STUDIES ON THE PATHOGENESIS OF TICK PARALYSIS

by

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Apart from the assistance acknowledged above this thesis represents my own work.

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GENERAL SUMMARY

1. The mechanisms involved in the pathogenesis of tick paralysis have been investigated \textit{in vivo} in paralysed dogs and \textit{in vitro} using nerve-muscle preparations removed from paralysed mice.

2. Neurologic and electromyographic examination of paralysed dogs indicated that tick paralysis involved a failure of neuromuscular transmission. No abnormality of conduction in the nerve trunk could be demonstrated and it was considered that the lesion was likely to be at or near the neuromuscular junction.

3. Nerve-muscle preparations from affected mice were found to be paralysed when examined \textit{in vitro}. The paralysis was found to be temperature dependent. Results of these experiments supported the contention that the lesion was near the neuromuscular junction.

4. Neuromuscular transmission was examined in preparations from paralysed mice. No abnormality of nerve conduction could be demonstrated. The release of acetylcholine in response to nerve stimulation was depressed due to a reduction in quantal content rather than quantal size. Lowering the temperature of the preparation partially reversed this effect. These results indicated that tick paralysis is due to an abnormality in the mechanism which couples nerve terminal depolarisation and acetylcholine secretion.

5. There is some indication that crude toxin extracted from partially engorged ticks could affect nerve-muscle preparations incubated in it.
6. Apart from some secondary changes no significant morphological abnormalities could be demonstrated in nerve fibres, muscle fibres or neuromuscular junctions from tick paralysed mice.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>General Summary</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1. Literature review—The distribution, aetiology and epidemiology of tick paralysis.</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 2. Maintenance of <em>I. holocyclus</em> in the laboratory.</td>
<td>12</td>
</tr>
<tr>
<td>Chapter 3. The neurological syndrome produced by <em>I. holocyclus</em>.</td>
<td>29</td>
</tr>
<tr>
<td>Chapter 4. Nerve conduction studies on dogs paralysed by <em>I. holocyclus</em>.</td>
<td>45</td>
</tr>
<tr>
<td>Chapter 5. Development of an <em>in vitro</em> preparation—Muscle tension experiments.</td>
<td>63</td>
</tr>
<tr>
<td>Chapter 6. The pathophysiology of the neuromuscular junction in tick paralysis.</td>
<td>79</td>
</tr>
<tr>
<td>Chapter 7. Morphology of the neuromuscular tissues in tick paralysis.</td>
<td>115</td>
</tr>
<tr>
<td>Chapter 8. Activity of the toxin <em>in vitro</em>.</td>
<td>133</td>
</tr>
<tr>
<td>Chapter 9. General discussion.</td>
<td>141</td>
</tr>
<tr>
<td>References</td>
<td>151</td>
</tr>
</tbody>
</table>
# CHAPTER 1

LITERATURE REVIEW—THE DISTRIBUTION, AETIOLOGY AND EPIDEMIOLOGY OF TICK PARALYSIS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>1.2</td>
<td>The distribution of tick paralysis throughout the world, the species of ticks involved and susceptible host species.</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>The aetiology of tick paralysis.</td>
<td>6</td>
</tr>
<tr>
<td>1.4</td>
<td>The epidemiology of tick paralysis in Australia.</td>
<td>9</td>
</tr>
<tr>
<td>1.5</td>
<td>Summary.</td>
<td>11</td>
</tr>
</tbody>
</table>
CHAPTER 1. LITERATURE REVIEW-THE DISTRIBUTION, AETIOLOGY AND EPIDEMIOLOGY OF TICK PARALYSIS.

1.1 Introduction

Many arthropods are capable of paralysing their victims (Karlsson, 1973). They may do this for purposes of defence or to capture their prey. In either case it is not difficult to understand why they possess this ability. Ticks, however, do not need to immobilise their hosts to feed, and to kill them is a positive disadvantage. Yet, strangely, many ticks are capable of both paralysing and killing their host, often before they have fed to completion. Why some ticks should have evolved with this ability is only one of the many questions relating to tick paralysis which are still to be answered.

Tick paralysis has been known as an entity since the late 19th Century, and from time to time has been investigated by scientists and clinicians. Considering its prevalence, it has received relatively little attention in Australia but in North America has been investigated fairly intensively. Despite these efforts, what might be regarded as one of the most important problems, namely the mechanism of the paralysis, remains to be solved.

The experiments to be described in this thesis were carried out in an attempt to provide some understanding of the pathogenesis of this puzzling disease.

1.2 The distribution of tick paralysis throughout the world, the tick species involved and susceptible host species

The highest incidence and most severe form of tick paralysis occur in Australia.
The disease is commonly encountered along a narrow coastal strip of eastern Australia (Seddon, 1951) and usually results from the engorgement, on the host, of *Ixodes holocyclus* Neumann (Dodd, 1921; Clunies Ross, 1926). Probably less commonly, it may be produced by *Ixodes hirsti* Hassall and *Ixodes cornuata* Roberts (Roberts, 1959, 1961, 1970; Mason, Kemp and King, 1974). As well as the adult female *I. holocyclus*, both the nymphs and the larvae can cause paralysis if present in sufficient numbers (Oxer and Ricardo, 1942).

Many species of warm blooded animals can act as host to this group of ticks (Roberts, 1970) and paralysis has been reported in man (Cleland, 1912; Eaton, 1913; Strickland, 1915; Hamilton, 1940), dogs (Dodd, 1921; Clunies Ross, 1926), cats (Clunies Ross, 1924; Roberts, 1961), foals (Bootes, 1962), sheep (Sloan, 1968), goats, pigs, calves, poultry (Seddon, 1951; Knott, 1961) and a variety of birds (Ranby, 1960; Derrick and Domrow, 1965; Roberts, 1970).

Amongst native fauna the tick has been found infesting a variety of species, in particular the short-nosed bandicoot (*Isoodon macrourus*) and the long-nosed bandicoot (*Perameles nasuta*) (Doube, 1972). Although these animals appear to be usually immune to the effects of the tick, they have occasionally been observed to be paralysed (Oxer and Ricardo, 1942).

Laboratory animals, including rats (Koch, 1967), mice (Murray and Koch, 1969) and guinea pigs (Oxer and Ricardo, 1942; Bagnall, 1975) are also susceptible to the disease.

Deaths due to tick paralysis are not uncommon and several fatal cases have been reported in humans, usually children (Ferguson, 1924; Hamilton, 1940).
Tick paralysis is by no means confined to Australia, and similar syndromes have been reported in several countries. In South Africa Stampa (1959) has classified the types of tick paralysis which occur there as Karoo tick paralysis, caused by *Ixodes rubicundus* Neumann, which affects sheep, goats, and cattle and less commonly dogs and jackals; Spring lamb paralysis, caused by *Rhipicephalus evertsi* Neumann, which affects lambs, occasionally adult sheep and calves; and tick paralysis in man caused by *Rhipicephalus simus* Koch, *Hyalomma truncatum* Koch and *I. rubicundus*. According to Neitz (1962) *R. evertsi* can also paralyse dogs and calves and *Rhipicentor nuttalli* Cooper and Robinson can paralyse dogs.

In Crete Blanc and Caminopetros (1924) reported paralysis of sheep, dogs and cats by *Ixodes ricinus* (L) and *Haemaphysalis punctata* (Can. and Fraz.), while in Macedonia and Bulgaria, *Haemaphysalis inermis* Birula has been reported as causing paralysis in sheep, goats, a calf and chickens (Pavlov, 1940; Pavlov and Miljowski, 1942). In Turkey, *I. ricinus* has caused paralysis in sheep, goats and calves (Kurtpinar, 1960). In Russia, *I. ricinus* has also paralysed foals (Balabekian, 1954) and *Ixodes crenulatus* Koch has paralysed sheep (Pomerantzev, 1950). *Dermacentor* spp. are said to have produced paralysis in cattle, goats and sheep in Italy (Tasselli, 1958) and, as well, Veneroni (1928) has reported two human cases in Italy, probably caused by *R. simus*. *Ixodes hexagonus* Leach has been reported, rarely, to cause paralysis in man in England (Neitz, 1962) and France (Garin and Bujadoux, 1923). In India *Haemaphysalis kutchensis* has affected rabbits (Singh, 1963) and *Dermacentor auratus* Supino has been reported as causing limping in domestic animals (Hoogstraal, 1970).
In Uruguay, Vogelsang (1925) reported two cases of paralysis in dogs caused by *Amblyomma maculatum* Koch and in Venezuela Viloria (1954) recorded a case in a dog caused by *R. sanguineus*.

Apart from Australia, the highest incidence of tick paralysis is certainly in North America. The most important species involved is *Dermacentor andersoni* Stiles (Gregson, 1973) which causes the disease in British Columbia and Alberta, in Canada, and in the north-western area of the United States (Muth, 1945; Jellison, Stoenner, Kramis and Beardmore, 1951). Occasional outbreaks have been reported in California, particularly in recent years, caused by *Dermacentor occidentalis* Marx (Emminger, 1951; Loomis and Bushnell, 1968) and cases due to *D. variabilis* Say occur in the eastern United States (Costa, 1952). Single reports of paralysis caused by *Amblyomma maculatum* Koch (Swartzwelder and Seabury, 1947), *Haemaphysalis cinnabarina* Packard (Todd, 1919), *Ixodes scapularis* Say and *Ixodes brunneus* Koch (Gregson, 1973) have appeared. British Columbia appears to have by far the highest incidence of the disease in North America and Gregson (1966) has reported on 189 outbreaks in that province.

Host species affected in North America include man (McCornack, 1921; Mail and Gregson, 1938; Phillips and Murphy, 1950; Costa, 1952; Adler, 1966) cattle, sheep, dogs (Gregson, 1966) guinea pigs, ground hogs (*Marmota* spp) ground squirrels (*Citellus* spp) (Gregson, 1958; Hughes and Philip, 1958) rhesus monkeys (Hughes and Philip, 1958) and buffalo (*Bison b. bison*) (Gregson, 1958).

Deaths due to *D. andersoni* are relatively common and Schmitt, Bowmer and Gregson (1969) have collected records of 30 deaths in humans in British Columbia between 1900 and 1968.
The true importance of this disease is difficult to assess. Certainly in Australia it is so common in the endemic areas that cases, at least in domestic animals, are usually not reported. In the opinion of the author the number of pet animals treated would amount to thousands each year.

1.3 The aetiology of tick paralysis

Ticks and other arthropods have long been recognised as important vectors of infectious diseases, and it is said that 80% of all infectious disease is arthropod borne (Soulsby and Harvey, 1972). It is known that ticks can transmit pathogenic organisms of various kinds, including protozoa, bacteria and viruses (Arthur, 1962). It is therefore not surprising that early investigators considered the possibility of an infectious agent, transmitted by the ticks, as the true cause of tick paralysis.

Early attempts to demonstrate an infectious agent were made by Hadwen (1913). Although he considered the disease most likely to be due to a toxin elaborated by the female tick, particularly during its period of rapid engorgement, he recognised that the delay in onset of symptoms could represent the incubation period of an infectious disease. The fact that some ticks, although feeding satisfactorily, do not produce paralysis was also considered to be suggestive of an infectious agent. To test this possibility, Hadwen inoculated lambs and guinea pigs with blood and homogenised tissue from lambs paralysed by *D. andersoni*, as well as with homogenised ticks, both fed and unfed. No infectious agent could be demonstrated.

Similar experiments were carried out in Australia using *I. holocyclus* (Dodd, 1921; Ferguson, 1924; Clunies Ross, 1926). Again, no direct evidence of an infectious organism was found.
The alternate view, namely that the tick produces a neurotoxin, has attracted considerable support and there is a good deal of circumstantial evidence in its favour. The period between the attachment of the tick and the onset of paralysis varies, and is related to the final rapid feeding activity of the tick (Hadwen, 1913; Clunies Ross, 1926). It was thought that this might be due to rapid secretion of toxin during that period. In addition, recovery is more rapid if the tick is removed early in the course of the paralysis and this also suggests that a toxin is responsible for the disease.

Still further evidence for a toxin produced by *I. holocyclus* was provided by Murray and Koch (1969). They showed that an *I. holocyclus* female, having killed one mouse, could be transferred to a second, which was killed more quickly than the first. This could be repeated until as many as five mice had been paralysed, and the time required to kill the final mouse had been reduced to 12 to 18 hours. A similar phenomenon is associated with *D. andersoni* (Emmons and McLennan, 1960). This finding has been taken as evidence of increasing toxin production as the tick engorges.

The finding that an emulsion prepared from the salivary glands of a partly engorged female *I. holocyclus* could produce paralysis and death in mice provided the first definitive evidence of the existence of a toxin secreted by the tick (Clunies Ross, 1926, 1935). The extract from two and a half to three glands from five-day engorged ticks was found to be the minimal lethal dose for mice. If three or four-day engorged ticks were used, more glands were required, supporting the contention that the onset of paralysis was related to increased toxin secretion in the later stages of engorgement.
Clunies Ross (1935) also demonstrated that large numbers of female *I. holocyclus*, applied to a small dog, could produce paralysis more quickly than usual.

Further knowledge of the nature of the toxin was not obtained until 1966, when Kaire described the isolation and partial purification of "tick toxin" from *I. holocyclus*. Replete ticks, fed on dogs being used to produce hyperimmune serum, were homogenised and the extract yielded a water soluble toxin. This toxin could be partially purified by chromatography on DEAE cellulose. The partially purified fraction was destroyed by heating at 100°C for 15 minutes but withstood 75°C for 10 minutes. It was reported to be stable in HCl at pH 3 and NaOH at pH 9. When the active fraction, which was originally non-dialysable, was treated with papain a proportion of the activity could be dialysed.

These findings suggested that the toxin might be a protein. Unfortunately, many of the findings have apparently not been confirmed by other workers. Goodrich (1976) has found that it is consistently possible to extract a toxic substance from ticks fed for four days on mice but has found some of Kaire's results impossible to repeat. It was found that there was only a small yield of toxin from replete ticks fed on immune bandicoots and that none at all could be extracted from replete ticks fed on hyperimmune dogs. It is not yet clear whether this failure is directly related to the immune status of the host but Goodrich is of the opinion that it is. Goodrich does, despite these inconsistencies, agree that the toxin is probably a protein or polypeptide and believes that it has a molecular weight in the region of 100,000.
Several workers have shown that extracts from the eggs of a wide variety of tick species are toxic (Regendanz and Reichenow, 1931; Mlinac and Oswald, 1936; Oswald, 1938; de Meillon, 1942; Riek, 1957; Gregson, 1973). The general conclusion of these authors was that the toxic substances present in tick eggs were not identical to that which produces tick paralysis.

Although it is apparent from these reports that current opinion favours a toxin as the cause of tick paralysis, its exact nature still remains to be elucidated.

1.4 The epidemiology of tick paralysis in Australia

The seasonal occurrence and geographic distribution of tick paralysis is determined by the biological requirements of the tick. As the detailed biology of *I. holocyclus* is discussed in Chapter 2 only the relevant factors will be mentioned here.

*I. holocyclus* is said to be found along a narrow coastal strip of Queensland and New South Wales, parts of Victoria and the east coast of Tasmania (Seddon, 1951; Roberts, 1960). This strip is usually no more than 10 miles wide but in southern Queensland and northern New South Wales the tick tends to extend a little further inland. There is some disagreement on the presence of *I. holocyclus* in Tasmania, some authors claiming that it does not occur there (Mason, Kemp and King, 1974).

The distribution of *I. holocyclus* is also strongly influenced by the presence or absence of its principal host, the bandicoot (either *Isoodon macrourus* or *Perameles nasuta*). Doube (1975) has discussed the biotope of bandicoots in relation to *I. holocyclus*. 
There is a marked seasonal incidence of the three instars of *I. holocyclyus* (Doube, 1975; Bagnall and Doube, 1975). Although present in low numbers at all times of the year the adult female is most common in spring and early summer, the nymphs in autumn and winter and the larvae in summer and autumn. As the adult female most commonly causes the disease (Clunies Ross, 1935) paralysis occurs most frequently in spring and summer. Only sporadic cases are seen at other times due either to adults or occasionally to nymphs (Clunies Ross, 1932).

Less still is known of the specific host-parasite interactions which determine whether a particular tick will paralyse a particular host. In Australia there is little evidence for non-pathogenic populations of *I. holocyclyus*, although individual ticks sometimes fail to produce paralysis. In North America and South Africa, however, *D. andersoni* and *I. rubicundus* commonly cause paralysis only in restricted parts of their overall distribution (Gregson, 1973; Stampa, 1959). The reasons for this are not known.

In Australia, host immunity plays an important role in determining the susceptibility of animals to the disease. Immunity may be against the toxin and requires multiple exposures to acquire (Clunies Ross, 1935). Cutaneous immunity, which affects tick feeding, may also occur (Bagnall, 1975). Cutaneous immunity appears to be of importance in protecting cattle (Doube, 1975). It is partly for this reason that calves, and not adult cattle are more often affected.

It is apparent that although much remains to be learned of this disease, an understanding of the factors influencing its occurrence can be useful in limiting losses. In particular, pastures should be kept clear of dense undergrowth such as lantana which provides a
refuge for bandicoots. Calving where possible should not correspond with the peak incidence of the adult stages of the tick. Allowing sensitisation of calves by larvae and nymphs should confer cutaneous immunity without producing paralysis.

1.5 Summary

1. Tick paralysis has occurred in many countries but is most common in Australia, North America and South Africa.
2. Many species of tick have been implicated in the disease, *Ixodes holocyclus*, *Dermacentor andersoni* and *Ixodes rubicundus* being the most important.
3. Available evidence favours a toxin as the agent causing paralysis.
4. The distribution of the disease and its seasonal incidence depend on the availability of natural hosts, the seasonal occurrence of the tick and its environmental demands.
CHAPTER 2
MAINTENANCE OF IXODES HOLOCYCLUS IN THE LABORATORY

2.1 Introduction 13
2.2 The biology of *Ixodes holocyclus* 13
2.3 Materials and methods 17
2.4 Observations 20
2.5 Discussion 26
2.6 Summary
CHAPTER 2. MAINTENANCE OF IXODES HOLOCYCLES IN THE LABORATORY

2.1 Introduction

Only limited accounts of the maintenance of Ixodes holocycles in culture have been published. The earliest of these was that of Clunies Ross (1924) in which he reported limited success in keeping the tick in the laboratory. Oxer and Ricardo (1942) were more successful. They maintained all stages in culture by feeding larvae on guinea pigs and nymphs on bandicoots. Bagnall (1975) also kept the tick successfully and reported on methods of producing known quantities of the larvae for experimental purposes.

As the prime purpose of keeping I. holocycles in culture in this case was to provide ticks for use in investigation of the pathogenesis of tick paralysis, detailed studies of culture procedures were not undertaken. However some observations are recorded which may assist future workers in establishing colonies.

The successful culture of I. holocycles in the laboratory depends on a thorough understanding of the biology of the tick which is reviewed in some detail.

2.2 The biology of Ixodes holocycles

Classification

According to Roberts (1959) I. holocycles forms part of a complex including also Ixodes cordipier Neumann, Ixodes cornuata Roberts, Ixodes hirsti Hassall and a new species since named Ixodes trichosuri Roberts. I. cordipier was reported to occur only in north Queensland and New Guinea, I. hirsti from Sydney south into Victoria and Tasmania, I. cornuata from south of Milton to Victoria (including
Lakes Entrance) and Tasmania while *I. trichosuri* had only been collected in the Sydney area. The distribution of *I. holocyclus* was described in Chapter 1.

In 1961, Roberts reported the occurrence of *I. hirsti* in south-east South Australia.

*I. holocyclus* was first described by Neumann (1899) but at this time the larvae were not described, and the nymphs described were collected in India.

Clunies Ross (1924) redescribed the species in detail including unfed and fed females, nymphs and larvae, and adult males. The descriptions vary somewhat from those of Neumann, particularly in relation to the nymphs. Descriptions have also been published by Roberts (1960, 1970).

**Life Cycle**

Clunies Ross (1924) showed that the tick had a three-stage life cycle, the stages being larvae, nymphs and adults, each engorging on a separate host. Although he was not successful in feeding the larvae in the laboratory Clunies Ross was able to work out most of the details of the life cycle. These are summarised below.

Replete females, after detaching from the host, fall to the ground and after 11 to 20 days begin to lay eggs. Oviposition continues for 16 to 34 days with the majority of the eggs being laid in the early stages. Clunies Ross found a maximum egg yield of about 2500 but Bagnall (1975) has observed as many as 6000 laid by a large female. After completion of oviposition the female dies.
Eggs hatch after 49 to 61 days and larvae, which are six-legged, emerge. After hardening for about one week the larvae become active and will attach to a host. Larvae feed for four to five days then detach and fall to the ground.

After a period of 20 to 40 days eight-legged nymphs emerge, and harden for six to eight days before being willing to attach to a host. Nymphs feed for four to seven days with the majority detaching after five days. Fed nymphs undergo the second moult after a resting period of 20 to 65 days.

Adults emerge as males and females, the first stage at which sexual dimorphism is apparent. Clunies Ross found that males emerged earlier than females. After a period of about seven days females will attach to a host. Males rarely attach to a host and Clunies Ross only observed this once, the host on that occasion being a human. The role of the male has since been elucidated by Moorehouse (1966). Although males apparently rarely feed on animal hosts it was shown that they parasitise the females, usually when the latter are partly fed, by piercing their cuticle on the ventral surface and feeding on haemolymph. Clunies Ross observed coupling (or mating) off the host and Moorehouse found that it also occurred on the host. Multiple coupling is common.

Adult females engorge for a period which varies a great deal depending on the temperature, and other less well-understood factors. The usual period is about six days. During the first four days of this time feeding is slow but on the fifth and sixth days there is a rapid increase in size, apparently due to an increase in feeding activity.
Temperature and humidity requirements

Clunies Ross found that the ambient temperature greatly influenced the rate of development of all stages. The minimum developmental periods reported above occurred at about $27^\circ$C while the maximum periods occurred at room temperature, which varied from $10^\circ$C to $20^\circ$C.

Clunies Ross found that all stages of the tick require high humidity to survive. Oxer and Ricardo (1942) confirmed this observation and added that extremes of temperature did not favour the tick. Above $32^\circ$C females failed to oviposit and temperatures below $7^\circ$C proved fatal after a few days.

Survival of unfed ticks

Clunies Ross found that larvae survived unfed for a maximum of 44 days. However by 23 days larvae were unable to feed to completion. In the case of nymphs he found a maximum survival time of 31 days. Survival times of 77 days, with subsequent successful feeding, were found for adult females and of 142 days for males.

Oxer and Ricardo (1942) found the maximum periods of viability of unfed ticks to be much longer. They found larvae to be alive and active after 162 days and nymphs after 275 days. Nymphs attached and fed at 225 days.

From his data Clunies Ross calculated a minimum life cycle of 135 days with a maximum of 379 days. Oxer and Ricardo extended the maximum to at least 741 days. The differences in survival found by these authors was probably due to inadequate control of humidity by Clunies Ross. His method of using glass tubes stoppered with moist
cotton wool might have allowed excessive fluctuations in humidity. Alternatively excessive condensation might have occurred and this wetness would shorten the lifespan of the ticks.

The method of holding ticks in a jar over damp sand used by Oxer and Ricardo provides a gradient of humidity in which the ticks can seek their own level, and seems to provide the best conditions for long term storage.

2.3 Materials and methods

The methods used to culture the ticks were adapted from those of Oxer and Ricardo (1942) and Bagnall (1975).

The cultures were started from fully engorged adult female *I. holocyclus* obtained from the Lismore district. These ticks had been collected from their natural environment and fed on hyper-immune dogs used for the production of tick antiserum. Each spring and summer new cultures were begun from wild caught ticks to avoid any possibility of reduction in virulence in laboratory maintained strains.

The engorged females were first gently washed in tap water at room temperature then placed on previously washed, boiled and cooled river sand in 1lb jars. As much water as possible was drawn off the sand to leave it moist, but not wet. The mouth of each jar was covered by fine terylene material held in place by a plastic screw cap in which a 5cm hole was cut. In order to minimise drying while allowing some air movement this was in turn covered by a plastic petri dish lid in which a 1cm hole was cut. Any ticks which died before oviposition had begun were removed.
Eggs laid by these females were not disturbed except to add a little deionised water once a week. Care was taken to avoid wetting the eggs directly.

After hatching, larvae were allowed to harden and when congregated at the top of the jar could be used for further propagation. Initially guinea pigs were used as hosts for larvae. However, as recommended by Bagnall (1975), after a short time a change was made to rats due to their greater resistance to paralysis and to the fact that they mount a minimal cutaneous response to repeated exposure to ticks. This allows the establishment of a colony of immune rats on which nymphs and adults may also be fed.

The rats used were large Wistar males, and were housed in groups of four. Each cage had a bottom of wire mesh of sufficient size to allow engorged ticks to fall through. The arrangement used for collection of engorged larvae (and nymphs) is shown diagramatically in figure 1.

Larvae were brushed onto the backs of the host animals on day 0 and normal food and water continued until day 3. At this time the blotting paper was changed and food and water withheld in preparation for collection.

Fed larvae, after detachment, fell onto the blotting paper and crawled into the water. Faeces and urine of the rats were retained on the paper thus reducing contamination of the water. The larvae were picked from the water by suction. For this a glass tube drawn to a tip diameter of about 2mm and connected by plastic tubing to a 2 litre filtering flask was used. Reduced pressure was applied to the side-arm of the flask in which the fed ticks collected.
Figure 1. Cage arrangement used for the collection of engorged larvae and nymphs. The cage stands on a sheet of blotting paper which is suspended over a moat of water into which the engorged ticks fall.
After collection engorged larvae were washed in three changes of tap water and placed in a heap in the centre of a disc of filter paper. This was suspended on an upturned petri dish, above a tray of water. The still active larvae were allowed to crawl off the paper into the water, from which they were strained through gauze mesh and placed on damp sand in 1 lb jars.

Nymphs which emerged from the fed larvae were allowed to harden and, when congregated at the top of the jar, could be used for experiments or for further propagation.

When adult ticks were required, nymphs were fed on rats using an identical procedure. The rats used were specimens on which larvae had already fed.

In the experiments to examine the effect of social grooming on the return of fed nymphs groups of four rats were used, one group being housed together and the other in individual cages so that no contact could be made between the rats. The daily return of fed nymphs from the two groups was counted during collection. In each experiment the nymphs applied were all from the same jar to minimise variation due to differences between batches of ticks.

After collection fed nymphs were cleaned in the same way as fed larvae. About 200 fed nymphs were placed in each jar.

2.4 Observations

The methods described above proved satisfactory in maintaining the tick in culture. The various stages of development of the tick are shown in figures 2, 3 and 4.
Figure 2. A large number of adult female *I. holocyclus* feeding on a hyperimmune dog. The top photograph was taken about 15 minutes after the application of about 400 ticks. The lower photograph was taken one week later and most of the ticks have completed engorgement and detached. This dog did not develop paralysis.
Figure 3. Stages in the life cycle of *I. holocyclus* (a) a batch of eggs laid by a single fully engorged female (b) unfed larvae (c) fed larvae (d) a mass of fed larvae after collection and washing. Calibration - 1mm.
Figure 4. Stages in the life cycle of *I. holocyclus*. (a) unfed nymphs (b) a group of fed nymphs after collection and washing (c) unfed adult female (d) fully engorged adult female. Calibration - (a) 1mm (c) 0.25mm (d) 1cm.
Paralysis in rats

Overzealous application of larvae to previously unexposed rats sometimes produced paralysis. In one experiment in which some rats died the fed larvae returned were counted and found to exceed 2000 per rat. Immune rats to which a similar number had been applied did not develop paralysis. Paralysis was not observed during the feeding of nymphs on immune rats although as many as 200 per rat were applied. Non-immune rats were sometimes paralysed by the application of 50 nymphs.

Period of feeding

Fed larvae always began to detach during the night of day 3 and it was obvious that most larvae were collected on the morning of day 4. A large number was also collected on the morning of day 5 and a rather low number on the morning of day 6. For example on one occasion when the fed larvae returned from 15 rats were counted the yields were 8950 on day 4, 5830 on day 5 and 229 on day 6. The trays were examined twice daily and it was quite apparent that the great majority of the fed larvae detached at night. For this reason collections were only made in the mornings.

Nymphs also tended to detach at night and were collected in the mornings. The majority of nymphs were collected on day 5. For example on one occasion the returns were 51 on day 4, 165 on day 5 and 10 on day 6.

The effect of social grooming was examined by comparing the return of fed nymphs from two groups of four rats. The rats of one group were housed in a single cage and those of the second group were housed in individual cages. Table 1 shows the return of fed nymphs from the two groups.
Table 1. Returns of fed nymphs from rats housed as a group of four, and from four rats housed individually. Fifty nymphs were applied to each rat. Rats had previously been exposed to larvae.
Although for any one batch of nymphs there was always a better return from rats housed individually, it is obvious that there is a large variation in percentage return, and the difference in yield in these experiments was not statistically significant. The return of fed nymphs was rather low and the maximum obtained from immune rats housed singly was 39.1%. It was obvious by the blood on their fur that even rats housed singly traumatised feeding nymphs by self-grooming.

On another occasion the return from immune rats housed individually over a single tray of water was compared to that from non-immune rats housed in the same way. Fifty nymphs were applied to each rat. The return from the immune rats was 39.1% while that from the non-immune was 70%. However two-thirds of the non-immune rats were paralysed by this dose of nymphs.

2.5 Discussion

Fully engorged adult females placed on sand showed considerable activity as described by Clunies Ross (1924). This activity, which was also exhibited by fed larvae and nymphs, continued for several days. Finally, engorged females became sedentary and tried to partly bury themselves in the sand. For this reason very fine sand was found somewhat unsatisfactory and one of average grain size of 0.5 to 1mm seemed best. It is important to provide a depth of 5 to 6cm to provide an adequate reservoir of moisture.

It was always difficult to prevent food and faecal material from contaminating the collected ticks. Previous workers (Bagnall, 1975; Goodrich, 1976) had recommended thorough washing to remove this debris. Even so it was found difficult to separate ticks from
particulate matter. Such debris, if placed on sand with the ticks, tended to act as a focus for fungal growth, with deleterious effects on the culture. The technique of placing washed ticks onto a disc of filter paper and allowing them to crawl off into clean water was therefore developed and proved very effective in removing particulate debris. Almost all debris was left behind when the ticks crawled off into the water. In addition, if the fed ticks were dropped in a heap in the centre of the sand, they tended to crawl away to the edges, any remaining debris being left behind.

In confirmation of Bagnall's suggestion the laboratory rat was found to be a suitable host for the propagation of I. holocyclus. Their major advantage is that, when immune rats are used, a much larger number of ticks can be fed on each animal than is the case with guinea pigs. There is some suggestion, however, that non-immune rats produce better yields of fed nymphs than those which are immune. The number of ticks used to sensitisise rats was much lower in Bagnall's experiments than that used here. It is possible that a large sensitising dose is required to produce cutaneous reactions in rats. Alternatively rats with high levels of antibodies against tick-derived antigens may simply scratch more, due to pruritis.

Social and self grooming were found to cause appreciable losses of feeding nymphs. This was possibly also precipitated by pruritis associated with immunity. Grooming seemed to cause greater losses of nymphs than larvae, probably due to the greater vulnerability of the larger partially fed nymphs.

Because of the relatively low numbers of ticks which can be applied to each animal and the necessity to use new animals for each feeding when guinea pigs are used, the low percentage yield obtained from
immune rats is probably outweighed by convenience and economy of animals. When only small numbers of ticks are to be fed, non-immune rats may offer the advantage of higher returns but the number applied per rat must be reduced to avoid paralysis. For large non-immune rats a maximum of 1000 larvae or 20 nymphs is suggested as an initial exposure.

Additional research is needed into the reasons for the low returns of fed nymphs, the role of immunity in reducing yields and methods of minimising losses due to grooming.

2.6 Summary

1. A coarse river sand was found to be the best substrate on which to keep all stages of I. holocyclus when large numbers are being propagated.

2. The cleaning of fed larvae and nymphs was greatly facilitated by allowing them to crawl away from debris.

3. The rat was superior to the guinea pig as a laboratory host for I. holocyclus.

4. Both self and social grooming caused losses of nymphs fed on rats. Losses were slightly lower when rats were housed individually and there is some evidence that returns are higher in non-immune rats.
3.1 Introduction

Although tick paralysis has been described and to some extent investigated in various parts of the world, it is by no means clear whether the different forms are related in their pathogenesis. The syndromes described in the various types of paralysis are basically similar (Clunies Ross, 1926), but there are certain differences which will be discussed in detail later in this chapter.

Despite the fact that a number of experimental studies of tick paralysis have been made, and the signs shown by animals with tick paralysis reported in a general way (Hadwen, 1913; Hadwen and Nuttall, 1913; Dodd, 1921; Clunies Ross, 1926) there does not appear to be any report of detailed neurological examination of a series of paralysed animals. Certainly reports of human cases have been published in which excellent neurological examinations were made (e.g. Eaton, 1913) but here also there are inconsistencies. For example, Lagos and Thies (1969) reported a case in which it was claimed there was no muscle weakness, a finding which is in disagreement with most other reports.

In the experiments to be described in this chapter a series of dogs, paralysed by *I. holocyclus*, was subjected to detailed neurological examination in an attempt to clarify the signs shown in the disease. In addition it was hoped, at least to some extent, to localise the possible site or sites of action of the toxin secreted by the tick.
3.2 Materials and methods

Dogs used in the experiments were crossbred males and females judged to be of young age (less than two years). Before experiments were undertaken each dog was vaccinated against canine distemper and hepatitis and treated with the antihelminthic, mebendazole (Telmin, Ethnor), then held for a period of observation of at least four weeks before use.

The ticks used were adult females collected from their natural environment near Lismore, New South Wales.

At the beginning of each experiment the dog to be studied was subjected to a thorough clinical and neurological examination. Any dog considered to be abnormal was rejected. The neurological examination was based on that described by de Lahunta (1971). Observations were recorded on a standard neurological report sheet (figure 5).

After the initial examination the dog was anaesthetised using thiopentone sodium (Pentothal, Abbott) and six active ticks were placed in the pinna of the ear. The ticks were observed until attached.

Daily neurological examinations were carried out until the first signs of abnormality appeared. After this, examinations were repeated several times daily to monitor progress of the paralysis. In two dogs paralysis was allowed to progress to death, but the remainder were killed by the intravenous administration of pentobarbitone sodium when the paralysis was in its terminal stages.
3.3 Results

In this series of experiments seven dogs were successfully paralysed and studied. Three dogs on which only a few ticks fed failed to become paralysed.

In confirmation of the observations of previous workers (Dodd, 1921; Clunies Ross, 1926, 1935) onset of paralysis was found to be closely related to the period in which rapid engorgement of all or some of the ticks occurred. Of the seven dogs successfully paralysed two showed first signs on the fifth day after attachment of the ticks and five on the sixth day. In some dogs some of the ticks had completed feeding and detached before the onset of paralysis. Figure 6 shows the appearance of the ticks at various stages of engorgement on experimental dogs.

The signs shown by affected dogs were found to be remarkably constant. The rate of progression of the paralysis however varied and the time required for the paralysis to progress from first signs to the terminal stages was of the order of 24 to 48 hours.

The earliest sign shown by the paralysed dogs was a stumbling hindlimb gait. At this early stage dogs remained bright and alert but appeared sometimes to "sink down" in the hindlimbs, and had difficulty in jumping up onto their back legs. Neurological testing demonstrated abnormalities in some postural reactions, namely hopping, hemistands and hemiwalks. These abnormalities were bilateral and, in the early phase of the disease, affected only the hindlimbs. Other postural reactions, spinal reflexes and cranial nerve function were normal.
GAIT AND POSTURE

MENTAL STATUS

CRANIAL NERVES

II  Menace
    Pupillary
    Ophthalmoscopic

III  Pupillary Strabismus

V  Motor
    Sensory

VI  Strabismus

VII

VIII Cochlear
    Vestibular - Head Tilt
    Nystagmus - Resting
    Positional

IX, X

XII

MUSCLE TONE

ADDITIONAL TESTS

POSTURAL REACTIONS

Wheelbarrowing
Hopping   LF   RF
          LH   RH
Extensor Postural Thrust
Hemistands L   R
Hemiwalks  L   R
Proprioceptive Positioning
              LF   RF
                  LH   RH
Placing
Optic Tactile
Tonic Neck and Eye

SPINAL REFLEXES

Patellar   LH   RH
Biceps     L   R
Triceps    L   R
Perineal
Flexor     LF   RF
              LH   RH
Crossed Extensor
Pain Perception

Figure 5. An example of the standard report sheet used to record the results of each neurological examination.
Figure 6. Changes in the appearance of *I. holocyclus* during feeding. (a) day 1 (b) day 4 (c) day 5 (d) day 6. Note the rapid increase in size occurring on days 5 and 6. The dog showed first signs of paralysis on day 6 when some of the ticks had already detached.
These signs progressed until the dogs were no longer able to support their weight on their hindlimbs. When the hindquarters were elevated then released, the dogs collapsed to a sitting position. There was a concurrent increase in impairment of postural reactions, including proprioceptive positioning which, in the hindfeet, could only be performed with difficulty. This difficulty appeared to be due to motor rather than sensory impairment as the dogs usually made an effort to return the foot to the normal position. The extensor postural thrust reaction remained normal. When the hindlimbs were supported and forelimb function assessed by walking and hopping, some impairment of those limbs was also noted at this stage. Cranial nerve function remained normal with the exception of a degree of pupillary dilation in some dogs. Pupillary dilation was noted in three of the seven dogs examined at some time during the course of paralysis and will be more fully discussed later in this section. At about this stage of the disease it was always noted that patellar reflexes were reduced. Initially withdrawal (flexor) reflexes remained intact and there was no indication of any reduction in pain perception.

Progression of the paralysis led to increased generalised weakness involving both forelimbs and hindlimbs. Patellar reflexes were absent and withdrawal reflexes, in the hindlimbs in particular, were palpably weaker. Similar signs were present in the forelimbs but were always less advanced than those in the hindlimbs. Pain perception, as indicated by struggling in response to a toe pinch, was intact. Hind limb muscles at this stage were obviously hypotonic. Dyspnoea had become evident and was characterised by a laboured respiration with an increased abdominal effort.
Flaccidity of the muscles of the head indicated involvement of the cranial nerves. A dog with advanced tick paralysis is shown in figure 7.

Terminal paralysis was characterised by extreme weakness affecting all limbs and trunk muscles. Muscle tone was markedly reduced, patellar and other tendon reflexes were absent and painful stimuli to the toes produced either no withdrawal effort or a very weak one. Painful stimuli did however evoke feeble struggling movements indicating that central pain perception was apparently still intact. Dyspnoea was severe and dogs tended to salivate freely, probably due to paralysis of the muscles of deglutition.

In those dogs in which the condition was allowed to progress to death, cyanosis of the visible mucosae preceded respiratory arrest.

Pupillary dilation, as mentioned previously, was seen in three experimental dogs and was also observed in a number of naturally occurring cases. When it occurred, its onset in relation to other signs was variable and it sometimes did not occur until paralysis was well advanced. In the early stages of pupillary dilation there was still a sluggish response of the iris to light, but this response rapidly disappeared. In two dogs (one a naturally occurring case) the pupils were observed to be dissimilar in size, one pupil being fully dilated while the other was less affected (figure 8). When a light was directed at the fully dilated pupil it showed no response, but the less affected pupil constricted indicating an intact consensual reflex. There was also mild prolapse of the nictitating membranes in dogs more than moderately affected by the disease.
Figure 7. A dog showing the muscle weakness and hypotonia characteristic of advanced tick paralysis.
Figure 8. Anisocoria shown by an experimental dog with advanced tick paralysis. The left pupil, which showed no reaction to light, is larger than the right. The prolapse of the nictitating membranes commonly seen in this disease is also apparent.
Vomiting, although not necessarily a sign of neurological dysfunction was a persistent feature of the disease. All paralysed experimental dogs vomited to some degree. Vomiting was usually first seen early in the disease and persisted throughout its course.

3.4 Discussion

Early descriptions of tick paralysis in Australia did not describe the characteristic signs fully, if at all. (Backhouse, 1843; Bancroft, 1884). These descriptions indicated only that affected animals became listless, inappetant and progressively weaker in the legs. Fainting, weak heart-beat and pallor of the mucous membranes were also described. Later descriptions by Anderson Stuart (1894) pointed out the vulnerability of young animals and the ability of repeated exposures to *I. holocyclus* to confer immunity in the host. The signs of tick paralysis were described as muscle weakness of an ascending nature and seizures were apparently observed. Death was attributed to heart and respiratory failure.

Both Dodd (1921) and Clunies Ross (1926) reported on experimental tick paralysis in dogs. Onset of signs occurred 5 to 13 days after attachment of the ticks and signs consisted of loss of appetite, dullness, vomition and incoordination of the hindlimbs, increasing in severity until there was flaccid paralysis of both hind and forelimbs. Convulsions were rarely seen. The pulse was not affected, but respiration was described as often of the Cheyne-Stokes type. Clunies Ross (1926) reported that reflexes were "modified", and that affected dogs remained bright throughout the course of the disease.
Several case reports have appeared describing the disease in man (Cleland, 1912; Eaton, 1913; Strickland, 1915) and from these, in particular that of Eaton, a good description of the neurological syndrome emerges. This consists of a flaccid paralysis, areflexia and often mydriasis with absence of pupillary light reflexes. In Eaton's case accommodation was paralysed although the patient could see.

Local signs have occasionally been reported associated with the attachment of a tick. These have been facial paralysis related to a tick attached in the ipsilateral auditory meatus (Crossle, 1932; Foster, 1931). It seems possible, or even likely, that these were associated with local inflammation and swelling, but local effects of a toxin secreted by the tick cannot be ruled out.

In North America tick paralysis was first produced experimentally by Hadwen (1913) and Hadwen and Nuttall (1913). Case reports are occasionally published describing tick paralysis in man (Temple, 1912; McCormack, 1921; Phillips and Murphy, 1950; Adler, 1966; Taylor, 1966; Gibson, 1966; Cherington and Snyder, 1968) and in animals (Muth, 1945; Jellison, Stoenner, Kramis and Beardmore, 1951; Emminger, 1951). A number of review articles discussing the disease have also appeared. (Mail and Gregson, 1938; Schmitt, Bowmer and Gregson, 1969; Abbott, 1943; Davidson, 1941; Loomis and Bushnell, 1968; Rich, 1971). These reports indicate that in North America the syndrome of tick paralysis is an ascending flaccid paralysis, beginning six to eight days after attachment of the tick (Hadwen, 1913; O'Rourke and Murnaghan, 1954). There is no pain associated with the condition, sensory function appears to be normal.
and the main feature is muscle weakness. Lagos and Thies (1969) however described a case in which they claimed that muscle strength was normal. Unlike the syndrome produced by *I. holocyclus* vomiting does not occur and recovery is usually rapid after the removal of the tick, although it occasionally can take up to seven days (Schmitt, Bowmer and Gregson, 1969). As sometimes occurs with *I. holocyclus*, signs can occasionally appear after removal of the tick (Mail and Gregson, 1938).

As for the South African forms of tick paralysis, it is considered characteristic of Karoo tick paralysis, caused by *I. rubicundus*, that all four limbs are affected simultaneously while in spring lamb paralysis, due to *R. evertsi*, only the hindlimbs are affected, at least initially (Stampa, 1959). A more detailed description of the signs seen in these forms of tick paralysis could not be found.

According to Clunies Ross (1926), the signs seen in the forms of tick paralysis occurring in other parts of the world resemble those encountered in Australia.

The observations made in this series of experiments support previous findings that tick paralysis is an ascending flaccid, motor paralysis. It is characterised by muscle weakness, hypotonia of the skeletal muscles, and reduction, then loss, of spinal reflexes. It is interesting to note that reduction and loss of tendon reflexes always precedes loss of withdrawal reflexes.

Loss of peripheral reflexes could be accounted for by a lesion anywhere along the reflex arc (de Lahunta, 1971). This includes the pain or stretch receptors, the sensory nerves, the spinal synapses,
the motor nerve, the neuromuscular junction and the muscle fibres themselves. However it seems clear that pain receptors and sensory nerves continue to function. As well, there is good evidence provided by the alertness of paralysed dogs and the central appreciation of, and attempted reaction to, pain that at least some central pathways are intact. The muscle hypotonia consistently observed in tick paralysis also suggests a lesion of the lower motor neurone rather than a central effect (de Lahunta, 1971). All of the neurological findings could be explained by an effect of the toxin on the lower motor neurone, the neuromuscular junction or directly on the muscle fibres. Although it cannot at this point be ruled out it would seem unusual if the toxin could affect the motor nerve trunk yet spare sensory nerves, as the propagation of impulses in these nerves is presumabbly by the same mechanism.

The observation of an effect on the iris and, in particular, the retention of an intact consensual reflex when this effect is not symmetrical, provides some interesting evidence. As dogs can still see, the sensory side of the pupillary reflex arc would appear to be intact. An intact consensual light reflex when light is directed at the "paralysed" eye also infers that the sensory limb of the reflex arc and the central synapses required for integration of the reflex are also functional. The iris receives a dual autonomic innervation, the adrenergic endings producing pupillary dilation and the cholinergic endings producing constriction (Mountcastle, 1974). The pupillary dilation observed could therefore be produced by increased adrenergic or reduced cholinergic activity. This observation, together with the fact that there is an effect on the voluntary muscles, makes attractive the hypothesis that the toxin
affects the cholinergic innervation of the iris. If this is true then it is an indication that the toxin affects not only skeletal muscle, but at least some parasympathetic pathways as well. This possibility is supported by Eaton's (1913) observation of paralysis of accommodation in an affected child.

During the course of this work the author has had the opportunity to examine more than one hundred clinical cases of tick paralysis in dogs. The signs seen in these cases were quite consistent with those described above, and no signs were seen in naturally occurring cases which were not observed also in experimental dogs. Occasionally apparent fear in an affected dog led to violent incoordinated struggling which could be confused with convulsions. True convulsions however were never observed.

The most attractive hypothesis to fit the evidence presented in this chapter is that there is, in tick paralysis, a lesion of the motor innervation of skeletal muscle and at least some smooth muscle supplied by cholinergic fibres. The site of this lesion could be the motor nerve, its terminals, the neuromuscular junction or the muscle fibres themselves. Direct examination of these components is needed to determine their functional status in tick paralysis.

3.5 Summary

1. Tick paralysis caused by *I. holocyclus* presents a neurological syndrome characterised by muscle weakness, hypotonia, and hyporeflexia progressing to areflexia.

2. The onset of signs occurred, in these experiments, five to six days after the attachment of the ticks and corresponded to the period of rapid engorgement of the fastest feeding of them.
3. Pupillary dilation was a common feature of the disease.

4. There was no evidence for any impairment of sensory function.

5. There was no direct evidence for an effect on the central nervous system.

6. There was evidence that at least some parasympathetic pathways were affected.

7. These results are best explained by postulating an effect of tick toxin on the lower motor neurone, the neuromuscular junction or the muscle fibres themselves.