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FELINE LEPTOSPIRAL INFECTION :

with particular emphasis on
leptospira interrogans serovar *ballum*.

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requirements for the degree of Master of Philosophy
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SUMMARY

Different aspects of feline leptospirosis including a study of the bacteriology, serology, histopathology and clinical pathology were investigated by inducing experimental infection by the oral and intra-peritoneal (I.P.) routes.

Experimental inoculation with five of the six *Leptospira interrogans* serovars which are known to exist in New Zealand, revealed a symptomless infection in cats. A carrier state developed with *pomona* and *ballum* whereas with *hardjo* and *tarassovi* the infected cats showed only a serological response. A cat inoculated with *balcanica* did not respond, even serologically, to infection. Susceptibility of cats to different serovars was thought to be due to individual cat variation.

A further series of experiments was conducted, infecting cats with serovar *ballum*. The infectivity studies using two-fold increases in dose from 10^2 to 10^8 by the oral route established a carrier state in cats given more than 10^2 leptospirae. Three cats with urinary carrier states did not have a detectable micro-agglutination (M.A.) titre at the time of euthanasia two and three weeks post-infection (p.i.) and there was a decrease in the titre with lowering the infective dose.

The longest duration of the carrier state was 122 days after oral infection and this was obtained with an infective dose of 10^8 leptospirae. Leptospiuria commenced at 13 days p.i. A direct correlation between serological titre and urinary carrier state was hypothesised after demonstrating a consistently high titre in one cat which was related to a long duration of leptospiuria and observing in another cat a low titre which disappeared by the 12th week p.i. and in which no leptospiuria could be detected. Serological response in the cat consists of the

primary production of IgM and subsequent establishment of IgG and this latter immunoglobulin appeared to be related to the carrier state.

Comparative studies of histological, serological and cultural findings revealed that the I.P. route of infection produced more severe infection than the oral route. Leptospiraemia was detected after the same time interval for both routes with the longest duration being seven days in an intraperitoneally infected cat. Leptospire were recovered from some lymph nodes and the brain only during the leptospiraemic phase.

All the cats had M.A. titres by the 10th day p.i. and higher initial titres were recorded in the cats infected by the I.P. route. By the third week p.i. the M.A. titres of the cats infected by the oral route reached the same level as those infected by the I.P. route. Elevation of rectal temperature coincided with the first isolation of leptospire from urine and kidney seven to nine days p.i. There was also a concurrent decrease in specific gravity of urine. This was thought to be due to invasion of the kidneys by leptospire and consequent tissue damage.

Histological studies showed disseminated foci of fatty change in the straight portion of proximal tubules in the inner cortex of the kidney one week p.i. Infiltration of lymphocytes occurred around the urinary tubules two and three weeks p.i. and early tubular necrosis was seen by the fourth week p.i. These histopathological changes are related to the migration of the leptospire into the kidney, invasion of the tubules by these organisms and subsequent reaction of the host.

Prey-predator transmission studies showed that more severe infections were produced in cats when small portions of infected mice were fed as compared with feeding whole infected mice. This is most

likely to be due to the longer contact of infected ingesta with the pre-gastric portion of the alimentary tract. All the cats showed 0.5 to 1 degree elevation of temperature 9-12 days p.i. which appeared to be due to the kidney invasion and related tissue damage by leptospire. Neutrophils were first seen in foci of interstitial nephritis at 47 days p.i. in addition to lymphocytes, macrophages and plasma cells. Duration of leptospiraemia was longer in the cats infected with the smaller portions of ingesta (infected mouse kidneys and bladder) than in the cats infected with whole infected mice.

No inhibitory effect was observed using the growth inhibition test even when the serum had a very low M.A. titre, these results are different from those obtained in other animals. Gel filtration tests on serum samples of the infected cats showed the specific antibody to be comprised of IgM and IgG in those cats which showed kidney lesions histologically, but in the cats with no detectable kidney lesions the only immunoglobulin detected was of IgM class. In all these experiments the cats showed a rapid decline in their M.A. titres after reaching a maximum level.

A serological survey of 225 cats from various sources in the North Island using 11 live leptospiral serovar antigens detected 25 (11.11%) of sera with an M.A.T. of 1/12 or greater. The prevalence of serological reactions to the different serovars which exist in New Zealand was determined. These were as follows: eight (3.55%) to *ballum*, six (2.55%) to *copenhageni*, five (2.22%) to *hardjo*, four (1.77%) to *pomona*, two (0.88%) to *balcanica* and one (0.44%) to *canicola*. No *tarassovi* titres were recorded at 1/12 or greater.

It is of interest that titres to *ballum* and *copenhageni* showed the greatest prevalence, both of which have rodents as their maintenance hosts. It is hypothesised that prey-predator transmission has been responsible for infection with these serovars, while with the other serovars direct contact with infected animals or a contaminated environment is presumed. There was no obvious relationship between the titre and the clinical disease as the majority of the cats with positive titres were apparently healthy. The results of this survey combined with experimental studies suggests that in feline serological surveys a low initial dilution should be used.

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INTRODUCTION

Following a review of the literature this thesis attempts to study the susceptibility of the cat to different leptospiral serovars. Particular studies were conducted to investigate the pathogenicity, development of the carrier state and the serological response of the cat infected by the oral and intraperitoneal route with *Leptospira interrogans* serotype *ballum*.

To investigate the hazard of the cat as a carrier and to simulate the field situation of prey-predator chain as a likely source of infection cats were fed infected mice. A serological survey was also conducted to determine the most commonly occurring leptospiral infection in the cat.

CHAPTER 1

LITERATURE REVIEW

The first isolation of leptospirae from a cat was reported in 1938 in Indonesia by Mertens according to Murphy *et al.* (1958).

Esseveld, Collier and Mochtar (1940) also conducted extensive serological and cultural studies of approximately 500 cats in Indonesia. They recovered 14 isolates from 343 cats' kidneys, eight of which were *Leptospira interrogans* serovar *bataviae* and six *L.javanica*. Approximately 30 per cent of the cats were serologically positive for *bataviae* and/or *javanica*. The results suggested that nearly one third of all adult cats in Java were infected, and over one quarter of all adult cats in Batavia were carriers of infection. The authors concluded that cats were only infrequently responsible for human leptospirosis.

Schmidt (1936) examined the kidneys of 40 cats which had died from various causes. All kidneys from these cats were considered to be not infected with leptospire.

Wirth (1937) observed no clinical symptoms in eight week-old kittens which were subcutaneously infected with *icterohaemorrhagiae* but three weeks after infection, leptospiruria was noted.

Klarenbeek and Winsser in 1938 elicited a serologic response in kittens experimentally infected with *canicola* and demonstrated leptospiruria in these cats 15 to 78 days post infection (p.i.). There was a slight serological reaction in a second group of cats which were inoculated with *icterohaemorrhagiae*. No clinical symptoms were noted in these infected animals. van den Brekel (1938) claimed a cat to be the source of infection for a human case of Weil's disease.

Greene (1941) in a survey of leptospirosis in Southern California using the macroscopic agglutination test examined sera from 100 cats, all were negative for *canicola* at 1:30 and one gave a positive reaction to *icterohaemorrhagiae* at 1:90.

Alicata and Breaks (1943) carried out a similar serological survey using *canicola* and *icterohaemorrhagiae* antigens on serum samples collected from 100 cats in Hawaii. All were said to be "negative". The sera of 165 cats from Hamburg were serologically examined by Weissflog (1952) for the presence of agglutinins against *icterohaemorrhagiae*. One hundred and twenty four of these serum samples were also tested for antibodies against *canicola* and *grippytyphosa* and 24 were tested for *bataviae* antibodies. No positive reactions were reported.

van der Hoeden (1953) infected a young cat by the subcutaneous route with a culture of *grippytyphosa* which was isolated from a goat in Israel. The cat excreted a large number of leptospire in the urine and when killed 26 days p.i. showed an agglutinin titre of 1:2000. *Leptospira* were cultivated from its urine but not from the liver or kidney. In the histological section the kidney showed a glomerulonephritis and clumps of *Leptospira* in the tubules.

Semmel (1954) found one of 128 serum samples taken from healthy and diseased cats in Munich district had a positive (1:640) titre to *canicola* and *sejroe*. He failed to produce clinical symptoms in six cats which he infected subcutaneously with *canicola*, *icterohaemorrhagiae*, *grippytyphosa*, *sejroe* and *pomona*, although there was a temporary rise in titre. He concluded that leptospirosis in cats was rare or could be overlooked as the infection can be symptomless.

The sera of 236 cats from Hamburg were tested for leptospiral antibodies by Otten, Henze and Goethe (1954) using a complement fixation test. None of these sera were positive however, when the M.A. test was used, eight out of 86 cats had titres of from 1:10 to 1:100 against *canicola* and *icterohaemorrhagiae*. These authors also failed to produce clinical symptoms in 21 cats experimentally infected with these same serovars and were unable to detect leptospiuria in the urine, bladder, pelvis or the kidney of their experimental cats. But in Levaditi stained sections the organisms were detected in two cases, on the 77th and 98th day p.i. In the opinion of these investigators, the epidemiological importance of leptospirosis in cats cannot be ignored, even though cats are apparently refractory to many leptospiral infections.

Mochmann (1955) cited by Murphy *et al.* (1958) recorded two positive serological reactions against *canicola* among 15 cats in Germany but the titres were not given.

In a survey by Broom (1955) cited by Hemsley (1956), 180 serum samples from cats, which were destroyed for various reasons during the period between 1949 to 1952, were tested against *icterohaemorrhagiae*, *canicola*, *sejroe*, *pomona*, *grippotyphosa* and *bataviae*. Five cats included in this survey showed evidence of nephritis, three of which gave negative agglutination reactions. Broom recorded 15 titres between 1:30 and 1:3000, five of those were 1:100 or higher. He suggested that titres of 1:100 and greater could be considered as significant.

Alston and Broom (1958) make mention of unpublished data from a survey by Joshua and Broom in which they found low agglutination titres to *icterohaemorrhagiae* or *canicola* but failed to isolate leptospires from a few cases of acutely jaundiced cats.

Amosenkova (1955) carried out a serological investigation of 358 cats in Leningrad, Russia, using *icterohaemorrhagiae* and *canicola* antigens. Three of the cats (0.83%) showed titres to *icterohaemorrhagiae* with the highest at 1:500. The results of an investigation of 176 kidneys from these cats were all negative microscopically and bacteriologically. In order to elucidate the susceptibility of cats to *icterohaemorrhagiae*, 12 kittens aged from three to six weeks were infected with this serovar. Infection in the kittens resulted in a less acute infection than puppies and no characteristic pathological changes were detected. As a result of her findings, Amosenkova (1955) claimed that cats are relatively insusceptible to *icterohaemorrhagiae*.

Bock (1954) described a case of *grippotyphosa* infection in a female human patient who apparently was infected from a cat.

Hemsley (1956) described six cases of chronic nephritis in cats. Serological examinations were carried out on four of these cases. Three of them gave a positive reaction to *canicola* by the M.A.T. He speculated that the prevalence of nephritis due to leptospirosis in cats is unknown but appears to be low.

Fennestad (1956) recorded infection of cats in Denmark, due to *icterohaemorrhagiae*, *poi*, *saxkoebing* and *bataviae* but this was based on serological evidence alone.

In an attempt to determine the prevalence of leptospirosis in America, Murphy *et al.* (1958) examined 350 sera collected from one to four year-old rural cats which were tested against five antigens, *pomona*, *djasiman*, *sentot*, *autumnalis* and *grippotyphosa*. Leptospiral agglutinins were present in 17 (4.9%) of these cats. The use of a further 12 antigens did not produce additional positive serological reactions.

Vysotskii *et al.* (1959) examined 30 cats' serum in Vladivostok using up to 11 antigens, one sample had a titre of 1:1000 to *grippotyphosa*. This cat was living with a herdsman who tended calves. Another cat, which was from a food warehouse in a town, had a *canicola* titre of 1:6400. *Grippotyphosa* was isolated from the kidney of a wild cat from a forest and had a titre of 1:100 to this serotype. These authors failed to obtain an isolate from any of the cats which they considered to have significant titres. They concluded that the cat is not important in the epidemiology of leptospirosis, but in isolated cases, because of close contact with man, could act as a source of infection.

In Kwangtung, China, 28 cats were examined in further studies of animal hosts of leptospirosis (Anon, 1960). Three of them gave positive C.F. tests but attempts to isolate leptospires from the kidney and liver failed.

Clark (1961) failed to isolate leptospires from 56 feral cats in Pennsylvania even though endemic *ballum* infection occurred in house mice in the same region.

Fessler and Mörter (1964) experimentally infected two groups of cats with *pomona* and *ballum* by the subcutaneous route. Clinical illness was not observed in either group of cats, but leptospires were recovered from the kidneys of all the cats which were infected with *pomona*. They concluded that cats are susceptible to experimental infections with both these serotypes of leptospire and that the establishment of a leptospiruria indicated that these animals could play a role in the transmission of *pomona*.

Rees (1964) reported a titre of 1:3000 to *pomona* from a cat in New Zealand, which was being treated for interstitial nephritis but no leptospire were seen in histological sections of the kidney.

Jones (1964) infected three groups of cats with formalin-killed preparations of *canicola*, *pomona* and *icterohaemorrhagiae*. There was no detectable antibody response to any of the antigens in the cats. Subsequently he used proven pathogenic strains of these serovars and even these failed to produce detectable antibody titres in cats. No leptospire were recovered from the urine or kidney cultures. Jones (1964) examined 139 cat sera and a titre of 1:100 to *pomona* was found in one cat, while another had a titre of 1:100 for *icterohaemorrhagiae*. He concluded from this evidence that the domestic cat possesses species immunity to these three serotypes.

Ferris and Andrews (1965) isolated *pomona* from the urine of one of 21 feral cats in Southern Illinois. This cat also had a microscopic-agglutination reaction to *pomona* (1:1000). They also infected three cats with their isolated ("intraperitoneally, on the conjunctival surface and conjunctival inoculation") and recovered leptospire from blood and urine of all three cats. No organisms were recovered from the kidneys of any of the cats at necropsy which was conducted 60 days p.i. They concluded from these findings that cats may be important in the spread of infection to domestic animals and humans.

Lucke and Crowther (1965) carried out a serological survey in the Bristol area during an investigation of spontaneous nephritis in cats. The sera from 118 cats were examined. The highest agglutination titres of 1:1000 to *canicola*, *mini-mini* and *bratislava* were found in three cases. Altogether eight of the cats (6.8%) had titres of 1:30 to 1:1000. They concluded that cats may be infected by a variety of leptospiral serotypes. The authors were undecided as to whether leptospirosis was

associated with nephritis or other clinical diseases although they believed that cats may act as carriers.

Freudiger (1969) tested 106 cats from Berne and its neighbourhood against different leptospiral serovars known to occur in Switzerland. Only four showed positive reactions, each one against three to four serotypes. He discussed the reasons for the infrequency of leptospirosis in cats compared with the dog and other animals. He suggested three possibilities :-

1. High natural resistance of the cat against *Leptospira* infection.
2. Less opportunity for exposure to infection as cats are more or less housebound.
3. Rapid decay of convalescent antibody titres.

He also discussed the result of his experimental infections with *pomona*, where he was not successful in isolating leptospires from the urine or kidneys of his two experimental cats.

The first time an isolation of *Leptospira* serovar *pomona* from cats has been recorded in New Zealand was by Harkness *et al.* (1970) who reported its isolation from a domestic farm cat. Three cases of human leptospirosis occurred on this farm and all the animals of this farm were bled and their serological reactions determined against eight serotypes of *Leptospira*. The cat was found to have a titre of 1:3000 against *pomona*. Leptospirae were seen on dark ground microscopy of the urine sediment. The cat which was in good condition was destroyed and leptospirae *pomona* isolated from both urine and kidney cultures. The cat had been brought to the farm recently from another farm on which an outbreak of bovine abortion had occurred and which had been attributed to *pomona*. It would appear that the cat was infected prior to June 1968 and remained a carrier of *pomona* until its death in December 1968.

(Katy had Hebd. SGA titres)
cattle had Hebd SGA titres - & the dogs were seroneg

Michna (1970) found agglutinins to *sejroe* in a dilution of 1:1000 in the serum of a Siamese cat from a rural district which became fatally ill showing fever and jaundice.

Leptospiral isolation was attempted on eight febrile and icteric domesticated cats by Carlos *et al.* (1971) in the Philippines. *Grippetypbosa* was isolated from one urine sample. The M.A.T. on the eight sera was negative for all cats except for the serum sample taken from the leptospiruric cat.

Mason *et al.* (1972) described two cases of suspected feline leptospirosis, one involved a dairy farm cat which became ill and showed jaundice. It had a titre of 1:1000 to *pomona* and a focal interstitial nephritis was detected histologically. In sections of kidney stained by a silver staining technique, some leptospiral-type organisms were observed. The second case was a Siamese cat which died after an acute illness. Both the clinical and clinical-pathological findings were indicative of nephritis. No serological reaction to any of six leptospiral serotypes tested was observed, but in the kidneys degenerated leptospiral bodies were observed.

Pavlov *et al.* (1972) studied the host-parasite immunologic relationships in cats and some other animals experimentally infected with *pomona* and *tarassovi*. Agar gel-immuno-electrophoresis was used to trace the quantitative and qualitative changes in the protein spectrum. In cats the most obvious changes were seen in the gamma globulin fraction.

The New Zealand National Health Institute (1972) examined sera from seven dogs and one cat in contact with a human case with evidence of both leptospirosis and toxoplasmosis. A titre of 1:200 to *hardjo* was obtained from the cat which also had a toxoplasma dye test titre of 1:32. Two of the dogs had positive titres to *hardjo*.

Watson and Wannan (1973) in Australia, tested the serum from 100 cats against 12 serotypes, one of the samples reacted with a titre of 1:30 to *grippotypbosa* while five others gave reactions at 1:30 to both *hebdomadis* and *hardjo*. These latter five also reacted with *wolfi*.

Modric (1974) experimentally infected cats using *icterohaemorrhagiae*, *pomona*, *sejroe* and *grippotypbosa*. His work described the clinical, immunological and pathoanatomical reactions. He isolated leptospire from the blood, kidney and urine. Infected cats produced agglutinins from the 4th to the 119th day p.i.

Bryson and Ellis (1976) isolated leptospire from thoracic fluid aqueous humour, and kidney of a cat which at necropsy had widespread haemorrhages and an excess of straw-coloured fluid in the thoracic and peritoneal cavities.

In the stained sections with fluorescein isothiocyanate conjugated antisera to five leptospiral serotypes known to occur in Britain namely, *ballum*, *bratislava*, *canicola*, *hardjo* and *icterohaemorrhagiae*, "strong fluorescence" was observed in lung, liver, brain and kidney with the *bratislava* labelled antiserum.

From this review of the literature it can be seen that most information relates to serological survey and field studies. They indicate that the cat appears to be liable to infection by a number of different leptospiral serotypes. The incidence and prevalence of serotypes differ considerably, according to the country and even from district to district. However, most of the workers are in agreement that the role of the cat in the epidemiology of leptospirosis is not very important.

The lack of published information on experimental infection of cats with different leptospiral serovars makes the interpretation of serological surveys difficult. The subsequent chapters of this thesis will attempt to provide some of this information.

CHAPTER 2

COMPARATIVE INFECTIVITY STUDIES WITH FIVE DIFFERENT SEROVARS

INTRODUCTION

Six pathogenic serovars of *Leptospira* have been isolated from farm livestock and wildlife in New Zealand. The serovars mainly associated with cattle and sheep are *hardjo* and *pomona*; with pigs *pomona* and *tarassovi*; with rodents *ballum* and *copenhageni*, and with possums *balcanica*. The latest report on the prevalence of these serovars is as follows:

In domestic animals *pomona* is the most common serovar. It was isolated from 45% of the kidneys of young pigs and from 2% of the adult pigs' kidneys from an abattoir with positive serological titres of 87% and 86% respectively in the lowest serum dilution of 1:12 (Ryan, 1978).

The prevalence of *tarassovi* as determined by cultural studies was found to be 1% in the young pigs and 5% in the adult pigs with a positive serological titre of 21% and 25% respectively (Ryan, 1978). The prevalence of titres at 1:24 or greater to *hardjo*, *pomona* and *tarassovi* titres in a random survey of New Zealand bovine sera was reported by Hellstrom (1978) to be 60%, 18% and 9% respectively.

In wildlife, Brockie (1977) reported that 12.7% of the Norway rats, 25% of the shiprats (*Rattus rattus*) and 14.9% of the mice (*Mus musculus*) which were tested had suffered a recent infection of the *ballum* serotype or were renal carriers. The serological evidence of *ballum* in hedgehogs (*Erinaceus europaeus*) from dairy farms was reported to be as high as 56% by Brockie and Till (1977). Hathaway (1978) isolated leptospires of the *Ballum* serogroups from 27% of Norway rats and 33% of shiprats. He also isolated it from housemice and hedgehogs.

The prevalence of *balcanica* in possums reported by Hathaway (1979) was as high as 85% (serological) and 65% (bacteriological).

Brockie (1977) reported evidence of current or recent infection with *copenhageni* in 32% of Waikato Norway rats (*Rattus norvegicus*), while Hathaway (1978) did not isolate this serovar in his survey of rats and mice conducted in the southern half of the North Island.

In New Zealand the role of farm livestock in the spread of leptospirosis to humans has been well demonstrated. In 1951, the first case of human leptospirosis reported by Kirschner and Gray was in a dairy farmer. Since then there have been yearly reports of human leptospirosis which have resulted from contact with farm livestock. The serotypes which have so far been isolated from human patients are *pomona*, *hardjo* and *ballum*.

Insufficient investigative work into leptospirosis in cats has been carried out to determine their role in the spread of this disease. As the cat is one of the most popular pets, diseases of this animal are of great importance, especially if they are transmissible to humans. Bock (1954) described a case of *grippotyphosa* infection in a human female patient who was apparently infected from a cat.

Harkness *et al.* (1970) reported the first isolation in New Zealand of serovar *pomona* from a cat which had been recently transferred from one farm to another. On the original farm an outbreak of bovine abortion had occurred which had been attributed to *pomona*. The cat appeared to introduce the disease to the new farm where three cases of human illness were diagnosed clinically as leptospirosis.

*Leish - by new
hardjo cases*

Because of lack of knowledge of the possible role of cats as a source of leptospiral infection, it was decided to initially investigate

the infectivity and pathogenicity to cats of five of the six serovars presently found in New Zealand, namely *pomona*, *balcanica*, *ballum*, *hardjo* and *tarassovi*. *Copenhageni* was excluded from this study because at present in New Zealand this serovar is less prevalent in animals than the others (Hathaway, 1978) and also occurs only rarely in man (Christmas *et al.*, 1974).

EXPERIMENT ONE

A PILOT STUDY WITH DIFFERENT SEROVARS

MATERIALS AND METHODS

This experiment was performed with five adult crossbred cats of different ages (Table 1). For two weeks prior to infection the cats were kept under the same conditions in an isolated room and fed with a canned commercial cat food. All the cats were examined for the presence of leptospiral agglutinins and all were negative pre-infection. The method used for detecting leptospiral antibodies was the microscopic agglutination test (MAT) using live leptospiral antigens with the lowest dilution of 1:12. Details of the procedure are described in Appendix II.

The cats were infected with five different serovars namely *pomona*, *ballum*, *hardjo*, *balcanica* and *tarassovi* by the intra-peritoneal (I.P.) route, using a dense 6-8 day old culture (approximately 10^8 leptospores/ml). (Table 1). Rectal temperatures were taken daily for the four weeks duration of the study, and the cats were observed closely for any change in behaviour. Blood and urine samples were collected on days 3, 5, 8, 11, 14, 17, 21 and 31 post-infection (p.i.). The blood was cultured during the first two weeks p.i. Haemoglobin, packed cell volume (PCV), white and red blood cell count, differential leucocyte count, total plasma protein, Icterus Index, and plasma fibrinogen were estimated. Micro-agglutination tests (M.A.T.) were performed for the duration of the experiment.

Urine samples were cultured. Other urine examinations included the measurement of pH, specific gravity, protein, glucose, blood, urobilinogen, bilirubin and ketones. Urinary sediment was also examined.

Table 1

DETAILS OF CATS AND LEPTOSPIRA SEROVARS
USED IN EXPERIMENT NO.1

Cat Reference Number	Sex	Age	Body Weight k.g.	Serovar Inoculated I.P. route
1	Female	Approx. one year	3	<i>pomona</i>
2	Male	More than six months	2	<i>ballum</i>
3	Female	Approx. six months	1.1	<i>hardjo</i>
4	Female	More than six months	2	<i>balcanica</i>
5	Male	More than six months	2	<i>tarassovi</i>

Urine and blood samples were taken under anaesthesia. The drug used was a 2% solution of Rompun* intramuscularly (i.m.) 0.15 mg per kilogram of body weight. This drug was found effective for inducing light anaesthesia. After injection the cats were returned to their cages for five minutes until anaesthesia had commenced and samples could be taken. With the regular use of Rompun, the dose required for anaesthesia had to be gradually increased. The animals were not fed for a half to one hour before the drug was administered to avoid vomiting.

Obtaining and processing the urine samples

Urine was obtained by manual pressure on the bladder. The area around the vulva or penis was swabbed with alcohol and the urine collected into a sterile bottle. One drop of fresh voided urine was inoculated into one of the three bottles of EMJH (Difco)** semi-solid media containing 5-fluorouracil (5Fu) (Sigma)***. Then a ten-fold dilution of urine was made into a 9 ml sterile Stuart's basal medium (SBM)** From this dilution (10^{-1}) two to three drops were inoculated into each of the other two bottles of media. Urine was also immediately examined for the presence of leptospire by direct darkfield microscopy.

Obtaining and processing the blood samples

The blood samples were collected while the animals were anaesthetised. Both sides of the neck were shaved and swabbed with alcohol and blood samples obtained from the jugular vein.

* Bayer Leverkusen, Germany. N.Z. importers : Henry H. York & Co. Ltd, P.O. Box 38-045, Petone.

** Difco Laboratories, Detroit, Michigan, U.S.A.

*** Sigma Chemical Co., P.O. Box 14508, St Louis, Missouri 63178, U.S.A.

** Div. Becton, Dickinson & Co., Cockeysville, Maryland 21030, U.S.A.

One drop of blood was inoculated into each of two bottles of EMJH semi-solid media containing 400 mg 5Fu (Appendix I) as a selective agent for growth of leptospirae (Johnson and Rogers, 1964). The cultures were incubated at 29°C and examined for leptospiral growth at two weekly intervals. The leptospiral antibody level of the serum was determined by the M.A.T. using live antigens (Appendix II).

Necropsy procedures and the cultural examination

After one month the experiment was terminated and the cats were killed by intra-peritoneal injection of Euthesate* (pentobarbitone sodium injection for euthanasia at a dose rate of 5 ml per 4.5 kg body weight).

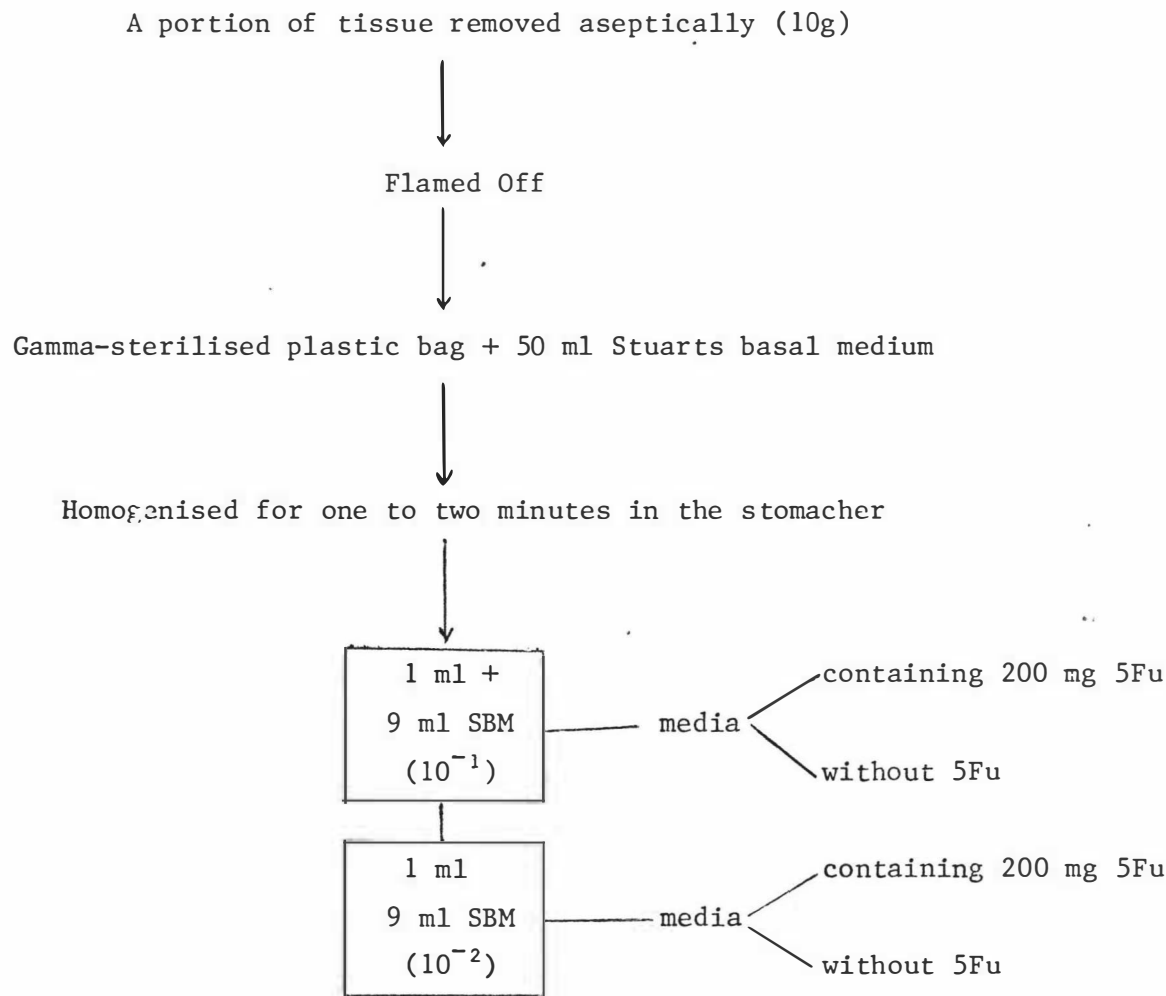
At necropsy the abdominal cavity was opened in all the cats using aseptic techniques to avoid contamination. The right kidney and a portion of liver (approximately 10 g) was removed for bacteriological examination. The skull was opened using an aseptic technique and half of the brain was removed for cultural examination. Urine samples were taken directly from the bladder. Aqueous humour from the right eye was removed using a sterile 16 gauge needle for cultural examination. A suspension of kidney, liver and brain were cultured using the method described by Hathaway *et al.* (1978) (Figure 1) : a portion of each tissue was removed aseptically, flamed, transferred to a sterile plastic bag to which was added approximately five times the tissues weight of Stuart's basal medium (SBM) and then homogenised for one to two minutes in the Stomacher**. For each suspension two bottles of 9 ml SBM and four bottles of EMJH semi-solid media were inoculated. Two of them contained 200 mg of 5Fu and the remaining two were without 5Fu. For each dilution one bottle of EMJH semi-solid media containing 200 mg of 5Fu and one without 5Fu were inoculated.

* Willows Francis, Westhoughton, England. N.Z. importers : VETCO Products Ltd, Wiri Station Road, Auckland.

** Colworth Stomacher. A. J. Stewart & Co. Ltd, London.

Figure 1.

CULTURE METHOD



One millilitre of the suspension prepared in the stomacher was added aseptically to 9 ml of SBM (10^{-1}). Two to four drops of this dilution (10^{-1}) were inoculated into each of two bottles of EMJH medium. A further dilution was made by transferring 0.5 ml of the (10^{-1}) dilution into 9 ml of SBM, and two to four drops of this medium were delivered into two more bottles of EMJH. Aqueous humour was cultured in the same manner as the urine, but using EMJH medium either containing 200 mg 5Fu or without 5Fu.

For histological examination, half of the left kidney, a portion of liver, a portion of the apical lobe of the lung, half of the brain, and the urinary bladder were fixed in 10% formalin. After fixation the tissues were trimmed and embedded in paraffin wax. Sections were cut at $5\mu\text{m}$ and stained with haemotoxylin and eosin. Liver and kidney sections were stained by the Warthin Starry method (Armed Forces Institute of Pathology, 1960) to demonstrate leptospire. One of the eyes, usually the left, was removed and fixed in the Bouin's fixative. After 24 hours this was changed to 75% alcohol and later the eye was trimmed, embedded in paraffin and was stained with haemotoxylin and eosin.

Haematological tests

Blood samples for haematological tests were collected into 4 ml vacutainers* containing 5.5 mg EDTA.

Red and white cell counts (RBC, WBC) were performed using a haemocytometer counting chamber and the usual associated techniques (Schalm *et al.*, 1975).

* Becton, Dickinson & Co., Rutherford, New Jersey 07070, U.S.A.

MacNeal (Tetrachrome stain*) was used for staining the blood smears for the differential leucocyte count.

Haemoglobin (Hb) was estimated by the cyanmethaemoglobin method. A microhaematocrit method used for packed cell volume (PCV) determination and the total plasma proteins were measured by refractometer. For the icterus index the colour of the plasma was compared to the colour of potassium dichromate standards.

The tests on the urine for pH, protein, glucose, ketones, bilirubin and the presence of blood were performed by using appropriate test strips (Bili-Labstix**). The specific gravity of urine samples was determined by using a refractometer.

* Curr Tetrachrome stain, Searle Diagnostic, High Wycombe, Bucks, England.

** Ames Company, Division, Miles Laboratories, Australia Pty Ltd.

RESULTS

Clinical symptoms

Lack of sheen and dull colour of the coat was observed in the cat inoculated with *pomona* (No.1). This occurred a few days after commencement of the study and lasted for the duration of the experiment.

Three cats (No.'s 1, 3 and 5) had mild respiratory symptoms a week p.i. which lasted a few days and resolved without any treatment. No other clinical signs were noted.

Temperature

Except for cat No.5 which had an elevated temperature (40.7°C) one day p.i. lasting only one day, no obvious changes in temperature were observed.

Haematological examination

Although the leucocyte counts of all the cats showed some fluctuation (Table 2) there was some indication of a leucocytosis between the 14th and 21st day p.i. in all the animals.

Urine analysis

The specific gravity of the urine of cat No.1 (*pomona*) increased from 1.012 to 1.045 after two weeks p.i. and stayed at this level until the end of the experiment. Although no abnormal urinary sediment was noted, the amount of sediment was greater for this cat than for the others.

Table 2

LEUKOCYTE COUNTS OF THE BLOOD SAMPLES PRE AND POST INFECTION

Cat Reference Number	Serovar Inoculated	Leukocyte count pre- infection	Days Post Infection									
			3	5	8	11	14	17	21	24	28	31
1	<i>pomona</i>	11.35x10 ⁹	11.5x10 ⁹	7.75x10 ⁹	11.35x10 ⁹	11.1x10 ⁹	20.7x10 ⁹	13.1x10 ⁹	7.95x10 ⁹	10.8x10 ⁹	14.7x10 ⁹	12.55x10 ⁹
2	<i>ballum</i>	13.0x10 ⁹	-	10.1x10 ⁹	10.32x10 ⁹	12.8x10 ⁹	14.25x10 ⁹	16.1x10 ⁹	10.75x10 ⁹	13.45x10 ⁹	9.55x10 ⁹	15.0x10 ⁹
3	<i>hardjo</i>	-	10.35x10 ⁹	-	9.65x10 ⁹	7.4x10 ⁹	11.3x10 ⁹	18.45x10 ⁹	13.45x10 ⁹	17.2x10 ⁹	10.95x10 ⁹	12.95x10 ⁹
4	<i>balcanica</i>	-	5.7x10 ⁹	8.55x10 ⁹	7.75x10 ⁹	7.75x10 ⁹	12.7x10 ⁹	8.75x10 ⁹	14.7x10 ⁹	12.35x10 ⁹	10.4x10 ⁹	9.4x10 ⁹
5	<i>tarassovi</i>	12.0x10 ⁹	9.1x10 ⁹	10.45x10 ⁹	9.8x10 ⁹	9.8x10 ⁹	21.9x10 ⁹	17.4x10 ⁹	12.2x10 ⁹	12.1x10 ⁹	12.3x10 ⁹	15.58x10 ⁹

Note: - = Blood samples were clotted and the results were not considered.

On the 11th day p.i. the urine of cat No.2 (*ballum*) was darker in colour than that of the other cats and appeared turbid. The specific gravity was 1.032 which was the highest recorded for this cat throughout the experiment. Cat No.3 (*hardjo*) had 0.5% glucosuria on the 11th and 24th day p.i.

Isolation of leptospires

The leptospire isolates from the cats inoculated are shown in Table 3. *Pomona* was recovered from the blood of cat No.1 three days p.i. *Ballum* was recovered from blood cultures in Cat No.2 three and five days p.i., positive urine cultures were also obtained 28 days p.i. from cat No.2. No further positive cultures were obtained from these two cats or from any of the others. Approximately half the cultures were contaminated in spite of the precautions taken. This obviously decreased the accuracy of these cultural examinations.

To confirm the identity of the isolates they were all tested against serial dilutions of all 12 sera of known serogroups, namely *australis*, *autumnalis*, *ballum*, *bataviae*, *biflexa*, *canicola*, *copenhageni*, *grippotyphosa*, *hardjo*, *pomona* and *pyrogenes* supplied to the laboratory from the Centre for Disease Control (C.D.C.), Atlanta, Georgia.

Serological response

All the cats except cat No.4 which was infected with *balcanica* responded serologically (see Table 4). In cat No.3 the micro-agglutination (M.A.) titre occurred earlier than in the other cats. The M.A. titres of all responding cats reached their maximum peak before 14 days p.i. with the serological titres of cat No.1 being the highest (Fig.2).

Table 3

ISOLATION OF LEPTOSPIRES FROM CATS

Cat Reference Number	Serovar Inoculated	Post Infection Day of Isolation			
		Blood	Urine	Kidney	Other Tissues
1	<i>pomona</i>	3	-	-	-
2	<i>ballum</i>	3 and 5	28	-	-
3	<i>hardjo</i>	-	-	-	-
4	<i>balcanica</i>	-	-	-	-
5	<i>tarassovi</i>	-	-	-	-

Table 4

SEROLOGICAL RESPONSE OF THE CATS DURING THE PERIOD OF EXPERIMENT

Cat Reference Number	Serovar Inoculated	Micro-Agglutination titres days post infection									
		3	5	8	11	14	17	21	24	28	31
1	<i>pomona</i>	-	1/96 *(1/12)	1/768 (1/192)	1/768 (1/96)	1/768 (1/24)	1/384 (1/24)	1/384 (1/24)	1/768	1/768	1/192
2	<i>ballum</i>	-	1/96	1/384	1/384	1/384	1/192	1/96	1/192	1/768	1/96
3	<i>hardjo</i>	1/24	1/384	1/192	1/192	1/192	1/192	1/96	1/96	1/96	1/96
4	<i>balcanica</i>	-	-	-	-	-	-	-	-	-	-
5	<i>tarassovi</i>	-	1/12	1/96	1/192	1/96	1/192	1/96 **(1/12)	1/96 (1/12)	1/48	1/48

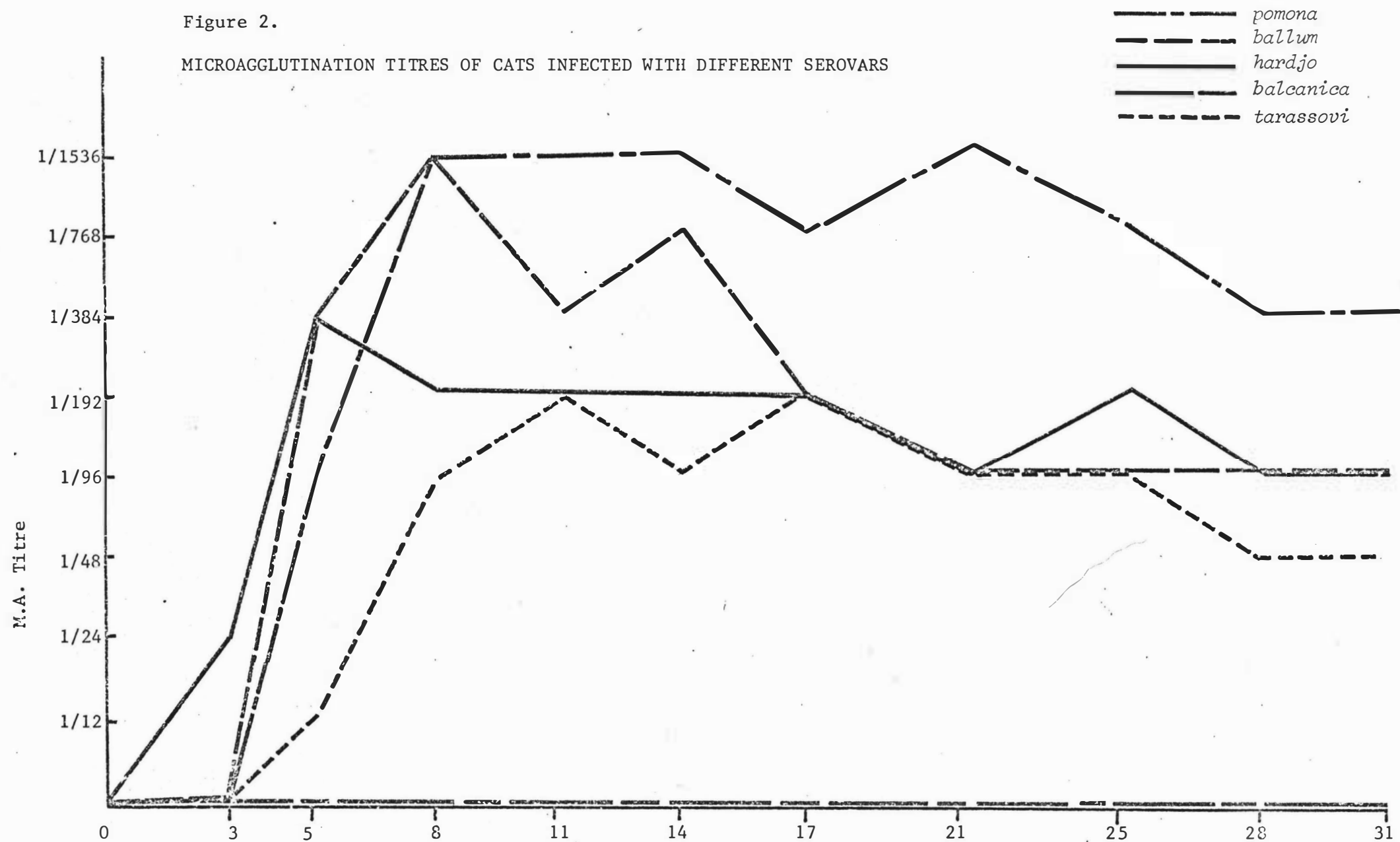
* Figures in parenthesis indicate cross-agglutination titres to *copenhageni*.

** Figures in parenthesis indicate cross-agglutination titres to *pomona*.

Note : All the cats showed no agglutination titres pre-infection to all serovars mentioned plus *copenhageni*.

Figure 2.

MICROAGGLUTINATION TITRES OF CATS INFECTED WITH DIFFERENT SEROVARS



Necropsy findings

At necropsy the cats appeared normal. There was no evidence of gross lesions in any of them.

Histological Findings

Liver

Small accumulations of inflammatory cells were seen in all five cats (Fig. 3). These changes were most severe in cat No.1 (*pomona*) where a mild diffuse neutrophil accumulation was seen around the portal area. The other cats showed mainly portal accumulation of small numbers of lymphocytes and plasma cells. Mild fatty change was seen in two of the cats (No.1 and No.5).

Kidney and Urinary Bladder

A subacute focal interstitial nephritis was seen in the cat inoculated with *pomona* (cat No.1)(Fig.4). This animal also had a small area of epithelial hyperplasia in the urinary bladder. Apart from occasional calcified casts in both the proximal and distal tubules of cat No.5, no significant changes were observed in the remaining animals. No leptospire were observed in the kidney and liver sections by silver stain.

Lung

The lung of cat No.3 was oedematous and congested with an increased number of macrophages in the interstitial tissue together with focal accumulation of lymphocytes and neutrophils, suggestive of a low grade pneumonia. There was also a bronchiolitis with necrotic leucocytes in the bronchioles and a small number of plasma cells around them.

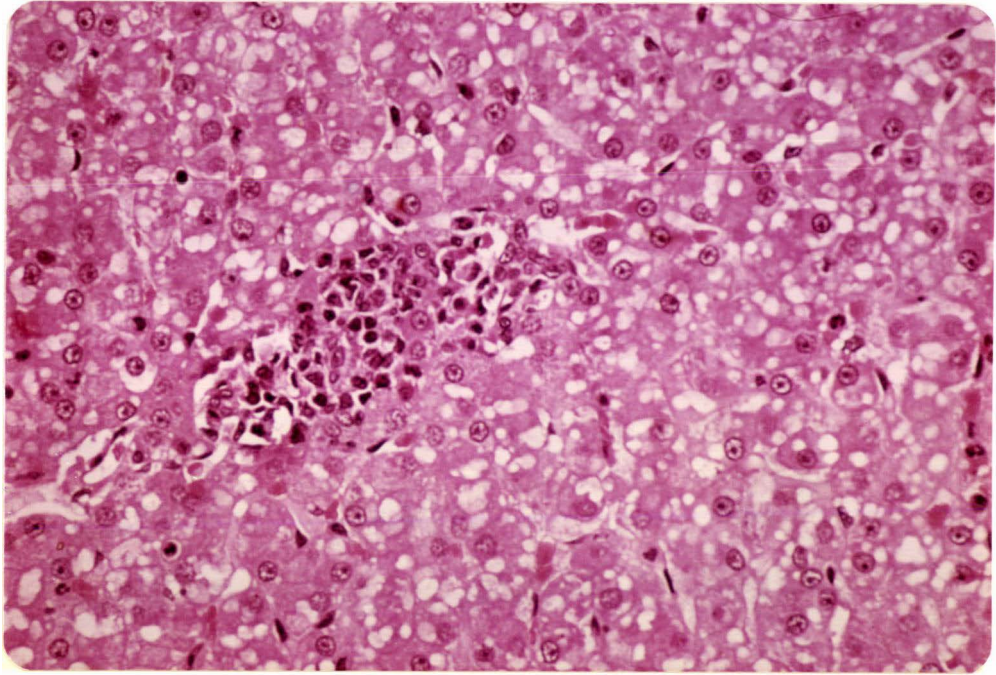


Figure 3. Cat 5 : Intraperitoneal *tarassovi* infection.
Small accumulations of mononuclear cells
around portal area in the liver. (HE X 250).

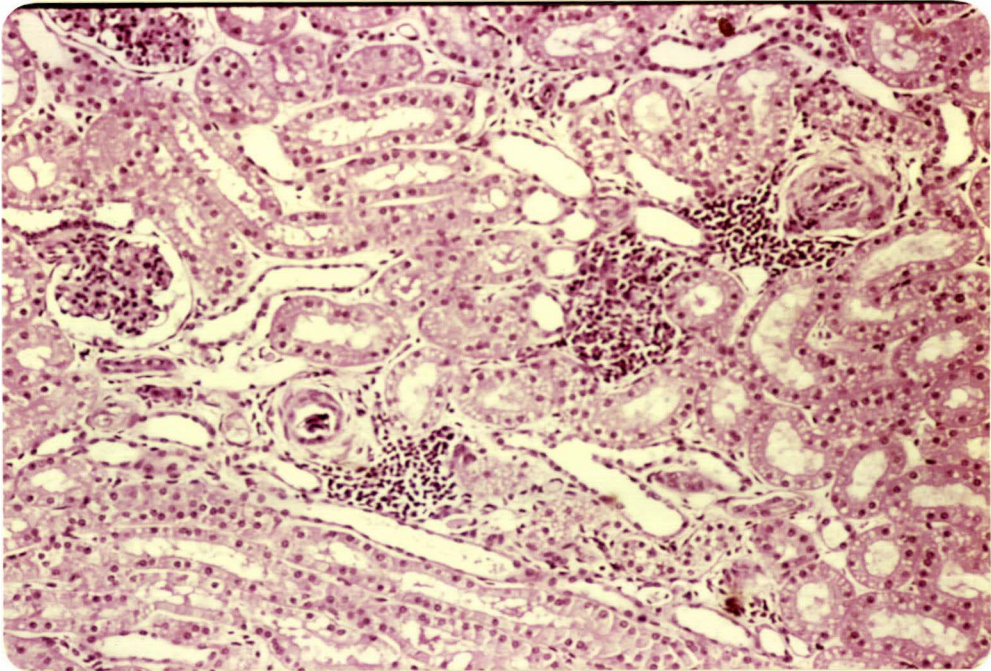


Figure 4. Cat 1 : Intraperitoneal *pomona* infection.
Subacute foci of interstitial nephritis.
(HE X 250).

Eye

Histological examination of eye tissue in cat No.1 revealed an acute necrotising conjunctivitis with infiltration of polymorphs and deposition of fibrin in the lymphoid follicles.

DISCUSSION

In discussing the results of this preliminary experiment, two important reservations must be considered:-

1. The value of infectivity studies based on the use of only one animal are obviously of extremely limited value and negative results must be treated with extreme caution.
2. The cats which were used in this experiment were not specific pathogen free (SPF) and their previous disease status was unknown. Therefore it is very difficult to interpret some of the histological findings.

Except for the cat which was inoculated with *balcanica*, all the cats responded serologically (Table 4). This response could be due to either :-

1. Infection which was not confirmed by blood or urine cultures owing to the high contamination rate.
2. Purely antigenic stimulation without infectivity.

The individual peak titres appeared at different times (Table II and graph 1) which may be explained by firstly, the differing ages of the cats involved and secondly, perhaps more importantly, by the different serovars used in inoculation. In the *pomona* infected cat, serological titres were higher than the others. Both *pomona* and *ballum* definitely caused infection. Positive blood cultures in the cat inoculated with *pomona*, and positive blood urine cultures in the cat inoculated with *ballum* prove that these cats were infected, although there were no clinical symptoms. Other researchers have also produced symptomless infections in cats with *pomona* (Fessler and Morter, 1964; Ferris and Andrews, 1965; and Modric, 1974).

It is possible that the leukocytosis observed in this experiment was due to infection however, physiological leukocytosis in which lymphocytes often equal or even exceed neutrophils is common in frightened or stressed young cats (Schalm *et al.*, 1975). In this experiment leukocytosis occurred on different days and in different degrees (Table II) and there was not an associated lymphocytosis. Therefore it was probably not related to excitement and stress. The increased production of leucocytes by an acceleration of leukopoiesis in the dog occurs only after a delay of three or four days (Schalm *et al.*, 1975). Assuming that dogs and cats have relatively similar responses, leukocytosis in these cats could have been related to active infection. In human leptospirosis, although the proportion of neutrophils often is in the range of 75-90%, the white blood cell count may be within the normal limits or below 5000/cu.mm. (Turner, 1969).

The only cat which exhibited pathological signs of disease likely to be related to leptospiral infection was the *pomona* infected cat. The subacute focal interstitial nephritis and histological changes in the

liver were similar to those described by Fessler and Morter (1964) although their cats were infected subcutaneously (s.c.). In this experiment leptospiraemia was recorded at an earlier stage (three days p.i.) which might be associated with an I.P. route of infection.

Lack of further recovery of leptospires in this study was probably due to contamination of the cultures. The common bacterial contaminants of cultures are known to prevent the growth of leptospires (Turner, 1970; and Ris, 1974). Fessler and Morter (1964) reported the isolation of leptospires from the urine of only two of their six experimentally infected adult cats, whereas the organisms were isolated from the kidney of all the six cats 12 to 61 days p.i.

Of the five serotypes tested in this experiment, *pomona* was shown to be a definite pathogen. The pathogenicity of *pomona* for cats is well documented (Fessler and Morter, 1964; Ferris and Andrews, 1965; and Modric, 1974). Fessler and Morter (1964) have also demonstrated the susceptibility of cats to experimental infection with *ballum*.

The lack of coat sheen which was observed in cat No.1 has not been reported by other researchers. It was more obvious in this cat than the others because of the black colour of the cat's coat. The only reference found which mentions any change in the condition of the cat's coat is by Modric (1974) who reported bristling of the hair in his experimental cats which were infected with different serotypes including *pomona*. According to him the hair bristling lasted for one day. The lack of sheen observed in the present case may have possibly been due to the regular use of Rompun. This lack of sheen would be less obvious in the cats inoculated with the other serovars because of their paler coat colours.

Body temperature is not a reliable indicator in leptospiral infections as the disease is often subacute and symptomless in cats. Stress will often cause a rise in temperature. Even normal cats which are not used to being handled by strangers may experience a rise in temperature greater than one degree celsius. It is therefore difficult to interpret the slight temperature changes which were recorded in the experimental cats. The rectal temperatures were within accepted normal ranges in experimentally infected cats with *pomona* in the studies by Fessler and Mörter (1964) and Ferris and Andrews (1965), whereas Modric's (1974) experimental kittens had elevated temperatures 1-2°C with *pomona*, 0.5-1.0°C with *icterohaemorrhagiae* and 0.5°C with *sejroe* recorded on the third and fourth day p.i.

Ocular and respiratory symptoms and related histological changes of the lungs were present in each experimental cat to a differing degree. As previously mentioned these cats were not SPF and the colony from which these cats were obtained had a record of respiratory disease.

The calcified casts which were seen in the kidney sections of one cat appeared too long standing to be related to the infection.

The findings obtained in this experiment indicate that *ballum*, *hardjo* and *tarassovi* are of low pathogenicity in the individual animals inoculated. Further experiments are needed to confirm whether the cat is susceptible to infection with *balcanica*.

However, a point well demonstrated by Fessler and Mörter (1964) is that individual variation related to the susceptibility of cats to different serovars is very important.

CHAPTER 3

INFECTIVITY STUDIES WITH *BALLUM*

INTRODUCTION

Leptospira interrogans serovar *ballum* was isolated for the first time from the urine of a mouse (*Mus musculus spicilegus*) by Borg-Peterson in 1943 near Ballum in Denmark. It has been found in other countries since then, chiefly in Europe.

The first human infected by this serovar was a laboratory worker in Amsterdam who handled a laboratory mouse. The mouse had superficially scratched his finger without making a visible wound. The illness developed nine days later producing a high fever and severe headaches. There were joint pains, mild conjunctivitis, exacerbation of labial herpes and severe dizziness. The fever subsided after five days whereas relief from the headache occurred only gradually and extended through the period of convalescence which took about three weeks. Further investigation revealed that practically all mice from this colony were excreting *ballum* in their urine (Wolff *et al.*, 1949).

So far *ballum* has been found in at least 29 mammalian species from all continents of the world (Anon, 1966).

In New Zealand *ballum* was first isolated as a human infection in Northland (1967). Later in 1973 it was isolated from healthy symptomless calves from the Hauraki Plains (Ris *et al.*, 1973) and Brockie and Till (1977) isolated it from five healthy hedgehogs (*Erinaceus europaeus*) out of the 76 caught on North Island dairy farms. Serological evidence showed a large portion (56%) of these hedgehogs had had contact with this serovar. They considered this animal was a major reservoir for *ballum*.

Brockie (1977) carried out a cultural and serological survey of *ballum* in rats and mice. He found ten (12.7%) of the Norway rats (*Rattus norvegicus*), nine (25%) of the shiprats (*Rattus rattus*), and ten (14.9%) of the mice (*Mus musculus*) tested were renal carriers or had suffered a recent infection with this serotype, although six of the thirteen infected Norway rats, three of four shiprats and eight of nine actively infected mice were seronegative (an original titre of $<1:100$ was used). Our laboratory serological test using an initial dilution of $1/12$ provides evidence that *Ballum* serogroup leptospires have low antigenicity and combined cultural and serological techniques are very important in surveying rodent populations for leptospirosis (Hathaway, personal communication). Birnbaum *et al.* (1972) discussed the effect of maternal antibodies in relation to establishment of the carrier state in mice and rats. The female mice with high titres of antibodies would transfer immunity to the young thus making them insusceptible to the disease for a relatively long period of their life. The transplacental or colostral transfer of antibodies confers immunity for several weeks. Mice which were infected at a very young age serve as a potential hazard in the maintenance of the disease in nature. When young mice are infected with low doses of leptospire they become carriers, but because of low immunocompetency their antibody titre soon drops thus they are unable to transfer any passive protection to their offspring.

Female mice infected at a later stage of life, although becoming carriers, are unable to transfer the disease to their offspring, because they have high titres of at least $1:10^4$ which would be transferred to the newborn as passive protection.

Hathaway (1978) found a high prevalence of infection with *ballum* amongst rat and mice populations in the North Island. He isolated *ballum*

from 27% of Norway rats, 33% of shiprats, from two of twelve housemice (*Mus musculus*) and two of fourteen hedgehogs. He considered the shiprat, the housemouse and hedgehog to be maintenance hosts for organisms of the *Ballum* serogroup. In Norway rats, he found the prevalence was directly related to population density. Although the percentage of leptospiral isolation was high, the prevalence of leptospiral titres in rats was very low (3% in Norway rats and 24% in shiprats). These results again emphasise the importance of combined serological and cultural studies.

Leptospirosis is a worldwide disease affecting people who come into contact with animals as part of their occupation as well as people who are engaged in outdoor field activities (Torten, 1970). It can be transmitted from many different animal carriers to humans (Anon, 1966).

However, because of the high percentage of rodents and hedgehogs infected with *ballum* in New Zealand and their close relationship with cats on the one hand and the close contact of cats with humans on the other hand, it is important to investigate the epidemiology of *ballum* infection in cats.

There have been few studies in New Zealand to determine dietary preferences of wild and semi-wild cats, however, it is well known that cats, even well fed domestic ones, will hunt wild rodents.

Marshall (1961) studied the habits of feral cats on Little Barrier Island in New Zealand. He found evidence of predation of the Maori Rat (*Rattus exulans*) in 73% of 44 cats droppings in winter, but in only 10% of 50 cats droppings in summer.

Daniel (1972) in studying the bionomics of shiprats (*Rattus rattus*) in New Zealand found out that feral cats are a significant predator and the major cause of mortality of shiprats in indigenous forest.

Collins (1978, personal communication) in a survey on feral cats found that they are widespread in New Zealand and that they breed in all types of terrain. In fact there are likely to be few rural areas without a resident population. Feral cat populations are formed in many very remote areas where it might be expected that escaped or abandoned cats would not occur. These cats feed on a wide variety of foods including the carcasses of domesticated and wild animals.

The climate and vegetation of the New Zealand countryside makes it very easy for the rodent and feral cat populations to survive and increase. For this reason any disease which is associated with these groups of animals are of great importance, especially if there is a possibility that these infections may spill over into the domestic animal or human populations.

As *ballum* is widespread in wildlife populations, especially rats, mice and hedgehogs, it was chosen for further research. The second experiment was undertaken to observe the clinical signs, leptospiral carrier state, immunological response, and the gross and histopathological changes in cats infected with different doses of *ballum*. Attempts were made to determine the infective dose of this organism for cats and the smallest dose which is capable of establishing the urinary carrier state in the cat. Any relationship between infective dose and serological and bacteriological response would also be of special interest. The oral route was chosen because it is considered to be the most likely natural route of infection. Parallel studies using the I.P. route of infection were carried out in order to compare these two routes of infection and to provide a known positive control.

EXPERIMENT TWO

DEFINITION OF INFECTIVE DOSE AND ITS RELATION TO CARRIER STATE

MATERIALS AND METHODS

Sixteen crossbred cats from domestic sources were used, 14 of which were four months old and the other two, 10 weeks old. These were kept and fed in the same manner as described in Experiment one.

The cats were divided into four groups of three, and two groups of two as shown in Table 5. Each cat was checked for the presence of agglutinins against *ballum* prior to infection.

The cats were infected with a seven day culture of *ballum* which originally was isolated from a Norway rat captured at the Longburn rubbish tip by Hathaway (1978). It had subsequently been passaged through hamsters. The culture was counted using a Petroff-Hauser counting chamber*. The original culture was then diluted with Stuarts basal medium to obtain the required dilutions. The infective dose and route of infection used are shown in Table 5.

In order to verify the viability of the inoculum, on the same day as the cats were infected, one hamster was injected by I.P. route with 0.1 ml of the original culture. This animal died six days post-infection. Another hamster was inoculated with a dilution of the culture estimated as having no organisms in it. This animal survived.

* C. A. Hauser and Son, Philadelphia, U.S.A.

Table 5

EXPERIMENT TWO: DETAILS OF EXPERIMENTAL ANIMALS,
DOSE AND ROUTE OF INFECTION

Group Number	No. of cats involved	Cat's Reference Number	Sex	Age	Infective dose of <i>ballum</i> *	Route of Infection
1	3	1	F	4 months	10^8 lep/ml	Orally
		2	M	4 months		
		3	F	4 months		
2	3	4	F	4 months	10^6 lep/ml	Orally
		5	M	4 months		
		6	M	4 months		
3	3	7	F	4 months	10^4 lep/ml	Orally
		8	M	4 months		
		9	M	4 months		
4	3	10	F	4 months	10^2 lep/ml	Orally
		11	M	4 months		
		12	M	4 months		
5	2	15	M	10 weeks	10^8 lep/ml	I/P
		16	F	10 weeks		
6	2	13	M	4 months	Control	-
		14	F	4 months		

* Each cat had 1 ml of the infective dose.

The temperatures of the cats were recorded daily and urine was collected from one or two cats in each group. The cats from which urine was collected were alternated each day. Their blood was collected for the M.A.T. at the termination of the experiment. The lowest dilution employed for the M.A.T. was 1/12.

Two weeks p.i. one cat from each group was killed by I.P. injection of Euthesate. Urine, aqueous humcr and suspensions of kidney, liver, brain and salivary gland were cultured by the same methods described in Experiment One.

The same procedure was used three and four weeks p.i. with the remaining cats. In the control and the I.P. infected groups, one cat was destroyed at a fortnight and another one a month after the beginning of the experiment.

RESULTS

Clinical findings

The temperature of both the cats which were infected by I.P. route within five days p.i. were higher than normal (40.5°C) but gradually returned to a normal temperature of 38.8°C . Cat No.3, which was infected by the oral route with the same number of leptospire (10⁸) as the I.P. infected group, also had a rise in temperature on the second and third day post infection.

One cat (No.6) from the second group showed a definite elevation in temperature nine days p.i. Three days p.i. this cat appeared depressed and the sclera of the eyes were jaundiced. There were no signs of icterus in the other cats and this cat recovered three to four days later.

No observable changes in behaviour or appetite were noted in any of the cats and they appeared normal throughout the experiment which lasted for a month. The only significant change was a slight absence of sheen on the fur which was not observed in the control cats.

In fresh voided urine from both of the I.P. infected cats, leptospire were seen by direct darkfield examination as early as seven days p.i. No leptospire were seen in the urine from the orally infected cats.

Cultural findings

The cultural findings are summarised in Table 6. Leptospire were isolated from the urine of two of the cats in group one, seven days p.i. Positive urine cultures were also obtained from two of the cats in group two and one of the cats in group three. Positive urine cultures were obtained from both the I.P. infected cats as early as three days p.i. The orally infected cat yielding a positive culture at necropsy was cat No.1 from group one (10^8 leptospire) in which organisms were recovered from kidney and retropharyngeal lymph node. However, both cats in the I.P. infected group yielded positive cultures from the kidney two and four weeks p.i. respectively. No positive cultures were obtained from any of the cats in group four or the control group. In this experiment the number of contaminated cultures was reduced to approximately 1-2 per cent.

Table 6

EXPERIMENT TWO: CULTURAL AND SEROLOGICAL FINDINGS AFTER INFECTION WITH *BALLUM*

Group Number	Cat Number	Infection route	Infective dose lep/ml*	Post infection day of isolation			Kidney	Other tissues	Time of euthanasia post-infection	Serological response
				Urine	Urine	Urine				
1	1	oral	10^8	7			+	retropharyngeal lymph node	2 weeks	1/768
	2	oral	10^8	7			-	-	3 weeks	1/96
	3	oral	10^8	-			-	-	4 weeks	1/384
2	4	oral	10^6	7			-	-	2 weeks	neg.
	5	oral	10^6	-					4 weeks	1/12
	6	oral	10^6	7	10	16	-	-	3 weeks	neg.
3	7	oral	10^4	-			-	-	2 weeks	neg.
	8	oral	10^4	7	20		-	-	3 weeks	neg.
	9	oral	10^4	-			-	-	4 weeks	neg.
4	10	oral	10^2	-			-	-	4 weeks	neg.
	11	oral	10^2	-			-	-	2 weeks	1/12
	12	oral	10^2	-			-	-	3 weeks	neg.
5	15	I.P.	10^8	3 days until P.M.			+	-	4 weeks	1/384
	16	I.P.	10^8	"			+	-	2 weeks	1/96
6	13	control							2 weeks	neg.
	14	control							4 weeks	neg.

* All the cats were infected with 1 ml

Serological titres

All the cats in groups one and five responded serologically (Table 6). In group two and four only one cat from each group had a positive titre. No positive titres were found in group three (infected with 10^4 orgs/ml) or group six (control). The M.A. titres in group one (orally infected cats) were higher than group five (I.P. infected cats), although both had the same infective doses. Three of the cats which had leptospiruria had no serological titres (Table 6).

Histological findings

The histological findings have been summarised in Table 7. In all groups the histological changes in the kidney and liver sections were most pronounced in the cats necropsied at two weeks p.i. The most severe kidney lesions were seen in the I.P. infected group. Cat 16 had several very diffuse foci of infiltrating lymphocytes, plasma cells and macrophages around the tubules in both the cortex and medulla (Fig.5). Liver sections from this cat had small foci of lymphocytic infiltration around the portal areas. In the orally infected group two cats showed focal interstitial kidney lesions, one of which (cat No.11) had the largest focal kidney lesion seen in this experiment (Fig.6). This cat also had liver lesions similar to those seen in the I.P. infected group. Cat No.2 had two foci of granulation tissue in the liver but these were not related to any particular part of the organ. Leptospores were demonstrated within the kidney tubules of one of the intraperitoneally infected cats (Cat No.15) using the Warthin-Starry staining technique (Fig.7).

Apart from the liver and kidney lesions the other tissues examined did not show any significant changes which could be related to the experimental infection.

Table 7

EXPERIMENT TWO : *BALLUM* INFECTION: SUMMARY OF HISTOLOGICAL FINDINGS

Group Number	Cat No.	Route of Infection	Infective dose lep/ml	Time of euthanasia week p.i.	Kidney			Liver
					Interstitial foci of cellular infiltration	Cloudy swelling of tubular cells	Focal fatty change of tubular cells	Scattered lymphocytes, plasma cells and neutrophils
1	1	oral	10^8	2	++	+	+	+
	2	oral	10^8	3	-	-	-	+
	3	oral	10^8	4	-	-	-	+
2	4	oral	10^6	2	-	+	+	+
	5	oral	10^6	4	-	-	-	+
	6	oral	10^6	3	-	-	-	+
3	7	oral	10^4	2	-	+	-	+
	8	oral	10^4	3	-	-	+	±
	9	oral	10^4	4	-	-	-	+
4	10	oral	10^2	4	-	-	-	±
	11	oral	10^2	2	+	+	-	±
	12	oral	10^2	3	-	-	-	±
5	15	I.P.	10^8	4	++	-	-	+
	16	I.P.	10^8	2	++++	-	-	+
6	13	control	-	2	-	-	-	-
	14	control	-	4	-	-	-	-

- No significant change
++ Moderate

± Very mild
+++ Severe

+ Mild
++++ Very severe

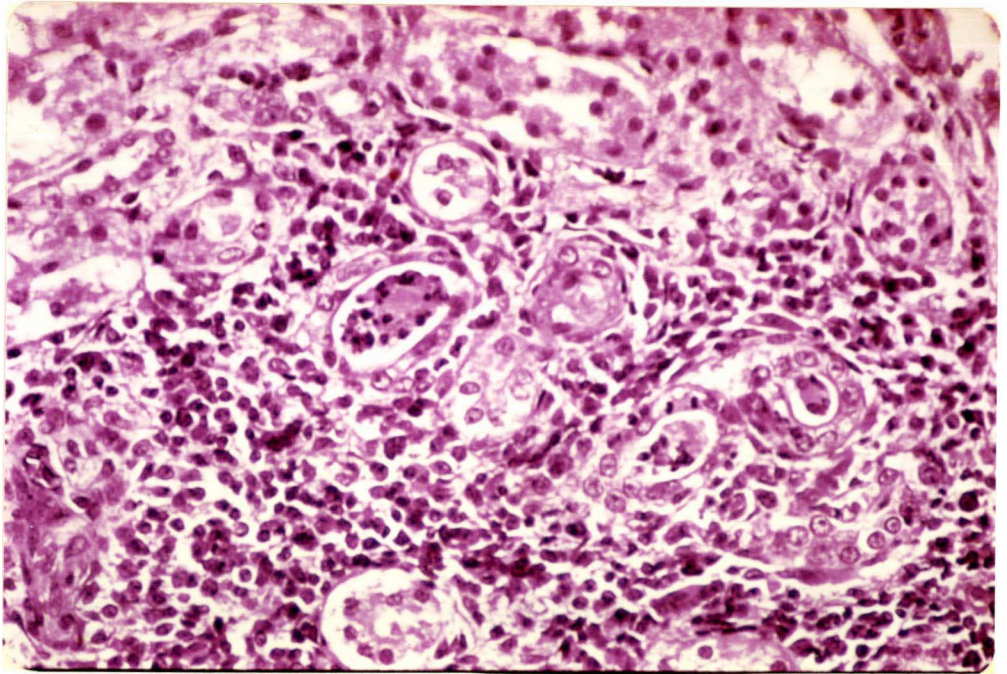


Figure 5. Intraperitoneal *ballum* infection, 10^8 leptospires (cat 16), two weeks post-infection. A large focus of infiltrating lymphocytes, plasma cells and macrophages around cortical tubules in the kidney. Several tubules contain necrotic leucocytes. (HE X 250).

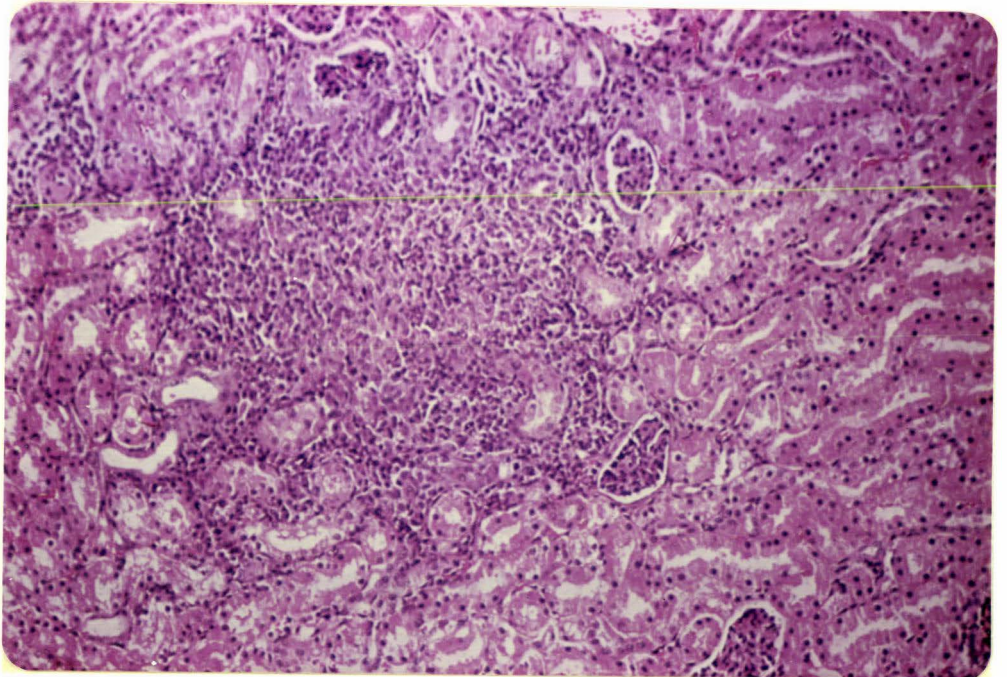


Figure 6. Oral *ballum* infection, 10^2 leptospires (cat 11) two weeks post infection. One of the largest foci of interstitial nephritis seen in this experiment. (HE X 125).

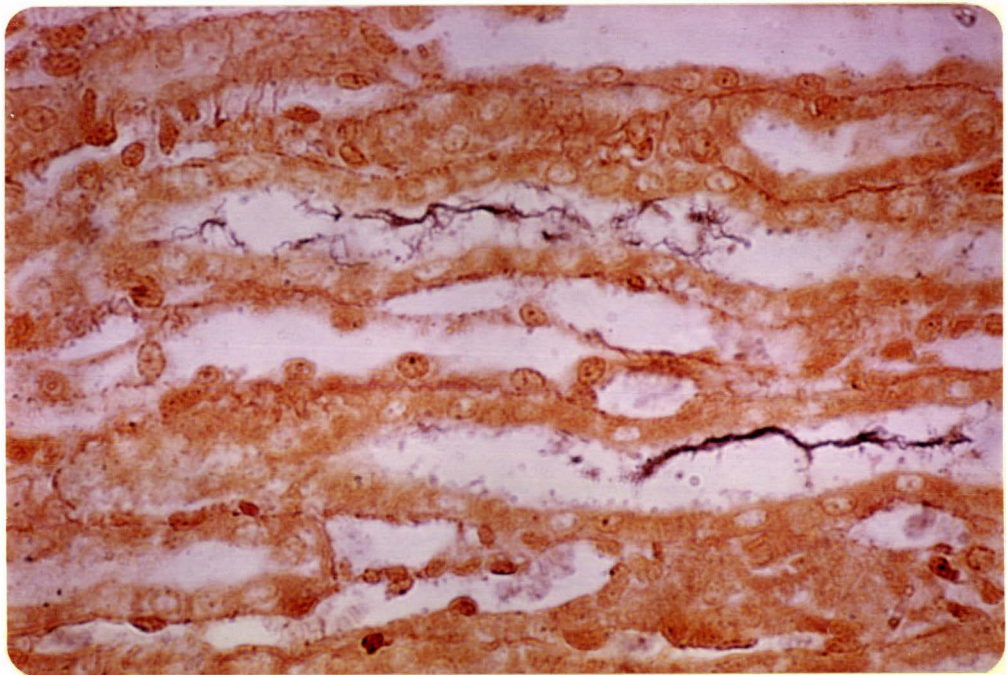


Figure 7. Intraperitoneal *ballum* infection, 10^8 leptospires (cat 15) one month post infection. Leptospiral organisms in the lumen of tubules of the kidney cortex. Warthin Starry X 900.

DISCUSSION

This experiment confirmed that leptospira of *Ballum* serogroup is capable of producing a carrier state in cats infected with more than 10^2 organisms. No clinical evidence of disease resulted from infection by either the oral or intraperitoneal route.

The results of the serological tests indicated that there was a decrease in agglutination titre with the lowering of the infective dose. However, an equal serological response was obtained with two different doses (10^2 and 10^6) thus emphasising the importance of individual variation in the immune response. In spite of the fact that the same infective dose was used, a higher serological response was obtained two weeks p.i. with orally infected cats (1/768) compared to the response obtained with the I.P. route (1/96). This might be due to firstly, the younger ages of the I.P. infected cats as it is known that younger animals are usually less serologically responsive than adults (Herbert, 1974); secondly, with the I.P. route of infection the serological response may begin earlier and decline more rapidly. Since the M.A. titre of each cat was only measured at the time of euthanasia it is not possible to reach a firm conclusion. However, a month after infection the serological response with both routes was the same (1/384).

The fact that three of the cats failed to produce a serological response even though they were shedding leptospores in their urine, demonstrates the importance of using both urine culture and serological technique to identify infected animals.

Another notable result of this study was the duration of the carrier state which appeared to be shorter in the orally infected cats than in those infected by the I.P. route. Leptospiruria started earlier in the cats infected by the I.P. route (3 days p.i.) and persisted until

the end of the experiment. The shedding of the leptospire was both heavier and more consistent so that after seven days p.i. the leptospire could be seen in direct urine examination by darkfield microscopy.

The lack of sheen on the coat observed in all of the infected cats in this experiment supports the hypothesis suggested in Experiment one that this was due to the effect of the regular use of Rompun for anaesthesia.

Jaundice was seen in only one cat (Cat No.6, infected with 10^6 orgs/orally) and cannot be clearly explained. However, jaundice with *Ballum* serogroup has been produced in hamsters (Frenkel, 1972; and Thompson, 1978).

With the I.P. route of inoculation, the histological changes observed were more severe than with oral exposure. The inflammatory response in cats infected by both routes and at all infective doses was greatest at two weeks post infection. Those cats necropsied one month p.i. showed comparatively less obvious histopathological changes, however, it cannot definitely be concluded that the tissue damage which occurred was transient and disappeared within this time or whether the variation seen was just due to individual response. In this regard it is worth noting that the foci of interstitial nephritis were small and limited in distribution, so some lesions could have been missed in the tissue sections selected.

This study agrees with the findings of Lucke (1968) who showed that chronic interstitial nephritis in cats which resembles that seen in dogs, is not caused by leptospiral organisms.

EXPERIMENT THREE

STUDIES ON THE DURATION OF THE CARRIER STATE IN CATS INFECTED BY THE ORAL ROUTE

This experiment was carried out to investigate the duration of leptospiraemia and to determine the length of time the cat can remain a carrier of serovar *ballum*. The experiment also included an examination of the serological changes which occur in the cat following infection.

MATERIALS AND METHODS

Five crossbred six month old cats were divided into two groups of two cats each (one male and one female) with one male cat remaining as a control. A month prior to infection all the cats were treated with piperazine. Serum samples were collected one week before infection and checked for leptospiral agglutinins. Infection was induced by the oral route. Two of the cats were infected with 10^8 leptospire (cats No.1 and No.2) and the other two with 10^4 organisms (cats No.3 and 4). These two infective doses were chosen after consideration of the results obtained in previous inoculation experiments (Chapter 3). The culture used was a seven day old culture of *ballum* from the same origin as the previous experiment, standardised by counting in a Petroff Hauser counting chamber.

Alternate cats from each group were bled daily until two weeks p.i. and the blood obtained was used for both serology and leptospiral culture. After two weeks the cats were bled weekly until one month p.i. and following this period blood samples were collected every fortnight for M.A.T. Gel-filtration tests were performed on all serum samples positive to the

M.A.T. using Sephadex G-200 (Pharmacia*) columns (Appendix III). The accuracy of the IgM and IgG fractions obtained by the gel-filtration technique was confirmed by immunoelectrophoresis (Appendix V). Growth inhibition tests (GIT) using the modified method of Tripathy *et al.* (1973) (Appendix IV) were performed on the last serum sample obtained at the time of euthanasia (19 weeks p.i.) from the two cats which produced a serological response. Blood samples for culture were obtained from the jugular vein but when a larger amount was required cardiac puncture was used. Urine was collected at the same time as the blood samples until two weeks p.i. after which it was collected twice weekly.

The study was carried out for 132 days. After this period of time the cats were killed with Euthesate and necropsied. All the procedures for culturing the tissues and urine and for histological processing were the same as previously described. Because of the contamination problem experienced in the first experiment, a modified technique was used for culturing blood. One drop of blood was inoculated into each of two bottles of EMJH semi-solid media containing 400 mg 5Fu. In addition, 0.2 - 0.5 ml of blood was diluted into a 9 ml sterile Stuarts basal medium (SMB) and from this dilution 3-5 drops were inoculated into each of two bottles of EMJH semi-solid containing 400 mg 5Fu.

The method of urine collection routinely used in these experiments was manual pressure on the bladder as described in Experiment one. In order to compare the effect of this manipulation on the bladder with the technique used by most overseas workers, namely; aspiration from the bladder through the abdominal wall (bladder tap), the latter technique was performed three to four times on the control cat in the last week of the experiment.

* Pharmacia Fine Chemicals AB, Uppsala, Sweden.

RESULTS

Blood and urine culture

Only one of the four cats (No.2) showed a leptospiraemia (Table 8). It commenced at seven days p.i. and lasted for a period of five days. Leptospiruria also occurred in this cat starting at the 13th day p.i. and shedding of organisms continued regularly until 122 days p.i. Urine specimens from the cat were always negative when examined by direct darkfield examination. None of the other cats had positive blood or urine cultures.

Serology

Both the cats that were infected with 10^8 organisms responded serologically but one of these (No.1) responded earlier than the other (Figure 8a&b). The serological titres of serum samples from cat No.1 were consistently low and became negative at 12 weeks p.i. The first positive titre in cat No.2 was detected three weeks p.i. and this persisted until the end of the experiment. The cats which were infected with 10^4 leptospores did not produce a serological response.

The gel-filtration technique established that the antibody response began with an IgM response (Figure 8a&b) and antibodies remained in the IgM peak until the 8th week p.i. In cat No.2 IgM reached its highest peak at the fourth week p.i. and thereafter decreased until 12 weeks p.i. In this cat IgG was detected from the 8th week p.i. with a IgG:IgM ratio of 3.5:1. At the 12th week p.i. the IgG:IgM ratio was 5:1 and at the end of the experiment (19th week p.i.) all the response was in IgG peak. In cat No.1 the serological response remained in the IgM peak throughout the experiment. The findings of immuno-electrophoresis confirmed the accuracy of the gel-filtration test (Figure 9).

Table 8

CULTURAL FINDINGS
FOLLOWING ORAL *BALLUM* INOCULATION

Cat's Reference Number	Infective dose	Route of Infection	Lepto- spiraemia days p.i.	Lepto- spiruria days p.i.	Kidney and tissue culture
1	10^8	Oral	-	-	-
2	10^8	Oral	7-11	13-122	-
3	10^4	Oral	-	-	-
4	10^4	Oral	-	-	-
Control Cat	Nil	Nil	N.D.	-	-

- negative

N.D. not done

Figure 8a.

MICROAGGLUTINATION TITRE OF WHOLE SERUM (—) AND M.A. TITRE OF IgM FRACTIONS
OF CAT 1.

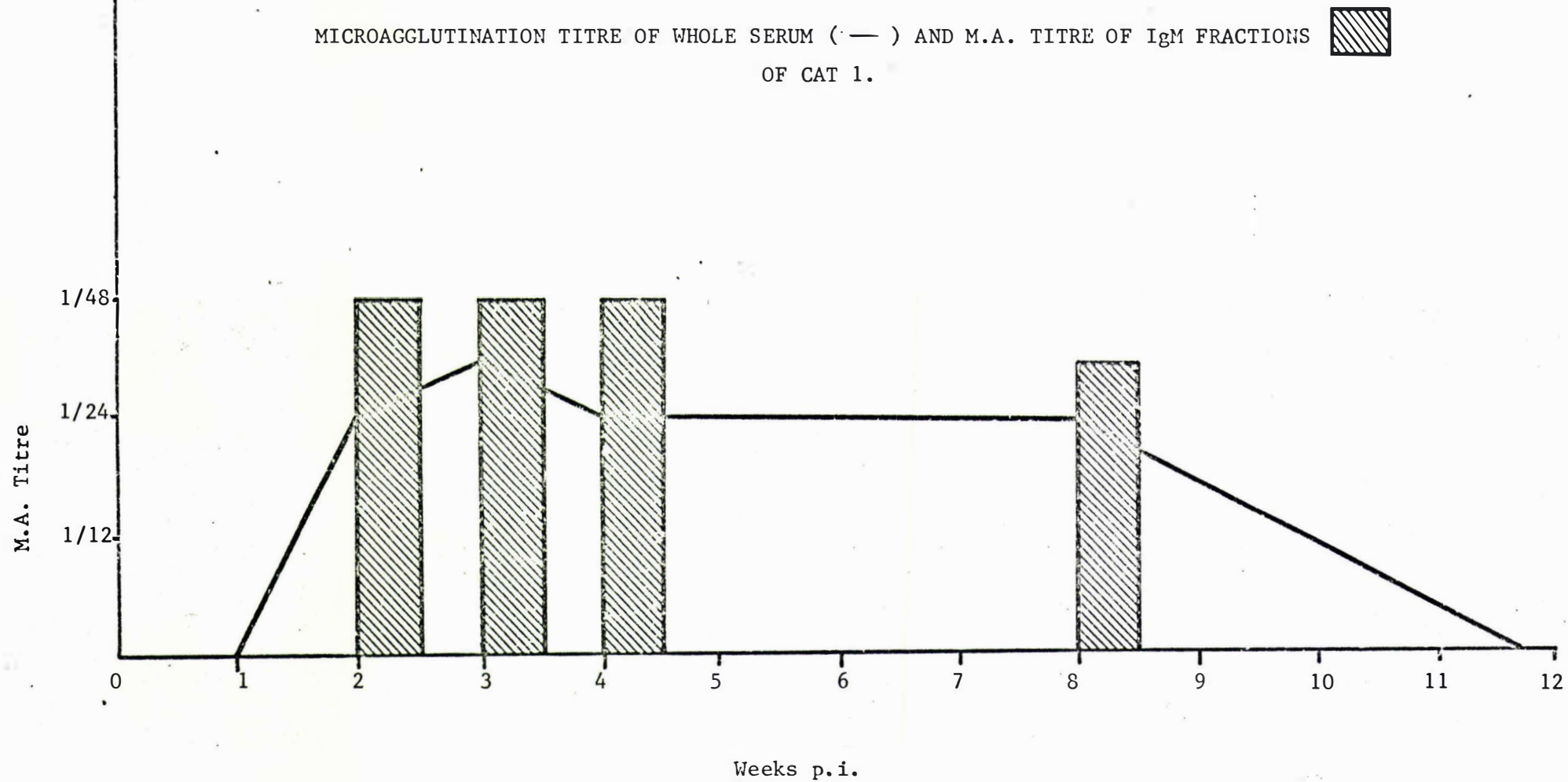


Figure 8b.

MICROAGGLUTINATION TITRE OF WHOLE SERUM (—) OF CAT NO.2 AND
IgM AND IgG FRACTION

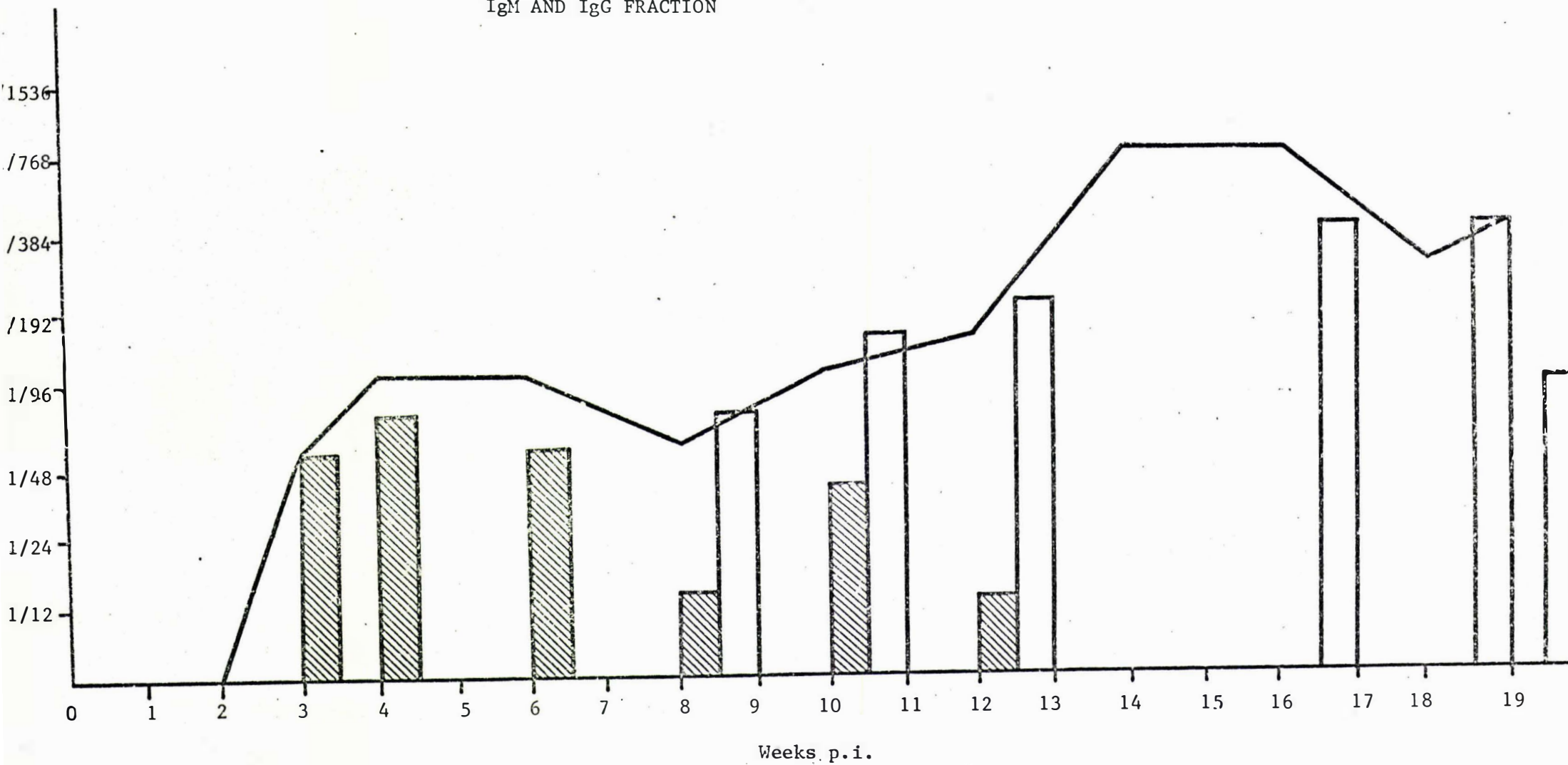
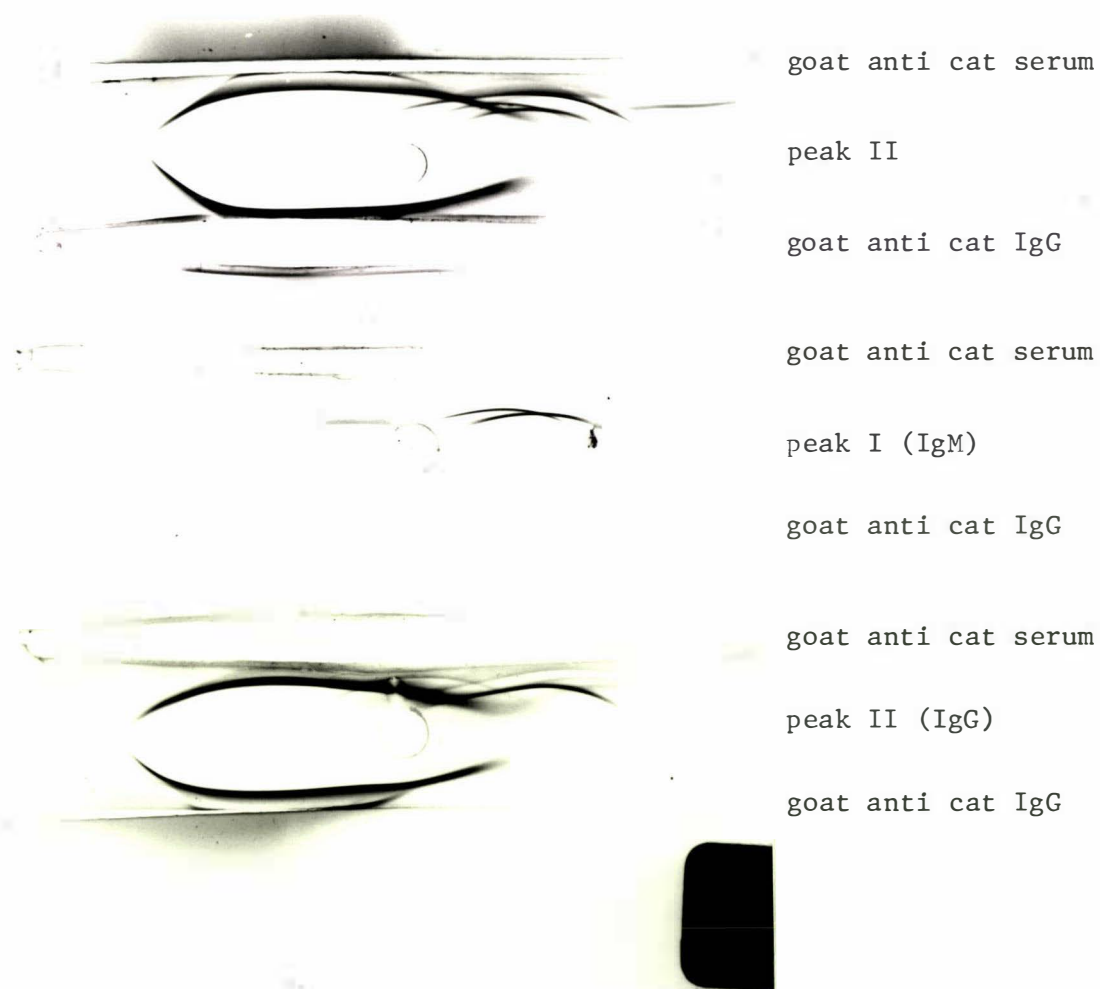


Figure 9.

Immuno-electrophoretic reaction of concentrated gel-filtration fractions of cat serum with goat anti cat serum and goat anti cat IgG.

Cathode

Anode



Cathode

Anode

The goat anti cat IgG was not specific for γ chains therefore, it cross reacted with IgM (peak I).

Growth inhibition tests (GIT) using sera obtained from cat No.2 inhibited the growth of leptospire completely whereas the serum from cat No.1 produced only partial clumping of the organisms without inhibiting their growth.

Necropsy findings

No gross lesions were found in any of the infected cats. The bladder of the control cat was moderately hyperaemic and diffuse haemorrhage was noted in the ventral wall around areas presumed to be sites of aspiration.

Histological findings

Despite long term shedding of leptospire cat No.2 showed no significant histological changes in the kidney.

The sections of bladder from the control cat showed congestion and oedema of the submucosa. There were also areas of diffuse haemorrhage containing occasional neutrophils in the lamina propria and a large focus of haemorrhage extended between smooth muscle bundles at one point in the bladder wall (Figure 10a).

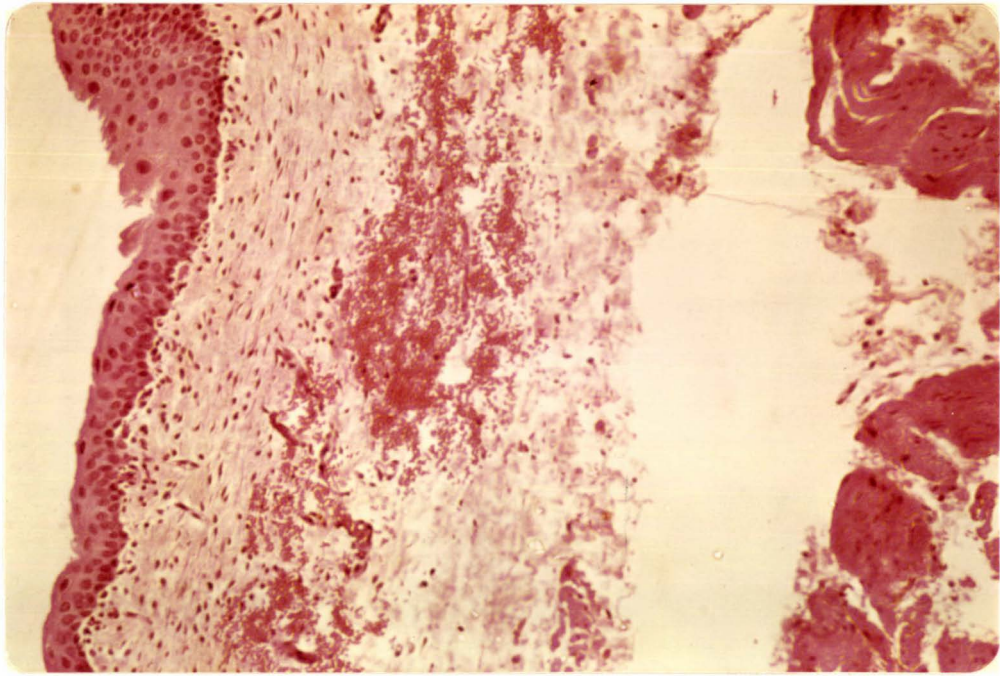


Figure 10a. Bladder from a cat following collection of urine by aspiration through the abdominal wall. Areas of diffuse haemorrhage and oedema are present in the submucosa. (HE X 125).

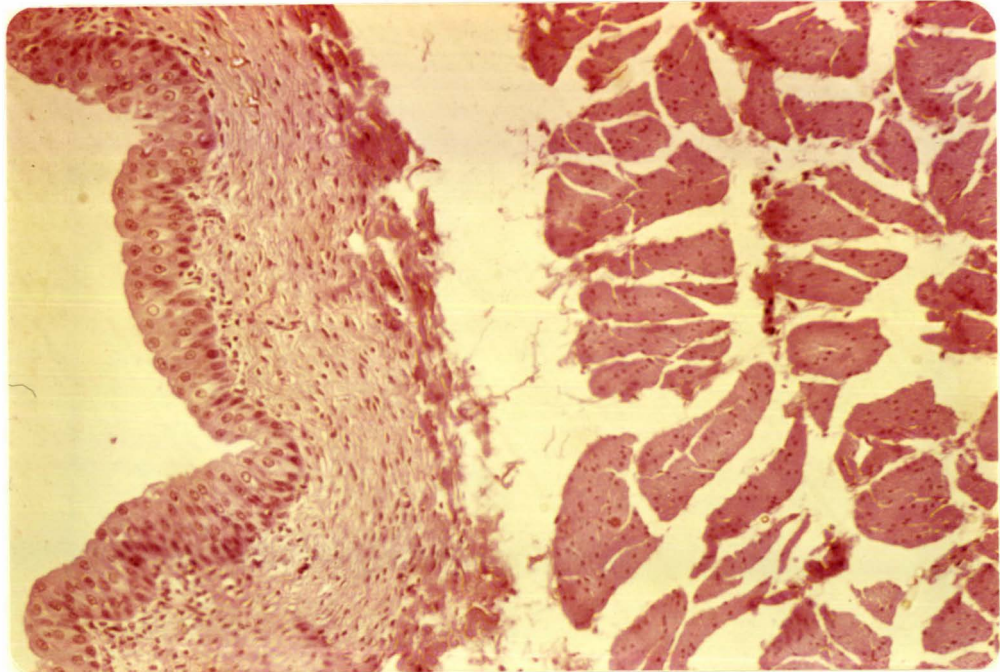


Figure 10b. Bladder of the cat No.2 following collection of urine by manual pressure throughout the 19 week period of the experiment. No histological abnormalities were observed. (HE X 125).

DISCUSSION

Oral infection of 10^8 leptospires successfully produced a carrier state in one of the two cats infected which lasted until 122 days p.i. Although several workers have previously produced carrier states experimentally in cats, different serovars and routes of infection have been used (Table 9). The infection with *ballum* in the present experiment has produced the longest carrier state as yet recorded in cats.

The cats which were infected with 10^4 leptospires did not respond serologically or yield positive cultures. This confirms the importance of the infective dose in producing an experimental infection as previously demonstrated in Experiment Two where only one of three cats became infected at this dose rate (10^4). One possible reason for failure of infection at this dose rate is the age of the experimental animal used and this has been discussed earlier in Experiment Two. Although the animals used in this experiment were six months old (two months older than those in the second experiment), their relatively young age cannot be discounted as a factor affecting their ability to be infected.

The serological results obtained in this experiment are rather difficult to interpret because two different patterns were obtained with the same infective dose. These results also emphasised the role of individual variation in response to infection (Hathaway, 1978; Hellstrom, 1978).

Fractionation of the sera using Sephadex G-200 demonstrated that both the cats responded to *ballum* infection initially with production of only IgM agglutinins. After the 8th week p.i. agglutinins of IgG class were identified in the serological response of cat No.2 with a decreasing IgM:IgG ratio and termination of the response with IgG. In one cat the serological response was 100% IgM.

Table 9

THE DURATION OF LEPTOSPIRURIA REPORTED
IN EXPERIMENTAL FELINE
LEPTOSPIROSIS

Author	Leptospira serovar	Route of Infection	Post-infection identification leptospire in urine
Wirth (1937)	<i>icterohaemorrhagiae</i>	*	3 weeks p.i.
Klarenbeek & Winsser (1938)	<i>canicola</i>	*	15-78 days p.i.
Otten <i>et al.</i> (1954)	<i>canicola</i> and <i>icterohaemorrhagiae</i>	*	⁺ 77 and 98 days p.i.
Fessler and Mörter (1964)	<i>pomona</i>	s.c.	**55 days p.i.
Ferris and Andrews (1965)	<i>pomona</i>	I.P. and conjunctivally	**45 days p.i.
Modric (1974)	<i>pomona</i>	I.P., s.c., i.m.	**14 and 34 days p.i.

* Not given

+ By Levaditi stain

** By culture

Hellstrom (1978) found that in cattle responding to natural *hardjo* infection the initial agglutinin was also only IgM, but later 90 to 100% of the agglutinins were in the IgG class. He also found that some low titred animals had a higher ratio of IgM:IgG than the higher titred animals.

In the rabbit, infection with different leptospiral serovars has also resulted in IgM as the initial agglutinin (Graves and Faine, 1970; Kadlcik *et al.*, 1973). Production of only IgM agglutinating antibody has been reported by Hocker and Bever (1965) in rabbits challenged repetitively over an 18 month period with serovar *biflexa*.

Since the cat in which a carrier state was established was also the only cat producing IgG it is tempting to postulate that the occurrence of a carrier state may be related to the production of IgG. However, it is difficult to draw firm conclusions in this experiment because of the limited number of cats used.

Roth *et al.* (1963) have described leptospiral infection with a specific serovar in a reservoir host as having the following characteristics:-

- 1) Rarity of clinical symptoms.
- 2) Long duration of leptospiruria with constant and heavy shedding of organisms.
- 3) Ratio of serological:bacteriological prevalence is approximately unity or below it.

They also state that a maintenance (reservoir) host for a particular serovar can only be catagorically defined as such by experimental infection. Roth *et al.* (1963) described the following definition for accidental (incidental) hosts:

- 1) They may or may not show symptoms following infection.
- 2) They usually become bacteriologically negative in a short time and as a result they are not effective shedders of leptospires.
- 3) They may remain serologically positive for several years.
- 4) They are not necessary for the continual existence of the disease.
- 5) They may be of minor epizootiological importance and serve as temporary transport hosts.

These criteria are mostly applicable to wild animals, but because of the cat's outdoor life and its relationship to other wildlife these criteria may be applied to all of the present experiments with *ballum*.

The ratio's of positive bacteriological and serological results to the numbers of animals infected are shown in Table 10. The combination of the results of Experiments Two and Three produced a mean bacteriological: serological ratio of 60% for an infective dose of 10^8 leptospires and 20% for 10^4 organisms. Combined analysis of the bacteriological and serological results, excluding the results of the infective doses less than 10^6 leptospires, reveals a 62.5% bacteriologic rate and 75% serologic rate for infected cats. These ratios according to the criteria of Roth *et al.* (1963) indicate that the cat could be a maintenance host for *ballum*. However, the field studies by Hathaway and the author of feral cats from areas with rodents having a high prevalence of *ballum* infection showed a very low prevalence of *ballum* titre in these cats. In fact only one of the eleven mostly mature cats examined by Hathaway (1978) had a titre (1:24) to *ballum* and no leptospiral isolates were obtained.

The results of the present investigation indicate that cats are potential carriers of leptospires of the *Ballum* serogroup, although the

Table 10

RATIO OF POSITIVE BACTERIOLOGICAL
AND SEROLOGICAL RESULTS.

Experiments two and three

Infective dose leps/orally	Bacteriological prevalence		Serological prevalence	
<u>Experiment 2</u>				
10 ⁸	2/3	66.6%	3/3	100%
10 ⁶	2/3	66.6%	1/3	33.3%
10 ⁴	1/3	33.3%	0/3	0
10 ²	0/3	0	1/3	33.3%
<u>Experiment 3</u>				
10 ⁸	1/2	50%	2/2	100%
10 ⁴	0/2	0	0/2	0

field studies do not support this view. This disagreement can be firstly due to the age of the experimental cats which were young, none of them being older than six months, while the feral cats were mostly mature. It has been reported by Chernuckcha *et al.* (1974) that young animals are more susceptible to the establishment of leptospiral infection than mature animals. Secondly, the longest duration of the carrier state was achieved in a cat receiving 10^8 organisms by the oral route. Challenge with this number of viable leptospores would be unlikely in the field.

To investigate the apparent disparity between the experimental results reported in this chapter and the results of field surveys, it is necessary to conduct more extensive experimental investigation in mature cats.

EXPERIMENT FOUR

COMPARATIVE STUDIES WITH INTRA-PERITONEAL AND ORAL ROUTES OF *BALLUM*

The recovery of leptospire from the retropharyngeal lymph node of a cat in Experiment No.2 highlighted the need for a further experiment to determine whether leptospire are found in the lymph node only during the leptospiraemic phase of infection or for a more prolonged period. The lymph nodes were cultured and examined for leptospire and for histopathological changes that might be consistent with *ballum* infection. An additional aim of this experiment was to define the serological changes more precisely than could be achieved in the second experiment.

MATERIALS AND METHODS

The ten cats which were used in this experiment were divided into three groups (Table 11). All cats were serologically negative to *ballum* pre-infection and also negative to the following serovars - *pomona*, *copenhageni*, *balcanica*, *tarassovi*, *hardjo*, *canicola*, *australis*, *javanica* and *pyrogenes*.

Both oral infection and intraperitoneal (I.P.) inoculation were performed to compare the results of these routes of infection. Four cats were used for each route; three of these cats were mature and one was between five and six months old. Cats number 1 to 4 (Group 1) were infected I.P. and cats 5 to 8 (Group 2) were infected by the oral route. Two further cats, one mature (cat No.9) and the other approximately four months old (cat No.10) (Group 3) were given a subcutaneous (s.c.) injection of formalised culture of *ballum* for serological study. All

cats were treated with piperazine one week before commencing the experiment except the two cats in Group 3.

A seven day old culture of *ballum* was used to infect all the cats. This culture was from the same origin as those used in the previous experiments, and had been passaged through hamsters.

One millilitre of the culture was used for I.P. inoculation and oral feeding. The cats were dosed orally using syringes without needles. No particular feeding regime or fasting was carried out before the infection. One millilitre of the formalised culture was injected s.c. To test the infectivity of the cultures one hamster was inoculated I.P. with 1 ml of the live culture. This animal died six days post-infection (p.i.). Another hamster inoculated I.P. with 1 ml of the formalised culture did not die.

The rectal temperatures of the cats were recorded every day and daily blood samples were obtained from the jugular vein for culture over a period of two weeks. Haematological tests such as haemoglobin, packed cell volume (PCV), white blood cell count, differential leukocytes, total plasma protein, icterus index, and plasma fibrinogen were carried out weekly. Serological tests were performed every alternate day for two weeks and thereafter weekly for the duration of the experiment.

One cat from both the I.P. and orally infected groups was killed every week and the following fluids and tissues were collected for culture; urine, aqueous humor, suspension of kidney, liver, brain and salivary gland. The following lymph nodes were also obtained for culture; mandibular, medial retropharyngeal, superficial cervical, accessory axillary, mesenteric and external iliac or popliteal.

The above tissues were also collected for histological study as well as culture. The procedures used for culture and histological processing were the same as used in the previous experiments.

RESULTS

Clinical findings

The temperatures of cat No.1 on the 8th and 11th day p.i. were higher than normal (40.2°C and 39.5°C respectively). (The normal temperature range established for cats in this study was 38.0°C to 39.0°C). Cat No.6 had a high temperature at seven days p.i. (39.7°C) and cat No.5 had an elevated temperature nine days p.i. (39.6°C).

Haematology

Three cats (No.'s 1, 5 and 6) had a neutrophilia one week p.i. (72%, 78% and 82% respectively). All the cats except cat No.8 had an increase in white blood cell count two weeks p.i.

Urine analysis

The specific gravity (S.G.) of the cats' urine, although fluctuating, showed a definite decrease at one week and then again at 28 days p.i. in two cats. Two weeks p.i. a decrease in S.G. was seen in the urine of cats No.'s 3, 4, 7 and 8.

Blood and Urine culture

Leptospiiraemia

Leptospiiraemia was detected by blood cultures in all the infected cats except cat No.7 (Table 11). In those cats infected by the I.P. route, leptospiiraemia was first detected two days p.i. while the orally infected cats had positive blood cultures on the third day p.i. In the cats infected by the I.P. route positive blood cultures were obtained for longer periods compared with the orally infected cats. The longest leptospiiraemic period of seven days occurred in cat No.1. Cat No.3

Table 11

CULTURAL AND SEROLOGICAL FINDINGS FOLLOWING I.P. AND ORAL *BALLUM* INOCULATION

Group Number	Cat's Reference Number	Route of Infection	Inoculum (1 ml)	Age of the cats	Post-infection day of isolation				Time of euthanasia week p.i.	Serological titres	
					Blood	Urine	Kidney	Other tissues		Initial titre (days p.i.)	Terminal titre at euthanasia
ONE	1	I.P.	live culture	5-6mth	2-8th	8th till the end of exp.	31	-	4 wks	1/384 6	1/384
	2	I.P.	live culture	mature	3rd	-	-	-	1 wk	1/384 6	1/384
	3	I.P.	live culture	mature	3-7th	13th, 19th, 21st	21	-	3 wks	1/384 7	1/1536
	4	I.P.	live culture	mature	3rd	-	-	-	2 wks	1/384 7	1/192
TWO	5	Oral	live culture	5-6mth	3rd	20th till end of exp.	31	-	4 wks	1/24 10	1/24
	6	Oral	live culture	mature	3-4th	-	7	brain, external iliac LN, mandibular LN, medial retropharyngeal LN	1 wk	1/24 7	
	7	Oral	live culture	mature	-	-	-	-	2 wks	1/24 7	1/384
	8	Oral	live culture	mature	6th	19th	-	-	3 wks	1/48 9	1/192
THREE	9	s.c.	formalised culture	mature	-	-	-	-	4 wks	1/48 7	1/96
	10	s.c.	formalised culture	4 mth	-	-	-	-	4 wks	1/48 7	1/96

had positive blood cultures for five days. Blood cultures of the two other cats in the I.P. group were positive only on the third day p.i. The longest period of leptospiraemia detected in the orally infected cats was two days (cat No.6).

Leptospiruria

The first urine cultures were positive on day eight p.i. (cat No.1) (Table 11) the last day of the leptospiraemia phase while leptospire were detected by darkfield microscopy in the fresh voided urine two weeks p.i. In cat No.3 positive urine cultures did not occur until 13 days p.i. In the orally infected group leptospiruria was detected in urine cultures from two of the cats on the 19th and 20th days p.i.

Serology

A serological response was first detected five days p.i. and all the cats which were infected I.P. were positive to the M.A.T. by seven days p.i. (Fig. 11). However, the orally infected cats had positive titres seven days p.i. and two of these (No.8 and 5) had positive M.A.T. at 9 and 10 days p.i. The I.P. infected cats had higher initial serological responses (1/384) than the orally infected cats whose titres started lower (1/24 and 1/48). By the third week p.i. these M.A. titres gradually increased to the same level as those in the I.P. group (Fig. 12). Although cat No.7 showed a very good serological response it had no positive cultures. Both of the cats which were inoculated with killed *ballum* also had positive titres seven days post s.c. injection. However, the mature cat (No.9) was serologically negative at 14 days p.i. while the kitten (cat No.10) which had the same dose of killed *ballum* as the mature cat had a positive M.A. titre of 1/65 until the end of the experiment (Fig.13).

Figure 11.

MICROAGGLUTINATION TITRES OF CATS INFECTED BY THE INTRAPERITONEAL ROUTE

MA Titre

— Cat 1
— Cat 2
— Cat 3
- - - Cat 4

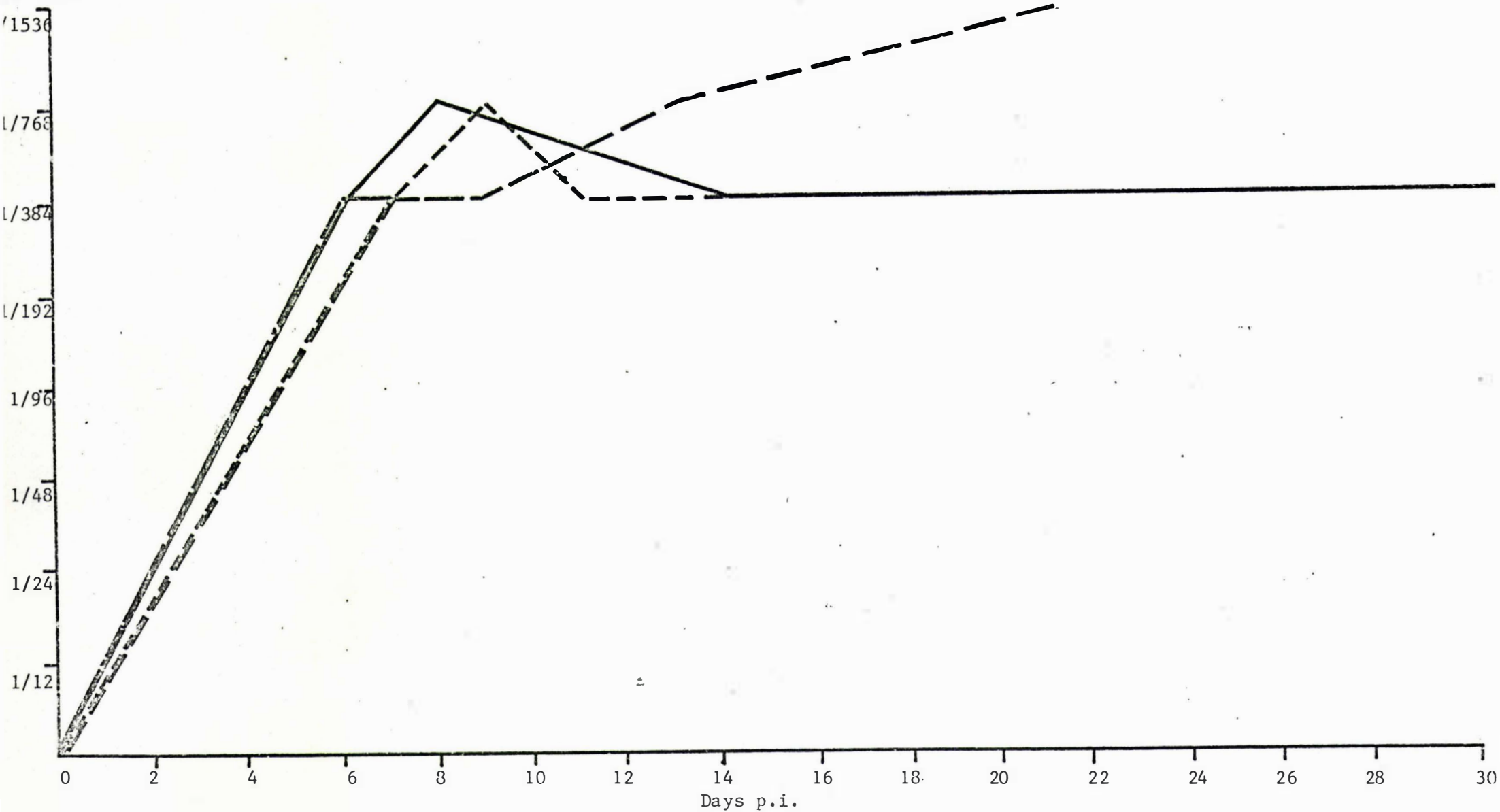


Figure 12.

MICROAGGLUTINATION TITRES OF ORALLY INFECTED CATS

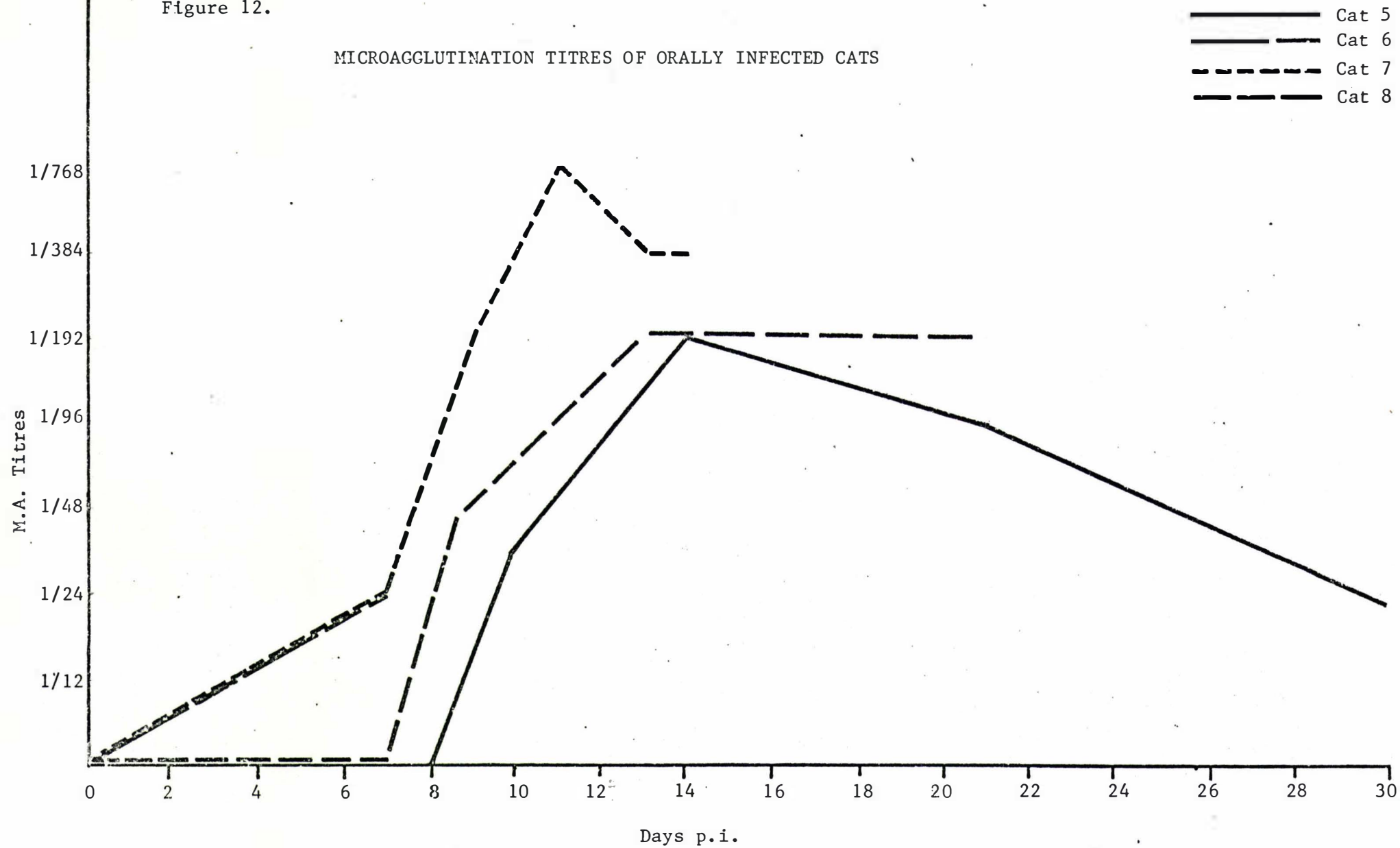
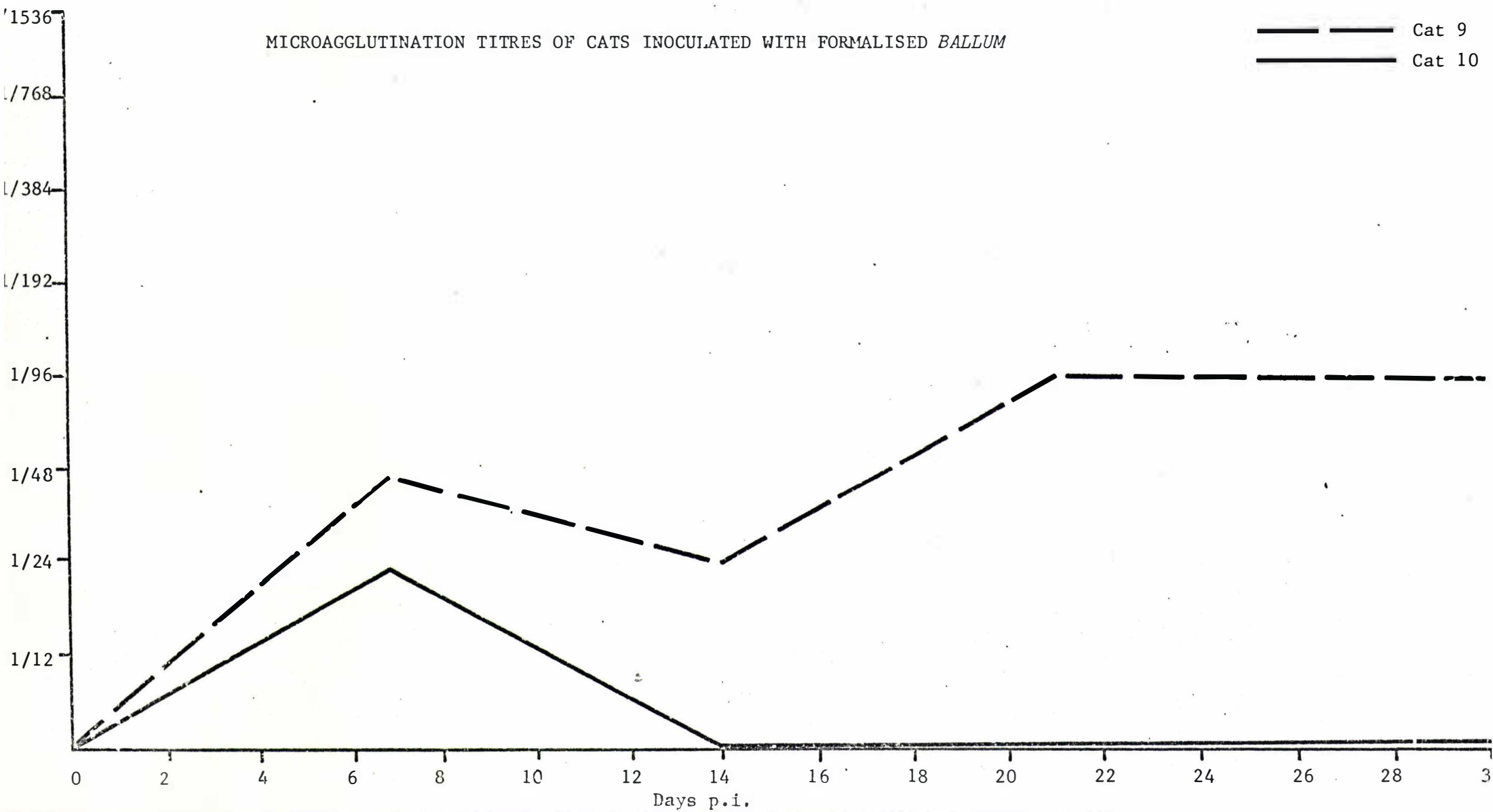


Figure 13.

Titres



Necropsy and cultural findings

The mesenteric and external iliac lymph nodes from one of the two cats killed at seven days p.i. (No.6) appeared swollen and hyperaemic. Leptospires were recovered from the cultures of kidney, brain and the following lymph nodes: mandibular, mediaretropharyngeal and external iliac of cat No.6, but no positive cultures were obtained from the tissue of cat No.2.

The two cats that were examined at two weeks p.i. showed no gross lesions apart from a pale fatty liver in one animal (No.4). No positive cultures were obtained from either of these cats.

At three weeks p.i. the kidney cultures from cat No.3 were positive, while those of cat No.8 were contaminated. One millilitre from each of the contaminated cultures was injected into hamsters I.P. The hamsters were killed after ten days and all cultures obtained from the kidneys of the hamsters were negative.

Cats No.1 and No.5 which were younger than the others were necropsied four weeks p.i. Positive cultures were obtained from their kidneys and urine but not from other organs.

Histological changes

The main histological findings are summarised in Table 12.

Kidney

All the kidneys examined showed a moderate degree of fatty change in the outer cortex which has been considered as normal (Bloom, 1954). In addition, focal areas of severe fatty change and moderate cloudy swelling were observed in the straight portion of the proximal tubules

Table 12

EXPERIMENTAL *BALLUM* INFECTIONS : SUMMARY OF HISTOLOGICAL FINDINGS

Group Number	Cat's Reference Number	Route of Infection	Time of Euthanasia	Focal fatty change in straight portion of proximal tubules	Kidney		Necrotic Tubules	Liver
					Cloudy Swelling of Tubular cells	Interstitial Focal of Cellular Infiltration		
ONE	1	I.P.	4 wks p.i.	±	±	±	++	+
	2	I.P.	1 wk p.i.	+++	-	-	-	±
	3	I.P.	3 wks p.i.	-	+	++	+	++
	4	I.P.	2 wks p.i.	+	±	±	++	++
TWO	5	Oral	4 wks p.i.	-	-	±	±	-
	6	Oral	1 wk p.i.	+++	±	-	-	+++
	7	Oral	2 wks p.i.	+	-	±	-	++
	8	Oral	3 wks p.i.	-	-	+++	-	++
	9	s.c.	4 wks p.i.	-	-	-	-	++
THREE	10	s.c.	4 wks p.i.	-	-	-	-	

- No significant change

± very mild

+ mild

++ moderate

+++ severe

in the inner cortex of both cats which were killed one week p.i. (Fig. 14a&b). One or two small foci of lymphocyte infiltration were seen around urinary tubules in the cats killed two weeks p.i. but at three weeks p.i. the kidney of one cat (No.8) contained several large foci of lymphocyte and macrophage infiltration in the interstitium (Fig. 15). Severe cloudy swelling, with disruption of the cytoplasm and early necrosis was also seen in some groups of urinary tubules in the I.P. infected cats at three and four weeks p.i. (Fig. 16).

Liver

The hepatocytes of both of the cats killed at one week p.i. showed a moderate degree of universal fatty change. A few small disseminated foci of lymphocytes and plasma cells numbering less than ten cells were also present around portal areas. A similar but more mild cellular reaction was seen in the cats killed at two, three and four weeks p.i. and this appeared to be consistently more severe in the I.P. infected animals.

The cats which were inoculated subcutaneously with formalised *ballum* both contained occasional focal granulomas consisting of lymphocytes, plasma cells and macrophages which were not related to any particular part of the liver.

Lymph nodes

At one week p.i. the germinal centres of the lymph nodes of both cats were active with proliferating blastocytes and macrophages. The germinal centres at two and three weeks p.i. were larger than those killed at one week and an early cellular response was visible in the paracortical area. Increased numbers of plasma cells were also seen in the medulla. The largest germinal centres seen in this experiment were found at four

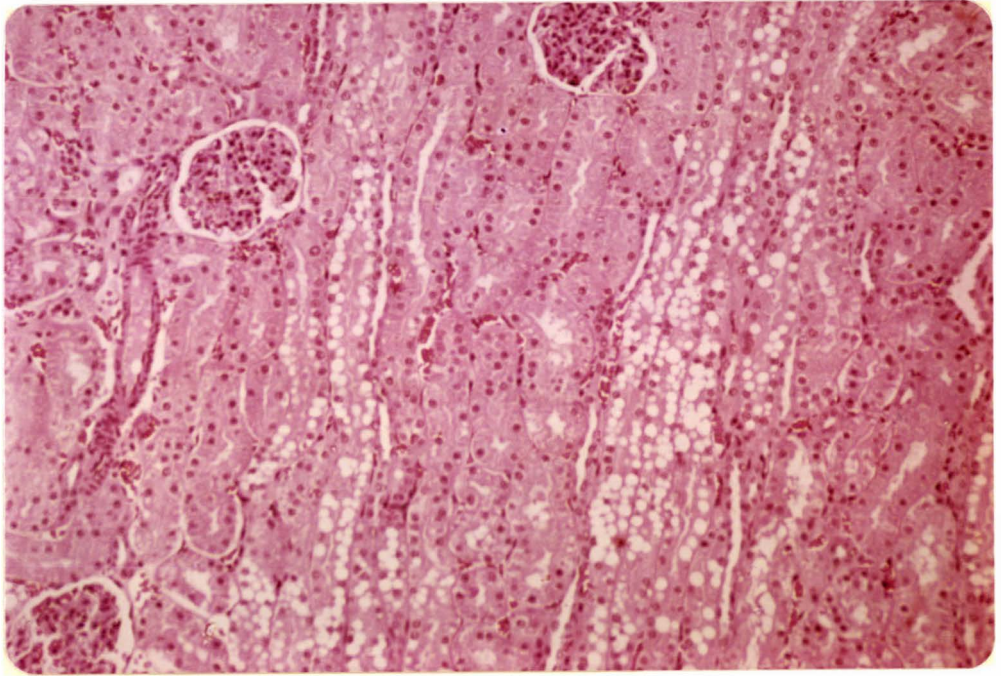


Figure 14a. Intraperitoneal *ballum* infection, one week post-infection (cat 2). Focal fatty change in the straight portion of the proximal tubules in the inner cortex. (HE X 125).

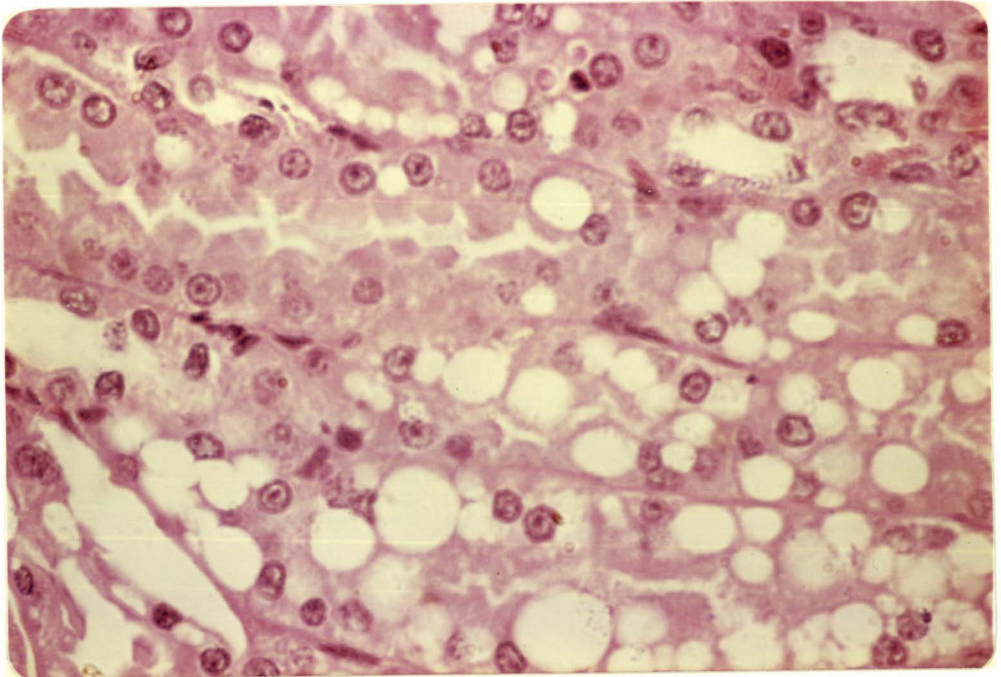


Figure 14b. Higher magnification of kidney shown above. (HE X 400).

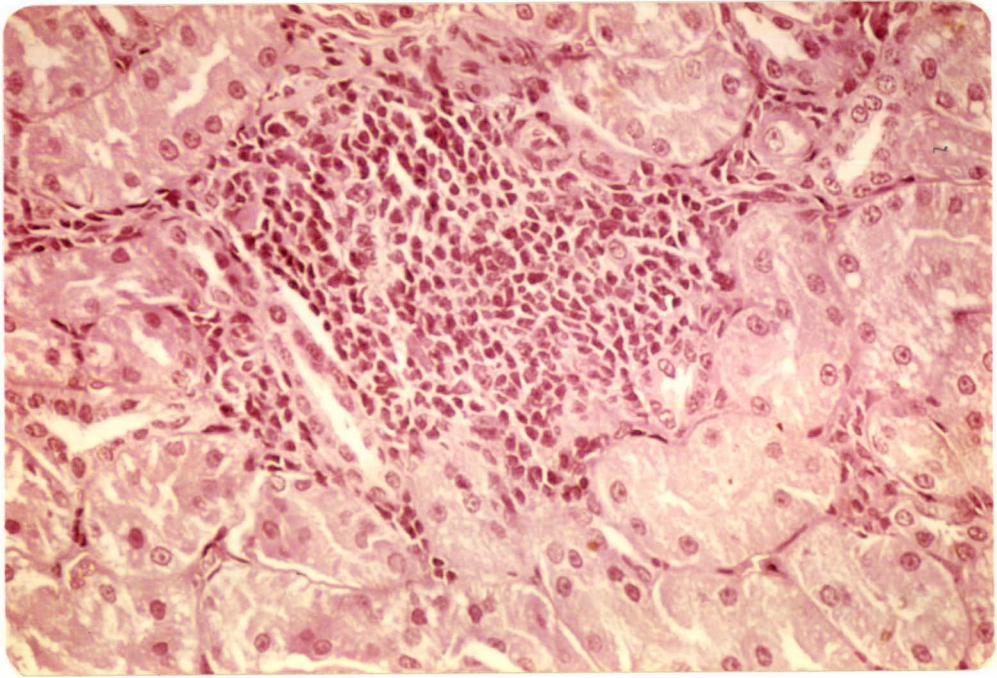


Figure 15. Oral *ballum* infection, three weeks post-infection (cat 8). A focus of lymphocyte and macrophage infiltration in the interstitium of the kidney cortex. (HE X 250).

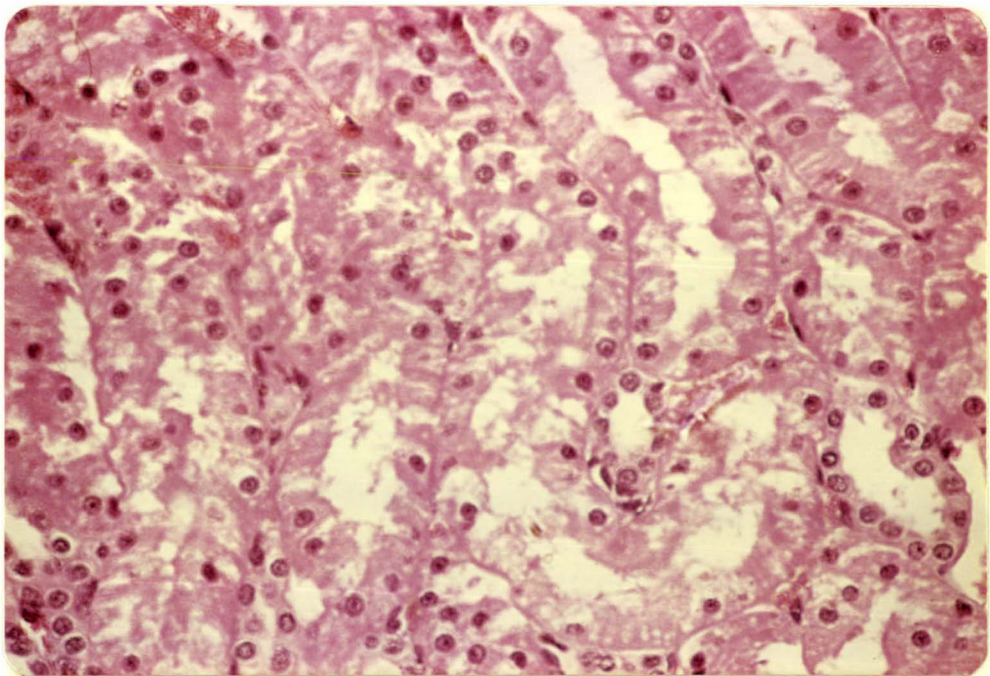


Figure 16. Intraperitoneal *ballum* infection, four weeks post-infection (cat 1). Severe cloudy swelling of cortical tubular epithelial cells with fragmentation of cytoplasm.

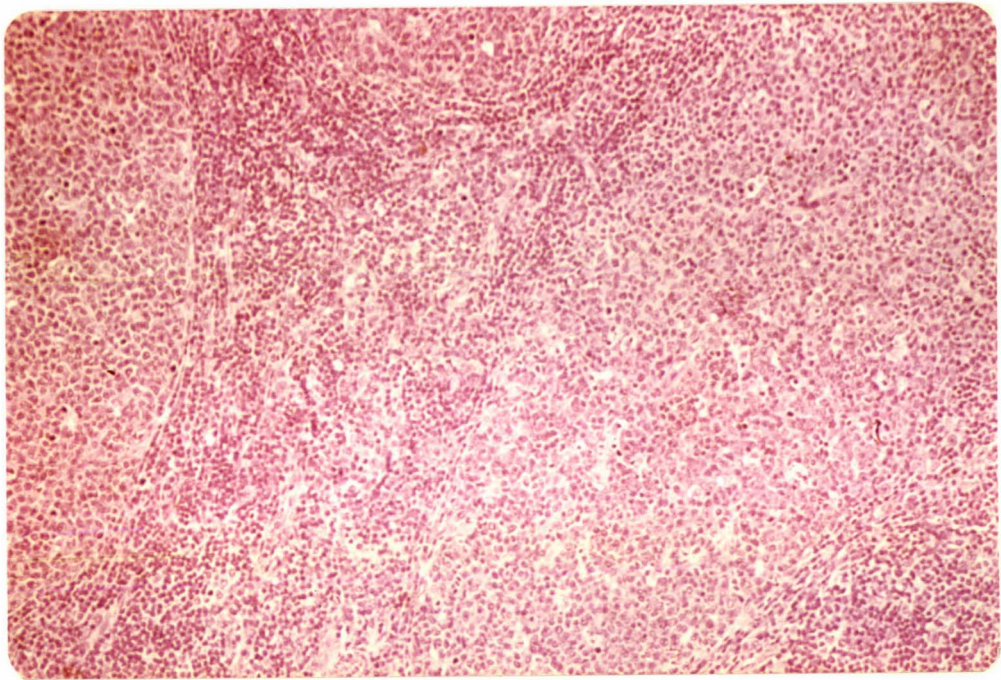


Figure 17. Intraperitoneal *ballum* infection, 4 weeks post-infection (cat 1). Hyperplasia of germinal centres in the mandibular lymph node. Macrophages make up a large proportion of the proliferating cells. (HE X 125).

weeks p.i. in cat No.1. Macrophages made up a large proportion of the proliferating cells (Fig. 17). The medulla was also very active and plasma cells extended up into the paracortical area. The lymph nodes from the second cat killed at four weeks p.i. (No.5) were also active but contained fewer plasma cells than cat No.1.

DISCUSSION

From the histological, serological and cultural findings it can be concluded that the I.P. route of *ballum* inoculation is more effective in producing an experimental infection than the oral route. The most obvious reason for this is that with the I.P. route of infection there is no loss of organisms, while with the oral route there are at least two host factors which may reduce the number of the viable organisms. These are firstly, saliva. Little is known of the specific effect of saliva on leptospires. However, it is known that saliva contains numerous antibacterial factors including lysozymes, local immunoglobulins, various enzymes and has a high pH. Leptospires can survive for longer periods in an alkaline rather than in acid pH. Gorden-Smith and Turner (1961) found that the survival time of a variety of leptospiral serotypes ranged from 21 to 152 days at pH 7.2 to 8.0 and 10 to 117 days at pH's 5.3 to 6.8. Okazaki and Ringen (1957) found that leptospires can remain motile at temperatures between 34°C to 36°C for 6.8 and 2.0 days at pH of 7.2 and 8.4 respectively.

The pH of saliva in the cat was estimated by the author to be between 7.5 and 8.0. It therefore seems unlikely that salivary pH has any effect on the viability of these organisms. If some of the other factors mentioned are responsible for reducing the viability of the organisms then the length of time that leptospire are exposed to saliva and the amount of saliva to which they are exposed will be important in reducing leptospiral infectivity. However, it is a common observation that the amount of saliva which each cat produces and the length of the time which a cat may keep a small volume of liquid in its mouth varies considerably between individual animals.

The second factor likely to affect viability of leptospire administered via the oral route is the acidic pH of the stomach. This is discussed more fully in the next chapter. Since only one ml of inoculum was used in this experiment the effect of this factor may be mitigated by the likelihood of absorption before the stomach is reached.

On the 7th to 9th days p.i. high temperatures were recorded in two cats, one from the orally and one from the I.P. infected groups. These temperature rises coincided with the initial isolation of leptospire from the kidney and urine of these cats on the 7th and 8th day p.i.

Marshall (1973) used the electron microscope to investigate leptospiral migration in kidneys and related tissues damaged during experimental infection of suckling mice. He demonstrated that migration of leptospire occurs between two and 14 days p.i. Leptospire migrated from the capillary lumen to the interstitial tissue within the first four days p.i. and this produced capillary endothelial damage. The migration of leptospire between the epithelial cell junction of the proximal convoluted tubules occurred by the 10th day after infection. By the 19th day p.i. many leptospire had arrived within the tubular lumen and those within the lumen had started to multiply.

In these experimental cats a similar pattern of tissue damage occurred. It can therefore be concluded that the rise in temperature of the cats at this time was due to invasion of leptospires into the kidney together with the tissue damage related to the multiplication of the organisms. The decrease in S.G. of urine samples observed at this time may also be related to this tissue damage.

Although Fessler and Mörter (1964) did not observe any changes in the temperature of experimentally infected cats in their studies with *ballum* and *pomona*, Modric (1974) has recorded some degree of elevation in the temperature three or four days p.i. in cats infected with four different serovars excluding *ballum*.

Leptospiraemia in both routes started at approximately the same time (two to three days p.i.). The duration of leptospiraemia by haemoculture was recorded to be as long as five and six days in two of the four cats in the I.P. infected group (Table 11), while in the orally infected group only one of the cats (cat No.6) had positive blood cultures for two days duration. However, recovery of leptospires from a number of tissues from cat No.6 at necropsy one week p.i., despite negative and contaminated blood cultures at this time confirmed a leptospiraemia of five days duration. This illustrates that recovery of leptospires using the present techniques is not one hundred per cent accurate, especially when dealing with a small sample such as a drop of blood containing relatively small numbers of organisms. For this reason it cannot definitely be concluded that the leptospiraemia in the orally infected cats was shorter than that observed with the I.P. route of infection.

If these results are considered in conjunction with those obtained in the previous experiment (Experiment 3) in which an orally infected cat yielded positive haemocultures for five days duration, then it seems

likely that the leptospiraemia produced by both routes of infection was of similar duration.

Leptospiuria appeared to start earlier in the I.P. infected cats, but since positive kidney cultures were obtained from one of the orally infected cats (No.6) one week p.i., it could be postulated that leptospiruria may start at the same time with both the routes of infection. Lack of positive urine cultures in the orally infected cats may have been due to fewer numbers of organisms in the urine.

All the cats had positive serological titres by the 10th day p.i. The I.P. infected cats had high initial titres while the orally infected cats started with low titres (1/24 and 1/48) but by the third week p.i. their titres had reached the same level as the I.P. infected group. Although one cat (No.7) had a very good serological response no positive cultures were obtained.

Fessler and Morter (1964) found demonstrable agglutinins in five of the six s.c. infected cats. They described the serological response as being moderate in degree but gave no specific details. They recorded a peak titre of 10^4 from one of the cats 26 days p.i. which was 12 days later than that obtained in the present experiment.

Positive serological results were obtained from cats inoculated with both killed *ballum* as well as those inoculated with live cultures. This differs from the results obtained by Jones (1964) who was not able to show positive titres in his experimental cats inoculated with either killed or pathogenic leptospiral serovars. A higher and longer serological response was obtained in the kitten inoculated with formalised *ballum* than in the mature cat. This could be explained by the age of the cat and the greater amount of the antigen administered in relation to its bodyweight and size.

According to Bloom (1954) fat occurs in the upper half or two thirds of the proximal convoluted tubules in the cat and the terminal portion is fat free. The presence of normal lipids in these areas of cat's kidney is relatively constant and they are not irregularly distributed as they are in the dog. The localised fatty change seen in the straight portion of the proximal tubules of both cats killed at a week p.i. was distinct in distribution and appearance from these normal lipids and was not observed in any of the other cats. It is possible therefore that this change may be related to leptospiral infection.

Based on Marshall's (1973) findings the following hypothesis may be suggested to explain the histological changes in the kidney.

- (a) At a week p.i. focal fatty changes and cloudy swelling were seen in the epithelial cells of proximal tubules which was likely to be due to systemic reaction and migration of leptospores into the kidney interstitium.
- (b) At two weeks p.i. early lymphocyte infiltration was seen around cortical tubules. This may be due to the arrival of leptospores in the tubular lumen.
- (c) At three and four weeks p.i. there was severe cloudy swelling, some necrotic changes in the tubules and foci of lymphocyte and macrophage infiltration. This was probably due to the reaction of kidney against leptospiral localisation.

During the bacteraemic phase the lymph nodes appeared to be swollen and congested at necropsy. Histologically the germinal centres at two weeks p.i. were more active than those examined at one week p.i. After this period all the lymph nodes were active and showed some degree of response in germinal centres, paracortex and medulla. It has been well documented that germinal centres and medullary areas are associated with humoral antibody production whereas paracortical areas are associated

with cell mediated immunity (Roitt, 1975). This evidence therefore suggests that both these forms of immunity contribute to the immune response to leptospiral infection in the cat.

The histological changes in the liver of the cats inoculated with formalized *ballum* culture did not appear to be related to leptospiral infection as they had an appearance characteristic of parasitic granulomas due to migrating toxocara larvae.

CHAPTER 4

EXPERIMENT FIVE

STUDIES OF THE PREY-PREDATOR CHAIN

INTRODUCTION

The acquisition of leptospiral infection by ingestion of food and water contaminated with the urine of carriers has been described in many reports. Experimental infection has been demonstrated in guinea pigs (Inada *et al.*, 1916), hedgehogs (van der Hoeden, 1958a), puppies (Cyss, 1951; Michna, 1962), cats (Cyss, 1951), wild marsupialia and carnivora (Reilly *et al.*, 1970). Natural infection by ingestion has been reported in silver foxes (Catchpale, 1934), wild carnivores (Vyostskii *et al.*, 1958), and aquatic birds (Ardeidae) (Babudieri, 1958).

Despite these reports it is generally agreed that the alimentary tract is an unimportant route of infection due to low pH of the stomach which is capable of destroying the leptospire (Alston and Broom, 1958).

Reilly *et al.* (1968) experimentally demonstrated the enteric route of infection with *grippotyphosa*. The leptospire was protected from gastric acidity with gelatin capsules and deposited into the duodenum where they were capable of invading and producing infection. By feeding infected mice the same researchers (Reilly *et al.*, 1970) were able to establish leptospiral infection in two of seven opossums, three of eight skunks and two of ten red foxes, but raccoons could not be infected.

In the previous experiments (Chapters 2 to 4) it was shown that the cat can easily be infected orally. However, it must be acknowledged that the experimental feeding of one ml of liquid media differs from natural

acquisition of the disease through the eating of prey. According to Alston and Broom (1958) leptospires given in liquid media may readily enter the tissues of the upper alimentary tract and produce infection.

The purpose of this study was to ascertain whether a cat can be experimentally infected via the alimentary tract and to determine whether or not an interspecies relationship is involved in transmission of *ballum* serovar from mouse to cat.

MATERIALS AND METHODS

Experimental animals

Four six-month-old cross-bred cats were kept in an isolated room from the age of one week, together with their seronegative dam. The cats were fed canned food and were dewormed before the experiment commenced.

Before infecting these cats, blood samples were collected for M.A.T. and growth inhibition tests (G.I.T.) and all were serologically negative to the M.A.T. and G.I.T. at the beginning of the experiment. One week before infection the cats were transferred to fibreglass cages in another isolated room. They were divided into two groups and their temperatures taken daily from one week pre-infection to the end of the experiment which lasted 47 days. A control cat (the same animals as used for the third experiment) was kept in the same environment.

Method of Infection

Six, young, white laboratory mice from an SPF group were inoculated with 1 ml of a 7-day culture of *ballum* as used in the previous experiments. To check the viability of the culture one hamster was inoculated I.P. with the same volume of culture. It died five days post-infection.

The mice were made to urinate on glass slides by applying slight abdominal pressure. Their urine samples were immediately examined by darkfield microscopy and at thirteen days post-inoculation when all were found to be shedding leptospire in their urine they were killed. Four of them were used for infecting the cats and the other two were cultured for leptospire and their kidneys processed for histology. The sections were stained by the Warthin-Starry technique as described previously (Chapter 2).

All cats were deprived of food and liquids for at least four hours before being fed the infected material. Two cats (cat No.'s 1 and 4) were fed one whole mouse each. The other two cats (No.'s 2 and 3) were each fed two kidneys and the bladder from one mouse. The cats were separated while they were fed the infected material after which each pair was kept in the same cage.

Collection of specimens

Alternate cats from each group were bled daily for two weeks p.i. and thereafter blood was collected weekly by cardiac puncture of *via* the jugular vein for M.A.T. and gel filtration test. The results of gel filtration test were confirmed with the immunoelectrophoresis technique. Growth inhibition tests were performed on serum samples obtained at the time of euthanasia.

Urine samples were obtained from the cats by manual pressure without the aid of anaesthesia. The urine samples were used for determination of the chemical properties of urine such as: pH, protein, blood, bilirubin, urobilinogen, ketone, glucose and nitrite which were estimated by using reagent strips*. The specific gravity of the urine samples was determined by using a refractometer. All procedures used for culturing blood, urine or tissues collected at necropsy were the same as in previous experiments.

* BM test, Combuar 7 Test Boehringer Mannheim GmbH, Mannheim, Germany.

RESULTS

Clinical findings

All cats showed a slight rise in temperature of 0.5 to 1.0°C at 9-12 days p.i., but apart from this the infection was symptomless. There was a significant drop in the specific gravity of the urine of all cats at two weeks p.i., although a mild degree of fluctuation was noted throughout the experiment.

Blood and urine culture

Leptospiraemia was detected in all the cats (Table 13). It was first seen in those cats which were each fed the kidneys and bladder from one mouse (No.'s 2 and 3) at six and seven days p.i. and the cultures remained positive for seven days. In those cats which were fed a whole mouse each (No.'s 4 and 1) leptospiraemia started at nine and ten days p.i. and the cultures were positive for a period of three days in one (No.4) and one day in the other.

Leptospiruria was also detected in all the cats (Table 13). It commenced at 12 days p.i. in the cats fed mouse kidneys and bladder but appeared at 15 days after ingestion in the animals fed whole mice. Cat No.13 was the first cat in which leptospires were detected by darkfield microscopy. This occurred at 18 days p.i. One to two leptospires were seen per darkfield examination at a 12.5 X objective (total magnification X 156). Subsequent counting and dilution of leptospiral cultures in the Petroff-Hausser counting chamber indicated that this number of leptospires was equivalent to 10^4 organisms per ml.

Table 13

CULTURAL FINDINGS FOLLOWING INGESTION OF *BALLUM* INFECTED PREY

Cat's Reference Number	Age	Sex	Infected Ingesta	Isolation of the leptospire			
				Days p.i.		Kidney	Other Tissues
				Blood	Urine		
						At euthanasia 47 days p.i.	
1	6 months	Male	One whole mouse	9th	16th till end of exp.	+	-
2	6 months	Male	Kidneys, bladder of one mouse	7,9,11, & 13th	15th till end of exp.	+	-
3	6 months	Female	Kidneys, bladder of one mouse	6,8,10, & 12th	12th till end of exp.	+	-
4	6 months	Female	One whole mouse	8, 10th	15th till end of exp.	+	-

Serology

Two weeks p.i. all the cats were positive to M.A.T. and their titres were similar (Table 14). Their titres reached a peak five weeks p.i. and declined thereafter (Fig. 18). In one cat (No.1) the titre declined more rapidly than the others.

The G.I.T.'s conducted on the blood samples obtained at euthanasia were positive for all cats except No.1 which had the lowest titre (1:48). The results obtained for this cat showed partial clumping and slightly less leptospiral growth than the control. The serum from this cat was compared with the serum from cat No.1 in Experiment 3 in which the titre declined rapidly and disappeared. Although both these sera were considered to be non-inhibitory the cat with no detectable titre (No.1 Exp.3) had slightly more leptospiral growth than the other (No.1 Exp.5). Fractionation of serum showed that all the cats initially produced IgM at two weeks p.i. (Fig. 19&20). Those cats which were fed infected kidneys and bladder subsequently produced IgG at five to six weeks whereas with those fed whole mouse, the response remained in the IgM peak. These results were confirmed by the immunoelectrophoresis technique (Fig.21).

Necropsy and cultural findings

No gross lesions were found in any of the cats. All the cats had positive kidney cultures, but all other tissues cultured were negative (Table 13).

Histological changes

The most severe histological changes were seen in the cats which were fed infected kidneys and bladder. These cats both showed focal areas of interstitial nephritis in the kidney cortex. They consisted of

Table 14

SEROLOGICAL RESPONSE OF THE CATS DURING THE PERIOD
OF EXPERIMENT

Cat's Reference Number	M.A. Titres day p.i.						
	7	14	21	28	35	42	47
1	-	1/192	1/768	1/384	1/1536	1/192	1/48
2	-	1/24	1/96	1/384	1/1536	1/1536	1/384
3	-	1/96	1/384	1/192	1/1536	1/1536	1/192
4	-	1/48	1/192	1/768	1/1536	1/192	1/192

All cats showed no agglutination titres pre-infection

Figure 18.

M.A. TITRE OF CATS IN THE PREY-PREDATOR CHAIN.

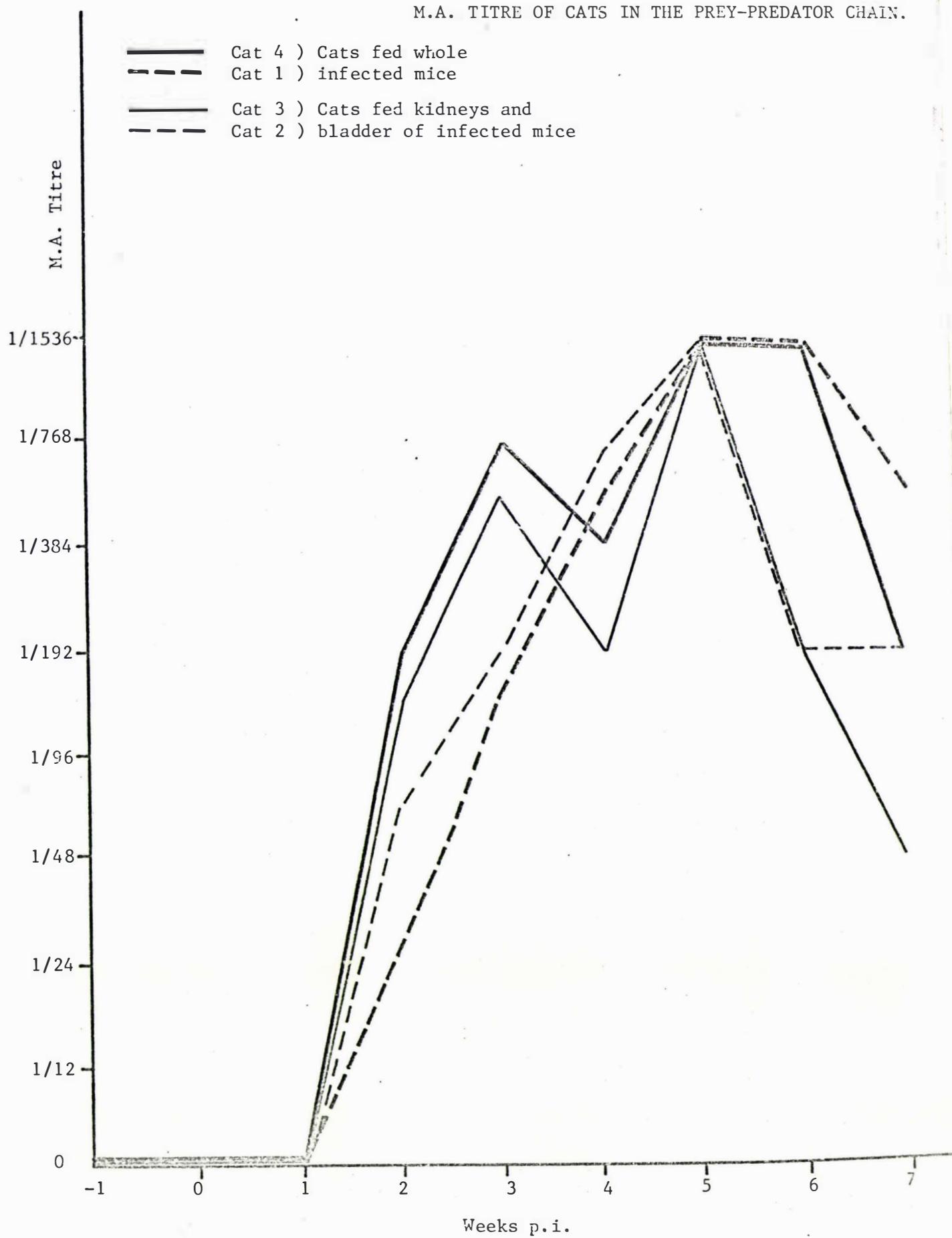


Figure 19.

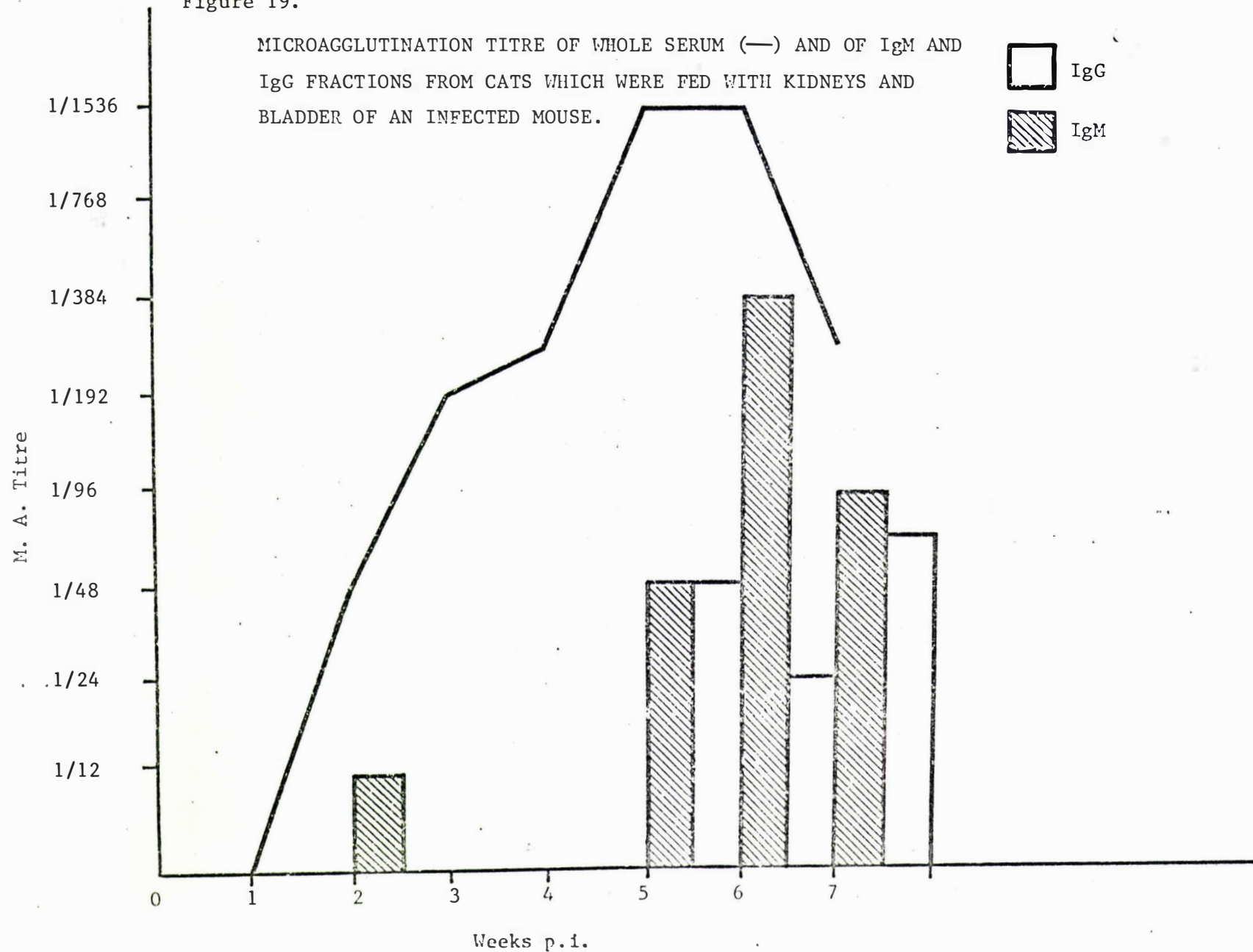


Figure 20.

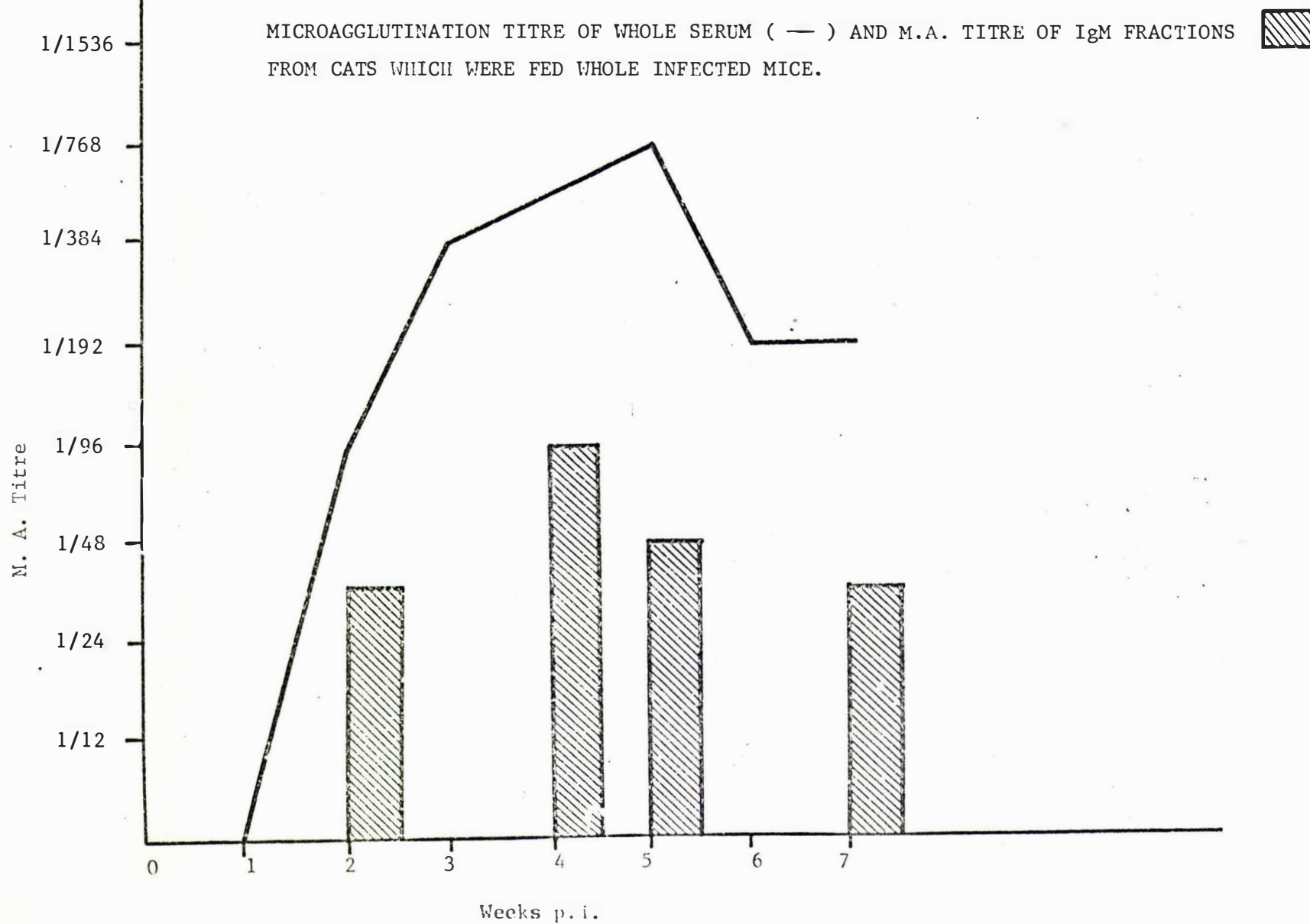
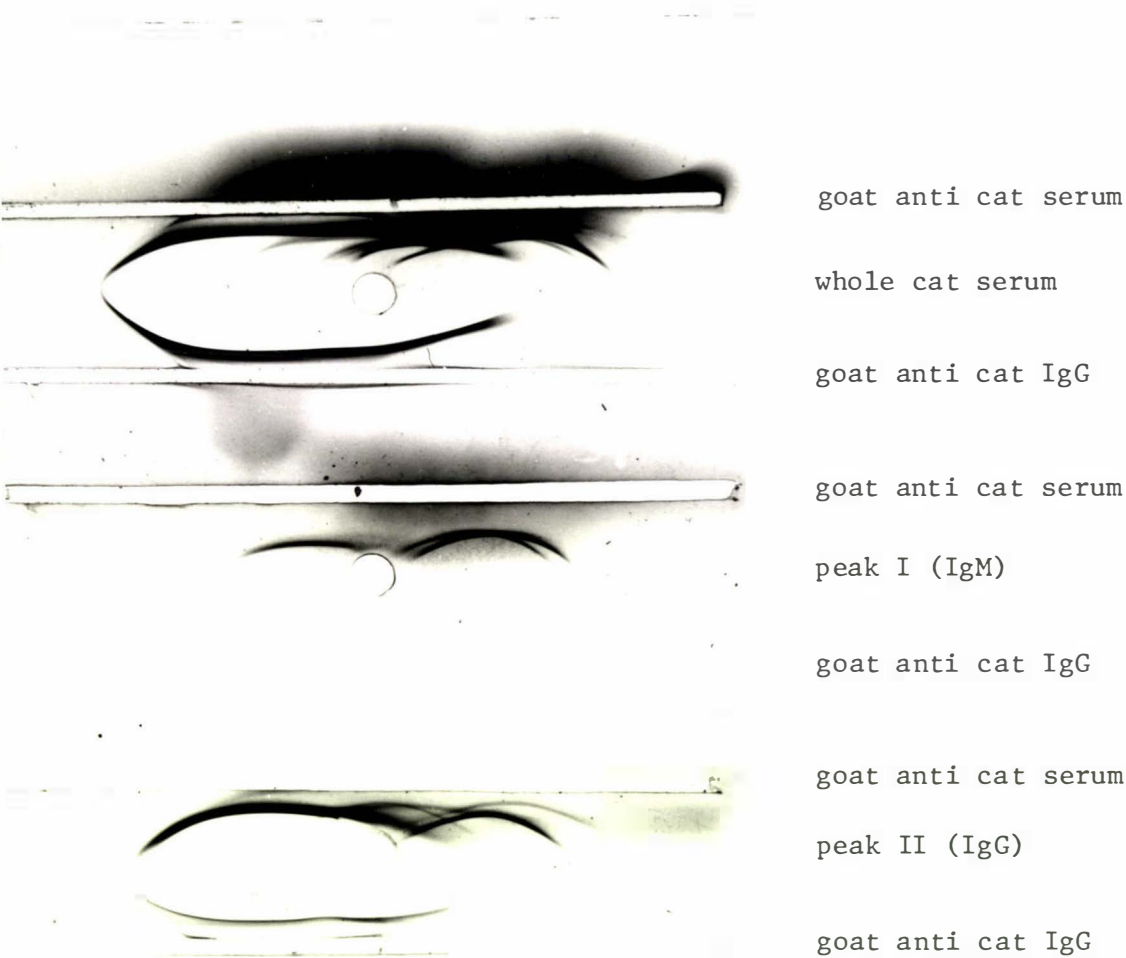


Figure 21.

Immuno-electrophoretic reaction of concentrated gel-filtration fractions of cat serum with goat anti cat serum and goat anti cat IgG.

Cathode

Anode



Cathode

Anode

The goat anti cat IgG was not specific for γ chains therefore it cross reacted with peak I (IgM).

macrophages, lymphocytes and plasma cells but those located in the outer cortex also contained neutrophils (Fig. 22). No foci of cell infiltration were seen in the kidneys of the cats fed whole mice and no leptospire were seen with Warthin-Starry stains of kidney sections from all cats.

The liver of one cat (No.2) fed infected bladder and kidney contained mild periportal infiltration of lymphocytes and plasma cells. The retropharyngeal and mesenteric lymph nodes from this animal also showed bigger germinal centres than those seen in the other cats.

DISCUSSION

This experiment has demonstrated that cats can become infected with *Ballum* serogroup by ingestion of infected prey. The infection was successful despite the inhibitory effect of low stomach pH induced by a period of starvation on the leptospire within the ingesta. The histological, serological and cultural findings showed that the cats fed whole mice exhibited less severe changes than those fed with infected bladder and kidney tissues. This may be due to two factors, firstly it is likely that a small portion of infected food would have greater contact with the pregastric mucosa during mastication and swallowing than a whole mouse. Secondly, a small portion of ingesta will pass more rapidly through the stomach than a large bolus of prey containing tissue which are difficult to digest (Thomas, 1957). Thus leptospiral organisms within a large bolus of prey will be exposed to gastric acids over a longer period than those within a small portion of tissue.

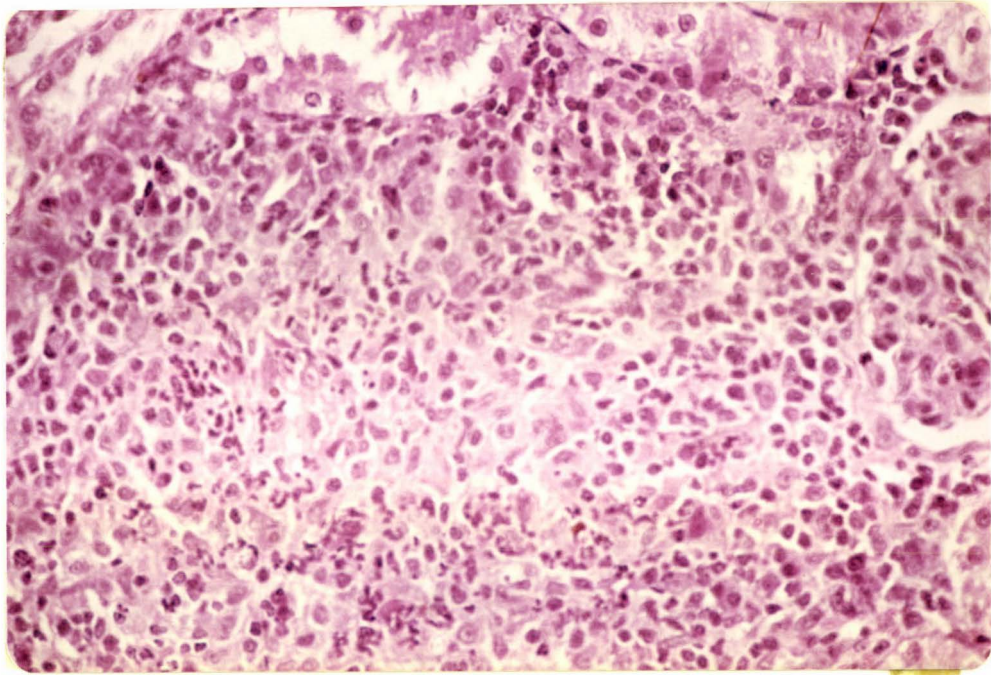


Figure 22. Cat 2, 47 days after ingestion of kidneys and bladder from an infected mouse. Neutrophils are present within the foci of interstitial nephritis as well as macrophages, lymphocytes and plasma cells.

The leptospiraemic phase of infection lasted seven days in the cats fed kidneys and bladder from infected mice but was of shorter duration in the cats which were fed a whole infected mouse each. These findings when taken in conjunction with the previous results (Experiment 4) indicate that the duration of leptospiraemia is likely to be related to the number of organisms which establish the infection. In the present experiment the microbiological results were more reliable than those previously obtained because contamination of blood cultures was avoided.

The carrier state began at 12 to 15 days p.i. and this coincided with a rise in body temperature of 0.5 to 1.0°C. This was presumably due to invasion of the kidney by leptospires and the associated tissue damage produced. The significance of this temperature elevation was greater than those obtained previously because the cats in this experiment were more adapted to handling and better acquainted with their environment.

By the third week p.i. all cats were microscopic shedders of the organism and the numbers seen were equivalent to those observed previously with the I.P. route of infection (Exp. 2 and 4).

All the cats had positive serological titres at two weeks p.i. Their titres were similar and showed a sharp decline after six weeks (Table 14). The serum samples examined for growth inhibition activity at the time of euthanasia showed that the sample with the lowest titre (1:48) had no inhibitory effect on the growth of leptospires. It can be concluded from these results and those in Experiment 3 that sera from cats with low declining serological titres or titres which have recently disappeared are not able to inhibit the growth of leptospires. This differs from the situation in other species which are able to inhibit leptospiral growth following immunisation although they no longer produce detectable MA titres (Tripathy *et al.*, 1972; Tripathy *et al.*, 1973; and Hathaway, 1978).

Although insufficient sera was available to carry out gel filtration tests on every sample the assumption regarding the relationship between IgG and the occurrence of a carrier state made in the third experiment was reinforced by the findings in the present experiment. The first cat to show leptospiuria culturally and microscopically also produced IgG agglutinins earlier than the others. In addition, those cats which exhibited the most severe histological changes also produced IgG agglutinins as well as IgM, whereas the two cats with insignificant lesions produced only IgM. The IgG response commenced three to four weeks earlier in this experiment than in Experiment 3. The earlier presence of IgG may be due to the greater numbers of organisms administered. On the other hand, as the length of the present study was only seven weeks the possibility that IgG could have subsequently been produced cannot be excluded although it is unlikely.

The histological changes were similar to those described in the previous studies with one significant difference namely the presence of neutrophils within the foci of interstitial nephritis. As this experiment was the only one of seven weeks duration, it is tempting to postulate that the presence of neutrophils may be related to the longer period of infection. However, the small numbers of animals involved makes it difficult to draw firm conclusions. The existence of the neutrophils in foci of interstitial nephritis has been reported with variety of serovars in animals such as cattle (Cordy and Jasper, 1952), and dogs (Bloom, 1941; Monlux, 1948; McIntyre and Montgomery, 1952).

In conclusion, this study has demonstrated the possibility of prey-predator transmission of leptospirosis from mice to cats and that despite the inhibitory effect of low pH in the stomach the number of the organisms which survive in the ingested prey are enough to produce infection which results in a carrier state.

CHAPTER 5.

A SEROLOGICAL SURVEY OF FELINE LEPTOSPIROSIS

INTRODUCTION

A number of serological studies have been undertaken to define the relationship between leptospirosis in the cat and wildlife (Esseveld and Collier, 1938; Clark, 1961; Ferris and Andrews, 1965; Hathaway, 1978) and these have revealed a prevalence of positive titres between 0 - 18% in the cats surveyed (Table 17). Other surveys have investigated the relationship between leptospirosis and feline nephritis (Hemsley, 1956; Lucke and Crowther, 1965; and Joshua, 1960).

The experiments described previously in Chapter 3 have shown that serological tests such as the M.A.T. are not by themselves sufficient to confirm leptospiral infection, because animals may shed the organism in their urine without having a significant serological titre. On the other hand these studies have also demonstrated that the cat does not respond to leptospiral infection with a very high titre and the titre tends to decline rapidly. This strongly suggests that a serological survey to determine the prevalence of feline leptospirosis should be based on lower serum dilutions than those used by earlier researchers in many overseas countries who have failed to identify any positive titres. Jones (1964) stated that the cat is resistant to infection by *canicola*, *pomona* and *icterohaemorrhagiae*. In his experimentally infected cats he did not obtain any positive titres even with proven pathogenic strains of these serovars. In a subsequent survey of 139 cats Jones (1964) recorded only two positive titres of 1:100, it seems likely that he missed low titres by choosing a high initial serum dilution of 1:100.

Broom (1955) suggested that in the cat a titre of 1:100 and greater indicates infection. Freudiger (1969) suggested that the infrequent finding of positive leptospiral titres in cats could be due to the rapid decline of the titre and the results obtained in previous experiments (Chapters 2, 3 and 4) support this suggestion. As infection is symptomless however, it is difficult to determine past or present infection and subsequent production of active immunity in the cat. Since no information is available on leptospiral titres in cats in New Zealand a serological survey was conducted to compare their prevalence with that reported from other countries.

MATERIALS AND METHODS

The blood samples were obtained from a number of sources in the North Island. Detailed histories of the cats were not available but the majority of samples were obtained from house cats and only occasional samples were from farm or feral animals. The serum samples were all subjected to the M.A.T. (Appendix 1) using the live antigens *pomona*, *ballum*, *hardjo*, *balcanica*, *tarassovi*, *copenhageni*, *canicola*, *pyrogenes*, *bataviae*, *javanica* and *australis*. The initial dilution in the M.A.T was 1:12. Six to ten day old cultures of leptospires in EMJH liquid media which contained approximately 1 to 5×10^8 leptospires per ml were used.

RESULTS

The positive serological reactions obtained together with the titres and details of the animals' samples are shown in Table 15. Twenty five of 225 cats (11.11%) were positive to one or more serovars ranging in titre from 1:12 to 1:768. Eight sera (3.55%) were positive to *ballum*, six (2.66%) to *copenhageni*, five (2.22%) to *hardjo*, four (1.77%) to *pomona*, two (0.88%) to *balcanica* and one (0.44%) to *canicola*. In those sera where multiple reactions were obtained, low titres were considered to be cross reactions and were disregarded (see discussion), whereas the equal titres were accepted for both serovars.

The other serovars for which positive titres were obtained were *javanica*, *pyrogenes* and *australis*. All positive titres to these serovars cross-reacted with others (Table 16). No titres were obtained with *tarassovi* and *bataviae*.

DISCUSSION

In interpreting the results, reactions with homologous antigen are considered to be the dominating ones (Borg-Peterson, 1949). The serological results of the previous experiments (Chapter 2 and 3) have shown that cross reaction is not very common in cats. The cross reactions seen in *pomona* and *tarassovi* infected cats were always in the lower titres (Chapter 2, Table 4) and in the *ballum* experiments cross reactions with heterologous antigens were seen only occasionally and did not follow a regular pattern. For this reason the highest titre obtained in multiple reactions was considered to represent the homologous antigen.

Table 15 TITRES OBTAINED IN LEPTOSPIRAL SEROLOGY
SURVEY OF NORTH ISLAND CATS

Sex	Age	Source and Health Status	Reciprocal of Titre								
			Bal.	Pom.	Cop.	Har.	Balc.	Can.	Jav.	Pry.	Aus.
M	8yrs	P.N.town cat- healthy	12								
M	-	healthy	48								12
M	3yrs	Feilding-healthy	12								
M	3yrs	dairy farm - healthy	48								
F	-	P.N. town cat	12								
F	12yrs	healthy	48								
M	9yrs	Tauranga town cat	12		12						12
NM	13yrs	P.N. town cat	768					48			
NM	-	Rangiwahia	24			48			12		
F	adult	Linton Army Camp - healthy	12		48						
M	-	P.N. town cat - healthy	12	192	12						
NM	7yrs	Feilding		192	48				12		
NM	12yrs	P.N. town cat - suspected nephritis		384	48				24		
F	adult	Piggery - healthy		24							
F	18mth	P.N. town cat - healthy			24						
FS	1yr	P.N. town cat - healthy			192						
M	3yrs	P.N. town cat - healthy			48						
F	6mth	Tauranga country area-healthy			48						
-	Aged	Feilding						48			
NM	16yrs	Feilding				192	96				
NM	8½yrs	P.N. town cat				24			24		
NM	12yrs	Rangiwahia				48			12		
N	16yrs	Feilding				192	96			12	
N	Aged	P.N. town cat					48				
-	-	healthy					12				

Bal. = ballum

Har. = hardjo

Jav. = javanica

Pom. = pomona

Balc. = balcanica

Pur. = pyrogenes

Cop. = copenhageni

Can. = canicola

Aus. = australis

M = Male

F = Female

NM = Neuter male

FS = Speyed female

P.N. = Palmerston North.

Table 16

REACTION OF POSITIVE TITRES TO DIFFERENT SEROVARs

Reactions to one serovar

<i>ballum</i>	5
<i>pomona</i>	1
<i>balcanica</i>	2
<i>copenhageni</i>	4

Reactions to two serovars

<i>hardjo/balcanica</i>	1
<i>hardjo/javanica</i>	2
<i>ballum/australis</i>	1
<i>ballum/australis</i>	1
<i>ballum/canicola</i>	1
<i>copenhageni/ballum</i>	1

Reactions to three serovars

<i>ballum/copenhageni/pyrogenes</i>	1
<i>hardjo/ballum/javanica</i>	1
<i>pomona/ballum/copenhageni</i>	1
<i>pomona/copenhageni/javanica</i>	1
<i>pomona/copenhageni/pyrogenes</i>	1
<i>hardjo/balcanica/pyrogenes</i>	1

Total number of sera with positive titres	11.11%
Reaction to one serovar	5.33%
Reaction to two serovars	3.11%
Reaction to three serovars	2.66%

1. *Ballum* serogroup

This was the most common serovar encountered in the present survey. More than three per cent (3.55%) of sera examined showed positive titres and this included the highest titre 1:768 recorded in the survey. The positive sera came from animals of different ages and from a variety of sources.

Hathaway (1978) recorded a positive titre 1:24 to *ballum* in one of eleven feral cats which he examined culturally and serologically. Initially he hypothesised that the titre may have been due to predator chain transmission from endemically infected rodents on the farm. But further work revealed no positive titres or cultures in six feral cats which he captured from the Feilding rubbish tip where the prevalence of *ballum* infection in the rats was 43%. In addition five of these cats had rat tissue and fur in their stomach content. He therefore considered the possibility of predator chain transmission was unlikely. Clark (1961) also failed to isolate *ballum* from 56 feral cats in Pennsylvania, although *ballum* occurred in house mice in that region.

The results obtained in the previous experiment (Chapter 4) have demonstrated that cats may easily become infected by ingesting infected mice. In view of these findings it may be hypothesised that the predator chain was the route of infection in the cats in the present survey. However, the number of feral cats which were examined from endemically infected areas in both the present study and by Hathaway (1978) were not large enough to reach a firm conclusion.

A further problem in interpreting these results is the possibility of fluctuation of titres as experienced in Experiment Three (Fig. 8b) and the consequent difficulty in relating the single sample obtained to the

stage of infection of the animal. Fluctuation in titre could also be expected with subsequent exposures to the organism but the serological response of the cat to repeated exposure has not been studied.

2. *Copenhageni* serovar

This was the second most common serovar detected in this survey with six (2.55%) positive titres recorded. The reservoir of *copenhageni* serovar in New Zealand is known to be rats, and it was first isolated from these animals by Kirschner and Gray (1951). These authors (1951) also recorded the first human infection with *copenhageni* in a farmer. This serovar has also been isolated from young calves showing sudden onset of clinical signs and acute toxæmia (Dodd and Brackenridge, 1960), as well as from healthy calves (Ris *et al.*, 1973). Hellstrom (1978) recorded sporadic evidence of positive sero-infection in a random survey of cattle in New Zealand and Mackintosh (1978, unpublished data) has observed positive seroreaction with *copenhageni* in a survey of hunt dogs. Brockie (1977) isolated *copenhageni* from the kidneys of *Rattus norvegicus* collected in rural rubbish tips near Tirau and Morrinsville.

Although Hathaway (1978) found no evidence of infection with this serovar in his wildlife survey covering the southern part of the North Island, it nevertheless seems likely that a predator chain was the cause of infection in the present study.

Infectivity of *copenhageni* serogroup to the cat has been experimentally demonstrated (Wirth, 1937; Otten *et al.*, 1954; and Modric, 1974).

3. *Hebdomadis* serogroup

Serovars *hardjo* and *balcanica* are members of the *hebdomadis* serogroup both of which have been isolated in New Zealand. They are antigenically closely related and routine serological tests cannot differentiate between the two organisms. In this survey 2.22% of sera were positive to *hardjo* and 0.88% positive to *balcanica*. *Hardjo* serovar has been isolated from cattle (Lake, 1973) and humans (Christmas *et al.*, 1974b). *Balcanica* serovar has so far only been isolated from the possum (*Trichosurus vulpecula*) (Marshall *et al.*, 1976) and this species is the maintenance host for the serovar (Hathaway, 1978). Three of the *hardjo* positive samples were obtained from a country area (Feilding) and *hardjo* has been reported to be prevalent in farm animals in this area (Hellstrom, 1978). A titre of 1:200 to *hardjo* has been recorded previously from a farm cat (Anon, 1972) in New Zealand and positive titres to *hebdomadis* serogroup have been reported in cats by numerous overseas workers (Table 17).

The two titres to *balcanica* which did not cross-react with *hardjo* were low 1:48 and 1:12 but may be regarded as indicative of present or past infection. In Experiment One the cat infected with *hardjo* produced a good serological response, but the cat infected with *balcanica* did not respond serologically and no positive cultures were obtained. Further experiments should therefore be undertaken to investigate the susceptibility of the cat to this serovar.

4. *Pomona* serovar

This serovar has not been found in any small free living species in New Zealand apart from cats. In this survey four cats (1.77%) showed positive titres to *pomona* and the highest titre of 1:384 was obtained

from an animal suspected to have nephritis by the referring veterinarian. *Leptospira pomona* was first isolated from a domestic cat in New Zealand by Harkness *et al.* (1970). A positive titre of 1:3000 has been reported by Rees (1964) from a cat being treated for nephritis and a titre of 1:96 was found in a feral cat by Hathaway (1978).

In New Zealand *pomona* infection is usually associated with farm animals particularly the pig (de Jong and Fowler, unpublished 1968) as well as cattle and dogs (Te Punga and Bishop, 1953), sheep (Hartley, 1952) and humans (Christmas *et al.*, 1974a). Ryan (1978) estimated that 53% of the pigs he sampled had been infected with *pomona*. In this survey one cat with a titre of 1:24 came from a piggery in which *pomona* had been isolated from the pigs. This suggests that *pomona* infection might occur through contact with infected animals or farm premises. It has been demonstrated that this serovar can survive in an acidic soil (pH 5.5) at least 42 days (Hellstrom and Marshall, 1978).

The infectivity of *pomona* has been discussed previously (Chapter 2). The titres which have been reported in the majority of experimental infections to date (Ferris and Andrews, 1961; Modric, 1974; and Chapter 2) appear to be lower than the titres which were observed in most naturally infected cats (Rees, 1964; Harkness *et al.*, 1970).

5. *Canicola* serogroup

Two sera were positive to *canicola*, one of which cross reacted with *ballum* and the other with *javanica* (Table 15). *Canicola* serotype has not been isolated in New Zealand but Kirschner and Gray (1951) recorded positive sero-reactions to *canicola* in three dogs.

van der Hoeden (1958b) has shown that in the early stages of some leptospiral infections, MA titres are encountered for one or more

heterologous serovars which are as high or higher than the homologous titre. In the present experiments *canicola* cross-reacted with *ballum* several times. It therefore seems likely the titres obtained represented agglutination with heterologous antigen.

In conclusion, the results of this survey show that *ballum* and *copenhageni* are the most prevalent serovars in cats in New Zealand. The reservoir hosts for both of these serovars in this country is known to be rodents while the maintenance hosts for all other serovars apart from *balcanica* are domestic animals. Although the majority of the cats from which sera were tested in this survey were house cats, these results strongly emphasise the relationship between the cat and rodents in transmission of leptospiral infection.

In comparison with the other surveys which have been conducted overseas (Table 17), the prevalence of the positive titres in cats in New Zealand is high. Cats in this country also appear to be unique in having a high prevalence of infection with *ballum* serogroup as well as being infected with a variety of other serovars. Since the majority of the cats with positive titres were apparently healthy the results are in agreement with those of the previous experiments (Chapter 2 to 4) in which no obvious relationship between positive titre and clinical disease could be established.

As the cat may become a carrier without having detectable antibodies and because titres when present tend to decline rapidly, a positive titre in the cat is likely to be significant, and a negative titre may not express negation of previous exposure.

Table 17

FELINE SEROLOGICAL SURVEYS OF LEPTOSPIRAL SEROVARS

Author	Country	District	Number of Cases tested	Serotype used for test	Positive Serotype	Total Positive	Percent.
Esseveld, Collier & Mochtar (1940)	Indonesia	Java	500 K + B A.T.	2,9	2,9	18/310 Agg+ 14/343 cul.+	5.80% 4.08%
Greene (1941)	U.S.A.	California	100 macro- scopic A.T.	1,3	1	1	1%
Alicata & Breaks (1943)	U.S.A.	Hawaii	100 A.T.	1,3		0	
Weissflog (1952)	Germany	Hamburg	165	1,3,7,9		0	
Semmel (1954)	Germany	Munich	128 C.F.T. 86 M.A.T.		3, 8a	1 8	0.78% 9.30%
Otten, Henze & Goethe (1954)	Germany	Hamburg	236 C.F.T. A.T.	1,3,6,7,8a,9	3,8a	2 8	0.84% 3.38%
Mochmann (1955)	Germany		15	3	3	2	13.3%
Broom (1955)	England		180	1,3,6,7,8a,9	1,3,7	15	8.33%
Amonsenskova (1955)	Russia	Leningrad	358	1,3	1	3	0.83%
Hemsley (1956)	England		180	1,3,6,7,8a,9	3	3	1.66%
Fennestad (1956)	Denmark				1,2a,8c,9		
Murphy <i>et al.</i> (1958)	U.S.A.	Pennsyl.	350 M.A.T	1,2,3,4,5,6,7, 8a,9,10,10a,10b	6,7,10,10a,10b	17	4.9%
Vysotskii <i>et al.</i> (1960)	Russia	Vladivostok	30	11 antigens	3,7	3	10.0%
Anon (1960)	China	Kwangtung	28			3	10.71%
Clark (1961)	U.S.A.	Pennsyl.	56	4		0	
Ferris & Andrews (1961)	U.S.A.	Sth. Illinois	21		6	1	4.76%
Jones (1964)	U.S.A.		139 M.A.T.	1,3,6	1,6	2	1.43%

(cont)

Table 17 (cont)

Author	Country	District	Number of Cases Tested	Serotype used for test	Positive Serotype	Total Positive	Percent.
Lucke & Crowther (1965)	England	Bristol	118	1,2,3,4,5,6,7,8a, 8b,9,10,11a,12	1,2,3,6,8b,11a	8	6.8%
Freudiger (1969)	Switzerland	Bern	106	1,3,11,6,7,8a,12	1,6,7	4	3.77%
Carlos <i>et al.</i> (1971)	Philippines		8	1,3,4,9,7,5,10,6, 8,11,13,2,10a,8a	7	1	12.5%
Watson & Wannan (1973)			100RSAT	1,3,6,7,8,8a,13	7,8d,8e	6	6.0%
Hathaway (1978)	New Zealand	P.N.	11 K + B		4,6	2 Agg+	18.18%

KEY

K = Kidney
 B = Blood
 A.T. = Agglutination test
 C.F.T. = Complement Fixation Test
 M.A.T. = Micro Agglutination Test
 Agg. = Agglutination
 Cul.+ = Positive tissue cultures

1. *icterohaemorrhagiae (copenhageni)*
2. *javanica*
3. *canicola*
4. *ballum*
5. *pyrogenes*
6. *pomona*
7. *grippotyphosa*
8. *hebdomadis*; serotype a) *sejroe*; b) *mini*;
c) *sarkoebing*; d) *hebdomadis*;
e) *hardjo*; f) *balcanica*
9. *bataviae*
10. *autumnalis*, serotype a) *djasiman*; b) *sentot*
11. *australis*, serotype a) *bratislava*
12. *hyos* 13. *tarassovi*

GENERAL DISCUSSION AND CONCLUSION

The main aim of the present study was to determine the role of the cat as a urinary carrier and in the transmission of leptospirosis to both man and other animals. The initial experiment with intraperitoneal (I.P.) infection of five of the six leptospiral serovars present in New Zealand established that the cats can become infected with a variety of these serovars. This finding was in agreement with the results of the survey of domestic and feral cats which showed serological reaction to all serovars that exist in this country except *tarassovi*. The prevalence of serological titre appeared to be high in New Zealand compared to other countries. This is probably related to the fact that all titres of 1:12 and above were recorded, also these results cannot be compared with most other surveys because firstly; many of the overseas studies have included feral cats only (Esseveld, Collier and Mochtar, 1940; Ferris and Andrews, 1965; and Clark, 1961) or cats with clinical disease such as nephritis (Hemsley, 1956; Lucke and Crowther, 1965) and in some cases there is no accurate data available about the cats which were surveyed. Secondly, the initial dilution of their serum samples were often not given. However, if it is assumed that the initial dilution in the overseas surveys had been 1:100 the number of positive animals obtained in this survey (Chapter 5) is reduced from 11.11% to 3.11% which is a similar figure to that found in some other countries (Table 17).

As *ballum* infection is common in New Zealand rodents, one would expect a high prevalence of this serovar in cats. Although the serological survey showed that *ballum* is the most prevalent serovar in cats, the percentage with titres to this serovar (3.55%) is not very high. An experimental food chain transmission (Chapter 4) was successfully demonstrated by feeding infected mice to cats. It was shown that small

pieces of viscera (bladder and kidneys of an infected mouse) produce a more severe infection than a whole infected mouse presumably due to closer contact with the upper alimentary tract. In the natural state, cats tend to eat their food greedily and consequently it is often poorly masticated. The large boluses of food produced by this type of feeding remain in the stomach for a longer period of time than smaller portions (Thomas, 1957), these boluses may be large enough to protect leptospires from complete destruction by gastric acids and the number of organisms which survive may be enough to produce a carrier state. The serological and cultural studies of a limited number of feral cats in which a prey-predator chain was clearly shown by the presence of rat fur and organs in their stomachs revealed a very low prevalence of leptospirosis (Hathaway, 1978 and the author). The cats did not yield any positive leptospiral cultures and only two low titres, 1:24 and 1:96 were obtained against *ballum* and *pomona* respectively.

The success in experimental prey-predator transmission studies may be due to the age of the cats used in the study, these were young animals (six months) and younger animals are more susceptible than older ones (Chernukcha *et al.*, 1974; and Herbert, 1974). On the other hand the laboratory mice which were infected by the I.P. route are different from the naturally infected rodents. The number of leptospires in the field infected rodents may be lower than in experimentally infected ones. According to the results of the second experiment (Chapter 3) by decreasing the infective dose the cats responded serologically with lower titres, although they became carriers even with this low infective dose.

The failure to obtain a higher prevalence of feline leptospirosis especially with *Ballum* serogroup in the field survey may be due to a

number of factors. Firstly; most of the cats surveyed were not very young and the majority were house cats. Secondly; cats generally do not respond to a leptospiral infection with a high serological titre and the titres decline rapidly. In the first experiment with different leptospiral serovars all the cats showed a drop in their titre a month p.i. Although the limited value of the results from one individual animal per treatment in this experiment was discussed, it is worth mentioning that this decline was more rapid for *ballum*, *hardjo* and *tarassovi* than for *pomona*. This rapid decline also has been previously suggested by Frendiger (1969) to be a major reason for the infrequent diagnosis of leptospirosis. *Ballum* studies (Chapters 3 and 4) showed that M.A. titres after reaching their maximum level declined rapidly. It is therefore possible for cats to become urinary carriers with no detectable M.A. titre or to have a detectable titre for a long period of time.

Sera from a variety of animal species remain capable of inhibiting the growth of leptospires after the disappearance of the M.A. titre (Tripathy *et al.*, 1972 and 1973; and Hathaway, 1979), whereas sera obtained from known infected cats in this study produced no inhibitory effect even when low titres were present. Therefore another factor which must be considered is the possibility of reinfection especially when considering the serological findings. Although this has not been studied in the cat it should be borne in mind.

With the knowledge of all these facts it can be concluded that the results of serological surveys are of limited value as an indicator of the prevalence of this disease as a negative titre might not indicate the absence of infection. However, serological findings should wherever possible be considered in conjunction with cultural findings. In the diagnosis of feline leptospirosis due to *Ballum* serogroup infection,

clinical and pathological data are of little value. The histological changes are not severe and the foci of interstitial nephritis are very small and can easily be missed in random sections of kidney. Although eleven kidneys yielded positive cultures, leptospires could be demonstrated in only one by the silver impregnation of tissue sections. Therefore demonstration of the leptospires using this method (Warthin Starry) is of very little value in detecting an infection.

The fact that infected cats are symptomless means they could be considered as a public health hazard. Babudieri (1958) postulated that in those species of animal which have a strongly acidic urine, the leptospires which are excreted may be either killed or rendered non-pathogenic. According to Kaufmann (1976) and to the annual report of the Centre for Disease Control (C.D.C.) (1976) a large number of cases of human leptospirosis are presumed to be transmitted from dogs. If this is true then cats may present an equal hazard since dogs and cats have the same urinary pH. The cat has also been reported to be a source of several cases of human leptospirosis (Bock, 1954; and Kaufmann, 1976). Infection in these circumstances could only be explained if one assumes that these animals were excreting sufficiently large numbers of leptospires in their urine to allow a few of them to survive and become the source of infection.

Different workers have attributed the survival of leptospires outside the host to a variety of environmental factors, and their individual estimates of the time of survival are dependent on which factors they considered to be important. Karaseva *et al.* (1973) demonstrated the survival of pathogenic leptospires in the humid soil up to 15 days. Hellstrom and Marshall (1978) found that *pomona* may survive for up to 42 days in the soil with a pH of 5.5. Leptospires are also very

susceptible to drying and do not survive more than 30 minutes in dry conditions (Okazaki and Ringen, 1957). Although most of the studies to date have concentrated on the survival of *pomona*, it seems likely that similar survival times apply for the *ballum* serogroup organisms.

Thus it is possible that carrier cats may transmit the disease to more susceptible animals or even humans and despite the fact that the cat is relatively susceptible to infection with *ballum* serogroup and may readily develop a carrier state there are several factors that minimise the hazard of cats from the public health stand point.

1. The cat is not a main reservoir host for *ballum* serogroup organisms although the results of experimental infection and field studies of feral cats suggest that this animal may be a potential host.
2. Intensity and duration of leptospiuria is less than for some other animals such as mice and rats.
3. Acid pH conditions of cats urine may reduce the infectivity of shed organisms.
4. Cats are very clean creatures in their habits and they usually cover their urine and faeces with soil. Rather than urinating on wet surfaces they appear to prefer dry places where leptospire would not survive for very long.

In conclusion it is evident that more research into the immunology and epidemiology of feline leptospirosis is needed to clarify the role of the cat as a specific host in transmitting different leptospiral serovars to susceptible animals and human beings.

APPENDIX I

EMJH MEDIUM

This medium was prepared according to the method of the Johnson-Harris modification of EMJH (Johnson and Seiter, 1977). All the glassware used for formulation of the medium and the cultivation of the leptospire was thoroughly washed in an automatic laboratory washing machine and finally rinsed with distilled water before being autoclaved at 121°C for 20 minutes.

Stock solution of the following chemicals were made for each batch of media:

Stock Solution		grams per 100 ml deionised distilled water
NH ₄ Cl	(B.D.H.)*	25.0
ZnSO ₄ . 7H ₂ O	(M&B)**	0.4
MgCl ₂ . 6H ₂ O	(Analar)***	1.5
CaCl ₂ 2H ₂ O	(Analar)	1.5
FeSO ₄ . 7H ₂ O	(Analar)	0.5
CuSO ₄ . 5H ₂ O	(Analar)	0.3
Sodium Pyruvate	(B.D.H.)	10.0
Glycerol	(B.D.H.)	10.0
Tween 80	(Sigma)****	10.0
Thiamine.HCl.	(Sigma)	0.5
Cyanocobalamin	(Sigma)	0.02

* British Drug House, Poole, England.

** May and Baker Ltd, Dagenham, England.

*** B.D.H. Chemicals Ltd, Poole, England.

**** Sigma Chemical Co., P.O. Box 14508, St Louis, Missouri 63178, U.S.A.

The pH of these stock solutions was not adjusted and the FeSO_4 solution were made freshly every time.

Albumin supplement was prepared by dissolving 100 g Bovine albumin fraction V powder (Pentex)* in 500 ml deionised water in a one litre conical flask. While the albumin solution was being stirred by using a magnetic stirrer the following stock solutions were slowly added.

CaCl_2	10 ml
MgCl_2	10 ml
ZnSO_4	10 ml
CuSO_4	1 ml
FeSO_4	100 ml
Cyanocobalamin	10 ml
Tween 80	125 ml

The pH was adjusted to 7.4 using a pH meter (Beckman^{***}) when the powder completely dissolved and the solution brought to one litre by the addition of distilled deionised water. This solution was then sterilised by filtration using a $0.22\ \mu\text{m}$ filter (Millipore)** and stored in 50 ml batches in sterile glass bottles.

Basal media : to 9960 ml deionised distilled water the following

salts were added :	KH_2PO_4	(Analar)	.3 g
	Na_2HPO_4	(Analar)	10 g
	NaCl	(Analar)	10 g

* Pentex Division, Miles Laboratories Inc.,

** Millipore Corporation, Bedford, Massachusetts 01730, U.S.A.

*** Beckman Instruments Inc., Palo Alto, California, U.S.A.

After attaining the salt solution the following stock solutions were added into it :

NH ₄ Cl	10 ml
Thiamine HCl	10 ml
Sodium Pyruvate	10 ml
Glycerol	10 ml

The pH was then adjusted to 7.4 and batches of 450 ml were autoclaved at 121°C for 20 minutes.

Liquid medium was prepared by adding 50 ml of albumin supplement to 450 ml of basal medium. Semisolid medium was made by addition of 1.5 g agar (Difco)* to one litre of basal medium and it was autoclaved at 121°C for 20 minutes, then cooled in a water bath to about 40°C before adding 50 ml of the albumin supplement with or without 5 fluorouracil (5Fu) (Sigma). All the media was dispensed in 5 ml sterile bottles under sterile conditions in lamina flow cabinet.**

Stock solution of 5Fu was prepared by adding 1.0 g of 5Fu to 50 ml of distilled water. To dissolve the 5Fu it was put in a 56°C water bath, then the pH was adjusted to 7.4 to 7.6 by the addition of 1N HCL. The solution was made up to 100 ml of adding distilled water and it was sterilised by filtration through 0.22 µm filter (Millipore). This solution was held at 4°C until required. A final concentration of 400 µg/ml and 200 µg/ml of 5Fu in semisolid media was prepared for use. All the media was incubated for three days at 37°C and three days at 30°C before use, to check for the presence of contamination.

All new batches of media tested for checking the growth of slow growing leptospirens such as *hardjo* and *balcanica*.

* Difco Laboratories, Detroit, Michigan, U.S.A.

** Bassaire, John Bass Ltd, Crawley, Sussex.

APPENDIX II

MICROSCOPIC AGGLUTINATION TEST (M.A.T.)

Each serum sample was initially diluted 1:3 with physiological saline in a flat bottom microtitre plate. These plates are called serum reference plates (SRP). They were kept at -20°C and sealed with cellophane until required.

Twenty five microlitres (μl) of saline were dispensed using a semi-automatic dispenser (minipipetter)* into a microtitre plate. An equal volume of serum ($25\ \mu\text{l}$) from the SRP's was added and mixed with the saline using a semi-automatic diluter (minidiluter)*. Eight doubling dilutions ranging from 1:12 to 1:1536 were made and each dilution was mixed for at least five seconds. After diluting the sera $25\ \mu\text{l}$ of six to ten day old leptospire antigen was dispensed with an antigen gun modified Cornwell syringe** which had a minipipetter dispensing head attached (Ryan, 1978) or by using disposable pipettes. The plates were then gently shaken to allow the antigen to mix with the diluted serum and then they were incubated for 90 minutes in the 37°C incubator. After this period of time the results were read by transferring a drop of each dilution onto a microscopic slide simultaneously by using a multiple dipper (Ryan, 1978). This was then read without a coverslip by direct darkfield examination at a magnification of 156 X. The dilution in which 50% of leptospire agglutinated was accepted as positive.

* Cook Engineering Co., Alexandria, Virginia, U.S.A.

** Becton and Dickinson & Co., Rutherford, New Jersey, U.S.A.

APPENDIX IIIGEL-FILTRATION METHOD

Cat sera were fractionated on Sephadex G-200* superfine (Pharmacia)* columns. The gel beads (1.5 - 1.7%) were swollen at 4°C for three days in phosphate buffered saline (P.B.S.) of pH 7.4 to 7.5. The stock P.B.S. was at 0.01M made of the following recipe:

1	- Na ₂ HPO ₄ (MW142)	60 g	BDH
2	- NaH ₂ PO ₄ ·2H ₂ O (MW156)	12.5 g	M&B
3	- NaCl	425 g	
4	- Five litre deionized distilled water		

The stock P.B.S. was diluted in 1/10 with deionized distilled water for using in column. During the swelling period fines were removed two or three times. The swollen gel was degassed under a vacuum of 700 mmHg 15 minutes.

The gel was then poured into a column measuring 70 cm high X 2.5 cm diameter (Pharmacia) using an attached reservoir (Pharmacia). A constant pressure head of five to ten centimetres high between outlet and inlet tubes was maintained during packing of beads using the mariote flask system. The column was operated at room temperature under constant pressure at a flow rate of approximately 20 ml/hr.

At the start of each fraction one ml of serum was dissolved in 200 mg of sucrose and carefully layered onto the top of the column. Approximately 24 fractions of 6 ml each were collected by using an automatic fraction collector (ISCO)**. The optimal density of protein in the eluent at 280 nm was continuously monitored and recorded graphically using a chart recorder (ISCO).

* Pharmacia Fine Chemicals AB, Uppsala, Sweden.

** Golden Retriever, Instrumentation Specialties Co., PO Box 5347,
Lincoln, Nebraska 68505, U.S.A.

Fractions were held at 4°C until tested using the M.A.T. test. For the M.A.T., eight serial doubling dilutions of each fractions were prepared in isotonic saline in microtitre plates and equal volume of live antigen were added to each dilution. Since on ml of serum had been applied to the column and six ml of fractions were collected the dilutions of each fraction ranged from 1:12 to 1:1572. Whole serum was also tested with each set of fractions. After incubating for 90 minutes at 37°C they were read the same as M.A. test.

APPENDIX IV

GROWTH INHIBITION TEST (G.I.T.)

The aim of this test was the investigation of the growth inhibition activity of serum for leptospira of *ballum* serogroup in cat post experimental infection with using modified method of Tripathy *et al.* (1973).

Method

All the serum samples were inactivated in water bath at 56°C for 30 minutes for removing the complement. Then they were sterilised by filtration through 0.22 µm membrane filter, and 0.05 ml of these sera put into each of a sterile culture tube. Five replicates were used for each serum sample. The media which was used was EMJH liquid media without 5Fu. One ml of this media was added into each culture tube. A five to seven day old *ballum* culture was counted with Petroff-Hausser* counting chamber and diluted with liquid media to a density of 1×10^7 organisms per ml. For each of the culture tube 0.05 ml of this culture was used.

For each set of tests five replicates of negative and positive controls were used. Negative control was one ml of EMJH liquid media plus 0.05 ml of leptospira culture and positive control was a serum of the experimentally infected cat with a high titre against *ballum* in M.A.T.

The turbidity and darkfield examination monitored after one and two weeks post inoculation at 29°C for presence or absence of leptospiral growth. More than ten leptospire per darkfield were considered as a negative and less than ten organisms per darkfield were considered as a positive for growth inhibition activity.

* C. A. Hauser and Son, Philadelphia, U.S.A.

APPENDIX V

IMMUNO-ELECTROPHORESIS (I.E.)

For antigen the serum fractions which were obtained on Sephadex G-200 gel-filtration were used. Antisera: anticat serum* and anticat IgG* produced in goats were used in this experiment. The modified technique of Scheidegger (1953), Graber, Burtin (1964) and Kulshrestha *et al.* (1969) were used as a procedure for I.E.

The 10 X 9 cm glass slides were thoroughly cleaned and rinsed in 70% alcohol to remove fatty materials. These slides were placed on a level board and 15 ml of agar gel (Ionagar**) 60°C was dispensed onto their surfaces. For preparation of the agar gel method of Williams and Chase (1971) was used.

After complete setting troughs of 5 cm long and 2 mm wide were cut in the slide parallel to the long axis. In the middle of each two troughs one well of 2-3 mm in diameter was made. These wells were filled with antigens. The electrophoresis using electrophoretic apparatus with contact wicks on 100 volts for 3½ to 4 hours.

After the electrophoretic run, the agar from the troughs were removed and they were charged with antiserum. The slides were incubated at room temperature in a moist atmosphere for 24 to 48 hours. By completing of the antigen-antibody reaction within this period of time the gels were washed with saline to remove the soluble non-reacting constituents. After washing which was continued up to seven days drying was done and the dried I.E. plates were stained according to Williams and Chase (1971) method with Amido-black.

* Miles Yeda Ltd, Kiryat Weizmann, Rehovot, Israel, distributed by Research Products Miles Laboratories Inc., Elkhart, Ind.46514, U.S.A.

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