SUMMARY

The purpose of the work recorded in this thesis was to investigate aspects of the several stages of the lungworm Aelurostrongylus abstrusus together with the effects of the parasite on intermediate, paratenic and final hosts.

With regard to first-stage larvae, an estimation of the number produced by infected cats revealed that millions of those forms were excreted over a patent-period of between nine and ten weeks. Furthermore, such larvae had the ability to survive dehydration and extremes of temperature for varying periods of time.

Aelurostrongylus abstrusus requires an intermediate host in which to complete its life-cycle and, in the past, there has been controversy as to the role of the mouse in that event. Experiments, herein described, indicated that, although the latter species was not a true intermediate host for the lungworm, it was able to act as a paratenic host and store third-stage larvae for at least four months. It was also confirmed that a snail, Helix aspersa, was a satisfactory intermediate host and that first-stage larvae invaded the sole of that mollusc, migrated into the deeper tissues during the course of the next twenty-four to forty-eight hours and, in twenty-three days, developed into third-stage infective larvae. The latter forms remained viable for the life-time of the snail. The majority of larvae was recovered from the muscular sole and,

although a substantial number was extracted from other parts of the snail, it was considered that infection of the mollusc resulted from penetration of the pedal epithelium by, rather than ingestion of, larvae. The possibility of the existence of molluscan immunity was discussed.

The effects of the parasite on the final host were ascertained. At least 100 third-stage larvae were necessary for successful parasitic infestation of the cat although, at that dosage, little clinical upset ensued. Numbers of 1,600 larvae, and more, produced increasingly severe clinical signs and pulmonary pathological changes while an infecting dose of 3,200 larvae was considered almost certain to be fatal. Because of difficulties inherent to the procedure of oral infection of cats, a parenteral method was tried but was found to produce inconstant results.

Many metazoan parasites induce, in their hosts, an immunity which persists for a varying period of time and protects against further attack by the same parasite. Aelurostrongylus abstrusus was found not to be an exception and cats infested by the parasite 388 days earlier withstood re-infection. The outcome of an experiment, in which resistance to lungworm disease was passively transferred to cats by means of serum obtained from recovered animals, emphasised the importance of humoral antibodies in parasitic immunity. Following upon the results of the above experiments, the possibility of inducing active immunity in cats was considered. To that end, small numbers

of unattenuated, infective larvae were administered to cats on three occasions and at monthly intervals and, at the end of the immunising regime, the animals were challenged with pathogenic doses of third-stage larvae. The results of that investigation showed that it was feasible to vaccinate cats against infection by Aelurostrongylus abstrusus. That section of the thesis was completed by an assessment of the effects of the lungworm on the pulmonary vasculature of the cat. Nine days after infestation, hypertrophic change was noticeable in the medial coat of branches of the pulmonary artery and the arteriopathy was found to persist for a period of at least two years. Such evidence confirmed the previously-held opinion of the author that spontaneous pulmonary arterial disease of the cat arose as a result of infestation, past or present, by Aelurostrongylus abstrusus.

The final part of the work was devoted to a study of diagnosis and treatment of feline parasitic pneumonia. The results of radiological examination showed that such a facility was of value in recognition of the disease. Of additional diagnostic worth was the Indirect Fluorescent Antibody technique by which procedure specific immunofluorescence was demonstrated to occur in sera taken from lungworm-infested cats. It was considered that the above examinations together with careful clinical observation were able to provide sufficient proof of the presence of lungworm disease.

With regard to therapy, diethylcarbamazine citrate was administered to a group of infected animals but examination of faecal larval output as well as post-mortem study of the lungs of the cats showed that the drug had been unable to control or cure the condition. When six oral doses of the anthelmintic, tetramisole, were given to infected cats it was discovered that all stages of the parasite had been either eliminated or destroyed. However, the substance proved to be unpalatable to cats and evoked excessive salivation. To overcome that problem, resort was made to parenteral administration. It was then found that fairly small amounts of the chemical were toxic to, and caused death in, a number of experimental animals. Numerous doses were still required to abolish excretion of first-stage larvae from the faeces of treated animals and it was decided that parenterally-administered tetramisole had little advantage over that given orally.

The thesis was concluded by a review of the work completed, and of the problems yet unsolved, with regard to the many facets of the lungworm, Aelurostrongylus abstrusus.

A STUDY OF *AELUROSTRONGYLUS ABSTRUSUS* WITH SPECIAL REFERENCE TO PATHOGENICITY

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THESIS

submitted to

THE UNIVERSITY OF GLASGOW

for the degree of

DOCTOR OF PHILOSOPHY

The Pathology Department,
University of Glasgow Veterinary School,
Glasgow.

April 1968

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ACKNOWLEDGMENTS

The author desires to record his gratitude to Professor J.W. Emslie for the facilities, generously made available, which enabled the investigations to be pursued and for advice and criticism given during preparation of this thesis. For general advice on the parasitological detail the author is indebted to Dr. A. Dunn, Senior Lecturer in Veterinary Parasitology. To Mr. W. Penny, F.I.M.L.T. and his technical staff thanks are due for the vast amount of histological preparations provided over the years. The author is grateful to Mr. A. McCaw and his staff for expert care and maintenance of the experimental animals. Mr. A. Finnie, with customary skill, provided the illustrations of morbid anatomical material and Mrs. E. Nisbet conscientiously typed the manuscript. Experiments One, Two and Three of Part One were carried out with the aid of Mr. A. McCaw, technician-in-charge of the animal house, and Experiment Two of Part Three was done in collaboration with Mr. R.J. Roberts, Lecturer in Veterinary Pathology. To all of the above persons, the author extends his sincere thanks.

INTRODUCTION

The presence of lungworms in the cat was first recorded by Leuckart (1867) although the author was of the opinion that he was dealing with Ollulanus tricuspis. Cobbold (1885), in the course of a lecture given in Great Britain, described the existence of lungworms in two cats and, in Germany, Muller (1890) recorded a new lungworm which he called Strongylus pusillus. Railliet (1898), on the grounds that the latter name was pre-empted, proposed the designation Strongylus abstrusus but, in the light of further investigation, Railliet and Henry (1907) transferred the species to the genus Synthetocaulus, which allocation was accepted until 1927 when Cameron created the currently approved genus Aelurostrongylus.

The various stages in the life-cycle of the nematode have been described by Cameron (1927), Fry and Stewart (1932), Hobmaier and Hobmaier (1935b), Gerichter (1949), Blaisdell (1952) and Mackerras (1957). While minor variations are to be found among recorded descriptions of the parasite, close agreement prevails with regard to the important anatomical features and the following account is based on that given by Mackerras (1957).

The adult forms are thin, delicate worms which are enclosed in a togmental sheath. On average, the males are 5.2 mm. in length by 0.07 mm. in breadth and females are 9.3 mm. long by 0.1 mm. broad. The short, club-shaped oesophagus is about 0.25 mm. in length while the anal orifice is located 0.26 mm., and the vulva

0.076 mm., from the tip of the tail. The female has two parallel uteri which unite to form a common vagina while, in the male, the spicules are identical, curved, striated rods 0.12 - 0.13 mm. in length. The gubernaculum consists of two parallel, uniform elements, 0.018 mm. long, which combine at the distal ends.

The egg, a delicate structure with a thin, clear shell, measures 63 - 84 microns long by 54 - 66 microns broad and may exhibit all stages of segmentation up to fully-formed larvae.

First-stage larvae from the cat are 0.36 - 0.4 mm. in length and 0.018 - 0.02 mm. in width while the oesophagus extends for about 0.15 mm. and is provided with median and posterior bulbs. A double-notched tail is of recognitory value.

Third-stage infective larvae, which develop in molluscs after two moults, are larger than the preceding form and measure 0.52 - 0.53 mm. long by 0.03 mm. wide. The oesophagus is 0.2 mm. in length and has a long, narrow anterior part with a clavate posterior section while chitinous thickenings and a knob-like anterior end are also prominent. The tail is 0.038 mm. long and ends in a characteristic rounded swelling.

Fourth-stage larvae are slightly larger still and approximate 0.88 by 0.045 mm., but the oesophagus is without chitinous thickenings and the tail has lost its rounded protuberance.

During the fifth, or final stage larvae develop bursae and spicules in the case of the male and vulva and vagina in the

instance of the female and growth in size continues until adulthood.

Aelurostrongylus abstrusus belongs to the family Metastrongylidae and with most other members of that group shares a complex life-cycle. Leuckart (1867) believed that lungworms required an intermediate host and attempted to pass the worms through mice and thence back to the cat. His experiments appear to have been of negative effect but, because of confusion between Ollulanus and what is now called Aelurostrongylus, the results are difficult to evaluate. Cameron (1927) claimed to have completed the life-cycle of the parasite by the use of mice as the intermediate host. Hobmaier and Hobmaier (1935a) failed to reproduce the disease in cats by the same procedure but, after much investigation, found that a variety of slugs and snails acted as suitable hosts and, in addition, discovered that animals, such as toads, frogs, lizards and snakes together with birds of various kinds and small rodents, behaved as auxiliary or storage hosts. The researches of Gerichter (1949), Blaisdell (1952) and Mackerras (1957) supported the above findings.

Opinions on the habitat of the adult nematodes gave rise to further controversy. Cameron (1928) was of the belief that they lived within branches of the pulmonary artery but Blaisdell (1952), Mackerras (1957) and Hamilton (1963 & 1966a) failed to confirm that notion and, instead, described the alveoli and alveolar ducts as

the residential sites of the adult parasites.

The occurrence of Aelurostrongylus abstrusus in the lungs of cats has been reported from many parts of the world, thus: in Britain by Cameron (1926, 1927, 1928 and 1932), Lewis (1927), McKenzie (1960) and Hamilton (1963 and 1966b); in America by Fry and Stewart (1932), Hobmaier and Hobmaier (1935b), Pritchett (1938), Bailey and Williams (1949), Bailey and Lowman (1952), Blaisdell (1952), Newberne (1953) and Suddath (1955); in France by Baron (1946); in the Netherlands by Baudet (1933); in Portugal by da Cruz and de Freitas (1948); in Denmark by Christensen et al. (1946); in Palestine by Gorichter (1949) and in Australia by Gordon (1933), Seddon (1947) and Mackerras (1957). An incidence-rate of 2.0%, 5.0%, 1.0% and 26.0% was reported by Hobmaier and Hobmaier (1935b), Baudet (1933), Christensen et al. (1946) and Gorichter (1949), respectively, while in Britain, Lewis (1927) recorded that 19.3% of 155 cats were affected and Hamilton (1966b) found that 6.6% of 256 animals had suffered from lungworm disease.

Many of the articles reflect the bionomics of the parasite rather than the effects on the final host and the most complete picture of the disease produced by Aelurostrongylus abstrusus has been given by Blaisdell (1952) and by Hamilton (1963 and 1966a). As a rule, clinical signs consist of coughing, particularly after handling, and some increase in respiratory rate. If infestation has been heavy, reduced appetite, increasing lassitude, loss of bodily condition, severe coughing and dyspnoea

may be noted and, on auscultation, adventitious pulmonary sounds are easily appreciable. Pyothorax may be present and, in extreme instances, death from respiratory failure may supervene. Haematologically, early leucopenia is followed by a leucocytosis in which eosinophil leucocytes play a major role and the sedimentation rate of erythrocytes is markedly increased. The prepatent period of the infestation lasts from five to nine weeks and patency, during which first-stage larvae may be recovered in large numbers from the faeces, lasts from eight to thirteen weeks although Blaisdell (1952) claimed to have recovered larvae for up to two years.

At autopsy, lesions are confined to the thoracic cavity and vary in character from animal to animal. At least, they consist of multiple, pale-white foci of pin-point size that are found throughout the substance and in all the lobes of the lungs. In more severe cases, larger lesions - up to two centimetres in diameter and caused by coalescence of adjacent foci - are to be found and, should they occur sub-pleurally, they project from the surface to impart a nodular appearance to the organ. In many instances, lobes may be entirely consolidated and the lungs enlarged. On section, lesions are often crumbly in character and from them a small amount of pus-like fluid may be expressed. Exceptionally and associated with involvement of the parietal pleura, a thick, milky exudate, rich in eggs, larvae and cellular

components, fills the thoracic cavity. Enlargement of the bronchial lymph nodes, sometimes considerable, is always present.

Histopathologically, the initial lesions are small foci comprised of lymphocytes, macrophages and eosinophil leucocytes that surround eggs and larvae in the parenchyma. Perivascular and peribronchial lymphocytic hyperplasia and slight hypertrophic changes in the medial coat of some branches of the pulmonary artery are also apparent. In other cases, the lesions consist of masses of developing eggs and larvae closely invested by a dense, cellular reaction in which eosinophil leucocytes and multinucleated giant-cells are particularly outstanding. Such foci often undergo contral necrosis. Adult parasites situated mainly in alveoli and alveolar ducts are appreciable while peribronchial, peribronchiolar and perivascular lymphocytic hyperplasia, sometimes follicular and at other times diffuse in type, is conspicuous. Many bronchi and bronchioles contain eggs, larvae and cellular exudate and ulceration and reactive hyperplasia of the bronchial mucosa may be present. Alveolar epithelialization together with emphysema and hypertrophy of the muscle of the walls of the bronchioles and alveolar ducts are common.

A prominent feature in the lungs of all animals infested by Aelurostrongylus abstrusus is the hypertrophic, sometimes hyperplastic, change induced in the media of the muscular pulmonary arteries. The mildest cases show thickening of up to several

times that of normality, while in the most severe instances, the alteration causes almost complete obliteration of the lumina. Sometimes, local occlusion of a vessel occurs but affected arteries are usually diffusely thickened and, although such changes are often widespread throughout the lungs, normal vessels are invariably present. Invasion by eosinophil leucocytes into the hypertrophied muscle and the intima as well as stretching and fragmentation of the internal and external elastic membranes together with endothelial proliferation are findings worthy of note.

The bronchial lymph-nodes show a diffuse increase in the lymphocytic elements associated, in some cases, with a moderate accumulation of eosinophil leucocytes.

The above changes are applicable to the active phase of the disease but Hamilton (1966a) has followed the condition up until six months after initial infection. After sixteen to twenty weeks, the bulk of the pulmonary tissue was found to be free of parasitic elements and by the end of twenty-four weeks the abnormalities in the branches of the pulmonary artery and the gross hypertrophy of the muscle of the bronchioles and alveolar ducts constituted the main pathological findings and only with great difficulty were portions of adult worms demonstrable by means of histopathological techniques. The conclusion reached, therefore, was that pulmonary arteriopathy in the cat resulted from an infestation, present or past, by lungworms.

If that hypothesis is accepted, it becomes apparent that the incidence of lungworm disease is considerably greater than that suggested by figures given earlier since pulmonary arterial disorder is, perhaps, the commonest pathological condition of the cat. Scratchard and Wright (1961) reported an incidence of 36.0% out of 111, Tashjian et al. (1965) a frequency of 68.8% out of 122, Stunzi et al. (1966) approximately 33.0% out of 150 and Hamilton (1966b) 34.7% out of 256 cats. Thus the nematode, Aelurostrongylus abstrusus, achieves prominence as one of the most important pathogens of the cat and cognizance of the associated disease is of importance not only to those who treat or keep cats as pets but also to those who use cats as experimental animals. Since the parasite readily induces vascular lesions, the mechanism by which it does so may also be of interest to those studying the pathogenesis of human pulmonary hypertension.

Unfortunately, there is a relative and, more often, a complete lack of information on many aspects of the parasite and of its effects on the intermediate and final hosts and the following thesis is a record of the work done towards elucidation of some of those problems.

PART ONE

1 THE OUTPUT OF FIRST-STAGE LARVAE BY CATS INFESTED WITH *AELUROSTRONGYLUS ABSTRUSUS*

2 THE LONGEVITY OF FIRST-STAGE LARVAE KEPT UNDER VARIOUS ENVIRONMENTAL CONDITIONS

3 A THE ROLE OF THE MOUSE AS AN INTERMEDIATE HOST IN THE LIFE-CYCLE OF *AELUROSTRONGYLUS ABSTRUSUS*

B THE ROLE OF THE MOUSE AS A PARATENIC HOST IN THE LIFE-CYCLE OF *AELUROSTRONGYLUS ABSTRUSUS*

4 THE DEVELOPMENT, MIGRATION AND LONGEVITY OF LARVAE OF *AELUROSTRONGYLUS ABSTRUSUS* WITHIN, AND PATHOGENICITY FOR, THE MOLLUSC *HELIX ASPERSA*

Experiment One

The Output of First-Stage Larvae by Cats Infested with *Aelurostrongylus abstrusus*

Introduction

Aelurostrongylus abstrusus is widely distributed throughout the world and the incidence of active infestation in Britain has been recorded as 10.3% (Lewis, 1927) and as 6.0% (Hamilton, 1966b). The parasite requires a mollusc as an intermediate host although it is possible that, under natural conditions, auxiliary hosts play an important part in the life-cycle. The average length of the patent period is from eight to thirteen weeks and, during that time, a considerable number of first-stage larvae are likely to be excreted whereby the parasite may survive. Information on that point is not available and the object of the following experiment was to ascertain the approximate number of larvae produced by cats in the course of typical infestations.

Materials and Methods

Third-stage larvae of Aelurostrongylus abstrusus were extracted from previously infected snails of the species, Helix aspersa, by the expedient of slicing the molluscs into small pieces, wrapping the proceeds in muslin and immersing the whole in a beaker of water kept at a temperature of 37°C in a water-bath. Within a few hours, larvae were able to be collected. Two kittens, twelve weeks of age and weaned in the animal house,

were fed with larvae in numbers of 1,000 (Cat A) and 500 (Cat B) and, nine weeks later, first-stage larvae were first noted in the faeces of both animals. Thereafter, and until samples had been free of larvae for seven days, the daily faecal output of each cat was collected, weighed and one gramme of each sample was bound in muslin and steeped in 100 ml. of water for twenty-four hours in order to extract the contained larvae. The latter were concentrated by centrifugation and counted. Where numbers were high, notably in the cat that had been given 1,000 larvae, the aggregate of first-stage forms was arrived at by the process of dilution.

Results

The output of larvae from each cat over the patent period is illustrated in Table 1 where, to avoid excessive detail, the figures have been computed on a weekly basis. First-stage larvae were noted in the faeces of both cats in the ninth week after infestation and continued to be excreted for the next seventy and sixty-seven days by Cats A and B, respectively. The peak of larval output was achieved in the fourth and fifth weeks of patency but, thereafter, there was a gradual diminution in numbers until in the tenth week the larvae finally disappeared from the faeces of both animals. A total of over seventeen and three-and-a-half millions was recorded for Cats A and B, respectively.

Table 1

Weekly Output of Larvae from Cats
Infected with Aelurostrongylus abstrusus

Post-patent week No.	Cat	Av. No. of larvae/gm. of faeces	Total weekly output of larvae
1	A	1,100	65,350
	B	360	15,900
2	A	5,040	277,350
	B	680	73,800
3	A	9,680	505,700
	B	2,840	182,250
4	A	24,750	5,300,500
	B	5,140	362,600
5	A	20,220	5,534,900
	B	7,220	1,241,550
6	A	14,320	3,404,000
	B	5,500	632,400
7	A	7,950	1,029,820
	B	3,390	266,000
8	A	4,930	287,750
	B	3,930	293,100
9	A	640	143,000
	B	630	44,100
10	A	140	8,550
	B	120	11,470

A = Cat given 1000 larvae

B = Cat given 500 larvae

Total A = 17,056,960

B = 3,623,170

Discussion

Aelurostrongylus abstrusus has an indirect life-cycle and to ensure successful parasitic reproduction a cat is required to ingest approximately one hundred third-stage larvae (*vide* Part Two, Experiment One) either from a molluscan or paratenic host, which latter may be a mouse or a bird. The nomadic habit of cats secures widespread dispersal of first-stage larvae during the patent period of the disease and such forms are known to survive out-of-doors for, at least, thirty-six days (*vide* Part One, Experiment Two). Successful contact with an intermediate host was presumed to depend upon excretion of considerable numbers of larvae and the above experiment has demonstrated that such is the case since, between them, the two cats produced in excess of twenty million larvae during a ten-week period. There was a marked discrepancy, however, in larval output in Cat B in contrast with Cat A, even allowing for the difference in quantity of the initial infecting dose, since the former produced but 20.0% of the number excreted by Cat A.

Rose (1959), with respect to *Muellerius capillaris* and Jarrett and Sharp (1963) in relation to *Dictyocaulus viviparus*, found that only 12.0 and 25.0%, respectively, of an infecting dose of larvae was eventually established in the lungs of experimental animals, the rest having failed to overcome the difficulties of the migratory pathways and the former author discovered that it was

possible for adult females in the lungs to be isolated in inflammatory nodules and thereby to be infertile. Such findings, applied to the lungworms of the cat, are sufficient to explain the variability of larval production in the two cats of the above experiment.

The females of Haemonchus contortus are considered to be examples of prolific egg-layers with daily outputs of between 5,000 (Martin and Ross, 1934) and 6,550 (Kelley, 1955). While little is to be gained by any attempt to assess, from the present experiment, the daily output of eggs by Aelurostrongylus abstrusus females, the productive capacity of the latter appears to be of comparable scale.

Experiment Two

The Longevity of First-Stage Larvae of *Aelurostrongylus abstrusus* Kept Under Various Environmental Conditions

Introduction

After five to nine weeks, and for a varying period thereafter, cats infested with Aelurostrongylus abstrusus excrete first-stage larvae in the faeces. Hobmaier and Hobmaier (1935b), Blaisdell (1952) and Mackerras (1957) collected larvae for eight weeks, two years and seven months, respectively, after patency. Cameron (1927) found that larvae, extracted from faeces, lived for eleven days but were killed by freezing. Since the fate of such larvae are of importance with regard to the incidence of lungworm disease of the cat and because of difference of opinion concerning the viability of first-stage larvae, it was considered necessary to establish the life-span of such larvae under various controlled conditions.

Materials and Methods

To assess the survival of first-stage larvae, the following procedures were adopted. Faeces from infested cats were placed out-of-doors, lightly covered with soil and maximum and minimum ground temperatures and rainfall recorded each day during the period of the experiment. Every three days, a sample of faeces was examined and a hundred larvae were judged as to whether they were dead, sluggish or highly active. Faeces were also kept at room temperature (18-21°C), in an incubator (37°C), in a refrigerator

(4°C) and frozen (0°C). Samples of faeces from each of those groups were examined every three days and the viability of the larvae was evaluated.

To form a second main group, larvae were extracted from infested faeces by placing the latter in a muslin bag in water. Within twenty-four hours most of the larvae had escaped from the faeces. After concentration, the larvae were divided into four lots and kept, still in water, at 18-21°C, 37°C, 4°C and 0°C. A third main group of larvae was similarly maintained except for the addition of antibiotic. As formerly, samples were examined at three-day intervals and the number of larvae dead, sluggish or active noted.

Results

Tables 2, 3, 4, 5 and 6 record the survival times of first-stage larvae under the conditions described above.

Discussion

With regard to the problem of natural infection, the results of the location of faeces out-of-doors were informative (Table 2). Larvae were found to be alive thirty-six days after exposure and the majority, until the last three-day period were still highly active. In all but two such periods, rainfall was registered.

The lowest ground temperature reached was -6°C and the highest 22°C while freezing and thawing of the faeces occurred on many occasions without apparent effect on the larvae. The latter may have been recovered alive for a longer period of time had not the faeces and, presumably, many of the larvae been gradually washed into the soil by the rain. Cameron (1927) stated that larvae were unable to resist actual freezing, Fry and Stewart (1932) found that out-of-doors in January, larvae lived for five days as well as for six days after freezing and Blaisdell (1952) showed that larvae survived for five days after thawing. The results of the present experiment revealed, however, that despite frequent freezing and thawing, first-stage larvae existed for thirty-six days.

When infested faeces were retained at 0°C (Table 3), larvae were recovered for thirty-three days. When larvae, extracted from faeces, were kept at the same temperature their life-span was prolonged for sixty-three days during which time there was a gradual reduction of mobility and an increasing death-rate. The difference in longevity between the larvae in faeces and those in water at 0°C was not the result of desiccation since the faeces remained moist throughout the time of the experiment. Possibly, some faecal constituent may have been toxic to the parasites.

Rose (1957a) studied Muellerius capillaris, a member of the same family as Aelurostrongylus abstrusus, and found that, from December to May in the open, first-stage larvae lived for twenty-

Table 2

Longevity of First-Stage Larvae
in Faeces kept Out-of-Doors

Date	Rainfall	3 - day Average Ground Temperatures °C	Active %	Sluggish %	Dead %
8/3	-	-	100	-	-
11/3	+	2 - 12	95	3	2
14/3	+	1 - 16	100	-	-
17/3	+	1 - 17	63	27	10
20/3	-	4 - 15	50	38	12
23/3	+	1 - 13	89	11	-
26/3	+	0 - 16	92	8	-
29/3	+	-2 - 14	80	20	-
1/4	+	-1 - 13	80	20	-
4/4	-	-3 - 16	91	9	-
7/4	+	4 - 10	87	13	-
10/4	+	4 - 10	73	27	-
13/4	+	1 - 10	-	100	-

Table 3

Longevity of First-Stage Larvae at 0°C in Faeces and in Water

Date	Faeces			Water		
	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %
13/3	100	–	–	100	–	–
16/3	25	66	9	12	85	3
19/3	4	82	14	11	69	20
22/3	3	85	12	–	74	26
25/3	19	43	38	1	70	29
28/3	4	66	30	–	60	40
31/3	14	74	12	1	54	45
3/4	10	70	20	–	47	53
6/4	–	67	33	–	56	44
9/4	2	60	38	–	62	38
12/4	1	58	41	–	60	40
15/4	–	38	62	1	61	38
18/4	–	–	100	–	63	37
21/4				–	65	35
24/4				–	54	46
27/4				–	60	40
30/4				–	66	33
3/5				–	60	40
6/5				–	27	63
9/5				–	41	59
12/5				–	28	72
15/5				–	28	72
18/5				–	–	100

Table 4

Longevity of First-Stage Larvae at Room Temperature (18-21°C) in Water, Water plus Antibiotic and in Faeces

Date	Water			Water + Antibiotic			Faeces		
	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %
2/3	100	–	–	100	–	–	100	–	–
5/3	67	26	7	75	12	13	54	44	2
8/3	48	45	7	15	56	29	3	96	1
11/3	12	78	10	8	52	40	–	24	76
14/3	23	70	7	13	70	17	–	30	70
17/3	6	52	42	2	52	48	–	53	47
20/3	2	48	50	1	40	59	–	42	58
23/3	1	33	66	–	22	78	–	36	64
26/3	13	22	65	2	16	82	2	34	64
29/3	9	20	71	1	13	86	–	45	55
1/4	11	24	65	2	14	84	–	50	50
4/4	8	19	73	1	11	88	2	42	56
7/4	3	24	73	–	7	93	–	40	60
10/4	1	10	89	4	17	79	–	22	78
13/4	4	7	89	13	17	70	–	25	75
16/4	3	20	77	1	16	83	–	28	72
19/4	–	–	–	–	–	–	–	17	83
22/4	3	25	72	2	39	59	–	–	100
25/4	1	29	70	4	26	70			
28/4	–	12	88	1	50	49			
2/5	1	28	71	–	33	67			
5/5	–	26	74	–	28	72			
8/5	–	26	74	–	28	72			
11/5	–	20	80	–	18	82			
14/5	–	8	92	–	–	100			
17/5	–	–	100						

Table 5

Longevity of First-Stage Larvae at 37°C in Water, Water plus Antibiotic and in Faeces

	Water			Water + Antibiotic			Faeces		
Date	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %
2/3	100	–	–	100	–	–	100	–	–
5/3	70	1	20	41	11	48	100	–	–
8/3	20	10	70	–	–	100	92	8	–
11/3	7	31	62	1	4	95	–	–	100
14/3	36	10	54	5	–	95			
17/3	1	49	50	–	–	100			
20/3	–	40	60						
23/3	1	18	81						
26/3	–	22	78						
29/3	3	8	89						
1/4	–	12	88						
4/4	–	8	92						
7/4	1	2	97						
10/4	–	5	95						
13/4	1	3	96						
16/4	–	–	100						
19/4									
22/4									
25/4									
28/4									
2/5									
5/5									
8/5									
11/5									
14/5									
17/5									

Table 6

Longevity of First-Stage Larvae at 4°C in Water, Water plus Antibiotic and in Faeces

Date	Water			Water + Antibiotic			Faeces		
	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %	Active %	Sluggish %	Dead %
28/2	100	–	–	100	–	–	100	–	–
3/3	96	2	2	95	–	5	85	15	–
6/3	71	23	6	40	41	19	40	53	7
9/3	65	32	3	28	59	13	39	53	8
12/3	68	24	8	47	49	4	–	78	22
15/3	60	38	2	29	66	5	4	53	43
18/3	42	37	21	8	76	16	9	36	55
21/3	91	–	9	60	38	2	–	52	48
24/3	89	11	–	53	42	5	–	55	45
27/3	76	16	8	60	34	6	–	60	40
30/3	74	24	2	62	29	9	–	57	43
2/4	69	29	2	66	19	5	–	65	35
5/4	88	8	4	70	20	10	–	43	57
8/4	67	23	10	63	15	17	–	28	72
11/4	75	22	3	38	48	14	–	20	80
14/4	75	20	5	50	27	23	–	18	82
17/4	76	21	3	50	27	23	–	12	88
20/4	44	53	3	43	42	15	–	–	100
23/4	44	53	3	43	42	15			
26/4	58	40	2	46	48	6			
29/4	58	32	10	49	40	11			
6/5	56	35	9	12	34	54			
13/5	67	24	9	6	21	73			
20/5	62	32	6	2	17	81			
27/5	54	46	–	7	9	84			
3/6	48	52	–	4	10	86			
10/6	40	60	–	2	7	91			
17/6	14	76	10	–	–	100			
24/6	20	70	10						
3/7	29	59	12						
21/7	60	38	2						
3/8	80	18	2						
10/8	84	12	4						
17/8	76	14	10						
24/8	82	10	8						
31/8	87	13	–						
7/9	90	10	–						
14/9	75	20	5						
21/9	70	22	8						
28/9	67	20	13						
5/10	42	38	20						
12/10	20	40	60						
19/10	–	–	100						

seven weeks during which period freezing occurred on a number of occasions. Larvae frozen in water lived for twelve days. Because of possible differences in metabolic requirements, collation between parasites, however closely related, is hazardous. In addition, it is difficult to compare exactly the climatic conditions of any two periods of time. Moreover, the physical character of the faeces of sheep and of cats differ, e.g. Rose stated that larvae were not, to any great extent, washed from the faeces of sheep by rain whereas, in the above investigation, the opposite was the case with those of the cat.

At room temperature, Cameron (1927) and Fry and Stewart (1932) kept first-stage larvae alive for eleven and eighteen days, respectively. Hobmaier and Hobmaier (1935a) recovered larvae after five weeks, Gorichter (1949) after seven weeks and Blaisdell (1952) after thirty-four days. Table 4 shows that, during the present experiment, at room temperature living larvae were recoverable from faeces for forty-eight days and from water for seventy-two days while the presence of antibiotic scarcely affected the period of survival. In each case where comparable figures are available, the longevity of the larvae was greater than that recorded by other authors.

At 37°C (Table 5), larvae in faeces remained active for six days but died quickly afterwards, probably as a result of desiccation. Larvae, in water at a similar temperature, survived

for forty-two days although during most of the time a high percentage of dead forms was found. The presence of antibiotic shortened larval life to such an extent that from the third to the twelfth days only the occasional living form was appreciable. That finding may have resulted from the breakdown of the antibiotic with the production of a larvicidal substance.

Another important finding was that, at 4°C (Table 6), larvae remained active for a considerable time and survived in water and in water plus antibiotic for 226 and 102 days, respectively. In faeces at 4°C, larvae lived for forty-eight days although during the last part of that time there was a high proportion of deaths. Comparable observations on the larvae of Aelurostrongylus abstrusus are lacking but Rose (1957a) found living larvae of Muellerius capillaris to be present in dry faeces kept at 3 - 6°C for fifty-two weeks.

Several conclusions may be drawn from the results of the experiment under report. It is clear that first-stage larvae are relatively resistant to changes in temperature and humidity. The life expectancy of larvae under natural conditions is at least thirty-six days although, under average climatic circumstances, they may live longer even if some time is spent in the soil. Of major importance with regard to experimental work is the fact that larvae may be stored at 4°C for a lengthy period. Those findings together with the knowledge gained from the previous experiment,

namely, that in the course of an average infestation millions of larvae are excreted by one cat, help to explain the ability of the parasite to survive despite its rather complex life-cycle. In that context too, it must be remembered that the incidence of active parasitic infection of the cat in Great Britain is high (Lewis, 1927 and Hamilton, 1966b).

A knowledge of the bionomics of the larval stages of all parasites is necessary and may lead to the introduction of measures of control. However, with respect to Aelurostrongylus abstrusus, the nomadic and preying habits of cats ensures continual contact with potentially infected intermediate or storage hosts and also causes widespread distribution of infested faeces. Accordingly, it is impossible to recommend any reasonable system of control which is likely to reduce parasitization of cats.

Experiment Three

A The Role of the Mouse as an Intermediate Host in the Life-Cycle of *Aelurostrongylus abstrusus*

Introduction

One of the controversial issues concerning Aelurostrongylus abstrusus is the role of the mouse in the life-cycle of the parasite. Cameron (1927), after a series of successful experiments, claimed that the intermediate host was the mouse, which finding appeared to be logical if only because of the close relationship of that species with the cat. Cameron's work, however, was not confirmed by Baudet (1933) nor were his results corroborated by Hobmaier and Hobmaier (1935a) whose investigations revealed that molluscs were the true intermediate hosts and that mice acted as auxiliary hosts. Later still, Gerichter (1949), Blaisdell (1952), Mackerras (1957) and Hamilton (1966a) found molluscs to be satisfactory intermediate hosts and, additionally, Mackerras reported failure to infect two mice with first-stage larvae procured from the faeces of a cat.

Thus, it appeared to the author that, because of the successful results reported in Britain by Cameron (1927) with the mouse as an intermediate host and since unsuccessful efforts to infest mice with first-stage larvae had been experienced in the Netherlands (Baudet, 1933), in America (Hobmaier and Hobmaier, 1935a), in Palestine (Gerichter, 1949) and in Australia (Mackerras, 1957), a further attempt should be made to ascertain whether, or not, the mouse may act as a true intermediate host for Aelurostrongylus abstrusus.

Materials and Methods

Young, laboratory-bred, white mice were used in the course of two experiments. During the first trial, eighteen mice were given, on average, several thousands of first-stage larvae that had been recovered from the faeces of an infected cat. One month later, the mice were autopsied and representative portions of practically every tissue of six mice were fixed in 10.0% corrosive-formol, embedded in paraffin-wax and sections cut at five microns were stained by haematoxylin and eosin for microscopical examination. The tissues of the remaining twelve mice were cut into small pieces, wrapped in muslin and immersed in water for forty-eight hours in an endeavour to recover larvae.

In the second experiment, twenty-four mice were used. Over a period of fourteen days, first-stage larvae were introduced into the drinking water whereby each mouse imbibed tens of thousands of larvae. Two months after the start of such infestation, the mice were sacrificed. Six animals were used to provide histopathological material and attempts were made to retrieve larvae from another six in the manner outlined above. The remaining twelve mice were fed in their entirety to two cats and the latter killed after a period of eight weeks.

Results

Experiment One

At autopsy of all eighteen mice, evidence of a parasitic infestation was wholly lacking. Histopathological examination of representative sections of all tissues also failed to reveal the presence of larvae. The extraction experiment, too, did not result in the isolation of any larvae of Aelurostrongylus abstrusus.

Experiment Two

Post-mortem examination of twelve of the mice failed to reveal evidence of parasitic infestation. Histopathological examination of the tissues of six mice and extraction investigations with other six were likewise negative. Autopsical and histopathological examinations of the tissues of the two cats that had been fed six mice each, eight weeks previously, did not disclose any sign of the presence of lungworm infestation.

Discussion

The completely negative findings of the above experiments confirm the work of Baudet (1933), of Hobmaier and Hobmaier (1935a), of Blaisdell (1952) and of Mackerras (1957), all of whom did not accept the view that the mouse was the true intermediate host. Controversy over the life-cycle of the parasite followed the apparently successful work of Cameron (1927) who fed four cats with first-stage larvae from infected cats and, at autopsy,

failed to find any pulmonary lesions. Again, of seven cats given infected mice, two died shortly after infection but all of the remainder were found to exhibit Aelurostrongylus in the lungs whereas control cats proved not to suffer from lungworm infestation. The discrepancy between the findings of Cameron (1927) and those of other workers is difficult to explain. It seems to be stretching coincidence too far to suggest that the experimental cats used by the former were already infested by lungworms since only those which had been fed with infected mice subsequently developed the disease. Mackerras (1957) compared the morphology of the parasite given by Cameron (1927) with that furnished by other authors as well as with her own findings and decided that, save for minor exceptions, the several descriptions tallied. It may, therefore, be accepted that Cameron was working with Aelurostrongylus abstrusus.

The main weakness in Cameron's work seems to lie in the forty-six white mice used for passage of the larvae, all of which were bred in captivity and the majority of which were obtained from the same source. Because of outbreaks of "sarcosporidiosis" and of "rat-bite fever", a number of the mice died before the experiments were completed. It is possible that such mice became infected with third-stage larvae as a result of the ingestion of parasitized slugs and snails contained, perhaps, in green food. Against that theory is the information that control mice from a similar source proved negative for the presence of the parasite.

The two versions of the life-cycle seem irreconcilable. Mackerras (1957) suggested that, in other parts of the world, there may be other species of parasites involved in lungworm infestation of the cat. However, despite considerable experience of that condition in Britain, the author of this thesis has recovered only Aelurostrongylus abstrusus and so has concluded that, in those Islands, the associated intermediate host is, as in other parts of the world, a mollusc.

Experiment Three

B The Role of the Mouse as a Paratenic Host in the Life-Cycle of *Aelurostrongylus abstrusus*

Introduction

It has been established that the mouse is not the true intermediate host of Aelurostrongylus abstrusus. Hobmaier and Hobmaier (1935a), however, found that a variety of animal species, including mice, may act as paratonic hosts for the parasite. That finding was supported by Mackerras (1957). In order to confirm the work of the latter authors and, more especially, to ascertain the length of time that larvae may survive within infected mice, the following experiments were performed.

Material and Methods

Third-stage larvae were extracted from infected snails by the method previously described and, in numbers of 500, were administered orally to eight mice. At the end of one, two, three and four months, two mice were autopsied and fed in their entirety to a kitten. Two months later, each cat was killed, examined and portions of lungs fixed in 10.0% corrosive-formol, embedded in wax, sectioned at five microns and stained by haematoxylin and eosin.

Results

Small, white nodules, about a millimetre in diameter and

containing third-stage larvae, were encountered on the serosal surface of the small intestine and within the omentum of infected mice. The larvae, which had lost their sheaths on release from the snails, were viable and had not undergone development beyond the third-stage.

Autopsical and histopathological examinations of the lungs of the four cats showed lesions characteristic of lungworm disease.

Discussion

Mice acted as paratenic hosts for Aelurostrongylus abstrusus. Third-stage larvae coiled up within the murine tissues and remained alive for four months although it is suspected that such forms may survive the lifetime of the mouse. It is highly probable, therefore, that the latter species plays an important part in the life-cycle of the lungworm.

The pulmonary lesions produced in the experimental cats, however, were less severe than those expected to result from oral administration of a thousand larvae and such findings suggested that a high percentage of the latter had failed to survive the additional passage through an auxiliary host. Nevertheless, in view of the fact that large numbers of first-stage larvae are excreted by infected cats and that first and third-stage forms are able to exist under varying conditions for a lengthy period, the latter loss is possibly of little significance to the ultimate survival of the parasite.

Experiment Four

The Development, Migration, Distribution and Longevity of Larvae of *Aelurostrongylus abstrusus* within, and Pathogenicity for, the Mollusc, *Helix aspersa*

Introduction

Hobmaier and Hobmaier (1935a) first showed that the intermediate host of Aelurostrongylus abstrusus was a mollusc. The authors found that the snails of the genus Epiphragmophora were best but that snails such as Helminthoglypta and Helix and slugs of the genera Agriolimax and Arionlimax were also suitable. Gerichter (1949) added other molluscs to the list of hosts: Chondrula, Helliocella, Monacha, Levantina, Retinella and Theba but considered that Helliocella, Monacha and Agriolimax were most appropriate since the parasite quickly developed within them. Blaisdell (1952) infected seven species of snails and five of slugs and introduced snails such as Zonitoides, Anguispira and Mesodon and slugs of the genera Deroceras, Arion and Fallifera. Mackerras (1957) and Hamilton (1966a) found that Agriolimax laevis and Helix aspersa, respectively, were convenient intermediate hosts.

Hobmaier and Hobmaier (1935a), Gerichter (1949) and Mackerras (1957) described the development of Aelurostrongylus abstrusus larvae within molluscs and the purpose of the following investigations was to confirm certain aspects of that cycle in Helix aspersa. In addition, it was proposed to investigate the route of entry of first-stage larvae into snails together with the distribution within, and the effects of such parasites upon, the host and, finally, to assess the longevity of third-stage

larvae in infected molluscs.

Materials and Methods

First-stage larvae were recovered from the faeces of infected cats in the manner earlier described. Water, containing large numbers of larvae, was placed in a shallow, glass dish to a depth of approximately six millimetres and snails of the genus, Helix aspersa (Fig. 1) were repeatedly immersed and allowed to wallow in the fluid for a period of six hours. In order to trace the process of invasion and migration of the larvae and the associated host reaction, at least two snails were killed at the end of one, two, four, six and twenty-four hours and one, two, four, eight, twelve, twenty-four, thirty-six, fifty-two and one hundred and four weeks and the tissues fixed in 10.0% formol-saline and embedded in paraffin-wax to be sectioned and stained by haematoxylin and eosin and picro-Mallory stains. In snails killed from twelve weeks onwards, a portion of sole was used for the provision of third-stage larvae. The development of the latter was followed over a period of thirty days. Every second day, larvae were teased from the foot of an infected snail, placed on a slide, a few drops of 0.01% cotton-blue-lactophenol added and the preparation gently warmed to fix and allow staining of the specimen. In addition, twenty, unstained first, second and third-stage larvae, immobilised by heat, were measured with the aid of an ocular micrometer

and an average figure computed for each stage. To assess what would constitute a fatal dose of larvae for a mollusc, a group of the latter was kept, as far as possible, in constant contact with first-stage larvae until death of the snails. At that time, larvae were extracted from the dead molluscs and the numbers counted.

Results

a Development of Larvae

First-stage larvae (Fig. 2) were, on average, 0.39 millimetres long by 0.02 millimetres wide. After penetration of, and migration within, the mollusc the larvae coiled up and movement ceased. Two days later, larvae showed the beginning of a gradual development of cells lining the intestine and until the time of the first moult there was, in those cells, an accumulation of refractile food-granules which, in some cases, masked the inner structure of the parasite. The exsheathing process originated at the head and tail regions on the twelfth day and had been completed in two days to form, still within the larval sheath, second stage forms. The latter (Fig. 3) measured, on average, 0.45 millimetres in length and 0.04 millimetres in breadth while the large intestinal cells were filled with granules which, however, decreased in amount as the time for the second moult approached. The latter occurred six to eight days after the first and resulted in the retention of the second sheath and the loss of most of the intestinal granules.

Fig. 1 Snails (Helix aspersa) along with a sixpenny-bit for comparison of size

Fig. 2 Smear taken from the lung of a cat infected with Aelurostrongylus abstrusus to show developing eggs, and first-stage larvae both free and contained within the egg-membrane.

x 300

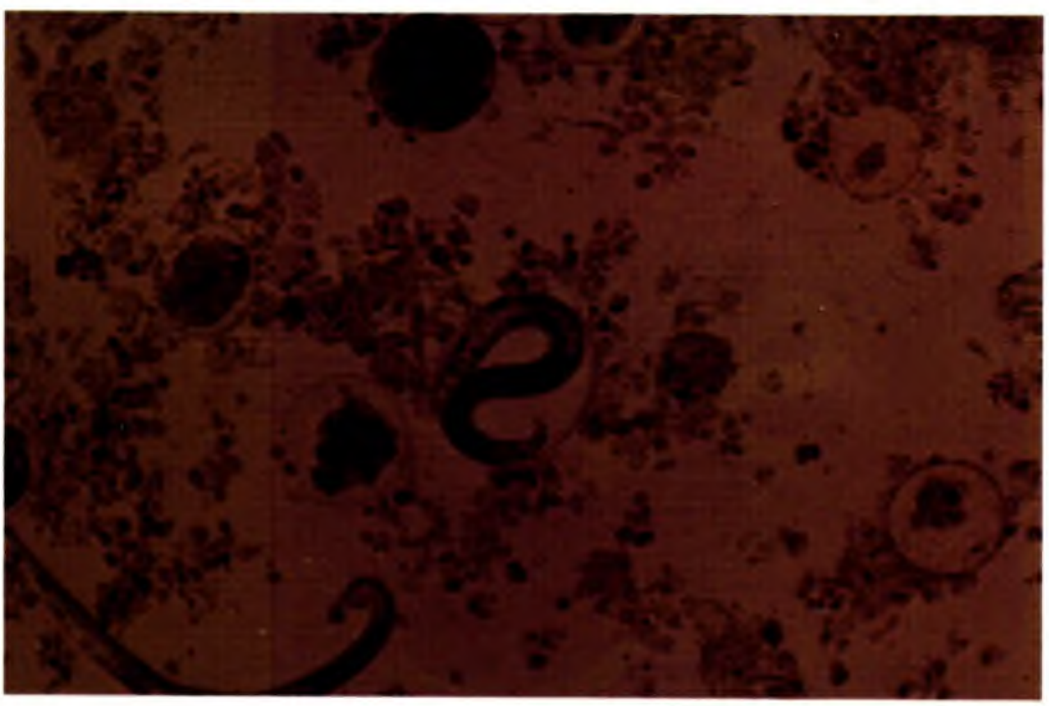
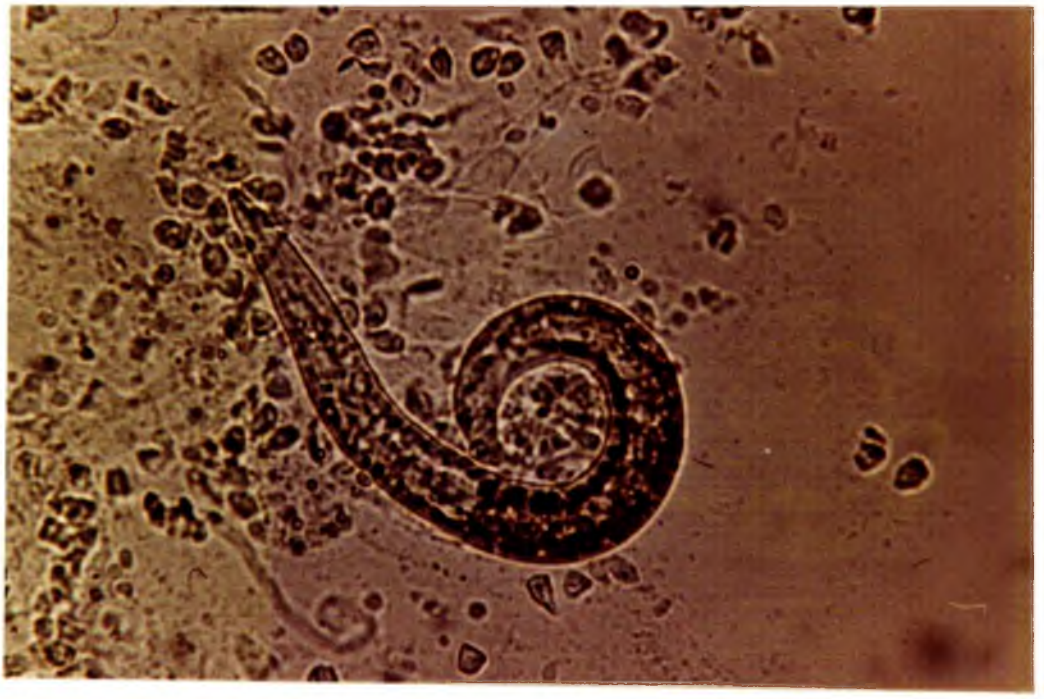


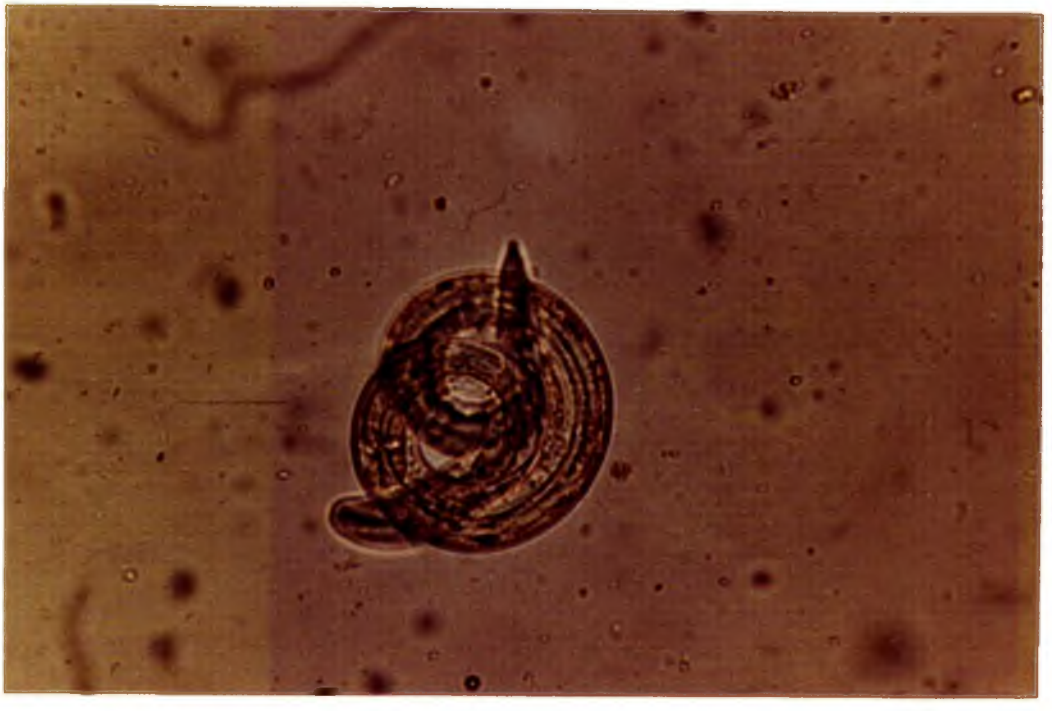
Fig. 3 Unstained, ensheathed second-stage larva which has been removed from molluscan tissue. The refractile food-granules are prominent.

x 900

Fig. 4 Unstained, ensheathed third-stage larva dissected from the sole of a snail.

x 900





Third-stage larvae (Fig. 4) were longer, at 0.53 millimetres, but slimmer, at 0.03 millimetres, than their predecessors and, after escape from the sheaths, displayed high motility. After a period of twenty-three days within snails, such larvae proved to be infective for the cat.

b Penetration, Migration and Host Reaction

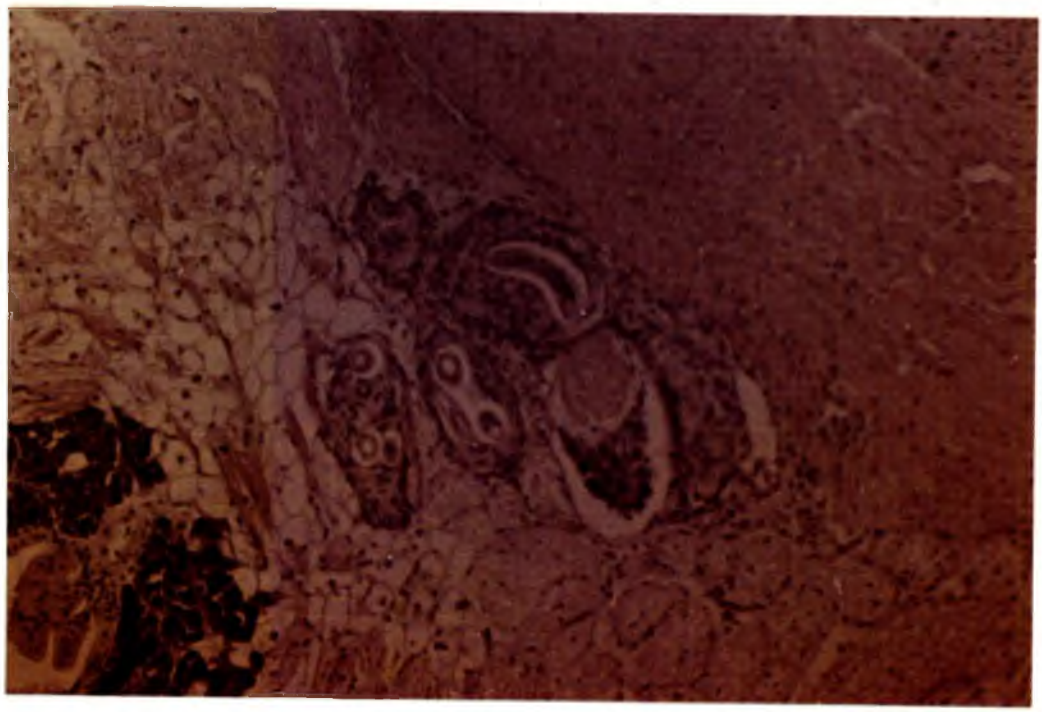
Larvae had not gained entrance to the snails after two hours but, two and four hours later, had appeared under the epithelium of the sole although their presence had not evoked any reaction on the part of the host. It was considered that migration within the mollusc had ceased by forty-eight hours and, at that time, numerous parasites were demonstrated in the deeper layers of, and organs adjacent to, the sole and were surrounded by an accumulation of macrophage-like cells (Fig. 5). One and two weeks after infection, the growth of larvae had caused the flattening of the investing cells and, by the latter period, there was evidence of a reduction in the cellular content of the parasitic nodules. Participation of connective tissue elements was minimal. One, two and three months after infestation, the lesions had decreased in size and the presence of a single-layered connective tissue capsule was noted. By six, twelve and twenty-four months, larvae presented a similar appearance (Fig. 6). The cellular reaction had disappeared with the exception of a single layer of fibroblasts

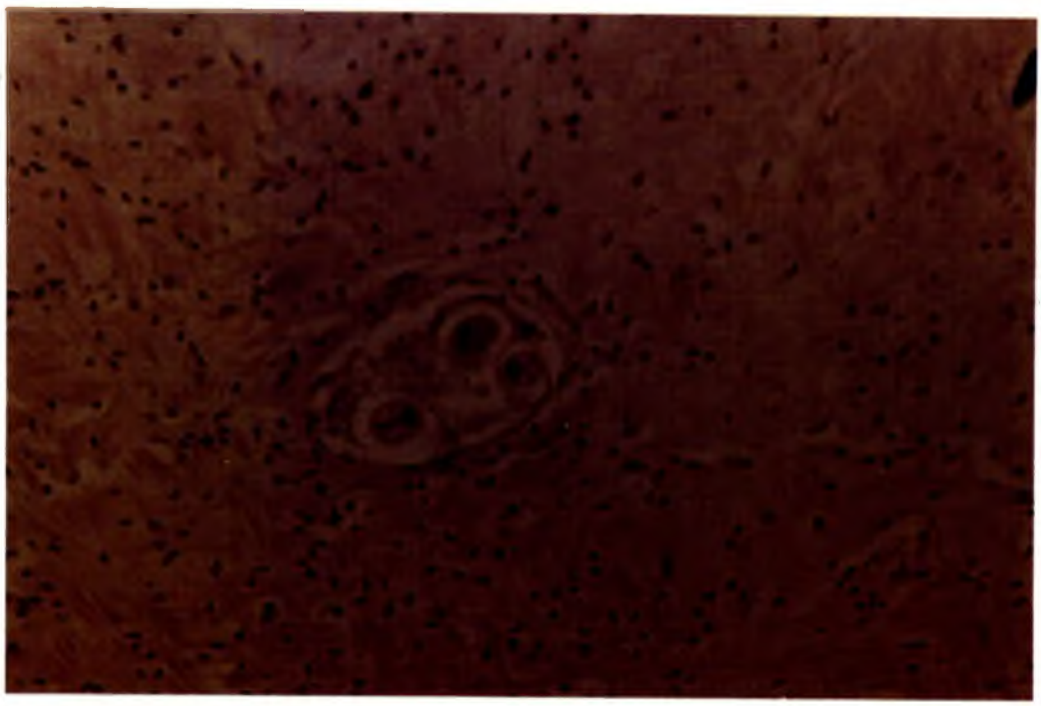
Fig. 5 First-stage larvae of Aelurostrongylus abstrusus forty-eight hours after invasion of a mollusc. There is marked macrophagic reaction around the parasites.

Haematoxylin and Eosin x 150

Fig. 6 Third-stage larvae of Aelurostrongylus abstrusus two years after invasion of a mollusc. The cellular reaction has disappeared and a thin layer of connective tissue separates the larvae from the molluscan tissues.

Haematoxylin and Eosin x 300





and their associated fibres but, in many instances, larvae lay in intimate contact with molluscan tissue. The number of parasites had apparently declined and, occasionally, degenerate forms were appreciable. Larvae liberated from snails after three, six, nine, twelve and twenty-four months were active and those recovered from the snails that had been infected for two years proved capable of causing lungworm disease in a cat.

c The Number and Distribution of Larvae in Molluscs

To assess the number and distribution of third-stage larvae within infected snails, thirty of the latter were killed, the foot of each mollusc separated from the viscera, and, by the method earlier described, larvae were extracted and counted from each of those areas. Table 7 records the results of the exercise and it may be observed that an average of 497 larvae was recovered from each snail. Sixty-four per cent of that number was isolated from the sole.

Table 7

The Number of Third-Stage Larvae of *Aelurostrongylus abstrusus* Recovered from Snails of the Species, *Helix aspersa*.

Snail No.	Foot	Viscera
1	174	47
2	410	239
3	667	273
4	133	52
5	441	36
6	362	00
7	266	00
8	357	30
9	275	150
10	497	271
11	117	150
12	120	120
13	343	752
14	222	461
15	311	50
16	147	100
17	100	150
18	570	325
19	658	200
20	130	100
21	107	200
22	368	509
23	323	170
24	368	100
25	250	108
26	303	424
27	511	168
28	120	-
29	02	-
30	115	14
Total	6472	5418
Average No. per Snail	316	181
% of Total	64	36

d Pathogenicity of Larvae for Molluscs

Of the twelve young snails that had been in constant contact with first-stage larvae, one died after fifteen, three after seventeen, three after nineteen and five after twenty-seven days. There was little difference in the number of larvae, of all stages, recovered from each of the molluscs and a mean rate of fifty-seven was achieved. It was notable, however, that only third-stage forms were retrieved from the latter five snails.

Discussion

The measurement and the stages of development of larvae recorded in the present experiment approximated the findings of Hobmaier and Hobmaier (1935a), Gerichter (1949) and Mackerras (1957). Larvae accumulated food-material at the expense of the host until the first moult and, thereafter, utilized these reserves for the production of third-stage forms. The latter authors described the first moult to occur ten, eleven and six days, respectively, after infection as opposed to the fourteen days of the present investigation and the second moult to take place four to five weeks, six and three days later, respectively, in contradistinction to the seven to eight days of this report. There is a two-fold explanation of those differences. Gerichter (1940) found that 30°C was the optimal developmental temperature for larvae and, as it grew colder, the maturation period increased. As the snails of

the present experiment were kept at a temperature that varied between 18 and 24°C, that factor may explain the longer time required for larval development. In addition, different species of snails may be less suitable intermediate hosts than Agriolimax agrestis in which Mackerras (1957) found the most rapid maturation, yet reported, of Aelurostrongylus abstrusus.

Hobmaier and Hobmaier (1934) considered that, if ingested by molluscs, first-stage lungworm larvae died and suggested that penetration of the foot was the only route of infestation. Richards and Merritt (1967) stated that, in the instance of Angiostrongylus cantonensis, molluscs became infected by the ingestion of larvae and received support for that hypothesis by the work of Knapp (1966) who recovered 98.7% of all larvae from, and around, the gut of snails infected by the same parasite. However, the results of the present work showed that after several hours of contact, larvae of Aelurostrongylus abstrusus invaded the sole of the mollusc and, over the next twenty-four to forty-eight hours, migrated into the depths of that organ and into the peri-visceral tissues. Larvae were never demonstrable in, or making their way through, the gut and it seems unlikely, therefore, that larval ingestion plays any part in infestation of snails by the latter parasite.

Hobmaier and Hobmaier (1934) reported that lungworm larvae were confined to the sole of infected snails but Table 7 shows that

no fewer than 36.0% of all larvae were recovered from the viscera of such hosts. From the experimental viewpoint, at least, the latter finding is of importance. The same Table also records that, from thirty snails, an average number of 497 larvae was retrieved although as few as ninety-two and as many as 1,500 were extracted from individual snails and, that, despite the fact that all of the latter had been subjected to the same treatment. It was surprising that more larvae did not enter the molluscs but whether, or not, that was a reflection of host resistance or a result of the inability of many of the parasites to breach the tough pedal epithelium is unknown. The failure may be connected with the use of inappropriate species as suggested by Gerichter (1948) who discovered that some snails were more susceptible to invasion than others. Mackerras and Sandars (1955), discussing the difference in molluscan liability to infection with larvae of Angiostrongylus cantonensis, noted that in one unfavourable species, Onchidium, the majority of the parasites had been surrounded by fibrous tissue and subsequently absorbed by the slug. That feature may be relevant to the longevity of the larvae of Aelurostrongylus abstrusus within Helix aspersa since the comparative lack of connective tissue participation was most marked. At best, it consisted of a single-layered capsule which, in many instances in the later stages of the infection, was absent and allowed the larvae to lie in intimate association with the molluscan tissues.

However, host susceptivity must be regarded in the light of the extent of opportunity given to the larvae to enter the snail and it is of obvious importance to provide adequate time for infection to take place.

With respect to the latter statement, the information gained from keeping snails in, more or less, constant contact with first-stage larvae is apposite. Young snails of six to eight hundred milligrammes in average weight had been utilized but despite the exposure time, as long as twenty-seven days in five individuals, the number of larvae that had gained entrance to the molluscs was not high, nor had it increased greatly as the period lengthened. If that result be compared with the larval recovery rate from adult snails (Table 7), of eight to ten times the body-mass of the juvenile forms, it will be appreciated that little numerical advantage accrued from the prolonged period of infection. In view of the paucity of larvae and with the knowledge that neither death nor apparent ill-health had affected other snails with heavier parasitic burdens, it was considered that those young molluscs had died from bacterial or protozoal infection or as a result of living under abnormal environmental conditions. In support of that theory, histopathological examination of portions of the dead snails showed that larvae had neither infiltrated nor destroyed any vital organ. It is distinctly possible that immunity, provoked by the entry of first-stage larvae, had

protected the snails against further invasion since, in the case of the five that had survived for twenty-seven days, only third-stage larvae had been recovered which seemed to indicate that the admission of first-stage larvae had been in abeyance for approximately fourteen days.

The current findings lead to the hypothesis that, under natural conditions, infected molluscs may carry small numbers of larvae and that cats suffering from lungworm disease require to have ingested numerous slugs or snails unless, of course, storage hosts play a more important part in the life-cycle of the parasite than has hitherto been suggested. Hobmaier and Hobmaier (1935a) found that frogs, toads, lizards and snakes, some species of birds and rodents acted as auxiliary hosts and it is possible that the latter two groups, with their propensity for eating molluscs, may accumulate reasonable numbers of third-stage larvae and so become an important link with the disease in the cat. A significant factor in that relationship is the length of time during which infective larvae may persist in intermediate and paratenic hosts. In the above experiment, although it did appear that the number diminished as time progressed, third-stage larvae were recovered for two years after infection of snails had been accomplished. Gerichter (1948), discussing the same parasite, described a period of seventeen months and, with Muellerius capillaris, Rose (1957b) recorded that larvae survived within molluscs for at least one year. These results

confirm the view of Gerichter (1948) that once such a host was infected, it retained that state for life. From the previous experiment has come the information that larvae remain viable in mice for at least four months.

That larval stages should persist for a lengthy period is important to any parasite but when longevity is reported for free first-stage larvae and for third-stage in intermediate and storage hosts, then such parasites are, indeed, favourably placed to overcome the vicissitudes of fortune which are inherent in any complex life-cycle. Aelurostrongylus abstrusus is in that propitious situation and its success in survival may be judged by the extent to which infestation has been achieved in the final host.

PART TWO

1 THE NUMBER OF LARVAE OF AELUROSTRONGYLUS ABSTRUSUS REQUIRED TO PRODUCE PULMONARY DISEASE IN THE CAT

2 PARENTERAL INFECTION OF THE CAT BY AELUROSTRONGYLUS ABSTRUSUS

3 RE-INFESTATION OF THE CAT WITH AELUROSTRONGYLUS ABSTRUSUS

4 PASSIVE IMMUNISATION IN LUNGWORM INFECTION OF THE CAT

5 THE PRODUCTION OF IMMUNITY AGAINST LUNGWORM DISEASE BY REPEATED ADMINISTRATION OF NON-PATHOGENIC NUMBERS OF THIRD-STAGE LARVAE OF AELUROSTRONGYLUS ABSTRUSUS

6 THE INFLUENCE OF INFESTATION BY AELUROSTRONGYLUS ABSTRUSUS ON THE VASCULATURE OF THE CAT

Experiment One

The Number of Larvae of *Aelurostrongylus abstrusus* Required to Produce Pulmonary Disease in the Cat.

Introduction

The number of third-stage larvae of Aelurostrongylus abstrusus required to produce disease in the cat is still unknown. Hobmaier and Hobmaier (1935b), Gerichter (1949), Mackerras (1957) and Hamilton (1966a) achieved such infestation but did not specify the number of larvae involved. Because of the lack of such information, of value both epizootiologically and experimentally, it was decided to ascertain the number of larvae necessary to produce lungworm disease and to investigate the course of any subsequent disorder in the cat.

Materials and Methods

Third-stage Aelurostrongylus abstrusus larvae were isolated from infected snails by the method described in Part One. The infective larvae were then fed to thirteen kittens, twelve to fourteen weeks of age, which had been weaned in the animal house. Each pair of kittens received fifty, 100, 200, 400, 800, and 1600 larvae, respectively, while the thirteenth animal was given 3,200. To obviate vomiting, the larvae were administered in milk or water, in small doses over several days. Eight weeks after the last dose, the cats were killed, a blood sample taken and a comprehensive autopsy performed. Euthanasia, in the present and in the following experiments, was performed by the

intraperitoneal administration of pentothal. Occasionally, in the case of a fractious animal, injection was given by the intrathoracic route. Portions of the lungs, myocardium, kidneys, liver, stomach and intestines, brain, bronchial and mesenteric lymph-nodes and spleen were taken for histopathological examination and fixed in 10.0% corrosive-formol, embedded in paraffin-wax, sectioned at five microns and stained by haematoxylin and eosin. Haematologically, haemoglobin, packed cell volume and erythrocyte sedimentation values were estimated for each cat and total and differential white cell counts were also made.

Results

a Clinical and Haematological

None of the cats displayed any clinical upset until approximately five weeks after infestation. At that time, the animals in receipt of 800, or more, larvae showed an accelerated respiratory rate, especially after exercise, and during the last three weeks the cat given 3,200 larvae was extremely dyspnoeic even at rest. Coughing, particularly after handling, was apparent in all of the cats from six weeks onwards and was worse in those that had been fed 800, or more, larvae. During the three weeks preceding euthanasia, the two animals given 1,600 as well as the cat fed 3,200 larvae had a reduced appetite which was associated with loss of bodily condition. The same animals evinced

diminished thoracic resonance on percussion and, on auscultation, adventitious pulmonary sounds were prominent.

The parasitic infection did not affect the red cells and normal values were obtained from haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration estimations. In eight of the thirteen cats, there was a leucocytosis which was due mainly to increase in the number of lymphocytes. Eosinophilia (more than 1000 cells/cu. mm.) was also noted in seven of the cats. In all but the animal given the greatest number of larvae, erythrocyte sedimentation rates were increased and values from 12 to 42 mm./hour were common (Table 8).

b Gross Post-Mortem Examination

The lungs of both of the animals infected with fifty larvae displayed a few pin-point, non-elevated, pale areas which were randomly distributed throughout all the lobes. In the two cats fed 100 larvae, the lungs were equally affected insofar as multiple lesions occurred in all lobes (Fig. 7). Such lesions varied from punctiform, pale white foci to those up to one millimetre in diameter and, in some areas, coalescence had created lesions, up to two centimetres in diameter, that were yellowish in colour and of fairly crumbly consistence. In one animal, the diaphragmatic lobes were more heavily infested while the cardiac lobes of the other cat were almost solid. However, the majority of the lesions were discrete

Table 8

Haematological Values in Cats infested with Aelurostrongylus abstrusus

No. of Larvae	Total W.B.C. per cu.mm.	Differential W.B.C.			Absolute Values			Hb Gm/100 ml.	P.V.C. %	M.C.H.C. %	E.S.R. mm/hr.
		Poly.	Lymph.	Eosin	Poly.	Lymph.	Eosin				
50	8,400	57	35	8	4,788	2,940	672	13	40	32	12
50	11,000	60	34	6	6,600	3,740	660	13	42	31	14
100	17,400	50	42	8	8,700	7,308	1,392	12.7	40	32	38
100	19,000	34	61	5	6,460	11,590	950	12.7	42	30	18
200	12,400	38	46	16	4,712	5,704	1,984	14.4	41	35	27
200	15,600	31	67	2	4,836	10,452	312	13.1	42	31	12
400	17,900	28	66	6	4,928	11,816	1,056	13.0	42	31	20
400	15,600	36	60	4	5,616	9,360	624	15	46	32	15
800	17,900	46	46	14	7,160	8,234	2,506	13.7	42	32	42
800	10,300	19	73	8	1,957	7,519	824	14.6	44	33	26
1600	15,000	52	41	7	7,800	6,150	1,050	13.5	40	33	40
1600	15,600	58	34	8	9,048	5,304	1,248	13	40	32	36
3200	10,900	43	43	14	4,687	4,687	1,526	16	52	31	5

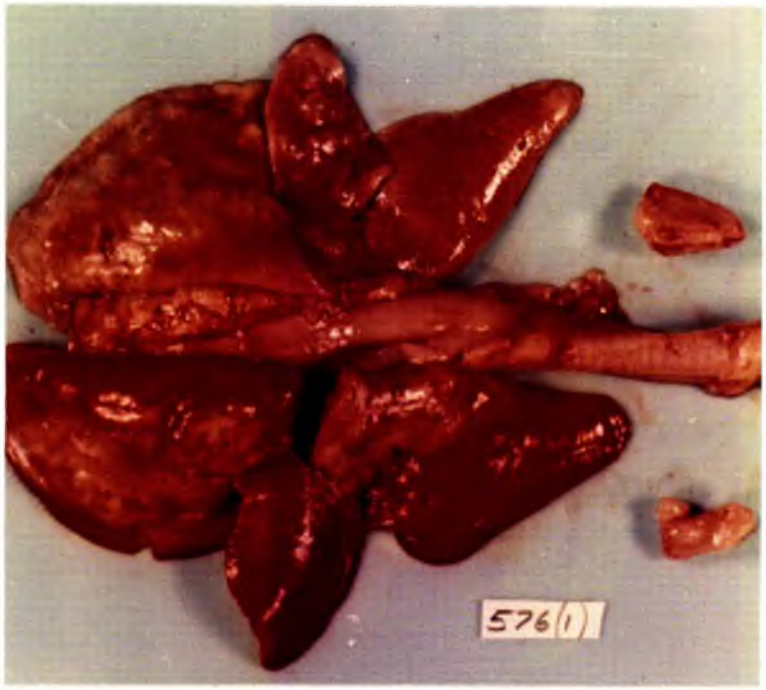
and plenty of normal pulmonary tissue remained. The appearance of the lesions in the lungs of the cats given 200 and 400 larvae was similar to that described above except that the foci were more numerous and widespread and had become less discrete to produce, by confluence, numerous elevated lesions of approximately two centimetres in diameter (Fig. 8). In one cat, the diaphragmatic lobes were markedly involved.

The pulmonary changes attained greater magnitude in the case of the cats fed with 800 and 1,600 larvae. The lungs of all four animals showed widespread and severe lesions (Fig. 9). The right apical and cardiac lobes of the lungs of one cat were entirely solid without a trace of functional pulmonary tissue. The other lobes, and those of the remaining three cats, were diffusely involved, firm to the touch and protusive, coalescent lesions imparted a rippled appearance to the surface of the organ. Because of the colour of the parasitic foci, the lungs had assumed a yellowish hue and a pus-like fluid was expressable from the cut surface. There were pronounced areas of emphysema. The lungs of the cat infected with 3,200 larvae were enlarged and virtually solid (Fig. 10). The lesions had involved practically all of the pulmonary tissue so that, on section, the lungs were of firm consistency because of the loss of elasticity. Emphysematous areas were prominent and creamy, viscid fluid, rich in eggs, larvae and cells, was procurable.

The bronchial lymph-nodes of every animal were enlarged, pale

Fig. 7 Gross appearance of the lungs and lymph-nodes of a cat given 100 third-stage larvae. The diaphragmatic lobes are notably affected.

Fig. 8 Widespread lesions in the lungs of the cat dosed with 400 larvae.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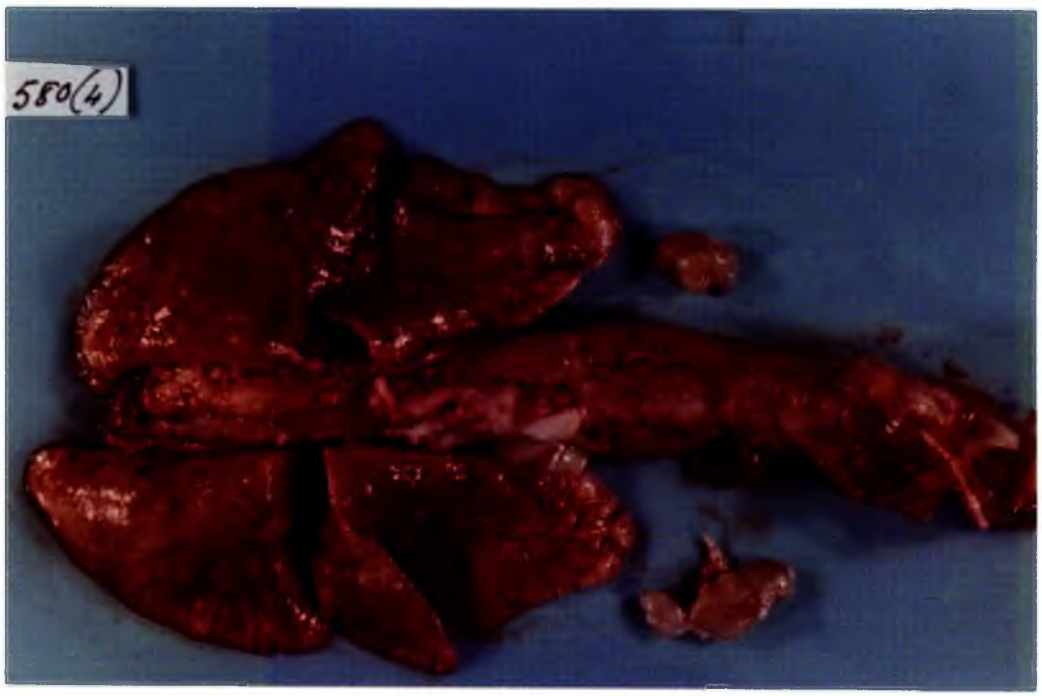
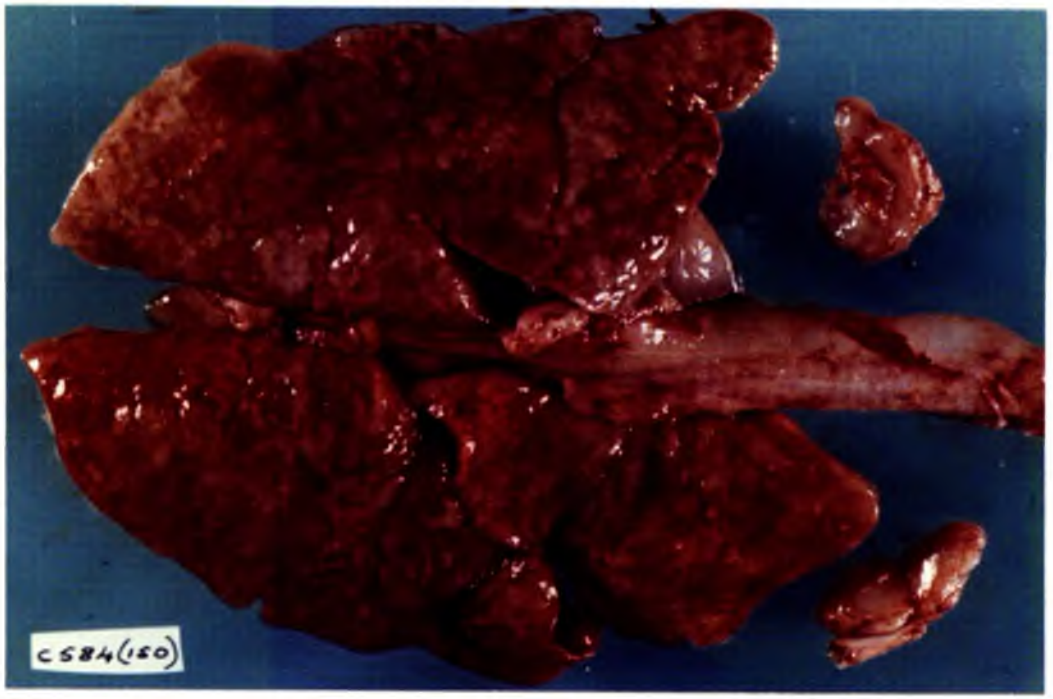

580(4)

Fig. 9 Cat given 1,600 larvae. The lungs are enlarged and every lobe is markedly affected. The bronchial lymph-nodes are grossly swollen.

Fig. 10 Cat dosed with 3,200 larvae. The lungs are enlarged with widespread involvement and consolidation of all lobes.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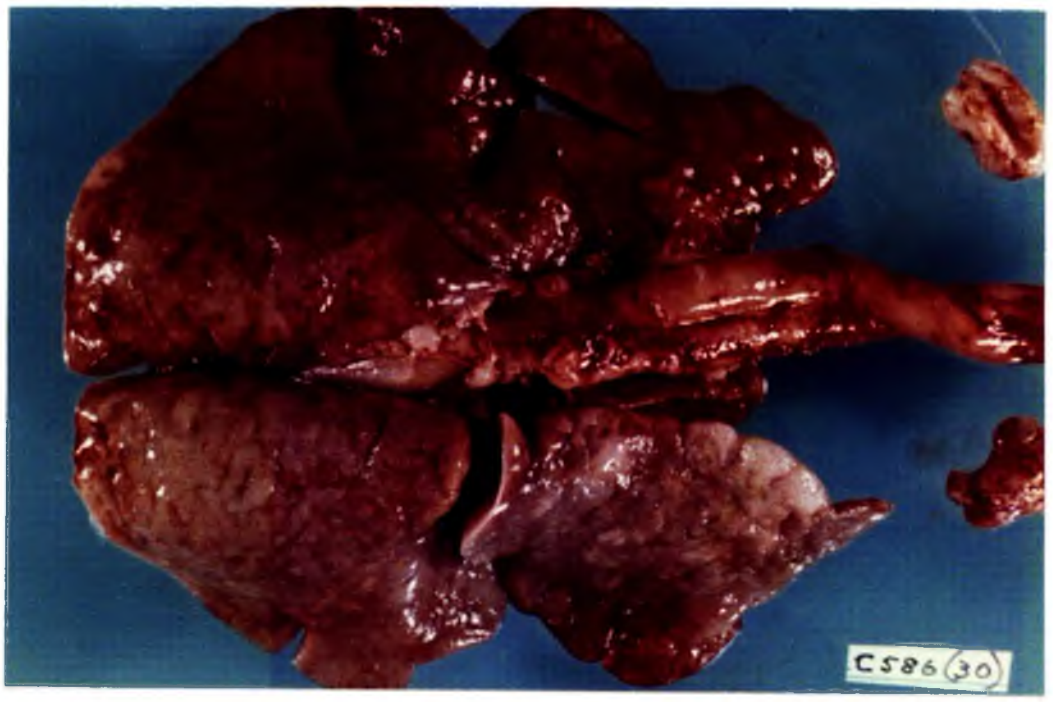

C586(30)

white, firm and homogeneous in character (Figs. 7 - 10). The degree of enlargement was roughly proportional to the infecting dose so that, in the animal given 3,200 larvae, the nodes were of maximal size and measured approximately two by two centimetres.

c Histopathology

Basically, the histopathological changes in the lungs of the thirteen experimental animals were similar although there were variations because of the different doses given.

Affected parts showed large areas of cellular reaction located around developing eggs and larvae. The main types of cells consisted of eosinophil leucocytes, lymphocytes, macrophages and numerous giant-cells. Adult worms were found in the bronchioles, alveolar ducts and alveoli and hypertrophic changes of the smooth muscle of the two former structures were present (Fig. 11). Cellular infiltration occurred into the interstitial tissues and alveoli while around the bronchi and bronchioles were many lymphocytes and eosinophil leucocytes. There were regions of alveolar epithelialization and emphysema. Bronchitis and bronchiolitis were associated with the presence of ova, larvae and eosinophil leucocytes within the affected lumina (Fig. 12). Migration of lymphocytes and eosinophil leucocytes into the arterial intima (Fig. 13) and the perivascular tissues was marked and, in some vessels, endothelial swelling and proliferation was apparent.

Hypertrophy of the medial coat of the muscular pulmonary arteries was pronounced but, although that change was often widespread in the vessels of lungs, normal arteries were still recognisable. Stretching, fragmentation and, even, disappearance of the elastic laminae of affected vessels were common. In the bronchial lymph-nodes, there was an increase of the lymphatic elements to a varying extent with, in some cases, a moderate accumulation of eosinophil leucocytes.

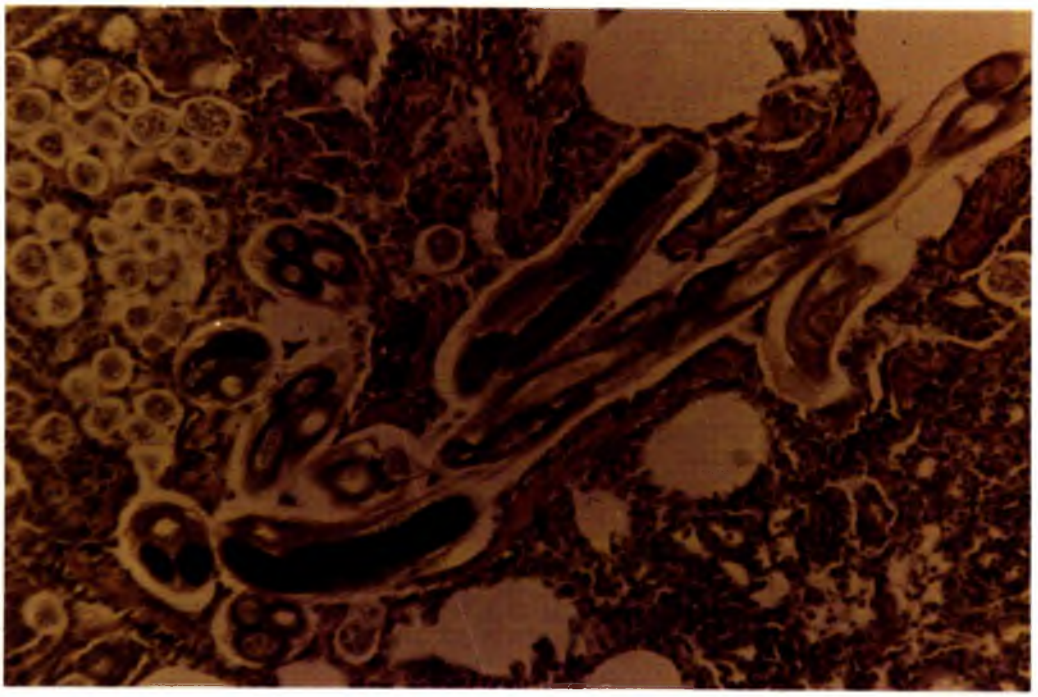
In the cats that had received fifty, 100, 200 and 400 larvae, there were some differences in the pulmonary pathology. The general reactions were similar to those described above but the number of eggs and larvae was considerably fewer and the latter were contained within a highly cellular area of reaction characterised by numerous lymphocytes, plasma cells, macrophages and giant-cells together with a smaller number of eosinophil leucocytes. Many of the eggs and larvae were degenerate. Alveolar emphysema was not widespread but was restricted to the scenes of parasitic activity. In the cats that had received 800 and, especially, in those given 1,600 and 3,200 larvae, the amount of surviving respiratory tissue was either considerably less or practically non-existent and emphysema was a prominent finding. In the same animals, a larger number of arteries were hypertrophic although the gravity of the change in individual vessels did not appear to be greater.

Fig. 11 Adult worm, associated with the presence of developing eggs, lying in an alveolar duct. Some hypertrophic change is evident in the wall of that structure.

Haematoxylin and Eosin x 150

Fig. 12 Bronchiolitis, periarterial lymphocytic reaction and hypertrophic change in the media of a small pulmonary muscular artery.

Haematoxylin and Eosin x 150



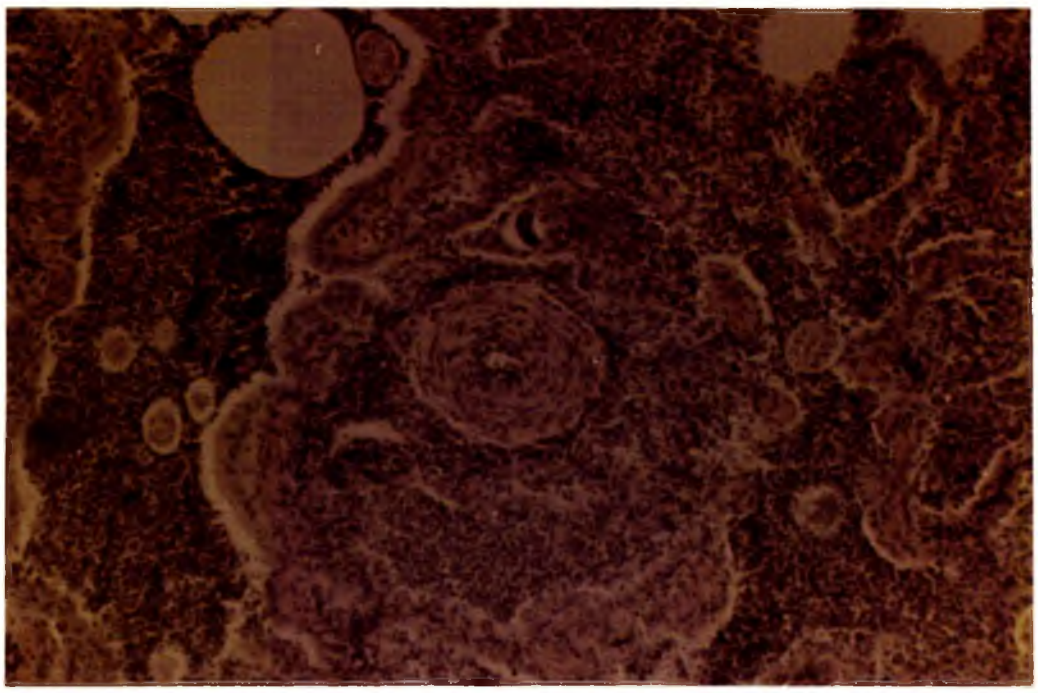
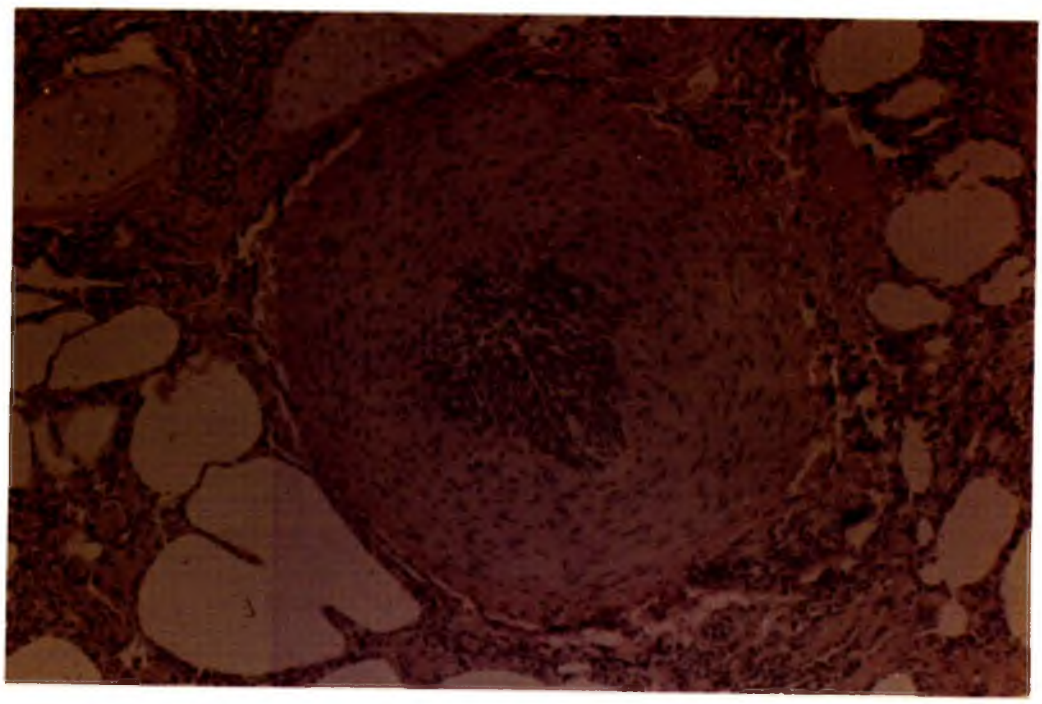


Fig. 13 A muscular pulmonary artery showing medial hypertrophy, endothelial proliferation and infiltration of the intima with eosinophil leucocytes and lymphocytes.

Haematoxylin and Eosin x 150



Discussion

There are a few recorded references to the clinical aspects of lungworm disease in the cat. Blaisdell (1952) reported occasional or frequent coughing in infected cats and, in three of her worst cases, loss of body weight, depression, increased pulse and respiratory rates and dyspnoea were recorded. Sudduth (1955) noted eczema, diminished appetite, loss of weight and dyspnoea in association with six cases of the disease. McKenzie (1960) observed slight unthriftiness in two kittens in which lungworms were found and Hamilton (1966a) found that experimentally infected animals coughed but appetite and bodily condition remained good. The same author (1963) described one spontaneously fatal case in which the cat became progressively emaciated and dyspnoeic and developed a marked pyothorax before death. However, the severity of the disease is related to the infecting dose of larvae, the immune state and the general condition of the infected animal. In the experiment under report, the kittens were in good bodily condition and had no immunity at the start of the experiment so that the only variable factor was the number of larvae given. Even in animals given the same number of larvae, there is some diversification of dosage inasmuch as it is not feasible to separate larvae into male and female forms hence, as the worms pair off, it may happen that a dose of 100 larvae may produce considerably fewer than fifty pairs of adult worms in the lungs of

the final host. It is also probable that only a small percentage of larvae eventually overcome the difficulties of migration and reach the lungs (Rose, 1959). Even with those reservations, the experiment has shown that as few as fifty third-stage larvae may cause lesions of the lungs although such infection is quickly overcome and, as few if any first-stage larvae are consequently excreted, the infestation does not tend to perpetuate the existence of the parasite.

Infection with 100, 200 and 400 larvae produced moderately severe disease but the defensive mechanisms of the animals seemed to cope adequately as, eight weeks from infection, there were to be found many degenerate eggs and first-stage larvae surrounded by a markedly cellular reaction. With doses of 800 larvae and upwards, there was a progressive increase in the amount of pulmonary tissue involved until, in the lungs of the cat fed 3,200 larvae, functional respiratory parenchyma was hard to find. The number of eggs and first-stage larvae was considerable and there was little discernible attempt on the part of the bodily defences to contain or remove the parasites. It is considered that the cat given 3,200 larvae was likely to have died as a result of the infestation since it was depressed, anorexic and showed notable respiratory distress before euthanasia was performed. How such heavily infected animals would fare outside a laboratory is not without interest. Certainly, they would be incapable of much exercise and, in the event of

superimposed infection by another agent, bacterial or viral, the prognosis would be grave.

In lungworm disease of the pig, McKenzie (1958) obtained a clear correlation between the extent of the pulmonary lesions and the infecting dose of larvae. The dissemination of lesions through all the lobes of the lungs was effected only with doses of 1,000 larvae or more, and clinical disease was to be seen in a pig given 3,000 larvae. The distribution of lesions in lungworm disease of the cat differed in that neither a lobe nor a part of a lobe was preferentially affected whatever the dose and, even in the lightest infection, all lobes of the lungs showed indiscriminately scattered foci. Occasionally, one lobe was more severely involved than the others, e.g. in one cat fed 800 larvae, the apical and cardiac lobes were entirely solid but, in another animal given a similar number, the latter lobes were not any more involved than the remaining parts of the lungs. A similar pattern of distribution was described by Jarrett and Sharp (1963) for the lesions arising from infection of calves by Dictyocaulus viviparus.

Hypertrophy of the medial coat of the muscular pulmonary arteries is of common occurrence in the cat in Britain (Scratchard and Wright, 1961 and Hamilton, 1966b). The latter author showed that the arterial condition was closely related to infestation, past or present, by Aelurostrongylus abstrusus. Such an association has been further proved by the experiment under report.

In the latter, the severity of individual lesions was not increased by the dose of larvae but the number of vessels affected was greater.

Haematologically, haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration values were within normal range and such findings are in accord with those recorded by Hamilton (1966a). However, in the case of animals infected for six to fourteen weeks, the latter author also described a leucopenia which was followed by a leucocytosis whereas, in the present experiment, a leucocytosis was prominent at eight weeks from infection in at least eight of the thirteen cats. The difference can not be adequately explained. The dose employed was approximately 400 larvae as opposed to the graded numbers of the above experiment but, even when the dose was the same, i.e. 400 larvae, the results still conflict. The ages of the animals and the conditions of husbandry were similar. Much of the difference may have resulted from the solitary nature of the blood-sampling of each cat but, while the dangers of that procedure are fully accepted, the results in each experiment were fairly constant. Eosinophilia was recorded by Hamilton (1966a) and was also detectable in the majority of cats in the present experiment although, in the latter, the number of eosinophil leucocytes was not entirely related to the dose of infective larvae. If it is accepted that the erythrocyte sedimentation rate of the blood of normal cats lies between 0 and 5 mm./hour, it is apparent that in every animal, except one, such values were increased. The degree

of acceleration was not commensurate with the number of larvae ingested and, therefore, to the amount of tissue damage. Indeed, the most severely ill cat had the lowest erythrocyte sedimentation rate although, in that particular animal, interpretation must be made in the light of the higher packed cell volume which may have been indicative of dehydration.

In summary, the results of the above experiment have shown that, while a small dose (fifty) of third-stage larvae administered to a non-immune animal may cause pulmonary lesions, the outcome is not propitious for the perpetuation of the parasite. It is considered that at least 100 larvae are necessary for successful parasitic infestation but that up to 1,600 larvae may be carried without great upset to the host. When more than that number has been ingested by the cat the clinical signs tend to be more obvious and serious and doses of the order of 3,200 larvae seem to be the level at which severe debility, and subsequent death, of the host may be expected.

Experiment Two

Parenteral Infection of the Cat by *Aelurostrongylus abstrusus*

Introduction

Lungworm disease of the cat may be reproduced either by the oral administration of third-stage larvae extracted from infected molluscs or by feeding the latter, themselves. For experimental purposes the former procedure is to be preferred but the main disadvantage is that an infecting dose must be given on several occasions and over a period of days if sickness, with consequent loss of larvae, is to be avoided. Parenteral administration would, therefore, be advantageous. Pulmonary infections have been produced in calves by the subcutaneous administration of larvae of Dictyocaulus viviparus (Wade and Swanson, 1966), in pigs by intravenous and intratracheal injection of Metastrongylus spp. (Kelley and Krous, 1961) and in sheep by intravenous inoculation of Dictyocaulus filaria larvae (Michel and Sinclair, 1963). As neither intravenous nor intratracheal administration is particularly desirable in cats, it was resolved to attempt the reproduction of lungworm disease in that species by the subcutaneous injection of third-stage larvae of Aelurostrongylus abstrusus.

Materials and Methods

Third-stage larvae of Aelurostrongylus abstrusus, recovered from infected snails, were placed in water to which a small amount

of antibiotic had been added. Eight kittens, twelve to fifteen weeks of age and weaned in the animal house, were used for the purposes of the experiment. Three of the animals (Nos. 1, 2 and 3) received 500 while another three (Nos. 4, 5 and 6) were given 1,000 larvae, all by subcutaneous administration. For control purposes, 500 and 1,000 larvae were dispensed orally to the remaining two cats, Nos. 7 and 8, respectively. On five occasions between the tenth and twelfth weeks after infection, and for each cat, the number of first-stage larvae per gramme of faeces was counted. At the end of the latter period, the cats were autopsied and portions of relevant tissues were removed and histopathological preparations produced by the methods described for the previous experiment.

Results

Table 9 shows the larval output per gramme of faeces from each cat during the period between the tenth and twelfth weeks after infestation. Larvae were recovered from only three of the animals that had been infected subcutaneously and, in general, the numbers were fewer than those excreted by the two orally-infected kittens.

At autopsy, the lungs of Subjects 1 and 2 were free from pathological change while those of No. 6 displayed a few, punctiform, pale white foci in all the lobes. The bronchial and axillary lymph-nodes, the latter only of the side of injection,

Table 9

Output of first-stage larvae in the faeces of cats infected subcutaneously and orally with Aelurostrongylus abstrusus

	Subcutaneous infection						Oral infection	
	500 Larvae			1000 Larvae			500 Larvae	1000 Larvae
	Larvae/gm.			Larvae/gm.			Larvae/gm.	
Sample No.	Cat No. 1	2	3	4	5	6	Cat No. 7	8
1	-ve	-ve	350	1,050	450	-ve	620	2,400
2	-ve	-ve	650	2,650	1,050	-ve	950	6,350
3	-ve	-ve	1,050	4,300	1,950	-ve	3,050	11,550
4	-ve	-ve	2,400	4,950	1,800	-ve	5,250	13,250
5	-ve	-ve	2,600	5,050	1,050	-ve	6,050	13,250

were slightly enlarged in the same animal. Histopathologically, the lungs of Nos. 1 and 2 were normal and those of No. 6 manifested a mild degree of perivascular and peribronchial lymphocytic reaction together with a few, scattered, cellular foci which were mainly lymphocytic in character and, in one section, portions of adult worms were appreciated.

Grossly, the lungs of kittens 3, 4 and 5 were of comparable appearance and showed multiple, cream-coloured lesions which were equally distributed throughout all the lobes and varied from pin-point to a few millimetres in diameter although, occasionally by confluence, foci of about two centimetres in size were produced. The bronchial lymph-nodes and the axillary node of the side of injection were enlarged, pale and homogeneous in character.

The two control animals, Nos. 7 and 8, displayed pulmonary changes which were similar in nature to those of the latter group but differed insofar as, particularly in No. 8, lesions were more widespread and severe and had affected large areas of parenchyma. The bronchial lymph-nodes in both cats were considerably enlarged and measured approximately two by two and a half centimetres.

Histopathological examination of the lungs of kittens 3, 4, 5, 7 and 8 revealed the changes characteristic of infestation by lungworms and described earlier in this thesis. Briefly, the lesions consisted of eggs, larvae and, occasionally, adult worms which were surrounded by lymphocytes, macrophages and giant-cells

together with many eosinophil leucocytes. Bronchitis and bronchiolitis in many areas and hypertrophic changes in the media of branches of the pulmonary artery and of the muscle of bronchioles and alveolar ducts were prominent. Perivascular and peribronchial lymphocytic reaction, sometimes gross in nature, was also a common finding. Enlargement of the bronchial and axillary lymph-nodes was the result, mainly, of lymphocytic hyperplasia.

Discussion

The above experiment has shown that third-stage larvae of Aelurostrongylus abstrusus migrate, possibly via the lymphatics, from the subcutaneous tissues to the lungs. However, the results were variable since only three of the animals showed active parasitological infestation and, in each of those cases, the number of first-stage larvae excreted in the faeces was considerably fewer than that produced by orally-infected cats which had been given a similar dose of larvae. While it is realised that the output of larvae may not always be a true indication of the number of adult females in the lungs (Rose, 1959), nevertheless, comparison of the pulmonary lesions suggested that a smaller number of adults had been present in the lungs of the cats infected by the subcutaneous route. The latter possibility was not discussed by Wade and Swanson (1958) with regard to subcutaneous infection of calves by Dictyocaulus viviparus.

All of the kittens were of the same age-group and had been weaned in the animal house so that it is unlikely that any cat had either an age or an acquired immunity against the parasite. In addition, all animals had been given larvae from the same sample so that the difference in the findings is safely attributable to the route of administration. The use of larger infecting numbers of third-stage larvae may have produced better results but is considered unlikely to have eliminated the variability in infection-rate or in the severity of the pulmonary disease, which latter problems are decreased to a large extent by use of oral dosage.

In summary, while infection of cats with third-stage larvae of Aelurostrongylus abstrusus by the subcutaneous route is easier and less time-consuming than oral administration, it must be accepted that, because of the inconstancy of the results, the procedure has distinct limitations in experimental work.

Experiment Three

Re-infestation of the Cat with *Aelurostrongylus abstrusus*

Introduction

Resistance to re-infection has been demonstrated in respect of many parasites including Haemonchus contortus (Stoll, 1929), Nippostrongylus muris (Taliaferro and Sarles, 1939), Trichostrongylus spp.(Stewart and Gordon, 1953), Dictyocaulus viviparus (Jarrett et al., 1957), Strongyloides papillosus (Turner, 1959) and Nematodirus battus (Thomas, 1959) and it is now generally accepted that antibodies are produced against metazoan antigens. Aelurostrongylus abstrusus is a common feline parasite and adult worms are known to survive in the lungs for six months (Hamilton, 1966a), up to two years (Blaisdell, 1952). Under these circumstances, and providing that the parasite induces an immune response, cats may be expected to be resistant to further infestation. Information on the latter subject, however, is not available and the object of the following experiment was to ascertain whether, or not, immunity follows primary infestation of cats with lungworms.

Materials and Methods

Seven kittens, twelve to fourteen weeks of age and weaned in the animal house, were infected orally with 800 third-stage larvae of Aelurostrongylus abstrusus. After nine weeks, and for four to seven months thereafter, the faeces of the animals were examined

for the presence of first-stage larvae and the clinical condition of the animals noted. Three hundred and eighty-eight days after initial infestation, five of the seven cats were given a further dose of 800 larvae while the remaining two animals were kept for control purposes (Control Group One). At that period two other cats, nine months of age and weaned in the animal house, were given a primary infection with a similar number of third-stage larvae (Control Group Two). All the cats were kept for a further twelve weeks and for the last four weeks of that time the faeces were examined for the presence of first-stage larvae. The nine cats were then autopsied and portions of relevant tissues were removed and histopathological preparations produced by the methods described in Experiment One. Additional staining methods for examination of the pulmonary tissues were a combined Lawson and Van Gieson procedure and Sudan IV stain.

Results

First-stage larvae were recovered in large numbers from the faeces of the original seven cats. Excretion was greatest in the ten to fourteen-week period after initial infection but larvae were found in one cat for seven months. Thereafter, and until re-infection, first-stage larvae were not isolated from the faeces. During the acute phase of the disease, clinical signs of coughing and increased respiratory rate were prominent and, on auscultation

of the chest, adventitious sounds were appreciated.

After re-infection, first-stage larvae were not isolated from the faeces of the five re-infects but all cats, including those of Control Group One, suffered from some degree of respiratory embarrassment, especially if exercised. First-stage larvae were recovered from the faeces of the two cats of Control Group Two and the same animals displayed the clinical signs of lungworm disease.

Post-Mortem and Histopathological Findings

a Control Group Two

The two cats given a primary infection with 800 third-stage larvae twelve weeks previously showed the characteristic pulmonary lesions of lungworm disease which have been described elsewhere in this thesis.

b Control Group One

This group consisted of two cats in which primary infestation had occurred 472 days previously. On palpation, the lungs of both were firmer than normal and a few, small, pale foci, of approximately one to two millimetres in diameter, were scattered throughout the substance of some of the lobes (Fig. 14). On closer inspection, more numerous, punctiform lesions were discernible. The bronchial lymph-nodes were moderately enlarged, pale and homogeneous in appearance.

Histopathological examination showed alterations consistent with

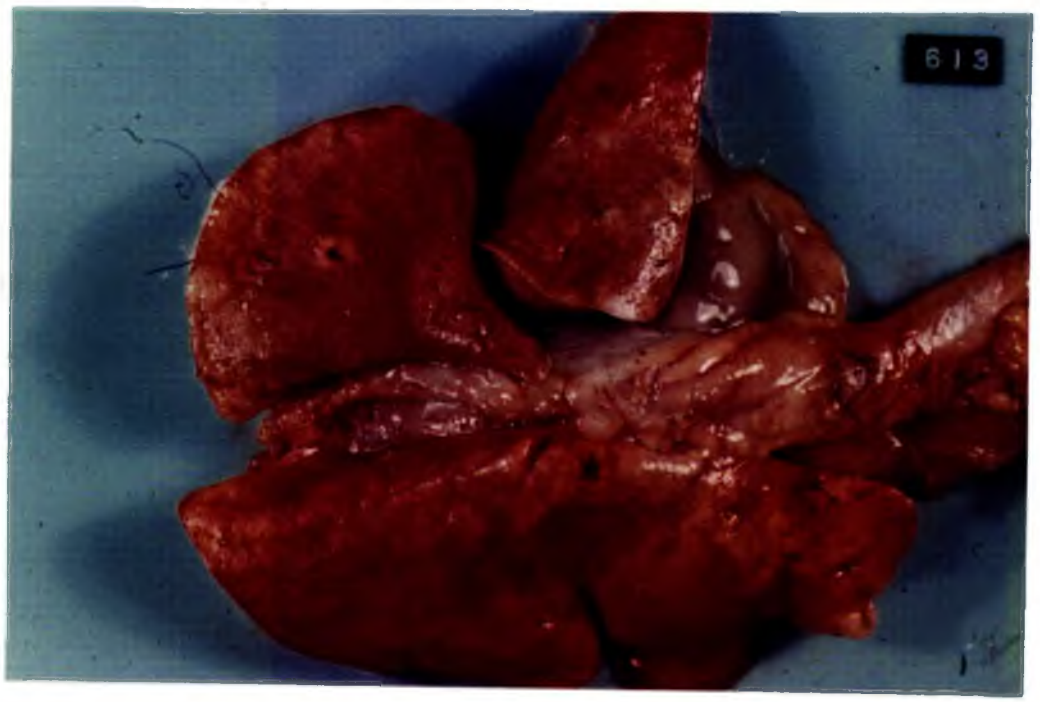
recovery from infestation. The outstanding changes were to be found in many of the muscular pulmonary arteries in which hypertrophy and, in some cases, hyperplasia of the medial coat had taken place. The affected vessels were considerably thickened and tortuous (Fig. 16) and, often, endothelial proliferation was present. Infiltration of the thickened vascular walls by eosinophil leucocytes, mild intimal fibrosis and fragmentation and, even, dissolution of the elastic membranes (Fig. 16) were conspicuous. Peribronchial and, especially, perivascular lymphocytic reaction and hypertrophy of the muscle of bronchioles and alveolar ducts (Fig. 17) were also prominent findings. There were a few large and numerous small, densely cellular foci which consisted mainly of lympho-reticular elements but, occasionally, eosinophil leucocytes predominated therein and it was in such areas that sections of adult worms were seen. Apart from one pulmonary histological section of one cat, in which a few degenerating eggs were found, neither eggs nor larvae were ever encountered. Proliferation of the lymphocytic elements of the bronchial lymph-nodes had taken place.

c Re-infected Animals

Grossly as well as histopathologically, the lungs of all five animals displayed lesions (Fig. 15) which, except for minor variations in individual cats, were wholly similar to those described above for the animals of Control Group One.

Fig. 14 Lungs of a cat of Control Group One 472 days after primary infestation. Gross lesions are minimal and consist mainly of punctiform, pale foci with occasionally a larger lesion.

Fig. 15 Lungs of a re-infected cat for comparison with Fig. 14. The gross appearance of the two sets of organs are similar.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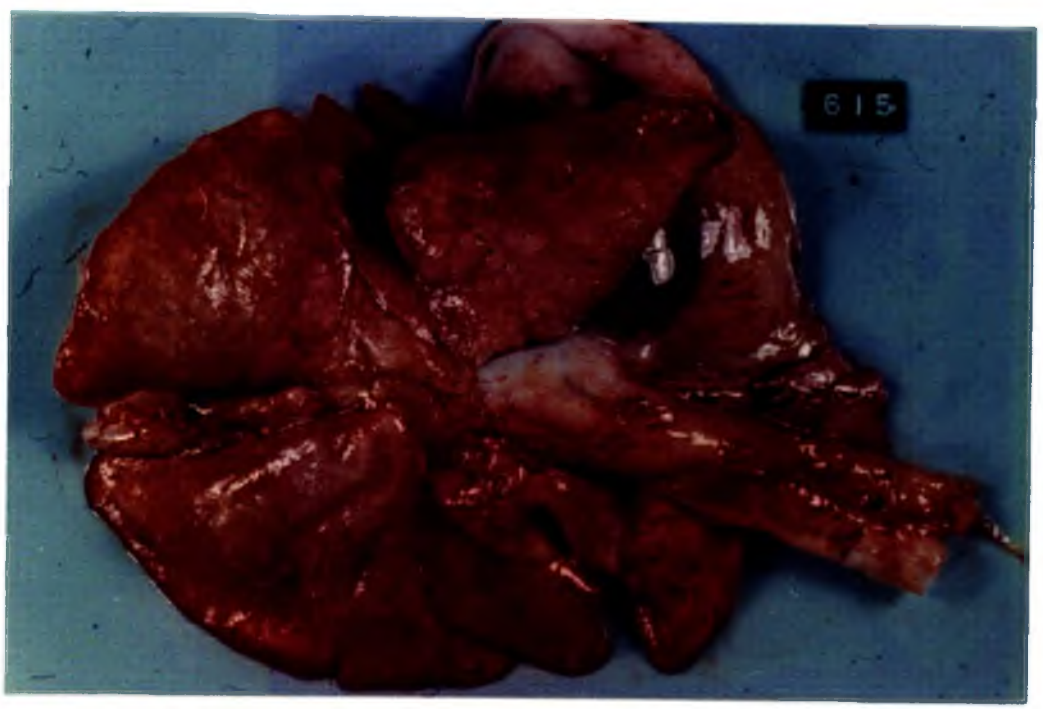
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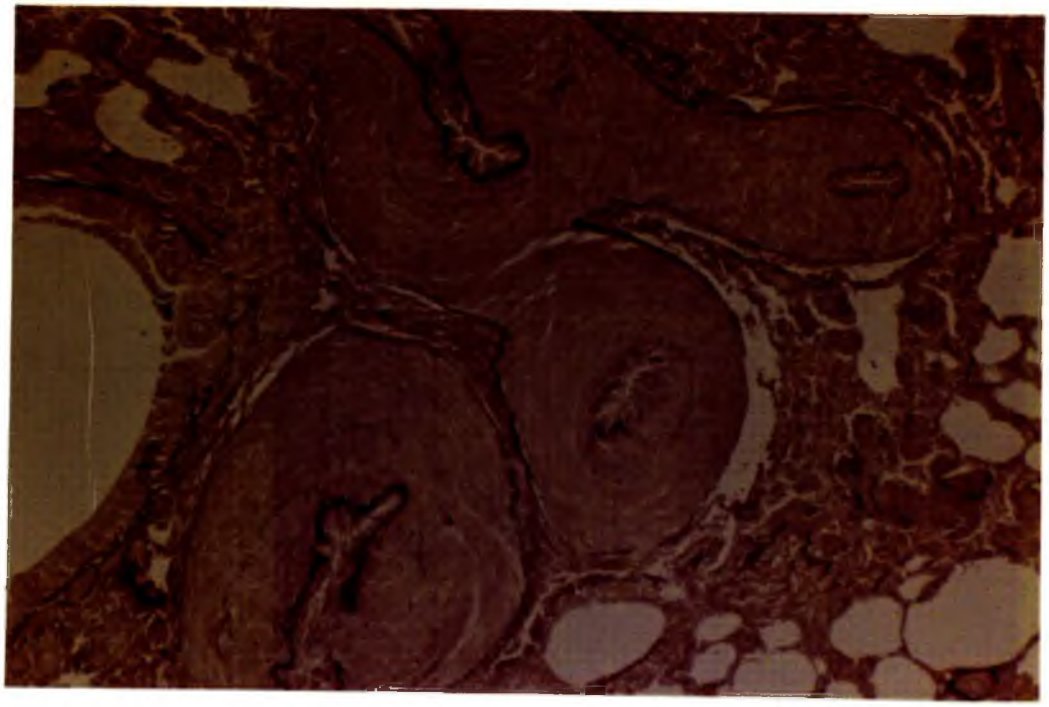
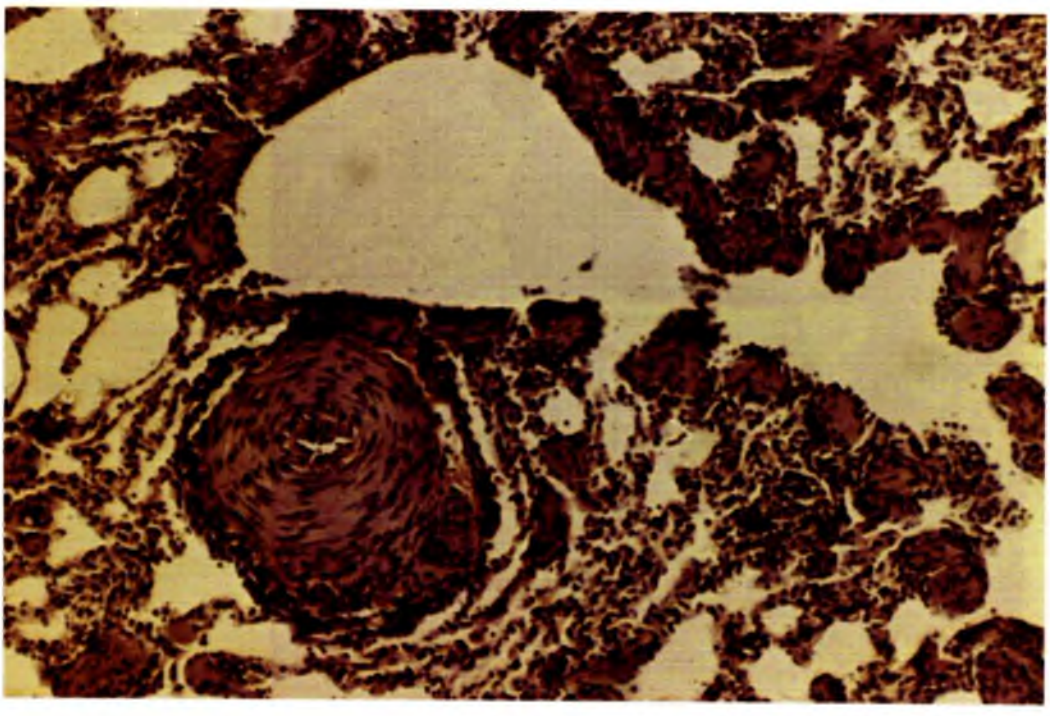
Fig. 16 Thickened and tortuous branch of the pulmonary artery. Fragmentation and incipient dissolution of the elastic laminae may be noted.

Lawson and Van Gieson x 150

Fig. 17 Hypertrophic change in the muscle of the wall of a bronchiole and alveolar duct. A thickened muscular pulmonary artery is also in evidence.

Haematoxylin and Eosin x 150





Discussion

The cost of housing and maintenance of cats for the long periods necessary before re-infestation tend to preclude the use of large numbers of animals. However, the results of re-infection of a small, but adequately controlled, group of cats was revealing. All five animals resisted re-infection and it is doubtful if third-stage larvae even reached the lungs. Since viable adult worms were present in the lungs of all the experimental cats, those to be seen in the lungs of the re-infected animals were probably survivors from the initial infestation. It was impossible to differentiate, on gross examination or on histopathological grounds, between the lungs of the re-infected cats and those of Control Group One, i.e., the animals that had been initially infected 472 days previously.

The cats of Control Group Two, infected at the same time and with the same number of third-stage larvae as were the re-infected animals, developed the clinical signs and autopsical appearances of lungworm disease.

That there is an acquired resistance to Aelurostrongylus abstrusus infestation is not altogether surprising since it is recognised that in other metazoan parasitic conditions, including lungworm disease of other species, an immunity is produced. Michel (1956) found that lambs previously infested with Dictyocaulus filaria were resistant to further infection and that the worms failed to reach, or to establish themselves, in the lungs. The

immunity so acquired was not the result of age and that finding seems to be equally relevant to the case of Aelurostrongylus abstrusus in the cat (Hamilton, 1966b). Soulsby (1965) suggested that an immunity was created by the presence of Metastrongyles in pigs and Michel (1965) as well as Jarrett et al. (1957) described unsuccessful attempts to re-infect calves with Dictyocaulus vivinarus. The latter authors also found that, even in heavy re-infection, clinical signs were mild and the disease did not become patent. In the latter respect, there is a similarity between the reactions to the bovine and feline lungworms.

With regard to infection by Dictyocaulus viviparus, Michel and Coates (1958) concluded that, in the absence of re-infection, protection did not last for long. Michel (1962) considered that immunity was present after ten days of infestation but that it declined, possibly as a result of loss of adult worms, from 100 days onwards. In a later publication, Michel and McKenzie (1965) confirmed that resistance to establishment of a challenging infection was reduced after three months and ceased to exist at twelve months, from initial infection. Weber (1953), however, suggested that the duration of immunity might be as long as twenty-four months.

The beginning and the duration of the immune period in the cat which has been infected by Aelurostrongylus abstrusus is, as yet, unknown but the present work has shown that, 388 days from initial infection and despite the lack of stimulus from constant re-infection,

an immunity was present which was sufficient to afford protection against a moderately heavy re-infecting dose of third-stage larvae. It is considered possible that the persistence of immunity was related to the continued presence of adult worms in the pulmonary tissues of the infected host.

Experiment Four

Passive Immunisation in Lungworm Infection of the Cat

Introduction

Passive immunisation against a number of helminths has been attempted with varying degree of success. Sarles and Taliaferro (1938) transferred resistance to Nippostrongylus muris by the administration of immune serum, Lawler (1940) did likewise with Strongyloides ratti and Otto (1940) and Miller (1967) successfully immunised dogs against Ancylostoma caninum. Jarrett et al. and Rubin and Weber (1955) showed that immune serum conferred a high degree of protection on calves experimentally infected with Dictyocaulus viviparus. Kelley and Nayak (1965a and 1965b) found that immunity against porcine ascariasis was transmissible via the colostrum as well as by administration of immune serum and Mulligan et al. (1966) produced resistance in rats against Nippostrongylus brasiliensis. Passive immunisation of cats against the lungworm, Aelurostrongylus abstrusus, has not yet been described. The preceding experiment, however, demonstrated that there is apparently a strong resistance to re-infection of cats by the parasite and the purpose of the experiment about to be described was to discover whether, or not, resistance is transferrable to other animals by means of immune serum.

Materials and Methods

Five kittens, twelve to fifteen weeks of age, were orally

infected with 800 third-stage larvae of Aelurostrongylus abstrusus. The animals developed clinical signs of lungworm disease, in the course of which disorder large numbers of first-stage larvae were excreted in the faeces. Six months after initial infection when all signs of the disease had disappeared, a second dose of 800 infective larvae was given. Twelve weeks later, the animals were killed and bled. The blood was allowed to clot and the serum removed, centrifuged and pooled. A total of 140 ml. of serum was so collected. By intraperitoneal injection and over three successive days, four kittens (Nos. 1 - 4), twelve - fifteen weeks of age and weighing an average of 925 gm., were each given a total of 35 ml. of serum. One day after the last injection, each cat was infected orally with 800 third-stage larvae of Aelurostrongylus abstrusus. At the same time, two control animals (Nos. 5 and 6) were given a similar infestation. On four occasions between the tenth and twelfth weeks after infection, the faeces of all kittens were examined for the presence of first-stage larvae and, where applicable, the number of larvae per gramme of faeces was counted. At the end of the experimental period, autopsy was performed and sections of relevant tissues were removed and treated in a manner similar to that described in Experiment One.

Results

Table 10 shows that larvae were not found in the faeces of three

Table 10

The Number of First-stage Larvae of Aelurostrongylus abstrusus Recovered per Gramme of Faeces from Immunised and Control Cats

Sample No.	Immunised cats 1	2	3	4	Control cats 5	6
1	-ve	-ve	-ve	250	9,300	6,050
2	-ve	-ve	-ve	310	12,000	8,000
3	-ve	-ve	-ve	290	14,600	8,950
4	-ve	-ve	-ve	350	16,500	12,300

of the immunised cats and only a small number was recovered from the fourth animal. The two control kittens, however, excreted large numbers of first-stage larvae and only in these cats were the clinical signs of increased respiratory rate and of coughing noted.

Post-Mortem and Histopathological Findings

a Control Cats

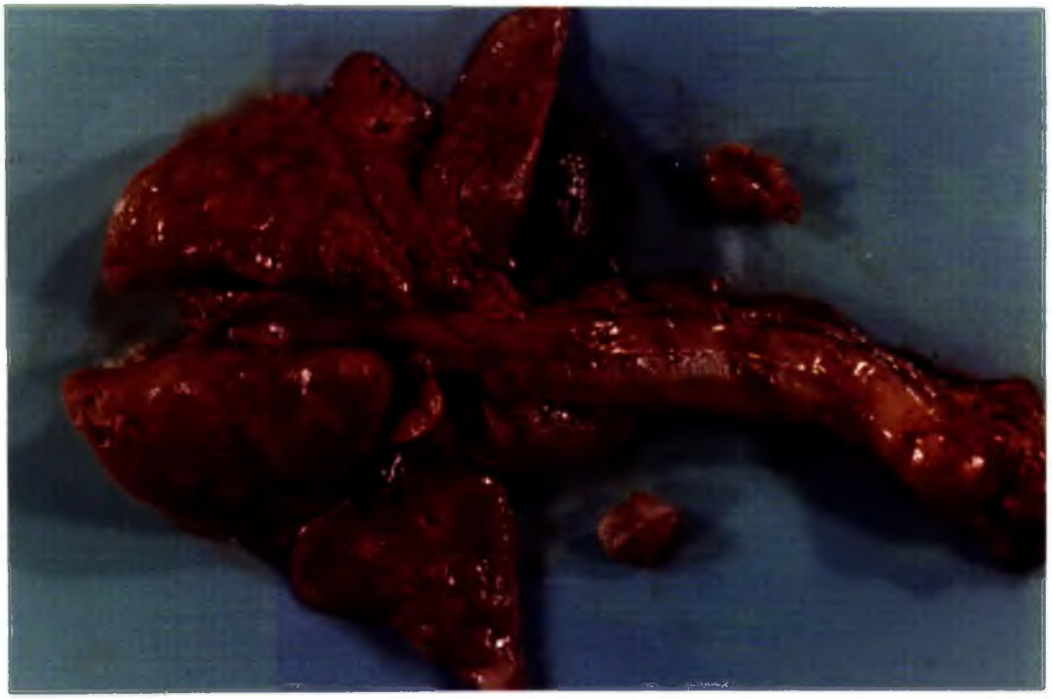
The lungs of both animals were severely affected and showed the characteristic gross and histopathological changes of lungworm disease which have been earlier described (Fig. 18).

b Immunised Cats

Gross examination of the lungs revealed the presence of a few pale, pin-point foci scattered throughout all the lobes while, in addition, those of one cat (No. 4) showed a number of lesions of up to five millimetres in diameter (Fig. 19 and 20). The bronchial lymph-nodes of all the animals were moderately enlarged. Histopathologically and in each cat, the pulmonary lesions were comparable. Small, cellular foci, mainly lymphocytic in nature and especially prominent around bronchi and arteries, were evident as was the occasional adult worm surrounded by a cellular reaction in which eosinophil leucocytes predominated. A small number of branches of the pulmonary artery manifested medial hypertrophy and there was some increase in thickness of the muscle of the bronchioles and alveolar ducts. The latter

Fig. 18 Lungs of a control cat twelve weeks after infection with 300 third-stage larvae. Lesions are widespread throughout the lungs and the bronchial lymph-nodes are notably enlarged.

Fig. 19 Lungs taken from two passively-immunised cats. Gross parasitic lesions are not obvious and the haemorrhagic focus in the left-hand set of organs was the result of intrathoracic injection of pentothal.



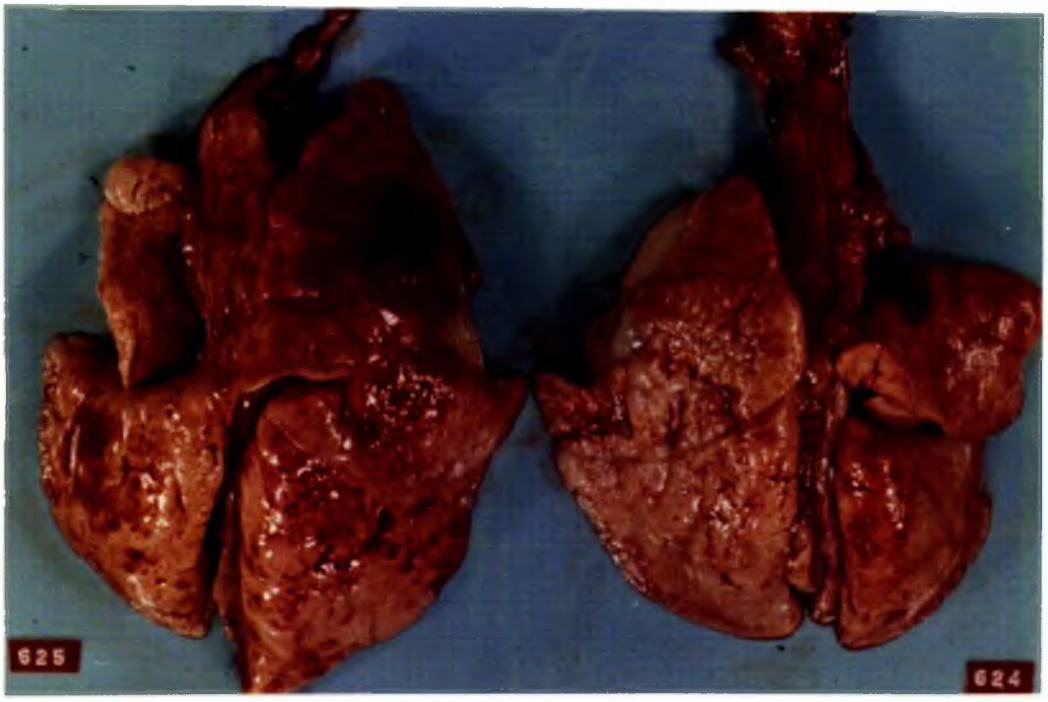

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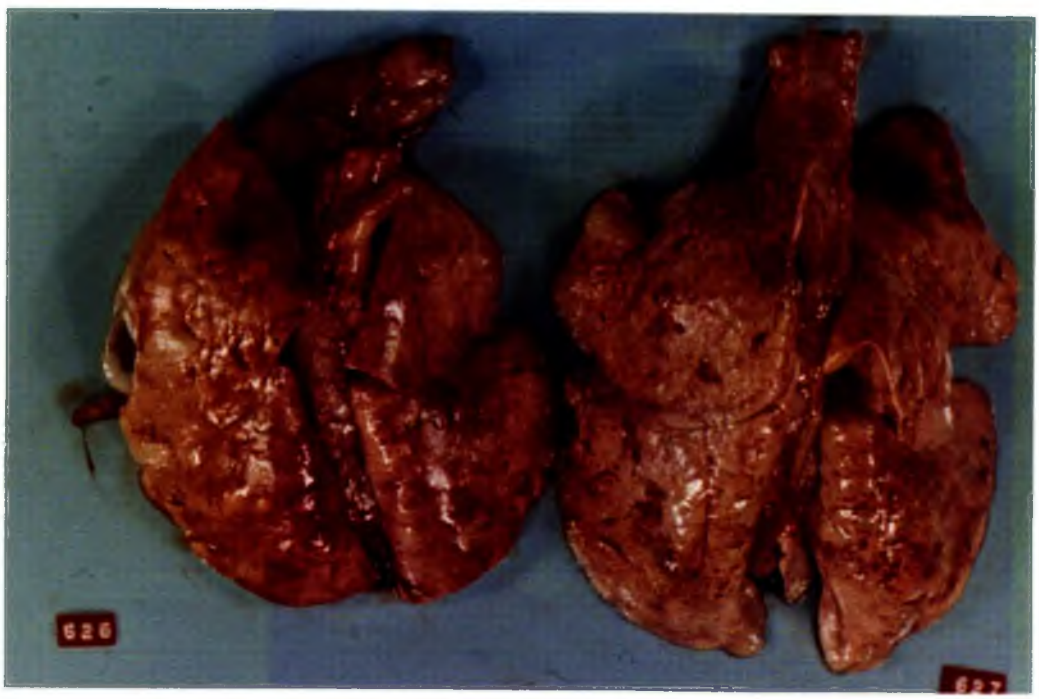
Fig. 20 Lungs taken from another two passively-immunised cats. A few lesions may be seen in the diaphragmatic lobes of the right-hand set of organs.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changes were more obvious in the lungs of Cat No. 4 where, too, several foci containing eggs and larvae were present. Such lesions were highly cellular and consisted of a mixture of macrophages, giant-cells, lymphocytes and eosinophil leucocytes but, despite those findings, the associated infection was of a mild nature.

Discussion

The blood volume of the cat varies between 65 and 69 ml. per kilogram of body weight (Schalm, 1965) and, on that basis, the experimental kittens received an amount of immune serum which was approximately equivalent to the total content of blood serum. It was considered that, if antibody was present in the donated serum, the dose given would effectively produce resistance to infection by Aelurostrongylus abstrusus. The results were not disappointing since, of four immunised cats, three were protected to high degree and the fourth only to a slightly lesser extent. The two control animals, which had received the same number of third-stage larvae, possessed pulmonary lesions which were widespread and severe.

The presence of adult worms in the lungs of the immunised cats indicated that third-stage larvae had reached the latter organ and, in one case, the parasites had matured sufficiently to produce eggs and to cause first-stage larvae to be excreted in the faeces. In Dictyocaulus viviparus infection, Jarrett et al.(1955) found that, of seven passively immunised calves, the lungs of three were free

of parasites while the total number of worms recovered from the other four animals was approximately 5,0% of that removed from five non-immunised calves. However, because of the difficulty of isolating adult lungworms from infected cats, it is impossible to decide either that third-stage larvae had been prevented from reaching the lungs in normal numbers or that larval development had been interfered with or, even, that oogenesis on the part of adult worms had been inhibited. Taliaferra and Sarles (1939), discussing cellular reactions against Nippostrongylus muris, concluded that the primary antiparasitic agent was humoral antibody and that the cellular response occurred after immobilisation of the parasite. The nature of pulmonary lesions in the animals of the present experiment seem to support that theory.

Whatever the mechanism involved, it was satisfactorily demonstrated that immune serum, whether, or not, it owed its protective effect to the presence of specific antienzymes as suggested by Chandler (1935) or the existence of antibodies against somatic antigen, conferred adequate protection against a pathogenic dose of third-stage larvae of Aelurostrongylus abstrusus.

Experiment Five

The Production of Immunity against Lungworm Disease by Repeated Administration of Non-Pathogenic Numbers of Third-Stage Larvae of *Aelurostrongylus abstrusus*

Introduction

Immunisation against parasitic disease has been effected by various means. It has been achieved by the use of secretions and metabolites of larvae (Thorson, 1953), by infection of animals with closely related strains of parasites (Parfitt and Sinclair, 1967), by administration of artificially attenuated larvae (Jarrett et al., 1960) and by the injection of standard doses of normal infective larvae. The latter method is easiest and has been employed against a variety of parasites with success e.g., with Dictyocaulus viviparus (Jarrett et al., 1959 and Weber and Lucker, 1959), with Nematodirus Spp. (Kates and Turner, 1953 and Gibson and Everett, 1963), with Haemonchus placei (Roberts and Keith, 1959), with Haemonchus contortus (Roberts, 1957), with Oesophagostomum radiatum (Roberts et al., 1962) and with Strongylus vulgaris (Enigk, 1951). The immunising larvae may be given on one occasion or as multiple small doses.

Earlier work has established that cats, previously infected with Aelurostrongylus abstrusus, are resistant to further infestation and that immunity may be passively transferred. Thus, it was considered that a method of vaccination which would protect susceptible animals was capable of development and the following report details the results of an attempt to produce immunity against the feline lungworm by means of repeated, oral

administration of unattenuated, third-stage larvae.

Materials and Methods

On three occasions and at monthly intervals, ten kittens, ten to fifteen weeks of age, were given orally fifty third-stage larvae of Aelurostrongylus abstrusus. Faeces were examined for the presence of first-stage larvae. One month after the final dose, two cats were autopsied (Control Group One), two kept for control purposes (Control Group Two) and six were challenged with 800 third-stage larvae. Two normal cats were infected with a similar number of parasites and retained as controls (Control Group Three). Nine to ten weeks later, first-stage larvae were excreted by one of the challenged animals and by both cats of Control Group Three. Thereafter, faecal larval counts were recorded on fifteen occasions over a period of four weeks. At the end of that time, the animals were killed and, at autopsy, representative portions of relevant organs were removed, processed and stained by the methods previously described (Experiment One) with the sole addition of a Prussian-blue method required for the demonstration of haemosiderin.

Results

Until the time of challenge, first-stage larvae were not recovered from the faeces of any of the cats that had been dosed

Table 11

The Number of First-Stage Larvae per Gramme of Faeces Excreted by Immunised and Control Cats between the 10th and 14th Week after Challenge

	Immunised Cats						Control Cats	
Sample	1	2	3	4	5	6	1	2
1	–	–	–	–	–	–	600	800
2	200	–	–	–	–	–	2,500	4,400
3	–	–	–	–	–	–	3,000	5,000
4	400	–	–	–	–	–	2,600	3,000
5	100	–	–	–	–	–	3,600	7,300
6	200	–	–	–	–	–	2,500	6,000
7	400	–	–	–	–	–	2,400	10,400
8	400	–	–	–	–	–	4,000	15,000
9	300	–	–	–	–	–	3,200	13,000
10	100	–	–	–	–	–	4,000	10,200
11	300	–	–	–	–	–	3,600	20,300
12	200	–	–	–	–	–	4,600	18,000
13	500	–	–	–	–	–	3,000	19,000
14	300	–	–	–	–	–	10,200	29,000
15	200	–	–	–	–	–	8,600	30,200

with fifty third-stage larvae on three occasions. One of the six challenged cats and both animals of Control Group Three excreted larvae (Table 11) and coughing and some degree of respiratory distress was noted in the animals of the latter group.

Post-Mortem and Histopathological Findings

a Control Group One

The lungs of both animals were pink and spongy but displayed a small number of pale white lesions - several millimetres in diameter - in all lobes of the lungs. A raised focus of approximately one centimetre in diameter was present in a diaphragmatic lobe. The bronchial lymph-nodes were moderately enlarged, homogeneously pale in appearance and of firm consistency. Histopathologically, densely cellular foci which contained degenerating eggs, macrophages, lymphocytes and eosinophil leucocytes together with some giant-cells were observed. Occasional degenerated nematodes, heavily invested by lymphocytes and macrophages, were recognised but whether, or not, the former structures were remnants of adult worms from the first, or larvae from subsequent, infection was unable to be ascertained. Around such parasites the presence of intracellular haemosiderin pigment was noted. Localised areas of bronchitis and bronchiolitis were appreciated but viable adult nematodes were not demonstrable. Peribronchial, but particularly perivascular, lympho-reticular proliferation was prominent and, especially in affected areas,

hypertrophic changes which were sometimes of an advanced nature affected the media of a number of branches of the pulmonary artery. A similar condition was apparent in the muscular walls of the alveolar ducts and bronchioles. An increase in the lymphocytic elements of the bronchial lymph nodes had occurred. Despite those alterations, the pulmonary disease was considered to be mild.

b Control Group Two

The two cats had received the same treatment as the animals of the previous group but had been allowed to live for a further fourteen weeks. At autopsy, lesions consisted of a small number of non-elevated, pale-white foci of one to several millimetres in size which were quite superficial in position. The bronchial lymph nodes were enlarged. Histopathologically, the main alteration was hypertrophy of the media of a number of muscular pulmonary arteries and of the walls of alveolar ducts and bronchioles. Compared with that found in the lungs of the animals of Control Group One, peri-vascular and peribronchial lympho-reticular reaction was less intense and, since neither eggs nor larvae were present, cellular infiltration of the pulmonary tissues was greatly reduced. Adult worms were demonstrated with great difficulty.

c Control Group Three

The two cats that had been infected with 800 third-stage larvae excreted large numbers of first-stage forms, (Table 11). Lesions

characteristic of severe lungworm disease were noted at post-mortem and histopathological examinations.

d Challenged Group

At autopsy, the lungs of three of the cats were pink and spongy and showed a small number of foci which were a few millimetres in diameter, pale cream in colour and fairly firm in consistency (Fig. 21). Lesions in the lungs of the remaining three animals were similar to the above except that they were more numerous and had fused in a number of areas, to produce foci of up to a centimetre in diameter (Fig. 22). The bronchial lymph-nodes of all cats were moderately enlarged and measured in the region of one centimetre in length by half a centimetre in breadth. The histopathological appearance of the pulmonary changes was comparable in all six cases. There were occasional small groups of degenerating eggs, sometimes larvae, which were confined by dense accumulations of macrophages and lymphocytes together with a few giant-cells and eosinophil leucocytes (Fig. 23). Hypertrophic changes in the medial coat of muscular pulmonary arteries associated, in some instances, by swelling and proliferation of the endothelium and infiltration of the intima by eosinophil leucocytes were more prominent in, but not exclusive to, the most affected regions of pulmonary tissue. On the other hand, hypertrophic change in the muscle of the walls of bronchioles and alveolar ducts was more widespread. Peribronchial

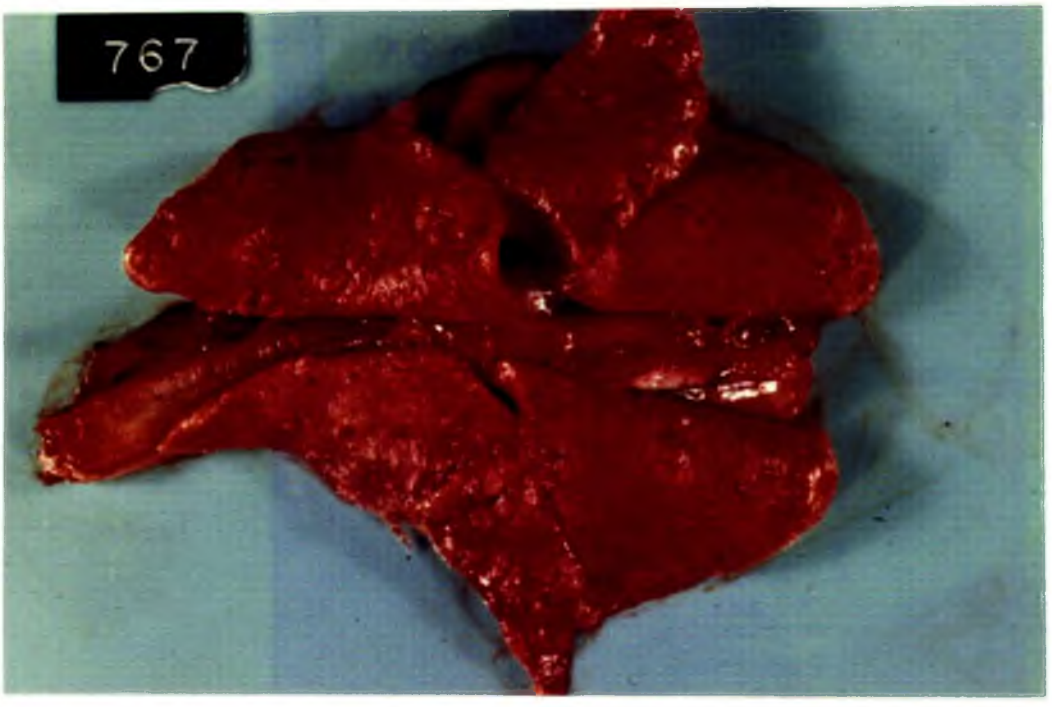
and perivascular lympho-reticular reaction was marked and, often, the latter consisted of follicular-like masses which exerted pressure on blood vessels (Fig. 24). Peribronchial lesions were most notable if associated with the presence of degenerating stages of the parasite (Fig. 25). Small numbers of adult worms, a large proportion of which were undergoing necrosis were found in pulmonary sections from all of the cats. The bronchial lymph-nodes showed proliferation of the lymphocytic elements with some slight eosinophil leucocytic infiltration of the hilar region.

Discussion

The administration of live, infective larvae in small numbers and on several occasions is the most convenient method of immunising animals against parasitic disease and such a method has been found to be reasonably successful in the case of the lungworm, Dictyocaulus viviparus (Jarrett et al., 1950 and Weber and Lucker, 1959). However, the procedure is not without disadvantage. The number of larvae given must be sufficient to stimulate the production of immunity but not to cause disease and, as animals may vary in health, nutritional status and response to infectious agents, it may be difficult to recommend a safe, standard dose. Even if clinical disease does not result, such a method may lead to the dissemination of first-stage larvae and, in the case of Aelurostrongylus abstrusus, cause

Fig. 21 Lungs from an immunised cat that had been challenged with 800 third-stage larvae. A small number of pale lesions varying between 1 and 5 mm. in diameter are appreciable.

Fig. 22 Lungs from the worst affected immunised cat after challenge with 800 third-stage larvae. It may be observed that lesions are more numerous, often raised and, by fusion, of larger size than those illustrated in Fig. 21.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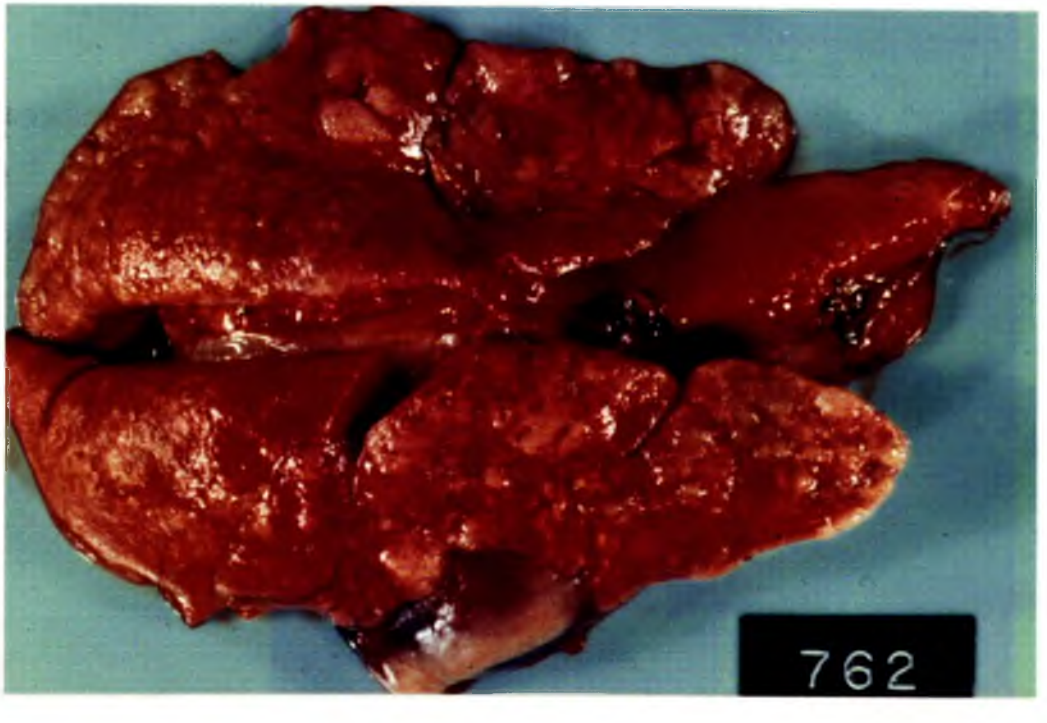
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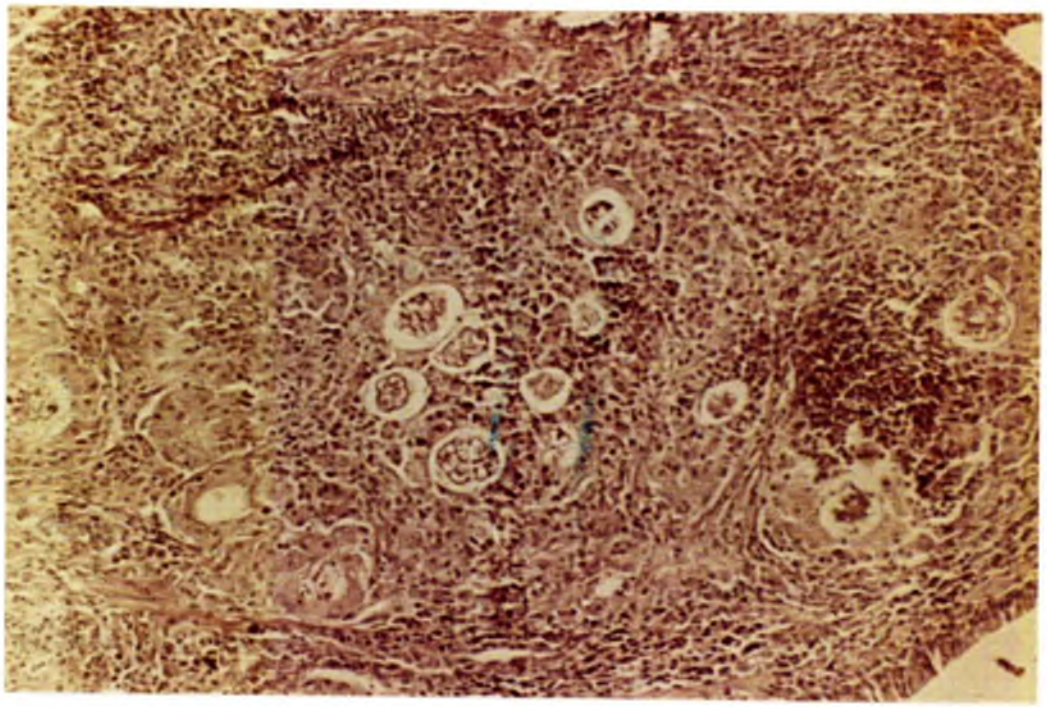
Fig. 23 Pulmonary section from an immunised cat that had been challenged with 800 third-stage larvae. A small group of degenerating eggs is surrounded by macrophages, lymphocytes, a few giant-cells and eosinophil leucocytes.

Haematoxylin and Eosin x 150

Fig. 24 Pulmonary section from a cat of the challenged group. There is a massive lympho-reticular reaction around a small, hypertrophied artery.

Haematoxylin and Eosin x 150



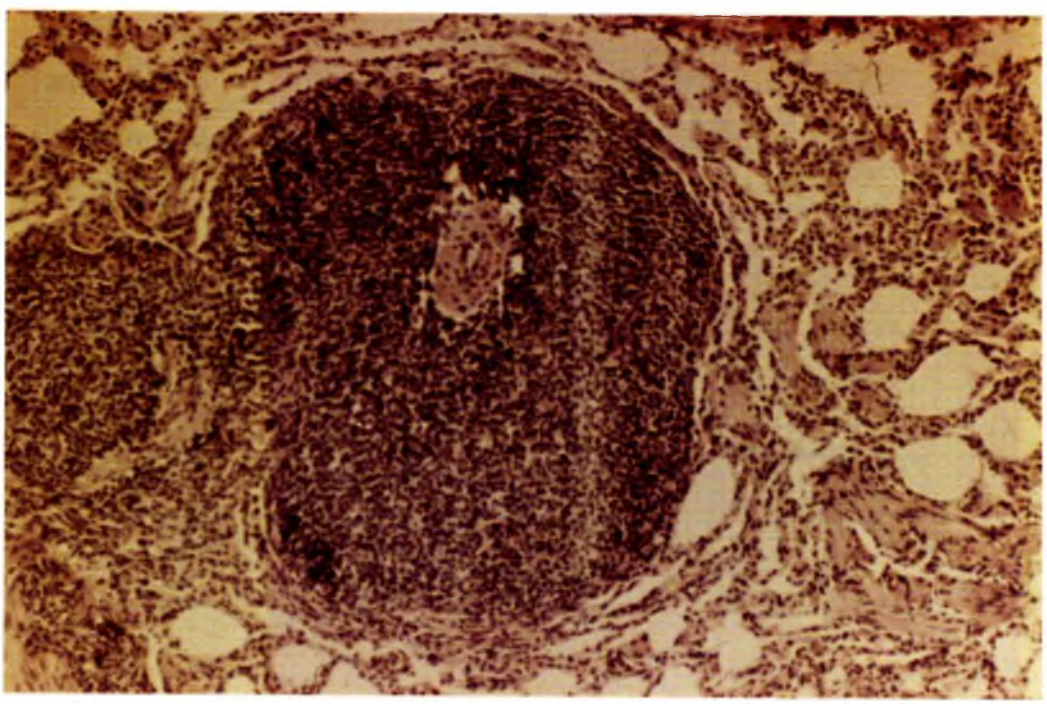
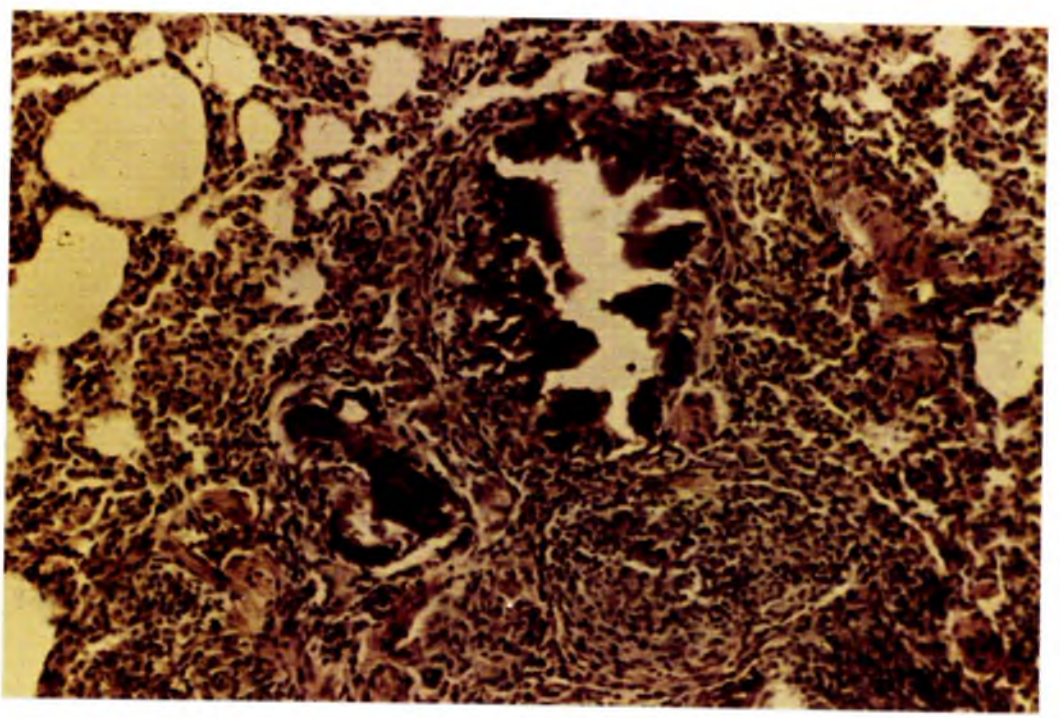


Fig. 25 Pulmonary section from the same animal as Fig. 24. Adjacent to a bronchus is a focus of lympho-reticular reaction which is associated with the presence of degenerated parasites.

Haematoxylin and Eosin x 150



the lungs of an animal to be parasitised for a considerable period of time.

In an earlier experiment (Part Two, Experiment One), it had been discovered that infection with fifty third-stage larvae had failed either to produce significant pulmonary disease or result in the excretion of first-stage larvae. Thus, a decision was made to use such a number on several occasions. Autopsical examination of the cats of Control Groups One and Two confirmed that a relatively mild pulmonary disease had been produced and it had been observed that neither clinical upset nor excretion of first-stage larvae had ensued. Degenerating parasites were appreciated in pulmonary sections of animals of Control Group One whereas, possibly because of fewness of numbers, adult worms were not demonstrable. The latter were seen occasionally in similar preparations from the cats of Control Group Two. In view of these findings, it was considered probable that adult parasites arising as a result of the first infection had survived while larvae of subsequent infestations had either been inhibited in their development or destroyed by the immune reactions of the immunised animals. Thus, such a method of vaccination may induce a light parasitisation of the lungs but, as the persistence of immunity may be related to the presence of adult worms (Parker and Roberts, 1958), such a state may be of advantage to the host. However, in lungworm disease in calves Michel (1955) suggested that inhibited forms subsequently matured and caused

intermittent excretion of larvae by the host. Such an event was considered, by that author, to constitute a possible hazard to other grazing animals. Should a similar incident occur in a cat, previously infected with small numbers of lungworms, insignificant harm would accrue to the host and, because of the complicated life-cycle of the parasite, there would be minimal danger to other members of the species.

The pulmonary lesions of the challenged animals were mainly of the nature of an immune response. There was little evidence of the presence of parasitic forms with the exception of small groups of degenerating eggs, rarely larvae, and around such structures as well as around blood-vessels there was an intense lympho-reticular reaction. A similar proliferation in the region of bronchi was seen notably in association with degenerating stages of the parasite. It is possible that such widespread and excessive cellular accumulation was concerned in the production of antibody although that, as suggested by Jarrett and Sharp (1963), may have been produced against somatic antigens and of little protective value. Another possible site of antibody formation was the bronchial lymph-nodes in which considerable lymphocytic proliferation was constantly recognised. The pulmonary lympho-reticular nodules, described by Jarrett and Sharp (1963) to occur in cattle infected with lungworm, were not a feature of the feline condition. Despite the heavy challenging dose, few adult worms were demonstrable in histopathological preparations, which

finding was presumably the result of the resistant state of the animals.

It is concluded, therefore, that immunisation of cats with small, repeated administrations of live, third-stage larvae of Aelurostrongylus abstrusus elicits the production of an immunity which is strong enough to prevent subsequent infection by the latter parasite.

However, the animals of Control Groups One and Two as well as those of the Challenged Group displayed pathological alteration to branches of the pulmonary artery and the importance of that change requires to be assessed. None of the cats had shown evidence of respiratory distress or of cardio-vascular deficiency, even when exercised whereas the members of Control Group Three, when similarly treated, coughed and became dyspnoeic. It may be suggested, therefore, that the arterial lesions of the immunised cats were of little immediate significance. Although it is known that such an arteriopathy may persist for as long as two years, the evidence suggests that, in young cats, the severity of the change does not increase and, indeed, may lessen (Part Two, Experiment Six). Previous results (Part Two, Experiment One) have also intimated that the extent of arterial involvement is dependent upon the number of infecting larvae. Other investigators of a similar condition in the same species (McKenzie, 1960; Dahme, 1960; Scratchard and Wright, 1961 and

Tashjian, 1965) did not describe any associated debility although Stunzi et al.(1966), by dissecting and weighing the chambers, established that hypertrophy of the right side of the heart frequently developed. As the latter authors were dealing with autopsical material, clinical correlation was not possible. There is, unfortunately, a complete absence of information on the effects of the vascular abnormality on pulmonary and systemic blood pressures but, on the basis of clinical observation and pathological studies of the average case, it may be suggested that the arteriopathy produces little disturbance in an affected animal. Possible exceptions to that statement are cats, infected with 1,500 or more, third-stage larvae, in which widespread and severe damage to the pulmonary tissues and vasculature has resulted. However, such levels of infection are beyond any contemplated immunising dose.

Although the pathological importance of the arterial change in vaccinated animals appears to be little, it is desirable to reduce, if possible, the extent and severity of the vascular damage. That may be achieved by the use of a number of larvae, similar to that recorded in the present experiment, on more numerous occasions or, alternatively, by employment of a smaller total amount over several doses. Cornwell (1962) found that parenteral injection of fourth-stage larvae of Dictyocaulus viviparus produced immunity in calves and a similar method, if applied to Aelurostrongylus abstrusus, may

be of value. The latter is likely to be reduced, however, since it has been established that subcutaneous infection of cats by the parasite is not a wholly reliable method (Part Two, Experiment Two). The use of irradiated larvae has proved to be a successful means of vaccination against lungworm infection of cattle (Jarrett et al., 1960; Jones et al., 1961 and Armour and Urquhart, 1965). The immunogenic larval stages of Dictyocaulus viviparus are probably the third and fourth (Jarrett and Sharp, 1963) and personal observations suggest that such is the case with Aelurostrongylus abstrusus. Irradiated larvae of the bovine parasite reach the lungs and although the majority has disappeared by the end of two weeks, stunted survivors may persist for a longer period (Urquhart et al, 1962). The findings of the subsequent experiment (Experiment Six) show that, after nine days of infection by the feline lungworm and at a time when fourth-stage larvae are present, pulmonary arterial change is evident. If, as appears likely, irradiated larvae of Aelurostrongylus abstrusus attained the lungs then, presumably, the chances of arterial damage are real and, accordingly, vaccination by the above method might show small advantage over that performed by the agency of live larvae. Confirmation of that hypothesis must await the outcome of further experiment.

In summary, it may be stated that repeated administration of small numbers of infective, third-stage larvae of Aelurostrongylus

abstrusus conferred a high degree of immunity to further infection, by the same parasite, on experimental cats. However, mild pulmonary lesions were produced but neither clinical disturbance nor excretion of first-stage larvae supervened. It is concluded, therefore, that active immunisation offers an effective method of control of feline pneumonia induced by infestation with the above parasite.

Experiment Six

The Influence of Infestation by *Aelurostrongylus abstrusus* on the Pulmonary Vasculature of the Cat

Introduction

Hypertrophy, and in some cases hyperplasia, of the medial coat of the pulmonary arteries of the cat has been described by Campbell (1927) in the case of animals suffering from oxygen deficiency and of cats injected intravenously with the dye, Janus green (Ettinger, 1932). Rubarth (1940), Marcato (1940), Olcott et al. (1946), Martin (1959), Dahme (1960), Scratchard and Wright (1961) and Tashjian et al. (1965) recorded the finding of an idiopathic form of medial hypertrophy and hyperplasia, intimal fibrosis and degeneration of the elastic laminae of pulmonary vessels in the same species and Neumann et al.(1942) and Kell et al.(1956) attributed similar changes to the injection of insulin and metrazol and the excitation of cerebral pressor mechanisms, respectively. Pritchett (1938), Blaisdell (1952), McKenzie (1960), Jubb and Kennedy (1963), Hamilton (1963 and 1966a) and Stunzi et al. (1966) remarked on the association of infestation by the lungworm, Aelurostrongylus abstrusus, with the pulmonary arteriopathy. Initially the attention of the author had been drawn to the above parasite because of its apparent effect on the pulmonary vasculature but, because of the lack of information and the conflicting nature of some of that already available, it was considered necessary to investigate various aspects of the parasite and its life-cycle, which task has been accomplished and

described in the preceding pages. Despite the variety of experiments, the effect of lungworms on the pulmonary vessels of the cat has constituted a link to many of those procedures and the following discussion is based on information gleaned from the foregoing experiments as well as from additional investigations.

Materials and Methods

Use was made of seventy-four kittens which had been infected with lungworms in the course of experimental work. In addition, twenty-three kittens were killed and examined at periods varying between one and forty-eight hours after infection, ten were autopsied between three and fourteen days, five between three and seven weeks and a further ten animals were retained for periods ranging from six to twenty-four months. In general, kittens killed up to fourteen days after infection had received 100 - 300, and the remainder were dosed with 800, third-stage larvae. A total number of 122 experimentally, and twenty-five spontaneously, infected cats was examined and the pulmonary lesions were compared with those of seventy-two animals of varying ages that had been found to suffer from pulmonary arterial disorder without apparent evidence of lungworm disease. All animals were subjected to post-mortem examination and portions of relevant tissues, comprising lungs,

gastro-intestinal tract, brain, myocardium and kidneys were fixed in 10.0% formol-saline or 10.0% corrosive-formol, embedded in paraffin-wax, sectioned at 5 microns and stained by haematoxylin and eosin. In addition, a combined Lawson and Van Gieson as well as Sudan IV stains were applied to selected parts of the lungs.

Results

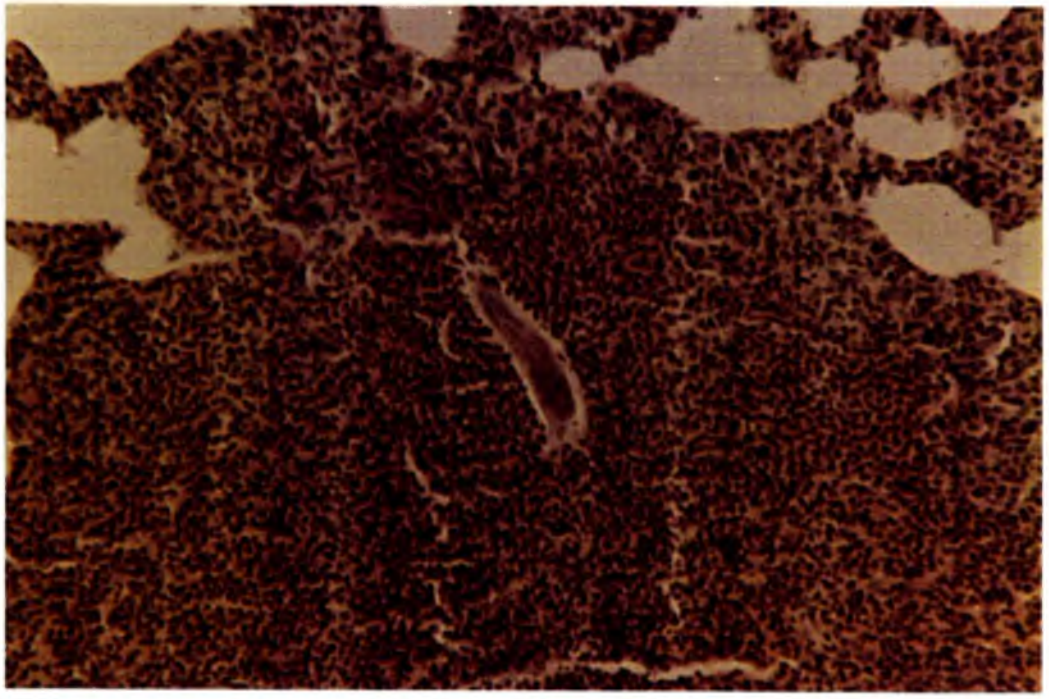
Neither gross nor microscopical pulmonary changes were noted one, two, six and sixteen hours after infection. By twenty-four hours, a mild peribronchial, and particularly perivascular, lymphocytic reaction had developed and the presence of a few small foci of mainly lymphocytic character were observed. At forty-eight hours, appearances were similar except for the prominence of a number of lesions, displaying central necrosis and consisting of lymphocytes, macrophages and a few eosinophil leucocytes. At three, five and seven days, the only further difference was the occurrence of an occasional larva at the centre of some cellular foci (Fig. 26) but, during that time, the pulmonary vessels had not undergone any pathological change apart from some congestion. Nine days after infection, the cellular reaction was still mainly mononuclear in nature but there was also evidence of thickening of the media of some muscular pulmonary arteries and of emigration from those vessels of eosinophil leucocytes (Fig. 27). Two days later, the

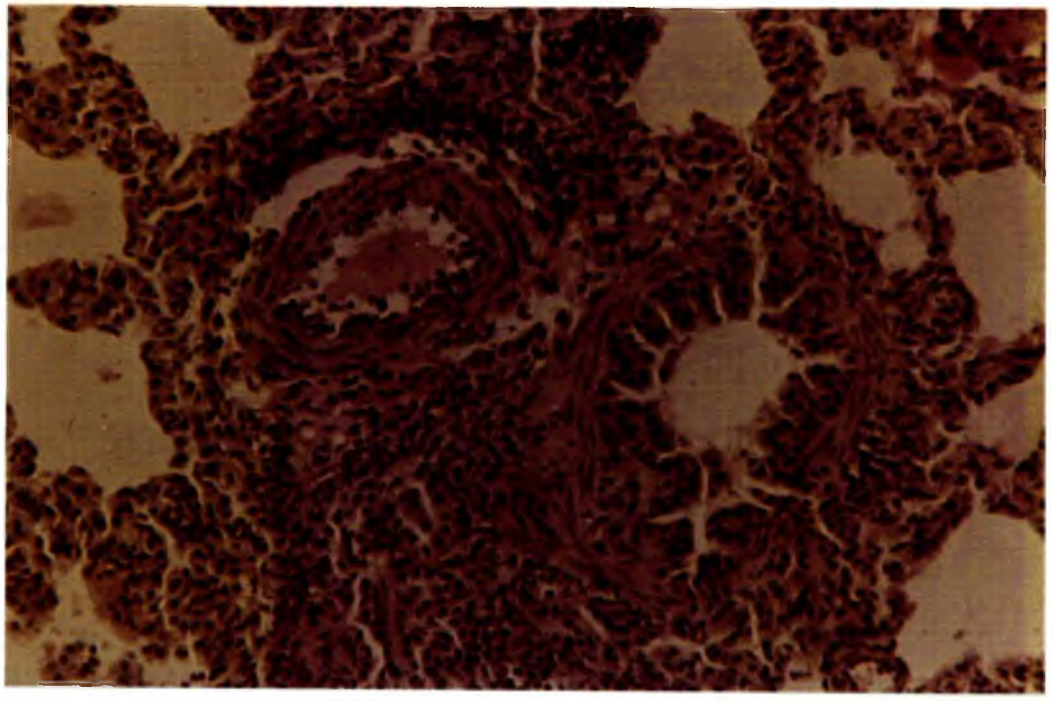
Fig. 26 Third-stage larvae of Aelurostrongylus abstrusus in the lungs of a cat three days after infection, surrounded by lymphocytes, macrophages and a few eosinophil leucocytes.

Haematoxylin and Eosin x 150

Fig. 27 Eleven days after infection. Hypertrophic change is apparent in the bronchiolar muscle. A similar state exists in the media of a muscular pulmonary artery along with emigration of eosinophil leucocytes through the vascular wall.

Haematoxylin and Eosin x 150





hypertrophic change was more advanced and had come to involve the muscle of the walls of the bronchioles and of the alveolar ducts. The lungs of animals killed between the thirteenth and twenty-first days showed few other features although the degree of perivascular and peribronchial reaction had increased and, occasionally, some bronchiolitis was apparent. Up to that time, too, gross changes had consisted of multiple, punctiform, pale areas scattered throughout the substance of both lungs.

Signs of sexual maturity on the part of the parasite were to be appreciated microscopically by the twenty-eighth day when a small number of eggs, surrounded by lymphocytes, macrophages, eosinophil leucocytes and some giant-cells, were noted (Fig. 28). Increased thickness of the media of a number of branches of the pulmonary artery was associated with the passage of eosinophil leucocytes through the vascular walls and peribronchial and perivascular lymphocytic accumulation was more conspicuous. Grossly, multiple, pale foci of up to one millimetre in diameter were observable but, as the disease progressed, enlargement and coalescence of those lesions gave rise to considerable pulmonary consolidation. At six to eighteen weeks, depending on the infecting dose of larvae, the pulmonary lesions were most marked and all the lobes showed large areas of cellular infiltration that were located around developing eggs and larvae and often showed central necrosis. The main types

of cell consisted of eosinophil leucocytes, lymphocytes, macrophages and numerous giant-cells. There were many areas of epithelialisation and, away from the main foci of activity, there was considerable cellular infiltration into the interstitial tissues and alveoli while around the bronchi and bronchioles there was marked accumulation of lymphocytes. In the most affected parts, ulceration of the mucosa of bronchi and bronchioles together with hyperplasia of the peribronchial glands were prominent. Bronchitis and bronchiolitis were associated with the presence within lumina of ova, larvae, eosinophil leucocytes and desquamated epithelial cells and adult worms were to be seen in alveoli, alveolar ducts and bronchioles. Vesicular emphysema was also conspicuous. Migration of lymphocytes and of eosinophil leucocytes into, and around, the arterial walls was marked and, in some vessels, there was swelling and proliferation of the endothelium. Hypertrophy and hyperplasia of the muscle of the media of some pulmonary arteries caused a thickening of three to twelve times that of normality and although lesions of the vasculature were widespread, particularly in the more heavily infected cases, normal arteries were always identifiable. Occasionally, serial sections revealed the presence of local occlusion of a vessel but, as a rule, an affected branch was entirely altered. Stretching, followed by fragmentation or even dissolution, of the elastic laminae accompanied the increase in

muscular content of the arterial walls. Simple hypertrophy of the muscle of alveolar ducts and of bronchiolar walls was commonly prominent (Fig. 28).

Regression of parasitic lesions occurred twelve to twenty weeks after initial infection when the number of larvae was reduced and often, then, groups of ova heavily invested by cells were the only form of the parasite present. In the majority of animals, at the end of six to nine months, eggs, larvae and most of the associated cellular reaction had disappeared and only a few adult worms remained. However, serial sections of lungs often revealed the presence of clusters of eggs, and rarely of larvae, which were in the process of phagocytosis by macrophages and giant-cells. The existence of those foci of reaction explained some of the lesions characteristic of the late post-patent stage of lungworm disease.

During resolution, and thereafter, the severity of the arteriopathy remained constant but, possibly because of the removal of the parasites and the accompanying cellular reaction, the changes became more obvious. Branches of the pulmonary artery were grossly thickened, had become quite tortuous and displayed abnormalities of the elastic laminae (Figs. 16 and 29). Vacuolation of hypertrophied muscle cells occurred and, in many areas, swelling and proliferation of the endothelium, often infiltrated with numerous eosinophil

leucocytes, were common and the presence of slight intimal fibrosis was noted. Marked hypertrophy of the smooth muscle of the walls of bronchioles and of the alveolar ducts persisted. Peribronchial and perivascular lymphocytic reaction (Fig. 30) was common and, in places, there was an increased cellularity of the alveolar septa.

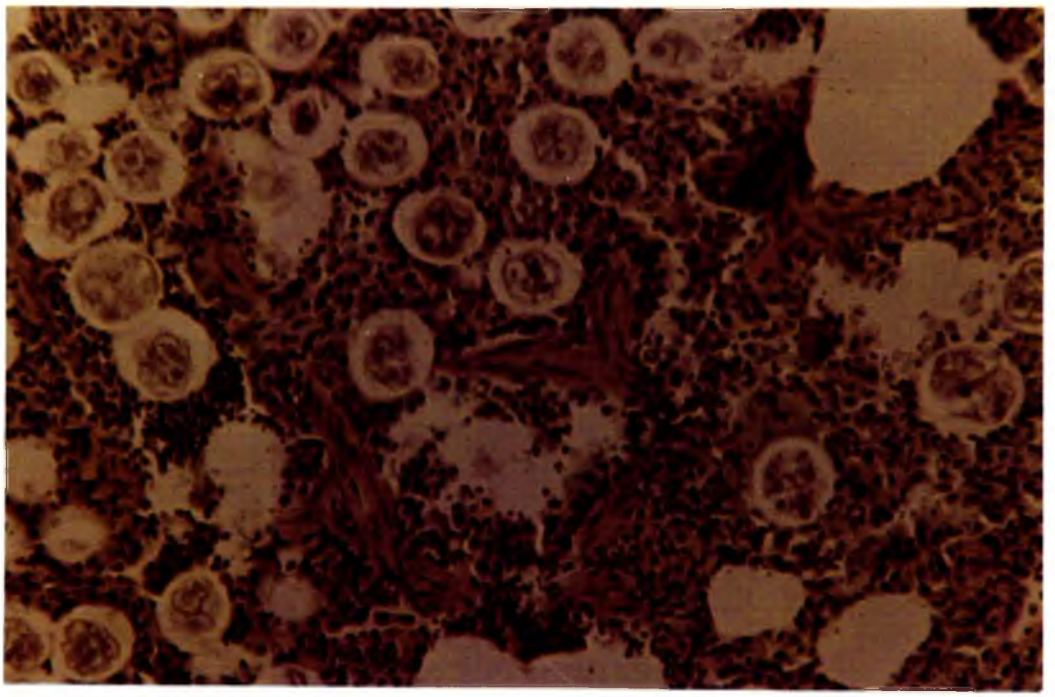
Sixteen, eighteen and twenty-four months after infection, gross pulmonary lesions consisted of a number of pale foci, one to three millimetres in diameter, scattered throughout the lobes. Histopathologically, the outstanding abnormality lay in branches of the pulmonary artery where the changes were similar to those already described for the earlier stages of the disease. Fewer vessels, however, seemed to be involved and some manifested a reduction in the amount of hypertrophied medial muscle (Fig. 31). Few arteries displayed intimal changes, including the eosinophil leucocytic infiltration, conspicuous in early lesions and there was much less hypertrophied muscle to be seen in the walls of the bronchioles and alveolar ducts. In general, there was little perivascular and peribronchial reaction but, occasionally adjacent to some of the larger bronchi, a large follicular lymphatic structure was apparent. Cellularity and thickening of the alveolar septa had decreased but sometimes a collection of cells, either of purely lymphocytic nature or consisting of a mixture of those cells and eosinophil leucocytes was to be appreciated in the pulmonary parenchyma along with, in a few instances, portions of adult worms.

Fig. 28 Twenty-eight days after infestation. Present is a group of developing eggs infiltrated by lymphocytes, macrophages, eosinophil leucocytes and a few giant-cells.

Haematoxylin and Eosin x 150

Fig. 29 Branches of the pulmonary artery showing marked medial hypertrophy twenty weeks after infection.

Picro-Mallory x 75



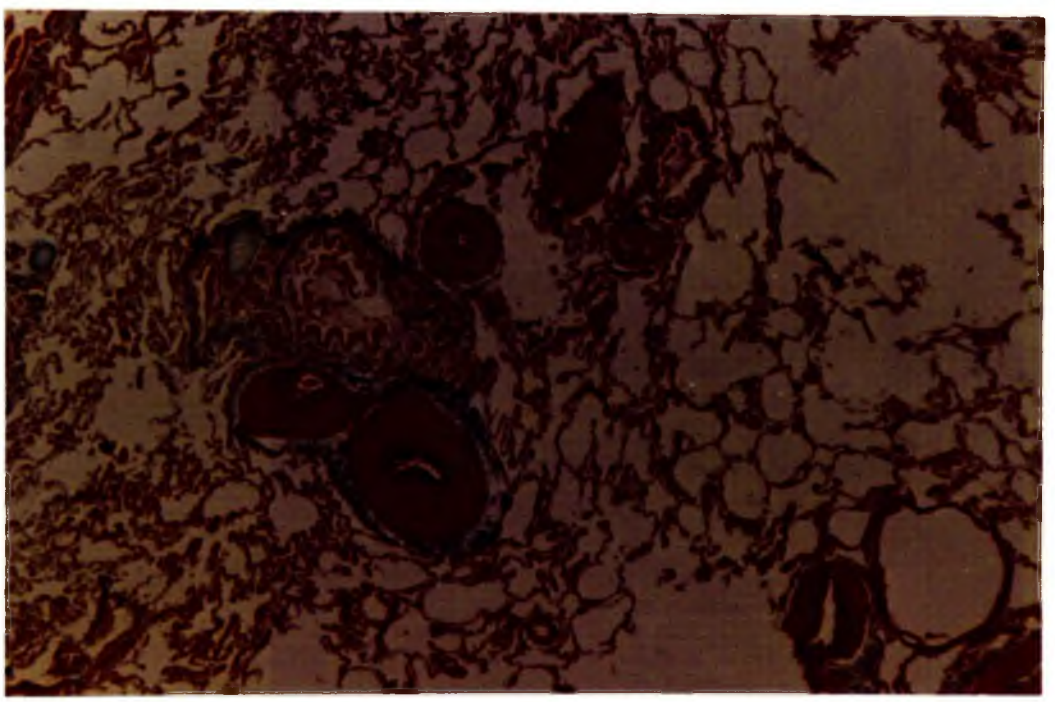
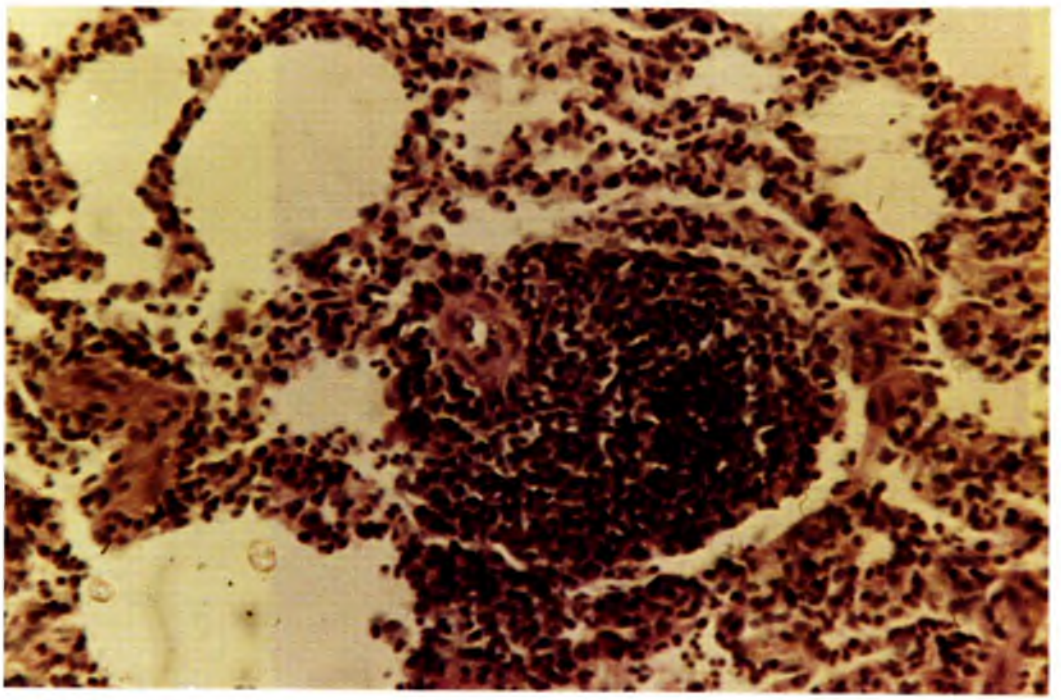


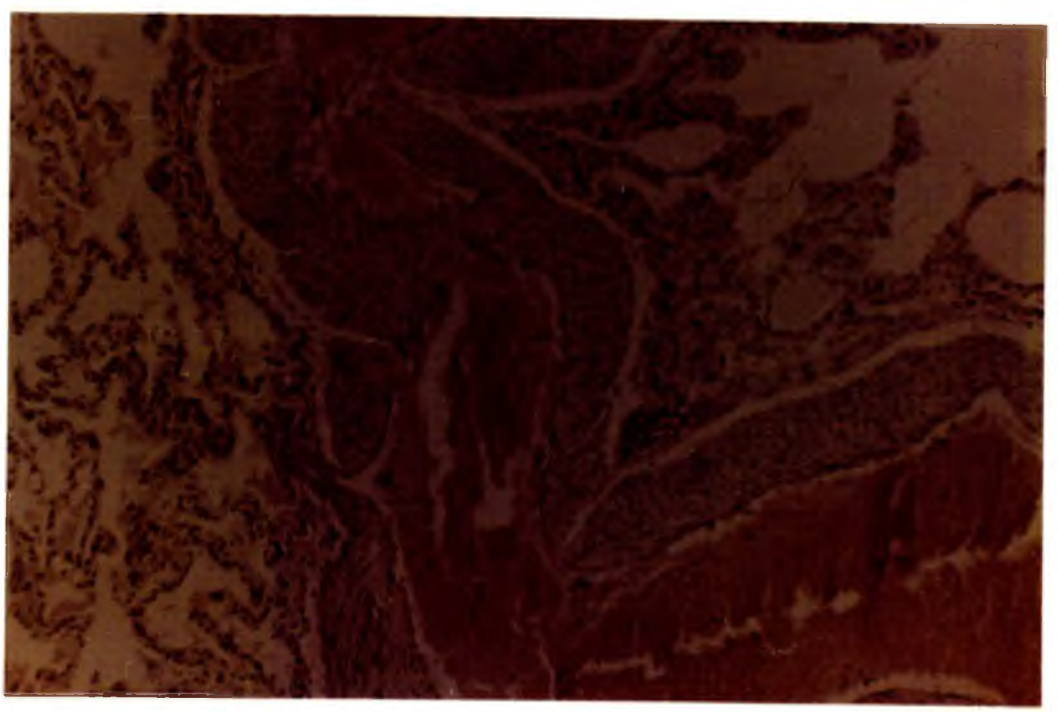
Fig. 30 Small hypertrophied muscular pulmonary artery with an associated lymphocytic reaction.

Haematoxylin and Eosin x 150

Fig. 31 Tortuous muscular pulmonary artery in which reduction in the amount of hypertrophied medial muscle has apparently taken place.

Haematoxylin and Eosin x 150





Discussion

Pulmonary arteriopathy is a common finding in cats and an incidence of 3.0% of 150, 36.0% of 111, 69.8% of 122, 33.0% of 150 and 34.7% of 256 cats has been reported by Olcott et al. (1946), Scratchard and Wright (1961), Tashjian et al. (1965), Stunzi et al. (1966) and Hamilton (1966b), respectively. Other authors have reported a similar condition in smaller numbers of animals (Marcato, 1940) as well as in a few cats (McKenzie, 1960). The aetiology of the condition has long been obscure and has been thought to be associated with ageing (Rubarth, 1940 and Marcato, 1940). McKenzie (1960) and Hamilton (1963 and 1966b) however, showed that the disease occurred in kittens. Neumann et al. (1942) and Kell et al. (1956) reported the existence of pulmonary arterial changes in cats in which stimulation of the cerebral centres had been effected by various means, including the use of the drug, metrazol. Hamilton (1966c) was unable to achieve a similar result in any of twelve cats injected with the latter substance and considered that through use of unselected groups of cats, the former authors had unwittingly employed animals already suffering from arterial changes. Scratchard and Wright (1961) postulated that intestinal parasites en route to the lungs were causatively involved and based their claim on the work of Harrison (1948), of Muirhead and Montgomery (1951) and of Barnard (1953 and 1957). Those workers had shown that arteriosclerotic changes may be produced in the lungs of rabbits

as a result of the injection of fragments of blood clots or of bubbles of oxygen, nitrogen or air. Generally speaking, the resultant alterations consisted of partially organised adherent thrombi in the larger arteries while, in smaller arteries and arterioles, varying degrees of fibro-elastic hyperplasia of the intima was recognisable together with endothelial proliferation and occasional fibrosis of the medial coat. The pulmonary lesions of the cat are difficult to explain on the basis of thromboembolism since the outstanding change is mainly medial in distribution, is accompanied by less marked intimal abnormality and the presence of pulmonary emboli is unrecorded. Furthermore, Boerema (1965) demonstrated that the lesions produced in rabbits by the inoculation of air or oxygen regressed shortly after injections ceased.

It seems unlikely that the dependent position of the lungs of cats alone leads to arterial changes as was suggested by Olcott et al. (1946). Tashjian et al. (1965) opined that the vascular alterations were part of an active process of infectious or toxic origin but discounted any relationship with infestation by Aelurostrongylus abstrusus, senile change or hypertensive pulmonary disease. The latter factor was dismissed as an aetiological potential by Hamilton (1966b) who failed to find any evidence of hypertensive disease in other organs of cats suffering from pulmonary arterial changes and pointed out that the probable

explanation for that finding lay in the focal distribution of the arterial lesions. Tashjian et al. (1965) are on less firm ground, however, when they hypothesise that lungworm infestation is without importance. Consistent changes in the arteries in spontaneous cases of lungworm disease has been noted by various observers including Pritchett (1938), McKenzie (1960), Jubb and Kennedy (1963), Hamilton (1963 and 1966b) and Stunzi et al. (1966) and in some instances of experimentally-induced disease (Blaisdell, 1952). Hamilton (1966a), in a study of the pathogenesis of feline lungworm disease, recorded a progressive increase in the severity of the arterial lesions over a period ranging from two weeks to six months after infection by the parasite. Furthermore, such pathological changes were exactly similar to those found in spontaneous arterial disease in the lungs of cats. The present investigation has served to confirm such findings and has yielded additional information. Alterations in the media of pulmonary arteries and in the smooth muscle of the walls of alveolar ducts as well as of the bronchioles were noted nine to eleven days after infection, at which time only small numbers of fourth-stage larvae were present in the lungs and the associated pathological changes were quite slight. As the parasite matured, produced its eggs and larvae thus to induce a massive cellular reaction, so did the severity of the arterial lesions increase but, when the pulmonary parenchymal

changes regressed, the hypertrophic and hyperplastic state of the vessels persisted. From fourteen months onwards, there was a diminution in the amount of hypertrophic muscle detectable in the walls of the alveolar ducts and of the bronchioles and, although fewer arteries appeared to be affected and a return to a more normal state on the part of others seemed to have occurred, at two years after initial infestation the arteriopathy was still a prominent histopathological feature. Certainly, the severity of the condition had not increased in the later stage of the disease nor was there any greater development of intimal fibrosis or any onset of longitudinal muscle production, both of which latter features had been reported to be present in a number of cases of the spontaneous disease (Hamilton, 1966b). That discrepancy, however, may be related to the fact that, in the latter report, the animals in which such lesions were present were considerably older than those of the current series. Other changes still apparent twenty-four months after infestation consisted of some degree of peri-vascular and peri-bronchial lymphocytic reaction, an occasional focus of cells in the pulmonary parenchyma and some infiltration of the intima of hypertrophied vessels by eosinophil leucocytes, all of which findings have been found to occur in spontaneous arterial disease. Of significance was the experience that the presence of adult forms of Aelurostrongylus abstrusus was seldom detectable

save by examination of serial sections of affected organs. If that technique was applied to lungs of cats suffering from so-called spontaneous arterial disease, adult worms would probably be demonstrable in an appreciable percentage of cases. Not without interest, too, is the fact that, of the 147 cases of spontaneous and experimentally-induced lungworm disease surveyed, with the exception of the twenty-five animals killed before the ninth day after infection all showed abnormality of the muscular pulmonary arteries.

The life-span of the cat is quite lengthy (ages of twenty-four years have been recorded). The duration of lesions caused by lungworms remains unknown and also problematical is the period of survival of the parasites within the pulmonary parenchyma. The present investigation has shown that pathological change, as well as adult worms, persisted for two years while Blaisdell (1952) recovered larvae from the faeces of one cat after two years and considered that, if the resistance of a cat harbouring such parasites was reduced, infection was likely to flare up. It is also possible, as earlier suggested (Part Two, Experiment Five), that the presence of adult worms plays a major part in maintaining immunity to re-infection. Complete solution of those problems is unlikely to be forthcoming until study of experimentally-infected animals has been pursued over many years, a procedure which is expensive of time, space and money.

It has been established that a form of pulmonary arteriopathy is produced in the cat following infestation by Aelurostrongylus abstrusus but the genesis of the lesion is uncertain. There is general agreement that, neoplasia apart, excess of muscle is an expression of increased muscular load and that arterial medial hypertrophy is a result rather than the cause of arterial constriction. Thus, medial hypertrophy implies an active contraction occurring intermittently or continually over a prolonged period (Harris, 1955). According to Harris and Heath (1962), the causation of muscular hypertrophy may be neural, humoral or myogenic in origin although the authors considered that the latter factor was the most important. Aviado (1960) hypothesised that anoxia caused constriction of the pulmonary vessels as a result of excitation of the chemo-receptive aortic and carotid bodies, a view which was shared by Naeye (1961). It has also been demonstrated that, in the cat, acute hypoxia causes increase of pulmonary arterial pressure brought about by constriction of the vessels (Duke, 1951). In the same species of animal, however, Weidman et al. (1965) have shown that chronic hypoxia does not produce any anatomical alteration of the pulmonary vasculature. Alexander and Jensen (1963), in relation to High Mountain disease of cattle, believed that the medial hypertrophic changes arose from hypoxia leading to vascular constriction. Further hypertrophy was attributed to a rise in intravascular pressure associated with hypertension, which

view seemed to combine the theories advanced by Harris and Heath (1962) and by Macye (1961). How far these hypothesis are applicable to the cat is debatable since, in the papers cited above, the pathological conditions productive of hypertension were located outside the lungs and affected all pulmonary vessels to a similar degree. The lesions of the cat were distinctly focal in character and involved only branches of the pulmonary artery and, in all cases, arteries of normal size were present. In consequence, the above theories do not seem entirely applicable to the causation of pulmonary arterial disease of the cat.

In view of the changes induced in the pulmonary vasculature of rats infected with Angiostrongylus cantonensis (Mackerras and Sandars, 1955) and in that of dogs infested with Angiostrongylus vasorum (Enzeby, 1961), it would be convenient to accept the opinion of Cameron (1928) that adult worms lived in the pulmonary vessels, caused irritation and gave rise to partial blockage. However, adult parasites have never been seen intravascularly but have been recognisable always in terminal bronchioles, alveolar ducts or in alveoli and such observations are in agreement with those of Hobmaier and Hobmaier (1935b), Gerichter (1949), Blaisdell (1952) and Mackerras (1957). Hamilton (1966a) considered that the occlusion of arteries and arterioles with disorganisation and even obliteration of the capillary bed, caused by the parasite

and the consequent cellular reaction, especially that located around blood vessels, resulted in increased resistance to flow of blood in the arteries of affected areas. Constriction of the arterial myofibrils over a period of time provoked hypertrophic changes in the media of the vessels which, in turn, led to further increase of intramural pressure. Emphysema is present, to a varying degree, in lungs affected by lungworms and Hicken et al. (1965) showed that, in some emphysematous conditions of man, muscularisation with thickening of pulmonary arterioles took place. With regard to the hypertrophy of the smooth muscle of the walls of the bronchioles and alveolar ducts, a process of gradual obstruction of the bronchiolar system by eggs, larvae and inflammatory exudate, as suggested by Pei-Lin-Li (1940) in explanation of a similar change in the lungs of sheep and goats infested by lungworms, may be of importance in the development of the lesion. However, while those factors probably operate significantly during the productive stage of the disease, they do not explain the muscular hypertrophy of the arteries and of the other structures notable as early as nine days after infection. At that period, fourth-stage, and possibly some third-stage, larvae were present but comparatively little cellular reaction had been produced. Release of a myo-stimulant as a result of pulmonary damage or, alternatively, production by the parasite of such a chemical must be considered. Substances of

that kind normally present in the body include acetylcholine, epinephrine, norepinephrine, angiotensin, histamine and serotonin. Acetylcholine acts as a dilator of the pulmonary circulation (Harris, 1957). Harris and Heath (1962) considered that epinephrine had little effect on the pulmonary circulation while, because of conflicting experimental results, doubt remains as to the effect of norepinephrine. Angiotensin constricts systemic arteries but the latter authors suggested that the substance had only a slightly constrictive effect on the pulmonary circulation. Histamine may be produced by metazoan parasites (Archer, 1963) but that substance has not been demonstrated to cause alteration of pulmonary arterial pressure (Nemir et al., 1954). Serotonin is a powerful vasoconstrictor and Comroe et al. (1953) described both reflex and cardiopulmonary effects of the drug on the cat and considered that the release of the substance from damaged vessels may cause additional vascular obstruction. Maddy (1960) remarked that serotonin consistently produced contraction of vascular beds of low tonicity, such as those of the lungs and kidneys, and Sanders et al. (1959) showed that the concentration of the drug in the blood of the cat was as much as thirty times that of man. As a result of injection of serotonin, Rossi and Zamboni (1958) and Ahmed and Harrison (1964) created pulmonary arterial lesions in rabbits. Hamilton (1966d), however, dosed ten cats twice a day for twenty-

eight days and at the end of that time serotonin was found not to have caused any alteration of the pulmonary arterial system. Again, in human beings suffering from malignant carcinoid tumours, pulmonary hypertension does not ensue as a result of the release of large quantities of serotonin by the neoplasm (Harris and Heath, 1962). On the basis of available evidence, serotonin seems unlikely to play a major part in the production of feline pulmonary arteriopathy and the proof as to whether, or not, a myo-stimulant, possibly of entirely different nature to the above chemicals, is produced by the lungworms must await the decision of further experimental work.

It must be concluded, therefore, that although some important participating factors have been recognised, much has still to be learned concerning the pathogenesis of the pulmonary arterial lesions associated with feline infestation by Aelurostrongylus abstrusus.

PART THREE

1 RADIOGRAPHY AS AN AID TO DIAGNOSIS OF LUNGWORM DISEASE OF THE CAT

2 IMMUNOFLUORESCENCE AS A DIAGNOSTIC PROCEDURE IN LUNGWORM DISEASE OF THE CAT

3 THE TREATMENT OF LUNGWORM DISEASE OF THE CAT WITH DIETHYLCARBAMAZINE CITRATE

4 THE TREATMENT OF LUNGWORM DISEASE OF THE CAT BY THE ORAL ADMINISTRATION OF TETRAMISOLE

5 THE TREATMENT OF LUNGWORM DISEASE OF THE CAT BY PARENTERAL ADMINISTRATION OF TETRAMISOLE

Experiment One

Radiography as an Aid to Diagnosis of Lungworm Disease of the Cat

Introduction

It has been established that infestation of the cat with Aelurostrongylus abstrusus is not uncommon in Great Britain (Lewis, 1927 and Hamilton, 1966b). The main clinical signs consist of increased respiratory rate and coughing, especially, after handling and exercise. In animals more severely affected, dyspnoea, reduced appetite, if not anorexia, and concomitant loss of bodily condition are apparent. Percussion of the chest reveals distinct dullness and, on auscultation, adventitious sounds are easily appreciable. Pyo-thorax has been recorded (Hamilton, 1963). The condition is afebrile and haematological examination may yield variable results since, at the height of the infestation, leucopenia is recorded whereas during the period of convalescence leucocytosis prevails. Eosinophilia is common and erythrocyte sedimentation rates are accelerated (Hamilton, 1966a). The disease is not acute and the most dangerous period for the host is the two months, or so, after patency when eggs and larvae are produced in great numbers. Animals of all ages may be affected. Radiological examination of the lungs of animals suspected of suffering from the disease may be of value as an aid to diagnosis and the following experiment was carried out to assess the worth of such a procedure.

Materials and Methods

Six kittens, ten to twelve weeks of age, were infected with 800 third-stage larvae of Aelurostrongylus abstrusus. Ten weeks later, the cats were X-rayed and lateral views of the thorax were obtained. Fast non-screen film was used with KV 42 and Mas 25 while the focal-film distance was 30". Following euthanasia, the lungs of the cats were examined and portions taken and subjected to the normal histopathological techniques.

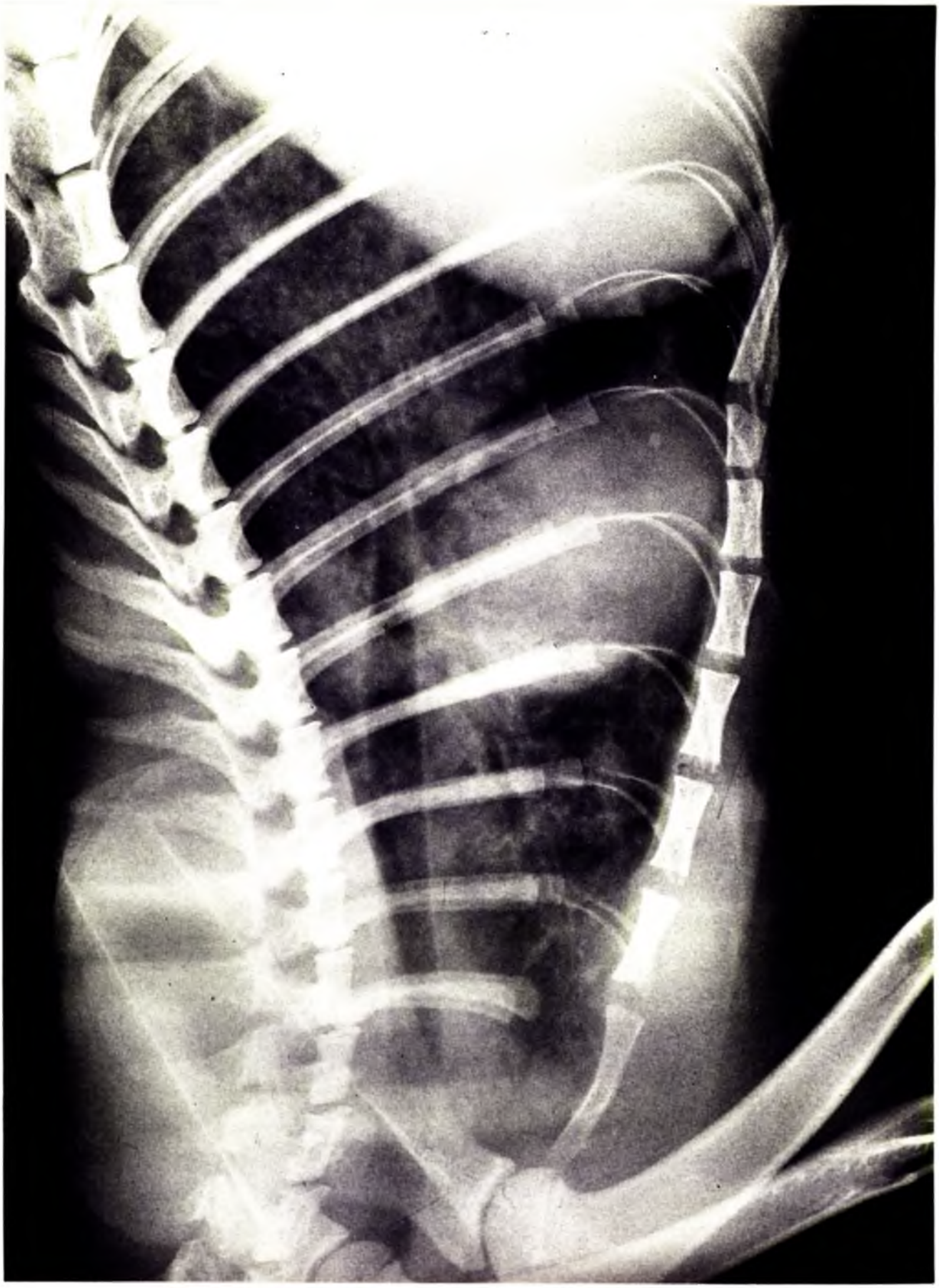
Results

On the X-ray plates, numerous small areas of increased opacity which were extensively distributed through the pulmonary substance of all lobes of the lungs were seen (Fig. 32). At autopsy, the multiple lesions characteristic of lungworm disease were found to conform with the changes demonstrable on the radiographs.

Discussion

The results of the above work show that, in lungworm infestation, radiological examination is a valuable additional aid to diagnosis since the widespread and diffuse appearance

Fig. 32 Lateral radiograph of the lungs of a cat infected with Aelurostrongylus abstrusus. Numerous small areas of increased opacity distributed throughout the pulmonary substance may be appreciated.



of the parasitic lesions is not likely to be confused with most of the other pathological conditions which, in this country, affect the pulmonary system of the cat. An acute and severe bacterial pneumonia, possibly a virus pneumonitis, may be radiologically similar but the clinical history and signs of such conditions are unlikely to be confused with those of lungworm disease. The presence of the parasitic condition may be confirmed by examination of the faeces of the cat. If the animal is affected, a simple smear will demonstrate a varying number, anything up to several hundreds, of very active first-stage larvae.

It is suggested, therefore, that if clinical, radiological and faecal examinations are undertaken in animals suspected of suffering from lungworm disease, such procedures will provide a diagnosis of the condition in the majority of cases.

However, none of the latter investigations will indicate the presence of lungworm disease until the fourth week, probably later, after infestation since gross pulmonary changes are not appreciable until the production of eggs and larvae has reached a reasonable level and larvae are rarely found in faeces, before the eighth week. Neither will such examinations be of value in the later stages of the disease when the excretion of larvae has ceased and pulmonary lesions are less dense but animals are still showing clinical signs of pulmonary disease. Some other diagnostic

aid is therefore demanded and the next experiment records the introduction of the fluorescent antibody technique to the diagnosis of the presence of lungworm infestation in the cat.

Experiment Two

Immunofluorescence as a Diagnostic Procedure in Lungworm Disease of the Cat

Introduction

The fluorescent antibody technique has been used in helminthological investigations since the late 1950's. Jackson and Levert (1957), Jackson (1959), Sadun (1963), Sulzer (1965), Scholtens et al. (1966) and Ruitenberg et al. (1967) used the method as a means of diagnosis of trichinosis. With Ascaris suum, Taffs and Voller (1963) demonstrated the incorporation of fluorescein-labelled antibodies into the precipitates at the mouth, excretory pore, anus and on the cuticle of third-stage larvae while Mitchell (1964), Hogarth-Scott (1966) and Roneus (1966) detected the presence of ascarid antibodies by the same means. The fluorescent antibody technique has also been utilised in the diagnosis of schistosomiasis in man (Sadun et al., 1960 and Sadun and Biocca, 1962), in hydatidosis (Pezzuoli et al., 1965), in hookworm infections (Zaman and Singh, 1965), in onchocerciasis (Lucasse, 1962) and in fascioliasis (Thorpe, 1965). A high degree of success has been claimed in the detection of antibodies against those various parasites. The demonstration of antibodies against Aelurostrongylus abstrusus by the above method has not been recorded and, as such a test may be of value in the diagnosis of lungworm disease in the cat, it was decided to assess the sensitivity and specificity of the technique as applied to sera obtained from cats suffering from infestation by the latter parasite.

Materials and Methods

The Indirect Fluorescent Antibody method (I.F.A.) was used. Third-stage larvae of Aelurostrongylus abstrusus were recovered from infected snails. About 200 larvae in 1 ml. of phosphate-buffered physiological saline (P.B.S.) at pH 7.1 were put into a test-tube and 10 ml. of acetone added. The mixture was shaken and allowed to sit for thirty minutes to allow fixation of the larvae to take place. After centrifugation and removal of the supernatant fluid, the larvae were rinsed twice in P.B.S. and re-suspended in that solution at a rate of 200 per ml. These constituted the antigen for immunofluorescence.

Labelled immune serum was prepared as follows: rabbit anti-cat globulin antiserum conjugated with fluorescein (Silvana) was adsorbed with moistened canine liver powder, in the ratio of 100 mg. of powder to 1 ml. of serum, in order to remove non-specific "electric charge" fluorescence. The mixture was shaken well and incubated overnight at 4°C. The liver powder was then sedimented by centrifugation at 4,000 rpm. for ten minutes and the supernatant serum removed. To the latter was added rhodamine-labelled normal guinea-pig albumen, in the ratio of 40/60, to act as a counterstain.

To perform the test, 0.1 ml. of the suspension of antigen was added to 0.5 ml. of each test serum, the mixture shaken well and incubated overnight at 37°C. The following day, the serum was

drawn off and, by means of centrifugation, the sediment of antigen was thoroughly washed on four occasions with P.B.S. The latter, in turn, was removed as completely as possible from the antigen to which five drops of labelled serum was added and the mixture was shaken and incubated overnight at 37°C. The supernatant was removed and, by means of centrifugation, the sediment was washed on four occasions with P.B.S. The antigen was then placed on a clean glass slide, covered by a cover-slip and examined under the high-dry objective (x40) of a Reichert 'Zetopan' fluorescence microscope fitted with a dark-ground condenser, an exciter filter UG1/1.5mm. and a barrier filter GG9/1mm. Unknown sera were examined in batches of ten and freshly-prepared 'known positive' and 'known negative' samples were viewed at the same time to ensure proper functioning of the technique. A test was considered positive when the cuticula of larvae fluoresced a strong, apple-green colour (Fig. 33). A dull blue-green or complete absence of fluorescence signified a negative result (Fig. 34). The mouth, gut and ovaries of the parasites took up the counterstain and fluoresced an orange colour in both positive and negative reactions. It was not considered feasible to titrate the level of antibodies in positive sera by use of the fluorescent technique.

Blood samples were taken, at the time of euthanasia, from seventy cats, twenty-four of which had suffered from either spontaneous or experimentally-induced lungworm disease, and the

serum separated and stored at -10°C. until required. At autopsy of the animals, portions of lungs and of any other infected tissue were removed, fixed in 10.0% corrosive-formol and processed to give sections of five microns in thickness to be stained with haematoxylin and eosin. Pulmonary preparations were viewed with regard for the existence of active or residual lesions of lungworm disease and the sera were examined in the manner described above. The presence of roundworms and tapeworms was recorded.

Results

Table 12 records the results of examination of the seventy sera. In summary, those from all twenty-four cats affected with active lungworm disease caused the production of positive fluorescence. Included in that number were sera taken from two cats, at three weeks, and from three cats, at four weeks, after infestation. Sera from thirteen other animals reacted in a similar manner but ten of those cats also displayed, on histo-pathological examination, pulmonary lesions consistent with post-patent parasitic pneumonia, namely, hypertrophic change in the media of pulmonary arteries and in the muscle of the walls of alveolar ducts and bronchioles together with the occurrence of occasional cellular foci in the tissues of the lungs. Samples from fourteen animals infested only with roundworms, from four

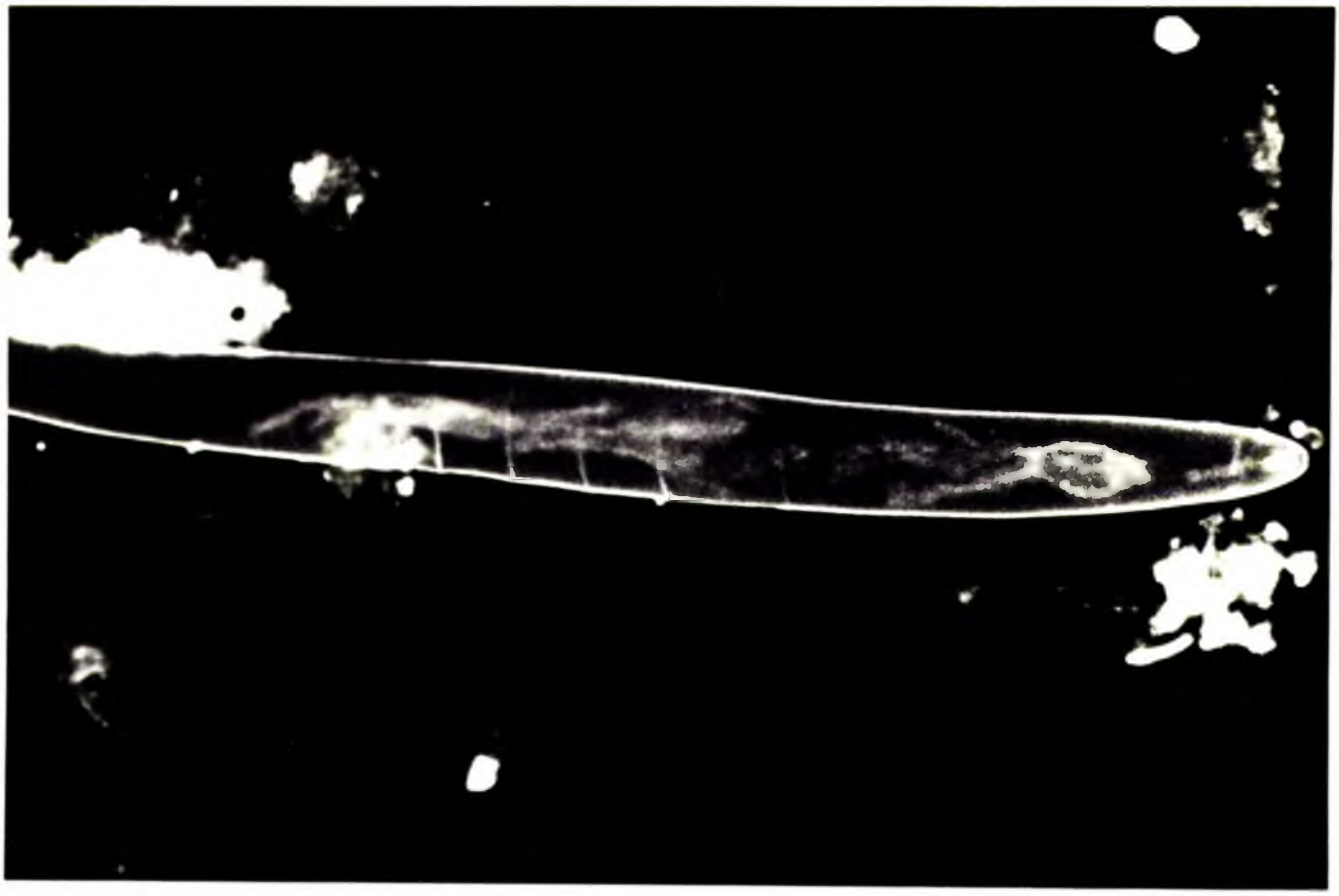
with tapeworms and from one which was infected with both of the latter parasites as well as sera from fourteen non-parasitised cats failed to cause fluorescence. Contained in the latter group were sera from four cats two of which had died as a result of virus enteritis and two from salmonellosis.

Fig. 33 Example of positive cuticular fluorescence of a third-stage larva of Aelurostrongylus abstrusus.

x 600

Fig. 34 Example of negative cuticular fluorescence of a third-stage larva of Aelurostrongylus abstrusus.

x 1500



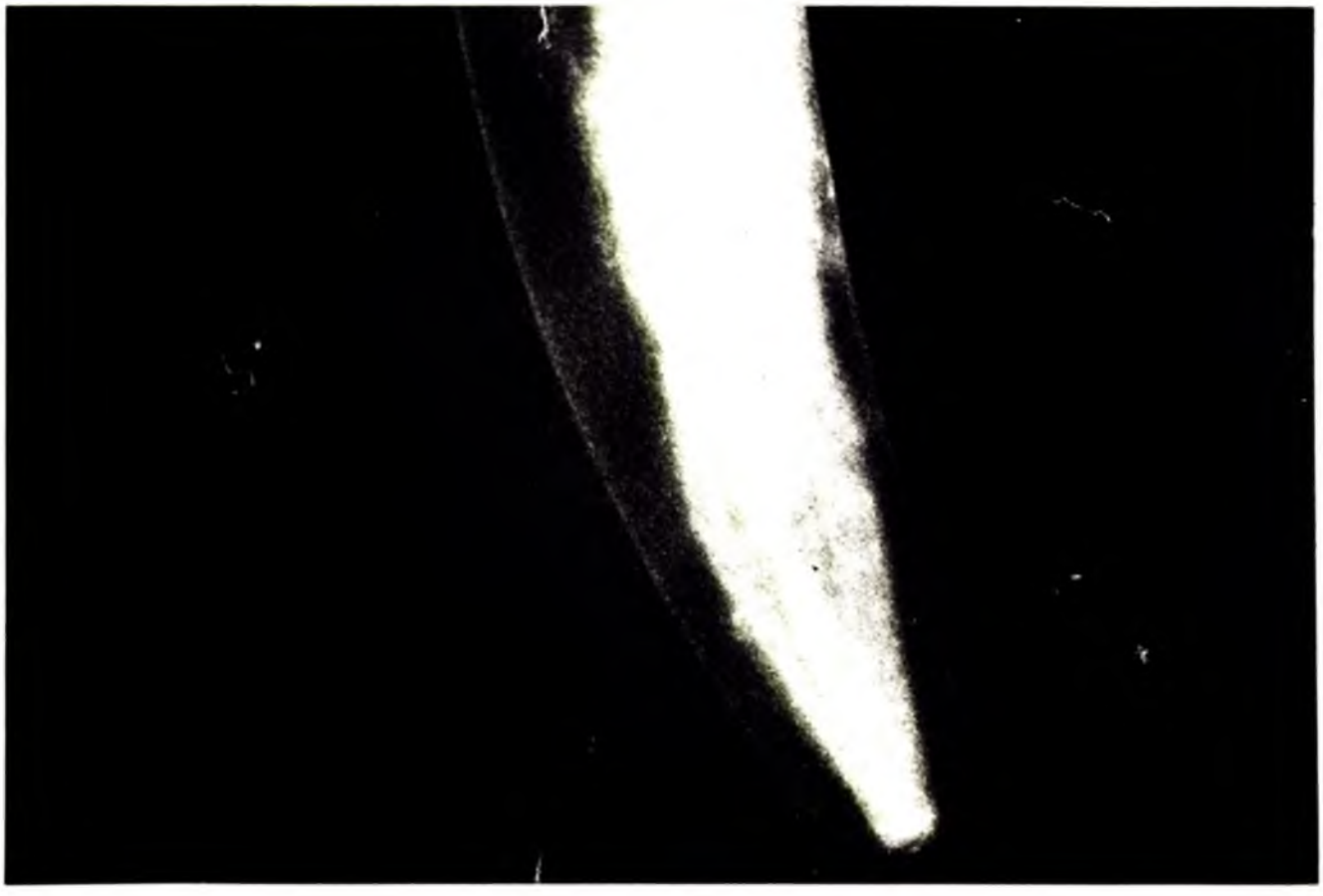


Table 12

Synopsis of Parasitic and Pulmonary Arterial State together with Serum Reaction of Seventy Cats

Cat No.	Infested with Lungworms	Roundworms	Tapeworms	Suffering from Arterial Lesions	Serum Fluorescence
1	+	+	-	+	+
2	+	-	-	+	+
3	-	+	-	-	-
4	+	-	-	+	+
5	-	-	-	-	-
6	+	-	-	+	+
7	+	-	-	+	+
8	+	-	-	+	+
9	-	-	-	-	-
10	-	-	-	+	+
11	-	-	+	+	+
12	-	+	-	+	+
13	-	-	-	-	-
14	-	+	-	-	-
15	-	-	-	-	+
16	-	-	-	+	+
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	+	-	-	-
22	-	+	-	-	+
23	-	-	-	+	+
24	-	+	-	-	-
25	-	-	-	+	+
26	-	-	+	+	+
27	-	-	+	-	-
28	-	+	-	-	-
29	+	-	-	+	+
30	-	+	+	+	+
31	-	-	+	-	-
32	-	+	+	+	+
33	-	+	+	-	-
34	-	+	+	+	+
35	-	-	-	-	-
36	-	-	-	-	+
37	-	-	-	-	-
38	-	-	+	-	-
39	-	-	+	-	-
40	-	+	-	-	-

Table 12 (Contd.)

Cat No.	Infested with Lungworms	Roundworms	Tapeworms	Suffering from Arterial Lesions	Serum Fluorescence
41	-	-	-	-	-
42	-	-	-	-	-
43	-	-	-	-	-
44	-	-	-	-	-
45	-	+	-	-	-
46	-	-	+	-	-
47	-	+	-	-	-
48	-	-	-	-	-
49	+	-	-	+	+
50	+	-	-	+	+
51	+	-	-	+	+
52	+	-	-	+	+
53	+	-	-	+	+
54	+	-	-	+	+
55	+	+	-	+	+
56	+	+	-	+	+
57	+	-	-	+	+
58	+	-	-	+	+
59	+	-	-	+	+
60	+	-	-	+	+
61	+	+	-	+	+
62	+	-	-	+	+
63	-	+	-	-	-
64	-	+	-	-	-
65	-	+	-	-	-
66	-	+	-	-	-
67	-	+	-	-	-
68	+	-	-	+	+
69	+	-	-	+	+
70	+	-	-	+	+

Discussion

Of the thirty-seven sera that had caused the production of fluorescence, only three came from cats that did not show either active, or residual lesions of, lungworm disease. It is impossible to state whether, or not, that result in the three sera represented a non-specific reaction since the absence of pulmonary lesions does not conclusively indicate lack of contact with Aelurostrongylus abstrusus. In support of that statement, the results of an earlier investigation (Part Two, Experiment Six) showed that in the early stages of infestation comparatively little pathological reaction may be appreciable and, additionally, information is not available on the long-term pulmonary changes which may be induced by a small number of parasites. One roundworm was recovered from the small intestine of one of the three cats, a finding which was without significance since negative fluorescence had been recorded after examination of the sera from nineteen animals infected, many heavily so, with roundworms, tapeworms or a mixture of both of those parasites.

It is considered, therefore, that the I.F.A test which uses third-stage larvae of Aelurostrongylus abstrusus as antigen is a highly specific measure for the demonstration of antibodies arising from infection of the cat by the latter parasite. Furthermore,

since the sera of cats infected with the parasite for only three weeks produced a positive result, the technique is able to provide a diagnosis of the presence of lungworm disease at an earlier stage than has hitherto been possible.

The sensitivity of the test is not an unexpected event in view of the reported work on other parasites. Buitenberg et al. (1967) found that, in pigs, although the charcoal-lecithin test, the latex-slide test and, to some degree, the complement-fixation test produced problems in interpretation because of cross-reaction due to other parasitic infections, the I.F.A. test was highly specific for the diagnosis of trichinosis. Mitchell (1964) was also able to demonstrate specificity for the method when it was applied to sera from rabbits and monkeys suffering from mixed parasitic and bacterial or virus diseases. In the present experiment neither virus nor bacterial infection influenced the fluorescence of sera.

It is accepted, however, that cross-reactions may occur, even with I.F.A. techniques, if closely-related parasites are present so that Zaman and Singh (1965) failed to distinguish between different hookworms, Sadun et al. (1960) found cross-reactions to occur between Schistosoma mansoni and Schistosoma japonicum or Schistosoma haematobium while Hogarth-Scott (1966) and Roneus (1966) described a similar relationship between Toxocara cati and Toxocara canis.

However, such cross-reaction does not constitute a practical problem for the cat. The common groups of parasites which infest that species are roundworms, tapeworms and lungworms, none of which are closely related and it has been satisfactorily demonstrated that it is possible to recognise specific antibodies to the latter group by the I.F.A. technique. Unfortunately, it was not found feasible to estimate the antibody titre by the latter method since dilutions of the test sera above 1/8 caused specific to merge with non-specific fluorescence so that results ceased to be of significance. Accordingly, it is impossible to discriminate, at the moment, between active and past infection by lungworms. A factor of indubitable importance in that respect is the longevity of the parasites in the lungs of the cat. Adult forms have been demonstrated two years after initial infection and their presence presumably encourages the retention of a degree of immunity. Such a state was represented by the ten cats, the sera of which fluoresced and the lungs of which had lesions of post-patent lungworm disease, since it was estimated that infestation had occurred approximately six to nine months earlier. Thus, there is need of refinement of the I.F.A. technique so that differentiation between the various stages of the disease caused by Aelurostrongylus abstrusus may be attempted. However, if the method is used in conjunction with clinical, radiological and faecal examinations it is of value in confirmation of a diagnosis of parasitic pneumonia in the cat.

Experiment Three

The Treatment of Lungworm Disease of the Cat with Diethylcarbamazine Citrate

Introduction

Diethylcarbamazine citrate (1-diethylcarbamoyl-4-methyl-piperazine dihydrogen citrate) is a white, crystalline, odourless powder with an acrid taste. The drug was developed for use in filariasis of man and has been successfully applied in the treatment of a similar condition in dogs (Hewitt et al., 1947). It is also effective against canine and feline ascariasis (Kanegis, 1948). Parker (1957) applied the drug to lungworm infection of cattle, since when it has been employed by many other workers against Dictyocaulus viviparus (Parker and Roberts, 1958; Parker and Vallely, 1960; Hollo, 1961; Rubin and Tillotson, 1962; Jarrett et al., 1962 and Cornwell, 1963). Treatment of sheep infected with parasitic bronchitis has been attempted by Ozerskaya (1959) and Ozerskaya et al.,(1962) and by Kassai and Hollo (1963). The drug has also proved effective against Metastrongyles in pigs (Sasaki, 1964 and Sisney, 1964). Because of the recorded value of the drug against lungworms of the above species, it was decided to assess the efficacy of the substance in experimentally-produced lungworm disease of the cat. Few records of such therapy are available although Sudduth (1955) treated two cases with diethylcarbamazine with insignificant result but found sodium iodide administered intravenously to be curative. In

another instance, Connan and Zurberg (1966) reported that diethylcarbamazine was efficacious and, again in a solitary feline case, Reed (1963) claimed therapeutic success with dithiazanine iodide. As the disease is not uncommon in Britain, some form of treatment is obviously desirable.

Materials and Methods

Eight kittens, twelve to fourteen weeks of age and weaned in the animal house, were used for purposes of the experiment. Each kitten was given orally 300 third-stage larvae of Aelurostrongylus abstrusus which had previously been extracted from infected snails. Within ten weeks, first-stage larvae appeared in the faeces of the infected cats. On four occasions during the twelfth and thirteenth weeks, the number of larvae excreted by each kitten in a gramme of faeces was counted and an average pre-treatment figure was computed. At the end of the latter period, three daily doses of diethylcarbamazine citrate amounting to 100 mg. per kilogram body-weight were administered orally to six of the animals on two occasions at an interval of three days. The two remaining kittens were kept as controls. Larval counts per gramme of faeces were performed for all eight animals throughout the period of the experiment. Fourteen days after the final dose of the drug, the cats were autopsied and portions of relevant tissues were taken, fixed in

10.0% corrosive-formol, embedded in paraffin-wax, cut at five microns and stained by haematoxylin and eosin.

Results

Table 13 shows the larval output per gramme of faeces before and after treatment with diethylcarbamazine citrate and, for comparison, that of the two control kittens. Reduction in the number of larvae occurred immediately after the first treatment and continued until approximately seven days after the second medication when, in the majority of the kittens, there was an increase in the numbers excreted. Two weeks later, it was obvious that the recovery in larval output was being maintained and, in some cases, even advanced.

Post-mortem examination of the lungs of all eight kittens revealed similar changes to be present. There were lesions of a few millimetres to two centimetres in diameter, which were cream-coloured, friable and widespread throughout the lobes. The bronchial lymph-nodes were enlarged - up to 2 by 2½ centimetres - and were firm, pale and homogeneous in appearance. Histopathologically, differences between the lungs of the treated and control groups were not apparent and the characteristic lesions of lungworm disease were present.

Table 13

No. of First-Stage Larvae Excreted in the Faeces of Control and Treated Cats

	Treated Cats						Control Cats	
	1	2	3	4	5	6	7	8
Pretreatment Nos.	35,000	17,320	5,400	1,980	1,445	18,650	20,500	1,350
Days Post-Treatment			3 x 100 mg/kilo body wt					
1	28,500	11,400	11,970	2,090	1,080	15,030	28,250	975
2	11,685	3,145	3,230	475	630	12,650	18,950	925
3	11,390	6,345	5,850	720	540	16,850	24,860	1,105
2nd Treatment			3 x 100 mg/kilo body wt					
1	7,280	1,900	1,785	595	270	11,865	25,750	1,500
2	9,990	360	5,800	1,080	900	13,030	22,980	1,345
3	8,100	2,160	4,860	720	980	10,560	19,875	1,690
4	9,000	900	6,300	1,350	1,090	7,340	23,650	1,985
7	8,900	4,960	7,200	1,530	1,800	9,780	21,480	1,880
14	9,500	7,550	6,350	1,725	2,050	12,825	15,340	1,760

Discussion

Each cat received a total of 600 mg. of diethylcarbamazine citrate per kilogram of body-weight. Having regard to dosages for other species of animals and in view of the fact that for treatment of ascariasis of cats the recommended amount is ten to fifty milligrams per kilogram it was considered that an adequate quantity of the drug had been administered. Side-effects were absent apart from profuse salivation manifest at the time of dosing.

Jarrett et al. (1962) used diethylcarbamazine against lungworms in cattle and found the drug to be extremely efficacious when it was given before patency but, if treatment was delayed until patency had supervened, the course of the disease remained unaltered. The drug, therefore, was presumed to be ineffectual against mature Dictyocaulus viviparus. Rubin and Tillotson (1962), in a complementary study, showed that the drug was effective mainly against two-week-old larvae and Kendall (1965) confirmed that observation. In reference to Metastrongyle infections of pigs, somewhat similar findings were recorded by Sasaki (1964) who found that, if the drug was administered eighteen days after infection, up to 98.0% of the worms were removed but, should treatment be delayed for a further seven days, the therapeutic effect was

minimal. However, Ozerskaya et al. (1962) reported diethylcarbamazine to be without value against immature Dictyocaulus filaria although, in an earlier report by Ozerskaya (1959), the substance was said to be efficacious against established lungworm disease in sheep. Parker (1957) and Hollo (1961) reported beneficial effects, including decreased faecal larval output, in field cases of husk while Sisney (1964) claimed favourable results in the treatment of lungworm infestation of pigs. Such a confusion of issues is probably due, in great measure, to the different species of lungworm involved.

In the course of the present investigation, for a period of seven days after the final treatment diethylcarbamazine was found to diminish the output of first-stage larvae but the associated mechanism by which that was achieved is still unknown. The possibility remains that the drug may have been lethal to mature adults, yet, histopathological examination did not reveal the presence of dead worms in the pulmonary substance nor did the available evidence suggest that eggs or first-stage larvae had been affected. Since none of the infected kittens showed signs of severe illness, it was impossible to determine whether, or not, clinical improvement had taken place, but at autopsy, it was clear that the severity of the pulmonary lesions of the treated cats matched that of the control animals.

To an extent, the failure of the therapeutic measures taken in the present experiment is confirmed by the experience of Sudduth (1955) who found the same drug not to influence the course of illness in two cases. Too much reliance cannot be placed on the report by Connan and Zurborg (1966) who recorded cure of a solitary case of feline lungworm disease by means of twenty-one doses of diethylcarbamazine administered over a period of twenty-eight days. As the authors themselves have pointed out, the stage of patency at which treatment was instituted was unknown and, since the average patent period of Aelurostrongylus abstrusus infection is thirteen weeks, or even less, the possibility of spontaneous recovery cannot be ignored.

In summary it may be concluded that, as treatment of patent lungworm disease of the cat, diethylcarbamazine citrate was found not to achieve a significant measure of success.

Experiment Four

The Treatment of Lungworm Disease of the Cat by the Oral Administration of Tetramisole

Introduction

In view of the failure of diethylcarbamazine, it was decided to assess the efficiency of a new anthelmintic, tetramisole, in the treatment of lungworm disease of the cat. Tetramisole (dl 2,3,5,6 - tetrahydro - 6 - phenyl - imidazo (2, 1b) thiazole hydrochloride) is a white, odourless, amorphous powder with an acrid taste and was first used by Thienpont et al. (1966) against nematodiasis of various animal species. The drug is soluble in water and in sterile solutions may be used for intraperitoneal, intramuscular or subcutaneous injection or, it may be administered by drenching. Walley (1966 and 1967), Forsyth (1966a and 1966b), Pankhurst and Sutton (1966), Reinders (1966), Guilhon (1966) and Gibson (1966) described the effects of the substance on metazoan parasites, including species of lungworm, of cattle, sheep, goats and pigs. Without exception, the results were favourable and so prompted hope of a similar outcome in the case of Aelurostrongylus abstrusus.

Materials and Methods

Six kittens, twelve to fourteen weeks of age, were fed with 300 third-stage larvae of Aelurostrongylus abstrusus. Eight to

nine weeks later, first-stage larvae appeared in the faeces. For five days before treatment began, the number of larvae per gramme of faeces was counted and an average computed (Table 14). Two animals were kept for control purposes and the remaining four were treated orally with tetramisole at a dosage of 15 mg. per kilogram of body-weight on three occasions at two-day intervals. At three and five days later, the dose was increased to 30 mg. and, two days later still, a final dose of 60 mg. per kilogram was administered. The kittens objected to the tartness of the drug and responded by profuse salivation but, otherwise, side-effects were not noticeable. During the period of treatment as well as thereafter, the number of first-stage larvae per gramme of faeces of all animals was estimated and recorded. Fourteen days from the start of therapy, one control and two treated animals were autopsied while the remaining three cats were kept for a further thirty-one days. After death, histopathological preparations of the lungs were prepared in the ways described in the preceding experiment.

Results

The larval output of the kittens is recorded in Table 14. To abolish excretion of larvae in the faeces of the four treated cats, from two to six doses of tetramisole were found necessary. In one of the two treated cats retained for forty-five days, larvae

reappeared six, eight and ten days after the final administration of the drug but subsequently disappeared.

At post-mortem examination, comparison of the lungs of the two treated animals and one control killed fourteen days after treatment commenced, failed to reveal any significantly different appearances. All the pulmonary lobes were affected and showed multiple, cream-coloured nodules, many of which had coalesced to produce lesions, up to one centimetre in diameter. Histopathological examination was more revealing inasmuch as, in the treated kittens, the eggs and larvae present showed evidence of degeneration.

At post-mortem and histopathological examinations of the three other cats, marked differences between the lungs of the control and the treated cats were to be noted. Thus, the control animal showed gross and microscopical lesions similar to those displayed by the first control cat (Fig. 35) apart from the fact that there was some reduction in the number of eggs and larvae. The lungs of the two treated animals, however, were pink, spongy and fairly normal in appearance except for the presence of a number of pin-point, or a few larger, lesions of several millimetres in diameter throughout the various lobes (Figs. 36 and 37). Histopathologically, such foci were found to consist of collections of lymphocytes, often of perivascular location, but neither eggs nor larvae were recognisable in the many sections of lungs examined. Any adult worms to be found

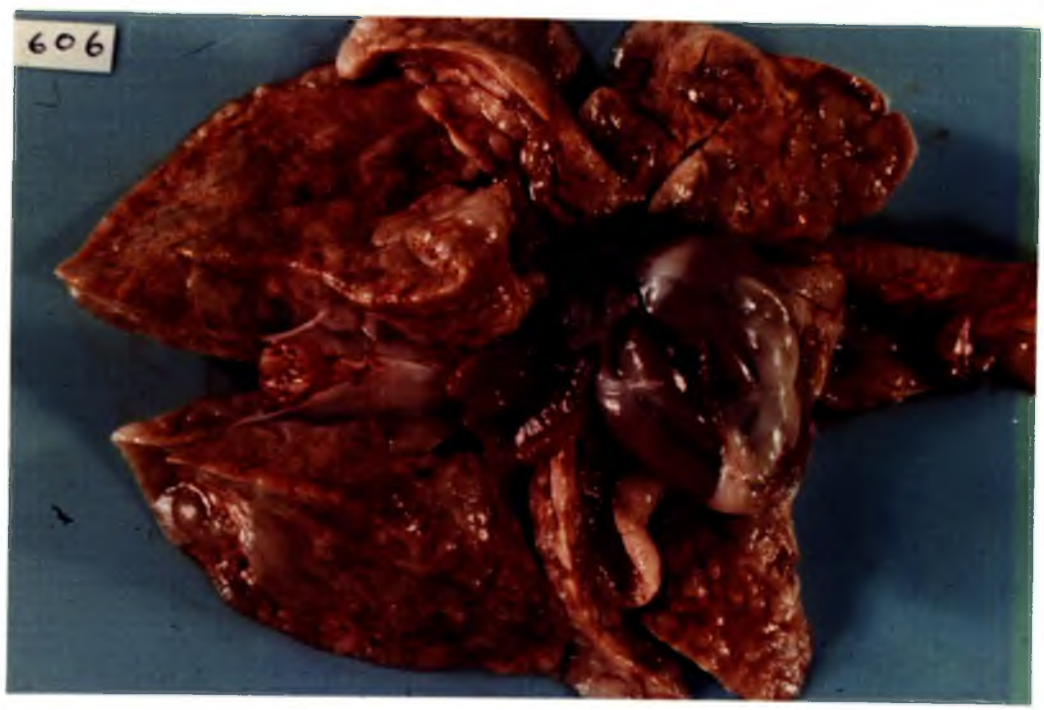
were fragmented, in an advanced state od degeneration and surrounded by a cellular reaction which included many lympho-reticular elements (Fig. 38).

Table 14

Number of Larvae Excreted per Gramme of Faeces by Control and Tetramisole-Treated Cats

Days after Treatment	Control Cats 1	Control Cats 2	Dosage Mg./Kilo	Treated Cats 3	Treated Cats 4	Treated Cats 5	Treated Cats 6
0	1,400	3,350	15	1,340	1,550	1,340	2,725
1	2,350			1,600	4,550	2,000	1,650
2	1,975	1,400	15	1,225		1,500	780
3	2,500	3,600		1,440	2,400	150	1,200
4	2,100	5,080	15	120	300	–	60
5	3,000	5,600		60	60	–	–
7	4,560	25,000	30	130	120	–	350
8	2,100	12,000		55	–	–	–
9	1,260	3,000	30	390	–	–	–
10	2,940	4,950		990	95	–	–
11		3,250	60	700	–	–	–
12	12,500	4,100		–	–	–	–
14	4,080	2,730		–	–	–	–
16	4,450					–	–
17	3,100					–	90
19	3,050					–	45
21	1,350					–	44
23	3,700					–	–
25	3,920					–	–
27	2,500					–	–
29	1,850					–	
31	1,500					–	–
33	1,820					–	–
35						–	–
37	1,300					–	–
39	1,050					–	–
41	850					–	–
43	400					–	–
45	320					–	–

Fig. 35 Lungs of control cat given 800 third-stage larvae. The lesions are extensive and involve large areas of the organ.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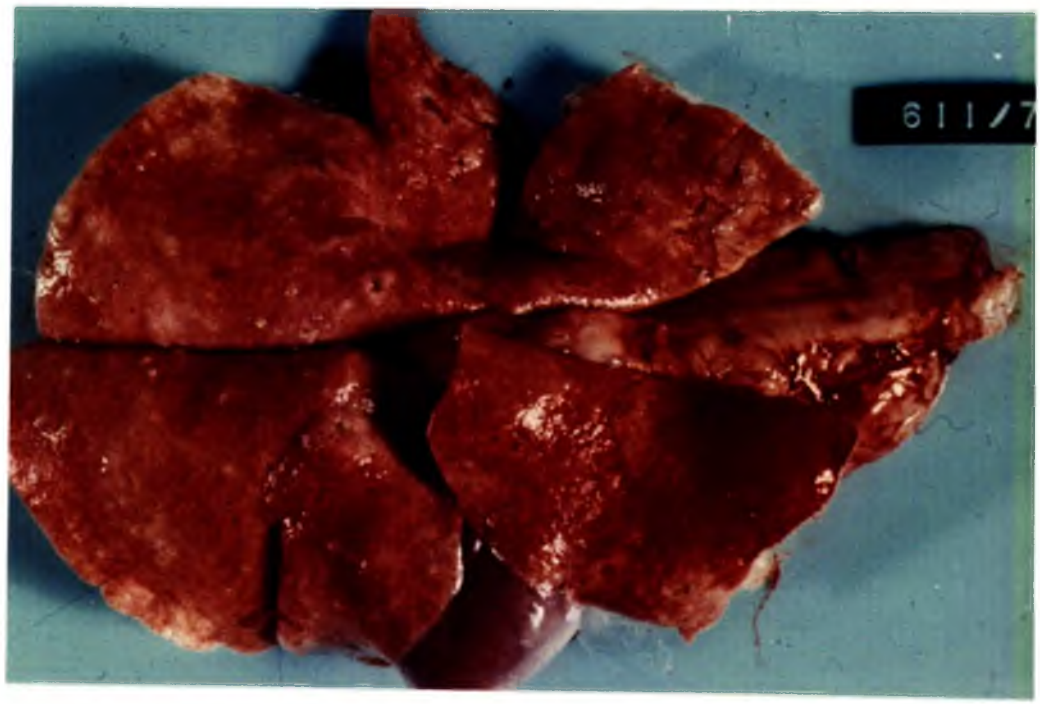
Figs. 36 and 37 Lungs of two cats 45 days after treatment with tetramisole. A number of lesions of a few millimetres in diameter, some protusive, may be seen in all lobes of the lungs.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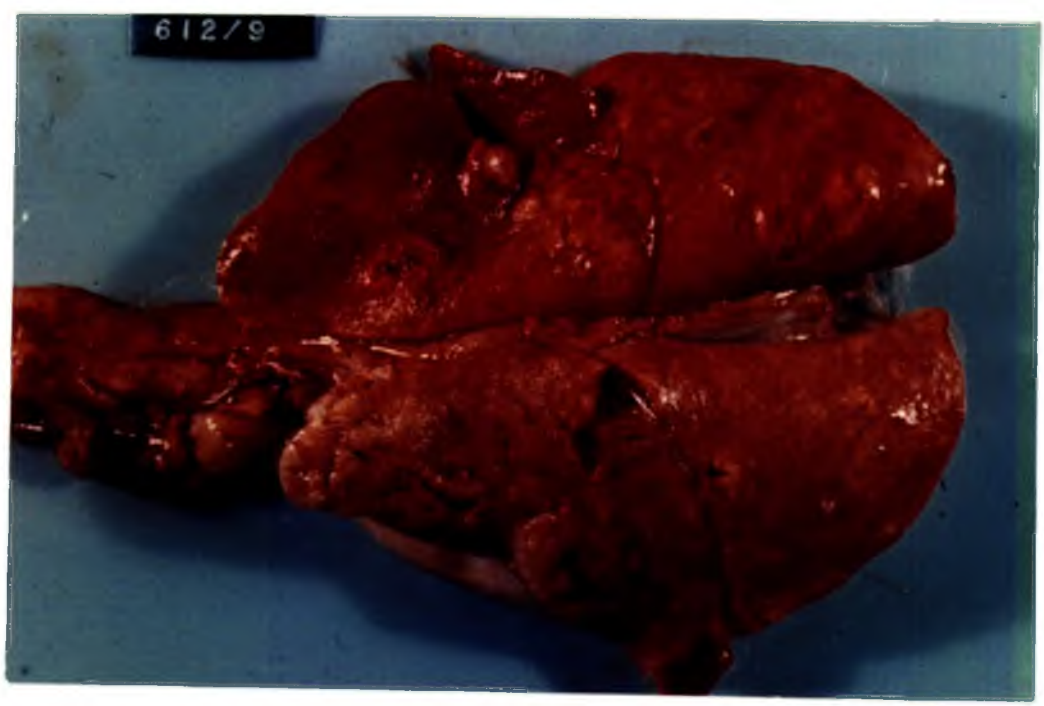
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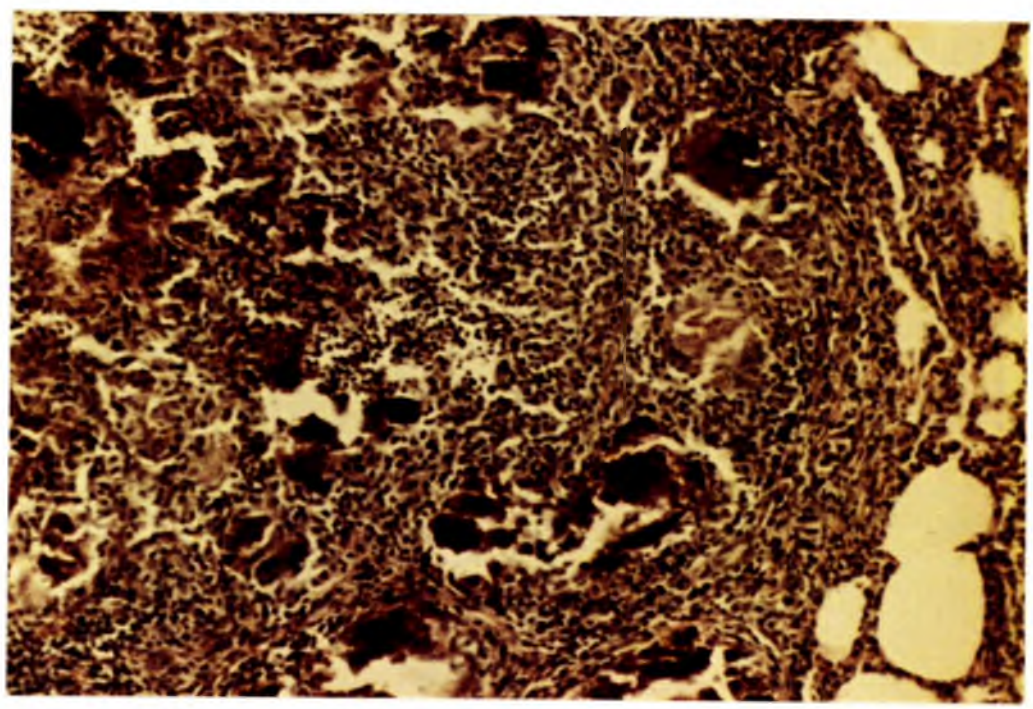
Fig. 38 Pulmonary section from a tetramisole-treated cat. Fragments of degenerated parasites are surrounded by a dense lympho-reticular reaction.

Haematoxylin and Eosin x 150



Discussion

Several conclusions may be stated. Tetramisole appeared to be lethal to adult forms of Aelurostrongylus abstrusus and successfully eliminated lungworm infestation from a small number of experimentally-infected cats. However, six oral administrations were necessary, in contrast with the single dose effective in other animal species, and the drug disquieted the kittens although toxic consequences were not appreciated. The latter fact is noteworthy since Kaemmerer and Budden (1966), in the instance of cats given, parenterally, doses in excess of 10 - 17 mg. per kilogram body-weight, reported the occurrence of fall in blood pressure attended by unrest, dyspnoea, muscular twitching, salivation, convulsions and sometimes death. The same authors also stated that cattle and sheep dosed parenterally with 10 mg. per kilogram displayed comparable side-effects whereas Forsyth (1966b) reported the use of oral amounts of between 5 and 20 mg. per kilogram body-weight in a large number of sheep to be without morbidity or mortality. The latter author (1966a) suggested too, that the minimum lethal dose for sheep was of the order of 90 mg. per kilogram which represents more than a four-fold margin of safety for the highest recommended therapeutic dose. Guilhon (1966) and Pankhurst and Sutton (1966) administered to sheep doses as high as 20 mg. per kilogram without

trouble although the former author warned that, in debilitated animals, the division between therapeutic optima and lethal dosage is slender. Walley (1967) by means of a single oral dose of 15 mg. per kilogram body-weight, eliminated Metastrongylus apri from the lungs of infected pigs.

In conclusion, it is suggested that Aelurostrongylus abstrusus is more resistant to the effects of tetramisole than are lungworms from other animal species and so, if the parasite is to be eradicated from the lungs of infected cats, numerous doses of the drug are essential. The latter factor together with the unpalatability of the substance leads to the view that heavier dosing on fewer occasions or an alternative route of administration may render the anthelmintic more acceptable in feline practice.

Experiment Five

The Treatment of Lungworm Disease of the Cat by Parenteral Administration of Tetramisole

Introduction

The preceding experiment has shown that the oral administration of tetramisole is of value in the treatment of feline lungworm infestation. However, numerous doses were necessary to achieve a successful outcome in all of the animals and, in addition, the drug was displeasing to the treated cats. To overcome those difficulties, therefore, it was thought advisable to resort to parenteral administration of the substance.

Materials and Methods

Nine kittens of eight to fifteen weeks of age were infected orally with 800 third-stage larvae of Aelurostrongylus abstrusus. Eight to ten weeks later, first-stage larvae appeared in the faeces. On five occasions thereafter, the number of larvae per gramme of faeces from each cat was counted and an average computed. Two cats (Nos. 1 and 2) were retained for control purposes while four (Nos. 3, 4, 5 and 6) were injected subcutaneously with 25 mg., followed a week later with 40 mg., per kilogram of body-weight of a 5.0% solution of tetramisole. Three of the latter cats died as a result of the second injection and, on four further weekly occasions, the survivor was given a dose of 25 mg. per kilogram body-weight. Of the three remaining cats, one (No. 7) died subsequent to a single injection of 25 mg. per kilogram body-

weight while the two others (Nos. 8 and 9) were given five daily injections of 5 mg. per kilogram body-weight on two occasions and at an interval of nine days. Tables 15 and 16 record the larval output of all cats over the period of the experiment. At post-mortem examination portions of relevant tissues were removed and treated in a manner similar to that described in Experiment Three.

Results

Of the cats treated weekly, all survived the initial dose of 25 mg. per kilogram body-weight although each animal showed marked salivation, muscular tremors and some degree of ataxia. There was a decrease in larval output two days after treatment but, five days later, the number of larvae had increased. Three cats died thirty minutes after receiving a dose of 40 mg. per kilogram body-weight and death was preceded by excessive salivation, impairment of locomotion and vision, muscular twitchings, dog-like panting, respiratory distress and convulsions. The survivor required a further four doses of 25 mg. per kilogram body-weight, at weekly intervals, in order to abolish excretion of larvae. Autopsy was performed on that animal fifty-six days after initial treatment.

In the case of animals treated on a daily basis (Table 16), larvae disappeared from the faeces after the fourth injection and

Table 15

Number of Larvae Excreted per Gramme of Faeces by Cats Treated Weekly with Tetramisole and by Control Cats

Days after Treatment	Control Cats 1	Control Cats 2	Dosage mg./kg.	Treated Cats 3	Treated Cats 4	Treated Cats 5	Treated Cats 6
0	12,600	18,900	25	5,380	10,050	25,280	13,930
2	15,400	17,800		600		6,888	
3	14,400	15,900		100	400	3,200	100
4	13,600	16,300		1,600	2,700	2,700	2,400
7	15,200	13,800		3,000	7,000	9,500	5,700
8	18,000	14,700	40	died	died		died
14	13,400	13,700				1,600	
15	12,600	13,900	25				
17	15,500	15,300				-	
20	17,300	17,100				600	
22	18,200	16,100	25			1,200	
24	12,500	18,400				200	
29	14,200	15,300	25			1,200	
34	19,200	12,200				2,000	
36	13,200	10,500	25			2,500	
41	9,600	7,800				-	
44	5,300	6,400				-	
48	4,900	3,300				-	
51	5,600	1,900				-	
56	3,300	950				-	

Table 16

Number of Larvae Excreted per Gramme of Faeces by Cats Treated Daily with Tetramisole

Days after Treatment	Dosage mg./kg.	Treated Cats 8	9
0	5	16,000	10,000
1	5	14,500	9,400
2	5	2,400	600
3	5	800	200
4	5	-	-
5		-	-
8		-	-
10		-	-
12		2,300	-
13	5	2,500	1,100
14	5	1,200	2,000
15	5	-	-
16	5	-	-
17	5	-	-
19		-	-
21		300	-
23		1,200	-
25		950	-
27		850	-
30		1,100	-
33		1,700	-

did not re-appear until the twelfth and thirteenth days, respectively, in the instance of Cats 8 and 9. The second dose of a further course of five injections of similar amounts of the drug eliminated larvae from the faeces of both animals and, in the case of Cat 9, that state was maintained until death eighteen days later. Four days after termination of treatment, Cat 8 re-excreted first-stage larvae and continued to do so, in increasing numbers, until euthanasia was performed twelve days later.

At post-mortem and histopathological examinations, the lungs of the four cats that had succumbed to the toxic effects of tetramisole showed lesions which were characteristic for lungworm disease. The lungs from the survivor of the weekly-treated group showed numerous, pale, punctiform foci together with a few protusive, roughened, moderately firm lesions, of up to one centimetre in diameter, scattered throughout the lobes. Histopathologically, there were accumulations of lymphocytes and macrophages located in the pulmonary parenchyma and around blood-vessels and bronchi but neither eggs nor larvae were demonstrable. Fragments of degenerated parasites, surrounded by lympho-reticular elements, were prominent. Hypertrophic change in the muscle of pulmonary arteries, bronchioles and alveolar ducts was appreciable.

Of the two animals that had been treated on a daily basis, the lungs of Cat 9 showed changes that were comparable in all aspects

to those described above while the same organs from Cat 8 displayed lesions which were larger, more widespread and softer in consistency and, on microscopical examination, eggs, larvae and adult worms were found to be present as well as degenerated parasitic forms.

Discussion

If given parenterally and on a sufficient number of occasions, tetramisole is lethal to Aelurostrongylus abstrusus but is is possible that the economics of feline practice may limit use of the drug. Thus, to eliminate parasitic infestation, one cat required six weekly doses, another needed seven daily injections and a further cat was not cured after ten daily doses of the chemical. However, during the period of treatment and probably as a result of the gradual destruction of all stages of the parasites in the lungs, faecal larval output was restricted. To that extent and as a similarly acting substance has not been found, tetramisole is considered to be of value in the control and treatment of lungworm disease of the cat.

Because of its toxicity, the amount of the drug given parenterally is unable to be increased although the need of, ever, administering large doses must be questioned because of the experience with Cat 5 which still excreted first-stage larvae after a dose of 40 mg. per kilogram body-weight. There appeared to be,

however, some degree of individual idiosyncrasy in regard to reaction to tetramisole. One cat recovered from a dose of 40 mg. per kilogram body-weight and four animals survived an amount of 25 mg. per kilogram whereas the latter dose caused the death of another cat and half of that amount induced severe clinical signs, including convulsions, in a test-animal. Only at a level of 5 mg. per kilogram body-weight were side-effects entirely absent. Such findings are supported, to some extent, by the work of Haemmerer and Budden (1966) who, after investigation of the physiological effects of the substance on cats, concluded that a parenteral dose of tetramisole beyond 10 to 17 mg. per kilogram body-weight was unsafe. In sheep and cattle, the same authors found that intramuscular injection of as little as 10 mg. per kilogram body-weight produced severe side-effects while Walley (1967) killed a pig with a dose of 100 mg. per kilogram body-weight and Ueno *et al.* (1967) in the same species, recorded a similar outcome with only half of the latter amount of the agent. It is clear, therefore, that parenteral treatment with tetramisole of diseased animals must be performed with the utmost care.

Of interest, too, is the fact that whether, or not, oral or parenteral administration is undertaken, frequent dosing is necessary for the cure of lungworm disease of the cat whereas, against comparable parasitic infestation of other species, a single dose of tetramisole is considered sufficient (Walley, 1967 and Ueno *et al.*,

1967). It is possible that the density of the investing, cellular response partially protects Aelurostrongylus abstrusus from the action of the drug, or more simply, the latter parasite may have a higher resistance than other lungworms to the toxic influence of the chemical.

Oral administration, as shown by the results of Experiment Four, eliminated lungworms from cats after a maximum of six doses and the main purpose of treating animals, parenterally, was the desire to limit the number of doses and, thus, make the anthelmintic more acceptable in feline practice. The results of the present experiment showed that, by the use of such a method, that end was not achieved and it was also discovered that, in some instances, the toxic/therapeutic ratio of the drug was quite narrow.

It is concluded, therefore, that parenteral injection of tetramisole to cats may be dangerous and, apart from ease of dosing, offers little advantage over oral administration. If the latter method of treatment is adopted it is further suggested that, because of potential toxicity, only small doses of tetramisole should be employed and careful watch maintained over the treated animals. If those criteria are observed, the drug offers a successful means of control and treatment of parasitic pneumonia of cats.

FINAL DISCUSSION

Most works of this kind set out to answer a series of questions and, in doing so, inevitably bring to light further problems which require solution. The preceding thesis is not an exception.

Part one of the work was concerned with larvae, free or within intermediate and paratenic hosts. It had been recognised, previously, that Aelurostrongylus abstrusus successfully parasitised the pulmonary tissues of cats and that achievement was understood with greater clarity as the result of an estimation of the total output of first-stage larvae by infested cats together with an assessment of the longevity and resistance of the larval forms under varying conditions of temperature and humidity. Associated with the latter factors and of equal importance to the survival of the parasite, was the arrangement by which molluscs acted as intermediate hosts. The latter are highly fertile, are distributed throughout the world and have a relatively long life-span during which time they provide a suitable medium for growth and persistence of the larval stages of numerous parasites, including the feline lungworm. First-stage larvae of the latter pierced the pedal epitholium and migrated into the sole and adjacent tissues of molluscs and yet, in comparison with the

number of larvae in contact with the snails, relatively few gained admission. It was also discovered that, at the end of three weeks constant contact with first-stage larvae, only third-stage larvae were recovered from infected molluscs, which finding indicated that further invasion by first-stage forms had been inhibited. It may be that immaturity on the part of the majority of larvae is sufficient explanation of those facts but it is also probable that molluscan immunological reactions played an important role. Aspects of molluscan immunity have been reviewed by Wright (1966) and, despite the fact that molluscs act as intermediate hosts to numerous parasites of animal and human importance, there is not a great deal of information on the subject and that which is available often conflicts. Accordingly, it is considered that investigation of the immune responses of molluscs infected with Aelurostrongylus abstrusus would be a valuable and productive exercise.

The work of investigators such as Knapp (1966) and Richards and Merritt (1967) leads to the conclusion that larvae of, even, closely-related parasites possess disparate properties. First-stage larvae of Angiostrongylus cantonensis appear to be incapable of invading the sole of molluscs and prefer, instead, to be ingested whereas the opposite position obtains with Aelurostrongylus abstrusus and certain other lungworms.

examination of those larval differences offers an interesting and probably a rewarding study.

In the course of investigation it became apparent that, under natural conditions, molluscs are unlikely to contain large numbers of infective larvae. Non-immune cats, therefore, would require to eat a reasonable quantity of infested slugs or snails within a period of 7 - 10 days, that is before the onset of feline immunity to the parasite, if moderately heavy infestations were to result. The alternative, and more likely, theory is that storage hosts play an important part in the life-cycle of the lungworm and one such host, namely, the mouse has been shown to harbour infective larvae for a period of four months. In contradiction of the work of Cameron (1927) and in agreement with the findings of Hobmaier and Hobmaier (1935a), it was found impossible to use the mouse as an intermediate host.

Part Two of the thesis described the effects of the lungworm on the final host. Cameron (1928) introduced a technique for the recovery of adult parasites from the pulmonary artery but other workers, including the present author, failed in attempts to repeat that manoeuvre but found adults in alveoli, alveolar ducts and bronchioles. It is re-emphasised, therefore, that the pulmonary vasculature is not the final habitat of the feline lungworm. It was also discovered that, although fifty third-stage

larvae induced pulmonary lesions, at least 100 were required for successful parasitic infestation and that over 1500 larvae were capable of causing marked clinical upset and, even, death.

The small dimensions and the fragility of adult forms of Aelurostrongylus abstrusus made it difficult to recover whole specimens from the pulmonary tissue so that it was impossible to assess the proportion of an infecting dose that reached, or matured within, the lungs. The absence of such information was particularly restricting in studies on re-infestation and on immunity and further efforts, with finer techniques, are necessary for the elucidation of that problem.

In the experimental work, a recurrent problem was the lack of a satisfactory route of infection. Larvae, given in milk or in water, induced various responses in the experimental animals. Some cats tolerated as many as 400 larvae per day whereas others would accept but a quarter of that number without ensuing sickness. It followed that the process of infection was often time-consuming and, in an attempt to overcome that difficulty, parenteral administration was employed but was found not to be entirely successful. The administration of gastric sedatives, feeding infected snails rather than free larvae to cats and a reduction, by cooling, of larval motility were methods tried, to little avail, in attempts to obviate vomiting.

Subsequent to the latter shortcoming was the failure to trace larval migration from the alimentary tract to the lungs of the cats. Fifteen animals were infected, killed at intervals of between one hour and three days and a wide selection of tissues examined. In not one instance were larvae, or lesions that may have been associated with such parasites, appreciated during examination of histopathological preparations from any organ, other than the lungs in which lesions were noted twenty-four hours after infestation. Other workers with the feline lungworm have been equally unsuccessful in the same respect and, there is little doubt that, the paucity of larvae made demonstration an extremely fortuitous task. Further effort to obtain information on the migratory path of the parasite requires to be expended.

It was difficult to re-infect cats with the parasite. Accordingly, it was not altogether surprising to find that immunity was able to be passively transferred but, although of academic interest, there is little occasion for the application of such a technique either from the prophylactic or therapeutic point of view. On the other hand, active immunisation is a highly practical means of control of feline lungworm disease, whether, or not, it may be induced by the administration of live or attenuated larvae. However, the high incidence of spontaneous disease in cats is not generally appreciated and, as only the occasional cat dies from

parasitic pneumonia, there is little demand for a vaccine. Nevertheless, it is certain that the condition, with its high morbidity, will receive more attention in the future and, at that time, the need for a safe, simple method of control will be appreciated. Another fact to emerge from the investigations was that approximately 1.0% of infected cats failed to develop more than minor lesions and to excrete first-stage larvae. In the absence of alternative explanation, it may be suggested that such animals possessed natural immunity to the parasite and further study of that type of cat would be of immunological interest.

A small experiment in which two pregnant cats, suffering from patent lungworm disease, were studied revealed that infection *in utero* did not take place. Unfortunately, it was not found practicable to test sera, from the eight kittens that were born to the animals, for the presence of antibodies against *Aelurostrongylus abstrusus*.

It was only recently that association between the lungworm and pulmonary vascular disease of the cat had been proved (Hamilton, 1966a). In the present series of experiments, arterial changes were recognised as early as nine days, and were still prominent two years after infection. The pathogenesis of the lesion was not fully clear but, basically, such changes may have resulted from vascular disorganisation and obstruction induced by the various

stages of the parasite and by the reactions of the host. Whether, or not, a myo-stimulant is released by the parasite has not been ascertained and research along that pathway may produce surprising results. Information on the systemic effects of the pulmonary arteriopathy is also required and the use of a technique such as cardiac catheterisation may aid the accumulation of knowledge. Finally, in the circulatory context, there are the comparative aspects of the condition. Gradations of pulmonary pressure may be achieved by varying the dose of larvae and such a system may be of interest to those concerned with the study of human pulmonary hypertension. Of some importance, too, is the fact that investigators, be they physiologists or pharmacologists, should be aware of the incidence of vascular disease in the cat otherwise false experimental results may follow the use of affected animals. Several publications in medical literature bear testimony to the need of such information.

The concluding part of the thesis was concerned with diagnosis and treatment of parasitic pneumonia in cats. There is nothing exceptional about thoracic radiography in that species and it was shown that, if combined with clinical and faecal examinations, the procedure was of diagnostic value. More complex is the problem of diagnosis by immunofluorescence. The latter is not a technique that is applicable outwith a laboratory but the success of the

method is gratifying. Refinement of the technique to allow of estimation of serum antibody titres may be possible since, although Roneus (1966) and Buitenberg et al. (1967) recorded such findings in the sera of pigs suffering from ascariasis and trichinosis respectively, similar results were not attainable in the course of the present investigations.

Treatment of parasitic pneumonia by diethylcarbamazine was unsuccessful. Tetramisole, however, was efficacious but required to be administered on several occasions. Nevertheless, if the need of the latter is accepted and provided that owners are prepared to pay for professional services, the anthelmintic will ensure clinical improvement provided that attention is paid to the potential toxicity of the drug.

The investigations recorded in this thesis were the result of the efforts of several years. The contained experiments encompassed the fields of biology, immunology, pathology and parasitology and were concerned with the discovery of new aspects of the parasite or of associated disease in intermediate, paratenic or final hosts. The exercise has been stimulating and has given the author a degree of satisfaction which will be magnified if it is accepted that the reported work has, in any way, advanced scientific knowledge.

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ADDENDUM

The following articles, arising from this thesis, either have been published or accepted for publication in the stated journals.

J.M. Hamilton (1966) — The Effects of Serotonin on the Pulmonary Arteries of the Cat. J. Path. Bact. 91, 249.

J.M. Hamilton and A.W. McCaw (1967) — The Role of the Mouse in the Life-Cycle of Aelurostrongylus abstrusus. J. Helminth. 41, 309.

J.M. Hamilton and A.W. McCaw (1967) — An Investigation into the Longevity of First-Stage Larvae of Aelurostrongylus abstrusus. J. Helminth. 41, 313.

J.M. Hamilton (1967) — The Number of Aelurostrongylus abstrusus Larvae Required to Produce Pulmonary Disease in the Cat. J. comp. Path. 77, 343.

J.M. Hamilton (1967) — The Treatment of Lungworm Disease in the Cat with Tetramisole. J. small anim. Pract. 8, 325.

J.M. Hamilton (1968) — Re-infection of the Cat by Larvae of Aelurostrongylus abstrusus. J. comp. Path. 78, 69.

J.M. Hamilton (1968) — An Assessment of the Value of Diethylcarbamazine in the Treatment of Cats Experimentally Infected with Lungworm. Vet. Rec. In Press.

J.M. Hamilton (1968) — Parenteral Infection of the Cat by Larvae of Aelurostrongylus abstrusus. J. Helminth. In Press.

J.M. Hamilton (1968) — Passive Immunisation in Lungworm Infection of the Cat. J. comp. Path. In Press.

J.M. Hamilton and A.W. McCaw (1968) The Output of First-Stage Larvae by Cats Infected with Aelurostrongylus abstrusus J. Helminth. In Press.