SOME OBSERVATIONS ON TICK PARALYSIS IN MARMOTS

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Infestation with certain species of ticks gives rise in a number of animals to a paralysis which if unrelieved may end in death. The effect of such parasites is felt particularly in the ranching areas of South Africa and along the east coast of Australia, where sheep and dogs respectively are principally affected, and in cattle ranches west of the Rocky Mountains in Canada and the north-western United States. In this last area the condition is produced by the bite of the ixodid tick *Dermacentor andersoni* Stiles (Gregson, 1953).

The attachment of a single feeding female *D. andersoni* may also bring about a paralysis in man which again can have a fatal result if the causative agent is not recognized. Removal of the tick results in a dramatic reversal of the paralysis with no apparent after effects. The paralysis is of a flaccid nature, and exhibits a general ascending character of the Landry type in that the hind limbs are affected before the fore. As will be noted below, however, it seems that a rigorous sequence of development is not strictly followed. In addition to dogs, sheep, cattle and man *D. andersoni* affects guinea-pigs, hamsters and certain of the wild marmots and ground squirrels indigenous to British Columbia. Cats, rabbits, rats and mice show no symptoms in spite of the prolonged attachment of numbers of ticks.

Data on the nature of the defect resulting in the paralysis are meagre. Rose & Gregson (1956) showed that a peripheral neuromuscular block appeared to be present since electrical stimuli applied through motor nerves failed to give contractions while direct stimulation of the muscles was effective. This finding was confirmed in dogs by Murnaghan (1958*a*) and in marmots by Emmons & McLennan (1959). The latter authors showed also that the neuromuscular block was associated with a diminished release of acetylcholine. Murnaghan (1958*b*) originally reported that conduction in the motor fibres during the paralysis was normal, but recently has observed that in fact there is a failure of conduction in the motor nerves (Murnaghan, 1960).

Information regarding the causative agent produced by the feeding ticks is even less available. It has been established that the paralysis is not due to a viral infection (see, for example, Stanbury & Huyck, 1945) and that it is not an anaphylactic reaction seems likely since there is neither increased sensitivity nor immunity conferred by one exposure to the ticks towards subsequent infestations. The most probable explanation is that the tick secretes into or produces within its host a toxin which causes the paralysis, but that this material must be continually

administered since the symptoms may be fairly rapidly reversed on removal of the feeding ticks.

The investigation reported herein was undertaken as an extension of our earlier work concerning the failure of acetylcholine production in paralysed limbs brought about by motor nerve stimulation. We have now shown that the capacity of excised tissue from paralysed animals to synthesize acetylcholine is unaffected, and that there is a depressed conduction in peripheral motor and sensory fibres, in heart muscle and of transmission processes in the central nervous system. We suggest that the toxin produced by the ticks causes a generalized depression of excitability in all excitable structures.

MATERIALS AND METHODS

We have used marmots (*Marmota flaviventris avara* (Bangs)) as experimental animals. The ticks employed were collected in the vicinity of Kamloops, B.C., during the months of April and May, and were kept cool (*c.* 8° C.) until used.

Since it has been shown that the production of paralysis is dependent on the length of time which the ticks have been feeding (Gregson, 1958), they were fed for 5 or 6 days on sheep or rabbits before being transferred to the experimental animals. In this way the period of observation and tick protection was kept to a minimum, for two to six pre-fed female ticks produced paralysis with respiratory involvement in 24–36 hr. The ticks were confined to the marmots by capsules held to the abdomen by a band of adhesive tape, and protected further by placing each animal in a narrow cylinder of metal mesh.

Records of electrical activity from peripheral nerve were made with the aid of electrodes applied under light general anaesthesia. These were formed of dental cement with four silver wires projecting 0·5 mm., or small silver plates (0·2 × 2 mm.) on the surface of the plastic. The desired nerve was exposed, the perineurium removed, and a small bit of polythene tubing slit longitudinally passed around it. The electrode assembly was fitted within the tubing in close contact with the nerve and the muscle and skin was sewed over it. The assembly remained in place for at least 24 hr., as judged by the unchanged responses in normal animals. One pair of electrodes was connected to a square wave stimulator, the other to an amplifier and oscilloscope. Simultaneous electromyographic records were obtained with a concentric needle electrode inserted into the belly of the muscle. Usually the electrode assembly was applied to the main trunk of the sciatic nerve and the muscle electrode to the gastrocnemius. Records were obtained photographically. The electrocardiogram was recorded with a conventional instrument (Sanborn 'Visocardiette') with lead 2.

Acetylcholine synthesis by excised tissues was measured by the method described by McLennan & Elliott (1950). The main trunks of the sciatic nerves were incubated whole; transverse slices (1 mm. thick) of the spinal cord in the region of the lumbar enlargement and 1 mm. thick slices of cerebral cortex were also used. The tissues were incubated for 2 hr. at 37° C. in a bicarbonate-buffered medium equilibrated with 95% O_2–5% CO_2, and in the presence of eserine as an inhibitor of cholin-

esterase. The amount of acetylcholine produced by the tissue and liberated into the medium ('free') and that fixed in the tissue ('bound') were separately determined. Assays were performed by observing the depression of blood pressure of cats or rats.

RESULTS

Gross symptoms of the paralysis. The first sign of the developing paralysis in marmots was a loss of the animal's normal piercing cry, which was replaced by a hoarse grunt and appeared to indicate paralysis of the vocal cords. This was rapidly followed by an ataxia and weakness in the hind limbs, and the animal ceased eating. Thereafter the condition progressed to involve the fore limbs until the animal was unable to move and lay on its side. It was noteworthy, however, that even at this advanced stage of paralysis movement and tone in the tail persisted, while small movements of the neck muscles continued after respiratory distress was apparent. Voluntary eye movements were unaffected. There was no loss of consciousness, although it appeared that a considerable sensory deficit was present. Body temperature was maintained until the paralysis was well advanced, when it often fell abruptly (to *c.* 29° C.); throughout, however, the skin felt unusually cold and dry. There was retention of urine and faeces.

Following removal of the ticks from a marmot in which the muscles of respiration had been involved, there was often a continued deterioration in the condition of the animal with death the result. In those cases where complete recovery did take place it was slow and required considerable care of the animals (warmth, assisted respiration and feeding by stomach tube). In this respect marmots differ from man, dogs and other animals affected, in whom recovery is normally rapid and complete following removal of the ticks. Subsequent infestations of the same animals resulted in precise repetitions of the above sequence of events, and there was no change either in severity or in the time course of development of the condition.

Table 1. *Acetylcholine synthesis* in vitro *by tissues from normal and paralysed marmots*

	Acetylcholine (μg./g.) at end of 2 hr. incubation					
	Sciatic nerve		Spinal cord		Cerebral cortex	
	Free	Bound	Free	Bound	Free	Bound
Normal	1·1	3·9	0·8	3·7	2·0	3·1
	0·7	3·6	1·0	3·8	1·4	3·0
	1·3	3·3	1·6	3·2	1·6	2·6
	—	4·8	1·8	3·0	1·3	2·9
Paralysed	1·5	6·0	1·9	4·7	3·4	—
	—	2·9	3·9	3·4	2·7	3·0
	1·4	4·6	3·3	—	0·8	2·3
	—	6·5	3·7	—	0·8	2·4

Acetylcholine synthesis in vitro. Table 1 sets out the results which were obtained when a comparison was made of the ability of peripheral nerve, spinal cord and cerebral cortex from normal and paralysed marmots to synthesize acetylcholine *in vitro*. It is evident that no diminished synthetic ability on the part of the tissues

from paralysed animals was found. The diminished output of acetylcholine following motor nerve stimulation in perfused limbs (Emmons & McLennan, 1959) is therefore not due to reduced synthesis of the substance.

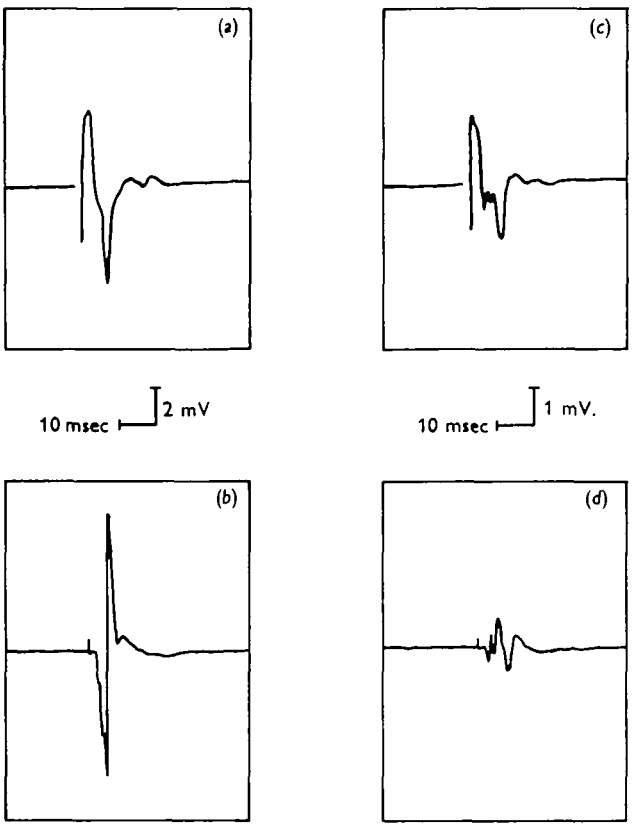

Fig. 1. Electrical responses of the sciatic nerve and gastrocnemius muscle elicited by maximal sciatic nerve stimulation, in a marmot before and after partial development of tick paralysis. Control (*a*) nerve and (*b*) muscle responses. Stimulus 7 V., 0·1 msec. Paralysed (*c*) nerve and (*d*) muscle responses. Stimulus 8 V., 0·1 msec.

Changes in conduction in peripheral nerve. Observations of the electrical responses recorded from the sciatic nerve when that nerve was stimulated, showed that marked changes had occurred in the paralysed state. The amplitude of all components of the compound action potential was reduced, which would indicate that conduction in both sensory and motor pathways was affected indiscriminately. Concomitantly there was a reduction in amplitude of the electromyographic response in the gastrocnemius muscle. These changes are illustrated in Fig. 1 by the responses of nerve and muscle before and after the development of partial paralysis in the same animal. In more severely affected cases the nerve and muscle responses were still further reduced in comparison with controls.

That conduction in both motor and sensory fibres was in fact depressed was demonstrated in acute experiments where the spinal roots were exposed. It has not been possible to compare the responses obtained in the same animal before and after paralysis had developed; nevertheless, the action potentials recorded in

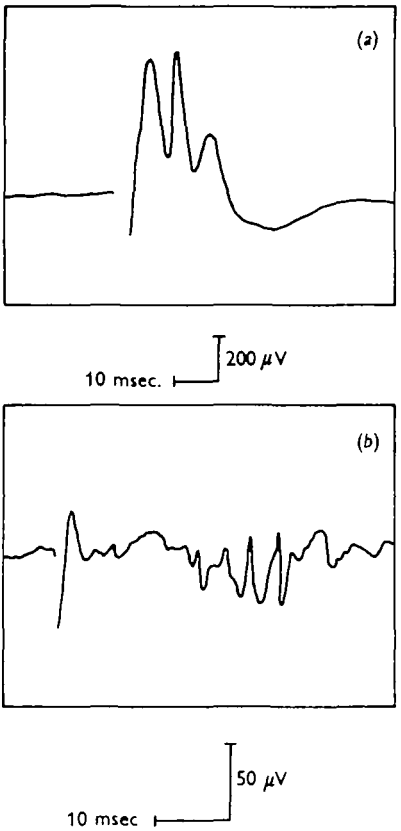

Fig. 2. Maximal reflex responses in marmots, obtained by stimulating a sensory root and recording from a motor root. (*a*) Normal, stimulus 8 V., 0·1 msec. (*b*) Paralysed, stimulus 10 V., 0·2 msec.

the sensory roots following sciatic nerve stimulation and those in the sciatic nerve as a result of motor root stimulation were invariably found to be much smaller than those obtained in control animals.

In view of this depressed conduction in both motor and sensory fibres it is somewhat difficult to assess the significance of the reduced reflex response recorded in motor roots upon stimulation of sensory roots, for it might be expected that the activity in the reflex arc would be small for this reason alone. However, almost no monosynaptic reflex response could be obtained at a time when there was still some conduction in both groups of root fibres (Fig. 2), and it would seem likely that the processes of transmission at the neurones of the spinal cord are affected during the paralysis as well as those of conduction in axons.

Effects on the heart. Changes in the electrocardiogram which we observed in paralysed animals fall into two groups. The first showed a sinus tachycardia in which the heart rate rose from its normal level of *c.* 190 per min. to *c.* 260 per min. The rhythm was regular and the intervals which have been measured were only

Table 2. *Electrocardiographic changes during tick paralysis in marmots*

	Average heart rate (beats/min.)	Intervals (sec)		
		P–R	*QRS*	*Q–T*
Normal	160	0·05	0·05	0·12
	170	0·07	0·03	0·11
	190	0·06	0·03	0·12
	220	0·05	0·04	0·12
	215	0·06	0·03	0·12
Average	190	0·06	0·04	0·12
Paralysed (tachycardia)	250	0·04	0·04	0·11
	250	0·06	0·05	0·09
	290	0·05	0·04	0·10
	260	0·06	0·04	0·12
Average	260	0·05	0·04	0·11
Paralysed (bradycardia and arrhythmia)	40	0·09	0·06	0·22
	90	0·10	0·06	0·27
	38	0·10	0·06	0·50
	75	0·09	0·05	0·26
	85	0·06	0·03	0·22
	85	0·06	0·04	0·26
	73	0·07	0·04	0·16
Average	70	0·08	0·05	0·27

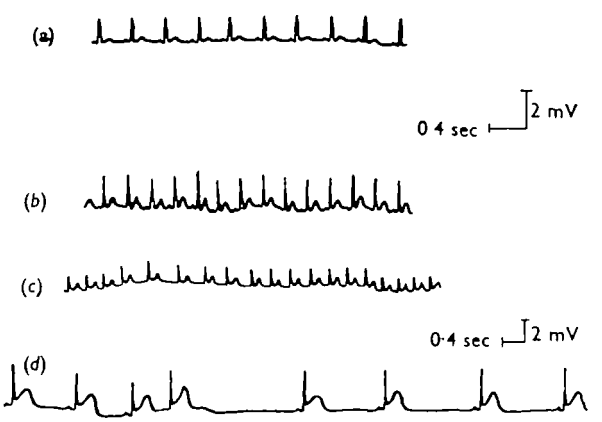

Fig. 3. Electrocardiographic changes during development of tick paralysis in marmots. (*a*) Normal; (*b*) tachycardia; (*c*) slight arrhythmia and bradycardia, intervals essentially unchanged; (*d*) severe arrhythmia and bradycardia, intervals prolonged and average heart rate very low. Note change in amplification in (*a*) and (*b*) compared with (*c*) and (*d*).

slightly below the normal (Table 2). The second group showed a pronounced bradycardia with some degree of sinus arrhythmia. The average rate fell to as low as 35–40 per min., with which was associated a much prolonged *Q–T* interval. The *P–R* interval was also somewhat extended in these cases (Table 2). Examples of these changes are shown in Fig. 3.

There were some indications that those animals showing arrhythmia were in a more advanced stage of the condition than those with tachycardia. It was noted above that the final stages of the paralysis were marked by a fall in body temperature and it was found that the deep temperature of all animals showing marked bradycardia was low, whereas those with tachycardia had a normal temperature.

DISCUSSION

Earlier work on the aetiology of the paralysis produced by the continued attachment of *D. andersoni* to experimental animals revealed that there was marked failure of neuromuscular transmission (Rose & Gregson, 1956; Murnaghan, 1958*a*) and that this was associated with a diminished release of the normal transmitter at the junctions (Emmons & McLennan, 1959). Murnaghan (1958*a*) deduced that the block was localized at the pre-junctional endings. The present experiments have demonstrated that there is an unimpaired ability of the tissues in paralysed animals to synthesize acetylcholine, from which it may be concluded that the absence of acetylcholine in the perfusate following stimulation may be due to a depressed release of the transmitter or to a failure of motor nerve conduction.

Other experiments described here show that there is in fact a diminished conduction in the spinal motor roots and hence in the peripheral motor nerve fibres. This finding adequately accounts for the flaccid motor paralysis which is the most pronounced symptom of the condition.

We have also shown that in marmots there is a loss of conduction in sensory fibres. This is borne out by the gross observation that paralysed animals are much less responsive to a painful stimulus than normals. Reports of the condition in humans on this point are far from consistent. Where changes in sensation have been specifically looked for or reported, the majority of cases are said to show none; however, numbness, paraesthesia and even complete sensory anaesthesia have been reported in a few instances (see, for example, Mail & Gregson, 1938; Stanbury & Huyck, 1945). In most human cases the condition does not progress to the extent usual in our experimental animals, and it may be that in these cases subjective sensory loss occurs later than does the motor defect and is not marked at the time of removal of the ticks. We have no experimental evidence to decide whether this is a true explanation or not.

The changes in the electrocardiogram observed during paralysis are not easy to interpret. Sinus tachycardia may occur in many toxic conditions. There is no indication of a defective conduction of impulses in the heart muscle at this stage. The sinus arrhythmia with bradycardia which develops later, with which is associated lengthened P–R and Q–T intervals, would indicate a depressed conduction and a slower rate of auricular and ventricular depolarization and repolarization. These results, then, are not inconsistent with the depressed conduction found in peripheral nerve. It should be noted that the defective circulation in late stages of the paralysis cannot be the cause of the condition, for all animals show complete paralysis of all four limbs before arrhythmia develops. The effects on the

heart therefore represent another manifestation of the widespread action of the toxin.

It is not possible on the basis of our experiments to decide absolutely whether the spinal neurones involved in the monosynaptic reflex arc are also depressed, or whether the areflexia is to be attributed entirely to the reduced conduction in the sensory and motor roots. The former would seem to be the case in instances like that illustrated in Fig. 2. It is reasonable by analogy with our other findings that a slower rate of depolarization of the neuronal soma and a longer absolute refractory period might be present, and that these contribute to the absence of activity in the reflex arc. Future experiments will be directed towards establishing this point.

In conclusion, the results which we have described show that the toxin produced by feeding ticks affects more structures than those of the motor pathways, and that all such structures show a lowered excitability. The effects of the heart indicate that the rates of depolarization and repolarization are slowed. It is possible that this may be the underlying cause of all observed changes and that all excitable tissues are affected to some extent.

SUMMARY

1. Observations have been made on marmots paralysed by the attachment of the ixodid tick *Dermacentor andersoni* Stiles.

2. Acetylcholine synthesis by excised tissues from paralysed animals is unaffected.

3. Conduction in both motor and sensory nerve fibres is markedly reduced. It is likely also that the excitability of neurones of the spinal cord is diminished.

4. There are changes in the electrocardiogram suggestive of a slowed rate of auricular and ventricular depolarization and repolarization.

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