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THE OXYGEN CONSUMPTION OF MAMMALIAN NON-MYELINATED NERVE FIBRES AT REST AND DURING ACTIVITY

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SUMMARY

1. A study has been made of the oxygen consumption of non-myelinated nerve fibres of rabbit desheathed cervical vagus nerves at rest and during activity.

2. The average resting oxygen consumption (Q_r) was 0·0924 μmole/g. min at 21° C. Stimulation for 1–3 min at 3/sec caused an extra oxygen consumption (Q_s) of 816 p-mole/g.shock.

3. When the frequency of stimulation was increased, to 10/sec and 30/sec, Q_s fell. When the frequency was decreased, to 1/sec and 0·3/sec, Q_s increased slightly.

4. When the temperature was decreased, Q_r fell; when the temperature was increased, Q_s also increased. Temperature similarly affected Q_s with high frequencies of stimulation, but had relatively little effect on Q_s at low frequencies of stimulation.

5. An isolated single shock seemed to produce an increase in oxygen consumption of about 1200 p-mole/g, and this value was largely independent of temperature.

6. When part of the sodium in the Locke solution was replaced by barium, Q_r decreased (by 12 %) whereas Q_s increased (by 87 %).

7. Veratrine (1 μg/ml.) increased both Q_r (by 142 %) and Q_s (by 361 %).

8. Acetylcholine (1·7 mM) increased Q_r (by 32 %).

9. When nerves were transferred to potassium-free solutions there was little change in Q_r, and Q_s fell slightly (by 8 %).

10. When the potassium concentration in the Locke solution was increased 4-fold, Q_r increased (by 27 %).

11. Salicylate (1–10 mM) increased Q_r (by 24 %) and abolished Q_s.

12. When the sodium of Locke solution was replaced by lithium, Q_r decreased (by 19 %) and Q_s was abolished.

13. In sodium-Locke solution ouabain (100 μM) decreased Q_r (by 26 %) and abolished Q_s. In lithium-Locke solution ouabain also decreased Q_r (by 28 %).

14. All or nearly all of the oxygen consumed at rest or during activity seemed to be used to pump potassium ions into, and sodium ions out of, the axoplasm.

15. The K/O_2 ratio during pumping was about 5·0.

INTRODUCTION

Because mammalian non-myelinated (C) fibres are the smallest nerve fibres in the body, and consequently have the largest ratio of surface membrane to nerve volume, they are eminently suitable for studying both the ionic and metabolic aspects of nervous activity (Ritchie & Straub, 1957; Greengard & Straub, 1959*b*, 1962; Keynes & Ritchie, 1965*a*). Recently, the heat production that accompanies a single impulse in these fibres has been examined (Abbott, Howarth & Ritchie, 1965; Keynes & Ritchie, 1965*b*; Howarth, Keynes & Ritchie, 1965). There is an initial heat that consists, as in crab nerve (Abbott, Hill & Howarth, 1958), of two phases: an evolution of heat of about 5–10 μcal/g, followed by a rapid reabsorption of most of it. The time courses of these two initial phases, the positive and negative initial heats, seem to correspond with the depolarization and repolarization phases of the action potential respectively (Howarth *et al.* 1965). The third and largest phase of heat production occurs during the 5–10 min period following stimulation; this is the recovery heat, which in mammalian C fibres at about 20° C amounts to about 50 μcal/g impulse (Howarth, Keynes & Ritchie, 1966). The recovery heat is presumably largely devoted to restoring the ionic imbalance across the nerve membrane that has resulted from the passage of the nerve impulse. It ought therefore (e.g. Hill, 1929; Brink, Bronk, Carlson & Connelly, 1952) to be quantitatively related to the amount of oxygen consumed during recovery. The oxygen consumption of nerve has already been investigated in detail (Meyerhof & Schultz, 1929; Fenn, 1927; Gerard, 1927, 1932; Brink *et al.* 1952; Connelly, 1962; Baker, 1965). But these experiments were carried out either on amphibian myelinated, or on crustacean non-myelinated, fibres and so cannot be readily compared with experiments on the recovery heat in mammalian C fibres. Furthermore, long periods of stimulation (20–40 min) were usually used—particularly in the studies on myelinated fibres in which the amount of active membrane is relatively small because it is restricted to the nodes of Ranvier; some degree of fatigue may therefore have occurred (see Hill, 1929). The present experiments were done to determine the

amount of extra oxygen consumed by mammalian C fibres in response to a relatively short period of stimulation and to compare it with known recovery heat production of these fibres (Howarth *et al.* 1966).

METHODS

Preparation. Large rabbits (about 5 kg) were killed by injection of air into an ear vein and both cervical vagi rapidly removed; this procedure took about 5 min. A length of about 80 mm of vagus nerve was then desheathed under a binocular microscope (× 40) and mounted in a perfusion chamber.

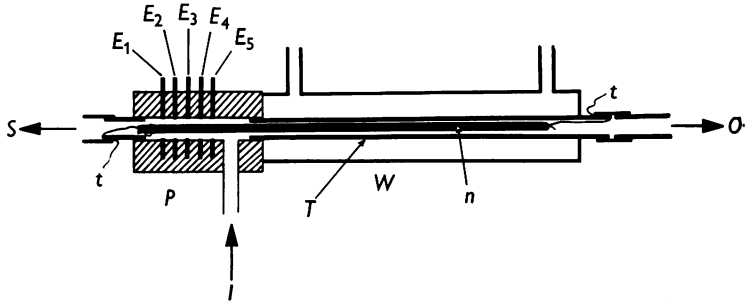

Fig. 1. Diagram of perfusion chamber. For detailed description, see text.

Perfusion chamber. Essentially, each of the several different chambers that were used (Fig. 1) consisted of a length of about 80 mm of fine stainless steel or platinum tubing (*T*) whose internal diameter was 0·65–0·75 mm. This tube fitted tightly into a slightly larger tube made by drilling a Perspex block (*P*) in which five sheets of platinum (E_1–E_5), 0·25 mm thick, had previously been embedded to serve as recording and stimulating electrodes. The nerve was pulled through the chamber, which was just wide enough to accommodate it, until about 10 mm of it lay on the electrode assembly while 70 mm remained in the metal chamber; it was held in position by fine threads (*t*) at either end. The metal part of the chamber was surrounded by a water jacket (*W*) so that the temperature of the nerve could be maintained at some chosen value.

The chamber was perfused at the side (through *I*) by a motor-driven syringe at a low rate usually 0·1–0·2 ml./min. Three-quarters of the perfusion fluid escaped, at *O*, through a short length of oxygen-impervious fine tubing and flowed past an oxygen cathode. The remaining quarter was sucked the other way past the stimulating electrodes by a second motor-driven syringe, through a second outflow (*S*) and discarded. In some experiments the inlet tube (*I*) was connected to a reservoir; the motor-driven syringe originally connected to *I* was then connected to *O* and used to suck the perfusion fluid through *O* and over the oxygen cathode instead of driving it forward. This latter method gave essentially the same results as the first.

Oxygen cathode. Because the metal chamber was impervious to oxygen, and all connexions between it and the oxygen cathode were oxygen-tight, any change in oxygen consumption by the tissue in the chamber necessarily led to a fall in the oxygen content of the fluid perfusing the oxygen cathode. The oxygen cathode (Carlson, Brink & Bronk, 1950; Connelly, Bronk & Brink, 1953; Davies, 1962) is a fine platinum electrode surrounded by an annular silver electrode. The current between the two electrodes, with the platinum about 0·65 V negative to the silver, is proportional to the oxygen concentration in the perfusing fluid. In the present experiments a commercially available oxygen cathode (Radiometer) was used. Both electrodes were protected from the external solution whose

oxygen content was to be determined by a thin, oxygen-permeable, polypropylene or polyethylene membrane. The current was recorded on a moving-paper ink recorder. The temperature of the oxygen cathode was maintained at the same temperature as the nerve chamber.

Calibration. The oxygen cathode was calibrated in each experiment by noting the deflexion produced when the solution perfusing the oxygen cathode was changed from an air-equilibrated Locke solution to a Locke solution (containing extra dextrose) to which a small amount of (+)-glucose oxidase had been added. The latter procedure reduced the oxygen content of the Locke solution to practically zero, as indicated by the fact that the oxygen current through the platinum electrode was scarcely detectable. The oxygen content of the air-equilibrated Locke solution at a given temperature was determined from tables in the *Handbook of Chemistry and Physics* (1962). No correction was applied to allow for the difference between the solubility of oxygen in distilled water and that in physiological salt solution; all values of oxygen consumption in the present paper are therefore probably about 5 % too large (Larrabee, 1958).

The guard-suck. As illustrated in Fig. 1, fluid that had been in contact with the stimulating electrodes was excluded from the oxygen cathode. This arrangement was necessary because the platinum stimulating electrodes (usually E_1 and E_2) themselves acted as oxygen electrodes, and the large current used for stimulating C fibres could remove a large fraction

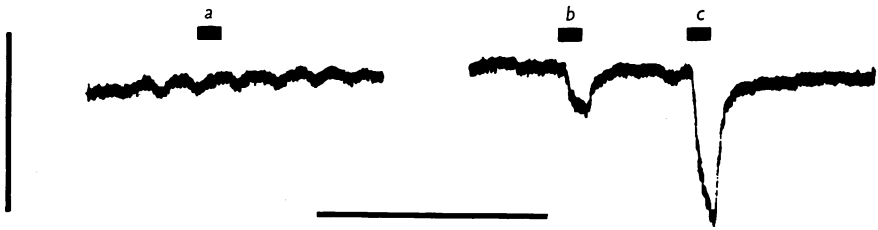

Fig. 2. The effect of the stimulating current on the oxygen content of the fluid reaching the oxygen cathode. The nerve in the chamber was inexcitable because it was being perfused by KCl–Locke solution. The left-hand record was taken with the usual perfusing arrangement, i.e. the inflow was at *I* (0·192 ml./min) and a fraction (0·042 ml.) was sucked through *O*. The right-hand record was taken with the reversed arrangement (see text). During the periods indicated by the solid bars above each record, shocks at a rate of 10/sec were applied through E_1 and E_2 at the same (*a* and *b*) current intensity used throughout the experiment to excite C fibres, and at 3 times (*c*) this intensity. The vertical bar represents a change in oxygen concentration of 10 μM; the horizontal bar represents 20 min. The temperature was 20° C. In this and all subsequent records the true orientation of the records has been preserved; a slight upward drift of the base line is evident in this particular record.

of the oxygen from the perfusing solution. This is illustrated by the two records of Fig. 2, which were obtained with a nerve that had been rendered inexcitable by perfusing it with a modified Locke solution in which all the NaCl had been replaced by KCl. In both the right- and left-hand records shocks were applied to the nerve at the rate of 10/sec for the 2 min periods indicated by the bars above the records. In the left-hand record (*a*) the usual perfusing arrangement (Fig. 1) was used. In the right-hand record (*b*, *c*) the connexions to the syringes were interchanged, so that perfusion was through *S* instead of *I* and the guard-suck solution was out through *I* instead of *S*. With this arrangement, fluid is passed over the stimulating electrodes and thence to the oxygen cathode; it can be seen that the application of electrical shocks, at two different intensities, caused a marked fall in the oxygen

content of the perfusion fluid. This response was completely absent when the usual perfusion arrangement was used (left-hand record).

The right-hand record of Fig. 2 can be used to estimate the volume of fluid in the nerve chamber. The interval between the onset of stimulation and the first sign of a fall in the oxygen content of the perfusion fluid was about 0·60 min. In this experiment the inflow at S was 0·192 ml./min and the outflow at I was 0·042 ml./min. Thus, the volume between E_1 and the oxygen cathode was $0{\cdot}15 \times 0{\cdot}60 = 0{\cdot}09$ ml. Because the diameter of tube T was 0·65 mm the volume of the portion containing the 70 mm of nerve can be calculated to be 0·02 ml. The bulk of the 0·09 ml. must therefore consist of the volume between E_1 and the end of tube T that is embedded in the Perspex, together with the dead space between the other end of the nerve and the oxygen cathode. This dead space can be determined from the results of the 'stop-flow' experiments (Fig. 3*d*) that were done to estimate the resting oxygen consumption. In such experiments the perfusion rate was 0·162 ml., of which 0·042 ml. was sucked through S; the deflexion signalling the arrival at the oxygen cathode of the oxygen-deficient solution that had been in contact with the nerve occurred about 0·35 min after resumption of flow. The volume between the end of the nerve and the oxygen cathode was $0{\cdot}12 \times 0{\cdot}35 = 0{\cdot}04$ ml.

Stimulation. The C fibres were excited by supramaximal shocks of 0·5 msec duration, applied through E_1 and E_2. The compound action potential was recorded through E_4 and E_5 whenever the possibility existed that the experimental procedure had blocked conduction, i.e. when using drugs. However, the action potential was not routinely recorded, in order that as long a length of nerve as possible could be mounted in the metal part of the chamber so as to get the greatest oxygen consumption.

Solutions. The composition of the Locke solution was (mM): NaCl, 154; KCl, 5·6; $CaCl_2$, 2·2; tris (hydroxymethyl) aminomethane buffer (pH 7·2), 2·5; dextrose, 5. Modifications of this solution are indicated at the appropriate places in the text. All perfusing solutions were equilibrated with atmospheric air at approximately the temperature at which the experiment was carried out; none of the procedures used in the present experiments greatly reduced the oxygen content of these solutions.

Wherever possible, means ± their standard errors are given.

RESULTS

The resting oxygen consumption

The resting oxygen consumption was estimated in two ways. In the first method the normal flow rate (v_1) was suddenly increased (usually 10-fold) to a new rate (v_2). Because the fluid was then passing more rapidly over the nerve, the oxygen concentration of the Locke solution leaving the chamber rose from C_1 to a higher value C_2. The oxygen consumption determined by this 'increase-flow' method, which is illustrated in Fig. 3*a*, was thus $[(C_2 - C_1)v_1v_2]/v_2 - v_1$. An allowance had usually to be made for the artifact caused by the change of flow itself in the oxygen recording system. This artifact was usually much smaller than in the experiment illustrated in Fig. 3*b*, and was often negligible. The artifact oxygen consumption could have been caused by rusting either of the stainless-steel chamber containing the nerve or of the stainless-steel chamber containing the oxygen cathode; alternatively, it could have been caused by bacterial action in the system. Whatever its cause, tests at the beginning

and at the end of each experiment showed that the artifact was relatively constant, and so it seemed justifiable to make a simple correction to the experimental results.

In the second method the perfusion of the nerve was stopped for a period of between 1 and 10 min; then, when the perfusion was restarted, the deficit in the oxygen content of the fluid passing over the oxygen cathode was noted. This 'stop-flow' method is also illustrated in Fig. 3. The

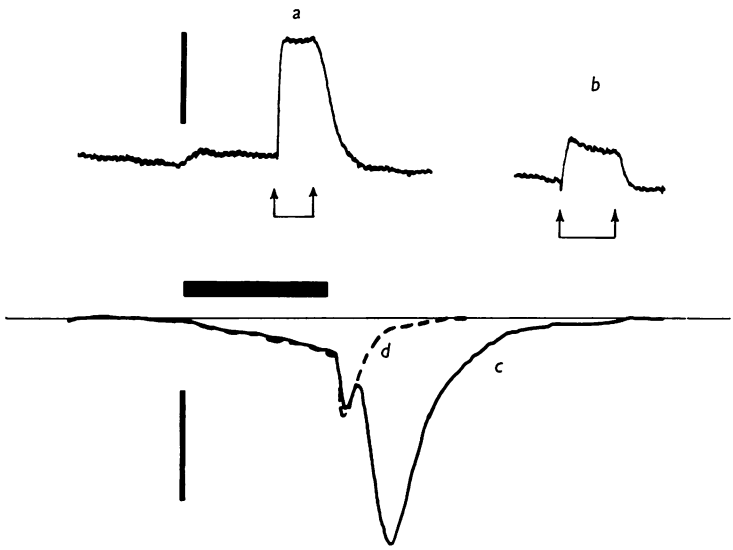

Fig. 3. Upper records. The 'increase-flow' method of determining the resting oxygen consumption of rabbit desheathed vagus nerve. In the left-hand record (*a*) the flow was increased (between the arrows) from 0·0764 to 0·764 ml./min; in the right-hand record (*b*) the experiment was repeated after the nerve had been removed. Lower records. The 'stop–flow' method of determining resting oxygen consumption. Record *c* was obtained when the flow through the nerve chamber (0·150 ml./min) was stopped for a period of 2 min, indicated by the solid bar; record *d* was obtained in a similar experimental procedure after the nerve had been removed from the chamber. The vertical calibration represents an oxygen concentration change of 20 μM (upper records) and 10 μM (lower records). The solid bar marking the stoppage of the flow indicates 2 min in the bottom set of records and 7½ min in the upper set. The temperature in the upper and lower set, respectively, were 22·6 and 21·6° C, and the weights of the preparations were 28·6 (two nerves together) and 14·9 mg, respectively.

oxygen consumed during the period that the fluid around the nerve was stagnant is taken to be equal to the perfusion flow rate times the area under record *c*, minus the area of the artifact produced by similarily stopping the flow in the chamber after the nerve had been removed (Fig. 3*d*).

Both methods gave essentially the same answer; for example, in three preparations where the two methods were compared directly, the oxygen

consumption with the 'increase-flow' method was 97% of that with the 'stop-flow' method. The results with the two methods were therefore combined in Table 1.

At 21° C the resting oxygen consumption of the rabbit vagus nerve was found to be 0·0924 μmole/g wet wt. min. Table 1 also shows how the resting oxygen consumption of the rabbit vagus nerve varies with temperature. Clearly, the Q_{10} of the oxygen consumption decreases as the temperature increases: between 20° and 30° C it was 1·97, whereas a slight extrapolation indicates that between 10° and 20° C it was 3·09. A similar increase in the

TABLE 1. The resting oxygen consumption (Q_r), and extra oxygen consumption with activity (Q_s), of rabbit desheathed vagus nerve at different temperatures. In this and subsequent tables the mean ± S.E. is given, the number of observations being in parentheses below each value

Temperature (° C)	11·6	14·8	21·0	28·0	35·8
Resting oxygen consumption, Q_r (μmole/g.min)	0·0349 ± 0·0033 (4)	0·0536 ± 0·0054 (5)	0·0924 ± 0·0043 (24)	0·1469 ± 0·0031 (12)	0·2407 ± 0·0195 (4)
Extra oxygen consumed per shock, Q_s, at 3/sec (p-mole/g. shock)	—	306 ± 26 (6)	816 ± 61 (30)	843 ± 95 (12)	—
Time after end of 2 min period of stimulation at 3/sec by which phase of extra oxygen consumption is over (min)	—	10·0 ± 1·0 (12)	8·4 ± 0·3 (49)	5·9 ± 0·3 (21)	—

temperature-dependence at lower temperatures is also observed in C fibres for the potassium efflux (Keynes & Ritchie, 1965*a*) and for the conduction velocity of the action potential (J. V. Howarth, R. D. Keynes & J. M. Ritchie, unpublished).

Unlike the stimulated oxygen consumption discussed below, the resting oxygen consumption of rabbit vagus nerve is not much different from that of other nerves. It is about 4 times that of frog myelinated fibres (Gerard, 1927; Connelly, 1959), 1½ times that of the non-myelinated fibres of *Maia* nerve (Baker, 1965), and only somewhat greater than the resting oxygen consumption of various mammalian nerves studied by Larrabee & Bronk (1952) at body temperature.

The extra oxygen uptake with activity

Figure 4 illustrates the effect of stimulation on the oxygen consumption of the non-myelinated fibres in the rabbit vagus nerve at about 20° C. Less than a minute after starting to stimulate the C fibres at a rate of 3 shocks/sec, the oxygen content of the solution leaving the nerve chamber fell and did not return to its prestimulation value until several minutes after the end of stimulation (Fig. 4*a*, *d*). When the experiment was repeated

at a lower frequency of stimulation, 1/sec, the response was less (Fig. 4*b*); at a higher frequency, 10/sec, the response was greater (Fig. 4*c*). When the intensity of the shocks was sufficient to excite the relatively few myelinated fibres in the vagus, but not the non-myelinated, no such responses were detected. The area under the oxygen-content/time curve, times the flow rate, gives the oxygen consumption of the nerve in response to the period of stimulation.

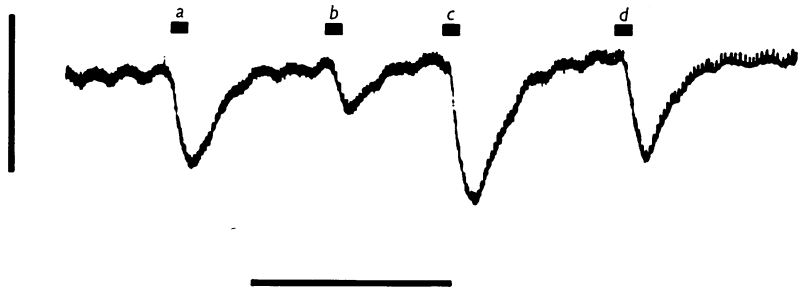

Fig. 4. The effect of stimulation on the oxygen consumption of mammalian non-myelinated nerve fibres. During the 2 min periods indicated by the solid bars above each record the C fibres of a rabbit desheathed vagus nerve were stimulated at a rate of (*a*) 3/sec, (*b*) 1/sec, (*c*) 10/sec, (*d*) 3/sec. The vertical bar represents a change in oxygen concentration of 10 μM; the horizontal bar represents 20 min. The temperature was 21·3° C. The wet weight of the nerve was 24·2 mg.

The 'stop-flow' method described in the previous section to estimate the resting oxygen consumption (Fig. 3*c*, *d*) was also used to estimate the extra oxygen uptake with activity in mammalian C fibres. Record *a* of Fig. 5 shows the oxygen deficit in the fluid perfusing the oxygen cathode after the fluid had been stopped for a period of 2 min, the nerve being left unstimulated. Records 5*b* and *c*, which were taken soon afterwards, show responses that were similar except that during the periods that the flow was stopped the nerve was stimulated at a rate of 0·3 and 3 shocks/sec respectively. Record *d* is a repeat of record *a*, i.e. the nerve was left unstimulated. The oxygen deficit in record *b* is slightly greater than that of records *a* and *d* and represents the extra oxygen taken up by the nerve as a result of the passage of 36 impulses; the oxygen deficit is clearly much greater in record *c*, in which 360 impulses were passed because of the higher frequency of stimulation. There was excellent agreement between the results obtained with the two methods of determining the extra oxygen uptake with stimulation, and the results with both methods were combined in Table 1.

Experiments at a frequency of 3 shocks/sec on thirty different preparations at about 21° C indicated that each shock increased the oxygen consumption of the vagus nerve by 816 ± 61 p-mole/g wet wt. At 14·8° C the

corresponding oxygen consumption was found to be 306 ± 26 p-mole/g.shock; at 28·8° C it was 843 ± 95 p-mole/g.shock (Table 1). The oxygen cost of a single impulse per gram of C fibres is thus much larger than that of myelinated fibres; for example, the extra oxygen consumption of frog myelinated fibres at this temperature (Brink *et al.* 1952, fig. 9) is only about 8·3 p-mole/g.shock, i.e. it is about 100 times less. (However, only the α fibres were stimulated by Brink *et al.* but the results were expressed per gram of nerve; the oxygen consumption expressed per gram of α fibre must thus be somewhat greater than 8·3 p-mole/g.shock.)

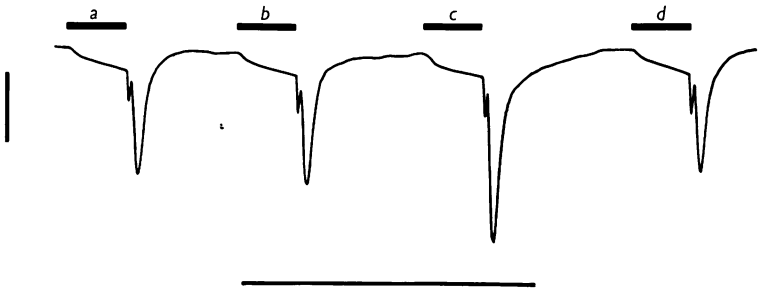

Fig. 5. Tracing of continuous record on ink writer illustrating the 'stop-flow' method of determining the effect of electrical activity on the oxygen consumption of rabbit desheathed vagus nerve. During the 2 min periods indicated by the solid bars above each record the perfusion flow (0·192 ml./min) was stopped. During this time the nerve was left unstimulated (*a*, *d*) or stimulated at a rate of 0·3/sec (*b*) or 3·0/sec (*c*). The vertical bar represents a change in oxygen concentration of 10 μM. The horizontal bar represents 10 min. The nerve temperature was 28·8° C and the weight was 17·5 mg.

In these experiments the extra oxygen consumption tended to fall in successive tests, perhaps because of the prolonged contact with the metal chamber. The results in Table 1 therefore give the results of only the first test made in each fresh preparation. Table 1 also indicates the duration of the period of increased oxygen consumption that follows stimulation. Since this time did not seem to depend obviously on how long the nerve had been mounted in the chamber, *all* results have been included. Clearly, the recovery process lasted longer at lower temperatures. It is difficult, however, to determine an exact Q_{10} for the process because a large part of this time probably represents the time required to flush out the system.

The duration of stimulation. Although at a given frequency of stimulation the oxygen consumption might reasonably be expected to increase in direct proportion to the duration of stimulation, Brink *et al.* (1952) found that the oxygen used per shock by frog myelinated fibres was greater the shorter the period of stimulation was. It seemed worth while therefore, to test this point for C fibres. The results of such a test at 20° C showed that

when the same preparation was stimulated at a frequency of 3/sec for successive periods of 2, 4, 1, 8 and 2 min the extra oxygen consumption per shock was relatively constant, being 606, 632, 556, 571 and 619 p-mole/g, respectively. Thus, the extra oxygen consumed does, in fact, seem to be directly proportional to the period of stimulation for periods up to 8 min in duration; in all the other experiments described here, stimulation was usually for only 2 min and seldom more than 4 min. The contrary finding by Brink *et al.* (1952), of a dependence on the duration of stimulation, seems to indicate that the long periods of stimulation (up to 1 hr) used to demonstrate oxygen consumption changes in myelinated fibres lead to fatigue.

TABLE 2. The extra oxygen consumption per shock at different frequencies of stimulation and at different temperatures in rabbit desheathed vagus nerves. In columns *a* the oxygen consumption is given relative to the oxygen consumption per shock when the nerve is stimulated 3/sec (immediately before and immediately after each test). Each value is the mean and standard error obtained from 8–10 different preparations (except the value for 30/sec, 28·0° C, which is the mean of 5). In columns *b* the relative values have been multiplied by the average values in Table 1 to give the absolute oxygen consumption in the different conditions in p-mole/g.shock

	Extra oxygen consumption per shock					
	14·8° C		21·0° C		28·0° C	
Frequency of stimulation (shocks/sec)	*a* (relative)	*b* (p-mole/g.shock)	*a* (relative)	*b* (p-mole/g.shock	*a* (relative)	*b* (p-mole/g.shock)
0·3	3·75 ± 0·48	1147	1·68 ± 0·36	1370	1·34 ± 0·13	1130
1	2·54 ± 0·65	777	1·66 ± 0·23	1354	1·19 ± 0·07	1106
3	1·00	306	1·00	816	1·00	843
10	0·584 ± 0·122	179	0·637 ± 0·032	520	0·807 ± 0·049	680
30	0·079 ± 0·010	84	0·256 ± 0·031	209	0·268 ± 0·027	226

The frequency of stimulation. The standard frequency of stimulation in these experiments was 3/sec. This frequency was chosen because it gave sufficiently large and consistent responses to measure, without, it was hoped, fatiguing the nerve. However, at 3/sec the nerve was clearly not responding maximally as far as oxygen consumption was concerned. When tests at 21° C at different frequencies of stimulation (e.g. Fig. 4) were interposed between two control responses at the standard frequency, it was found that with a stimulation frequency of 1/sec the oxygen consumed per shock rose to 1·66 ± 0·23 (ten experiments) times the average of the two control values at 3/sec; i.e. it was 1354 p-mole/g.shock; with 0·3/sec it was 1·68 ± 0·36 (eight experiments) times that at 3/sec, being about 1370 p-mole/g.shock. On the other hand increasing the frequency to 10/sec and to 30/sec decreased the oxygen consumption to 520 and 209 p-mole/g.shock respectively (Table 2). A similar decrease in the oxygen

consumption per shock with increasing frequency has also been observed for rat cervical sympathetic ganglia (Larrabee, 1958) and for frog myelinated nerve fibres (Brink *et al.* 1952).

The effect of temperature. The experiments described above were done at about 21° C. Similar experiments were carried out at other temperatures. At high frequencies of stimulation (e.g. 10/sec and 30/sec, Table 2), the oxygen consumption per shock was found to increase markedly, as the temperature went up; it increased about 3-fold between 14·8 and 28° C. However, at low frequencies (0·3/sec and 1/sec) the extra oxygen consumed per shock hardly depended on temperature at all.

It seems clear from Table 2 that as the frequency of stimulation is reduced, or as the temperature is increased, the extra oxygen consumed with stimulation reaches a limiting value of about 1200 p-mole/g.shock (mean of the five highest values in Table 2), which agrees well with the findings both that the extra potassium efflux on stimulation (Keynes & Ritchie, 1965*a*) and the size of the action potential (Ritchie & Straub, 1956) are largely independent of temperature in mammalian C fibres.

The effect of various compounds on the resting and stimulated oxygen consumption

A variety of drugs and procedures (chosen because of their known effects on the electrical, metabolic or thermal activity of nerve fibres) were examined for their effect on the oxygen consumption of C fibres.

Potassium-free solutions. In the absence of potassium in the bathing medium the mechanism for the active extrusion of sodium ions is considerably depressed in invertebrate nerve (Hodgkin & Keynes, 1955). However, there is no clear evidence that C fibres are similarly affected. Ritchie & Straub (1957) did find that potassium-free solutions reduce the post-tetanic hyperpolarization in C fibres indicating that the mechanism for the active extrusion of sodium ions had been depressed. However, Holmes (1962) has subsequently shown that this is not a consistent finding (see also Straub, 1961). Furthermore, the recovery heat in C fibres is not reduced in potassium-free media; indeed it is somewhat enhanced (Howarth *et al.* 1966). The results of experiments to test the effect of the absence of potassium on the oxygen consumption of C fibres are shown in Table 3. Clearly, the absence of potassium has little or no effect on the resting uptake of oxygen, and only slightly reduces the stimulated uptake. Furthermore, the phase of extra oxygen consumption in potassium-free solutions was complete within $7{\cdot}0 \pm 1{\cdot}0$ min (five measurements) after stopping the 2 min period of stimulation at 3/sec, which is not much different from the corresponding value of $8{\cdot}2 \pm 1{\cdot}0$ min in the same nerves in normal Locke solution. Mammalian non-myelinated fibres thus behave differently from

crab non-myelinated fibres (Baker, 1965) and from frog myelinated fibres (Connelly, 1959) in which potassium-free solutions abolish or greatly slow the extra oxygen consumption with activity.

TABLE 3. The effect of various procedures on the resting and stimulated oxygen consumption of rabbit desheathed vagus nerves

Temperature (° C)	Treatment or modification	Ratio (treated/control) Resting	Stimulated
20·7	K_{free}	0·897	0·829
20·7		1·003	—
20·8		—	0·913
20·6		1·090	1·036
20·5		1·003	0·897
Means ± S.E.		0·998 ± 0·041	0·919 ± 0·42
15·0	4 × K	1·375	—
15·7		1·162	—
Mean ± S.E.		1·269 ± 0·107	
20·5	ACh 1·7 mM	1·135	—
14·4		1·949	—
35·8		0·986	—
15·7		1·209	—
Mean ± S.E.		1·320 ± 0·166	
28·9	46·8 mM Ba 92·4 mM Na	0·893	1·394*
28·9		1·025	4·690†
28·8		0·976	0·646†
28·8		1·061	0·811*
14·4		—	1·822†
20·9		0·556	—
21·2		0·744	—
Means ± S.E.		0·876 ± 0·079	1·873 ± 0·735
14·4	Veratrine 1 μg/ml.	1·55	2·52†
14·4		1·61	6·92†
14·4		1·63	1·66†
14·4		2·88	—
14·4		4·41	7·32‡
Means ± S.E.		2·42 ± 0·55	4·61 ± 1·46
20·2	0·2 mM	0·96	0·95
20·2	1·0 mM	1·03	0·99
20·2	1·0 mM	1·187	—§
21·4	Salicylate 1·0 mM	1·014	—§
21·4	10·0 mM	1·237	—§
21·1	10·0 mM	1·482	—§
21·1	10·0 mM	1·182	—§
Mean ± S.E. (last 5 observations)		1·238 ± 0·065	

* Tested at 1/sec.
† Tested at 0·3/sec.
‡ Tested at 0·1/sec.
§ Active oxygen consumption too small to be detected, virtually abolished.

Potassium-rich solutions. Potassium-rich solutions enhance the resting heat production of skeletal muscle (Hill & Howarth, 1957) and presumably also increase its oxygen consumption. Two experiments (Table 3), done to test the effect of a 4-fold increase in the potassium concentration of the

Locke solution, showed that potassium-rich solutions also increase the resting metabolism of the rabbit vagus nerve. Such high concentrations of potassium block conduction; no measurement of the stimulated oxygen consumption could be made therefore.

Acetylcholine. Acetylcholine, like excess potassium ions, depolarizes C fibres (Armett & Ritchie, 1960; Ritchie, 1965). Furthermore, acetylcholine (Lorente de Nó, 1944), or some such acetylated compound (Greengard & Straub, 1962), may well affect the general metabolism of nerve. It is interesting therefore that acetylcholine (1·7 mM), like excess potassium, was found also to enhance the resting oxygen consumption of rabbit vagus nerve (Table 3) by about 32 %. The effect of acetylcholine on the stimulated oxygen consumption was not measured.

Barium. Barium ions markedly prolong the action potential of C fibres (Greengard & Straub, 1959*a*; Keynes & Ritchie, 1965*a*), and greatly augment the heat production after a single stimulus (Abbott *et al.* 1965; Keynes & Ritchie, 1965*b*; J. V. Howarth, R. D. Keynes & J. M. Ritchie, unpublished). Barium ions also decrease the resting and stimulated effluxes of potassium ions (Keynes & Ritchie, 1965*a*). Table 3 shows the effect of stimulation on the oxygen consumption of nerves that had been previously equilibrated in a modified Locke solution in which 40 % of the NaCl had been replaced by 46·8 mM-$BaCl_2$. On the average, the extra oxygen consumption per shock is greatly increased in this solution. This increase, however, was not obtained consistently; in two out of five experiments it was reduced. A likely explanation for the variability lies in the observation (Armett & Ritchie, 1963*b*) that prolonged exposure to, or stimulation in, barium solutions may lead to conduction block. This was confirmed in the present experiments in which the action potential, prolonged by barium, was usually depressed or absent by the end of the period of stimulation required to measure the stimulated oxygen consumption. Thus although the oxygen consumption per impulse may increase, the net oxygen consumption may decrease because of a decrease in the number of conducting fibres. Low frequencies of stimulation (0·3/sec and 1/sec) were therefore used in the experiments with barium (and with veratrine) in order to minimize this effect. In contrast to the variable effects obtained with stimulation, barium had a more consistent effect on the resting oxygen consumption, and reduced it by about 12 % (Table 3).

Veratrine. Veratrine, like barium, greatly increases the duration of the action potential of mammalian C fibres. However, unlike barium, which decreases both the resting and stimulated effluxes of potassium ions, veratrine markedly increases the efflux on stimulation (Keynes & Ritchie, 1965*a*). The present experiments show that veratrine acts like barium and greatly increases the oxygen consumption with nervous activity; however,

unlike barium, which decreases the resting oxygen consumption, veratrine more than doubles it.

Salicylate. Oxidative phosphorylation in various tissues is uncoupled by the salicylate ion (for references, see Hurlbut, 1965). This is why it almost completely abolishes the active transport of sodium and potassium ions in frog nerve fibres (Hurlbut, 1965), which is dependent on an adequate supply of high-energy phosphates. In keeping with these observations, the present experiments (Table 3) show that salicylate in concentrations that do not affect resting metabolism (0·2–1·0 mM) has little or no effect on the stimulated oxygen consumption of C fibres; on the other hand, concentrations that do increase the resting metabolism (1·0–10 mM) almost completely abolish the extra oxygen consumption with electrical activity.

Lithium. Lithium ions can substitute for sodium ions as far as the action potential is concerned. However, they do not seem to be extruded from the

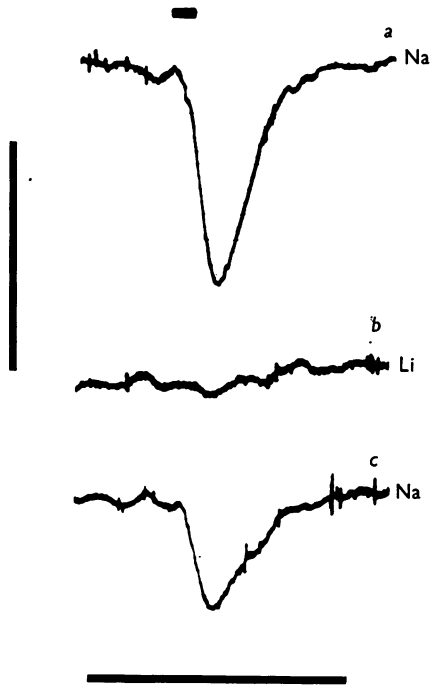

Fig. 6. The effect of lithium on the extra oxygen consumption with electrical activity in rabbit desheathed vagus nerve. In all records during the period indicated by the solid bar the nerve was stimulated at a rate of 3/sec for 2 min: (*a*) in sodium–Locke solution; (*b*) after soaking for about 30 min in lithium–Locke solution; (*c*) about 20 min after the nerve had been returned to sodium–Locke solution. The vertical bar represents change in oxygen concentration of 10 μM; the horizontal line represents a period of 20 min. The temperature of the nerve was 20·5° C. The weight of the nerve was 14·0 mg.

axoplasm during recovery nearly as rapidly as sodium (Ritchie & Straub, 1957). Furthermore, the recovery heat in C fibres is practically abolished in lithium solutions (Howarth *et al.* 1966). Figure 6 shows that if C fibres are equilibrated for 10–30 min in a Locke solution in which all the NaCl has been replaced by LiCl (Fig. 6*b*) stimulation no longer causes a rise in oxygen consumption. Returning the nerve to sodium-Locke solution largely restores the response.

Table 4 shows that replacing sodium by lithium also reduces the resting oxygen consumption, by 19 %.

Ouabain. Caldwell & Keynes (1959) showed that ouabain markedly reduces the sodium efflux in squid giant axons. Ouabain also abolishes the post-tetanic hyperpolarization in C fibres, apparently by greatly slowing down the post-tetanic active reabsorption of potassium ions (Ritchie & Straub, 1957). Not unexpectedly, therefore, it was found in the present experiment that ouabain (100 μM) reduces to negligible amounts the extra oxygen consumption of C fibres on stimulation.

Ouabain also produces a 26 % reduction in the resting oxygen consumption (Table 4).

Ouabain and lithium. The reductions in resting oxygen consumption in nerves exposed separately to ouabain and to lithium solutions can each be readily explained by a turning-off of the basal activity of the mechanism for the active extrusion of sodium ions from nerve fibres. However, the depression of oxygen consumption by ouabain seemed much greater than that with lithium (see Table 4), i.e. ouabain appeared to depress some metabolic mechanism not depressed by substituting lithium ions for sodium. It seemed worth while therefore to test this point directly. Nerves were exposed, unstimulated, for 4–6 hr to lithium-Locke solution. At the end of this time most of the sodium would be expected to have been washed out of the fibres; indeed direct analysis by an atomic absorption spectrometer of four such nerves kept at 27·9° C showed that any sodium present was less than 3 % of normal. However, subsequent exposure of these lithium treated nerves to ouabain still caused a reduction in the resting oxygen consumption, of 27·9 %, i.e. a percentage reduction of similar size to that produced by ouabain alone in nerves in sodium-Locke solution. The converse experiment, replacing the sodium of Locke solution by lithium in a ouabain-poisoned nerve, had only a slight effect on oxygen consumption (an increase of 5·7 %, Table 4).

The action potential. During periods of stimulation in lithium-Locke solution, and in solutions containing ouabain or salicylate, a decrease in the amplitude of the compound-C potential, sometimes to about half its initial size, was often observed. This decrease occurred, presumably, because the mechanism for the active extrusion of sodium ions had been

depressed so that sodium or lithium had accumulated inside the fibres, in some of which, in consequence, conduction block had occurred. Nevertheless, the separation between electrical activity and oxygen consumption remains clear; for the latter was always nearly completely abolished at a time when the electrical activity, though reduced, was still clearly present.

TABLE 4. The effect of ouabain, and of replacing sodium by lithium, on the resting oxygen consumption of a rabbit desheathed vagus nerve. The values in parentheses indicate an increase in oxygen consumption

	Temperature (° C)	Treatment or modification of Locke solution	Effect of treatment on Q_r: Relative (treated/control)	Effect of treatment on Q_r: Absolute decrease (μ mole/g.min)
	21·3	Li	0·692	0·0160
	21·7		0·831	0·0183
	21·7		0·876	0·0168
	35·8		1·064	(−0·0122)
	35·8		0·810	0·0451
	35·8		0·798	0·0577
	27·9		0·627	0·0271
Mean ± S.E.			0·814 ± 0·053	
	35·8	Ouabain 100 μM	0·686	0·0897
	27·9		0·751	0·0348
	27·9		0·889	0·0184
	27·9		0·593	0·0605
	27·9		0·751	0·0294
	27·9		0·907	0·0122
	11·4		0·517	0·0124
	11·4		0·636	0·0125
	11·8		0·887	0·0045
Mean ± S.E.			0·735 ± 0·047	
	27·9	Ouabain 100 μM in Li	0·843	0·0144
	27·9		0·895	0·0087
	27·9		0·750	0·0300
	27·9		0·751	0·0358
	27·9		0·595	0·0350
	27·9		0·635	0·0426
	21·5		0·662	0·0257
	21·5		0·751	0·0188
	21·5		0·651	0·0373
	21·5		0·672	0·0272
Mean ± S.E.			0·721 ± 0·029	
	27·9	Li after Ouabain 100 μM	1·048	(−0·0050)
	27·9		1·054	(−0·0080)
	27·9		1·069	(−0·0061)
Mean ± S.E.			1·057 ± 0·006	

DISCUSSION

In preliminary experiments Howarth *et al.* (1966) found that recovery heat in mammalian C fibres following a brief period of stimulation at 2·5 shocks/sec was just over 50 μcal/g wet wt.shock. To obtain for comparison the extra oxygen consumption at this frequency of stimulation it is necessary to interpolate between the values at 1/sec and at 3/sec in

Table 2; such an interpolation indicates that the extra oxygen consumption with stimulation at 2·5 shocks/sec is about 900 p-mole/g wet wt.shock, which, on the assumption that the utilization of 1 l. of oxygen corresponds to the liberation of 5 cal of heat, corresponds to a value of 101 μcal/g. wet wt.shock. Thus the recovery heat predicted on the basis of the oxygen consumption experiments is larger than that determined directly in thermal experiments. Nevertheless, they are both of the same order of size. The discrepancy between the two values is reduced slightly when the difference between the dry weight/wet weight ratios in the thermal experiments ($0{\cdot}235 \pm 0{\cdot}010$; nineteen determinations: J. V. Howarth, R. D. Keynes & J. M. Ritchie, unpublished), and in the present experiments ($0{\cdot}262 \pm 0{\cdot}002$; forty-six determinations) is taken into account. A more detailed comparison of the results of the oxygen and thermal experiments is pointless, however, until a more directly obtained value for the recovery heat per gram dry weight becomes available.

The ionic imbalance that occurs after activity is particularly large in C fibres. However, the oxygen consumption in nerves *in vitro* is clearly adequate for complete recovery. Thus, the free energy change involved in transporting 1 mole of sodium ion out of the cell and 1 mole of potassium ion into the cell is $RT\ln[([Na_o][K_i])/([Na_i][K_o])]$. The values of $[K_i]$ and $[Na_i]$ for rabbit C fibres are 144·9 and 63·4 m-mole/kg fibre water, respectively (Keynes & Ritchie, 1965*a*); $[K_o]$ and $[Na_o]$ are 5·6 and 154 mM, respectively; and R, the molar gas constant, is 1·986 cal/° K. At 21° C (294° K), therefore, the total free energy change is 2408 cal/mole. Taking the amount of sodium and potassium to be transported after each stimulus as 6000 p-mole/g (Keynes & Ritchie, 1965*a*), the free-energy change becomes 14·5 μcal/g.shock; even with a low efficiency of conversion, therefore, the energy released by the utilization of 1354 p-mole oxygen following each shock (Table 2), 152 μcal/g.shock, seems to be quite adequate for active transport of the cations.

At 21° C the extra oxygen release per shock when C fibres are stimulated at the rate of 1/sec is 1354 p-mole/g (Table 2). Under similar conditions the amount of extra potassium lost is 6000 p-mole/g (Keynes & Ritchie, 1965*a*). If there is no exchange diffusion, this is the amount of potassium that has to be pumped back into the cell during recovery from a single impulse; presumably, a similar amount of sodium has also to be pumped out of the cell. The K/O_2 ratio is thus 4·4, which is similar to the value obtained in myelinated nerve (Connelly, 1959) and a variety of other tissues (Whittam & Willis, 1963) for the active transport of potassium. An alternative method of determining the K/O_2 ratio in C fibres is based on the fact that ouabain decreases both the resting efflux of potassium and the resting oxygen consumption. Thus, ouabain (100–140 μM,

at about 21° C causes a decrease in resting K influx of 0·152 μmole/g.min (Table 10, Keynes & Ritchie, 1965*a*) and a decrease in resting oxygen consumption of 0·0245 μmole/g.min (i.e. $0{\cdot}265 \times 0{\cdot}0924$, Tables 4 and 1); the K/O_2 ratio obtained in this way is thus 5·6, which agrees reasonably well with the value obtained by the other method. These results suggest that K/O_2 ratio is about 5 in rabbit C fibres. Since the resting efflux of K is 0·590 μmole/g.min (Keynes & Ritchie, 1965*a*), it seems that all, or nearly all, of the energy derived from the resting oxygen consumption of nerve (0·0924 μmole/g.min) may well be devoted to the active transport of sodium and potassium ions.

The finding that ouabain depresses the oxygen consumption in nerves in which the sodium has been replaced by lithium is somewhat puzzling. There is now strong evidence that the sodium and potassium-dependent ATPase described by Skou (1957, 1965) forms part of the sodium-pump mechanism in intact nerve (Baker, 1965). There is also evidence (see Skou, 1960) that it is not entirely insensitive to lithium, although lithium seems to be acting at the potassium rather than the sodium site on the enzyme. Thus, the possibility exists that lithium is indeed being pumped out of the cell, but at a slow rate. The rate of pumping, however, would have to be relatively insensitive to the internal concentration of lithium, since stimulation in the lithium-Locke solution does not lead to an increase in oxygen consumption.

Another possible explanation exists: the extracellular fluid in preparations that have soaked for about 6 hr in lithium–Locke solution cannot contain any sodium. The sodium in such preparations that was in fact detected by direct analysis, $2{\cdot}2 \pm 0{\cdot}6$ m-mole/kg (nerve), must therefore have been bound to the fibre stroma or have been left in the fibre water. If all of it were in the fibre water, there could be as much as 8·6 m-mole/kg (fibre water), which is about 15 % of the value obtaining in sodium–Locke solution (tables 12 and 14, Keynes & Ritchie, 1965*a*). This amount of intracellular sodium might conceivably account for the ouabain-sensitive oxygen consumption in the lithium treated nerves. Such an explanation would, however, require that any extruded sodium is not washed away, but rather is trapped by the fibre. This is indeed possible because the electrical potential gradient, and the high permeability of C fibre membranes to sodium (Armett & Ritchie, 1963*a*), might force the sodium back into the fibre again.

In a theoretical analysis, Rushton (1951) has pointed out that, from the teleological point of view, non-myelination of nerve fibres may be desirable because it allows more fibres to run in a given nerve trunk without too great a sacrifice in conduction velocity. On theoretical grounds this arrangement might well have proven uneconomical because of the much

larger amount of nerve membrane participating in conduction in C fibres than in myelinated fibres where ionic interchange is restricted to the nodes of Ranvier. Thus in a 2 mm stretch of nerve, containing just one node, a single large myelinated fibre has only between 6 and 60 μ^2 of active membrane (Abbott *et al.* 1958), whereas a single vagal non-myelinated fibre of average diameter 0·75 μ (Keynes & Ritchie, 1965*a*) has $4{\cdot}7 \times 10^3 \mu^2$, i.e. it has about 100–1000 times more than the myelinated fibre. It seemed interesting therefore to compare the oxygen cost of a single impulse in the two kinds of fibre. The α fibres of the unbranched portion of the sciatic nerve used by Connelly (Brink *et al.* 1952; Connelly, 1959) comprise about 0·28 of the total population of myelinated fibres in the nerve (estimated from fig. 10 of Gasser & Erlanger, 1927), which, according to tables III and VI of Dunn (1909), contains about 3700 fibres. There are therefore about 1040 α fibres in this portion of the nerve. Brink *et al.* (1952) give the weight of the frog sciatic nerve trunk as 3·51 mg/cm. Thus, a sciatic nerve that was $10^3/3{\cdot}51$ (i.e. 285) cm long would weigh 1 g and hence (Brink *et al.* fig. 9) would consume about 8·3 p-moles of oxygen for a single, isolated, shock. The oxygen consumed when a single impulse travels over a distance of 1 cm in a single myelinated fibre is thus $(8{\cdot}3 \times 10^{-12})/(1040 \times 285)$, i.e. $2{\cdot}8 \times 10^{-17}$ mole. According to Keynes & Ritchie (1965*a*) there are about 80,000 non-myelinated fibres in the rabbit cervical vagus nerve; the linear weight of the desheathed vagus is $1{\cdot}78 \pm 0{\cdot}05$ mg/cm (sixty-two observations, present experiments). This means that the oxygen consumption associated with the conduction of one impulse in a single C fibre over a distance of 1 cm is about $(1200 \times 1{\cdot}78 \times 10^{-12})/(80{,}000 \times 10^3)$ mole, i.e. $2{\cdot}7 \times 10^{-17}$ mole. The oxygen cost of a single elementary impulse is thus about the same in the two types of fibre. This suggests that the area of nodal membrane participating in the impulse in the myelinated fibre is greater than is usually assumed, a possibility that Abbott *et al.* (1958) have already considered. Alternatively some degree of specialization of the C-fibre membrane may have occurred to reduce the cost of an impulse. That this is indeed a factor is suggested by the finding (Keynes & Ritchie, 1965*a*) that the measured amount of potassium lost per unit area per impulse is smaller for C fibres than for any other nerve examined so far. Furthermore, the low resting potential of these fibres (Keynes & Ritchie, 1965*a*) would also reduce the free-energy changes involved in the extrusion of sodium; this, together with the associated high internal sodium and low internal potassium concentrations, would tend to reduce the energetic cost of an impulse in C fibres. Whatever the cause, however, an interesting example of economy in nature seems to be revealed by this calculation.

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