University of Neuchâtel, Institute of Zoology
and
Pharmaceutical Research Division, F. Hoffmann-La Roche and Co., Ltd.,
Basle

Litomosoides carinii
(Travassos, 1919) Chandler, 1913 (Filarioidea)
in Cotton Rats
(Sigmodon hispidus, Say et Ord, 1825)
and Jirds
(Meriones unguiculatus, Milne-Edwards, 1867):
Comparison of the Infection in Relation to the Immune Response

Thesis submitted to the Faculty of Science of the
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by
Catherine Jaquet

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UNIVERSITÉ DE NEUCHÂTEL
FACULTÉ DES SCIENCES

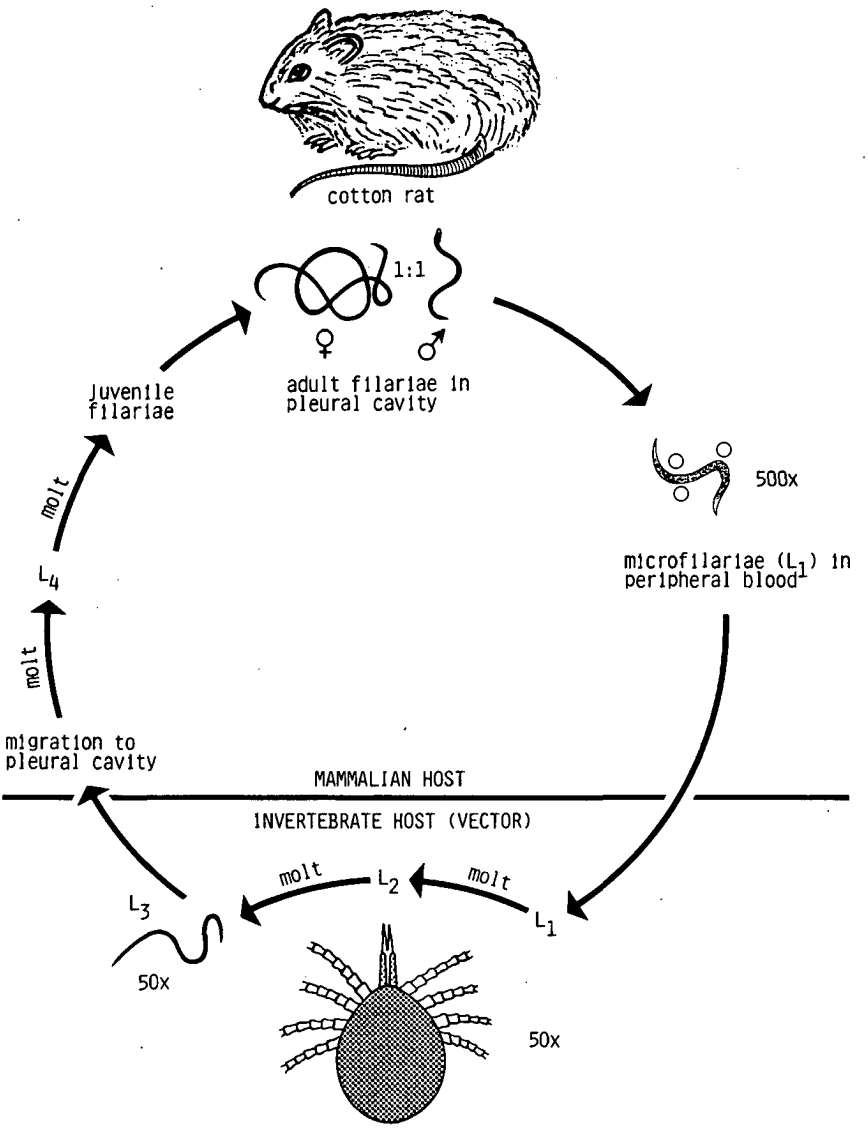
La Faculté des sciences de l'Université de Neuchâtel,
sur le rapport des membres du jury,
Messieurs A.Aeschlimann, M.Brossard, H.Stohler (Bâle),
N.Weiss (Bâle) et P.Wenk (Tübingen)
autorise l'impression de la présente thèse.

Neuchâtel, le 17 mars 1980

Le doyen:

K.Bernauer

Life cycle of Litomosoides carinii



Bdellonyssus bacoti:

development from microfilariae to infective third stage larvae (L_3)

List of Abbreviations

BBSS	buffered balanced salt solution
BSA	bovine serum albumin
BTA	blast transformation assay
cpm	counts per minute
FITC	fluorescein-iso-thiocyanate
IFAT	indirect immunofluorescent antibody test
IHA	indirect haemagglutination
L_3	infective, third stage larvae
mf	microfilariae
PBS	phosphate buffered saline
PHA	phytohaemagglutinin
RBC	red blood cells
SD/SE	standard deviation / standard error
SI	stimulation index
SRBC	sheep red blood cells
TCA	trichlor-acetic acid
WBC	white blood cells

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INTRODUCTION

The course of a filarial infection depends on species-specific characteristics of the parasite as well as those of the host, and it is also dependent upon the host's response to the parasite. The biology of filariae as regards the habitat of the adult worms (mainly the subcutaneous tissue, the lymphatics and the body cavities), their metabolism and the number and distribution of microfilariae produced by the females varies quite a lot according to the parasite species, and it may, in part, also differ according to the host species (e.g. in any other than the natural host). The same applies to the pathogenicity of filarial infections. There can be species and even strain specific variations.

Experimental studies on filariasis usually have to be carried out with non-human filariae because most species which are parasitic in man cannot be transmitted to laboratory animals. However, relating experimental results to human filariasis gives rise to a number of problems, since neither the parasite nor the host are reliable indicators of the response to, and the possible control of a filarial infection in man. Accordingly, experimental studies are directed to either substituting the parasite with a species which has a closer resemblance to human pathogenic forms, or to substituting the natural host in order to attain a situation which is similar to the pathogenesis of the infection in man.

The pathological processes in filariasis are associated with the body's response to live and dead parasites and with the immune reactions against them. Chronic infections with a parasite which lives in constant, close contact with the host's tissues and blood can lead to immunopathological conditions. Death of a parasite as a consequence of either immune reaction or successful chemotherapy may result in pathological changes and can even be fatal in a host which is sensitized against the homologous antigen.

Efforts to improve the control of filariasis go together with the need for further information about the kinetics and the pathogenicity of the infection in the laboratory models available. Since treatment with

filaricidal substances involves the risk of immunopathological reactions by the host, more has to be known about the host's immune response during the course of an experimental infection.

Among the few animal filariae which can be successfully maintained in the laboratory, Litomosoides carinii is widely used for the screening of potentially filaricidal substances. The usefulness of this model in chemotherapy was shown by the discovery of the microfilaricidal activity of diethylcarbamazine by Hewitt et al. in 1947. It is so far the only relatively non-toxic drug available and is effective against all species of human filariae. Yet, its efficacy and side effects differ considerably according to the parasite species. Thus treatment of human filariasis is still far from being satisfactory.

The complete life cycle of L.carinii in its final and intermediate host was first shown by Williams (1945,1946,1948). It is a filarial parasite naturally occurring in the cotton rat, Sigmodon hispidus, native to the southern United States. The adult male and female worms live in the pleural cavity of the rodent host. Occasionally a few worms can also be found in the peritoneum. The females give birth to the first stage larvae, the microfilariae, which have not yet undergone the first molt, but are still in the sheath (i.e. the external egg membrane). They gain access to the blood circulation mainly via the lungs and the heart. The vector and intermediate host is the bloodsucking tropical rat mite, Bdellonyssus (Synonyms: Liponyssus or Ornithonyssus) bacoti [Hirst, 1913]. The microfilariae (average length 73 microns) undergo two molts and develop into the infective third stage larvae (average length 853 microns). During the mite's next bloodmeal the infective larvae invade the final host, migrate to the pleural cavity and molt to a fourth stage larvae within about 8 days. A fourth molt takes place about 24 days later, liberating the juvenile filariae which then develop into fertile adult worms (Cross and Scott, 1947; Scott et al., 1951). About 7 - 8 weeks post infection the females start to produce microfilariae.

Cotton rats whether infected in nature or in the laboratory may contain a few or several hundred adult worms, and microfilarial density may range from a few to over 3000/mm^3 of blood.In the natural host adult worms of both sexes can live for several months to over a year. When

they begin to die they become increasingly embodied in a 'lobulate mass of encapsulation' (Bertram, 1966). Microfilaraemia can persist throughout the infection. There is a tendency for rising numbers of circulating microfilariae during the early patent (i.e. microfilaraemic) period, followed by declining values towards the end of the infection.

The pathology of the infection in the cotton rat has been described in detail by Wharton (1947). Changes are restricted mainly to the tissues of the pleural cavity. Proliferative reactions of the mesothelium which lead to the formation of papillary nodules that protrude from the surface of the pleura (including heart and lungs) are observed. The nodules are infiltrated by lymphocytes, neutrophils, eosinophils and later plasma cells and fibrocytes. In heavy infections the hyperplasia of the mesothelium leads to thick, laminated fibrosis and enlargement of the lymphatics of the pleura. The lung tissue can show hypertrophy of the alveolar cells and pseudo-asthmatic changes of the bronchioles. The tissue reactions are considered to be of a predominantly allergic nature and to be caused mainly by adult worm products; they are not seen to be locally associated with the presence of penetrating microfilariae. Apart from the pleuropulmonary pathology, which bears some resemblance to the reactions in human filariasis, some degree of splenomegaly is observed.

The difficulties in breeding and handling of cotton rats soon gave rise to the search for other adequate laboratory hosts. Today the multimate rat, Mastomys natalensis, the jird, Meriones unguiculatus and, with the restriction to certain strains (Singh and Raghavan, 1962) the albino rat are successfully used as experimental hosts of L.carinii.

However certain differences are observed in the course of the infection and in the response caused by the parasite:
- In the albino rat the average duration of microfilaraemia is about 18 weeks as compared to a year or more in cotton rats even though adult worms remain alive and active for a long time after the disappearance of microfilariae from the blood. This latent condition of the infection is evidence for an acquired immunity to microfilariae and for an active suppression of microfilaraemia in this host. Latent infections (i.e. filariasis without microfilaraemia) are seldom seen in either cotton rats or the other experimental hosts of L.carinii. However, they are known to occur in man. They are typical in hamsters used as experimental host of

Dipetalonema viteae (Weiss, 1970), and are also observed in Brugia pahangi infected cats following repeated re-infection (Denham et al., 1972). Experimentally, they can be induced by repeated injections of microfilariae of Dirofilaria immitis into dogs (Wong, 1974).

- In M.natalensis, on the other hand, only minor differences as compared to cotton rats in the course of the L.carinii infection are observed (Lämmler et al., 1968; Pringle and King, 1968). Maximum microfilarial levels are reached earlier than in cotton rats and a tendency to undergo 'natural cure' by the death of adult worms and the disappearance of microfilariae from the blood can already be seen around 9 months post infection.
- No investigations on the entire course of a L.carinii infection in the jird, M.unguiculatus, have previously been published. A comparative description by Schneider et al. (1968) is limited to the first four months, which is long before the infection comes to an end. No significant difference as compared to cotton rats was observed during this early period of the infection.

Essentially the same pathological changes as reported for cotton rats are observed in all experimental hosts. However, the extent of the pleuropulmonary tissue reactions is variable. In albino rats (Bagai and Subrahmanyam, 1968) and jirds (Schneider et al., 1968) they are consistently greater than in cotton rats. In Mastomys (Pringle, 1974) they are reported to be less severe.

Growing interest in immunological phenomena and their role in the host-parasite compatibility and the pathogenesis of the infection has led to studies on the development and nature of the immune response to L.carinii in its natural and experimental hosts. Quantitative serological tests such as indirect haemagglutination, complement fixation and indirect immunofluorescence have successfully been used to demonstrate antibodies in L.carinii infected cotton rats (reviewed by Tanaka et al., 1970). However, these studies have so far mainly been limited to the early period of the infection. Thus, data on the kinetics of the humoral immune response over the entire course of the infection is still incomplete.

For Mastomys the development of antibodies reacting in indirect haemagglutination and complement fixation tests as well as the occurrance of precipitating and homocytotrophic antibodies has been reported on in detail by Zahner et al. (1970), Zahner (1974) and Soulsby et al. (1976).

Other immunological investigations deal mainly with the nature of the acquired immunity to microfilariae which is typical for the infection in the albino rat. Cell mediated factors are considered to be involved together with humoral antibodies (Bagai and Subrahmanyam, 1970; Subrahmanyam et al., 1976).

Nothing is known about the immune response of L.carinii infected jirds.

In general, it can be said that the development of humoral antibodies is related to certain stages of the parasite's development in the host. Yet, the immune factors influencing the course of the infection are still largely unknown. The problems brought about by a parasite that can survive through several developmental stages with possible different metabolic activities and antigenic properties in spite of constant close contact with the tissues and the blood of the host are very complex. The mechanisms involved in the immune response to filarial infections are still not very well understood and seem to vary from one host-parasite system to another.

Furthermore, the activity of certain filaricidal drugs, known to be different according to the filarial species is not even always identical in the various hosts of L.carinii (Stohler, unpublished). The interpretation of experimental results and their possible relation to the pathogenesis of human filariasis is difficult and is further impeded by great variations in the reactions of individual animals which are typical for this type of infection.

The present experiments were carried out with regard to obtain a better understanding of the complexity of the problems which are encountered in chemotherapeutic trials with experimental filariasis. Improved knowledge of the processes going on in an unadapted host as compared to those in the natural, well-adapted host is essential. Jirds were chosen as experimental hosts because the course of a L.carinii infection in this host was still not known well enough and because the severer pathological changes of the pleural cavity suggested a strong immune response by this host. In addition, jirds are the natural host of D.viteae and can also be infected with B.pahangi and the human pathogenic sub-periodic form of B.malayi. Thus the ability of this host and the mechanisms by which it responds to a filarial infection is of much interest. As direct

comparative studies with the natural host of L.carinii are scarce and limited to the early patent period, the experiments were performed simultaneously with cotton rats and jirds, including the chronic phase, until the infection was seen to come to an end.

The experiments were carried out in partial fulfillment of the requirements for the degree of Ph.D. at the University of Neuchâtel.

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MATERIALS AND METHODS

1. Animals

Male and female cotton rats and jirds from the outbred colonies of the Institute of Biological and Medical Research Ltd (CH - 4414 Füllinsdorf) were either infected in groups of 10 - 20 animals at a time at 5 - 8 weeks of age or kept as age-matched controls.

L.carinii was maintained in a colony of B.bacoti according to Hawking and Sewell (1948) by cotton rat - cotton rat passages.

2. Infection

14 days after the exposure of uninfected mites to infected cotton rats infective larvae (L_3) were collected by pulling single mites apart in sterile halfstrength Tyrode solution (Scott and MacDonald,1953). 100 L_3 were taken up in 0.2 - 0.3 ml of liquid under a stereomicroscope and injected intradermally (i.d.) into the thighs of the hind legs on either side of the body.

3. Parasitological examinations

3.1. Microfilaraemia

The number of microfilariae (mf)/mm^3 of blood withdrawn from the retro-orbital sinus was determined in a Fuchs-Rosenthal counting chamber for liquor cells, starting at week 8 post infection (p.i.) in 2 week (8 - 16 weeks p.i.) or 4 week (from week 16 p.i.) intervals respectively.

For bleeding, animals were anaesthetised with aether.

3.2. Post mortem examinations

Animals were killed at 1 - 2 months intervals p.i.

3.2.1. Adult worms

Live adult, encapsulated worms and nodules were recovered from the pleural cavity, living male and female worms were counted and en-

capsulated worms and nodules were weighed. As the transition from the entangled mass of encapsulated worms to nodules is indistinct, no attempt was made to separate the two, and thus they will further be referred to as nodules only.

In order to measure the length of male and female worms from the two host species, intact worms were put into a drop of NaCl (0.8%) on a slide and spread out while the liquid was drawn off with a filter paper. Their length was then followed up with a thin glass-marker and copied onto transparent paper, where their size was finally measured with a map-measure.

For an estimation of the normal development of mf during the course of the infection the percentage distribution of the various embryonic egg stages was determined. Egg expulsion in hypotonic solution (Weiss,personal communication) from single female worms was induced by a 1% aqueous formaline solution (100 µl). This prevented the eggs from immediate deterioration by osmotic shock without disturbing the egg expulsion. After 15 minutes the eggs were fixed by the addition of 20 µl of 10% formaline. Eggs were counted differentially in a Fuchs-Rosenthal counting chamber under the phase-contrast microscope. Differentiation was made according to McFadzean and Smiles (1956) and Taylor (1960). The stages were combined after the following criteria (cf.Fig.14):

1. single cell to multiple cell stage
2. beginning of gastrulation as shown by a lateral indentation to embryo stage showing head and tail (referred to as 'embryo')
3. fully developed microfilaria still coiled up in the vitelline membrane and extended mf
4. non-viable eggs: all stages with atypical appearance.

3.2.2. Microfilariae and cells in the pleural cavity

Before the adult worms were recovered, free mf and cells of the pleural exudate were obtained by washing out the pleural cavity with 1 ml of buffered balanced salt solution (BBSS ; Shortman et al.,1972) containing heparin. The cells were counted together with the mf in an improved Neubauer haemocytometer as is classically done for white blood cell (WBC) counts. Smears were prepared with the fresh cell suspension in a Shandon Elliot cytocentrifuge and subsequently stained with Giemsa for further differentiation.

The percentage of dead (i.e. immobile) mf in relation to live (i.e. mobile) mf was determined under the phase-contrast microscope. Cell adhesions on mf were recorded.

4. Immunological examinations

4.1. Serology

About 0.3 ml of blood was withdrawn from the retro-orbital sinus with a pasteur pipette in two week intervals until week 8 p.i. and afterwards according to the time intervals of mf counts. Uninfected animals of age-matched control groups were bled accordingly. Serum was stored at -20°C until use.

Pooled serum from several infected cotton rats or jirds served as a standard positive control for all serological tests, and pooled normal sera from both host species served as negative controls. Sera from uninfected control animals were checked at random.

4.1.1. Indirect haemagglutination test (IHA)

Twofold serum dilutions in 25 µl phosphate buffered saline (PBS, unless otherwise stated pH 7.2) starting with a titer of 1/10 were carried out on V-shaped Cooke microtiter plates with 25 µl microdiluters. An equal amount of a 1% suspension of antigen-sensitized sheep red blood cells (SRBC) was added to each well. The plates were left at room temperature (r.t.) for two hours and then kept at 4°C overnight. One hour before the readings were taken the plates were shaken up once more and left at r.t. (Weiss and Degrémont, 1976).

4.1.1.1. Preparation of antigen

Adult male and female worms from cotton rats (14 - 20 weeks p.i.) were washed three times in NaCl (0.8%) , dried on a filter paper and stored at -80°C until further processing. The worms were then thawn, put into about twice their volume of PBS, refrozen in a convenient plastic bag and processed 3 - 4 times through a french pressure cell (cell-disintegrator, LKB-Biotech) at 200 bar in order to break up tissue and cells. After elution at 4°C for 16 - 18 hours and centrifugation at 35,000 g for 45 minutes at 4°C the supernatant was dialysed against PBS. Protein determination after Lowry was carried out before storage at -80°C in aliquots.

4.1.1.2. Sensitization of sheep red blood cells

Fresh SRBC were fixed with formaline according to Herbert (1973). Tanning of the fixed SRBC (Boyden, 1951) was carried out in 10^{-4} %

tannic acid (Gerbsäure, Fluka) in PBS for 15 minutes in an ice-bath (4°C). Subsequent sensitization was carried out at an antigen concentration of 200 µg protein per ml of 3% SRBC-suspension in PBS pH 6.4 for 30 minutes at 37°C. The sensitized SRBC were stored frozen at -20°C as a 1% suspension in PBS pH 7.2 with the addition of 1% bovine serum albumin (BSA). The aliquots were thawn just before use (Weiss and Degrémont, 1976).
SRBC which were not coated with antigen but which had otherwise been processed in the same way served as control and were prepared with each sensitization batch.

4.1.2. Indirect immunofluorescent antibody test (IFAT)

IFAT was carried out on slides using dried papain-treated mf as antigen according to Gonzaga dos Santos et al. (1976), with the following modifications: a 1/50 dilution in H_2O of the papain solution described (1% papain, 0.2% cysteine-HCl, 3.6% Na_2HPO_4) was added to an equal volume of mf-suspension in PBS. After incubation for 5 minutes at 37°C the reaction was stopped in an ice-bath. The suspension was then pipetted into small untreated circles of siliconised slides and left to dry at r.t.

The IFAT was carried out according to the classical technique. Drops of twofold serum dilutions starting with a titer of 1/20 were placed on the pretreated mf and incubated for 30 at 37°C, followed (after 2 washes in PBS) by a 30 minute incubation period with conjugate (anti-cotton rat or anti-jird Ig fluorescein-isothiocyanate [FITC] conjugated antisera) diluted to 1/40 in Evansblue 1/40,000 in PBS.

4.1.2.1. Collection of microfilariae

Mf were isolated from heparinised whole blood of cotton rats either by agglutination of the red blood cells (RBC) with phytohaemagglutinin P (PHA, Difco) as described by Wong (1964) or with lymphocyte separation medium (LSM solution, Bionetics) according to the instructions for lymphocyte separation, leading to a concentration of mf in the lymphocyte layer. Mf were washed four times in PBS and stored at -20°C in aliquots until treatment with papain and subsequent use for IFAT.

4.1.2.2. Preparation of conjugates

The gamma-globulin fraction was isolated from normal cotton rat or jird serum by precipitation with ammonium-sulphate. 3.2 M $(NH_4)_2SO_4$ was

added to an equal volume of serum on a vibromixer and left on a magnetic stirrer at 4°C for one hour. The precipitate was washed twice with 1.6 M $(NH_4)_2SO_4$ (10 min., 3000 g), re-dissolved in PBS (1/3 of the initial serum volume) and re-precipitated with 3.2 M $(NH_4)_2SO_4$. The procedure was repeated four times for further purification of the globulin fraction. The final sediment was again dissolved in PBS and dialysed against PBS for 48 hours at 4°C. After protein determination the gamma-globulin solution was stored at -20°C until use.

New Zealand white rabbits were immunized with either cotton rat or jird gamma-globulin with a single dose of 500 µg protein in 2 ml of complete Freund's adjuvant into the footpads and under the shoulder-blades, followed by five subcutaneous (s.c.) injections of 500 µg protein in 2 ml of incomplete Freund's adjuvant at weekly intervals. Four weeks later the rabbits were boosted with another 500 µg protein in incomplete Freund's adjuvant s.c. and bled on day 7.

The rabbit sera had an antibody titer of 1/64 against 1 mg protein/ml gamma-globulin solution as shown by Ouchterlony double diffusion tests.

Conjugation with FITC: Rabbit anti-cotton rat and anti-jird sera were precipitated with $(NH_4)_2SO_4$ as described above. Conjugation with FITC (Merck) was carried out at a final protein concentration of 10mg/ml at pH 9. After appropriate dilution of the globulin solution with isotonic saline the pH was adjusted with carbonate buffer (0.5 M) in a volume equal to one-tenth of the required final volume. Dry FITC was added to the chilled solution at a concentration of 0.8 mg/10 mg of protein and the mixture gently rotated at 4°C for 18 hours. Unbound dye was removed by dialysis against several changes of PBS until the dialysing fluid no longer showed visible fluorescence. The conjugate was stored at -20°C.

4.2. Cell-mediated reactions

4.2.1. Cellular morphology of the pleural exudate and cell adhesions on microfilariae

Procedures as described under 3.2.2.

4.2.2. Blast transformation assay (BTA)

4.2.2.1. Culture conditions

Lymphocyte suspensions from spleen and lymph nodes (axillary, inguinal and pharyngeal) were prepared in BBSS by gentle teasing with anatomical forceps. Cell aggregates were removed by sedimentaion for 5 minutes. The cells were washed twice with BBSS (10 min., 160 g at 4°C) and re-suspended in medium RPMI 1640 (Gibco, with 25mM HEPES and l-glutamine) supplemented with 100 µg/ml streptomycin (Novo), 100 U/ml penicillin (Novo) and 5% heat inactivated (30 min., 56°C) fetal calf serum (Difco). The cell suspension was adjusted to 2.5 x 10^6 cells/ml and contained at least 95% viable cells as determined by the exclusion of Trypanblue.

Antigens were added in concentrations of 10 µg and 50 µg of protein /ml medium (2 µg and 10 µg /well) . Phytohaemagglutinin P (PHA, Difco), diluted to final concentrations of 0.5 µl and 2 µl /ml, was used as unspecific T-cell mitogen.
Quadruplicate cultures of 200 µl/well were set up in round bottom tissue culture microtiter plates (Cooke) and incubated at 37°C in an atmosphere of 6% CO_2 in air and 95% relative humidity. The cultures were incubated for 4 days, after which 0.4 µCi of ^{3}H-thymidine (specific activity 5.0 Ci/mM, Amersham) in 10 µl BBSS was added to each well for 16 - 18 hours . Cultures were harvested onto glassfiber filters with a semiautomatic multiple cell culture harvester (Titertek Flow) by precipitation with 10% trichloracetic acid (TCA, cold) followed by a water wash. The filters were dried, suspended in 10 ml toluene-Permablend III (Packard) scintillator solution and counted in a liquid scintillation counter.
Lymphocyte reactivity was expressed as stimulation index (SI) by calculating the ratio of counts per minute (cpm) of stimulated to cpm of unstimulated cultures.

4.2.2.2. Preparation of antigens

Male and female adult worms from cotton rats (12 - 20 weeks p.i.) were separated and processed as described under 4.1.1.1.
Mf were separated from cotton rat blood in several batches with LSM as described under 4.1.2.1. In a further step mf were isolated from lymphocytes by gradient centrifugation in Path-o-Cyte 4 (Miles). The washed mf-lymphocyte mixture separated from whole blood with LSM was

resuspended in 2 ml Path-o-Cyte 4 50% (=concentrated) and carefully layered over with equal volumes of 29%, 26%, 23% and 10% dilutions in BBSS. After centrifugation at 20,000 g for 20 minutes at 5°C in a swinging bucket rotor (Beckman JS-13) mf were removed with a pipette from the 10% - 23% interphase, whereas WBC and remaining RBC were kept back at the 23% - 26% and 29% - 50% interphases respectively. Differential counts revealed 2 - 4 WBC/100 mf.

Mf were then washed 5 times with PBS (10 min., 3000 g at 4°C) and stored at -80°C until processing through the french pressure cell as described for adult worm antigen. (1 mg protein by the Lowry determination method was obtained from approximately 10^9 mf.)

All the antigens used for BTA were additionally dialysed against RPMI 1640 and sterilized by filtration (0.22 µm, Millipore).

The same batches of antigens were used throughout the experiments.

5. Statistical analysis

Spearman rank correlation coefficients between the extent of microfilaraemia, adult wormburden and nodule formation as well as between humoral antibody response (negative logarithm of the last positive dilution) and parasitological findings were calculated according to Siegel (1956). Significant coefficients with an absolute value of ≥ 0.4 were taken as strong, those of < 0.4 were taken as weak correlations.

The course of microfilaraemia in all animals examined per time was expressed as median and quartiles, calculated as described in Documenta Geigy (1969).

Mean antibody titers represent the geometric mean, all other mean values represent the arithmetic mean, and standard deviation (SD).

T-tests were performed to evaluate significant differences.

RESULTS

1. Influence of the infection on the host

1.1. Bodyweight

The weight of normal male jirds (Fig.1, top) remained more or less stable (mean ~70 g) from around 3 months after birth onwards. Young females were rather lighter (mean 53 g) than young males (mean 59 g). A steady increase in bodyweight to a final mean of 80 g was observed in females only.

Normal male and female cotton rats (Fig.1, bottom) showed an increase in bodyweight until about 8 - 9 months of age. The mean values of uninfected females kept as age-matched controls were higher than those of males, namely 230 g for about 9 months old females compared to 200 g for males of the same age (corresponding week 32 p.i.). This seems somewhat unlikely, but is probably due to an insufficient number of control animals being weighed regularly.

Compared to uninfected age-matched controls (Fig.1), infected males of both host species did not show any difference in development and maintenance of bodyweight. The weight of females though seemed to be affected by the infection. Particularly female cotton rats and to some extent also female jirds showed reduced development from at least week 16 p.i. when the mean difference between infected and uninfected females was 43 g for cotton rats and 4.5 g for jirds.

1.2. Spleen

A high degree of splenomegaly was observed in infected jirds (Tab.1). As early as 12 weeks p.i. their spleens were greatly enlarged, followed by a further increase up to week 24 p.i., when the average weight was about 10 times higher than in control animals.

Spleens of cotton rats were found to be slightly enlarged in older infections only. In no instance did the weight exceed twice the weight of the normal organ.

The splenic index, representing the ratio of spleen weight to body-weight x 1000, increased from 0.9 to 8.0 between weeks 4 and 24 p.i. in infected jirds but from 0.8 to only 1.2 in infected cotton rats. The index did not differ significantly between males and females of either host species.

1.3. Mortality of the host

Pathological changes of the pleural cavity of early L.carinii infections are, as already described by Schneider et al.(1968), more severe in jirds and resemble those of older infections in cotton rats. Following the course of the infection over a longer period of time revealed that the mortality rate was also much higher in jirds (Tab.2). About one third died between weeks 24 and 32 p.i. Thus the observation period for the study of immune reactions in a sufficient number of surviving animals was limited to 40 weeks p.i. Death was usually preceded by a 10 g to 15 g weight loss within 2 to 4 weeks.

The mortality rate of cotton rats, though also higher between weeks 24 and 32 p.i., was much lower than in jirds and the observation period could thus be extended to one year p.i.

Even though the reduced development of bodyweight under the influence of the infection would indicate greater impairment of the females, no difference in the mortality rate with regard to the sex of the host could be found.

Summary of chapter 1.

Influence on the host	cotton rats	jirds
bodyweight: males females	- unimpaired - reduced	- unimpaired - reduced
splenomegaly	moderate	marked
mortality rate	slightly increased between weeks 24-32 p.i.	high between weeks 24-32 p.i.

2. Biology of the parasite

2.1. Recovery of adult worms

Adult worms developed in all the animals studied. No significant difference in the total number and distribution of male and female worms with regard to the sex of the host was observed.

The mean recovery rate, i.e. the number of worms recovered as a percentage of injected larvae, was, as regards the total wormburden (male and female worms), generally higher in jirds than in cotton rats (Tab.3), namely 21.5% for cotton rats and 31.0% for jirds. Slightly more female worms were found in most animals of both host species, mean values being 12.1% females and 9.4% males for cotton rats and 17.2% females and 14.2% males for jirds.

A decrease in wormburden with time by encapsulation of worms (see below) through either host reaction or death of the worms became statistically significant ($P < 0.05$) in infections exceeding 44 weeks in jirds and exceeding one year in cotton rats. In jirds the decrease in male wormburden became manifest earlier than the decrease in female and total wormburden.

In spite of the fact, that all animals were infected with the same number of L_3 and care was taken to maintain equal conditions for the infection of all animals, there was considerable variation in the infection rates of individual animals. Absolute minimum and maximum values were 3% and 66% for cotton rats and 5% and 55% for jirds. The number of adult worms found within the same infection group (animals infected at the same time and with the same batch of infected mites) could range from 7% to 42% in cotton rats and from 15% to 55% in jirds. Accordingly, to some extent variable mean values resulted from different infection groups (Tab.4). However, only two groups (a and b) of cotton rats and one group (E) of jirds showed significant ($P < 0.05 - 0.01$) differences from the other infection groups of the respective host species.

As mentioned above, the average number of worms found in jirds was usually about 10% higher than in cotton rats. This difference could also be shown in a single trial, where cotton rats and jirds were infected at the same time and with the same batch of L_3 (groups d and D).

Four animals each were examined 16 weeks p.i. The mean recovery rates were 24.5% for cotton rats (12.5% females and 12.0% males) and 36.7% for jirds (17.5% females and 19.2% males). T-test revealed a significant difference of $P < 0.001$ between the two host species.

In those instances where animals of the same infection group were examined at intervals of several months (Tab.5) again no significant decrease in female wormburden with time could be observed in the earlier infections in either host species. However, for male wormburden in jirds a slight significance ($P < 0.05$) was seen in two cases (groups C and D) between weeks 16 to 28 and 20 to 34 p.i. This observation corresponds to the results mentioned earlier (Tab.3) where most of the mean values given include animals derived from different infection groups. There also a decrease in male wormburden becomes apparent before a decrease in female or total wormburden becomes significant.

2.2. Encapsulated worms and nodules (also referred to as nodules only)

As early as 4 - 8 weeks p.i. distinct nodules with a mean weight of 21 mg (5 - 78 mg) were present in 5 out of 17 jirds. From 12 weeks p.i. onwards nodule formation became very pronounced in most animals, but remained more or less at the same level throughout the rest of the observation period. Mean values were between 20 mg and 40 mg; maximum values of 100 - 130 mg were observed several times.

In cotton rats, on the other hand, nodule formation was quite unimportant until week 40 p.i. Occasionally small nodules weighing between 2 mg and 10 mg (max.20 mg) were found. An increase in nodule material up to values already found in early infections in jirds could not be observed until later than 52 and 60 weeks p.i., when mean weights of 18 mg and 39 mg respectively (max. 60 - 85 mg) were found.

In order to gain an indication of to what extent nodules are a sign of an elimination of worms, the percentage of nodule material was calculated from the total weight of live worms and nodules found. As some females of most animals were used for observations on embryogenesis, the actual weight of the live wormburden of the animals studied was not determined. Instead, a standard weight for single worms was used

to determine the weight of the live wormburden per animal. The average weight of single male worms from both host species was 0.13 mg, the weight of single females was 2.47 mg and 2.24 mg for worms derived from cotton rats and jirds respectively. The values were obtained from known numbers of weighed adult (20 weeks p.i.) worms derived from 62 cotton rats and 41 jirds with a total wormburden of between 3 and 70.

In jirds the proportion of nodule material (Fig.2) reached a mean of about 6% as early as 4 weeks p.i. and increased considerably between weeks 8 and 12 p.i. No significant further increase was observed thereafter. Median values remained between 30% and 40%. The rise to nearly 60% in infections exceeding 40 weeks was not significant, but went along with the decrease in wormburden.

In cotton rats the mean percentage proportion of nodule weight (Fig.2) remained below 10% until week 28 p.i. An increase then became apparent but the values were still about two times lower than in jirds for the same times of the infection. A median of over 30%, comparable to the values found in jirds from week 12 p.i. onwards, was seen between weeks 52 and 56 p.i. only. A steady rise to a final value of nearly 100% (i.e. practically no more live worms) was observed in infections of more than one year.

The definite increase in nodule material found in cotton rats in late infections (52 weeks p.i. and onwards) seems to be related to the decrease in wormburden. The latter became significant around the same time period (cf.Tab.3). Accordingly Spearman-correlations (Tab.12) between the number of adult worms and the extent of nodule formation were strongly negative, indicating a decrease in wormburden with increasing amounts of nodules found. The correlations were more pronounced for female worms and for female host animals; male cotton rats usually showed only weak correlations (correlation coefficient significant, but < 0.4).

In jirds, on the other hand, the significant rise in nodule material (week 12 p.i.) was apparently not related to a decrease in wormburden. Nodules therefore did not seem to be a sign of an elimination of worms. This was further substantiated by Spearman-correlations (Tab.12), which in contrast to cotton rats proved to be positive in jirds. Positive correlations were seen in male host animals only, and were found

only between male worms and nodules. This would indicate an increase (stimulation ?) in nodule formation with increasing numbers of male worms, and might explain the fact, that in spite of the increasing amounts of nodule material found in early infections no decrease in total wormburden could be seen.

2.3. Length of adult worms

In most cotton rats adult (12 weeks p.i. and onwards) female worms were, by appearance, longer than those found in jirds. Measuring the worms revealed a statistically significant ($P < 0.001$) difference in length of about 10 mm. No difference in the length of male worms from the two host species could be found (Tab.6).

An influence on the length of female worms in relation to the wormburden of individual animals could be shown in cotton rats only. A decrease in length was observed with increasing numbers of female worms. The difference in mean length was about 12 mm between females coming from animals with a wormload of 5 - 10 and those with a load of 21 - 25 females (Tab.7). No similar influence could be seen with worms which had developed in jirds.

2.4. Development of microfilariae

The various developmental stages are shown in Fig. 14. Young female worms contained around 28% still coiled up and extended mf, 22% 'embryos' and around 45% single to multiple cell stages (Fig.3). These mean values were regularly observed in worms which had developed in jirds from week 8 until week 20 p.i. In worms from cotton rats slightly less (mean 20%) coiled up and extended mf were found in 8 week old females; older worms showed the mean percentage distribution mentioned above until week 28 p.i.

Some non-viable eggs were found in most females as early as 8 weeks p.i., but they were always more abundant in worms which had developed in jirds. An increase of these eggs with time (age of the worms) together with a decrease of the later developmental stages followed by a decrease of single to multiple cell stages was observed in worms from both host species. The onset of this change became evident two months earlier in

jirds (week 24 p.i.) than in cotton rats (week 32 p.i.).

As early as 16 weeks p.i. an increasing number of females which had no more viable eggs (Fig.4) was found in jirds. In cotton rats the first females with 100% non-viable eggs were seen only at week 32 p.i. Whilst steadily increasing numbers of these females appeared in jirds, a sudden rise between weeks 32 to 40 p.i. was observed in cotton rats.

The total number of eggs decreased with the age of the worms (Tab.8). For females which had developed in jirds the decrease became significant ($P < 0.01$) between weeks 20 and 24 p.i., and for females from cotton rats between weeks 32 and 40 p.i. This decrease correlated with the beginning change in the percentage distribution between the apparent viable and non-viable embryonic egg stages (cf.Fig.3), and was again observed earlier in jirds than in cotton rats.

Total egg counts were usually higher in worms from jirds (difference not significant). Since there was a tendency towards lower egg counts in shorter worms of the same host species (Tab.9) also lower counts might be expected from the (in general shorter) females which had developed in jirds. The discrepancy can probably be explained by the higher incidence of eggs, observed in these worms, which showed abnormal development.

2.5. Microfilaraemia

All animals studied developed a microfilaraemia which became evident from the 8th week onwards in all jirds and in 56% of cotton rats. In 44% of the cotton rats the first mf were detected in the blood 10 to 12 weeks p.i. only.

Considerable variations in the extent of microfilaraemia were observed not only between individual animals, but also between the two host species (Fig.5). For jirds median values showed a marked rise in mf counts with the onset of patency, reaching a distinct plateau-like peak with 700 mf/mm^3 between weeks 20 and 24 p.i., followed by a sudden decrease in mf (but not a disappearance from the blood). In cotton rats the increase in mf at the beginning of microfilaraemia was slower, and although median values also revealed a peak around week 20 p.i., it was with only 250 mf/mm^3 much less pronounced and not followed by a

sudden decrease as observed in jirds.

Even though median values showed a peak between the 20th and the 24th week for both host species, the individual times for peak microfilaraemia (Tab.10) varied greatly in cotton rats. Mf counts could reach a maximum at any time during the infection, or be raised repeatedly at irregular intervals. In jirds, on the other hand, the course of microfilaraemia was more predictable since the times for peak mf counts were restricted mainly to the period between weeks 20 and 28 p.i.

Maximum mf counts could range between less than 100 mf/mm^3 and nearly 2000 mf/mm^3 in individual animals of both host species, but were usually lower in cotton rats (mean 408 mf/mm^3) than in jirds (mean 942 mf/mm^3).

Only two jirds reached a stage (beginning week 20 and 28 p.i.) where no more mf could be detected in the blood (Tab.11). In cotton rats microfilaraemia could last from 4 weeks (i.e. week 12 p.i.) to over one year. Amicrofilaraemia was observed in 19 animals. However, 8 of these were examined later than 52 weeks p.i. and had no more live female worms at autopsy. Of the remaining 11 cotton rats 2 had no more male worms, and their female worms contained only non-viable eggs (duration of microfilaraemia 24 and 40 weeks; time of autopsy 40 and 60 weeks p.i. respectively). All other amicrofilaraemic animals (9) still had live female worms with viable eggs at autopsy.

Spearman rank tests (Tab.12) revealed positive correlations between mf counts and the number of adult female worms in both host species, indicating higher microfilaraemia with increasing female wormburden. This might explain to some extent the higher total amount of mf found in jirds which, generally, also had a higher wormburden. It does not explain the marked decrease after peak microfilaraemia, though, as decrease in wormburden around that time had not yet been observed. The correlations were obtained for weeks 14, 16, 24 and 32 p.i. in cotton rats, and for weeks 16, 20 and 24 in jirds. However, these values were obtained mainly from female host animals. Male cotton rats showed no correlation except for week 14 p.i., and male jirds showed only weak correlations for the above mentioned times. Yet, no significant difference in the course or the extent of microfilaraemia with regard to the sex of the host was shown by rank tests (Mann-Whitney U-test).

As mentioned above, mf counts were generally much higher in jirds. Solely the difference in wormburden between the two hosts (an average of 12 females in cotton rats and 17 females in jirds) could not account for the elevated number of mf in jirds. Yet, this phenomenon could, apart from a possible higher productivity of the females, also be due to the difference in size of the two hosts. Since a jirds is about 1/3 the weight of a cotton rat, the mf produced by a same number of female worms would, accordingly, be dispersed in two distinctly different volumes of blood. This discrepancy was therefore taken into account in a second calculation of the median course of microfilaraemia. For this purpose the difference in bodyweight between the two host species was considered large enough to allow calculations without the inclusion of the exact factor for the percentage of blood volume to bodyweight ($F = \frac{\text{blood volume}}{\text{bodyweight}}$). The number of mf/mm^3 found per time was therefore simply multiplied by the host's bodyweight. Further multiplication of the resulting values by $F \times 10^3$ (for conversion of mf/mm^3 into mf/ml) would give the approximate median number of mf per total blood volume per animal (Fig.6, top).

As was to be expected, the great difference in actual mf counts between the two hosts became less evident. The overall shape of the curves remained the same, but at the beginning of microfilaraemia the total amount of mf was now seen to rise just as quickly in cotton rats as in jirds. Peak median values remained higher in jirds and the decrease in mf after the 24^{th} week p.i. was still more pronounced than in cotton rats.

In order to gain an indication as to whether the remaining higher values that resulted in jirds might be due to the difference in wormburden between the two hosts, the median values were divided by the mean number of females recovered from either cotton rats (12 females) or jirds (17 females). Knowing that the number of mf is not strictly proportional to the number of females and that, already, bodyweight is not an absolute measure for blood volume, calculations including the actual wormburden per individual animal were omitted for simplicity. The resulting total number of mf from single female worms proved to be nearly identical in both host species (Fig.6, bottom). Only the plateau-like peak and a slightly faster decrease remained in jirds. It therefore seems probable that in both host species essentially the same number of mf gains access to the blood circulation throughout the infection.

2.6. Microfilariae in the pleural cavity

The total number of free mf (mobile and dead or immobile) found in the pleural cavity (Fig.7) from week 8 until week 24 p.i. was generally at least two times higher in jirds (median 6 - 10 x 10^5) than in cotton rats (median 0.5 - 3 x 10^5). A certain decrease was observed in jirds between weeks 24 to 28, which became significant ($P < 0.05$) from week 32 p.i. onwards (week 24/40 $P < 0.01$). In cotton rats mf counts were very low at the beginning of patency. From week 16 p.i. higher values which remained at a more or less even level throughout the observation period were found. Except for two animals (week 52 p.i.) the comparatively high numbers of mf found in early infections in jirds were never seen in cotton rats.

However, it has to be taken into account that as early as 4 weeks after the onset of mf production (week 12 p.i.) around 60% of the mf in the pleural cavity of jirds were found dead or immobile (Fig.8). In cotton rats dead and immobile mf increased only to 20% between weeks 12 and 20 p.i., and towards the end of the observation period (week 52 p.i.) to 40%. Even as late as 60 weeks p.i. the percentage was much lower than in jirds during early infection.

Deducting dead and immobile mf from the total amount revealed that as regards living mf alone (Fig.9, top) the difference between the two hosts was limited to the period between weeks 8 and 12 p.i. Shortly after the onset of mf production their number was about 10 times higher in jirds (median 5 x 10^5) than in cotton rats (median 0.5 x 10^5). Living mf then decreased in jirds and increased in cotton rats, remaining in both hosts within the same range of values.

Essentially the same picture was obtained when the number of living pleural mf was put in relation to the number of female worms per animal (Fig.9, bottom). The difference between the two hosts was clearly restricted to week 8 p.i., when a median value of 40,000 mf per single female worm was found in jirds in contrast to only 6,500 mf per female in cotton rats.

Summary of chapter 2.

Biology of the parasite	cotton rats	jirds
recovery rate of live worms (mean):	- 9.4% males - 12.1% females	- 14.2% males - 17.2% females
decrease in live wormburden:	after week 52 p.i.	after week 44 p.i.
nodule formation:	- moderate until week 40 - related to decrease in live wormburden - negative correlations with the number of female worms	- marked from week 12 p.i. - not related to decrease in live wormburden - positive correlations with the number of male worms
length of adult worms (mean):	females: 103.4 mm males : 26.8 mm	females: 92.9 mm males : 25.4 mm
total number of eggs/female worm:	significant decrease between weeks 32-40 p.i.	significant decrease between weeks 20-24 p.i.
number of non-viable eggs:	- low in early infection - marked increase from weeks 32-40 p.i.	- higher in early infection - marked increase from weeks 20-24 p.i.
female worms with 100% non-viable eggs	none until week 28 p.i.; sudden increase between weeks 32-40 p.i.	steadily increasing numbers from week 16 p.i.
course of micro-filaraemia (median):	- moderate peak (250 mf/mm^3) at week 20 p.i. - slow decrease in mf after peak	- marked peak (700mf/mm^3) between weeks 20-24 p.i. - abrupt decrease in mf after peak
	- calculations including bodyweight and the mean number of female worms per host species indicate that in both hosts about the same number of mf gains access to the blood circulation .	

Biology of the parasite	cotton rats	jirds
time of maximum mf counts of individual animals:	any time between weeks 12 and 52 p.i.	concentrated on weeks 20-28 p.i.
amicrofilaraemia:	occasionally	rare
total number of pleural mf (dead and live):	higher in jirds than in cotton rats from week 8 until week 24 p.i.	
number of live pleural mf:	higher in jirds at week 8 p.i. only	
percentage of dead pleural mf:	low (< 20%) until week 40 p.i.	60% from week 12 p.i.

3. Immunology

3.1. Humoral antibody response

Both methods used (IFAT and IHA) revealed rising mean antibody titers until weeks 8 - 12 p.i. Except for an increase in IHA titers in late infections in cotton rats, no significant further increase was observed thereafter. Antibody titers were generally higher and did rise faster in jirds, where mean values were already definitely positive at week 4 p.i. In cotton rats mean titers were not above the background level of normal sera (1/20 for IHA and 1/40 for IFAT) before week 10 p.i.

3.1.1. Indirect haemagglutination tests (IHA)

Animals of both host species were tested at two week intervals until week 16 p.i. and at four week intervals until the end of the observation period. Starting with a total of 60 - 70 sera per time in early infections, gradually less sera were tested as more and more animals were being killed for either parasitological examinations or cellular immune reactions, ending with 20 sera each for the last time (weeks 40 and 52 p.i. for jirds and cotton rats respectively).

Even though mean values for cotton rats remained negative until week 10 p.i. (Fig.10) an increasing number of animals became positive during the first two months of the infection (5%, 30%, 50% for weeks 4, 6, 8 respectively). But mean reciprocal titers from weeks 10 to 32 p.i. were still not higher than 20 to 40, i.e. just slightly above background level only. In late infections (weeks 36 - 52 p.i.) an increase to 80 and finally 320 was observed.

With a mean value of 80, reciprocal titers of jirds were already distinctly positive at week 4 p.i., rising quickly until week 8 p.i. (reciprocal titer 640). With the onset of mf production (week 8 p.i.) a further but slower increase was seen during the following 8 weeks, levelling out with a mean reciprocal titer of 2560 (Fig.10).

All of the jird sera tested had positive IHA titers from week 8 p.i. onwards (40%, 80%, 90% were positive at weeks 2, 4, 6 respectively). Fluctuations over two to three dilution steps were frequent,

but invariably remained within the positive range. Maximum reciprocal titers of 20480 and 40960 were seen in over 20% of the animals tested from week 12 p.i. onwards.

As regards the cotton rats, fluctuations were much more pronounced. 20% to 40% of the sera tested per time after week 10 p.i. did not even show antibodies that could be traced with IHA. However, every animal examined for a period exceeding 28 weeks, made a positive antibody response at least for some time during the infection. Positive titers could appear at irregular intervals or over several consecutive times, being either preceded or followed by negative phases. Cotton rats showing positive titers continuously from early infection (weeks 8 - 12 p.i.) until the end of the observation period (week 52 p.i.) were fairly rare (5/22).

Neither mean values nor the results obtained from individual animals showed any clear relations between IHA response and parasitological findings. Statistical analysis though revealed some relation both with wormburden and with microfilaraemia. In cotton rats Spearman tests (Tab.13) showed strong rank-correlations with wormburden at weeks 20 and 28 p.i., and with microfilaraemia at weeks 14, 36 and 52 p.i. In jirds strong correlations with wormburden were seen at week 12 p.i., and with microfilaraemia at weeks 18, 28, 32 and 36 p.i. However, all correlations (both with adult worms and mf) which became manifest in cotton rats were negative, whilst in jirds they were always positive. This would indicate higher antibody titers with decreasing mf counts and wormburden in cotton rats, but increasing antibody titers together with increasing parasitological values in jirds.

3.1.2. Indirect immunofluorescent antibody tests (IFAT)

A total of 20 jirds and 22 cotton rats were tested throughout the observation period at the same times as mentioned for IHA.

Staining of mf with conjugate was only possible after pretreatment with papain. Reaction with strongly positive sera at low serum dilutions led to intense staining of the whole mf making a distinction of antigenic sites impossible. With increasing dilution steps immunofluorescent reaction just along the outer edge (subcuticular cells) became visible,

the sheath remaining unstained. A concentration of immunofluorescence around the excretory cell and the anal pore was often observed. However, these sites were also frequently subject to unspecific staining.

Antibodies to the cuticle of intact mf (papain untreated mf as antigen) were never observed. Anticuticular antibodies could also not be demonstrated in the sera of those cotton rats and jirds which had become amicrofilaraemic.

Even though the test was carried out on mf it was not specific for this stage of the parasite since a rise in antibody titers was traced long before the onset of mf production. As early as 2 weeks p.i. weak positive titers were frequently observed in both host species. 20% of the cotton rats and 60% of the jirds tested had reciprocal titers of 80 to 160 by the end of the 4^{th} week.

As was the case with IHA, IFAT (Fig.11) also revealed an increase in mean antibody titers in early infections, levelling out from week 10 to week 12 p.i. in jirds. Cotton rats showed a further increase until week 16 p.i. followed by a decrease until week 20. Mean reciprocal titers remained between 80 and 160 thereafter. Negative titers after week 8 p.i., frequent in IHA, were seen only occasionally in IFAT and were concentrated mainly to weeks 20 and 24, when 8 out of the 22 cotton rats tested showed, temporarily, no antibody response with this test. All of the jird sera tested were continuously positive from week 6 p.i. onwards, and again antibody titers were generally higher in jirds than in cotton rats.

Spearman rank-correlations (Tab.13) between IFAT titers and microfilaraemia were strongly negative for week 10 p.i. in both host species, indicating a relation between the number of circulating mf and the amount of humoral antibodies shortly after the onset of mf production. There was a difference with regard to the sex of the host though. Correlations became evident only in male cotton rats and in female jirds. For weeks 20, 32 and 36 p.i. strong positive correlations with adult female worms were revealed in jirds, but none at all were revealed in cotton rats.

Summary of chapter 3.1.

humoral immune response	cotton rats	jirds
IHA and IFAT titers:	generally lower	generally higher
main increase in antibody titers:	with onset of patency	during pre-patent period
IFAT with intact mf (papain untreated):	no reaction	no reaction
Spearman rank-correlations with parasitaemia:	negative correlation coefficients	positive correlation coefficients

3.2. Cell-mediated immune response

3.2.1. In vivo observations

At autopsy the pleural exudate was checked for cell-adhesions on mf by phase-contrast microscopy. The cells of the exudate of infected and uninfected age-matched controls were counted and stained with Giemsa for morphological differentiation. Pleural exudate usually being scarce, was obtained by rinsing the cavity with 1 ml of BBSS. Yet, between 0.5 ml and 2 ml exudate was found in some cotton rats (6/11) in late infections (weeks 52 - 60 p.i.) and 0.3 ml to 1 ml in 7 of the 8 jirds killed at weeks 8 and 24 p.i.

In spite of the use of anticoagulants(heparin) clotting of the fluid could not be avoided completely. Therefore cell counts were impeded by cell-clumps and accordingly a certain inaccuracy has to be taken into account. Nevertheless an increase in the total number of cells (Tab.14) during the first half of the observation period was noticed in jirds. Counts became lower in older infections (except for week 20 p.i. from week 28 p.i. onwards), but were still about two times higher than in uninfected controls. Cotton rats did not reveal this difference between infected and uninfected animals.

An invasion of large cells (blast cells and activated macrophages, see below) was observed particularly in cotton rats as early as 4 weeks p.i. Thus, together with total cell counts an approximate differentiation into 'large' cells and cells of the size of normal WBC was made. Throughout the infection the percentage of these large cells was higher in infected cotton rats than in jirds (Tab.14).

Morphologically cell smears of the pleural exudate (Tab.15) showed a distinctly different picture if derived from infected or uninfected animals. There also was a marked difference between infected animals of the two host species.

Most of the cells found in uninfected animals belonged to the monocyte - macrophage group. Lymphocytes were fairly rare (2 - 10%) and of the polymorphonuclear cell line only basophils or mast cells were found in jirds, whereas cotton rats additionally had about 10% eosinophils. Neutrophils were practically absent in both species.

In infected cotton rats large, activated macrophages, blast cells and giant cells (Fig.15) were observed from week 4 p.i. and throughout the infection. The number of eosinophils was about two times higher than in uninfected controls (Tab.15) until week 24 p.i. At the same time the number of activated macrophages increased.

In infected jirds on the other hand necrotic polymorphonuclear leucocytes became predominant, and activated macrophages often showed signs of degeneration (Fig.16). Blast cells were less frequent than in cotton rats and giant cells were rare. No significant change in the percentage distribution of the various cell types (Tab.15) between weeks 4 and 40 p.i. was seen in this host. Whether or not some of the necrotic polymorphonuclear cells were eosinophils could not be determined with certainty.

By week 4 p.i. mast cells had lost their basophil granules and subsequently disappeared from the pleural cavity of both host species.

In cotton rats and jirds an exact distinction between blast cells and macrophages was often difficult since most cells seemed to be in various stages of either differentiation or activation. The possibility that some of the cells were also mesothelial cells, as described for the pleural exudate of white rats by Mohan (1974), cannot be completely excluded.

Cell-adhesions on mf in the pleural exudate were observed only occasionally in cotton rats (5/54) at varying times during the infection and, with one exception, never in jirds. Latent infections (i.e. amicrofilaraemia) had developed in 7 out of the 54 cotton rats by the time their pleural exudate was examined (weeks 32 - 60 p.i.). Cell - adhesions on mf were seen in patent infections (4 animals) rather than in latent ones (1 animal). No significant difference in the total cell counts or cell morphology in amicrofilaraemic animals, compared to those with patent infections killed at the same times, was noticed.

As mentioned earlier only two jirds developed latent infections. Their pleural exudate was examined at weeks 28 and 34 p.i. respectively. Again no difference in cell counts or cell morphology could be seen. The single jird whose pleural exudate contained adherent cells on mf still had a patent microfilaraemia.

In addition, humoral antibody response of amicrofilaraemic animals did not show any significant alteration in antibody titers either before or after the beginning of latency.

3.2.2. In vitro experiments

Blast transformation assays (BTA) were performed with lymph node cells and spleen cells at weeks 4, 12, 32 and 56 p.i. from cotton rats and at weeks 4, 12, 20, 28 and 36 p.i. from jirds. Six infected animals and at least two uninfected age-matched controls were studied per time. Lymph-node cells were stimulated with female and male adult worm antigen, as well as with microfilarial antigen; spleen cells were stimulated only with female adult worm antigen. The protein concentrations used for all antigens were 2 µg and 10 µg per well (200 µl).

Under the given experimental conditions no blastogenic response to any of the antigens used was seen with lymphocytes derived from cotton rats at any of the times tested. Some positive results were obtained from jirds only.

Lymph node and spleen cells of infected jirds were stimulated by male and female adult worm antigen (Fig.12) at the beginning of the infection, mainly at week 4 p.i. and to a lesser extent at week 12 p.i. The blastogenic response was about two times higher in spleen cell cultures, and all individual stimulation indices (SI) were above the background stimulation of uninfected controls, whereas lymph node cells of every second infected animal only were stimulated by adult worm antigens. By week 20 p.i. the reactivity of both lymph node and spleen cells had decreased and was, with the exception of one animal per time, no longer different from the uninfected controls. Towards the end of the observation period (week 36 p.i.) lymph node cells, but this time not spleen cells, again showed antigen reactivity.

Male adult worm antigen usually led to higher SI than female antigen (Tab.16), but at the same time non-specific stimulation of the cells of uninfected animals was increased. Usually better results were obtained from the higher protein concentration (10 µg/well) with either type of adult worm antigen.

No marked difference in ^{3}H-thymidine incorporation into stimulated or unstimulated cultures of uninfected controls was noticed as regards the age of the animals.

Neither lymph node nor spleen cells were ever stimulated by microfilarial antigen. On the contrary, a rather inhibitory effect was observed on the cells of infected and uninfected animals. Incubation of cultures (jird lymph-node cells) with microfilarial antigen plus phytohaemagglutinin (PHA) revealed a dose-dependent inhibition of the PHA response (Tab.17).

The mitogenic response to PHA of jird lymph node cells remained unchanged throughout the infection, and in spite of variations between individual animals ^{3}H-thymidine incorporation remained within the range of uninfected controls. However jird spleen cells showed a marked unresponsiveness to the unspecific T-cell mitogen from week 20 p.i. onwards. A significant ($P < 0.001$) change was already observed between weeks 4 and 12 p.i. (Fig.13). Cotton rats did not exhibit this phenomenon; a slightly reduced responsivenes after week 12 p.i. was not significant.

The observed decrease of reactivity to PHA of the spleen cells derived from jirds coincided in time with the results obtained from stimulation with adult worm antigen. Furthermore, those 3 jirds whose spleen cells still showed mitogen reactivity (Fig.13) at weeks 20, 28 and 36 p.i. also had stimulation indices above control values by incubation with adult worm antigen (Fig.12).

No correlation though could be made between the extent of antigen and PHA response of either lymph node or spleen cells and parasitological findings (microfilaraemia, wormburden or nodule formation) in individual animals. Also humoral antibody titers (IHA) in animals whose cells were submitted to BTA were within the normal range for the times tested.

Summary of chapter 3.2.

Cell-mediated immune response	cotton rats	jirds
cells of the pleural exudate: - cell count	mainly large, activated macrophages, giant cells and blast cells - unchanged	mainly necrotic polymorphonuclear cells and macrophages with signs of degeneration - increased
cell adhesions on pleural mf:	occasionally	rare
blastogenic response to adult worm antigens:	no response	- lymph node & spleen cells are stimulated at weeks 4 and 12 p.i. - no response at weeks 20 and 28 p.i. - week 36 p.i.: lymph node cells are again stimulated
blastogenic response to mf antigen	no response	no response (mf antigen cytotoxic in vitro ?)
mitogenic response to PHA:	- response of lymph node and spleen cell unchanged throughout the infection	- response of spleen cells markedly reduced from week 12 p.i. - response of lymph node cells remains unchanged

DISCUSSION

1. Biology of the parasite

All animals studied developed a patent infection, i.e. adult worms were recovered from all animals and microfilariae (mf) were present in the peripheral blood for a sufficient time to allow natural transmission of the parasite in both host species. However, certain differences in the extent of parasitaemia as well as in the development and the survival time of the parasite were observed between the natural and the experimental host.

Recovery of live worms

The mean recovery rate of live immature and adult worms (Tab.3) was about 10% higher in jirds (31.4%) than in cotton rats (21.5%). This would indicate that jirds are more susceptible to a L. carinii infection than cotton rats. However, it has to be taken into consideration that the animals were infected by intradermal injection of infective larvae (L_3). This form of administration was chosen in an attempt to keep quantitative infections as close as possible to the natural situation. (Wenk (1967) was able to prove that in natural infections the L_3 migrate from the biting site of the mite to the regional lymph nodes via the lymphatic spaces of the skin. From the lymph nodes they probably reach the pleural cavity via the venous system by finally penetrating the tissues of the lungs.) The possibility that i.d. injection is more harmful to the larvae cannot be completely excluded, as Scott and MacDonald (1953) and Zein-Eldin (1965) found higher recovery rates following subcutaneous implantation (53.3%) or intravenous injection (44.4%). Viewed in this light, the lower recovery rates generally observed in cotton rats during the present experiments could be a result of this species' firmer skin as compared to that of jirds. The larvae would then be subject to a higher pressure during injection. Yet, it can be said that jirds are perhaps not more, but at least equally susceptible to the L. carinii infection as cotton rats.

Length of adult worms

Female worms which had developed in cotton rats were significantly longer

than those found in jirds (Tab.6). An influence on the length of the females by the number of worms as described by Bertram (1966) and Wenk and Neef (1970) was observed in cotton rats but not in jirds (Tab.7). Thus the higher recovery rates which were usually obtained from jirds as well as the smaller space of their pleural cavity can certainly not be the only reasons for a stunting effect on worms which developed in jirds. A deficiency in some selective nutritional factors required by developing and adult worms might be responsible. Zein-Eldin and Scott (1961) e.g. studying the plasma-protein composition of susceptible and resistant hosts of L. carinii, found that the concentration of α-2-globulins was two times lower in jirds than in cotton rats. In resistant hosts the level of this fraction was about six times lower.

Development of microfilariae

The percentage distribution of embryonic egg stages at various times of the infection was, with the exception of week 8 p.i., similar in both host species (Fig.3). Young adult females (8 weeks p.i.) which had developed in cotton rats showed higher numbers of early developmental stages (single to multiple cell stages) and lower numbers of fully developed mf compared to females of the same age in jirds. Zein-Eldin (1965) made the observation that L. carinii reaches maturity about six days earlier in jirds than in cotton rats and assumed that the difference in the plasma-protein composition between the two host species leads to a change in the rate of development.

As early as 8 weeks p.i. young adult females contained a certain number of non-viable eggs. These eggs were always more abundant in worms from jirds, particularly from week 16 p.i. onwards. After an initial phase of unchanged distribution of the abnormal and the various normal developmental stages, increasing numbers of non-viable eggs appeared in older worms (Fig.3). Simultaneously a decrease was observed in the total number of eggs per female (Tab.8). It seems likely that this reduction in fertility is a sign of the beginning of ageing of the worms. These changes were observed earlier in jirds (weeks 20-24 p.i.) than in cotton rats (weeks 32-40 p.i.).

Another sign for the beginning of ageing of the worms can be concluded

from the percentage of females which contained no more viable eggs (Fig.4). In cotton rats no such females were seen until week 28 p.i. Yet, beginning week 32 p.i. their number rose rapidly to nearly 60% of the total number of females examined. In jirds on the other hand, the first females containing only non-viable eggs were already observed at week 16 p.i. A slow but steady rise in the number of these females followed from week 20 to week 40 p.i. In other words, ageing of the worms sets in rather rapidly in the natural host, whereas in the experimental host the ageing of the worms seems to be a more gradual process, which begins comparatively early in the infection.

Microfilariae in the pleural cavity

In early patency (week 8 p.i.) the number of living pleural mf per single female worm was about ten times higher in jirds than in cotton rats (Fig.9). This observation corresponds with the observations made on embryogenesis, where at week 8 p.i. a higher percentage of the later developmental stages was found in worms from jirds. Yet, as early as 4 weeks later (week 12 p.i.) a high percentage of mf was found dead or immobile in jirds (Fig.8) and the number of living mf started to decrease to the values found in cotton rats (Fig.9). However, the total number of live and dead mf from week 8 until at least week 24 p.i. constantly remained about three times higher as compared to the number in cotton rats (Fig.7). Therefore, the possibility of a greater productivity of the females in jirds cannot be excluded. Since it is not known how long it takes until dead mf are eliminated, it can only be assumed that the decrease in the total number of dead and live mf would again be due to the ageing of the females which was suggested from the lower total egg counts and the increasing numbers of non-viable eggs.

The sudden increase in dead mf from practically nil to 60% between weeks 8 and 12 p.i. might amongst other reasons be due to unfavourable physiological conditions for mf in the pleural cavity of the experimental host.

Survival of adult worms

Even though some worms were found dead and encapsulated throughout the observation period, a significant decrease in live wormburden was ob-

served in both hosts only in late infections. As the decrease in wormburden was not a gradual process, probably only a few worms are eliminated by encapsulation due to possible immune mechanisms. The reduction in their number observed in both hosts only in late infections could then again mainly be due to the physiological ageing and subsequent death of the parasite. In agreement with this presumption is the fact that the decrease in live wormburden was observed somewhat earlier in jirds than in cotton rats (Tab.3).

Signs of earlier ageing of the worms, as judged by the onset of lower total egg counts, higher numbers of non-viable eggs and the appearance of females containing no more viable eggs have been discussed above. The results concerning mf in the pleural cavity suggest a possible greater productivity of female worms in jirds. Earlier ageing and subsequent earlier death of the worms in jirds might therefore be the result of this increased productivity. It remains unexplained as to why this phenomenon should occur in an experimental host. It is, however, possible that the parasite, being in an unnatural environment, reacts with a certain 'overproductivity' in order to guarantee survival over the next generation.

In cotton rats the decrease in wormburden coincided approximately with the increase in nodule material (Fig.2). Furthermore, negative correlation coefficients between the number of female worms and nodules were consistently obtained from week 10 to week 28 p.i. (Tab.12). Thus an elimination of dying worms by increasing encapsulation could also statistically be demonstrated in the natural host.

In contrast, nodule formation in jirds was very pronounced as early as week 12 p.i. (Fig.2) and thus began long before a decrease in wormburden was noticed. Nodule formation therefore does not seem to have any relation to an effective destruction or elimination of worms in this host. In addition, positive correlations resulted consistently from week 10 to week 36 p.i. between the number of male worms and nodules. This suggests that the formation of nodules might be stimulated by the presence of live (male) worms, and that it has to be regarded as a host reaction to parasitic antigen which has nothing to do with a control of parasitaemia.

The correlations were more often obtained from female cotton rats and from male jirds. Without further investigations no conclusions can be drawn on the importance of this sex-linked difference revealed by statistical analysis in both host species.

Microfilaraemia

In spite of considerable individual variations, observed particularly in cotton rats, the median course of microfilaraemia with steadily rising numbers of mf until week 20 p.i. and declining values thereafter proved to be similar in both host species. The decline in mf counts in jirds coincided with the above-mentioned beginning of ageing of the female worms and could therefore be due to a reduced production of viable mf. It is more difficult to explain why also in cotton rats the median course of microfilaraemia shows a decline following week 20 p.i. even though the ageing of the worms sets in later in this host. Considering the greater individual variations observed in cotton rats as regards the time of peak microfilaraemia (Tab.10), it becomes questionable as to what extent any calculation of a mean or median course of microfilaraemia is adequate. The variations in individual wormburden and the corresponding extent of microfilaraemia which could only occasionally be statistically correlated are a sign for the certainly complicated balance between the two parasitological values. However, it has to be kept in mind that in cotton rats correlation coefficients between female worms and nodules were consistently negative from weeks 10 to 28 p.i. thus indicating a certain suppression of adult worms during patency and the time before the worms show obvious signs of ageing. This might make plausible an average slow decrease in mf counts during a relatively early phase of patency.

The most striking difference between the two host species was the median extent of microfilaraemia. Jirds showed a rapid rise to a clear peak followed by an abrupt decline in mf counts, whereas cotton rats showed a gradual rise until week 20 p.i. and a gradual fall in the median number of mf thereafter (Fig.5). The sudden decrease in mf counts in jirds might (apart from the ageing of the female worms) be interpreted as an active suppression of microfilaraemia in this host. However, calculations which included the host's bodyweight and wormburden led to an almost complete elimination of the difference between

the two host species (Fig.6). Microfilaraemia no longer seemed to be more actively suppressed in jirds. Admittedly, these calculations are rather theoretical since bodyweight instead of actual blood volume was used, and the mean number of female worms per host species was taken instead of individual wormburden. Furthermore, it had to be presumed falsely that mf are evenly distributed in the blood. Yet, in spite of these reservations, it can be concluded that essentially the same total number of mf is circulating in the blood of both hosts throughout the infection. Thus the marked difference in the extent of microfilaraemia including the much more rapid decrease in mf counts after week 24 p.i. in jirds is mainly due to the difference in size of the two hosts. The above-mentioned calculations further confirm that microfilaraemia is actually more severe in jirds, as they are exposed to roughly the same number of mf as cotton rats. Relative to their size this is about three times as much. In comparison, L. carinii infections in Mastomys natalensis (a host of about the same size as jirds) lead, according to Lämmler et al. (1968), generally to a lower microfilaraemia than that observed in cotton rats. Thus mechanisms which prevent an excessive mf density in the blood circulation seem to function in that host but not in jirds.

Amicrofilaraemia

Proven latent infections, i.e. amicrofilaraemia in spite of the presence of fertile female worms, were more often observed in cotton rats (9 animals) than in jirds (2 animals). This is a further indication that jirds cannot more actively control their microfilaraemia. On the contrary, it seems that they might be even less capable of an active suppression than cotton rats.

2. Host reactions

2.1. Humoral immune response

Discussing immune reactions in helminth infections the inadequacy of the antigen and the possible inadequacy of the test procedures chosen have to be kept in mind. The commonly used soluble worm antigen is in fact a mixture of an unidentified number of worm components which can be brought

into aqueous solution. Specific and / or cross-reacting antibodies can be present to any of these components. It is largely unknown which of these components react with antibodies and to what extent others may disturb a reaction in a given test procedure. Yet, fractionation and purification of this 'antigen' is only sensible once the primary reactions of a host to the whole worm extract have been established. The use of corpusculate antigen e.g. in immunofluorescent assays allows to some extent an identification of antigenic sites of the parasite, provided the host has formed antibodies which react in this test. Yet, the specificity of the test to a particular worm stage is doubtful. Thus during the present experiments antibodies to somatic mf antigen (papain treated mf) could clearly be demonstrated before the hosts have been exposed to this stage of the parasite. The results obtained with indirect immunofluorescent antibody tests (IFAT) using enzymatically disintegrated mf as antigen have therefore nothing to do with an mf specific immune response. Antibodies to common antigens of the different worm stages are also known to exist in Dipetalonema vitaea infections (Weiss, 1978).

Another problem on working with blood or tissue dwelling parasites is the possibility that antibodies, although present, cannot be detected because they might be absorbed by the parasite or be neutralized by metabolic products. As regards filariae Pacheco (1966) already suggested that humoral antibodies could be absorbed by circulating mf. Capron et al. (1968) found an inverse relation between the immune response and microfilaraemia in D. viteae infected hamsters and also in the diagnosis of human filariasis. Yet, experimentally antibodies to the mf surface cannot be detected in patent infections. Hence the unsuccessful attempts to use intact mf for IFAT in the diagnosis of filarial infections (Mantovani and Sultzer, 1967). Since mf survive only for a limited time in the mammalian host it is perhaps possible that antibody is rather absorbed by dying mf which are subsequently drawn out of the circulation.

Apart from a possible antibody absorption by mf which could so far not be proved, the observations of Ishii (1970) suggest that a neutralization of antibodies to adult L. carinii may occur in the pleural cavity of cotton rats by excretory and secretory products of the worms. These metabolic products proved to be antigenic themselves and there was

evidence that at least some of these antigens have common components with somatic adult worm antigens. Thus antibodies directed against adult worms may directly be complexed in the pleural cavity. According to Zahner et al. (1970) important stimulation of antibody production is caused by the increased metabolic activity of the female worms with the onset of fertility, and by antigenetically active products which are set free together with mf-shedding. Together with the observation of Ishii this implies that at least during the most active phase of the parasite's life, the amount of detectable and perhaps also effective antibody could be greatly reduced. The possibility of antibody absorption and neutralization (together with the above-mentioned complexity of the antigens used for the tests) may therefore hide the actual situation of the immune response in the course of the infection.

The present experiments were carried out simultaneously using the same antigen preparation and the same test procedures for both host species in order to allow as far as possible a direct comparison of the reactions of the natural and the experimental host.

The humoral antibody response was generally much higher in jirds than in cotton rats. Furthermore, the main increase in antibody titers was observed during the pre-patent period in jirds, whereas in cotton rats mean antibody titers rose above the background level only after the onset of microfilaraemia (Figs.10 and 11). Fairly regular titers which remained within the positive range throughout the infection were obtained from all jirds. Cotton rats showed marked individual variations with stronger fluctuations from one time to the next. Negative values were observed frequently, particularly with indirect haemagglutination assays (IHA). A relation to parasitological data like adult wormburden or the course of microfilaraemia in individual animals was not clear in either host species. However, statistical analysis occasionally revealed correlations between parasitological and immunological results. In addition to the differences already mentioned between cotton rats and jirds, all correlations between immunological and parasitological data obtained from cotton rats were negative, whereas those obtained from jirds were, with one exception, positive. It therefore seems that there are major differences not only in the extent and development, but also in the mechanisms of the antibody response between the natural and the adequate (but experimental) host.

Cotton rats

The fact that in cotton rats the significant rise in mean antibody titers was observed after the onset of microfilaraemia is evidence for the new and strong antigenic stimulus presented by the worms with the onset of fertility and mf-shedding. Since, by the end of the pre-patent period (week 8 p.i.), about 50% of the animals still did not react with either IHA or IFAT or even with both together, migrating larvae and immature worms are possibly only slightly antigenic. This was also suggested by Fujita and Kobayashi (1969) who observed that the development of the antibody response was faster following transplantation of adult worms than following natural infection of the host. The possibility of common antigens between the parasite and its natural host (Damian 1964) and the incorporation of host antigens, known from Schistosoma mansoni (Damian 1967), have to be considered. Direct evidence for these phenomena in filarial infections is still missing, yet the apparent inability of the majority of cotton rats to react to the invading and developing parasite might be an indication of a strong host - parasite adaptation. Consequently, antibody production would not be stimulated so much by the worm's surface but rather by metabolic products and substances they release together with mf-shedding once they have reached maturity.

On the other hand, the mean recovery rate of juvenile and adult worms was only 21.5%. Antibodies to migrating larvae and to those which died during migration ought to be expected, but could not be detected in a high percentage of cotton rats with the test methods and antigens used.

The second increase in mean IHA titers of cotton rats from week 32 p.i. onwards could be due to liberation of somatic antigen with the onset of ageing and subsequent death of some worms (as judged by increased numbers of non-viable eggs and nodule formation). The rise in mean IHA titers and the ageing of the worms fall within the same time period.

In contrast to IHA, mean IFAT titers showed, after an initial rise in the early patent period, declining values from weeks 14 to 20 p.i., and remained at that level for the rest of the observation period (Fig.11).

Fujita and Kobayashi (1969) were able to show that the haemagglutination activity of the serum of L.carinii infected cotton rats can first be detected between weeks 6 to 7 p.i. in the 19S antibody fraction, but that it disappears after week 13 p.i. The activity is then replaced by 7S antibodies until the end of the infection. Since Weiss (1978) found a different immunofluorescent activity of the 19S and 7S fractions to either mf or adult worm antigens, there is probably a relation between the results of Fujita and the development of IFAT titers observed in cotton rats during the present experiments. The observed rise and decline in mean IFAT titers fall within the period of 19S antibody activity. 7S antibodies might have a lower affinity to the antigen used and would then be partly washed away during the test procedure.

The negative correlation coefficients obtained from cotton rats between antibody titers and microfilaraemia and wormburden suggest, apart from a possible antibody absorption, a certain control of the host - parasite interactions. Correlations with the extent of microfilaraemia were obtained in early patency (week 10 p.i. with IFAT and week 14 p.i. with IHA) and again during the period of decreasing numbers of mf (weeks 36 and 52 p.i. with IHA). During the time of peak median microfilaraemia, correlations were obtained only with the number of adult worms (weeks 20 and 28 p.i.). Since, at the same time, positive correlations existed between the number of adult worms and the number of circulating mf, it is somewhat astonishing that correlations between antibody titers and the two parasitological values do not occur simultaneously. However, the balance between the metabolic activity of the worms, the release of mf and antibody response is probably very delicate. In addition, the possibility of antibody neutralization and absorption, which could be increased with maximum mf levels, might hide the actual situation. Together with the marked individual variations it becomes questionable whether accidental results, as far as the times of the correlations are concerned, can be completely excluded. What is probably more important is that, in cotton rats, correlations between antibody response and parasitaemia were always negative, independent of when they appeared, indicating a possible balance between antibody production and suppression or control of microfilaraemia and adult worms.

Jirds

In contrast to cotton rats, antibody response in jirds could clearly be demonstrated during the pre-patent period (Figs.10 and 11). By week 4 p.i. 80% of the animals showed positive titers with IHA and 60% with IFAT (compared to 5% and 20% of the cotton rats for IHA and IFAT respectively). Thus the unadapted host showed a stronger reaction to the migrating larvae and immature worms. On the other hand, the recovery rate of adult worms was rather higher in jirds than in cotton rats. It is therefore either possible that with the antigens used for the serological tests (adult worm extract and papain-treated mf) specific reactions to immature worm stages were not traceable, and that in cotton rats the higher amount of antibodies reacting with IHA or IFAT was complexed by migrating and dying worms. Or since i.d. injection of L_3 might have been more harmful to the larvae when injected into cotton rats, the smaller number of L_3 would have molted to the L_4 stage. It seems that the antigens which are set free during the molt are a better antigenic stimulus than dying L_3 (Weiss, personal communication).

With the onset of microfilaraemia mean antibody titers (both IHA and IFAT) started to level out. It can be presumed that the increased metabolism of fertile females and mf-shedding present a similar stimulus for antibody production in any host. Bearing in mind that mf density in the blood of jirds is about three times greater than in cotton rats, a higher rate of antibody absorption by circulating mf is possible. This might be the reason why mean antibody titers do not rise significantly after patency. On the other hand, it may also be that either a maximum of immune responsiveness or an inability to respond further to parasitic antigens (see below) is reached during early patency.

During the pre-patent to early patent period negative correlations were obtained between IFAT titers and wormburden (week 8 p.i.) and the number of mf (week 10 p.i.). This may indicate a certain active suppression of parasitaemia during this early stage of the infection. Yet, as already mentioned, all correlations obtained for any later time during the infection were positive. It can be expected that a stimulation for an increased antibody response is caused by higher wormburden. But at the same time (since the correlations were positive) it can also be said that

the antibodies produced do not exert a suppressing effect on the parasite. Even though antibody titers were generally much higher than in cotton rats, parasitaemia was not more actively suppressed in jirds.

2.2. Cell-mediated immune response

Cell-mediated immune reactions examined by means of blast transformation assays (BTA) could be followed up only in jirds. Blastogenic response to any of the antigens tested was never observed in cotton rats. The lack of a 'positive control' makes it impossible to say whether lymphocytes derived from cotton rats are actually unable to show specific antigen-induced blast transformation activity in vitro. Other culture conditions such as purification of the cell suspension or the substitution of the medium by a different serum (or even serum batch) can lead to different results in this test. The problems of the complexity of the antigens encountered already in serological tests apply even more so for BTA. Different batches of antigen preparations may alter the results. It is therefore important that the same antigen batch is used throughout a given test series. Since the stimulation indices (SI) obtained from jirds were rather low, the results of the present experiments on blastogenesis to filarial antigens have to be considered as preliminary only.

Blastogenic response of jirds to filarial antigen.
Jird-derived lymph node and spleen cells were stimulated by male and female adult worm antigen at week 4 p.i. and to some extent at week 12 p.i.; spleen cells showed greater reactivity than lymph node cells (Fig.12). No blastogenic response in either of the cell populations was observed at weeks 20 and 28 p.i.

The decline in lymphocyte reactivity to filarial antigen was parallel to the increase in serum antibodies during the prepatent and early patent period. Weiss (1978) observed the same phenomenon in D. viteae infected hamsters. However, so far, no information is available on cell interactions and collaborations in the response to filarial antigen.

Independent of the humoral antibody response, lymph node cells again showed transformation activity in late infections (week 36 p.i.). The

question of whether this is a response to somatic antigen liberated by dying worms or of whether a change in lymphocyte responsiveness occurs cannot be answered.

Microfilarial antigen never induced lymphocyte stimulation. ^{3}H-thymidine incorporation in these cultures was generally less than in unstimulated control cultures. Combination of this antigen with phytohaemagglutinin (PHA) led to a dose-dependent inhibition of the PHA response. It is therefore possible that this mf-antigen had an inhibitory or cytotoxic effect in vitro. A specific unresponsiveness of lymphocytes to mf antigen is therefore questionable. On the other hand, since the response to the unspecific T-cell mitogen was affected by this antigen in vitro, it remains to be answered if mf might have a similar effect on T-cell activity in vivo.

Mitogenic response to phytohaemagglutinin

The reactivity to PHA was strongly depressed in the spleen cells of jirds during patency (Fig.13). The response to the unspecific T-cell mitogen was reduced at week 12 p.i. and reached a minimum at week 20 p.i. In late infections (weeks 28 and 36 p.i.) PHA response appeared to be slightly restored but was still significantly below the values of uninfected controls or infected animals during early infections.

The normal PHA response at week 4 p.i. and the first sign of depressed activity at week 12 p.i. suggest a possible association between microfilaraemia and immunodepression. The same situation was found by Dalesandro and Klei (1976) in the response of D. viteae infected hamsters and jirds to bovine serum albumin and to sheep red blood cells. The question of whether or not there is a relation to the above-mentioned in vitro effect of the mf antigen cannot, without further experiments, be answered.

The unresponsiveness of splenocytes to PHA was parallel to the blastogenic activity to filarial antigen during the course of the infection. Furthermore, the three jirds whose spleen cells still showed some PHA reactivity at weeks 20, 28 and 36 p.i. also reacted to adult worm antigen. Depressed mitogen reactivity to PHA of the spleen cells of Brugia pahangi infected jirds was described by Portaro et al. (1976). Yet, PHA response

in his experiments correlated inversely with the activity to filarial antigen. This suggests that different mechanisms to a cell-mediated response might be evoked by different filarial species in the same host. Weiss (1978) found a depressed PHA response in lymph node cells but not in spleen cells of D.viteae infected LSH hamsters. Furthermore he found no evidence of suppressor cell activity in unresponsive lymph node cultures, whereas Portaro's results suggest an inhibition of the PHA response by adhering suppressor cells. On the other hand, strain differences in the loss of PHA response have been observed in hamsters (Weiss, 1978) and mice (Pelley et al.,1976). It is not known whether such differences also exist in jirds.

Immunosuppression is also observed in human filariasis. Ottesen et al. (1977) were able to show that the in vitro responsiveness of peripheral lymphocytes to B.malayi and Dirofilaria immitis antigens was markedly reduced in chronic filariasis (Wuchereria bancrofti), particularly in children with persistent microfilaraemia. Grove and Forbes (1979) describe an impaired antibody response to tetanus and typhoid vaccines and a suppression of delayed hypersensitivity skin reactions to Candida, mumps and streptococcal antigens by W.bancrofti infected patients.

Immunosuppression in association with parasitic infections has been reviewed by Terry (1977). The cellular mechanisms leading to this phenomenon are still not entirely understood. T-cell suppression, antigenic competition, clonal exhaustion of antigen-sensitive cells, lymphocytotoxic factors and the influence of macrophages are discussed. However, the significance of immunosuppression in the host-parasite relation certainly lies in the possibility that the host makes less than a fully effective immune response to the homologous parasite, thus enabling the parasite too to evade the host's immune response.

The results of the present experiments show that immunosuppression in L.carinii infected jirds has to be considered. It might be an explanation for the (compared to cotton rats) high antibody response without any apparent suppressive effect on the parasite.

The PHA response of cotton rat derived lymphocytes was generally much lower than the response of jird lymphocytes under the same culture

conditions (data not presented), yet there was no evidence for a depressed mitogen reactivity of either spleen or lymph node cells due to the infection.

2.3. Immune response and amicrofilaraemia

Antibodies to cuticular antigens of mf (IFAT with intact mf as antigen) which were shown to exist in the sera of amicrofilaraemic hamsters infected with D.viteae (Weiss, 1978) and amicrofilaraemic cats infected with B.pahangi (Ponnudurai et al., 1974) were not observed in the sera of the few cotton rats and jirds which developed latent infections. Furthermore, cell-adhesions on mf in the pleural cavity can be observed in latent L.carinii infections of albino rats (Bagai and Subrahmanyam, 1970). This phenomenon was seen occasionally but could not be related to amicrofilaraemia in the animals studied during the present experiments. Thus, a specific immune response to mf as judged by the above-mentioned criteria could not be shown in latent L.carinii infections of cotton rats and jirds.

Since amicrofilaraemia was seldom observed in cotton rats and was very rare in jirds, it may well be that mf evoke little immune response in these two hosts. In accordance with Smithers (1968) the phenomenon of the acquired immunity to mf with an active suppression of microfilaraemia as seen in L.carinii infected albino rats and D.viteae infected hamsters, may arise mainly in certain abnormal host-parasite systems. In a normal host-parasite system such as D.immitis in dogs, amicrofilaraemia and the formation of mf specific antibodies can only be induced by repeated injections of mf (Wong, 1964). Also in cotton rats the course of microfilaraemia can, to some degree, be influenced by mf injections (Wegerhof, 1977; Wegerhof and Wenk, 1979). Thus it is potentially possible that antibodies against mf can develop in the natural host. Wegerhof (1977) was further able to show that uterine mf of L.carinii have a higher antigenicity when injected into cotton rats than blood mf. He assumes that blood mf may be coated with host antigens and thus become less antigenic. It is possible that this is the case in the natural and some experimental hosts. In certain other experimental hosts, however, the coating with or incorporation of host antigens may not work or not be sufficient for an 'immunological disguise'. Resistence to mf could then

develop more readily in these hosts. Yet in human filariasis certain amicrofilaraemic conditions can be associated with the presence of mf specific antibodies (Wong and Guest, 1969). Thus in man, antibodies against mf can be formed in spite of a 'normal situation'.

2.4. Unspecific host reactions

General pathology

Splenomegaly was very pronounced in jirds, whereas in cotton rats the spleens were only slightly enlarged with the infection (Tab.1). Already Wharton (1947) considered splenic enlargement in L.carinii infections to be caused by antigenic adult worm products. Crowell et al.(1973) were able to show that splenomegaly in jirds as a result of a L.carinii infection is due to amyloid formation. The pathogenesis of amyloidosis is said to be associated with chronic antigenic stimulation. According to Pringle (1974) enlargement of the spleen in L.carinii infected M.natalensis begins with the onset of microfilaraemia, and the severity depends on the number of mf. Yet Vincent and Ash (1978) could not find a consistent relationship between splenic enlargement and the level of microfilaraemia. They interpret splenomegaly as a response to mf production and to chronic antigenic stimulation by excretory and secretory adult worm products. Additionally, the spleen is considered to have little or no effect on the course of the infection, since mf do not seem to be destroyed in the spleen (Hawking, 1962; Pringle, 1974). Also Weiss (1978) found no influence on the course of microfilaraemia in splenectomized D.viteae infected hamsters.

The much higher degree of splenomegaly observed in jirds during the present experiments indicate that the response to the chronic antigenic stimulation is much more intense in this host than in cotton rats. The greater mf density and the higher wormburden in the also smaller host animal cannot solely account for the difference in the extent of splenomegaly. The severer splenic reactions of jirds confirm the presumption that this host actually responds more strongly to the parasitic antigen than the natural host, even though parasitaemia does not seem to be more actively controlled.

A high mortality rate was observed in jirds beginning week 24 p.i. (Tab.2). Apart from the severer pleuro-pulmonary reactions (Schneider et al.,1968)

and the severer splenomegaly which are more likely to be fatal for this host, the association with the time of peak microfilaraemia (Fig.5) suggests a relation between increasing numbers of mf and death. The mortality rate of cotton rats was also slightly higher during the same period. Therefore the possibility of a mechanical blocking of capillary vessels by increasing numbers of mf ought to be considered.

On the other hand, the rather higher wormburden in jirds in spite of a stronger immune response, and positive correlations between parasitaemia and immunological data, suggested a certain inability of this host to effectively cope with the infection. The balance in the host-parasite relation may thus (with time) more easily be changed against the favour of the unadapted host.

No explanation can be given as to why reduced development of bodyweight was observed in infected females but not in males of both host species (Fig.1). The positive correlations between the number of adult worms and the number of mf which were seen in females rather than in males cannot solely explain this phenomenon since no sex-linked difference in the extent of microfilaraemia or wormburden could be proved. Furthermore, there was no difference in mortality rate with regard to the sex of the host.

Cells of the pleural exudate

Together with the infection, marked changes in the cell population of the pleural exudate were observed as early as week 4 p.i. Activated macrophages and blast cells appeared in both hosts, and mast cells disappeared. The percentage of giant cells and eosinophils increased in cotton rats only (Tab.15). Jirds, on the other hand, showed a high percentage of necrotic polymorphonuclear cells (in spite of eosinophils and neutrophils being absent in uninfected animals) and many of the macrophages and blast cells showed signs of degeneration. Additionally, the total number of cells increased significantly in jirds but not in cotton rats (Tab.14). Thus, in infected cotton rats the picture was dominated by large activated macrophages, blast cells and giant cells (Fig.15), whereas in infected jirds the appearance of degenerating cells was the most striking feature (Fig.16).

The importance of these probably unspecific host reactions to the host-parasite relation is uncertain. Both hosts show signs of a strong reaction to foreign protein. Yet, since the total number of cells does not increase with the infection in cotton rats and large activated macrophages become predominant, it seems that the natural host is better able to cope with the parasitic antigen. The significant increase in the total cell count in infected jirds together with the presence of mainly degenerating cells may again indicate a certain inability of this host to effectively react against the parasite. In spite of the heavy invasion of cells into the pleural cavity already during early infection, the worm recovery was not affected and even higher than in cotton rats.

CONCLUSIONS

Jirds showed an overall stronger specific and unspecific reaction to L.carinii than cotton rats. All the same, they were at least equally susceptible to the infection. The recovery rate of adult worms was even rather higher in the experimental host. Apart from a higher mf density the median course of microfilaraemia also proved to be similar in both host species. Calculations including the difference in size and the difference in wormburden between the two hosts showed that roughly the same total number of mf gets access to the blood circulation in both hosts, and that microfilaraemia is not more actively suppressed in jirds. Additionally, amicrofilaraemia was more often observed in cotton rats.

However, worms which developed in jirds were shorter, and signs of physiological ageing of the worms were observed earlier than in cotton rats. A deficiency in some selective nutritional requirements may be responsible for a stunting effect. The stronger humoral immune response generally observed in jirds might additionally expose the worms to greater 'stress'. On the other hand, mf counts in the pleural cavity suggest a greater productivity of the female worms in jirds. The faster ageing of the worms could therefore mainly be a result of this increased fertility.

A significant decrease in the number of live worms (once they have reached

the pleural cavity) was observed in both hosts in late infections only, yet again earlier in jirds than in cotton rats. As there was no gradual decrease in live wormburden in either host species it has to be presumed that the worms die mainly by simple physiological ageing.

In cotton rats the increase in nodule material coincided with the ageing of the worms. Furthermore, Spearman rank-correlations between the number of live worms and nodules were consistently negative from week 10 to 28 p.i., suggesting a certain control over the parasite before the worms begin to die. In jirds on the other hand, nodule formation was already very pronounced in early patency and did thus not seem to be related to a significant elimination of worms by encapsulation. In addition, rank-correlations in this host were consistently positive, indicating rather a stimulation to nodule formation by live worms. It can therefore be presumed that nodule formation in jirds is mainly a host reaction to parasitic antigen, which has, unlike in cotton rats, nothing to do with a possible control over the parasite.

The generally stronger humoral immune response of jirds thus did not seem to lead to an effective control of parasitaemia. This was further substantiated by rank-correlations. Correlation coefficients between immunological data and parasitaemia were, when they appeared, positive in jirds but negative in cotton rats. It therefore seems that the natural host has a better control over the parasite than the experimental host. The results rather indicate a certain inability of jirds to effectively cope with the infection.

SUMMARY

The course of a Litomosoides carinii infection in the jird, Meriones unguiculatus was followed for a period of 40 weeks (until the infection was seen to come to an end). The development of the humoral immune response of the host to the infection was examined with indirect haemagglutination and indirect immunofluorescence. Cellular immune reactions were tested with blast transformation assays. All experiments were performed simultaneously with cotton rats, Sigmodon hispidus (for a period of 52 weeks), with the aim of a direct comparison of the course of the infection and the development of immune reactions in the natural host (cotton rat) and the experimental host (jird).

1. The mean recovery rate of live worms (as a percentage of injected larvae) was rather higher in jirds than in cotton rats.

2. Adult female worms were shorter in the experimental host. They also contained higher numbers of non-viable eggs. Increasing numbers of these non-viable eggs appeared with time (age of the worms) in both host species. At the same time reduced fertility of the females, as judged by lower total egg counts, was observed. These phenomena were considered to be signs of ageing of the worms. They were observed earlier in jirds (from week 24 p.i.) than in cotton rats (from week 40 p.i.).

   A decrease in the number of live worms found at autopsy became significant in late infections only, but again earlier in jirds than in cotton rats.

   Earlier ageing and death of the worms in jirds, as well as the stunting effect, might be due to a deficiency in nutritional factors. A stronger humoral immune response generally observed in this host may also expose the worms to greater 'stress'. Yet, microfilarial counts in the pleural cavity suggested the possibility of a greater productivity of the female worms in jirds. Earlier ageing might, therefore, also be due to this enhanced fertility.

3. There was no evidence for a significant elimination of worms in the pleural cavity by encapsulation during early infections. In cotton rats nodule formation became important in late infections only and coincided

with the decrease in wormburden. In jirds, on the other hand, the percentage of nodule material was high as early as week 12 p.i. and was thus not related to the decrease in live wormburden. In agreement with these observations Spearman rank correlation coefficients between nodules and the number of live worms were negative in cotton rats but positive in jirds. This leads to the conclusion that nodule formation in jirds is mainly a host reaction to live worms which, unlike in cotton rats, has nothing to do with a control over the parasite.

4. The median course of microfilaraemia showed peak microfilariae (mf) counts between weeks 20-24 p.i. in both host species. Jirds had a higher mf density than cotton rats and the median peak microfilaraemia was followed by an abrupt decrease in the number of mf. Yet, calculations including the host's bodyweight and wormburden indicate that essentially the same number of mf gains access to the blood circulation in both hosts and that the rapid decline in mf counts in jirds does not seem to be a sign of a more active suppression of microfilareamia in this host. Furthermore, amicrofilaraemia was more often observed in cotton rats than in jirds. This also indicates a less active control of microfilaraemia in the experimental host.

5. The humoral immune response was generally much higher in jirds than in cotton rats. After an initial increase mean antibody titers began to level out in both host species. However, the main increase in antibody titers was observed during the prepatent period in jirds but only after the onset of microfilaraemia in cotton rats. The lack of an increased antibody production with the onset of patency in jirds might be explained by a depression of certain immune reactions (e.g. jird spleen cells showed a marked unresponsiveness to PHA from week 12 p.i.).

   Spearman rank correlation coefficients between humoral antibody response and parasitaemia were negative in cotton rats and positive in jirds, indicating an active control of parasitaemia in cotton rats, but a rather ineffective response to the parasite in jirds.

6. Cell-mediated reactions were examined with blasttransformation assays. Under the given experimental conditions, lymph node and spleen cells from cotton rats did not respond to filarial antigen. Jird lymph node cells were stimulated by male and female adult worm antigens at weeks

4, 12 and 36 p.i., spleen cells only at weeks 4 and 12 p.i. The blastogenic response correlated inversely with the humoral immune response.

Microfilarial antigen never induced lymphocyte stimulation.

7. Jird spleen cells showed a marked reduction in their mitogenic activity to phytohaemagglutinin during patency. This phenomenon was parallel to the responsiveness of splenocytes to filarial antigen. It is a further sign of the jirds' possible inability to make a fully effective immune response against L. carinii.

8. Jirds showed stronger unspecific reactions to the parasite than cotton rats. Apart from the severer pleuro-pulmonary changes a much higher degree of splenomegaly and a higher mortality rate during peak microfilaraemia were observed.

9. A change in the cell population of the pleural exudate observed shortly after infection, indicated a strong reaction to foreign protein in both host species. Large activated macrophages, giant cells and blast cells appeared in infected cotton rats. In infected jirds necrotic polymorphonuclear leucocytes became predominant. Blast cells and activated macrophages were also present, but often showed signs of degeneration. The total number of cells increased significantly in infected jirds, but not in infected cotton rats. The importance of these reactions on the host-parasite relation is uncertain.

10. In spite of stronger specific and unspecific reactions, jirds were at least equally susceptible to the infection. It, therefore, seems that jirds are less capable of an effective control of parasitaemia than the natural host.

TABLES

Table 1

Comparison between splenomegaly in infected jirds and infected cotton rats expressed as a mean weight in mg and as the mean ratio of spleen weight to bodyweight x 1000 (splenic index). The values for uninfected controls include animals of various age groups.

Time p.i. in weeks	Weight of spleen in mg (mean) jirds(n)*	cotton rats(n)	Splenic index jirds	cotton rats
4	52.7 (4)	ND**	0.9	-
12	243.0 (4)	115.2 (4)	4.1	0.8
24	573.3 (3)	165.5 (4)	8.0	1.2
34	622.5 (4)	ND	10.5	-
40	567.5 (4)	220.0 (1)	8.4	1.1
52	ND	226.5 (7)	-	1.2
Uninfected controls	60.4 (9)	113.0 (4)	0.8	0.7

* (n) = number of animals

** ND = not done

Table 2

Mortality rate of infected jirds compared to infected cotton rats and uninfected age-matched controls in relation to time after infection.

Time p.i. in weeks	Jirds		Cotton rats	
	infected dead/total	uninfected dead/total	infected dead/total	unifected dead/total
1 - 8	9/132	0/42	8/107	2/33
9 - 16	4/106	1/38	1/85	1/22
17 - 24	8/91	2/33	0/76	0/16
25 - 32	21/69	0/17	7/66	1/15
33 - 40	16/40	1/11	2/42	2/11
41 - 48	7/13	0/4	0/36	1/7
49 - 60	-	-	9/38	0/5

Table 3

Recovery rate of live juvenile and adult worms (mean ± SD; as a percentage of injected larvae) in relation to time after infection, and host species.

Time p.i. in weeks	Cotton rats			Jirds		
	% females	% males	% total (n)*	% females	% males	% total (n)
4	10.8 ± 4.7	8.6 ± 4.9	19.5 ± 8.9 (8)	15.7 ± 7.8	15.3 ± 6.6	31.0 ± 13.2 (11)
8	8.8 ± 4.9	8.2 ± 5.2	17.0 ± 9.5 (5)	15.7 ± 6.7	15.7 ± 5.8	31.4 ± 11.7 (7)
12	9.2 ± 5.9	7.4 ± 3.6	16.6 ± 9.1 (8)	14.5 ± 8.1	14.4 ± 8.1	28.9 ± 14.6 (10)
16	12.5 ± 5.3	12.0 ± 2.5	24.5 ± 7.8 (4)	17.5 ± 4.9	19.2 ± 2.9	36.7 ± 4.0 (4)
20	10.2 ± 3.2	6.4 ± 2.9	16.6 ± 6.1 (5)	19.7 ± 4.7	16.5 ± 5.4	36.2 ± 8.7 (12)
24	15.5 ± 4.2	9.5 ± 3.2	25.0 ± 6.6 (6)	16.6 ± 4.7	12.4 ± 4.6	29.0 ± 8.4 (8)
28	17.1 ± 9.9	14.2 ± 8.0	31.2 ± 17.2 (8)	18.6 ± 5.2	12.9 ± 5.6	30.4 ± 11.3 (17)
32 - 36	12.7 ± 4.8	11.5 ± 4.5	22.7 ± 8.9(12)	17.5 ± 5.7	12.3 ± 5.4	29.9 ± 9.2 (24)
40 - 44	9.2 ± 4.7	7.4 ± 8.5	16.6 ± 13.0 (5)	16.3 ± 7.2	9.0 ± 6.5	25.3 ± 12.9 (15)
48 - 52	12.7 ± 3.3	9.0 ± 3.2	21.7 ± 5.5 (8)	6.8 ± 4.2	4.6 ± 3.3	11.4 ± 7.1 (5)
56 - 60	6.9 ± 5.4	4.9 ± 4.1	11.8 ± 9.2(15)			
> 60	2.6 ± 3.5	2.5 ± 3.3	5.1 ± 6.6(10)			
Total (4 - 36)				17.2 ± 6.3	14.2 ± 6.3	31.0 ± 11.5 (93)
Total (4 - 52)	12.1 ± 6.1	9.4 ± 5.6	21.5 ± 10.9(69)			

* (n) = number of animals examined

Table 4

Recovery rate of live worms (mean ± SD; as a percentage of injected larvae) in relation to infection groups, i.e. animals infected at the same time with the same batch of L_3.

Inf. group	Host species	% Females	% Males	% Total	Number of animals*
a	cotton rat	12.8 ± 5.8	12.2 ± 4.4	25.1 ± 9.7	9
b	"	14.4 ± 4.7	10.2 ± 5.4	24.6 ± 9.0	15
c	"	9.8 ± 3.0	7.2 ± 3.7	17.0 ± 6.1	9
d	"	10.7 ± 4.5	6.5 ± 3.6	17.2 ± 6.9	10
e	"	10.0 ± 4.5	9.7 ± 5.1	19.7 ± 9.1	6
f	"	7.2 ± 4.8	5.8 ± 2.5	13.0 ± 6.7	6
g	"	11.0 ± 5.0	9.7 ± 4.8	20.7 ± 9.1	6
A	jird	22.6 ± 2.0	13.7 ± 6.0	36.4 ± 7.1	8
B	"	19.8 ± 3.5	15.1 ± 6.3	34.9 ± 7.1	12
C	"	16.0 ± 5.5	13.2 ± 6.7	29.2 ± 10.6	10
D	"	17.2 ± 3.8	15.1 ± 5.0	32.3 ± 6.0	10
E	"	12.6 ± 2.4	11.5 ± 4.8	24.4 ± 4.8	8
F	"	15.5 ± 5.2	14.8 ± 6.0	30.3 ± 10.2	10
G	"	16.4 ± 7.9	17.2 ± 6.1	33.6 ± 13.1	8
H	"	19.6 ± 5.5	14.2 ± 6.8	33.8 ± 10.6	5
I	"	18.7 ± 6.5	14.7 ± 7.3	33.4 ± 13.4	7
K	"	19.7 ± 6.5	15.6 ± 4.3	35.8 ± 10.0	7

* excluding animals examined later than 40 weeks p.i. (jirds) or 52 weeks p.i. (cotton rats).

Table 5

Mean recovery rate of live worms from animals of the same infection group (cf. table 4) examined at intervals of several months.

Inf. group	Host species	Time p.i. in weeks	% Females	% Males	% Total	Number of animals
b	cotton rat	24 52	16.2 12.8*	10.6 8.7*	26.8 21.5	5 7
c	"	20 40	10.7 8.0	7.3 7.0	18.0 15.0	6 3
d	"	16 32	10.0 11.2	7.7 5.7*	17.7 16.9	4 6
C	jird	20 34	19.0 13.0*	18.0 8.4**	37.0 21.4	5 5
D	"	16 28	17.5 17.0	19.2 12.3**	36.7 29.3	4 6
F	"	4 12	15.0 16.2	14.7 15.0	29.7 31.2	6 4

* Decrease not significant;

** decrease significant ($P < 0.05$)

Table 6

Length of adult (week 12 p.i. and onwards) male and female worms which had developed in either cotton rats or jirds.

Length in mm (mean ± SD) females	males	Host species	Number of worms measured females	males
103.4 ± 16.8	26.8 + 2.8	cotton rat	150	155
92.9 ± 10.6	25.4 ± 5.1	jird	108	136

Table 7

Length of female worms (week 12 p.i. and onwards) in relation to female wormburden.

Number of female worms per animal	Length of female worms in mm (mean ± SD) from cotton rats	from jirds
a) 5 - 10	108.3 ± 11.3	-
b) 11 - 15	103.8 ± 11.8	91.5 ± 9.7
c) 16 - 20	104.1 ± 14.6	94.3 ± 10.8
d) 21 - 25	96.0 ± 6.6	93.5 ± 13.9
e) 26 - 30	-	90.5 ± 6.3

t-test:

Females derived from cotton rats: groups a) - c) and d) $P < 0.001$
groups b) - d) $P < 0.05$

Females derived from jirds : groups b) - e) not significant

Table 8

Total number of eggs in relation to age of worms (excluding females with 100% non-viable eggs) which had developed in either cotton rats or jirds.

Time p.i. in weeks	Egg count (mean ± SD x 10^3) of females from	
	cotton rats(n)*	jirds(n)
8	303.2 ± 116.8 (18)	456.4 ± 166.7 (16)
12	226.1 ± 76.9 (26)	313.2 ± 169.1 (22)
16	349.5 ± 102.1 (17)	395.8 ± 160.3 (21)
20	336.7 ± 103.4 (23)	374.7 ± 134.3 (26)
24	300.6 ± 135.6 (25)	269.7 ± 76.9 (24)
28	310.8 ± 145.4 (20)	274.4 ± 138.3 (26)
32	368.9 ± 138.7 (21)	259.6 ± 110.2 (21)
40	144.3 ± 59.3 (11)	190.0 ± 127.9 (19)
52	185.0 ± 71.3 (17)	-

* (n) = Number of female worms examined

Table 9

Total number of eggs of adult female L. carinii which had developed in either cotton rats or jirds in relation to the length of the worms. Females containing only (100%) non-viable eggs are excluded.

Host species	Length of adult females in cm	Total egg count (mean x 10^3)	t-test
cotton rat (weeks 16-32 p.i.)	8 - 10	260.0	$P < 0.01$
	10 - 12	379.8	
jird (weeks 8-20 p.i.)	7 - 9	318.4	$P < 0.001$
	9 - 11	459.2	

Table 10

Time of maximum microfilarial counts of individual animals during patency. The number of animals with peak microfilaraemia is expressed in percentage of the total number of animals examined throughout the whole observation period.

Time p.i. in weeks	Cotton rats		Jirds	
	%	(n)*	%	(n)
12	12.8	39	0	55
16	7.7	"	1.8	"
20	20.5	"	38.2	"
24	15.4	"	32.7	"
28	7.7	"	23.6	"
32	2.6	"	4.6	43
36	5.1	"	0	27
40	12.8	"	0	16
44	7.7	"	0	9
52	7.7	"	0	5

* (n) = number of animals

Table 11

Duration of microfilaraemia in cotton rats and jirds. (Number of female worms recovered at autopsy are indicated in brackets.)

Time after onset of microfilaraemia (weeks)	Cotton rats number of animals		Jirds number of animals	
	neg.*	pos.**	neg.	pos.
4	1 (0)	80	0	106
8	2 (12/14)	78	0	106
12	1 (0)	73	1 (14)	101
16	0	69	0	84
20	3 (0/14/11)	56	1 (6)	68
24	3 (4/5/7)	45	0	43
28	1 (12)	34	0	27
32	0	33	0	16
40	5 (6/2/3/2/0)	26	0	9
52	3 (0/0/0)	8	-	-

neg.* = reaching the end of patency;

pos.** = still patent.

Table 12

Spearman rank-correlations between parasitological data.

Host	Correlation between: Female adult worms & microfilariae	Female adult worms & nodules	Male adult worms & nodules
Male cotton rat	+ ** (14)	- ** (14)	0
Female cotton rat	+ ** (14,16,24,32)	- ** (10 - 28)	- ** (24,28)
Male & female cotton rat	+ ** (14,16,52)	- ** (10 - 28,44)	- ** (24,28)
Male jird	(+) * (14,18,20)	0	+ ** (10-36)
Female jird	+ ** (16,20,24)	0	0
Male & female jird	+ ** (18,20,22,24)	0	+ ** (10,14,18)

+ ** = strong positive correlation;

- ** = strong negative correlation;

0 = no correlation;

(+)* = weak positive correlation;

Weeks p.i. in brackets.

Table 13

Spearman rank-correlations between parasitological data and humoral antibody response.

Host	Correlation between IHA and:				Correlation between IFAT and:			
	Male adult worms	Female adult worms	microfilariae	nodules	Male adult worms	Female adult worms	microfilariae	nodules
Male cotton rat	- ** (20)	0	- ** (14,52)	0	0	0	- ** (10)	0
Female cotton rat	- ** (20)	- ** (20)	0	0	0	0	0	- ** (44)
Male & female cotton rat	- ** (20,28)	- ** (28)	- ** (14,36,52)	0	0	0	0	0
Male jird	0	0	+ ** (28)	0	0	+ ** (20,32)	0	0
Female jird	+ ** (12)	+ ** (12)	+ ** (36)	+ ** (32,36)	0	0	- ** (10)	0
Male & female jird	+ ** (12)	0	+ ** (28,32,36)	+ ** (32,36)	0	- **/+ ** (8)/(32,36)	(-) * (10)	0

+ ** = strong positive correlation; - ** strong negative correlation;

(+) * = weak positive correlation; (-) * weak negative correlation;

0 = no correlation;

weeks p.i. in brackets

Table 14

Cells of the pleural exudate of infected animals and uninfected age-matched controls. Total cell counts (mean ± SD) and percentage of 'large cells' (possible activated macrophages, giant and blast cells).

Time p.i. in weeks	Host species	Infected		Uninfected	
		total cell count x 10^6	'large cells' %	total cell count x 10^6	'large cells' %
4	cotton rat	12.9 ± 2.1	62.5 (6)*	ND**	ND
8	"	6.8 ± 0.6	58.9 (4)	7.6	7.8 (2)
12	"	2.0 ± 0.4	55.3 (2)	6.3	11.8 (3)
16	"	4.9 ± 3.0	74.4 (4)	ND	ND
20	"	3.9 ± 1.8	68.1 (5)	7.6	37.1 (2)
24	"	3.5 ± 1.4	73.8 (5)	ND	ND
28	"	3.2 ± 2.1	69.4 (4)	ND	ND
32	"	3.4 ± 1.7	77.7 (5)	5.1	35.3 (2)
40	"	4.4 ± 2.4	65.2 (4)	ND	ND
52	"	13.0 ± 3.7	58.6 (6)	ND	ND
60	"	7.7 ± 5.6	55.5 (5)	6.8	32.2 (3)
4	jird	11.5 ± 3.0	37.8 (4)	1.3	15.6 (2)
8	"	28.2 ± 11.9	40.6 (5)	1.8	54.2 (1)
12	"	11.6 ± 7.1	38.8 (4)	1.4	16.3 (3)
16	"	10.1 ± 3.1	45.3 (4)	ND	ND
20	"	3.2 ± 0.8	26.1 (5)	1.6	32.9 (2)
24	"	11.8 ± 5.6	51.7 (3)	1.8	36.9 (2)
28	"	4.6 ± 1.6	42.2 (4)	2.9	19.7 (3)
34	"	5.2 ± 2.1	41.1 (5)	ND	ND
40	"	4.1 ± 2.8	37.3 (4)	2.3	33.8 (3)

* number of animals

** ND = not done

Table 15

Cell differentiation in the pleural exudate of infected and uninfected cotton rats and jirds (mean of 4-5 animals per point).

Time p.i. in weeks	Host species	% blast cells	% giant cells	% normal macrophages	% degenerated macrophages	% lympho-cytes	% normal polymorphonuclear eosinophils	neutrophils	basophils	% necrotic poly-morphonuclear cells
4	Cotton rat	32.8	1.5	38.7	0	1	25.4	0	0	0.6
8		19.8	4.6	39.9	0.25	2.5	32.0	0.5	0.25	0.75
12	infected	23.7	5.1	37.0	1.9	3.9	23.1	0.6	0	1.3
24		18.7	9.6	51.1	0.3	4.6	14.2	0.6	0	0.9
40		15.7	9.4	44.6	1.5	6.9	16.9	1.7	0	3.0
60		24.8	3.2	52.5	0	7.1	8.7	1.0	0	2.3
4-8	age-matched controls	2.3	0.2	78.7*	0	4.8	10.2	0.2	3.7	0
24		4.8	0.2	79.5*	0	4.8	6.8	0.2	3.3	0
52-60		1.0	0.5	83.0*	0	1.0	12.5	0	3.0	0
4	Jird	2.5	0.4	41.6	14.0	1.5	0	0	1.25	39.0
8		2.9	0.4	46.6	7.1	24.0	0	0	0.4	18.6
12	infected	11.7	0.5	42.0	18.0	6.0	0	0	0.5	21.3
20-24		2.9	0.3	51.2	15.7	4.3	0	0	0	25.8
34-40		7.9	0.1	44.2	5.1	9.4	0	0	0	33.2
4-8	age-matched controls	0.7	0	91.0*	0	2.0	0	0	6.3	0
24-28		0	2.3	83.0*	0	10.3	0	0	4.3	0
40		0.5	4.5	79.0*	0	11.5	0	0	4.5	0

* monocytes and/or macrophages

Table 16

Blastogenic response of jird lymph-node cells and spleen cells to filarial antigens and phytohaemagglutination (PHA)
Stimulation indices: mean ± SD.

Time p.i. in weeks	Cells derived from	Stimulation with : PHA	Female adult worm antigen (10µg)	Female adult worm antigen (2µg)	Male adult worm antigen (10µg)	Male adult worm antigen (2µg)	microfilarial antigen (10µg)	microfilarial antigen (2 µg)
	lymph-node:							
4	infected (6)*	18.3 ± 16.1	3.4 ± 1.0	3.0 ± 0.9	4.4 ± 2.4	2.7 ± 1.3	0.7 ± 0.2	0.9 ± 0.3
	uninfected (3)	22.5	2.0	1.9	2.8	1.4	0.9 0.9	0.9
12	infected (6)	21.6 ± 18.8	2.7 ± 1.0	1.8 ± 0.4	3.2 ± 1.0	1.7 ± 0.4	0.9 ± 0.1	1.2 ± 0.3
	uninfected (2)	28.0	1.2	1.2	2.2	1.5	0.6	0.6
20	infected (6)	16.3 ± 8.2	1.8 ± 0.8	1.3 ± 0.6	2.4 ± 0.9	1.5 ± 0.4	0.5 ± 0.4	0.7 ± 0.3
	uninfected (3)	10.5	1.2	1.2	1.7	1.3	0.3	0.4
28	infected (6)	46.1 ± 68.3	1.9 ± 0.6	1.6 ± 0.4	2.2 ± 0.6	1.4 ± 0.3	0.9 ± 0.6	0.9 ± 0.4
	uninfected (2)	18.2	0.6	0.7	0.9	0.6	0.5	0.4
36	infected (5)	8.8 ± 5.9	4.6 ± 0.9	3.4 ± 1.1	4.5 ± 1.4	3.4 ± 1.3	1.0 ± 0.5	1.4 ± 0.6
	uninfected (4)	24.2	1.5	1.9	2.8	1.9	0.5	0.6
4 - 36	all uninfected controls	20.6 ± 15.3	1.4 ± 0.7	1.5 ± 0.5	2.2 ± 1.0	1.5 ± 0.6	0.6 ± 0.3	0.6 ± 0.3
	spleen:							
4	infected (6)	80.0 + 28.4	10.5 ± 5.5	6.0 ± 2.3				
	uninfected (3)	71.7	1.0	1.0				
12	infected (6)	22.0 ± 12.3	4.7 ± 2.3	4.0 ± 2.2				
	uninfected (2)	62.0	1.8	1.6				
20	infected (6)	8.8 ± 13.9	2.4 ± 2.6	2.0 ± 1.2				
	uninfected (3)	44.0	1.2	1.2				
28	infected (6)	8.0 ± 6.7	1.1 ± 0.6	1.4 ± 0.7				
	uninfected (2)	77.5	1.2	-				
	infected (5)	10.0 ± 8.1	1.3 ± 0.6	1.7 ± 1.0				
	uninfected (4)	108.7	1.0	1.0				
4 - 36	all uninfected controls	77.8 ± 38.9	1.4 ± 0.6	1.4 ± 0.9				

* number of animals

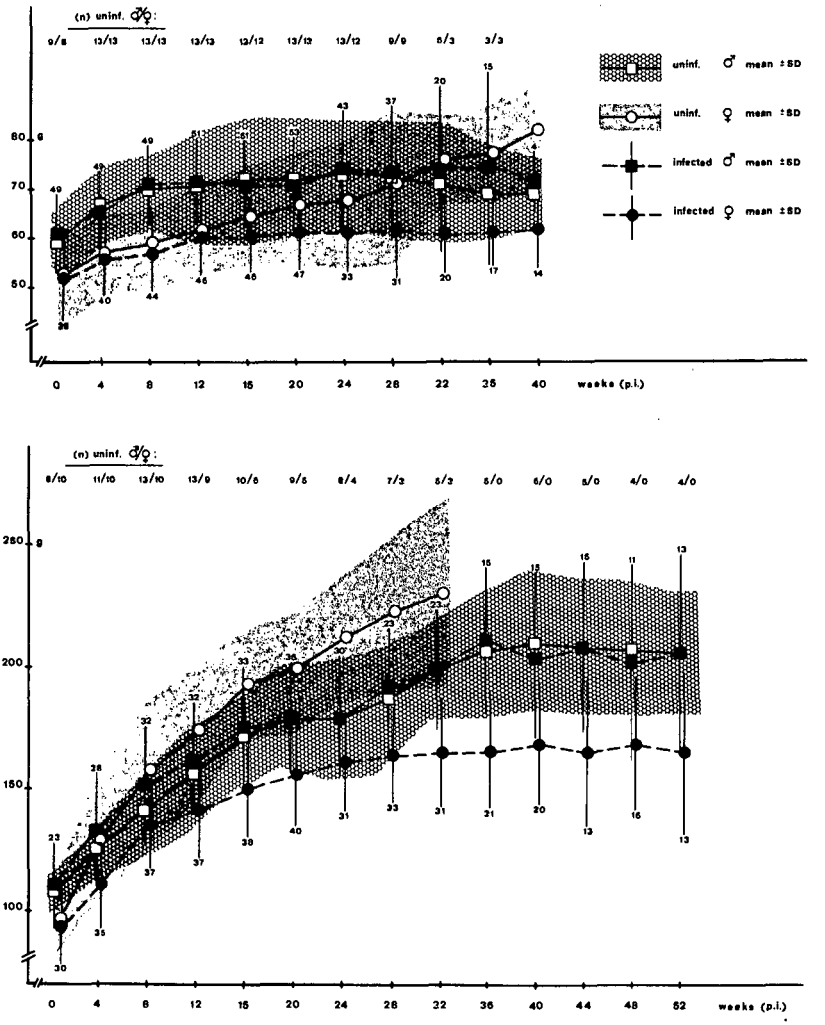
Table 17

Blast transformation assays with microfilarial antigen plus PHA: Stimulation indices (mean of quadruplicate cultures)

Assay no.	mf antigen		PHA	mf antigen + PHA	
	10 µg	2 µg		5 µg	1 µg
1	0.6	0.9	21.7	11.7	17.2
2	1.1	1.2	40.2	29.0	44.1
3	0.9	1.4	5.6	3.5	6.6
4	1.2	1.0	9.0	6.7	9.8

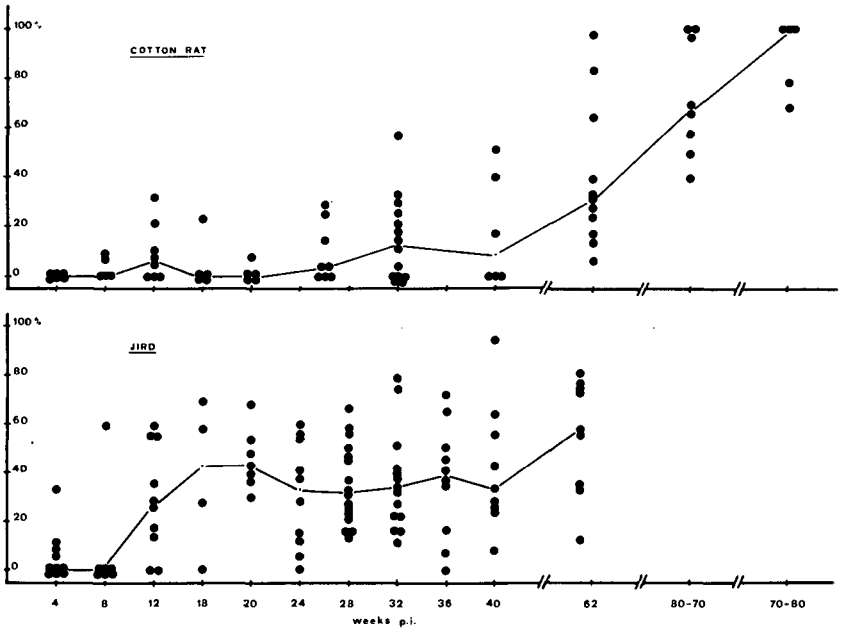
FIGURES

Figure 1



Bodyweight of infected and uninfected age-matched male and female jirds (top) and cotton rats (bottom). Number of animals (n) indicated in figure.

Figure 2



Encapsulated worms and nodules expressed as a percentage of the total weight of live worms and nodules found at autopsy. Symbols represent the values for individual animals and curves connect the medians.

Figure 3

Percentage distribution (mean ± SE) of embryonic egg stages from worms which had developed in cotton rats (top) and jirds (bottom) in relation to the age of the worms.

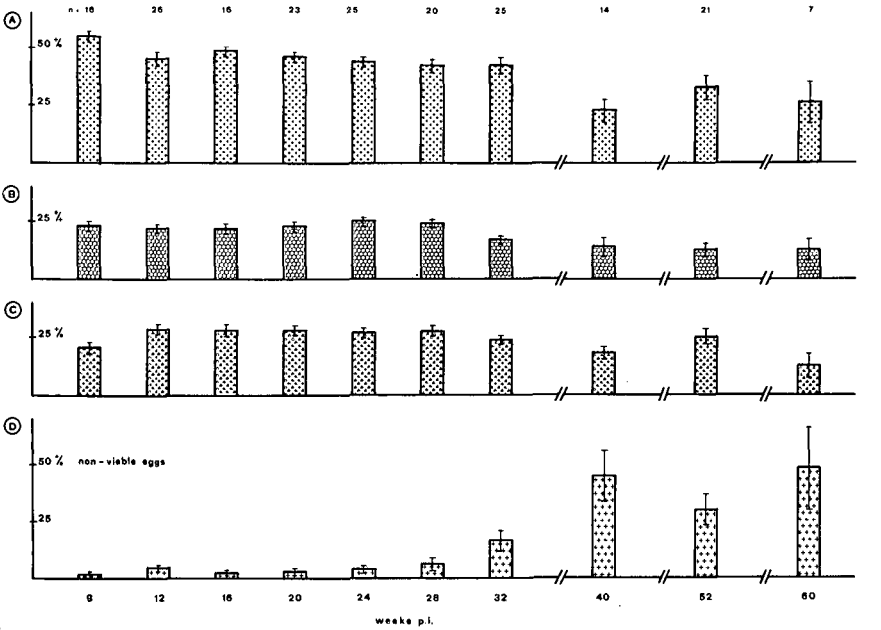
Ⓐ single to multiple cell stages (cf.Fig.14 b and c)

Ⓑ beginning of gastrulation to embryo stage showing head and tail (cf.Fig.14 d, e and f)

Ⓒ fully developed microfilariae still coiled up in vitelline membrane and extended microfilariae (cf.Fig.14 g and h)

Ⓓ non-viable eggs (cf.Fig.14 i, k and l)

Figure 3


n = 16 26 16 23 25 20 25 14 21 7
A
50 %
25
B
25 %
C
25 %
D
50 % non-viable eggs
25
8 12 16 20 24 28 32 40 52 60
weeks p.i.

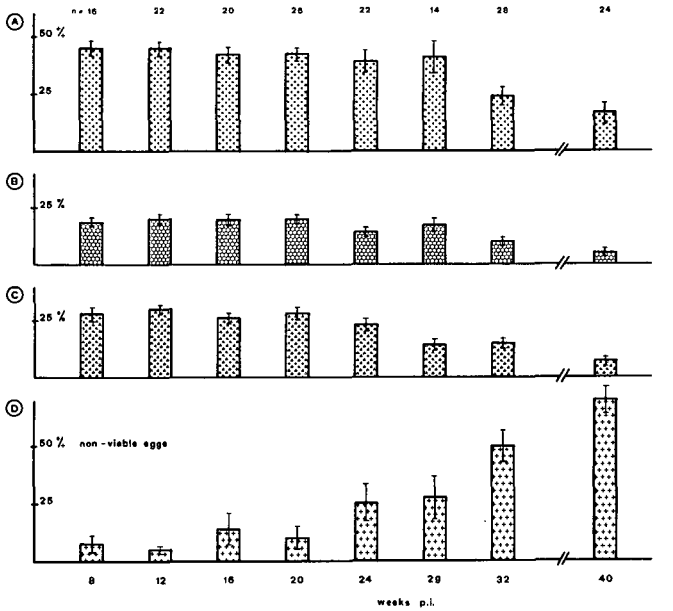
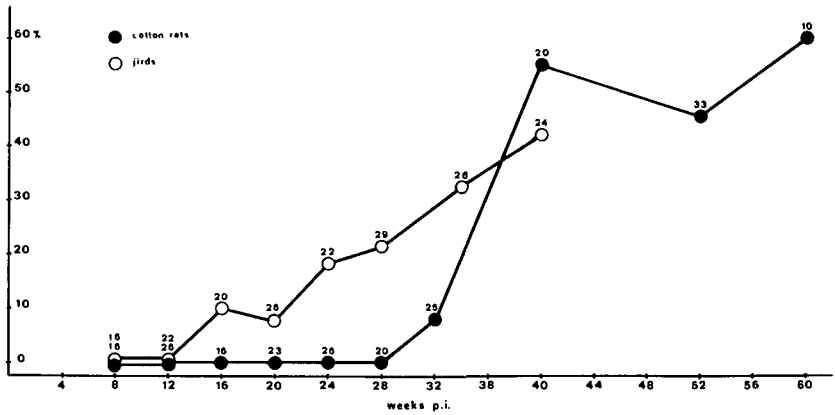
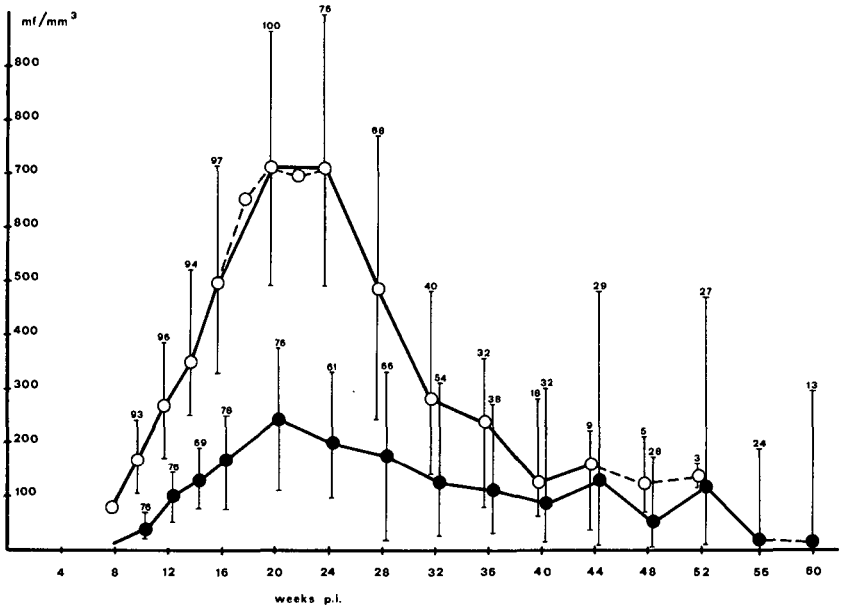

n = 16 22 20 26 22 14 28 24
A
50 %
25
B
25 %
C
25 %
D
50 % non-viable eggs
25
8 12 16 20 24 28 32 40
weeks p.i.

Figure 4



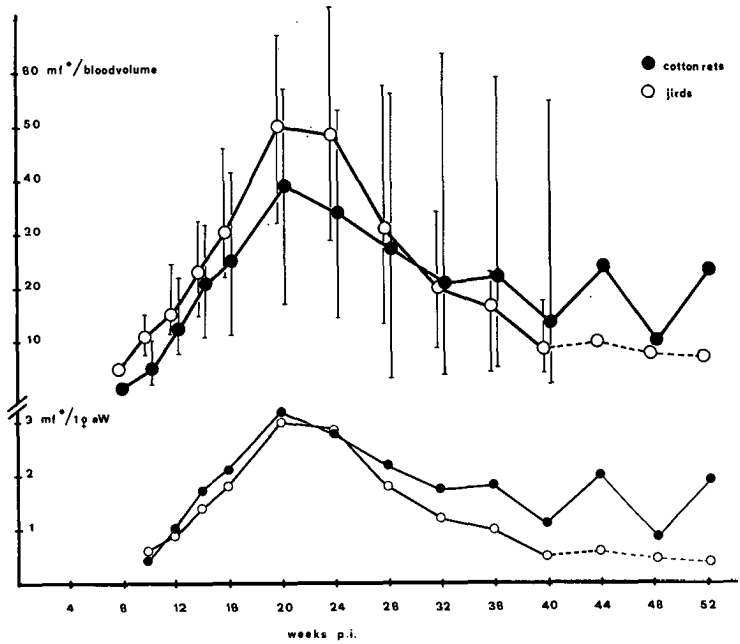
Percentage of female worms (mean) which contained only non-viable eggs calculated from the total number of females (n, indicated in figure) examined per time. Open symbols represent worms from jirds and closed symbols represent worms from cotton rats.

Figure 5



Course of microfilaraemia expressed as the median number of mf/mm^3 (round symbols) and quartiles (bars) in jirds (open symbols) and cotton rats (closed symbols). The number of animals examined per time is indicated in the diagram.

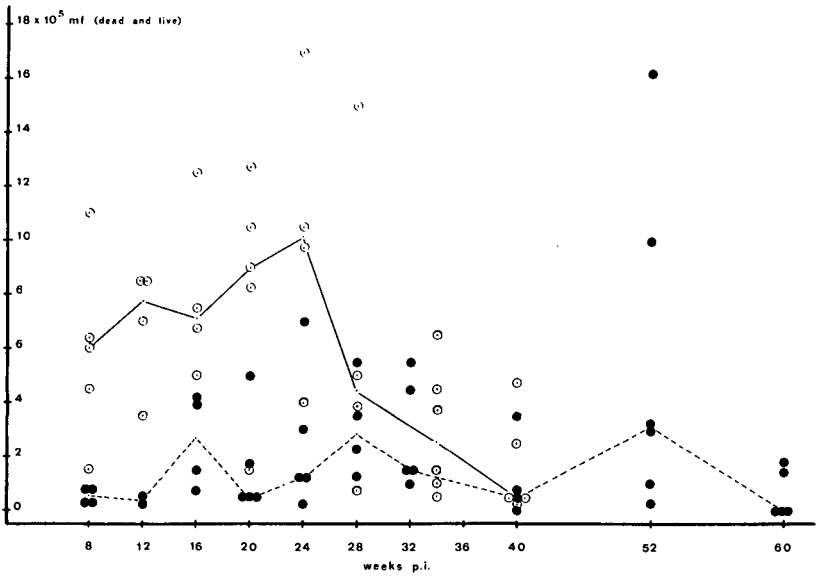
Figure 6



Course of microfilaraemia in relation to bodyweight (median and quartiles) of cotton rats and jirds (top), and median course of microfilaraemia in relation to bodyweight plus the mean number of female worms per host species (bottom). Symbols and number of animals as indicated in Fig.5.

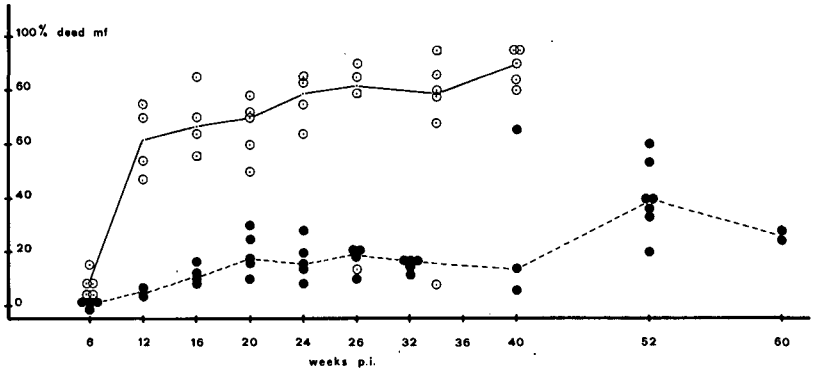
mf* = number of mf x 10^6 x F = median number of microfilariae in the entire blood volume (top) and approximate median number of microfilariae derived from a single female worm in the total blood volume (bottom). ($F = \frac{\text{blood volume}}{\text{bodyweight}}$)

Figure 7



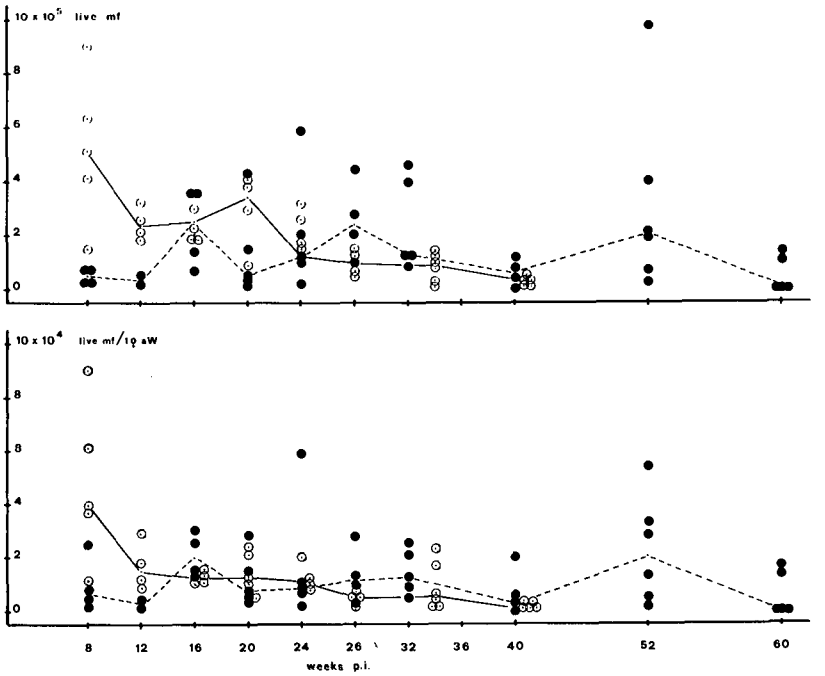
Total number of live and dead pleural microfilariae found at autopsy in individual jirds (open symbols) and individual cotton rats (closed symbols). Curves connect the medians of all jirds (———) and all cotton rats (-----) examined per time.

Figure 8



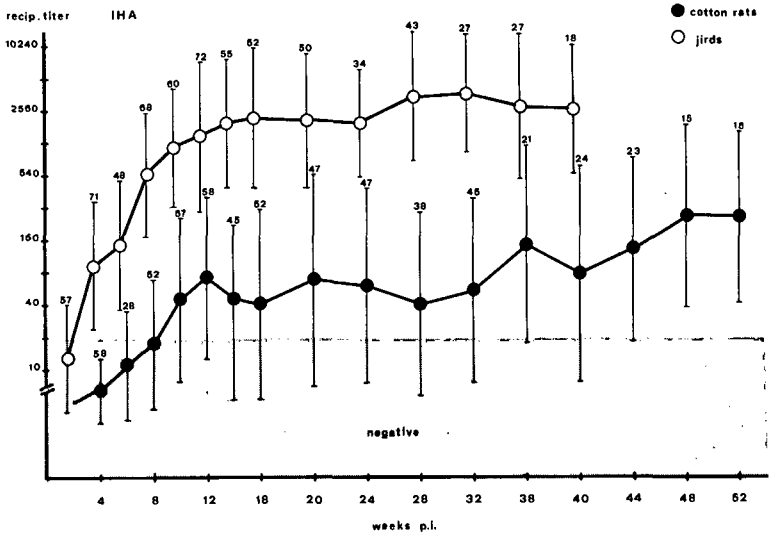
Percentage of dead pleural microfilariae in individual jirds (open symbols) and individual cotton rats (closed symbols). Curves connect the medians of all jirds (———) and all cotton rats (-----) examined per time.

Figure 9



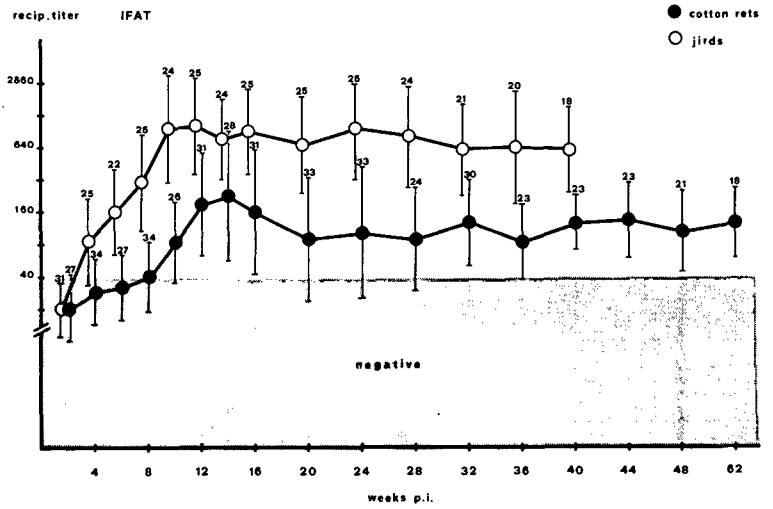
Total number of live microfilariae found at autopsy in the pleural cavity of each animal (top) and relative number of microfilariae per single female worm of each animal (bottom). Symbols represent individual values for jirds (open symbols) and for cotton rats (closed symbols). Curves connect the medians of all jirds (———) and all cotton rats (-----) examined per time.

Figure 10



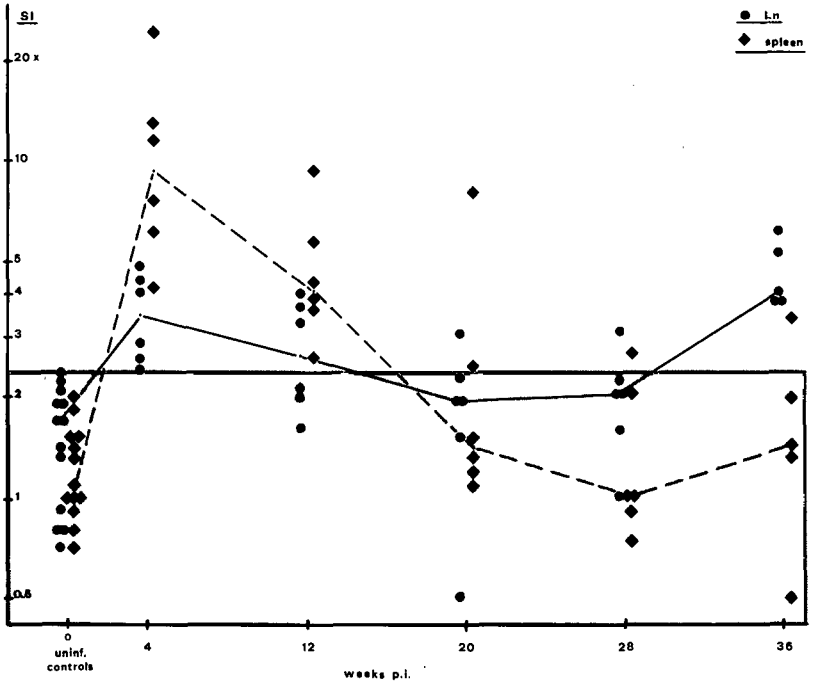
Development of the humoral antibody response tested by indirect haemagglutination (IHA) of infected cotton rats (closed symbols) and infected jirds (open symbols): geometric mean ± SD of reciprocal titers of the last positive dilution. The number of animals (n) tested per time is indicated in the diagram. The shaded area gives the background level of normal sera.

Figure 11



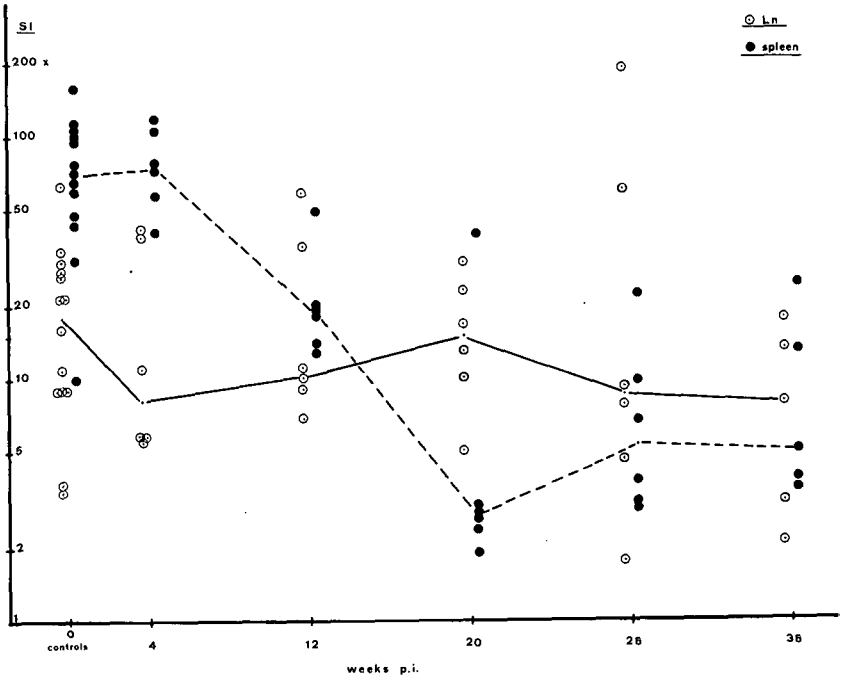
Development of the humoral antibody response tested by indirect immunofluorescence (IFAT) of infected jirds (open symbols) and infected cotton rats (closed symbols): geometric mean $\pm$ SD of reciprocal titers of the last positive dilution. The number of animals (n) tested per time is indicated in the diagram. The shaded area gives the background level of normal sera.

Figure 12



Blastogenic response of jird lymph node (Ln) cells and spleen cells to female adult worm antigen. Symbols represent the stimulation index (SI) of individual animals (mean of quadruplicate cultures) and curves represent the median course of the response of lymph node cells (———) and spleen cells (-----). The shaded area gives the range of stimulation indices obtained from uninfected age-matched controls. The individual values of these animals are plotted at point O.

Figure 13



Mitogenic response to phytohaemagglutinin of jird spleen cells and lymph node (Ln) cells. Symbols represent the stimulation index (SI) of individual animals (mean of quadruplicate cultures) and curves represent the median course of the response of spleen cells (-----) and lymph node cells (———). Values for all uninfected controls are plotted at point 0.

Figure 14

Development of microfilariae shown by phase-contrast micrographs.

a = oocytes

b = 2 - 4 cell stages

c = multiple cell stages

d, e, f = beginning of gastrulation to embryo stage showing head and tail

g = fully developed but still coiled up microfilaria

h = extended microfilaria

i, k, l = non-viable eggs

Magnification: a - g and i - l ~ 1420 x

h ~ 900 x

Fig. 14

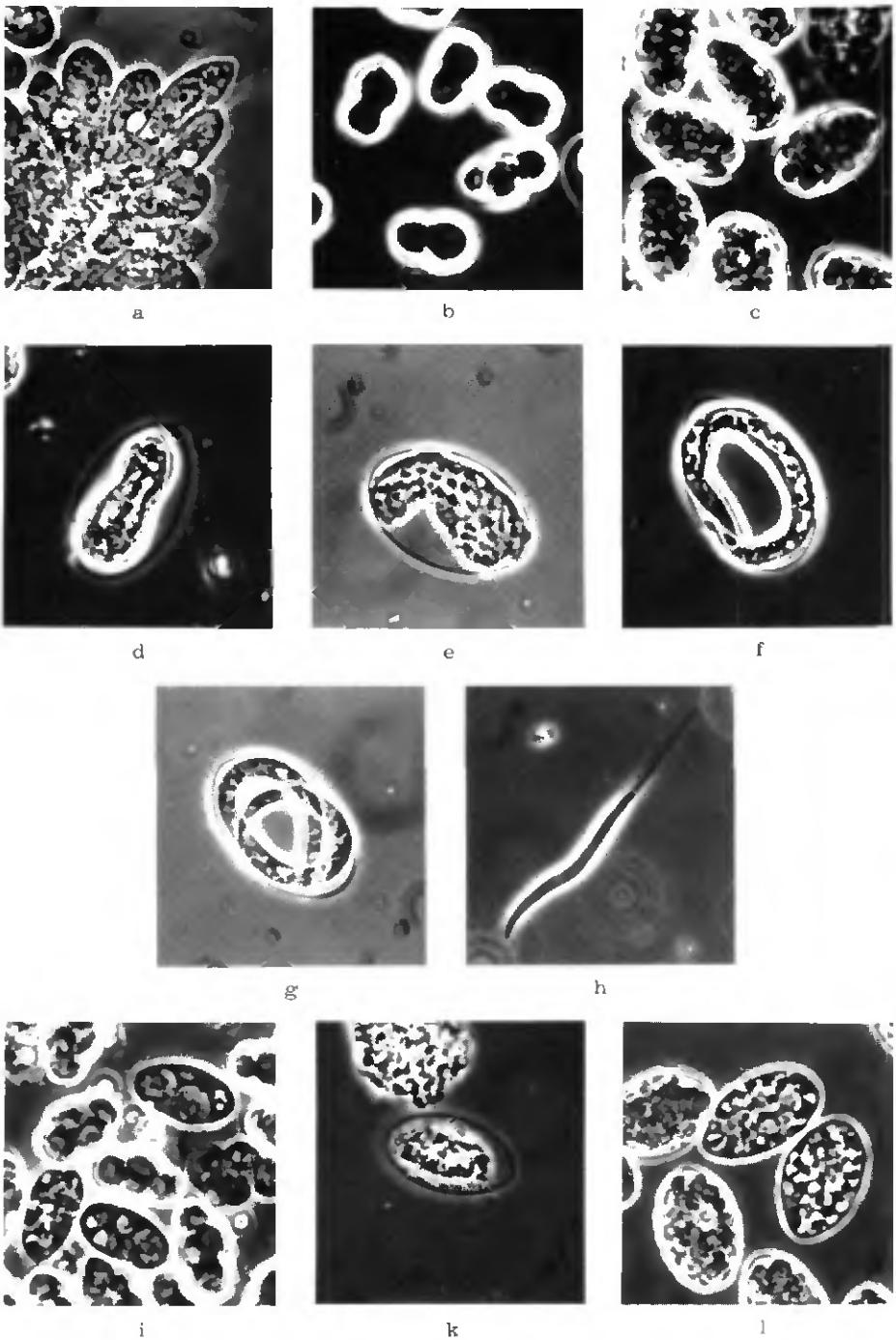


Figure 15

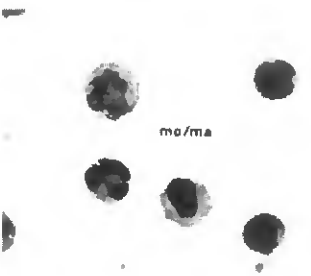
Cells of the pleural exudate of cotton rats stained with Giemsa.

mo/ma = monocytes and/or macrophages
am = activated macrophage
g = giant cell
bl = blast cell

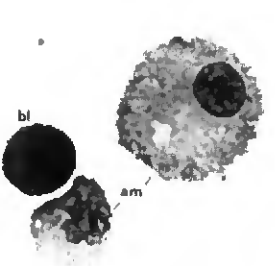
Magnification: ~ 1420 x

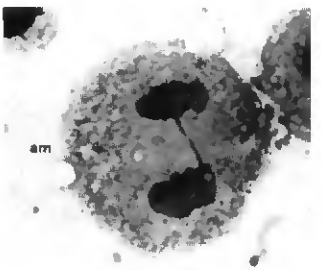
Fig. 15

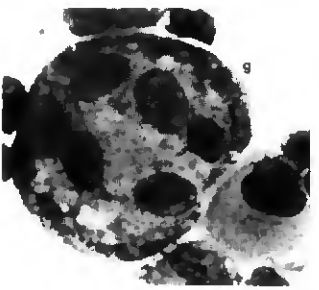
normal cotton rat



infected cotton rat







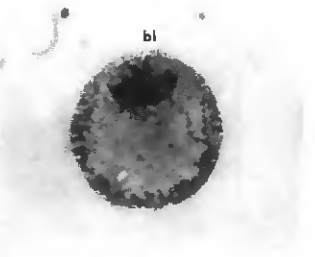


Figure 16

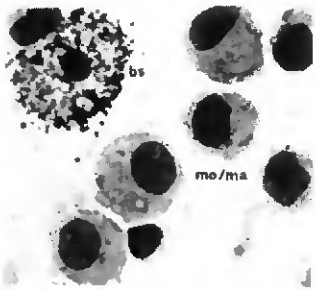
Cells of the pleural exudate of jirds stained with Giemsa.

mo/ma = monocytes and/or macrophages
bs = basophil (mast cell)
ly = lymphocyte
am = activated macrophage
g = giant cell
bl = blast cell
dm = degenerating macrophage
np = necrotic polymorphonuclear cell

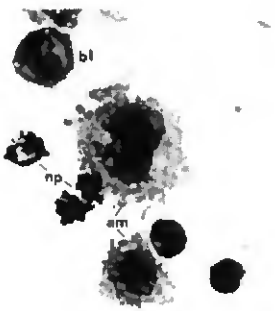
Magnification: ~ 1420 x

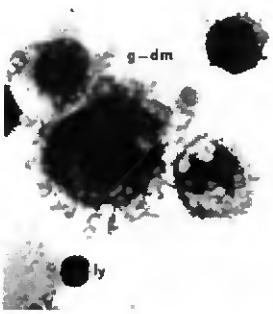
Fig. 16

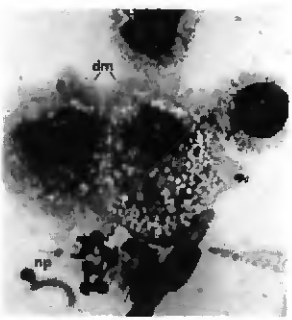
normal jird



infected jird







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Résumé

L'évolution d'une infestation à Litomosoides carinii chez Meriones unguiculatus (le mérion) a été suivie pendant 40 semaines. Parallèlement, le développement de la réponse immunitaire humorale a été étudié par hémagglutination et immunofluorescence indirectes. Enfin, les réactions immunologiques cellulaires au cours de l'infestation ont fait l'objet de tests de blastogenèse réalisés avec des antigènes filariens.

Toutes les expériences parasitologiques et immunologiques ont été entreprises simultanément chez le rat du coton, également infesté par L. carinii, afin de permettre la comparaison la plus directe possible de l'évolution de l'infestation et du développement de la réponse immunitaire chez l'hôte naturel (rat du coton) et chez l'hôte expérimental (mérion). Chez le rat du coton, la période d'observation a été étendue à 52 semaines p.i.

1. En règle générale, plus de larves infectieuses se sont developpées en vers adultes chez le mérion que chez le rat du coton.

2. Chez le mérion, les vers femelles adultes étaient plus courts que chez le rat du coton. En outre, les femelles qui se sont développées chez le mérion avaient également eu un plus grand nombre d'oeufs non viables. Chez les deux espèces d'hôtes, il a été observé qu'une augmentation de la quantité de ces oeufs anormaux allait de pair avec le vieillissement des vers; toutefois, ce phénomène a été plus précoce chez le mérion (à partir de la 24^{e} semaine p.i.) que chez le rat du coton (à partir de la 40^{e} semaine p.i.). En outre, il a été constaté une baisse simultanée du nombre total d'oeufs par ver. On peut en conclure que la fertilité des femelles a commencé à baisser aux moments indiqués ci-dessus et que cela correspondait à leur vieillissement (physiologique probablement).

C'est seulement à un stade avancé de l'infestation que l'on a observé une baisse significative du nombre des vers trouvés vivants à la dissection; toutefois, là encore, cette baisse est intervenue plus tôt chez le mérion (> 44 semaines p.i.) que chez le rat du coton (> 52 semaines p.i.). Il se pourrait qu'un manque d'aliments spécifiques soit à l'origine d'un vieillissement plus rapide et d'une mort plus précoce des vers chez l'hôte expérimental. En outre, il a été observé chez le mérion une réponse im-

munitaire à l'infestation plus forte que chez le rat, ce qui a pu constituer à la longue un stress supplémentaire pour le parasite.

A la dissection, il a été trouvé beaucoup plus de microfilaires libres dans la cavité pleurale du mérion que dans celle du rat du coton. Cela permet de supposer que la productivité des filaires femelles était plus élevée chez le mérion. Cette plus grande fertilité pourrait aussi expliquer le vieillissement plus rapide des vers.

3. Parmi les observations faites, rien n'indique qu'un nombre significatif de vers s'encapsulent déjà à un stade précoce de l'infestation et se trouvent ainsi éliminés. Toutefois, à un stade plus avancé de l'infestation, il a été constaté chez le rat du coton un rapport chronologique entre l'augmentation des encapsulations et la diminution du nombre des vers trouvés vivants à la dissection. De plus, les coefficients de corrélation de rang de Spearman entre le poids des capsules (calculé en % du poids global des vers vivants et des capsules) et le nombre de vers vivants étaient négatifs. Cela confirmait donc l'élimination de filaires adultes par encapsulation chez le rat du coton. Mais, chez le mérion, une augmentation significative du matériel d'encapsulation a été constatée dès la 12^{e} semaine p.i., donc bien avant qu'une diminution du nombre de vers vivants puisse être enregistrée (> 44 semaines p.i.). En outre, les coefficients de corrélation de rang entre les capsules et les vers mâles ont été positifs. Cela permet de supposer que chez le mérion la proportion importante de matériel d'encapsulation - décelée dès un stade précoce de l'infestation - n'a rien à voir avec l'élimination de parasites. La positivité des coefficients de corrélation tendrait plutôt à prouver que les vers vivants incitent (stimulent) l'hôte expérimental à former des capsules.

4. Pour pouvoir représenter l'évolution moyenne de la microfilarémie, il a été procédé au calcul de la médiane. Les deux types d'hôtes ont présenté une microfilarémie maximale entre la 20^{e} et la 24^{e} semaine p.i. L'évolution de la microfilarémie a été plus régulière chez le mérion puisque, chez la plupart des animaux de cette espèce, ses valeurs maximales étaient groupées entre la 20^{e} et la 24^{e} semaine p.i., alors que chez le rat du coton, des valeurs maximales pouvaient être observées à tout moment de l'infestation.

Le mérion a présenté une densité de microfilaires plus élevée (valeur médiane maximale 700 mf/mm^3) que celle du rat du coton (valeur médiane maximale 250 mf/mm^3). En outre, chez le mérion, le nombre des microfilaires a baissé rapidement après la 24^e semaine p.i. Toutefois, des calculs faisant intervenir le poids corporel de l'hôte et le nombre moyen de vers femelles permettent de supposer que le nombre de microfilaires qui passent dans la circulation sanguine pendant toute la durée de la microfilarémie est à peu près le même chez les deux hôtes. C'est pourquoi il n'est guère possible d'interpréter la chute rapide du nombre des microfilaires chez le mérion après la 24^e semaine p.i. comme la conséquence d'une suppression de la microfilarémie plus active. De plus, une amicrofilarémie a été plus rarement observée chez les mérions que chez les rats du coton. Cela indique également une suppression de la microfilarémie moins active chez l'hôte expérimental.

5. Le titre des anticorps humoraux était en général plus élevé chez le mérion que chez le rat du coton.

Après une augmentation initiale, les valeurs moyennes se sont stabilisées en plateau chez les deux espèces animales. Chez le mérion, l'augmentation du titre a déjà été observée au cours de la période de prépatence, tandis que chez le rat du coton elle ne l'a été qu'au début de la patence. Chez les deux variétés d'hôtes, on devrait pouvoir s'attendre au début de la patence à ce que le métabolisme intensifié des femelles fécondes et l'expulsion des microfilaires déclenchent une augmentation de la production des anticorps. Toutefois, cela n'a pu être mis en évidence que chez le rat du coton. On suppose que chez le mérion la patence s'accompagne d'une certaine incapacité à réagir plus fortement à des antigènes parasitaires supplémentaires (par exemple des cellules spléniques de mérion ont présenté une forte réduction de leur aptitude à réagir à la phytohémagglutinine à partir de la 12^e semaine p.i.).

Les coefficients de corrélation de rang de Spearman entre la réponse immunitaire humorale et la parasitémie ont été négatifs chez le rat du coton mais, à une exception près, positifs chez le mérion. Ce pourrait être un indice tendant à prouver l'existence d'un contrôle actif de la parasitémie chez le rat et d'une réponse immunitaire plutôt inefficace chez le mérion.

6. Les réactions immunologiques cellulaires ont été suivies grâce aux tests de blastogenèse. Chez le rat du coton (dans les conditions données d'expérimentation), il n'a été constaté de réaction aux antigènes filariens à aucun moment de l'infestation.

   En revanche des cellules de ganglions lymphatiques et de rate du mérion ont pu être stimulées la 4^e et la 12^e semaine p.i. par des antigènes filariens mâles et femelles. Au cours de la 20^e et de la 28^e semaine p.i. aucun de ces deux types de cellules n'a plus présenté de réaction. A la 36^e semaine p.i., les cellules des ganglions lymphatiques réagissaient de nouveau aux stimulations.

   La réduction de l'aptitude des cellules à subir une blastogenèse - réduction enregistrée au début de l'infestation - s'est accompagnée d'une augmentation de la réponse immunitaire humorale.

   Ni les cellules des ganglions lymphatiques, ni celles de la rate n'ont réagi aux antigènes microfilariens.

7. Pendant la patence, les cellules spléniques du mérion infesté ont présenté, parallèlement à leur aptitude à être stimulées par des antigènes filariens, une capacité de réaction à la phytohémagglutinine fortement réduite. Peut-être est-ce là un nouvel indice tendant à prouver que l'infestation déclenche chez le mérion un effet immunosuppresseur et empêche ainsi qu'il se produise une réponse immunitaire pleinement efficace.

8. Les réactions non spécifiques contre le parasite étaient plus fortes chez le mérion que chez le rat du coton. Mis à part les sévères altérations pleuro-pulmonaires, on a observé une splénomégalie plus importante et une plus forte mortalité.

9. Des altérations importantes de la population cellulaire de l'exsudat pleural ont été constatées dès le début de l'infestation (4 semaines p.i.) ce qui indique une forte réaction aux protéines étrangères chez les deux espèces. Chez le rat du coton, de grands macrophages activés, des cellules géantes et de grands blastocytes ont fait leur apparition. Chez le mérion, en revanche, outre des macrophages activés, il a été observé essentiellement des granulocytes nécrotiques. En outre, de nombreux macrophages présentaient des signes de dégénérescence. Une

augmentation significative du nombre des cellules a été constatée chez le mérion mais pas chez le rat du coton. L'influence de ces réactions sur la relation hôte-parasite n'est pas définie.

10. Malgré les réactions spécifiques et non spécifiques plus fortes, l'infection s'est développée tout aussi bien chez le mérion. On peut ainsi supposer que le mérion est moins capable de contrôler efficacement une infestation à L. carinii que l'hôte naturel.

Zusammenfassung

Der Verlauf einer Litomosoides carinii-Infektion in Meriones unguiculatus wurde über die Dauer von 40 Wochen verfolgt. Gleichzeitig wurde die Entwicklung der humoralen Immunantwort mit Hilfe von indirekter Haemagglutination und indirekter Immunfluoreszenz untersucht. Zur Abklärung von zellulären Immunreaktionen im Verlauf der Infektion wurden Blasttransformationstests mit Filarienantigen durchgeführt.

Sämtliche parasitologischen und immunologischen Experimente wurden gleichzeitig auch an mit L. carinii infizierten Baumwollratten (Sigmodon hispidus) unternommen, um soweit wie möglich den Infektionsverlauf und die Entwicklung der Immunantwort im natürlichen Wirt (Baumwollratte) und im experimentellen Wirt (Meriones) direkt miteinander vergleichen zu können, wobei die Beobachtungszeit bei Baumwollratten auf 52 Wochen p.i. ausgedehnt wurde.

1. Die Infektionsrate (Anzahl der bei der Sektion gefundenen Makrofilarien in Prozent der injizierten Larven) war bei Meriones allgemein eher höher als bei Baumwollratten.

2. In Meriones waren die adulten weiblichen Makrofilarien kürzer als in Baumwollratten. Weibchen, die sich in Meriones entwickelt hatten, enthielten auch vermehrt anomale Eistadien. Eine Zunahme dieser anomalen Eier mit zunehmendem Alter der Würmer wurde bei beiden Wirtstierarten festgestellt; jedoch früher bei Meriones (ab 24. Woche p.i.) als bei Baumwollratten (ab 40. Woche p.i.). Eine gleichzeitige Abnahme der Gesamt-Eizahl pro Wurm lässt auf eine beginnende verminderte Fertilität der Weibchen zu den oben erwähnten Zeitpunkten und damit auf ein (wahrscheinlich physiologisch bedingtes) Altern schliessen.

   Eine signifikante Abnahme der bei der Sektion gefundenen lebenden Makrofilarien wurde erst zu einem späten Zeitpunkt der Infektion beobachtet; jedoch wiederum früher bei Meriones (> 44 Wochen p.i.) als bei Baumwollratten (> 52 Wochen p.i.). Ein Mangel an spezifischen Nahrungsangeboten im experimentellen Wirt könnte ein rascheres Altern und den früheren Tod der Würmer bewirken. Die im allgemeinen stärkere humorale Immunantwort der Meriones auf die Infektion könnte auf die Dauer auch einen zusätz-

lichen Stress für den Parasiten bewirken. Andererseits wurden bei der Sektion in der Pleuralhöhle der Meriones viel mehr freie Mikrofilarien gefunden als bei Baumwollratten. Damit kann eine grössere Produktivität der weiblichen Filarien in den Meriones vermutet werden. Das raschere Altern der Makrofilarien könnte daher auch das Resultat dieser gesteigerten Fertilität sein.

3. Es konnte kein Anhaltspunkt dafür gefunden werden, dass bereits in einem frühen Stadium der Infektion eine bedeutende Anzahl Würmer durch Einkapselung (Kapselbildung) eliminiert werden. Bei Baumwollratten bestand in älteren Infektionen ein zeitlicher Zusammenhang zwischen vermehrter Kapselbildung und der Abnahme der bei der Sektion gefundenen lebenden Makrofilarien. Bei Meriones hingegen wurde eine signifikante Zunahme an Kapselmaterial bereits in der 12. Woche p.i. festgestellt, d.h. lange bevor eine Abnahme der Makrofilarienzahlen beobachtet werden konnte. Entsprechend ergaben Spearman Rank-Korrelationen zwischen dem Kapselgewicht und der Anzahl lebender Makrofilarien negative Korrelationskoeffizienten bei Baumwollratten, jedoch positive Werte bei Meriones. Dies lässt vermuten, dass die Kapselbildung bei Meriones hauptsächlich eine Wirtsreaktion auf lebende Würmer ist, die nicht, wie dies bei Baumwollratten der Fall zu sein scheint, mit einer Kontrolle über den Parasiten in Verbindung gebracht werden kann.

4. Zur Darstellung des mittleren Verlaufs der Mikrofilaraemie wurde der Median berechnet. Dabei zeigten beide Wirtstierarten maximale Mikrofilaraemiewerte zwischen der 20. und 24. Woche p.i. Der Verlauf der Mikrofilaraemie war regelmässiger bei Meriones, indem bei den meisten Tieren die höchsten Mikrofilarienwerte auf die Zeit zwischen der 20. und der 24. Woche p.i. konzentriert waren. Bei Baumwollratten konnten maximale Mikrofilarienzahlen zu praktisch jedem Zeitpunkt der Infektion auftreten.

   Meriones zeigten eine höhere Mikrofilariendichte als Baumwollratten. Zusätzlich zeigte sich bei den Meriones ein rapider Abfall der Mikrofilarienzahl nach der 24. Woche p.i. Jedoch, Berechnungen bei denen das Körpergewicht des Wirtes und die durchschnittliche Anzahl weiblicher Würmer miteinbezogen wurden, lassen vermuten, dass für die ganze Dauer der Mikrofilaraemie bei beiden Wirten ungefähr die gleiche Anzahl Mikro-

filarien in die Blutzirkulation gelangt. Daher kann der rapide Abfall der Mikrofilarienzahlen bei Meriones nach der 24. Woche p.i. kaum als eine aktivere Unterdrückung der Mikrofilaraemie interpretiert werden.

Amikrofilaraemie wurde bei Baumwollratten häufiger beobachtet als bei Meriones. Dies kann ein weiterer Hinweis auf eine möglicherweise schwächere Kontrolle des experimentellen Wirtes über die Mikrofilaraemie sein.

5. Humorale Antikörper waren bei Meriones generell höher als bei Baumwollratten. Nach einem anfänglichen Anstieg erreichten bei beiden Wirtstierarten die Durchschnittswerte ein Plateau. Bei Meriones wurde der Titeranstieg während der Präpatenz beobachtet, bei Baumwollratten hingegen erst zu Beginn der Patenz. Bei beiden Wirtstierarten sollte mit beginnender Patenz eine erhöhte Antikörperproduktion erwartet werden können, die durch den gesteigerten Stoffwechsel fertiler Weibchen und die Ausschüttung von Mirkrofilarien ausgelöst wird. Es könnte jedoch sein, dass bei Meriones im Zusammenhang mit der Patenz eine gewisse Unfähigkeit stärker auf zusätzliches Parasitenantigen zu reagieren einsetzt (z.B. zeigten Meriones-Milzzellen eine stark verminderte Reaktionsfähigkeit auf Phytohaemagglutinin von der 12. Woche p.i. an).

   Spearman Rank-Korrelation zwischen humoraler Immunantwort und Parasitaemie waren negativ bei Baumwollratten, jedoch (mit einer Ausnahme) positiv bei Meriones. Dies könnte ein Hinweis auf eine aktive Kontrolle der Parasitaemie bei Baumwollratten und auf eine eher wirkungslose Immunantwort bei Meriones sein.

6. Zelluläre Immunreaktionen wurden mit Hilfe von Blasttransformationstests verfolgt. Bei Baumwollratten wurde (unter den gegebenen experimentellen Bedingungen) zu keinem Zeitpunkt der Infektion eine Reaktion auf Filarienantigen festgestellt.

   Lymphknoten - und Milzzellen von Meriones hingegen konnten 4 und 12 Wochen p.i. mit männlichem und weiblichem Makrofilarienantigen stimuliert werden. Während der 20. und 28. Woche p.i. zeigten beide Zelltypen keine Reaktion mehr. 36 Wochen p.i. waren Lymphknotenzellen erneut stimulierbar.

Die Abnahme der Blasttransformations-Fähigkeit der Zellen zu Beginn der Infektion fiel zeitlich mit der Zunahme der humoralen Immunantwort zusammen.

Weder Lymphknoten noch Milzzellen zeigten je eine Reaktion auf Mikrofilarienantigen.

7. Parallel zur Stimulierbarkeit mit Filarienantigen zeigten die Milzzellen infizierter Meriones eine stark verminderte Reaktionsfähigkeit auf Phytohaemagglutinin während der Patenz. Dies kann ein weiteres Anzeichen dafür sein, dass die Infektion bei Meriones einen immunsuppressiven Effekt auslöst und damit eine vollwirksame Immunantwort verunmöglicht wird.

8. Meriones zeigten stärkere unspezifische Reaktionen als Baumwollratten; insbesondere eine starke Splenomegalie und eine hohe Sterberate zwischen der 20. bis 28. Woche p.i., also zum Zeitpunkt der durchschnittlich höchsten Mikrofilaraemie-Werte.

9. Schon zu Beginn der Infektion (4 Wochen p.i.) wurden bedeutende Veränderungen der Zellpopulation des Pleuralexsudates festgestellt, die eine starke Reaktion auf Fremdprotein vermuten lassen. Grosse aktivierte Makrophagen, Riesenzellen und Blastzellen erschienen bei den Baumwollratten. Bei Meriones hingegen waren neben aktivierten Makrophagen hauptsächlich nekrotische Granulozyten zu beobachten. Zudem zeigten viele Makrophagen Degenerationserscheinungen. Die Gesamtzahl der Zellen nahm bei Meriones signifikant zu, bei Baumwollratten jedoch nicht. Die Bedeutung und der Einfluss dieser Reaktionen auf das Wirt-Parasit-Verhältnis sind ungewiss.

10. Da Meriones trotz stärkerer spezifischer und unspezifischer Reaktionen mindestens ebenso empfänglich für die Infektion waren wie Baumwollratten und die Mikrofilaraemie nicht aktiver unterdrückt wurde, kann vermutet werden, dass die Meriones zu einer effektvollen Kontrolle über den Parasiten weniger fähig sind als der natürliche Wirt.