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EXPERIMENTS ON THE BLOOD SUPPLY OF NERVES

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In a recent review Adams [1942] has drawn attention to the fact that there is but little experimental evidence regarding the part played by the circulation in maintaining the functions of nerves. Much work has, however, been done on the reaction of isolated nerve to asphyxia, and reference need only be made to the important paper by Gerard [1930]. It seems reasonable to assume that the facts elicited by such experiments apply also to nerve *in situ*, and that its behaviour will consequently be modified by the presence and extent of the circulation.

Almost the only direct experimental approach along these lines is that of Koch [1926] who investigated the effect of ischaemia on the current of injury, though Fröhlich & Tait [1904] had demonstrated the dependence of the excitability of nerves upon blood supply. Reference should also be made to the work of Forbes & Ray [1923] on the conditions of survival of mammalian nerve trunks. No less important, if less direct, is the evidence of Lewis, Pickering & Rothschild [1931], whose experiments, carried out with pneumatic cuffs applied to the arm of the human subject, demonstrated a centripetal spread of anaesthesia.

It is sufficiently obvious that the blood supply of nerves has a considerable functional importance. It has been our object in this work to investigate directly the effects of ischaemia upon conduction in nerve and to assess as far as possible the significance of a blood supply.

Methods

The experiments were done on cats anaesthetized by the intraperitoneal injection of 0·7 c.c./kg. body weight of 'Dial Liquid Compound'. Experiments were carried out in a chamber at 34–36° C., and the atmosphere was almost saturated with water vapour to prevent cooling and drying of the exposed parts of the nerve. The animal was allowed to breathe air cooled by passing through a copper spiral immersed in iced water; respiratory valves ensured the correct direction of the air flow. By this means the rectal temperature was kept constant at 37° C. The nerve employed was the external popliteal division of the sciatic, and it was stimulated supra-maximally through silver electrodes delivering shocks induced in the secondary of an air-cored transformer by the discharge of a condenser through the primary. Action potentials were recorded monophasically from non-polarizable Ag-AgCl-saline-wick electrodes by means of a condenser coupled valve amplifier of conventional design, and a cathode-ray oscillograph. It is concluded that these recording electrodes are not in fact polarized by the current of injury because identical results have been obtained both with and without an input condenser. The system had an over-all time constant of 100 msec. Photographic records were made of single traverses, which were suitably timed in relation to the action potentials by means of a Keith Lucas pendulum. The rate of the traverse and its exponential character were checked with a beat-frequency oscillator.

Unless there is a statement to the contrary the nerve in each experiment was divided close to the sciatic notch and its motor branches at the knee were cut. There was thus available a conduction distance of from 20 to 25 cm. If the nerve trunk had to be exposed for experimental purposes the muscles and the skin were always sutured over the nerve and the ends of the trunk only exposed at intervals for stimulation and recording, after which they were buried again in the limb.

The record of the action potential taken at the ankle after stimulation at the sciatic notch remained constant for at least 6 hr.; it showed the *A* wave of Gasser [1934] due to impulses in the larger fibres, with a conduction rate of 35–75 m./sec. The effects of ischaemia on the *A* wave only were studied. The altered state of the nerve was reflected in the changed action potential whose height is proportional to the voltage developed; this in its turn was accurately determined by the use of a suitable calibrator.

It has been thought desirable not to lengthen the description which follows by including protocols; the experiments which are described have all been repeated several times with the same results. Where there have been variations in the times recorded in repetitions of the same experiment the extreme limits found are given in the text.

Results

The sciatic nerve and its external popliteal continuation have a well-defined blood supply. One or more longitudinal vessels run the whole length of the nerve, supplied from above by a relatively large arterial branch running on to the trunk in the gluteal region. This branch will be referred to as the blood supply from above. In addition, vascular twigs run on to the nerve at different levels in the limb and, dividing into ascending and descending branches, join the main longitudinal vessel. There is one such lateral vessel at the level of the hamstring branches of the internal popliteal nerve, another in the lower third of the thigh, one at the level of the head of the fibula, and several tiny twigs in the leg and at the ankle.

This blood supply provides a wide margin of safety for the nerve, and it was found that several of the lateral vessels could be cut without interfering with conduction; but the following experiment shows that a blood supply is essential. The supply to that part of the external popliteal nerve lying in the thigh was reduced by dividing the sciatic nerve close to the sciatic notch together with the blood supply from above. The lateral vascular twigs running on to the nerve trunk in the thigh were also cut. Therefore there remained only such blood supply as reached the nerve in the thigh along the longitudinal vessel coming up from, and supplied by, the lateral twigs in the leg. The internal popliteal nerve was cut in the leg to prevent distortion of records by a motor response to stimulation. The sciatic nerve was exposed in the upper part of the thigh and the external popliteal nerve at the ankle every 30 min. for stimulation and recording respectively. The action potential at the ankle began to fall after 2 hr. until at the end of about 6 hr. the state of the nerve was as follows. While stimulation in the upper third of the thigh was without effect, an action potential appeared at the ankle and increased as the stimulating electrodes were moved peripherally until they reached the lower third of the thigh, when a normal response was obtained. Thus the blood supply ascending from the leg along the longitudinal vessel only sufficed, after the lapse of 6 hr., to maintain the nerve in a normal state in the lower third of the thigh.

The relative importance of the blood supply from above and of the lateral vascular branches was investigated as follows. All the lateral vessels running on to the nerve between the upper part of the thigh and ankle were cut, but the blood supply from above was left intact. The external popliteal nerve was stimulated in the upper part of the thigh. At the end of 3 or 4 hr. the action potential at the ankle was 80% of the original. The blood supply from above was now cut off. In half an hour the potential had fallen almost to zero. Control experiments have shown that the action potential does not undergo any significant change during this half hour if the blood supply from above be left intact.

It is clear that the blood supply from above must have sufficed to maintain at a high level the excitability of the nerve under the stimulating electrodes. But it is by no means so clear that conduction throughout the nerve was being similarly maintained. It is possible that it depends upon diffusion from the surrounding tissues of the limb, which are of course normally vascularized.

To explore this possibility a tourniquet was applied to the limb so as to leave out the sciatic nerve together with its blood supply from above. This was done by tunnelling through the soft tissue of the upper part of the thigh and passing two lengths of rubber tubing through the hole. These were then tightly stretched and tied around all the tissues in front of and behind the sciatic nerve. When such a nerve is stimulated above the level of the double tourniquet the action potential at the ankle disappears in 45 min.; it is still normal at the level of the head of the fibula in 120 min., though only a very small one remains in the leg. It would appear therefore that conduction ceases in a partially devascularized nerve much earlier when it lies in an avascular than in a vascular limb.

The dependence of continued nervous activity upon the environment was further demonstrated by experiments in which a complete tourniquet was applied. Three turns of tightly stretched rubber tubing were applied at the root of the limb and were obviously effective. The external popliteal nerve was divided distal to the tourniquet and stimulated just below the level of section. The action potential at the ankle began to diminish in 18 min. and had fallen to zero in 30 min. It was immaterial whether the nerve was stimulated just below the tourniquet or at knee level, and the same result was obtained when the tourniquet was applied just above the knee. The period of 30 min. is a very constant one under these experimental conditions and may be called the 'ischaemia time'. Though the nerve below the knee seems to be completely out of action it is found that a considerable action potential can be recorded in the thigh in response to stimulation at the sciatic notch even 70 min. after the application of the tourniquet. The nerve at this level is of course quite a large one and therefore not directly comparable with that used at the ankle. This objection can be overcome by stimulating the sciatic trunk just below the tourniquet and recording from the branch of the internal popliteal nerve to the lower part of the biceps muscle. This nerve is somewhat more slender than that used at the ankle, but it continues to produce an action potential for an hour and a half after that at the ankle has disappeared. The longer survival of conduction in the nerve in the thigh is again suggestive of an environmental effect, and the following experiment finally establishes it.

In a limb with a rubber tube tourniquet at its root the external popliteal nerve was cut at the ankle and freed from its bed up to the lower third of the thigh, where it was laid alongside the main trunk. The nerve was stimulated just below the tourniquet and records were made, at intervals. The action potential at the transposed ankle level disappeared in from 100 to 135 min. The time involved is much longer than the 'ischaemia time' referred to above, but it must be borne in mind that the nerve was exposed to the air during the dissection. Control experiments undertaken to assess the importance of this factor showed that when the nerve was freed from its bed and replaced in its normal position the action potential disappeared in 50 min. (Fig. 1). The nerve similarly freed and replaced in a limb without a tourniquet continued to conduct normally for at least 2 hr. This is undoubtedly an environmental effect on conduction. It might be thought that the effect is due to reduced excitability, but it is clear that the excitability of the ankle fibres at the point of stimulation was maintained for nearly twice as long as the nerve survives in its normal situation.

The environmental effect upon conduction which has been demonstrated invites further analysis. The experiments now to be described are in the nature of a preliminary exploration of the phenomenon.

A possible criticism that the longer survival in the thigh might be due to the passage of small quantities of blood under the tourniquet in particular through vessels in the bone can be met by the fact that no such blood leak can be detected when the limb is amputated distal to the tourniquet. Moreover, in an experiment in which the limb was amputated proximal to the tourniquet the same time relations between nerve block in the leg and in the thigh were observed.

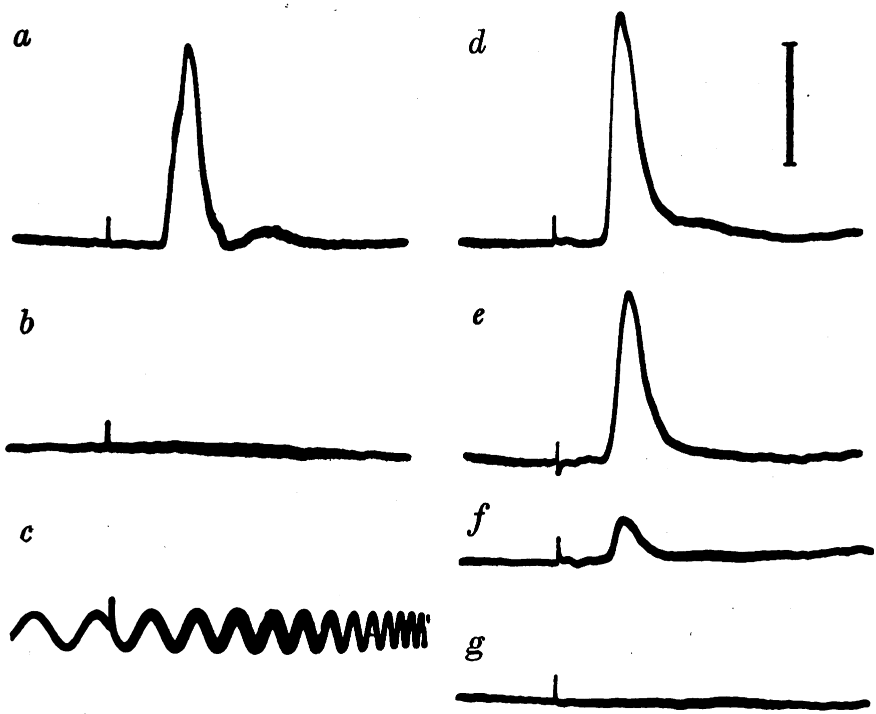

Fig. 1. (*a*) Left leg. Nerve freed to lower third of thigh and replaced in its bed. Record at ankle 14 min. after application of tourniquet. (*b*) As (*a*). Record at ankle 39 min. later. (*c*) 500 c./sec. (*d*) Left leg. Nerve freed to lower third of thigh and placed alongside sciatic trunk. Record at transposed ankle level 14 min. after application of tourniquet. (*e*) As (*d*). Record at transposed ankle level 36 min. later. (*f*) As (*e*). 88 min. later. (*g*) As (*f*). 106 min. later. Calibration for all action potential records in (*d*) = 250 μV.

Again, it might be thought that the survival of the nerve in the thigh should be attributed to a temperature difference. Temperatures taken with a thermometer embedded in the limb under the conditions described above are higher by 1° C. in the thigh than at the ankle. Such a difference is unlikely to be significant, and in any event if it were significant the nerve at the ankle should survive the longer. Experiments with tourniquets carried out at room temperature, but otherwise under identical conditions, show that nerve survives longer in the cold, and this confirms an observation which has already been made by Allen [1938]. The longer survival in the thigh cannot be explained by a temperature difference; it remains to be determined whether the factor concerned is exercising a beneficial influence in the thigh or an adverse one in the leg.

A rubber tube tourniquet was applied to a limb, the sciatic trunk divided immediately distal to the tourniquet and freed to knee level, along with the branch to biceps which was used for recording. The nerve was stimulated at its cut end. When the initial record had been made the nerve and its branch were wrapped in a sheet of thin rubber and laid back in the nerve bed, and the muscles and skin sutured. Thirty-five minutes later, and 1 hr. after the application of the tourniquet, the action potential had disappeared. This is a considerably shorter period of survival than is found when the nerve remains in direct contact with the tissues of the thigh. In a control experiment in which no tourniquet was applied, the action potential was little changed in 90 min.

On the other hand, the period of survival of the nerve in the leg is not altered by enclosing it in rubber tubing. For this experiment tourniquets were applied on both limbs and the nerves were freed from the ankle level to the knee. On one side the nerve was threaded through a rubber tube. Muscles and skin were then sutured over as usual. The action potential at the ankle disappeared simultaneously on both sides in about 50 min.

These experiments, in which the nerve was isolated from its environment by a rubber membrane, show that while the procedure is without effect in the leg, the survival time of the nerve in the thigh is considerably reduced. This again is probably best explained in terms of anoxia, and it is suggested that the fleshy tissues of the thigh supply oxygen to the nerve by diffusion, while in the leg the character of the tissues—predominatingly tendinous, bony, and of less bulk—does not provide such a large reservoir.

It is obvious from the experiments so far described that if nerves of the cat are to function at all there must be a supply of blood either directly or to the surrounding tissues. The impression gained is that the blood supply need not be a large one, and this is confirmed by experiments making use of a pneumatic cuff.

Such a cuff, 4 cm. wide, was applied in the middle of the thigh and surrounded with a plaster of Paris bandage to prevent slipping and stretching; it was distended with air at a pressure of 150 mm. Hg, some 30–40 mm. higher than the carotid blood pressure previously determined with a mercury manometer. The nerve was stimulated above the cuff at the sciatic notch and records were made at the ankle. Within 15 min. the action potential began to fall and it disappeared very constantly in 25–30 min.—approximately the 'ischaemia time' referred to above. But if at this time the nerve was stimulated distal to the cuff a normal response was obtained at the ankle for at least 1 hr., after which time the pressure in the cuff was released so that recovery might be studied.

It is clear that during the application of pressure with the pneumatic cuff conduction in the nerve under it was blocked, for the distal part was shown to be functioning and the central part had not been interfered with. It seemed likely that some blood was passing into the limb in spite of the distension of the cuff. The truth of this explanation was confirmed by the oozing of blood when the tibial artery was opened. This is not surprising when it is borne in mind that fallacious results in human blood-pressure measurements may easily be obtained by the use of too narrow a sphygmomanometer cuff. In the present experimental conditions it was necessary to have the pressure in the cuff at least 50 mm. Hg in excess of the carotid blood pressure before all oozing from a severed tibial artery ceased. When the cuff was distended with a pressure of 240–280 mm. Hg, it could be shown that the action potential at the thigh disappeared in 30 min. whether stimulation was above or below the cuff. The explanation of the results obtained with the lower pressure lies in the fact that the large arteries of the limb allow some blood to pass to the periphery though the pressure under the cuff is sufficient to stop the circulation in the nerve itself and in the surrounding tissue.

Finally, some reference must be made to the recovery which ensues when the tourniquet is removed from the limb. If the circulation has been stopped for no more than 1–1½ hr., a small action potential can be detected at the ankle in response to stimulation in the thigh within 30 sec. of removal of the tourniquet; and this holds also in the case of the pneumatic cuff for both the lower and the higher pressures. Recovery becomes complete in 5 or 6 min., by which time it is obvious from the appearance of the limb that the circulation has been restored. When, however, the tourniquet is applied for longer periods, the recovery becomes slower and of less degree. At the same time it is clear from the appearance of the limb that the circulation is being restored more slowly and less completely. Indeed, so far as can be judged from inspection recovery of the nerve occurs *pari passu* with recovery of the circulation in the limb. From the fact that nerves of limbs to which a pneumatic tourniquet has been applied for 3 or 4 hr. have recovered completely in from 1 to 2 hr. it can be concluded that the former period in ischaemic conditions does not damage the nerve permanently.

Discussion

It is well-known that in experiments with isolated nerves conduction is maintained in an atmosphere of pure oxygen. Our experiments demonstrate that in the animal body a nerve requires an active circulation to maintain normal conduction. The anatomical arrangement of the blood supply provides a wide margin of safety; but in conditions of complete ischaemia a nerve ceases to conduct in about 30 min. at 25° C. This time is in accordance with the results of other workers who have subjected nerves to anoxia or to ischaemia.

TABLE 1

Author	Nerve used	Temp. ° C.	Result
Gerard [1930]	Dog peroneal *in vitro*	25	Blocked after 60 min in nitrogen
Gerard [1930]	Dog peroneal *in vitro*	28	Blocked after 24 min. in nitrogen
Lehmann [1937]	Cat peroneal *in vitro*	37	Blocked in 35 min. in hydrogen
Clark, Hughes & Gasser [1935]	Cat saphenous compressed by pneumatic cuff *in vivo*	Not stated	A and B waves blocked in 45 min.
Lewis, Pickering & Rothschild [1931]	Human arm nerves compressed by pneumatic cuff *in vivo*	35–36	Blocked after 16 to 35 min.

The time taken for cat peroneal nerve to be blocked by anoxia at 37° C. (Lehmann) corresponds closely with that taken for ischaemia to produce a block in the nerve at ankle level in our experiments, and also with the times for ischaemia block reported by the last two authors listed in the table. It does not appear likely that tourniquet ischaemia introduces any factor other than anoxaemia, for anoxia *in vitro* and tourniquet ischaemia *in vivo* produce their effects in the same time.

The small oxygen requirements of isolated nerve have led to the view that the circulatory requirements must also be small, and this view is supported by our experiments. It has been questioned of late by Bülbring & Burn [1939]. It should be noted that the experiments they describe were carried out on limbs perfused with defibrinated blood, and it would be well to exercise care in applying results obtained in this way to the problem of the normal circulatory requirement. Indeed, until there is more conclusive evidence to the contrary it must continue to be assumed that the circulatory requirements of nerve though definite are small.

It has been shown that if the hind limb of a cat be rendered ischaemic by a tourniquet applied at the root, conduction fails first in the periphery. A rather similar phenomenon was described by Lewis *et al* [1931], who showed that in the human subject the application of a pneumatic cuff or of a special clamp (designed to press on the nerve without interfering with the circulation to the limb) caused an anaesthesia which spread centrally from the finger tips. In our experiments the degree of block produced appears to be dependent upon the degree of anoxia which develops. It certainly does not depend on the length of the nerve fibre rendered ischaemic, for the 'ischaemia time' of 30 min. is unaltered when the tourniquet is applied at the level of the knee though the length of the ischaemic part of the nerve has thereby been halved. Our experiments with the pneumatic cuff show further that when the circulation to the nerve is cut off locally the peripheral part continues to conduct normally. We are satisfied that this nerve block is not due to direct pressure. It will be recalled that Gasser [1934] has taken the same view. He showed that the block produced by direct pressure developed more slowly and that recovery is also slow, an observation which we have been able to confirm in experiments to be described elsewhere.

Our results, so far as they go, do not support the view of Lewis *et al.* that the nerve in the proximal part of the limb is more susceptible to ischaemia than in the distal, or that a sensory nerve is more rapidly blocked by ischaemia the farther away it is from its sensory endings. We conclude that given equal anoxaemia the nerves in the leg and in the thigh are blocked in much the same time. It is necessary, however, to bear in mind a point they have emphasized, for their 'observations were carried out upon very long nerves lying completely undisturbed by dissection in their natural surroundings and tested by natural stimuli'. The length of nerve rendered ischaemic did not exceed 4 cm. in our cuff experiments and 25 cm. in our tourniquet experiments. It may be that the explanation of the discrepancy is to be found in this fact. Indeed, our observations are much more in accord with their 'uniformly developing anaesthesia resulting from pressure rendering short stretches of nerve trunks ischaemic...'.

Summary

1. The blood supply of the external popliteal nerve of the cat consists of a branch running down the nerve from above and of lateral branches running on to it at approximately constant levels.

2. A small though definite blood supply is essential for the maintenance of conduction in the nerve. The anatomical arrangement of the blood supply provides a wide margin of safety. Even if all the lateral branches are divided conduction in the nerve is unaffected in a normally vascularized limb. In a limb rendered avascular by a tourniquet from which the arterial branch to the nerve from above is excluded, conduction is maintained in the nerve to the upper part of the leg. Below this level the nerve is inactive.

3. In a limb rendered ischaemic by a complete tourniquet at its root the nerve in the leg and foot becomes inactive in about 30 min., though the nerve in the thigh survives for at least two hours.

4. If in such a limb the nerve in the foot and leg be freed from its bed and laid alongside the main trunk in the thigh, it survives much longer in the transposed situation than in the normal one. The effect cannot be attributed to a difference of temperature, or to leakage of blood under the tourniquet.

5. The survival time of the nerve in the thigh may be curtailed by enclosing it in rubber, a procedure which has no effect on the survival time in the leg. It is suggested that a greater supply of oxygen is available in the thigh to supply the nerve by diffusion.

6. A pneumatic cuff, 4 cm. wide, applied to the limb and distended to a pressure equal to that in the carotid artery interrupts conduction in the nerve immediately under it in 30 min. Conduction in the nerve distal to the cuff is unaffected. This state of affairs must be due to local ischaemia. The pneumatic cuff does not become a completely effective tourniquet till it is distended to at least 50 mm. Hg in excess of the carotid pressure. Under such conditions conduction fails also in the nerve distal to the cuff.

7. On the release of a tourniquet recovery begins in 30 sec. and is complete in 5 or 6 min. if the circulation has been interrupted for no more than $1\frac{1}{2}$ hr. Recovery is slower after longer applications but the nerve is able to recover from ischaemia of 4 hr.'s duration produced by a pneumatic cuff. It is suggested that the factor governing recovery is the return of the circulation.

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