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BLOCK OF CONDUCTION IN MAMMALIAN MYELINATED NERVE FIBRES BY LOW TEMPERATURES

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Low temperatures are used extensively for blocking various respiratory and cardiovascular reflexes on the presumption that cold must have a differential effect on the nerve fibres of different diameters. However, there is no unanimity of opinion regarding the nature of this differential effect (Paintal, 1963).

One group of studies, in which the compound action potential has been used as a guide, has indicated that the smaller myelinated fibres are blocked at a higher temperature than that needed to block conduction in the larger fibres (Dodt, 1953; Douglas & Malcolm, 1955). On the other hand, Torrance & Whitteridge (1948), Whitteridge (1948), and Widdicombe (1954), who recorded impulses in individual fibres, have arrived at the opposite conclusion. Some support for the latter conclusion was obtained recently by comparing the distribution of the blocking temperatures of pulmonary afferent fibres with the distribution of their conduction velocities (cf. Fig. 8 in Paintal, 1963). Experiments were begun with the expectation that this conclusion would be easily confirmed but it turns out, as shown in this paper, that there is no relation between the conduction velocities of myelinated nerve fibres and their blocking temperatures. In the following paper, the results of a systematic study of the effects of different temperatures on conduction in nerve fibres will be presented (Paintal, 1965).

METHODS

Experiments were done on twenty-six adult cats anaesthetized with chloralose (75 mg/kg). Filaments from the vagus, the saphenous, and the cervical sympathetic nerves were dissected, and the conduction velocities of individual nerve fibres determined, as described before (Paintal, 1953*b*). While recording from vagal afferent fibres, the e.c.g. (lead II) and intratracheal pressure were recorded simultaneously.

Methods of cooling the nerve

In the first four experiments a conventional type of thermode in which the vagus rested at the bottom of a silver-foil trough, 13 mm long, was used. The pattern of this thermode

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was similar to that used by Partridge (1939) and subsequently by Torrance & Whitteridge (1948) and Whitteridge (1948). It was placed between the stimulating electrodes low in the neck and the recording electrodes near the nodose ganglion. Chilled brine or alcohol was run through the thermode. The temperature of the vagus was recorded with a thermocouple that lay at the bottom of the groove in the thermode, i.e. between the thermode and the vagus. The thermocouple was connected to a suitable needle galvanometer. The reference junction was fixed to the bulb of a mercury thermometer that was immersed in water at a suitable temperature, mostly round about 10–15° C contained in a vacuum flask, fixed inside another vacuum flask also containing water at about the same temperature, so as to ensure that the temperature inside the inner flask rose at a very slow rate, e.g. less than 0·5° C/hr. The calibration of the thermocouple which remained constant was checked periodically against a mercury thermometer.

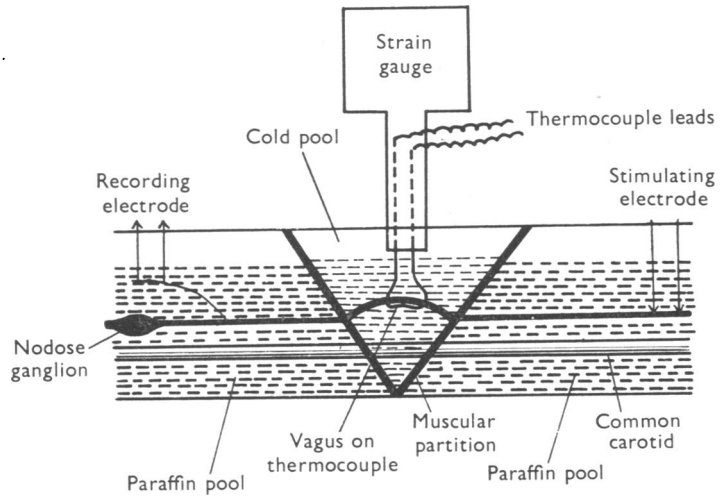

Fig. 1. Diagram showing experimental arrangement for immersion cooling of the vagus and studying the effects of various temperatures on responses of nerve fibres.

The chief source of error in this method is the possibility that nerve fibres near the exposed surface of the thermode and not in contact with it may be at a different temperature from those in direct contact with the thermode. This source of error, which could not be measured accurately, had to be eliminated as far as possible, and so a method of cooling, in which the nerve was bathed in the coolant (Ringer's solution or 0·9 % NaCl solution) itself was devised (Fig. 1).

Immersion cooling. In this method the nerve is bathed in the coolant which surrounds all parts of the nerve. The nerve was lifted away from the surrounding tissues by the thermocouple (Fig. 1) and supported on it. The cold pool was formed by making use of skin, muscle and connective tissues. In the case of the vagus and cervical sympathetic the pool, which was triangular in shape, was bounded by the oesophagus, the trachea and the sternothyroid muscle medially; the two sides of the triangle consisted of the sternomastoid muscle which was pulled away with a ligature and some connective tissue (Fig. 1). The bottom of the pool was formed by the common carotid artery, other muscles, and connective tissue. The width of the pool at its widest portion varied from 22 to 30 mm in different experiments and its capacity was about 1 ml. This pool which was in between two paraffin pools was surprisingly watertight, because the coolant did not leak into either of the paraffin pools.

In the case of the saphenous nerve the cold pool was formed only by skin and connective tissue. It was about 30 mm wide and roughly quadrilateral in shape. The stimulating and

recording electrodes were applied in two paraffin pools, very similar to those shown in Fig. 1 for the vagus and sympathetic.

The coolant, which consisted either of Ringer's solution or 0·9 % NaCl solution, was kept at about 0° C by surrounding the bottle containing it with ice. The Ringer's solution contained NaCl, 0·9 %; $CaCl_2$, 0·025 %; KCl, 0·045 % and $NaHCO_3$, 0·015 %. The rate of flow of coolant was regulated by a stop-cock so as to ensure a particular temperature of the cold pool. The excess coolant was continuously aspirated. By this means it was possible to keep the temperature of the nerve constant at any desired level for any length of time.

The length of nerve cooled, which was 9–13 mm in nineteen experiments, and 16 mm in two experiments, was measured with a pair of calipers. The measurement was made with the nerve *in situ* on the thermocouple by measuring off the length of nerve that was separate from the surrounding tissue and which was clearly bathed in the coolant. The errors arising from this procedure, which are discussed in the accompanying paper, do not affect the results presented in this paper.

The temperature of the thermocouple, and therefore of the nerve, was read off directly from galvanometers. In the first seventeen experiments the temperature could be read accurately to 0·5° C; in the last nine to 0·1° C. The galvanometers and thermocouples were accurately calibrated from time to time, most often at the end of each experiment.

In some experiments in which the nerve was stretched, the tension exerted by the thermocouple on the nerve was measured by fixing the thermocouple to the end of a strain gauge (Fig. 1). The strain gauge was calibrated by using gram weights. No attempts were made to measure the precise amount of tension exerted on the nerve since the aim was only to determine the qualitative effects of tension on the blocking temperature of the nerve fibres. The amount of pressure exerted on the nerve by a glass rod was measured in a similar manner.

The compound action potential of the saphenous nerve was recorded in a conventional way in all four experiments on this nerve. In two of these it was recorded simultaneously with the recording of impulses in individual fibres (Figs. 8 and 9). This was achieved by dividing the saphenous nerve near the recording electrodes into two parts, one for recording the compound action potential and the other for providing filaments for recording unitary activity simultaneously. The conduction velocities of individual fibres were determined as described earlier (Paintal, 1953*b*). It was proved that slowly conducted potentials were not due to repetitive firing in faster conducting fibres. The method for doing this has been described previously (Paintal, 1960).

RESULTS

Preliminary experiments with the thermode

The experiments were begun with the aim of finding out if there was any relation between the conduction velocities of vagal afferent fibres and the temperature at which conduction was blocked in them. Initially, therefore, afferent fibres were identified by their natural pattern of discharge, their conduction velocities were determined, and the temperature at which conduction was blocked was established by noting the temperature at which all impulses produced by natural activity were blocked. Altogether seventeen fibres obtained from four cats were examined, thirteen from pulmonary stretch receptors, two from aortic baroreceptors and two from atrial receptors.

The blocking temperatures of these fibres which ranged from 5 to 15° C are plotted in Fig. 2 which also shows that all the five fibres with blocking

temperatures greater than 13° C were from pulmonary stretch receptors. The results obtained are therefore similar to those obtained by others using a similar technique (Torrance & Whitteridge, 1948; Whitteridge, 1948; Widdicombe, 1954). If all the points are taken together, Fig. 2 gives the impression that faster fibres are blocked at higher temperatures. On the other hand, if the results of individual experiments are considered, then this is true in only one experiment (open circles); in the others there is no clear relation between conduction velocity and blocking temperature.

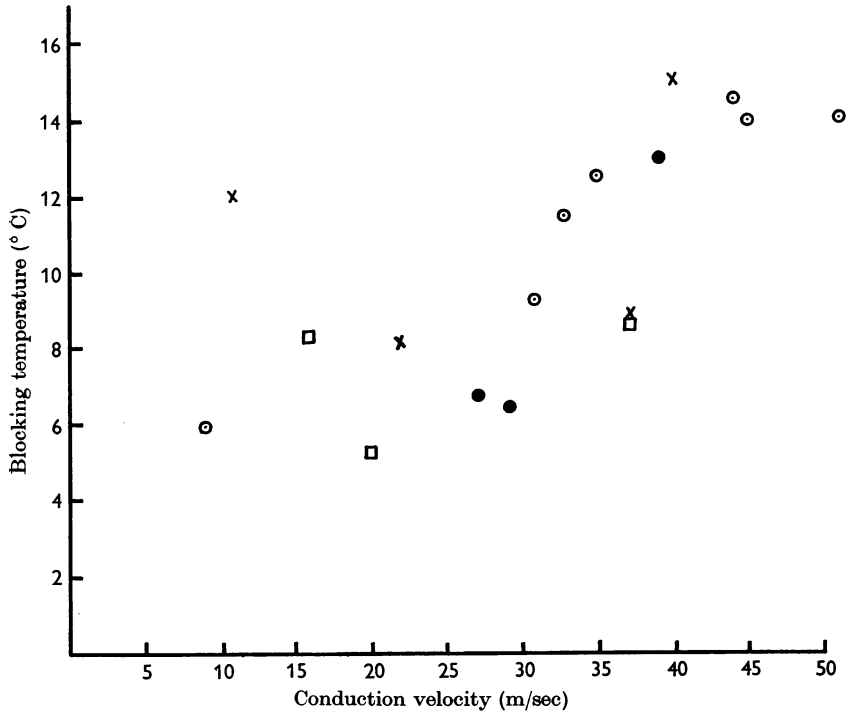

Fig. 2. Graph showing temperatures at which naturally produced impulses were blocked in vagal afferent fibres when the temperature of the vagus was lowered with a thermode. Results obtained from each of four cats are depicted differently. All the five fibres with blocking temperatures greater than 13° C were from pulmonary stretch receptors.

From the above, it may be concluded that, using natural discharges as an index of activity, there is a poorly defined relation between the conduction velocity of afferent fibres and their blocking temperatures, and that the discharges of faster conducting fibres tend to be blocked at higher temperatures in some experiments. However, as shown below, natural discharges always give an over-estimate of the actual blocking temperatures of the fibres. For this reason, the actual blocking temperatures have

been determined by using an electrically evoked response as an index of activity in the fibres in all the remaining twenty-two experiments described below. In these experiments the nerve was cooled by bathing it in the coolant, so as to ensure that all parts of the nerve were at the same temperature.

Factors influencing blocking temperature

Definition of blocking temperature. Using the experimental arrangement shown in Fig. 1, it was found that on cooling the nerve locally, the latency of an electrically evoked response in a normally silent fibre increased progressively. At first, it increased very slowly and finally rapidly, just before conduction in the fibre was blocked (Fig. 4*A–C* and Fig. 8). For any particular temperature, the increase in latency was much greater in the slowly conducting fibres than in the faster conducting ones, provided the blocking temperatures of the two fibres was the same or that of the slower one was higher (compare response of fibres 1 and 2 in Fig. 8).

When the fibre was cooled to near its blocking temperatures, the responses in it appeared in only some of the trials (Fig. 4*C* and *H*). At this stage, the number of times that a response appeared relative to the number of trials depended on the repetition rate of the stimulus; the higher the rate, the less frequent the number of responses relative to the trials. Finally, a stage was reached when only an occasional response appeared, even though the repetition rate of the stimulus was low, say 0·5/sec (Fig. 4*C* and *H*). This, then, was the closest approximation to the actual blocking temperature of the fibre, and for the purposes of this paper the actual blocking temperature would be taken to be 0·1° C less than this temperature, i.e. 9·6° C for the fibre of Fig. 4*C*, and 4·7° C for the fibre of Fig. 4*H*. Therefore, in the rest of this paper and in the following one (Paintal, 1965), the blocking temperature of a nerve fibre is defined as being 0·1° C less than the lowest temperature at which the fibre is able to conduct an electrically evoked impulse (repeated at about 0·5/sec) in the absence of any other discharge of impulses.

Effect of natural trains of impulses. If the natural train of impulses is short lived and there are long enough gaps of silence between trains, then the electrically evoked responses at various temperatures are unaffected if they are elicited during the natural silent periods. Thus, in the case of some pulmonary stretch fibres that are active only during inspiration, the evoked response is unaffected when it is elicited during expiration, especially if the silent gaps between the trains of impulses last about 2–4 sec. This will ensure that enough time elapses after a train before the evoked response is elicited. The blocking temperature obtained under these circumstances will then approach the true blocking temperature.

The same result will be obtained if one is guided by the first impulse of each train.

On the other hand, if the evoked response is elicited during the period of natural activity, then the latency is much increased (e.g. in third sweep of Fig. 3*A*), and at lower temperatures the response may be altogether abolished (Fig. 3*B*). This is due to the incomplete recovery of the fibre (cf. Paintal, 1965). This is what happens in the case of fibres with a con-

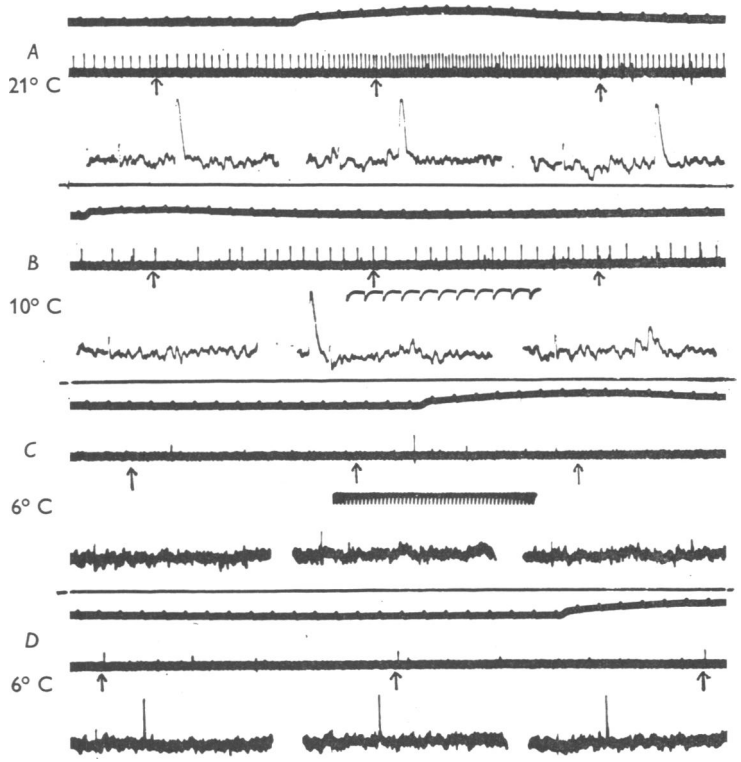

Fig. 3. Effect of lowering the temperature on the evoked responses in a pulmonary stretch fibre. The third sweep in *A* shows that the latency is prolonged owing to relative refractoriness. At 10° C in *B* the evoked responses are absent although some naturally produced impulses still pass through the cooled nerve. At 6° C in *C* there are no natural or evoked impulses. The single impulse in the middle of the trace is from another fibre. *D* shows the reappearance of evoked responses at the same temperature after cutting the vagus distal to the stimulating electrodes. The sweeps and the continuous record in *C* therefore show that block of natural impulses is not a reliable guide to the actual blocking temperature of the fibre. From above downwards in each record, 0·1 sec time marks superimposed on trace of intratracheal pressure; impulses in a slowly adapting pulmonary stretch fibre and sweeps of evoked responses recorded simultaneously. Msec time marks in *B* apply to sweeps in both *A* and *B*; those in *C* to sweeps in *C* and *D*. Arrows indicate position of stimulus artifacts in the continuous records.

tinuous discharge with or without superimposed rhythms, e.g. slowly adapting pulmonary stretch receptors with very low threshold to inflation (Fig. 3). In such fibres it has been frequently noted under suitable conditions that the natural discharge (and also evoked responses) is completely abolished (Fig. 3*C*) although the nerve fibre can still conduct electrically evoked impulses at this temperature if the natural discharge is eliminated by some means, e.g. cutting the vagus as in Fig. 3*D*. In these fibres it is therefore impossible to determine with certainty the true blocking temperature, unless the natural discharge is either blocked by crushing or cutting the nerve distal to the stimulating electrodes (Fig. 3*D*), or is made periodic by suitable manoeuvres.

For the same reason, it is not possible to determine the correct blocking temperature of afferent fibres with a cardiac rhythm. However, an approximation may be possible, if the heart rate is slow and there is only a short burst of impulses as in type A atrial receptors, so that an evoked response elicited before the atrial systolic burst of impulses can be used as a guide to the presence of conduction in the nerve fibre. The same information can be obtained by using the first impulse of each burst as a guide, but, in either case, the blocking temperature obtained even under these conditions is a little higher than the actual blocking temperature of the fibres.

Effect of local pressure, tension or asphyxia. Nerve fibres may be blocked at higher temperatures if any pressure or tension is applied on the cooled nerve (Fig. 4). An increase of 2° C is easily produced by light tension (e.g. 4*G*) and the increase may be even 10° C if considerable tension of say 50–100 g is put on the nerve. Marked tension will produce block at 37° C (Gray & Ritchie, 1954). The application of direct pressure on the nerve produces the same effect. Such effects were observed in ten fibres with conduction velocities ranging from 11 to 57 m/sec. Figure 4*I* shows that stretching the nerve on the thermocouple, as shown in Fig. 1, so as to register a tension of 16 g for 5 sec on the strain gauge, raised the blocking temperature of a fibre with a conduction velocity of 17 m/sec by 3·5° C. Similarly, application of 20 g tension in the case of a faster fibre with a conduction velocity of 39 m/sec raised the blocking temperature by 4·3° C (Fig. 4*F*). Fast and slow fibres are therefore affected similarly. The time taken for the blocking temperature to return to normal varied in different fibres; this was greater with application of greater tension. In the case of the fibre shown in Fig. 4*I*, the blocking temperature was still 7·4° C 21 min after the application of the tension, i.e. it was still raised by 2·7° C.

Asphyxia had no effect on the blocking temperature. Thus, switching off the respiratory pump leading to cardiac standstill did not raise the blocking temperature of nerve fibres. As shown in Fig. 4*J*, every stimulus yielded a response at 6·3° C, 23 min after such asphyxia. These results also

indicate that, since the circulation was at a standstill, local ischaemia also does not raise the blocking temperature of the fibres. This is consistent with the observation that occluding the common carotid artery at the root of the neck did not affect the blocking temperature of the fibres.

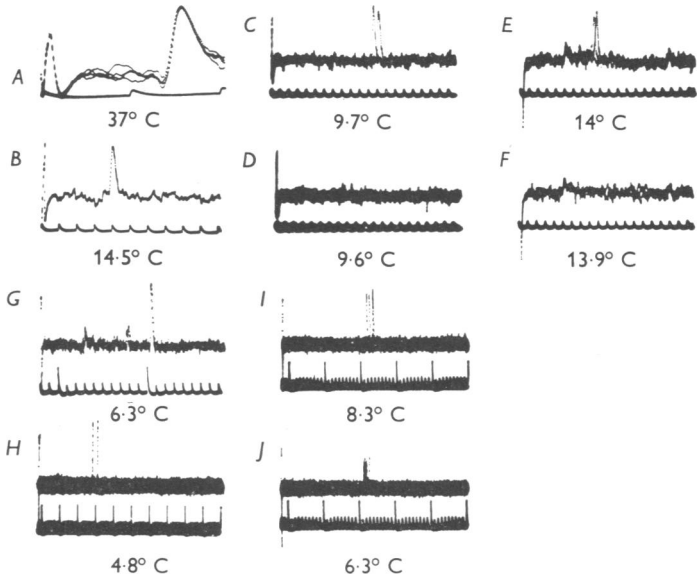

Fig. 4. Effect of application of tension on the vagus on the blocking temperature of individual nerve fibres. *A* to *D* show the normal evoked responses at different temperatures in a fibre with a conduction velocity of 39 m/sec; six and eight superimposed sweeps were recorded in *C* and *D*, respectively, but there were only two evoked responses in *C*. Both *E* and *F*, which consist of superimposed sweeps, were recorded after application of 20 g tension on the nerve. They show that tension increased the blocking temperature of the fibre by 4·3° C. *G* and *H* show the normal responses in another fibre with a conduction velocity of 17 m/sec; eight superimposed sweeps in *H*. Eight superimposed sweeps were recorded in *I*, 6 min after application of 16 g tension for 5 sec to the nerve. Blocking temperature was raised by 3·5° C. *J* shows responses in the same fibre 23 min after asphyxia at 6·3° C; blocking temperature was unchanged by asphyxia.

So far, no means have been found for lowering the blocking temperature of nerve fibres other than by eliminating a natural discharge of impulse in them (Fig. 3*D*). In view of this and the above results, it will therefore be assumed that if the blocking temperature of nerve fibres is higher than normal, then this must be due to local abnormal conditions, such as the presence of tension or pressure or possibly some invisible damage to the nerve sheath.

Relation of blocking temperature to conduction velocity

Vagal fibres. In view of the effect of natural impulses described above, it follows that it is possible to determine the blocking temperature of only those sensory fibres that are either normally silent, e.g. rapidly adapting tracheobronchial receptors (Widdicombe, 1954), or those which have a silent period of at least 2–3 sec between rhythmic discharges. Such discharges are found in the higher threshold group of pulmonary stretch

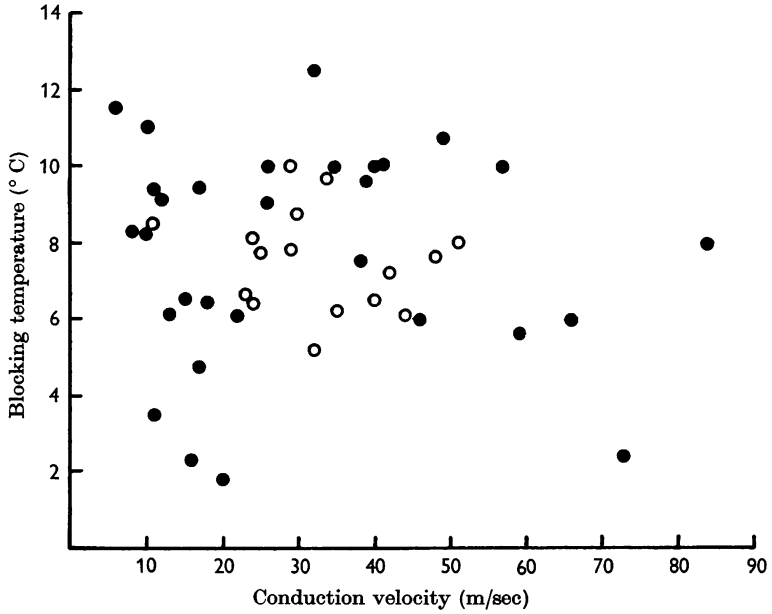

Fig. 5. Graph showing temperatures at which conduction was blocked in fibres with different conduction velocities. Open circles, fibres from pulmonary stretch receptors with impulses only during inflation of the lungs. The cats were artificially ventilated. Closed circles, fibres from normally silent vagal fibres.

receptors, especially in the presence of artificial ventilation which can be adjusted in depth and frequency to provide the desired silent periods in the afferent fibres. Complete silence in sensory fibres is obtained by crushing or cutting the vagus distal to the stimulating electrodes; by this means it was therefore possible to determine the blocking temperatures of fibres from pulmonary stretch receptors with a respiratory rhythm superimposed on a steady discharge (Fig. 3).

The open circles in Fig. 5 show the temperatures at which conduction was blocked in fibres from pulmonary stretch receptors. In this, no relation between blocking temperature and the conduction velocity of the fibres is visible and from it one can conclude that conduction in myelinated

vagal sensory fibres is blocked between 5 and 10° C, the mean blocking temperature being 7·5° C (S.E. 0·3) (Table 1).

The filled circles in Fig. 5 show the blocking temperature of normally silent myelinated vagal fibres. These were silent either because they were motor, or if they were sensory they were inactive normally; once again, there is no relation between conduction velocity and blocking temperatures. This conclusion is supported by the following analysis of the results of Fig. 5: the mean blocking temperature of ten of the fastest vagal fibres with an average conduction velocity of 64 m/sec was 6·9° C (range,

TABLE 1. Blocking temperatures of myelinated vagal and saphenous nerve fibres

Type of fibres	No. of fibres	Range (° C)	Mean (° C)	S.E.
Vagal fibres				
All fibres studied	47	1·8–12·5	7·6	0·4
Slowest fibres with conduction velocities of 6 to 15 m/sec (mean = 11 m/sec)	10	2·3–11·5	7·6	1·0
Fastest fibres with conduction velocities of 46 to 84 m/sec (mean = 64 m/sec)	10	2·4–10·7	6·9	0·8
Pulmonary stretch afferent fibres with conduction velocities of 11–51 m/sec	16	5·2–10·0	7·5	0·3
Saphenous fibres				
(Normal fibres)				
All fibres isolated from two cats	31	4·7–11·9	9·1	0·3
Slowest fibres with conduction velocities of 11–21 m/sec (mean = 17·4 m/sec)	10	5·5–11·9	10·0	0·6
Fastest fibres with conduction velocities of 51 to 77 m/sec (Mean = 67 m/sec)	10	4·7–11·0	8·4	0·6
(Abnormal fibres)				
All fibres isolated from two cats	25	12·5–22·7	15·9	0·4

2·4–10·7° C; S.E. 0·8). Similarly, the mean blocking temperature of the ten slowest fibres with an average conduction velocity of 11 m/sec was 7·6° C (range, 2·3–11·5° C; S.E. 1·0) (Table 1). It is therefore clear that the fastest and the slowest myelinated fibres in the vagus have nearly the same mean blocking temperature which also corresponds to the mean blocking temperature of sensory fibres from pulmonary stretch receptors.

These results are open to the criticism that they are the pooled results from different cats and they might be obscuring a relation between conduction velocity and blocking temperature. There is no doubt that the mean blocking temperatures differed markedly from cat to cat in experiments on the saphenous nerve (Fig. 7), but the variation in the vagus was small. Thus the mean blocking temperatures of vagal fibres in each of four

cats were 5·0° C (nine fibres), 8·8° C (five fibres) 7·1° C (four fibres) and 9·0° C (seven fibres).

However, proof that the blocking temperatures of the fibres did not vary with their conduction velocities was obtained by noting repeatedly that fast and slow fibres in the *same* filament were blocked at the same temperature (Figs. 6*A* and 8). In such cases all experimental factors, such as position of the fibres in the nerve and the tension exerted on them, must be common to all the fibres of the filament, since they must all be positioned close together in the cooled part of the nerve that was only about 15 mm distal to the recording electrodes.

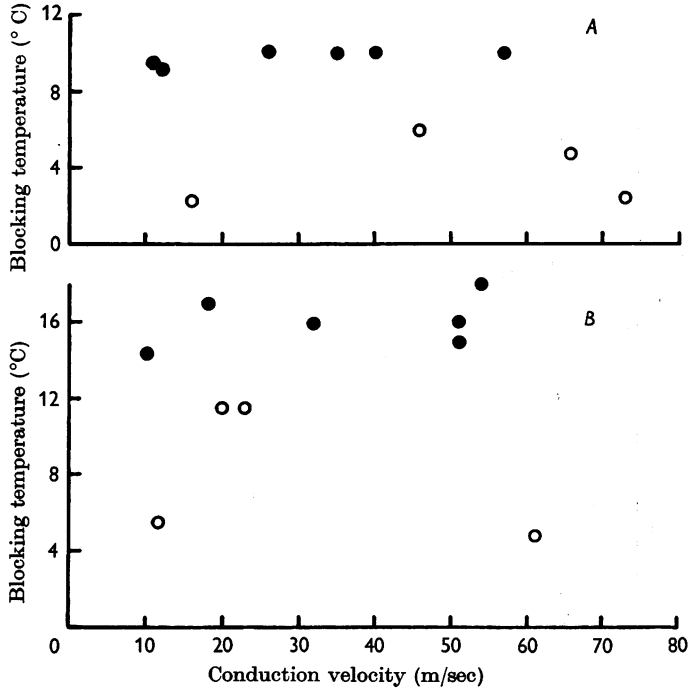

Fig. 6. Temperature at which conduction was blocked in different fibres of the same filament. *A*, fibres of two vagal filaments from two different cats. *B*, fibres in two filaments of the saphenous nerve of two cats. In *B*, closed circles, abnormal nerve; open circles, normal nerve.

Fibres of cervical sympathetic. In two experiments it was found that the blocking temperature of fifteen myelinated fibres of the cervical sympathetic nerve were similar to those of the medullated fibres of the vagus. The conduction velocities of these fibres ranged from 4 to 16 m/sec; their blocking temperatures averaged 6·1° C (range, 10–4·1° C; S.E. 0·4).

Fibres of saphenous nerve. The myelinated fibres of the saphenous nerve are all sensory (Heinbecker, O'Leary & Bishop, 1933). In this nerve,

Douglas & Malcolm (1955) obtained apparently clear-cut evidence that the slower fibres were blocked at higher temperatures than those needed to block the faster ones. Since no such differential effect was found in the vagus, either by them or in the present investigation, it was, therefore, necessary to study the fibres of the saphenous nerve using the same techniques as used in the vagus.

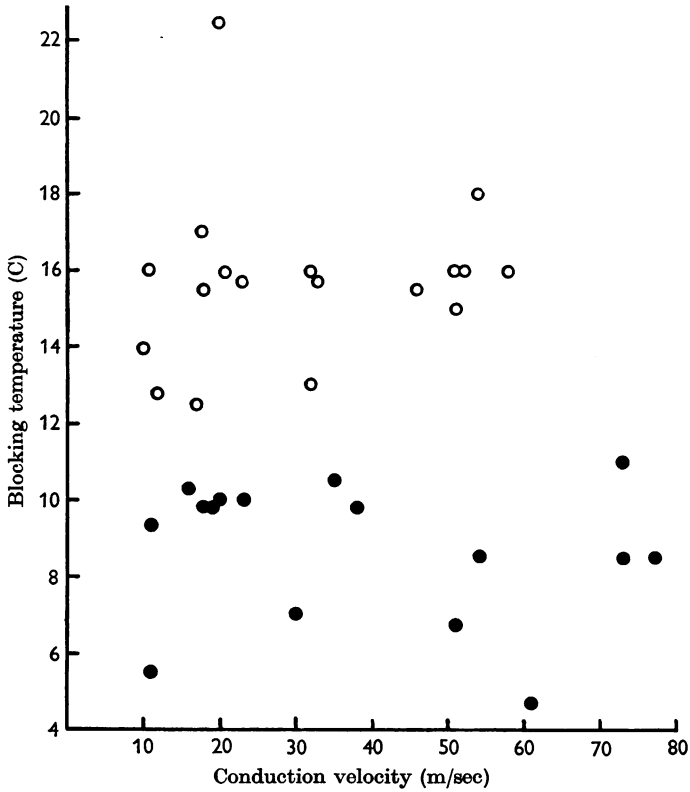

Fig. 7. Temperatures at which conduction was blocked in saphenous nerve fibres of two cats. Closed circles, normal nerve; open circles, abnormal nerve.

Four experiments were done on the saphenous nerve and the blocking temperatures of fifty-six fibres determined. The results obtained were qualitatively similar to those observed in vagal fibres, all the experiments showing unequivocally that there was no relation between blocking temperature and conduction velocity (Fig. 7). However, a notable difference was that the mean blocking temperature of the fibres differed markedly in different cats (Fig. 7) (Table 1). In the four cats it was respectively 10·0° C (10 fibres), 8·5° C (16 fibres), 16·4° C (7 fibres) and 15·7° C (18 fibres). The values in the last two cats, which are significantly

higher than those obtained in the first two cats, correspond to the blocking temperatures of alpha fibres as reported by Douglas & Malcolm (1955).

Since the mean blocking temperatures in the first two cats are almost similar to those obtained in the vagus, it is concluded that the blocking temperatures of fibres of the saphenous nerve are similar to those of vagal nerve fibres. The values in the last two cats are regarded as abnormal because, as already shown (Fig. 4), the effect of experimental factors, such as inadvertent application of tension or pressure, leads to a rise in the blocking temperatures. Besides, the fibres in these two cats showed other abnormal features, e.g. raised minimum conduction velocity just before block of conduction and unusual recovery curves (Paintal, 1965).

Figure 7 shows that conduction velocity bore no relation to the blocking temperature of the fibres in the same cat. This was true whether the fibres of the saphenous nerve were normal (Fig. 7*B*) or abnormal (Fig. 7*A*). As in the case of vagal fibres, it was noted repeatedly that blocking temperatures bore no relation to the conduction velocity of the fibres in the *same* filament (Fig. 6*B*). This, therefore, proves once again that there is no relation between the conduction velocity of the fibres and their blocking temperatures. Similar results were obtained in five other filaments with four to eight fibres in each.

Effect of low temperatures on the compound action potential

The effect of lowering the temperature on the compound action potential of the saphenous nerve was examined in all four cats. In two cats the compound action potential was recorded simultaneously with a record of impulses in individual fibres (Figs. 8 and 9*F*).

In all experiments the delta wave disappeared at a temperature that was several degrees higher than that required to block conduction in individual fibres with delta conduction velocity of say 10–25 m/sec. This difference was usually about 6° C, but in some instances it was as much as 9° C (Fig. 8); the minimum difference was 4° C (Fig. 9). It is, therefore, clear that the compound action potential gives an erroneous impression about conduction block in the fibres. This is due to the large increase in conduction time in these delta fibres on lowering the temperature, which causes a pronounced dispersion of the delta wave that makes it indistinguishable from the noise level of the tracing (Figs. 8 and 9).

As reported by Douglas & Malcolm (1955), all experiments showed conclusively that the alpha component of the compound action potential was reduced or it disappeared at a lower temperature than that needed to reduce or abolish the delta component (Figs. 8 and 9). However, this difference between the fast and slow myelinated fibres is not due to a difference in their respective blocking temperatures, but to the greater

increase in the conduction time of the delta fibres leading to a greater dispersion of the delta elevation of the compound action potential relative to the alpha elevation. Thus, Fig. 8 shows that in fibre no. 1, with a normal conduction velocity of 60 m/sec, the conduction time had increased only by about 1 msec at 12° C (Fig. 8*E*), whereas the conduction time of the three fibres marked *x* (with delta conduction velocity) had increased by

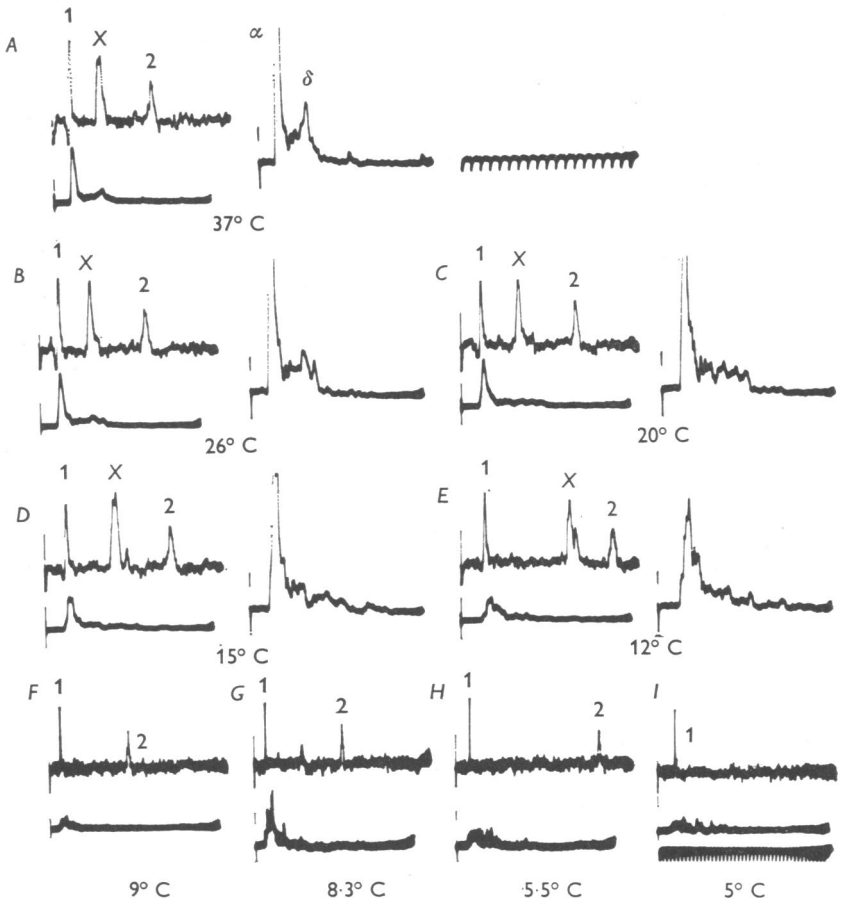

Fig. 8. Simultaneous records of compound action potential of the saphenous nerve and responses in two individual fibres of the saphenous nerve, labelled 1 and 2. Potential labelled *x* is the summed potential from three fibres. In *A*, *B*, *C*, *D* and *E* the compound action potential has been recorded at two amplifications, one 5× the other. In *F* the compound action potential is shown only with the lower amplification; in *G*, *H*, and *I* only at the higher amplification. Msec time marks in *A* apply to all sweeps from *A* to *E*; those at the bottom of *I* to sweeps *F* to *I*. Conduction velocity of fibre 1 was 61 m/sec; that of fibre 2 was 11 m/sec. Conduction in fibre 2 was blocked at 5° C although the delta wave disappeared at 14° C. Length of nerve cooled was 13 mm.

8 msec. Similarly, at 5·5° C, the conduction time of fibre no. 1 had increased by less than 3 msec, but that of no. 2 had increased from 10 msec at 37° C (Fig. 8*A*) to 37 msec at 5·5° C (Fig. 8*H*). These results, therefore, demonstrate that the lower the conduction velocity of the fibre, the greater is its increase in conduction time on reduction of temperature. It therefore follows, as shown in Figs. 8 and 9, that the dispersion of the delta

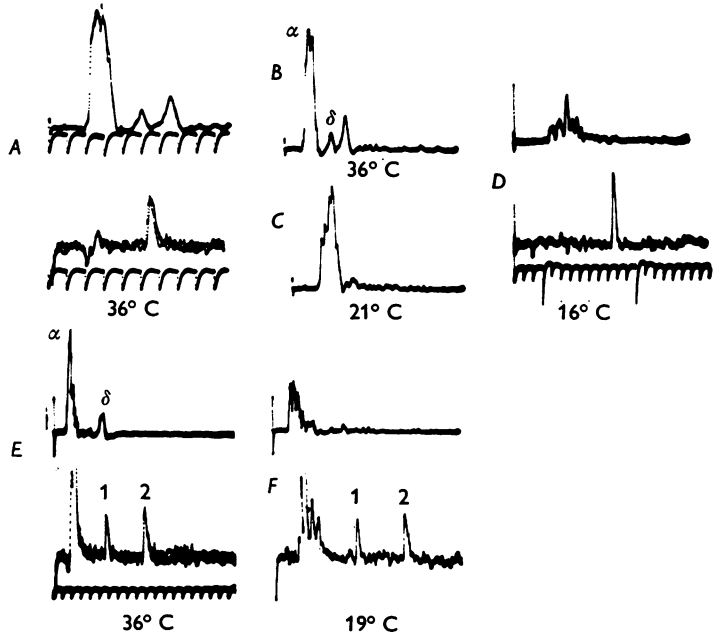

Fig. 9. Responses in individual fibres and compound action potential of 'abnormal' saphenous nerves of two cats. The compound action potentials in *A* to *D* were recorded a few minutes after recording the responses in the single fibre shown in *A* and *D* with a normal conduction velocity of 20 m/sec corresponding to the delta elevation in *A*. Blocking temperature of this fibre was 15·8° C, i.e. 6° C less than that required to almost eliminate the delta elevations at 21° C in *C*. Time marks in *D* apply to *B* and *C* as well. Sweeps in *E* and *F* were obtained from another 'abnormal' saphenous nerve. In *F* the responses in the individual fibres and the compound action potential were recorded simultaneously. In this case the delta elevation disappeared at 20° C, but as shown in *F* the responses in the two individual fibres, marked 1 and 2, with delta conduction velocity (19 and 11 m/sec respectively) were still present at 19° C. Conduction in no. 1 was blocked at 15·5° C, that in no. 2 at 16° C. Length of nerve cooled in both experiments was 13 mm.

elevation will be much greater than that of the alpha elevation, and this explains why the alpha component can still be seen at temperatures at which the delta component is no longer visible.

It is obvious from Figs. 8 and 9 that owing to the existing low signal (single spikes) to noise ratio, measurement of the areas under various com-

ponents of the compound potential rather than their respective amplitudes can do little to correct the erroneous impression about the relative amount of block in the different fibre-groups.

DISCUSSION

The results have shown beyond doubt that there is no relation between the conduction velocity of myelinated nerve fibres and the temperatures at which conduction is blocked in them. It can, therefore, be concluded that if experimental conditions could be kept constant then all myelinated nerve fibres would be blocked at nearly the same mean temperature. Since the effect of various factors, such as pressure or tension, is to raise the blocking temperature, it can also be concluded that the more favourable the experimental conditions the lower the blocking temperature. In the present experiments on vagal and saphenous nerve fibres one may therefore conclude from Table 1 that the experiments on vagal nerve fibres were better than those on saphenous nerve fibres. In fact, it follows that the best experiment was the one in which the mean blocking temperature of the vagal fibres was 5·5° C. The mean blocking temperature of all vagal fibres was 7·6° C (Table 1). Although every effort was made to avoid injury or stretching or kinking the nerve, one cannot assume that the experimental conditions were ideal, and it is, therefore, possible that the mean blocking temperature might be slightly lower under still more improved conditions.

If natural discharges are used as an index of activity in nerve fibres, then it is certain that the blocking temperatures recorded will always tend to be higher than the actual blocking temperatures. The difference between the two will depend on the pattern and frequency of discharge, as described in the following paper (Paintal, 1965). This, therefore, explains why previous investigations have recorded rather high blocking temperatures in some pulmonary afferent fibres (Torrance & Whitteridge, 1948; Whitteridge, 1948; Widdicombe, 1954), a feature also noted in the present investigation (Fig. 2). On the other hand, the lowest values for blocking temperatures reported in this paper (Fig. 5; Table 1) correspond to the lowest values reported by Whitteridge, who found that pulmonary vascular fibres (i.e. type B atrial receptors (cf. Paintal, 1953*a*)) were blocked at 3–4° C (Whitteridge, 1948). However, the actual blocking temperatures of these fibres in the absence of natural discharges would probably have been a little lower, as already pointed out. Probably, the same thing applied to Widdicombe's observations; he found the lowest blocking temperature of pulmonary afferent fibres to be 6° C (Widdicombe, 1954). In one experiment he recorded values as low as 3° C.

The experiments on the effect of tension, pressure and asphyxia were done in order to find out why the blocking temperature was raised in two experiments on saphenous nerve fibres (Table 1). It would now appear from the results that this raised blocking temperature could be due to undue stretching of the nerves which had actually taken place in one experiment and the same could have happened in the other. Furthermore, the nerves may have been kinked against the sharp and firm fascia at the two ends of the cooled nerve. Such conditions would raise the blocking temperature. In the case of the vagus there is no fascia but only loose connective tissue which yields readily on gentle separation, except in old male cats. This may account for the more satisfactory experiments on vagal nerve fibres.

The above observations show that in experiments with cold block it is important to keep in mind that the blocking temperature of nerve fibres can be raised by inadvertent application of tension or pressure through electrodes, thermodes, etc.

The present experiments on the saphenous nerve have demonstrated a serious shortcoming in the compound action potential as an index of activity in the nerve, because the unequal dispersion of its fast and slow components leads to the erroneous impression about the reduction of activity in the nerve (Figs. 8 and 9). The results in the accompanying paper (Paintal, 1965) show quantitatively the relative amount of dispersion of the alpha and delta waves to be expected. Thus, at about 3–4° C above the blocking temperature, the conduction velocity of the fibres is about 10 % of that at 37° C (cf. Fig. 4 in Paintal, 1965). If it is assumed that the length of cooled nerve is 10 mm it follows that the conduction time of the delta fibres with a conduction velocity of 10–20 m/sec will have been increased by 5–10 msec. On the other hand, the conduction time of the alpha fibres with a conduction velocity of about 80–50 m/sec will have been increased only by about 1–2 msec. The relative amount of dispersion of the delta elevation will therefore be much greater than that of the alpha elevation, and this will lead to the erroneous impression that the delta fibres are blocked before the alpha fibres, because the delta elevation becomes so dispersed as to be indistinguishable from the noise level of the amplifier (Figs. 8 and 9).

It might be argued that the present results with immersion cooling differ from those obtained by other investigators because of a different method of cooling the nerve. This is not so, because in the present investigation qualitatively similar results were obtained by using a conventional thermode about 13 mm long, i.e. similar to that used by Torrance & Whitteridge (1948) and Whitteridge (1948). Widdicombe used a thermode 10 mm long and Douglas & Malcolm (1955) used 6 mm-long cooling heads

in most experiments but 20 mm-long ones in the experiment which they believed showed the differential effect conclusively. In the present experiments with immersion cooling the length of nerve cooled was 9–13 mm in nineteen experiments and 16 mm in two experiments.

In view of the fact that immersion cooling provides a more uniform cooling of the nerve fibres, it would appear to be the method of choice owing to its simplicity because all that is required is cold Ringer's solution and some arrangement for suction.

SUMMARY

1. The temperatures at which conduction was blocked in myelinated nerve fibres of the cat were determined on forty-seven vagal, fifty-seven saphenous and fifteen cervical sympathetic nerve fibres. The nerves were cooled locally by immersing them in a pool of cold Ringer's solution formed by skin, muscles and connective tissue.

2. The presence of natural discharges in the nerve fibres raised their blocking temperatures. This was also raised by any adverse experimental factor such as tension or pressure on the nerve; moderate tension raised it by about 4° C. Asphyxia or ischaemia had no effect.

3. The blocking temperatures of the fibres bore no relation to their normal conduction velocities. This was demonstrated conclusively by noting in several filaments that conduction was blocked at the same temperature in slow and fast fibres of the *same* filament. The mean blocking temperatures of vagal and normal saphenous nerve fibres were 7·6 and 9·1° C, respectively; that of abnormal saphenous fibres 16° C. This raised temperature was probably due to increased tension on the nerve.

4. The delta elevation of the compound action potential of the saphenous nerve, recorded simultaneously with records of impulses in individual fibres, was always abolished at a higher temperature than that needed to block conduction in individual fibres with delta conduction velocities; sometimes the difference was as much as 9° C. The delta elevation was abolished at a higher temperature than that needed to abolish the alpha elevation owing to the much greater dispersion of the former; this was due to the greater increase in conduction time in the slower fibres as compared to the faster ones.

5. It is concluded that conduction is blocked in all myelinated nerve fibres at about the same temperature.

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