

A STUDY OF THE EFFECTS IN THE DOG OF

IXODES HOLOCYCLUS

by

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Doctor of Philosophy

The University of Sydney

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PREFACE

The work presented in this thesis was carried out in the Department of Veterinary Clinical Studies (Sydney) to fulfil the requirements for the degree of Doctor of Philosophy of the University of Sydney.

I would like to thank all the people in that department for their assistance and time. In particular I would like to thank:

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Finally I would like to thank Mr. D.M. Turner, Department of Veterinary Clinical Studies, for his valued assistance throughout the entire project. The work was such that without his help the project would never have been accomplished.

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

January, 1979.

Jan E. Ilkiw.

GENERAL SUMMARY

1. A number of ticks were reared in the laboratory but were found to be unsuitable since no signs of the disease were produced when up to ten were attached to a dog. All experiments reported in this study were conducted using wild-caught unfed adult female ticks.
2. The period to onset of clinical signs from attachment varied from 5.5 to 9 days, with most dogs showing signs on day 6 or 7.
3. Mild hypoxaemia was present when paralysed dogs reached the stage of lateral recumbency with no withdrawal reflexes. Just prior to death, moderate hypoxaemia with acute ventilatory failure and a mild metabolic acidosis was present.
4. The biochemical abnormalities found were unremarkable and difficult to interpret but could represent the biochemical response to release of catecholamines and/or corticosteroids.
5. Histological examination of sections from heart, lung, liver and kidneys demonstrated moderate to severe congestion.
6. There was a progressive fall in respiratory rate with no change in tidal volume. Expiratory time was prolonged due to closure of the vocal cords during expiration. A progressive increase in the difference between alveolar and arterial oxygen tensions was observed.

7. The electrocardiographic changes were extremely variable between animals and even in an individual animal. The abnormalities in rhythm recorded were sinus tachycardia, ventricular tachycardia, sinus arrest and sinus bradycardia. The sinus bradycardia observed terminally was probably due to increased vagal tone.

8. There was an increase in peripheral vascular resistance leading to a significant elevation in mean arterial pressure at all stages of the disease. Heart rate was elevated until two hours before death when it tended to decrease. Mean cardiac output decreased throughout the disease. The elevation of pulmonary arterial pressure despite the reduction in cardiac output indicated an increase in pulmonary vascular resistance. Although cardiac stimulation was present, there was no increase in measured contractility. These changes appear to be due to central sympathetic stimulation.

9. The value of three drugs, promethazine hydrochloride, dexamethasone and phenoxybenzamine hydrochloride, in combination with hyperimmune serum and hyperimmune serum alone, were compared in the treatment of dogs at an advanced stage of the disease. Each drug when combined with hyperimmune serum was found to be more beneficial than hyperimmune serum alone. Phenoxybenzamine hydrochloride, a drug which reverses many of the cardiovascular abnormalities found, was the most beneficial. No dog survived without the use of hyperimmune serum.

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INTRODUCTION

In Australia, tick paralysis is a disease produced by the three-host tick *Ixodes holocyclus*. It has intrigued and captivated both observers and students for almost a century, defied understanding by scientists and evaded treatment by medical practitioners. Mortalities have been reported in man (principally infants), foals, calves, pigs, sheep, dogs, cats and poultry. In contrast, death is rarely observed in native fauna.

Cases of tick paralysis are seen only along the coastal regions of eastern Australia. This very limited distribution is due to the susceptibility of *Ixodes holocyclus* to slight variations in temperature, and particularly humidity. In the vicinity of Sydney, the rainfall 20 miles inland may be a little more than half that in the metropolitan area, and with this decline the incidence of the disease falls very rapidly. Another factor governing the incidence of the disease is the presence of the bandicoot (*Perameles sp.* and *Isodon sp.*). These small marsupials are always heavily parasitized and are important hosts for the tick.

The disease is characteristically described as a rapidly-ascending flaccid motor paralysis. The first signs in the dog are usually an altered voice and slight incoordination of the hind-quarters. The disease progresses to an inability to walk, then to stand and finally to right. The muscles of the head and neck then become affected and respiratory embarrassment is a marked feature of severe cases. If the animal is not treated early and the ticks removed, death is inevitable.

Clinical examination and acid-base studies carried out on tick-affected dogs presented at the University of Sydney Veterinary

Hospital have provided data suggesting that death from tick poisoning should not be attributed solely to respiratory paralysis.

This study was undertaken to establish a background of comprehensive clinical and experimental data essential to an understanding of the clinico-pathological course of the disease. Accordingly studies of the respiratory and cardiovascular systems, electrolytes, biochemistry, haematology and post-mortem examination were carried out on experimental dogs infested by *Ixodes holocyclus*. The results obtained were then applied to develop a rational method of treatment for tick paralysis, especially for those animals presenting in an advanced stage where current methods of treatment usually fail to prevent death.

CHAPTER 1
LITERATURE REVIEW

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1.1 EARLY HISTORY

The first reference to tick paralysis is from the diary of Hovell, the explorer, describing his journey from Lake George to Port Phillip in 1824, and published in 1921. He wrote about "the small insect called the tick, which buries itself in the flesh, and would in the end destroy either man or beast if not removed in time".

Bancroft (1884) drew attention to a disease produced by ticks in dogs. He observed that ticks found in the thickly wooded districts were very toxic to introduced animals, but that native animals appeared to be unaffected. The disease produced in dogs and cats was so severe that if ticks were not removed, death could be expected within three or four days. In the adult human, however, the symptoms seen were those of weakness, a fear of falling when stooping or getting up and blindness. These symptoms gradually disappeared over a period of a month after removal of the tick.

A decade later, Stuart (1894) presented a summary of 100 cases of tick bites in animals. The question of immunity was raised, and it was noted that in some districts where ticks abounded, dogs were rendered immune by allowing the ticks acquired naturally to remain on the animal until the first signs appeared. These signs, he claimed, could sometimes be seen within a few hours of attachment of the tick.

Naming of the tick *Ixodes holocyclus* was by Neumann in 1899. He described the adult and nymphal stages, but no mention was made of the larval stage.

It was not until 1912 that a case of paralysis was reported in man. Cleland described paralysis and death in a 13 month old girl in which a large tick was removed from behind the left ear. On examination she was unable to stand and appeared agitated. Her

temperature and pulse rate were elevated, respiration was rapid and shallow, and she had great difficulty in coughing up mucus. Later that evening she collapsed suddenly after failing to bring up mucus and died. Another case discussed by Cleland (1912) was a patient who showed signs of faintness within an hour of attachment of 200 ticks. On examination he had a weak pulse and syncope, and although there were no signs of paralysis, the patient remained ill for a week with signs referable to the heart.

In a severe non-fatal case of tick poisoning in a child (Eaton, 1913) the first signs were those of refusal to eat, restlessness and an unsteady gait. The signs progressed to an inability to stand and frequent vomiting and then to a state bordering on delirium. Her temperature and pulse rate were elevated and respiration was rapid and shallow and performed mainly by the accessory muscles of respiration. Pupillary dilation that was unresponsive to light and pilocarpine was observed. The leg muscles were flaccid and knee jerks, wrist jerks and plantar reflexes could not be elicited. The next day the patient was more alert, temperature had returned to normal and breathing was easier. There was no return of voluntary movements, but the muscles did not appear as flaccid as before. The pupils were still dilated and did not respond to light but responded weakly to pilocarpine. The child continued to improve and had completely recovered within a week.

In another non-fatal case of tick paralysis reported by Strickland (1915) the child complained of feeling very sick and giddy four days after walking in the bush. His condition deteriorated until he could no longer walk without assistance, and his face was asymmetrical. The examining doctor found a large tick in the ear, and after removal the boy had fully recovered in ten days.

It was not until 1921 that a definite link between the disease and the agent was established when Dodd proved experimentally that a causal agent of tick paralysis was *Ixodes holocyclus* (Neumann). By sending his own dog into the bush and searching it afterwards he collected adult ticks before attachment. One female tick was placed on each of three dogs, which subsequently became paralysed, died and were subjected to a post-mortem examination. The ticks were removed from the dead dogs and identified. This experiment established two facts:

- 1) there was a period of 5-6 days from the time of attachment to the time of appearance of clinical signs; and
- 2) the signs were those of a motor paralysis with the sensory pathways apparently unaffected.

A review of deaths in humans from tick paralysis was published by Ferguson in 1924. The case reported by Cleland (1912), as well as seven new cases, were listed. Information was obtained in each case from the attending medical practitioner, but it contained little detail.

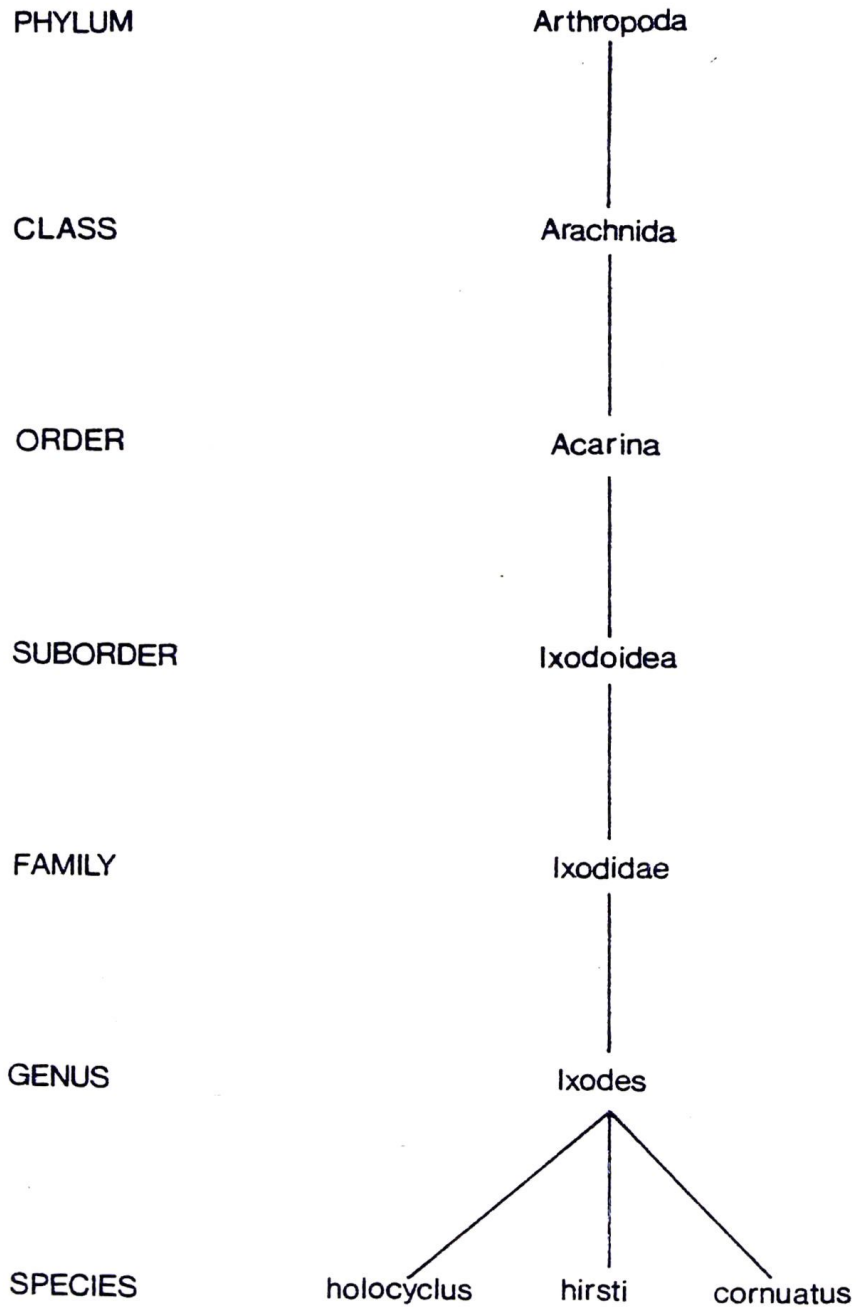
Following these early reports, more comprehensive experimental work concerning the tick and the disease it produced was undertaken, and this is discussed in the next sections.

1.2 CLASSIFICATION

Ticks, together with mites, belong to the order Acari or Acarina, class Arachnida, phylum Arthropoda (Fig. 1 i). Ticks are placed in the suborder Ixodoidea. They are characterized by an elongate hypostome furnished with rows of recurved teeth, a pair of spiracles

FIGURE 1 i

CLASSIFICATION OF TICKS



either posterior or anterior to coxa IV, cheliceral digits with the dentate faces directed externally and working in a horizontal plane, and of a conspicuous sense organ, Haller's organ, on tarsus I (Roberts, 1970).

Two families are recognized, Ixodidae and Argasidae.

Ixodidae are the "hard" ticks. They possess a dorsal scutum in all stages, which covers practically the entire dorsum in the adult male, but only the anterior portion in the adult female, nymph and larva. The capitulum is terminal in all stages, and in the female the basis is furnished with porose areas. Articulation of the palpi is variable, and the fourth article is much reduced. Eyes, when present, are situated laterally on the scutum. The spiracles are posterior to coxa IV. The coxae are frequently provided with spurs, and sexual dimorphism may be marked. The total number of known world-wide species of ixodid ticks is around 590 (Roberts, 1970).

Family Ixodidae consists of the Prostriata and Metastriata. The Prostriata includes the genus *Ixodes*, in which the anal grooves embrace the anus anteriorly and usually unite in an arch or point. The external morphology of the male and female ixodid tick is illustrated in Figures 1 ii and 1 iii. There are at present 22 species (Roberts, 1970) of this genus occurring in Australia.

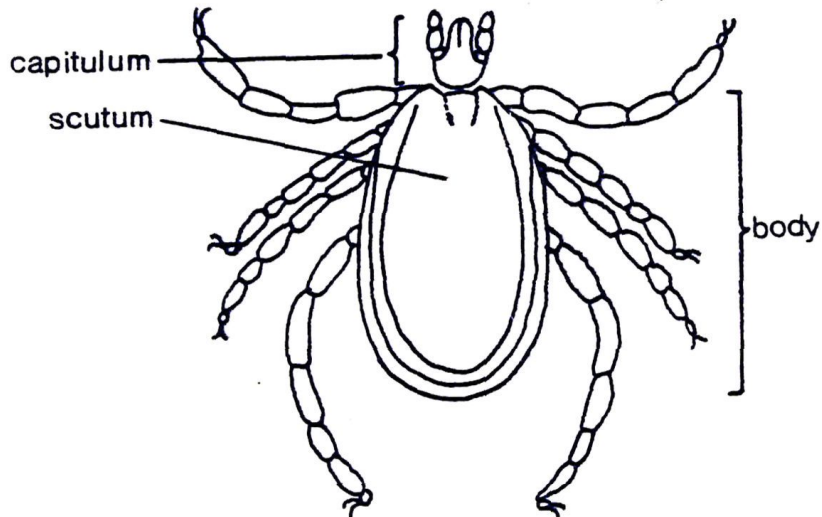
Ixodes holocyclus (Neumann), illustrated in Figures 1 iv and 1 v, is commonly recognized as causing death due to paralysis in domestic and native animals (Dodd, 1921; McMichen, 1934).

Ixodes hirsti (Hassall), illustrated in Figures 1 vi and 1 vii, was reported by Roberts (1961) associated with a case of paralysis in a cat.

Roberts (1960) recorded an association between *Ixodes cornuatus* (Roberts) and tick paralysis in a dog at Lakes Entrance, Victoria.

FIGURE 1 ii EXTERNAL MORPHOLOGY OF AN IXODID TICK

PROSTRIATA, IXODES, MALE (DORSAL VIEW)



PROSTRIATA, IXODES, MALE (VENTRAL VIEW)

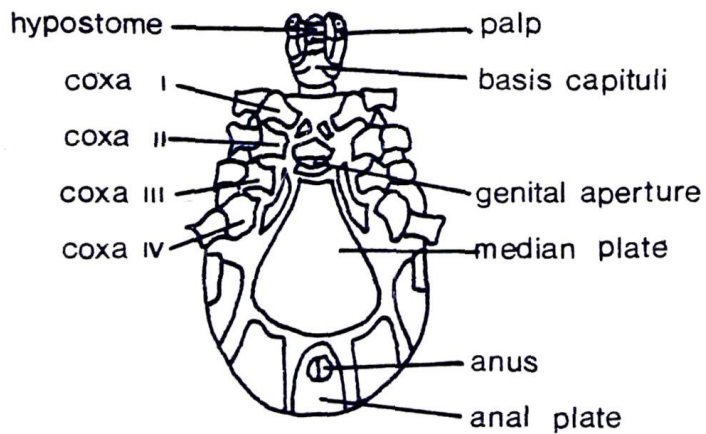
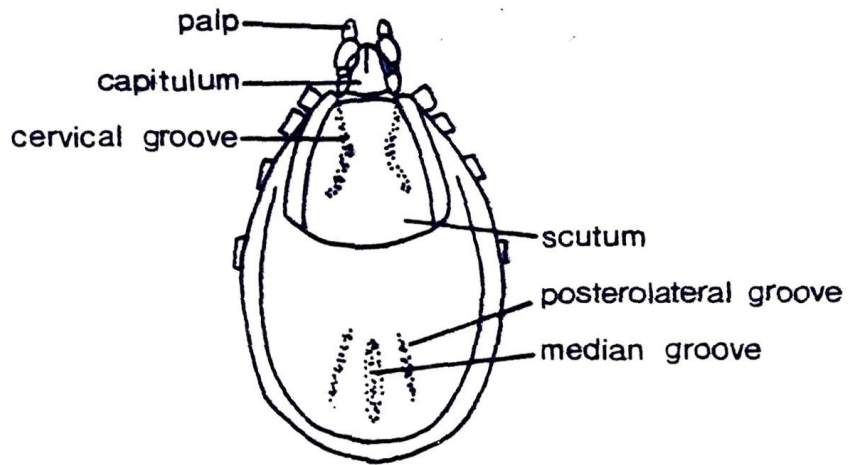


FIGURE 1 iii

EXTERNAL MORPHOLOGY OF AN IXODID TICK

PROSTRIATA, IXODES, FEMALE (DORSAL VIEW)



PROSTRIATA, IXODES, FEMALE (VENTRAL VIEW)

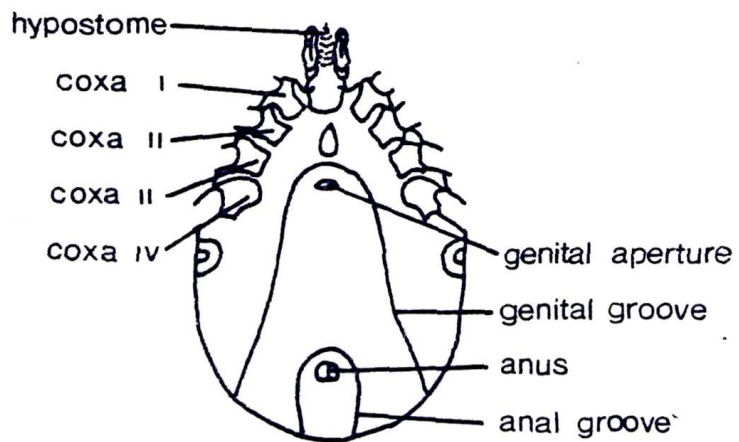
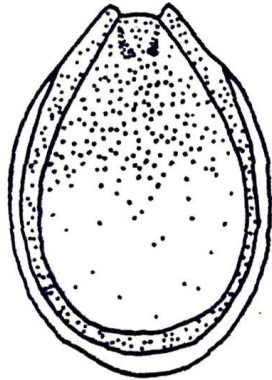
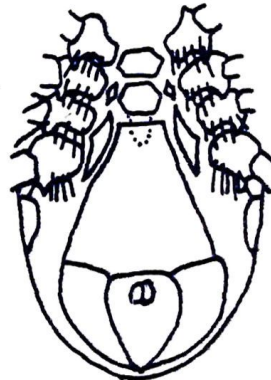


FIGURE 1 iv
IXODES HOLOCYCLUS - MALE

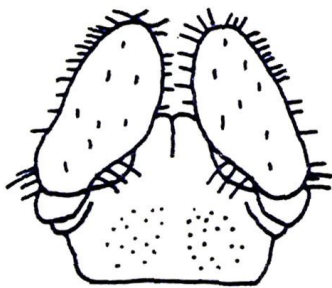
BODY (DORSAL VIEW)



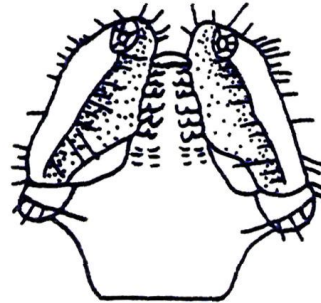
BODY (VENTRAL VIEW)



CAPITULUM (DORSAL VIEW)



CAPITULUM (VENTRAL VIEW)



TARSUS IV

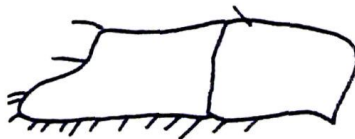
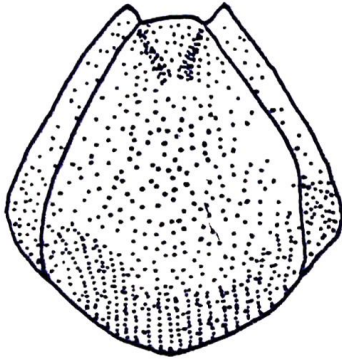


FIGURE 1 v
IXODES HOLOCYCLUS - FEMALE

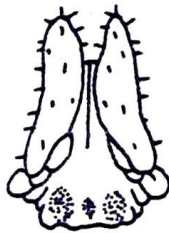
SCUTUM



HYPOSTOME



CAPITULUM (DORSAL VIEW)



CAPITULUM (VENTRAL VIEW)



PALPAL ARTICLE I



TARSUS I

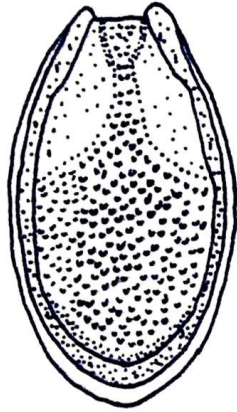


TARSUS IV

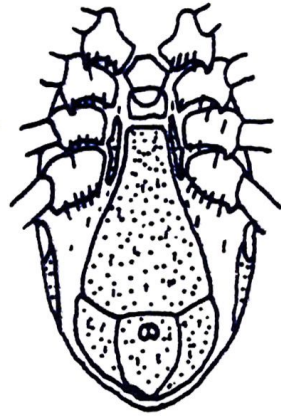


FIGURE 1 vi IXODES HIRSTI - MALE

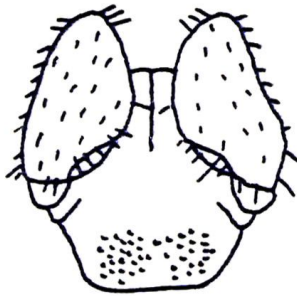
BODY (DORSAL VIEW)



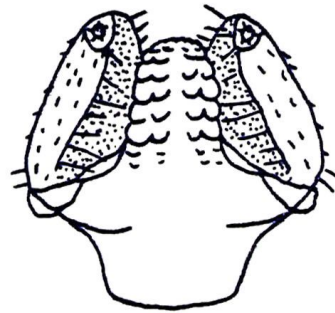
BODY (VENTRAL VIEW)



CAPITULUM (DORSAL VIEW)



CAPITULUM (VENTRAL VIEW)



TARSUS I



TARSUS IV

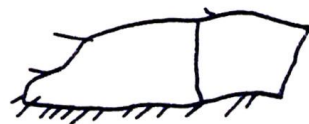
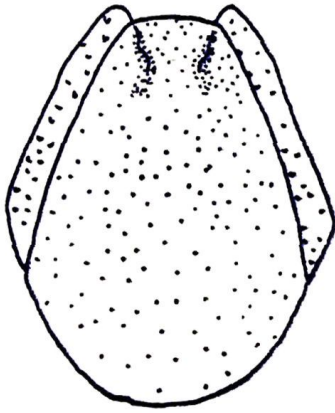
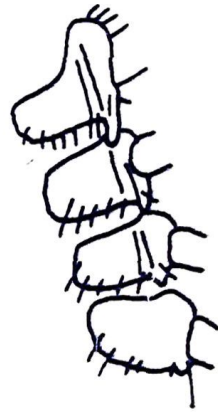


FIGURE 1 vii
IXODES HIRSTI - FEMALE

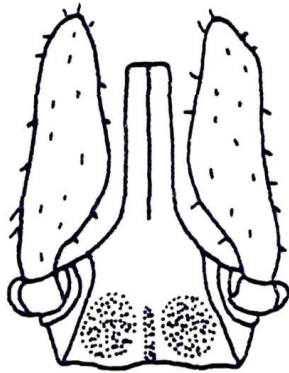
SCUTUM



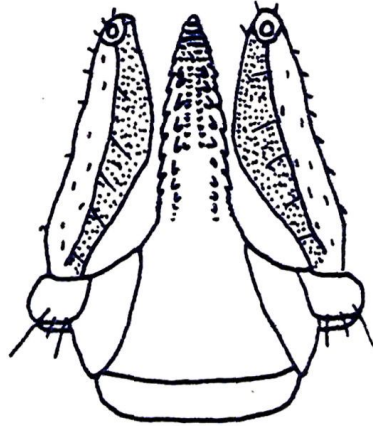
COXAE I - IV



CAPITULUM (DORSAL VIEW)



CAPITULUM (VENTRAL VIEW)



TARSUS I

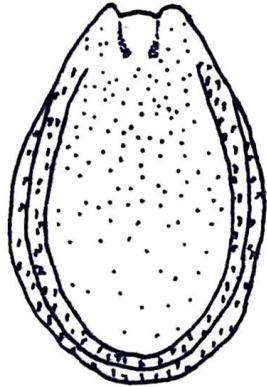


TARSUS I - IV

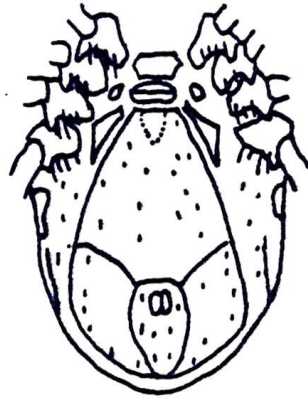


FIGURE 1 viii
IXODES CORNUATUS - MALE

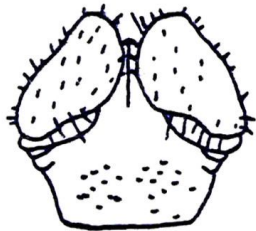
BODY (DORSAL VIEW)



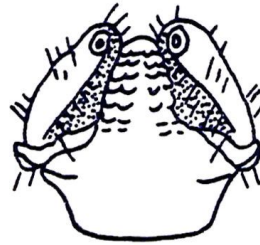
BODY (VENTRAL VIEW)



CAPITULUM (DORSAL VIEW)



CAPITULUM (VENTRAL VIEW)



TARSUS I



TARSUS IV

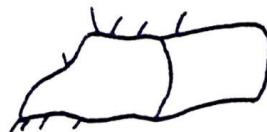
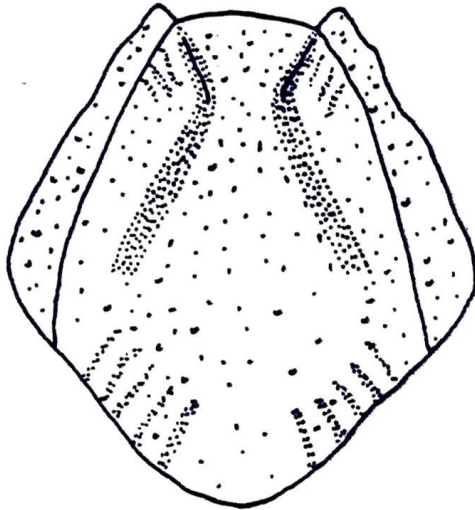
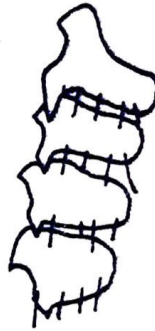


FIGURE 1 ix IXODES CORNUATUS - FEMALE

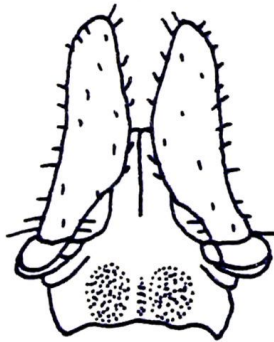
SCUTUM



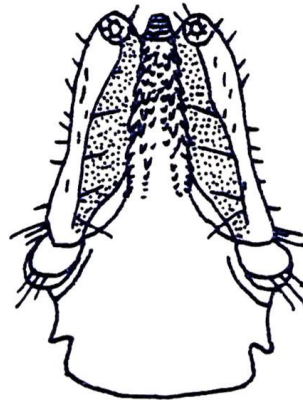
COXAE I-IV



CAPITULUM (DORSAL VIEW)



CAPITULUM (VENTRAL VIEW)



TARSUS I



TARSUS IV



Further evidence that this species may induce paralysis has since been encountered in dogs in northern Tasmania, sheep at Orbost, and a calf at Tostaree near Orbost, Victoria (Roberts, 1970). For comparison with *Ixodes holocyclus*, the external morphology of *Ixodes cornuatus* is illustrated in Figures 1 viii and 1 ix.

1.3 DISTRIBUTION

Ixodes holocyclus is not indigenous only to Australia and has been recorded in New Guinea, Indonesia, Kei Island and India. In Australia it is restricted to bush and scrub country and is found in the coastal area of Queensland, New South Wales and northern Victoria (Fig. 1 x). Usually it is found within a few miles of the coast, but in southern Queensland and northern New South Wales it extends somewhat further inland.

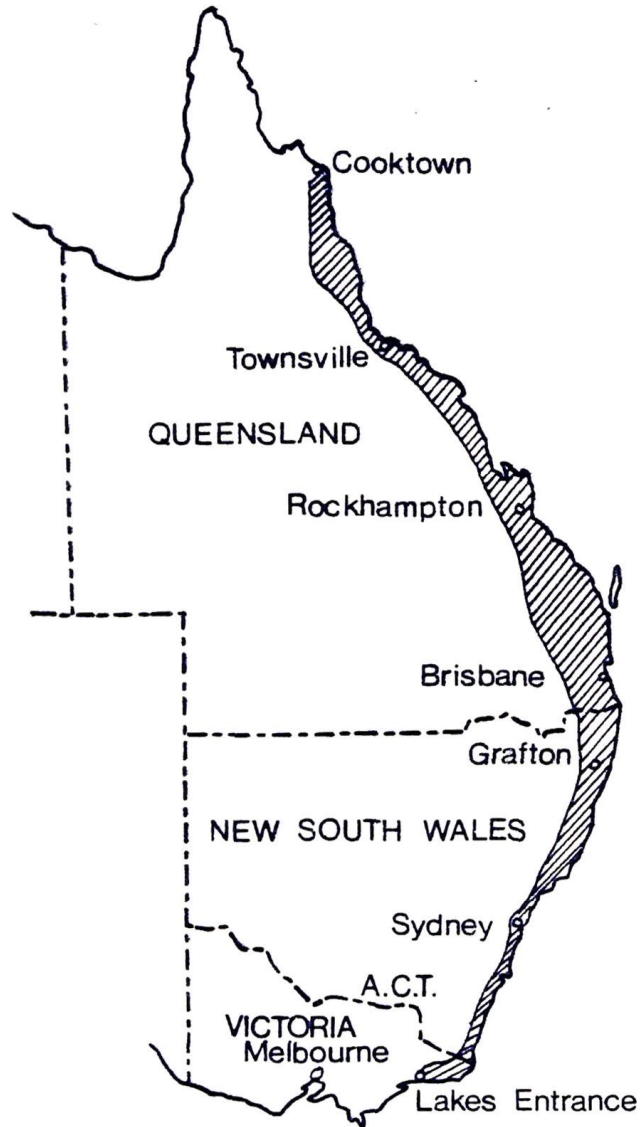
In Queensland where it is most prevalent in rain forest country, it has been found in the south as far inland as Warwick and Bunya Mountains near Dalby (Roberts, 1970).

In New South Wales it rarely ranges more than ten miles or so from the coast, but there are some records from the Armidale area (Roberts, 1960). It is abundant in the vicinity of Sydney.

In Victoria the most southern locality from which *Ixodes holocyclus* has been collected was Bairnsdale, near Lakes Entrance (Roberts, 1960).

No specimens of *Ixodes holocyclus* have been seen in Tasmania, and although it was recorded by Nuttall, Warburton, Cooper and Robinson (1911) in Western Australia, Roberts (1960) did not find it in this state, and there are no records of tick paralysis from this

FIGURE 1 x
THE DISTRIBUTION OF IXODES HOLOCYCLUS
IN AUSTRALIA



Ticks endemic 

part of Australia.

Ixodes hirsti was first described under the name *victoriensis* (Hirst, 1930), from Lower Tarwin, Victoria. Roberts (1960 and 1964) added the localities Selby, Cape Otway and Sassafras (Victoria), Mount Irvine, Taronga Park Zoo and Colo Vale (New South Wales), and King Island, Mount Wellington and Smithton (Tasmania). Roberts (1961) also recorded it from Reedy Creek, south-eastern South Australia. New locality reports (Roberts, 1970) are Allambee, Sandy Point, South Gippsland, Sperm Whale Head, Forrest, San Remo (Victoria), Deviot, Clarence, Blue Mountains (New South Wales), Bald Island, near Albany (Western Australia), and Kangaroo Island (South Australia).

Latest records (Roberts, 1970) show *Ixodes cornuatus* to be present in the southern coastal areas of New South Wales, eastern Victoria as far west as Dandenong and Mount Buffalo, and in Tasmania.

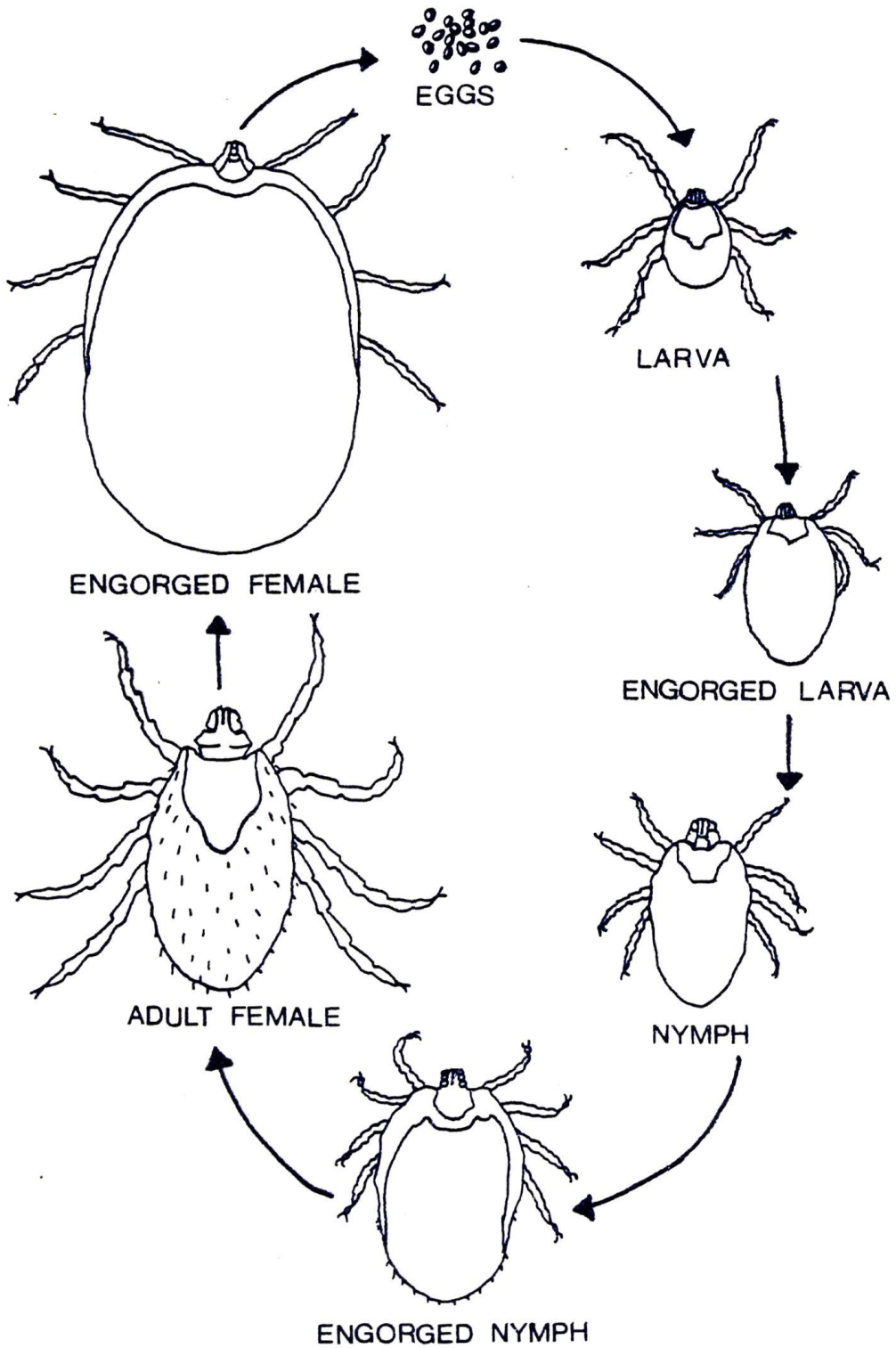
1.4 LIFE CYCLE

Ixodes holocyclus is a three-host tick which engorges and then falls off the host between each successive stage of the life cycle, as illustrated in Figure 1 xi. The following description is taken from work carried out by Ross in 1924.

Larvae collected from captured bandicoots indicated that larval engorgement usually took from 4 to 5 days, but could take as long as six days.

Following engorgement, larvae fell off the host and subsequently moulted to the nymphal stage. The period of time occupied by the first moult was greatly influenced by the degree of heat and moisture at which the engorged larvae were maintained. Under suitable

FIGURE 1 xi
THE LIFE CYCLE OF AN IXODID TICK



conditions of moisture and optimum temperature (27.5°C) the quiescent period was about 20 days. At room temperature, however, it was extended to about 40 days.

Nymphs which had just emerged after moulting showed little tendency to move about for two or three days, but then started to climb up the walls of the tube in which they were kept. In four days the majority were clustered about the top of the tube, and in 5-6 days they became very active when disturbed. When placed on a bandicoot eight days after moulting, the nymphs attached. The period that nymphs remained attached to and engorged on the host varied from 4 to 7 days, although most fell off on the fifth day. As with the larvae, cold lengthened the time taken before the moult, but did not prevent it from taking place. The shortest period taken was that of 20 days when the nymphs were incubated at a temperature which ranged from 24 to 27°C . If the nymphs were kept at room temperature (falling as low as 10°C at night), and in moist conditions, the adults emerged in 53-65 days. Dry atmosphere was either fatal to the engorged nymphs or prolonged the moulting period.

When they first emerged, the adult males were shiny-black with rounded bodies, slightly convex on both dorsal and ventral surfaces. After one or two days they tended to become yellowish-brown in colour due to the passage of large quantities of black faecal material, and their ventral surface became less convex. The legs at the same time changed from a pale yellow to a reddish colour. With time the ventral surface became markedly concave and the scutum transparent so that the outline of the black intestinal caeca could be seen clearly.

Upon emerging, the body of the female was much lighter in colour than that of the male, with a semi-transparent greyish appearance. It was thick and rounded posteriorly and somewhat convex on both

dorsal and ventral surfaces. The legs also were very pale and transparent. The body darkened rapidly in the first day or two and became a dull greyish-yellow, while the scutum was yellow in the median field with dark lateral borders. For the first two or three days after moulting there was little movement, then the newly-hatched adults commenced to move up the walls of the container in which they were kept, and in six or seven days they became quite active if disturbed. Females placed on dogs nine days after emerging attached themselves.

Coupling is the period during which the male and female remain attached to each other to enable copulation to take place. Coupling was thought to occur away from the host. It occurred readily in the laboratory, usually about five days after the females emerged. One male could copulate with more than one female and *vice versa*. The duration of coupling varied considerably from a few hours to three or four days. Coupling was thought not to be essential for engorgement.

The preferential site of attachment of females on dogs was around the anterior part of the body, especially behind the ears, on the jaws and the neck. Males were not found attached to the host. Attachment to the host did not produce pain or discomfort. Not all females engorged and some died *in situ*.

The period occupied by engorgement varied greatly according to the temperature at which the host was maintained. Adults remained attached for as long as 21 days in winter weather when the temperature fell to 6°C during the night. The shortest period for engorgement was six days in warm summer weather.

The process of engorgement usually followed well defined steps. For the first 24 hours there was little, if any, increase in size,

but there was a slight dorso-ventral thickening of the posterior part of the body. On the second day this thickening became more pronounced and there appeared to be a slight increase in length. This gradual increase in size continued on the third day, the body was now greyish in colour and noticeably lengthened. On the fourth day there was again an increase in length, but little increase in thickness. On the fifth day the tick increased considerably in length and breadth and became darker in colour. The period of final rapid engorgement had then been entered, and the following day the tick was found to have increased enormously in all dimensions and to have become a glossy greenish- or bluish-black. It completed engorgement and fell off in the next few hours.

After the females fell off, they were active for several days, but then became inactive and sought cover.

The period during which oviposition took place was subject to considerable variation, ranging from a minimum of 16 to a maximum of 34 days. The number of eggs laid per day was a maximum of 200 during the early period of oviposition, while towards the end only 20 or less per day were laid. Usually females died within a day or two after oviposition. The total number of eggs laid by a female varied from about 1,800 to 2,500.

In view of the susceptibility of eggs, engorged larvae and nymphs to a dry atmosphere and the decreased susceptibility of unfed adults to dryness, Ross (1924) concluded that the influences of heat and moisture account for the seasonal incidence of the various stages under natural conditions. During the cold winter months the various metamorphoses and hatchings of eggs are greatly prolonged. The warmer spring and early summer weather with heavy dew at night, however, causes rapid metamorphoses to the adult stage in large numbers.

As the summer advances and conditions become hotter and drier, ticks become less numerous, but because the adult stages are less affected by environmental conditions, cases of tick paralysis continue to be seen through November and December. Then with autumn rains and cooler weather, conditions are again more favourable for development, and a second smaller peak of activity may be found in May.

1.5 NATURE OF THE PARALYSIS

The disease produced by *Ixodes holocyclus* is described as a rapidly ascending motor paralysis and may follow infestation with adults (Dodd, 1921), nymphs (Ross, 1932) and larvae (Oxer and Ricardo, 1942). Larvae, however, have only been incriminated experimentally when 500 were found to be fatal to a guinea pig.

Dogs

The disease is seen most commonly in the dog. Ross (1926) demonstrated that one tick was sufficient to cause paralysis with progression to death, but that not all adult female ticks were capable of producing the disease.

Where one or two ticks only were attached, there was no risk of paralysis developing before the fifth day. Mass infestation with adults in a small pup produced signs of incoordination of the hind-quarters early on the fourth day (Ross, 1934).

Many investigators (Bancroft, 1884; Dodd, 1921; McKay, 1928; Ross, 1935; Knott, 1961) have described the clinical signs in the dog, but the most detailed observations were those of Ross (1926). Some dogs became depressed before definite signs developed, and

sometimes there was an appreciable rise in temperature. Altered voice, cough and some dysphagia were early signs noted by Seddon (1968) while Ross (1926) claimed that the appetite was usually unaffected.

The first consistent clinical sign is a slight incoordination of the hindquarters. Whether the ticks are removed at this stage or not the incoordination becomes more marked, and soon the dog is unable to stand. Movements of the limbs, although vigorous, are uncoordinated. The motor disturbance ascends rapidly to involve the muscles of the forelimbs so that the dog cannot raise itself and lies in sternal recumbency; respiration is slow and embarrassed. Violent retching occurs after taking food or water, but vomiting is difficult. In fatal cases the muscles of the head, neck, tongue and larynx become affected, swallowing is difficult and retching is marked. Some movement of the limbs is usually possible, so that the dog makes feeble convulsive movements, usually associated with respiration.

The reflexes which can be elicited in the dog are either modified or abolished early in the disease. The pupil is widely dilated but may still react to light till shortly before death.

In contrast to the severity of the motor disturbance, other signs are slight. There is often an initial fever, but the temperature in severe cases becomes subnormal. Even in severely affected animals consciousness is not lost and the animal is aware of any activity in its immediate surroundings and can always recognize its owner. There appears to be little depression of the cardiovascular system and a strong regular pulse is maintained till shortly before death (Ross, 1926).

Defaecation and urination are normal while the animal is able to

stand. When unable to stand, it is difficult to determine whether the retention which subsequently occurs is voluntary.

Severe forms of the disease are associated with a rapid onset of signs after attachment of the tick. According to Ross (1926), death in all cases appeared to be due to respiratory paralysis. The severity of the respiratory signs appeared to run parallel to the degree of paresis affecting the limbs, and without treatment no experimental dogs recovered if the stage of inability to stand was reached.

Pulmonary oedema, as well as aspiration pneumonia, have long been suspected as a cause of death, and myocarditis was also thought to occur in severe cases where vomiting was a prominent sign and recovery was slow. In several cases sudden and violent exercise within a day of complete recovery from severe paralysis resulted in collapse and death within a matter of minutes (Allan and Pursell, 1971).

Four cases of "autonomic" dysfunction of the bladder associated with tick paralysis were reported by Gordon (1972). In each case the dogs continued to dribble urine for 1-2 weeks after complete recovery from the paralysis.

Cats

The signs of tick paralysis in cats are similar to those in dogs, but vomiting is uncommon (Dodd, 1921). The mortality rate according to Knott (1961) is much lower than in dogs, and there is a greater tendency to develop an immunity from natural infestations and to retain such an acquired immunity. The favourite sites for attachment of the ticks are behind the elbows, between the shoulder blades and on the head and neck, but they have also been found on

the flanks, the tail and in the anus (Seddon, 1968).

Horses

Heavy mortalities in foals have been reported on the Atherton Tablelands in Queensland (Knott, 1961) and in north-western New South Wales (Seddon, 1968). The disease may be fatal in adults if mass infestation occurs (McCarthy, 1958).

Bootes (1962) reported the death of five yearling standard-bred foals on a property near Newcastle. First signs, seen six days after introduction to the property, were a posterior paralysis which quickly ascended and was fatal within 24 hours. Up to 40 ticks in various stages of engorgement were removed from each foal and no treatment was attempted.

Sheep

Considerable losses (up to 42%) have been reported in sheep on the Atherton Tablelands (Legg, 1927) and Lake Tyers at Victoria (Sloan, 1968).

All degrees of incoordination from partial ataxia of the hindlimbs to complete paralysis were observed (Sloan, 1968). Several of the moribund sheep were in sternal recumbency, but most were lying on their sides. Respiratory exertion was very marked, with expiratory groaning detectable in some cases at a distance of ten metres. All recumbent sheep had pupillary dilation and several produced copious quantities of clear viscid mucus from the mouth and nostrils. Three sheep that died during observation showed extreme respiratory distress immediately prior to death. Ticks were found predominantly around the eyes, ears, face and lateral aspects of the neck. Keratitis was a common feature described by Knott (1961) and often preceded paralysis.

Goats

Goats were reported by Knott (1961) to develop paralysis, but they generally recovered. Keratitis and eye discharge were noticed in association with the paralysis.

Cattle

Graziers in coastal New South Wales and southern Queensland claim that the losses from tick paralysis have increased over the years (Doube, 1975). They attribute this to effective 1080 poisoning campaigns against dingoes and foxes, which have allowed bandicoots and hence the tick population to increase significantly.

From field experience it appeared that the paralysis problem in cattle was limited to young calves born in spring and susceptible cattle introduced into tick-infested country in spring. The susceptibility of unexposed cattle was a function of their size and age, and the number of ticks engorging on them. Between three and ten females were required to paralyse calves 2-3 weeks of age, weighing 30-40 kg, whereas 20-25 females were necessary to paralyse weaner steers weighing between 80 and 160 kg (Doube and Kemp, 1975).

The clinical signs are similar to those described in the dog. The onset of posterior incoordination is usually sudden, and death may occur shortly after the animal becomes recumbent or may be delayed for a period up to three weeks (Roberts, 1946).

Poultry

Tick paralysis has been reported in various species of duck. Muscovy ducks in the northern Sydney district developed paralysis but recovered. Brown Rouen ducks kept on the same property were unaffected, an "immunity" which was thought to be related to their

colour which was not as attractive to the ticks (Turner, 1950).

Native fauna

Tick paralysis is not usually observed in native fauna in the wild state, although the common host, the bandicoot, may carry many engorging female ticks. Native fauna, however, when introduced from a tick-free area to a tick-infested area, or when kept in a laboratory for 4.5-5 months, succumb to one adult female tick (Smith, 1942; Koch, 1967). It is probable that, in the wild, native fauna develop an immunity as a result of repeated and light infestations with larvae and nymphs.

Grey and red kangaroos and koala bears kept at Koala Park near Pennant Hills in New South Wales often carry ticks which engorge and drop off without causing ill effects. Death, however, was reported by McMichen (1934) in a wallaby which was introduced from Tasmania to Koala Park, and examination of the carcass revealed 16 engorged ticks.

Humans

Up to 1945 about 20 deaths from tick paralysis had been reported in Australia, mostly in children under the age of three years (Banfield, 1966).

The most comprehensive description of the symptoms was published by Hamilton (1940) who reported that the disease consists of a widespread lower motor neurone paralysis that commences after the tick has been feeding for some days and gradually increases in severity. The child is noticed to be unsteady when walking, the gait at first being ataxic rather than weak. Soon weakness is apparent, with staggering gait, falling and difficulty in rising. Within the next

day or two this weakness increases and spreads so that the child can no longer walk. The arms become tremulous on effort and then obviously weak. The trunk and neck muscles become limp and the child has difficulty sitting up and holding up his head. Constipation may be severe due to flabby abdominal muscles. The muscles of the face may be affected, the cheeks are smooth, the mouth sags open and the face is expressionless. Involvement of the external eye muscles produces a transient squint or diplopia if muscle imbalance exists. Usually there is no squint, but if the eyes are turned to one side, nystagmus appears, for the weakened muscles are unable to hold the eye steady in this strained position. Weakness of the intrinsic eye muscles produce photophobia or blurring of near vision. Usually the muscles of deglutition are involved, the child cannot swallow and food regurgitates through the mouth and fluids through the nose. The voice becomes thick and indistinct, but not nasal unless the palatal movement is impaired. If the respiratory muscles are involved, the respiratory rate increases, the breathing is laboured and the child restless and anxious. The external nares become dilated and cyanosis may develop. The expansion of the chest from intercostal action is noticeably lessened, or the bulge of the epigastrium from the downward movement of the diaphragm during inspiration disappears. As the restlessness of the child increases, the pulse becomes increasingly rapid and feeble. The temperature frequently rises several degrees, but the patient may remain apyrexial throughout the disease. The height of the weakness is reached approximately 48 hours after the ticks fall off or are removed. If death occurs it is from respiratory failure, or later from aspiration pneumonia as a complication of feeding at the height of the paralysis. Once this period of maximum paralysis is passed,

a gradual recovery sets in and becomes complete in every respect after a variable period.

In some cases a purely local paralysis develops, especially in adults. Foster (1931) examined a case of well-marked facial paralysis on the left side, inability to close the left eye, wrinkle the forehead or whistle. An engorged tick was removed from the left auditory meatus. Crossle (1932) reported the same condition in a two year old child where a dead tick was again found in the auditory meatus.

Three cases of acute systemic sensitivity reactions to tick bites were described by Banfield (1966). Two cases showed acute allergic reactions with syncope, while the third presented in acute anaphylactic shock.

1.6 AETIOLOGY OF TICK PARALYSIS

Three concepts have been considered as possibilities for explaining the aetiology of tick paralysis:

- 1) a toxic metabolic product of the tick is produced;
- 2) a toxic metabolite may be formed from the interaction of tick saliva and host fluid and tissues; or
- 3) an organism within the tick may be the source of the disease.

Dodd (1921) found that no organism, protozoan or bacterial, could be demonstrated in the blood or other body fluids or organs of naturally and experimentally affected animals. No febrile reaction or pronounced histological change was seen, and, furthermore, attempts to transmit the disease by inoculation of blood or cerebrospinal fluid failed. The only remaining hypothesis of a living

entity transmitted by the tick was that of an ultra-visible virus which, while being non-transmissible by artificial inoculation, required passage through an intermediate host, the tick, before the ultimate host could be infected.

Emulsions of brain and spinal cord taken from a child that died from tick paralysis were made by Ferguson (1924) and injected into laboratory animals. Two rabbits and four white mice were injected both intracranially and intraperitoneally with the two emulsions. Two white mice died and cultures of *Staphylococcus aureus* were isolated and held responsible for the deaths.

In view of the above work and failure of his own experiments to isolate an organism or transmit the disease, Ross (1926) felt that the latent period was not an incubation period as in infective disease. Onset of signs was observed to depend directly upon the stage of engorgement attained by the tick, so that if ticks engorge slowly, signs were not observed for 12-13 days, whereas in those cases where ticks engorge rapidly, signs were most frequently seen 5-6 days after attachment.

This work clearly defined the close relationship of the stage of engorgement of the tick to the onset of tick paralysis. It would appear, therefore, that some change in the physiological activity of certain organs of the tick during the final rapid stage of engorgement was responsible for the production of the toxic agent. Although toxic materials could be present in various parts of the tick, in order to affect the host they must pass through its mouth parts. The salivary glands with their ducts which run forward to open into the base of the buccal capsule in close proximity to the tissues of the host suggest themselves as a likely source of the toxin, and it was probably for this reason that Ross (1926) carried out dissections

of numerous ticks.

This work revealed in all cases enormous development of the salivary glands from ticks which had engorged for 4-5 days, compared with the size of those from ticks which had only engorged for 2-3 days. In the later stages of engorgement the glands appeared as prominent pearly-white masses occupying the greater part of the anterior half of the body cavity. In partially engorged ticks the glands were much less conspicuous and the alveoli very much smaller. Two separate types of alveoli were described: a large pear-shaped alveolus and a much smaller alveolus limited in its distribution to the main salivary duct.

Emulsions of salivary glands taken from partially engorged ticks prevented coagulation when mixed with equal or double quantities of blood (Ross, 1926), thus establishing that an anticoagulant was present.

It remained to be shown whether a definite toxin could be demonstrated in the salivary glands. By dissection of numerous ticks which had engorged for five days on dogs and emulsification of the salivary glands, Ross (1935) obtained a solution which, if injected into white mice, would regularly produce death after characteristic signs of tick paralysis. The minimum lethal dose of this pooled salivary emulsion for white mice was the equivalent of 2.5-3 glands. A larger dose of glands from three and four day engorged ticks was required to produce similar signs, and Ross concluded that the onset of paralysis was determined by quantitative rather than qualitative factors. However, even when 30 ticks were placed on a 4.2 kg pup, clinical signs were not seen before the fourth day.

As tick paralysis was not noted until egg development had started in the female tick, it was necessary to determine whether

the toxin was associated with ovarian development. Riek (1957), after studying the eggs of various species of Australian ticks, including *Ixodes holocyclus*, stated that toxæmia, a rise in temperature and death, but no paralysis, followed parenteral injection of egg extracts. However, in spite of this observation, he added the interesting notation "that paralysis may be observed in some experimental animals, if the extract is injected subcutaneously in the dorsal part of the neck. In such cases, paralysis results from the inflammatory reaction associated with the injection, and not from any specific effect of the injected material on the nervous system".

Success in finally isolating the toxin, which is believed to be similar to that which causes natural tick paralysis, was achieved by Kaire (1966). Replete *Ixodes holocyclus* were homogenized and the protein precipitated from the supernatant fluid by acetone at -40°C . The precipitate was then dissolved in water and dialysed. The resulting supernatant was freeze-dried, then fractionated in diethylaminoethyl (DEAE)-cellulose columns. Fraction 2 had a lethal dose for 50% of the population (LD_{50}) of 200 milligrams per kilogram (mg.kg^{-1}) in mice. The fatal dose for dogs was $30-40 \text{ mg.kg}^{-1}$. In dogs the first clinical signs of poisoning appeared about 48 hours after injection of the toxin and were followed by characteristic signs: weakness, paralysis of the hindquarters, loss of voice, respiratory distress, vomiting and death. Mice became affected within 6-8 hours and usually died within 48 hours. Both types of animal were protected from the fraction 2 by hyperimmune serum, and immunization of dogs with the fraction protected them from paralysis by live ticks. No information regarding the nature of the toxin was obtained. Results of rechromatography of fraction 2 suggested that it contained much inert material to which the toxin was loosely bound.

The toxin was resistant to the action of pepsin, trypsin and papain, perhaps because it lacked bonds attacked by these enzymes or only a part of the molecule, not associated with the toxicity, was split off.

Koch (1967) demonstrated that with *Ixodes holocyclus* it was possible to produce signs in white mice as early as 9-12 hours after attachment. When an unengorged adult was placed on a mouse, it killed it in four days. Transfer of this partially engorged female to other mice killed the second host within 48 hours, the third in approximately 36 hours, the fourth within 29 hours and the fifth in 9-12 hours after attachment.

Fully engorged detached female adult ticks excrete a deep wine-red coloured substance through the cuticular canals before oviposition takes place. This material was collected by Koch (1967) and injected intraperitoneally into white mice. The white mice died from respiratory paralysis 9-10 hours after the injection. Examination of the material revealed the presence of at least nine different substances belonging to the group of simple poly-phenols. The substances responsible for the toxicity of the mixture and their relationship to the partially purified toxic fraction prepared by Kaire (1966) has yet to be elucidated.

Three aspects, in particular concerning the aetiology of tick paralysis, are still not understood:

- 1) the manner in which the toxin is disseminated from the site of tick attachment;
- 2) the reason for the delay of paralysis following injection of the toxic fraction (48 hours in the dog and 6-8 hours in the mouse); and
- 3) the mechanism by which a paralysed animal rids itself of the toxin.

1.7 MECHANISM OF PRODUCTION OF TICK PARALYSIS

Physiological studies

The first physiological studies of paralysis induced by *Ixodes holocyclus* were carried out by Ross (1926). He stimulated the *peroneus communis*, femoral and median nerves of an anaesthetized paralysed dog, and when he saw the normal and powerful response, he concluded that the toxin did not act peripherally. Furthermore, as the cortex did not seem to be affected, he concluded that the motor neurones in the anterior horns and nerve cells of the cranial nuclei were chiefly involved.

Most work concerning nerve conduction and transmission at myoneural junctions has been carried out in North America on animals paralysed by the tick *Dermacentor andersoni* (Stiles). This tick produces a paralysis which appears similar to that seen with *Ixodes holocyclus*. However, three features are strikingly different:

- 1) the signs seen with *Ixodes holocyclus* are more bizarre; vomiting is common and the animal appears acutely ill;
- 2) hyperimmune serum can be produced in dogs to *Ixodes holocyclus*, whereas no immunity to *Dermacentor andersoni* has been observed; and
- 3) when *Dermacentor andersoni* is removed from the host, signs of recovery are apparent immediately. Death is unusual unless the patient is moribund before removal of the tick. With *Ixodes holocyclus* the disease progresses and reaches a peak about 48 hours after removal of the tick. Death is common and recovery is slow and may take weeks.

A short summary of the physiological studies carried out on animals paralysed with *Dermacentor andersoni* is included, as future

studies with *Ixodes holocyclus* may show similar neurological defects to be present.

Initial studies by Murnaghan (1955,1956) showed conduction to be present in the motor nerves. However, indirect stimulation through the peroneal nerve or reflex stimulation through the posterior tibial nerve did not result in contraction of the anterior tibial muscle, and thus he suspected a block at the neuromuscular junction and in the spinal cord synapses. As the muscle contracted when acetylcholine was injected rapidly into the femoral artery, he concluded that the condition was probably due to a decreased liberation or synthesis of acetylcholine at the nerve end-plate. Later (1958) he showed that the end-plates were functionally active by depolarizing them with succinylcholine and thus the defect seemed to be a failure of acetylcholine liberation at the nerve terminals.

The results of further experiments (Murnaghan, 1960) indicated that tick paralysis was due to a defect in conduction of motor nerve fibres and that the tick "toxin" exhibited a predilection for the slower-conducting, smaller-diameter fibres. Since direct stimulation of the perfused paralysed muscle failed to liberate acetylcholine, a conduction block in the small-diameter terminal motor fibres was also thought to be present. These studies were substantiated by the report (Murnaghan, 1961) that, although the amplitude of the potential recorded from the fastest-conducting fibres was reduced to 30% of normal by moderate paralysis, the potential from the slower-conducting fibres was reduced to approximately 7% of normal. Furthermore, moderate paralysis was associated with a significant reduction in the conduction velocity of the slower-conducting fibres.

Other workers (Emmons and McLennan, 1959,1960) demonstrated

that, although acetylcholine was not released in the paralysed animal, the tissues could still synthesize it. In addition, depressed conduction existed not only in the spinal motor roots and hence the peripheral motor nerve fibres, but also in the sensory fibres and in the myocardium. Furthermore, the transmission processes of the central nervous system were also depressed.

Cherington and Synder (1968) also found reduced nerve conduction velocities but felt that no defect was present at the neuromuscular junction.

Esplin, Philip and Hughes (1960) suggested that the venom has a biphasic action. Initially there is impairment of the stretch reflexes, then paralysis at the neuromuscular junctions. Suggestions that tick paralysis may involve the stretch reflexes is supported by recent findings that levodopa-compensated Parkinsonian patients can suffer acute Parkinsonian crisis after tick invenomation (Sax and Mejszenkier, 1971). Culebras (1971) reported that if the pathologic alteration of motor control in Parkinsonism was the result of an altered alpha-gamma balance with augmented alpha-motor activity and depressed gamma-motor activity, then tick paralysis could precipitate decompensation of Parkinsonian manifestations by selective depression of the gamma loop, presumably at the muscle spindle level as suggested by Esplin, Philip and Hughes (1960).

Neurophysiological experiments performed in animals paralysed with *Ixodes holocyclus* by Cooper, B.J. (Cooper, Cooper, Ilkiw and Kelly, 1976) demonstrated no change in the amplitude of nerve action potentials or in maximum conduction velocities. The muscle response, however, showed a marked reduction in amplitude of the compound muscle action potential. These results suggested a failure of neuromuscular transmission, and further studies which were conducted using

a nerve-muscle preparation taken from mice paralysed by nymphal *Ixodes holocyclus* demonstrated a reduction in acetylcholine output from the nerve terminals. This effect was found to be markedly temperature dependent. Lowering of the temperature of isolated tissue reduced the effect.

Biochemical studies

Few biochemical studies of paralysed animals have been made. Koch (1967) examined tissue homogenates and sub-cellular particle preparations from gastrocnemius muscle, central nervous system and liver tissue of paralysed mice. The biochemical abnormality found in these tissues was an inhibition of those reactions, both extra- and intra-mitochondrial, which use nicotinadeninedinucleotide (NAD) coenzyme. The addition of NAD to the reaction mixtures restored the reaction rates to normal. Oxidative phosphorylation was also depressed.

Acetylcholine-esterase and the acetylcholine synthesizing enzyme of the central nervous system were found to function normally.

A decrease in the amount of pyruvic acid production in the muscle of mice paralysed by *Ixodes holocyclus* was also found by Koch (1973).

Immunological studies

Although many authors (Bancroft, 1884; Dodd, 1921; Ross, 1926) mentioned the phenomenon of immunity to *Ixodes holocyclus*, especially among native fauna and dogs living in tick-infested areas, no experimental work was carried out to substantiate this until 1935. It was established by Ross (1935) that certain dogs possess a high degree of immunity to tick paralysis and could tolerate 40-50

engorging ticks without becoming paralysed. He found that 1 ml of the serum taken from two such animals was capable of neutralizing 20-40 minimum lethal doses of tick salivary secretion in mice, while 10 ml of the serum was of value in curing paralysis induced by a single tick in pups. In naturally occurring cases evidence pointed to a 75% recovery of all cases treated as against a recovery rate of 10-60% without the serum. In the serum-treated cases, those which showed mild or non-progressive signs were excluded from the results.

To determine whether immunity was due to natural or inherited resistance, Ross (1935) mated a hyperimmune female with a hyperimmune male and placed ticks on the pups. These pups were found to be no less susceptible to paralysis than controls, suggesting that immunity was wholly acquired.

On request by Ross, hyperimmune serum was then produced by the Commonwealth Serum Laboratories, and the method used was described by Oxeer and Ricardo (1942) and Oxeer (1948). Preparation was carried out in three stages:

- 1) basal immunization;
- 2) hyperimmunization; and
- 3) bleeding and preparation of the serum.

The following table outlines the programme for basal immunization:

APPLICATION	INTERVAL BETWEEN EACH APPLICATION	NUMBER OF TICKS	DEGREE OF ENGORGEMENT ALLOWED
1	7 days	1	Half engorgement
2	"	1	" "
3	"	1	" "
4	"	1	Three-quarter engorgement
5	"	1	" "
6	"	1	Full engorgement
7	"	1	" "
8	"	2	" "
9	"	2	" "

Such a programme occupies from 2 to 3 months, and once commenced it must be continued since acquired immunity is not permanent.

To obtain a hyperimmune serum of sufficient potency for therapeutic use, the number of ticks applied is increased to 6, then to 12 and finally to about 30, with 7 days elapsing between each application.

Hyperimmune serum is collected 18 days after the application of the final dose of ticks, that is, about 10 days after the majority of ticks have fully engorged. The dogs are anaesthetized with pentobarbitone sodium and bled from the jugular vein into bottles containing potassium oxalate. Each bottle is centrifuged and the plasma siphoned off. The potassium oxalate is then neutralized with calcium chloride and the plasma allowed to clot. The serum is separated from the clot, a preservative is added and the serum filtered and bottled ready for use.

Although the potency of the serum can be measured against an emulsion of tick salivary glands, this was found impractical as a

routine measure because of the very large number of ticks that must be dissected to carry out even one titration.

Kaire (1965) described a method for assay of hyperimmune serum using the toxic fraction 2 which he isolated from *Ixodes holocyclus* homogenates. Results indicated that 1 ml of hyperimmune serum neutralized 2.3-6.7 minimum lethal doses of toxin in white mice.

Despite the availability of these assay methods, they are not used in the production of hyperimmune serum in Australia.

CHAPTER 2

GENERAL MATERIALS AND METHODS

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This chapter describes in detail the techniques used throughout the study. The protocols and any additional methods are outlined in a separate methods section in each chapter.

2.1 PROTOCOL SUMMARY

Ninety cross-bred dogs ranging in weight from 9.5 to 32.0 kg were used in these studies. The seasonal incidence of the tick and the nature of the disease prevented multiple measurements to be taken simultaneously on many dogs. Although ticks were theoretically available from October to January, practically this was not always so, as availability depended on local weather conditions. The dogs which were used in these experiments had to be prepared and held until arrival of the ticks, as it was found that the longer the period between arrival and attachment of ticks, the greater the number of ticks needed to produce the disease. The nature of the disease was such that continuous observations of the animals were necessary, and although in most cases this was achieved, not all measurements at all stages in every animal were obtained.

As a result the study was divided into several sections:

1. The initial experiment consisted of the measurements of haematological, biochemical and arterial blood-gas and pH values. The clinical course of the disease was documented so that it could be divided into stages to facilitate the statistical analysis of the results. At death the animals were subjected to post-mortem examination and tissues were collected for histological examination. Ten dogs with surgically implanted catheters in the carotid artery were used in this experiment.

Two dogs were not infested and served as control animals for analysis of time and individual animal changes.

2. In the next experiment a detailed study of the effects of *Ixodes holocyclus* on respiration was undertaken. Respiratory rate, tidal volume, expiratory time, mixed expired carbon dioxide tension and arterial blood-gas and pH values were measured. From these values minute respiratory volume, the ratio of dead space to tidal volume and the difference between alveolar oxygen tension and arterial oxygen tension were calculated. Fourteen dogs with surgically implanted catheters in the carotid artery were used in this study.
3. An examination of the electrocardiographic changes observed in tick paralysis was undertaken in the next experiment. A description of the electrocardiogram and, where relevant, the values of arterial oxygen tension, plasma potassium concentration, plasma calcium concentration and body temperature of each dog at each stage of the disease is presented. Ten dogs with surgically implanted catheters in the carotid artery were used in this study.
4. In the next experiment a detailed study of the effects of *Ixodes holocyclus* on the cardiovascular system was investigated. Ten dogs were surgically prepared via a left thoracotomy with catheters in the pulmonary artery, left atrium and aorta, an electromagnetic flow probe around the ascending aorta and a micromanometer in the left ventricle. Following surgery the dogs were allowed two weeks for return to a physiologically normal state before application of the ticks. Throughout the various stages of the disease systolic and diastolic arterial pressure, mean pulmonary arterial and left atrial pressure, mean

cardiac output, myocardial contractility and arterial and mixed venous oxygen, carbon dioxide tension and arterial and mixed venous pH were measured. Mean arterial pressure, mean cardiac output per kilogram, systemic vascular resistance per kilogram, pulmonary driving pressure and pulmonary vascular resistance per kilogram were calculated.

5. The final experiment consisted of a comparison of the effects of various treatments commonly used in tick paralysis with no treatment or a treatment derived from the previous experiments on survival rate. Forty-six dogs were used in this experiment.

2.2 SELECTION OF DOGS

Cross-bred dogs were obtained from the Sydney University Animal House and, as prior history was not available, there was no knowledge of previous tick infestation or immunity.

On arrival all dogs were subjected to a full clinical examination, vaccinated against distemper and hepatitis and held for at least two weeks before inclusion in an experiment.

Prior to experimentation all dogs were again given a full clinical examination and any showing signs of disease or unpleasant disposition were not used.

2.3 HOUSING OF DOGS

All dogs were housed individually in ventilated kennels and fed once daily. They were exercised for one hour night and morning until

signs of paralysis were observed.

Once signs of paralysis appeared the animals were taken to an air-conditioned laboratory where they were constantly observed, and throughout this period all food and water was withheld.

2.4 ADULT FEMALE TICKS

An attempt to breed ticks in the laboratory was made to try to obviate the seasonal dependence of the experiments. This is described in detail in the appendix (Chapter 9). The laboratory-reared adult female ticks were found to be unsuitable since no signs of disease were produced when up to ten ticks were placed on a dog. All experiments reported in this thesis were conducted using wild-caught unfed adult female ticks.

Source of ticks

By arrangement with Lismore Serum Products, wild-caught ticks were made available from October to January. Fifty ticks were placed in a 1.5 cm diameter perspex container on moistened moss and sent on the afternoon plane to Sydney. Immediately upon arrival at the airport the ticks were taken to the laboratory where they were placed in sterilized jars on moistened sand. In all experiments arrival of the ticks and placement on the dogs occurred within 48 hours.

Application of the ticks

The dogs were restrained by anaesthesia with halothane, nitrous oxide and oxygen during application of the ticks (day 0 of attachment). The inside of the ear was chosen as the site of attachment of the

ticks for two reasons:

- 1) under natural conditions most ticks are found on the head and neck region, and therefore no trouble was anticipated in obtaining attachment; and
- 2) when recording the rate of engorgement and onset of clinical signs, this site facilitated finding and measuring the ticks.

The ticks were placed at the site of intended attachment and a blunt-ended probe dipped in water was used to stimulate the under-surface of the body of the tick. Once the palps parted, tipping the tick on its head induced initial penetration, and gentle stroking, simulating removal, assured firm attachment. If attachment was not observed within five minutes, the ticks were replaced by a fresh batch.

Ticks obtained from the serum laboratory were very viable, attached easily and fed aggressively, so that six ticks could be placed on each of 20 dogs over a period of an hour.

2.5 CATHETERS FOR IMPLANTATION

All catheters for implantation were manufactured from medical grade polyethylene tubing (internal diameter (ID) 1.00 mm, outer diameter (OD) 2.00 mm) and silicone-rubber tubing (Silastic, Medical-Grade ID 1.57 mm, OD 3.18 mm, Dow Corning). To allow passage of the polyethylene tubing within the silicone-rubber tubing, the latter was immersed in toluene for three hours to allow expansion. The polyethylene tubing was then inserted into the silicone-rubber tubing and the assembled catheter was dried to promote shrinkage of the silicone-rubber to its original size. The tip of the catheter was

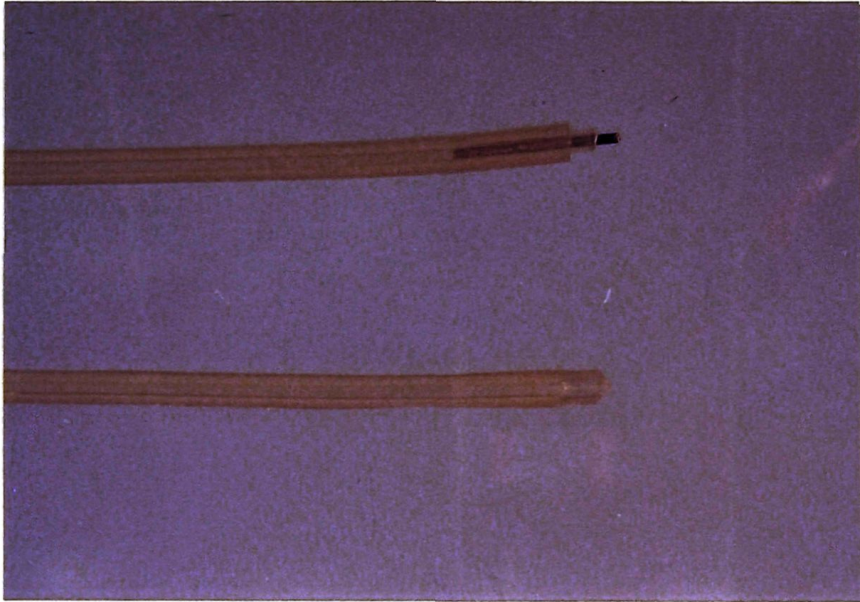
cut as illustrated in Figure 2 i, and the silicone-rubber tubing advanced 5 mm beyond the polyethylene tubing. The completed catheter was kept a further seven days for vaporization of all toluene before implantation. The catheters were prepared as above because both materials have distinct advantages and disadvantages, which when used together approach the ideal catheter. Silicone-rubber is less thrombogenic and reactive than polyethylene, is more flexible and can be secured more firmly in place, but it is more pervious to external agents, such as bacteria, has a poor frequency response, and its lumen is occluded if tied tightly. By combining the two types of tubing and adapting the tip, the frequency response was excellent, the catheter impervious, it could be secured firmly in place without obstructing flow and it produced minimal thrombus formation.

Similarly constructed catheters were implanted into the pulmonary artery and left atrium. However, as these catheters were placed directly into these structures, an adaptation was made to the catheter to allow it to be held securely in place. Two rings of silicone-rubber medical adhesive (Silastic type A, Dow Corning) and a flange of silicone-rubber reinforced medical-grade sheeting 0.5 mm thick (Silastic, Dow Corning) were added to the tip of the catheter as illustrated in Figure 2 ii.

The finished catheters were sterilized by boiling for 20 minutes prior to implantation.

FIGURE 2 i
ARTERIAL CATHETER

ACTUAL



DETAILED STRUCTURE OF TIP

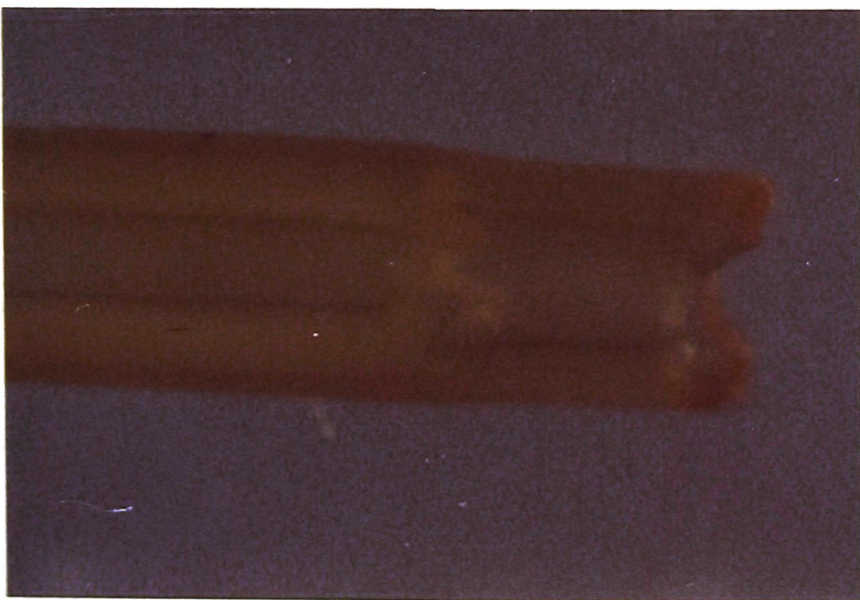
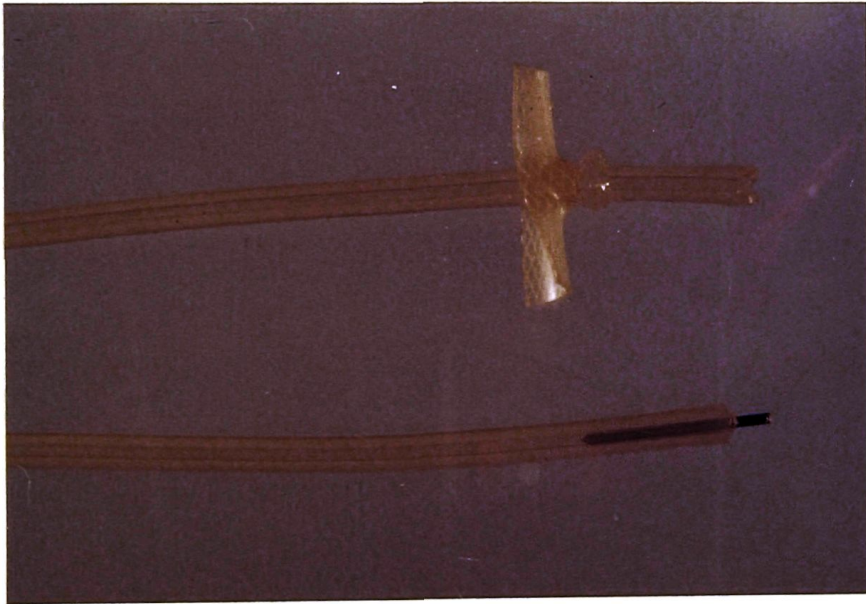


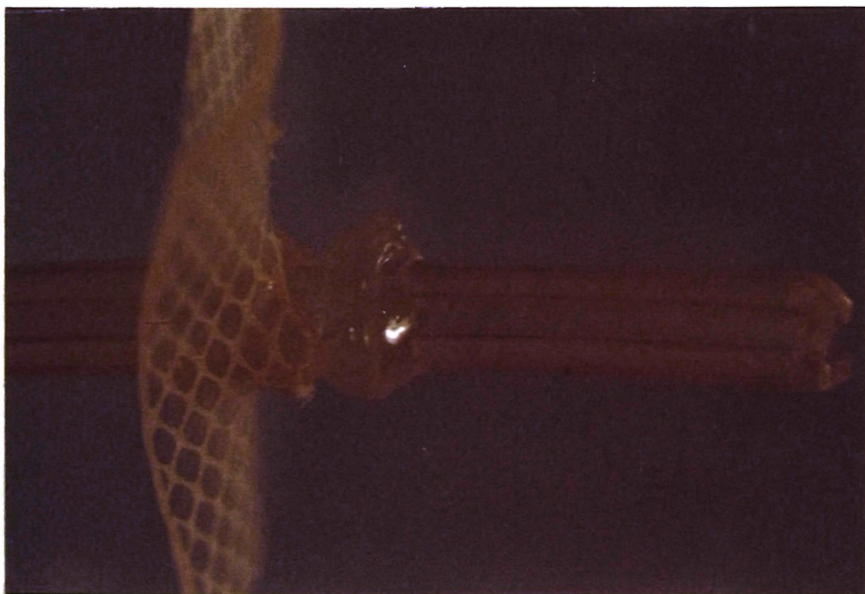
FIGURE 2 ii

ATRIAL AND PULMONARY ARTERIAL CATHETER

ACTUAL



DETAILED STRUCTURE OF TIP



2.6 IMPLANTATION OF CATHETERS

Implantation of the catheters was carried out under general anaesthesia with halothane, nitrous oxide and oxygen. For arterial pressure and acid-base measurements a surgical approach with aseptic technique was made in the jugular furrow. The carotid artery was isolated and a small transverse incision made. The catheter was advanced until the tip was in the arch of the aorta and was secured by ligation using metric 2.5 black braided silk (B.B. silk, Ethnor) in the carotid artery. It was filled with sterile heparin, 1:1000 international units per millilitre (IU.ml^{-1}), and sealed with a stainless steel obturator (Fig. 2 i).

The placement of catheters directly into the pulmonary artery and left atrium is described in the next section.

2.7 THORACOTOMY TECHNIQUE AND POST-SURGICAL CARE

The animals requiring thoracotomy for placement of catheters and instruments employed in the cardiovascular studies were pre-medicated with atropine sulphate, 0.02 mg.kg^{-1} . Anaesthesia was induced with thiopentone sodium, 20 mg.kg^{-1} , and maintained using halothane, nitrous oxide and oxygen. Intermittent positive-pressure ventilation was accomplished using a Pulmoflater 5050 (Blease Medical Equipment Limited). Hartmann's solution (Abbott Laboratories), 1-3 litres, was administered during the surgery.

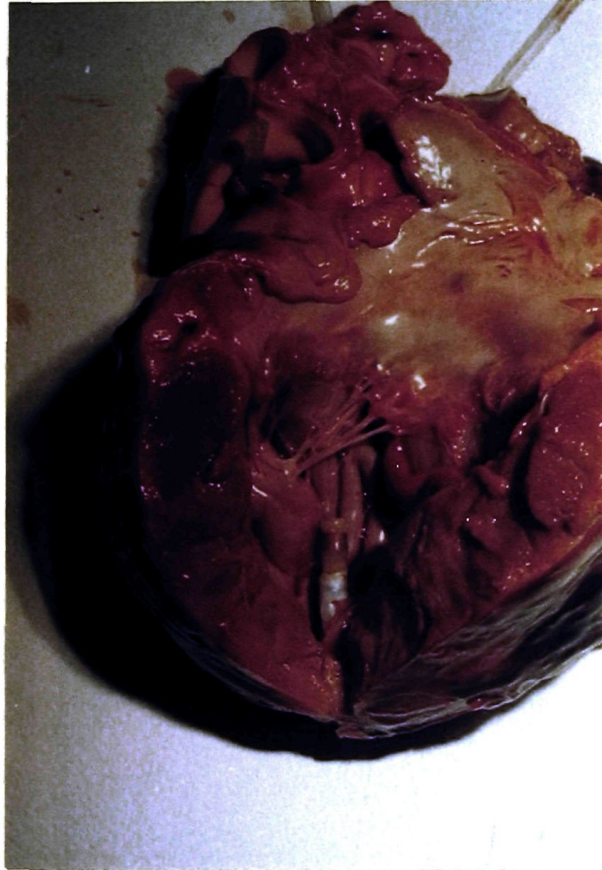
The surgical approach to the thorax was via a left fourth interspace incision. The skin, subcutaneous tissue, *cutaneous trunci*, *latissimus dorsi*, *scalenus*, *serratus ventralis*, and internal and external intercostal muscles were transected. Retraction of the

ribs was achieved with self-retaining retractors. The pericardium was incised ventral to and parallel with the phrenic nerve and the heart gently lifted from the pericardial sac. A purse-string suture of metric 2.5 braided polyester (Mersilene, Ethnor) was placed in the apex of the left ventricle. A stab incision through the myocardial wall was made in the centre of the area enclosed by this suture, enlarged with haemostats, and a micromanometer was carefully inserted and sutured in place (Fig. 2 iii). The left ventricular pressure (LVP) wave was visualized to ensure correct placement of the manometer. Purse-string sutures of metric 2.5 braided polyester (Mersilene, Ethnor) were placed in the *conus arteriosus* of the pulmonary artery and the *auricle* of the left atrium. Stab incisions were made in the centres of the areas enclosed by the sutures and the catheters inserted so that the first of the two rings lay within the vessel. The suture was ligated and the flange sutured to the vessel with metric 2.5 braided polyester (Mersilene, Ethnor). The catheter in the left atrium was further held by a ligature of umbilical tape because of the fragility of the atrial wall. The arterial catheter, as described in 2.6, was passed down the carotid artery and the tip was positioned by palpation in the aortic arch. Finally an electromagnetic flow probe was placed around the ascending aorta as illustrated in Figure 2 iii. The pericardium was repositioned (not closed) using metric 3.5 chromic catgut (Ethnor), and the leads of the micromanometer, flow probe and two catheters were passed subcutaneously to be exteriorized on the dorsal midline (Fig. 2 iv). Crystalline penicillin, 600 mg, was instilled in the thorax before closure. The thoracotomy was closed using 4-5 stay sutures of metric 5 braided polyester (Mersilene, Ethnor) around the ribs, and a continuous suture with metric 4 chromic catgut

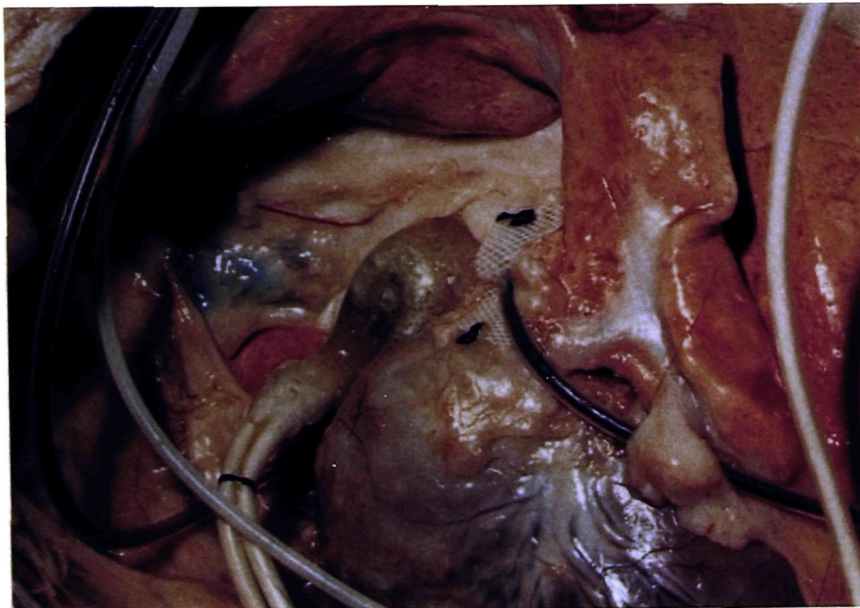
FIGURE 2 iii

PLACEMENT OF CARDIOVASCULAR MEASURING DEVICES

MICROMANOMETER



ELECTROMAGNETIC FLOW PROBE



(Ethnor) in the intercostal muscles. Collapsed lung was reinflated by increasing ventilatory pressure prior to final closure of the thorax and a thoracic drain was not used. The overlying muscle layers were closed with metric 4 chromic catgut (Ethnor), the subcutaneous tissues with metric 3.5 chromic catgut (Ethnor), and the skin with metric 3 black braided silk (B.B. silk, Ethnor).

The thorax was bandaged with combine dressing roll and elasto-plast. This was changed daily until replaced by a terylene coat at six days (Fig. 2 iv). The dogs received $40,000 \text{ IU.kg}^{-1}$ of procaine benzyl penicillin and 10 mg.kg^{-1} of dihydrostreptomycin by intramuscular (IM) injection every 12 hours for five days. The catheters were flushed daily and the dead space filled with $1:1000 \text{ IU.ml}^{-1}$ of heparin.

Following surgery, the dogs were brought to the laboratory each day and trained to lie with minimal restraint on a table. The table was covered with a foam bed and, as illustrated in Figure 2 iv, the dogs rested their heads on a pillow.

The dogs were exercised twice daily for at least one hour from the third post-operative day.

2.8 BLOOD-GAS AND pH MEASUREMENTS

The partial pressure of oxygen (P_{O_2}) in millimetre of mercury (mmHg), the partial pressure of carbon dioxide (P_{CO_2} , mmHg) and the pH of the blood were measured electrometrically with a blood-gas analyzer (BMS3, Radiometer). The span of the pH electrode (G298A) was calibrated with precision buffer solutions (S1500 and S1510, Radiometer) of pH 6.841 and pH 7.383. The carbon dioxide electrode

FIGURE 2 iv
INSTRUMENTED DOGS



(E5037) was calibrated with gases containing carbon dioxide concentrations of 2.82% and 8.3% and the oxygen electrode (E5046) with gases containing oxygen concentrations of 0% and 20.9%. The calibrations were checked twice weekly against known gas and pH concentrations (Versatol Acid Base, General Diagnostics). A 2 ml sample of blood was collected anaerobically over a period of 15 seconds (sec) into a glass syringe, the dead space of which was filled with 1:1000 IU.ml⁻¹ of heparin, and analysis performed immediately. All measurements were made at 37°C and the results were corrected for body temperature (temp) using a blood-gas calculator (BGCl, Radiometer). Standard bicarbonate (Std HCO₃ in milliequivalents per litre, mEq.l⁻¹) was calculated using a blood-gas calculator (BGCl, Radiometer).

CHAPTER 3

A CLINICAL, CLINICO-PATHOLOGICAL AND HISTOPATHOLOGICAL
STUDY OF THE EFFECTS OF *IXODES HOLOCYCLUS* IN THE DOG

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3.1 INTRODUCTION

Dogs with *Ixodes holocyclus* toxæmia show such characteristic signs that the only detailed examination carried out by most veterinarians is to find the offending parasite.

The first step in the investigation of any disease is a thorough clinical examination. Aids to diagnosis include measurements of haematological and biochemical values and acid-base status. If the animal dies, a post-mortem examination is carried out and pathological tissues are taken for histological examination.

Therefore, the first stages in this study were the measurement of the above parameters and the recording of clinical signs and the time taken between attachment of *Ixodes holocyclus* and onset of signs under laboratory conditions.

3.2 MATERIALS AND METHODS

Ten healthy cross-bred dogs, five males and five females, ranging in weight from 20.7 to 32.0 kg were used in this study. The dogs were randomly numbered 1-10, and two dogs which were not infested served as control animals.

One week prior to attachment of the ticks, the dogs were surgically prepared by implantation of a catheter in the carotid artery to enable collection of arterial blood.

Arterial blood-gas and pH, haematological and biochemical estimations, together with a clinical examination were carried out on all dogs on the two days preceding attachment of the ticks to serve as baseline control data.

Arterial pH, partial pressure of oxygen in arterial blood (Pa_{O_2} , mmHg) and the partial pressure of carbon dioxide in arterial blood (Pa_{CO_2} , mmHg) were measured.

Haematological estimations were carried out on 2.5 ml of blood collected into a tube containing dipotassium ethylenediamine tetraacetic acid (EDTA) as anticoagulant. Packed cell volume (PCV), total plasma protein (TPP), haemoglobin (Hb), total red blood cells (RBC) and total white blood cells (WBC) were measured. Haemoglobin (grams per decilitre, g.dl^{-1}) was estimated by the cyan-methaemoglobin method as described by Wintrobe (1964). Total red blood cells ($\times 10^6$ per microlitre, $\times 10^6 .\mu\text{l}^{-1}$) and white blood cells ($\times 10^3 .\mu\text{l}^{-1}$) were counted using an electronic counter (Coulter Counter model F, Coulter Electronics). Packed cell volume (%) was determined by the microhaematocrit centrifuge technique and total plasma protein (g.dl^{-1}) measured using a refractometer (Series PRB, American Optical Company).

Biochemical estimations were carried out on plasma obtained by spinning 10 ml of heparinized blood at 3000 revolutions for five minutes. Measurements of sodium (Na , mEq.l^{-1}), potassium (K , mEq.l^{-1}), phosphate (PO_4 , milligrams percent, mg%), total protein (TP, grams percent, g%), albumin (ALB, g%), total carbon dioxide (TCO_2 , mEq.l^{-1}), blood urea nitrogen (BUN, mg%), cholesterol (CHOL, mg%), glucose (GLUC, mg%), total bilirubin (TBIL, mg%) and alkaline phosphatase (AP, units per litre, u.l^{-1}) were made using an autoanalyzer (SMA 12/60, Technicon) and standard Technicon 12/60 methods. Calcium (Ca, mg%) and magnesium (Mg, mg%) were determined using a spectrophotometer (303, Perkinelmer) with lanthanum as diluent and creatine phosphokinase (CPK, u.l^{-1}) by the Rosalki ultraviolet kinetic method.

Four ticks were attached to six of the dogs, while two dogs were infested with three ticks. Two dogs were not infested with

Ixodes holocyclus and served as control dogs to enable between-dog and day-to-day variations in the measurements throughout the experimental period to be documented.

In the two dogs that were not infested arterial blood was collected for blood-gas and pH measurements on days 2, 4, 6 and 8 of the experimental period. Blood for haematological measurements was collected on day 2, 4 and 8 of the experimental period, while blood for biochemical estimations was collected on days 1, 2, 4, 6 and 8 of the experimental period.

In the tick-infested dogs clinical examinations were carried out each morning and night, and blood was collected for the various estimations on alternate days until signs of the disease were observed. Once signs were evident, clinical examinations were carried out, and blood was collected at the various stages of the disease until death occurred. After death the ticks were collected and measured. A full post-mortem examination excluding brain and spinal cord was carried out on all dogs, and sections of tissue from the heart, lungs, liver and kidneys were fixed in 10% buffered formaldehyde for histological examination.

A two-way analysis of variance as described by John and Quenouille (1977) was carried out on the measurements obtained from the two control dogs throughout the experimental period.

The results of the blood-gas, pH and biochemical measurements were analysed by pairing the control and test values in each animal, and the mean differences at each stage were compared with zero using the Student's t-distribution. Simple logarithmic transformations were carried out on the data of glucose, cholesterol and creatinine phosphokinase at all stages of the disease, because these values were distributed over a very wide range. The transformed data were

then analysed by Student's t-test.

The results of the haematological measurements were analysed by pairing the control and test values in each animal and the mean differences at an advanced stage of the disease compared with zero using the Student's t-distribution.

3.3 RESULTS

3.31 Arterial blood-gas and pH, haematological and biochemical measurements in control dogs

The arterial blood-gas and pH, haematological and biochemical measurements found in the control dogs are presented in Table 3 i. The results of a two-way analysis of variance carried out on the two control dogs over the experimental period is illustrated in Table 3 ii.

There were no significant changes in arterial oxygen tension, arterial carbon dioxide tension, pH and standard bicarbonate between dogs or between sampling times. However, temperature showed a significant variation between dogs. Haematological examination revealed no significant changes between dogs or between sampling times.

Within the biochemical values calcium and phosphate showed both between dog and between sampling time variations. Alkaline phosphatase and creatinine phosphokinase showed only between dog variations, while potassium, total carbon dioxide and total bilirubin showed only between sampling time variation.

TABLE 3 i

ARTERIAL BLOOD-GAS AND pH, HAEMATOLOGICAL AND BIOCHEMICAL
MEASUREMENTS IN CONTROL DOGS

		CONTROL DOGS	
		MEAN	± SEM*
Pa _{O₂}	mmHg	n=8	90.87 ± 3.70
Pa _{CO₂}	mmHg		28.90 ± 1.21
pH			7.355 ± 0.031
Std HCO ₃	mEq.l ⁻¹		18.23 ± 1.11
Temp	°C		38.83 ± 0.38
PCV	%	n=6	41.0 ± 1.2
TPP	g.dl ⁻¹		6.68 ± 0.50
Hb	g.dl ⁻¹		13.75 ± 0.66
RBC	x10 ⁶ .μl ⁻¹		5.78 ± 0.19
WBC	x10 ³ .μl ⁻¹		20.48 ± 4.24
Na	mEq.l ⁻¹	n=10	143.8 ± 1.1
K	mEq.l ⁻¹		4.24 ± 0.09
Ca	mg%		10.06 ± 0.13
Mg	mg%		1.81 ± 0.04
PO ₄	mg%		4.04 ± 0.18
TP	g%		6.54 ± 0.13
ALB	g%		2.98 ± 0.05
TCO ₂	mEq.l ⁻¹		17.00 ± 1.13
BUN	mg%		13.8 ± 1.0
CHOL	mg%		204.8 ± 23.2
GLUC	mg%		103.0 ± 13.4
AP	u.l ⁻¹		73.9 ± 19.1
TBIL	mg%		0.24 ± 0.04
CPK	u.l ⁻¹		18.4 ± 2.6

* Standard Error of the Mean (SEM)

TABLE 3 ii ANALYSIS OF VARIANCE OF CONTROL DOGS

	SOURCE OF VARIATION					
	DOGS		DAYS		ERROR	
	DF*	1	DF	3	DF	3
	F	P	F	P		
Pa _O ₂	0.30	NS†	0.26	NS	123.00	
Pa _{CO} ₂	0.03	NS	4.78	NS	6.99	
pH	3.711	NS	0.342	NS	0.004	
Std HCO ₃	5.38	NS	1.42	NS	4.37	
Temp	12.93	<0.05	0.95	NS	0.24	
	DF	1	DF	2	DF	2
	F	P	F	P		
PCV	0.63	NS	3.49	NS	9.5	
TPP	1.06	NS	0.48	NS	0.76	
Hb	0.86	NS	3.63	NS	0.86	
RBC	4.57	NS	3.97	NS	0.06	
WBC	0.56	NS	0.65	NS	76.82	
	DF	1	DF	4	DF	4
	F	P	F	P		
Na	3.66	NS	3.21	NS	5.35	
K	0.21	NS	8.63	<0.05	0.02	
Ca	30.12	<0.05	22.53	<0.05	0.01	
Mg	1.88	NS	1.35	NS	0.01	
PO ₄	47.52	<0.05	15.30	<0.05	0.02	
TP	0.02	NS	0.32	NS	0.22	
ALB	1.11	NS	0.82	NS	0.02	
TCO ₂	6.30	NS	8.12	<0.05	4.38	
BUN	0.40	NS	2.70	NS	12.15	
CHOL	2.83	NS	1.06	NS	3733.75	
GLUC	0.52	NS	1.53	NS	49.1	
AP	13.47	<0.05	0.88	NS	1055.4	
TBIL	1.00	NS	25.00	<0.05	0.001	
CPK	0.10	<0.05	1.46	NS	37.6	

* Degrees of freedom (DF)

† Not significant (NS)

3.32 Period elapsing between attachment of ticks and onset of clinical signs

The number (n) of days from attachment of *Ixodes holocyclus* to onset of clinical signs is shown in Table 3 iii. The duration of the disease, illustrated by arrows in Table 3 iii, was quite variable. The mean duration of the disease was 23.3 hours. The mean duration of the disease in those animals showing earliest clinical signs was 20.8 hours. In those animals not showing clinical signs until at least 12 hours after the first dog showed signs, the mean duration of the disease was 26.7 hours.

In this study the minimum duration of the disease was 15 hours while the maximum duration was 30 hours.

The number of ticks attached to each dog at the various stages of the disease is also shown in Table 3 iii.

3.33 Size of ticks at full engorgement or if attached at death of the dog

The length and breadth of the ticks, in mm, were measured either at full engorgement if they could be found, or if still attached at death of the animals. These values are shown in Table 3 iv.

3.34 Stages of the disease

From the clinical signs observed, the course of the disease was divided into five stages.

Stage 1: Ataxia

The first signs observed were a slight ataxia of the hindlimbs. The dogs appeared alert, were eating and drinking normally, and no

TABLE 3 iii

PERIOD ELAPSING BETWEEN ATTACHMENT OF *IXODES HOLOCYCLUS*, ONSET
AND DURATION OF CLINICAL SIGNS AND NUMBER OF TICKS ATTACHED

DOG NUMBER		DAYS FROM ATTACHMENT						
		5	6			7		8
1	TICKS	3	3	3		1	1	
	SIGNS							
3	TICKS	4	4	4				
	SIGNS	←————→						
4	TICKS	4	3	2	1	1	1	1
	SIGNS					←————→		
5	TICKS	4	4	3		3		
	SIGNS		←————→					
6	TICKS	4	4	4		3	2	
	SIGNS					←————→		
7	TICKS	2	2	2				
	SIGNS	←————→						
9	TICKS	4	4	4		4	3	3
	SIGNS					←————→		
10	TICKS	4	4	4		4		
	SIGNS		←————→					

TABLE 3 iv

SIZE OF *IXODES HOLOCYCLUS* AT FULL ENGORGEMENT
OR IF ATTACHED AT DEATH OF THE DOG

DOG NUMBER	TICK SIZE (mm)			
	TICK 1	TICK 2	TICK 3	TICK 4
3	14 x 10	14 x 10	6 x 4	5 x 3
4		12 x 9	10 x 7	5 x 3
5		8 x 6	8 x 5	7 x 4
6		13 x 10	12 x 10	12 x 9
7			11 x 8	10 x 7
9	13 x 10	7 x 5	6 x 4	4 x 3
10	13 x 10	12 x 9	9 x 6	8 x 5

vomiting was seen. Coughing was observed in one dog. The character of respiration was normal.

Stage 2: Unable to walk

The animals were in lateral recumbency but could right themselves and hold this position if prompted. They crawled round the floor, were alert and responded with tail wagging when approached. An artery forcep applied to the webbing between the toes resulted in withdrawal of that leg (withdrawal reflex). Positive withdrawal reflexes were present in both fore- and hindlimbs, although the dogs did not appear to feel a painful stimulus to the neck or along the forelimbs. Painful stimuli to other areas resulted in movement and the dog would look round at that area. The eyes were normal in appearance and the pupils were normal in size and responded to light (pupillary light reflex). The dogs appeared to be able to swallow, as no pooling of saliva around the mouth was noticed, although the gag reflex (pharyngeal movement in response to fingers placed in the pharyngeal area) was diminished. Two dogs vomited throughout this stage; one vomited white-frothy material, while the other vomited bile-stained material. There was a slight change in respiratory pattern, with respiration becoming more noticeable.

Stage 3: Unable to right

The dogs lay in lateral recumbency and despite attempts to right themselves they were unable to do so. Withdrawal reflexes were usually present in both fore- and hindlimbs, but the response was slow and less forceful. There was no response to a painful stimulus to the neck, a quarter-way down the back and along the forelimbs. The pupils were normal to dilated and the nictitating membrane was half-way across the eye. Pupillary light reflexes were

present. The gag reflex was again depressed but still present and saliva pooled around the mouth. Respiration was noticeably changed. A "grunting" type of respiration was observed with a forced expiration.

Stage 4: Unable to right and absence of withdrawal reflexes in fore- and hindlimbs

There were spontaneous movements of the fore- and hindlimbs. No response to a painful stimulus was observed on the neck, thorax, forelimbs, down the hindlimbs and half-way along the back. Painful stimulus further down the back or on the tail caused attempts at movement. In most dogs the pupils were dilated, the pupillary light reflex was absent in some cases, and the nictitating membrane more than half-way across the eye. The gag reflex was depressed and pooling of saliva in front of the dogs was marked. There appeared to be loss of bladder control with urinary incontinence. Respiration was forced and "grunting" in type.

Stage 5: Prior to death

These animals were within two hours of death. All withdrawal reflexes had disappeared and the distal half of the tail was the only area which responded with movement to a painful stimulus. The gag reflex was still depressed and there was marked pooling of saliva in front of the dogs. The pupils were dilated and pupillary light reflexes were absent. Respiration was forced and "grunting", the animals appeared agitated and the limbs seemed to move with respiration. As this stage progressed the lips were drawn back with each breath and the colour of the mucous membranes appeared grey. The dogs would then pass into a stage of occasional gasps. The pupil was widely dilated and the cornea dry.

3.35 Arterial blood-gas and pH measurements

Arterial oxygen tension, arterial carbon dioxide tension, pH, standard bicarbonate and temperature at the control period and at the various stages of the disease are shown in Table 3 v. The range considered by the Sydney University Veterinary Clinic to be normal for these measurements is also included.

Stages 1, 2 and 3 revealed no significant changes in values from control.

Stage 4 demonstrated a significant elevation in arterial carbon dioxide tension, while arterial oxygen tension, pH, standard bicarbonate and temperature did not change significantly from control.

During stage 5 all values except temperature showed significant changes from control. Arterial oxygen tension, pH and standard bicarbonate fell significantly, while arterial carbon dioxide tension rose significantly.

3.36 Haematological measurements

The change in haematological values between control data and dogs showing advanced signs of paralysis is shown in Table 3 vi. The range considered by the Sydney University Veterinary Clinic to be normal for these measurements is also included in the table.

Haemoglobin rose significantly, while all other values did not differ significantly from control.

3.37 Biochemical measurements

The changes from control in biochemical values at the various stages of the disease are demonstrated in Table 3 vii. The range considered by the Sydney University Veterinary Clinic to be normal for these measurements is also included in the table.

TABLE 3 v CHANGES IN ARTERIAL BLOOD-GASES AND pH MEASUREMENTS AT THE VARIOUS STAGES OF PARALYSIS
CAUSED BY *IXODES HOLOCYCLUS*

	NORMAL RANGE	CONTROL MEAN \pm SEM n = 10	STAGE 1 MEAN \pm SEM n = 4	STAGE 2 MEAN \pm SEM n = 4	STAGE 3 MEAN \pm SEM n = 4	STAGE 4 MEAN \pm SEM n = 4	STAGE 5 MEAN \pm SEM n = 5
Pa _{O₂} mmHg	80 - 105	92.22 \pm 1.63	97.45 \pm 3.69	88.13 \pm 1.64	89.88 \pm 2.73	74.47 \pm 5.26	55.64 \pm 5.97 ^{***}
Pa _{CO₂} mmHg	35 - 45	30.78 \pm 0.98	25.40 \pm 2.45	31.73 \pm 2.45	31.00 \pm 1.83	38.84 \pm 4.75 [*]	51.36 \pm 3.35 ^{***}
pHa	7.350- 7.450	7.368 \pm 0.008	7.395 \pm 0.035	7.378 \pm 0.010	7.390 \pm 0.011	7.318 \pm 0.016	7.203 \pm 0.015 ^{***}
Std HCO ₃ mEq.1 ⁻¹	20 - 30	19.61 \pm 0.47	19.20 \pm 0.87	20.08 \pm 0.45	19.98 \pm 0.20	19.75 \pm 0.78	17.74 \pm 0.54 ^{***}
Temp °C	37.8 - 39.2	38.61 \pm 0.16	38.70 \pm 0.11	38.58 \pm 0.06	38.38 \pm 0.21	37.92 \pm 0.21	37.16 \pm 1.01

* P<0.05

*** P<0.01

TABLE 3 vi

CHANGES IN HAEMATOLOGICAL VALUES IN DOGS
PARALYSED BY *IXODES HOLOCYCLUS*

	NORMAL RANGE	CONTROL MEAN \pm SEM n = 10	PARALYSED MEAN \pm SEM n = 5
PCV %	30 - 50	46.4 \pm 1.0	50.0 \pm 2.4
TPP g.dl ⁻¹	5.5 - 7.5	6.78 \pm 0.11	7.58 \pm 0.43
Hb g.dl ⁻¹	10 - 15	14.71 \pm 0.34	16.68 \pm 0.77**
RBC $\times 10^6$. μ l ⁻¹	5 - 7	6.36 \pm 0.16	6.80 \pm 0.30
WBC $\times 10^3$. μ l ⁻¹	7 - 12	15.38 \pm 1.15	20.82 \pm 4.84

** P<0.02

TABLE 3 v11

CHANGES IN BIOCHEMICAL VALUES AT THE VARIOUS STAGES OF PARALYSIS CAUSED BY *IXODES HOLOCYCLUS*

	NORMAL RANGE	CONTROL MEAN ± SEM n = 10	STAGE 1 MEAN ± SEM n = 4	STAGE 2 MEAN ± SEM n = 4	STAGE 3 MEAN ± SEM n = 4	STAGE 4 MEAN ± SEM n = 4	STAGE 5 MEAN ± SEM n = 5
Na mEq.l ⁻¹	137 -149	146.6 ± 0.4	145.5 ± 1.3	146.3 ± 0.5	146.3 ± 0.6	144.5 ± 0.9	144.0 ± 2.6
K mEq.l ⁻¹	3.2- 5.2	4.51± 0.07	4.48± 0.11	3.90± 0.12 ^{**}	4.08± 0.14 ^{**}	3.83± 0.13 ^{**}	4.04± 0.40
Ca mg%	8.5- 11.5	10.44± 0.05	10.35± 0.14	10.10± 0.08	10.10± 0.32	10.18± 0.19	10.38± 0.28
Mg mg%	2	1.83± 0.04	1.78± 0.10	1.70± 0.04	1.68± 0.08	1.88± 0.18	2.18± 0.28
PO ₄ mg%	2.5- 5.0	4.14± 0.12	4.78± 0.48	4.00± 0.32	4.48± 0.45	5.68± 0.34	6.98± 0.37 ^{***}
TP g%	5 - 7	6.55± 0.07	6.78± 0.20 [*]	6.63± 0.09	6.68± 0.13	6.98± 0.39	7.46± 0.48
ALB g%	2.3- 3.9	2.94± 0.04	2.95± 0.12	2.73± 0.10 ^{**}	2.83± 0.17	2.83± 0.13	2.98± 0.16
TCO ₂ mEq.l ⁻¹		20.96± 0.77	15.32± 1.92	17.18± 0.39	17.40± 0.73 ^{***}	19.70± 0.80	17.86± 0.99
BUN mg%	10 - 20	18.2 ± 1.4	11.8 ± 1.0	10.0 ± 1.1 [*]	9.8 ± 1.3 ^{**}	12.5 ± 1.4 [*]	13.6 ± 2.2
CHOL mg%	100 -300	159.8 ± 6.7	170.3 ± 4.8	187.0 ± 17.0 [*]	171.3 ± 8.0	233.0 ± 33.4 ^{***}	228.4 ± 27.3 [*]
GLUC mg%	60 -100	110.4 ± 2.6	112.3 ± 9.3	125.8 ± 0.6 ^{***}	118.0 ± 4.4 ^{**}	179.0 ± 43.3 ^{***}	202.6 ± 47.6 ^{***}
AP u.l ⁻¹	<50	52.8 ± 6.7	63.8 ± 16.9	82.3 ± 14.8	64.3 ± 19.6	58.3 ± 7.6	64.8 ± 8.8
TBIL mg%	0.07-1.00	0.34± 0.01	0.23± 0.03	0.20± 0.00 ^{****}	0.15± 0.03 ^{****}	0.13± 0.06	0.10± 0.06 [*]
CPK u.l ⁻¹	<100	22.3 ± 1.9	19.3 ± 5.3	27.3 ± 4.8	57.8 ± 35.0	22.8 ± 5.7	223.0 ± 152.4 ^{***}

* P<0.05

** P<0.02

*** P<0.01

**** P<0.001

During stage 1 protein rose significantly from control. The other measurements did not change significantly.

During stage 2 potassium, albumin, blood urea nitrogen and total bilirubin fell significantly, while glucose and cholesterol rose significantly from control. There was no significant difference from control in the other measurements.

Stage 3 demonstrated a significant fall in potassium, total carbon dioxide, blood urea nitrogen and total bilirubin, while glucose and cholesterol were significantly elevated from control. The other measurements did not change significantly from control.

Stage 4 revealed a significant fall in potassium and blood urea nitrogen, while glucose and cholesterol rose significantly from control. There was no significant difference from control in the other measurements.

At stage 5 phosphate, cholesterol, glucose and creatinine phosphokinase rose significantly, while total bilirubin fell significantly. The other measurements did not differ significantly from control.

3.38 Post-mortem and histological examination

Histological examination of the tissues removed at post-mortem revealed similar changes in all dogs.

In the myocardium the small blood vessels and in some dogs the large blood vessels appeared moderately to severely congested with slight disassociation of the muscle bundles.

The liver sections, illustrated in Figure 3 i, showed moderate to severe acute passive venous congestion. In one dog early centrilobular necrosis was noted throughout the section.

Patchy collapse with moderate to severe generalized pulmonary

FIGURE 3 i
POST-MORTEM FINDINGS IN DOGS WITH
IXODES HOLOCYCLUS PARALYSIS

LIVER

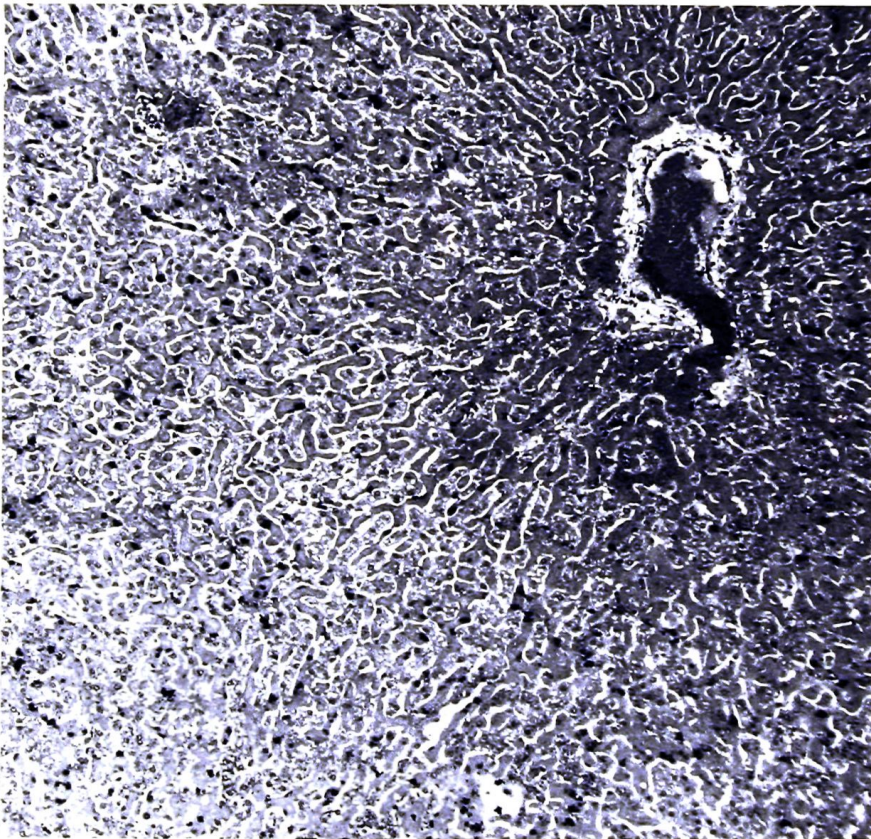
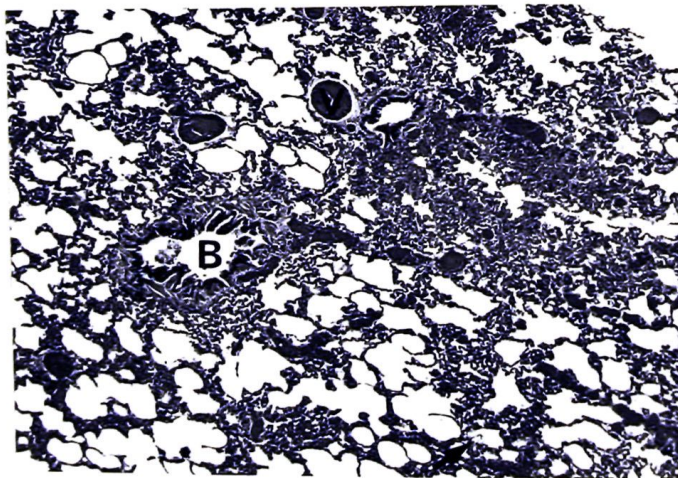
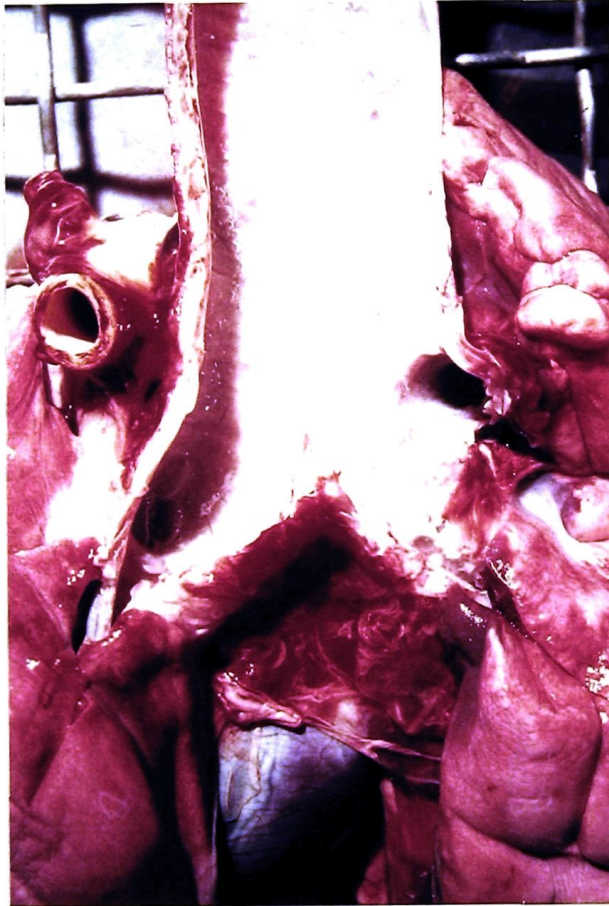


Figure 3 i demonstrates acute passive congestion of the liver. The sinusoids are very apparent and their extreme congestion has caused some atrophic changes in the adjacent hepatocytes. Deposit of formalin pigment has occurred over most accumulations of red blood cells. (Haematoxylin and Eosin stain x 125)

Figure 3 ii shows the gross and histological changes in the lungs. Clear, frothy material can be seen in the trachea and bronchi. Histologically the pulmonary vessels (**V**) are extremely dilated. The epithelium of some bronchioles (**B**) is thrown up into contorted folds suggestive of pre-mortem constriction. The dilated alveolar spaces are filled with a serous transudate in which protein is occasionally encountered (**↑**). (Haematoxylin and Eosin stain x 43)

FIGURE 3 ii
POST-MORTEM FINDINGS IN DOGS WITH
IXODES HOLOCYCLUS PARALYSIS
LUNG



congestion and in some animals pulmonary oedema was evident in the lung sections (Fig. 3 ii).

In the kidney there was severe congestion of all blood vessels and glomeruli.

3.4 DISCUSSION

Arterial blood-gas and pH, haematological and biochemical measurements in control dogs

Analysis of these measurements in the control dogs revealed significant between-sampling time variations in serum levels of potassium, calcium, phosphate, total carbon dioxide and total bilirubin. Changes in these measurements at the various stages of the disease, when within the normal range, were probably due to day-to-day variations and are unlikely to be of clinical significance.

Onset of clinical signs and engorgement of ticks

It was intended to attach four ticks to each dog, but because of the scarcity of ticks, two dogs received only three ticks. One of these dogs did not show any signs of the disease. These findings support the work of Ross (1926) who found that, although a single female tick is capable of causing a fatal paralysis in even the largest dog, not every female causes paralysis. For future experiments it was therefore decided that, if available, 4-8 ticks would be placed on each animal.

In this experiment the period elapsing between the attachment of the ticks and the onset of clinical signs was variable and ranged

from 5 to 7 days. The duration of the disease was also variable, although animals which showed earliest clinical signs tended to pass through the stages at a faster rate than those in which the onset of the disease was later. This resulted in loss of some valuable measurements, as dogs which were observed late at night and were normal may have progressed through most stages by early next morning.

Ross (1926) found that the incubation period corresponded to the state of engorgement of the tick rather than to a time interval. In his experimental dogs the first definite signs might not have been seen until 13 days after attachment, whereas massive infestation (30 ticks) in a ten-week old pup weighing 4.2 kg produced signs of the disease at the end of the fourth day (Ross, 1934). Thus the onset of clinical signs can occur any time between 5 and 13 days.

In this experiment the ticks were found to engorge at different rates. From Tables 3 iii and 3 iv it can be seen that at the death of the dogs some ticks were fully engorged and had dropped off, while other ticks were only partially engorged and still attached. As all dogs were housed under identical conditions of temperature and humidity, it appears that other factors might also influence engorgement. Doube, Kemp and Bird (1977) found that the length of time from moulting to attachment influenced the rate of engorgement and that rate of engorgement did not necessarily coincide with signs of the disease. Newly moulted laboratory-reared ticks grew slowly and weighed only 30-50 mg at the time of paralysis, whereas laboratory-reared ticks which were stored for 3-4 months had fully engorged (600 mg) at the time of paralysis. Four stored ticks did not paralyse 2-3 week-old calves, whereas four newly moulted ticks caused signs in all calves in 9-13 days.

Clinical signs

Accurate documentation of clinical signs was carried out so that they could be used as a basis for dividing the disease into stages to facilitate analysis of the data.

The main clinical signs observed in this experiment were of a rapidly ascending flaccid motor paralysis.

An early sign observed was a change in the character of the bark from the normal to a "gruff" hoarse type. This became a reliable sign in predicting that ataxia would be observed in the next 12-24 hours.

Initially slight ataxia or weakness of the hindlimbs, which always became more evident on exercise, was observed. This progressed to involve the forelimbs until the dogs lay in lateral recumbency and could no longer right themselves.

The vomiting which was observed was accompanied by violent prolonged retching with little production of vomited material. Although food and water were withheld, it was noticed that if food was placed in front of the animal, the sight of food often resulted in pronounced retching.

The dogs remained alert throughout all stages of the disease (until just prior to death), and would respond by tail-wagging when approached.

There appeared to be a diminution in awareness to a painful stimulus which was progressive throughout the stages, indicating some involvement of the sensory pathways. This loss of sensation was rarely noticed in further experiments.

Although a detailed neurological examination was not carried out, the withdrawal reflex, gag reflex and pupillary light reflex were examined. The withdrawal reflex is elicited by pressure on the

pain receptors of the interdigital tissue between the pads. This reflex tests the integrity of peripheral nerves and the spinal centres, resulting in limb flexion, and the cord and thalamus, resulting in evidence of feeling pain. The pupillary light reflex depends on the functional integrity of the optic system, on the parasympathetic portion of the oculomotor nerve with its associated autonomic nuclei and on the sympathetic innervation to the eye. The gag reflex depends on the integrity of the vagus and glossopharyngeal nerves. These reflexes, which were examined in the dogs in this experiment, became less pronounced throughout the stages of the disease.

The diminished reaction and final loss of the withdrawal reflex could be accounted for by a lesion anywhere along the reflex arc, including the pain or stretch receptors, the sensory nerves, the spinal synapses, the motor nerves, the neuromuscular junctions and the muscle fibres themselves.

Pupillary dilation with no pupillary light reflex indicated that paralysis of the oculomotor nerves or stimulation of the cervical sympathetic nerve was present.

Involvement of the glossopharyngeal and vagus nerves was suggested by a diminished gag reflex. Excessive salivation, dysphagia and difficulty in barking indicated partial paralysis of the vagus nerve.

A more extensive neurological examination was not performed, as at the same time as this study was being carried out a colleague (Cooper, B.J.) was also studying the effects of *Ixodes holocyclus* with particular reference to its effect on the peripheral nervous system.

The clinical signs of the disease are well documented by Dodd (1921), Ross (1926), Knott (1961) and are similar to those observed

in this study. Division of the disease into different stages based on clinical signs has been described by Hindmarsh and Pursell (1935) and Furneaux (1969). Furneaux (1969), who assumed that the clinical changes could be attributed to a neurotoxin, divided animals with the disease into four groups:

- 1) Harsh expiratory sound which immediately followed manual pressure on the lateral walls of the larynx. This "pseudo-laryngitis" in some cases was accompanied by signs of limb incoordination and/or posterior paresis.
- 2) Limb incoordination and posterior paresis. Vomiting was frequently associated with these signs of muscular weakness.
- 3) Respiratory distress which was particularly noticeable with excitement of the patient. This distress took the form of rib fixation and diaphragmatic respiratory efforts.
- 4) Prostration, cyanosis and Cheyne-Stokes respiration.

Hindmarsh and Pursell (1935) suggested that two distinct types of clinical signs may be exhibited by tick-infested dogs:

- 1) Motor paralysis, indicated by incoordination leading to complete loss of control of the hindlegs, followed by paralysis of the forelegs and finally the respiratory system. Vomiting did not occur.
- 2) Vomiting and loss of voice without evidence of paralysis of the limbs.

Whilst cases showing only one type of signs were uncommon, the authors felt that some significance should be placed on the fact that these two types of signs could be observed independently.

The diminution in awareness to a painful stimulus found in this experiment has not been reported previously. Dodd (1921), Ross (1926), Hamilton (1940) all reported that the integrity of the afferent

sensory pathways was maintained. It is interesting to note that, although the majority of reports in the literature concerning *Dermacentor andersoni* state that changes in sensation were not seen, Emmons and McLennan (1960) found that conduction in sensory as well as motor pathways was depressed in marmots paralysed by this tick. They also commented that this was borne out by the observations that paralysed animals were much less responsive to a painful stimulus than normal animals.

Cooper (1976), during neurological examination of tick-infested dogs, found muscle weakness, hypotonia of the skeletal muscles and reduction, then loss, of the spinal reflexes. A reduction followed by loss of the tendon reflexes always preceded loss of withdrawal reflexes. Cranial nerve involvement was not found except for pupillary dilation, although later it was stated that flaccidity of the muscles of the head indicated involvement of the cranial nerves. Cooper felt that the pupillary dilation could be due to blockage of the cholinergic innervation of the iris. From the neurological examination he proposed that the lesion caused by *Ixodes holocyclus* was in the motor nerve, its terminals, the neuromuscular junctions or the muscle fibres.

From the clinical signs observed in this experiment it can be concluded that tick paralysis produces its effects on the peripheral nervous system by a lesion somewhere in the reflex arc. While disturbed function of the efferent motor system appears to be the most prominent feature of the neurological findings, it appears that there is also some disturbance of the afferent pathways. In addition pupillary dilation, excessive salivation, difficulty in barking and a diminished gag reflex suggest some involvement of the autonomic nervous system.

Arterial blood-gas and pH measurements

Arterial blood-gas and pH measurements in cases of tick paralysis have not been documented previously. Measurements of these indices indicated that there was no significant change from control until the fourth stage of the disease was reached. From this point until death the dogs showed a progressive decrease in ventilatory capacity, illustrated by a fall in arterial oxygen tension and a rise in arterial carbon dioxide tension (respiratory acidosis) and a slight fall in standard bicarbonate (metabolic acidosis).

Shapiro, Harrison and Walton (1977), in their approach to interpretation of blood-gas results in man, classify:

- 1) Hypoxaemia as
 - mild, when $Pa_{O_2} < 80$ mmHg
 - moderate, when $Pa_{O_2} < 60$ mmHg
 - severe, when $Pa_{O_2} < 40$ mmHg
- 2) Alveolar ventilation as
 - hyperventilation (respiratory alkalosis),
when $Pa_{CO_2} < 30$ mmHg
 - normal ventilation when Pa_{CO_2} is between
30 mmHg and 50 mmHg
 - ventilatory failure (respiratory acidosis),
when $Pa_{CO_2} > 50$ mmHg

When ventilatory failure is present this is classified as

- acute, if the pH is below 7.300
- chronic, if the pH is within the normal range
indicating that the condition has existed for
long enough to allow renal compensation.

Analysis of the results in this experiment by these criteria shows that there was a mild hypoxaemia at stage 4 which progressed to moderate hypoxaemia with acute ventilatory failure during stage 5.

Ventilatory failure is seen in a variety of pathological conditions. Comroe, Forster, Dubois, Briscoe and Carlsen (1968) list these as:

- 1) depression of respiratory centres;
- 2) interference with neural conduction or with neuromuscular transmission to the respiratory muscles;
- 3) limitation of movement of the thorax or lungs;
- 4) pulmonary diseases which cause a decrease in functioning lung tissue, a decrease in the distensibility of lung tissue or obstructive lesions in the upper or lower respiratory tracts.

While this experiment demonstrated that ventilatory failure occurred in the late stages of the disease, the cause was unclear. Although respiratory muscle paralysis (Hamilton, 1940; Roberts, 1941; McCarthy, 1958) has been blamed for death in this disease, the dogs in this experiment appeared to be making considerable respiratory effort during the stages when blood-gas analysis showed ventilatory failure to be developing. Moist râles and post-mortem specimens suggestive of pulmonary congestion and oedema were also found. This supports the findings of Hindmarsh and Pursell (1935) and Calder (1974) who found post-mortem evidence of pulmonary oedema in dogs paralysed by *Ixodes holocyclus* and who suggested that this might have been the cause of death despite treatment in severe cases.

Haematological measurements

Haematological measurements in animals with tick poisoning have not been described previously, and the measurements in this experiment were unremarkable. Haemoglobin concentration was significantly elevated. This could possibly be due to slight dehydration, as the total red cells, the plasma protein and the packed cell volume also

demonstrated similar elevations, although they were not significant. Mild dehydration would be expected in animals that were not drinking and eating and that had been vomiting.

Pearn (1966) reported the haematological picture in a child with tick paralysis and associated myocarditis. The indices measured were within the normal range. Examination of the blood smear showed the erythrocytes to be of normal morphology, the leucocytes normal except for mild toxic changes in the neutrophils and the presence of a moderate number of plasma cells, and the platelets normal in character.

Biochemical measurements

It was anticipated that many biochemical values were unlikely to be altered by the disease. However, since these indices have not been measured previously, it was felt that a detailed analysis of blood biochemistry should be performed to establish what changes, if any, occur. Many of the significant changes in the biochemical measurements are difficult to interpret individually but taken together could represent the biochemical response to sympathetic stimulation of the adrenal medulla, causing liberation of noradrenaline and adrenaline or release of adrenocorticotrophic hormone (ACTH) which stimulates the adrenal cortex to secrete corticosteroids.

The metabolic changes caused by noradrenaline and adrenaline are similar. Hyperglycaemia is attributed in part to activation, via cyclic adenosine 3', 5'-monophosphate (AMP), of hepatic glycogen phosphorylase. This enzyme converts glycogen to glucose-1-phosphate. In addition the action of cyclic AMP results in the inactivation of glycogen synthase, the enzyme that catalyzes the transfer of glycosyl units from uridine diphosphate glucose to glycogen. These two effects summate to increase the output of glucose from the liver. Other

factors leading to hyperglycaemia are inhibition of insulin secretion predominantly via α receptors and decreased uptake of glucose by peripheral tissues. Both these catecholamines raise the blood concentration of free fatty acids by activation of triglyceride lipase, which accelerates the breakdown of triglycerides to form free fatty acids and glycerol. In addition increased plasma concentrations of cholesterol, phospholipid and low-density lipoproteins are seen. Noradrenaline and adrenaline initially produce a transient rise in plasma potassium concentration, mainly due to release of potassium from the liver. Then a prolonged fall in plasma potassium concentration is seen as the potassium is taken up by the muscle. Increased erythrocyte and plasma protein concentrations are seen, due to a decreased circulating plasma volume by loss of protein-free fluid to the extracellular space (Goodman and Gilman, 1975).

Release of ACTH results in secretion of cortisol and corticosterone. These hormones cause hyperglycaemia by promoting the conversion of protein to glucose (gluconeogenesis), by inhibiting utilization of glucose (possibly because they inhibit the action of insulin) and by increasing glycogen deposition in the liver. When insulin secretion is inhibited, an increase in cholesterol concentration is found, due to increased mobilization of peripheral fat deposits. Glucocorticoids also induce sodium retention and potassium excretion. The sodium retention is due to increased resorption by the kidney, while the mechanism for potassium excretion is unclear. Glucocorticoids also tend to increase the haemoglobin and red cell content of the blood (Goodman and Gilman, 1975).

Thus the increase in glucose, cholesterol and haemoglobin and the decrease in potassium found in this experiment could be explained by increased release of catecholamines or glucocorticoids, or both.

Although phosphate showed a time variation in the control dogs, the elevation at stage 5 of the disease was clinically abnormal. Severe muscle cell damage, with liberation of phosphate into the blood, could explain the elevated level (Hensley, 1978).

Creatinine phosphokinase, which was significantly elevated at stage 5 of the disease, is the enzyme responsible for the phosphorylation of adenosine diphosphate by creatinine phosphate, producing adenosine triphosphate. Adenosine triphosphate is necessary for muscle contraction. Elevated levels of creatinine phosphokinase suggest active muscle lysis and tie in well with the elevated phosphate. Creatinine phosphokinase elevation was found in a case of tick paralysis produced by *Dermacentor andersoni* (Boffey and Paterson, 1973). The elevation was thought to be due to release of the enzyme through damaged muscle cell membranes, and they postulated that this damage resulted from interference by the tick toxin with cellular energy metabolic pathways.

Histological examination

The moderate to severe congestion of all tissues examined was suggestive of both left- and right-sided cardiac failure (Howlett, 1978).

Pulmonary congestion (Ross, 1926) and pulmonary oedema (Hindmarsh and Pursell, 1935; Calder, 1974; Cooper, 1976) have been reported previously in dogs dying from tick paralysis. Although these pathological changes have been thought to contribute to death, the cause has been unknown and heart failure has not been suggested.

The brain and spinal cord were not examined in this study, as a detailed histological examination of these tissues was conducted by Cooper (1976). He concluded that the small focal brain haemorrhages,

pulmonary congestion and oedema and an increase in lipid droplets in muscle fibres which he found in tick paralysis occurred as secondary changes, probably to hypoxaemia.

The other microscopic findings reported in tick paralysis have also been in the brain and spinal cord (Ferguson, 1924; Ross, 1926). Sections from the brain of a 16 month-old child revealed intense engorgement of the vessels with the presence of numerous small, newly formed capillaries. Diffuse infiltration with small round cells, probably plasma cells, was noted in some places, but there was no perivascular cuffing of these cells. Some of the vessels contained polymorphonuclear leucocytes. In the spinal cord similar congestion was present with some small haemorrhages (Ferguson, 1924). Sections from the brain and spinal cord of dogs examined by Ross (1926) showed considerable congestion of both the anterior and posterior horns and in some cases numerous capillary haemorrhages both into the adventitial sheath and around the nerve cells, accompanied by an excess of mononuclear cells, with some perivascular infiltration. No suggestions were put forward to explain the significance of these changes.

The consistent finding of congestion of all tissues in this experiment, as well as those of other workers, supports the diagnosis of subacute congestive cardiac failure.

CHAPTER 4

THE RESPIRATORY EFFECTS OF *IXODES HOLOCYCLUS* IN THE DOG

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4.3	Results	94
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4.1 INTRODUCTION

Although no studies have been undertaken to observe the effects of *Ixodes holocyclus* on respiration, many authors (Anderson, 1894; Dodd, 1921; Ross, 1926; Ross, 1935; Hamilton, 1940; Roberts, 1941; McCarthy, 1958) concluded that death was due to respiratory failure. Hamilton (1940), Roberts (1941) and McCarthy (1958) stated that death was due to paralysis of the muscles of respiration.

During the investigations undertaken in Chapter 3 it was observed that an early sign of the disease was a change in respiratory pattern. The normal was initially replaced by a "grunting" type respiration, which progressed to a laboured slow form with feeble limb movements and grunts accompanying each respiration.

As this type of respiration had not been noticed in dogs with neuromuscular paralysis disorders (polyradiculoneuritis, myasthenia gravis and tiger snake bite), it was decided to study in detail the effects of *Ixodes holocyclus* on respiration in the dog.

4.2 MATERIALS AND METHODS

Fourteen healthy cross-bred dogs ranging in weight from 11.0 to 23.5 kg were used in this study.

One week prior to attachment of the ticks the dogs were surgically prepared by implantation of a catheter in the carotid artery to enable collection of arterial blood.

The dogs were trained to lie quietly on a foam-rubber padded table and to breathe normally through a facemask. Expiratory volume (\dot{V}_E), expiratory time (tE), expiratory minute volume (\dot{V}_E) and

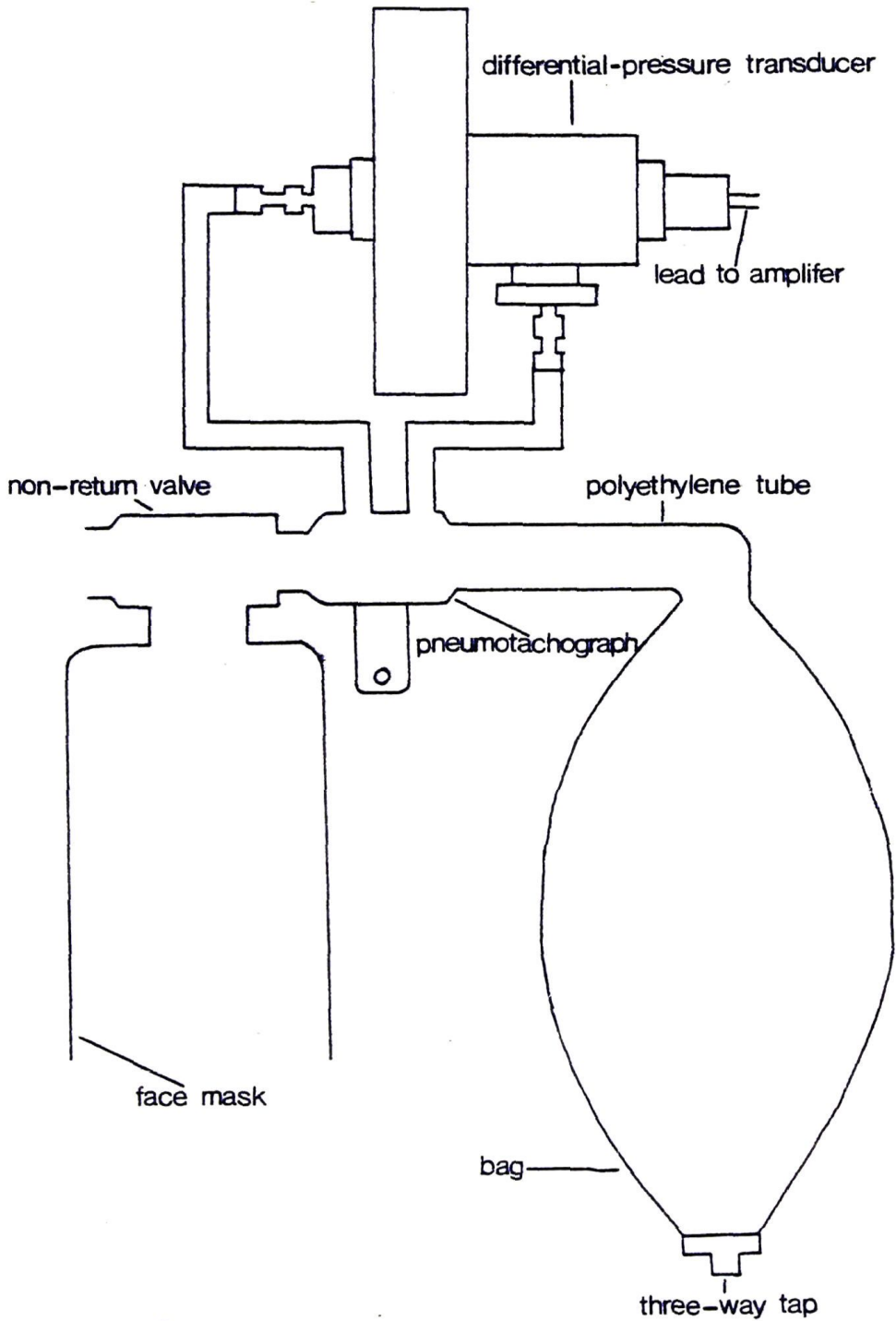
respiratory rate (f) were measured with the apparatus illustrated in Figure 4 i. The facemask was manufactured from polyethylene, and variable amounts of foam-rubber sheeting were placed inside to obtain an air-tight fit and to decrease apparatus dead space. The facemask was connected to a non-rebreathing valve (Ruben, Ambu) which in turn was joined to a pneumotachograph (Fleisch type 1). A smooth-walled polyethylene tube conveyed expired gases from the pneumotachograph to a bag.

The pneumotachograph records the velocity of air flow at each instant during the respiratory cycle. The principle of measurement is based on Poiseuille's law, which states that the decrease in pressure along a rigid tube is proportional to the velocity of flow per unit length when such flow is laminar.

The pneumotachograph used in this experiment contained a mesh screen to avoid turbulence of the air flow and an electrical heating system to preheat and therefore stop water condensation on the apparatus which could alter the resistance to air flow. Two leads ran from the pneumotachograph to a differential pressure transducer (type UP 1/TC, Pye Ethnor Limited). The signal from the differential pressure transducer was passed to a system containing a high-gain directly coupled amplifier (3550, Devices), an integrator (3620, Devices), a timer (3680, Devices) and a limit switch (3655, Devices). There the signal from the differential pressure transducer (expiratory flow velocity) was amplified and its amplification integrated with respect to time to give the volume of air moved per unit time. The pneumotachograph was calibrated using a 200 ml syringe to pass known volumes of gas through the system. The deflections recorded were then used to construct a volume scale.

The expired gas collected in the bag during the recording of

FIGURE 4 i
APPARATUS FOR MEASUREMENT OF
RESPIRATORY PARAMETERS



the above parameters was passed through a carbon dioxide medical gas analyser (LB-1, Beckman) with a micro-catheter sample cell to record the fractional concentration of carbon dioxide in the expired gas (FE_{CO_2}). The gas analyser was calibrated each day with known concentrations (2.89%, 4.8% and 8.3%) of carbon dioxide.

A six channel direct-recording oscillograph (Visigraph-P, type PR101, San-ei) was used to record respiratory parameters.

Measurements of the gas volumes VE (ml) and $\dot{V}E$ (l) were made at $21^\circ C$ and the results were corrected for body temperature and pressure saturated with water vapour (BTPS). Expiratory time was calculated by measuring the time in seconds from the onset to the end of expiration and respiration rate (breaths per minute, BPM) was counted from the respiratory traces.

Arterial pH, partial pressure of oxygen in arterial blood and the partial pressure of carbon dioxide in arterial blood were measured.

The dead space/tidal volume ratio (VD/V_T) was calculated from the standard formula:

$$\frac{VD}{V_T} = \frac{Pa_{CO_2} - PE_{CO_2}}{Pa_{CO_2}}$$

where the partial pressure of carbon dioxide in the expired gases (PE_{CO_2}) was obtained from FE_{CO_2} .

$PE_{CO_2} = FE_{CO_2} \times (\text{barometric pressure (PB)} - \text{pressure of water vapour at body temperature (PH}_2\text{O, } 37^\circ C))$. Arterial blood was collected over the sampling period of the expired gases.

The alveolar oxygen tension (PA_{O_2}) was calculated from the formula:

$$PA_{O_2} = PI_{O_2} - PA_{CO_2} \times \frac{FI_{O_2} + 1 - FI_{O_2}}{R}$$

- 1) $P_{I_{O_2}}$ is the partial pressure of oxygen in the inspired air, which is 20.93% of $(P_B - P_{H_2O})$ and equals 149 mmHg.
- 2) $P_{A_{CO_2}}$ is equal to $P_{a_{CO_2}}$.
- 3) the fraction of oxygen in the inspired air ($F_{I_{O_2}}$) is 0.2093.
- 4) the respiratory exchange ratio (R) is considered to be normal (0.8).

The difference between the alveolar oxygen tension and the arterial oxygen tension ($P_{A_{O_2}} - P_{a_{O_2}}$) was then calculated.

Control measurements were taken on the two days preceding attachment of the ticks. Six ticks were attached to each dog, and once clinical signs were evident, measurements were carried out at stages 1, 2, 3 and 4 of the disease as outlined:

Stage 1 - the dogs showed ataxia when walked.

Stage 2 - the dogs were unable to stand but could right.

Stage 3 - the dogs were unable to right.

Stage 4 - within 4 hours of death.

The results were analysed by pairing the control and test values in each animal, and the mean differences at each stage were compared with zero using the Student's t-distribution.

4.3 RESULTS

Although 14 dogs were infested with *Ixodes holocyclus*, only seven dogs showed signs of the disease.

The mean and the SEM of the respiratory measurements during the control period and at the various stages of the disease are illustrated in Table 4 i.

Stage 1 revealed a significant fall in respiratory rate and standard bicarbonate and a significant rise in expiratory time from

TABLE 4 1 CHANGES IN RESPIRATORY MEASUREMENTS AT THE VARIOUS STAGES OF PARALYSIS CAUSED BY *IXODES HOLOCYCLUS*

		CONTROL		STAGE 1		STAGE 2		STAGE 3		STAGE 4	
		MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
		n = 14		n = 5		n = 6		n = 3		n = 6	
f	BPM	29.9	± 2.5	20.2	± 1.4 ^{***}	18.3	± 1.9 ^{***}	12.7	± 0.7 ^{**}	13.3	± 1.1 ^{***}
VE	ml	213.6	±34.9	200.6	±53.4	193.4	±38.3	224.7	±84.4	199.8	±51.6
VE	l	5.686 ± 0.791		3.912 ± 0.937		3.651 ± 0.554		2.954 ± 1.263 ^{**}		2.556 ± 0.592 ^{***}	
tE	sec	1.29	± 0.09	1.90	± 0.09 ^{***}	1.87	± 0.28 [*]	2.53	± 0.30	2.68	± 0.44 ^{***}
VD/VT		0.5260± 0.0210		0.5309± 0.0564		0.5595± 0.0326		0.6517± 0.0338		0.5662± 0.0351	
FE _{CO₂}	mmHg	15.02	± 0.44	12.50	± 0.83	13.80	± 1.08	11.40	± 1.40	16.00	± 1.07
Pa _{CO₂}	mmHg	32.32	± 1.03	31.33	± 1.10	32.10	± 2.66	32.63	± 0.49	38.04	± 2.42
Pa _{O₂}	mmHg	92.86	± 1.18	78.18	± 6.05	73.18	± 4.20 [*]	79.07	± 7.64	57.94	± 4.53 ^{***}
pH		7.389 ± 0.006		7.358 ± 0.018		7.359 ± 0.025		7.329 ± 0.008		7.314 ± 0.017 [*]	
Std HCO ₃	mEq.l ⁻¹	21.23	± 0.60	19.45	± 0.91 ^{****}	20.35	± 1.45	18.60	± 0.85	19.55	± 0.61 ^{**}
PA _{O₂} - Pa _{O₂}	mmHg	17.35	± 1.03	33.25	± 5.90	37.35	± 1.48 ^{***}	30.77	± 7.82	45.42	± 2.71 ^{****}

* P<0.05 ** P<0.02 *** P<0.01 **** P<0.001

control.

During stage 2 respiratory rate and arterial oxygen tension fell significantly, while expiratory time and alveolar-arterial oxygen tension difference rose significantly from control. There was no significant change from control in the other measurements.

Stage 3 demonstrated a significant fall in respiratory rate and minute respiratory volume from control. The other measurements did not change significantly from control.

During stage 4 respiratory rate, minute respiratory volume, arterial oxygen tension, pH and standard bicarbonate fell significantly, while expiratory time and alveolar-arterial oxygen tension difference were significantly elevated from control. There was no significant change from control in the other measurements.

4.4 DISCUSSION

Analysis of the respiratory measurements revealed a progressive fall in respiratory rate throughout all stages of the disease. Although respiratory rate fell, tidal volume did not change, and therefore it was only during stages 3 and 4 that a significant fall in minute respiratory volume was observed. Expiratory time was prolonged and the alveolar-arterial oxygen tension difference increased at all stages of the disease.

A progressive fall in respiratory rate is not encountered in other diseases and therefore it is difficult to explain in tick paralysis. It is found in animals under barbiturate anaesthesia, where there is progressive central depression of the inspiration centre of the medulla oblongata. A decrease in respiratory rate is

FIGURE 4 ii
RESPIRATORY MEASUREMENTS

CONTROL

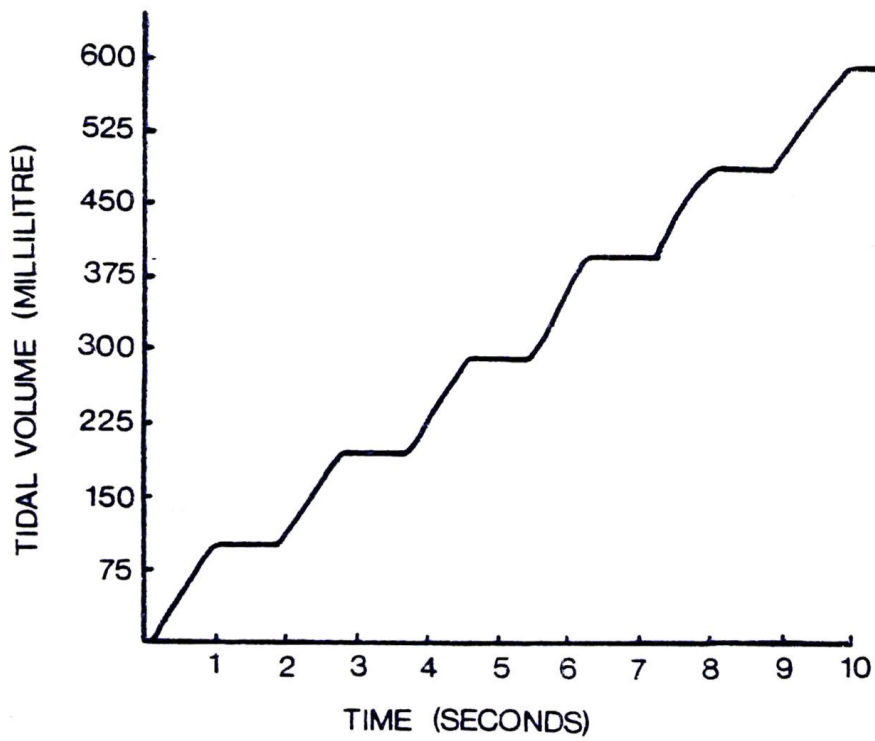


FIGURE 4 iii
RESPIRATORY MEASUREMENTS

STAGE 1



STAGE 3



also found if expiratory time is prolonged due to increased expiratory airway resistance (Bartlett, Jeffrey, Sant'Ambrogio and Wise, 1976). The expiratory traces obtained from dogs with tick paralysis demonstrated that increased expiratory airway resistance was present and after tracheotomy respiratory rate increased slightly. This increase, however, was but temporary. It appears, therefore, that central depression is responsible for the progressive fall in respiratory rate.

For comparative purposes a control respiratory trace is illustrated in Figure 4 ii.

Analysis of the respiratory traces revealed that the prolonged expiratory time was due to closure of the vocal cords during the expiratory phase of respiration. Initially this closure was abrupt and only altered the expiratory flow slightly (Fig. 4 iii), but as the disease progressed the expiratory flow changed markedly (Fig. 4 iii). Reflex closure of the vocal cords due to the presence of foreign material has been observed in poliomyelitis (Taylor, 1955) and could be responsible for the changes in expiratory flow in tick paralysis. However, because respiration in tick paralysis is accompanied by a "grunting" noise, it seems more likely that this closure is similar to that observed in babies with pulmonary congestion and oedema where the vocal cords are closed during expiration to assist re-expansion of collapsed portions of the lungs (Read, 1978). Respiration in these babies is also accompanied by a "grunting" noise.

Although the alveolar-arterial oxygen tension difference was elevated at all stages of the disease, statistical significance was found only at stages 2 and 4. The cause of this elevation cannot be determined without more extensive respiratory measurements.

Hypoventilation is unlikely to be the cause, as an elevation of arterial carbon dioxide above 50 mmHg was not found at any stage of the disease. A combination of uneven ventilation and venous-to-arterial shunts caused by pulmonary congestion, hypostatic congestion and the inability to clear secretions from the lungs could be responsible for the alveolar-arterial oxygen tension difference.

Although respiratory measurements in cases of tick paralysis have not been described previously, comments of the respiratory type and rate have been published. In man it appears that as the disease progresses respiration becomes fast and shallow (Cleland, 1912; Eaton, 1913; Ferguson, 1924; Hamilton, 1940; Pearn, 1966), whereas in the dog the rate progressively decreases (Ross, 1926). The type of respiration in man following tick envenomation is more consistent with that observed in diseases such as poliomyelitis, where there is partial or complete paralysis of the muscles of respiration. In these cases respiratory rate increases while tidal volume decreases and the accessory muscles contribute to respiration (Hobes, 1955; Taylor, 1955). It seems likely, therefore, that in the dog important factors other than neuromuscular paralysis affect respiration. These factors are central respiratory depression and pulmonary congestion and oedema.

CHAPTER 5

THE EFFECT OF *IXODES HOLOCYCLUS* ON THE ELECTROCARDIOGRAM
IN THE DOG

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5.1 INTRODUCTION

Pearn (1966) documented the only electrocardiographic changes reported in the literature. In this case a child suffering from tick paralysis developed acute cardiac failure, and with the aid of radiology, haematology, biochemistry and electrocardiography a diagnosis of toxic myocarditis with concomitant cardiac failure was established. This led Pearn to conclude that mortality from tick bite might not be simply due to anoxia following respiratory failure or pneumonia, but that a hitherto unrecognized myocarditis may contribute significantly to the clinical picture in severe cases.

Veterinarians working in tick-infested areas advise that dogs must be kept quiet for at least one week following full clinical recovery. In convalescent animals sudden collapse and death following extreme exercise have been reported (Allan and Pursell, 1971), suggesting acute cardiac failure or cardiac arrest.

During the course of the disease described in Chapter 3, pulse rates were recorded, and it was found that some dogs developed an arrhythmia or tachycardia and terminally a bradycardia.

These findings prompted an investigation of the electrocardiographic changes in dogs at various stages of the disease produced by *Ixodes holocyclus*.

5.2 MATERIALS AND METHODS

Ten healthy cross-bred dogs ranging in weight from 11.0 to 30.0 kg were used in this experiment.

One week prior to attachment of ticks the dogs were surgically

prepared by implantation of a catheter in the carotid artery to enable collection of arterial blood.

Arterial pH, arterial oxygen tension and arterial carbon dioxide tension were measured.

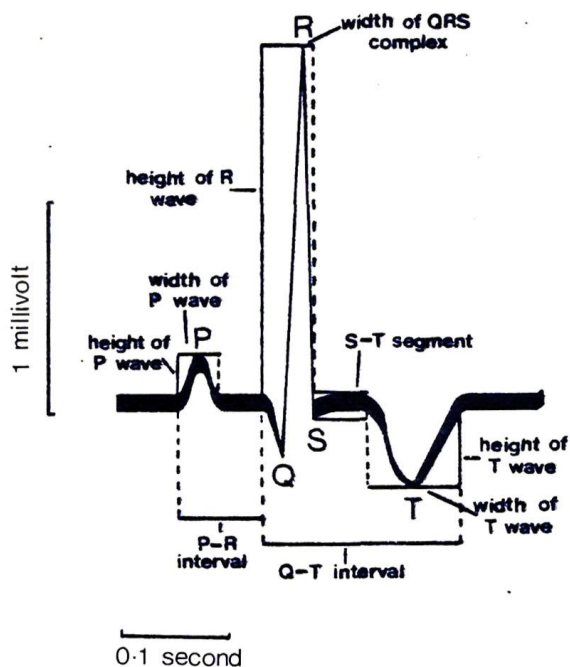
Plasma potassium concentration and plasma calcium concentration were determined. Electrolyte values were included in the results only where they might account for changes in the electrocardiogram.

Electrocardiograms (ECG) were recorded using an electrocardiographic unit (Watson Victor, Heat Mark II). Before each recording the machine was standardized so that an input of 1 millivolt (mv) produced a deflection of 1 cm, and electrocardiograms were taken at a paper speed of 25 millimetre per second ($\text{mm}\cdot\text{sec}^{-1}$). The leads I, II, III, aVR, aVL and aVF were recorded to obtain a standard six-lead electrocardiogram.

All dogs were trained to lie quietly on a rubber-topped table in right lateral recumbency and were held so that their forelimbs and hindlimbs were at right angles to their body and parallel to each other. Alligator clips were used to attach the leads to the skin, and the clips and skin were moistened with alcohol to establish good clip-to-skin contact and prevent electrical interference. The arm leads were attached to the appropriate forelimb on the caudal aspect just proximal to the olecranon. The leg leads were attached to the appropriate hindlimb on the cranial aspect just proximal to the patella.

When examining the electrocardiograms, lead II was looked at in detail, while the other leads were used to provide confirmatory or additional information. Analysis of the electrocardiograms was carried out as illustrated in Figure 5 i and the heart rate (beat per minute, bpm) was counted from the ECG.

FIGURE 5 i
THE NORMAL CANINE LEAD II
ELECTROCARDIOGRAPHIC COMPLEX



The electrocardiograms were considered normal if all parameters lay within the criteria for the normal canine electrocardiogram as described by Bolton (1975). The normal heart rate in an adult dog is 70-160 bpm with a sinus rhythm or sinus arrhythmia. The P wave is measured from the beginning to the end and from the top to the bottom (baseline), and has a maximum width of 0.04 sec and a maximum height of 0.4 mv. The P-R interval is measured from the beginning of the P wave to the beginning of the QRS complex and has a width of 0.06-0.13 sec. The QRS complex is measured from the beginning to the end and from the baseline to the top of the R wave. The maximum width

is 0.06 sec, while the maximum height is 3.0 mv. The S-T segment is the area between the end of the S wave and the beginning of the T wave. There is no S-T segment depression (more than 0.2 mv below the baseline) or S-T segment elevation (more than 0.15 mv above the baseline). The T wave can be positive, negative or biphasic, but should not change in polarity between recordings from the same dog. The height is less than 0.25 x (R wave amplitude). The Q-T interval is measured from the beginning of the Q waves to the end of the T wave, and the width is 0.14-0.22 sec.

Control electrocardiograms were taken on the two days preceding attachment of the ticks. Six ticks were attached to each dog as described in section 2.4.

Once clinical signs were evident measurements were carried out at five stages of the disease as outlined:

- Stage 1 - the dogs showed ataxia when walked.
- Stage 2 - the dogs were unable to stand but could right.
- Stage 3 - the dogs were unable to right.
- Stage 4 - within four hours of death.
- Stage 5 - just prior to death.

These stages were similar to those already described in Chapter 3, except for the criteria used to establish stage 4. This was necessary because of the inconsistency in disappearance of the withdrawal reflex.

Analysis of the electrocardiograms revealed changes which were not consistent from dog to dog. Therefore these changes are documented separately for each dog with an illustration of lead II.

5.3 RESULTS

5.31 Electrocardiograms recorded during the control state

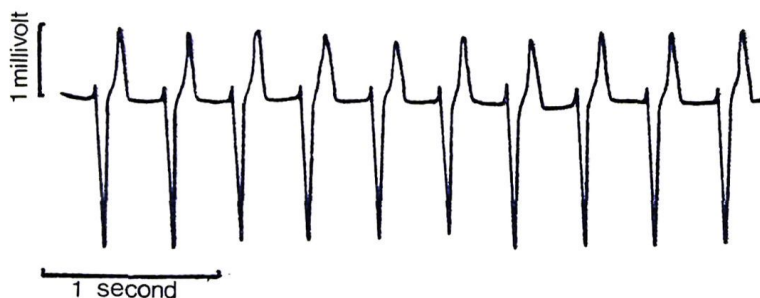
Electrocardiograms recorded from the dogs before application of the ticks satisfied the criteria for the normal electrocardiogram as described in section 5.2.

5.32 Electrocardiograms recorded during the various stages of the disease

Dogs 3, 9 and 10 did not show signs of tick paralysis, although six ticks were attached to each dog.

Dog 1

FIGURE 5 ii



The electrocardiogram illustrated in Figure 5 ii and recorded at stage 1 of the disease shows a continuous ventricular tachycardia with a heart rate of 160 bpm. The ventricular tachycardia is unifocal in origin. The arterial oxygen tension was 105.8 mmHg, the plasma potassium concentration 4.7 mEq.l^{-1} and the temperature 38.7°C .

FIGURE 5 iii



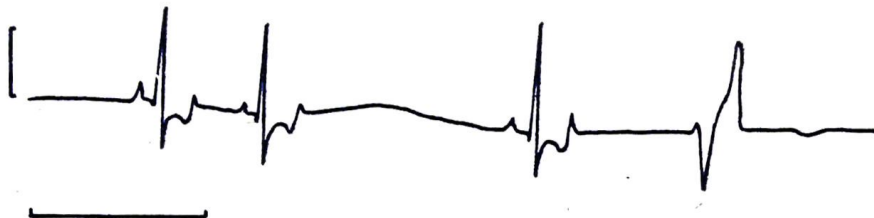
At stage 2 of the disease the electrocardiogram (Fig. 5 iii) showed a marked sinus arrhythmia with a heart rate of 110 bpm. The rhythm is interrupted by episodic ventricular escape beats. S-T segment depression (-0.3 mv) can also be seen. The arterial oxygen tension was 84.0 mmHg, the plasma potassium concentration 4.2 mEq.l^{-1} , the plasma calcium concentration 10.1 mg% and the temperature 38.7°C .

FIGURE 5 iv



Sinus arrhythmia with sinus arrest is found on the electrocardiogram illustrated in Figure 5 iv and recorded at stage 3 of the disease. The heart rate is 100 bpm and there is S-T segment depression (-0.3 mv). The arterial oxygen tension was 94.5 mmHg, the plasma potassium concentration 4.2 mEq.l^{-1} , the plasma calcium concentration 10.2 mg% and the temperature 38.5°C .

FIGURE 5 v



The electrocardiogram illustrated in Figure 5 v and recorded at stage 4 of the disease shows a sinus bradycardia and sinus arrest with a heart rate of 60 bpm. The rhythm is broken by occasional ventricular escape beats. The arterial oxygen tension was 69.5 mmHg, the plasma potassium concentration 4.1 mEq.l^{-1} and the temperature 37.9°C .

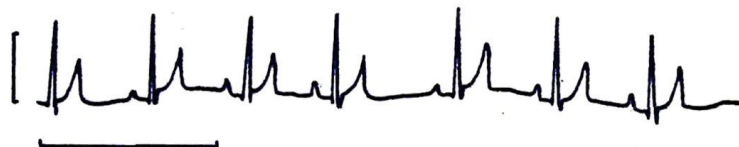
FIGURE 5 vi



Figure 5 vi shows an electrocardiogram recorded 60 minutes after that in Figure 5 v. Second degree heart block, followed by first degree heart block (P-R interval 0.14-0.18 sec) can be seen in the trace. The change in the P wave polarity is indicative of a wandering pacemaker. The arterial oxygen tension was 61.5 mmHg, the plasma potassium concentration 3.6 mEq.l^{-1} and the temperature 37.9°C .

Dog 2

FIGURE 5 vii



An increase in the amplitude of the T waves can be seen in the electrocardiogram in Figure 5 vii, recorded at stage 1 of the disease. The heart rate is 110 bpm, the arterial oxygen tension 91.5 mmHg, the plasma potassium concentration 4.6 mEq.l^{-1} and the temperature 38.5°C .

FIGURE 5 viii



The electrocardiogram illustrated in Figure 5 viii was recorded at stage 3 of the disease. There is a sinus tachycardia with a heart rate of 165 bpm. The amplitude of the T waves is still increased. The arterial oxygen tension was 92.0 mmHg, the plasma potassium concentration 3.9 mEq.l^{-1} and the temperature 38.1°C .

FIGURE 5 ix

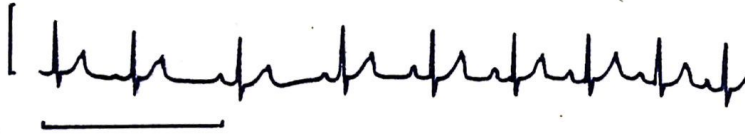
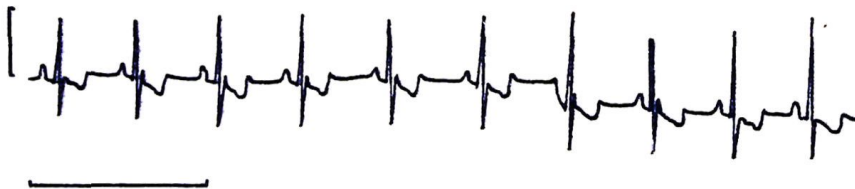


Figure 5 ix shows an electrocardiogram recorded three hours after that in Figure 5 viii. There is a sinus arrhythmia with a heart rate of 130 bpm. The T waves are again increased in amplitude.

FIGURE 5 x



The heart rate and rhythm of the electrocardiogram illustrated in Figure 5 x are the same as shown in Figure 5 ix. However, the T waves are now biphasic. This electrocardiogram was recorded at stage 4 of the disease. The arterial oxygen tension was 71.5 mmHg, the potassium concentration 3.1 mEq.l^{-1} and the temperature 37.9°C .

FIGURE 5 xi

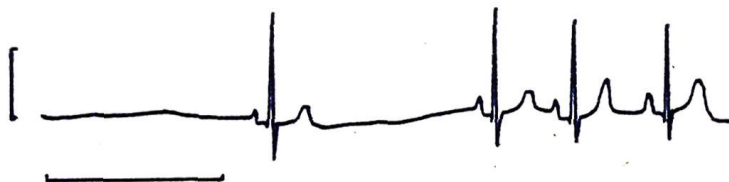
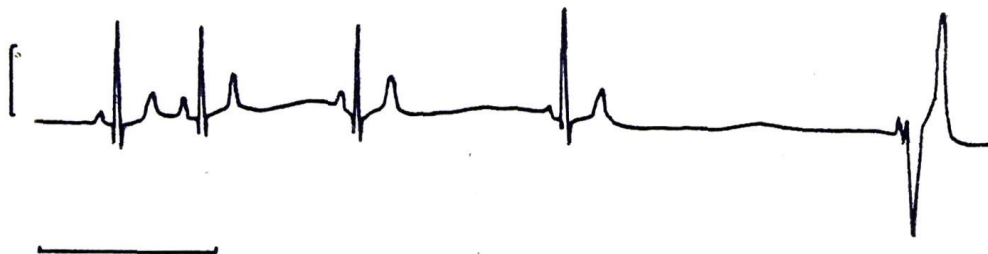


Figure 5 xi shows an electrocardiogram recorded at a later part of stage 4. There is a sinus bradycardia with sinus arrest and a heart rate of 60 bpm. The arterial oxygen tension was 64.5 mmHg, the plasma potassium concentration 3.2 mEq.l^{-1} and the temperature 38.0°C .

FIGURE 5 xii



The electrocardiogram illustrated in Figure 5 xii was recorded 30 minutes after that in Figure 5 xi. Ventricular escape beats can be seen and the T waves are increased in amplitude. The arterial oxygen tension was 59.0 mmHg, the plasma potassium concentration 3.6 mEq.l^{-1} and the temperature 37.5°C .

FIGURE 5 xiii

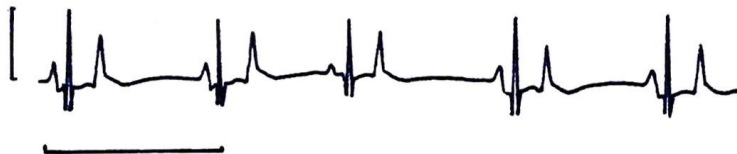
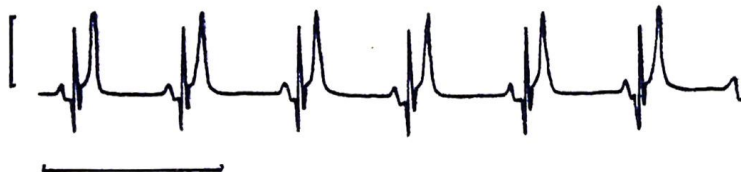
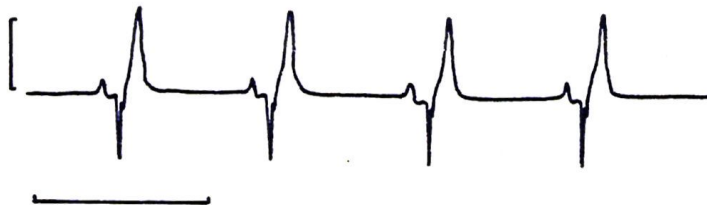


FIGURE 5 xiv



Figures 5 xiii and 5 xiv show electrocardiograms recorded at stage 5 of the disease. Sinus bradycardia is prominent and the T wave amplitude is increasing, while the R wave amplitude is decreasing.

FIGURE 5 xv



The R wave has virtually disappeared in the electrocardiogram illustrated in Figure 5 xv, which was recorded at death of the animal. The arterial oxygen tension was 25.2 mmHg, the plasma potassium concentration 5.1 mEq.l^{-1} and the temperature 37.1°C .

Dog 4

FIGURE 5 xvi

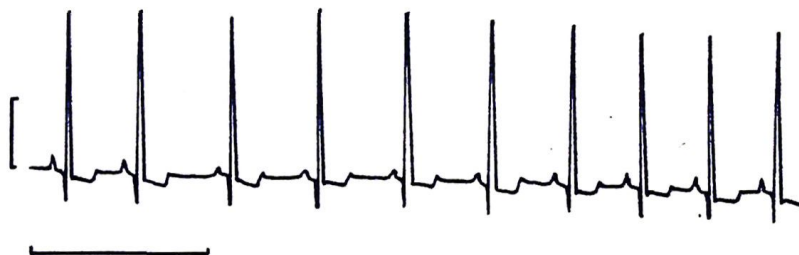


Figure 5 xvi shows an electrocardiogram recorded at stage 2 of the disease. The heart rate is 130 bpm and the electrocardiogram is normal.

FIGURE 5 xvii



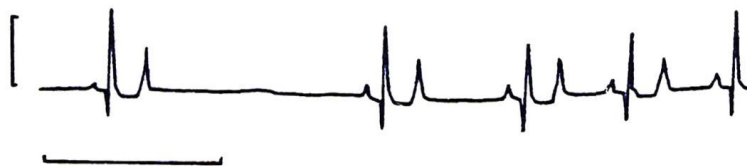
A marked overall decrease in amplitude of the electrocardiogram is illustrated in Figure 5 xvii, recorded at stage 3 of the disease. The heart rate is 100 bpm with sinus arrest. The arterial oxygen tension was 84.1 mmHg, the plasma potassium concentration 3.3 mEq.l^{-1} and the temperature 38.8°C .

FIGURE 5 xviii



Figure 5 xviii shows an electrocardiogram recorded at stage 4 of the disease. The overall amplitude has returned to normal and the heart rate is 110 bpm. There is a mild Q-T segment prolongation (0.24 sec). The arterial oxygen tension was 71.5 mmHg, the plasma potassium concentration was 4.0 mEq.l^{-1} , the plasma calcium concentration 10.1 mg% and the temperature 37.8°C .

FIGURE 5 xix



The electrocardiogram illustrated in Figure 5 xix was recorded at stage 5 of the disease. There is a marked sinus bradycardia and sinus arrest with a heart rate of 50 bpm. Mild Q-T segment prolongation (0.24 sec) and an increase in the amplitude of the T waves can also be seen. The arterial oxygen tension was 32.8 mmHg, the plasma potassium concentration 3.8 mEq.l^{-1} , the plasma calcium concentration 10.2 mg% and the temperature 33.3°C .

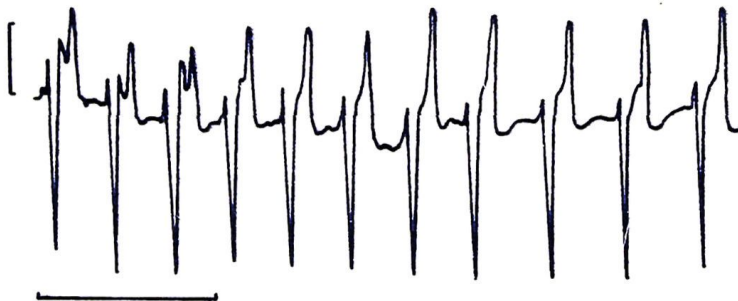
Dog 5

FIGURE 5 xx



A normal electrocardiogram can be seen in Figure 5 xx, recorded at stage 1 of the disease. The heart rate is 100 bpm.

FIGURE 5 xxi



The electrocardiogram illustrated in Figure 5 xxi was recorded at stage 4 of the disease and shows a ventricular tachycardia with a heart rate of 160 bpm. The arterial oxygen tension was 67.6 mmHg and the temperature 39.3°C.

FIGURE 5 xxii

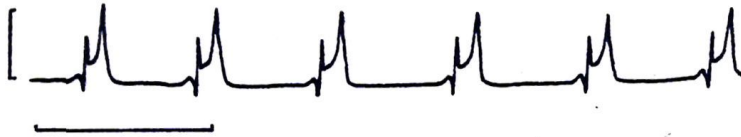
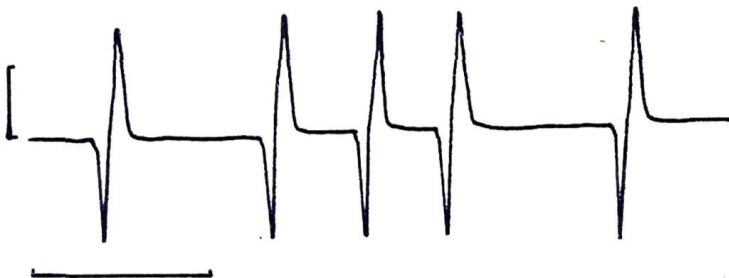


Figure 5 xxii shows the electrocardiogram recorded at stage 5 of the disease. There is atrioventricular dissociation with synchrony and a heart rate of 80 bpm.

FIGURE 5 xxiii



Atrial standstill with multifocal ventricular ectopic beats can be seen in the electrocardiogram illustrated in Figure 5 xxiii. This trace was recorded at death of the animal. The heart rate has slowed to 60 bpm, the arterial oxygen tension was 19.4 mmHg, the plasma potassium concentration 3.4 mEq.l^{-1} and the temperature 39.3°C .

Dog 6

FIGURE 5 xxiv



The electrocardiogram illustrated in Figure 5 xxiv and recorded at stage 1 of the disease shows a sinus tachycardia with a heart rate of 170 bpm. The arterial oxygen tension was 93.9 mmHg and the temperature 39.1°C.

FIGURE 5 xxv



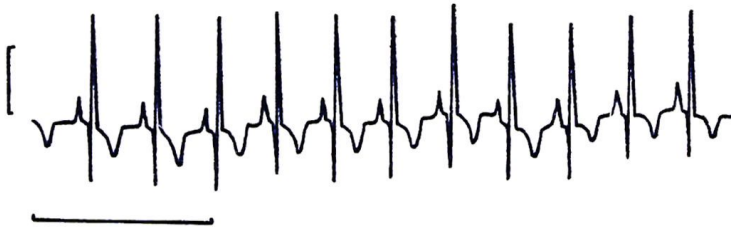
Figure 5 xxv shows an electrocardiogram recorded at stage 2 of the disease. There is a sinus tachycardia with a heart rate of 170 bpm. The arterial oxygen tension was 86.0 mmHg and the temperature 39.1°C.

FIGURE 5 xxvi



Sinus tachycardia with a heart rate of 165 bpm is illustrated in the electrocardiogram in Figure 5 xxvi, recorded at stage 3 of the disease. The arterial oxygen tension was 76.2 mmHg and the temperature 38.4°C.

FIGURE 5 xxvii



The electrocardiogram in Figure 5 xxvii was recorded at stage 4 and demonstrates a sinus tachycardia with a heart rate of 165 bpm. There is an increase in amplitude of the T waves. The arterial oxygen was 47.0 mmHg and the temperature 37.9°C.

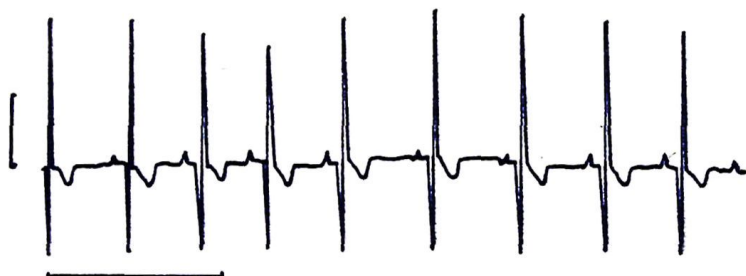
FIGURE 5 xxviii



Figure 5 xxviii shows an electrocardiogram recorded at a later time during stage 4. There is a sinus arrhythmia with a heart rate of 135 bpm and the T waves are within normal limits.

Dog 7

FIGURE 5 xxix



A normal electrocardiogram recorded at stage 1 is illustrated in Figure 5 xxix. The heart rate is 140 bpm.

FIGURE 5 xxx



The electrocardiogram illustrated in Figure 5 xxx and recorded at stage 2 of the disease shows a sinus tachycardia with a sinus arrhythmia and a heart rate of 165 bpm. The arterial oxygen tension was 67.3 mmHg and the temperature 39.1°C.

FIGURE 5 xxxi

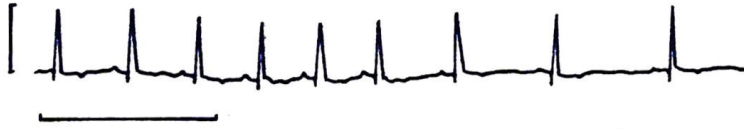


FIGURE 5 xxxii

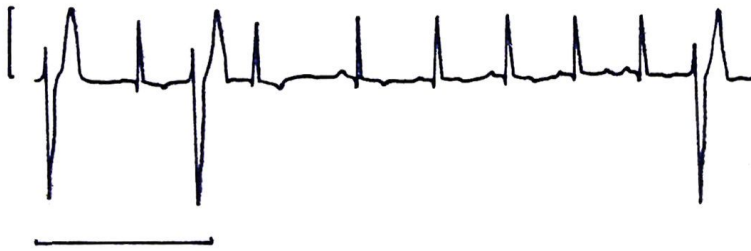


FIGURE 5 xxxiii



Figures 5 xxxi, 5 xxxii and 5 xxxiii show electrocardiograms recorded on half sensitivity at stage 3. These were taken from an electrocardiogram recorded continuously for three minutes to show various changes in the rhythm.

Initially (Fig. 5 xxxi) there is a sinus arrhythmia with sinus arrest and a heart rate of 70 bpm. This progresses (Fig. 5 xxxii) to single premature ventricular contractions which are unifocal in origin. The dog then goes in and out of ventricular tachycardia (Fig. 5 xxxiii), which is multifocal in origin, and in between these rhythms there are capture beats. The arterial oxygen tension was 67.5 mmHg and the temperature 38.0°C.

FIGURE 5 xxxiv

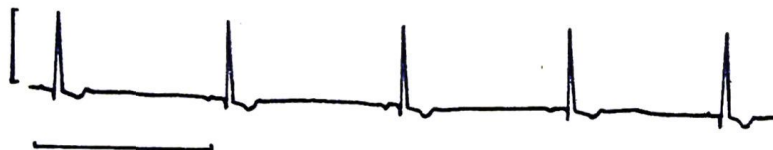
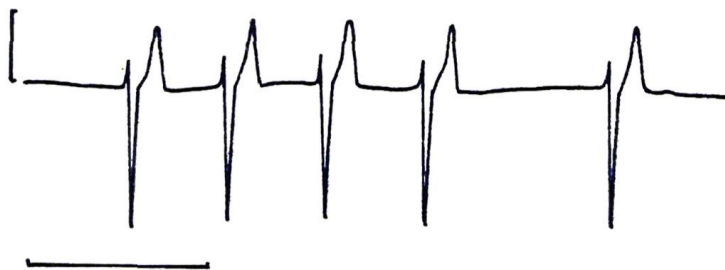


FIGURE 5 xxxv



The electrocardiograms illustrated in Figure 5 xxxiv and 5 xxxv were recorded on half sensitivity before the administration of 0.6 mg atropine sulphate intravenously (IV). The heart rate is 70 bpm with a wandering pacemaker (Fig. xxxiv). Ventricular premature contractions occur singly or grouped together (Fig. 5 xxxv). The arterial oxygen tension was 67.2 mmHg and the temperature 37.8°C.

FIGURE 5 xxxvi

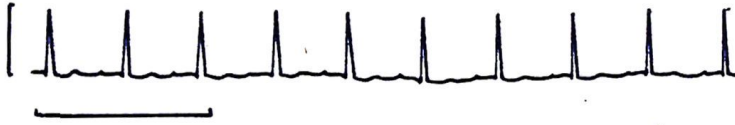
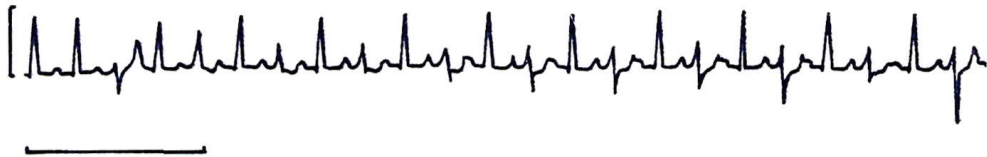


Figure 5 xxxvi illustrates the electrocardiogram taken at the completion of atropine sulphate administration. The heart rate is 140 bpm and normal in character.

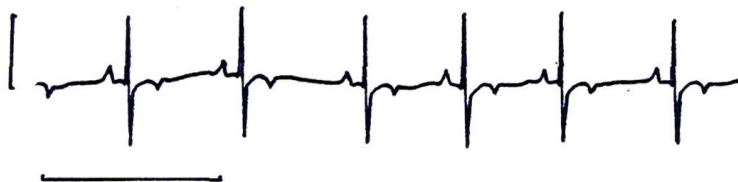
FIGURE 5 xxxvii



The electrocardiogram illustrated in Figure 5 xxxvii was recorded 10 sec after that in Figure xxxvi. There is a ventricular tachycardia with bigeminal rhythm and a heart rate of 250 bpm.

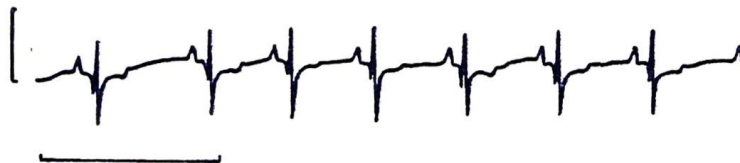
Dog 8

FIGURE 5 xxxviii



A normal electrocardiogram recorded at stage 1 is illustrated in Figure 5 xxxviii. The heart rate is 100 bpm.

FIGURE 5 xxxix



The electrocardiogram illustrated in Figure 5 xxxix and recorded at stage 2 of the disease is normal in character with a heart rate of 115 bpm.

FIGURE 5 xl

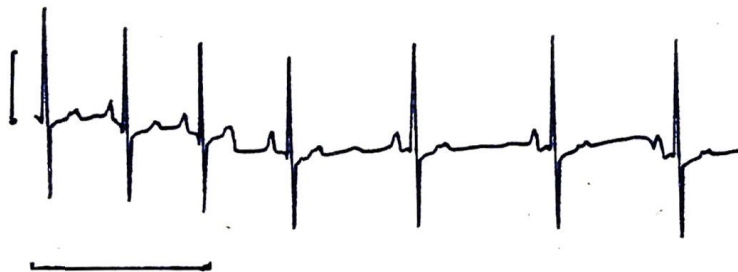
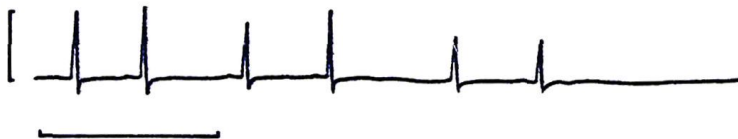


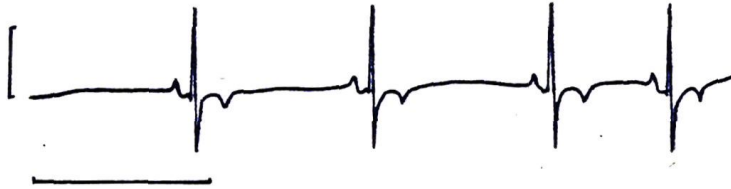
Figure 5 xl shows an electrocardiogram recorded at stage 3. There is a sinus arrhythmia with a heart rate of 115 bpm.

FIGURE 5 xli



There is a marked decrease in the amplitude of the electrocardiogram illustrated in Figure 5 xli and recorded at stage 4 of the disease. There is sinus arrhythmia with sinus arrest and a heart rate of 80 bpm. The arterial oxygen tension was 93.5 mmHg and the temperature 38.5°C.

FIGURE 5 xlii



The electrocardiogram illustrated in Figure 5 xlii was recorded before the administration of 0.6 mg atropine sulphate IV. The overall amplitude has increased and there is a sinus arrhythmia with a heart rate of 70 bpm.

FIGURE 5 xliii

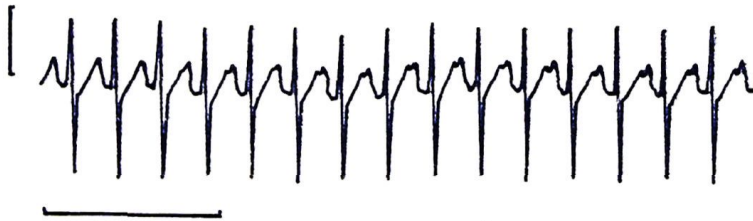


Figure 5 xliii shows the electrocardiogram after the administration of atropine sulphate. There is a ventricular tachycardia with a heart rate of 230 bpm.

5.4 DISCUSSION

The electrocardiographic changes found were extremely variable and this fact makes interpretation difficult. The arrhythmias observed were not consistent even for the individual animal, so that one dog showing a sinus tachycardia at one stage may have a normal rhythm or sinus arrest at the next stage. Generally if an abnormality in rhythm occurred in stages 1, 2 or 3, it tended to be a sinus tachycardia, a ventricular tachycardia or a sinus arrest. During stage 4 the abnormality in rhythm tended to be a sinus arrest, a sinus bradycardia, a sinus tachycardia or a ventricular tachycardia. While in stage 5 sinus bradycardia was the predominating rhythm.

Abnormalities, other than in rhythm, occurred in two dogs during stages 1, 2 and 3. One dog demonstrated S-T segment depression, while an increase in amplitude of the T waves was seen in another dog. During stage 4 an increased amplitude of the T waves occurred in two dogs, biphasic T waves in one dog and a mild Q-T prolongation in another. Increased amplitude of the T waves was present in two dogs, mild Q-T prolongation in another and atrial standstill with multifocal ectopic ventricular beats in the third during stage 5 of the disease.

From the changes in the electrocardiographs recorded in the individual dogs from one stage to another and even in some cases while the electrocardiogram was being recorded, continual electrocardiographic monitoring would probably have revealed more abnormalities than were recorded in this experiment.

The measurements of arterial oxygen tension, plasma potassium concentration, plasma calcium concentration and body temperature were included where changes in these could be responsible for the

electrocardiographic abnormalities found.

Myocardial hypoxia caused by hypoxaemia produces specific electrocardiographic changes. Initially there is a sinus tachycardia and abnormalities in the S-T segment and the T waves. The S-T segment shows depression or elevation while the T waves change in shape, polarity or increase in height. A more advanced sign is the development of an arrhythmia. The most common arrhythmias that occur are ventricular premature beats, although ventricular tachycardia, ventricular fibrillation and cardiac standstill also occur. Since mild hypoxaemia is represented by an arterial oxygen tension of less than 80 mmHg (Shapiro, Harrison and Walton, 1977), the electrocardiographic changes recorded during the first three stages were probably not due to hypoxaemia. It is possible that the electrocardiographic changes recorded in those animals in which the arterial oxygen tension was between 61.5 and 71.5 mmHg at stage 4 could have been due to hypoxaemia. Myocardial hypoxia from hypoxaemia is the most probable cause of the electrocardiographic changes just prior to death of the dogs where the arterial oxygen tension was between 19.4 and 32.5 mmHg. Hyperkalaemia, in which mild elevations ($6.0-6.5 \text{ mEq.l}^{-1}$) produce tall peaked T waves, moderate elevations ($6.5-7.0 \text{ mEq.l}^{-1}$) produce a decrease in heart rate, flattening of the P waves and tall T waves, and high elevations (7.5 mEq.l^{-1} or greater) cause a bradycardia and atrial standstill, was not responsible for the electrocardiographic changes observed in this experiment. Hypokalaemia (plasma potassium concentration below 3.0 mEq.l^{-1}), which produces bradycardia, prolonged Q-T interval and small biphasic T waves, was not observed in this experiment. As the plasma calcium concentrations remained within the normal range at all times, the abnormalities found could not be attributed to changes in this electrolyte. An elevation

in body temperature will frequently produce a sinus tachycardia, but no elevations were observed.

Dysfunction of the autonomic nervous system could explain the electrocardiographic changes found when arterial oxygen tensions were normal. Excessive sympathetic activity produces sinus tachycardia, ventricular tachycardia, ventricular premature contractions and changes in the S-T segments and T waves, while excessive parasympathetic activity produces sinus arrest, wandering pacemaker and heart block.

The electrocardiographic changes of sinus arrest, ventricular escape beats and wandering pacemaker in some cases during the early stages of the disease and the fall in heart rate during the later stages of the disease could be explained by increased vagal tone. This was demonstrated by the 300% increase in heart rate after the administration of 0.6 mg atropine sulphate intravenously. This marked response in heart rate to such a small dose of atropine indicates that sympathetic tone was still increased at this stage, although masked by the predominant vagal tone. At all stages when vagal tone was increased the rhythm was regularly irregular, demonstrating the normal changes in heart rate produced by the respiratory cycle. These changes in heart rate are produced by both central and peripheral mechanisms such that during inspiration heart rate increases and during expiration heart rate decreases. The normal rate is that which is seen in expiration. Reflexes operating during inspiration cause a decrease in vagal tone and an acceleration in heart rate.

Both excessive sympathetic activity and excessive parasympathetic activity with appropriate electrocardiographic changes have been reported in polyradiculoneuritis (Lichtenfeld, 1971) and tetanus (Hollow and Clarke, 1975).

The cardiotoxic effects of catecholamines are well documented (Raab, 1966; Szakacs and Mehlman, 1960). They are thought to produce their effects by intensifying myocardial oxygen consumption to a degree which exceeds both the oxygen demands made by simultaneous increased muscular action and the increase of oxygen supply due to simultaneous coronary dilation (Raab, 1948).

Electrocardiographic changes similar to those produced by catecholamines are reported in poliomyelitis (Weinstein and Shelokov, 1951), phaeochromocytomas (Sode, Getzen and Osborne, 1967), emotional and sensory stress (Raab, 1966) and subarachnoid haemorrhage (Greenhoot and Reichenbach, 1969). These changes have been attributed to respiratory insufficiency, involvement of the myocardium and lesions in the autonomic nervous system. Although hypoxaemia and hypercapnia were probably responsible for some of the earlier reported abnormalities, these arrhythmias are still found in patients under intermittent positive pressure ventilation where hypoxaemia and hypercapnia are known not to be present.

Histopathological lesions in the myocardium have been reported in poliomyelitis (Dolgopol and Cragan, 1948), polyradiculoneuritis (Klein, 1954), tetanus (Kerr, Corbett, Prys-Roberts, Crampton Smith and Spalding, 1968), phaeochromocytomas (Sode, Getzen and Osborne, 1967) and subarachnoid haemorrhage (Greenhoot and Reichenbach, 1969). These changes have been termed toxic myocarditis and are characterized by a patchy myocardial necrosis with or without inflammatory infiltration.

Sympathetic overactivity has been confirmed in tetanus (Kerr, Corbett, Prys-Roberts, Crampton Smith and Spalding, 1968) and suggested in polyradiculoneuritis (Lichtenfeld, 1971). Increased urinary excretion of catecholamines and 17-hydroxycorticosteroid has

been reported in polyradiculoneuritis (Davies and Dingle, 1972) and tetanus (Corbett, Kerr, Prys-Roberts, Crampton Smith and Spalding, 1969).

Bradycardia has been reported in polyradiculoneuritis (Lichtenfeld, 1971) and sinus arrest with nodal escape rhythm which progressed to nodal bradycardia in tetanus (Hollow and Clarke, 1975). Both authors felt that these changes were due to dysfunction of the parasympathetic nervous system. However, excessive vagal tone need not necessarily be due to central dysfunction but may be reflex in origin. Arterial hypertension stimulates the baroreceptors, principally those in the carotid sinus and aortic arch, and results in a slowing of heart rate mediated by the parasympathetic nervous system. Therefore the increased vagal tone could be reflex in origin, if arterial hypertension is present. The pronounced bradycardia seen prior to death is probably due to hypoxaemia. Arterial oxygen tensions below 30 mmHg lead to a circulatory crisis consisting of bradycardia, augmented venomotor tone and elevated systemic vascular resistance. This crisis is initiated by reflexes from the chemoreceptors (Cross, Rieben, Barron and Salisbury, 1963) and terminates in cardiac failure.

The electrocardiographic changes caused by *Ixodes holocyclus* reported by Pearn (1966) consisted of a prolonged Q-T interval, S-T segment depression, a low amplitude of the P and T waves and a heart rate of 164 bpm. A diagnosis of toxic myocarditis with concomitant cardiac failure was made, and with conservative treatment the condition of the child improved and the electrocardiogram recorded three days later was normal.

In view of the electrocardiographic changes found in this experiment, it is interesting to note the changes found by Emmons and McLennan (1960) while studying nerve conduction velocities in

marmots paralysed by *Dermaacentor andersoni*. They found that the changes in the electrocardiogram fell into two groups. The first group showed a sinus tachycardia in which heart rate rose from its normal level of about 190 bpm to about 260 bpm. The rhythm was regular and the intervals which were measured (P-R, QRS, Q-T) were only slightly less than normal. The second group showed a pronounced bradycardia with some degree of sinus arrhythmia. The average rate fell to as low as 35-40 bpm and the Q-T interval was very prolonged. In some cases the P-R interval was also prolonged. A fall in body temperature was noticed in the final stages of the paralysis. As all animals with marked bradycardia had low body temperatures while those with tachycardia had a normal temperature, they felt that those animals showing bradycardia were in a more advanced stage of the condition than those with tachycardia. They attributed these later changes to depressed conduction and a slower rate of atrial and ventricular depolarization and repolarization.

In conclusion the electrocardiographic changes produced by *Ixodes holocyclus* are extremely variable and difficult to interpret. Those arrhythmias characterized by sinus tachycardia and ventricular tachycardia could be a result of increased cardiac sympathetic stimulation. In view of the biochemical changes found in Chapter 3 it is likely that this increased stimulation is the result of overall excessive sympathetic activity. Those arrhythmias characterized by sinus arrest and sinus bradycardia could be reflex in origin or again due to autonomic dysfunction. The bradycardia observed at death of these animals is probably due to circulatory crisis triggered by hypoxaemia.

CHAPTER 6

THE CARDIOVASCULAR EFFECTS OF *IXODES HOLOCYCLUS* IN THE DOG

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6.1 INTRODUCTION

Although specific investigations of disturbances of cardiovascular function produced by *Ixodes holocyclus* infestation have not been described previously, there have been several reports suggesting that in some cases the disease affects the heart. Acute heart failure with myocarditis together with signs of paralysis have been described by Pearn (1966). Allan and Pursell (1971) and Calder (1974) found pulmonary oedema at autopsy in dogs dying of tick paralysis, and acute respiratory distress and death (Hindmarsh and Pursell, 1935) and death after extreme exercise (Allan and Pursell, 1971) have been reported in dogs after recovery from tick paralysis.

The electrocardiographic changes documented in Chapter 5 demonstrated that in some cases of tick paralysis there appeared to be increased cardiac sympathetic stimulation, while in other cases vagal tone was increased. This chapter describes experiments designed to investigate overall cardiovascular function throughout the course of tick paralysis in the dog, to assess further the role of the autonomic nervous system in the response to infestation and to determine whether disturbances of cardiac function were a consistent feature of the disease.

6.2 MATERIALS AND METHODS

Ten healthy cross-bred dogs ranging in weight from 24.6 to 31.2 kg were used in this study.

Two weeks prior to attachment of the ticks the dogs were prepared by surgical implantation of catheters in the carotid artery,

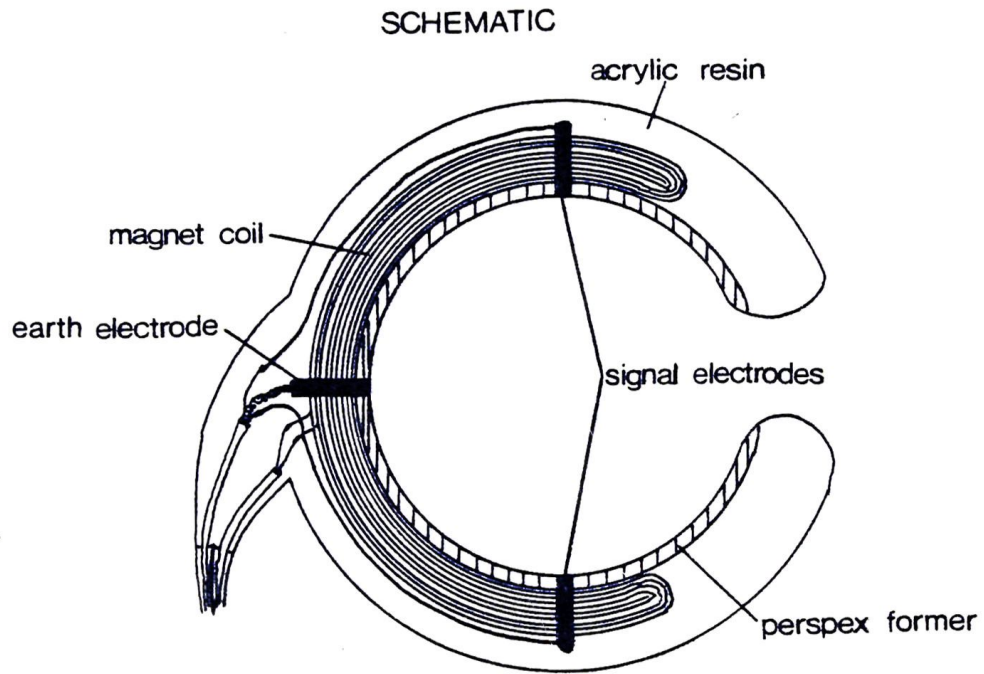
pulmonary artery and left atrium, a micromanometer in the left ventricle and an electromagnetic flow probe around the ascending aorta.

Arterial pH, partial pressure of oxygen in arterial blood, partial pressure of carbon dioxide in arterial blood, mixed-venous pH ($pH\bar{v}$), partial pressure of oxygen in mixed-venous blood ($P\bar{V}_{O_2}$) and the partial pressure of carbon dioxide in mixed-venous blood ($P\bar{V}_{CO_2}$) were measured.

Systolic and diastolic arterial pressure, systolic and diastolic pulmonary arterial pressure and left atrial pressure were measured in mmHg using strain-gauge pressure transducers (Statham P23AA, P23Db and P23BB). The pressure transducers were calibrated against a mercury manometer. Zero pressure was referred to mid-sterum. Mean arterial pressure ($\bar{A}P$) in mmHg was calculated by addition of one-third of the pulse pressure to the diastolic pressure measurement. Mean pulmonary arterial pressure ($\bar{P}A$) and mean left atrial pressure ($\bar{L}A$) in mmHg were obtained by mechanical damping. All catheters used for pressure measurements were connected to the pressure transducers using sterile three-way taps and manometer connection tubing.

The phasic aortic flow in litre per minute ($l.min^{-1}$) and the mean aortic flow ($\bar{C}O$) in millilitre per minute ($ml.min^{-1}$) were measured with an electromagnetic flowmeter (EMI Australia Limited). The flow transducer was placed at the base of the aorta just distal to the aortic valve. This technique gave a measurement of total cardiac output minus the coronary blood flow, since the *coronary ostia* lie proximal to the position of the flow probe. Light-weight flow probes were constructed as described by Goodman (1969), Figure 6 i. These probes were individually chosen for each animal so that

FIGURE 6 i ELECTROMAGNETIC FLOW PROBE



ACTUAL



they fitted loosely on the aorta. The internal diameter of the probes varied from 19 to 22 mm. More tightly fitting probes were not implanted, as the pulsating aorta rubbed against the edges of the transducer causing local necrosis, aortic rupture and early death. Although loose probes prevented stable flow recordings with minimal electrocardiographic interference until fibrous tissue had formed around the probe (usually 4-7 days), selection of probes in this manner allowed most dogs to survive four weeks before aortic rupture. Both phasic and mean aortic flow were recorded. Mean flow was obtained by damping the phasic aortic flow signal using a resistance-capacitance (RC) filter with an appropriate time constant. Phasic flow during diastole provided the zero flow reference.

All aortic flow probes were calibrated *in vitro*. Saline 0.9% was passed under hydrostatic pressure from a ten litre reservoir through a section of bovine aorta (preserved in 10% formaldehyde in saline) and collected in a measuring cylinder over a timed period. The outflow from the aorta was led through a resistance before being collected to ensure that the aorta was pressurized. The sensitivity of square-wave electromagnetic flowmeters has been reported to fall with an increasing haematocrit (Spencer and Denison, 1959; Goodman, 1969). When fresh artery and whole blood are used for calibration *in vitro*, however, flowmeter sensitivity appears to be within 5-10% of that determined using preserved artery and saline (Goodman, 1978). It is likely, therefore, that the measurements of cardiac output in these experiments have consistently underestimated absolute flow values by no more than about 5-10%.

In the determination of myocardial contractility using the index $\frac{dP/dt}{IIT}$ it is necessary to obtain a high fidelity recording of the left ventricular pressure pulse. The catheter-tip manometer used to

measure left ventricular pressure in this work was similar to that designed by Goodman, Angus, Einstein and Cobbin (1972a) and is illustrated in Figure 6 ii. The index $\frac{dP/dt}{IIT}$ is obtained from an analogue computer (Goodman, Angus, Einstein and Cobbin, 1972b). Peak dP/dt (maximum rate of rise of left ventricular pressure) is divided by the integrated isovolumic ventricular pressure (IIT) measured from the R wave of the electrocardiogram to peak dP/dt to obtain $\frac{dP/dt}{IIT}$. The value of this index as a measure of cardiac inotropism has been described by Angus, Richmond, Cobbin and Goodman (1977).

The data were recorded on a twelve-channel direct-recording oscillograph (Visigraph-F type FR 301, San-ei). The recordings were made on ultra-violet photosensitive paper (Oscilloscript D, Agfa-Gevaert).

Cardiac output in millilitres per minute per kilogram ($\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) was obtained by dividing mean cardiac output by the weight in kilograms of the dog.

Stroke volume (SV) in millilitres per kilogram ($\text{ml} \cdot \text{kg}^{-1}$) was obtained by dividing mean cardiac output per kilogram by the heart rate per minute.

Systemic vascular resistance (SVR) in arbitrary units was calculated by dividing mean arterial pressure by mean cardiac output per kilogram.

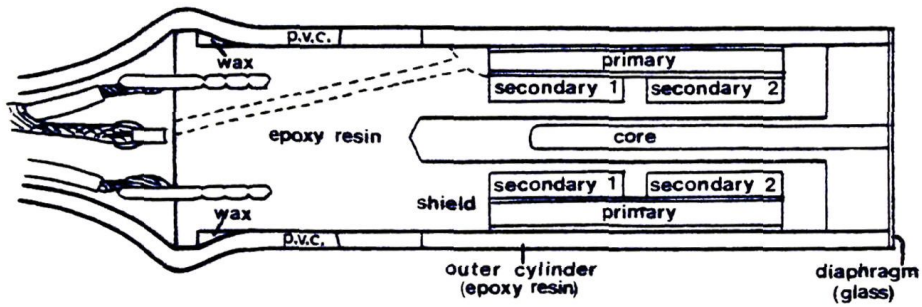
Pulmonary driving pressure (PDP) in mmHg was obtained by subtracting left atrial pressure from pulmonary arterial pressure.

Pulmonary vascular resistance (PVR) in arbitrary units was calculated by dividing pulmonary driving pressure by mean cardiac output per kilogram.

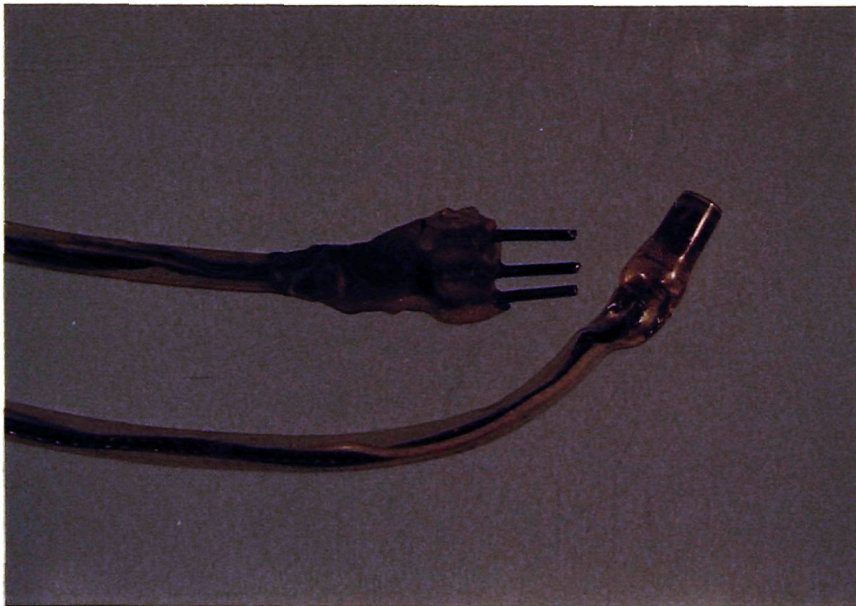
Control measurements were taken on the two days preceding attach-

FIGURE 6 ii MICROMANOMETER

SCHEMATIC



ACTUAL



ment of the ticks. Eight ticks were attached to each dog as described in section 2.4. Once clinical signs were evident measurements were carried out at stages 1, 2, 3, 4 and 5 of the disease as outlined below:

Stage 1 - the dogs showed ataxia when walked.

Stage 2 - the dogs were unable to stand but could right.

Stage 3 - the dogs were unable to right.

Stage 4 - the dogs were unable to lift their heads.

Stage 5 - within two hours of death.

The results were analysed by pairing the control and test values in each animal and the mean differences at each stage were compared with zero using the Student's t-distribution.

6.3 RESULTS

Although ten dogs were used in this experiment, the results of six dogs only are presented. The results of the other four dogs were discarded due to rupture of the aorta during stage 1 of the disease.

The mean and the SEM of the cardiovascular measurements during the control period and at the various stages of the disease are illustrated in Table 6 i.

Although all dogs were prepared with left atrial catheters, it was very difficult to keep this catheter functioning throughout the duration of the experiment. The control values for left atrial pressure, pulmonary driving pressure and pulmonary vascular resistance represent three dogs only. From stage 1 to stage 5 only two dogs had functioning left atrial catheters, and therefore the values

TABLE 6 1 CHANGES IN CARDIOVASCULAR MEASUREMENTS AT THE VARIOUS STAGES OF PARALYSIS CAUSED BY *IXODES HOLOCYCLUS*

		CONTROL MEAN SEM n = 6	STAGE 1 MEAN SEM n = 5	STAGE 2 MEAN SEM n = 5	STAGE 3 MEAN SEM n = 4	STAGE 4 MEAN SEM n = 4	STAGE 5 MEAN SEM n = 4
HR	bpm	102 ± 9	135 ± 16 *	128 ± 15	128 ± 16	111 ± 10	78 ± 3
$\bar{A}P$	mmHg	94.5 ± 1.9	111.8 ± 2.1 ***	122.4 ± 4.2 ***	122.8 ± 5.6 *	135.5 ± 6.0 **	134.3 ± 7.4 *
$\bar{P}A$	mmHg	12.5 ± 0.7	12.6 ± 1.3	16.2 ± 1.2 *	18.8 ± 2.8 *	20.5 ± 1.2 ***	16.5 ± 1.4
$\bar{L}A$	mmHg	4.7 n=3	5.0 n=2	4.5 n=2	5.0 n=2	4.5 n=2	9.5 n=2
$\bar{A}F$	l.min ⁻¹	3.5 ± 0.2	3.0 ± 0.4	2.7 ± 0.2 *	2.6 ± 0.5	3.2 ± 0.3	3.0 ± 0.8
$\bar{C}O$	ml.min ⁻¹ kg ⁻¹	123 ± 9	105 ± 17	93 ± 11 *	93 ± 22	112 ± 13	107 ± 34
SV	ml.kg ⁻¹	1.23 ± 0.09	0.85 ± 0.22	0.78 ± 0.15 *	0.86 ± 0.36	1.07 ± 0.24	1.33 ± 0.37
SVR	arbitrary units	0.80 ± 0.07	1.15 ± 0.14 *	1.39 ± 0.16 *	1.56 ± 0.36	1.26 ± 0.14 *	1.65 ± 0.43
PDP	mmHg	7.3 n=3	9.5 n=2	10.5 n=2	9.5 n=2	14.0 n=2	6.0 n=2
PVR	arbitrary unit	0.06 n=3	0.10 n=2	0.12 n=2	0.12 n=2	0.15 n=2	0.10 n=2
$\frac{dP}{dt}$ IIT	sec ⁻²	2950 ± 190	2860 ± 190	2700 ± 280	2200 ± 480	2510 ± 130	3060 ± 300
Pa _{O₂}	mmHg	85.9 ± 2.3	85.9 ± 2.6	82.9 ± 4.2	80.5 ± 2.5	72.6 ± 4.4 *	62.4 ± 2.8 **
Pa _{CO₂}	mmHg	33.9 ± 2.1	32.2 ± 2.6	33.3 ± 2.6	36.9 ± 1.7	36.8 ± 3.9	44.6 ± 1.5 ***
pHa		7.405 ± 0.004	7.424 ± 0.009	7.395 ± 0.017	7.385 ± 0.009	7.353 ± 0.011 **	7.302 ± 0.025 *
$\bar{P}\bar{V}_{O_2}$	mmHg	38.1 ± 1.0	41.3 ± 0.8 *	40.7 ± 1.3	41.0 ± 1.8	41.5 ± 1.2 *	39.4 ± 0.5
$\bar{P}\bar{V}_{CO_2}$	mmHg	37.1 ± 2.1	36.4 ± 2.1	36.9 ± 2.6	40.5 ± 2.5	40.0 ± 3.1	47.0 ± 2.2 ***
pH \bar{v}		7.385 ± 0.004	7.397 ± 0.012	7.372 ± 0.014	7.364 ± 0.005	7.316 ± 0.011 **	7.295 ± 0.016 **

* P<0.05 ** P<0.02 *** P<0.01

of left atrial pressure, pulmonary driving pressure and pulmonary vascular resistance are included for interest only as they do not represent true values of the group.

One dog died suddenly during stage 1 of the disease, and his measurements are not included in the mean or SEM for stage 1. Another dog, while vomiting during stage 3, inhaled the vomitus, and his measurements are not included in the mean or SEM for stage 3.

Mean arterial pressure rose during stage 1 and continued to increase until just before death. This was accompanied by a rise in systemic vascular resistance and a small (but not statistically significant) fall in cardiac output. Pulmonary arterial pressure rose during stage 2 and continued to increase until just before death. Heart rate was significantly elevated at stage 2, but then fell slowly until it was below control levels at stage 5. Myocardial contractility did not significantly differ from control at any stage of the disease. Arterial oxygen tension, arterial and mixed-venous pH fell during stages 4 and 5, while arterial and mixed-venous carbon dioxide tensions were elevated at stage 5. Mixed-venous oxygen tension, which tended to be slightly elevated at all stages, was significantly elevated at stages 2 and 4.

6.4 DISCUSSION

The most consistent finding in this experiment was the increase in peripheral resistance leading to a significant elevation of mean arterial pressure at all stages of the disease. Heart rate was significantly elevated only at stage 1 of the disease, although there was a trend towards an increase in stage 2, 3 and 4 and a

trend towards a decrease in stage 5. Although mean cardiac output was significantly decreased at stage 2 only, there was a general trend towards a decrease throughout the disease. The elevation of pulmonary arterial pressure despite a tendency for cardiac output to fall indicated an overall rise in pulmonary vascular resistance. (In the two dogs with patent left atrial catheters, left atrial pressure remained unchanged until shortly before death.)

Although all dogs showed the clinical signs outlined in Chapter 3, the onset and progression of signs in one dog was atypical. In this dog the first signs of the disease were not observed till nine days after attachment of the ticks and progression through the stages to death took four days. The cardiovascular changes observed in this dog differed from those in the other dogs and were responsible for the failure of some of the results to reach significance. While cardiac output fell through the disease in the other dogs, in this dog it was similar to, or elevated above, control at stages 1, 3, 4 and 5. Similarly systemic vascular resistance, which was elevated at all stages of the disease in the other dogs, fell below control at stages 1 and 5 in this dog.

From the results of these cardiovascular measurements it appears that there was increased sympathetic stimulation of the cardiovascular system producing a tachycardia during stage 1, 2 and 3 and an increase in peripheral vascular resistance. In addition this increased sympathetic drive occurred in the presence of normal arterial oxygen and carbon dioxide levels. As the disease progressed, the rise in arterial pressure appears to have caused reflex vagal stimulation of the heart, resulting in a tendency towards a bradycardia. Although cardiac stimulation was present, there was no increase in measured contractility. This could indicate that there was a reduction in

the inotropic state of the heart (heart failure), but that it was being masked by the large increase in sympathetic tone.

At stage 4 of the disease the mean arterial oxygen tension had fallen to 72.6 mmHg, indicating that mild hypoxia was present. The cardiovascular changes found in hypoxia are determined by an increased sympathetic drive in response to central hypoxia and by peripheral vasodilation in response to tissue hypoxia. Usually there is an elevation in heart rate, a slight elevation in arterial pressure and dilation in the coronary vessels, the cerebral vessels and in the skeletal muscle vasculature (Korner, 1959).

The dog which died during stage 1 of the disease died with a high output cardiac failure. The measurements recorded three hours before death are shown below:

		Control	Measurements three hours before death
HR	bpm	101	150
\overline{AP}	mmHg	90	129
\overline{PA}	mmHg	10	37
\overline{LA}	mmHg	6	26
\overline{CO}	ml.min ⁻¹ kg ⁻¹	130	400
SVR	arbitrary units	0.72	0.33
PDP	mmHg	4	11
PVR	arbitrary units	0.03	0.03
$\frac{dP/dt}{IIT}$	sec ⁻²	3330	3860
Pa _{O₂}	mmHg	91.4	65.0
Pa _{CO₂}	mmHg	27.4	16.4

These results indicate a marked sympathetic stimulation of heart rate. The rise in mean left atrial pressure from 6 mmHg to 26 mmHg is excessive in relation to the modest three-fold increase in cardiac output and indicates a serious decline in left ventricular function. This is borne out by the relatively insignificant rise in measured contractility despite the increase in heart rate. Histopathological examination of the lungs revealed severe acute congestion and oedema superimposed on a chronic pleurisy. Some diffuse inflammatory changes were also developing. Although this form of death is uncommon in tick paralysis, during this study two other dogs in the early stages of the disease developed acute respiratory problems and died suddenly. At death blood flowed freely from the lungs. It is probable that this death was not a complication of instrumentation but another entity which the tick is capable of producing. As reported in Chapter 3, varying degrees of pulmonary congestion and oedema were present in dogs affected by tick paralysis. In the cases mentioned above the pulmonary changes were rapid and severe and resulted in early death.

Although specific studies of the cardiovascular system in cases of tick paralysis have not been undertaken previously, Murnaghan (1958), while studying the neurophysiology of tick paralysis produced by *Dermacentor andersoni*, measured the blood pressure in five paralysed dogs. He found the blood pressures to be 130, 150, 100, 170 and 190 mmHg, and although the method of measurement was not described and control values were not given, nor was it stated whether the animals were conscious or anaesthetized, some of these values appear to be elevated above the values which are considered normal in the dog.

Arterial hypertension has been reported in other diseases which

affect the nervous system such as poliomyelitis (Grulee and Panos, 1948; McDowell and Plum, 1951; Kemp, 1957), polyradiculoneuritis (Mitchell and Meilman, 1967; Lichtenfeld, 1971; Davies and Dingle, 1972) and tetanus (Kerr, Corbett, Prys-Roberts, Crampton Smith and Spalding, 1968; Hollow and Clarke, 1975).

In poliomyelitis arterial hypertension was more common in those patients with the bulbospinal form or with paralysis involving all four extremities. These patients were the most severely ill and many needed artificial respiration. Arterial hypertension was of a considerably greater degree and of longer duration in patients requiring ventilation than in non-respirated patients (McDowell and Plum, 1951). The mechanism for production of arterial hypertension in poliomyelitis is unknown. Involvement of the brain stem autonomic functions, however, was thought to be a cause by McDowell and Plum (1951) and Kemp (1957).

Hypertension occurred in 61% of the patients with polyradiculoneuritis studied by Lichtenfeld (1971). In this study all blood pressure elevations were transient lasting from 2 to 21 days, and a widely fluctuating blood pressure was a common finding. Increased urinary excretion of catecholamines was found in cases of polyradiculoneuritis with associated hypertension by Mitchell and Meilman (1967) and Davies and Dingle (1972). When hypertension was present, the administration of the α -adrenergic blocking agent phentolamine mesylate resulted in a drop in both diastolic and systolic pressures (Mitchell and Meilman, 1967; Lichtenfeld, 1971). The mechanism for the production of arterial hypertension in polyradiculoneuritis is unknown, but pathological alteration of the sympathetic nervous system is thought to play a role.

The hypertension reported in cases of tetanus has been found to

be due to sympathetic activity (Corbett, Kerr, Prys-Roberts, Crampton Smith and Spalding, 1968). The blood pressure, as with polyradiculoneuritis, was subject to wide fluctuations (Hollow and Clarke, 1975). Prys-Roberts, Kerr, Corbett, Crampton Smith and Spalding (1969) found that treatment directed towards suppressing sympathetic activity resulted in a successful outcome in cases which, on the basis of previous experience, carried a gloomy prognosis.

In conclusion, the disease produced by *Ixodes holocyclus* was accompanied by significant changes in cardiovascular function. Increased peripheral resistance, producing increased arterial pressure and a trend towards a decrease in cardiac output, together with an initial rise in heart rate indicated increased sympathetic stimulation. Within the pulmonary circulation it appeared that the rise in pulmonary arterial pressure resulted from increased pulmonary vascular resistance. Myocardial contractility was not increased, suggesting a reduction in the inotropic state of the heart, which was masked by sympathetic drive. These changes were demonstrated at all stages of the disease, whereas hypoxaemia was not found until stage 4 of the disease. The reason for the increased sympathetic stimulation is unclear, but it is likely that, as in other neurological diseases, it is a manifestation of central autonomic dysfunction.

CHAPTER 7

A COMPARISON OF THE EFFECTS OF FIVE TREATMENTS ON SURVIVAL
OF DOGS WITH *IXODES HOLOCYCLUS* PARALYSIS

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7.1 INTRODUCTION

From the first case reports of paralysis caused by *Ixodes holocyclus* numerous treatments have been advocated. It was not until Ross (1935) published his work on the value of hyperimmune serum that a specific treatment for tick paralysis was established. Ross found that about 75% of all animals showing definite paralysis prior to treatment recovered when given hyperimmune serum.

It is the 25% which still die, however, that has encouraged many people to try other drugs in combination with hyperimmune serum. The problem in assessing the benefits of these combinations is pointed out by Gray, Trevena and Perry (1974) who stated that one has no controls to compare the response with and that every single case of tick paralysis has so many variables that the value of any treatment is hard to assess accurately.

Therefore the final experiment in this study of *Ixodes holocyclus* was to compare, under controlled conditions, the value of other drugs in combination with hyperimmune serum in the treatment of dogs showing advanced signs of the disease. Two of the drugs were chosen because they are commonly used by veterinary practitioners in the treatment of tick paralysis. The third drug was chosen because it counteracts many of the cardiovascular abnormalities reported in Chapter 6.

7.2 MATERIALS AND METHODS

Forty-six healthy cross-bred dogs ranging in weight from 9.5 to 31.0 kg were used in this experiment.

Six ticks were attached to each dog and the dogs watched closely for onset of clinical signs.

Once ataxia was noticed the dogs were taken to an air-conditioned laboratory and observed until they could no longer right themselves. This time was noted, together with the animals' heart rate, respiration rate and type, temperature, and whether the animal was vomiting or not. The dogs were then randomly allocated a treatment.

The treatments were:

Treatment 1: Control - no treatment given.

Treatment 2: Hyperimmune serum (Canine Anti-tick Serum, Lismore Serum Products) - at a dose of 0.5 ml.kg^{-1} body weight, intravenously.

Treatment 3: Hyperimmune serum - at a dose of 0.5 ml.kg^{-1} body weight, intravenously;
Promethazine hydrochloride (Phenergan, May and Baker) - at a dose of 1 mg.kg^{-1} body weight, slowly, intravenously every eight hours.

Treatment 4: Hyperimmune serum - at a dose of 0.5 ml.kg^{-1} body weight, intravenously;
Dexamethasone (Dexadreson, Intervet) - at a dose of 0.5 mg.kg^{-1} body weight, intravenously every 12 hours.

Treatment 5: Hyperimmune serum - at a dose of 0.5 ml.kg^{-1} body weight, intravenously;
Phenoxybenzamine hydrochloride (Dibenylene, Smith Kline and French Laboratories Limited) - at a dose of 1 mg.kg^{-1} body weight, intravenously as a 0.1% solution over 10-15 minutes.

Treatment 6: Promethazine hydrochloride - at a dose of 1 mg.kg^{-1} body weight, slowly, intravenously every eight hours;

Dexamethasone - at a dose of 0.5 mg.kg^{-1} body weight, intravenously every 12 hours;

Phenoxybenzamine hydrochloride - at a dose of 1 mg.kg^{-1} body weight, intravenously as a 0.1% solution over 10-15 minutes.

After the treatment was administered heart rate, respiration rate and type and temperature were monitored every two hours. The dogs were also watched carefully for signs of recovery or deterioration and the time of death or ability to walk ten metres unaided was recorded.

7.3 RESULTS

Although 46 dogs were infested with *Ixodes holocyclus*, only 24 dogs showed signs of paralysis. Therefore four dogs only were represented in each treatment group.

The results were recorded by comparing survival to death rates in each of the treatment groups.

The number of dogs which survived or died, together with the average time in hours to walking or death for each of the treatment groups, is shown in Table 7 i.

In group 1, which received no treatment, all dogs died in an average of 3.3 hours from the time when a treatment would have been administered.

One dog survived and three dogs died, with 57.5 hours to walking and an average of 38.8 hours to death in group 2, which received hyperimmune serum alone.

In group 3, which received hyperimmune serum and promethazine

TABLE 7 i

THE EFFECT OF VARIOUS TREATMENTS ON THE SURVIVAL
OF DOGS WITH *IXODES HOLOCYCLUS* PARALYSIS
(4 DOGS IN EACH GROUP)

TREATMENT	SURVIVED		DIED	
	NUMBER OF DOGS	AVERAGE HOURS TO WALK	NUMBER OF DOGS	AVERAGE HOURS TO DEATH
1	0	-	4	3.3
2	1	57.5	3	38.8
3	2	28.0	2	41.0
4	3	48.0	1	11.8
5	4	34.9	0	-
6	0	-	4	21.8

hydrochloride, two dogs survived and two dogs died, with an average of 28.0 hours to walking and 41.0 hours to death.

Three dogs survived and one dog died, with an average of 48.0 hours to walking and 11.8 hours to death, in group 4, which received hyperimmune serum and dexamethasone.

In group 5, which received hyperimmune serum and phenoxybenzamine hydrochloride, all dogs survived, with an average of 34.9 hours to walking.

All dogs died in an average of 21.8 hours in group 6, which received promethazine hydrochloride, dexamethasone and phenoxybenzamine hydrochloride, but no hyperimmune serum.

Heart rate, respiration rate and temperature are not listed in the results, as there were no consistent changes within any of the treatment groups.

7.4 DISCUSSION

Treatment 1

From the results it can be seen that treatment was not attempted until the disease was at an advanced stage. The average time from this stage to death in the untreated dogs was 3.3 hours. The control group therefore demonstrated that, once the disease has reached this stage, recovery does not occur without some attempt at treatment.

Treatment 2

The results with hyperimmune serum alone at this stage of the disease showed that it was of limited value. In the dog that

survived the time to walking was 57.5 hours. Within the mortality group, hyperimmune serum greatly prolonged the time to death. Although it is not reported in the literature, it is generally acknowledged amongst veterinarians treating tick-poisoning cases that any signs of recovery from this disease may not be seen for up to 12 hours after treatment with hyperimmune serum. Therefore there is a delay period from administration to onset of action of the hyperimmune serum. This would explain the difference in survival after treatment with hyperimmune serum in this experiment and that found by Ross (1935) of 75%. In this experiment the animals were in a more advanced stage of the disease, and although hyperimmune serum must make some improvement to the animal from the time of administration, as shown by the longer time to death, the improvement is not sufficient to result in survival. Allan and Pursell (1971) examined the records of 100 cases of tick paralysis. Of the 100 dogs examined, 58 presented with a straightforward ascending motor paralysis with no vomiting or respiratory distress. Following treatment with hyperimmune serum, all these cases recovered. The remaining 42 dogs examined had varying degrees of motor paralysis and also varying degrees of vomiting, laryngeal paralysis, hypersalivation and pulmonary oedema. Following treatment with hyperimmune serum and symptomatic treatment, seven dogs of this group died, representing 16.6% of those with the vomiting/respiratory syndrome. These results are in accord with those published by Hindmarsh and Pursell (1935) who reported death from respiratory failure in four dogs that were recovering from motor paralysis. They hypothesized that the tick may secrete two toxins, one responsible for the motor paralysis which was treated by hyperimmune serum, and another which acted on the vagal centre of the medulla oblongata and was not

reversed by hyperimmune serum. The vomiting associated with tick paralysis does not cease with administration of hyperimmune serum, and dogs that have recovered completely from the motor paralysis may continue to vomit for one or two days when water or food is taken.

When placed in an open area with healthy dogs, the dog which survived with hyperimmune serum alone showed decreased exercise tolerance for at least four days after an apparently otherwise complete recovery. This observation was substantiated in further animals treated with hyperimmune serum alone and also in the literature where it is reported that sudden exercise within a day of complete recovery from severe paralysis has resulted in collapse and death within a matter of minutes (Allan and Pursell, 1971).

Treatment 3

The results obtained with this treatment demonstrated that it was beneficial in two dogs, and that the recovery time in these cases was rapid (average 28.0 hours). In the other two dogs, death occurred after a prolonged period (41.0 hours). The reason for including this treatment in the study was that many veterinarians advise sedation in tick cases, and they also premedicate animals, especially cats, with an antihistamine before administration of hyperimmune serum to prevent sensitivity reactions to the serum.

Promethazine hydrochloride is a phenothiazine derivative with potent antihistamine activity. In clinical doses it causes sedation and has an anti-emetic action. Its antihistaminic action is derived from a competitive antagonism of histamine and its effects are confined to suppression in varying degrees of symptoms attributable to the pharmacological activity of histamine released by the antigen-

antibody reaction (Goodman and Gilman, 1975). In the dog, Goldberg, Linde, Wolfe, Griswold and Momma (1969) found that promethazine infused into the pulmonary artery caused a significant increase in calculated pulmonary vascular resistance despite a marked increase in cardiac output. This increase in pulmonary vascular resistance was thought to be due to active vasoconstriction. Heart rate increased but stroke volume decreased. Systemic arterial pressure remained relatively unchanged during the first five minutes, but then was significantly elevated between the fifth and tenth minute post-injection.

Observations after the administration of promethazine hydrochloride to dogs with tick paralysis indicated that, whereas it was advantageous in some cases, in others it caused death very quickly (minimum 5.5 hours). Those animals which died quickly developed marked moist râles and pronounced respiratory difficulty. The reason for this difference between animals in their response to promethazine could perhaps depend on the state of the pulmonary circulation at the time of administration and also the degree of pulmonary vasoconstriction caused by the drug. In view of the unpredictable responses to promethazine, it is probably inadvisable to administer this drug to animals with tick paralysis.

Treatment 4

The results obtained using a combination of dexamethasone and hyperimmune serum indicated that it was beneficial in the treatment of dogs at the advanced stage of the disease.

Dexamethasone was selected as a treatment because veterinary practitioners using it have claimed better survival rates in clinical cases. Dexamethasone is the synthetically produced glucocortico-

steroid 9 α -Fluro-16 α -methylprednisolone. It increases gluconeogenesis and inhibits peripheral glucose utilisation, which leads to an increase in tissue stores of glycogen, especially in the liver. Hyperglycaemia and glucosuria occur. It causes catabolism of proteins leading to negative nitrogen balance and increased urinary elimination of nitrogen and uric acid. Dexamethasone is a very potent anti-inflammatory agent. It suppresses the connective tissue response to injury, whether traumatic, anaphylactic or infective. It stabilises lysosomal proteolytic enzymes from escaping to damage surrounding cells. In addition there is an increased capillary tone and selective permeability that diminish plasma exudation into the tissues (Jones, Booth and McDonald, 1977).

The reason for the beneficial result with dexamethasone is difficult to interpret. The biochemical measurements studied in Chapter 3 suggested that glucocorticoids may be released in dogs with tick paralysis. Since dexamethasone is a glucocorticoid, it may augment the effects of these naturally occurring hormones to allow greater survival. Yard and Kadowitz (1972) demonstrated that dexamethasone, unlike hydrocortisone, does not augment the vasoconstrictor responses to adrenaline, and therefore blood pressure would not be expected to increase further.

Treatment 5

The combination of hyperimmune serum and phenoxybenzamine hydrochloride appeared to be highly beneficial at this stage of the disease.

The reason for including phenoxybenzamine in this study was based on the cardiovascular abnormalities demonstrated in Chapter 6. Phenoxybenzamine hydrochloride is a post-synaptic α -adrenergic

blocking agent of the haloalkylamine series. In addition it exerts important effects on catecholamine metabolism. Phenoxybenzamine increases the rate of peripheral noradrenaline turnover, which is associated with increased tyrosine hydroxylase activity. It also inhibits the uptake of catecholamines into both adrenergic nerve terminals and extraneuronal tissues. Its effect on blood pressure is highly dependent on the state of activity of the system on which it acts. Thus the vasodilation induced by phenoxybenzamine may vary markedly in different vascular beds, depending on their degree of adrenergic vasomotor tone, and may vary over a wide range in a single vascular bed, depending on its physiological stage. Transmission of the nerve impulse to the α receptors of the blood vessel cells is prevented and the interaction of circulating adrenaline with α receptors is blocked. If sympathetic vasoconstriction is present, a fall in blood pressure occurs. Phenoxybenzamine produces a considerable progressive decrease in total peripheral resistance, increase in tissue perfusion and increase in cardiac output. A higher percentage of the total blood flow is directed through channels that exchange metabolites effectively with tissue cells, and there is movement of fluid from the interstitial to the vascular compartment. Phenoxybenzamine administration causes a reflex tachycardia, but may inhibit cardiac arrhythmias that involve catecholamines in their genesis. In the presence of phenoxybenzamine the inhibiting response of adrenaline on insulin secretion is blocked and thus glucose uptake is facilitated. Mild to moderate sedation commonly results from a slow intravenous infusion of phenoxybenzamine. The wide variety of responses to catecholamines that are mediated by α -adrenergic receptors are antagonised. Stimulation of the radial fibres of the iris is readily blocked and miosis is a prominent

component of the response to phenoxybenzamine. Blockade develops relatively slowly and the peak effect is not obtained in less than one hour after intravenous administration. The blockade produced by a single dose of phenoxybenzamine disappears with a half-life of roughly 24 hours (Goodman and Gilman, 1975).

After the administration of phenoxybenzamine and hyperimmune serum, a tachycardia was noticed in all cases, whereas no change in heart rate was observed in those dogs treated with hyperimmune serum alone. No arrhythmias other than sinus tachycardia were observed after treatment. Sedation appeared within 5-10 minutes after administration and continued for up to 24 hours. Respiration became easier and less forceful, but there was no change in rate. Miosis of the pupil was observed in all cases treated with this combination but did not occur in those dogs treated with hyperimmune serum alone. This observation indicates that the pupillary dilation seen in dogs with tick paralysis is due to increased adrenergic activity, not reduced cholinergic activity as proposed by Cooper (1976). After recovery, exercise tolerance in this group was considered normal, as they would run and play continuously in an open area, whereas the dog treated with hyperimmune serum alone was either reluctant to run or tired easily.

Treatment of tick paralysis with phenoxybenzamine as well as hyperimmune serum has not been described previously. The use of adrenergic blocking drugs to control the hypertension which occurs in tetanus has been advocated by Prys-Roberts, Corbett, Kerr, Crampton Smith and Spalding (1969). Mitchell and Meilman (1967) used an α -adrenergic blocking drug to demonstrate that the hypertension in polyradiculoneuritis was in fact due to increased amounts of circulating sympathetic amines. In their study, after administra-

tion of this drug, blood pressure fell to 90/0 mmHg and an increase in heart rate occurred.

Although measurement of blood pressure should be carried out in cases of tick paralysis before and during the administration of phenoxybenzamine, this cannot be done in a practice situation. From the information gained in this study and its use in clinical cases, it appears that no detrimental effects, such as profound hypotension, occur when it is administered in the manner outlined here.

Treatment 6

The results obtained using promethazine hydrochloride, dexamethasone and phenoxybenzamine hydrochloride in combination but without hyperimmune serum indicated that death was not prevented. The time to death was increased, although not to the extent found with hyperimmune serum alone. Instead of combining these drugs, it would have been better to study each drug separately. The small number of dogs that became paralysed, however, prevented this. Observations after the administration of this combination indicated that respiration became more dyspnoeic and moist râles more evident after each dose of promethazine hydrochloride. If the dogs were able to clear this fluid, they survived till the next dose; if not, they died quickly. It appears therefore that unless hyperimmune serum is used in the treatment of dogs with advanced signs of tick paralysis, death will occur.

In conclusion it appears that in the treatment of dogs with advanced signs of tick paralysis, all the drugs compared in this study when administered in combination with hyperimmune serum increased the survival rate. The best survival rate was obtained with phenoxybenzamine and in this group all dogs survived. Prometha-

zine gave unpredictable results and therefore it is probably inadvisable to administer this drug to animals with tick paralysis. It was found that at this stage of the disease no dog survived without the use of hyperimmune serum.

CHAPTER 8
GENERAL DISCUSSION

Although tick paralysis is a very common entity in certain areas, little experimental work has been carried out on the disease itself. Cooper (1976) studied in detail the pathophysiology of the paralysis, but there is no published work other than case reports to suggest that the toxin may affect body systems other than the nervous system.

The aim of this study was to undertake a series of experiments to evaluate the effect of the disease on other body systems, in particular the respiratory and cardiovascular systems.

As the literature suggests (Ross, 1926), it was found that onset of the disease from attachment of the ticks to first clinical signs was inconstant and ranged from 5.5 to 9 days. In those dogs which showed earliest clinical signs the progression of the disease was most rapid (minimum six hours). Exercise was noted to hasten the onset of clinical signs. All dogs did not show identical clinical signs, but it appeared that the signs most closely resembled each other in dogs in which the ticks used came from the same batch. This could suggest that more than one toxin is secreted by the tick and that the clinical signs are dependent on the quantities of each of the toxins.

Although Ross (1926) stated that not every female tick caused paralysis, it was quite unexpected to find that, even when four to eight ticks were applied to each animal, only approximately 50% became affected. The ticks on the unaffected dogs engorged normally and dropped off when fully engorged. Although the previous history of the experimental dogs was unknown, it seems unlikely that so many dogs could be immune to four or more ticks. Because of this problem, the difficulty in obtaining a larger number of ticks and the rapidity with which some dogs passed through the different stages, the significance of some of the results is lessened by insufficient numbers.

The results obtained from the various experiments conducted in this study suggest that the disease produced by *Ixodes holocyclus* in the dog has far-ranging effects on many body systems and that death may not be due simply to neuromuscular paralysis, but that central respiratory depression, pulmonary congestion and oedema and central sympathetic overactivity may all contribute to a fatal outcome.

The arterial blood-gas and pH measurements demonstrated that, in spite of early changes in the character of respiration, there was no change in these values until the animal was recumbent and unable to lift its head. At the stage of the disease just prior to death moderate hypoxaemia, with acute ventilation failure, was present. This was probably caused by the progressive fall in respiratory rate, which resulted in a fall in minute respiratory volume at the later stages. This fall in respiratory rate was possibly central in origin and was accompanied by an increase in alveolar-arterial oxygen tension difference from pulmonary congestion and oedema. The "grunting" character of respiration seen in dogs with tick paralysis was due initially to complete closure and lateral partial closure of the vocal cords during expiration and could represent an attempt by the animal to re-expand collapsed parts of the lungs caused by pulmonary congestion and oedema.

The biochemical, electrocardiographic and cardiovascular abnormalities suggest overactivity of the sympathetic nervous system. These changes are brought about by the release of noradrenaline from adrenergic nerve endings and release of adrenaline and noradrenaline into the circulation from the adrenal medulla.

As the sympathetic neurotransmitter, noradrenaline acts predominantly on α receptors and has little effect on β receptors,

except in the heart. Sympathetic nerve activity causes an increase in systolic, diastolic and mean arterial pressure. Usually pulse pressure is increased. Cardiac output is unchanged or decreased and systemic vascular resistance is increased. Peripheral vascular resistance increases in most vascular beds and blood flow through the kidney, brain and liver and usually skeletal muscle is reduced. A marked venoconstriction contributes to the increased resistance. An increase in heart rate is uncommon as the raised arterial pressure causes reflex vagal activity that opposes sympathetic chronotropic stimulation.

As a circulating hormone, adrenaline acts on both α and β receptors. It causes a rise in systolic arterial pressure, but diastolic arterial pressure may rise slightly, remain normal or fall slightly. Mean arterial pressure is not greatly elevated and, therefore, compensatory reflexes do not antagonize appreciably the direct cardiac actions. Heart rate and myocardial contractility are increased due to direct stimulation. The action on blood vessels depends on the activation of α and β receptors, since when both are present stimulation of β receptors causes vasodilation while stimulation of α receptors causes vasoconstriction. The blood vessels of the skin, mucosae and kidneys are constricted, while in skeletal muscle the net response depends on activation of both α and β receptors. The overall effect of maximum activation of both α and β receptors in the skeletal muscle vasculature is vasoconstriction and an increase in peripheral resistance.

The biochemical, electrocardiographic and cardiovascular abnormalities were present at stages of the disease when the arterial oxygen and carbon dioxide tensions were normal and, therefore, hypoxaemia or hypercapnea are unlikely to be the cause of the

sympathetic stimulation. It is possible that this overactivity is similar to that observed in other diseases which affect the nervous system and is due to central autonomic dysfunction.

In view of the cardiac stimulation present, it is surprising that there was no increase in measured contractility. This could indicate that there was a reduction in the inotropic state of the heart (heart failure), but that it was being masked by the large increase in sympathetic tone. This finding is consistent with the pathological changes in the myocardium, liver, kidney and lung, indicating left- and right-sided heart failure.

As the disease progressed the elevated arterial pressure caused reflex vagal stimulation and heart rate tended to fall. Atropine sulphate administered at this stage of the disease resulted in a doubling of arterial pressure, a 300% increase in heart rate and usually death of the animal.

When a number of drugs in combination with hyperimmune serum were compared in the treatment of dogs at an advanced stage of the disease, it was found that phenoxybenzamine hydrochloride, a drug which reverses many of the cardiovascular abnormalities, was the most beneficial. Treatment at this stage with hyperimmune serum alone was of little value, but no dog survived without the use of hyperimmune serum.

The classical description of the disease resulting from tick infestation in the dog is a rapidly ascending motor paralysis. Although Cooper (1976) described in detail the neuromuscular abnormality in this disease, the data from the present experiments show that it is not the prime cause of death and confirm the findings of other workers who have suggested involvement of the cardiovascular and respiratory systems. Death in tick poisoning is the result of

cardiac failure augmented by profound cardiac loading from severe systemic hypertension and poor tissue perfusion, combined with central respiratory depression. The biochemical changes appear to reflect the general systemic response to stress that the disease imposes on the animals.

In view of the progression of the disease after removal of the causative ticks, it appears that an essential component of therapy must always be the administration of hyperimmune serum. The results of combining this with the α -adrenergic blocking agent, phenoxybenzamine hydrochloride, have been striking and are probably attributable to the reduction of hypertension and the associated cardiac unloading, together with a profound improvement in tissue perfusion. The successful application of this combined therapy has proved a satisfying outcome to the investigations described in this thesis.

EPILOGUE

The frustrations and disappointments of studying a disease produced by a tick where the toxin or even toxins have yet to be identified can best be understood by those who have taken up the challenge. The following statement taken from a monograph of all experimental work carried out on *Dermacentor andersoni* (Gregson, 1973), however, expresses this far better than I am able.

"After this widely scattered flurry of work, interest in studies on the effect of *Dermacentor andersoni* on the physiology of various laboratory animals subsided. One physiologist wrote, 'the tick paralysis problem is so complex that I am not quite sure what I am going to do next'. Another had 'run out of ideas', and having tried a number of drugs in an attempt to diminish or intensify paralysis in hamsters, 'had no clues regarding the nature or the mechanism of action of the toxin'. And still another 'was confused by the whole b----- thing' and, after a series of infesting misadventures, admitted that 'life among the ticks had been hell'."

CHAPTER 9

APPENDIX

BREEDING OF TICKS IN THE LABORATORY

To ensure an adequate supply of unfed female ticks for at least five months of the year, artificial rearing of ticks was undertaken in the laboratory (Fig. 9 i).

Source of engorged ticks

The engorged female ticks were obtained from a laboratory (Lismore Serum Products) where commercial canine hyperimmune serum is produced. Up to 30 live engorged ticks were gently packed between two layers of slightly moistened cotton wool inside a 5 cm diameter plastic screw-top container, which was then packed for postage and sent by airmail to Sydney. The ticks generally arrived in excellent condition unless they were delayed for more than four days by the postal service. This source of ticks was available only from October to December.

Conditions for egg-laying

Engorged *Ixodes holocyclus* females were placed on the surface of sterilized moist sand inside gauze-topped glass jars and kept at room temperature. These ticks crawled on the sand for several weeks and usually attempted to dig shallow holes where they laid their eggs. Oviposition normally started in 1-2 weeks and continued for another 3-4 weeks after which the ticks died.

Conditions required for hatching of eggs

There was a variable mortality among the engorged ticks. Any engorged females which died before oviposition were removed immediately. The bodies of remaining ticks were also removed six weeks after being placed in the oviposition jars. Hatching began 3-6 weeks later. During this interval the eggs were examined twice

FIGURE 9 i LABORATORY CULTURE OF IXODES HOLOCYCLUS

LARVAE, NYMPHS AND ADULTS

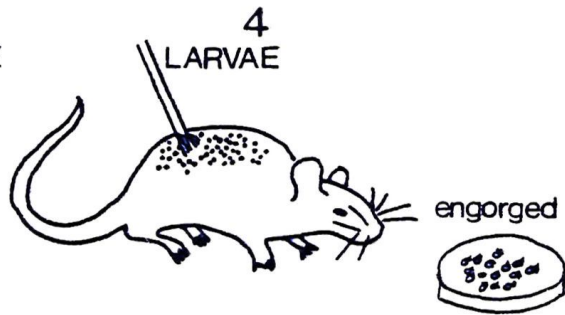
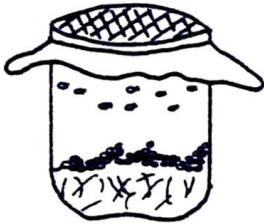
1
ENGORGED FEMALES



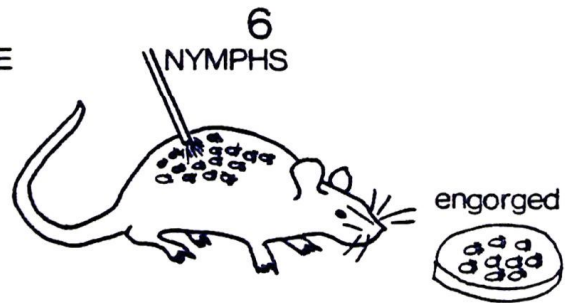
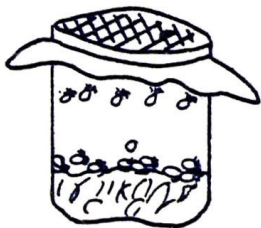
2
OVIPOSITION



3
HATCHING AND STORAGE



5
HATCHING AND STORAGE



7
HATCHING AND STORAGE



weekly for signs of impending hatchment, characterized by a change in the colour to a lighter opaque brown with a visible white spot.

Ixodes holocyclus eggs require virtually 100% relative humidity for their survival and subsequent hatching. Once weekly distilled water was added to the sand to prevent it drying. Larvae hatched in moist sand and slowly moved up the sides of the jars to cluster around the neck or on the gauze lid, where they survived for five months or longer. The larvae were considered ready for attachment when they crawled on the gauze and moved actively if disturbed.

Feeding of larvae

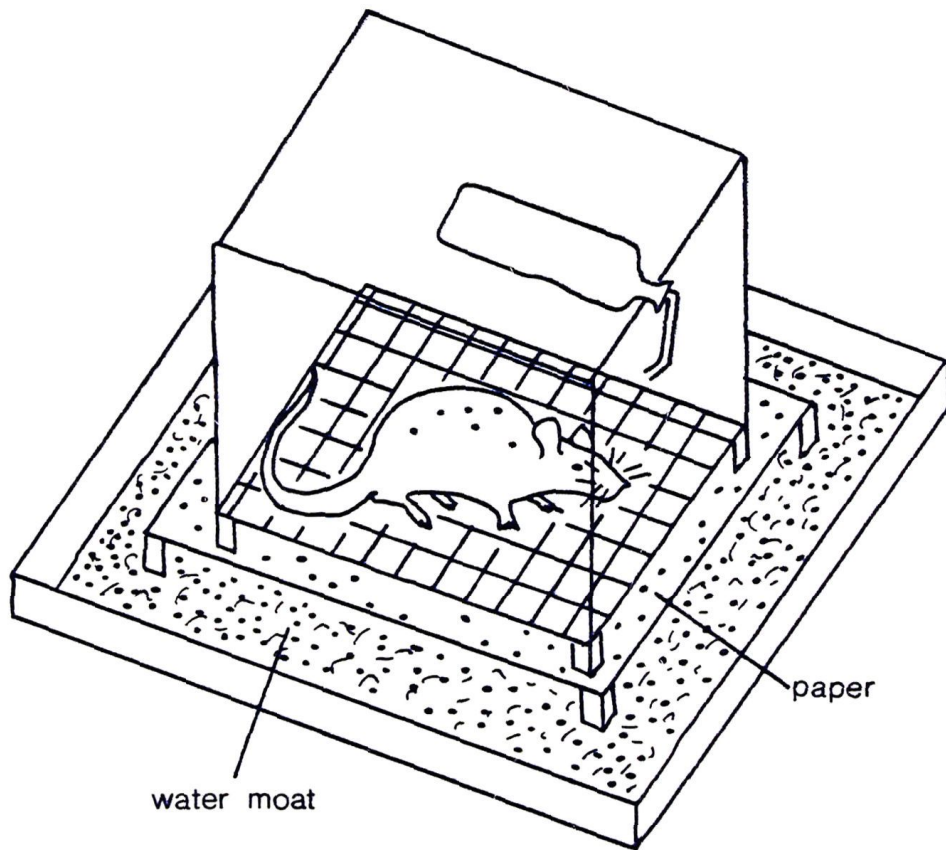
Larvae were fed by allowing them to attach and engorge on white adult rats. About 400-500 larvae were applied, using a paint brush directly to the hair along the backline of each rat. Any larvae which remained on the gauze were removed by wiping the fabric against the hair. After 5-10 minutes the larvae had penetrated the hair coat and begun feeding.

Sixty-five hours after application of the larvae the rats were placed in a wire cage which was kept 3 cm above a tray covered with blotting paper. The whole apparatus was suspended in a large plastic tray containing water to a depth of about 1 cm so as to form a moat (Fig. 9 ii). They were kept in the cage for two days.

During this period the engorged larvae detached and fell onto the blotting paper where they crawled to the water and were immobilized. They were collected by suction using a sawn-off Pasteur pipette attached to a pump and conveyed to a flask. Collection of hair and faeces was avoided to reduce contamination. The ticks were washed with water, sieved through gauze fabric and dried on filter paper in a petri dish.

FIGURE 9 ii
LABORATORY CULTURE OF IXODES HOLOCYCLUS

CAGE FOR COLLECTION OF ENGORGED TICKS



Emergence, storage and feeding of nymphs

The engorged larvae were placed on moistened sand in sterilized glass jars at room temperature. After four weeks the nymphs emerged from the moult. Excessive moisture prevents development of the moult and fungal growth kills the ticks. It was difficult to avoid excessive moisture on the sand, but with care the nymphs remained viable for up to four months.

Nymphs were considered ready for attachment to rats when they clustered on the gauze lids and moved actively if disturbed.

Approximately 50 nymphs were painted along the back of each rat. Rapid penetration was observed and the engorged nymphs detached in 72-120 hours. The method of collection was similar to that used for the larvae, except that the engorged nymphs were harvested with a small spoon rather than by suction.

Emergence, storage and feeding of adults

The engorged nymphs were placed on moist sand for the moulting period of 3-5 weeks. As the adults emerged they were placed in new jars under the same conditions.

Long-term storage under these simulated natural conditions at 22-25°C allowed survival for over six months. Adult males, recognized by their smaller size and shorter mouthparts, quickly began coupling with females. The adult females were considered ready for attachment when they clustered on the gauze lids and were attracted to a finger drawn across the gauze.

Six unfed laboratory-reared ticks were placed on each of ten dogs, but signs of paralysis were not observed after three weeks and the animals discarded. Another batch of ticks was tried together with those artificially reared under similar conditions in Commonwealth

Scientific and Industrial Research Organization (CSIRO) and the Department of Veterinary Pathology. Again no sign of the disease was observed.

Due to the inability to produce the disease with artificially reared ticks, it was decided to use wild-caught unfed females in all experiments. The author did not undertake any experiments to determine if the laboratory-reared ticks did, in fact, secrete a toxin, although of decreased potency. It seems likely that this was the case, as during the breeding program it was observed that, if excessive numbers of larvae of nymphs were placed on the rats, paralysis and death occurred.

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