Heat Production in Nerve and Electric Organ

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Our knowledge of the physical events associated with the passage of an action potential along a nerve probably represents the most precise and detailed information available for any biological process. Yet there is an almost complete absence of knowledge of the specific biochemical changes involved or of the energetics of the action currents. Measurement of thermal changes during such activity can furnish information which may provide clues as to the sorts of chemical processes which may be occurring; while studies on the electric organs of fishes in which the electrical work available can be extracted into an external system, permit thermal and electrical energy changes to be measured under widely differing conditions of loading and so give information on the dynamics of the energy source.

The resting heat of nerve, and the heat of the recovery processes, have been studied in detail with an accuracy and precision which cannot at present be improved upon (Feng, 1936). Valuable information could certainly be obtained from further work on these events particularly from comparisons under aerobic and anaerobic conditions. A small burst of heat occurs before the recovery begins, amounting to only 1 or 2 per cent of the recovery heat. The experiments of Beresina and Feng (1933) showed this early outburst of heat in crab nerve at 16.5°C. to be $0.7 \times 10^{-6}$ cal./gm./impulse, and Feng (1936) estimated that the corresponding value for frog's medullated nerve at the same temperature would be $0.067 \times 10^{-4}$ cal./gm. But the slow response of the apparatus made it uncertain whether this heat was really an "initial" heat or only a first part of the recovery process.

The experiments I wish to review are those which have been carried out in recent years, using more rapid recording techniques. Thermopiles are still the most suitable detectors of temperature changes for most of the experiments described, although thermistors were used in some instances. The use of rapid stable galvanometers with photoelectric amplification of galvanometer movement, and cathode rayoscillographic recording, provides considerable improvement in time resolution. It must be remembered that, in general, improvement in time resolution and (temperature) sensitivity move in opposite directions, so that our sensitivity is no greater than it was in the older work referred to; but the time resolution then was poor. With the present equipment the time resolution has been improved so that we can resolve the heat
production to within 20 msec. blocks of time, with a temperature resolution of better than 10⁻⁶°C. using the special thermopiles and galvanometers developed for this work (Abbott, Hill, and Howarth, 1958). In the studies of the initial events in nerve excitation, the changes at 0°C. were followed for about \( \frac{3}{2} \) second. The positive recovery heat begins later than this and was not studied.

The choice of nerve is important. Since the action potential and the heat are thought to be essentially membrane events, and since the thermal changes are very small, it is necessary to have as much active membrane area as possible per unit length of nerve. This criterion argues for the use (a) of a non-medullated nerve, and (b) of nerves with as many small fibres as possible.

The leg nerve of the spider crab *Maia squinado* was used. In this nerve there are a few large fibres, but the majority are very small. A typical distribution among 10,000 fibres would be: 123 greater than 10 \( \mu \); 801 between 4 and 10 \( \mu \); 1790 between 2 and 4 \( \mu \), and 7286 less than 2 \( \mu \). This distribution, although giving a large surface-to-volume ratio (of probably about 10,000) produced, in fact, a great complication for two reasons: (a) from such a distribution it is very difficult to estimate a true value for the membrane area involved, (b) because with such a distribution of sizes the speed with which the action potential spreads along the fibre will vary widely with fibre diameter. There is no clear evidence on the relation between speed of propagation and fibre diameter in such small fibres—almost all the relevant studies of conduction speed have been made on larger fibres (20 \( \mu \) diameter and upwards). But in order to estimate the thermal changes at a given point in the membrane, it is necessary to correct for the different speeds of conduction. The complexity of the fibre size distribution forced the final analysis of heat production in nerve to be one of trial and error.

**Experiments on Nerve Heat Production**

The experimental procedures have been described in detail (Abbott, Hill, and Howarth, 1958) and will not be elaborated here. The leg nerves from *Maia* were drawn out, not dissected. A leg is removed and cracked at one of the joints. When the portions of the leg are gently pulled apart the nerve slips out through the upper part of the limb (Furusawa, 1929). Half a dozen nerves, 7 cm. long were used, with 4.4 cm. of nerve lying on the thermopile element. This allowed several millimeters of nerve to lie between the last thermal junction and the stimulating electrodes, and eliminated any immediate interference from the stimulus (whose energy is much greater than the heat produced by the nerve). The temperature changes were measured following a single impulse in the nerve at 0°C., and recorded on a cathode ray oscilloscope.
The results of a single impulse were immediately apparent and surprising. The traces showed clearly and without any analysis, a rapid increase in temperature (and so of heat production) followed by a drop in temperature almost back to the base-line (Fig. 1). This drop in temperature was far more rapid than could be explained by heat losses and implied a reabsorption of much of the heat produced. Since apparent absorption of heat, or "negative heat" can easily occur during thermal measurements by such artefacts as an inexcitable outer layer of tissue, the results obtained were treated with considerable suspicion. However, continued repetition of the experiments under various experimental conditions (e.g. repositioning the nerves) always gave the same results, and forced the conclusion that the initial rapid burst of heat was followed by a slower negative heat phase.

Analysis of the results allowing for heat loss, inertial delays, and velocity of propagation over the nerve showed that at 0°C. the time course of the heat production at a point could best be described in Fig. 2a: a positive burst of heat of $14 \times 10^{-6}$ cal./gm. occurs in the first 20 msec. after the stimulus, and $12 \times 10^{-6}$ cal./gm. of this is then reabsorbed over the next 100 to 200 msec.

The inertia of the system limits the analysis to blocks of heat lasting 20 msec., so that although it is not possible to identify the positive heat with action potential (which lasts more than 20 msec. in the crab leg at 0°C.), it is certain that this heat is associated with initial events rather than recovery.

No direct evidence is available on the origin of the heats, but apart from the obvious suggestion that unknown exothermic reactions are followed by
endothermic ones, there are certain physical possibilities. Among these are
the following:
(a) The Joule heat of the currents which flow during the impulse.
(b) The heat associated with the discharging and recharging of the capacity
of the excitable membrane.
(c) The heat associated with the mixing of Na and K ions between axoplasm
and outside fluid.
Each of the three alternatives must be considered.
(a) The value for the Joule heat was calculated by Hill (1921) but more
precisely by Hodgkin (1951). The values which can be estimated for the dis-
sipation in crab nerve are of the same order as the positive heat measured.

![Figure 2. Positive and negative heat during and after the passage of an impulse. The positive heat is assumed to occur in a single 20 msec. block, the negative heat in successive 20 msec. blocks after it. The figures show analysis assuming that fibre diameter and velocity of propagation is correlated (α) for $v d^{1/2}$, (β) for $v d^{2/4}$, and (γ) for $v d^{3/4}$. The relation (α) is the most satisfactory. (Modified from Abbott, Hill, and Howarth, 1958).]

But in the discussion of the results on crab nerve (Abbott, Hill, and Howarth, 1958) this heat was discounted for a specific reason: on the present view of nerve activity the power driving the action current is derived solely from the free energy associated with two electrolytes separated by the nerve mem-
brane. Thus a concentration cell exists from which currents can be obtained. The currents which flow would produce Joule heat, but the driving energy would come from the heat of the system. Since the currents flow through the fluid surrounding the nerve fibres the thermopile measures the sum of the
Joule heat and the expected heat decrease of the source, and this sum is expected to be zero apart from the heat of mixing of Na and K ions.

An extension of this argument was discussed by Bernstein and Tschermak (1906) showing that if the electrical work were dissipated in an external load, there would be cooling of the source equivalent to the external work. Bernstein and Tschermak (1906) carried out experiments on electric organs which supported this concept, but experiments are described below in which this is reinvestigated. The results are such that it is doubtful if the Joule heat in nerve can in fact be dismissed as completely as at first believed.

(b) The condenser theory is very attractive because the energies involved are of the right size and both positive and negative heats would be possible. The energy liberated from the capacitor during discharge could heat the system, while the recharge energy might be taken from the heat of the system. The rate of energy liberation during discharge is \( CV \frac{dV}{dt} \) in which \( C \) is capacity and \( V \) is the voltage across the condenser. In a \( Maia \) nerve a surface-to-volume ratio of about 10,000 and a membrane capacity of about 1.2 \( \mu \)F/cm.\(^2\) can be expected. During an action potential the membrane is discharged from its resting value of probably \(-80\) mv. with an energy release of \( 9.2 \times 10^{-4} \) cal./gm. nerve, and if there is an overshoot of \(+25\) mv. this would mean an absorption of \( 0.9 \times 10^{-4} \) cal./gm. So up to the peak of the action potential there could be about \( 8.3 \times 10^{-4} \) cal./gm. liberated. During the falling phase this heat would be reabsorbed; which represents an appreciable fraction of the actual positive heat.

The difficulties are that there is no evidence for such thermal changes, particularly in the recharging process, and the time relationships do not seem to fit. The negative phase lasts for more than 100 msec. and it is unlikely that the action potential lasts that long. The shape of the falling phase of the action potential in the small nerve fibres is not known, or whether a complex after-potential exists. But the evidence suggests that the action potential is over within 20 msec. at \(0°C\). This means that any thermal changes due to the discharging and recharging of the capacity would be complete within the resolution time of the equipment and could not be detected. But it means that further experiments are needed with even more rapid equipment to analyze thermal events within the duration of the action potential.

(c) The heat of mixing results from the fact that during each impulse Na ions enter the nerve and mix into the axoplasm which has a high concentration of K ions, while some K ions move out into the high Na concentration of the external fluid. Heats of mixing occur in both cases, and account for a considerable portion of the initial positive heat produced. It was shown (Abbott, Hill, and Howarth, 1958) that the K lost in a single impulse at \(2°C\) is about \(7.6 \times 10^{-4}\) mol/gm. nerve or about \(9 \times 10^{-8}\) mol/gm. at \(0°C\). The heat of mixing of Na or K ions between 0.6 m solutions of NaCl and KCl...
is 35 cal./mol for each ion (see reference and acknowledgment, Abbott, Hill, and Howarth, 1958, p. 166). The exchange per impulse would thus account for about $6 \times 10^{-6}$ cal./gm./impulse, or half the positive heat produced.

The negative heat might be associated with the reversal of this process; i.e., with the separation of the ions. This seems unlikely as the restoration is believed to be a much slower process involving aerobic recovery metabolism (Hodgkin and Keynes, 1955). But it may be that the negative heat is an early anaerobic phase of recovery as has been reported by D. K. Hill (1940) in skeletal muscle.

The possibility that the negative heat might merely represent temporary cessation of the resting heat was considered (Abbott, Hill, and Howarth, 1958). The resting heat at 0°C. extrapolated from the results of Beresina and Feng (1933) is about $2.8 \times 10^{-4}$ cal./gm. x sec. The maximum rate of negative heat production is at least $10 \times 10^{-4}$ cal./gm. x sec., or four times greater than could result from cessation of resting heat.

More recent experiments by Hill and Howarth (1958) have reexamined the initial heat changes in frog medullated nerves. The heat production in these nerves is less than in crab nerve—according to Feng (1936) the resting heat is three times smaller, while the initial heat was thought to be only $0.26 \times 10^{-4}$ cal./gm./impulse at 0°C. Hill and Howarth were able to measure the initial heat production in frog's sciatic nerve at 0°C. in a tetanus but not after a single shock. The positive heat per impulse was estimated as $0.8 \times 10^{-4}$ cal./gm./impulse but there was no indication of any negative heat. This may mean that no absorption occurs but it is also possible that the events are too rapid to record with existing equipment, and all that is seen is the net heat. The magnitude of the positive heat here is of interest, for it is generally assumed that activity occurs only at the nodal region, and the nodal material constitutes about $1.6 \times 10^{-5}$ of the weight of the nerve. If heat production is confined to the membrane it would require the enormously high rate of heat production of 0.05 cal./gm. nodal material. This is fifteen times the heat produced per gram of muscle in a twitch and twenty times the corresponding value estimated per gram of crab nerve membrane. Hill and Howarth suggest that this high value for medullated nerve may indicate that the metabolic activity may spread further than just at the nodes. It is by no means certain, however, that the heat production measured in nerve is limited to the membrane. The results to be described below on electric organs show that the simple Bernstein hypothesis of heat production from a concentration cell is not obeyed, and so the thermal processes may involve much more of the cell than just the membrane. The action potential in the frog's nerve rises much more rapidly and the duration is shorter than in the crab nerve at the same temperature; this may reflect differences in the gearing of the metabolic processes during activity.
Much more information would be readily available if a long nerve consisting of very small fibres of approximately uniform calibre were available. One such nerve is the olfactory nerve of the pike, but this is too short; and an alternative is a cat or dog vagus nerve, although this has a sheath which must be removed and is not very uniform in fibre size. The influence of the heat of mixing could also be readily tested if some other ion such as lithium replaced Na in the bathing fluid. Unfortunately the crab nerve seems to be blocked rapidly albeit reversibly in a LiCl bathing solution.

Studies on Electric Organs

An attempt has been made to study the assumptions about the Joule heat made in paragraph (a) using the electric organ of the ray Torpedo. A piece of electric organ from the wing of the ray can be separated out with its nerve supply intact. If the organ is in air with plates of Ag-AgCl in contact with the two body surfaces, then the electrical discharges can be led out from the organ into an external resistor. This removes the electrical work from the tissue, allows the external electrical work to be measured, and the thermal changes to be recorded by means of a thermistor or thermopile inserted into the piece of organ.

The preliminary results (Abbott, Aubert, and Fessard, 1958) in Fig. 3 show several interesting properties both in single shocks and repeated stimulation. There is always both positive heat production of short duration and negative heat which is long lasting. As indicated above the negative heat may represent early anaerobic recovery. But the positive heat does not behave in the manner which would be expected from the consideration of a simple concentration cell.

In these experiments the temperature changes were followed for longer periods than in the nerve experiments (up to 20 seconds). So the slow positive recovery heat is very apparent, especially in the case of multiple stimuli. The initial positive burst of heat and the slower absorption of heat (the drop in the curve) can be seen in all cases. The figure shows the heat production of the organ when loaded either with a 5 ohm resistance or with an infinite resistance, both after a single stimulus and after 11 rapid shocks. In each case the heat absorbed is independent of the load (i.e. of external work done), and seems to depend only on the number of impulses at constant temperature. Its value is between 50 and 100 μ cal./gm./impulse and at room temperature can last up to 15 seconds. On the other hand the positive heat produced varies considerably with the external load. Contrary to the suggested theory the heat produced is greater when the current is dissipated within the tissue than when it is on open circuit (100 μ cal./gm. with the organ short circuited; 20 to 50 μ cal./gm. on open circuit; about 80 μ cal./gm. when a load of 20 ohms is used).
Now the current which flows through the external load must, of course, also pass through the source of current. This source has an internal resistance and so the current passing will heat the tissue. The internal resistance of the tissue was found to be between 20 and 40 ohms at the peak of the discharge and under the conditions used. When the organ was matched by an external resistance equal to its own internal resistance, the external work was maximal, at about 100 μcal./gm., while the heat liberated in the organ was about 80 μcal./gm. Thus in this case, the heat is not negligible compared with the work, but there is no sign of the cooling which might have been anticipated.

The value most relevant to the nerve (where the action currents run in fluid remaining between the nerve fibres) is the sum of external and internal heats. It can be seen that neither in the short circuited condition nor in the

![Graph showing heat production over time](image)
matched condition does the total heat (internal plus external) become negligible. There is in some cases a minimum value for the positive heat in the organ, and this occurs when the organ is connected to a resistance somewhat higher than that for which maximum work is done. But there also the summated heat is quite considerable. So there seems to be little evidence in favor of an internal cooling equal to electrical work performed externally.

Probably the most striking curve is that shown for the single impulse on open circuit (Fig. 3, \( n = 1, R = \infty \)) in which the positive heat is small and the negative heat cools the organ below the equilibration temperature, i.e., the result is an absorption of heat from the surroundings with no external work being done. The results are not actually in disagreement with those of Bernstein and Tschermak (1906), for with their slow instruments they would register the slow negative phase but not the early rapid positive events.

From these results no certain conclusions can yet be drawn. It seems that the results on the crab nerve should be reconsidered since it is doubtful if the considerations by which the Joule heat was discounted are valid. Similar experiments must be performed on the electric organ of the electric eel, in which the discharge is shorter in duration, and consists of a spike with reversal of the membrane potential rather than the slower end-plate potential wave form of the Torpedo discharge (Keynes, 1957). These results could be compared more directly with the thermal changes which occur as a result of the spike discharges in the crab nerve. And it is also obvious that measurements should be made on the level of the various phosphate compounds during activity. But it is in any case difficult to find a simple explanation which can conform to Bernstein's hypothesis.

REFERENCES