The ecology of helminth parasites of laboratory bred and wild mice

Thesis

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THE ECOLOGY OF HELMINTH PARASITES OF
LABORATORY BRED AND WILD MICE

by

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B.Sc. Hons.

Ph.D. Thesis
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ACKNOWLEDGEMENTS

ABSTRACT
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ABSTRACT

A long term study of the monthly incidence and intensity of infection of *Apodemus sylvaticus* (L) with *Syphacia stroma* (von Linstow 1884, Nematoda: Oxyuridae) and *Nematospiroides dubius* (Baylis 1926, Nematoda: Heligmosomidae) has been made. A systems analysis approach to the host parasite relationship has characterised *S. stroma* and *N. dubius* in terms of an r- and K-strategy respectively.

The controls within the host parasite systems of *S. stroma: A. sylvaticus* and *N. dubius: A. sylvaticus* have been considered with respect to the structure of the parasite populations, spatial distribution within the host and time lags inherent in the systems. Control by density independent environmental variables and the intrinsic density dependent mechanisms of competition, parasite pathogenicity and host immune response has also been considered. A period of stress and lowered immune response has been identified in the wild populations of *A. sylvaticus*.

Systems analysis of egg production of a primary infection of *N. dubius* in CD1 mice from day 10-55 has revealed a changed pattern of gastrointestinal motility and cyclicity in egg production and size.

A syndrome of low intensity of infection, reduced fecundity and non-viability of eggs associated with a possible bacterial contaminant in the bedding material has been described for *N. dubius* in ASH/CSI S.P.F. mice. Manipulation of the host parasite system with oxytetracycline hydrochloride has emphasised the importance of microbial environment to *N. dubius*. 
The response of ASH/CSI S.P.F. mice to a primary infection of *N. dubius* has revealed qualitative and quantitative changes in those parameters indicative of host immune status. The response of the ASH/CSI S.P.F. mouse system under varying regimes of stress is different in male and female hosts and it is concluded that *N. dubius* has the ability to exploit the host parasite relationship at all levels of stress.

The findings of the laboratory and field studies are integrated and the balance of the regulatory and destabilising processes in the relationship of *N. dubius* to its host is discussed.
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the splenic capsule (b) are no longer basophilic. 
H and E x 200.
GENERAL INTRODUCTION

The origin of this work lies in a paper written by Elton, Ford and Baker in 1931 who studied the health and parasites of small mammal populations with respect to the age structure of the host. The research undertaken projects that study further and attempts to identify the strategies and controls in the host parasite systems established in the small mammal *Apodemus sylvaticus* by the oxyurid nematode *Syphacia stroma* and the trichostrongylid nematode *Nematospiroides dubius*. The host parasite relationship is considered in terms of system theory which employs a basic universal model of a system with an input and an output. The input is regarded as the initial parasite population in the form of eggs or infective larvae and the number of eggs produced by adults developing from these eggs or larvae as the output of the system (Kennedy 1975). The approach is used to analyse the structure of the systems established by *S. stroma* and *N. dubius* with *A. sylvaticus* in the field situation.

*Nematospiroides dubius* Baylis, 1926 (= Heligmosomoides polygyrus Dujardin, 1845) is a trichostrongylid nematode belonging to the family Heligmosomatidae (Chitwood 1969). It has a direct life cycle involving a free living phase and a parasitic phase. Infective ensheathed third stage larvae enter the host by mouth and after a period in the intestinal mucosa in the mid and lower portion of the small intestine, they take up a position in the lumen of the duodenum when adult. The minimum generation time for the species is fourteen days (Spurlock 1943; Ehrenford 1945; Dobson 1961; Rainbow 1972; Bryant 1973). A number of different strains of *N. dubius* are known to exist in field systems (Forrester 1971; Forrester and McL. Neilson 1973) but the assumption made in the field studies is that the strain of *N. dubius* is the same by reason of the relatively small area of the trapping site. The strains of
N. dubius used in the later laboratory systems were obtained from the Wellcome Laboratories, Beckenham, Kent and Beecham Pharmaceuticals, Reigate, Surrey. The life span of N. dubius in the laboratory mouse is of the order of eight to nine months (Scott, Cross and Dawson, 1951).

Syphacia stroma (Von Linstow, 1884) is an oxyurid nematode (York and Maplestone 1926). The life cycle is direct and infection is through eggs laid on the perianal fur which are transferred and ingested by the host during grooming. Transfer can also take place through physical contact of individuals and will therefore be partially dependent on the density of the host population. Infective eggs are rarely found in faecal pellets and infective stages only remain viable for several hours (Rainbow 1972). An accurate description of the life cycle does not exist due to the difficulty in culture of the eggs of S. stroma. Morgan (1932) has described S. stroma and notes that male worms are far fewer in number than females, even when the latter are present in abundance. It is probable that the females are fertilised before reaching full size and after impregnation the males die and soon pass out of the small intestine. The life span of S. stroma in small mammal hosts is approximately twelve days (Lewis 1966).

The two nematodes are contrasted in terms of r- and K- selection and the effect of abiotic factors on the host parasite relationships is assessed in terms of the environmental variables of temperature, rainfall and humidity. In addition, the regulation of parasite population numbers is considered with respect to the intrinsic density dependent mechanisms of host density, interspecific and intraspecific competition between parasites and specific host age for parasite mortality (Anderson 1978; Anderson and May 1978). The roles of overdispersion and time lags in the stability of the field systems (Anderson 1978; May and Anderson 1978, 1979) as well as that of stress are also analysed.
The stress induced in the field systems is measured by changes in the adrenal gland weight of the host and the response to the increased adrenocortical function considered with respect to changes in weight of the kidneys, heart, liver and gonads. Change in the immune status of the host as a consequence of stress is monitored by the weights of the thymus, spleen and mesenteric nodes as well as a derived index for Peyer's patch area. The results of the field study from June 1973 to December 1975 identify a period of stress which was associated with an increased egg output from both host parasite systems. Consequent to these findings a laboratory study was planned to examine the behaviour of these systems under changing regimes of stress.

During preliminary work at Royal Holloway College, however, difficulty was experienced in the initiation of an infection of ASH/CSI S.P.F. mice with N. dubius and subsequent culture of the eggs of N. dubius to viable L3 larvae. The problem required experimental analysis of the sequence of events which occur during the egg production of N. dubius during a primary infection of the laboratory mouse. Consideration of the contribution of open loop and closed loop control towards stability of the host parasite system reveals that N. dubius possesses the ability to use both types of control. The techniques of cusum analysis and autocorrelation are used to follow the behaviour of the system and locate events during the establishment of the system.

A similar approach is adopted to identify the parameters responsible for the prevention of the establishment of the host parasite relationship between N. dubius and the ASH/CSI S.P.F. mice described above. The hypothesis is that the environment of the small intestines is in some way hostile to the establishment of the worms and that the probable cause is microbial in origin. Manipulation of the system with
oxytetracycline hydrochloride improves the egg output of the parasite and the viability of the larvae. The source of the contamination is located and microbiological assay indicates the presence of members of the chitinase bearing group of the Bacillaceae in the bedding material of the mice.

Changes in the controls that exist at the level of the host individual due to stress are related to the output of the N. dubius: ASH/CSI S.P.F. mouse system and the time sequence of the development of the immune response of laboratory mice to N. dubius is compared to the field data on A. sylvaticus. In addition histological examination of the changes induced in the thymus, spleen, mesenteric nodes and Peyer's patches of the ASH/CSI S.P.F. mouse by an infection of N. dubius is carried out. The results of this quantitative and qualitative analysis are used as a baseline for the stress experiments described in the last chapter.

Stress in the N. dubius: ASH/CSI S.P.F. laboratory system is simulated with the use of injections of a placebo preparation and ACTH is graded in terms of mild, moderate and chronic stress. The effects of stress on the general metabolic function of the host are quantified with respect to changes in the morphophysiological indices of heart, kidneys, liver and gonads and immune status is assessed through the histology and weight of the thymus, spleen and mesenteric nodes as well as the area of Peyer's patches. The variation in total egg output of the N. dubius ASH/CSI S.P.F. mouse system induced by the three regimes of stress is also considered.

The general discussion integrates the findings of the field and laboratory studies and develops ideas as to the role of the habitat in the evolution of the life strategies (Southwood 1977) of S. stroma and N. dubius. It also analyses the balance of regulatory and destabilising processes important in the maintenance and stability of the N. dubius: A. sylvaticus system.
CHAPTER 1


Introduction

The earliest work on the population dynamics of the helminth populations of Apodemus sylvaticus studied the incidence and intensity of infection with respect to the age structure of the host population (Elton, Ford and Baker 1931). Later work by Sharpe (1964) and Lewis (1968 (a) and (b)) correlated the age structure of the host population with seasonal changes in the levels of parasitism and emphasised the importance of the density and ecology of the host population. Rainbow (1972) extended this work in a study of the helminth parasites of Apodemus sylvaticus and Clethrionomys glareolus and related the variation in incidence and intensity of infection to the sex, age and feeding habits of the host population and the types of life cycles of the most commonly occurring species. In addition the effect of environmental variables of rainfall, temperature and relative humidity on the parasitic population was studied in detail.

The present survey was carried out on a private estate situated near Poole, Dorset and repeats the temporal studies of the incidence and intensity of infection of Syphacia stroma and Nematospiroides dubius in small mammals, in particular A. sylvaticus, from June 1973 to December 1975. It also analyses the structure and growth of the populations of these two parasites at individual and population level of the host and contrasts the two nematodes in terms of r- and K-selection (Dobzhansky 1950; MacArthur and Wilson 1967; Pianka 1970, 1978). The r- K- continuum is one in which the r- end point represents a situation in which the optimum strategy is to place all the resources
of the organism into reproduction with a minimum into individual offspring so as to produce the maximum number of progeny. At the other end of the spectrum, K-selection leads to the efficient utilisation of environmental resources and the population is at capacity for a given amount of space and nutrient level. Competition is keen and energy are shunted into fewer but highly fit progeny. The r-strategist therefore exhibits exponential increase in population which acts as positive feedback and causes the population to depart further and further from equilibrium and unstable systems are the consequence. The K-strategist incorporates some form of negative feedback into the system which tends to restore the population to its original steady equilibrium state.

**Materials and Methods**

(a) **The study area**

Trapping was carried out in the study area which was bounded on all sides by roads and occupied an area of approximately 3 square miles. The area contained a variety of habitats, among them being deciduous woodland, conifer plantation and agricultural land (Fig. 1). All the habitats were sampled in the space of a year and the same sequence of trapping was adopted each year during the period June 1973 to December 1975.

(b) **Trapping**

For eight trap nights of each month thirty Longworth mammal traps were laid out in a line transect, the distance between each trap being six feet. The trapping was not consecutive but split into four sessions of two nights each and these coincided with the weekend. They were baited with rolled oats only and bedding material was provided. No prebaiting was carried out because of time constraints. Traps which had been sprung and contained animals, were transported back to
Fig. 1 The study area of Lytchett Matravers Estate, Poole, Dorset showing the variety of habitats available to the small mammal hosts from June 1973 to December 1975.
THE STUDY AREA.

KEY.
A: Deciduous Woodland.
B: Conifer Plantation.
C: Bracken and Heath.
D: Agricultural Land.
E: Larch Plantation.
F: Mixed Woodland.
G: Hedges.

Scale: 2" = 1 mile.
the laboratory and anaesthesia of the animals with chloroform took place within the trap on the same day of capture. Chloroform was used in preference to ether or cervical dislocation as it caused least stress in the animals. Traps which had been removed were replaced. While this method gave no estimate of the population density of the small mammal populations, it provided a relative abundance index and allowed the culling to be random and non-intensive.

(c) Ageing of small mammals

The small mammals were aged according to their weight in grams (Baker 1930; Morris 1972). The population of _A. sylvaticus_ was divided into adult and juvenile cohorts. The line of demarcation between the adults and juveniles in the male population was 20 gms weight. Previous studies have identified adults at 15 gms weight because the testes are descended and sperm is present in the cauda epididymis (Lewis 1968 (a)) but _A. sylvaticus_ is cyclomorphic and recognition of this fact is important in a long term study. Animals born in the spring grow in a single rapid spurt, become sexually mature and reproduce in a short time but those born in the autumn show a biphasic pattern of growth and a delay in sexual maturity (Anderson 1970). Thus an animal born in the autumn, although it may be the same weight as one born in the previous summer will not be reproducing. By spring, however, most of the males have reached the 20 gm mark and entered the breeding sector of the population. Involution of the lymphatic tissue, particularly that of the thymus and Peyer's patches also takes place at this weight and is another indicator of sexual maturity (Comes 1965, Weir 1977). The division between the juvenile and adult cohorts of the female population was made at 15 gms this being the weight when first pregnancies begin to make their appearance. Lactating females were distinguished by swollen mammary glands.
(d) Examination of Hosts for Parasites

The animals were weighed and their sex and state of reproductive maturity recorded. A ventral median incision was made into the abdominal cavity and the alimentary canal from the cardiac sphincter of the stomach to the terminus of the rectum was removed. Dissection first removed the pancreas and mesenteric nodes which served as a focal support to the coils of the alimentary canal. The stomach, liver and pancreas were then examined for parasites as also was the remainder of the alimentary canal. One centimetre lengths of the small intestine were cut open under water and systematically examined under the x5 objective of a light microscope. Each visible helminth was removed with fine forceps and the wall of the small intestine was gently scraped. Further identification of the sex and the life history stages of *S. stroma* and *N. dubius* under a x10 objective then followed and these were recorded along with the fecundity of all female worms present. The length of the small intestine was noted.

A similar procedure for the location of parasites was used in the examination of the caecum, colon and rectum.

(e) Ageing of Parasite populations

The parasite populations of *S. stroma* and *N. dubius* were divided into adult and juvenile cohorts dependent on the presence of testes and ovaries. Adult males of *S. stroma* vary from being tightly rolled on themselves to an uncoiled state with the mamelons everted and free from contact with the body wall. Larval males are small and tightly rolled. Adult females of *S. stroma* include gravid and spent female worms and those that have distinct ovaries but as yet no fertilised eggs. The juvenile stages include sub adult females without distinct ovaries but just reaching sexual maturity and the larval females which are small, coiled and obviously a fresh infection. The adult males and females of *N. dubius* were divided
into adults and larvae using the same criteria and a distinction was made between gravid and non gravid females.

(f) Analysis of results

Mean intensities of infection are expressed on a logarithmic scale. The input of the host parasite system was derived from the number of larval worms present in the host and the output from the product of the number of fecund worms present in the host and their mean fecundity. The equations for the population growth of the parasite populations are as described by Esch (1975) and Krebs (1978), where:

\[ \frac{dN}{dt} = \text{rate of population increase} \]
\[ r = \text{intrinsic rate of natural increase} \]
\[ N = \text{initial population size} \]
\[ K = \text{maximum carrying capacity of habitat} \]

The population growth curve of an r- strategist is described by the equation

\[ \frac{dN}{dt} = rN \]

and the population growth curve of a K- strategist by the equation

\[ \frac{dN}{dt} = rN \left( \frac{K - N}{K} \right) \]

The term \( \frac{K - N}{K} \) describes how the instantaneous rate of increase changes in relation to the changing levels of competition.

Results

(a) The host population

A total of 486 A. sylvaticus were trapped from June 1973 to December 1975 (Figs. 2 and 3). The sex ratio (♂: ♀) of the population for the duration of trapping was 1.35:1. There was variation in the sex ratio from year to year as well as during the years. The figures for the complete years of 1974 and 1975 were 1.87:1 and 1.31:1 respectively which may reflect a depletion of males by trapping because of their greater success in intraspecific competition.
The population of *A. sylvaticus* trapped on Lytchett Matravers Estate, Poole, Dorset, from June 1973 to December 1975. The demarcation between adult and juvenile mice was 20 gm and 15gm weight for male and female hosts respectively.
POPULATION OF APodemus SylVaticus
JUNE 1973 TO DECEMBER 1975
□ Juveniles

Number of individuals

1975
Fig. 3 The numbers of pregnant and lactating female *A. sylvaticus* trapped on Lytchett Matravers Estate, Poole, Dorset from June 1973 to December 1975.
POPULATION OF APODEmus SYLVAТИCUS
JUNE 1973 TO DECEMBER 1975
PREGNANT AND LACTATING FEMALES

1973 - 74

[Bar chart showing the number of pregnant and lactating females by month for 1973-74, with peaks in June and December.]

1974 - 75

[Bar chart showing the number of pregnant and lactating females by month for 1974-75, with a peak in January.]

1975

[Bar chart showing the number of pregnant and lactating females by month for 1975, with a peak in June.]

Number of Individuals
competition for food in the traps (Ashby 1967). The sex ratio peaked in the period April to June in 1974 and 1975 and was 2.1:1 in both years, a feature indicative of the greater wandering activity of the male during the spring breeding season (Baker 1930). Sex ratios were lowest in the latter six months of the period being of the order of 1.12:1 and it may be that as the population diminished in size more females took to wandering in the search for mates during the autumn breeding season of 1975. The peak breeding months were October, September and August in 1973, 1974 and 1975 respectively. The gradual bringing forward of the peak autumn breeding period as the trapping progressed suggests that some density dependent factor has lessened its effect on the population of mice. Pregnant and lactating mice appeared in the traps from March to November in 1974 and from April to December 1975 so the breeding period in 1975 commenced and finished later. The number of embryos and placental scars per female mouse in 1974 was 2.57±1.82 and in 1975 the count was 2.82±1.75. Although the figure was slightly higher in 1975 it was not significantly different. Juvenile males appeared in the traps in all the months of trapping except May 1974 and June 1975. Only two female juveniles were caught in the period February to July in 1974 and 1975 and this lesser figure for juvenile females may in part be due to the different behaviour of the sexes at the various seasons (Baker 1930). The juveniles constituted 55.3% of the total population. Other small mammals trapped included 186 Clethrionomys glareolus (Schreb), 14 Apodemus flavicollis (Melch), 7 Microtus agrestis (Bellamy) and 1 Micromys minutus (Pallas). These were dissected and the number of larval and adult worms of S. stroma and N. dubius found in the alimentary canal counted. Shrews also appeared occasionally but were usually dead and this caused decomposition of their internal
helminth populations. It became apparent in December 1974 that the numbers of *S. stroma* and *N. dubius* were small in *C. glareolus* and so trapping of this species was discontinued. The sex ratio of the *C. glareolus* population during the period June 1973 to December 1974 was 1.27:1 and the juveniles constituted 57% of the population.

(b) **The parasite composition**

Seven species of parasite were recovered from the alimentary canal of *A. sylvaticus*.

**List of Helminth Species Recovered**

**June 1973 - December 1975**

**Nematoda**

*Nematospiroides dubius* (Baylis 1926) Small intestine  
*Syphacia stroma* (von Linstow 1884) Small and large intestine

**Cestoda**

*Cysticercus taenia taeniaeformis* (Batsch 1786) Liver  
(= *Cysticercus fasciolaris* (Rudolphi 1808))  
*Catenotaenia pusilla* (Goeze 1782; Janicki 1904) Small intestine  
*Hymenolepis sps.* Small intestine

**Trematoda**

*Corrigia vitta* (Dujardin 1845) Pancreas  
*Brachylaimus recurvum* (Dujardin 1845) Lower small intestine

*N. dubius* and *S. stroma* were also found in *C. glareolus* and *A. flavicollis* while *Cysticercus taenia taeniaeformis* was present in *C. glareolus*, *A. flavicollis* and *M. agrestis*.

(c) **The incidence and intensity of infection of *A. sylvaticus* with *S. stroma***

(i) **Incidence of infection**

The incidence of infection in male and female *A. sylvaticus* was 82.1% and 67.6% respectively. The seasonal variation in the incidence of infection of *S. stroma* in *A. sylvaticus* is
shown in Table I. There was a gradual rise in the incidence of infection during the winter of 1973-74 until in January 1974 the whole population of mice was infected. This high level of infection was maintained in male mice until July 1974 apart from a small decrease in April 1974; the decline in incidence occurred earlier in female mice in June 1974.

The incidence of infection was lower in the summer months of 1974 but it did not fall below 50%. The incidence of infection began to rise again in October 1974 in the male mice but 100% incidence of infection only occurred in December 1974. In female mice the incidence of infection began to rise in August 1974 and, apart from the anomaly of November 1974 due to a small catch of adult females, it continued to rise until 100% infection was achieved in February 1975. The incidence levels thereafter fluctuated between 65% and 90% for the remainder of the study period except for June 1975 when all female mice were infected and July 1975 when all male mice carried the infection. The lower levels of incidence of infection in the winter of 1974-75 may reflect the gradual depletion of the mice population as the study progressed.

The incidence of infection was significantly higher in male mice than female mice ($P = 0.05$). The incidence of infection in adult and juvenile mice was 78.7% and 75.3% respectively. The incidence of infection was higher in adults but the difference was not significant and it was generally lower in the summer months in all the population. The lower levels of incidence in the winter of 1974-1975 as compared to the previous year was more marked in the adults. The incidence of infection in 1975 as compared to 1974 is not significantly different in either adults or juveniles.
<table>
<thead>
<tr>
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<th>FEMALE</th>
<th>ADULT</th>
<th>JUVENILE</th>
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</thead>
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<td>75</td>
<td>88.8</td>
<td>66</td>
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19
(ii) Intensity of infection

The intensity of infection of *S. stroma* in male *A. sylvaticus* over the study period was 127.7±110.5 worms and in the female mouse was 98.5±110.2. The difference in the intensity of infection is not significant and the high variance:mean ratio indicates overdispersion in both sexes. The mean monthly worm burdens are shown in Table II and show some seasonal variation in intensity of infection. A slow build up of infection took place in the winter of 1973-74 in all mice, with a peak of infection in February and March in female and male mice respectively. Infection levels were generally lower in April-August 1974 although there was a temporary rise in July. During the winter months of 1974-75 the initial rise in infection was not maintained and there was a steady decline in intensity of infection in both sexes. The low points of infection in February and March of 1975 are in sharp contrast to the same months in 1974 and again may be explained by a decrease in the number of hosts available for infection. Apart from temporary peaks in April, June and August 1975 the steady decline in intensity of infection occurred in male and female mice. Male mice had their highest worm burdens in March and July 1974 and in January and April 1975. Female mice had their highest worm burdens in October 1974 and June 1975.

The mean worm burden of adult and juvenile was 141±117 and 95.5±100 respectively and the difference was not significant. The intensity of infection was lower in 1975 but the difference was significant in the adult sector only (P = 0.01).
**TABLE II**

The intensity of infection of *S. stroma* in *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset from June 1973 to October 1975

<table>
<thead>
<tr>
<th>MONTH</th>
<th><strong>MALE</strong> Log 10</th>
<th><strong>FEMALE</strong> Log 10</th>
<th><strong>ADULT</strong> Log 10</th>
<th><strong>JUVENILE</strong> Log 10</th>
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The incidence and intensity of infection of A. sylvaticus with N. dubius

(i) Incidence of infection

The incidence of infection in male and female A. sylvaticus was 86.9% and 76.7% respectively. The seasonal variation in the incidence of N. dubius in A. sylvaticus is shown in Table III. It was higher in the winter months of both years in all mice and indicates that an overwintering population of the parasite within the host is important in the survival of N. dubius. The incidence of infection in the summer months of the study was lowest in 1973. The incidence of infection in juvenile and adult mice was 64.4% and 90.24% respectively and this difference was significant (P = 0.002). There was greater variation in the incidence of infection in juveniles than in adults and all adults were infected during the winter months of 1973-74 and 1974-75. The incidence of infection was generally lower in the summer months.

(ii) Intensity of infection

The intensity of infection of male and female mice over the study period was 21.72 ± 16.2 and 16.1 ± 16.3 but the difference was not significant. The mean worm burdens of N. dubius in A. sylvaticus are shown in Table IV. They are higher in the winter months than in the summer and during both winters there is a slow build up of infection until it peaks in the spring when juveniles enter the population. This slow increase in intensity of infection is well marked in female mice over a period of ten months in 1973-74.

The intensity of infection in juveniles and adults was 11.1 ± 16.1 and 22.82 ± 15.6 respectively and the difference was significant (P = 0.01). The juveniles exhibit slow rises in mean worm burdens during the winter months of 1973-74 and
The incidence of *N. dubius* in *A. sylvaticus* on Lytchett Metravers Estate, Poole, Dorset
from June 1973 to October 1975

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<th>JUVENILE %</th>
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The intensity of infection of *N. dubius* in *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset from June 1973 to October 1975

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1974-75 and the intensity of infection in adults is higher in these months too.

(e) The structure of the parasite population of S. stroma

(i) S. stroma in A. sylvaticus

The intensities of infection of the individual cohorts of the population of S. stroma in male and female A. sylvaticus during the period January 1974 to October 1975 are shown in Figs. 4 and 5. The rapid rise and fall in numbers of S. stroma which occurs in both sexes of mice is characteristic of an r-strategy or opportunist infection. The patterns of behaviour of the individual cohorts of the parasite population, however, differs in male and female A. sylvaticus. In the female host they assume a broad wave-like pattern with peaks of infection during the winter months of 1974 and 1975. Fluctuations in intensity of infection are not so extreme in the male host and there is more stability in the system. This may in part be due to a slow build up of larval male worms and gravid females in the male host between August 1973 and January 1974 which more nearly approaches a logistic growth curve of a K-strategist. If the assumption is made that 570 and 1110 worms constitute the maximum carrying capacities of the small intestine for larval male worms and gravid females the slopes of the curves are 1.11 and 1.44 respectively. All cohorts show a gradual decline over the study period and this is most marked in the larval input into the male system.

The intensities of infection of the individual cohorts of the population of S. stroma in juvenile and adult A. sylvaticus during the period January 1974 to October 1975 are shown in Figs. 6 and 7. In juvenile mice the cohorts of S. stroma...
Fig. 4 The intensity of infection of individual cohorts of the population of S. stroma in male A. sylvaticus on Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
POPULATION STRUCTURE OF S. STROMA IN MALE A. SYLVIATICUS.

Log.10 Intensity

- Gravid ♀
- Non gravid ♀

- Adult ♂

- Larval ♀
- Larval ♂

1973 1974 1975
Fig. 5 The intensity of infection of individual cohorts of the population of *S. stroma* in female *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset.

POPULATION STRUCTURE OF S. STROMA
IN FEMALE A. SYLVATICUS.

Log. 10 Intensity.

- Gravid ♀
- Non gravid ♀
- Adult ♂
- Larval ♀
- Larval ♂
Fig. 6  The intensity of infection of individual cohorts of the population of *S. stroma* in juvenile *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
POPULATION STRUCTURE OF S. STROMA
IN JUVENILE A. SYLVATICUS.

Log. 10 Intensity

- - Gravid ♀
- Non gravid ♀

ΔΔ Adult

Δ Δ Larval ♀
Δ Δ Larval ♂

1973 1974 1975
Fig. 7 The intensity of infection of individual cohorts of the population of *S. stroma* in adult *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
POPULATION STRUCTURE OF S. STROMA IN ADULT A. SYLVATICUS.

Log 10 Intensity

- Gravid ♀
- Non gravid ♀

Δ Δ Adult

- Larval ♀
- Larval
follow the same pattern of growth as in female hosts and this suggests primary infection in the nest and some instability in the system. The numbers of worms in adult mice follow more closely the smaller fluctuations seen in the male mouse parasite system.

The fecundity of *S. stroma* in *A. sylvaticus* during the period January 1974 to October 1975 is shown in Fig. 8. The fecundity of the worms was 27.8±5 and 26.04±6 in male and female mice respectively but the difference is not significant. The fecundity of *S. stroma* in juvenile and adult mice was 30.1±6.52 and 26.9±5 respectively but again this difference is not significant.

The structure of the parasite population of *S. stroma* in *A. sylvaticus* from January 1974 to October 1975 is shown in Table V. Two thirds of the population of *S. stroma* reside in the male mice but the percentage of each cohort is approximately the same in both sexes of *A. sylvaticus*. The higher sex ratio achieved in the female mice indicates better survival of male worms in this host. The egg output per female host parasite system was two thirds of that of the male system but egg output from all female hosts constituted only 47% of the total egg output of the parasite. Twenty eight per cent of the worms reside in juvenile *A. sylvaticus* and the larval cohorts constitute 51% of this total. Both the primary and secondary sex ratios of *S. stroma* are higher in juvenile mice which may be due to insufficient time for numbers of adult female worms to build up in these younger members of the mouse population. Juvenile mice contributed 35% of the total egg output of *S. stroma*.

(ii) *S. stroma* in *A. flavicollis*

A total of 568 worms were found in 14 specimens of
Fig. 8 The fecundity of *S. stroma* in *A. sylvaticus*
on Lytchett Matravers Estate, Poole, Dorset.
THE FECUNDITY OF S. STROMA IN A. SYLVATICUS

Fecundity

Male Host

Female Host

Juvenile Host

Adult Host

1973 1974 1975
### TABLE V: Population structure of S. stroma in A. Sylvaticus

Total number of worms = 57,559

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<th>Female</th>
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<th>Adult</th>
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**S. stroma**

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<td>0.19</td>
<td>0.21</td>
<td>0.24</td>
<td>0.17</td>
</tr>
<tr>
<td>Intensity</td>
<td>127±111</td>
<td>98.3±110</td>
<td>95.5±100</td>
<td>141±117</td>
</tr>
<tr>
<td>Fecundity</td>
<td>27.8±5</td>
<td>26.04±5</td>
<td>30.1±6.5</td>
<td>26.9±5</td>
</tr>
<tr>
<td>Egg output per host</td>
<td>840.8</td>
<td>531.4</td>
<td>336.7</td>
<td>1196.7</td>
</tr>
<tr>
<td>Total egg output</td>
<td>234,493</td>
<td>109,993</td>
<td>90,571</td>
<td>259,693</td>
</tr>
</tbody>
</table>

**Location:** Lytchett Matravers Estate, Poole, Dorset.

**Time:** January 1974 to October 1975

37
A. flavicollis. The primary and secondary sex ratios were 0.33 and 0.054 respectively which indicates poorer survival of male worms in this species. The mean fecundity was 22.7±10.7 and total egg output was only 1.7% of that of the output of the S. stroma: A. sylvaticus system.

(f) The structure of the parasite population of N. dubius

(i) N. dubius in A. sylvaticus

The intensities of infection of the individual cohorts of the population of N. dubius and the fecundity of N. dubius in male and female A. sylvaticus during the period January 1974 to October 1975 are shown in Figs. 9 and 10. The individual cohorts of N. dubius also exhibit different patterns of behaviour in male and female mice. In contrast to the S. stroma: A. sylvaticus relationship there is less stability in the male system and the intensities of infection of gravid females and adult males show greater fluctuation than that seen in the female host. In female mice the populations of the gravid female worms and adult male worms are characterised by a slow build up of infection through the autumn and winter months of 1973-1974 and 1974-1975 and more nearly match a logistic growth curve of a K-strategist. Making the assumption that 120 is the maximum carrying capacity of the small intestine of the female mouse for gravid female worms, the slopes for the growth curves of this sector of the parasite population in 1973-1974 and 1974-1975 are 0.65 and 0.38 respectively. Similarly with a maximum carrying capacity of 100, the slopes of the growth curves of adult male worms are 0.48 and 0.89 respectively. The smaller slope of the growth curve of adult male worms in 1973-1974 is followed by remarkable stability of this cohort in the female host during the winter months of 1973-1974. The growth curves of
Fig. 9  The intensity of infection of individual cohorts of the population of *N. dubius* and the fecundity of *N. dubius* in male *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset.

POPULATION STRUCTURE OF N. DUBIUS IN MALE A. SYLVATICUS

Log 10 Intensity

Gravid ♀
Non gravid ♀

Adult ♀

Larval ♀

Fecundity

J J A S O N D J F M A M J J A S O
1973 1974 1975
Fig. 10  The intensity of infection of individual cohorts of the population of *N. dubius* and the fecundity of *N. dubius* in female *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
POPULATION STRUCTURE OF N. DUBIUS IN FEMALE A. SYLVATICUS.

Log.10 Intensity

- O Gravid ♀
- ● Non gravid ♀

Adult

Larval ♀

Larval

Fecundity

gravid female worms and adult male worms in the male host only approach the logistic growth curve in 1973-1974. Making the assumption that 360 and 220 represent the maximum carrying capacity of the small intestine of the male mouse, the slopes for the growth curves of gravid female worms and adult male worms are 0.94 and 0.86 respectively. These sharper slopes are characterised by more instability in the male mouse parasite system.

All cohorts of *N. dubius* in male and female hosts show a decline over the study period and this is most marked in the larval input. Larval input into the male mouse system is higher than in the female mouse system and infection of female mice appears to take place in the autumn and winter periods of the year. Larval input is low in both sexes and the intensities of infection seen in all hosts must be due to good larval survival in *A. sylvaticus*.

The intensities of infection of the individual cohorts of the population of *N. dubius* and the fecundity of *N. dubius* in juvenile and adult *A. sylvaticus* during the period January 1974 to October 1975 are shown in Figs. 11 and 12. The intensities of infection of gravid female worms and adult male worms show a slow build up in juvenile mice in the winter months of 1973-1974 and 1974-1975, and suggest infection in the nest during the autumn breeding season.

The fecundity of *N. dubius* in male and female *A. sylvaticus* was 21.9±6.4 and 21.7±7.1 respectively but this difference is not significant. The lower intensities of infection in the autumn months of 1974 and 1975 are marked by rises in fecundity and indicates some intraspecific interaction. The fecundity of *N. dubius* in juvenile and adult *A. sylvaticus* was 23.1±9 and 21.2±4.7 respectively but the difference is not significant.
Fig. 11  The intensity of infection of individual cohorts of the population of *N. dubius* and the fecundity of *N. dubius* in juvenile *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
POPULATION STRUCTURE OF N. DUBIUS IN JUVENILE A. SYLVATICUS.

- Gravid ♀
- Non gravid ♀
- Larval ♀
- Larval ♂

Fecundity

1973 1974 1975
Fig. 12 The intensity of infection of individual cohorts of the population of *N. dubius* and the fecundity of *N. dubius* in adult *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
POPULATION STRUCTURE OF *N. DUBIUS* IN ADULT *A. SYLVATICUS*

Log 10 Intensity

- **Gravid ♀**
- **Non gravid ♀**

Adult Male

Larval Female

Larval Male

Fecundity

- 20
- 10
- 2

1973 1974 1975
The structure of the parasite population of *N. dubius* in *A. sylvaticus* from January 1974 to October 1975 is shown in Table VI. Sixty-nine per cent of the worms reside in the male host and the percentages of cohorts differs from that of the female host. There is a higher percentage of adult and larval males in female mice but a lower percentage of gravid females. Thus it would appear that the small intestine of the female mouse affords a better environment for male worm survival but some factor may come into operation to keep the number of gravid female worms lower in the female host. The egg output of the female system was 55% that of the male system but egg output from all female hosts over the study period constituted only 29.3% of the total egg output of the parasite. Twenty per cent of the worms reside in juvenile *A. sylvaticus* and the larval cohorts of *N. dubius* constitute only 2.34% of this total. The percentage of larval male worms is higher in juveniles than in adults and suggests some bias against this cohort as the mouse matures.

(ii) *N. dubius* in *A. flavicollis*

A total of 194 worms were found in 14 specimens of *A. flavicollis*. The primary and secondary sex ratios were 1:1 and 0.65:1 respectively which indicates a similar survival rate for adult male worms as seen in *A. sylvaticus*. The mean fecundity was 26.2±11.2 and the total egg output amounted to 2.85% of that of the output from the *N. dubius* : *A. sylvaticus* system.

(iii) *N. dubius* in *C. glareolus*

A total of 266 worms were found in 186 hosts of *C. glareolus*. The primary and secondary sex ratios were 0.42 and 0.87 respectively which suggests lower survival values for larval males as compared to adult male worms. The mean fecundity was 14.7±5.4 and total egg output was only 1.9% of that of
TABLE VI: Population structure of *N. dubius* in *A. sylvaticus*

Total number of worms = 8,200

<table>
<thead>
<tr>
<th>Population</th>
<th>Male</th>
<th>Female</th>
<th>Juvenile</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of hosts</td>
<td>279</td>
<td>207</td>
<td>269</td>
<td>217</td>
</tr>
<tr>
<td><em>N. dubius</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>5658</td>
<td>2542</td>
<td>1640</td>
<td>6560</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravid ♀</td>
<td>57.6</td>
<td>53.6</td>
<td>57.4</td>
<td>55.12</td>
</tr>
<tr>
<td>Non gravid ♀</td>
<td>5.3</td>
<td>7.8</td>
<td>8.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Larval ♀</td>
<td>1.1</td>
<td>0.59</td>
<td>1.2</td>
<td>1.46</td>
</tr>
<tr>
<td>Adult ♀</td>
<td>35.3</td>
<td>36.8</td>
<td>31.8</td>
<td>36</td>
</tr>
<tr>
<td>Larval ♂</td>
<td>0.71</td>
<td>1.2</td>
<td>1.3</td>
<td>0.88</td>
</tr>
<tr>
<td>Primary Sex Ratio</td>
<td>0.66</td>
<td>2.1</td>
<td>1.05</td>
<td>0.6</td>
</tr>
<tr>
<td>Secondary Sex Ratio</td>
<td>0.61</td>
<td>0.6</td>
<td>0.48</td>
<td>0.58</td>
</tr>
<tr>
<td>Total Sex Ratio</td>
<td>0.56</td>
<td>0.61</td>
<td>0.49</td>
<td>0.59</td>
</tr>
<tr>
<td>Intensity</td>
<td>21.7±16.2</td>
<td>16.1±16.3</td>
<td>11±16</td>
<td>22.8±15.6</td>
</tr>
<tr>
<td>Fecundity</td>
<td>21.9±6.4</td>
<td>21.7±7.1</td>
<td>23.1±9</td>
<td>21.2±4.7</td>
</tr>
<tr>
<td>Egg output per host</td>
<td>255.9</td>
<td>142.8</td>
<td>80.8</td>
<td>353.3</td>
</tr>
<tr>
<td>Total egg output</td>
<td>71,415.9</td>
<td>29,555.4</td>
<td>21,737.1</td>
<td>76,659.2</td>
</tr>
</tbody>
</table>

Location: Lytchett Matravers Estate, Poole, Dorset

Time: January 1974 to October 1975
the egg output from the *N. dubius: A. sylvaticus* system.

**Discussion**

In this chapter the incidence and intensity of infection of some of the parasites of small mammals have been studied in the Poole area during a period from June 1973 to October 1975. In some respects it repeats and enhances work of previous authors (Elton, Ford and Baker 1939; Thomas 1953; Sharpe 1964; Lewis 1968; Rainbow 1972; Healing 1981; Hominick and Aston 1981) but it differs in that the period of study is more than twice as long as any other, the structure of two of the parasite populations are studied in detail and the results are analyzed in terms of system theory and r- and K- strategy. The two host parasite relationships studied in this way are those of *S. stroma: A. sylvaticus* and *N. dubius: A. sylvaticus*. The longer period of study has revealed changes in the two systems which have resulted in variation of output and consequent change in system behaviour (Ratcliffe, Taylor, Whitlock and Lynn 1969; Crofton 1971; Kennedy 1975).

The *S. stroma: A. sylvaticus* is an unstable system with wide oscillations in intensity of infection particularly in female and juvenile hosts and this characterizes an r- strategy for the parasite. The more constant levels of infection in the male host maintain the parasite in the host population throughout the year and probably serve as a source of reinfection of females and juveniles during the breeding season. The relative stability of the male system may in part be due to a closer approach to a logistic growth curve in the winter of 1973-1974.

Fluctuations in intensity of infection in the *N. dubius: A. sylvaticus* system are not so extreme and more nearly represent a K- strategy. The rates of increase in the population growth of the adult cohorts are slower and peaks of intensity of infection have smaller values. Fluctuations as a whole are smaller in the female mouse than in the male and it is probable that this system is more important in the survival of *N. dubius*. 

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Approximately forty four per cent of the total population of *S. stroma* are larvae and this high larval input is responsible for the rapid increases in the populations of the adult cohorts. Larval input follows a seasonal pattern in female and juvenile hosts but maintains a more constant pattern in male and adult hosts. The uninterrupted larval input into the male system results in two thirds of the worms residing in the male host. Resource allocation is in favour of adult female worms in both sexes of host and they outnumber male worms in an approximate ratio of 6:1. The large numbers of worms which build up in the *S. stroma*: *A. sylvaticus* system probably contribute to the instability of the system for the greater the number of interactions between the components of a system the greater the variety of behaviour exhibited by the system (Open University T1001 1976). This coupled with differing times of larval input into female and juvenile hosts predict the system behaviour typical of an *r*-strategy.

In contrast, the larval input into the *N. dubius*: *A. sylvaticus* system is low and constitutes only 1.8% of the total worm population. It allows a slow rate of increase in the population growth of the adult cohorts and therefore lesser fluctuation around an equilibrium level. Most of the larval input is into the male host parasite system and as a consequence 69% of adult worms reside in the male host. Resources are concentrated in the maintenance of adult worms within the host and the high percentage of adult males accounts for the small number of non-gravid females.

The fecundity of *S. stroma* does not vary significantly in any sector of the mouse population and ranges from 26-43 per adult worm. Fecundity also appears to bear little relationship to the intensity of infection with *S. stroma* and food resource is probably not a limiting factor in reproduction of the nematode. The fecundity of *N. dubius* is lower than that of *S. stroma* and ranges from 2-40 per adult worm. It is lower in the autumn and winter months of 1974 and 1975 as the intensity of
infection builds up in the mouse population and such a strategy of competitive ability in gravid female worms for a possible food resource is further evidence of K-selection.

Two thirds of the egg output of *S. stroma* and *N. dubius* is from the male host parasite system and a similar partitioning of egg output also occurs between adult and juvenile mice in a ratio of 3:1. However, seven times as many worms of *S. stroma* only produce 3.4 times as many eggs as *N. dubius* so in terms of efficiency in the utilisation of resources the *N. dubius: A. sylvaticus* system is superior by 50%.

Both species of parasite employ a survival strategy where their ecology overlaps with other species of small mammals. *S. stroma* and *N. dubius* are able to establish in *A. flavicollis* but egg output from these systems were only 1.7% and 2.35% of that of the *A. sylvaticus* system respectively. *N. dubius* was also present in *C. glareolus* but the host parasite relationship is only a tenuous one for 186 hosts only produced an egg output equivalent to 1.9% of that of the *N. dubius: A. sylvaticus* system. Similar diminished egg output has been described for *N. dubius* in the abnormal laboratory host (Cross and Duffy 1963).

The changes that occur in egg output of both systems in 1975 are associated with changes in intensity of infection rather than changes in fecundity. In 1975 the larval input and egg output of the *S. stroma: A. sylvaticus* system began to decrease as a result in a decrease in intensity of infection with adult male and gravid female worms. As spread of infection only occurs if hosts come into direct contact with each other this is a probable consequence of the depletion of hosts throughout the study period. The mode of transmission of the parasite must in part determine the type of system operation for eggs laid on perianal fur will have a high survival value and contribute a high larval input into the system. In the *N. dubius: A. sylvaticus* system the infective larvae are subjected to the external environmental variables of temperature and rainfall as well as being dependent on
spatial distribution. Such a system will have a low larval input and
greater stability will result. It will, however, still be dependent
on the number and type of hosts available for infection as well as the
length of life in the host. *N. dubius* can persist for eight months
or more in the laboratory mouse (Scott, Cross and Dawson 1951) and it
is likely that persistence in *A. sylvaticus* is similar. Any alteration
in system behaviour and output will therefore be accompanied by a time
lag. Changes in the *S. stroma: A. sylvaticus* system occur earlier in
the study and suggest a shorter life span for *S. stroma* in *A. sylvaticus*.
The host *A. sylvaticus* is thus being exploited by parasites employing
r- and K- strategies. *S. stroma* has a variable population size with
high rates of increase and rapid falls in intensity of infection. The
system so created leads to productivity and instability and is typical
of an r- strategy. *N. dubius* on the other hand relies on small numbers
and slower rates of increase to produce a more stable system with
greater efficiency and this is more representative of a K- strategy.
Such a hypothesis is not inconsistent with niche theory, for keen
competition between two K- strategists in the same host would probably
lead to the extinction of one or both species. Further partitioning
of the resource spectrum is indicated by the behaviour of the parasites
in the different sexes of the host. *S. stroma* establishes its most
stable system in the male host while *N. dubius* utilises the female host
for this purpose.
CHAPTER 2
REGULATION OF THE HOST PARASITE SYSTEMS OF SYPHACIA STROMA: APODEMUS SYLVAUTICS AND NEMATOSPIROIDES DUBIUS: APODEMUS SYLVAUTICS

Introduction

The previous chapter has identified parameters in the host parasite systems of Syphacia stroma: Apodemus sylvaticus and Nematospiroides dubius: Apodemus sylvaticus which indicate an r- strategy for S. stroma and a K- strategy for N. dubius. They were associated with oscillation in egg output as well as changing equilibrium levels of the system during 1974 and 1975. The present chapter examines the controls within the host parasite relationships that may have been responsible for these observations.

A host parasite system which employs an r- strategy will have lax controls, for its operation is dependent on the continual input of large numbers of infective larvae and the use of time lag within the system to maintain itself. A host parasite system which employs a K- strategy will possess tighter controls for the population size of the parasite is fairly constant in time with small fluctuations around an equilibrium level. The controls upon both types of system may be in the external environment or exist within the system itself.

Controls in the external environment will include temperature, humidity and rainfall, all of which are essential in the early development of the free living infective stages of parasites. The oxyurid S. stroma gains entry into the host when infective eggs laid on the perianal fur are transferred to the mouth during grooming behaviour. As such the eggs are relatively protected from the external environment by the more equable microclimate of the mouse fur and since transmission between hosts is through host
contact, control in this compartment of the *S. stroma: A. sylvaticus* system will be relatively weak. In contrast *N. dubius* gains entry into the host through the ingestion of infective L3 larvae which have hatched from embryonated eggs laid on pasture and environmental variables will therefore assume greater importance in the *N. dubious: A. sylvaticus* system control.

Regulatory controls of the parasite population within the systems of *S. stroma: A. sylvaticus* and *N. dubius: A. sylvaticus* will include the density dependent mechanisms of host population density, intraspecific and interspecific competition between parasites and the threshold level of parasite density at which the host immune response is triggered (Anderson 1978; Anderson and May 1978). Individual host differences in diet, susceptibility and behaviour combined with heterogeneity in the external environment will cause aggregated distributions of the parasites to occur within their hosts. Over-dispersion of this nature can give rise to excessive regulation of the parasite population if it is accompanied by the death of a heavily infected host at a time when reproduction and contact of hosts is at a minimum. The net reproductive rate of the parasite may be so reduced that it is no longer able to maintain itself within the host population (May and Anderson 1979). In this respect, *S. stroma* will be more vulnerable than *N. dubius* for the death of a heavily infected host in the *N. dubius: A. sylvaticus* system is balanced by the survival of trickle and aggregated infections on the pasture. The regulation of the parasite population will be the more complete if the parasite has a short life span and high pathogenicity. Parasites which invoke the host immune response too early in the life of the host fail to reach the requisite threshold level of intensity of infection essential to their establishment in the susceptible host population.

Selection in host parasite systems will therefore be for moderate pathogenicity and those factors which lower the immune response of the host and thereby enhance the survival and transmission of the parasite.
Stress in the mammalian host due to competition for resources and habitat, disease and pregnancy can significantly lower host immune response to parasitic infection (Josephine 1958; Noble 1961; Oliver 1962; Esch 1967; Behnke 1975; Esch, Gibbons and Bourque 1975; Hall and Gross 1975; Selby and Wakelin 1975). The mediation of the stress response is initially through the sympathetic nervous pathway and invokes the fight or flight defence mechanism of the alarm reaction. It is characterised by an enlargement of the adrenal glands, the loss of corticoid laden lipids from the adrenal cortex, haemoconcentration, hypochloremia, loss of body weight due to tissue catabolism and emphasis on the production of catecholamines from the adrenal medulla. If stress is prolonged the host response is switched to the endocrine pathway and control through the pituitary-adrenal cortex axis predominates (Henry 1980). The switch is achieved by the release of cortico-trophin releasing factors from the hypothalamus (CRF) to which the pituitary is sensitive and the subsequent production of the adrenocorticotropic hormone (ACTH) by the pituitary gland. The effect of ACTH on the adrenal cortex is to cause still further enlargement of the adrenal gland and to stimulate the production of glucocorticoids, mineralocorticoids and sex hormones (Brain 1972). The strategy becomes one of conservation and withdrawal and the host successively passes through the resistance and exhaustion stages of Selye's General Adaptation Syndrome (1973).

Glucocorticoids rapidly mobilise nitrogen pools in the body, in particular those that reside in lymphatic tissue. This has an important influence on the ability to mount an immune response to infection for it affects such organs as the spleen and thymus as well as the more scattered lymph nodes which occur in Peyer's patches and the mesenteric nodes (Dougherty 1952). The mineralocorticoids have their chief action on the kidney tubules and promote the retention of sodium and the excretion of potassium. The manufacture of sex hormones by the adrenal cortex causes the
production of sex hormones by the testes to decline and this is
accompanied by a deterioration in function, inhibition of spermatid
maturation and a decrease in the weight of the testes and the accessory
glands of the male reproductive system (Gürtner, Schüller and Reznik
1973). The interaction of the sex hormones from the adrenal cortex
and ovarian androgens makes the situation more complex with respect
to ovarian size.

The central position of the adrenal gland in the stress response and its
proportional enlargement with increasing levels of stress has led to the
use of adrenal gland weight as an indicator of stress in small mammal
populations in a number of laboratory and field studies (Davis and Christian
1957; Chitty 1961; Brain and Nowell 1969; McKinney and Pasley 1973).
Adrenal gland weight in this chapter is considered with respect to certain
morpho-physiological indices of general metabolic function and immune
status of the host. The general response of the host to stress in the
external environment is measured by changes in the weight of the specific
target organs of stress, viz the kidneys, heart, liver and gonads and the
immune status of the host is considered in the context of the weights of
the thymus, mesenteric nodes and spleen and the area of Peyer's patches
in the small intestine. These parameters are then related to the egg
output of the two host parasite systems of S. stroma: A. sylvaticus and
N. dubius: A. sylvaticus. In addition the parasite intensities of
infection in A. sylvaticus are correlated with the environmental variables
of temperature, rainfall and humidity and host regulation of the parasite
population is assessed in terms of overdispersion, intrinsic density,
dependent mechanisms and host age of immune response.

Materials and Methods

The trapping procedure, the mode of sacrifice of A. sylvaticus and the
examination of hosts for parasites have already been described in Chapter 1.
The daily environmental variables of temperature, humidity and rainfall were recorded on a site approximately one mile away from the study area. The intensity of infection of *S. stroma* and *N. dubius* in male and female *A. sylvaticus* is correlated with the environmental variables of the corresponding and preceding month. Statistical analysis uses the approximation of the $\hat{k}$ exponent of the negative binomial distribution (Elliot 1977) to describe the frequency distribution of *S. stroma* and *N. dubius* in male and female *A. sylvaticus*. Overdispersion is described by a low value for $\hat{k}$ and a less aggregated distribution is described by a high value for $\hat{k}$. In addition the parasite intensities of infection are correlated with the fecundity of gravid female worms, the fecundity of *S. stroma* and *N. dubius* is compared in single and concurrent infections and the intensities of infection are related to the age of the host with respect to age classes of 2 grams weight.

The dissection of the adrenal glands, kidneys, liver, heart, testes and accessory glands, spleen, thymus and mesenteric nodes took place after the removal of the alimentary canal from the host. They were placed in 70% alcohol and later dissected free from their surrounding tissue. After drying lightly with Whatman No. 1 filter paper, they were weighed on a Torbal balance Model ET1. Fresh weights of the organs were not used because of the drying of the body cavity which occurred during the initial dissection. The weights of these organs are described in terms relative to the weight of the animal.

If $x = \text{weight of organ}$,

Then

$$\text{Relative weight of } x = \frac{\text{Weight of } x \ (g)}{\text{Total Body weight \ (g)}}$$

Multiplication of the relative weight up to 100g is usual, but it is not made in this study for it assumes a linear relationship between organ and body weight and will exaggerate discrepancies if organ weight and body weight fail to follow such a regression (Brain and Nowell 1969).
Peyer's patches showed up as white areas and were present throughout the small intestine. Their length was measured and their approximate area calculated using the widest point of the patch as the width. Hence no distinction was made between oval and square Peyer's patches. The area of circular patches was calculated from their diameter. The Peyer's patch area was summed and expressed as a percentage of the length of the small intestine. This value is called the Peyer's patch area.

The mean monthly egg output of the S. stroma: A. sylvaticus and the N. dubius: A. sylvaticus system is used as a measure of system function with respect to the morphophysiological parameters indicative of general metabolic state and immune status of the host. It is derived from the product of the number of gravid female worms present and their mean fecundity and as such represents an instantaneous value.

Results

1. The environmental variables

The environmental variables of temperature, relative humidity and rainfall during the study period are shown in Fig. 1 and correlation analysis between the intensity of infection and environmental variables is shown in Table 1. Correlation of the environmental variables of the month preceding the intensity of infection of the parasite give higher correlation coefficients with respect to rainfall for S. stroma and for temperature and rainfall for N. dubius. Temperature would appear to be more important to N. dubius than rainfall. The mean monthly maximum temperature for 1974 and 1975 were 14.3°C ± 4.5 and 18.9°C ± 5.96 respectively. Similarly the mean monthly minimum temperatures were 6.9°C ± 3.4 and 7.5°C ± 4.3 and mean monthly rainfalls were 3.63 ins ± 2.8 and 2.46 ins ± 1.93. Only the difference in the mean monthly maximum temperature was significant (P = 0.1).
Fig. 1  The environmental variables of temperature, humidity and rainfall on Lytchett Matravers Estate, Poole, Dorset from June 1973 to October 1975
ENVIRONMENTAL VARIABLES - JUNE 1973 TO OCT. 1975.

MAXIMUM TEMPERATURE.

MINIMUM TEMPERATURE.

MAXIMUM RELATIVE HUMIDITY.

MINIMUM RELATIVE HUMIDITY.

RAINFALL.
The correlation coefficients between the intensities of infection with S. stroma and N. dubius and environmental variables

June 1973 to October 1975

<table>
<thead>
<tr>
<th>Environmental Variable</th>
<th>Concurrent Correlation Coefficient</th>
<th>Lag Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. stroma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum Temperature (°C)</td>
<td>-0.265</td>
<td>-0.251</td>
</tr>
<tr>
<td>Minimum Temperature (°C)</td>
<td>-0.156</td>
<td>-0.138</td>
</tr>
<tr>
<td>Maximum Relative Humidity (%)</td>
<td>+0.035</td>
<td>+0.069</td>
</tr>
<tr>
<td>Minimum Relative Humidity (%)</td>
<td>+0.009</td>
<td>+0.079</td>
</tr>
<tr>
<td>Rainfall (ins)</td>
<td>+0.338</td>
<td>+0.407</td>
</tr>
<tr>
<td><strong>N. dubius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum Temperature (°C)</td>
<td>-0.297</td>
<td>-0.505</td>
</tr>
<tr>
<td>Minimum Temperature (°C)</td>
<td>-0.334</td>
<td>-0.492</td>
</tr>
<tr>
<td>Maximum Relative Humidity (%)</td>
<td>-0.145</td>
<td>+0.104</td>
</tr>
<tr>
<td>Minimum Relative Humidity (%)</td>
<td>-0.182</td>
<td>+0.074</td>
</tr>
<tr>
<td>Rainfall (ins)</td>
<td>-0.081</td>
<td>+0.369</td>
</tr>
</tbody>
</table>
2. The frequency distributions of *S. stroma* and *N. dubius* in *A. sylvaticus*

The frequency distributions of *S. stroma* and *N. dubius* in male and female *A. sylvaticus* are shown in Tables II and III respectively. The values for the approximation of the $\hat{k}$ exponent of the negative binomial distribution for the male and female *S. stroma: A. sylvaticus* systems are 0.23 and 0.16 respectively. The female system therefore exhibits the greater over-dispersion. The values for the $\hat{k}$ exponent in the male and female *N. dubius: A. sylvaticus* systems are 0.35 and 0.32 respectively. Overdispersion is therefore more marked in the *S. stroma: A. sylvaticus* system and may be a consequence of an *r*-strategy while the less aggregated distribution of *N. dubius* in *A. sylvaticus* indicates that overdispersion as a regulatory control of the parasite population is of less importance in this system.

The spatial distribution of both parasites as indicated by the approximation to the $\hat{k}$ exponent varied during the course of the study and the changes are shown in Fig. 2. Overdispersion of *S. stroma* in the summer of 1973 moves to a less aggregated distribution in the winter of 1973-1974. The slope of the change in the $\hat{k}$ exponent is such that the $\hat{k}$ value oscillates in time around an equilibrium and some stability in spatial distribution is achieved. Overdispersion is not as extreme in the summer of 1974 but the sharp change to the less aggregated distribution of the parasite in the winter of 1974 indicates a possible disturbance in the system. The changes that follow are such that overdispersion occurs at a time when the loss of a host with a heavy parasite burden could have important consequences for the parasite population. If the hosts are not replaced by host reproduction or immigration then the net reproductive rate of the parasite will show a steady decline.
<table>
<thead>
<tr>
<th>Number of Parasites</th>
<th>Male Host % Total Population</th>
<th>Female Host % Total Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 19</td>
<td>39.7</td>
<td>58.9</td>
</tr>
<tr>
<td>20 - 39</td>
<td>11.6</td>
<td>6.9</td>
</tr>
<tr>
<td>40 - 59</td>
<td>6.5</td>
<td>6.9</td>
</tr>
<tr>
<td>60 - 79</td>
<td>5.8</td>
<td>2.5</td>
</tr>
<tr>
<td>80 - 99</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>100 - 119</td>
<td>3.97</td>
<td>3.5</td>
</tr>
<tr>
<td>120 - 139</td>
<td>3.97</td>
<td>0.5</td>
</tr>
<tr>
<td>140 - 159</td>
<td>2.2</td>
<td>1.98</td>
</tr>
<tr>
<td>160 - 179</td>
<td>1.1</td>
<td>0.99</td>
</tr>
<tr>
<td>180 - 199</td>
<td>1.8</td>
<td>0.99</td>
</tr>
<tr>
<td>200 - 219</td>
<td>1.8</td>
<td>1.98</td>
</tr>
<tr>
<td>220 - 239</td>
<td>2.2</td>
<td>0.99</td>
</tr>
<tr>
<td>240 - 259</td>
<td>1.1</td>
<td>0.99</td>
</tr>
<tr>
<td>260 - 279</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>280 - 299</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>300 - 319</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>320 - 339</td>
<td>0.3</td>
<td>0.99</td>
</tr>
<tr>
<td>340 - 359</td>
<td>0.3</td>
<td>0.99</td>
</tr>
<tr>
<td>380 - 399</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>420 - 439</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

(continued..)
<table>
<thead>
<tr>
<th>Number of Parasites</th>
<th>Male Host % Total Population</th>
<th>Female Host % Total Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>440 - 459</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>460 - 479</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>480 - 499</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>500 - 519</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>520 - 539</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>540 - 559</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>560 - 579</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>580 - 599</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>600 - 619</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>620 - 639</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>640 - 659</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>660 - 679</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>680 - 699</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>700 - 719</td>
<td>0.36</td>
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</tr>
<tr>
<td>720 - 739</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>740 - 759</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>800 - 819</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>860 - 879</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>880 - 899</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>920 - 939</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>940 - 959</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>1000 - 1019</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Number of Parasites</td>
<td>Male Host % Total Population</td>
<td>Female Host % Total Population</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>1040 - 1059</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>1060 - 1079</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1180 - 1199</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1220 - 1239</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1280 - 1299</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>1380 - 1399</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1480 - 1499</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>1760 - 1779</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>
### Table III: The frequency distribution of N. dubius in A. sylvaticus

<table>
<thead>
<tr>
<th>Number of Parasites</th>
<th>Male Host % Total Population</th>
<th>Female Host % Total Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 9</td>
<td>48.03</td>
<td>64</td>
</tr>
<tr>
<td>10 - 19</td>
<td>16.8</td>
<td>14.8</td>
</tr>
<tr>
<td>20 - 29</td>
<td>12.9</td>
<td>9.4</td>
</tr>
<tr>
<td>30 - 39</td>
<td>8.96</td>
<td>3.5</td>
</tr>
<tr>
<td>40 - 49</td>
<td>2.87</td>
<td>3.5</td>
</tr>
<tr>
<td>50 - 59</td>
<td>2.87</td>
<td>0.49</td>
</tr>
<tr>
<td>60 - 69</td>
<td>2.51</td>
<td>2.46</td>
</tr>
<tr>
<td>70 - 79</td>
<td>1.43</td>
<td>0.98</td>
</tr>
<tr>
<td>80 - 89</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>90 - 99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 - 109</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>110 - 119</td>
<td>0.35</td>
<td>0.98</td>
</tr>
<tr>
<td>120 - 129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>130 - 139</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>180 - 189</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>260 - 269</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2  The spatial distribution of *N. dubius* and *S. stroma* in *A. sylvaticus* on Lytchett Matravers Estate, Poole, Dorset

June 1973 to October 1975
THE SPATIAL DISTRIBUTION OF N. DUBIUS AND S. STROMA IN A. SYLVATICUS.


N. dubius.

S. stroma.
Overdispersion in the *N. dubius*; *A. sylvaticus* occurs less frequently than it does in the *S. stroma*; *A. sylvaticus* system but there is a marked oscillation in the spatial distribution of the parasites within the hosts towards the end of the study period and this suggests a sudden introduction of instability into the system.

3. The fecundity of *S. stroma* and *N. dubius*

The correlation coefficients between the intensities of infection and the fecundity of *S. stroma* in male and female *A. sylvaticus* are +0.175 and +0.347 respectively. Similarly the correlation coefficients between the intensities of infection and the fecundity of *N. dubius* in male and female *A. sylvaticus* are -0.32 and -0.38 respectively. Some positive cooperation between worms is therefore indicated in the *S. stroma*; *A. sylvaticus* system and a modicum of intraspecific competition may exist in the *N. dubius*; *A. sylvaticus* system. The coefficient does not improve in either system when the intensity of infection with gravid female worms alone is correlated with their fecundity.

The fecundity of *S. stroma* in single and concurrent infections with *N. dubius* in male *A. sylvaticus* is 26.2 \(\pm\) 9.8 and 28.6 \(\pm\) 3.6 respectively. In the female host the fecundity of *S. stroma* in single and concurrent infections with *N. dubius* is 35.5 \(\pm\) 11.2 and 25.04 \(\pm\) 5.4 respectively and the difference is significant \((P = 0.01)\). Some competitive superiority for *N. dubius* over *S. stroma* in the female host is therefore indicated. Similarly the fecundity of *N. dubius* in single and concurrent infections with *S. stroma* in the male host is 22.73 \(\pm\) 12.4 and 21.96 \(\pm\) 6.4 but the difference is not significant. Neither is there a significant difference in the fecundity of *N. dubius* in single and concurrent infections with *S. stroma* in the female host where the values for the fecundity of *N. dubius* are 28.5 \(\pm\) 18.4 and 21.7 \(\pm\) 7.1 respectively.
4. Age of the host and intensity of infection

The intensities of infection of *S. stroma* and *N. dubius* in *A. sylvaticus* with respect to the age of the host are shown in Tables IV and V. The variance on the mean worm burdens is high but both parasites show a general trend of increasing worm burden up to the peak of the host breeding period followed by a loss of worms as the host undergoes senescence. There is some loss of *S. stroma* in the 24 - 25.9 g and 26 - 27.9 g weight classes in male and female hosts respectively. In both hosts this is followed by a further increase in intensity of infection and then a dramatic decrease in worm burden as the host passes reproductive age. The intensity of infection of *N. dubius* in male and female *A. sylvaticus* decreases in the 26 - 27.9 g weight classes. In the female host the intensity of infection remains lower than the initial values but shows a return to higher values in the male host. This may indicate a parasite evasion of the male host immune response.

5. Host status

(a) Adrenal gland weight

The mean monthly relative adrenal gland weights of male and female *A. sylvaticus* during the period from January 1974 to October 1975 are shown in Figs. 3 and 4. Stress as indicated by adrenal gland weight is most evident during the months of January to June in 1974 and 1975, a period when the onset of breeding comes as a climax to the stress already experienced during winter. These months are also the period when all juveniles of the autumn breeding season become adult and experience social interaction with adults of the preceding year. The mean adrenal gland weights of the adult male mice for January-June 1974 and January-June 1975 are $3.97 \times 10^{-4} \pm 9.4 \times 10^{-5}$ and $3.21 \times 10^{-4} \pm 8.7 \times 10^{-5}$ and
<table>
<thead>
<tr>
<th>Age of Host $g.$</th>
<th>MALE HOST Mean Worm Burden</th>
<th>FEMALE HOST Mean Worm Burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 - 9.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 - 11.9</td>
<td>$27.5 \pm 73$</td>
<td>$50.8 \pm 114$</td>
</tr>
<tr>
<td>12 - 13.9</td>
<td>$33.3 \pm 34.1$</td>
<td>$87.14 \pm 192$</td>
</tr>
<tr>
<td>14 - 15.9</td>
<td>$70.4 \pm 178.2$</td>
<td>$69.7 \pm 190$</td>
</tr>
<tr>
<td>16 - 17.9</td>
<td>$103.1 \pm 227.3$</td>
<td>$65.7 \pm 133.6$</td>
</tr>
<tr>
<td>18 - 19.9</td>
<td>$123.3 \pm 190.1$</td>
<td>$103.2 \pm 238$</td>
</tr>
<tr>
<td>20 - 21.9</td>
<td>$169.6 \pm 336$</td>
<td>$99.8 \pm 159$</td>
</tr>
<tr>
<td>22 - 23.9</td>
<td>$161.6 \pm 290$</td>
<td>$113 \pm 291$</td>
</tr>
<tr>
<td>24 - 25.9</td>
<td>$153.1 \pm 206$</td>
<td>$283 \pm 442$</td>
</tr>
<tr>
<td>26 - 27.9</td>
<td>$214.7 \pm 294$</td>
<td>$65 \pm 48$</td>
</tr>
<tr>
<td>28 - 29.9</td>
<td>$250.3 \pm 293.6$</td>
<td>$141.8 \pm 211.8$</td>
</tr>
<tr>
<td>30 - 31.9</td>
<td>$10$</td>
<td>$2.7 \pm 3.1$</td>
</tr>
<tr>
<td>32 - 33.9</td>
<td>$16$</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE V  The intensity of infection of N. dubius with respect to the age of A. sylvaticus

<table>
<thead>
<tr>
<th>Age of Host $G$</th>
<th>MALE HOST Mean Worm Burden</th>
<th>FEMALE HOST Mean Worm Burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>$8 - 9.9$</td>
<td>$0.56 \pm 1.3$</td>
<td>$0.2 \pm 0.4$</td>
</tr>
<tr>
<td>$10 - 11.9$</td>
<td>$2.2 \pm 2.68$</td>
<td>$3 \pm 4.7$</td>
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<tr>
<td>$12 - 13.9$</td>
<td>$6.9 \pm 9.03$</td>
<td>$7.6 \pm 12.8$</td>
</tr>
<tr>
<td>$14 - 15.9$</td>
<td>$4.64 \pm 4.6$</td>
<td>$14.3 \pm 21.9$</td>
</tr>
<tr>
<td>$16 - 17.9$</td>
<td>$18.9 \pm 19.2$</td>
<td>$14.1 \pm 13.2$</td>
</tr>
<tr>
<td>$18 - 19.9$</td>
<td>$19.8 \pm 20.6$</td>
<td>$19.6 \pm 26.4$</td>
</tr>
<tr>
<td>$20 - 21.9$</td>
<td>$26 \pm 25.8$</td>
<td>$18.4 \pm 17.9$</td>
</tr>
<tr>
<td>$22 - 23.9$</td>
<td>$31.2 \pm 29.8$</td>
<td>$24.1 \pm 30.7$</td>
</tr>
<tr>
<td>$24 - 25.9$</td>
<td>$23.2 \pm 21.4$</td>
<td>$11 \pm 7$</td>
</tr>
<tr>
<td>$26 - 27.9$</td>
<td>$63.5 \pm 72.4$</td>
<td>$12.6 \pm 8.3$</td>
</tr>
<tr>
<td>$28 - 29.9$</td>
<td>$17$</td>
<td>$17 \pm 21.4$</td>
</tr>
<tr>
<td>$30 - 31.9$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$32 - 33.9$</td>
<td>$6$</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3

Morphophysiological indices of male *A. sylvaticus*
on Lytchett Matravers Estate, Dorset from January 1974 to October 1975.
MORPHOPHYSIOLOGICAL INDICES OF MALE
A. SYLVATICUS.

Relative Wt. (gms)

Adrenal gland.

Thymus.

Peyer's Patch Area.

1974 1975

**Figure 4**
MORPHOPHYSILOGICAL INDICES OF FEMALE A. SYLVATICUS.

Relative Weight (gms)

- - - Adults
- - - Juveniles

Adrenal gland

Thymus

Peyers Patch Area

J F M A M J J A S O N 1974
F M A M J J A S O N 1975
the difference is highly significant \((P = 0.001)\). Similarly the mean adrenal gland weights for adult female mice during the same periods are \(4.4 \times 10^{-4} \pm 1.3 \times 10^{-4}\) and 
\(3.7 \times 10^{-4} \pm 9.2 \times 10^{-5}\) but the difference is not so significant \((P = 0.05)\). The mean adrenal gland weights for juvenile males in these same periods are \(3.6 \times 10^{-4} \pm 1.2 \times 10^{-4}\) and 
\(3.3 \times 10^{-4} \pm 4.9 \times 10^{-4}\) and for the female juveniles they are 
\(2.5 \times 10^{-4} \pm 7.4 \times 10^{-5}\) and \(4.4 \times 10^{-4} \pm 1.3 \times 10^{-4}\). The difference is only significant in female juveniles \((P = 0.05)\).

The adrenal gland weight is generally higher in female mice and may be due to high titres of ovarian hormones which bring about a vacuolation of the cells between the medulla and cortex (Brain and Nowell 1971(b)). Adrenal gland size is also increased in pregnancy and lactation and this again is attributed to high levels of androgens being produced at the time (Chester Jones 1952).

(b) Morphophysiological indices of general metabolic function

The relative weights of the kidney, liver and heart for the periods January-June 1974 and January-June 1975 are shown for both sexes of mice in Table VI. Significant differences occur in the weights of the kidney and liver in male adult mice \((P = 0.001)\) and in the kidney and heart of female adult mice \((P = 0.002)\). Correlation of adrenal gland weight and kidney weight give a positive correlation coefficient of 0.569 and 0.206 in male and female mice. The correlation coefficients for adrenal gland weight and liver weight are also positive and are 0.31 and 0.38 in male and female mice respectively. These results suggest that stress causes kidney and liver enlargement. The relationship between adrenal gland weight and heart weight is a negative one and the correlation coefficients for these parameters are -0.411 and -0.28 in male and female mice respectively. These latter
<table>
<thead>
<tr>
<th>Relative Weight (g)</th>
<th>ADULT</th>
<th>JUVENTILE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan-June 1974</td>
<td>Jan-June 1975</td>
<td></td>
</tr>
<tr>
<td>MALE</td>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Kidney</td>
<td>$1.1\times10^{-2}$</td>
<td>$9.5\times10^{-3}$</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$\pm1.5\times10^{-3}$</td>
<td>$\pm1.2\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>$5.3\times10^{-2}$</td>
<td>$4.7\times10^{-2}$</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$\pm7.3\times10^{-3}$</td>
<td>$\pm6.5\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>$7.1\times10^{-3}$</td>
<td>$7.4\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\pm1.18\times10^{-3}$</td>
<td>$\pm1.17\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>$2.5\times10^{-2}$</td>
<td>$2.4\times10^{-2}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\pm1.2\times10^{-2}$</td>
<td>$\pm6.8\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>*vs/cg</td>
<td>$1.16\times10^{-2}$</td>
<td>$9.6\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$-6.2\times10^{-3}$</td>
<td>$-5.9\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>FEMALE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>$1.17\times10^{-2}$</td>
<td>$1.06\times10^{-2}$</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>$\pm1.97\times10^{-3}$</td>
<td>$\pm1.28\times10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>$5.7\times10^{-2}$</td>
<td>$5.2\times10^{-2}$</td>
<td></td>
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<tr>
<td></td>
<td>$\pm1.1\times10^{-2}$</td>
<td>$\pm1.03\times10^{-2}$</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>$7.4\times10^{-3}$</td>
<td>$8.13\times10^{-3}$</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>$\pm9.3\times10^{-4}$</td>
<td>$\pm1.18\times10^{-3}$</td>
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*vs/cg vesicula seminalis and coagulating gland
results are in contradiction with the enlarged heart normally associated with stress (Selye 1950). There is no significant difference in the weight of the testes and vesicula seminales and coagulating glands during the two periods of stress.

(c) Morphophysiologica indices of immune status

The relative weights of the thymus, spleen and mesenteric nodes and Peyer's patch area for the same periods of January-June 1974 and January-June 1975 are shown in Table VII. Significant differences between the two periods occur in the weight of the spleen and Peyer's patch area in the adult male host and in the thymus weight and Peyer's patch area of the adult female host. In the male the weight of the thymus and Peyer's patch area increase in 1975 when stress as indicated by adrenal gland weight is less. This suggests that more lymphatic tissue is present in male mice and that the immune status of *A. sylvaticus* has improved. Larger amounts of lymphatic tissue are also present in female hosts in 1975 for the weights of the thymus in adult females are consistently higher (*P* = 0.001) and the area of Peyer's patches is also greater (*P* = 0.05). The mesenteric nodes are only significantly heavier in adult females in January-June 1975 (*P* = 0.01).

An inverse relationship exists between the weights of the adrenal gland and the thymus and also between adrenal gland weight and Peyer's patch area (Figs. 3 and 4). The correlation coefficients for adrenal gland weight and the thymus are -0.35 and -0.36 in male and female hosts respectively. Similarly the correlation coefficients for adrenal gland weight and Peyer's patch area are -0.57 and -0.55. Although there are limitations to the interpretation of correlation coefficients, these results
TABLE VII The morphophysiological indices of the immune status of
A. Sylvaticus January - June 1974 and January - June 1975

| Relative Weight (g) | ADULT | | JUVENILE | |
|---------------------|-------|---------------|--------|---------------|--------|
|                     | Jan-June 1974 | Jan-June 1975 | P      | Jan-June 1974 | Jan-June 1975 | P      |
| MALE                |       |               |        |               |        |        |
| Thymus              | 2.8x10^{-4} | 3.1x10^{-4} | 3.61x10^{-4} | 6.93x10^{-4} | 0.001 |
|                     | ±1.47x10^{-4} | ±1.4x10^{-4} | ±1.2x10^{-4} | ±3.4x10^{-4} |        |
| Spleen              | 3.5x10^{-3} | 2.6x10^{-3} | 2.5x10^{-3} | 2.1x10^{-3} |        |
|                     | ±1.8x10^{-3} | ±1.1x10^{-3} | ±1.6x10^{-3} | ±8.7x10^{-4} |        |
| Mesenteric nodes    | 1.09x10^{-3} | 1.23x10^{-3} | 1.1x10^{-3} | 1.3x10^{-3} | 0.01   |
|                     | ±4.5x10^{-4} | ±1.2x10^{-4} | ±2.5x10^{-4} | ±3.1x10^{-4} |        |
| Peyer's patch area  | 1.016 | 1.426 | 0.001 | 1.308 | 1.578 |
|                     | ±0.56 | ±0.52 | ±0.71 | ±0.5 |        |
| FEMALE              |       |               |        |               |        |        |
| Thymus              | 4.6x10^{-4} | 6.9x10^{-4} | 0.02 | 9.15x10^{-4} | 5.56x10^{-4} |
|                     | ±1.8x10^{-4} | ±4x10^{-4} | ±1.6x10^{-4} | ±4.76x10^{-4} |        |
| Spleen              | 2.85x10^{-3} | 2.33x10^{-3} | 2.65x10^{-3} | 2.32x10^{-3} |        |
|                     | ±1.2x10^{-3} | ±1.54x10^{-3} | ±2.13x10^{-3} | ±2.1x10^{-3} |        |
| Mesenteric nodes    | 1.45x10^{-3} | 1.43x10^{-3} | 8.7x10^{-4} | 9.6x10^{-4} |        |
|                     | ±7x10^{-4} | ±5.4x10^{-4} | ±2.57x10^{-4} | ±3.86x10^{-4} |        |
| Peyer's patch area  | 1.030 | 1.78 | 0.001 | 0.765 | 0.884 |
|                     | ±0.602 | ±0.75 | ±0.56 | ±0.22 |        |
are consistent with the involution of lymphatic tissue during stress. The relationship between adrenal gland weight and those of the spleen and mesenteric nodes are not so clear.

6. The egg output of the host parasite systems

The egg output per male and female *A. sylvaticus* for the *S. stroma: A. sylvaticus* and the *N. dubius: A. sylvaticus* systems is shown in Tables VIII and IX. The egg output of *S. stroma* per male host in 1974 and 1975 was $1311 \pm 985$ and $563.2 \pm 410$ respectively and the difference is significant ($P = 0.05$). The lower egg output of *S. stroma* per male host in 1975 is associated with a lower intensity of gravid female worms and lowered larval input. The intensity of infection of male *A. sylvaticus* with gravid female worms of *S. stroma* in 1974 and 1975 was $45.3 \pm 30$ and $20 \pm 15$ respectively and the difference is significant ($P = 0.05$). The larval input of *S. stroma* into the male *S. stroma: A. sylvaticus* system in 1974 and 1975 was $919.5 \pm 1061$ and $294.5 \pm 265$ and the difference is significant ($P = 0.1$). The female host parasite system of *S. stroma: A. sylvaticus* which possesses less stability than that of the male shows no such significant changes and is therefore not subject to the same level of control.

The egg output per male and female host of the *N. dubius: A. sylvaticus* system is also lower in 1975. The mean egg output of *N. dubius* per male host for 1974 and 1975 was $372 \pm 206$ and $223.7 \pm 160$ respectively and the difference is significant ($P = 0.1$). The mean egg output of *N. dubius* per female host is $286.5 \pm 246$ and $123.8 \pm 83$ and the difference is more marked ($P = 0.05$). As in the *S. stroma: A. sylvaticus* system the lower egg output of *N. dubius* is associated with fewer adult female worms and lowered larval input into the system. The intensity of infection per male host with gravid female worms of
The egg output of *S. stroma* per host *A. sylvaticus*

January 1974 to October 1975

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<tr>
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<td>January</td>
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<td>828</td>
<td>1902</td>
<td>420</td>
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<tr>
<td>February</td>
<td>1266</td>
<td>343</td>
<td>930</td>
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<tr>
<td>March</td>
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<td>495</td>
<td>1044</td>
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<td>520</td>
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<tr>
<td>May</td>
<td>540</td>
<td>233</td>
<td>127</td>
<td></td>
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<td>June</td>
<td>338</td>
<td>356</td>
<td>8</td>
<td>3006</td>
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<td>July</td>
<td>3406</td>
<td>598</td>
<td>687</td>
<td>146</td>
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<tr>
<td>August</td>
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<td>1382</td>
<td>328</td>
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<tr>
<td>September</td>
<td>2257</td>
<td>264</td>
<td>1589</td>
<td>460</td>
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<tr>
<td>October</td>
<td>2364</td>
<td>69.4</td>
<td>2037</td>
<td>189</td>
</tr>
<tr>
<td>November</td>
<td>1002</td>
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<tr>
<td>December</td>
<td>387</td>
<td></td>
<td>1359</td>
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</table>
### TABLE IX  The egg output of *N. dubius* per host *A. sylvaticus*

January 1974 to October 1975

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>January</td>
<td>222</td>
<td>220</td>
<td>81.5</td>
<td>139</td>
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<tr>
<td>February</td>
<td>535</td>
<td>258</td>
<td>389</td>
<td>120</td>
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<tr>
<td>March</td>
<td>414</td>
<td>297</td>
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<td>April</td>
<td>427</td>
<td>547</td>
<td>539</td>
<td>333</td>
</tr>
<tr>
<td>May</td>
<td>453</td>
<td>336</td>
<td>843</td>
<td>-</td>
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<tr>
<td>June</td>
<td>707</td>
<td>71</td>
<td>117</td>
<td>84</td>
</tr>
<tr>
<td>July</td>
<td>353</td>
<td>48</td>
<td>221</td>
<td>154</td>
</tr>
<tr>
<td>August</td>
<td>373</td>
<td>310</td>
<td>42</td>
<td>53</td>
</tr>
<tr>
<td>September</td>
<td>126</td>
<td>51</td>
<td>68</td>
<td>49</td>
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<tr>
<td>October</td>
<td>661</td>
<td></td>
<td>426</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td>105</td>
<td></td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>94</td>
<td></td>
<td>130</td>
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</tr>
</tbody>
</table>
N. dubius in 1974 and 1975 was 17.7 ± 11 and 10.7 ± 7 respectively and the difference is significant (P = 0.05). In the female host the intensity of infection with gravid female worms of N. dubius in 1974 and 1975 was 15.1 ± 1.3 and 7.2 ± 4.1 (P = 0.1). Significant differences are also present in the larval input into the N. dubius: A. sylvaticus system in 1974 and 1975. Larval input into the male host parasite system in 1974 and 1975 was 5.9 ± 3.4 and 1.3 ± 1.3 per host respectively. Larval input into the female host parasite system was almost non existent in 1975.

Discussion

Host parasite systems require controls if they are to maintain viability and be capable of evolution towards stability. The controls may be in the external environment in the form of climatic environmental variables or within the system itself. External environmental variables will act on the system in a density independent manner but internal processes may involve density dependent mechanisms. The regulatory processes important within host parasite systems include overdispersion of parasite numbers per host, density dependent parasite reproduction and survival and a non linear relationship between host death rate and parasite burden (Anderson 1978; Anderson and May 1978). The latter control may be described by the degree of pathogenicity of the parasite and this can affect the timing of entry into the susceptible host population. If pathogenicity is high then the parasite will be unable to attain the requisite net reproductive rate essential to its initial establishment and subsequent maintenance in the host population (May and Anderson 1979). Destabilising processes which prevent too severe regulation are essential to balance within host parasite systems and these include time delays in parasite reproduction and transmission as well as a critical host density to provide continuity in parasite transmission (May and Anderson 1978;
May and Anderson 1979).

The effects of the environmental variables on the systems under study show that the use of the variables of the preceding month enhance the correlation with the intensity of infection of both parasites. Only rainfall of the previous month shows a relationship with the intensity of infection with *S. stroma* and this is difficult to explain for the infective eggs are viable for a few hours only. It may be that rain confined *A. sylvaticus* to its burrow system and increased host contact and transmission of the parasites and if this is so definitive positive changes in the system were not detected until after a time delay of the order of one month. These results are not in agreement with those of Rainbow 1972 where the incidence and intensity of infection of *A. sylvaticus* with *S. stroma* was found to be related to a decrease in concurrent relative humidity and rainfall respectively. Similarly correlation analysis of environmental variables of the previous month and the intensity of infection of *A. sylvaticus* with *N. dubius* indicates time delay in the effects of temperature and rainfall on the infective L3 larva in the external compartment of the system. Extremes of temperature would appear to act as negative feedback factors on the *N. dubius: A. sylvaticus* system serving to restrict transmission whilst rainfall enhances the survival of the L3 larva and acts as positive feedback to the system. The intensity of infection of *N. dubius* in *A. sylvaticus* was correlated with concurrent relative humidity in Rainbow's study (1972) and the explanation for the differences may be in the closer proximity of recording and trapping site as well as the less refined nature of the correlation analysis of this study.

Overdispersion is present in both the *S. stroma: A. sylvaticus* and *N. dubius: A. sylvaticus* systems and this concurs with a number of other field studies of host parasite systems (Lewis 1968 (a) and (b); Pennycuick 1971; Randolph 1975). Overdispersion is greater in the female
host and male host respectively and it is these systems which exhibit
the greater oscillatory nature during 1974 and 1975. The changes in
spatial distribution observed in the S. stroma: A. sylvaticus system
during the course of the study reflect the changing behaviour of the
hosts and emphasise the necessity to synchronise the less aggregated
distribution to the time when the host reproduction is at a minimum
and host mortality is high due to predation and other causes. The lack
of replacement of hosts by host reproduction and the steady trapping
of hosts in the field studies must have contributed to the decline in
larval input observed in 1975. The origin of the greater stability of
spatial distribution of N. dubius in the host A. sylvaticus with respect
to time may be in environmental control of larval survival as well as
spatial heterogeneity in the compartment external to the system. Spatial
distribution as a single factor is important in both systems but
interrelated with time it can assume an even greater potency as a control
factor. Similar time changes in dispersion but of a different nature
have been shown to occur in experimental studies on the infection
dynamics of Transversotrema patialense where there is a change from a
random distribution to one of overdispersion as cercarial density and
duration of host exposure to infection increases (Anderson, Whitfield
and Dobson 1978).

Some density dependence in parasite reproduction is evident in both host
parasite relationships. The positive correlation of the intensity of
infection of S. stroma with the fecundity of gravid female worms indicates
positive feedback in the system and may be explained by the presence of
greater numbers of the shorter lived male worms in the mouse small
intestine. The negative relationship between the intensity of infection
of N. dubius and the fecundity of gravid female worms indicates a modicum
of intraspecific competition for this parasite. The correlation does not
improve when the intensity of infection with gravid female worms alone is used and this is to be expected for all worms browse on the intestinal mucosa and contribute to any shortage of nutrients that must occur. Interspecific interaction between *N. dubius* and *S. stroma* in the female *A. sylvaticus* indicates a competitive superiority for *N. dubius* in a habitat of possible lower carrying capacity than that of the male host. The entry of *S. stroma* into the susceptible host population occurs before juveniles leave the nest, for the youngest male and female hosts caught in this study carry mean worm burdens of 27.5 *±* 73 and 50.8 *±* 114 respectively. The high variance on the mean worm burdens is consistent with larval recruitment from a patchy spatial distribution of adult parasites in the female host. A lack of specific host age mortality for the parasite is indicated by the general trend of increasing infection as the host passes into sexual maturity and finally undergoes senescence. The continual build up of the parasite population in the male host is in contrast to the fluctuation in levels of intensity of *S. stroma* in the female host. Control in terms of a host immune response must therefore be small in the *S. stroma*: *A. sylvaticus* system and this is the expected outcome from a host parasite relationship where the parasite lies at the interface of the small intestine and external environment and exploits only the temporary food resource afforded by each feeding period of the mouse.

The entry of *N. dubius* in the susceptible host population appears to be a slower process and the intensities of infection are lower. The steady build up of infection in male and female mice until the peak of the host breeding period followed by a decrease in intensity of infection imply a specific host age for parasite mortality in the 26 to 27.9 g weight class. The threshold level for the mounting of this host immune response may lie between mean worm burdens of 26-30 and 18-24 for male and female hosts respectively. The incorporation of this time delay in the longer
evolving host parasite relationship of *N. dubius* and *A. sylvaticus* will allow the requisite larval input into the system essential to the establishment and maintenance of the parasite in the host population. Possible evasion of the male host immune response is indicated by a later tripling of the mean worm burden but in the female host the later intensities of infection remain lower than the initial values.

The use of the adrenal gland weight as an indicator of stress in the host suggests that the stress input varied in time and the high variance on the parameter indicates an unequal spread of stress throughout the host population. Stress was most evident in the periods of January-June 1974 and January-June 1975 when the spring breeding period came as a climax to the stress already experienced during the winter. Competition between hosts for space and resources will be strong in these periods particularly in male mice. Social hierarchies have been shown to exist in feral populations of *A. sylvaticus* (Brown 1966) and it is the subordinate animals that will bear the greater part of the stress or input. If subordinates are equal in rank then the presence of numbers of subordinates will dilute the effect (Brain and Nowell 1970). Although the adrenal gland weights were higher in female mice they were not always accompanied by higher levels of infection with parasites. Hypertrophy of the adrenal gland may be due to a number of causes in female mice and these include hypertrophy of steroid producing tissue in response to stress, increased storage of lipid, vasodilation of the gland, vacuolation of non-cortical tissue or increased size of the connective tissue capsule (Brain and Nowell 1969). Adrenal gland weight, therefore, is not a good stress indicator in female mice. The lower adrenal gland weights in 1975 suggest that stress in the host population lessened during the study period and this may have been due to the distortion of social hierarchies that must have occurred consequent
to the depletion of males by trapping for subordinates may have been released from the aggression and fighting of social interaction.

The higher levels of stress experienced by the host in January-June 1974 are associated with kidney and liver enlargement in the male mouse and with kidney enlargement only in the female mouse. Kidney enlargement and malfunction are known to occur in stress because of the high blood pressure generated by the catecholamines (Selye 1950; 1973). Seasonal changes in the kidney weight of voles, shrews and mice have also been linked with the activity of alkaline phosphatase (Hyvärinen 1968), the normal growth pattern of the animal (Berry, Jakobson and Triggs 1973; Schvarts 1975) and oligosyndactyly which causes diabetes insipidus (Falconer, Latyzewski and Isaacson 1964). It is difficult to explain the liver enlargement for it would be expected that when food resource was limited in the external environment, food stores within the liver would be used. The negative correlation between the weights of the adrenal gland and heart is in contradiction with the enlarged heart normally associated with stress (Selye 1950; 1973). It may be due to a normal component of the growth process for similar changes in size have been observed in A. agrarius (Schvarts 1975).

The lower weights of the testes and the vesiculae seminales of male hosts in 1975 when stressor input had lessened are not in agreement with other studies on stress and social interaction in male mice (Davis and Christian 1957; Lloyd 1973) and lend support to the hypothesis of immigration of new male hosts into the area.

The lower weights of lymphatic tissue in the period of greater stress in January-June 1974 are consistent with the generally accepted theory of involution of lymphatic tissue with adrenocortical hormones (Dougherty 1952). These lower values for the morphophysiological indices of immune status, in particular those of thymus weight and Peyer's patch area during 1974 are associated with significantly higher egg output from both host parasite systems. The greater output is due to significantly
more gravid female worms rather than an increase in the fecundity of
the parasites. Such results are consistent with lowering of host
immune response with respect to the parasitic infection.

No significant change in spleen weight occurred in any sector of the
population during the two selected periods of stress and this is not
surprising for the spleen is involved in other activities as well as
the immune response. Seasonal changes in weight have been observed in
C. glareolus (Newson 1962) and M. agrestis (Dawson 1956) and they have
been accompanied by changes in blood profile. Such changes are also
implemented by ectoparasitic infection and availability of Vitamin C
(Liebovitz and Siegal 1981). The situation becomes even more complex
with stress for the normal action of glucocorticoids is to cause
involution of lymphatic tissue in the spleen but this is offset by
increased haematopoiesis in mice and voles subjected to stress (Dawson
1956; Elaine and Conway 1969).

Transmission in terms of the egg output from the host parasite systems
is therefore enhanced by host stress and will constitute a destabilising
process in the host parasite relationship. The resultant positive
feedback into the system will be more severe in the S. stroma: A. sylvaticus
system by reason of the short life span of twelve days for the parasite
(Lewis 1966) as well as direct transmission by host contact. Positive
feedback into the N. dubius: A. sylvaticus system as a consequence of
stress will be delayed by time required for the development of the egg
to the infective L3 larva as well as by the external location of the
infective stage. Further time delay will result whilst the immune
response is mounted by the host, the evolutionary origin of which may
be a consequence of a longer life span of eight to nine months for this
parasite (Scott, Cross and Dawson 1959) as well as the less aggregated
distribution within the host population. Some reduction of the time lag
will be introduced into the system by way of the refection practised by
A. sylvaticus (Rainbow 1972). Any increase in transmission from this behavioural activity will have the equivalence of increased fecundity.

The continual decline in the egg output of the two host parasite systems as the field study progressed was paralleled by a decrease in larval input into the system. This was not due to a critical host density for the numbers of mice trapped did not significantly change, although the sex ratio of the host population reflected a depletion of male mice. The decline in larval input may have been due to the ecological vacuum created by the monthly removal of hosts and the consequent immigration into the area of new hosts with relatively low levels of infection.

In summary, this chapter has shown that the control of parasite numbers associated with the r-strategy of S. stroma includes overdispersion with respect to time, short life span, low parasite sex ratio, some interspecific competition with N. dubius in the female host and unpredictability of host behaviour. Destabilising processes are the mode of contagious transmission, low pathogenicity, lack of specific host age for parasite mortality and host stress. Regulation in the K-selected host parasite system of N. dubius: A. sylvaticus is more severe and includes density independent environmental control due to the external compartmentalisation of the infective larva, density dependent intraspecific competition, specific host age for parasite mortality as a consequence of host immune response and to a lesser extent overdispersion in the host population. Destabilising processes are the time lags which occur during the development of the egg to the infective larva and the mounting of the host immune response, the effect of stress on the host immune response and the behavioural activity of refection. Critical host density for the establishment and maintenance of the parasite population was not detectable in either system.
Chapter 3

THE HOST PARASITE RELATIONSHIP OF THE LABORATORY MOUSE AND NEMATOSPIROIDES DUBIUS

Introduction

The host parasite system of the laboratory mouse and N. dubius is widely used in drug and anthelmintic studies because of the relative ease with which it is maintained under laboratory conditions. During preliminary work at Royal Holloway College, however, difficulty was experienced in the initiation of an infection in ASH/CSI S.P.F. mice and the subsequent culture of eggs to viable L3 larvae. Such "die offs" have been known to occur but little work has been done as to the causes of the condition. It is important that such parameters be identified for they must influence not only the intensity of the infection but also the ensuing behaviour of the system. Hence experimental procedures were designed to analyse the sequence of events which occur during the egg production of a primary infection of N. dubius in the laboratory mouse. As the ASH/CSI S.P.F. mice were not suitable, a system which used the closely related CD1 strain of mouse was set up in an area away from the normal animal house.

The life cycle of N. dubius is direct and involves free living and parasitic stages. Infection occurs after ingestion of ensheathed third stage larvae by the host. During the initial period of infection on days 4-6 the worms occupy up to 50% of the small intestine (Lewis and Bryant 1976). They burrow into the intestinal mucosa and undergo a period of development. By Day 7 the worms leave the mucosa and take up a position in the anterior part of the duodenum. On arrival here, copulation occurs and the first eggs are detected in the host faeces 10 days after infection (Bryant 1973). Egg production then continually occurs for approximately 8 months in the laboratory mouse (Scott et al, 1964). In this experiment a systems approach is adopted to analyse the events which
occur during egg production in a primary infection of *N. dubius* in CD1 mice from day 10-58. The technique views the host parasite relationship as a system into which there is continual input by the host and parasite followed by a corresponding change in the output of the system (Kennedy 1975).

The immune response of the mouse initiated by the larval antigens of *N. dubius* and the demand for resources from the host can be regarded as the initial inputs into the system. Manifestations of immune response by the mouse will include a reduction in the fecundity of the worms and their final expulsion (Fenner 1969; Bartlett and Ball 1974; Behnke and Parish 1981). Excessive demand for resources will also have an effect on fecundity.

The output of the system is a population of eggs actively extruded by the parasite and passively carried outside the system through faecal production. Such an output will include viable and non viable eggs, the proportions of which will depend on the presence of male worms, essential nutrients and the magnitude of the immune response of the mouse. Each worm represents a sub-system within the main host parasite system and the contribution that each makes to the output of eggs will be according to their competitive ability. Interaction between the worms will give the total system a variety of function and behaviour.

Stability of the system will be dependent on control which can be of two types - open loop control or closed loop control. An open loop control is one in which the control action is independent of the output. Control is achieved by varying the input and relying on the inertia of the system to average out the fluctuations of the input. Closed loop control is one in which the control action is somehow dependent on the output. A feedback element operates from the output and influences the actuating signal to the control element (Millsum 1966; Beishon & Peters 1972).

Open loop control in the *N. dubius* mouse system is evidenced by cyclicity in the rise and fall of egg production of *N. dubius* during the course of the experiment. While egg production is rising the system will be in the "ON" period and as egg production falls the system will be in the "OFF"
The rate of switching is not dependent on the output and can be measured by the number of days which occur between the peaks of egg production. These days will represent the inertia or lag in the system.

Closed loop control will operate when total egg output of *N. dubius* becomes high and certain nutrients required for oogenesis, particularly bases for RNA and DNA, become in short supply. It is the demand for resources which acts as a feedback element to the system and as such represents an input into the system. Some measure of the input can be derived from the rate at which egg production falls. Closed loop control will also be in evidence during the immune response of the host.

The mode of operation of the host parasite system will be indicated by the variation in egg production and faecal production that occurs from day to day. This variability in output can be measured by retrospectively cumulating the daily variation around the mean of the total number of observations of the experimental procedure. The technique is known as cusum analysis (Chatfield 1970) and the chart so produced is called a cusum chart. Cusum charts do not show total values and are not to be confused with cumulative ogives. They serve to show how a system is functioning at a particular point in time. The larger the cusum around the mean the greater the oscillation of the system and the points at which the cusum graph changes slope or crosses the base line indicates the limits of discrete units or components within the system.

Most systems are characterised by inertia and exhibit a time lag before input into the system affects the output. This results in a cyclicity of the system which can be identified by using correlation analysis. Auto-correlation correlates the initial members of a population with those of increasing lag. When it is applied to the output variables of a system it defines output in terms of a coefficient. A declining positive correlation coefficient indicates decrease in output and an increasing negative correlation coefficient denotes increase in output (D. Piggot,
personal communication). The existence of cyclicity in a system is characteristic of one which is under control and capable of evolution. Auto correlation is used on the data on faecal production and egg output of the host parasite relationship of the present study.

The efficiency of a system is described by the ratio of input:output of the system. Some loss of efficiency occurs if there is a high proportion of non viable eggs in the output of a host parasite system.

Materials and Methods

1. The Host:Parasite System

Six week old CDI male and female mice supplied by Charles River Ltd., Kent, were housed in baskets of area 102 sq. ins. and volume 663 cu.ins. All baskets, grids and water bottles were sterilised in an autoclave at 16 lbs/sq.ins. for 20 minutes (117°C) prior to the experiment. The housing material was fresh vermiculite bought from a builders' merchant and housing density was 4 animals per basket up to Day 12 of the experiment and thereafter 3 animals per basket until autopsy on Day 58. Sterilised 4RF diet was supplied by Charles River Ltd. Barrier care was adopted throughout the experiment and all possible precautions were taken to prevent contact with other animals. Separate washing facilities were used.

The mice were infected orally with a dose of 70 infective larvae of N. dubius using a blunt wide gauge needle. The larvae were obtained from the Wellcome Laboratories, Beckenham, Kent. Control animals with the same housing density were set up alongside the experimental animals. On Day 10 the animals were removed from the vermiculite and put on to paper to facilitate the collection of the faecal output of the mice at 08.00 hours each day. Egg production of N. dubius was measured using the MacMaster technique (Whitlock and Gordon 1939) and counts expressed as the number of eggs per gram of faeces. The percentage of viable eggs in the sample was calculated by removing 0.1 ml aliquots from the saline solution and placing them on a microscope slide. Those
eggs showing complete chitin and ascaroside layers and embryogenesis were counted as viable.

Autopsy was carried out on 2 male and 2 female mice on Day 12 and the remaining mice were sacrificed on Day 58.

2. Inputs into the host parasite system

(a) Input can be measured by the rate at which total egg production falls.

Let $I_T = \text{input against total egg production}$

$N$ = total egg production

$t$ = time interval

then

$$I_T = \log e \frac{N_t}{N_{t+1}}$$

(b) The measure of input against viability is similar.

Let $I_V = \text{input against viable egg production}$

$N$ = viable egg production

$t$ = time interval

then

$$I_V = \log e \frac{N_t}{N_{t+1}}$$

Total input into the system will be the sum of these two inputs, $I_T$ and $I_V$.

3. Statistical techniques

(a) Cusum analysis

Each observation in the experiment was compared with the mean.

Let $x$ denote the system variable at time $p$ and $\bar{x}$ the mean of a number of observations. Then at time $t$ the cumulative sum of deviations $S$ about $\bar{x}$ is given by

$$S_t = \sum_{p < t} (x_p - \bar{x})$$

The calculation of the cusum is as follows.

$$S_1 = x_1 - \bar{x}$$

$$S_2 = (x_1 - \bar{x}) + (x_2 - \bar{x})$$

$$= S_1 + (x_2 - \bar{x})$$

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(b) The correlation analysis was completed on an ICL 2903 computer located at Dorset Institute of Higher Education.

Results

The results are grouped under five headings:

1. The inputs of the laboratory mouse: *N. dubius* system
2. The output of the laboratory mouse: *N. dubius* system
   (a) Faecal production  (b) Egg output
3. Cyclicity in the laboratory mouse: *N. dubius* system
4. Analysis of Output
5. Autopsy

1. **THE INPUTS OF THE LABORATORY MOUSE: *N. DUBIUS* SYSTEM**

   Egg production expressed as the number of eggs per mouse passing to the exterior per hour is listed in Tables I and II. A distinction is made between total egg output and viable egg output. Egg output from the male host exceeds that of the female except on days 16, 19, 31 and 41. All eggs are viable on Days 10, 15, 24, 31 and 45 in the male host. In the female host all eggs are viable on Days 10, 15, 24, 25, 31, and 45.

   Sequences of rising and falling egg production occur throughout the period. The time when egg production is falling represents a brake on the system and as such can be regarded as negative feedback in the system. The rate at which it falls measures the magnitude of the input. The inputs against total egg production and viability are generally higher in the female system. There would appear to be a series of seven periods of input during the experiment and they correspond broadly in time in the male and female host.

   The days when egg production is rising and the system is "ON" are shown in Tables III and IV. The percentage of total time it is rising is greater in the female mouse. It may represent an endogenous neurosecretory rhythm of the worm and as such be open loop control.
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TABLE II

Egg production of *Nematospiroides dubius* in female CD1 mice infected at six weeks

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**TABLE III**

Open loop control of the fecundity of Nematospiroides dubius in male CD1 mice

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<th>Day</th>
<th>Number of Days OFF</th>
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<td>27-30</td>
<td>4</td>
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<table>
<thead>
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<th>Total Days ON</th>
<th>Total Days OFF</th>
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<tbody>
<tr>
<td>24</td>
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<table>
<thead>
<tr>
<th>% Total time</th>
<th>% Total time</th>
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<tr>
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Table IV

Open loop control of the fecundity of *Nematospiroides dubius* in female mice

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of Days ON</th>
<th>Day</th>
<th>Number of Days OFF</th>
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<tbody>
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<td>14</td>
<td>1</td>
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<td>15-19</td>
<td>5</td>
<td>20-23</td>
<td>4</td>
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<td>25</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>27-29</td>
<td>3</td>
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<td>30-31</td>
<td>2</td>
<td>32-33</td>
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<td>7</td>
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</tr>
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<td>47-48</td>
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<tr>
<td>54-56</td>
<td>3</td>
<td>57-58</td>
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</tr>
</tbody>
</table>

| Total Days ON | 28 | Total Days OFF | 21 |

| % Total Time | 57 | % Total Time | 43 |
Cusum of total egg production (Fig. 1) indicates that the system has three components which correspond to the establishment of the system, adjustment within the system and eventual stabilisation of the system.

Component I Day 10-26
Day 26 is the day of maximum egg production and represents the end of the initial phase of establishment

Component II Day 27-40
The rate of egg production is increased in male and female systems

Component III Day 41-58
The male and female systems stabilise on Day 55 and Day 40 respectively.

Cusum analysis of the total egg production per hour shows that the rate of egg output follows the same general trend in male and female mice until Day 26-29 but begins to show differences in Components II and III of the system. The initial negativity of the cusum indicates that the system is evolving towards the process mean. The fluctuations around the process mean are greater in the male than in the female system and indicate less stability in the male system. The smaller oscillations around the mean at about Day 40 in the female system are evidence of tighter control in the female system.

The sum of the mean inputs of the seven periods against total egg production ($I_n$) and viability of the eggs ($I_v$) are shown in Table V and the following can be deduced from the data.

(a) The sequence of size of input in each component is different in male and female hosts. In the male host decrease in the size of input occurs in a sequence of Components I→III→II. In the female host a decrease in the size of input occurs in the sequence of Components II→I→III.
Fig. 1 Cusum analysis of the total egg production of an infection of 70 larvae of *N. dubius* in adult male and female CD1 mice shows three components in the system:

<table>
<thead>
<tr>
<th>Component</th>
<th>Days</th>
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<tr>
<td>Component I</td>
<td>10-26</td>
</tr>
<tr>
<td>Component II</td>
<td>27-40</td>
</tr>
<tr>
<td>Component III</td>
<td>41-58</td>
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</table>
CUSUM OF TOTAL EGG PRODUCTION
OF NEMATOSPIROIDES OUBIAS IN C.D.I. MICE
<table>
<thead>
<tr>
<th>MALE CD1 Mice</th>
<th>FEMALE CD1 Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>INPUT</td>
<td>I_T</td>
</tr>
<tr>
<td>Day 14-23</td>
<td>1.19</td>
</tr>
<tr>
<td>Day 27-37</td>
<td>0.69</td>
</tr>
<tr>
<td>Day 40-50</td>
<td>0.74</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2.62</td>
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</table>

<table>
<thead>
<tr>
<th>OUTPUT Male</th>
<th>Total Eggs per Hour</th>
<th>% Viable</th>
<th>Viable Eggs per hour</th>
<th>OUTPUT Female</th>
<th>Total Eggs per Hour</th>
<th>% Viable</th>
<th>Viable eggs per Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10-58</td>
<td>691</td>
<td>71.7</td>
<td>495</td>
<td>Day 10-58</td>
<td>412</td>
<td>71.5</td>
<td>295</td>
</tr>
</tbody>
</table>

**TABLE V**  Inputs and output of the laboratory mouse and Nematospiroides dubius system
(b) Input against viability is greater than it is against total egg production. The percentage viability is the same in male and female host.

(c) The lower total input of the male host parasite system results in a higher output of non viable and viable eggs.

2. THE OUTPUT OF THE LABORATORY MOUSE: N. DUBIUS SYSTEM

(a) FAECAL PRODUCTION

The faecal production of individual control and infected mice is listed in Table VI. The use of the cusum chart for faecal production (Figs. 2a and 2b) shows three components, both in the control and experimental systems. They correspond broadly in time with those of total egg production and it suggests an external factor in the system which is affecting the pattern of defaecation. Possible factors are changing weight as the mouse passes into maturity and external temperature. It is unlikely to be the former as there is a drop in faecal production from Day 33-50. The decline in faecal production in both control and infected mice coincided with a doubling of external temperature and it may be that temperature acted as a trigger to different activity or feeding behaviour in the mice. The mean faecal production from Day 10-58, however, is significantly different in control and infected mice of both sexes. Mean faecal production per hour in control and infected male mice was 0.120 g ± 0.17 and 0.132 g ± 0.018 respectively (P = 0.001) and mean faecal production per hour in control and infected female mice was 0.114 g ± 0.017 and 0.119 g ± 0.018 respectively (P = 0.001).
**Table VI**

Faecal production (FaP) per hour in control and infected CD1 male and female mice.

*Infection: Nematospirooides dubius*

<table>
<thead>
<tr>
<th>Day</th>
<th>Sex</th>
<th>FaP per Hour Control</th>
<th>FaP per Hour Infected</th>
<th>Sex</th>
<th>FaP per Hour Control</th>
<th>FaP per Hour Infected</th>
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<tr>
<td>58</td>
<td></td>
<td>0.150</td>
<td>0.158</td>
<td></td>
<td>0.136</td>
<td>0.104</td>
</tr>
<tr>
<td>59</td>
<td></td>
<td>0.133</td>
<td>0.153</td>
<td></td>
<td>0.125</td>
<td>0.126</td>
</tr>
</tbody>
</table>
Fig. 2  Cusum analysis of the faecal production of adult CD1 male and female mice infected with 70 larvae of *N. dubius* shows three components in the system

(a) **Male host**
- Component I  Day 10-26
- Component II Day 27-36
- Component III Day 37-58

(b) **Female host**
- Component I  Day 10-22
- Component II Day 23-32
- Component III Day 33-58
CUSUM OF FAecal PRODUCTION OF C.DI. MALE MICE INFECTED WITH 70 LARVAE OF NEMATOSPIROIDES DUBIUS.
Temperature may be eliminated as a contributory factor to the system if the cusum of faecal production of infected mice is derived from the control experiment. Let \( x \) denote the system variable and \( \bar{xc} \) the mean of the control faecal production.

Then

\[
S_1 = x_1 - \bar{xc} \\
S_2 = x_1 - \bar{xc} + (x_2 - \bar{xc}) = S_1 + (x_2 - \bar{xc})
\]

Note \( \bar{xc} \) must be changed as the system passes from one component into the next.

The resultant cusum chart (Fig. 3) shows total faecal production to be greater in infected mice than the controls. It is more marked in males than females. The change of slope around Day 25 and Day 45 in both male and female systems indicates three components which are independent of the control system.

Auto correlation and partial correlation analysis of faecal production of infected mice (Fig. 4) reveal differences in the behaviour of male and female systems. In both systems initial infection maintains steady faecal production as shown by the high correlation coefficient on a lag of one day. It remains steady until Day 18 in the male system but is depressed in the female system. Between days 19-21 there is an indication of an event taking place which alters the pattern of output completely. Faecal production begins to increase but the increase is not so marked in the females.

Sections of the upper duodenum during a primary infection with \( N. dubius \) reveal extensive browsing on the mucosa with muscle layer involvement (Fig. 5). Damage to the nerve plexi may account for the increased peristaltic activity of the alimentary canal.

(b) EGG OUTPUT

In male mice minimum and maximum egg production were on Days 10 and 55 respectively. In female mice minimum and maximum
Fig. 3 Cusum analysis of the faecal production of adult male and female CDI mice infected with 70 larvae of *N. dubius*. The cusum chart is derived from the use of the mean faecal production of control CDI mice. Faecal production is greater in infected mice and the increase is more marked in the male host.
CUSUM OF Faecal Production in Male and Female C.O.I. mice derived from the control mean \( \bar{X}_c \).
Fig. 4  Autocorrelation and partial correlation analysis of the faecal production of CD1 mice infected with 70 larvae of *N. dubius*.

There is an indication of an event taking place between Days 19-21 as the correlation coefficients move across the base line and change sign.
MALE C.O.I. MICE

AUTOCORRELATION.

PARTIAL CORRELATION.

FEMALE C.O.I. MICE.

AUTOCORRELATION.

PARTIAL CORRELATION.

AUTOCORRELATION AND PARTIAL CORRELATION OF FAECAL PRODUCTION OF C.O.I. MICE INFECTED WITH N. OUBIUS
Fig. 5  (a)  T.S. Duodenum of uninfected ASH/CSI S.P.F. female mouse x 140

(b) T.S. Duodenum of a female ASH/CSI S.P.F. mouse infected with 70 larvae of *N. dubius*. x 345
TS Duodenum of Uninfected ASH/CSI Female Mouse (x140)

TS Duodenum of ASH/CSI Female Mouse Infected with N. dubius (x345)
egg production were on Days 23 and 52 respectively. The same disturbance indicated by autocorrelation of faecal production and which occurs on Day 19 is apparent in autocorrelation on total egg production (Fig. 6(a)). The correlation coefficients become small and move about the base line. Auto correlation of viable egg output (Fig. 6(b)) shows no such interpolation and the correlation coefficients change smoothly from positive to negative sign. It follows, therefore, that the input to the system is affecting oogenesis rather than the maturation of eggs. Partial correlation (Figs. 6(a) and (b)) shows a three day cyclicity in the total egg production of the female mouse which is interrupted on Day 23 and changes rhythm. Caution must be used in the interpretation of partial correlation coefficients as they are only small and may reflect variance in the system.

3. CYCLICITY IN THE LABORATORY MOUSE: N. DUBIUS SYSTEM

Patterns of faecal production in control male mice are irregular (Fig. 7(a)). Cycles of peak faecal production can vary between not less than 5 days and not greater than 10 days. Faecal production patterns in female control mice (Fig. 7(b)), however, exhibit marked regularity, predominantly of 5 day length. Although faecal production shows a decrease from Day 32 due to increase in temperature, the cycle is maintained.

Evidence that this cycle is shortened in an infected mouse is provided if faecal production is linearly correlated with increasing lag in egg production. Although the correlation coefficients are small they exhibit a regularity which cannot be denied. The cycle in the infected male mouse becomes increasingly reduced with the duration of the experiment whilst the cycle of an infected female mouse shows a marked regularity of 4 days.
Fig. 6 Autocorrelation and partial correlation of total egg production and viable egg production of C57 mice infected with 70 larvae of *N. dubius*.

(a) Total egg production.
    The correlation coefficients become small and remain near the base line on Days 19-26

(b) Viable egg production.
    The change of sign of the correlation coefficients is smooth in the male host but tends to fluctuate around the base line from Day 19-27 in the female host.
MALE C.O.I. MICE

AUTOCORRELATION

0 - 6

PARTIAL CORRELATION

FEMALE C.O.I. MICE

AUTOCORRELATION

0 - 6

PARTIAL CORRELATION

AUTOCORRELATION AND PARTIAL CORRELATION
TOTAL EGG PRODUCTION OF N. OUUBUS IN C.O.I. MICE.

Fig. 6 (a)
AUTOCORRELATION AND PARTIAL CORRELATION OF VIVABLE EGG PRODUCTION OF N. OUBIUS IN CDI. MICE.

Fig. 6(b)
Fig. 7 Patterns of the faecal production of control CDL mice and CDL mice infected with 70 larvae of N. dubius.

The system cycle of infected mice shown below the faecal production of control mice is derived by linear correlation of faecal production with increasing lag in total egg production.

(a) The male CDL mouse
(b) The female CDL mouse
Fig. 7 (b)
4. **ANALYSIS OF OUTPUT**

Egg sizes are listed in Tables VII and VIII. Chi squared evaluation shows that the distribution of length and width approximates to normal. The distributions belong to the positive Binomial family with a tendency to regular dispersion. The distributions are compared in terms of skew and coefficients of variation. The skew on length distribution in female eggs is more positive than the male. The skew on width distribution in female eggs is more negative than the male. Coefficients of variation in the female system tend to be larger both in length and width (Tables 10-12). The length and width of the egg are not significantly correlated in the separate components or total population, although correlation tends to be stronger in the female. Maximum correlation between the length and width of the egg in the female host occurs between Days 10-26 \( r = +0.414 \) and Days 27-40 \( r = -0.339 \).

There is no significant difference between the lengths and widths of viable eggs in any component of male and female systems. There is, however, a significant difference in length between viable and non viable eggs in female hosts \( (P = 0.001 \text{ Student's t test}) \). The differences between viable and non viable eggs are shown in Fig. 8. A comparison of egg populations in male and female hosts shows there to be a significant difference in length from day 27-58 (Day 27-40 \( P = 0.05 \), Day 41-58 \( P = 0.001 \)). Eggs are smaller in the female host and will therefore develop more rapidly. Some cyclicity in length also appears to be present (Fig. 9) and the figures indicate the period is shorter in the female.

5. **AUTOPSY**

The mean intensity of infection in male and female mice on Day 58 was 34.8% and 33% respectively and the mean fecundity of *N. dubius* in male and female mice was \( 91 \pm 11.3 \) and \( 95 \pm 9.6 \).
<table>
<thead>
<tr>
<th>MALE CD1 MICE</th>
<th>Mean Length</th>
<th>Mean Width</th>
<th>Skew on Length</th>
<th>Skew on Width</th>
<th>Coeff. of Var Length</th>
<th>Coeff. of Var Width</th>
<th>Cyclicity of Length (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10-26</td>
<td>80.6 ±4.65</td>
<td>50.2 ±2.5</td>
<td>-0.403</td>
<td>+0.239</td>
<td>5.76</td>
<td>5.01</td>
<td>6, 7, 7, 7</td>
</tr>
<tr>
<td>Day 27-40</td>
<td>80.2 ±4.03</td>
<td>50.1 ±2.3</td>
<td>+0.168</td>
<td>+0.130</td>
<td>5.02</td>
<td>4.59</td>
<td></td>
</tr>
<tr>
<td>Day 41-58</td>
<td>81.1 ±4.8</td>
<td>49.83 ±2.6</td>
<td>-0.094</td>
<td>-0.204</td>
<td>5.88</td>
<td>5.17</td>
<td></td>
</tr>
<tr>
<td>Day 10-58</td>
<td>80.73 ±4.4</td>
<td>50.04 ±2.5</td>
<td>+0.494</td>
<td>+0.052</td>
<td>5.45</td>
<td>4.93</td>
<td></td>
</tr>
<tr>
<td>Non Viable</td>
<td>79.63 ±4.55</td>
<td>49.95 ±2.55</td>
<td>-0.204</td>
<td>-0.059</td>
<td>6.92</td>
<td>5.11</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE VII** Size of eggs of *Nematospiroides dubius* in male CD1 mice
<table>
<thead>
<tr>
<th>FEMALE CD1 Mice</th>
<th>Mean Length</th>
<th>Mean Width</th>
<th>Skew on Length</th>
<th>Skew on Width</th>
<th>Coeff. of Var Length</th>
<th>Coeff. of Var Width</th>
<th>Cyclicity of Length (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10-26</td>
<td>80.25 ±1.75</td>
<td>50.3 ±2.4</td>
<td>+0.157</td>
<td>+0.319</td>
<td>5.9</td>
<td>4.8</td>
<td>6, 6, 4, 5</td>
</tr>
<tr>
<td>Day 27-40</td>
<td>78.92 ±4.98</td>
<td>49.38 ±2.43</td>
<td>+0.105</td>
<td>-0.77</td>
<td>6.3</td>
<td>4.9</td>
<td>6, 5, 5, 6</td>
</tr>
<tr>
<td>Day 41-50</td>
<td>79.2 ±4.18</td>
<td>49.7 ±2.65</td>
<td>+0.23</td>
<td>-0.297</td>
<td>5.3</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Day 10-58</td>
<td>79.48 ±4.65</td>
<td>49.83 ±2.5</td>
<td>-0.34</td>
<td>-0.21</td>
<td>5.9</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Non Viable</td>
<td>77.88 ±5.98</td>
<td>49.68 ±2.2</td>
<td>+0.188</td>
<td>-0.445</td>
<td>7.7</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE VIII**  
Size of eggs of Nematospioides dubius in female CD1 mice
Fig. 8  The egg of *N. dubius* in the CD1 mouse host.

(a) Viable egg x 560

The outer uterine layer, the middle chitin layer
and the inner ascaroside layer are complete around
the developing embryo

(b) Non viable egg x 560

Non viability or abnormal larvae development is
associated with a rumpled or broken chitin layer
or incomplete ascaroside layer.
Non viable eggs tend to be smaller than viable eggs. They show greater variation in length but less variation in width in the female host.

Fig. 8 (a) Viable Egg of *N. dubius* (x560)

Fig. 8 (b) Non Viable Egg of *N. dubius* (x560)

Non viability or abnormal development is associated with a rumpled or broken chitin layer or incomplete ascaroside layer.
Fig. 9  The length and width of the eggs of *N. dubius* in CDL mice infected with 70 larvae of *N. dubius*.

Some cyclicity in length indicated by the figures above the peaks may be present in the system.
SIZE OF EGGS OF NEMATOSPIROIOS OUBLIS IN C.D.I. MICE.
Discussion

Systems analysis provides an interesting approach to the host parasite relationship. It is a tool that can be used to locate events and quantify the interactions that exist between host and parasite (Kennedy 1975). The system boundary of the laboratory mouse: *N. dubius* system is the environment of the upper small intestine. Both mouse and nematode contribute inputs into the system which alter the output of the system.

Cusum analysis, a useful technique in monitoring the performance of a system has shown that the CDL mouse: *N. dubius* system falls naturally into three components. An attempt has been made to quantify the inputs and constraints in each component in terms of days of inertial lag, decrease in rate of egg production and decrease in the viability of the eggs. These inputs act upon the system in the form of open loop and closed loop control to produce an output of eggs. There is a lower total input into the male host parasite system than in the female host parasite system. This is reflected in a higher output of both viable and non viable eggs from the male system. The inputs occur roughly at the same time in male and female hosts but the sequence of size of input is different. In the male host the input is greatest from Day 10-26 but in the female maximum input occurs from Day 26-40. The greater input into the female mouse system results in smaller oscillations in the egg output of *N. dubius* and thus this system has greater stability than that of the male.

It is interesting to note that the percentage of viable eggs in the egg output is the same in the male and female host. This suggests that the input against the viability of the eggs of *N. dubius* may be of the same nature in the male and female mouse and it is the input against total egg production that differs.
The cyclical production of eggs by *N. dubius* seen in the experiment may be a form of open loop control which relies on the rate of switching and inertia in the system to maintain constancy of egg output. The rate of switching in the *N. dubius* : CD1 laboratory mouse system may be determined by an endogenous neurosecretory rhythm of the worm which can be modified by the micro environment of the small intestine. The extent of this modification is reflected in the days of inertia in the system and the environment of the female host small intestine must therefore have a greater input against oogenesis than that of the male mouse.

Closed loop control will begin to operate when the demand for certain nutrients for oogenesis enters into a competitive situation. The negative feedback that results will dampen down the oscillations of the system. These essential nutrients may have several origins. They may be provided by the bacteria acquired by the worms during day 4-6 while they were in the lower part of the small intestine (Lewis and Bryant 1976) or bacteria in the paramucosal lumen of the upper small intestine may form an oscillating system and furnish a continual source of precursors. Alternatively the gut mucosa with its own intrinsic flora may supply the nutrients when the worms browse on the intestinal tissues (Donaldson 1968; Tannock and Savage 1974). Nutrients in the diet were unlikely to be limiting factors for food was supplied ad libitum.

Significantly more adult worms of *N. dubius* are known to develop in conventional mice than in germ free mice and infections persist longer in conventional hosts (Wescott, 1968). Prolonged monoassociation with *Lactobacillus* sps also enhances the development and survival of *N. dubius* in gnotobiotic mice (Chang and Wescott, 1972). Mice maintained in the germ free state show no difference in infection in male and female hosts and the sex difference only appears outside the germ free system (Newton, Weinstein and Sawyer 1962). Thus it may be the microbial environment of the small intestine which is important in the negative feedback of the system.
Autocorrelation and partial correlation of faecal and egg production have located events which occur between days 19 to 21 in both hosts and they are followed by interruption of the cycle and an increase in faecal production. The assumption has been made that the eggs are randomly distributed within the sample and if this is so a rise in total egg production occurs. Some decline in the viability of the eggs is present in the male system during this period.

The system begins to stabilise on Day 40 in the female mouse and viability of eggs begins to improve in the male mouse at the same time. The female system exhibits greater input than the male and this is reflected in the smaller oscillations around the mean egg output. The male system, however, continues to undergo a series of oscillations, some of which can be quite large. Negative feedback is not necessarily producing a decrease in error at the output. Instability in a system allows it to exhibit a greater variety of behaviour and output. Such variability would be of adaptive value to the parasite in wild populations of mice for a sudden upsurge of viable eggs would give it the option of both trickle and heavy infection.

The abnormal mobility pattern of the alimentary canal associated with an *N. dubius* infection may be due to a variety of reasons. Aliphatic amines, fermentation acids, etc. have been demonstrated as metabolites of *Trichinella spiralis* (von Brand, Weinstein, Mehlman and Weinbach, 1952; Castro, Ferguson and Gordon, 1973) and it is possible that similar compounds produced by *N. dubius* have a pharmacological action on the motility of the small intestine. It could be due to the release of 5 HT from the argentaffan cells in the crypts of Lieberkuhn but this is unlikely as the gut has been shown to exhibit a 5 HT insensitivity after a period of time (Hukhura et al 1960). Microbial mechanisms are known to contribute to defence by stimulating peristaltic emptying (Abrams & Bishop 1967; Donaldson, 1974) and the introduction of a new flora...
from the lower small intestine, protected within the tissues of
N. dubius, could cause disturbance in the normal bacterial equilibrium
of the gut.

It is likely however that the abnormal motility pattern is the result of
the interference with the nervous control of the small intestine.

N. dubius feeds on the mucosa of the duodenum from day 10 onwards and
microscopic examination of the duodenum of the closely related strain
of ASH/CSI mice and indicates that the damage is extensive and penetrates
below the plexi of Auerbach and Meissner. Not only are sensory receptors
in the mucosa which trigger peristaltic activity destroyed (Haverbrack
and Davidson 1958) but also the plexi themselves. Several workers have
shown the existence of a pacemaking area in the duodenum and suggest that
there may be several pacemakers with different natural frequencies,
one for each functional unit of the small intestine. There is a stepwise
decrement of electrical activity and frequency of contraction in the
anterior posterior direction and these may be destroyed by N. dubius
as it browses on successive lengths of the mucous membrane. This would
account for the initial rise in faecal production being followed by a
slowing down of that rate. The effect of the changed motility pattern
may have a regulatory effect and represent a response of the alimentary
canal aimed at altering the environment around the parasite and thereby
rendering survival less likely (Larsh and Hendricks 1949; Kelly and

The existence of a cyclicity in the relationship between faecal production
and egg production indicates a relationship between the two processes.
It may be that the demands for the prerequisites of egg production require
an alteration in peristaltic activity and it is this input which shortens
the normal faecal production pattern of female mice. The existence of
a four to five day cycle in control female mice could be a reflection of
oestrous. Such a regular cycle is not present in control males but
appears in the infected males. Cyclicity in egg length is also
indicated.
Non viability of eggs was associated with smaller eggs, rumpled or broken chitin layer or an incomplete ascaroside layer. The incomplete ascaroside layer was due to a failure of separation of this layer from the chitin layers at the poles of the egg rather than the middle. The failure to maintain larval homeostasis by reason of entry of external agents was reflected in abnormal development. Occasionally very large eggs, 140μ in length and 55μ in width appeared in the faeces particularly in those obtained from male mice.

The significant difference in the size of egg in male and female hosts in Component III has a number of implications. Crofton and Whitlock 1965 clearly showed that the volume of a number of sheep nematode eggs determines the length of time required for development to the hatching stage. The minimum volume of eggs of a particular species determines the minimum time for hatching at any temperature and the maximum volume determines the theoretical maximum required. The smaller eggs of *N. dubius* in the female host will therefore develop more quickly than the male. The distribution of size of eggs approximates to the normal distribution both in length and width and this will ensure a longer infectivity for a given temperature in the external environment.

The intensity of infection in male and female hosts on Day 58 showed no significant difference, neither did fecundity. The end result was an established host parasite system which had evolved through a sequence of components differing in the male and female host. The two systems exhibited a variety of behaviour which can only be of benefit to the parasite in the wild situation.
CHAPTER 4

THE EFFECTS OF MICROBIAL ENVIRONMENT ON THE HOST PARASITE SYSTEM
OF NEMATOSPIROIDES DUBIUS AND THE LABORATORY ASH/CSI S.P.F. MOUSE

Introduction

The preceding chapter has described the systems approach to the host parasite relationship of laboratory CD1 mice and Nematospiroides dubius. It has revealed differences in the behaviour of the system in male and female hosts as well as an abnormal motility pattern of the alimentary canal during infection. A similar approach is now adopted in an attempt to identify the parameters important in the failure of N. dubius to establish in the laboratory ASH/CSI S.P.F. mouse in the animal house of Royal Hollway College. The difficulty experienced in the initiation of an infection in ASH/CSI S.P.F. mice was compounded by an inability to culture the eggs of N. dubius to the infective L3 stage of development. The analysis of primary infections of ASH/CSI S.P.F. mice with N. dubius revealed low intensities of infection, reduced fecundity and distortions in the cuticle of the head and vulva regions of those worms which had been successful in the anterior migration. This suggested that the environment of the small intestine was in some way hostile to the establishment of the worms and that the probable cause was microbial in origin (Newton, Weinstein and Sawyer 1962; Wescott 1968; Przyjalkowski and Wescott, 1969). The hypothesis is tested in this chapter by treating male ASH/CSI mice with the antibiotic oxytetracycline hydrochloride to change the microbial populations of the small intestine.
Materials and Methods

(a) System measurement and data analysis

Male and female ASH/CSI mice of varying age were infected orally using a blunt wide gauge needle with 70 larvae of *N. dubius* obtained from the Wellcome Laboratories, Beckenham, Kent. The mice were of a colony maintained at Royal Holloway College for a number of years. Light ether anaesthesia was used during infection which took place between 12.00 to 14.00 hours. All mice were kept in an area designated as the New Animal House and had spent all their lives on a bedding material of Irish peat obtained from Dordmamona in Southern Ireland. The area and volume of the baskets were as previously described (Chapter 3) and housing density was 4 mice per basket.

Mice in the experimental room were subjected to a 14 hours light and a 10 hours dark regime, this being nearest to the conditions prevailing in Spring when infection levels are highest in wild populations of *A. sylvaticus*. The temperature was maintained at 68°F throughout the experiments and food and water was supplied ad libitum. The diet used was Diet No. 86 manufactured by E. Dixon and Sons, Ware, Herts. Mice were sacrificed on Day 14.

When eggs were required for culture, faeces were collected overnight from infected mice. The mice were given water but no food during the period. The faeces were mixed with sufficient deionised water to form a thick paste and then washed through muslin with more deionised water. The liquid was allowed to settle for at least two hours. The greater part of the supernatant was then discarded and the remainder thoroughly mixed with the sediment and centrifuged for 3-5 minutes at 2,500-3,000 r.p.m. The supernatant was discarded again and the faecal sediment was put onto strips of Whatman 54 filter paper, the ends of which were tucked under a small dish within a petri dish containing a shallow layer of deionised water. Larvae thus cultured
were able to migrate from the faecal mass into the surrounding deionised water.

Another method of larval culture mixed the faeces with sterilised BDH charcoal. The mixture was kept moist for four to five days and the Baermann technique used to extract the larvae. This method prevented the growth of fungi and the unpleasant smell associated with culture. Separate larval cultures of faeces mixed with 2% copper sulphate as a fungicide and 0.04% oxytetracycline hydrochloride as a bacteriocide were also used.

10% carbol fuschin, a stain for chitin, was used in the microscopic examination of eggs for viability. If the embryos were stained they were judged to be non viable. Eggs were also sent to the Veterinary Establishment at Weybridge for viral detection and electron microscopy.

In addition, infected LACA mice were obtained from outside sources and placed in the same area and on the same bedding material as the ASH/CSI mice. They had been infected with 100 larvae of the Beecham Pharmaceutical strain of N. dubius one month and two months previously. Eggs from infected female MFI mice were also kindly made available by Miss S. Hopkins of Royal Holloway College. The MFI mice had been housed in two areas away from the New Animal House. The areas were designated as the Old Animal House which contained wild populations of mice and voles and an Isolated Area away from all the other animals.

Once the syndrome had been identified a second set of experiments placed the problem on a more quantitative basis. Six week old imperforate female ASH/CSI mice and six week old male ASH/CSI mice were infected per os with 70 larvae of the Wellcome strain of N. dubius under conditions described previously. Total faecal output was recorded daily and egg production measured from Day 10-40.
using the MacMaster technique (Whitlock and Gordon 1939). Infected ASH/CSI mice were sacrificed on Day 10, 12, 14, 16 and 18 post infection.

(b) The experiments with oxytetracycline hydrochloride

Four six week male ASH/CSI mice were infected with 70 larvae of *N. dubius* and housed as before. One day after infection they were given 0.04% oxytetracycline hydrochloride as drinking water for three days and the antibiotic was changed daily. The antibiotic was given therefore while *N. dubius* was in the intestinal phase of development and would change the milieu of the lumen of the small intestine when the worms emerged to make the anterior migration. Another male mouse was given 0.04% oxytetracycline hydrochloride from Day 6-10 of infection.

(c) System Optimization

Four 8 week ASH/CSI mice were removed from the Irish Peat and housed on paper for 10 days prior to infection with 70 larvae of *N. dubius*. Egg production and total faecal production were measured each day. Two mice were sacrificed on Day 14 and two mice on Day 40. Severe fighting took place between the two male mice from Day 30-35 so they were separated on Day 35 and housed in individual baskets. The peat was tested for the presence of insecticide using the carabid beetles *Nebra bevicollis* and *Abax parallel epilepis*. It was tested for herbicide by growing seedlings of *Sinapsis alba* on filter paper continually moistened with water that had been in contact with the peat for 14 days. Controls of both systems using normal habitat soil for the beetles and distilled water for the seedlings of *S. alba* were set up and run in parallel with the experimental systems. Cultures of the faeces were also made up with the peat water and left at room temperature for 5 days while samples of the peat were sent to the Microbiology Department of Poole General Hospital, Dorset, for assay.
Results

(a) System Measurement and Data Analysis

The intensities of infection and the fecundity of *N. dubius* in ASH/CSI mice of varying age are shown in Table I. The mice have low intensities of infection apart from the six week old males but fecundity is low in this group. Where intensity of infection is relatively lower then there is a corresponding increase in fecundity and this would seem to indicate some competition for nutrients for egg production.

Parasite nodules were only observed in a few specimens and when present were well down the small intestine. The appearance of a nodule in section (Fig. 1) shows an apparent disintegration of the larva within the nodule and is to be contrasted with that of a normal nodule (Fig. 2). Although L3 larvae have been successful in the penetration of the small intestinal wall, they are unable to leave it. The lack of nodules in other specimens suggest that the larvae have not even survived the first few hours. The cuticles of those worms which had been successful in the anterior migration were distorted in the head and vulva region (Figs. 3 and 4).

Cultures of eggs from infected mice yielded few L3 larvae, if any at all. Normal eggs of *N. dubius* are characterised by complete uterine, chitin and ascaroside layers (Anya 1976; Lee 1976). Symptoms of the syndrome of the non viable eggs included a thin uterine layer to which debris became attached, a thin rumpled and broken chitin layer and an incomplete ascaroside layer which showed adhesion to the outer layers at the middle or poles of the egg. The latter two features were the most critical factors because they were always associated with an abnormal embryo or one that was completely disorganised.

Experiments involving leaving the eggs in deionised water overnight
<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>wt. (gms)</th>
<th>Intensity of Infection (%)</th>
<th>Mean Fecundity</th>
<th>Number of Nodules</th>
<th>Position Small Intestine (cms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>3 wks</td>
<td>20</td>
<td>0</td>
<td>0</td>
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TABLE I

Intensity of infection and fecundity of N. dubius in ASH/CSI Mice.

Syndrome Condition
Fig. 1  TS. Wall of the small intestine of the laboratory male ASH/CSI S.P.F. mouse infected with 70 larvae of 
N. dubius. Syndrome condition.
The parasite nodule contains the remains of a disintegrating larva.
\[ x \times 140 \]

Fig. 2  TS. Wall of small intestine of the laboratory male ASH/CSI S.P.F. mouse infected with 70 larvae of 
N. dubius. Normal condition.
Sections of the developing larva are clearly visible within the nodule.
\[ x \times 345. \]
Fig. 3  The cuticular distortion of the head region of *N. dubius* in the laboratory male ASH/CSI S.P.F. mouse. Syndrome condition. x 560.

Fig. 4  The cuticular distortion of the vulva region of *N. dubius* in the laboratory male ASH/CSI S.P.F. mouse. Syndrome condition. x 345.
revealed differences in eggs derived from male and female hosts. The embryos of eggs derived from male hosts swelled in size which indicated a permeable ascaroside layer, while eggs from female hosts showed a significant increase in length with little increase in the size of the embryo. These features suggest that the precursors for both egg layers are lacking in the male host but that only the precursors for chitin manufacture are lacking in the female host. Staining with dilute carbol fuschin showed the presence of more chitin on the surface of eggs derived from female hosts. The eggs sent to Weybridge revealed no viral contamination and those cultured with 2% copper sulphate and 0.04% oxytetracycline hydrochloride still failed to yield any larvae.

The second group of experiments with the successfully infected LACA mice which had been placed on the same bedding material as the ASH/CSI mice were housed in the same area, showed a drop in egg production and loss of viability of eggs approximately 14 days later. The eggs from the female MFI mice in the old Animal House were also showing non viability early post infection (Hopkins, S. - personal communication) whilst those eggs from the isolated system had incomplete shell layers on Day 17 post infection.

The results of the ASH/CSI: *N. dubius* system when placed on a more quantitative basis are summarised in Tables II and III. Inputs and outputs of the system are calculated as described previously and the results obtained in CDL mice, which are the most closely related strain to ASH/CSI mice, are inserted for comparison. Input into the ASH/CSI system is approximately three times as great as that in the CDL system in both male and female mice. It is matched by a nil or miniscule output of viable eggs from the male and female host. The bulk of the increase in input of the ASH/CSI: *N. dubius* system comes from an increase in input against the total egg production rather than input against viability. There is also evidence that the normal rhythmic rise and fall in egg production is lacking,
<table>
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<tr>
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<th>Total log e Nt</th>
<th>Mean Rate Eggs Nt</th>
<th>Viable log e Nt+1</th>
<th>Mean Rate Nt+1 of Decrease</th>
<th>Mean Rate Nt+1 of Decrease</th>
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<th>Mean Rate of Decrease</th>
<th>Viable Eggs per Hour</th>
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<tr>
<td>2.36</td>
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</table>

| OUTPUT            | Mean Total Eggs per Hour | Viability % | Mean Viable Eggs per Hour | | Mean Total Eggs per Hour | Viability % | Mean Viable Eggs per Hour |
|-------------------|--------------------------|-------------|---------------------------| |--------------------------|-------------|---------------------------|
| Component I       | 11                       | 0.6         | 0                         | | 61                       | 6.2         | 4                          |
| ASH/CSI Male      | 691                      | 71.7        | 459                       | | CD1 Male                | 412         | 71.5                       | 295          |
| CD1 Male          |                          |             |                           | |                          |             |                            |

**TABLE III**  
Input and output of the laboratory mouse: *N. dubius* system.
particularly in the female host.

Cusum analysis of egg production and faecal production (Fig. 5) indicates the components I and II seen in the CDI system but there is no switch on in faecal production around Day 19. Worms that have survived are unable to trigger the system. The mean faecal production per hour in components I and II of the male system was 0.075 gm and 0.082 gm respectively. For the female host the figures were 0.084 gm/hr and 0.086 gm/hr. It is interesting to note that the cusum of egg production in the male host and the cusum of faecal production of the female host are evolving towards the mean in an opposite direction to that seen in the CDI system. Maximum egg production occurred on day 15 in male mice and on Day 25 in female mice. The intensity of infection, sex ratio and fecundity of _N. dubius_ at time of autopsy are shown in Table IV. There is a loss of worms and reduction in fecundity within the time course of the experiment.

The statistics of egg output in terms of size, distribution and coefficients of variation are shown in Table V. A comparison of the ASH/CSI male and female system shows there to be no significant difference in the length of the egg but there is a significant difference in width (P = 0.01). The skew on length is more negative and the skew on width is more positive in the female host. There is also more variation in the length of the egg in the female host. Comparison of the ASH/CSI system with the CDI system shows there to be highly significant differences of length and width of eggs in the two systems (P = 0.001 in all cases). A 3 or 4 day cyclicity in length of egg contrasted with the 7 day cyclicity seen in the CDI system (Chapter 3). No viable larvae were produced by the ASH/CSI system.
Figure 5. Cusum analysis of egg production and faecal production of the laboratory male ASH/CSI S.P.F. mouse infected with 70 larvae of *N. dubius.*

Syndrome condition.
CUSUM ANALYSIS OF EGG PRODUCTION IN ASH/CSI. MICE

CUSUM ANALYSIS OF FAECAL PRODUCTION IN ASH/CSI. MICE
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<th>FEMALE</th>
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**TABLE IV**

Autopsy of ASH/CSI Mice infected with 70 larvae of *N. dubius*.

Syndrome condition.
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<th>Strain</th>
<th>Sex</th>
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<th>Mean Width</th>
<th>Skew of Length</th>
<th>Coeff. to Mean of Length</th>
<th>Var. to Mean</th>
<th>Coeff. to Var.</th>
<th>Skew of Width</th>
<th>Coeff. to Var.</th>
<th>Linear Correlation Coefficient of L and W</th>
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<tr>
<td>ASH/CSI</td>
<td>Male</td>
<td>74.73 ±5.8</td>
<td>48.5 ±2.35</td>
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<td>7.66</td>
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<td>80.65 ±4.65</td>
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<td>5.76</td>
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<tr>
<td>CD1</td>
<td>Male: N.V.</td>
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<tr>
<td>CD1</td>
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<td>80.25 ±4.75</td>
<td>50.3 ±2.4</td>
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<td>5.9</td>
<td>-10.4 Reg</td>
<td>+0.319</td>
<td>4.8</td>
<td>-12.3 Reg</td>
<td>+0.414</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=122</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD1</td>
<td>Female N.V.</td>
<td>78  ±6</td>
<td>49.8 ±2.5</td>
<td>+0.188</td>
<td>7.7</td>
<td>-9.32 Reg</td>
<td>-0.443</td>
<td>4.4</td>
<td>-12.65 Reg</td>
<td>-0.049</td>
</tr>
<tr>
<td></td>
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<td>n=133</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

V = viable, NV = non viable

**TABLE V**  Size of eggs of N. dubius in ASH/CSI and CD1 mice

*Infection regime. 70 larvae*
The influence of oxytetracycline hydrochloride on *N. dubius*

The daily egg production of *N. dubius* in ASH/CSI male mice treated with 0.04% oxytetracycline hydrochloride Day 2-5 post infection is shown in Table VI. There is more evidence of a cyclical production of eggs and hence possible open loop control. A comparison of input and output in the three systems is shown in Table VII. Input in the ASH/CSI mouse system manipulated with oxytetracycline hydrochloride is less and mean egg production and viability have increased. The number of days when egg production is rising in Component I is 9 and these represent inertia in the system.

Cusum analysis of total egg production (Fig. 6) using the experimental mean shows a temporary breakthrough into Component II of the system. If the mean egg production of the untreated ASH/CSI mice is used as a process mean or control instead of the experimental mean, then continued rise in egg output is apparent. Cusum analysis of faecal production (Fig. 6) locates an event taking place between Days 18-24 as in the CDL system but by Day 28 the cusum begins to change slope. The worms have lost control of faecal production and are unable to maintain input into the system. The mean faecal production of the untreated male ASH/CSI mice and those treated with oxytetracycline hydrochloride was 0.075 gm/hr and 0.089 gm/hr respectively. The difference is significant (*P* = 0.02). Faecal production in male CDL mice was 0.125 gm/hr and this is also significantly different from the values indicated for both ASH/CSI systems (*P*=0.001).

The statistics of egg size of *N. dubius* in ASH/CSI mice treated with oxytetracycline hydrochloride are shown in Table VIII. There is no significant difference in length or width of egg from the untreated ASH/CSI mice, although the skew on length and width is of opposite sign. Coefficients of variation on width are higher in the treated mice but the correlation coefficient between length and width is very low.
### TABLE VI

Egg production of *N. dubius* in male ASH/CSI mice treated with 0.04% oxytetracycline hydrochloride Day 2-5

<table>
<thead>
<tr>
<th>Day</th>
<th>Total Eggs per Hour</th>
<th>log e Nt</th>
<th>Mean Rate of Decrease Viable Eggs per Hour</th>
<th>log e Nt+1</th>
<th>Mean Rate of Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12</td>
<td>61</td>
<td></td>
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<tr>
<td>13</td>
<td>105</td>
<td></td>
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<td>14</td>
<td>141</td>
<td></td>
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<tr>
<td>15</td>
<td>15</td>
<td>2.24</td>
<td>2.24</td>
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<td></td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>18</td>
<td>309</td>
<td></td>
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<td></td>
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<tr>
<td>19</td>
<td>179</td>
<td>0.55</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td>256</td>
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<td></td>
</tr>
<tr>
<td>21</td>
<td>99</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>353</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>229</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>163</td>
<td>0.34</td>
<td>1.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>TOTAL</td>
<td>4.33</td>
<td>4.95</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>9</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>8</td>
<td>0.12</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>4</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE VII

Input and output of laboratory mouse/*N. dubius* systems, Day 10-26

<table>
<thead>
<tr>
<th>Strain</th>
<th>I_T</th>
<th>Mean Egg Output</th>
<th>% Viable</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 0'</td>
<td>1.19</td>
<td>691</td>
<td>81.3</td>
</tr>
<tr>
<td>ASH/CSI 0' OT</td>
<td>4.33</td>
<td>124</td>
<td>5.13</td>
</tr>
<tr>
<td>ASH/CSI 0' No OT</td>
<td>6.4</td>
<td>11.2</td>
<td>0.6</td>
</tr>
</tbody>
</table>

OT = oxytetracycline hydrochloride
Figure 6  Cusum analysis of total egg production and faecal production of the laboratory male ASH/CSI S.P.F. mouse infected with 70 larvae of N. dubius. Treatment with 0.04% oxytetracycline hydrochloride Day 2-5 post infection.
ASH/CSI MALE MICE

OXYTETRACYCLINE HYDROCHLORIDE (0.04%).

**Cusum Total Egg Production**

- Experimental Mean
- Control Mean

**Cusum Faecal Production**

- Experimental Mean
- Control Mean
<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean Length</th>
<th>Mean Width</th>
<th>Skew of Length</th>
<th>Coeff. Var. to Mean Length</th>
<th>Var. Dispersion of Length</th>
<th>Skew of Width</th>
<th>Coeff. Var. to Mean Width</th>
<th>Var. Dispersion of Width</th>
<th>Linear Correlation Coefficient of L and W</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASH/CSI</td>
<td>75 ± 5.5</td>
<td>49.2 ± 3.13</td>
<td>+0.016</td>
<td>7.3</td>
<td>-12.7</td>
<td>Reg.</td>
<td>-0.049</td>
<td>6.35</td>
<td>-9.12</td>
</tr>
</tbody>
</table>

**TABLE VIII**

Egg size of N. dubius in ASH/CSI Mice treated 0.04% oxytetracycline hydrochloride

Day 2-5 post infection

OT = oxytetracycline hydrochloride
The mean intensity of infection was 62.9% and the mean fecundity of gravid female worms was 84.11. Eggs in the oviducts appeared to be larger than those which appeared in the faeces and abnormal cleavage had taken place as they passed down the oviduct. The eggs were characterised by a better developed chitin coat than that seen previously. There was no cyclicity in egg length. Culture of eggs from the mouse treated with antibiotic on Day 6 yielded abnormal larvae. The alimentary canal was empty and dilated at the anterior and posterior end. Movements were jerky and not typical of nematode locomotion and the cuticle was characterised by crenulations as the animal moved. Also present in some cultures was the protozoan Colpidium which is normally found in the soil. Its origin was therefore probably from the peat on which the mice were housed.

(c) System Optimization

The sequential appearance of the syndrome is shown in Fig. 7. The only common feature of all three systems was the bedding material. It was significant that removal from Irish Peat and housing on paper brought about a recovery of MFI mice, which had been on peat for three weeks, more slowly than that of others which had been on peat for only five days.

The results for egg production of *N. dubius* in male ASH/CSI mice removed from peat ten days prior to infection are shown in Table IX. There is more evidence of cyclicity and viability has improved. The input and output of the system is shown in Table X and the results of the other systems are included for comparison. The lesser input against total egg production, $I_T$, is not reflected in greater total output in Component I. However, the worms have crossed the interface between Component I and II and maintained egg output in Component II, a phenomenon not seen in the other two systems. This input therefore must not only be directed against cogenesis but in some way impair the ability of *N. dubius* to manipulate the
<table>
<thead>
<tr>
<th>SEQUENCE OF IDENTIFICATION</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYSTEMS</td>
<td>NEW ANIMAL HOUSE</td>
<td>OLD ANIMAL HOUSE</td>
<td>ISOLATED SYSTEM</td>
</tr>
</tbody>
</table>
| SYSTEM FEATURES           | 1. ASH/CSI Mice Lifetime Housing on Irish Peat Delivery 4.10.76  
3. No link with other two systems | 1. Wild populations of mice, voles, etc.  
2. Failure of MFI/N. dubius system housed on Irish Peat for 3 weeks.  
3. Identification of syndrome Feb. 1977 | 1. MFI mice housed on Irish Peat approx. 6 days.  
2. New delivery of peat (6.1.77) used thereafter.  
| OUTCOME                   | Failure of Mouse/N. dubius system | Failure of mouse/N. dubius system | Initial Failure followed by Recovery. |

The only common factor was the Irish Peat delivered 4.10.76.

Fig. 7 SEQUENTIAL APPEARANCE OF SYNDROME IN THREE SYSTEMS
### TABLE IX  Egg production of *N. dubius* in male ASH/CSI mice removed from peat 10 days prior to infection

<table>
<thead>
<tr>
<th>Day</th>
<th>Total Eggs per Hour</th>
<th>log e Nt</th>
<th>Mean Rate of Decrease Hour</th>
<th>Viable Eggs per Hour</th>
<th>log e Nt+1</th>
<th>Mean Rate of Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>59</td>
<td></td>
<td></td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>154</td>
<td></td>
<td></td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>1.12</td>
<td></td>
<td>25</td>
<td>1.26</td>
<td>2.23</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>2.3</td>
<td>1.71</td>
<td>0 ± 1</td>
<td>3.2</td>
<td>2.23</td>
</tr>
<tr>
<td>14</td>
<td>263</td>
<td></td>
<td></td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>34</td>
<td>2.05</td>
<td></td>
<td>3</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>87</td>
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<td></td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>1.58</td>
<td></td>
<td>2</td>
<td>2.14</td>
<td>1.67</td>
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<tr>
<td>18</td>
<td>24</td>
<td></td>
<td></td>
<td>0 ± 1</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>24</td>
<td></td>
<td></td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td></td>
<td></td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
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<td></td>
</tr>
<tr>
<td>22</td>
<td>9</td>
<td>1.61</td>
<td></td>
<td>1</td>
<td>3.14</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>172</td>
<td></td>
<td></td>
<td>52</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>36</td>
<td>1.56</td>
<td>1.06</td>
<td>4</td>
<td>2.56</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td>158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>395</td>
<td></td>
<td></td>
<td>TOTAL 3.98</td>
<td></td>
<td>TOTAL 5.8</td>
</tr>
<tr>
<td>27</td>
<td>472</td>
<td></td>
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<td>156</td>
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</tr>
<tr>
<td>28</td>
<td>161</td>
<td>1.08</td>
<td></td>
<td>27</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>556</td>
<td></td>
<td></td>
<td>306</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>266</td>
<td>0.74</td>
<td>0.61</td>
<td>97</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>367</td>
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<td></td>
<td>147</td>
<td></td>
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</tr>
<tr>
<td>32</td>
<td>134</td>
<td>0.64</td>
<td></td>
<td>135</td>
<td>0.085</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>515</td>
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<td>419</td>
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<tr>
<td>34</td>
<td>552</td>
<td></td>
<td></td>
<td>221</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>43</td>
<td>2.55</td>
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<td>4</td>
<td>4.01</td>
<td>1.49</td>
</tr>
<tr>
<td>36</td>
<td>172</td>
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<td>113</td>
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<td>0.99</td>
<td>11</td>
<td>1.32</td>
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<td>35</td>
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<td>28</td>
<td>1.10</td>
<td>0.90</td>
<td>6</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td>TOTAL 3.14</td>
<td></td>
<td>TOTAL 3.34</td>
</tr>
</tbody>
</table>
### TABLE X  
Input and output of the laboratory mouse: *N. dubius* system

<table>
<thead>
<tr>
<th>System</th>
<th>( I_T )</th>
<th>Mean Egg Output</th>
<th>% Viable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 10-26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD1 ♂</td>
<td>1.19</td>
<td>691</td>
<td>81.3</td>
</tr>
<tr>
<td>ASH/CSI ♂</td>
<td>3.98</td>
<td>92</td>
<td>29.2</td>
</tr>
<tr>
<td>ASH/CSI ♀</td>
<td>4.33</td>
<td>124</td>
<td>17.5</td>
</tr>
<tr>
<td>OT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASH/CSI ♀ untreated</td>
<td>6.4</td>
<td>11</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Component II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 27-40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD1 ♂</td>
<td>0.69</td>
<td>737</td>
<td>59.4</td>
</tr>
<tr>
<td>ASH/CSI ♂</td>
<td>3.14</td>
<td>264</td>
<td>34.3</td>
</tr>
</tbody>
</table>

OT = oxytetracycline hydrochloride
Cusum analysis of total egg production (Fig. 8) shows more deviation around the process mean and change is more marked and rapid than in the CDI system, so instability is present. The mean faecal production in Component I is 0.094 gm/hr and in Component II is 0.091 gm/hr. Cusum analysis of faecal production (Fig. 8) using the mean faecal production of the untreated mice as a process mean or control reveals the system being triggered at Day 16, somewhat earlier than in the oxytetracycline hydrochloride and CDI systems.

The statistics of egg output are summarised in Table XI. The egg is larger in size than in the ASH/CSI untreated male mice, the difference in length being significant ($P = 0.001$). The skew on length is markedly negative in Component I and the coefficients of variation are fairly high. There was a 3–4 day cyclicity in length.

Autopsy of mice on Day 14 and 40 showed intensities of infection of 56.4% and 44% respectively and the fecundity of *N. dubius* on these days was $78^\pm 28$ and $57^\pm 36$. The large variance on fecundity may be the result of the small number of mice involved or may reflect less selective pressure on oogenesis.

The beetles *Nebra brevicollis* and *Abax parallelepilepis* were apparently normal after living on the peat for 14 days and the seedlings of *Sinapsis alba* continually watered with a distillate from the peat showed no abnormality.

Culture of the faeces from the experimental mice using deionised water and peat water revealed a stepwise pattern of development of larvae consistent with the work of Crofton and Whitlock (1965). Only very few L3 larvae hatched and those only in cultures with deionised water (Fig. 9). Development appeared to be faster in cultures with peat water revealing a peak of L1 development two hours earlier than in cultures with deionised water. Rumpling
Figure 8  Cusum analysis of egg production and faecal production of *N. dubius* in ASH/CSI S.P.F. male mice removed from the contaminant in the peat 10 days prior to infection with 70 larvae of *N. dubius*. 
ASH/CSI MALE MICE
REMOVAL PEAT 10 DAYS

- Experimental Mean
- Control Mean

Cusum Total Egg Production

Cusum Faecal Production
<table>
<thead>
<tr>
<th>Sex</th>
<th>Strain</th>
<th>Mean Length</th>
<th>Mean Width</th>
<th>Skew of Var on Length</th>
<th>Coeff. Var. of Length to Mean Dispersion</th>
<th>Skew of Var on Width</th>
<th>Coeff. Var. of Width to Mean Dispersion</th>
<th>Linear Correlation Coefficient of L and W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>ASH/CSI</td>
<td>78.3 ±5.2</td>
<td>49 ±2.8</td>
<td>-0.96</td>
<td>6.67</td>
<td>-11.58 Reg</td>
<td>+0.214 5.7 -13.8 Reg</td>
<td>-0.045</td>
</tr>
<tr>
<td>Day-10-26</td>
<td></td>
<td>n=172</td>
<td>n=172</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off Peat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 27-40</td>
<td></td>
<td>78.6 ±6.2</td>
<td>49.65 ±2.25</td>
<td>-0.032</td>
<td>7.92</td>
<td>-10.3 Reg</td>
<td>-0.47 4.53 -14.76 Reg</td>
<td>+0.073</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=172</td>
<td>n=172</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE XI**  
Egg size of *N. dubius* in ASH/CSI Mice removed from Irish Peat ten days before infection
Developmental stages of *N. dubius* from male ASH/CSI S.P.F. mice removed from the peat source 10 days prior to infection with 70 larvae of *N. dubius*. Cultures using deionised water and a distillate from the peat differ in the rate of development.
DEVELOPMENTAL STAGES IN CULTURE OF N. DUBIOUS.

% Deionised Water

- --- Unhatched LI

- - Blastula and Gastrula

- --- Up to 64 cell

% Peat Water

L3 = 0.83%
and breakage of chitin was noticed at 43 hours, the broken ends of the chitin sticking outwards from the surface of the egg. This would seem to indicate that pressure in or on the perivitelline space and then on the chitin finally ruptured it. Support for this theory is derived from the fact that rumpling appeared at the poles of the egg first where pressure would be greatest due to the smaller radius at this point (Laplace's Law).

Microbiological assay of the peat sent to the Microbiology Department of Poole General Hospital isolated a number of organisms. They included *Bacillus pumilis*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus sphaericus*, *Staphylococcus citreus* and spp of *Candida*, *Mucor* and *Penicillium*. 
Discussion

A systems approach to the problem of the initiation of infection in the ASH/CSI mouse system with subsequent failure of viable egg production by the parasite has identified a number of differences from the CDI system. These include fewer parasitic nodules in the lower small intestine, a lower intensity of infection, distortion of the cuticle in adult worms, reduced fecundity, failure of eggs to develop and an inability to cross the interface between components I and II of the system and trigger increased faecal production.

Since the initial larvae had every appearance of being normal the hypothesis was that their failure to develop may have been due to a hostile microbial environment in the small intestine of the mouse. Lack of the right host microflora has been shown to influence the development and pathogenicity of an N. dubius infection (Newton, Weinstein and Sawyer 1962, Wescott 1968, Przyjalkowski and Wescott 1969) and microflora are also essential to the survival of the related Nippostrongylus brasiliensis (Wescott and Todd 1964). Bacterial flora are also known to be an important determinant of mucosal structure (Abrams et al 1963) and an abnormal flora may have rendered the wall of the small intestine less easy to penetration by the larvae. Such changes in microbial environment do occur naturally and are therefore not unlikely (Schaedler et al 1965), but once inside the mucosal wall it is likely they would have been able to discount the bacterial environment of the lumen of the small intestine as does Trichinella spiralis (Stefanski and Przyjalkowski 1965).

Intensities of infection in the 6 week ASH/CSI mice did not differ appreciably from the CDI system and fecundity was not significantly different yet egg output was considerably lower in the ASH/CSI system. This reduced egg output and the associated non viability could have been due to a number of factors among them being damage to the reproductive
system of the worm in the anterior migration, failure to obtain the
requisite nutrients from the host, lack of flora or an inhibitory effect
on embryogenesis by resident bacteria. There may also have been an
abnormal flora in the gut of the nematode which was either lacking in
bacteria essential to the worm or was sufficiently different to upset
equilibrium in the anterior small intestine. An associated flora has
been described for Aspiculurus tetraptera (Tannock and Savage 1974) and
the presence of B. mesentericus and B. cereus var. mycoides can inhibit
the cleavage of the eggs of Ascaris suum (Przyjalkowski and Jaskoski
1968). An ovostatic effect of cultures of bacteria in Ascaridia galli
and Aspiculurus tetraptera has also been observed (Przyjalkowski
1973).

The increased faecal production seen in CDI: N. dubius system in spite
of the anorexia induced by an N. dubius infection (Baker 1954), did
not take place in the ASH/GSI system. The distortion of the cuticle
in the head and vulva region of N. dubius may have been related to the
reason why the parasite was unable to establish in the host.

The use of oxytetracycline in the microbial manipulation of the ASH/GSI
system was of benefit to N. dubius for it resulted in increased total
egg production, viability and a temporary breakthrough into Component
II of the system. Oxytetracycline hydrochloride is a broad spectrum
antibiotic not effective against viruses. In vitro studies show it to
be effective against Clostridium spp., Escherichia coli, Aerobacter
aerogenes, Bacillus anthracis, non hemolytic streptococci and \( \alpha \) and \( \beta \)
hemolytic streptococci. Nearly all strains of Proteus vulgaris,
Pseudomonas aeruginosa and freshly isolated staphylococci and enteroc-
cocci are resistant. The antibiotic is incompletely absorbed from the
intestinal tract and within 48 hours the enteric flora is markedly
altered. The normal populations of lactobacilli, anaerobic streptococci
and bacteriodes rapidly disappear and tetracycline resistant organisms
overgrow, particularly species of *Proteus*, *Pseudomonas*, staphylococci and yeasts (Dubos et al 1965; Goodman and Gilman 1965; Hawker 1972). This was apparent when cultures of faecal pellets of the single mouse yielded staphylococcal overgrowth when compared to controls. Also microbiological assay of the small intestine of another mouse which had died previously revealed the presence of a *Proteus* sps and *Escherichia coli*.

The changed microbial environment induced by oxytetracycline resulted in eggs with a thicker chitin layer and there were fewer embryos stained with carbol fuschin. Either the precursor acetylglucosamine units (Tarr and Fairbairn 1973) and protein for the chitin layer were in better supply or pre-existing disturbance to the metabolic function of the worm had lessened. The fact that abnormal cleavage of the egg had occurred suggested that the influence was still there. Abnormal larvae seen in culture of the eggs were similar to those described in the faeces of germ free mice (Weinstein et al 1969). The death of the larvae could have been due to a lack of bacteria or an inhibitor may have been present which is normally degraded by bacteria in the faecal pellet.

The location of the factor causing disturbance in the ASH/CSI male mouse: *N. dubius* system and the removal of the mice from the peat bedding material prior to infection improved system behaviour. The increase in faecal production and egg output occurred as in the CDI mice but at a lower level. The system maintained operation and the worms were able to control faecal production at the interface of components I and II. Although there was cuticular distortion in the worms on Day 14, the agent responsible for it did not seem able to damage this switching mechanism of the system.

The microenvironment of the small intestine also had the capacity to alter eggs in terms of shape and size. The significantly larger egg and the marked negative skew on length in Component I of the system where peat is removed is noteworthy. Examination of the eggs during development revealed an outward rupture of chitin which suggests that, despite an
apparently normal chitin and ascaroside layer, the egg was still permeable. Slow absorption of water would increase pressure within the egg and cause the type of break observed in the experiment. If the membranes were permeable then the embryonic cortex which carries information for future developmental processes would enter a control space of larger dimension. No filtering into the interior of the embryo would take place and external variables would be internalised. An environmental variable entering at the wrong instant in time into the genetic programme of the egg would upset it. The faster development of eggs in the peat water and the abnormal cleavage seen in the oviduct of worms in the antibiotic experiment may be an indication that this process is occurring. The effect on development is seen in the very low hatch to 13 larvae even when the system is optimised.

The presence of a number of Bacillaceae in the peat and the fact that the mice were observed to eat it may be another cause for chitin rupture. The Bacillaceae are a group of gram positive aerobic rods able to secrete chitinase and it may be that this enzyme was attacking the chitin. These bacteria are also able to spore in adverse conditions and it is possible that storage in black plastic bags during the very hot summer of 1976 may have eliminated all those bacteria unable to spore. Given the right conditions the spores would germinate immediately and multiply rapidly with little competition. Such a situation would arise when the peat was used as a bedding material for mice. Faecal pellets and urine would supply nitrogen and other nutrients to an already optimal environment. The optimised system, however, requires still further manipulation to achieve an output comparable to the CDI system.
CHAPTER 5
THE RESPONSE OF THE HOST TO NEMATOSPIROIDES DUBIUS

Introduction

Field studies of the Nematospiroides dubius: Apodemus sylvaticus system have characterised *N. dubius* in terms of a K-strategy with regulation of parasite numbers at host individual level (Bradley 1972). A host parasite system with this type of strategy requires an integration of controls if efficiency is to be achieved at population level. Integration in the host parasite system of *N. dubius*: *A. sylvaticus* is programmed at population level by the genetic characteristics of host and parasite (Spurlock 1943; Liu 1965; Brindley and Dobson 1981) and it possesses a time sequence dependent on the age and sex of the host (Dobson 1961, 1966) as well as the availability of larval input (Lewis 1968 (a)). All of these factors will initiate a series of stages through which the system passes until it is fully operational first at individual and then at population level. Regulation using the inputs of sex and age of the host is predictable but not the availability of larval input. The latter is dependent on host behaviour, diversity in egg output and dispersion on pasture. Thus the compartment of the system within the mouse will possess the tighter controls and the compartment on the pasture will be subject to pressures of a more random nature. Both types of control will lead to a slow build up of system operation and therefore provide a relatively stable strategy for the continued survival of the parasite.

The work described in this chapter examines the controls that exist at the level of the host individual and relates them to the output of the system. It also provides a baseline for the interpretation of the results of the next chapter which describes the disturbance of the host parasite system by stress.

Regulation at the host individual level is achieved by an immune response.
of the host and the availability of lymphatic tissue and its components will determine the level of control of parasite numbers within the host individual. The spleen, mesenteric nodes, thymus and Peyer's patches are all part of this system and that the first three are involved in the immune response of laboratory mice to *N. dubius* is well documented (Spurlock 1943; Baker 1955; 1962; Liu 1965; Cypess 1972; Jones and Rubin 1974; Bartlett and Bell 1974; Prowse, Mitchell, Ey and Jenkin 1978) but little work has been completed on the histology of these tissues during an infection. Neither are there any reports in the literature as to the status of Peyer's patches in an infection of mice by *N. dubius*.

The functional organisation in lymphatic tissue delineates B and T cell dependent areas in the lymph nodes and spleen (Inchley 1981). The cortex of a lymph node is divided into an outer B dependent area characterised by dense clusters of B lymphocytes known as lymphoid follicles and an inner T dependent paracortex in which few B cells are found. Similarly in the spleen a cross section through the white pulp reveals a central arteriole around which is a core of T lymphocytes while beyond this is the B dependent area and a marginal zone which separates the areas of white pulp from the red pulp. Antigen is maintained on dendritic reticular cells which reach in among clustered lymphocytes in the B dependent area and many dividing lymphocytes form the germinal centres associated with the generation and maintenance of immunological memory. Peyer's patches in the mouse can be circular, oval, rectangular or irregular in shape. They are situated on the antimesenteric border of the intestine and extend through the lamina propria to the external muscle sheath. In correctly cut sections the domes of the patches are always found above the follicles they contain and there are mushrooms of villi above the interfollicular areas between the domes (Waksman 1973). Thoracic duct B cells labelled with $^3$H-uridine home to these domes (Howard, Hunt and Gowans 1972) and $^3$H-thymidine perfused via the thymic artery of calves results in labelled T cells appearing almost exclusively in the interfollicular or
thymus dependent area (Waksman 1973).
The thymus of the mouse possesses typical mammalian structure and consists of a number of lobes, each lobe being surrounded by a thin capsule of connective tissue (Ham 1950; Bloom and Pawcett 1968). The connective tissue extends into incomplete septa and divides the lobes into lobules. The thymic tissue of each lobule is therefore continuous. Lymphocytes are concentrated in the periphery or cortex of the lobules near the capsule and interlobular septa so that they mask the epithelial network in which they lie. The medulla contains fewer lymphocytes but more numerous blood vessels.

Materials and Methods

1. Morphophysiological indices indicative of immune status

The availability of lymphatic tissue with respect to age was studied in female ASH/CSI S.P.F. mice with a housing regime of 4 per basket of area 102 sq. ins. and volume 663 cu. ins. Mice of 3-8 weeks old were sacrificed and the mesenteric nodes, spleen and thymus were removed and weighed as described previously. Peyer's patches and the number of follicles they contain were identified by placing the alimentary canal in dilute acetic (water: acetic acid, 3:1) for 24 hours. Peyer's patches contain much nuclear material and this has the effect of making the patches stand out clearly as superficial plaques (Cornes 1965). The effect of the dilute acetic acid on the rest of the alimentary canal is to make the thin gut of ASH/CSI mice firmer to cut. Each patch was measured and the total area of Peyer's patches expressed as a percentage of the length of the small intestine. The number of follicles in each patch were counted with the aid of a hand lens x 10 magnification. Peyer's patches were also excised and weighed. These experiments served as the controls to the infection experiments described below. In addition the relative weights of the mesenteric nodes, spleen, thymus and the area of Peyer's patches with
respect to the length of the small intestine of 279 male and 207 female *A. sylvaticus* were grouped into age classes of 2 gm differences in body weight.

2. **Morphophysiological indices in infection with N. dubius**

Female ASH/CSI S.P.F. mice of ages 3 to 6 weeks and subjected to the same housing regime as the controls were infected with 70 larvae of *N. dubius* as described elsewhere. Sacrifice took place on Day 14 and an autopsy procedure identical to the controls was adopted.

3. **Egg output of the N. dubius: mouse system**

The egg output of each mouse was calculated from the product of the number of gravid female worms present and their mean fecundity.

4. **Histology of the Morphophysiological indices**

The mesenteric nodes, spleen, thymus and Peyer's patches from the anterior, middle and posterior small intestine were removed from uninfected and female ASH/CSI S.P.F. mice infected with 70 larvae of *N. dubius*. They were placed in Bouin's fixative, carried through the usual paraffin method, sectioned at 7 micra and stained with haematoxylin and eosin. The sections were examined under a Leitz Orthoplan microscope with camera attachment.

**Results**

1. **Morphophysiological indices indicative of immune status**

The weights of all the tissues and organs are described relative to body weight and in this respect they become comparable. The relative weights of the mesenteric nodes, spleen and thymus and the Peyer's patch area of female ASH/CSI S.P.F. mice at different ages are shown in Fig. 1. Steady involution of lymphatic tissue in the spleen and thymus as indicated by relative weight occurred between the fourth and seventh week. The range in spleen and thymus weight was $2.8 \times 10^{-3}$ gm to $6.16 \times 10^{-3}$ gm and $1.2 \times 10^{-3}$ gm to $4.3 \times 10^{-3}$ gm respectively. The relative weight of the spleen is at a minimum in week 6 and that of the thymus in week 7. The mesenteric nodes show no clear trend but exhibit small fluctuations. The range of mesenteric node weight was from
Fig. 1

The relative weights of the mesenteric nodes, spleen and thymus and the Peyer's patch area (P.P.A.) of female ASH/CSI S.P.P. mice with respect to age.
MORPHOPHYSIOLOGICAL INDICES OF FEMALE ASH/CSI S.P.F. MICE.

- Spleen
- Thymus
- Mesenteric nodes

**Weight (mg)**
- $6 \times 10^{-3}$ to $1 \times 10^{-3}$

**Age**
- 3 wks., 4 wks., 5 wks., 6 wks., 7 wks., 8 wks.

**P.P.A.**
- 1 to 2

**Age**
- 3 wks., 4 wks., 5 wks., 6 wks., 7 wks., 8 wks.

**Peyers Patch. Area.**
1.9 x 10^{-3} \text{ gm.} \text{ to } 5.2 \times 10^{-3} \text{ gm.} \text{. In contrast the area of Peyer's patches was maximal at 6 weeks of age and may compensate for the decline in lymphatic tissue elsewhere. The mean number of Peyer's patches in 4-8 week old ASH/CSI S.P.F. mice ranged from 8.75 to 10.25 and they compare well with figures of 10.7 in C3H mice and 8-9 in Swiss and dba laboratory strains (Richter and Hall 1948). The number of follicles in each Peyer's patch varied from 3 to 13 and the numbers of follicles in Peyer's patches are greatest in the regions corresponding to 10-20% and 90-100% of the length of the small intestine (Table I). The size of the follicles varied and sometimes they were restricted as a dark brown margin at the periphery of the patch, the rest of the patch being a whitish colour. The mean number of follicles in 4, 5, 6, 7 and 8 week ASH/CSI S.P.F. mice were 57.3, 71, 72.8, 71.8 and 62.8 respectively.

The relative weights of the mesenteric nodes, spleen, thymus and the Peyer's patch area of wild male and female A. sylvaticus are shown in Figs. 2 and 3. Steady involution of the thymus takes place with increasing body weight and the relative weight of the thymus is higher in the female mouse than the male in the early age classes. The range of relative thymus weight was from 1.3 x 10^{-4} \text{ gm.} \text{ to } 1.5 \times 10^{-3} \text{ gm.} \text{ and } 1.1 \times 10^{-4} \text{ gm.} \text{ to } 1.8 \times 10^{-3} \text{ gm.} \text{ in male and female mice respectively. Steady increase in spleen weight occurs in the male mouse up to the 26-27.9 gm. age class and may represent involvement of the spleen in natural infections. The spleen weight of the female mouse peaks in the 24-25.9 gm. age class. Ranges of spleen weight in male and female mice were 1 \times 10^{-3} \text{ gm. to } 8.7 \times 10^{-3} \text{ gm. and } 2.5 \times 10^{-4} \text{ gm. to } 6.9 \times 10^{-3} \text{ gm. respectively. The mesenteric node weights of wild A. sylvaticus are comparable to the laboratory ASH/CSI S.P.F. mice in the small fluctuations that occur with increasing age but they differ in that an increase begins to occur in older mice. The range of mesenteric node weight in male and female A. sylvaticus was from } 5 \times 10^{-4} \text{ gm. to}
### Table 1
Position of Peyer's patches and the number of follicles they contain in female ASH/OSI S.P.F. mice

<table>
<thead>
<tr>
<th>% Small Intestine</th>
<th>Total Number of Peyer's Patches</th>
<th>Number of Follicles in Peyer's Patches</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 9.9</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>10 - 19.9</td>
<td>34</td>
<td>217</td>
</tr>
<tr>
<td>20 - 29.9</td>
<td>11</td>
<td>80</td>
</tr>
<tr>
<td>30 - 39.9</td>
<td>13</td>
<td>106</td>
</tr>
<tr>
<td>40 - 49.9</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>50 - 59.9</td>
<td>21</td>
<td>143</td>
</tr>
<tr>
<td>60 - 69.9</td>
<td>16</td>
<td>115</td>
</tr>
<tr>
<td>70 - 79.9</td>
<td>23</td>
<td>160</td>
</tr>
<tr>
<td>80 - 89.9</td>
<td>28</td>
<td>179</td>
</tr>
<tr>
<td>90 - 100</td>
<td>35</td>
<td>219</td>
</tr>
</tbody>
</table>

Total No. of Mice: 18

Age: 4-8 weeks
Fig. 2

The relative weights of the spleen and mesenteric nodes of male and female *A. sylvaticus* with respect to age.

Age is expressed in terms of body weight in grams.

Lytchett Matravers Estate, Poole, Dorset.

MORPHOPHYSIOLOGICAL INDICES OF A. SYLVATICUS.

Spleen

Mesenteric Nodes.

Female

Male

Weight (mgm)

Age of A. sylvaticus

Age of A. sylvaticus.
Fig. 3

The relative weight of the thymus and Peyer's patch area (P.P.A.) of male and female *A. sylvaticus* with respect to age.

Age is expressed in terms of body weight in grams.

Lytchett Matravers Estate, Poole, Dorset.

Morphophysiological Indices of A. sylvaticus.

**Thymus.**

Peyers Patch Area.

**Age of A. sylvaticus.**

**Age of A. sylvaticus.**

**Weight (mgm)**

- $2 \times 10^{-3}$
- $1.5 \times 10^{-2}$
- $1 \times 10^{-3}$
- $5 \times 10^{-4}$
- $0$
2.8 x 10^{-3} \text{ gm.} and 5 x 10^{-4} \text{ gm.} to 3.2 x 10^{-3} \text{ gm.} respectively. The Peyer's patch area of *A. sylvaticus* peaked at approximately the same time in male and female mice, that is in the 16-17.9 gm. age class. There is some indication of involution of Peyer's patch area with age but it is not a clear cut decrease. The presence of two very high Peyer's patch areas in the oldest mice suggests the presence of two phenotypes in the population. The range of Peyer's patch area in male and female mice was 0.078 to 3.14 and 0.04 to 3.597 respectively. No counts of follicles in the Peyer's patches of wild mice were made during the study. However, intussusception of involution of the small intestine on itself was associated with Peyer's patches in 6.1% of male mice and 3.9% of female mice. The size of involution ranged from 0.2 cm to 2 cm and in some cases blockage of the alimentary canal occurred.

Significance testing of the morphophysiological indices of male and female ASH/CSI S.P.F. mice at 7 weeks of age showed a larger thymus in the female mouse (P = 0.02) and greater Peyer's patch area in the male mouse (P= 0.05). There was no significant difference in the weight of the mesenteric nodes and spleen of male and female ASH/CSI S.P.F. mice. Neither was there any significant difference between any of the morphophysiological indices of male and female wild *A. sylvaticus*.

2. Morphophysiological indices in infection with *N. dubius*

The mean relative spleen weight of all the female ASH/CSI S.P.F. mice used in the experiments is the only parameter to show a significant difference in weight during an infection with *N. dubius*. The mean relative spleen weights of uninfected and infected mice were $2.15 \times 10^{-3}$ gm $\pm$ 8.4 x $10^{-4}$ gm and $2.55 \times 10^{-3}$ gm $\pm$ 8.6 x $10^{-4}$ gm respectively (P= 0.001). The results present a different picture if the infection regimes are considered in the context of individual age classes. Infection at 5 weeks produces significant difference in the weight of the spleen (P = 0.05) but not in that of the thymus or
mesenteric nodes. Infection at 6 weeks, however, produces significant increase in the weight of the spleen and mesenteric nodes \((P = 0.05)\) but no significant difference in the weight of the thymus and Peyer's patches. This would seem to suggest that it is at this age that the immune response begins to mature in female mice. The immune response in male ASH/CSI S.P.F. mice is not mature at a 5 week infection for there are no significant differences in any of the morphophysiological indices in uninfected and infected mice.

3. Egg output of the *N. dubius*: mouse system

The mean egg output of *N. dubius* per female host of ASH/CSI S.P.F. mice in 4, 5 and 6 week infections are 567, 2835 and 1258 respectively. The mean egg output of *N. dubius* per host of *A. sylvaticus* with respect to age is shown in Table II. Egg output of *N. dubius* is lower in wild mice and it only approaches a similar value to that of the laboratory mice in male *A. sylvaticus* in the 28-29.9 gm. weight class. It is also lower in young laboratory and wild mice and this may reflect an unsuitable microenvironment for the establishment of the worms. The sex of the wild host also plays a part in the control of egg output particularly in the older age groups of female *A. sylvaticus*. The oscillations in egg output from the male *N. dubius*: *A. sylvaticus* system are more extreme than those of the female system and may reflect lesser stability.

4. Histology of the morphophysiological indices

The photographs of the histology of the morphophysiological indices appear in Figures 4 - 23.

(a) The thymus (Figs. 4 - 9)

The thymus of the uninfected control female ASH/CSI SP.P. mouse exhibits the typical mammalian structure already described in the introduction to this chapter. Mitotic figures are present in the large lymphocytes near the margin of the gland and numbers of small lymphocytes increase in the mid cortex and in areas adjacent
### TABLE II

**The age of A. sylvaticus and the egg output of N. dubius**

<table>
<thead>
<tr>
<th>Age Class Weight (gms)</th>
<th>Mean Egg Output per host MALE</th>
<th>Mean Egg Output per Host FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 - 9.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 - 11.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 - 13.9</td>
<td>36 $\pm$ 32.5</td>
<td>43 $\pm$ 84.7</td>
</tr>
<tr>
<td>14 - 15.9</td>
<td>117 $\pm$ 164.1</td>
<td>124 $\pm$ 135.6</td>
</tr>
<tr>
<td>16 - 17.9</td>
<td>73 $\pm$ 77.3</td>
<td>162 $\pm$ 201.7</td>
</tr>
<tr>
<td>18 - 19.9</td>
<td>284 $\pm$ 361.5</td>
<td>112 $\pm$ 115.9</td>
</tr>
<tr>
<td>20 - 21.9</td>
<td>371 $\pm$ 405.8</td>
<td>303 $\pm$ 285.3</td>
</tr>
<tr>
<td>22 - 23.9</td>
<td>354 $\pm$ 382.6</td>
<td>207 $\pm$ 204.6</td>
</tr>
<tr>
<td>24 - 25.9</td>
<td>324 $\pm$ 360.3</td>
<td>311 $\pm$ 333.1</td>
</tr>
<tr>
<td>26 - 27.9</td>
<td>257 $\pm$ 263.5</td>
<td>112 $\pm$ 82.6</td>
</tr>
<tr>
<td>28 - 29.9</td>
<td>827 $\pm$ 903.9</td>
<td>235 $\pm$ 64.7</td>
</tr>
<tr>
<td>30 - 31.9</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>32 - 33.9</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>
to the medulla. Blood vessels are more numerous in the medulla and the reticuloendothelial cells lining them are clear (Figs. 4-6). Lymphocytes are not so tightly packed in the cortex of the thymus of a mouse infected with *N. dubius* and more are present in the medulla than in the control. Lymphocytes in the medulla tend to be concentrated around blood vessels and some are present in the reticuloendothelial cells lining the blood vessels. The junction of the cortex and medulla is not clearly demarcated as in the control mice (Figs. 7-9). Corpuscles of Hassal are relatively more frequent in the medulla of infected mice than they are in the control. These are centres of non living material which contain pyknotic and broken up nuclei, keratin and non-descript material. They can vary in size between 30-100 \(\mu m\) in diameter; a typical measurement in ASH/CSI S.P.F. mice infected with *N. dubius* is 33 \(\mu m\). They stain with acid dyes and the cells of the central part may die completely leaving a cyst in the centre. One such cyst is visible in Fig. 8. These centres of activity may be concerned with the cycling of nucleic acids.

(b) Peyer's patches

The domes and thymus dependent areas of the Peyer's patches in the control ASH/CSI S.P.F. mice are heavily laden with lymphocytes and a germinal centre with large lymphocytes is situated excentrally towards the thin external muscle layer of the dome (Fig.10). It is associated with a small lymphocyte zone on one side only and at the top of the dome is a mixed population of small and medium sized lymphocytes (Fig.11). The dome is covered by an epithelium which lacks villi, intestinal glands and goblet cells. The cells of the epithelium are less columnar than the generalised intestinal epithelium around them and this may be due to distortion by the lymphocytes they contain. The follicle-associated epithelium also differs at the apex in the possession of a fuzzy border
The thymus of the ASH/CSI S.P.F. female mouse.
The thymus is divided into lobes, each lobe being surrounded by a thin capsule of connective tissue. The connective tissue extends into incomplete septa and divides the lobes into lobules. Each lobule is divided into cortex (a) and medulla (b).

H and E x 80

Fig. 5

The thymus of the ASH/CSI S.P.F. female mouse. Lymphocytes are concentrated in the periphery or cortex of the lobules near the capsule and interlobular septa (a). Note the large blood vessel in the medulla (b) and the reticuloendothelial cells adjacent to the lumen (c).

H and E x 200
Fig. 6

The thymus of the ASH/CSI S.P.F. female mouse.

Lymphocytes in the cortex mask the epithelial network in which they lie. Mitotic figures are present in the large lymphocytes near the margin of the gland (a) and numbers of small lymphocytes increase in the mid cortex and adjacent to the medulla (b).

H and E x 320

Fig. 7

The thymus of the ASH/CSI S.P.F. female mouse.

Infection 70 larvae N. dubius.

Lymphocytes are not so tightly packed in the cortex of the thymus (a) and more are present in the medulla (b) where they tend to be concentrated round the blood vessels. Note the presence of lymphocytes in the reticuloendothelial cells lining the blood vessels (c).

H and E x 80
Fig. 8  The thymus of the ASH/CSI S.P.F. female mouse.
Infection 70 larvae of *N. dubius*.
Junction of cortex and medulla. A corpuscle of
Hassall (a) is located at the top right hand corner;
the central part has died and a cyst has been left.
H and E x 200

Fig. 9  The thymus of the ASH/CSI S.P.F. female mouse.
Infection 70 larvae of *N. dubius*.
Corpuscles of Hassall are more frequent in the
medulla of an infected mouse.
H and E x 320.
Fig. 10  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.

The domes (a) and thymus dependent areas (b) are heavily laden with lymphocytes and a germinal centre (c) with large lymphocytes is situated excentrally towards the thin external muscle layer of the dome (d).

H and E x 80

Fig. 11  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.

The follicle associated epithelium (a) of the dome lacks villi, intestinal glands and goblet cells. The cells of the epithelium (b) are less columnar than the generalised intestinal epithelium and contain lymphocytes.

H and E x 320
adjacent to the intestinal lumen. The generalised intestinal epithelium and the follicle associated epithelium are continuous at the base of the dome (Fig. 11). In the thymus dependent area there are blood vessels just under the crypts and near the external muscle layer. Lymphocytes are present in the intact reticuloendothelial cells and lumen of the post capillary venule in the intervening region (Figs. 13-14). It is through the post capillary venules that lymphocytes pass from Peyer's patches; they leave via an efferent lymphatic vessel and drain into the mesenteric nodes and thence to the systemic circulation by way of the thoracic duct (Tilney 1971; Inchley 1981). The passage of T lymphocytes through the endothelium of the post capillary venule is thought to be the crucial step in their recirculation from blood to the lymphatic tissue (Syrjaenen 1978; 1979).

The generalised intestinal epithelium in the upper jejunum of mice infected with N. dubius shows extensive damage due to the mucosal browsing of the worm. The follicle associated epithelium of the domes of Peyer's patches has been stripped away and the sub epithelial zone is devoid of lymphocytes apart from the odd one or two which appear to be migrating through the zone. All that is left is the meshwork of reticular cells on which the structure of the dome is based. The epithelium of the villi above the thymus dependent area has also been removed and although the post capillary venule is still intact, the lining endothelial cells have lost their integrity (Figs. 15-17). Peyer's patches in the mid region of the ileum are unaffected in control and infected mice. There are marginally larger germinal centres in the dome areas of the Peyer's patches of infected mice and there are also numerous mitotic figures in the thymus dependent areas.
Fig. 12  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.
Germinal centre with large lymphocytes.
H and E x 320

Fig. 13  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.
Thymus dependent area with post capillary venule (a).
H and E x 320
Fig. 14  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.
Thymus dependent area.
Lymphocytes are present in the intact reticuloendothelial cells and lumen of the post capillary venule.

H and E x 320.

Fig. 15  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.
Infection 70 larvae of N. dubius.
Extensive damage (a) is present due to the mucosal browsing of N. dubius.

H and E x 80
Fig. 16
Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.
Infection 70 larvae of *N. dubius*.
The follicle associated epithelium (a) of the dome has been stripped away and the sub epithelial zone (b) is almost devoid of lymphocytes.
H and E x 320.

Fig. 17
Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine.
Infection 70 larvae of *N. dubius*.
The post capillary venule (a) in the thymus dependent area is still in tact but the lining endothelial cells (b) have lost their integrity.
H and E x 320.
(c) The mesenteric nodes

Germinal centres and mitotic figures are present in the dense lymphatic tissue at the periphery of the mesenteric nodes of the control and infected mice. The lymphocytes in the infected mice may be undergoing change because they are more acidophilic than those in the control mice but it is not easy to distinguish if their number has changed (Figs. 18-19).

(d) The spleen

The spleen of the uninfected control mouse shows a clear distinction between the white and red pulp (Figs. 20-21) and both types of pulp contain many lymphocytes. Small follicular arteries are surrounded by sheaths of lymphocytes which are denser in the area adjacent to blood vessels. Blood from the follicular artery will pass through capillaries in this white pulp into the sinusoids seen in the red pulp. The function of the meshwork of the sinusoids in the red pulp is to filter the blood before it leaves the spleen via the splenic vein. The white and red pulp of the spleen of the infected animal are less densely packed with lymphocytes (Figs. 22-23). The areas of white pulp, the splenic corpuscles, appear to be larger in the infected animal because of the active proliferation of cells in the germinal centres. The sheaths of the lymphocytes around the small arteries do not show the same dense accumulations seen in the control.

Discussion

The parasitic life style of *N. dubius* requires an efficient strategy to initiate and maintain a system capable of exploiting the habitat and resource provided by the mouse. In turn the mouse is required to prevent the establishment of the parasite and the consequence of the interaction of these two opposing drives produces a system which varies both in space and time. Stability of the system is an essential requisite for the
Fig. 18 The mesenteric nodes of the ASH/CSI S.P.F female mouse. Germinal centres (a) and mitotic figures (b) are present in the dense lymphatic tissue at the periphery.

H and E x 80

Fig. 19 The mesenteric nodes of the ASH/CSI S.P.F. female mouse.

Infection 70 larvae of *N. dubius*.

Note that the cells in the medulla are more acidophilic than they are in the control.

H and E x 80
Fig. 20 The spleen of the ASH/CSI S.P.F. female mouse.
Many lymphocytes are present in the red (a) and white pulp (b).

H and E x 50

Fig. 21 The spleen of the ASH/CSI S.P.F. female mouse.
There is a small follicular artery (a) surrounded by a sheath of lymphocytes (b) at the bottom right hand corner.

H and E x 200
Fig. 22
The spleen of the ASH/CSI S.P.F. female mouse. Infection 70 larvae of *N. dubius*.
The white (a) and red pulp (b) are less densely packed with lymphocytes than the control.

H and E x 80

Fig. 23
The spleen of the ASH/CSI S.P.F. female mouse. Infection 70 larvae of *N. dubius*.
There is active proliferation of cells in the germinal centres (a) and the sheaths of lymphocytes around the small arteries do not show the same accumulations seen in the control.

H and E x 200
parasite and this is dependent on a series of inputs into the system before it becomes operational. These inputs can be in sequence as they are in the host or run parallel with each other if the compartment in the external environment is taken into account. The inputs in the external environment that run parallel to the sequential events in the mouse are those factors essential to the development of infective L3 larvae and will include the environmental variables of temperature, rainfall and humidity as well as the food resource within the faecal pellet. This compartment of the system must be at the correct stage of development if infective larvae are to be available at the appropriate time. Once within the host the success of *N. dubius* depends on the ability to establish itself within the wall of the small intestine and close proximity of host and parasite then sets in motion a series of events involving the lymphatic tissue of the mouse. The level of antigenic challenge and the immune status of the host will determine whether or not the system comes into existence. If it does then it will be a system in which there is a one way flow of resource to the parasite and a continued output of eggs to the external environment.

The involvement of lymphatic tissue in the immune response of the mouse to *N. dubius*, in particular that of the spleen and mesenteric nodes, has been described by a number of authors. The splenomegaly seen in ASH/CSI S.P.F. mice with an infection of *N. dubius* in this study has been observed and related to the blood profile in other laboratory mice, also infected with *N. dubius*, by Spurlock (1943) and Baker (1955, 1962). Lymphadenitis and hyperplasia of the reticuloendothelial tissue in the mesenteric nodes and splenomegaly were noted in Webster and C3H mice by Liu (1965) and were assumed to be due to mechanically produced tissue damage in the intestine resulting from larval invasion and substances released by the parasite. In-depth studies of the blood profile of mice undergoing an immune expulsion of *N. dubius* have reported a faster response to a challenge infection in terms of an earlier onset of leucocytosis, a sharp reversal of the
lymphocyte neutrophil ratio and a distinct eosinophilia (Cypess 1972) whilst macrophages and eosinophils seen around the larval stages of N. dubius have also been associated with larval dissolution (Rainbow, 1972; Jones and Rubin 1974).

The involvement of the thymus in the immune response of the mouse to N. dubius was first reported by Bartlett and Ball (1974) who observed that immune mice depleted of thymus derived cells formed no inflammatory nodules and they were unable to delay the maturation of the larvae. More recent work has shown that athymic mice cannot expel worms but can do so when reconstituted with thymus cells (Prowse et al 1978). Mice recipients of sensitised thymus cells alone can cause significant expulsion of worms on the 1st and 5th day after challenge and that this effect is enhanced if bone marrow cells are also transplanted indicates a co-operation between T and B cells (Vyas et al 1981). Passive and adoptive transfer methodology have also shown co-operation between immune serum and immune mesenteric lymph node cells which is directed at the trapping and destruction of the worms in the intestinal wall (Behnke and Parish 1981).

The part played by Peyer's patches in an immune response to N. dubius has not yet been ascertained but their high frequency in the anterior small intestine of female ASH/CSI S.P.F. mice may be indicative of an importance in response to infection. In particular they contain large numbers of follicles packed with B cells and interfollicular areas populated with T cells, all of which can easily pass through the specialised follicle-associated epithelium.

Thus all the morphophysiological indices in this study are intimately concerned with the immune response to N. dubius and the regression that occurs with age in ASH/CSI S.P.F. mice and wild A. sylvaticus has a number of interesting features. The regression of lymphatic tissue in female ASH/CSI S.P.F. mice is stepwise in time for minimal weights of spleen and thymus are in weeks 6 and 7 respectively. Some compensatory
mechanism for this loss of lymphatic tissue may be present for the area of Peyer's patches is maximal at 6 weeks. In the wild mice there is a similar involution of the thymus with age but the process is slow and continues through the life of both male and female mice. Emphasis on immune status with respect to these indices would appear to be on the thymus in female *A. sylvaticus*, particularly in the early age groups, while the male mouse maintains a fairly steady level of lymphatic tissue in the gut in terms of Peyer's patches. Thus the pathway of the immune response to *N. dubius* may be different in the sexes for it has been shown that the immunity which develops in male ICR mice is slower and weaker than the immune response of female ICR mice to infection (Hosier and Peller 1973; Hosier 1974).

The increasing involvement of the mesenteric nodes and spleen in the immune response of *A. sylvaticus* to infection with *N. dubius* and other parasites illustrate that the immune system of the mouse also oscillates in time, the spleen showing the greater fluctuation around an equilibrium level. The closer these oscillations are to the fluctuations in larval input of *N. dubius*, the more efficient will be the regulation of the parasite population at host individual level. The infection regimes of ASH/CSI S.P.F. mice considered in the context of the age classes show that there is a critical age for the development of an immune response to *N. dubius* and this is in agreement with the findings of Cypess, Van Zenát and Zidian (1973). The timing of larval input with respect to age will therefore have some influence on the behaviour of the system.

If the regulation of the population of *N. dubius* is at host individual level, then three types of regulation can occur. Regulation may be through modification of either the number of parasites which become established or the proportion of surviving parasites which reproduce, or through changes in the fecundity of the worms (Holmes, Hobbs and Leong 1977). The studies described in Chapter 2 have shown that in the wild it is the number of gravid females which determines the egg output of the system rather than
the fecundity of the worms. The mode of action would therefore appear to be the establishment of a ceiling for the number of gravid females in the small intestine. The egg output as calculated in this chapter is an instantaneous value but it is related to time in terms of the age of the host. Maximum egg output occurred in week 5 in female ASH/CSI S.P.F. mice before the immune response had matured. Further experiments with male ASH/CSI S.P.F. mice may reveal a difference in the timing of the maturity of the immune response in female mice.

The lower egg output of *N. dubius* per host in wild *A. sylvaticus* as compared to the laboratory mice is due to lower fecundity of the gravid female worms in the wild host and not to intensity of infection. The fecundity of *N. dubius* in ASH/CSI S.P.F. mice was 73.9 ± 40.3 as compared to 21.3 ± 10.3 and 20.3 ± 13.6 in male and female *A. sylvaticus* respectively. This may have been due to the greater variety and flux of microbial populations in the small intestine of the wild mouse and not due to modification by the immune response of the mouse. The fluctuation in egg output of *N. dubius* in *A. sylvaticus* provides an aggregated distribution with peak of egg output occurring in the breeding sector of the mouse population. Overdispersion of egg output in the context of time as well as space will allow fuller exploitation of the *N. dubius*: mouse system. The variance on the mean egg output of *N. dubius* per host *A. sylvaticus* is large and indicates inequality of parasite flow.

Histological examination of the lymphatic tissue of female ASH/CSI S.P.F. mice infected with *N. dubius* appears to set in motion a series of events leading to a transport of lymphocytes through the thymus. The thymus is essential to the development of Peyer's patches and bacteria in the lumen of the small intestine are required before the development of their germinal centres (Guy Grand, Griscelli and Vassalli 1975; Pollard and Sharon 1970; Carter and Collins 1975). An increase in the weight of Peyer's patches occurs with an oral infection of *Salmonella paratyphi A* due to the influx of inflammatory cell types (Carter and Collins 1975) but this did not occur with the infection of female ASH/CSI S.P.F. mice with *N. dubius*. The presence of *Salmonella paratyphi A* in Peyer's patches
requisite for T and B cell co-operation is in dispute but recent work has shown that their previously described absence (Kagnoff and Campbell 1976) is due to a preferred distribution below the epithelial and sub epithelial layers of the patch at the site of antigenic stimulation (Lause and Bookman 1981). IgA precursor cells and IgM and IgG memory cells have also been identified in Peyer's patches (Cooper and Turner 1969; Henry 1970; Craig and Cebra 1971) and the role of Peyer's patches as a pathway of intestinal drainage into the site of maturation of IgA plasma cells has been proposed by Roux et al (1981).

Immune reaction in Peyer's patches with respect to helminths has been described for *Trichinella spiralis* (Levin et al 1976) where initial transient reactivity is only replaced by maximum reactivity two to three weeks later in parallel with other elements of the immune system.

Immune response by Peyer's patches is dependent on the specialised follicle-associated epithelium of the dome. Recent work suggests that the association of lymphocytes with epithelial cells, in particular the specialised M cells in some way processes antigen in the lumen of the small intestine before presentation to those cells responsible for the immune response (Buckman and Cooper 1973; Owen and Jones 1974; Owen 1977; Smith and Peacock 1980; Smith, Jarvis and King 1980; Hamilton, Keren, Yardley and Brown 1981). If this is so then such processing is not possible in mice infected with *N. dubius* for the specialised follicle-associated epithelium is stripped away by the mucosal browsing of the adult worms. The situation is more akin to that described by Cooper and Turner (1967) where the injection of red cell antigens directly into Peyer's patches resulted in a substantial proportion being retained in the injected patch and its draining mesenteric node. The immune response of the gut is dependent on the migration of lymphoblasts from the small intestine to the mesenteric nodes for further maturation and then transfer to the systemic circulation as plasmablasts and redistribution along intestinal mucosal surfaces where they produce secretory IgA antibodies.
in response to the intestine absorbed antigen (Walker and Isselbacker 1977). It may be that the direct contact of unprocessed antigens from *N. dubius* with the lymphocytes of the Peyer's patches and the lamina propria of the small intestine results in their retention and will not allow the normal sequence of events to take place. It may explain why adult worms fail to elucidate an immune response on the part of the mouse host as well as prolong concurrent infections of *Trichinella spiralis* (Behnke, Wakelin and Wilson 1978) and *Trichuris muris* (Jenkins and Behnke 1977).

The histological picture of mitotic figures in T cell and B cell areas of Peyer's patches is repeated in the mesenteric nodes but the change in the staining character of the lymphocytes in the medulla of the nodes suggests they have been altered in some way. The role of the spleen in the immune response is marked by active proliferation of the cells in the germinal centres and there is a general depletion of lymphocytes. Thus the picture with respect to the thymus, mesenteric nodes and spleen during an infection of the mouse with *N. dubius* is one of a generalised mobilisation of lymphocytes but the situation in Peyer's patches is not so clear. Peyer's patches may provide a point in space or time of the system where disturbance in the sequential events of the immune response will cause a changed behaviour of the system. The feeding behaviour of the worms which strips off the specialised follicle associated epithelium from Peyer's patches may prevent the integration of the events of the immune response and thereby lower the efficiency of the control on the parasite.

The intussusceptions associated with Peyer's patches in *A. sylvaticus* have been reported in other vertebrates as well, including man (Nissan and Levy 1966; Bennett 1973; Forrester et al 1975). The causes quoted include ingestion of foreign bodies, intestinal neoplasms and hypertrophy of Peyer's patches. In *A. sylvaticus* the intussusceptions were associated with Peyer's patches and were at time so severe that the lumen of the gut was occluded. Although only a small percentage showed this condition it
was present in most age classes of male and female mice and it will act as a lesser control on *N. dubius* for not only does it cause anorexia but it will obviously limit egg output of the parasite.
CHAPTER 6
STRESS AND THE HOST PARASITE SYSTEM OF NEMATOSPIROIDES DUBIUS

Introduction

The parasite, host and external environment form three compartments in the Nematospiroides dubius: Apodemus sylvaticus system in which information flows in a one way direction from the external environment to host and parasite and is then interchanged between host and parasite. Information is in the form of change in the environment with respect to habitat and resource and will act as a stressor input first on the host and then on the parasite. Homeostasis in the system is only restored by the adaptation of host and parasite. Genetically based life strategies of host and parasite evolve with changes that are seasonal and predictable (Southwood 1977) but some stressor inputs may arise unexpectedly and are made more complex by way of the interaction which occurs between host and parasite and because of the time lag in their responses. If the disturbance to the system is such that an integration of response is not achieved then parasite population numbers are altered with consequent change in egg output.

The population numbers of a parasite may be altered at two levels of organisation, that is at infrapopulation level or suprapopulation level (Esch, Gibbons and Bourque 1975). The population of parasites within the individual host is regarded as the infrapopulation of the parasite and this contributed to the larger suprapopulation of parasites which includes all individual parasites at all stages of development both inside and outside the host. The regulation of N. dubius at suprapopulation level has already been identified with environmental variables and overdispersion in the system (Chapter 2). The death of a host with a heavy parasitic burden will have an important regulatory effect on the egg output of the N. dubius: A sylvaticus system but the survival of a smaller number of parasites in a large number of hosts controlled at infrapopulation level
by the immune response of the host will ensure continued output from the
system. Egg output will also be prolonged over a period of time for the
life span of *N. dubius* in laboratory mice can be as long as nine months
(Scott, Cross and Dawson 1957). The interconnections between the infra-
populations of *N. dubius* in *A. sylvaticus* is provided by the behavioural
activity and social organisation of the mouse population. While these
features tend to bind the mouse population into a cohesive unit, they can
give rise to excessive stress in certain genetically predisposed individuals.
Social organisation in the male sector of the population is mediated
through the hormone testosterone which controls fighting and mating
behaviour of the male mouse. Lower levels of testosterone are required
to initiate aggressive behaviour than those which control sexual activity
but both types of behaviour act as stressors on the mouse. The stressor
of aggressive social interaction is stronger even more so than actual
wounding (Bronson and Eleftheriou 1965). Aggression establishes non
linear hierarchies in laboratory mice (Brain and Nowell 1970; Bronson
1972; Brain 1975) with one dominant animal and a number of equally ranked
subordinates. Social hierarchies are also known to exist in feral pop-
ulations of *A. sylvaticus* so that one dominant animal restricts the activities
of several subordinate animals (Brown 1966). It is the subordinate animals
which are subjected to the most stress but the effect of equality in
subordinates may dilute the effect (Brain and Nowell 1970). Aggression and
social interaction among male mice therefore makes its own contributory
input into the hypothalamo-pituitary-adreno-cortico axis with consequent
release of ACTH.

ACTH production due to stress also occurs in the female mouse but that
the corticosterone so produced is a consequence of the release of ACTH
is more difficult to demonstrate. Ovarian and adrenocortical hormones
interact in pregnancy and lactation and high titres of oestrogen, although
they cause increase in adrenal gland size are not accompanied by an
increased corticosterone output (Brain and Nowell 1971). In addition
the foeto-placental unit makes its own contribution of corticosterone as well as providing a stimulus to the adrenal cortex to produce still more (Barlow, Morrison and Sullivan, 1973).

The effect of stress in the host individual is to cause involution of lymphatic tissue (Dougherty and White 1945; Baker, Ingle and Li 1951; Dougherty 1952) and therefore a depression of the immune response (Brayton and Brain 1973, 1974; Glenn and Becker 1974; Vessey 1964). Earlier in this study a period of stress was identified in the field studies of the _N. dubius:_ _A. sylvaticus_ system and this coincided with changed morphophysiological indices as well as increased egg output from the system.

The increase in egg output of the _N. dubius:_ _A. sylvaticus_ system was due to a higher level of intensity of infection with female gravid worms (Chapter 2) whilst the higher egg output of the _N. dubius:_ ASH/CSI S.P.F. mouse system is due to the greater fecundity of the worms (Chapter 5).

In this chapter the effect of simulated stress is related to the egg output of a specified _N. dubius:_ ASH/CSI S.P.F. mouse system. The egg output is an instantaneous value and is graded in terms of mild, moderate and chronic stress. The morphophysiological indices indicative of stress are examined both in wild populations of _A. sylvaticus_ and laboratory ASH/CSI S.P.F. mice. In addition immune status is assessed with respect to weight and histology of the thymus, mesenteric nodes, spleen and Peyer's patches during an infection with _N. dubius_.

**Materials and Methods**

1. **The Stress Experiments**

24 male and 24 female ASH/CSI S.P.F. mice aged 6 weeks were used in the experiments. They were weaned at 3 weeks of age and housed in opaque plastic cages of area 102 square inches and volume 663 cubic inches with wire grid tops. The food was Diet 86 supplied by E. Dixon and Sons, Ware, Herts, and both food and water were freely available. The animals were kept in a quiet room at 18 to 22°C with 10 hours of fluorescent lighting alternating with 14 hours of darkness. The
light dark regime was chosen as being akin to conditions prevailing in the spring. Initial housing density was 8 per basket from 3 to 4 weeks and thereafter 4 per basket from 6 to 9 weeks of age. Stress was simulated in the mice with a long acting ACTH preparation. The injection schedule was such that animals were injected intramuscularly in the thigh on alternate days with 0.1 ml of a long acting ACTH preparation (Cortotrophin/zn, Organon Laboratories Ltd., Lanarkshire, Scotland), this volume being equivalent to 4 International Units (IU) of ACTH. At the same time control animals were injected with a placebo solution made up to the manufacturer's specifications containing 2mg/ml colloidal zinc hydroxide at a pH of 8 (Mills, Brain and James 1972). The time of injection was 09.30 to 10.00 hours to maintain constancy in the phase of the circadian rhythm of adrenal steroids.

The mice were split into three groups of 16 animals and each group contained 8 male and 8 female mice. In each group 4 male and 4 female mice were injected with ACTH and the remaining animals were injected with the placebo preparation. The duration of stress in each of the three experiments was measured by the number of injections received. In mild, moderate and chronic stress the mice received 3, 6 and 11 injections respectively before infection with 100 larvae of N. dubius of the Beechem's Pharmaceuticals strain. Infection with N. dubius took place between 12.00 to 13.00 hours and the sacrifice of mice in each experiment was at 14.00 hours on Day 14 post infection.

The autopsy procedure was as described elsewhere. The relative weights of the adrenal glands, kidneys, heart and liver were judged to be indicative of the general metabolic function of the mouse while the relative weights of the spleen, mesenteric nodes, thymus and the area of Peyer's patches were regarded as indicative of immune status.
2. **The morphophysiological indices of A. sylvaticus and ASH/CSI S.P.F. laboratory mice**

The relative weights of the adrenal glands, kidneys, heart and liver of 208 male and 132 female *A. sylvaticus* were also examined with respect to age classes of 2 gm weight. They are included for comparison with the same morphophysiological indices of ASH/CSI S.P.F. mice under different stress regimes. In addition the relative weights of the testes and their associated glands were examined in *A. sylvaticus* and compared to those of male ASH/CSI S.P.F. mice.

3. **Histology of tissues**

The tissues of one female ASH/CSI S.P.F. mouse injected with ACTH and of one placebo mouse in the moderate stress experiment were used for the histological examination of the thymus, Peyer's patches, mesenteric nodes and spleen.

**Results**

1. **The Stress Experiments**

Stress in the ASH/CSI S.P.F. system is assessed in terms of efficiency and egg output. Efficiency is expressed as the percentage of worms which establish from the initial input of 400 larvae into each of the placebo and ACTH systems of male and female hosts. In this respect, the sex ratio of the worms, their ability to persist until sexual maturity and the number of hosts which fail to survive the stress or infection are important. The egg output of the system is the product of the number of gravid female worms present and their mean fecundity.

All mice survived the mild stress but a number of hosts died in the moderate and chronic stress regimes. In moderate stress, severe fighting broke out in the male placebo group and the subordinate animals were separated from the dominant animal by a wire mesh. They were not removed to separate baskets as it was important to maintain social contact as well as uniform area in each system. As
a consequence, one male mouse died after the second placebo injection before infection with *N. dubius* and a second male mouse died on Day 4 post infection. Similarly moderate stress in the female ACTH system caused two mice to die on Day 2 and Day 11 post infection respectively. The female mouse which died on Day 11 post infection was sent to Weybridge Veterinary Establishment for investigation of the microbial flora of the gut but no unusual flora were detected. In the chronic stress regime one male mouse and one female mouse died after the tenth and eleventh injection of ACTH respectively and one female mouse of the ACTH system died on Day 6 post infection. The intensity of infection of *N. dubius*, the sex ratio and the percentage take of the initial 400 larval input is shown in Table I.

In the male host parasite system, maximum efficiency occurs in the ACTH and placebo systems under mild stress and least efficiency is attained in the placebo system where severe fighting took place. In the female *N. dubius*: ASH/CSI S.P.F. mouse system, more worms establish in the placebo systems than in those which employ ACTH. This may indicate a greater alteration of microbial populations in the small intestine by ACTH than that induced by the placebo preparation. All sex ratios are lower in the female mouse systems with one exception (the chronic stress placebo system) and this suggests poorer survival of male worms in the female host.

The egg output of the *N. dubius*: ASH/CSI S.P.F. mouse system under mild, moderate and chronic stress is shown in Table II. In the male host parasite system maximum egg output occurs in the placebo system under a mild stress regime. There are signs of instability in the male host parasite system under chronic stress for the egg outputs of both the ACTH and placebo systems are lower than they are in mild stress. The female host parasite system appears to be more predictable for there is increasing egg output from the
<table>
<thead>
<tr>
<th>STRESS REGIME</th>
<th>MALE</th>
<th>FEMALE</th>
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<tbody>
<tr>
<td></td>
<td>A.C.T.H. PLACEBO</td>
<td>A.C.T.H. PLACEBO</td>
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<tr>
<td>MILD STRESS</td>
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<td></td>
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<tr>
<td>(a) Intensity of Infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male worms</td>
<td>152</td>
<td>186</td>
</tr>
<tr>
<td>Female worms</td>
<td>276</td>
<td>272</td>
</tr>
<tr>
<td>Total</td>
<td>428</td>
<td>458</td>
</tr>
<tr>
<td>(b) Sex Ratio</td>
<td>0.55</td>
<td>0.68</td>
</tr>
<tr>
<td>(c) % Efficiency</td>
<td>100</td>
<td>100</td>
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<tr>
<td>MODERATE STRESS</td>
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<tr>
<td>(a) Intensity of Infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male worms</td>
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<td>78</td>
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<tr>
<td>Female worms</td>
<td>185</td>
<td>94</td>
</tr>
<tr>
<td>Total</td>
<td>307</td>
<td>172</td>
</tr>
<tr>
<td>(b) Sex Ratio</td>
<td>0.66</td>
<td>0.83</td>
</tr>
<tr>
<td>(c) % Efficiency</td>
<td>76.8</td>
<td>43</td>
</tr>
<tr>
<td>CHRONIC STRESS</td>
<td></td>
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<tr>
<td>(a) Intensity of Infection</td>
<td></td>
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<tr>
<td>Male worms</td>
<td>88</td>
<td>131</td>
</tr>
<tr>
<td>Female worms</td>
<td>122</td>
<td>222</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>353</td>
</tr>
<tr>
<td>(b) Sex Ratio</td>
<td>0.72</td>
<td>0.59</td>
</tr>
<tr>
<td>(c) % Efficiency</td>
<td>52.5</td>
<td>88.3</td>
</tr>
</tbody>
</table>

Infection regime: 100 larvae per mouse
Total no. of mice: 48
The egg output of the *N. dubius* ASH/CSI mouse system under stress

<table>
<thead>
<tr>
<th>STRESS REGIME</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A.C.T.H.</td>
<td>PLACEBO</td>
</tr>
<tr>
<td>MILD STRESS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) No. of gravid female worms</td>
<td>$51^{\pm}22.4$</td>
<td>$67^{\pm}19.3$</td>
</tr>
<tr>
<td>(b) Fecundity</td>
<td>$69.5^{\pm}53$</td>
<td>$110.7^{\pm}17.5$</td>
</tr>
<tr>
<td>(c) Total egg output</td>
<td>$17466.7$</td>
<td>$29937.2$</td>
</tr>
<tr>
<td>(d) % total egg output</td>
<td>$36.8$</td>
<td>$63.2$</td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) No. of gravid female worms</td>
<td>$46^{\pm}12.1$</td>
<td>$43^{\pm}26.9$</td>
</tr>
<tr>
<td>(b) Fecundity</td>
<td>$144.9^{\pm}20$</td>
<td>$68.2^{\pm}16$</td>
</tr>
<tr>
<td>(c) Total egg output</td>
<td>$26072.4$</td>
<td>$5436.5$</td>
</tr>
<tr>
<td>(d) % total egg output</td>
<td>$82.8$</td>
<td>$17.2$</td>
</tr>
<tr>
<td>CHRONIC STRESS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) No. of gravid female worms</td>
<td>$39.7^{\pm}8.1$</td>
<td>$59.5^{\pm}29.1$</td>
</tr>
<tr>
<td>(b) Fecundity</td>
<td>$73.9^{\pm}29.5$</td>
<td>$74.1^{\pm}31.5$</td>
</tr>
<tr>
<td>(c) Total egg output</td>
<td>$9215.5$</td>
<td>$20292.7$</td>
</tr>
<tr>
<td>(d) % total egg output</td>
<td>$31.2$</td>
<td>$68.8$</td>
</tr>
</tbody>
</table>
placebo systems with increasing stress. The situation in the female host parasite system where ACTH is used is not so clear and this may be due to the host death described above. The use of the placebo appears to be more effective than ACTH in the induction of stress for the egg output of all the placebo systems with the exception of the male group where severe fighting took place is higher than in those systems where ACTH was used. The increase in egg output from the individual systems is accompanied by significant differences in fecundity only. In the mild stress regime significant differences in fecundity occur between male placebo and ACTH systems (P = 0.02) and between placebo male and female systems (P = 0.01). In moderate stress there is a highly significant difference in fecundity in the male placebo and ACTH systems (P = 0.001) and in chronic stress there is a significant difference in fecundity between the placebo systems of the male and female host (P = 0.05).

2. Morphophysiological indices of A. sylvaticus and ASH/CSI S.P.F. laboratory mice

All weights of the morphophysiological indices are expressed in relative terms to enable comparisons to be made. The morphophysiological indices of the relative weights of the adrenal glands, kidneys, heart and liver of wild A. sylvaticus with respect to age are shown in Figs. 1 and 2. Ageing is accompanied by increasing stress and this is indicated by the rise in adrenal gland weight which occurs with age. The rise is greater in the female mouse than it is in the male mouse and the relative weights of the kidney, heart and liver are also higher in wild female A. sylvaticus during the reproductive period of life. Both male and female mice show a decline in the relative weight of the kidney and heart with age but the relative weight of the liver appears to be more stable. The relative weights of the morphophysiological indices of ASH/CSI S.P.F. laboratory mice indicative of stress are shown in Tables III and IV.
The morphophysiological indices of *A. sylvaticus* with respect to age.

(a) The adrenal gland

(b) The kidney
MORPHOPHYSIOLOGICAL INDICES OF A. SYLVATICUS.

Adrenal gland

Kidney
- Female
- Male

Weight (gm).

Age of A. sylvaticus (gm).
The morphophysiological indices of *A. sylvaticus* with respect to age

(a) Heart

(b) Liver
MORPHOPHYSIOLOGICAL INDICES OF A. SYLVATICUS.

Female

Male

Liver

Heart

Age of A. sylvaticus.

Weight (gms).

Weight (gms).
<table>
<thead>
<tr>
<th>STRESS REGIME</th>
<th>Adrenal glands gm</th>
<th>Kidneys gm</th>
<th>Heart gm</th>
<th>Liver gm</th>
<th>Thymus gm</th>
<th>Spleen gm</th>
<th>Mesenteric Nodes gm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLACEBO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD STRESS</td>
<td>1.73x10^-4</td>
<td>0.012</td>
<td>8.3x10^-3</td>
<td>0.048</td>
<td>3.53x10^-4</td>
<td>3.9x10^-3</td>
<td>9.5x10^-4</td>
</tr>
<tr>
<td></td>
<td>±3.4x10^-5</td>
<td>±0.012</td>
<td>±4x10^-4</td>
<td>±0.012</td>
<td>±2.6x10^-4</td>
<td>±4.7x10^-4</td>
<td>±3.2x10^-4</td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td>2.23x10^-4</td>
<td>0.0135</td>
<td>6.2x10^-3</td>
<td>0.045</td>
<td>6.1x10^-4</td>
<td>2.4x10^-3</td>
<td>1.53x10^-3</td>
</tr>
<tr>
<td></td>
<td>±4.2x10^-4</td>
<td>±7.9x10^-4</td>
<td>±7.9x10^-4</td>
<td>±7.9x10^-4</td>
<td>±7x10^-4</td>
<td>±7.9x10^-4</td>
<td>±7.9x10^-4</td>
</tr>
<tr>
<td><strong>ACTH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD STRESS</td>
<td>1.87x10^-4</td>
<td>0.0122</td>
<td>7.5x10^-3</td>
<td>0.051</td>
<td>2.9x10^-4</td>
<td>2.8x10^-3</td>
<td>1.55x10^-3</td>
</tr>
<tr>
<td></td>
<td>±3.6x10^-5</td>
<td>±9.2x10^-4</td>
<td>±6.4x10^-3</td>
<td>±1.73x10^-4</td>
<td>±6x10^-4</td>
<td>±1.9x10^-4</td>
<td>±6.5x10^-4</td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td>2.07x10^-4</td>
<td>0.0138</td>
<td>6.2x10^-3</td>
<td>0.045</td>
<td>6.1x10^-4</td>
<td>2.4x10^-3</td>
<td>1.53x10^-3</td>
</tr>
<tr>
<td></td>
<td>±1.1x10^-4</td>
<td>±3.1x10^-3</td>
<td>±3.8x10^-3</td>
<td>±4.2x10^-4</td>
<td>±7.9x10^-4</td>
<td>±7.9x10^-4</td>
<td>±7.9x10^-4</td>
</tr>
</tbody>
</table>

**TABLE III**  The relative weights of morphophysiological indices in the N. dubius Male ASH/GSI mouse system under stress
<table>
<thead>
<tr>
<th>STRESS REGIME</th>
<th>Adrenal glands (gm)</th>
<th>Kidneys (gm)</th>
<th>Heart (gm)</th>
<th>Liver (gm)</th>
<th>Thymus (gm)</th>
<th>Spleen (gm)</th>
<th>Mesenteric Nodes (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLACEBO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD STRESS</td>
<td>0.017 ± 9.6x10^-3</td>
<td>5.4x10^-3</td>
<td>0.063 ± 7.6x10^-3</td>
<td>2.8x10^-3</td>
<td>6.63x10^-3</td>
<td>4.51x10^-3</td>
<td></td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td>3.7x10^-4 ± 1.7x10^-4</td>
<td>9.1x10^-3 ± 2.6x10^-3</td>
<td>7.2x10^-3 ± 3.8x10^-3</td>
<td>0.055 ± 0.0104</td>
<td>1.49x10^-3 ± 5.8x10^-4</td>
<td>3.5x10^-3 ± 8.8x10^-4</td>
<td>2.62x10^-3 ± 1.2x10^-3</td>
</tr>
<tr>
<td>CHRONIC STRESS</td>
<td>1.93x10^-5 ± 5.5x10^-5</td>
<td>8.4x10^-3 ± 8.2x10^-4</td>
<td>5.8x10^-3 ± 1.5x10^-3</td>
<td>0.049 ± 2.5x10^-3</td>
<td>8.6x10^-4 ± 2.8x10^-4</td>
<td>4.2x10^-4 ± 5.9x10^-4</td>
<td>1.78x10^-3 ± 4.3x10^-4</td>
</tr>
<tr>
<td>ACTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD STRESS</td>
<td>0.020 ± 4.4x10^-3</td>
<td>6.4x10^-3</td>
<td>0.062 ± 5.5x10^-3</td>
<td>2.5x10^-3</td>
<td>6.4x10^-3</td>
<td>4.2x10^-3</td>
<td></td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td>4.24x10^-4 ± 1.2x10^-4</td>
<td>0.0113 ± 2.9x10^-3</td>
<td>8.1x10^-3 ± 1.6x10^-3</td>
<td>0.057 ± 0.020</td>
<td>1.35x10^-3 ± 7.3x10^-4</td>
<td>3.5x10^-3 ± 2.9x10^-3</td>
<td>2.9x10^-3 ± 2.6x10^-3</td>
</tr>
<tr>
<td>CHRONIC STRESS</td>
<td>2.78x10^-4 ± 7x10^-5</td>
<td>0.0104 ± 1.4x10^-3</td>
<td>5.7x10^-3 ± 1.03x10^-3</td>
<td>0.044 ± 4.1x10^-3</td>
<td>3.8x10^-4 ± 3.9x10^-4</td>
<td>1.7x10^-3 ± 8.9x10^-4</td>
<td>1.92x10^-3 ± 1.44x10^-4</td>
</tr>
</tbody>
</table>

**TABLE IV** The Relative Weights of Morphophysiological Indices in the N. dubius: female ASH/CSI mouse system under stress
As in the wild mice, adrenal gland weight is higher in female laboratory mice than in male mice and it is also greater in those systems which employ ACTH to create the stress than those which rely on the placebo. The fact that there is an actual decrease in the weight of the adrenal gland in high levels of stress in three out of the four systems under study may be due to the depletion of the contents of the adrenal gland during excessive stimulation.

Kidney weight is greater in the male mouse than in the female mouse but there is a steady decline in kidney weight with increasing stress in both sexes. Kidney enlargement appears to take place in laboratory male mice under chronic stress. Temporary heart enlargement induced by moderate stress in both sexes is replaced by a reduction in size in chronic stress. Liver weight is greater in the female mouse than in the male mouse and in the female there is a steady decline in weight with increasing levels of stress.

The morphophysiological indices indicative of immune status in laboratory ASH/CSI S.P.F. mice are also shown in Tables III and IV. There is steady involution of lymphatic tissue in the thymus of male and female mice with increasing stress except in the male system using ACTH. There is a similar relationship between spleen weight and stress except in the female placebo system. Decrease in mesenteric node weight is present in the female mouse only. The Peyer's patch area of male and female mice is lower in the ACTH groups than those in the placebo groups at all levels of stress but the difference is not significant.

A decline in reproductive function as stress rises is indicated by the decline in the weight of the gonads and their associated glands (Table V). The anomalies seen in the relative weights of the vesiculae seminales and the ovaries in the placebo system under chronic stress suggests there is a limit to the relationship. In wild *A. sylvaticus* there is a steady increase in the weight of the testes and the vesiculae seminales with age with a plateau being reached in the 22 to 23.9 gm
<table>
<thead>
<tr>
<th>STRESS REGIME</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt of Body gm</td>
<td>Wt of Testes gm</td>
</tr>
<tr>
<td>PLACEBO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD STRESS</td>
<td>29.24 ±3.96</td>
<td>0.034 ±4.3x10^-3</td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td>24.6 ±7.1</td>
<td>2.7x10^-3 ±1.2x10^-3</td>
</tr>
<tr>
<td>CHRONIC STRESS</td>
<td>40.25 ±2.6</td>
<td>2.28x10^-3 ±1.09x10^-3</td>
</tr>
<tr>
<td>ACTH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD STRESS</td>
<td>29.03 ±2.4</td>
<td>0.0287 ±1.86x10^-3</td>
</tr>
<tr>
<td>MODERATE STRESS</td>
<td>31.96 ±1.6</td>
<td>2.8x10^-3 ±5.1x10^-4</td>
</tr>
<tr>
<td>CHRONIC STRESS</td>
<td>31.16 ±5.6</td>
<td>2.23x10^-3 ±4.6x10^-4</td>
</tr>
</tbody>
</table>

**TABLE V** The indices of reproductive status in the *N. dubius* ASH/CSI mouse system under stress
weight age class. The mean relative weight of the testes and the vesiculae seminales in this age group are $0.0285 \text{ gm} \pm 8.9 \times 10^{-3}$ and $0.013 \text{ gm} \pm 6.2 \times 10^{-3}$ respectively. The relative weight of the testes is comparable to that of the laboratory mouse and the presence of a number of individuals with low testes weight in the breeding groups of *A. sylvaticus* suggests the existence of stress in the wild populations of mice. Regression of the testes and vesiculae seminales with age does not appear to occur in *A. sylvaticus*.

3. The histology of the adrenal glands, thymus, Peyer's patches, mesenteric nodes and spleen

The photographs of the histology of these tissues are shown in Figures 3 - 24.

(a) The adrenal gland (Figs. 3-7)

A connective tissue capsule encloses the adrenal gland which is divided into a cortex and medulla. There are three zones in the adrenal cortex; the outermost zona glomerulosa, a middle zona fasciculata and an inner zona reticularis (Fig. 3). The zona glomerulosa is thicker in the ACTH stressed mouse than it is in the placebo and is consistent with induced stress (Fig. 4). The cells composing the zone are roughly columnar in shape in both groups but those of the ACTH treated mouse are strongly basophilic in the nuclei and cytoplasm and there are also mitotic figures present (Figs. 5 and 6). The extension of the zona glomerulosa in the ACTH treated mouse obscures the regular arrangement of cells in the cords of cells seen in the zona fasciculata of the placebo mouse (Fig. 7). Cells in the zona reticularis show variation in shape but they have the same acidophilic character in the placebo and ACTH treated mouse. Capillaries in this zone appear to be wider in the ACTH stressed mouse (Fig. 6). The parenchymal cells of the medulla of the adrenal gland of the placebo mouse are more loosely
Fig. 3 The adrenal gland of the ASH/CSI S.P.F. female mouse.
Placebo injection.
The adrenal gland is divided into the cortex (a) and the medulla (b).
There are three zones in the adrenal cortex: the outermost zona glomerulosa (c), a middle zona fasciculata (d) and an inner zona reticularis (e).
H and E x 80

Fig. 4 The adrenal gland of the ASH/CSI S.P.F. female mouse.
Simulation of moderate stress by injection of Cortotrophin/Zn. (ACTH)
The zona glomerulosa is thicker in the ACTH stressed mouse than in the placebo.
H and E x 80
Fig. 5 The adrenal gland of the ASH/CSI S.P.F. female mouse. Placebo.

The cords of cells in the zona reticularis are regularly arranged (a). The parenchymal cells in the medulla are loosely packed and acidophilic (b).

H and E x 200.

Fig. 6 The adrenal gland of the ASH/CSI S.P.F. female mouse. Cortotrophin/Zn, moderate stress.

The nuclei and cytoplasm of the cells comprising the zona glomerulosa (a) are basophilic and there are more mitotic figures present (b) than in the placebo. Capillaries in the zona reticularis (c) are wider than they are in the placebo mouse.

H and E x 200
packed than their counterpart as well as being more acidophilic (Fig. 5).

(b) The thymus (Figs. 8-13)

The thymus of the infected placebo mouse has a thicker capsule, thinner cortex and wider medulla than either a control or normally infected mouse (see Chapter 5) (Fig. 8). The junction between the cortex and medulla is sharp and the pitting evident in the cortex is related to relatively fewer lymphocytes in the cortex. The numbers of mitotic figures in the large lymphocytes is low and the lymphocytes themselves have a clumped appearance. The structural framework of the processes which support them appears to be incomplete (Fig. 9). Some of the reticular cells in the cortex have become large and ovoid. The thinning of the cells in the cortex is reminiscent of the involution of the thymus with age where the epithelial reticular cells are gradually compressed and replaced with adipose tissue. No such replacement has occurred here and the spaces which are present are due to a breakdown of the reticulum. The reticulum has greater integrity in the medulla but the corpuscles of Hassal seen in a normally infected mouse are absent (Fig. 10).

The thymus of the ACTH treated mouse shows no sharp distinction into cortex and medulla. The nuclei of the lymphocytes are present but they lack the basophilic stain seen in normal animals (Fig. 11). Pitting in the cortex is not so marked as in the placebo mouse and the framework of the supporting reticular processes is more complete (Fig. 12). The numbers of large lymphocytes and mitotic figures in the cortex is reduced when compared to control and normally infected mice. In the medulla the reticuloendothelial cells lining the blood vessels are indistinct and do not contain lymphocytes (Fig. 13). Corpuscles
SCHEMATIC CROSS SECTION THROUGH A LYMPH NODE AND PART OF A SPLEEN TO SHOW THE ARRANGEMENT OF 'T'-DEPENDENT AND 'B'-DEPENDENT AREAS.

- 'B'-dependent outer cortex with lymphoid follicles.
- Medulla with sinuses lined with macrophages.
- 'T'-dependent deep cortex site of post capillary venules.

Lymphocyte traffic.

- Splenic capsule
- Red pulp with macrophages and haematopoietic cells
- Marginal zone surrounding white pulp.
- Periarteriolar lymphoid sheath, the T dependent area.
- 'B'-dependent follicular area.

(After Inchley 1980)
Fig. 7  The adrenal gland of the ASH/CSI S.P.F. female mouse. Cortotrophin/Zn, moderate stress.
The extension of the zona glomerulosa obscures the regular arrangement of cells in the cords of the zona fasciculata seen in the placebo mouse.
H and E x 320.

Fig. 8  The thymus of the ASH/CSI S.P.F. female mouse. Placebo.
The thymus has a thicker capsule (a), thinner cortex (b) and wider medulla (c) than either a control or normally infected mouse.
H and E x 80.
Fig. 9  The thymus of the ASH/CSI S.P.F. female mouse.

Cortex, placebo.

The pitting evident in the cortex is related to relatively fewer lymphocytes in the cortex (a). The numbers of mitotic figures in the large lymphocytes is low and the structural framework which supports them appears to be incomplete (b).

H and E x 200

Fig. 10  The thymus of the ASH/CSI S.P.F. female mouse.

Medulla, placebo.

The reticulum has greater integrity than the cortex but the corpuscles of Hassal seen in a normally infected mouse are absent.

H and E x 200
Fig. 11  The thymus of the ASH/CSI S.P.F. female mouse.
Corticotrophin/Zn, moderate stress.
There is no sharp distinction into cortex and medulla. The nuclei of the lymphocytes lack the basophilic stain of the normal thymus.
H and E x 80

Fig. 12  The thymus of the ASH/CSI S.P.F. female mouse.
Cortex, Corticotrophin/Zn, moderate stress.
Pitting in the cortex is not so marked as in the placebo mouse and the framework of the supporting reticular processes is more complete.
H and E x 200
of Hassal are absent as in the placebo mouse.

(c) Peyer's patches (Figs. 14-18)

Damage due to mucosal browsing is severe in the placebo mouse. There appears to be little structure in the residual network in the domes of Peyer's patches and the thymus dependent area has lost the villi normally above it. In the ACTH treated mouse, the thymus dependent area is still recognisable as also the dome area (Fig. 15) but the open meshwork of the dome is totally devoid of lymphocytes (Fig. 16) and there are large open spaces in the tissue of the thymus dependent area (Fig. 17). The reticuloendothelial cells lining the blood vessels have disappeared and their place is taken by fibres of a brownish red nature similar to those seen in the tissue adjacent to the external muscle layer. Dougherty and White (1945) report similar brown staining of thymic reticular cells with haematoxylin. The same reddish brown staining is seen in the domes of Peyer's patches in both placebo and ACTH treated mice. The Peyer's patches and villi in the mid ileum of placebo and ACTH treated mice show the same degeneration of structure (Fig. 18) as seen in the anterior region which suggests a larger area of exploitation of mucosal tissue by the worms than in normally infected mice. These Peyer's patches show negligible basophilic staining apart from sparsely populated open germinal centres in the dome of the ACTH stressed mouse, but the nuclei of the lymphocytes that they contain are abnormal in shape. The supporting meshwork of the thymus dependent area carries cells in the placebo mouse but there are none in the ACTH stressed mouse suggesting that a localised immune response is no longer possible in the absence of T cells.
SCHEME TO ILLUSTRATE IDENTIFICATION OF TISSUE ELEMENTS IN PEYER'S PATCHES.

2. F.A.E. Follicle associated epithelium
3. G.C. Germinal centre ('B' lymphocytes)
4. P.C.V. Post capillary venule
5. T.D.A. Thymus dependent area ('T' lymphocytes)
6. V. Villus.

POST CAPILLARY VENULES.

- High endothelium.
- Basement membrane Type I.
- Low endothelium. Type III.

Fig. 13  The thymus of the ASH/CSI S.P.F. female mouse. Medulla, Cortotrophin/Zn, moderate stress. The reticuloendothelial cells lining the blood vessels (a) are indistinct and do not contain lymphocytes. H and E x 200

Fig. 14  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine, placebo. Damage due to mucosal browsing by N. dubius is severe and there is little structure in the residual network of the dome (a). The villi above the thymus dependent area have disappeared (b). H and E x 50.
Fig. 15  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine, Cortotrophin/Zn, moderate stress.

The dome (a) and thymus dependent area (b) are still recognisable.

H and E x 50.

Fig. 16  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine, Cortotrophin/Zn, moderate stress.

The open meshwork of the dome is devoid of lymphocytes.

H and E x 320.
Fig. 17  Peyer's patch of the ASH/CSI S.P.F. female mouse, anterior small intestine, Cortotrophin/Zn, moderate stress.

There are open spaces (a) in the thymus dependent area. The reticuloendothelial cells lining the blood vessels have disappeared and their place is taken by fibres of a reddish brown nature (b) similar to those in the tissue adjacent to the external muscle layer (c).

H and E x 320

Fig. 18  Peyer's patch of the ASH/CSI S.P.F. female mouse, middle small intestine, placebo.

There is the same extensive damage to the dome (a) and thymus dependent area (b) as seen in the anterior small intestine.

H and E x 80
(d) The mesenteric nodes (Figs. 19-20)

The mesenteric nodes of the placebo and ACTH stressed mouse are almost devoid of lymphocytes and the open meshwork of blood sinuses is visible. The few lymphocytes and mitotic figures seen in the germinal centres of the nodes of the placebo mouse have abnormal shaped nuclei. The small solid areas in the medulla of the nodes of the placebo mouse are where sinuses have collapsed and a dense area of meshwork results. Such areas are not present in the mesenteric nodes of the ACTH treated mouse.

(e) The spleen (Figs. 21-24)

Few lymphocytes are present in the splenic corpuscles of the placebo mouse and the reticular connective tissues appears to be in a state of degeneration (Figs. 21-22). Some large swollen reticular cells similar to those in the thymus can also be seen. The nuclei of the lymphocytes in the outer zone of the splenic corpuscle are basophilic and possess irregular shapes. Lymphocytes are absent from the red pulp although the general impression is one of slight basophilia. The amount of blood traversing or being stored in the red pulp is reduced because the sinusoids contain little blood. Any erythrocytes remaining are not normal because there is a failure to stain with cosin. The intervening reticular structure is so densely packed that differentiation of cell type and determination of structure is difficult. The small dark red areas visible in the red pulp areas suggest the collapse of the sinusoids.

The structure of the white pulp of the spleen in the ACTH stressed mouse is similar to that of the placebo but the number of lymphocytes in the outer zones of the splenic corpuscles are still further reduced and their nuclei are no longer basophilic (Fig. 23 and 24).
Fig. 19  The mesenteric nodes of the ASH/CSI S.P.F. female mouse. Placebo.

There are few lymphocytes present and these have abnormal nuclei. The small solid areas in the medulla (a) are where the sinuses have collapsed and a dense area of meshwork results.

H and E x 80

Fig. 20  The mesenteric nodes of the ASH/CSI S.P.F. female mouse. Cortotrophin/Zn, moderate stress.

The blood sinuses have not collapsed as in the placebo mouse.

H and E x 80
Fig. 21  The spleen of the ASH/CSI S.P.F. female mouse.
Red and white pulp, placebo.
Lymphocytes are absent from the red pulp (a) and the small dark areas visible in the red pulp areas (b) are where the sinusoids have collapsed.
H and E x 80

Fig. 22  The spleen of the ASH/CSI S.P.F. female mouse.
Red and white pulp, placebo.
The nuclei of the lymphocytes in the outer zone of the splenic corpuscle are basophilic and possess irregular shapes.
H and E x 200
Fig. 23  The spleen of the ASH/CSI S.P.F. female mouse.  
Red and white pulp, Cortotrophin/Zn, moderate stress.  
Lymphocytes are more reduced in number than in the placebo.  
H and E x 80

Fig. 24  The spleen of the ASH/CSI S.P.F. female mouse.  
Red and white pulp, Cortotrophin/Zn, moderate stress.  
The nuclei of the lymphocytes (a) in the outer zone of the splenic corpuscle (b) are no longer basophilic.  
H and E x 200
Discussion

Factors important in the regulation of the host population of *A. sylvaticus* in the *N. dubius: A. sylvaticus* system are both external and internal to the system. External factors include food availability (Smythe 1966; Watts 1969, 1970; Flowerdew 1972), predation and disease (Elton, Ford and Baker 1931) whilst the internal control mechanisms are mediated through the physiology, behaviour and social organisation of the species (Wynne Edwards 1962). Parasitism is a density dependent process in the population dynamics of both host and parasite and the measure of regulation achieved in the host population by these factors will determine the stability or otherwise of the host parasite system. It is the intrinsic population controls that are the concern of this study for the hypothesis of intrinsic population control is based on the stress induced when certain individuals are restrained from breeding or suffer increased mortality at times when population density is high. The stimuli which signal density stress are those received in social interaction and they produce endocrine response in terms of the general adaptation syndrome and reduction in breeding success (Christian 1963).

The mediation of the intrinsic control of population size is through the adrenal cortex which responds to stress in terms of an increased output of testosterone. This has the effect of decreasing the output of testosterone from the testes (Christian et al 1965; Tissell and Angervall 1969) probably because the adrenal cortex provides the precursors of androgens to the testes (Kniewald et al 1974). The decline in testicular testosterone is accompanied by a deterioration in function of the testes (Gärtner et al 1973), inhibition of the maturation of the spermatids as well as a decrease in the weight of the testes and their associated glands (Christian 1963; McKinney and Desjardins 1973 (a) and (b); McKinney and Pasley 1973; Lloyd 1973). A reduction in fertility also occurs in female mice under stress (Christian 1963; Paris and Ramaley 1973 (a), (b)).

In the experimental situation of this study the animals were stressed by
injection with a placebo and a long acting ACTH preparation for varying periods of time corresponding to mild, moderate and chronic periods of stress prior to infection with *N. dubius*. The results show a decline in the weight of the gonads and their associated structures with increasing levels of stress and are therefore consistent with other findings. The anomaly of a slight increase in the weight of the vesiculae seminales of male mice under chronic stress in the placebo system suggests an adaptive mechanism inherent in the genotype of the mouse. The fact that the testes and seminal vesicles of wild *A. sylvaticus* do not appear to regress with age is surprising but the variance on these parameters is high in all breeding age groups and this may indicate the existence of above optimal stress in some individuals. No weights of ovaries were made during the field studies of *A. sylvaticus* but there are indications of lowered fertility of female mice with respect to the number of embryos and placental scars. Although the difference is not significant these were fewer in number during the stress period of January - June 1974.

The response to stress is through the pituitary-adrenal axis and the enlargement of the zona glomerulosa in the adrenal cortex of the female mouse injected with the long acting preparation of ACTH is consistent with a stress situation. The closer packing of the medullary tissue and its contrasting basophilia to that of the placebo suggest that the medulla of the adrenal gland is also being stimulated. The fact that the zona glomerulosa of the adrenal gland of the placebo mice is small and yet all the effects of stress are evident in the placebo mice suggests that there may be another pathway of response to stress other than through the adrenal cortex. Recent work by Popova and Koryakina (1981) supports this hypothesis for they showed that the reactivity of the adrenal cortex of mice to ACTH stimulation was not consistent with a number of different stressors.

The decrease in adrenal gland weight that occurred in male and female laboratory mice with increasing stress may be due to a depletion of its
contents in excessive stress, particularly that of ascorbic acid (Christian 1963). The effect of changing levels of stress induced by differential grouping of male and female mice also has the same effect for hypertrophy of the adrenal gland would only seem to occur in the smaller group sizes of male mice (Brain and Nowell 1970) and the degree of hypertrophy is also dependent on age (Brain and Nowell 1971 (a)). Hypertrophy of the adrenal gland in the female mouse occurs in isolation probably due to the elevation of oestrogen levels and a time scale is in operation (Brain and Morris 1971). The adrenal glands of male ASH/CSI S.P.F. mice under stress are smaller than those of the female laboratory mice as well as wild male A. sylvaticus. The adrenal glands of wild male A. sylvaticus are also smaller than those of wild female A. sylvaticus and these are in accord with other findings with respect to wild and domestic Mus musculus (Seabloom and Seabloom 1974). The existence of a number of individuals with high adrenal gland weights in all age groups of wild A. sylvaticus suggests that some individuals experience more stress than others, particularly in the male population. The differential response of the adrenal cortex to ACTH in wild and domestic Mus musculus is thought to be genetic (Seabloom and Seabloom 1974) and it is a reasonable assumption that it may be so in A. sylvaticus. Inequality in relative adrenal gland weight may also derive from the hierarchical social organisation in wild mice (Brown 1966, 1969, Flowerdew 1974, 1978) and it may be that this parameter serves to identify subordinate mice. The situation in female mice is not so clear for adrenal hypertrophy may be caused by the interaction of ovarian and adrenocortical hormones during pregnancy and lactation (Chester Jones 1952). Relative adrenal gland weight can also vary in time in the wild populations of A. sylvaticus and this may represent a cyclicity of stress in the system.

Stress also produces a significant reduction in the size of the kidneys in both male and female laboratory mice. The changes that occur in the shift from mild to moderate stress are significant in placebo male and female mice (P = 0.01) and in ACTH treated male and female mice (P = 0.001 and P = 0.01 respectively). The kidney enlargement which occurs in male
mice during the switch from moderate to chronic stress is in contrast to the further reduction in kidney size which occurs in female mice during the same change. Similar enlargement of the kidney of male and female laboratory mice under a chronic stress regime of ten consecutive daily injections of ACTH has been reported by Christian (1967) and it is accompanied by a marked sclerosis of the renal glomeruli and ultimate destruction of the glomerular tufts. The destruction of the glomeruli in chronic stress may either be due to an autoimmune reaction caused by the involution of the thymus during stress (Teague et al 1970) or high blood pressure generated by the release of catecholamines from the adrenal medulla during the initial fight or flight reaction of the stress syndrome. In support of the latter hypothesis is the temporary heart enlargement which occurs when mild stress changes to moderate stress in both male and female mice. The difference in size is only significant in the male placebo (P = 0.01) and male ACTH treated mice (P = 0.1) and is consistent with the more frequent mediation of the sympathetic adrenal medullary pathway of the fight or flight reaction during social interaction with other mice. The response to changing levels of stress in female mice would appear to emphasise the mobilisation of reserves from the liver consequent to the release of glucocorticoids in the endocrine pathway of the pituitary adrenal cortical system for the differences in liver weight in the change from mild to chronic stress are significant in the female placebo (P = 0.002) and ACTH systems (P = 0.05).

The effects of the differing regimes of stress on the general metabolic function of the mouse reflected in the changes that occur in the kidney, heart and liver of the laboratory mouse are repeated in the indices used to measure immune status viz the thymus, spleen, mesenteric nodes and Peyer's patches. Involution of lymphatic tissue in the thymus occurs in male and female mice when mild stress changes to chronic stress. There are significant differences in weight in male (P = 0.01) and female placebo systems (P = 0.05) and involution is significantly greater in the female ACTH system than it is in the female placebo system (P = 0.001). The loss in
weight of the thymus during administration of ACTH has been reported elsewhere (Weaver 1955) and the increase in size of the thymus that occurred in male mice in chronic stress induced by ACTH is therefore not in agreement with these findings. Gonadectomy in rats allows significant increase in the weight of the thymus (Bellamy, Hinsull and Phillips 1976) and it may be that the regression of the testes that occurred in the stress regimes of these experiments was sufficient to allow the thymus to increase in size. The presence of pitting in the cortex of the thymus of the placebo mouse due to a breakdown of the structural framework of the reticular cells is reminiscent of the replacement of epithelial reticular cells by adipose tissue that occurs with age. The large reticular cells visible in the spaces are similar to those described by Baker, Ingle and Li (1951). They may be concerned with the breakdown of worn out nuclei for some of them contain droplets and corpuscles of Hassall are absent from the medulla or they may be the large thymic nurse cells responsible for thymocyte maturation and exposed by the lack of lymphocytes (Ritter, Sauvage and Cotmore 1961). The general depletion of lymphocytes and lack of mitotic figures in the cortex of the placebo thymus is paralleled in the thymus of the mouse treated with ACTH and suggests that injection with the placebo preparation may have been a stress within itself. It may even have constituted a greater stress for pitting in the cortex is not so marked in the ACTH treated mouse and the numbers of lymphocytes are generally higher. Large reticular cells are present in the thymic cortex and there is a corresponding lack of corpuscles of Hassall in the medulla. Similar histological changes have been reported in the thymus of rats treated with ACTH for the induction of chronic stress (Weaver 1955) and loss of lymphocytes occurs in cortisol treated C57/EL mice (Bellamy and Alkafaishi 1972). This loss of T lymphocytes due to stress will play an important part in the response of ASH/CSI S.P.F. mice to an N. dubius infection (Bartlett and Ball 1974).
There is also involution of lymphatic tissue in the spleens of male and female laboratory mice with increasing levels of stress. The diminished spleen weight may not reflect the true effect of stress for it is balanced by the splenic enlargement associated with low status and the regression of the testes and seminal vesicles that occurs in subordinate mice (Blaine and Conway 1969; Rapp and Christian 1963). It must also be seen in the context of the splenic enlargement associated with an *N. dubius* infection (Liu 1965). The effect of the stress induced by the placebo and ACTH injections has been to reduce the amount of blood traversing the spleen. The sinusoids of the spleen contain little blood and in the placebo mouse they have collapsed not only in the spleen but also in the mesenteric nodes. Mitotic figures in the outer ring of the lymphocytes of the lymphatic nodules indicate that the production of lymphocytes is still taking place in the spleen but their nuclei are abnormal and it is doubtful if they are functional. Whether the disorganisation of the reticuloendothelial cells lining the blood vessels has the same effect on T lymphocyte circulation in the spleen as it has in Peyer's patches has yet to be demonstrated.

Stress has also affected the lymphatic tissues in Peyer's patches for lymphocytes are absent from the thymus dependent areas but the presence of small abnormal germinal centres in the dome areas indicates that the effects of stress on B lymphocytes is less severe. The loss of the reticuloendothelial cells lining the post capillary venules implies loss of regulation of the circulation of any remaining T lymphocytes. The removal of the specialised follicle associated epithelium and the lamina propria in the middle regions of the ileum in the placebo and ACTH treated mouse suggests that the microenvironment of the gut is so altered that *N. dubius* is able to leave its preferred position in the anterior small intestine (Lewis and Bryant 1976) and exploit the resources of this region as well as delay a possible immune response from these Peyer's patches. Cells draining from the gut into the mesenteric nodes are more acidophilic.
in the placebo mouse than they are in the ACTH treated mouse and it is further evidence of different pathways of the response to physical and chemically induced stress. The proximity of *N. dubius* to the mesenteric nodes may explain the lack of regression in the weight of these lymph nodes of the male host to be expected from the stress regime involved. The depletion of T and B lymphocytes and the degenerative changes in the lymphatic tissue of ASH/CSI S.P.F. laboratory mice caused by stress is reflected in an increased egg output from the host parasite system. Egg output from the host parasite system is dependent initially on the number of worms which become established and later on the fecundity of the gravid female worms. The experimental systems in this study are compared at these two points in time in terms of efficiency or the percentage of worms which develop from the initial larval input and a value for instantaneous egg output derived from the product of the number of gravid female worms in the infection and their mean fecundity. The efficiency of a normal control infection of six week old female mice with *N. dubius* is approximately 80% and this compares favourably with the efficiency of all the systems under stress. Efficiency is higher in the placebo systems than those which employ ACTH and it may be that stimulation of the adrenal cortex by ACTH causes changes in the microbial environment of the small intestine which makes it less suitable than that of the placebo for the establishment of the worms. The lower sex ratios of the worms in the female host parasite system under placebo and ACTH induced stress as compared to the male host parasite system under the same stress regimes emphasizes the importance of microbial flora in the lumen of the small intestine. Host death occurred in the ACTH systems only, with the exception of the male placebo system under moderate stress where severe fighting broke out among the group. Stress induced by fighting would therefore seem similar in quality and quantity to that induced by ACTH and it is clear that if stress becomes too severe it will impose limits...
to the efficiency of the *N. dubius* : ASH/CSI S.P.F. mouse system by reason of host death.

The egg output of the host parasite system of a control infection of six week old female ASH/CSI S.P.F. mice with similar housing density and under the same lighting regime is approximately 5,000. The increase in egg output of the systems under stress is due to an increased fecundity of the gravid female worms and suggests that stress in some way makes the requisites for egg production more easily available to the worms.

The increase in egg output from the *N. dubius* : ASH/CSI S.P.F. systems under stress is higher in the placebo systems than those which employ ACTH. When comparing the placebo and ACTH systems the increase is achieved through the establishment of more gravid female worms as well as through greater fecundity. In the stress situation therefore, *N. dubius* is able to combine the strategy adopted in wild *A. sylvaticus* where the number of gravid female worms is the determinant of egg output and the strategy adopted in laboratory mice where it is the fecundity of the worms which has the greater importance. The differences in the intensity of infection with gravid female worms in the systems under stress are not significant but there are significant differences in fecundity between male placebo and ACTH systems in mild and moderate stress and between male and female placebo systems under mild and chronic stress.

The significant differences in fecundity that occur in the male placebo and ACTH systems in mild and moderate stress indicate that the *N. dubius* male ASH/CSI S.P.F. mouse system is more labile than that of the female host parasite system. The lesser egg output of the male system under chronic stress as compared to that under mild stress also suggests limits to the extent to which *N. dubius* is able to manipulate the system. Thus there may be some threshold of stress where adaptation in the mouse occurs and new mechanisms are brought into play. The temporary enlargement of the heart in moderate stress and the kidney enlargement of male mice in chronic stress have already been described and indicate the induction of
other body changes not so intimately concerned with the immune response.

In contrast, the female host parasite system appears to be more predictable for there is continued increase in egg output with increasing levels of stress in both placebo and ACTH systems.

The significant differences in fecundity which occur in male and female placebo systems under mild and chronic stress suggest that stress in itself is a resource spectrum which is exploited by the parasite at different points in time in male and female hosts. Mild stress in the male host allows an immediate and rapid rise in egg output from the system and this is continued in the female host under moderate stress to reach an even higher peak in egg output from the female system under chronic stress. Both male and female systems are vulnerable to stress but the timing and level of response differs in the sexes.

It is a reasonable assumption that wild *A. sylvaticus* will respond to a stress situation in a similar way to the laboratory mice and if this is so then individuals will differ in their response according to their sex and susceptibility. The cyclicity of stress in the social organisation of *A. sylvaticus* must be matched by larval availability of *N. dubius* if maximum exploitation by the parasite is to be achieved. In this respect there will be a delay in the system while the intensity of infection with gravid female worms builds up in the individual hosts, a process which is aided by lowered immune response due to social interaction within the mouse group.

The conclusions of this chapter, therefore, are that the host parasite system which *N. dubius* establishes with either the ASH/CSI S.P.F. mouse or wild *A. sylvaticus* is one that shows flexibility of response to changing levels of stress and that such flexibility allows maximum exploitation of the host as well as the continued maintenance of the system.
GENERAL DISCUSSION

The objectives of this study have been to examine the host parasite relationships that *Syphacia stroma* and *Nematospiroides dubius* establish with the wild host *Apodemus sylvaticus* and to analyse in more controlled conditions the specific relationship that *N. dubius* establishes with the laboratory ASH/CSI S.P.F. mouse. The approach has been to view the interaction of host and parasite as a system with infective stages supplying the input and egg output serving as an indicator of system function. Both wild and laboratory host are considered as habitat for the parasites and it is suggested that the differences in system function are a consequence of their respective life strategies in the exploitation of the food resource spectrum and shelter provided by the mouse.

The habitat has been used by the parasites as a template for the evolution of different life history strategies (Southwood 1977) and this has allowed the coexistence of two potential competitors with little overlap of niche. Food resource in time is unpredictable for *S. stroma* for it is primarily dependent on the intermittent food intake consequent to feeding activity of the mouse. It is also patchy in space by reason of the mode of transmission by host contact and the possession of a short life span.

Habitats that are unpredictable and patchy experience environmental fluctuation and require rapid reproduction to maximise the use of resource in the shortest possible time. Such a strategy is characterised in terms of r-selection and is reflected in the wide fluctuations in the numbers of *S. stroma* that occurred during 1974 and 1975. In contrast, *N. dubius* exploits the more continuous food resource of mouse tissue and has the ability to exploit the spatial distribution of two habitats using only the food resources of one. Survival of *N. dubius* is not restricted to the single host and habitat in the context of space is therefore more continuous. This greater continuity of habitat in terms
of time and space has led to the development of a K- strategy for *N. dubius*, with consequent longer life span and the production of fewer but highly fit young.

High larval input into the *S. stroma: A. sylvaticus* system is responsible for the rapid increases in the populations of the adult cohorts of *S. stroma*. The subsequent high egg output serves as positive feedback to a system which is akin to an open loop system. Such a system relies on the rate of switching in terms of intermittent high larval input and inertial lag to maintain the stability of egg output seen during the study. Inertia is incorporated into the system by way of the developmental time of the infective egg to adult within the host and the time intervals elapsing between the host contact essential to transmission. It would seem therefore that time delays are not always destabilising processes in host parasite systems (May and Anderson 1978) but can act as stabilising factors if they are part of open loop systems and associated positive feedback mechanisms. The regulation of parasite numbers by the rate of transmission or Type I regulation as defined by Bradley (1972) is an inefficient way of life but seen in the context of an r- strategy and open loop control, system function is somewhat improved. Control of parasite population size by way of changing levels of recruitment of infective stages has also been shown in the *Pomphorhynchus laevis: goldfish* system but in this latter system it was temperature which determined the number of cystacanths able to establish in the host (Kennedy 1974).

Other controls in the *S. stroma: A. sylvaticus* system include overdispersion with respect to time, low parasite sex ratio, some interspecific competition with *N. dubius* in the female host and unpredictability of host behaviour. Controls are balanced by the destabilising processes of low pathogenicity, lack of specific host age for parasite mortality and host stress. The latter observation is surprising in view of the
low pathogenicity of the parasite and the explanation may be in the concurrent infections with *N. dubius*. The greater intensity of infection with gravid female worms of *N. dubius* during the stress period of January to June 1974 may have facilitated a greater resource of nutrients for *S. stroma* by way of an intensified browsing on the host intestinal mucosa.

In contrast, the system which *N. dubius* establishes with *A. sylvaticus* is one that exhibits smaller fluctuations in number around an equilibrium level. The existence of parasite population numbers near asymptotic density over periods of time characterises *K*-selected organisms and they are dependent on closed loop systems with controls incorporated in the form of negative feedback mechanisms. The negative feedback in the *N. dubius*: *A. sylvaticus* system includes density independent environmental control of larval survival, density dependent intraspecific competition, specific host age for parasite mortality as a consequence of host immune response and to a lesser extent overdispersion in the host population. Destabilising processes are the time lags which occur during the development of the egg to the infective larva and the mounting of the immune response and the behavioural activity of refection.

The evidence provided by indices of host immune status during the stress period of January to June 1974 showed that the involution of lymphatic tissue which accompanied the stress syndrome was reflected in the greater egg output of *N. dubius* per host. This serves to identify an important role for host immune response in terms of control of the parasite population numbers. Regulation of this nature is designated as Type III regulation by Bradley (1972) and it is regarded as a highly efficient process if it is accompanied by a net reproductive rate continually well above the minimum for the persistence of the parasite.

It is unlikely that the control of the egg output of the *N. dubius*: *A. sylvaticus* system during 1974 and 1975 was through the mechanism described by Kerboeuf (1978 (a) and (b)). The higher egg output of the
system in 1974 was not due to an increase in fecundity of the worms as a consequence of temperature ageing in the external compartment, loss of antigenicity of surviving worms and consequent less stimulation of host immune response. Rather the involution of lymphatic tissue which accompanied the greater egg output of the system appeared to result in an increased ability of the nematode to establish in the small intestine of the host.

It was not possible to quantify the effect of a heavy infection on *A. sylvaticus* and one can only speculate on the cumulative effects of a number of worms continually browsing on the intestinal tissue for a possible life span of eight to nine months duration. The evidence is that the relationship between host death rate and parasite burden is non linear (Forrester 1971) but in this study the intensities of infections were such that it is unlikely they would have had serious effects on the wild populations of mice. Recent work by Freeland (1981), however, shows that the effects of a heavy parasite burden can be sufficient to cause a reversal of status in the dominance hierarchies of populations of mice and this may have had relevance for host gene pools.

The host *A. sylvaticus* is thus being exploited by two parasites with different life history strategies. The r-selected strategy of *S. stroma* has the greater stability in the male host parasite system which is consistent with a more readily exploited food resource and shelter spectrum. Stability in the K-selected strategy of *N. dubius* is located in the female host and this identifies the system with the stronger regulation. Both parasites have the additional option of survival in other small mammal hosts and these systems may act as support systems should the destabilising processes in *A. sylvaticus* proceed too far. The presence of *S. stroma* in hosts other than *A. sylvaticus* was easy to identify but some difficulty was experienced with infections of *N. dubius*. Worms of the latter species were generally smaller and thinner in these
other hosts and the lower fecundity was accompanied by a smaller size of egg. This suggests the existence of a number of different strains of *N. dubius* present in the field system and is consistent with the work of Durette-Desset (1969), Forrester (1971) and Forrester and McL. Neilson (1973).

The pattern of behaviour of the *N. dubius* laboratory mouse system is similar to that of the *N. dubius: A. sylvaticus* system. The preliminary problems encountered in the initiation of an infection in ASH/CSI S.P.F. mice at Royal Holloway College with subsequent failure of culture of eggs to viable L3 larvae required analysis of the sequence of events which occur during the egg production of a primary infection of the laboratory mouse with *N. dubius*. As the ASH/CSI S.P.F. mice were not suitable and contamination was suspected, a system using CDI mice was set up in an area away from the normal animal house. The input of larvae into this system produced an oscillating output of eggs similar to that described elsewhere (Scott, Cross and Dawson 1959; Bartlett and Ball 1974).

Oscillatory behaviour of this nature will produce trickle and aggregated infections in the wild situation and give the parasite an additional survival strategy. Rhythmic patterns of egg production have been described for *Fasciola hepatica* (Dorsman 1956), *Aspiculuris tetraptera* (Phillipson 1974) and *Syphacia muris* (Lewis and D'Silva 1980). The process underlying such rhythms is that of oogenesis which is programmed in the genotype of the worm. The control of oogenesis will therefore be the same in male and female hosts and conceptually it represents the switching mechanism of an open loop control system. It is the different microenvironments encountered by the worms in the small intestine of male and female hosts that modifies the rhythm and creates a difference in the inertia of the system. The male host parasite system would appear to have fewer constraints than the female system for the number of days of inertia is less and egg output is significantly greater by 15% (*P* = 0.01). This compares with a 40% greater output of the male.
host parasite system of *N. dubius*: *A. sylvaticus* as compared to that of the female system. Negative feedback in terms of rates of fall in egg production are indicative of additional control and regulation by a closed loop system. It may represent a depletion of nutrients essential to oogenesis and if this is so the micro-environment of the small intestine of the female mouse is less suitable for the reproduction of *N. dubius*. The rates of fall in egg production are faster in the female host and this combined with increased inertia of the system produces a lower output of eggs.

Cumulative analysis and autocorrelation used to monitor the operation of the system located events between days 19-21 indicative of adjustment within the system and this was followed by increased faecal production from both male and female hosts. Abnormal motility patterns of the host alimentary canal have been reported for a number of parasitic infections which include *Nematodirus fillicollis* and *Nematodirus spathiger* (Tetley 1949) *Trichinella spiralis* (Castro et al. 1976, 1977) and *Nippostrongylus brasiliensis* (Farmer 1981). It is likely that the abnormal motility pattern of the alimentary canal associated with an infection of *N. dubius* is due to the mucosal browsing of the worms which destroys the plexi of Auerbach and Meissner. Located within these plexi are pacemaking areas with different natural frequencies, one for each functional unit of the small intestine (Hasselbrack and Thomas 1961; Milton and Smith 1965) and these may be successively removed by the parasite. The changed motility pattern observed may be an adaptive response aimed at altering the environment around the parasite and thereby rendering survival less likely (Larsh and Hendricks 1948, Kelly and Dineen 1976) or it may be an adaptive mechanism of the worm to ensure the transport of the eggs to the exterior.

The existence of a cyclicity in the relationship between faecal and egg production is based on the assumption that the dispersion of eggs in the samples followed a Poisson distribution (Hunter and Quenouille 1952). It
may indicate that the demands for the prerequisites of egg production require an alteration in peristaltic activity. A weak cyclicity in egg length, not matched by that of width, gives the parasite the option of altering development time of egg to larva for the minimum and maximum volume of the eggs set the minimum and maximum hatching time (Crofton and Whitlock 1965). The smaller eggs of *N. dubius* in the more stable female system will therefore hatch more quickly than those from the male system and the approximate normal distribution of the size of the eggs will ensure the maximum time of infectivity in the external environment. Similar distributions of egg size for *Trichostrongylus axei* and *Trichostrongylus vitrinus* have been described by Cunliffe and Crofton (1953).

The results of this analysis of the sequence of events leading to egg production in *N. dubius* were used in an attempt to identify the source of disturbance in the *N. dubius*: ASH/CSI S.P.F. mouse system. The symptoms of the syndrome in the ASH/CSI S.P.F. mice included fewer parasitic nodules in the lower small intestine, a lower intensity of infection, distortion of the cuticle in adult worms, reduced fecundity, failure of eggs to develop and an inability to trigger the increased faecal production which characterised the *N. dubius*: CD1 system. These features suggested that the microenvironment of the small intestine of the ASH/CSI S.P.F. mouse was in some way hostile to the normal establishment and development of the L3 larva and this hypothesis was tested with the use of the broad spectrum antibiotic of oxytetracycline.

Manipulation of the system with oxytetracycline resulted in increased faecal and egg production as well as improved viability. Abnormal cleavage of the eggs during their passage through the small intestine of the mouse occurred and this suggested that the influence was still present. This was confirmed when the eggs from the oxytetracycline system yielded abnormal larvae. The results suggested the presence of
some contaminating agent common to all three systems and the only such feature was the bedding material of peat on which the mice were housed. Experiments designed to detect the presence of insecticide and herbicide were negative but that the agent was soluble was seen in the faster development of cultures of eggs using a distillate from the peat. Microbiological assay of the peat revealed the presence of a number of bacteria belonging to the group of the Bacillaceae which are able to secrete chitinase and spore in adverse conditions. The mice were observed to eat the peat and the ingestion of these bacteria may have been the cause of the imperfect chitin layers of the egg seen during the syndrome. The peat had been stored in black plastic bags during the hot summer of 1976 and it is probable that the high temperature caused these organisms to spore while others died. Given the right conditions they would germinate immediately and multiply rapidly with little competition from other organisms. Such a situation would arise when the peat was used as a bedding material for the mice. Faecal pellets and urine would supply nitrogen and other nutrients to an already optimal environment. The combined deleterious effects of lower fecundity of the adult worms and reduced viability of eggs on the output of the disturbed *N. dubius*: ASH/CSI S.P.F. mouse system was compounded by the ability of the microbial populations to alter the shape and size of the eggs. This will have important implications in the external compartment of the *N. dubius*: *A. sylvaticus* system for the time of availability of the infective L3 larva will be less extended. It is apparent that microbial environment is all important to *N. dubius* in the wild host for the fecundity of gravid female worms is much lower than it is in laboratory based systems and any increased egg production is dependent on an increasing intensity of infection with gravid female worms. The size of egg and the time of availability of the L3 larva for input into the *N. dubius*: *A. sylvaticus* system therefore assumes greater importance.
This study has also shown that the balance of stabilising and destabilising processes in the host parasite system of *N. dubius; A. sylvaticus* may be disturbed by the effect of stress on the host. The use of adrenal gland weight as an indicator of stress in the wild situation has its limitations particularly with respect to the female host but other methods which use changes in adrenal ascorbic acid, lipids, cholesterol, corticosteroids and blood profile reflect only the status of the animal at that specific moment in time. Provided the animal is killed quickly and the adrenal glands are fixed to facilitate easier dissection from surrounding tissue then adrenal gland weight is an acceptable indicator of adrenocortical activity (Christian 1963; Christian and Davis 1964).

The stress response of the adrenal gland is mediated through the release of corticotrophic releasing factors (CRF) by the hypothalamus to which the pituitary is sensitive. The pituitary responds with the production of ACTH which is transported to the adrenal cortex and causes the production of the glucocorticoids, corticosterone and cortisone (Brain, 1972). Glucocorticoids stimulate gluconeogenesis from proteins, increase glycogen deposition in the liver, depress protein anabolism, and suppress mitosis as well as connective tissue growth, inflammation, phagocytosis, granulation, and antibody formation (Christian 1963). These effects markedly affect wound healing and produce depression in host immune response to parasitic infection (Josephine 1958; Cross 1960; Noble 1961; Oliver 1962; Villarejos 1962; Campbell and Collette 1962; Cross and Duffy 1963; Campbell 1963; Briggs 1963; Ogilvie 1965; Mills, Brain and James 1973; Wakelin and Selby 1974; Ray, Bhopale and Shrivastava 1975).

Corticosterone, which is the major glucocorticoid of the mouse does not have a marked immunosuppressive action (Brayton and Brain 1974) but its possible role in a diminished response to larval invasion of the wall of the mouse small intestine by *N. dubius* is obvious. The glucocorticoids
are secreted by the zona fasciculata of the adrenal gland and homeostasis in their production is controlled by negative feedback to the hypothalamus with respect to their plasma levels (Brain 1972). Adaptation to chronic stress in rats can override this cortical feedback and increase drive to the ACTH secreting mechanism with consequent hypersensitivity to additional stimuli (Sakellaris and Vernikos-Danellis 1975). There are indications that such an adaptation may have taken place in the experimental stress system which employed ACTH to stimulate the stress.

To a lesser extent ACTH is able to stimulate the zona glomerulosa of the adrenal gland to produce aldosterone which acts primarily to maintain fluid and electrolyte balance through its effect on the tubular cells of the renal nephron. ACTH can also stimulate the growth of the preputial glands and other sex accessories although not as strongly as testosterone (Christian 1963).

The monitoring of stress in the wild population of A. sylvaticus revealed that ageing is accompanied by greater stress. The increase is more severe in the female mouse and associated with higher relative weights of the kidney, heart and liver during reproductive life than in the male mouse. Levels of stress were highest at the beginning of the study but with the progress of time these declined and were accompanied by a significantly smaller size of the kidney and liver in male mice and a significantly smaller size of kidney and heart in female mice. These changes may perhaps be explained in terms of lower ACTH production and consequent lower levels of glucocorticoids and aldosterone in these mice. The statistically significant lesser weights of the spleen and Peyer's patches of male mice and of the thymus and Peyer's patches of female mice during the stress period of January to June 1974 are also consistent with this hypothesis of stress. They suggest the involution of lymphatic tissue in these morphophysiological indices which is reflected in the greater egg output of the N. dubius; A. sylvaticus and the S. stroma:
A. sylvaticus systems.

The use of the adrenal gland weight greater than $5 \times 10^{-4} g$ and $6 \times 10^{-4} g$ for male and female mice respectively identifies those animals of the wild population of A. sylvaticus most susceptible to stress. The mean body weight of these susceptibles is $19.45g \pm 4.2$ for male mice and $18.1g \pm 5.4$ for female mice and they are therefore situated at the younger end of the breeding spectrum. When compared to the population as a whole the male mice are characterised by a larger kidney ($P = 0.05$) and smaller thymus ($P = 0.1$) and liver weight is greater in the female mouse ($P = 0.1$). The results suggest that the stress response may be mediated through different pathways in the sexes. They carry an intensity of infection slightly higher than the rest of the population but with time this margin must increase. The immune response of the laboratory mouse to N. dubius develops between 3 to 4 weeks of age and is dependent on multiple immunising infections (Cypress, van Zandt and Zidian 1973). It may be that the immune response of the young wild host develops more quickly and is strongly regulatory and N. dubius therefore uses these stressed hosts to make entry into the N. dubius: A. sylvaticus system.

The body weights of those animals with more than sixty worms per host are $24.1g \pm 3.41$ and $19.63g \pm 3.9$ for male and female hosts respectively. They carry $31.3\%$ of the total worm population and $82\%$ of these carriers have an associated infection of S. stroma. In addition, $25\%$ of the carriers have an infection with the other parasites observed in this study. The differences in male mice when compared to the whole population include a heavier testis weight ($P = 0.001$) and a smaller thymus ($P = 0.02$) while the only significant difference in female mice is a heavier mesenteric node weight. It is a reasonable assumption, therefore, that it is the dominant males and mature breeding females that maintain the genetic diversity of the worm within the group and contaminate the local habitat by reason of their greater contribution to the egg output of the
N. dubius: A. sylvaticus system. The subordinate animals provide for the contamination of a wider habitat during their enforced dispersal and form a focal point for the promotion of fresh influx into the gene pool of the worm through infection from a greater area of activity.

The adrenal gland weight of the heavily infected hosts is no different from the rest of the population and most of the morphophysiological parameters of immune status show no response to the high worm burdens. The decrease in thymus weight observed in male mice may be due to high testis weight (Bellamy, Hinsull and Phillips 1976) and the increase in mesenteric node weight of female mice can be explained as part of the normal ageing process. The features point to a lack of immune response in older animals and may indicate the immunosuppression described for N. dubius (Chowaniec, Wescott and Congdon 1972; Shimp, Crandall and Crandall 1975; Jenkins and Behnke 1977; Wescott and Colwell 1980).

Continual infection combined with the long generation time of N. dubius and possible immunosuppression must play some part in the build up of the high worm burdens. Evidence of a self cure as described for N. dubius (Cypess and van Zandt 1973; Cypess and Zidian 1975) does not appear to be present.

The histological examination of the lymphatic tissue of female ASH/CSI S.P.F. mice infected with N. dubius appears to set in motion a series of events leading to the transport of lymphocytes through the thymus, mesenteric nodes and spleen but the situation in Peyer's patches is not so clear. The feeding behaviour of N. dubius which strips off the specialised follicle-associated epithelium of the dome may delay the integration of the immune response and even contribute to the immunodepression already described. On the other hand the browsing may facilitate the contact of the worm with the lymphocytes bearing the specific serum antibodies of IgA and IgG (Crandall, Crandall and Franco
Cooperation between T and B lymphocytes is important in the immune response to *N. dubius* (Bartlett and Ball, Prowse et al, 1978; Vyas et al 1981; Behnke and Parish 1981) and the average life span of these cells in the mouse is of the order of several months and several weeks respectively (Sprent and Basten 1973). Their rapid disappearance from the lymphatic tissue of the stressed host confirms that the mouse is a steroid sensitive species (Claman 1972) and suggests that their life is considerably shortened in stress. The depletion of T lymphocytes is more complete than that of the B cells and indicates a greater sensitivity. T lymphocytes are still present in the thymus but the lack of basophilia in their nuclei, the presence of few mitotic figures and absence of corpuscles of Hassall in the medulla leads to the conclusion that nucleic acids are in short supply. Disorganisation of the reticuloendothelial cells lining the post capillary venules of the lymph nodes must also hinder the recirculation of any remaining T lymphocytes (Syrjaenen 1978; 1979). B lymphocytes when they are present in the germinal centres have abnormal nuclei. Other degenerative changes include collapsed sinusoids in the spleen and mesenteric nodes as well as the presence of abnormal erythrocytes. The effect of stress on the lymphatic and circulatory systems of the laboratory mouse is therefore severe, particularly with respect to the immune response.

Recent work with rats shows that graded stress produces progressively greater suppression of lymphocyte function and suggests that immunity is suppressed in proportion to the intensity of the stressor (Keller et al 1981). These findings are consistent with the increasing egg output of *N. dubius* in the *N. dubius*: female ASH/CSI S.P.F. mouse system as levels of stress rise. The lesser egg output of the *N. dubius*: male ASH/CSI S.P.F. system under chronic stress as compared to that under mild
stress in both the placebo and ACTH systems suggests that there are
limitations to this relationship in the male host. The increase in the
egg output of *N. dubius* in the host under stress is achieved by a
strategy that combines a greater intensity of infection with gravid
female worms seen in the field situation with that of the increased
fecundity characteristic of laboratory based systems. It is accompanied
by a general involution of lymphatic tissue in the thymus and Peyer's
patch area of the laboratory mouse and this is consistent with the
observations of the field studies. Involution of lymphatic tissue in
the spleen in the experimental system is not matched in the wild and the
relationship between stress, infection and the changes induced in the
mesenteric nodes requires further study.
The effect of the different regimes of stress on the lymphatic tissues
of mice is paralleled by changes that occur in the kidney, heart and
liver. The temporary heart enlargement which occurs in male mice when
mild stress changes to moderate stress is accompanied by a permanent
kidney enlargement as the system changes to the chronic stress state.
Similar kidney enlargement took place during the stress period of the
field studies and is consistent with a more frequent mediation of the
sympathetic adrenal medullary pathway of the fight or flight reaction
during social interaction with other mice. The response to changing
levels of stress in female mice appears to emphasise the endocrine
pathway of the stress response for liver involvement is indicated in
both the laboratory and field studies.
The general conclusions concerning stress and the systems that *N. dubius*
establishes with *A. sylvaticus* and the ASH/CSI S.P.F. mouse are that both
host parasite systems show flexibility of response to changing levels of
stress. Mild stress in the male host allows an immediate and rapid rise
in egg output from the system and this is continued in the female under
moderate stress to reach an even higher peak in egg output from the
female system under chronic stress. Both male and female systems are vulnerable to stress but the timing and level of response differs in the sexes as well as between individuals. If stress becomes too severe then host death occurs and regulation of parasite numbers will follow. Genes that programme response to stress may interact with those that control immunity to infection with *N. dubius* (Liu 1966; Forrester and Neilson 1973; Lueker and Hepler 1975; Behnke and Wakelin 1975, Brindley and Dobson 1981; Jenkins and Carrington 1981) and further work on such an interaction would provide useful information on the processes which underlie the host parasite relationship.

Environmental stress can also produce change in the microbial populations of the lactobacilli and coliforms in the murine gastro intestinal tract (Tannock and Savage 1974) which may affect the establishment and survival of *N. dubius* (Dobson 1961). The effect of altered gastrointestinal motility during stress on these microbial populations will also interact with the altered pattern of intestinal emptying associated with an infection of *N. dubius*. Complex interactions of this nature placed in an ecological framework compound the difficulties which beset field studies of parasitic infection and it is felt that the systems analysis approach adopted in this thesis may be one of the best ways of providing a solution to some of the problems involved. System theory furnishes a conceptual structure in which host, parasite and environment are not seen as separate entities but as a number of components interacting to produce systems of infinite variety and behaviour.

These particular studies have shown that the relationship of *N. dubius* with its host is one that combines innovatory behaviour with finely balanced inbuilt controls to ensure the system stability essential to its survival. A deeper understanding of the relationship between the size and infectivity of larval input to system function, in-depth studies of the cyclicity within the system as well as its response to stress
would provide a logical sequential analysis of some of the factors which may have been important in the evolution of stability in the *N. dubius: A. sylvaticus* system. Such stability is associated with evolutionary time and the system may therefore represent a late successional stage in the exploitation of *A. sylvaticus* by indigenous parasites.
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ADDENDUM I

The incidence of infection of small mammal hosts with digeneans and cestodes from Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.
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The incidence of infection of small mammal hosts with digeneans and cestodes from Lytchett Matravers Estate, Poole, Dorset. June 1973 to October 1975.

Five species of parasite are emphasised, namely:-

a) Cysticercus taeniae-taeniaeformis
b) Corrigia vitta
c) Brachylaimus recurvum
d) Catenotaenia pusilla
e) Hymenolepis species

a) Cysticercus taeniae-taeniaeformis is the larval stage of the cestode Taenia taeniaeformis belonging to the family Taeniidae. It has been reported in Apodemus sylvaticus (Elton, Ford and Baker 1931), Microtus sps and rats (Rausch and Tiner, 1949), Clethrionomys glareolus (Sharpe 1964; Lewis and Twigg 1972) and Microtus arvalis (Tenora 1972). The morphology of C. taeniae-taeniaeformis has been described by Rees (1951).

The definitive host is the cat and its relatives the stoat and fox. Rats and mice are normally the intermediate hosts but the rabbit can occasionally harbour a cysticercus. The larval stage appears as a large cyst on the liver and contains a strobilocercus. When it is eaten by the definitive host the segments, neck and bladder are digested off and the scolex forms a new chain of segments (Lapage 1968). The adult stage can live for three years in the domestic cat without clinical symptoms of the infection. (Williams and Shearer 1981).
Cysticercus taenia taeniaeformis in this study was found in
A. sylvaticus (8.1%), C. glareolus (10.2%), Microtus agrestis
Bellamy (12.5%) and Apodemus flavicollis Melch (7.1%). The
incidence of infection with respect to time is shown in
Table I. It was higher in male mice than in female mice and
it was lower during the summer months of the year. The
total population of the parasite recovered from A. sylvaticus
was 61, giving intensities of infection for male and female
mice of 0.09 and 0.06 respectively throughout the study period.
The approximation of the k exponent of the negative binomial
distribution is 0.12 and this indicates overdispersion of the
parasite population. Overdispersion is greater in the male
host (k = 0.12) than it is in the female host (k = 0.13).

b) Corrigia vitta

The dicrocoeliid digenean has been reported in A. sylvaticus
(Lewis 1968; Rainbow 1972) and Corrigia sps in A. sylvaticus
have been described by Sharpe (1964). It has also been
reported in C. glareolus and Microtus agrestis (Lewis and
Twigg 1972). It occurs in the interlobary canals of the
pancreas and in the duodenum. The life history is unknown
but it is assumed to parallel the life histories of other
dicrocoeliid digeneans. (Lewis 1968 a).

In this study C. vitta was found only in A. sylvaticus and the
incidence of infection with respect to time is shown in
Table II. The incidence of infection was higher in female
mice than male mice and was more prevalent in the summer months.
The incidence of infection was significantly lower in juvenile
mice than adult mice and this may reflect the difference in
the type of food taken by the two age groups. Young mice eat
less animal and more vegetable food than adults (Southern 1964)
Table I  The incidence of infection of *Apodemus sylvaticus* with *Cysticercus taenia taeniaeformis* from the Lytchett Matravers Estate, Poole, Dorset, from June 1973 to October 1975

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Table II The incidence of infection of *Apodemus sylvaticus* with *Corrigia vitta* from the Lytchett Matravers Estate, Poole, Dorset, from June 1973 to October 1975

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and these findings with respect to host age are in agreement with Rainbow (1972). Data collection of the numbers of parasites in hosts was not obtained.

c) **Brachylaemus recurvum**

This trematode parasite has been recorded by Baylis (1927) and has been reported in *A. sylvaticus* (Thomas 1953). In this study it was found in *A. sylvaticus* and *A. flavicollis*. The incidence of infection of *A. sylvaticus* with *B. recurvum* in male and female mice was 1.43% and 0.48% respectively. Similarly the incidence of infection in adult and juvenile mice was 1.11% and 0.46% respectively. During the study period the intensity of infection in male mice was 0.02 per host and in female mice it was 0.019 per host. The incidence of infection in *A. flavicollis* was 7.1% and the intensity of infection was 0.28 per host.

d) and e) **Catenotaenia pusilla and Hymenolepis sps**

*Catenotaenia pusilla* has been reported in *C. glareolus* (Elton, Ford and Baker 1931; Sharpe 1964; Kisielewska 1970; Lewis and Twigg 1972), *A. sylvaticus* (Sharpe 1964; Lewis and Twigg 1972), and *M. agrestis* (Lewis and Twigg 1972).

In this study it was positively identified in *A. sylvaticus*. The incidence of infection of *A. sylvaticus* with *C. pusilla* and *Hymenolepis* sps was 8.6% and 8.2% in male and female hosts respectively. Twenty seven per cent of the female mice were either pregnant or lactating. Similarly the incidence of infection in adult and juvenile mice was 11.8% and 4.1% respectively. No data with respect to the number of tapeworms was collected but occasionally the numbers were such that severe blockage of the small intestine occurred.
ADDENDUM II

The effects of stress on helminth infection
The effects of stress on helminth infection

The aims of this addendum on the effects of stress on helminth infection are:

1. To include details of other studies pertinent to the effects of environmental stress on the host parasite interaction.

2. To update the relevant information on the immune response of the laboratory mouse to Nematospiroides dubius.

3. To survey the range of immunosuppressive methods available in stress research and to place these in the context of the immune response of the laboratory mouse to N. dubius.

1. Studies pertinent to the effects of environmental stress on the host parasite interaction

The effect of stress on parasite population numbers can be (a) direct or (b) indirect.

(a) Direct Effect There is a direct correlation between the intensity and duration of exposure to thermal effluent and the diversity and dynamics of the parasite infrapopulations within the yellow bellied turtle, Pseudemys scripta. (Bourque and Esch 1974). As the intensity and duration of exposure increases so the diversity of the parasite fauna decreases while the density of the remaining infrapopulation increases. Similarly thermal stress is also responsible for the higher incidence of the acanthocephalan Neoechinorhynchus cylindratus in the large mouthed bass, Micropterus salmoides (Eure and Esch 1974).
(b) **Indirect Effect** Thermoal stress may affect parasite population numbers indirectly through the stress on the host. The effect of high thermal stress in male mice is to significantly reduce the numbers of non-budding larvae of *Taenia crassiceps* but heat and cold stress appears to have little effect on the parasite in the female host. (Novak 1978).

Environmental stress probably acts directly on the host parasite relationship of *N. dubius: Apodemus sylvaticus* for it has been shown in this thesis that the larval populations are sensitive to temperature and rainfall. The survival time of the larvae of *N. dubius* is reduced with temperature (Misra and Katiya 1980) and artificial stressing of larvae by irradiation with a Cobalt 60 source shows that male worms are more susceptible to stress than female worms (Behnke and Parish 1980).

Adaptation to environmental stress in the *S. stroma: A. sylvaticus* system may be indirect through female host density. If female mice become a limiting resource because of depletion of the host population due to environmental stress, then increased social interaction among male mice will serve to increase the rate of transmission and ensure dispersal of the parasite by way of enhanced male migratory behaviour.

2. **The immune response of the laboratory mouse to *N. dubius***

The immune response of the laboratory mouse is directed not against the adult worm, but against the 3rd stage larva following penetration into the wall of the small intestine (Chaicumpa, Prowse, Ey and Jenkin 1977). Inflammation and the formation of granulomata around the larva are part of the non
specific defense mechanism of the host and requires the presence of the thymus (Prowse, Mitchell, Ey and Jenkin 1979). Peritoneal exudate cells, which are predominantly macrophages, infiltrate the granulomata and have specific cytophilic antibody on their surface membrane (Chaicumpa and Jenkin 1978). Their action requires intimate contact between the cells and larva (Chaicumpa, Jenkin and Fischer 1977). Eosinophil penetration of the cyst and the activation of complement by the cuticular surface of the larva through the alternative pathway help the adherence of the mouse peritoneal exudate cells to the larva during incubation in vitro. (Prowse, Ey and Jenkin 1979).

The specific immune response of the laboratory mouse to *N. dubius* requires both humoral and cell mediated immunity. Humoral immunity can be conferred on female NIH mice by immune serum administered before Day 6 of the infection and is apparent in the establishment of fewer worms and a reduction in fecundity (Behnke and Parish 1979a). The passive transfer of protective immunity to mice with immune serum is directly proportional to the number of infection experiences by the serum donor mice. Male worms are more susceptible to immune serum than female worms and the serum from female donors is more efficacious than that from the male in the protection of mice against infection. Also passively immunised males harbour more worms than females given the same serum (Dobson 1982). Cell mediated immunity requires the presence of immune mesenteric lymph node cells and it is thought that they act synergistically with immune serum in mediating resistance to infection (Behnke and Parish 1979c). It is possible that the immune serum contains antitoxins which are formed as a consequence of the immunopotency of the worm E/S (excretory/secretory) products (Day et al 1979) and that the presence of other antibodies protects the transferred
immune cells from the immunosuppression activity of the parasite (Behnke and Parish 1979). Host immunoglobulin has been identified in cuticular precipitates (Hagan et al 1979) and the specificity of response to the antigens of the living larva is manifested by specific T lymphocytes secreting interleukin II which drain from the site of infection (Prowse 1982).

The effect of sex hormones on an *N. dubius* infection appears to be complex. Estradiol stunts the growth of adult worms and treatment with testosterone favours the growth of male worms but stunts that of female worms (Dobson 1966). Administration of β estradiol can also reduce the number of *N. dubius* in male mice (Hosier and Durning 1975). Serum from immune female mice protects both male and female recipients better than immune serum from male donors and the female environment enhances the protective qualities of male and female immune mesenteric lymph node cells but the male environment suppresses these effects (Dobson and Owen 1977).

3. The effect of stress on the immune response of the laboratory mouse to *N. dubius*

A number of immunodeficiency models in the characterisation of the immune response to parasites are in existence. Some models use antisera, others achieve immunodepression with the use of steroids, ionising radiation and congenitally immunodeficient animals. (Jacobson 1982).

Heterologous antilymphocyte serum potentiates primary non lethal infection of *Toxoplasma gondii* so that it causes death (Stahl et al 1978) and antithymocyte serum immunosuppresses
acquired resistance to *Trichuris muris* (Tanabe et al 1979). Steroids have also recently been used in a number of immunodepression models including those of the rat and *Trichinella spiralis* (Zayats 1980), hamsters and *Leishmania mexicana* (Chinchilla et al 1980) and lambs and *Dictyocaulus filaria* (Dhar et al 1981). Additional models have used the mouse as host to *Strongyloides ratti* (Grove and Dawkins 1981), *Giardia muris* (Nair, Gillon and Ferguson 1981) and *Hymenolepis nana* (Ito 1982). The use of sub lethal irradiation of the host as a means of immunodepression is not as effective as steroids. Whilst sub lethal irradiation can delay rejection of *Hymenolepis nana* by immune mice (Hopkins and Zajac 1976), its effects on the immune response to *Necator americanus* are less specific than those produced by steroids (Chao 1982).

Depression of the immune response of the laboratory mouse to *N. dubius* has primarily been dependent on steroids. The effect of the steroid may be a blocking of the response of the macrophages and eosinophils thus preventing their normal accumulation at antigenic sites (Hepler et al 1976). Cortisone treatment reactivates arrested larvae in the small intestine and a period 9 - 11 days is required for worms in immune mice to complete their development to the adult lumen dwelling stage (Behnke and Parish 1979b). Other effective steroids are promethazine hydrochloride and hydrocortisone acetate (Srilakshmi et al 1982). The use of the latter drug with tremisol allows the exploitation of a greater length of the small intestine (Lakshmi and Johri 1980) by the parasite and this is in accordance with the results of the stress experiments in the thesis.

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In retrospect, the use of ACTH to simulate the different regimes of stress as described in Chapter 6 may have been too drastic for the sensitivity of the adrenal cortex may vary in different mice and cause different levels of glucocorticoids to be released into the circulation. A set dose of an immuno-suppressive drug for each mouse would have ensured a more controlled experiment. Alternatively the use of crowding might have been just as effective for this simple method is more realistic with respect to the field situation. Recently it has been shown that the crowding of mallard ducklings enhances their susceptibility to infection by the nematode *Echinuria uncinata* by reason of the involution of their lymphatic tissue (Ould and Welch 1980).

The more usual forms of stress induced by social interaction, pregnancy and lactation in wild populations of *A. sylvaticus* provide for a sequential input of stress into the host parasite system. Social interaction in male mice competing for females can potentiate gastric lesions (Fukushima et al 1981) which will enhance the gastritis caused by an *N. dubius* infection (Lui, Cypess and van Zandt 1974) and pregnancy and lactation can have direct and indirect effects on host and parasite population numbers by way of transfer of corticosterone across the placenta (Dupont et al 1975). Stress induced in pregnant rats can severely impair the copulatory behaviour of male offspring (Rhees and Fleming 1981) and this will be of consequence in the number of hosts available for infection in the succeeding generations. Stress during lactation would appear to increase egg output from host parasite systems more than during pregnancy (Michel et al 1979; Jayapragsam 1982). Lactation in mice infected with *N. dubius* causes an increased susceptibility to newly acquired infection and a periparturient relaxation of the
immune response in female mice. Immunity, however, can still be transferred to the offspring by way of passive transfer of antibodies (Shubber, Lloyd and Soulsby, 1981). Pregnant and lactating mice constituted 17.2% of the total population of *A. sylvaticus* and their destabilising role in the ultimate egg output of the host parasite system is apparent.

Such relaxations of the immune response are counterbalanced by the genotype of the host. Recently it has been suggested that innate immunity is due to a partial linkage of genes and that adaptive immunity is controlled by a few dominant genes (Sitepu and Dobson 1982). High responding mice are characterised by lower worm burdens and less fecund parasites and the presence of high responders in wild populations of *A. sylvaticus* is not unlikely. The control exerted by these individuals will act in parallel with the density dependent checks on parasite population numbers already described in chapter 2 of the thesis.
BIBLIOGRAPHY


