail nutritional stress or starvation can reduce (Anderson, Whitfield & Mills, 1977; Becker, 1971; Coles, 1973; Erickson & Caldwell, 1961; Eveland & Ritchie, 1972; Kendall, 1949) or terminate (Anderson *et al.*, 1977; Kendall, 1949) cercarial development and emergence. Placing the snails on inadequate diets 4 weeks after infection similarly produced a marked reduction in cercarial output (Erickson & Caldwell, 1961). Mechanisms implicated include competition for nutrients between host and parasite and between individual parasites (Coles, 1973; Eveland & Ritchie, 1972; Kendall, 1949), and limitations on the space available to developing parasites (Coles, 1973; Eveland & Ritchie, 1972). Furthermore, *in vitro* evidence suggests that during host starvation, late-stage larvae may autolytically resorb developing cercariae (Whitfield, 1981).

Regardless of their precise mechanisms, these effects are reversible. Following suppression of cercariogenesis during snail starvation, refeeding can lead to the resumption of larval emergence (Anderson *et al.*, 1977; Erickson & Caldwell, 1961; Kendall, 1949) and, indeed, enriching normal host diets with highly nutritious supplements can actually increase cercarial production above normal levels (Coles, 1973). In addition to the quantitative success of larval survival and replication within the snail host, individual cercarial quality, manifest as infectivity, is likely to be a major determinant of schistosome success.

Cercarial Infectivity

One of the major factors limiting schistosome transmission is host availability. Under field conditions, the probability that each cercaria will encounter a suitable host is small, however the chances are greatly increased by adaptive cercarial behaviors and through time.

Adaptive behaviors consist of general temporal and spatial orientation in the environment which optimize the chances of encountering a suitable host (Jourdane & Théron, 1987; Wilson, 1987). Once general orientation is achieved, more specific responses are deployed to conserve energy, thus enabling the parasite to maintain position for as long as possible. For example, the use of flotation devices, complex swimming and dropping behaviors, and physical attributes that reduce sinking rates can all be regarded as mechanisms for conserving glycogen (Jourdane & Théron, 1987; Whitfield, Anderson & Bundy, 1977; Wilson, 1987).

Finally, attachment to and penetration of the vertebrate host precede successful transformation to the schistosomule. The longer cercariae can maintain the ability to perform these behaviors, the greater the chance of encountering and successfully entering a potential host. And so it follows that the probability of a cercaria finding and challenging a host is

related to the longevity and vigor of its behaviors (Lawson & Wilson, 1983; Olivier, 1966; Wilson, 1987).

Under natural conditions, the activity involved means that each of these behaviors is energetically expensive (Coles, 1973; Lawson & Wilson, 1983; Wilson, 1987). Given that cercariae do not feed (Lawson & Wilson, 1983; Whitfield, 1981) and that vigor and longevity are advantageous for host acquisition (Lawson & Wilson, 1983; Olivier, 1966; Wilson, 1987), the amount of glycogen stored during intramolluscan development (Becker, 1971) is likely to be a major determinant of cercarial infectivity (Evans & Stirewalt, 1951; Whitfield, 1981) and establishment (Lawson & Wilson, 1983).

Other physical variables, such as water temperature and ionic concentration, influence the temporal value of this energy (Krakower, 1940; Lawson & Wilson, 1980b; Whitfield, 1981). All other things being equal, it follows that cercarial infectivity will be some function of snail nutrition. It might therefore be expected that host nutritional stress during cercariogenesis compromises the longevity and hence the infectivity of cercariae.

The observation that snail starvation which reduces cercarial production does not compromise the infectivity of individual cercariae (Erickson & Caldwell, 1961; Eveland & Ritchie, 1972) suggests that whilst snail undernutrition produces a decline in cercarial quantity, quality is not significantly affected. However the fact that these, and other experimental infections generally make use of cercariae immediately after, or within a few hours of emergence, indicates that longevity is rarely a determinant of infection success in the laboratory. In the field, however, where suitable hosts are likely to be scarce, longevity might be expected to play a major role in cercarial success (see above). Clearly, snail nutritional status is not only a major determinant of the number of cercariae produced, but also potentially influences their infectivity under natural conditions.

5.4: IN THE FIELD: SCHISTOSOMES & SNAIL NUTRITION

Under natural conditions found in areas endemic for schistosome transmission, there is likely to exist wide temporal and spatial variation in snail habitats. As a result, snail survival strategies are also likely to vary. It has been suggested that observed reductions in schistosome replication during times of nutritional stress, such as occur during snail aestivation, ensure maximum host, and therefore parasite, survival during times when unabated parasite exploitation of a weakened host might kill the snail (Kendall, 1949). It might also be expected that reversing this stress might restore parasite exploitation and thus lead to resumption of cercarial release. Thus seasonal and man-made factors which have an impact on snail nutritional status may provide an additional source of heterogeneity in schistosome transmission.

5.5: HYPOTHESIS

That snail nutritional status is a significant determinant of schistosome transmission success and snail population dynamics.

5.6: MATERIALS & METHODS: THE INVERTEBRATE HOST

This section describes the invertebrate host and the methods used in the experimental work comprising this chapter. Details of the design of the individual experiments are given in the Experimental Design section of each experiment.

Snail

All snails used in these experiments and in schistosome maintenance were albino strain *Biomphalaria glabrata* Say, permissive for the PR-1 strain of *S. mansoni*. Snails were 3-6 weeks old (04-07 mm in maximum shell diameter) at the beginning of each experiment. Constant temperature ($28 \pm 0.2^\circ$ C) and a 12:12 h light:dark photoperiod were maintained throughout the experiments. Breeding colonies were housed in large aerated aquaria (500 mm x 300 mm x 300 mm) containing preconditioned water (as described in Chapter 2). Infected snails used to passage the parasite were kept in groups of 10 in large plastic containers (2 liter) whilst those in experiments were housed individually in small plastic containers (100 ml).

Maximum shell diameter and snail wet weights were measured each week by using calipers (Mauser, Germany) and by blot-drying the snail on absorbent paper and weighing on an electronic balance (Sartorius), respectively.

Snail Diets

Snails used to passage the parasite were fed a standard diet (SD) of TetraMin® (TetraWerke, Mellen, Germany) (constituents shown in Table 1, overleaf) and freeze-dried lettuce (Commercial Freeze Drying Limited, Clitheroe, Lancashire, UK) *ad libitum*. Snails used in experiments were fed TetraMin, a commercial fishfood, in the following manner: well-fed animals received food *ad libitum* (flakes were sprinkled onto the water surface each day, and the amount adjusted to ensure a small excess when next feeding the animals), whilst snails fed restricted diets received 0.3 g TetraMin.snail⁻¹.week⁻¹ fed evenly throughout the week. The latter level was known to be inadequate for normal snail requirements based on observations of impaired growth and reproduction in uninfected snails (see Experiment 5.I).

<i>Constituent</i>	<i>Composition (%) (weight for weight)</i>
Crude Protein	min. 45.0%
Crude Fat	min. 05.0%
Crude Fibre	max. 05.0%
Sodium Chloride	max. 03.0%
Moisture	max. 08.0%

TABLE 1: Weender Analysis of TetraMin (TetraWerke), provided by Dr. H. Kürzinger, TetraWerke, Germany.

Miracidial Infection of Snails

To infect snails both for parasite maintenance and for experiments, miracidia were obtained as follows: chronically infected stock mice (fed SLC *ad libitum*) were sacrificed by cervical dislocation. Livers and intestines were homogenized in a mortar and pestle containing 5 ml saline¹. The homogenate was then placed in a 500 ml side-arm erhlenmeier flask which was filled to 10 mm below the side-arm with preconditioned water (see above). Water was slowly added through the side-arm, onto which a 50 mm length of clear plastic tubing had been fixed, until the elevated tube was full of water to the level of the flask lip. The flask was totally covered with opaque cloth and a 100 W light source placed above the side-arm tubing.

After 1 hour, the required number of miracidia were drawn off individually with a pasteur pipette and placed into a small plastic cup (100 ml) containing 20 ml of preconditioned water. A single snail was added and held in the cup for 12 hours. Snails were then either returned to groups for parasite maintenance (see above) or used as described in individual experiments.

Cercarial Shedding

To obtain cercariae for use in mouse experiments or parasite passage, snails were placed in groups of 40 in small glass containers under 100 W bench lighting for 1.0 hour. Cercariae were then counted as they were pipetted off.

To quantify cercarial output during the experiment, containers were cleaned and refilled with fresh water and snails removed after 24 h. 25 ml of 70% alcohol was added to the container and left for 5 minutes. After the supernatant had been carefully decanted, the dead cercariae were counted and measured under a dissecting microscope using an ocular micrometer.

Statistical Analyses

Statistical analyses were performed, and are presented, as described in Chapter 2.

FOOTNOTE

¹ Saline consisted of 0.85% NaCl in distilled H₂O.

5.7: EXPERIMENTS

The hypothesis was tested by manipulating the quantity of food which infected snails received. Different levels of the same diet were used to remove the confounding effects of different foods on host and parasite. The restricted dietary régime was arbitrarily set at a level providing inadequate nutrition for normal snail growth and reproduction. Hosts received 10 miracidia.snail⁻¹, a level found to ensure infection, yet minimize acute snail mortality (D. Dale, unpublished results).

THE INFLUENCE OF SNAIL NUTRITION ON *SCHISTOSOMA MANSONI* IN *BIOMPHALARIA GLABRATA*: I. PRIMARY INFECTION

Abstract

This experiment examines the influence of inadequate snail diets on the parasite and host following a single *S. mansoni* infection. Results show that parasites invading poorly fed snails suffer from suboptimal conditions during cercariogenesis, resulting in the production of fewer, smaller cercariae. This had the effect of reducing host injury associated with cercarial emergence, which led to greater survival of underfed hosts when compared with well-fed, infected controls. These results have implications for the survival of host, and thus parasite, in areas where snail nutrition fluctuates seasonally or as a result of human activities.

INTRODUCTION

Evidence presented above suggests that miracidial establishment may be facilitated during a fall in snail defences accompanying nutritional stress. Despite this, previous studies have shown that cercarial production is significantly reduced in undernourished snails (see above). These results indicate that, although their chances of surviving to establishment are increased, the replicative potential of invading miracidia is reduced in undernourished snails. This is likely to reflect the deleterious effects of suboptimal conditions found within the poorly fed host on the developing parasite.

No previous study has examined the effects of snail nutrition on cercarial output and size, despite the value of larval size as an indicator of endogenous energy reserves (Lawson & Wilson, 1980b; Wilson, 1987) and the pivotal role miracidial success plays in schistosome transmission. This experiment examines the influence of snail nutrition on *Biomphalaria glabrata* following a single exposure to *S. mansoni* miracidia, as well as the influence of snail nutrition on the quantity and quality of emergent cercariae.

Hypothesis

That changes within the snail host, such as those produced by fluctuations in host nutritional status, influence schistosome exploitation and thus host survival.

EXPERIMENTAL DESIGN

Eighty snails of similar age and size (8-14 mm) were chosen at random from a large population of naïve juveniles and fed on the SD for two weeks. Forty individuals were then assigned at random to each of two dietary régimes; either restricted (Dr) or *ad libitum* (Dal) feeding with Tetramin. Each dietary group was subdivided into two cohorts: one cohort from each group was exposed to schistosome infection by placing each snail in a plastic cup with 10 miracidia (I.10), as described above. Twelve hours later, the water level in each pot was increased to 100 ml and the appropriate experimental feeding régime begun. Snails from the second cohort of each dietary group experienced identical treatment in the absence of

miracidia. The position of pots on the laboratory work surface was randomized every other day during the experiment.

At weekly examinations (E), snail shell diameter and wet weight were measured, and mortality and fecundity recorded. All pots were then cleaned, fresh water added and the surviving snails replaced. Twenty-four hours later, snails were removed to fresh pots and remaining cercariae counted under a dissecting microscope. All snails were handled identically throughout the experiment regardless of dietary and/or infection treatment.

Final examinations (FE) were made six weeks after exposure to miracidia. At this time, a sample of cercariae less than 2 h old was taken at random from infected snails in each dietary group at mid-photoperiod, killed by the addition of 70% alcohol and maximum dimensions measured using an ocular micrometer. The experimental design is shown in Table 1.

Week Group (n)	-2	-1	0	1	2	3	4	5	6
A (20)	SD	→	Dr	E→	E→	E→	E→	E→	FE
B (20)	SD	→	Dr I.10	E→	E→	E→	E→	E→	FE
C (20)	SD	→	Dal	E→	E→	E→	E→	E→	FE
D (20)	SD	→	Dal I.10	E→	E→	E→	E→	E→	FE

TABLE 1: Design of Primary Infection experiment (n=number of snails in group).

RESULTS: HOST

Mortality

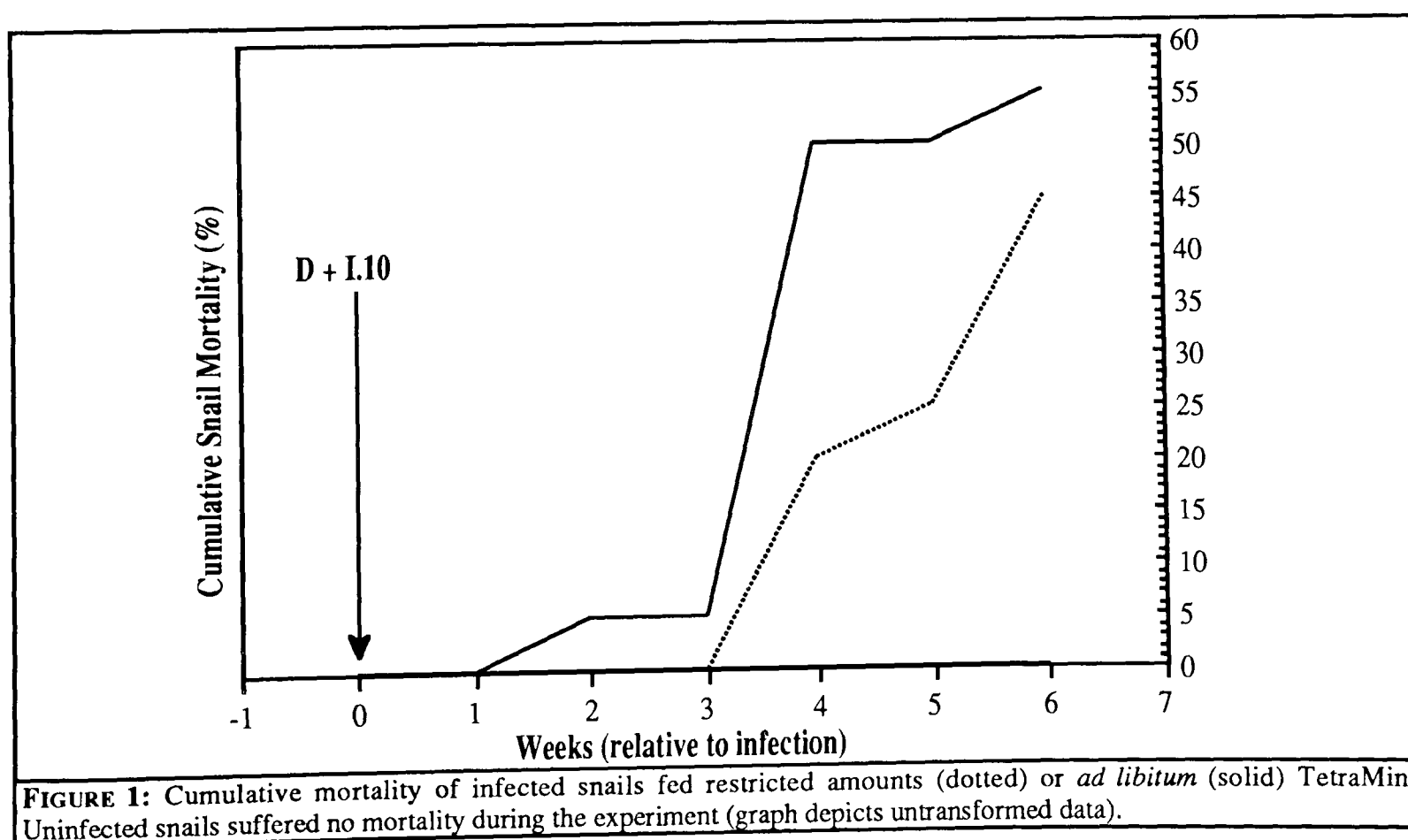
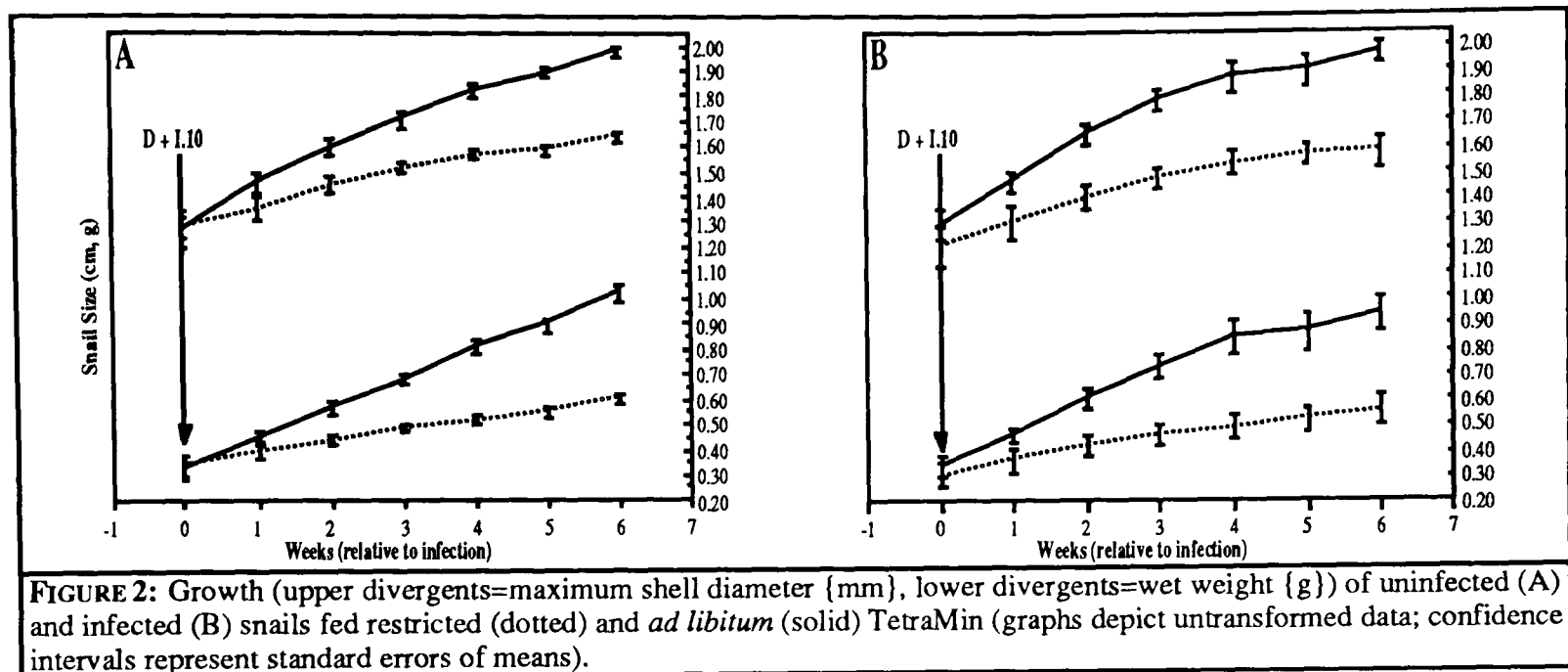


FIGURE 1: Cumulative mortality of infected snails fed restricted amounts (dotted) or *ad libitum* (solid) TetraMin. Uninfected snails suffered no mortality during the experiment (graph depicts untransformed data).

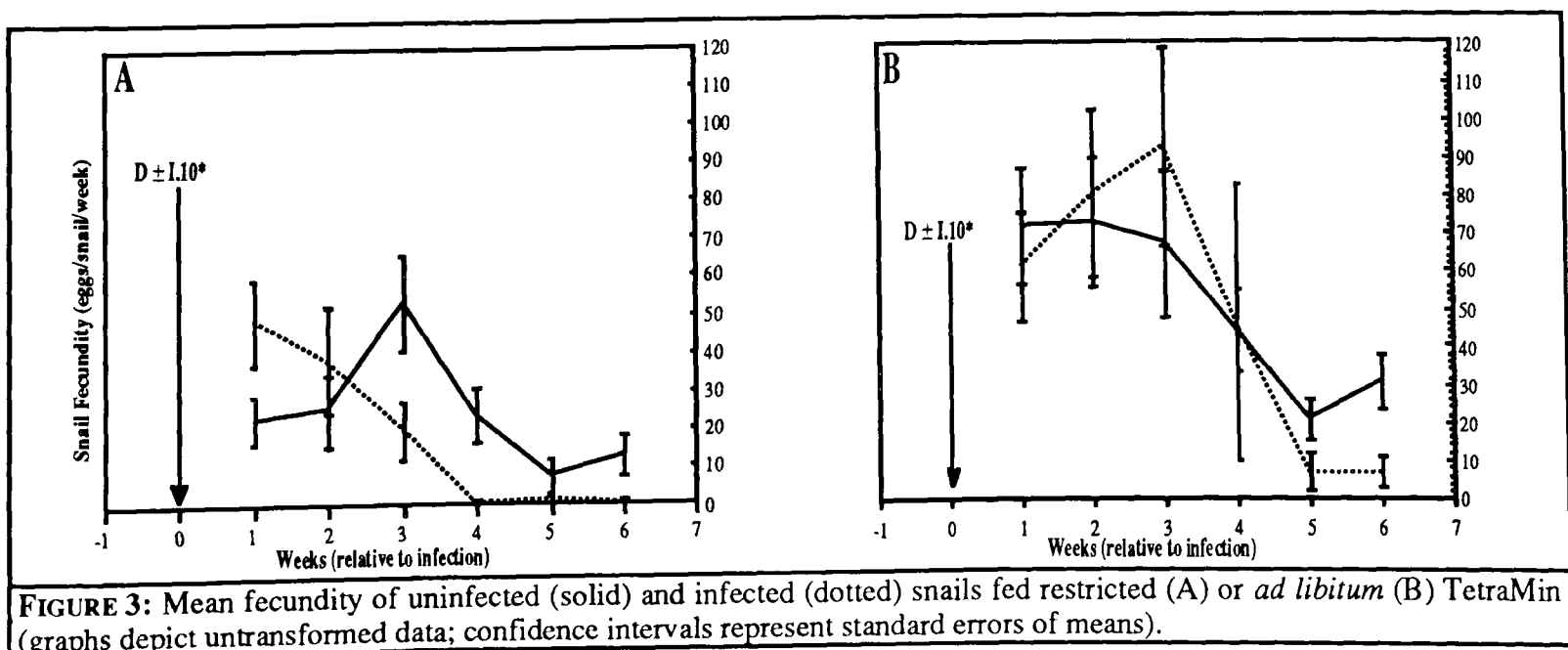
Uninfected snails suffered no mortality regardless of dietary treatment. Miracidial challenge markedly reduced snail survival in both dietary groups, however mortality tended to be earlier and heavier in well-fed snails when compared with those fed restricted diets (Figure 1, previous page).

Growth



Miracidial challenge did not significantly influence snail growth in either dietary group, however dietary treatment had marked effects on growth, regardless of infection (Figure 2). In unchallenged snails, those fed *ad libitum* grew faster and to a larger size than underfed snails; differences in both shell diameter (df=38, $t=-3.15$, $p=0.002^*$) and weight (df=38, $t=-3.48$, $p=0.001^*$) became significant from week 2 and continued to diverge for the remainder of the experiment (Figure 2A). Snails exposed to miracidia showed similar growth patterns to unchallenged snails (Figure 2B); differences in shell diameter (df=38, $t=-2.19$, $p=0.035^*$) and weight (df=38, $t=-3.58$, $p=0.001^*$) between dietary groups differed significantly from weeks 1 and 2 onwards, respectively.

Fecundity: Influence of Infection



Individual snail fecundity was influenced by exposure to miracidia. In snails fed restricted diets, miracidial challenge was associated with a significant increase in fecundity during the first week after exposure ($df=38$, $t=-2.06$, $p=0.047^*$) which fell rapidly to be significantly lower than the fecundity of unchallenged snails in weeks 3 ($df=38$, $t=2.31$, $p=0.027^*$) and 4 ($df=38$, $t=2.84$, $p=0.008^*$) (Figure 3A, previous page). In snails fed *ad libitum* (Figure 3B, previous page), exposure had no significant effect on fecundity until week 6, when challenged snails produced fewer eggs than unchallenged snails ($df=27$, $t=2.09$, $p=0.046^*$).

Fecundity: Influence of Diet

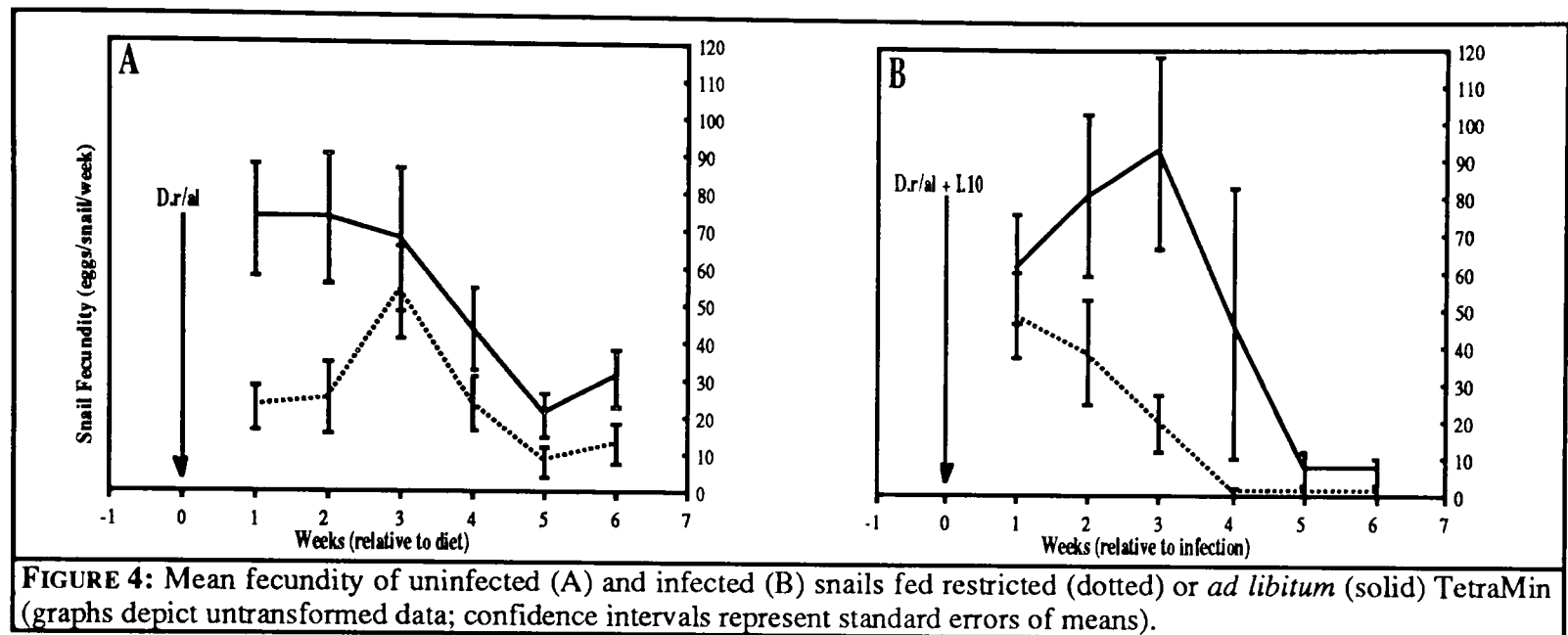
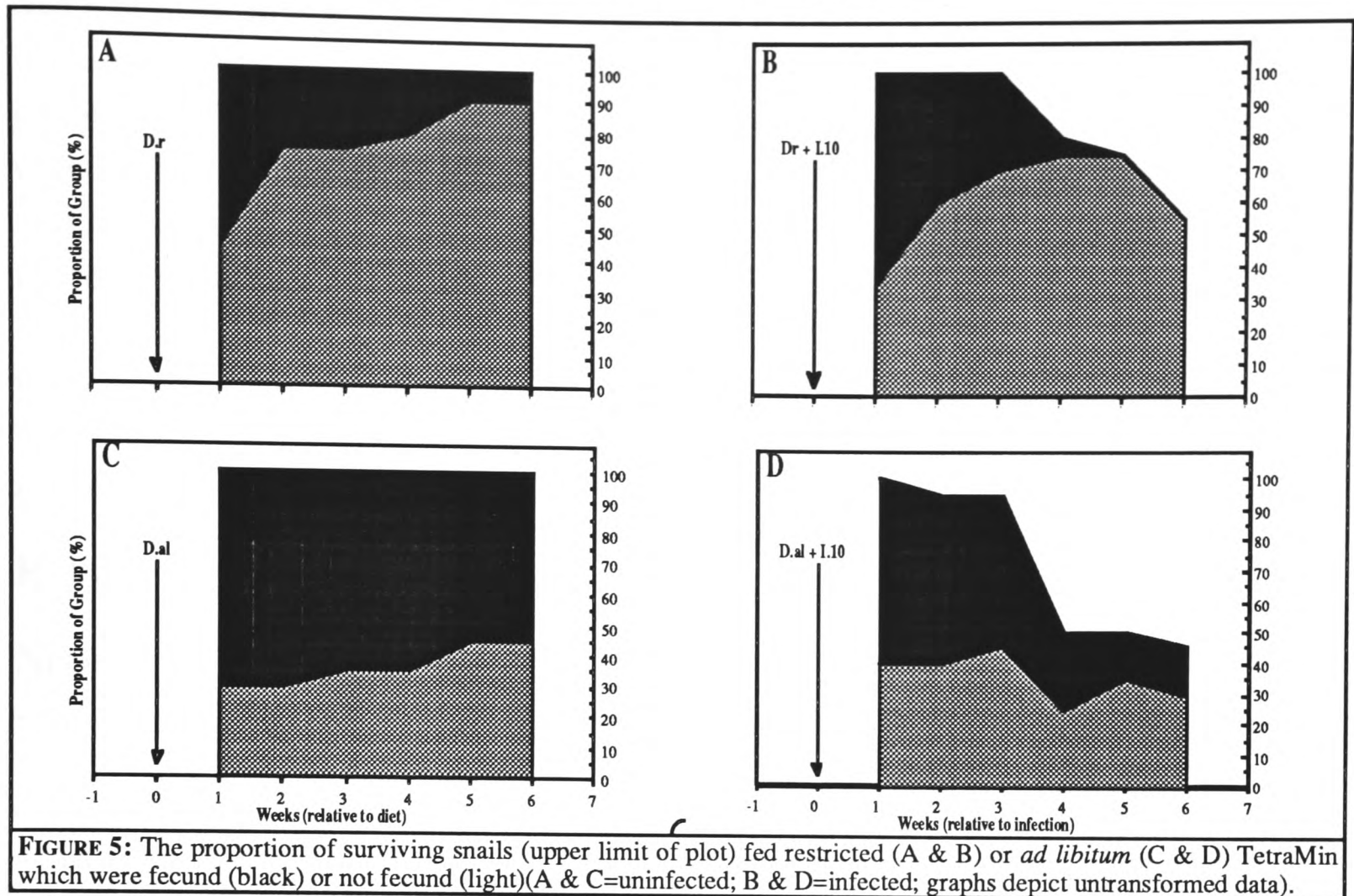


FIGURE 4: Mean fecundity of uninfected (A) and infected (B) snails fed restricted (dotted) or *ad libitum* (solid) TetraMin (graphs depict untransformed data; confidence intervals represent standard errors of means).

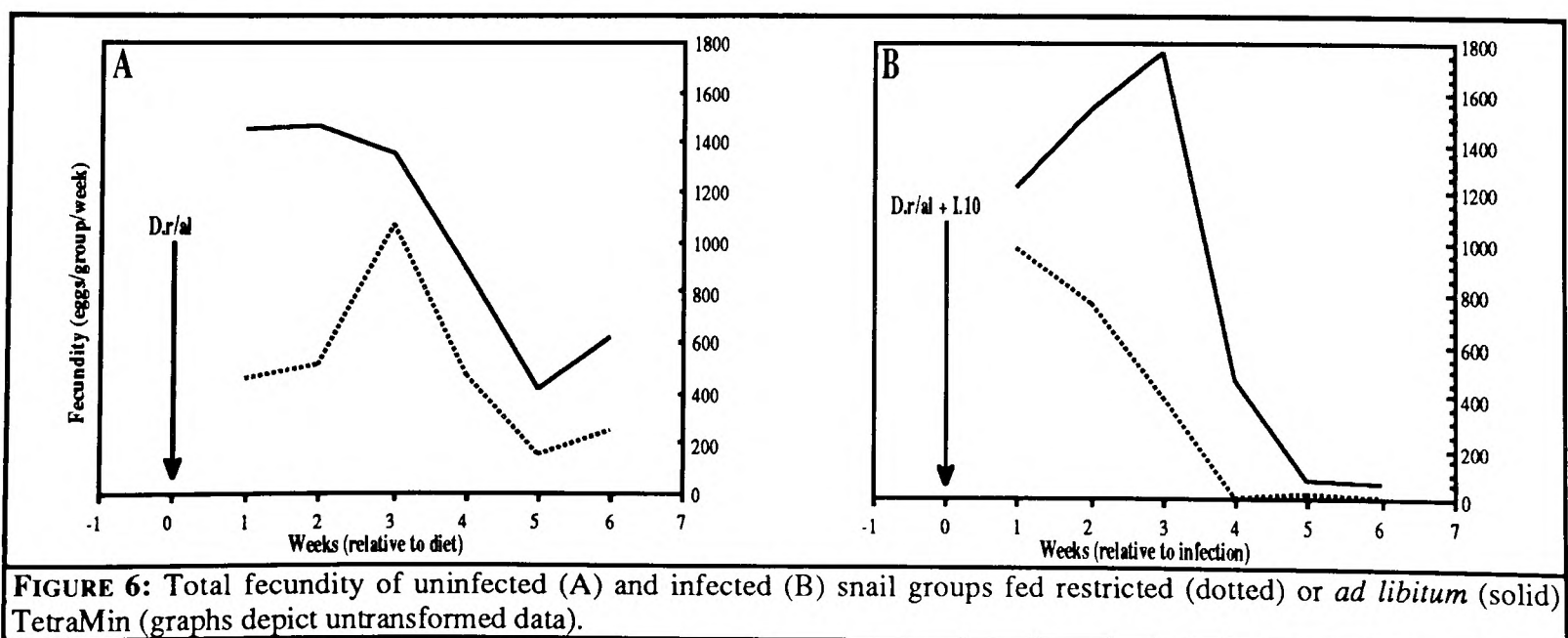
Snail fecundity was also influenced by dietary treatment. In unchallenged snails, dietary stress led to a significant decrease in egg production in weeks 1 ($df=38$, $t=-3.00$, $p=0.005^*$) and 2 ($df=38$, $t=-2.44$, $p=0.02^*$) (Figure 4A) when compared with well-fed controls, after which both groups saw a similar decline in fecundity. In snails exposed to miracidia, dietary stress led to a significant reduction in *per capita* egg output (Figure 4B) in week 3 ($df=37$, $t=-2.76$, $p=0.009^*$) only, after which fecundity fell in both dietary groups.

Fecundity: Proportion of Snails Producing Eggs



The proportion of snails producing eggs fell rapidly in both dietary groups following miracidial challenge. Egg production ceased almost completely in underfed snails (Figure 5 A & B), however despite increased mortality, exposure to miracidia did not lead to a cessation of egg production in well-fed snails (Figure 5 C & D). In unchallenged snails, dietary stress led to a reduction in the proportion of individuals producing eggs (Figure 5 A & C). In challenged snails, a greater proportion of well-fed snails remained fecund despite high mortality, with some snails remaining fecund throughout the experiment (Figure 5 B & D); the proportion of underfed snails producing eggs fell rapidly despite greater survival.

Fecundity: Group Egg Production



The total egg production of each snail cohort (the product of survival and mean fecundity) was plotted (Figure 6, previous page). Over the 6 week experimental period, uninfected snails fed *ad libitum* produced 6067 eggs, and uninfected snails receiving restricted diets produced 2898 eggs; thus, dietary stress led to a 52% decrease in the fecundity of unchallenged snails. Challenged snails fed *ad libitum* produced 5144 eggs, whilst challenged snails receiving restricted diets produced 2141 eggs; thus, dietary stress led to a 58% fall in the egg production of challenged snails. Exposure to infection in snails fed restricted and *ad libitum* rations led to apparent declines in total egg production of 26% and 15% respectively when compared with uninfected controls. Snails both fed a restricted diet and exposed to infection saw a decrease of 65% in egg production when compared with unchallenged, well-fed controls.

RESULTS: PARASITE

Number of Emergent Cercariae

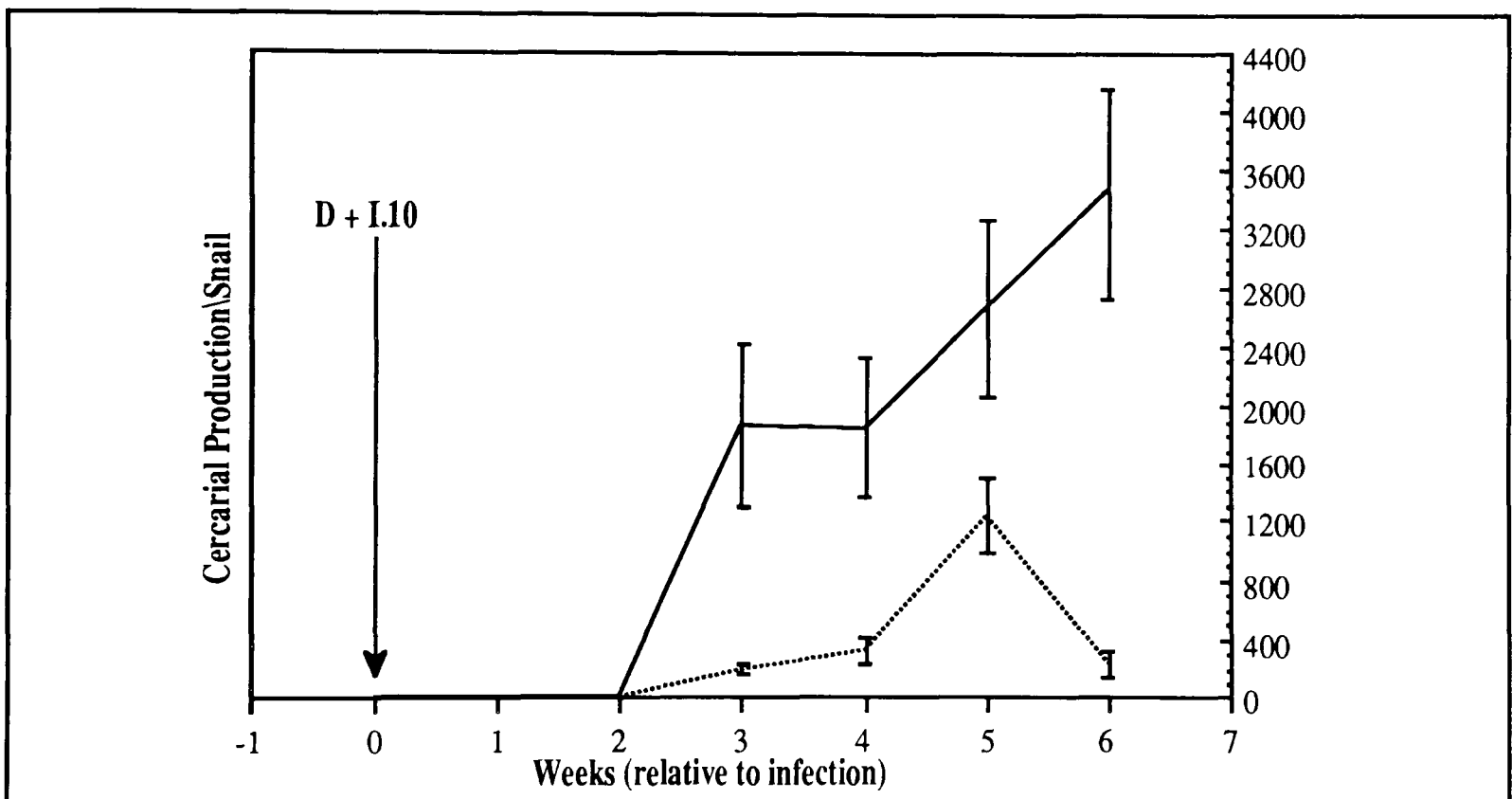
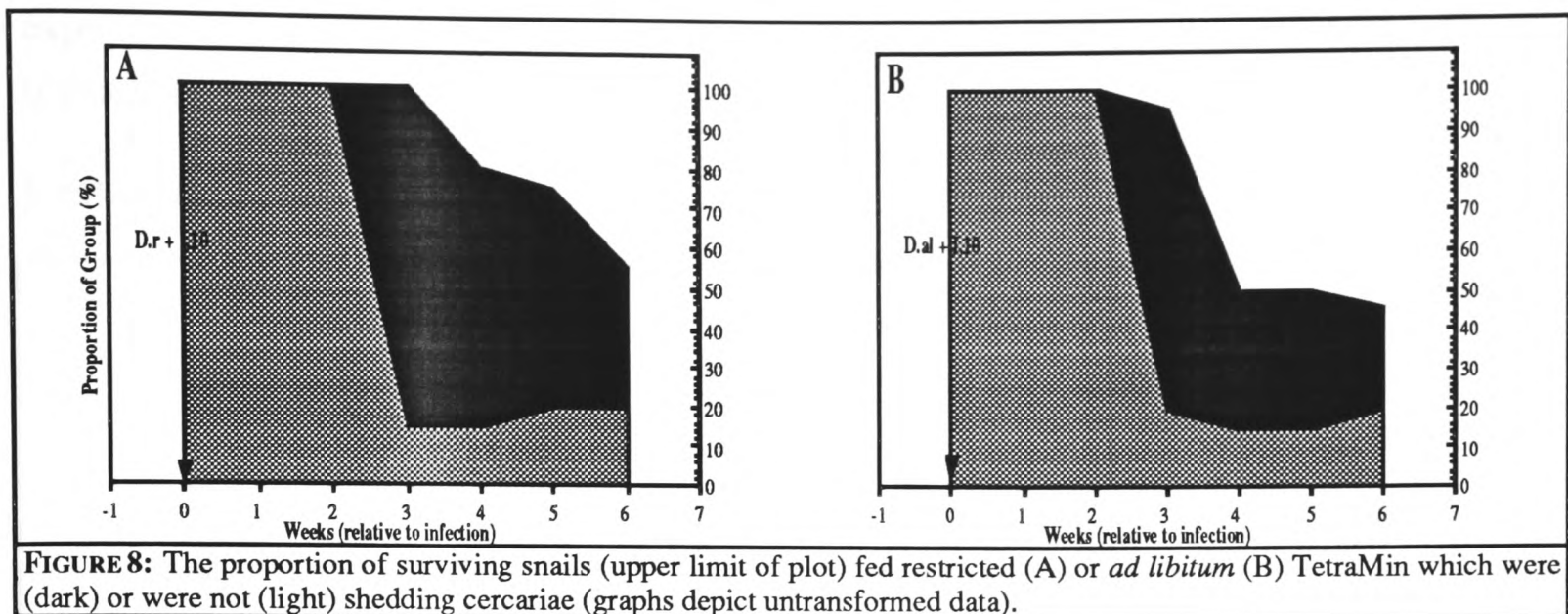


FIGURE 7: Mean 24 hour cercarial emergence (6 weeks after infection) from experimental snails fed restricted (dotted) or *ad libitum* (solid) TetraMin (graph depicts untransformed data; confidence intervals represent standard errors of means).

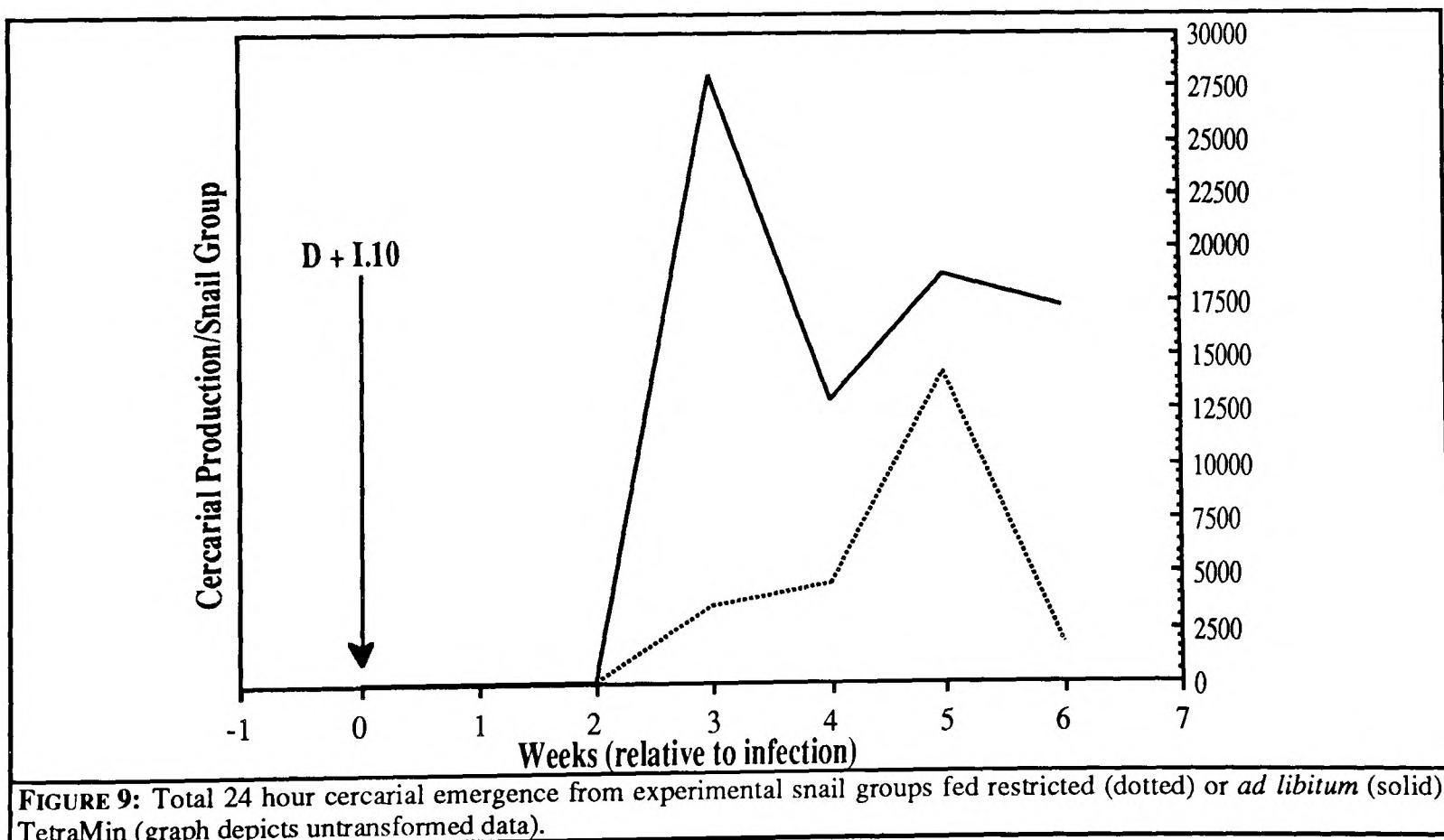
Of those snails which became infected, underfed individuals produced fewer cercariae than snails fed *ad libitum* (Figure 7). These differences were statistically significant from first emergence and remained so for the duration of the experiment (week 3 $df=30$, $t=-3.16$, $p=0.004^*$ to week 6 $df=12$, $t=-6.11$, $p=0.0001^*$).

Proportion of Snails Shedding Cercariae



Although *per capita* cercarial production was reduced by dietary stress (see Figure 7), differences in the mortality of challenged snails with respect to diet (see Figure 1) meant that more underfed snails survived to produce cercariae (Figure 8), albeit at a lower rate. The product of the number of snails shedding and their mean shedding density represents the total cercarial contribution made by each cohort during the 24 h shedding period monitored each week.

Cercarial Production by Snail Groups



Despite higher mortality among well-fed snails following challenge (see above), as a cohort they produced more cercariae than did underfed snails (Figure 9). During the six 24 h periods in which cercarial production was monitored, well-fed snails produced an estimated

total of 76644 cercariae, compared with 26325 cercariae produced by snails fed restricted diets. The replicative potential of each 10 miracidia infecting individual snails for the experimental period was thus reduced by approximately two-thirds as a result of host dietary restriction.

Cercarial Size

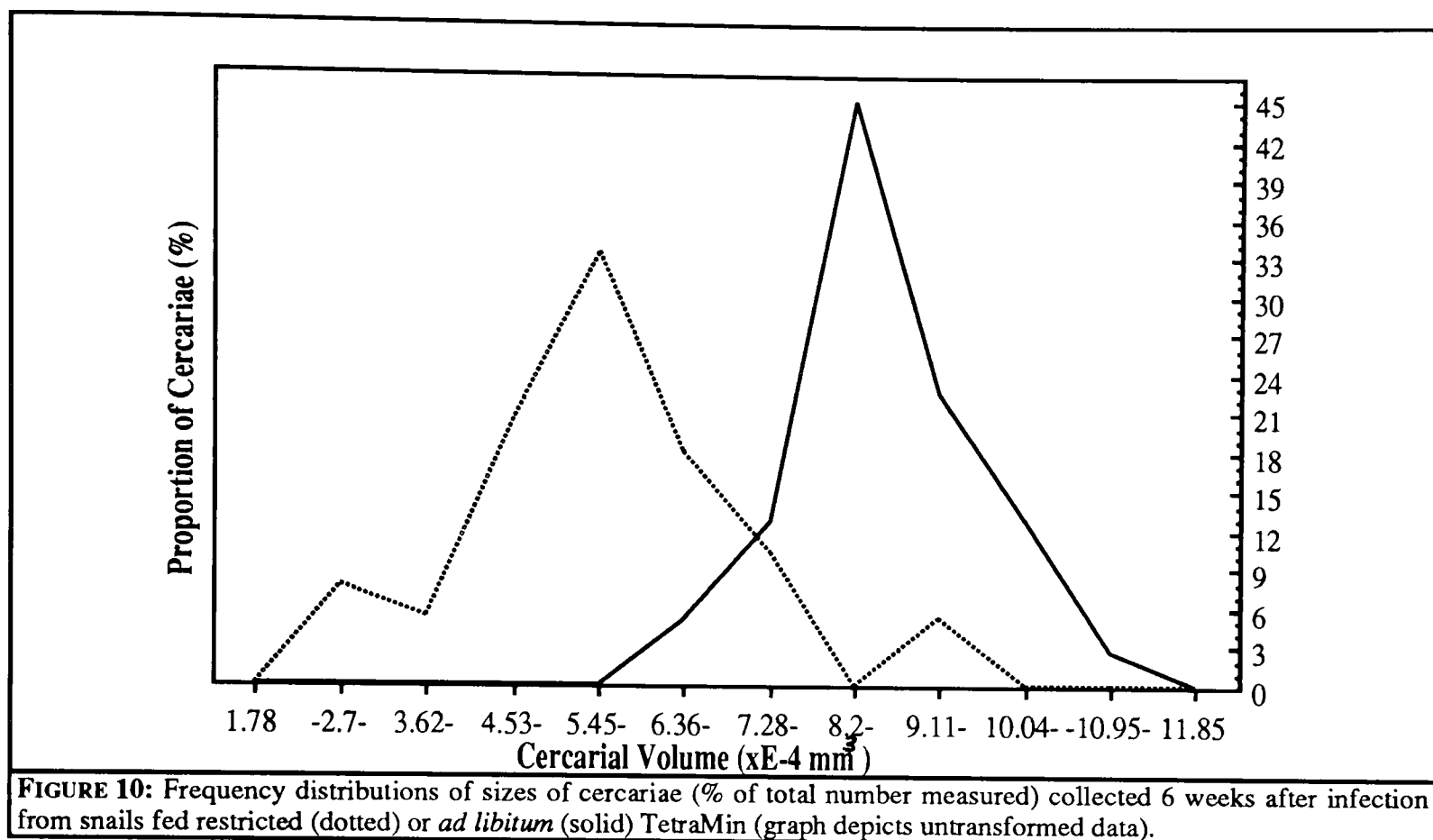


FIGURE 10: Frequency distributions of sizes of cercariae (% of total number measured) collected 6 weeks after infection from snails fed restricted (dotted) or *ad libitum* (solid) TetraMin (graph depicts untransformed data).

Host diet also affected the size of emergent cercariae. Measurements of the heads and tails of cercariae shed from snails fed *ad libitum* were significantly greater than those recorded from underfed snails (Table 2); distributions verify these results (Figure 10). Calculations of cercarial volume (assuming the head and tail are two cylinders) show that cercariae shed by well-fed snails have a significantly greater body volume than those from snails fed the restricted diet (Table 2).

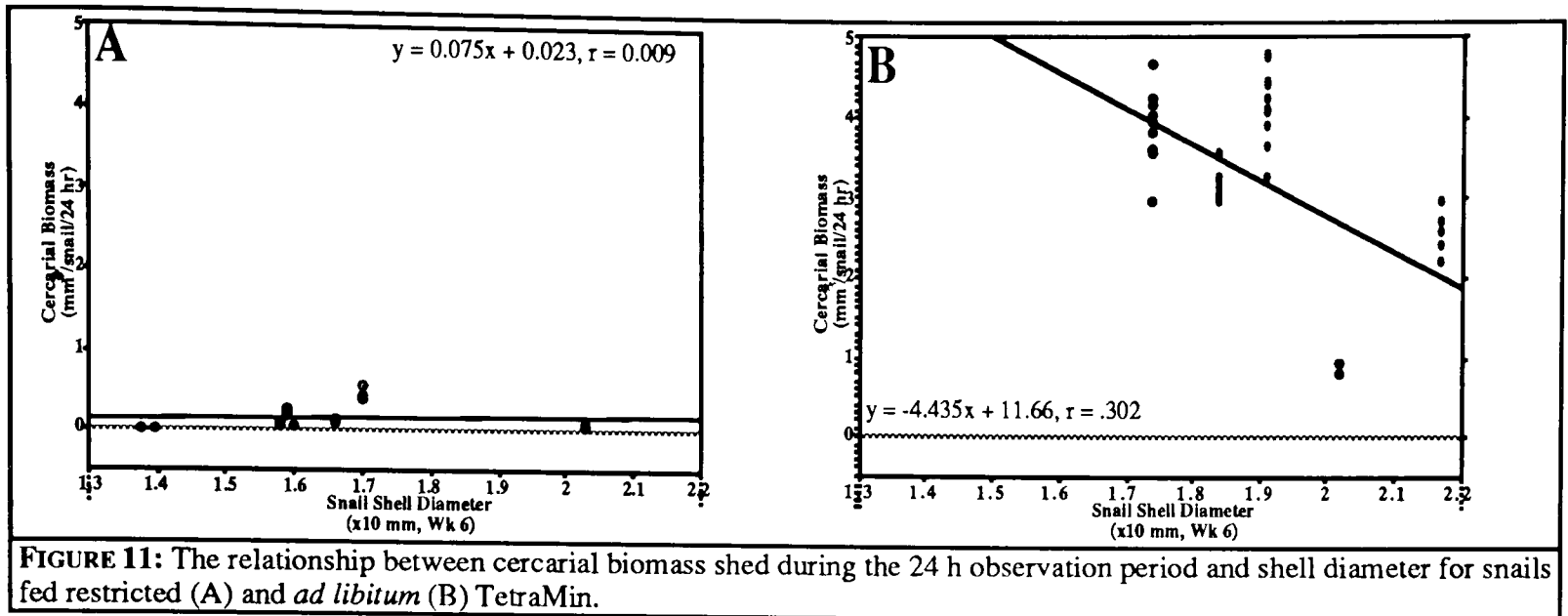
	A	s.e. §	B	s.e. §	degrees of freedom	t value	p value
Head: length(mm)	0.16	0.001	0.171	0.002	132	-5.333	0.0001 *
width (mm)	0.052	0.001	0.06	0.001	132	-9.168	0.0001 *
Tail: length (mm)	0.244	0.002	0.266	0.002	132	-7.105	0.0001 *
width (mm)	0.031	0.001	0.037	0.001	132	-7.752	0.0001 *
Volume($E-4\text{mm}^3$)	5.36	0.158	9.27	0.158	132	-11.448	0.0001 *
Biomass($E-1\text{mm}^3$)	0.146	0.022	3.25	0.174	77	-17.439	0.0001 *

TABLE 2: The mean dimensions, volume and biomass of cercariae shed (during the 24 hr period 6 weeks after infection) from snails fed (A) restricted or (B) *ad libitum* TetraMin (Student's t-test of untransformed data; §=standard error of mean).

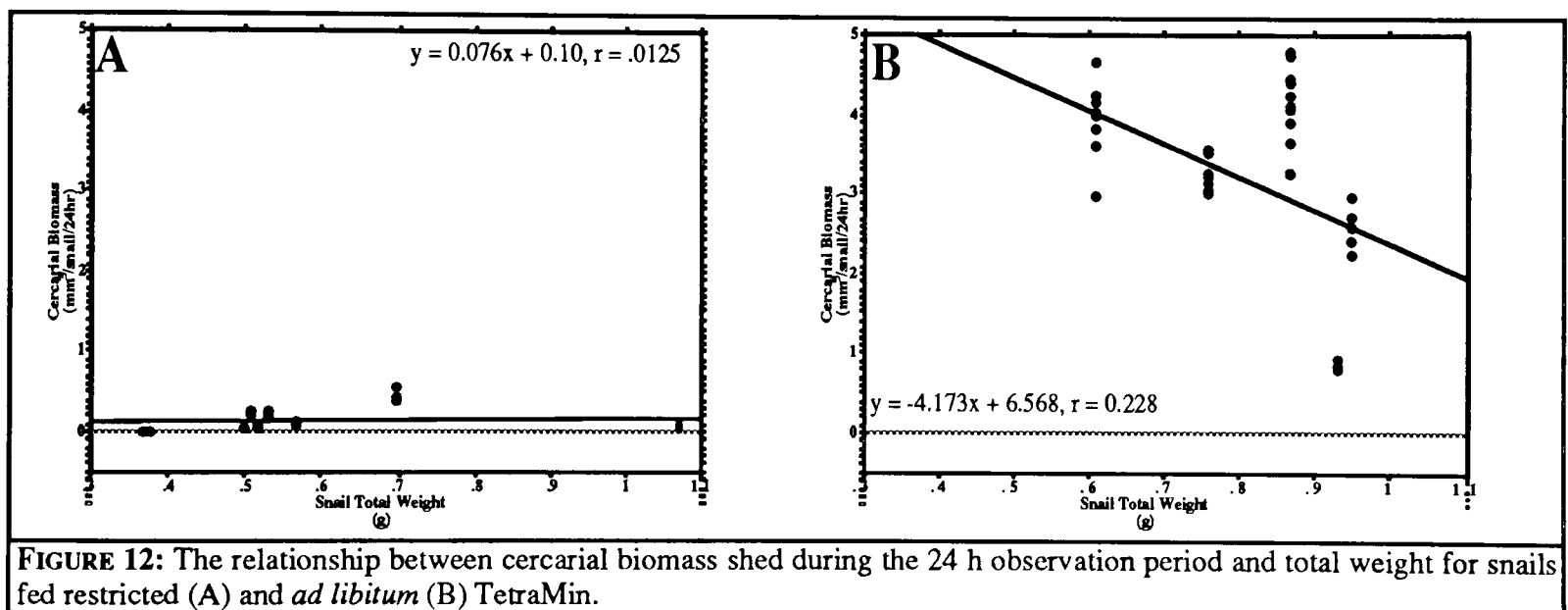
Cercarial Biomass

The product of 24 h shedding density and mean cercarial volume yields a crude relative estimate of the parasite biomass shed in the observed 24 h period of the sixth week following infection. From this estimate, well-fed snails produce a significantly greater biomass of parasites than do underfed snails (Table 2). The relationship between the size of well-fed snails and parasite biomass shed during this period shows that increasing shell diameter

(Figure 11; $r^2=0.302$, $df=39$, $p<0.0002^*$) and total weight (Figure 12; $r^2=0.228$, $df=39$, $p=0.002^*$) are significantly and negatively associated with the cercarial production: there was no significant relationship between parasite production and host size in underfed snails (Figures 11 & 12; shell diameter $r^2=0.009$, $df=38$ $p=0.569$; total weight $r^2=0.0125$, $df=38$,



$p=0.498$).



DISCUSSION

The results presented in this experiment are in general agreement with previously published evidence (see Introduction), and confirm the hypothesis generated above that changes within the snail host, such as those produced by fluctuations in host nutritional status, influence schistosome exploitation and thus parasite and host survival.

The Host

Experimental feeding was begun immediately after infection, and therefore all miracidia encountered similarly fed snails. The poor growth and reproduction observed in uninfected snails maintained on the restricted diet suggests that miracidia developing within similarly fed, infected animals did so under increasingly deficient conditions. The present experiment

shows that the harmful effects of schistosome infection differ markedly between snails fed different amounts of the same diet.

Mortality

Well-fed, infected snails suffered greater mortality than infected animals fed restricted diets. This is likely to be a result of greater tissue damage accompanying the tissue migrations (Pan, 1965) of larger numbers of cercariae emerging from well-fed hosts (see below).

Growth

Infection had little impact on snail growth, as measured by shell diameter and wet weight, although there was slight reductions 4 weeks after infection which tended to be greater in well-fed animals. This probably reflects the onset of cercarial emergence from infected snails, which was greater in well-fed animals (see below).

Fecundity

Infection produced a significant rise, followed by a rapid fall, in the fecundity of poorly fed snails. As there was no appreciable increase in the fecundity of well-fed snails following miracidial exposure, it appears that this previously reported response to schistosome challenge (Minchella, 1985; Minchella & Loverde, 1981) occurs most when resources are limited, perhaps resulting in maximal reproductive output before parasitic castration. Consistent with this notion is that a proportion of well-fed, infected snails remained fecund throughout the experiment, whilst fecundity ceased in infected snails maintained on the restricted diet.

Dietary restriction produced a significant increase in egg output in uninfected animals at week 3 of the experiment. Although egg production was initially higher, this increase did not occur in well-fed animals. Infected animals on both diets produced an increase in fecundity, followed by a more rapid decline in snails fed the restricted diets. It appears that both infection and dietary restriction elicit an increase in reproductive output in the weeks immediately following this stress. It may be that this response allows the snail to maximize reproductive output at a time when future conditions become increasingly stressful, a notion consistent with the theory that parasitism imposes starvation conditions on the snail host (see above).

The Parasite

Cercarial Number

Results clearly show that snails fed restricted diets produced significantly fewer cercariae during the experimental period than well-fed snails. As dietary restrictions were only

imposed after exposure to miracidia, it appears that these results reflect reduced replication of miracidia rather than reduced establishment.

In the presence of substantial snail tissue (which can be almost entirely consumed by the parasite during replication), the observed reductions in cercarial output suggest that suboptimal conditions, rather than a paucity of nutrients, within the undernourished snail limited fulminant parasite replication.

Cercarial Size

At the time of final examinations (6 weeks post-infection), cercariae which emerged from undernourished snails were significantly smaller than those leaving well-fed animals. As suggested above, it is likely this effect results from suboptimal host internal conditions. If cercarial size was limited by nutrient availability only, it is unlikely that size would fall whilst host tissue remained to fuel their production.

Cercarial Biomass

Decreased production and size of emergent cercariae from undernourished snails clearly illustrates the disadvantage snail stress imposes on miracidia which infect them. However at times when stress, such as undernutrition, might weaken the host, the ability of schistosomes to reduce exploitation and avoid killing the host, may allow the parasite to maximize use of the snail resource. Thus schistosome susceptibility to suboptimal host conditions may be advantageous in hosts subject to nutritional stresses, such as aestivation in snails (Kendall, 1949).

As interesting addendum is the increased survival observed in nutritionally stressed hosts, which likely resulted from reductions in tissue damage during cercarial migration and emergence (see above). Although the brevity of this experiment precluded examination, it may be interesting to follow the survival and reproductive success of undernourished snails in comparison to well-fed animals during their entire life spans.

THE INFLUENCE OF SNAIL NUTRITION ON *SCHISTOSOMA MANSONI* IN *BIOMPHALARIA GLABRATA*: II. AGE-RELATED CERCARIAL INFECTIVITY

Abstract

This experiment examines the influence of snail nutritional status on the age-related infectivity of emergent *S. mansoni* cercariae. It was hypothesized that, under natural conditions, the size of cercariae emerging from the snail host will be a primary determinant of their longevity, and thus infectivity. Results show that the infectivity of cercariae shed from snails fed restricted diets is significantly inferior to that of cercariae shed from well-fed snails. It is suggested this effect has an impact on schistosome transmission dynamics.

INTRODUCTION

Under natural conditions, it is thought that the ability of cercariae to maximize the longevity of orientation behaviors will be advantageous (see above). As cercariae do not feed once they have emerged from their snail host, it follows that the nutritional resources central to the temporal value of these behaviors must be acquired during intramolluscan development. It has been shown previously (Experiment 5.I) that cercariae emerging from snails fed restricted diets are smaller in all body components than those shed from well-fed snails. It is therefore suggested that, under natural conditions, snail nutritional status during cercariogenesis may be a major determinant of the ability of cercariae to successfully acquire a vertebrate host.

No previous study has examined the effects of snail nutrition on cercarial infectivity, despite the pivotal role miracidial success plays in schistosome transmission (see above). This experiment examines the influence of snail nutrition on the quality of emergent *S. mansoni* cercariae.

Hypothesis

That fluctuations in snail nutritional status influence the infectivity of emergent cercariae by limiting the energy resources required to fuel vertebrate host acquisition.

Emergent cercariae were collected, at a single time point, from snails fed differing dietary régimes and used to challenge *ad libitum*-fed mice after 2, then every 4 hours for 16 hours (total of 5 time points). The number of worms recovered from mice at autopsy 6 weeks later provided an estimate of the age-related change in infectivity of cercariae which had developed in snails fed either restricted amounts or *ad libitum* food.

EXPERIMENTAL DESIGN

The procedure culminating in the production of cercariae has been described previously (see Experiment 5.I). Five weeks following infection, cercariae were collected from snails in each of two dietary groups: snails fed restricted (0.03 g TetraMin.snail⁻¹.week⁻¹ (Dr)) or *ad libitum*

on TetraMin (TetraWerke) (*Dal*). The surviving infected snails from each of the two dietary groups (15 snails receiving restricted, and 10 receiving *ad libitum* TetraMin) were placed in two small glass containers (100 ml) and placed under 100 W bench lighting in 75 ml of preconditioned water. After 1.5 hours, the snails were removed; immediately and every 4 hours thereafter for 16 hours, the cercarial suspension was gently mixed and 100 cercariae drawn off (D) each container to paddle infect (Meier & Meier-Brook, 1981) each of five mice (all fed SLC *ad libitum* previous to, and for the duration of the experiment) for 0.5 h in 20 ml of preconditioned water (I.100; range 96-104). After 6 weeks, all mice were autopsied (At) as described in the Materials and Methods section of Chapter 2. The design of this experiment is presented in Table 1.

In the Snail (from Experiment 5.I) (n=number of snails in group)

Week Group (n)	-2	-1	0	1	2	3	4	5	6
A (20)	SD	→	Dr	E→	E→	E→	E→	E→	FE
B (20)	SD	→	<i>Dal</i>	E→	E→	E→	E→	E→	FE
C (20)	SD	→	Dr I.10	E→	E→	E→	E→	D↓E→	FE
D (20)	SD	→	<i>Dal</i> I.10	E→	E→	E→	E→	D↓E→	FE

Time Origin ^a	Age ^b					Group ^c (n)	Weeks ^d				
	0-2	4-6	8-10	12-14	16-18		0	1	2	~	6
Snl GrpC	100	→	-	-	→	1 (5)	I.100	→	-	→	At
Snl GrpC		100	→	-	→	2 (5)	I.100	→	-	→	At
Snl GrpC			100	→	→	3 (5)	I.100	→	-	→	At
Snl GrpC				100	→	4 (5)	I.100	→	-	→	At
Snl GrpC					100→	5 (5)	I.100	→	-	→	At
Snl GrpD	100	→	-	-	→	6 (5)	I.100	→	-	→	At
Snl GrpD		100	→	-	→	7 (5)	I.100	→	-	→	At
Snl GrpD			100	→	→	8 (5)	I.100	→	-	→	At
Snl GrpD				100	→	9 (5)	I.100	→	-	→	At
Snl GrpD					100→	10 (5)	I.100	→	-	→	At

^a Dietary group of snails from which cercariae originated (see Experiment 5.I).

^b Age of cercariae (in hours) post shedding from snails.

^c Groups of mice exposed to cercariae.

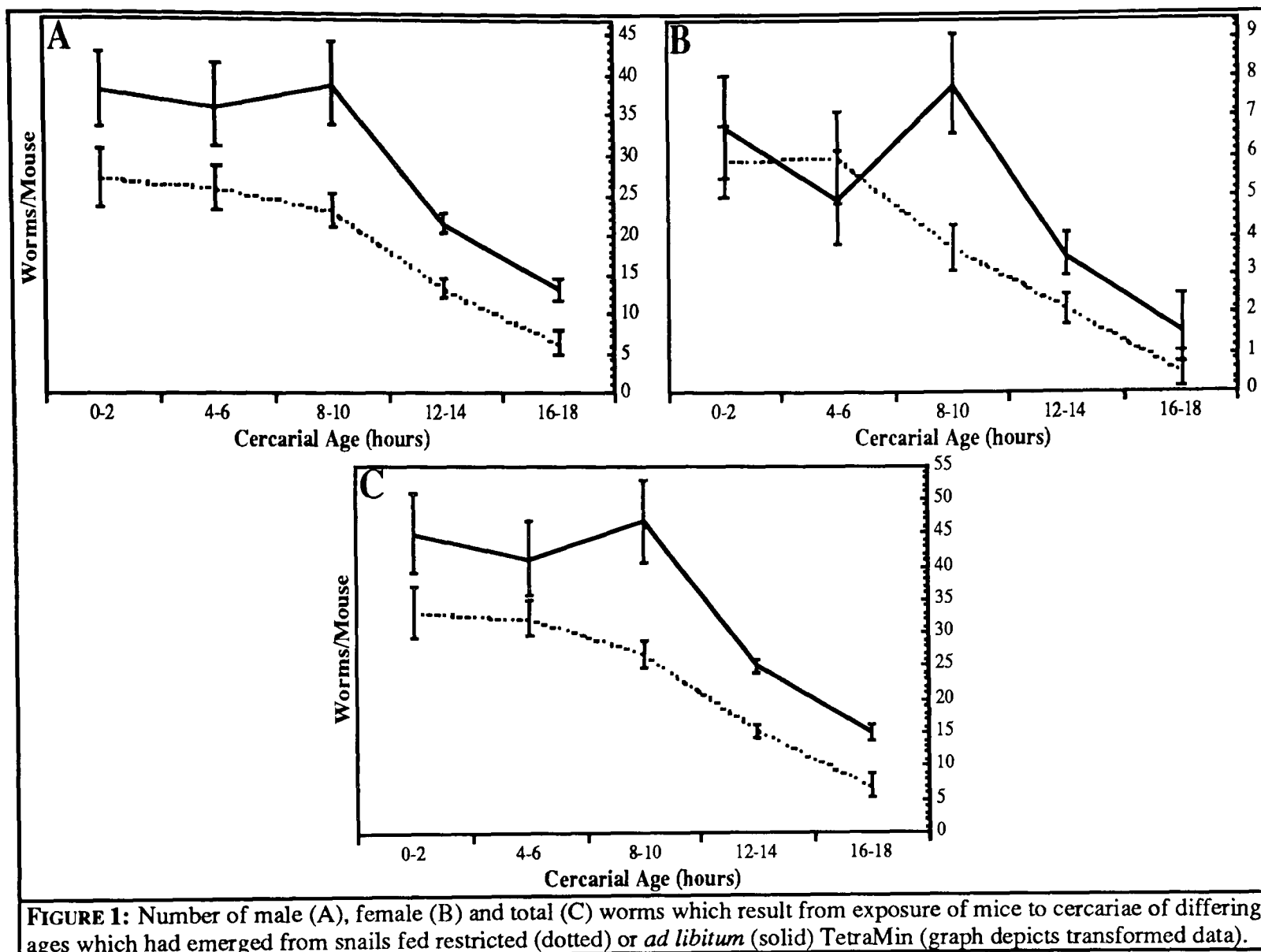
^d Weeks after exposure to cercariae.

n Number of mice in groups exposed to cercariae.

TABLE 1: Design of Cercarial Infectivity experiment

RESULTS: PARASITE

Number of Cercariae Developing to Worms



The proportion of cercariae emerging from well-fed snails which reached maturity in the experimental mice did not begin to fall appreciably until 8-10 hours post-shedding (Figure 1). Thereafter, the ability of aging cercariae to develop to adult worms fell markedly. This pattern was observed in male, female and total worm numbers originating from well-fed snails.

In contrast, the infectivity of cercariae (both male and female) shed from poorly fed snails began to decline from the first time point studied, and continued to fall sharply for the duration of the experimental period. Combined with the age-independent, general tendency for cercariae from poorly fed snails to be less successful at reaching maturity in the mouse (Figure 1), this steeper fall in infectivity produced statistically significant differences between cercarial groups (Table 2, overleaf); from 8 hours post-shedding onwards, cercariae shed from poorly fed snails were less likely to reach maturity in the vertebrate host than those which had emerged from well-fed snails.

Cercarial Age (hours)		0-2	4-6	8-10	12-14	16-18
Low Diet	Male range ¹ (n)	17-36 (5)	19-35 (5)	16-28 (5)	9-16 (5)	3-12 (5)
	Female range (n)	3-8 (5)	3-10 (5)	2-5 (5)	1-3 (5)	0-2 (5)
	Total range (n)	23-43 (5)	25-40 (5)	20-30 (5)	12-19 (5)	3-13 (5)
Comparison	Males	0.051(-1.84;8)	0.06(-1.74;8)	0.007*(-3.09;8)	0.001*(-4.51;8)	0.01*(-2.90;8)
	Females	0.29(-0.58;8)	0.27(0.64;8)	0.006*(-3.24;8)	0.03*(-2.15;8)	0.15(-1.12;8)
	Total Worms	0.07(-1.67;8)	0.09(-1.48;8)	0.005*(-3.40;8)	0.0002*(-6.21;8)	0.006*(-3.28;8)
High Diet	Male range (n)	27-53 (5)	23-52 (5)	28-54 (5)	19-26 (5)	9-17 (5)
	Female range (n)	5-12 (5)	2-7 (5)	4-11 (5)	2-5 (5)	0-4 (5)
	Total range (n)	32-65 (5)	29-59 (5)	32-65 (5)	23-28 (5)	11-20 (5)

TABLE 2: T-test comparisons (using transformed data) of number of worms recovered from mice exposed to cercariae shed by snails receiving differing amounts of TetraMin (central values represent p value followed by t value and degrees of freedom).

Worm Size

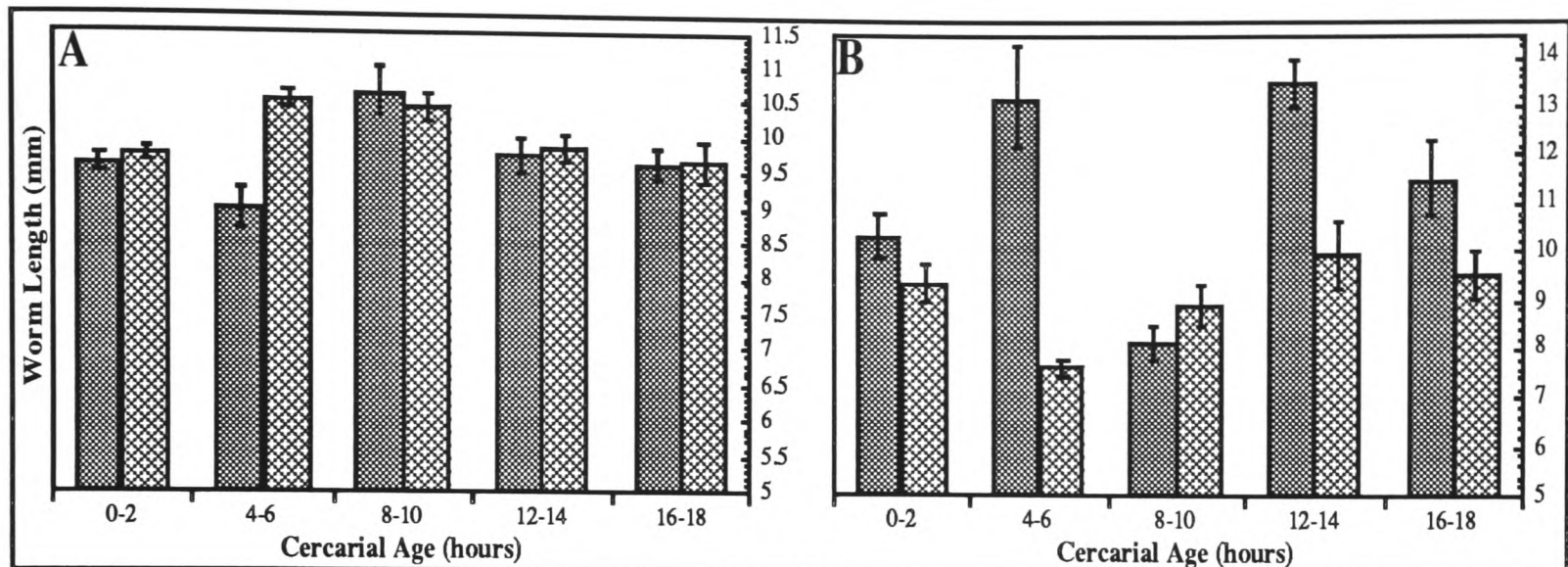


FIGURE 2: Length of male (A), female (B) worms which result from exposure of mice to cercariae of differing ages which had emerged from snails fed restricted (solid) or *ad libitum* (hatched) TetraMin (graph depicts transformed data).

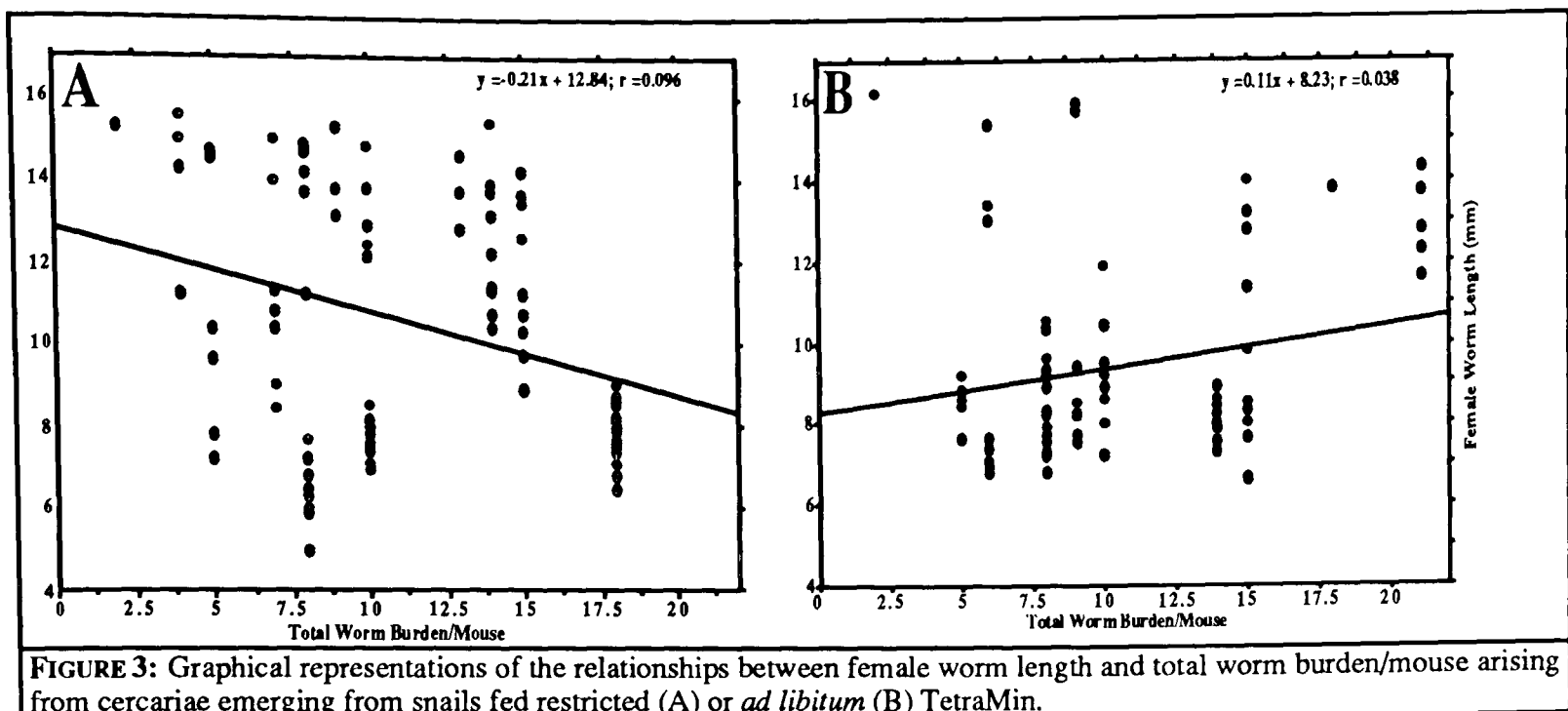
The nutritional status of snails in which cercariae developed had a variable effect on the sizes of resultant worms in the vertebrate host; male worms derived from cercariae shed 4 to 6 hours earlier by snails fed restricted diets were significantly smaller at autopsy than worms derived from cercariae of the same age produced by well-fed snails (Figure 2; Table 3). There were no differences in male sizes at any other time point. Conversely, female worms derived from cercariae shed by snails fed restricted diets were significantly longer than those derived from cercariae shed by well-fed snails at 4-6, 12-14 and 16-18 hours post-shedding.

Cercarial Age (hours)		0-2	4-6	8-10	12-14	16-18
Low Diet	Male range (mm) (n)	6.5-12.3 (20)	7.8-11.3 (20)	9.5-11.9 (20)	8.2-11.3 (20)	8.2-11.2 (20)
	Female range (mm)(n)	5-15.4 (20)	10.4-15.6 (20)	6.5-14.2 (19)	12.1-15.0 (10)	8.4-15.1 (4)
Comparison	Males ANOVA (df=199)	0.08	2.26*	0.03	0.01	0.001
	Females	0.06(1.61;90)	0.0001*(7.36;11)	0.23(-1.21;8)	0.004*(3.07;16)	0.02*(2.22;26)
	T-test					
High Diet	Male range (mm) (n)	8.1-12.1 (20)	9.4-12.2 (20)	9.3-12.0 (20)	8.2-11.1 (20)	7.8-13.4 (20)
	Female range (mm)(n)	6.6-15.4 (20)	6.8-8.3 (20)	6.1-14.8 (20)	7.2-16.2 (10)	7.5-15.9 (10)

TABLE 3: Statistical comparisons of sizes of worms (using transformed data) recovered from mice exposed to cercariae shed by snails receiving differing amounts of TetraMin (top central values represent ANOVA F values for male comparisons; lower central values represent t-test p values followed by t values and degrees of freedom for female comparisons).

FOOTNOTE

¹ Refers to range of untransformed data.



The tendency for female worm lengths to differ between groups, and the clear differences in the numbers of cercariae successfully reaching maturity prompted a comparison of female worm length with respect to total worm burdens using data from all cercarial ages. There was a significant negative correlation between the length of female worms arising from cercariae which had developed in poorly fed snails and total worm burden/mouse (Figure 3A; $p=0.003^*$); although clearly a tendency to the positive, no significant relationship existed for worms arising from cercariae shed by well-fed snails (Figure 3B; $p=0.08$).

RESULTS: HOST

No mice died during these experimental infections.

DISCUSSION

The results presented in this experiment support the hypothesis that fluctuations in snail nutritional status influence the infectivity of emergent cercariae by limiting the energy resources required to fuel vertebrate host acquisition.

The Parasite

Infectivity

True to the predictions which led to the formulation of the above hypothesis, there were significant differences recorded in the age-dependent infectivity of cercariae emerging from hosts maintained on different dietary regimes. In addition to being generally smaller than cercariae from well-fed snails (see Experiment 5.I), cercariae emerging from snails fed the restricted diet produced fewer worms in mice, with differences becoming more pronounced with cercarial age post-shedding.

It is likely that larger cercariae contain more metabolic resources needed to fuel the energetically expensive behaviours required to locate, attach to and penetrate a suitable vertebrate host. These resources are used initially to position the cercaria in the environment in a way to make host contact most likely; for example, cercariae can be seen actively maintaining their position in the water column. The longer such behaviours can be maintained, the higher the chances of the cercaria contacting a suitable host. Clearly, the larger cercariae emerging from well-fed snails are able to maintain host locating behaviours for a longer period of time than the smaller cercariae emerging from poorly fed snails. Thus their infectivity is maintained longer, as shown above.

It should also be noted that it is probable that the results presented in this experiment underestimate the differences in cercarial infectivity likely to exist in the field. As the paddle infection method of host exposure involves a shallow infecting chamber containing a mouse in contact with the bottom of the container, cercariae which were unable to maintain their position in the water column, having fallen to the bottom, may still have been able to infect the host, as cercariae lose the ability to swim before losing the ability to crawl and to penetrate host skin (Lawson & Wilson, 1983).

In addition to an age-related fall in cercarial infectivity, there appeared an underlying trend, consistent across all cercarial age classes, for the cercariae from poorly fed snails to slightly lower survival during infection of and maturation in the vertebrate host. This suggests that a higher proportion of cercariae shed from poorly fed snails are not viable; regardless of their age, some of these cercariae were not able to successfully reach maturity in a host, even if they did contact one. The design of this experiment was such that the relative contribution of failure at any specific point in this process could not be determined. Further study examining different periods of the host location and infection processes could provide more insight into this phenomenon.

Worm Size

Male worms developing from cercariae shed from snails fed differing diets were generally indistinguishable in size, although 4-6 hour old cercariae emerging from snails fed restricted diets grew to be smaller than male worms developing from cercariae shed from well-fed snails. In the absence of any trend, this result is difficult or impossible to explain.

Conversely, female worms developing from cercariae shed by snails fed restricted diets were generally longer than female worms developing from cercariae shed from well-fed snails. As the vertebrate hosts exposed to the two groups of cercariae were identical, it appears that either the size of adult female schistosomes may be related to their cercarial characteristics in some way or density-dependent constraints affected female sizes. As there was a negative relationship between female worm length and total worm burden in mice harboring worms

which developed from cercariae shed by poorly fed snails, it may be that the smaller number of worms in such mice led to reduced density-dependent constraints on female growth, allowing these worms to grow to a greater length than those in mice harboring a greater number of the worms which had developed from more infective cercariae originating from well-fed snails.

The Host

The development of schistosomes arising from cercariae emerging from snails fed differing diets had no effect upon the manifestations of infection observed in the vertebrate host.

5.8: CHAPTER DISCUSSION

This chapter examined the hypothesis that snail nutritional status is a major determinant of schistosome transmission success and snail population dynamics.

Experiments Summary

Results support the hypothesis and show that, in experimental infections, snail diet was a major determinant of the number of cercariae produced. This was thought to be due to nutritional deficiency producing conditions within the snail which were suboptimal for parasite development.

In addition, cercarial infectivity through time post-shedding was significantly influenced by snail diet, confirming indications provided by size differences between those produced by well-fed and those produced by poorly fed snails.

The suppressive effects of host deficiency on parasite emergence (both number and size) allowed for increased snail survival in the face of infection, however snail reproduction fell during the experimental period as a result.

In the Field

The above results suggest that factors which alter the conditions within infected snails can influence the outcome of the host/parasite relationship in terms of cercarial output and host survival and reproduction.

It has been previously suggested that the suppression of cercarial replication during times of host nutritional stress, such as snail aestivation, provides a mechanism ensuring maximal survival of both host and parasite when unrestrained parasite exploitation may be expected to kill the host and thus the parasite (Kendall, 1949). The results reported above confirm this suggestion.

It is interesting to imagine that, if host nutritional stress can protect the snail from parasite-induced mortality, the ability to induce this state might infer some advantage to snails which do. One way to reduce nutritional status is to alter feeding, and this has been well documented (Bayne & Loker, 1987). Alternatively, a burst of reproduction could rapidly induce deficiency. It may be that snails can actively induce internal changes in an effort to avoid parasite-induced death, albeit at the cost of impaired, short-term fecundity following the reproductive burst.

The observation that cercarial production is influenced by snail nutrition indicates that changes in snail habitats, such as seasonal fluctuations in food availability, may contribute to

the heterogeneity observed in infection dynamics. In a similar way, human activities which alter snail habitats may influence the production of cercariae.

Further, suppression of parasite development during snail aestivation may permit resurgence of infection following snail emergence, enabling the persistence of schistosome foci in seasonal areas.

Conclusions

Reduced parasite replication within the undernourished snail host is likely to influence parasite transmission dynamics. On the individual host level, this reduction during times of host stress may maximize both host and parasite survival.

CHAPTER SIX

MALARIA & HOST NUTRITION



“Show him death and he will be content with fever”

Old Persian Proverb



Chapter Summary

This chapter reviews experimental and field studies which examine the influence of host nutritional status on the parasite and host during malarial infection. The review indicates that a number of different mechanisms operate to suppress or promote malarial survival and replication in hosts suffering various nutrient deficiencies, and that refeeding commonly reverses the effects of deficiency. These observations have implications for the effective treatment and control of malaria in human populations suffering poor nutritional security and endemic plasmodial infection.

6.1: INTRODUCTION

Malarial parasites of the genus *Plasmodium* are sporozoan protozoa which undergo asexual replication in their vertebrate hosts. The present chapter examines the nutritional interaction between the plasmodia and their vertebrate hosts. Although this review refers largely to work on human plasmodia, the broad similarities in parasite development and disease aetiology observed between these and other apicomplexan species (Beaver, Jung & Cupp, 1984) permit some general extrapolation of the findings.

The plasmodia are well studied due to their medical and economic importance. By virtue of their wide distribution and the acute and debilitating nature of the diseases they induce, the plasmodia is generally considered to be the most damaging parasites of man.

Malaria & Host Nutrition: Mutually Inclusive

It is well established that the plasmodia have a significant impact on the nutritional status of their hosts (McGregor, 1982; 1988): in addition to competing with the host for essential nutrients, plasmodia may induce disease which impairs the host's ability to satisfy nutritional requirements.

As obligate endoparasites, the plasmodia are entirely dependent upon their hosts to provide suitable food and shelter. However malarial infection and disease, and the nutritional stress which results, commonly affect the characteristics of the resource upon which the parasites themselves depend (Harinasuta & Bunnag, 1988). The complex, and fundamentally nutritional nature of their relationship suggests that to view either malarial infection or host nutrition in isolation is artificial. In attempting to understand the complex relationship between the plasmodia and host nutrition, it is useful to begin by examining the effects each has upon the other.

6.2: THE EFFECTS OF MALARIA ON HOST NUTRITIONAL STATUS

Malaria is extremely costly to the host in terms of nutrition. The majority of this cost is a result of host-mediated changes in tissue structure and function which conspire to radically reduce host nutritional status. Reviews of the influence of malarial infection and disease on host nutritional status have appeared previously (McGregor, 1982; 1988). The converse effect, that of host nutritional status on malaria, has received much attention during the past 50 years. Although previous studies have clearly shown that host nutritional status has a marked impact on plasmodial success and host disease, there currently exists little, if any consensus. The following review seeks to consolidate the evidence on this aspect of malarial infection in order to facilitate further study. The present chapter has been specifically included in this thesis so that this exhaustive literature search is accessible to workers in this field.

6.3: THE EFFECTS OF HOST NUTRITIONAL STATUS ON MALARIA

Plasmodial Survival & Replicative Success

The following sections review the influence of specific nutritional conditions on the development and expression of malaria and discuss them in relation to possible implications for health policy. The five nutrients (*para*-aminobenzoic acid, riboflavin, protein, vitamin E and iron), which have received the major share of attention, are dealt with separately.

PARA-AMINOBENZOIC ACID

P-aminobenzoic acid (*p*-ABA) is one of three precursors to folic acid, which in its enzyme form is essential for many important biosynthetic reactions (Garnham, 1966).

Evidence that *p*-ABA is important for the malarial parasite first arose in the 1940s with the demonstration that malarial inhibition by sulphonamides *in vivo* (Maier & Riley, 1942) and sulphadiazine *in vitro* (Ball, 1946; Ball, Anfinsen, Geiman & Ormsbee, 1945) was reversed by the addition of *p*-ABA. Geiman & McKee (1948) subsequently showed that malarial suppression resulting from 1-2 days of host starvation was similarly reversed by *p*-ABA supplementation. Therefore not only was the activity of sulpha drugs dependent upon host *p*-ABA status, but this nutrient was essential for malarial growth.

The first applied indication of malaria's dietary dependence was furnished by Maegraith (1953) and colleagues (Maegraith, Deegan & Jones, 1952) with the demonstration that rats fed diets comprised solely of milk developed lower parasitaemias when inoculated with *P. berghei* than animals maintained on complete diets. Although the parasites were not completely eliminated in the deficient hosts, a potentially fatal disease was converted into an unapparent infection.

Other workers have since confirmed these results by feeding milk diets to various experimental hosts and observing malarial suppression (Adler & Gunders, 1965; Bray & Garnham, 1953; Ferraroni, 1983; Hawking, 1953; 1954; Kretschmar, 1966; Mackerras, 1953; Nowell, 1970; Raffaele & Carrescia, 1954; Ramakrishnan, 1954b; Ramakrishnan, Bhatnagar, Prakash & Misra, 1953; Refaat & Bray, 1953; Schneider & Montezin, 1953). However it was Hawking (1954) who first showed that *p*-ABA deficiency plays a key role in this phenomenon. Working with *P. berghei* in rats and *P. cynomolgi* in monkeys, malarial suppression which resulted from feeding solely milk diets was reversed by dietary supplementation with *p*-ABA. Hosts regained their susceptibility to the parasite following refeeding (Hawking, 1954), implicating this nutrient as essential for parasite replication.

Other workers have since shown that specific *p*-ABA deficiencies in various experimental hosts confer protection against malaria, both when infections are begun in hosts with established deficiencies (Adler & Gunders, 1965; Al-Aday, 1983; Ferraroni, 1983; Gilbertson, Maegraith & Fletcher, 1970; Jacobs, 1964; Kretschmar, 1966; Rindi & Azimi, 1980; Zainal-Abidin & Cox, 1988) and in hosts experiencing dietary changes during infection (Zainal-Abidin & Cox, 1988). Suppression of malaria in *p*-ABA deficient hosts has now been demonstrated in *P. berghei*, *P. chabaudi*, *P. cynomolgi*, *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. vinckei*, and *P. yoelli* (Al-Aday, 1983), however exclusion of a species from this list does not indicate that the *p*-ABA effect does not occur.

Despite reductions in parasite density as a result of dietary suppression, it appears that the development of acquired immunity to malaria proceeds normally (Adler & Gunders, 1965; Ferraroni, 1983; Gilbertson *et al.*, 1970; Kretschmar, 1966; Zainal-Abidin & Cox, 1988). The unique study of Ferraroni (1983) showed that immunoglobulin activity was unaffected by *p*-ABA deficiency of mice, despite marked suppression of parasitaemias. Immunity in deficient animals was acquired regardless of initial inoculum. Blood taken from *p*-ABA-deficient mice exhibiting low grade, sub-clinical infections produced severe malaria and high mortality when inoculated into naive, well-fed mice (Ferraroni, 1983). These results suggest that the plasmodia maintain virulence during nutritional suppression, and are capable of inciting fulminant disease when host conditions permit.

Jacobs (1964) has shown that feeding mice diets devoid of *p*-ABA led to almost total suppression of *P. berghei*, whilst boosting dietary levels of *p*-ABA saw associated increases in parasitaemias. Other workers have demonstrated similar reversal of dietary suppression upon supplementation (Hawking, 1954; Refaat & Bray, 1953; Zainal-Abidin & Cox, 1988).

Despite obvious success in the nutritional suppression of malaria both *in vitro* and *in vivo*, other results have clearly demonstrated the potential for parasites to circumvent dietary suppression. For instance, some sulphonamide-resistant strains of *P. berghei* grow well in mice fed milk diets (see Ferone, 1977). It may be that selection favours plasmodia more able

to deal with *p*-ABA deficiency, when differences in the ability to use low levels of *p*-ABA exist between parasite strains. In such cases, supplementation of chronically deficient hosts, in which parasites are adapted to optimal replication limited by the availability of *p*-ABA, might lead to higher than normal densities of plasmodia and hence disease.

Correcting deficiencies also potentially reduces the useful life of antimalarials, as increasing levels of *p*-ABA have been shown to accelerate the development of *P. berghei* resistance to sulphadoxine and pyrimethamine (Merkli & Richle, 1983). Such an observation might be explained by a simple dietary-induced increase in parasite replication leading to a greater rate of selection for drug resistance.

Human Evidence

Shortly after the early experimental demonstration that milk diets suppressed malaria, a number of workers attempted to demonstrate these effects in human subjects fed exclusively on milk (Chaudhuri & Chakravarty, 1953; Chaudhuri & Dutta, 1955; Kretschmar, 1966; McGregor, 1956; Miller, 1954). These studies failed, however, to produce consistent and convincing results.

Despite the suggestions of several authors (Edington, 1967; Hawking, 1953; McGregor, 1988) that neonates fed diets comprised solely of breastmilk develop *p*-ABA deficiencies, which in concert with other factors such as the passive transfer of maternal antibodies, inhibit the growth of infections during the first few months of life, confirmatory evidence of this effect is still lacking.

Whilst Colbourne and Sowah (1956) did not record significantly increased malarial prevalences during studies of breast-fed neonates supplemented with *p*-ABA, yeast supplements containing *p*-ABA led to increased susceptibility to malaria in 3-6 month old children.

Mechanisms

The mechanisms involved in these observations appear straightforward. That *p*-ABA deficiencies suppress, and supplements increase malaria suggests that the parasite has a greater requirement for this nutrient than the host. It appears that unlike their hosts, malarial parasites are unable to use intact folates and must synthesize their requirements *de novo* from folate precursors. As humans can use intact folates from the diet, *p*-ABA is not an essential nutrient provided dietary folate intake is adequate (Ferone, 1977). In theory then, hosts suffer less from *p*-ABA deficiency than does malaria, whilst the parasites exhibit immunity to folate deficiencies which can affect humans. This notion is supported by studies in which avian (Seeler & Ott, 1945a; 1946; Trager, 1958) and rodent malarial (Brabin, 1982;

Ramakrishnan, 1954a) exhibit increased growth in hosts deficient in folic acid, indicating that the host has a greater requirement for this nutrient than the parasites.

Implications for Refeeding

Food stuffs with high *p*-ABA content are therefore unsuitable supplements for populations where malaria is present. Examples of such foods are yeast supplements which are commonly given to young children (Colbourne & Sowah, 1956). Where dietary folate deficiencies exist, intact folates should be supplemented.

Implications for Chemotherapy

It follows then that any condition, such as the hormonal disorder producing hypothyroidism, which blocks folate synthesis will act to suppress malarial growth whilst having little effect upon the host. The activity of sulpha drugs has thus been linked to their abilities to compete with *p*-ABA for active enzyme binding sites (Ferone, 1977). Therefore host *p*-ABA status can influence the activity of antimalarials which employ competitive inhibition of malarial folate synthesis.

Given that the activity of some antimalarials relies on competitive blocking of folate synthesis, it is recommended that such drugs be administered to individuals with low precursor levels and/or together with other analogues to folate precursors. Their use is not recommended in individuals exhibiting high folate precursor levels. Despite such caution, the extent of the problem remains unknown and demands further study.

In summary, though non-essential for humans, *p*-ABA appears essential for malarial survival. Clearly, where folate deficiencies occur, it is imperative that foods rich in intact folates, and devoid of *p*-ABA and other folate precursors be administered, as a dietary increase in *p*-ABA potentially increases malarial disease, increases neonatal susceptibility to infection, compromises drug use and accelerates the development of drug resistance. In contrast, supplementing intact folates will lower deficiency disease without exacerbating malaria, thus producing a net reduction in disease.

RIBOFLAVIN

In its coenzyme forms, riboflavin (vitamin B₂) is an essential component of various cellular oxidative enzyme systems (Sauberlich, 1985).

Experimental Observations

Seeler and Ott (1944) were the first to demonstrate experimentally the suppressive effect of riboflavin deficiency on the development of *P. lophurae* in chickens. These results were

subsequently confirmed by Rama Rao and Sirsi (1956b) who also showed that deficiency-induced suppression was reversed by administration of parenteral riboflavin.

Human Evidence

Renewed interest in the relationship between riboflavin status and malaria appeared in the early 1980s with the observation that riboflavin status in children correlated positively with malarial parasitaemia and disease (Das, Das, Satpathy, Patnaik & Bose, 1988; Thurnham, Oppenheimer & Bull, 1983). The observed negative correlation between riboflavin deficiency and parasitaemia in rats infected with *P. berghei* (Kaikai & Thurnham, 1983) lends support to these observations.

Given the indications that riboflavin deficiencies are extensive throughout tropical countries, and that riboflavin deficiency protects young children from malaria (Thurnham *et al.*, 1983), it has been suggested that the apparently severe riboflavin deficiency at birth in some tropical countries may be more than simply a coincidence, but a physiological adaptation in areas where riboflavin deficiency and malaria are common (Thurnham *et al.*, 1983). Increasing riboflavin levels in the presence of disease potentially removes this advantage. Differences in nutritional oxidative stress may help explain variable levels of human resistance to malaria observed in the field (Friedman, 1979).

Mechanisms

The mechanisms putatively involved in malarial suppression during riboflavin deficiency have been the subject of recent reviews (Dutta, Pinto & Rivlin, 1985; 1986), and chiefly comprise the premature lysis of deficient, infected erythrocytes. Due to riboflavin's role as an essential erythrocytic antioxidant, deficiency of this nutrient predisposes erythrocytes to oxidative stress (Friedman, 1979). As malaria increases oxidative stress in infected erythrocytes by the production of free oxygen radicals, it is thought that concomitant riboflavin deficiency and malarial infection lead to lysis of the erythrocyte before the plasmodia are mature and infective for other cells, thereby inhibiting continued replication (Thurnham, 1985a). Therefore, any reduction of host antioxidant, or increase in pro-oxidant status, potentially inhibits normal malarial development.

Implications for Refeeding

As limited amounts of riboflavin are stored in the body, and dietary items rich in this vitamin are often in short supply, riboflavin deficiency is relatively common throughout the world (Sauberlich, 1985). Given the evidence described above, it is suggested that correction of riboflavin, or other antioxidant deficiencies in the presence of active infection can exacerbate disease. The provision of riboflavin-rich foods, such as milk and other dairy products

(Sauberlich, 1985), in response to deficiency should be accompanied by antimalarial drugs which do not employ the generation of free radicals to effect cure.

Implications for Chemotherapy

Recent work (Dutta, Gee, Rivlin & Pinto, 1988; Dutta *et al.*, 1986; Dutta, Pinto, Raiczuk & Rivlin, 1987) has attributed the antimalarial properties of some drugs to their generation of free oxygen radicals, which exacerbate erythrocytic oxidative stress and promote premature cell lysis. Given that antioxidants mediate erythrocytic oxidative stress, and that certain drugs act as pro-oxidants, it follows that the host's riboflavin status will not only influence the progression of malaria but also influence the activity of drugs which employ the lytic mode of action. It follows then that host antioxidant levels must be monitored during drug trials. Further, the use of riboflavin analogues to induce deficiency and hence increase the probability of infected cells lysing prior to malarial maturation has been suggested as a potentially fertile area for future antimalarial drug research (Dutta *et al.*, 1986).

Thus, antimalarial drugs which effect cure by the generation of free oxygen radicals should not be administered to populations with high cellular antioxidant status. In contrast, the administration of dietary pro-oxidants such as cod liver oil may synergistically increase the efficacy of drugs inducing cellular oxidant stress (Levander, Ager, Morris & May, 1989d).

PROTEIN

Protein is an essential nutrient for all manner of biological functions, and has been the subject of numerous reviews (see Torún & Viteri, 1985). It is well known that man's physiology is influenced by protein intake. There is much evidence too, from both the field and the laboratory, that protein deficiencies influence the development and expression of malaria.

Experimental Observations

The first recorded effects of protein status and malaria were observed in avian malaria, and showed a positive correlation between host deficiency and parasitaemia (Seeler & Ott, 1945b; 1946). Al-Aday (1983) has since shown a similar effect in rodent malarias. All other studies have indicated that whilst the induction of protein undernutrition in experimental hosts protects them from severe malaria and death (Edirisinghe, Fern & Targett, 1981a; 1981b; 1982; Fern, Edirisinghe & Targett, 1984; Keshavarz-Valian, Alger & Boissonneault, 1985; Platt, Dema & Miller, 1959; Refaat & Bray, 1953; Targett, 1981), the development of acquired immunity proceeds normally, appearing more dependent upon duration of infection than parasite density (Edirisinghe *et al.*, 1982).

As the induction of specific amino acid deficiencies by the administration of analogues can suppress malarial parasitaemias (Taylor, 1956), it can be concluded that the composition of dietary protein is central to these effects. Conversely, the addition of specific amino acids, such as isoleucine (Edirisinghe *et al.*, 1981a; Fern *et al.*, 1984), methionine (Fern *et al.*, 1984; Keshavarz-Valian *et al.*, 1985; McKee & Geiman, 1948), pyridoxine (Ramakrishnan, 1954a), threonine (Edirisinghe *et al.*, 1981a; Fern *et al.*, 1984; Keshavarz-Valian *et al.*, 1985), valine (Edirisinghe *et al.*, 1981a; Fern *et al.*, 1984) and methionine and threonine together (Keshavarz-Valian *et al.*, 1985) to deficient host diets produces rapid reversal of deficiency-induced malarial suppression, leading to marked increases in infection intensities. The speed with which reversal of parasitaemias proceeds following the provision of supplementary amino acids suggests that increases in parasite numbers are not due to changes in host immunity, but result from the correction of specific deficiencies which had been restricting parasite replication (Thurnham, 1985b).

An additional factor in this complex interplay is host immunity, which is under dietary influence. That the intra-uterine transfer of maternal antibodies to offspring suffers during parental protein deficiency, increasing susceptibility to malarial parasitaemias in offspring (Salimonu, Adeiga & Renu, 1989), suggests that an increase in host immune susceptibility to malaria may be overcome by the constraints on parasite replication imposed by host protein deficiency.

Human Evidence

Anecdotal evidence linking protein undernutrition to malarial suppression in humans is provided by observations reporting reductions in clinical malaria during times of famine (Edington, 1967; Hendrickse, Hasan, Olumide & Akinkunmi, 1971; McGregor, 1982; 1988; Moore, Heyworth & Brown, 1974). Suppressed parasitaemias, whilst protecting the hosts from severe malaria, allowed the development of specific acquired immunity to proceed normally (Brown & Opio, 1966; McGregor, Williams, Voller & Billewicz, 1965). However the multiple deficiencies characteristic of famine (Rohde & Chen, 1985) make specific nutritional cause and effect difficult.

Mechanisms

It has been suggested that the suppression of malarial parasitaemias during protein deficiencies results from physical changes in the host internal milieu and a paucity of available nutrients to the parasite. For example Ahmad, Moonis, Shahab, Khan and Jilani (1985) have mooted that the fatty infiltration of the liver seen in acute protein-energy undernutrition might adversely influence the pre-erythrocytic phase of plasmodial development.

In summary, it appears that dietary protein deficiencies inhibit the development of high parasitaemias, whilst refeeding sees recrudescences of infection. In addition, despite the suppression of parasite densities and disease, the development of acquired immunity proceeds normally.

Implications for Refeeding

Protein and energy undernutrition is the most important deficiency in developing countries. Humans are able to adapt to chronic deficiency by altering metabolic activities. Despite increased susceptibility to malarial infection, during chronic deficiency, the clinical manifestations of infection tend to be mild. Upon refeeding, rapid changes in protein status can seriously affect health (Torún & Viteri, 1985). It is suggested then that rapid increases in chronically protein deficient nutrition be avoided in the presence of malaria. Food containing high levels of protein should be avoided for these reasons.

VITAMIN E

Vitamin E is an essential nutrient and an important biological antioxidant, protecting cells from oxidative stress imposed by excessive activity of oxygen, iron, unsaturated fatty acids and other agents.

Experimental Observations

The early demonstration that the suppressive effect of dietary cod liver oil on malaria could be eliminated by the addition of vitamin E to the diet (Godfrey, 1957) suggested that this nutrient was essential to the malarial parasite, and that the antimalarial properties of cod liver oil were related to the antagonism of vitamin E.

A number of laboratory studies have demonstrated that induced vitamin E deficiency suppresses the development of malaria (Ager, Levander, Fontela & Morris, 1990; Ager, Levander, May & Morris, 1988; 1989; Eaton, Eckman, Berger & Jacobs, 1976; Eckman, Eaton & Jacob, 1976; Levander, Ager, Morris & May, 1988; 1989a; 1990a; 1990b; 1989b; 1989c; Levander *et al.*, 1989d; Levander, Ager, Morris & May, 1989e; 1990c; Omwega, Bates & Millward, 1988). Further, the addition of supplementary vitamin E to animals during deficiency-induced malarial suppression saw recrudescences of malaria, with consequent increases in disease (Ager *et al.*, 1989; Eckman *et al.*, 1976).

Human Evidence

Little was thought of these effects until epidemiological evidence arose suggesting that vitamin E deficiency may limit the severity of malaria in deficient African populations (Allison, 1984; Eckman *et al.*, 1976). Support for these studies recently came from Africa, where Docampo and Mareno (1984) noted that severely undernourished nomadic Africans,

whose dietary intake of vitamin E might have been low, showed evidence of *P. falciparum* recrudescence following the provision of cereal-based diets. The increase in vitamin E status accompanying the feeding of wheatgerm, a rich source of vitamin E, was thought to reverse the dietary suppression of malaria (Docampo & Mareno, 1984), although other nutrients present in the cereals may have contributed to the observed effects.

Mechanisms

The mechanisms involved in malarial suppression during vitamin E deficiency are similar to those responsible for suppression during riboflavin deficiency. Vitamin E is a potent antioxidant, and deficiency can therefore lead to unchecked oxidant stress in infected erythrocytes which lyse before malarial maturation is complete (Levander *et al.*, 1988).

Implications for Refeeding

The implications for populations suffering endemic malaria are obvious. Increases in vitamin E may enhance survival of intraerythrocytic stages of malaria, and allow clinical intensities to develop. Conversely, reductions will see less malarial replication and disease. However vitamin E deficiency is rare in human populations (Fitch, 1985) and thus vitamin E supplementation is also rare. The protective effect of antioxidant deficiencies can be paralleled by the provision of pro-oxidant foods, such as fava beans (Huheey & Martin, 1975), or certain seed and fish oils (Levander, personal communication) which increase oxidant stress and thus act synergistically to lower malaria .

Implications for Chemotherapy

It appears that some new antimalarial drugs owe their activity to the generation of intraerythrocytic free oxygen radicals, which exacerbate the increased oxidant stress induced by malarial presence, leading to lysis of oxidant-stress sensitized infected erythrocytes before the parasite merozoites are fully formed and infective to a new cell (Crompton, 1989). The status of host pro- and antioxidants, such as vitamin E, which mediate the oxidant stress in the cells, have been shown to affect the activity of these drugs (see work by Levander and colleagues).

The possible future effectiveness of drugs which induce erythrocytic lysis of oxidant stress-sensitized infected cells, thereby limiting parasite replication, will rely on host anti- and prooxidant status. For example, antimalarial drug activity was observed to fall following the addition of α -tocopherol (a form of vitamin E) to cultures (Krungkrai & Yuthavong, 1987). Conversely, drug activity (for example qinghaosu) can be potentiated by the inducing host vitamin E deficiency (Levander *et al.*, 1988; 1989e).

IRON

Iron is an indispensable micronutrient to almost all living organisms, and today, iron deficiency is the one of the commonest nutrient deficiencies of man (Oppenheimer, 1989). A number of detailed reviews have well described the close association between host iron status and infection (Hershko, Peto & Weatherall, 1988; Keusch & Farthing, 1986; Oppenheimer & Hendrickse, 1983; Peto & Hershko, 1989), noting that both hypo- and hyperferraemic states affect the host/parasite association.

Experimental Observations

Experimental work has shown a substantial reduction of mortality in hypoferraemic mice infected with *P. berghei* (Murray, Murray, Murray & Murray, 1978a). *In vitro* investigations, using iron chelators (reviewed by Peto & Hershko, 1989) such as desferrioxamine to induce deficiency, have been able to demonstrate malarial suppression (Clark, Cowden & Butcher, 1983; Fritsch, Dieckmann & Menz, 1987; Hershko *et al.*, 1988; Peto & Thompson, 1986; Pollack, Rossan, Davidson & Escjadillo, 1987; Raventos-Suarez, Pollack & Nagel, 1982), and similar effects have been seen *in vivo*, followed by malarial recrudescence when mouse iron status was enhanced by supplementation (Harvey, Bell & Nesheim, 1985; Smith, Hendrickse, Harrison, Hayes & Greenwood, 1989).

Human Evidence

Despite long held anecdotal evidence associating iron repletion and resurgence of infection (Murray *et al.*, 1978a), current interest in the applied effects of iron status on malaria was initially stimulated by Byles and D'Sa (1970) who reported that iron supplementation in pregnant women led to increased incidence of malaria, putatively due to the blockage of the reticuloendothelial system by the large iron dextran molecule. Other reports of increased malarial disease (by recrudescence of low grade, subclinical infections and also by increased prevalence) in undernourished Africans following provision of iron therapy (Brabin, 1983; Masawe, Muindi & Swai, 1974; Murray, Murray, Murray & Murray, 1975; 1978a; 1978b), and similar long-term indications from infants in Papua New Guinea (PNG) receiving intramuscular supplements (Oppenheimer & Hendrickse, 1983; Oppenheimer, Gibson, Macfarland, Moody & Hendrickse, 1984; Oppenheimer, Gibson, MacFarlane, Moody, Harrison, Spencer & Bunari, 1986a; Oppenheimer, MacFarlane, Moody, Bunari & Hendrickse, 1986b) and pregnant African women (Brabin, 1983), have led Oppenheimer (1989) and colleagues (1986a; 1986b) to warn that iron therapy, especially in neonates, infants and pregnant women, may increase the risk of clinical malaria. The similarity in those infected and those likely to receive supplements made this point more important.

Oppenheimer and colleagues (1986a; 1986b) have reported increased prevalences of malarial parasitaemias, and splenomegaly, as well as other infections, in children 6 and 12 months after iron supplementation. They have also correlated birth haemoglobin with malarial disease. Similar work in PNG did not support this concern (Harvey, Heywood, Nesheim & Alpers, 1987a; 1987b; Harvey, Heywood, Nesheim, Galme, Zegans, Habicht, Stephenson, Radimer, Brabin, Forsyth & Alpers, 1989), as no difference was detected between groups receiving iron therapy and those receiving placebos.

Mechanisms

Reviews of the mechanisms involved in malarial inhibition during host iron deficiency have been presented elsewhere (Hershko *et al.*, 1988; Keusch & Farthing, 1986; Oppenheimer & Hendrickse, 1983). Although still not fully understood, it is believed that these generally involve disruption of erythrocyte dynamics and survival during coincident deficiency and infection.

Implications for Refeeding

It has been suggested that in Somali nomads, observed iron deficiency may be part of an "ecological compromise", permitting optimal cosurvival of host and infecting agent (Murray *et al.*, 1978a), however a predominantly milk diet may have also predisposed them to less malaria by creating *p*-ABA deficiency (see above). The evidence presented by Murray and colleagues (1978a) is the best so far by a prospective, randomized clinical study.

Given the global prevalence of iron deficiency, its close antagonistic relationship with malaria, and the extent of global iron supplementation policies, it must be seen as an immediate priority to include the coincidence and effects of malaria upon interventions designed to increase iron status (Hershko *et al.*, 1988; Oppenheimer & Hendrickse, 1983). As much of the anaemia associated with iron deficiency is secondary to infection with malaria, and given that subclinical malaria may respond to host iron supplementation, it seems essential to treat individuals prior to iron therapy (Oppenheimer & Hendrickse, 1983). Since those groups of individuals with a high risk of anaemia secondary to malaria (infants, toddlers and mothers) are also selectively those more likely to receive iron either as a supplement or as presumptive treatment for anaemia, presupplementary treatment appears obligatory (Oppenheimer & Hendrickse, 1983). Indeed, it may be that the removal of infection will allow such improvements in iron status that the provision of iron supplements may be unnecessary. Treatment will, in any case, remove the dangers of recrudescence malaria following iron therapy. Despite the above evidence to the contrary, McGregor (1982) stated that he has given iron to many people with asymptomatic parasitaemias and noted no exacerbation of malaria.

Implications for Chemotherapy

The possible use of iron chelating drugs as effective antimalarials is an exciting challenge to future clinical research, of which malaria may be the first example (Peto & Hershko, 1989).

OTHER SINGLE NUTRIENTS

Much evidence exists that malaria is influenced by numerous other specific nutrients. However, from the few studies done, and their inconsistent nature, it appears that much important work remains to be done. The following lists nutrients which have been associated with malarial success.

Antagonism with Undernutrition

A number of studies have shown that deficiencies of various nutrients suppress malaria.

It appears that high levels of exogenous ATP are required for the *in vitro* maintenance of malaria, and that the parasite has, in its extracellular stages, a need for glutathione, coenzyme A, and high levels of nicotinamide (Trager, 1977; Trager & Jensen, 1980). Brewer and Powell (1965) have shown that the time required for *P. falciparum* to reach a parasitaemia of 1000 parasites/mm³ in human volunteers was significantly correlated to erythrocytic ATP levels, which led them to speculate that low cellular levels of ATP might be adaptive in the presence of malarial infection.

Malaria has demonstrated a need for *in vivo* biotin. Induced biotin deficiencies have been shown to suppress the rate and extent of parasite replication in experimental animals (Rama Rao & Sirsi, 1956b; Ramakrishnan, 1954a; 1954b; Trager, 1943b; 1947), whilst supplementation favours reversal of suppression, and accelerated plasmodial growth (Rama Rao & Sirsi, 1956b; Siddiqui, Schnell & Geiman, 1969). It appears that malaria requires biotin at concentrations higher than those at which the host is adversely affected. Hence analogues of biotin would appear to be potentially effective antimalarial drugs.

Calcium, and factors such as quinine which affect ion availability, appear to be nutritional factors affecting malaria, as reviewed by Scheibel, Colombani, Hess, Aikawa, Atkinson, Igarashi, Matsumoto and Milhous (1989). Menadione (vitamin K) increased oxidant stress and therefore malarial suppression which was blocked by dithiothreitol (Friedman, 1979).

Rats fed diets high in unsaturated fats and oils were observed to have reduced parasitaemias (Ramakrishnan, 1954a), and plant and fish oils high in unsaturated fats have also been effective in malarial suppression, a subject reviewed by Levander and colleagues (Levander *et al.*, 1989a; 1989b; 1989c; 1989d). Saturated oils do not protect when vitamin E is lacking as do diets rich in unsaturated oils (Levander *et al.*, 1989a). Cod liver oil (a vitamin E

antagonist and prooxidant) so increases oxidant stress on RBCs that drugs which generate free oxygen radicals do not have further therapeutic benefits (Levander *et al.*, 1989d).

Pantothenate has long been recognized as an essential factor for malarial growth. Trager (1943a; 1943b; 1977) has shown that withdrawal of coenzyme A, the active form of pantothenate, or the addition of pantothenate antimetabolites (Trager & Jensen, 1980), will inhibit *in vivo* and *in vitro* replication, sexual activity and infectivity of avian and primate malarias. It appears that malarial parasites rely completely on the biosynthesis of coenzyme A by the host erythrocytes. This "biosynthetic lesion" points to the potential exploitation of these parasite inabilities, by reducing the availability of essential metabolites to parasites.

The results of Brackett, Waletzky and Baker (1946), which showed differential reductions in parasitaemia resulting from blood- or sporozoite-induced infections in pantothenate-deficient hosts illustrates the stage-specific differences in parasite nutrient requirements. Further, it appears the productivity of asexual reproduction within the malarial schizont in the host blood is reduced by pantothenate deficiency (Huff, Marchbank & Shiroishi, 1958).

Malaria also appears sensitive to host thiamine intake. Despite severe deficiencies leading to increased parasitaemias, marginal deficiencies saw reduced parasitaemias when compared with controls (Rama Rao & Sirsi, 1956a; 1956b; Seeler & Ott, 1946) or when thiamine was added to the diets (Rama Rao & Sirsi, 1956a; 1956b).

Synergy with Undernutrition

Roos, Hegsted and Stare (1946), studying avian malarias, demonstrated the parasites low requirements for dietary choline and niacin relative to the host, by showing that deficiencies led to increased parasitaemia.

Several studies have observed the effects of feeding malarious hosts diets deficient in folic acid. Both avian (Seeler & Ott, 1945a; 1946; Trager, 1958) and primate malarias (Brabin, 1982; Ramakrishnan, 1954a) exhibit increased growth in hosts deficient in folic acid, indicating that the host has a greater requirement for this nutrient than the parasites. This agrees with work (above) showing malarial parasites to produce their folate requirements *de novo* from the precursor *p*-ABA and cannot use intact folates, and hence their independence of host folate status, and explains the suppressive effect of *p*-ABA deficiency on parasitaemia, and the lack of effect during folate deficiency (Ferone, 1977).

Vitamin A deficiency has been associated with reduced avian malaria in chickens, however this effect was not evident in ducks (Roos *et al.*, 1946). A study by Krishnan, Krishnan, Mustafa, Talwar and Ramalingaswami (1975) showed that *P. berghei* multiplication was higher in vitamin-A deficient rats, however their controls were thought to be protein and energy undernourished, which may have led to their lower parasitaemias (Thurnham, 1985b).

A recent field study has shown serum retinol (vitamin A) levels in Tanzanian children to be inversely correlated with malaria intensity, and positively correlated with antibody titres to specific malarial antigen preparations (Sturchler, Tanner, Hanck, Betschart, Gautschi, Weiss, Burnier, Del-Giudice & Degremont, 1987). Vitamin A deficiency is widespread and one of man's greatest nutritional problems (Crompton, 1989). These observations suggest that the host has greater requirements for vitamin A than the parasite. Conversely, laboratory studies have shown vitamin C deficiency in monkeys leads to suppression of parasitaemia during *P. knowlesi* infection, which is rapidly reversed with supplementation (McKee & Geiman, 1946).

MULTIPLE NUTRIENTS

Several reports of dietary effects on malaria involve combinations of nutrients, making cause and effect difficult, however all studies have shown that multiple deficiencies lead to malarial suppression.

Birds on diets deficient in biotin, folic acid, thiamine, choline and other food factors developed greater malarial intensity than well-fed controls (Brooke, 1945). And the addition of vitamin B complex nutrients partially reversed milk diet or low protein diet suppression of *P. berghei* in rats (Refaat & Bray, 1953). The addition of both methionine and threonine to mice fed diets low in protein increased malarial indices to levels similar to those seen in mice fed diets high in protein (Keshavarz-Valian *et al.*, 1985). The change in cellular lipid and oxidant environment for the parasite has been implicated in the protection against severe malaria furnished by diets rich in fish oils (Ager *et al.*, 1988; Godfrey, 1957; Levander *et al.*, 1988; 1989a; 1989b; 1989c; 1989d), which are potent tocopherol (vitamin E) antagonists.

The fall in malarial counts with increasing PEM could be due to decreased RBC survival time & reduced haematocrit & Hb reported to occur in PEM, or fatty infiltration of the liver affecting pre-erythrocytic development of malaria (Ahmad *et al.*, 1985).

Coatney and Greenberg (1961) fed mice diets deficient in Factor 3 (=Selenium), vitamin E and cystine, and noted suppressed parasitaemias in deficient mice, but decreased survival time, implicating age of erythrocytes and preferences.

Inanition and starvation have also been associated with malarial inhibition. Studies of *P. knowlesi* (McKee & Geiman, 1946) and *P. cynomolgi* (Ray, 1957) in monkeys have shown starvation to markedly inhibit parasitaemias. Several days of starvation have shown marked reductions of parasitaemia in monkeys (McKee & Geiman, 1948) and rats (Ramakrishnan, 1954b), which after 10 days approached elimination (Ramakrishnan, 1954b). Return to a full diet saw recrudescence of malaria in starved monkeys (McKee & Geiman, 1948). Concurrent undernutrition has been associated with increases in the rate and extent of hepatic fibrosis development in mice infected with *P. berghei* (Macdonald, 1960). The observation

that malaria is suppressed in tuberculous as compared with non-tuberculous monkeys has been attributed to the former's tuberculosis-induced protein and vitamin C deficiencies (Singhe, Ray & Nair, 1956). Similar findings have been presented by the Murrays (1977) who have observed that concomitant clinical ascariasis reduces the effects of malarial infection. Chemotherapeutic removal of ascarids saw a recrudescence of malaria in infected children. *Ascaris* is well known to affect host nutritional status (Stephenson, 1980; Stephenson & Holland, 1987), and the effects were attributed to removal of ascarid nutrient competition with malaria, and/or removal of worm-induced host changes suppressing malaria (Murray *et al.*, 1977).

In man, it is generally considered that malaria is suppressed during famine (Garnham, 1971; McGregor, 1988; Ramakrishnan, 1954a), and remains quiescent until a better of plane of nutrition is achieved (Crompton, 1989; Heywood & Harvey, 1986; Ramakrishnan, 1954a). Results of a number of field studies support this hypothesis. The distribution of grain supplies to African nomadic peoples following famine conditions in Ethiopia led to recrudescences of malaria, brucellosis and tuberculosis, precipitating increased disease severity in adults, and increases in severe cerebral malaria in children (Murray *et al.*, 1975; 1978b). Other studies, in recording gross nutritional status through weight-for-age, have shown a significant positive relationship between nutritional status and malarial intensity (Ahmad *et al.*, 1985; Hendrickse *et al.*, 1971), whilst the provision of an iron and vitamin supplement, including riboflavin, thiamine and vitamin C, has been shown to increase the mean intensity of malarial infection in Gambian children (Bates, Powers & Lamb, 1986; Bates, Powers, Lamb, Gelman & Webb, 1987).

Plasmodial Success & Host Nutrition: Counterintuitive

From the evidence reviewed above, it is clear that host nutritional status can affect malarial replication and the consequent evolution of disease. The majority of cases show malaria to act R_0 - and patho-antagonistically with host deficiencies, however other nutrients show synergy. The simple fact that different nutrients affect malaria in different ways suggests that further study should be carried out to establish the effects of dietary changes of human populations exposed to malarial infection.

Plasmodia & Invertebrate Host Nutrition

Interestingly, a number of studies have also demonstrated the relationship between invertebrate host nutrition and the success of malarial infection. The nutritional requirements of the invertebrate host stage of malarial development have been reviewed by Schneider and Vanderberg (1980). It appears that the quality of nutrient intake affects the development of malaria in the mosquito host, as a blood meal allowed increased malarial replication over that seen from glucose fed mosquitoes (Ghosh & Ray, 1957). Terzian (1947; 1950) and

colleagues (Terzian & Stahler, 1960; Terzian, Stahler & Miller, 1952; 1953) have shown the mosquito's immune dependence on numerous nutrients, and the resultant change in oocyst presence within the mosquito. It also appears reductions of nutrients in the mosquito may further help explain these results, as the presence or elimination of endoparasites thought to compete with malaria for nutrients in the mosquito alters the ability of malaria to replicate (Garnham, 1956; 1966; Micks & Ferguson, 1961). In fact, it has been suggested that invertebrate host specificity depends upon the presence or absence of certain amino acids (Garnham, 1966).

6.4: MALARIA IN THE FIELD

Malaria far exceeds all other parasites in public health significance (Beaver *et al.*, 1984; Latham, 1982; Wernsdorfer & Wernsdorfer, 1988). Recent estimates have placed the number of people exposed to malarial infection at 2.2 billion, a figure currently representing more than one half of the world's population. Estimates suggest that over 900 million infections occur worldwide, of which some 500 million produce clinical disease. In addition to the obvious economic burden such illness imposes, each year more than 2.5 million people are killed by malaria (Rogers, 1986; Scheibel *et al.*, 1989; Sturchler, 1989; Wernsdorfer & Wernsdorfer, 1988).

6.5: DIETARY INTERVENTIONS

The frequent co-existence of malaria and undernutrition suggests that those most likely to harbor infection are also those most likely to receive nutritional support in response to deficiency.

The Unknown Effects of Dietary Interventions

Given the dichotomy of disease outcomes following the refeeding of deficient hosts harboring malaria, it is suggested that clarification of the net disease effects of nutritional supplementation programs operative in areas endemic for malaria should be a priority.

6.6: TREATMENT & CONTROL INTERVENTIONS

Interventions designed to treat or control malaria are likely to be largely delivered in impoverished communities. Given the apparent intimacy between the activities of various antimalarial drugs (see above), it is suggested that the planning of such interventions should proceed in the presence of firm evidence of the influence of various levels of host nutrients on the drugs involved.

The Unknown Effects of Undernutrition & Refeeding

Antimalarial drugs vary widely in their modes of action, however evidence of their sensitivity to host nutritional status suggests that more work needs to be done to clarify the effects of various nutritional deficiencies on the therapeutic benefits of chemotherapy.

6.7: HYPOTHESIS

That changes in host nutritional status may make conditions within the host suboptimal for malarial proliferation. This has serious implications for the effective interventional control of malaria.

6.8: CHAPTER DISCUSSION

Although incomplete, this review of the nutritional aspects of malarial infection is included so that the collection of published evidence herein is placed in the public domain.

Further Work

If increases in malaria are to be avoided during refeeding programs, and if treatment and control interventions are to have maximum benefit, it is imperative that the influence of changing host nutritional status on the expression of malaria be examined.

CHAPTER SEVEN

...CONCLUSIONS...



Chapter Summary

From previous evidence, and after reviewing the results of experimental investigations, this dissertation emerges with the thesis that undernutrition which produces changes in the host, such as that which may arise as a result of poor food quality and/or availability, and/or via the metabolic response to infection, may constitute a non-immunological host defence against exploitation by some parasites. And that the occurrence of this previously unreported form of defence has profound implications for the effective interventional control of parasitic diseases.

7.1 SUMMARY OF FINDINGS

This Thesis examined nutritional aspects of the host/parasite association. A review of previous, scattered evidence led to the generation of the thesis that host nutritional status has a significant influence on the outcome of the host/parasite interaction. Three host/parasite systems were investigated and provided results which were generally supportive of the thesis.

Schistosomes & Vertebrate Host Nutrition

The influence of vertebrate host nutrition on schistosome infection and disease was investigated by experimentation employing a permissive mouse model. Investigations concentrated on three areas:

Single & Multiple Infections

Host protein deficiency during single infections demonstrated that schistosome survival and development are compromised by suboptimal conditions encountered within protein deficient hosts. These effects appeared to occur at, or soon after penetration, suggesting that parasite metamorphosis at this stage may render new schistosomula susceptible to attrition or deleterious effects which produce residual stunting of developing worms. In addition, the fecundity of worms inhabiting deficient hosts was impaired relative to those in well-fed animals, and fewer eggs reached the external environment. As the egg stimulus central to pathogenesis was reduced by host deficiency, and in the presence of reduced immune activity producing disease, hosts which were protein deficient at the time of infection, or which became so during the experimental period, suffered significantly less pathology than those which remained protein replete.

Exposure of mice to multiple infections produced similar results to those above, and showed that, despite their reduced ability to acquire immunity in the face of continuing infection, protein deficient mice remained less susceptible to reinfection, and indeed became increasingly more resistant to reinfection compared with well-fed animals. This is consistent with the thesis that suboptimal conditions secondary to undernutrition can limit parasite

infection, and suggests that in schistosome infections, this response may constitute an appreciable component of host defence.

Dietary Interventions

Manipulation of host protein status during mature infection demonstrated that suboptimal conditions within the host can also limit the success of established adult schistosomes. A period of protein refeeding of mice harboring schistosomes which had matured under deficiency largely reversed the deleterious effects of protein undernutrition on the parasite. The resultant rise in antigenicity, in the presence of immune activity restored by repletion, produced a marked increase in disease severity. Conversely, a period of acute protein deficiency induced in hosts harboring schistosomes which had matured under conditions of protein repletion significantly reduced parasite reproductive success and led to the amelioration of chronic disease. These results indicate that fluctuations in host nutritional status may be important determinants of both parasite reproductive success and the severity of schistosomal disease.

Treatment & Control Interventions

Vaccination of protein deficient hosts produced lower rates of protection against reinfection than vaccination of well-fed animals. Results indicate this is due to the appreciable rise in immune activity occurring in challenged well-fed animals which did not appear in deficient hosts. However reinfection in well-fed hosts which had received vaccine protection saw the establishment of larger, more fecund worms than in unvaccinated controls. This shows that, in the face of continued reinfection, vaccination may complicate matters by reducing density-dependent constraints on schistosome growth and reproduction.

The therapeutic value of praziquantel was reduced when administered to protein deficient hosts when compared with well-fed controls. This was likely due to a fall in immune activity central to the drugs success. Reinfection was reduced by the residual presence of worms surviving drug treatment in deficient hosts; this is consistent with the notion that infection produces host conditions suboptimal for invading schistosomes.

Schistosomes & Invertebrate Host Nutrition

Experiments which show underfed snails produced fewer cercariae than well-fed hosts are likely to reflect the suppressive effect which suboptimal conditions secondary to undernutrition have on parasite replication. As reduced cercarial production lowers potentially fatal damage to host tissues, an ability on the host's part to induce deficiency could provide some protection from fulminant exploitation. Alterations in snail nutritional status were also shown to alter cercarial infectivity, an observation with clear implications for schistosome and host population dynamics.

Malaria & Host Nutrition

A survey of the available literature on the effects of host nutritional status on malaria reveals that various forms of host deficiency generally suppress malarial replication, and the evolution of disease. This is consistent with the thesis, and suggests that hosts which develop undernutrition during infection may gain some advantage in the face of severe disease pressure. It is suggested that this subject may provide a fertile area for future study.

In summary, it appears that the general fall in parasite success observed in each of the three systems examined during times of host nutritional stress results from the physico-chemical conditions within the host becoming increasingly unsuitable for fulminant parasite exploitation. If net disease falls as a result, it follows that those hosts which become malnourished during infection with parasites susceptible to such changes may enjoy a selective advantage over hosts which do not. An intriguing question at this point may ask is the host able to make use of this suppression in defence against parasites?

7.2 THESIS: IS HOST DEFENCE A TWO-EDGED SWORD?



“It is an error to imagine that evolution signifies a constant tendency to increased perfection. That process undoubtedly involves a constant remodeling of the organism in adaptation to new conditions; but it depends on the nature of those conditions whether the direction of the modifications effected shall be upward or downward”

Thomas Henry Huxley, *The Struggle for Existence in Human Society* (1888)

The Ambient Defence

Chapter 1 of this thesis demonstrated that undernutrition can significantly reduce parasite success and the expression of disease. Undernutrition can be exogenously imposed (by a reduction in food quantity and/or quality) or hosts can induce undernutrition by behavioral and physiological changes accompanying the release of soluble mediators by activated macrophages (see Chapter 1). These changes, which we perceive as disease, may in fact constitute a form of passive defence by reducing the parasite- and host-induced components of parasitic disease.

The protection which host deficiency provides against some parasites consists of changes within the host which render conditions suboptimal for fulminant parasite exploitation. As infection is well documented to induce largely host-mediated changes in the ambient conditions within the host, it is suggested that the ability of the host to respond to infection in this way may provide an effective form of defence against parasites susceptible to fluctuations in ambient conditions.

It is suggested that hosts exposed to infection respond in two ways: activated macrophages release various lymphokines which coordinates and amplifies immune activity designed to attack and destroy invading organisms. Simultaneously, lymphokines induce a wide range of metabolic changes which conspire to radically reduce host nutritional status. This produces a change in the conditions within the host, which in turn may impair the survival and reproductive success of parasites which are sensitive to such changes. Although this “ambient defence” involves the cost of this change, manifest as deficiency disease, it is suggested that the protection it provides in the face of severe parasitic exploitation could provide a mechanism for its retention.

The total number and range of activity of lymphokines are still incompletely known, however one major effector, IL-1 has biological activity close or identical to Tumor Necrosis Factor (also known as Cachectin), a powerful monokine highly active against tumor cells (Beutler & Cerami, 1990; Dinarello, 1990). It may be that other lymphokines act in concert with IL-1 to rapidly alter the conditions within the host following exposure to infection.

Clearly host undernutrition can lead to a significant reduction in parasite survival and reproductive success. Thus parasites invading hosts which respond by mobilizing immunity and inducing endogenous undernutrition are likely to be at a reproductive disadvantage. It is interesting to speculate that the ability of parasites to actively immunosuppress their hosts (for example, schistosomes blocking lymphocyte transformation and inhibiting mast cell degranulation {Cohen, 1985}) may not only protect the parasite from immune attack, but also suppresses those mechanisms (chiefly macrophage/lymphokine-mediated) which enable the host to effect the metabolic response to infection which may negatively influence parasite success.

This suggests that non-immunological defences can be acquired by the host in response to infection in the form of an immune-mediated metabolic response which produces a reduction in host suitability. By limiting parasite exploitation, the ability to rapidly enter deficit when infected may therefore present a considerable advantage to those hosts by which it is employed. Therefore hosts which exist close to nutritional deficit may enjoy an advantage over those unable to rapidly induce deficiency when exposed to infection. Refeeding hosts to standards set in the absence of such stresses as infectious disease may therefore remove the advantages leanness provides.

The term "malnutrition" implies that nutritional deficits are undesirable. However in those cases where low nutritional status protects the host from fulminant parasitic disease, it may in fact be preferable to repletion. In such cases, being well-fed and unable to make use of the ambient defence may expose the host to fulminant parasitic disease; under such circumstances, to be well-fed is to be, by definition, "malnourished". As the true definition of malnutrition clearly depends upon other factors such as infection, it is suggested that nutritional deficit sufficient to cause changes in the infected host should henceforth be referred to as undernutrition.

Parasites, Disease & Host Nutrition

Parasites enjoy a vast reproductive advantage over their hosts, and therefore exposure to infection in endemic areas is virtually inevitable, regardless of host defences. Hosts defend themselves from exploitation as best they can: clearly immunity is a major component of their defences, however it is well known that many parasites can largely circumvent host immunity (Behnke, 1990; Bloom, 1979).

However there may be another way in which hosts can defend themselves against parasites other than to attack and destroy them via the immune system, which involves the difficulty of finding and killing them, and which parasites can frequently evade. By employing the Ambient Defence, hosts can turn the endoregulatory fragility of their parasites into a form of defence. The ability of the host to alter itself in response to infection demands that the

parasite spend resources coping with this change, just as parasites must trade off the cost of immune evasion against reproductive success (Behnke, 1990).

An analogy may be useful at this point. If a well-stocked restaurant (the host) wishes to remove an unwanted diner (the parasite), there are two ways to do so: first, the unwanted guest can be attacked and removed, much as hosts attempt to attack and kill parasites employing the immune system. However this presupposes that the intruder can be found and overcome; if the intruder is invisible to detection, or can evade attack (immune evasion), he or she can continue to exploit the restaurant. There is another way, however, to stop the parasite.

Simply changing the menu, or altering the ambient conditions (via the metabolic response to infection) could disrupt a diner who is well accustomed to this restaurant and unable to cope well with change (the dependent parasite). This strategy does not require that the restaurant be able to find the parasite. The unwanted guest may be unable to exploit to the full if he or she cannot cope with change, or must expend effort trying to do so. Although this form of defence involves the cost of disrupting the restaurant (deficiency disease), the beneficial effects of reducing parasite exploitation (parasitic disease) may warrant this cost. In this case, the restaurant (the host) simply takes the lesser of two evils - disruption over exploitation, illness over death.

This theory introduces non-immunological changes in the host milieu in response to infection as the second edge of the sword of host resistance to parasites.

Future Work

The theory that hosts deliberately respond to infection by producing potentially damaging change within the body demands further research. Given the proposed immune-mediated initiation of the metabolic response, useful lines of investigation could involve selective removal of soluble effectors using monoclonal antibodies raised specifically to them (Dr. P Whitfield, personal communication).

In summary, it appears that, in order to avoid greater morbidity or even death, the host settles for a compromise, and takes deficiency. Just as deficiency alters the conditions within the body, and may provide some protection from parasites, so too host-induced pathology in response to infection may contribute to host resistance. Host-mediated disease may simply be a form of defence against exploitation, or the vestiges of a former defence. This could provide a mechanism whereby susceptibility to disease is maintained through host selection, a previously unexplained phenomenon.

Whilst purely speculative, it is thought that these suggestions may provide interesting avenues for future work which views the symptoms and disease accompanying infection as a compromise enabling survival, rather than an unavoidable evil.

THESIS

That undernutrition, such as that arising secondary to infection, which produces changes within the host may constitute an acquired, non-immunological host defence against parasite exploitation. And that the occurrence of this previously unexplored form of defence has implications for the effective interventionary control of parasitic diseases.

7.3 PARASITES & HOST NUTRITION: OPTIMIZING DISEASE MANAGEMENT



“... it is necessary ... to ensure that, in our attempts to improve health, we do no harm.”

Keusch & Migasena (1982)

The implications which follow the above suggestions are serious: refeeding chronically deficient humans in the presence of active infection can remove the protection ambient defences may provide against some diseases by restoring conditions suitable for parasite survival and reproduction. Although refeeding is likely to remove deficiency disease, resurgence of parasitic disease which had been suppressed may increase total disease - thus interventions may exacerbate disease by replacing undernutrition with parasitic disease. It is therefore recommended that refeeding be avoided in the presence of active infection. This is not to condone starvation, but to recommend caution when seeking to correct deficiencies in the presence of potentially resurgent infections.

Although the ideas proffered in this Thesis appear counterintuitive, it is felt that further examination of the interpretations contained herein will yield novel, and potentially helpful information relevant to the effective management of disease in Developing Countries.

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APPENDICES



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APPENDIX I. EXPERIMENTAL DIET COMPOSITIONS:

Except where otherwise indicated, mice were fed SLC *ad libitum*. SLC composition appears in Table 1. Mice used in experiments, unless otherwise indicated, were fed synthetic diets containing either 3% protein by weight, or 16% protein by weight *ad libitum*. The synthetic diets were blended in a hand-operated mixer (20 l) to the specifications shown in Table 2, stored at 02-06°C in sealed, plastic containers and used within 1 week of preparation.

Constituent	Composition (%)
Digestible crude oil	2.6
Digestible crude protein	17.7
Total dietary fibre	16.3
-Pectin	1.7
-Hemicellulose	8.6
-Cellulose	4.8
-Lignin	1.2
Starches	35.5
Sugars	9.1
Supplementary minerals & vitamins	2.5

TABLE 1: Theoretical composition of SLC. Information supplied by Special Diets Services.

Constituent	$g.kg^{-1}$	Supplier
Casein (soluble light white)	varied as required ^A	BDH ^B
Corn oil	100	Sainsbury Limited
alpha cellulose	100	Sigma ^C
Starch (maize)	makes up to 1.0 kg ^A	BDH
D L-methionine	1.5	BDH
L-threonine	1.5	BDH
Choline chloride	1.5	BDH
Mineral mix ^D	45	
Vitamin mix ^E	10	

^A If it is assumed that the metabolizable energy values of casein and starch are each approximately $16.8 kJ.g^{-1}$ (Crompton, Walters & Arnold, 1981), the two synthetic diets (3 & 16% protein by weight) will remain isoenergetic if starch is used to replace casein (and vice versa) on a weight for weight basis, whilst the proportion of other constituents remains unchanged.

^B BDH Chemicals (Poole, Dorset, UK). ^D See Table 3.

^C Sigma Chemical Company (Poole, Dorset, UK). ^E See Table 4 (overleaf).

TABLE 2: Theoretical composition of the synthetic diets used experimentally in Chapters 2, 3 & 4.

Mineral ^F	Composition (%)
Calcium hydrogen phosphate	29.4
Calcium carbonate	18.9
Sodium hydrogen phosphate	16.9
Potassium chloride	13.0
Magnesium sulphate	12.2
Potassium hydrogen carbonate	7.8
Manganous sulphate	0.8
Ferric citrate	0.74
Zinc carbonate	0.13
Cupric sulphate	0.03
Potassium iodate	0.005

^F All minerals were supplied by BDH Chemicals (Poole, Dorset, UK).

TABLE 3: Theoretical composition of the mineral mixture used in the synthetic diets described in Table 2.

<i>Vitamin</i> ^G	<i>Quantity.kg⁻¹ diet</i> ^H
Thiamine HCl	10 mg
Riboflavin	15 mg
Pyridoxine HCl	15 mg
Nicotinic acid	50 mg
d-Cal-pantothenic acid	50 mg
Folic acid	10 mg
Biotin	1 mg
Menadione	2 mg
Butylated hydroxytoluene	100 mg
Vitamin A	5000 IU ^I
Vitamin D ₃	1000 IU
Vitamin E	20 IU

^G All vitamins were supplied by Sigma Chemical Company (Poole, Dorset, UK), excepting Vitamins A & D₃, which were provided by Roche Products (Dunstable, Bedfordshire, UK).

^H These ingredients were mixed in cellulose (952.7 g cellulose.kg⁻¹ of vitamin mixture) to ensure uniform distribution in the diet. Vitamin B₁₂ was added to the diet in solution (as aerosol through a 1.0 ml syringe and fine gauge needle), giving a concentration of 0.02 mg.kg⁻¹ of vitamin mixture.

^I International Units

TABLE 4: Theoretical composition of the vitamin mixture used in the synthetic diets described in Table 2.

APPENDIX II. ELISA PROCEDURE:

A standard ELISA was used to quantify antibodies specific to SSA preparations in plasma collected from experimental mice. The following outlines the procedure as employed by the Parasite Immunology group at NIMR, London.

1. Antigen Preparation

SSA, prepared by previously described methods (Payares & Simpson, 1985), was provided by Drs Paul Hagan and S. Ron Smithers at the NIMR, London.

2. Antigen Coating

Assays were performed in Falcon[®] Microtest III[™] polystyrene, 96 well (flat-bottomed wells) tissue culture treated plates (Becton Dickinson Labware, Lincoln Park, New Jersey, USA). SSA was diluted at 10 µg.ml⁻¹ of Carbonate Coating Buffer¹ and 50 µl placed in each well of the plate. The plate was incubated overnight at room temperature. The plates were shaken vigorously to remove unattached SSA solution and washed 3 times by immersion in Tween Saline Washing Solution² (TSWS) (allowing 3 min for each immersion) and blot dried on absorbent paper.

3. Antibody Addition

Phosphate Buffered Tween Incubation Solution³ (PBTIS) was added to each well at the rate of 50 µl, but for the second column, which received 100 µl. Five µl of plasma, collected as described in Chapter 2 and pooled from

FOOTNOTE

¹ Carbonate Coating Buffer = 1.59 g Na₂CO₃ + 2.93 g NaHCO₃ dissolved in 1.0 l of distilled H₂O (0.05 M carbonate at pH 9.6).

² Tween Saline Washing Solution = 45 g NaCl + 2.5 ml Tween 20 (Dissolve NaCl in 5 l of distilled H₂O, then add Tween 20).

³ Phosphate Buffered Tween Incubation Solution = 900 ml Phosphate Buffered Saline (pH 7.6; PBS = 170 g NaCl + 25.6 g Na₂HPO₄ + 3.12 g NaH₂PO₄.2H₂O dissolved in 20 l distilled H₂O) + 0.45 ml Tween 20.

each experimental animal group, was added to each well in column 2 and the solutions double diluted across the plate. The final 50 μ l was discarded. The plate was incubated for 1.0 h at room temperature, washed 3 times by immersion in TSWS (allowing 3 min for each immersion) and blot dried on absorbent paper.

4. Conjugate Binding

Commercial goat anti-mouse whole molecule IgG peroxidase conjugate (Sigma Chemical Company, St. Louis, Missouri, USA) was diluted at $\frac{1}{2000}$ in PBTIS and 50 μ l added to each well. The plate was incubated for 1.0 h at room temperature, washed 3 times by immersion in TSWS (allowing 3 min for each immersion) and blot dried on absorbent paper.

5. Substrate Reaction & Data Collection

The Substrate Solution¹ was prepared fresh on the day of use. Fifty μ l of this solution was added to each well and left to incubate at room temperature for 20 min. To arrest the reaction, 50 μ l of 4.0 M sulphuric acid was added to each well. Absorbance was immediately read at 492 nm on a Titertek Multiskan[®] MC microELISA plate reader (Eflab, Helsinki, Finland) in the Department of Zoology, Oxford.

The first column on each plate was used to blank the microELISA reader for substrate absorbance (ie. by omitting the addition of plasma containing antibody to column 1 of the plate in step 3, above). Negative controls (plasma from uninfected mice) from each dietary group were included in each experiment. All of the chemicals used in the various solutions were obtained from BDH Chemicals (Poole, Dorset, UK) and were of reagent grade. Results are expressed as log (1+O.D.) of plasma samples at each of 11 double dilutions, from $\frac{1}{20}$ to $\frac{1}{20480}$.

APPENDIX III. LIVER HISTOLOGY:

The relative damage to liver tissue from the presence of schistosome eggs was compared by estimating the size of granulomata surrounding eggs trapped in host tissue following the criteria of von Lichtenberg (1962). Immediately following excision, the anterior lobes of mouse livers were fixed in formal saline² for more than 7 days. A series of tissue sections was prepared from full sections through the middle of each anterior lobe using standard histological techniques as follows. One half of the lobe was dehydrated for 20 min in 70% then 80% alcohol, followed by 30 min in each of 95% and two changes of absolute alcohol. The tissues were cleared by two changes through HistoClear[™] (National Diagnostics, Manville, New Jersey, USA) of 30 min each, then embedded through two wax changes of 30 min each. After drying, six 7 μ m serial sections were cut on a Spencer 820 microtome (American Optical Company, USA), mounted on a microscope slide and air dried.

FOOTNOTE

¹ Substrate Solution = 100 ml Substrate Phosphate-Citrate Buffer (7.19 g Na₂HPO₄ + 5.19 g citric acid; dissolve in 1.0 l of distilled H₂O) + 1.0 ml Substrate Stock (100 mg orthophenylene diamine + 10 ml methanol; store in dark at 4°C for a maximum of 1 week) + 10 μ l 30% H₂O₂.

² Formal saline=10% formaldehyde + 0.85% NaCl in distilled H₂O.

Mounted sections were then cleared with HistoClear™ and hydrated through two 30 min changes of absolute alcohol followed by one 30 min change in each of 95%, 80% and 70% alcohol followed by distilled water. The slide was then placed in Ehrlich's Haematoxylin for 15 min at 56° C. After washing under tap water, the stain was differentiated through 1.0% acid alcohol and again rinsed under tap water. The slide was then stained in Eosin for 30 sec and allowed to air dry. The stained preparation was again dehydrated through 30 min immersion in each of distilled water, 70%, 80%, 95% and 2 changes of absolute alcohol. After final clearing with HistoClear, the slide was permanently mounted in DPX™ mountant (BDH Chemicals, Poole, Dorset, UK). Granuloma dimensions were recorded and volumes estimated as described in the text.

