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# THE INITIAL HEAT PRODUCTION ASSOCIATED WITH THE NERVE IMPULSE IN CRUSTACEAN AND MAMMALIAN NON-MYELINATED NERVE FIBRES

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The passage of a single impulse in non-myelinated nerves of the spider crab, *Maia squinado*, at 0° C is associated with a positive heat production of about 9 $\mu$cal/g; this is followed by a slower heat absorption of about 7 $\mu$cal/g (Abbott, Hill & Howarth, 1958). Although the diphasic character of the heat production cannot at the moment be accounted for satisfactorily, it may eventually provide an important clue to the physical and chemical mechanisms underlying the nerve impulse. It seemed important, therefore, to determine whether or not similar heat changes occur in nerves of species other than *Maia*. We have therefore examined the heat production of the nerve of two other crustaceans, namely the Pacific Spider crab, *Loxorhynchus crispatus* and the lobster, *Panulirus interruptus*. In addition, we have studied the heat production of mammalian non-myelinated nerve fibres. The latter experiments were done on rabbit vagus nerves (Ritchie, Abbott & Howarth, 1961) which are composed mainly of non-myelinated fibres of more uniform and smaller average diameter than those of the crab.

These experiments have confirmed the presence in all the nerves examined of the early phases of positive and negative heat production which occur during, or soon after, the spike. In addition, a previously unsuspected third phase of prolonged cooling was found to occur in the rabbit nerve under certain experimental conditions.

## METHODS

Nerves from the claws or walking legs of the Atlantic and Pacific species of spider crab (*Maia squinado* and *Loxorhynchus crispatus*, or of the Pacific lobster (*Panulirus interruptus*), were drawn out, not dissected by the method described by Furusawa (1929) and by Abbott, Hill & Howarth (1958). Cervical vagus nerves were rapidly dissected from rabbits (killed by the injection of air into an ear vein) and then desheathed under the dissecting microscope (×40). A bundle of these nerves (usually two crab-claw nerves plus four walking-limb

nerves, or two lobster-claw nerves, or eight rabbit vagus nerves) was mounted on a thermopile similar to that described by Abbott, Hill & Howarth (1958). The output from the thermopile was fed into a sensitive galvanometer/photocell amplifier and recorded. Three different thermopiles were used, with outputs of about 3500, 3000 and 2000 $\mu$V/°C respectively. Electric shocks about 0·5 msec in duration were applied through two pairs of electrodes at either end of the thermopile to minimize conduction time. The characteristics of the system were such that a block of heat of 20 msec duration, applied to the thermopile by passing a pulse of current for 20 msec through a dead nerve mounted on it, caused a deflexion that took 60–100 msec (depending on which thermopile was used) to reach half its maximum value and about 250 msec to reach its maximum. The slowness of this response was almost entirely the result of the thickness of the insulation on the face of the thermopile, for the galvanometer itself was extremely rapid. The deflexion of the galvanometer in response to a suddenly applied voltage reached half its maximum value in under 20 msec and was virtually complete in 70 msec. The galvanometer used was, therefore, unnecessarily fast and much of the noise on the heat traces could have been avoided by using a galvanometer whose speed of response was more closely matched to that of the thermopile.

The composition of the crab and lobster Ringer's solution was (mM): NaCl, 520; KCl, 13; $CaCl_2$, 14; $MgCl_2$, 24; urea, 0 or 17. The composition of the Locke's solution for the rabbit nerve was (mM): NaCl, 154; KCl, 5·6; $CaCl_2$, 2·2; glucose, 5; tris(hydroxymethyl)aminomethane buffer (pH 7·0), 1·0.

Wherever possible, means $\pm$ the standard error are given.

## RESULTS

Figure 1 shows three consecutive traces of the temperature changes in nerves of the Pacific spider crab at 0° C in response to single maximal shocks applied at the arrows. The large random fluctuations in the base line make it difficult to determine the precise time course of the changes in any individual record. However, these fluctuations disappear when the deflexions in each trace are measured at intervals (of 16·7 or 20 msec) and the responses in 10 or 20 traces averaged. This is shown in Fig. 2 which illustrates the average response of a bundle of lobster nerves (*A*) and of crab nerves (*B*). Record C is the 'heating control' obtained when a piece of wet filter paper, about the same weight as the nerves and placed on the thermopile instead of them, was suddenly heated by passing a brief current through it during the 20 msec after the arrow; in all the subsequent heat analyses in this paper the heating control was obtained by passing the current through dead preparations. It is clear, from a comparison of the nerve heat records (particularly *B*) with the heating control, that the nerve temperature declined much more rapidly than could be accounted for by the passive diffusion away of the heat. As Abbott, Hill & Howarth (1958) have already pointed out for *Maia*, there must have been an active reabsorption of the positive heat that was produced initially. The present experiments thus show that the heat production in the nerves of both the Pacific spider crab and the lobster is also diphasic as it is in *Maia* nerves. In four experiments on the Pacific spider crab at 0° C the average initial maximum rise of temperature was $5·2 \pm 0·6\ \mu$° C which occurred about

150 msec after the stimulus. Because of the reabsorption of heat the average temperature 1 sec after the stimulus was only about 1·0 $\mu$° C above the initial temperature.

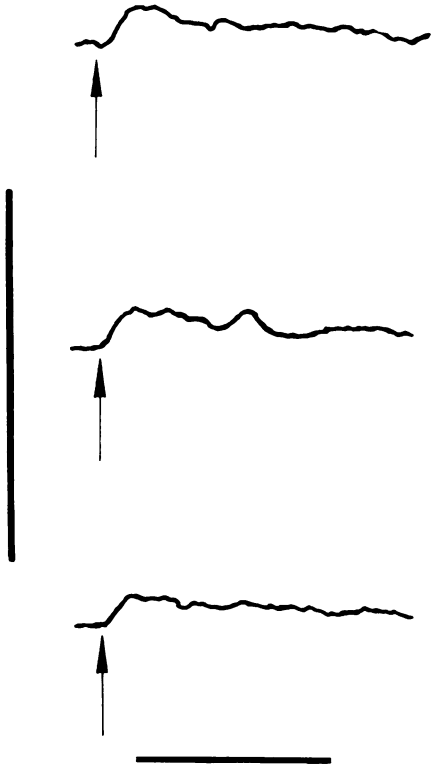

Fig. 1. Consecutive traces of the temperature changes in nerves of the Pacific spider crab at 0° C. Single maximal shocks were applied at the arrows. The vertical bar represents 100 $\mu$° C, the horizontal bar 1 sec.

In the lobster experiment shown in Fig. 2 the maximum rise in temperature was 1·0 $\mu$° C; in two experiments on *Maia* nerves it was 12·3 and 8·3 $\mu$° C/impulse respectively.

*Heat analysis of the crab and lobster records*

Because of the characteristics of the galvanometer and the thermopile (see Fig. 2*C*) the deflexions in the recording system lagged behind the actual temperature changes. A true time course of the heat production can be obtained by the method of analysis described by Abbott, Hill & Howarth (1958) in which the heat records are analysed in 'blocks' of heat of some predetermined duration, usually 1/50 or 1/60 sec. Because of the large amount of numerical analyses entailed by this procedure, the analyses

were carried out only in a few typical experiments. One such analysis of an experiment on crab nerve is illustrated in Fig. 3. There were two phases of heat production; 3·8 $\mu$cal/g of heat was evolved during the first 50 msec and then nearly 80% was reabsorbed, equivalent to a negative heat production of about 3 $\mu$cal/g.

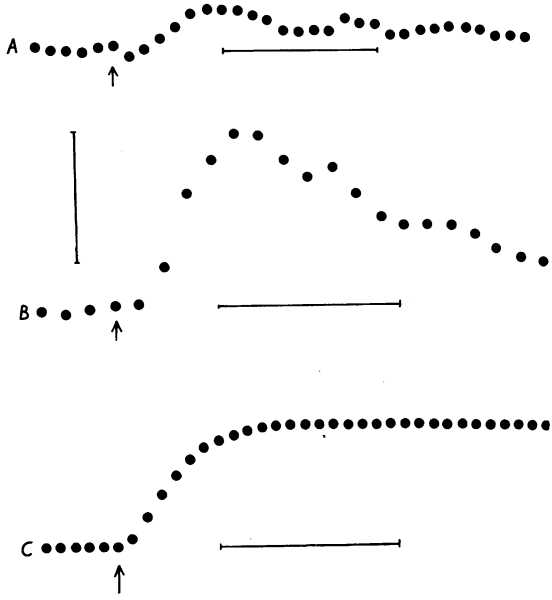


Fig. 2. The average temperature change in response to a single shock (applied at the arrow) in (*A*) lobster, and (*B*), Pacific spider crab nerves at 0° C. The vertical bar represents 4 $\mu$° C and the horizontal bars 250 msec. The bottom trace, which is the heating control, is the response to a 20 msec burst of heat applied beginning at the arrow.

*Temperature changes and heat analyses in rabbit nerves*

Figure 4 shows three consecutive traces of the changes in temperature of a bundle of rabbit vagus nerves at 17° C associated with the passage of a single impulse. Because the temperature changes in the mammalian nerve were smaller than in the crab nerve, and often could barely be discerned above the random fluctuations in the base line, in preliminary experiments the thermal response to a train for five stimuli was determined. Such stimulation produced a simple rise in temperature of $2{\cdot}5 \pm 0{\cdot}3$ $\mu$° C/impulse (five experiments). There was no rapid fall in temperature. Indeed, the initial temperature rise was often followed by a longer period of increasing temperature at the rate of about 1 $\mu$° C/sec, presumably due partially to recovery heat but perhaps largely to stimulus heat slowly

diffusing down from the stimulating electrodes. In later experiments stimulus heating was quite small, presumably because of the different design of the thermopile and stimulating electrodes.

The temperature change in the rabbit nerve was most clearly seen by averaging up to 50 consecutive traces of the response to single maximal shocks. The laborious procedure of numerical averaging used to obtain

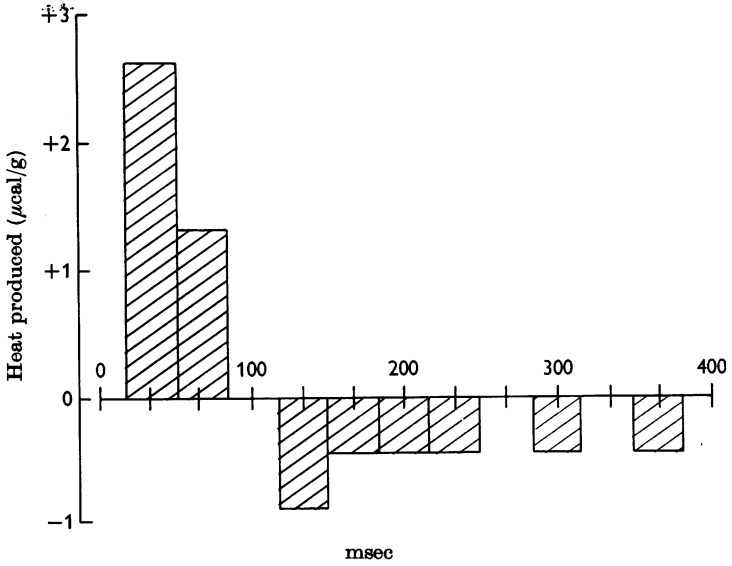


Fig. 3. The analysis of the heat production of Pacific spider crab nerves at 0° C. A single maximal shock was applied at zero time. The record is analysed in blocks of heat of 33·4 msec duration. The stimulus artifact occurred at zero time, and the delay between the artifact and the appearance of the positive heat is largely accounted for by the conduction time between the stimulating electrodes and the thermopile.

Fig. 2 (see Abbott, Hill & Howarth 1958) was replaced by an electronic averaging procedure using a CAT computer. In this procedure the time base of each trace is divided into four hundred parts and the corresponding ordinates at each interval in consecutive traces are electronically averaged. Typical records obtained by this procedure are shown in Fig. 5. A single impulse at about 15° C (Fig. 5*A*) produced a rise in temperature to a maximum value $3{\cdot}9 \pm 0{\cdot}6\ \mu°$ C (six experiments) above the initial temperature. Although there was often a subsequent slow decline in temperature, the rapid fall in temperature obtained with the crab nerve at 0° C was absent. However, the maximum temperature was attained much more rapidly than the characteristics of the recording system would have allowed unless there was a phase of heat reabsorption. Indeed, analysis of four of

these experiments showed that the temperature changes in the nerve were produced by an evolution of $7{\cdot}2 \pm 0{\cdot}5$ $\mu$cal/g during the first 80 msec after the stimulus and by a subsequent reabsorption, in the next 65 msec, of $4{\cdot}8 \pm 1{\cdot}1$ $\mu$cal/g. These temperature changes were obtained only when the vagal non-myelinated fibres were stimulated; weaker stimuli, exciting only the myelinated fibres, did not produce any thermal changes that could be detected.

The two phases of heat production in rabbit nerve became even clearer when experiments were carried out at a lower temperature. Even before analysis, the records of the temperature changes in experiments carried out at about 5° C (Fig. 5*B*) clearly indicated the presence of a phase of

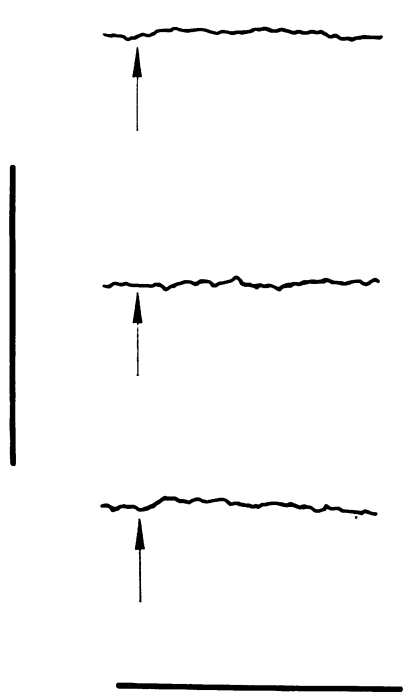

Fig. 4

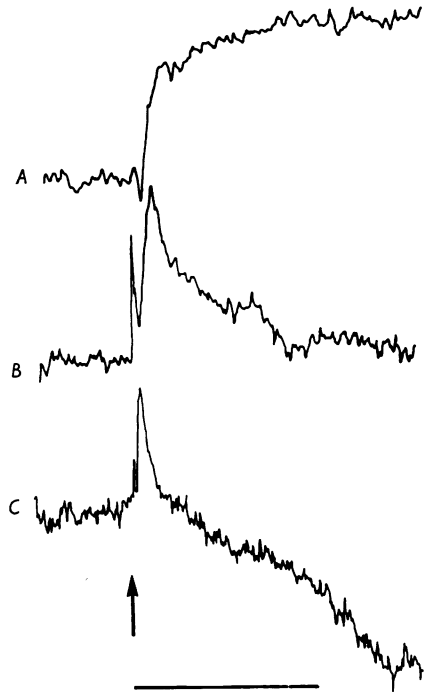


Fig. 5

Fig. 4. Consecutive traces of the temperature changes in a bundle of rabbit desheathed vagus nerves at 17° C in response to single maximal shocks applied at the arrows. The vertical bar represents 100 $\mu$° C and the horizontal bar 5 sec.

Fig. 5. Records of the changes in temperature of three different preparations of rabbit desheathed vagus nerves at: *A*, 14° C; *B*, 6·1° C; *C*, 0° C. An upward deflexion indicates an increase in temperature. The two top records were obtained by averaging, with a CAT computer, the responses to 50 single impulses; the bottom record is the average of five responses. The stimulus was applied at the arrow and the first deflexion is the stimulus artifact. The horizontal bar represents 2 sec in the top two records and 8 sec in the bottom record. The vertical bar represents 3 $\mu$° C.

rapid heat reabsorption. Analysis of four such experiments showed that $7{\cdot}4 \pm 0{\cdot}6$ $\mu$cal/g of heat was evolved during the first 113 msec after the stimulus and that $5{\cdot}8 \pm 1{\cdot}1$ $\mu$cal/g of heat was reabsorbed during the next 85 msec.

Figure 6 shows the heat analysis of the record shown in Fig. 5*B*. The 'latent' period of 40 msec appearing in this record corresponds to the conduction time between the stimulating electrode and the thermopile. The corresponding values at 15° and 0° C were 20 and 80 msec respectively.

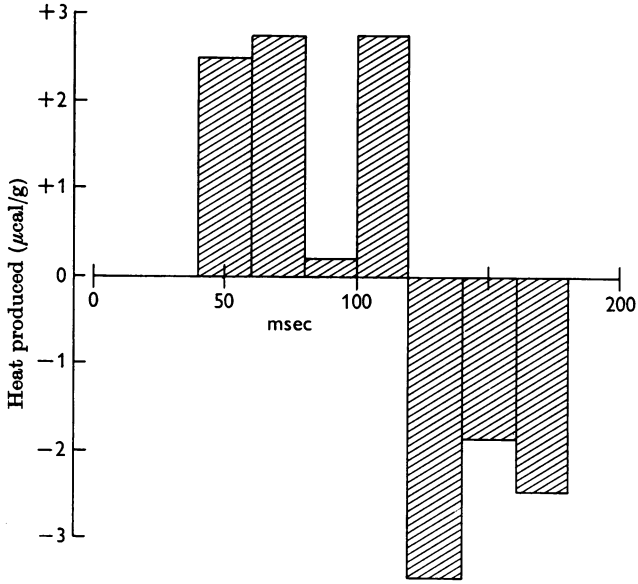


Fig. 6. The analysis of the heat production (in 20 msec blocks) in a preparation of rabbit desheathed vagus nerves at 6·1° C; same experiment as shown in Fig. 5*B*. The stimulus was applied at zero time.

When the experiments were carried out at 0° C the presence of a phase of rapid reabsorption of heat was again clear in the uncorrected records of the temperature changes (Fig. 5*C*). Analysis of two such experiments showed that 8·2 and 10·4 $\mu$cal/g, respectively, were evolved during the first 360 msec after the stimulus and that 5·4 and 5·5 $\mu$cal/g, respectively, were reabsorbed during the next second.

The most interesting finding in the experiments at 0° C was that, following the initial positive and negative phases of heat production, there was a third phase, of prolonged cooling, that resulted in the nerves becoming $9{\cdot}4 \pm 4{\cdot}0$ $\mu$° C (four experiments) colder than their initial temperature during the next 12 sec after the stimulus (Fig. 5*C*). The temperature changes during this phase occurred so slowly that it is less necessary to

correct for the slowness of the recording system than in the study of the rapid early heat changes. However, it is more necessary to correct for the heat that is lost by radiation and by conduction along the wires of the thermopile. Experiments on dead nerves showed that, after a nerve had been suddenly heated by passing a brief pulse of current through it, the temperature deflexion declined by 35% per sec. When the records, such

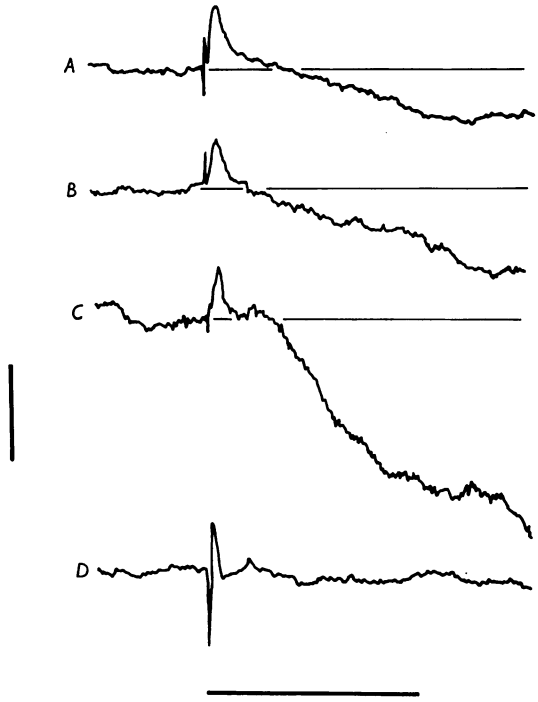


Fig. 7. Records of the changes in temperature of three different preparations (*A*, *B* and *C*) of rabbit desheathed vagus nerves at 0° C. Records *A* and *B* are the average of 10 responses to single maximal shocks. Record *C* is a single trace. Record *D* is a repeat of *A* after the preparation had been killed by HCl. The vertical bar represents 10 $\mu$° C and the horizontal bar 8 sec.

as those in Fig. 5*C*, were corrected to allow for this passive exchange of heat with the surroundings, it was found that a single stimulus caused the four preparations to cool by $31{\cdot}1 \pm 15{\cdot}5$ $\mu$° C during the 12 sec following the stimulus.

Figure 7 shows records of the temperature changes in three other preparations of rabbit vagal C fibres at 0° C. The bottom record in this figure shows the response of the same nerves after they had been killed by adding a small amount of concentrated HCl to the bathing medium. Clearly the phase of prolonged cooling is absent. It was also absent in

other experiments at 0° C in nerves in which conduction had been blocked by treatment with a local anaesthetic (1–3 mM lidocaine).

*The effect of barium ions*

When the axons of crab nerves or mammalian nerves are treated with barium the action potential becomes markedly prolonged and may last for several seconds (Greengard & Straub, 1959; Armett & Ritchie, 1963; Keynes & Ritchie, 1965). Because the heat production in nerve is presumably associated with the events that underlie the action potential, it seemed interesting to determine the effect of barium ions on the nerve heat production. The following experimental procedure was adopted. The heat production was determined in the untreated nerve in response to a single stimulus, the preparation and thermopile necessarily being in air during the time the measurements were made. Modified bathing solutions

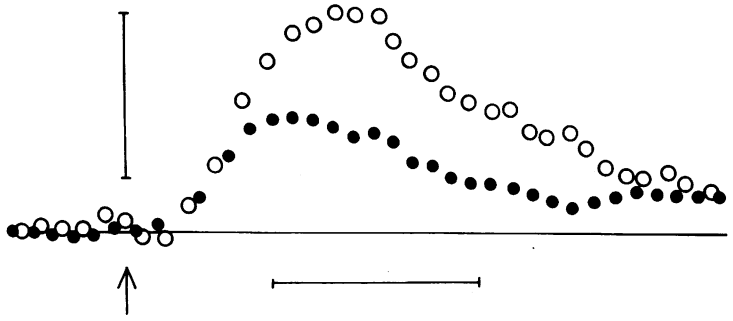

Fig. 8. The effect of barium ions on the heat production of crab nerve at 0° C (average of 10 traces). The closed circles show the response in a nerve equilibrated in normal crab Ringer's solution and the open circles after barium treatment (see text). The vertical bar represents 5 $\mu$° C and the horizontal bar 250 msec.

were then run into the chamber containing the thermopile; all the sodium chloride of these solutions had been replaced by isosmotic amounts of barium chloride (400 mM in the crab and lobster experiments and 113 mM in the rabbit experiments). Although conduction was not maintained in these solutions, it was quickly restored by briefly exposing the preparations (for $\frac{1}{2}$–1 min) to the normal bathing solution. In this condition not only had conduction been restored, but the action potential was markedly prolonged. The heat production of a barium-treated crab nerve is illustrated in Fig. 8. The filled circles show the response in the normal nerve and the open circles the response in the nerve after treatment with barium. It is clear that both phases of heat production were very much enhanced after treatment with barium. The heat analysis of such an experiment (same experiment as illustrated in Fig. 3) indicated that the positive heat production increased from 3·8 to 6·3 $\mu$cal/g impulse and the heat reabsorption

from 3 to 4·6 $\mu$cal/g impulse. A similar enhancement of the positive heat production was obtained in the analysis of another experiment on crab nerve and of another on lobster nerve; there was however no marked enhancement of the negative heat production in these experiments. Thus, in these three experiments, barium increased the positive heat $2{\cdot}5 \pm 0{\cdot}04$ times and the negative heat only $1{\cdot}18 \pm 0{\cdot}18$ times.

Fig. 9. Consecutive traces of the temperature changes in response to a single shock (applied at the arrows) in a preparation of barium-treated rabbit desheathed vagus nerves at 17° C (same experiment as Fig. 4). The vertical bar represents 100 $\mu$° C, the horizontal bar 5 sec.

In mammalian nerves, barium had such a marked effect that the temperature changes associated with the impulse became quite distinct even in the individual heat races, as illustrated in Fig. 9. The temperature either reached an early maximum and then declined, or after an early rapid rise continued, as in Fig. 9, to rise more slowly. In five experiments at about 15° C the barium treatment increased the early temperature rise of rabbit nerves $6{\cdot}6 \pm 1{\cdot}8$ times.

Heat analyses were not carried out on the records of experiments at room temperature. In a single experiment at 0° C in which a modified

experimental procedure was used, exposure to 64 mM-$BaCl_2$ increased the positive heat from 8·2 to 10·4 $\mu$cal/g and the negative heat from 5·2 to 5·4 $\mu$cal/g. This concentration of $BaCl_2$ markedly enhances the duration of the action potential and does not produce conduction block.

*The effect of lithium ions*

Lithium ions may replace sodium ions in carrying the inward depolarizing current during the action potential; however, they are probably not lost from the axoplasm as rapidly as sodium is extruded by the sodium pump (Ritchie & Straub, 1957). The effect of equilibrating crab nerves in

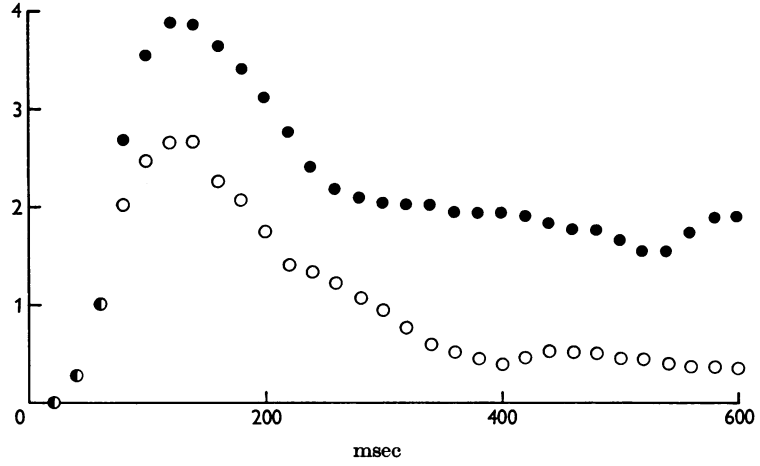


Fig. 10. The effect of lithium on the heat production of *Maia* nerve at 0° C in response to a single maximal shock at zero time. The closed circles show the response in a normal nerve and the open circles the response after the nerve had been equilibrated in lithium-Ringer's solution. The ordinate is in arbitrary units.

solutions in which all the sodium had been replaced by lithium is illustrated in Fig. 10. The most striking effect of the lithium treatment was to reduce the net temperature change almost to zero. This could have resulted either from a decrease in the positive heat production or an increase in the amount of heat reabsorbed. Since the early part of the rising phase of the temperature record seemed to be about the same in both solutions (Fig. 10) it seems likely that the positive phase is unchanged. Indeed analysis of a typical record showed that the positive phase was exactly the same in lithium- as in sodium-Ringer's solution. However, the negative heat in lithium solution early equalled the positive heat (98%), whereas it was only about 74% of the positive heat in sodium-Ringer's solution.

## DISCUSSION

It is a commonplace that thermal experiments are beset with artifacts, sometimes of such subtle nature as to escape detection for a long time. The phase of heat reabsorption originally observed by Abbott, Hill & Howarth (1958) was, however, unlikely to be an artifact. In their original experiments, Abbott, Hill & Howarth (1958) seemed to have done every conceivable control; and, anyway, most of the spurious deflexions that might have been expected (for example, from stimulus heating) would have given a positive rather than a negative heat. The possibility of an artifact has been rendered even more unlikely by the present findings of a similar negative heat in three different species of animals (Pacific spider crab, lobster and rabbit) with three different sets of recording apparatus. The differences between *Maia* nerves on the one hand and those of the Pacific spider crab and lobster on the other (namely that the heat changes in the latter are much smaller) seem much less important than the similarity between them. In all crustacean nerves examined, and in the mammalian nerves, about 80% of the heat released is actively reabsorbed within a short period of time. It seems likely that the initial phases of positive and negative heat production will prove to be characteristic of all types of non-myelinated nerve fibre.

Perhaps the most disappointing finding in the present experiments was that the heat produced in the mammalian nerves was about the same as the net heat produced in the crab nerves, namely 2·8 $\mu$cal/g (average of 10 analysed experiments at 14, 4 and 0° C). A greater heat had been expected in the mammalian preparation because of the presumed larger amount of membrane in the smaller mammalian non-myelinated fibres. From the spectrum of fibre diameters obtained from electron microscopic studies, Abbott, Hill & Howarth (1958) had calculated that 1 g of *Maia* nerve (whose fibres are between 20 and 0·3 $\mu$ in diameter) contained $10^4$ cm$^2$ of membrane. Similar calculations for mammalian C fibres, whose average diameter is about 0·8 $\mu$, seemed to give a surface/volume ratio of $5 \times 10^4$ cm$^{-1}$ (Keynes & Ritchie, 1963). Therefore, if the nerve heat were proportional to the area of nerve membrane, five times as much heat per g would have been expected from a mammalian nerve consisting solely of C fibres. Even allowing for the fact that in the cervical vagus nerve there is about as great a cross-section of myelinated fibres as non-myelinated fibres, one might have expected more than twice as much heat per g in mammalian nerve as in crab nerve. However, the extracellular space in mammalian desheathed nerves has been found to be extremely high, about 60% (Keynes & Ritchie, 1965). On this basis the area of membrane per gram of vagus is only $0{\cdot}6 \times 10^4$ cm$^2$ (Keynes & Ritchie, 1965). The areas of

membrane in both the mammalian and crustacean nerves (expressed per gram of tissue) are therefore about equal, and this might well account for the near equality of the heats.

The exact time course of the heat production during an impulse is obscured in the experimental records because of the relatively large time of conduction of the impulse from one end of the thermopile to the other. The effective conduction distance was reduced in the present experiments to 14 mm, and at 5° C the propagation velocity of the impulse was found to be about 0·12 m/sec (0·14 m/sec was taken for the convenience of the analyses). This means that 100 msec after the beginning of the heat production, the deflexion would be composed partly of heat from an element of nerve in which the action potential had just occurred, and partly of heat from a series of elements in which the action potential had occurred successively at intervals from 0 to 100 msec previously. The exact time course of the heat production at a given point on the length of the nerve can, nevertheless, be obtained from the experimental records by the method of analysis described below.

The heating control, such as that illustrated in Fig. 2*C*, shows the result of releasing a block of heat of 20 msec duration uniformly over the face of the thermopile. If this curve is successively displaced 20, 40, 60 and 80 msec to the right and the five curves summed, a new heating control is obtained that gives the form of the deflexion that would have been produced by a block of heat of 20 msec duration travelling at a velocity of 0·14 msec from one end of the thermopile to the other. As Professor A. V. Hill (personal communication) has pointed out, the same result would have been obtained more simply just by heating for 100 msec. The experiment shown in Fig. 6 was reanalysed with this 'conducted' heating control and the result is shown in Fig. 11. The procedure used provided a simpler method of analysis than that used by Abbott, Hill & Howarth (1958), and was made possible by the relatively uniform conduction velocity of the mammalian, compared to the crustacean, fibres. Two features of the analysis should be noted. First, as already noted by Abbott, Hill & Howarth (1958) for crab nerve, the positive heat production in mammalian nerve at 5° C is virtually complete in the time occupied by the rising phase of the action potential (Fig. 12). Secondly, between the positive and the negative phases there is 'silent' period during which either no heat changes occur or the positive and negative changes are equal; this seems to correspond with the time during which substantial recovery of the membrane potential occurs. Similar results were obtained in the analysis of three other experiments at this temperature. However, it might be unwise to draw firm conclusions from these analyses because of the large fluctuations in the experimental records and the fact that the conduction velocity

was not determined in the same experiments as the heat production. Further experiments are clearly required to establish the critical relation between the several phases of heat production and those of the action potential.

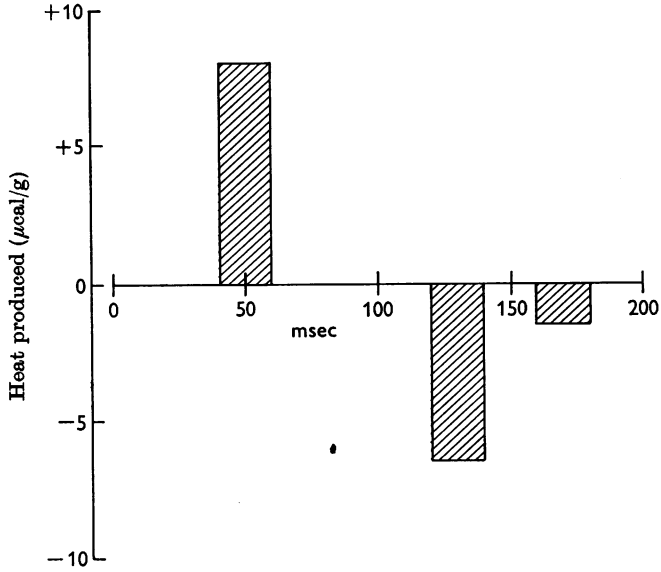


Fig. 11. Analysis of the heat production (in 20 msec blocks) of a preparation of rabbit desheathed vagus nerves at 6·1° C using 'conducted' heating control (see text).

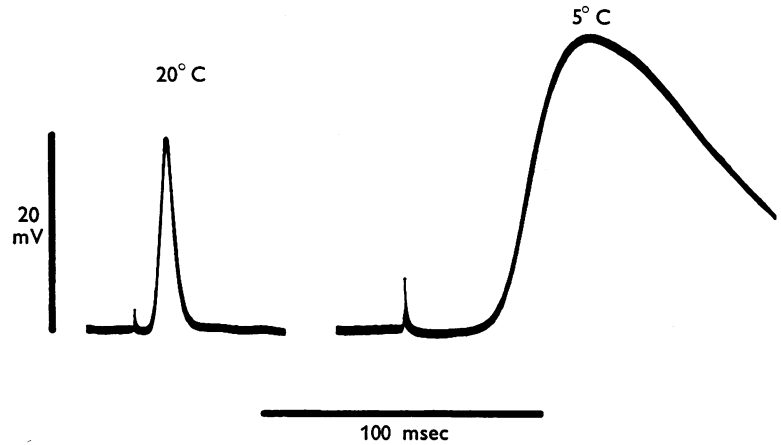


Fig. 12. Record of the compound action potential of the non-myelinated fibres of a rabbit desheathed vagus nerve obtained by the sucrose gap method (see Armett & Ritchie, 1963, for references) at 20° C (left-hand record) and at 5° C (right-hand record). The effective conduction distance was about 3 mm.

The origin of the nerve heat is at present obscure. One possibility considered by Abbott, Hill & Howarth (1958) was that the early positive and negative phases were due to the heats of mixing of the ions that crossed the membrane during the action potential. On this basis, the near equality of the net heat per unit area of membrane in mammalian and crustacean nerves would mean that the net heat of mixing of the ions is the same in both types of nerve. This is interesting in view of the large differences in the ionic composition of both the intracellular and extracellular fluids in the two types of nerve. However, the experiments in which lithium replaced sodium seem to argue against the heat of mixing being wholly responsible for the initial heat. For the component of heat production most closely associated with the inward movement of sodium seems to be the early positive phase. Nevertheless, although the heat of mixing of lithium chloride with potassium chloride is nearly twice that of mixing sodium chloride with potassium chloride (Abbott, Hill & Howarth 1958), the positive heat produced does not seem to be increased when lithium replaces sodium as the carrier of inward depolarizing current during the action potential. Whatever the origin of the heat, it is interesting that agents, such as barium (present experiments) and veratrine (Hill, 1933), which markedly prolong the duration of the action potential, also greatly increase the net positive heat production.

The finding in the present experiments of the late phase of prolonged cooling in rabbit nerve at 0° C, causing the preparation eventually to become colder than its initial temperature, was entirely new and unexpected. It removes the one outstanding difference between the thermal events in nerve and those in the electric organs of various electric fish in which a phase of cooling to below the initial temperature is well established (Abbott, Aubert & Fessard, 1958; Aubert & Keynes, 1961; Keynes & Aubert, 1964). The origin of this phase of late cooling, like those of the previously described phases, remains unknown. The fact that so far its presence has been detected only in rabbit nerve at 0° C, and not in crab nerve at 0° C or in rabbit nerve at higher temperatures, does not necessarily exclude the possibility that it is a constant accompaniment of nervous activity. In the latter preparations the phase of late cooling may well occur, but be masked by the simultaneous presence of recovery heat.

## SUMMARY

1. A study has been made of the heat production associated with the passage of a single impulse in the non-myelinated fibres in the vagus nerve of the rabbit, and in the leg nerves of the lobster *Panulirus interruptus*, and of the Atlantic and Pacific species of spider crab *Maia squinado* and *Loxorhynchus crispatus*.

2. In the mammalian nerve at 0, 5 and 14° C, and in the crustacean nerve at 0° C, the temperature of the nerve rises by a few micro-degrees centigrade after a single stimulus.

3. Analysis has shown that the initial temperature changes are caused by two phases of heat production. First, there is an evolution of about 1–10 $\mu$cal/g. Secondly, there is a phase of negative heat production during which about 80% of this heat is reabsorbed.

4. In rabbit nerve at 0° C there is a third phase of prolonged cooling which results in the preparation becoming about 30 $\mu$° C colder than its initial temperature within 10 sec after the stimulus.

5. The positive heat in mammalian nerve and crustacean nerve is augmented by replacing the sodium of the bathing solution with barium.

6. The negative heat in crustacean nerve seems to be augmented by replacing the sodium with lithium.

7. Analysis suggests that the positive heat is associated with the rising phase of the action potential.

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