liver is a major site of butyrate utilization in ruminants, and the present results strongly suggest the absence of net synthesis of carbohydrate from butyrate in this tissue.

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PHYSIOLOGY

Longitudinal Direct-Current Gradients of Spinal Nerves

In a previous publication[1] some evidence was presented for the existence of a steady-state longitudinal charge carrier flow in the peripheral nerves of Amphibia. At that time this flow of current was considered to be unidirectional in the nerves with the necessary circuit completion path through some non-neural tissue. This concept required the presence of measurable longitudinal direct-current potential gradients along the peripheral nerves with a uniform polarity. The return path would then be evidenced by a gradient of opposite polarity in some other tissue. This communication presents the results of an evaluation of this hypothesis.

Large bullfrogs (*Rana catesbeiana*) were used as the experimental animals, and atraumatic surgical approaches were devised for exposure of the structures of the posterior thigh. Potential gradients were measured on the surface of intact structures, dissection being limited to gentle separation only. The electrodes were saline-agar filled silver–silver chloride with thin terminal cotton wicks. Potentials were amplified with either an electrometer amplifier (input impedance 10^{14} ohms) or a chopper-type direct current amplifier (input impedance 10^{6} ohms). Electrical isolation was secured at the measurement site by elevating the structure on a thin sheet of polyethylene.

In the first series of 12 animals, the direct-current gradients on the surface of muscle, blood vessel, bone and fascia were noted to be consistently distally negative. The gradients were generally uniform, although lower in magnitude than the surface direct-current gradients across comparable areas. Measurements on nerve utilized the sciatic nerve, which was exposed from its entrance into the thigh to the knee, a distance averaging 6 cm. The nerve sheath was left intact, the nerve being dissected from its bed sufficiently to secure electrical isolation. The gradient along the sciatic was found to be distally negative (-2 to -5 mV/cm) and linear down to the point of bifurcation. Distal to this point, the gradient either changed in value or in some cases reversed polarity. It was then found that the sheath could be partially removed without altering the gradient along the main trunk, thus permitting measurements of each of the branches separately. The tibial branch was found to be invariably distally negative with a gradient of greater amplitude (-4 to -15 mV/cm) than the main sciatic.

The peroneal branch, however, showed a direct-current gradient of opposite polarity with amplitudes always lower than those of the tibial ($+3$ to $+10$ mV/cm). On most occasions the algebraic sum of the gradients on the two branches was equal to the 1-cm gradient along the main sciatic. These gradients were noted to change with the depth of anæsthesia. If anæsthesia was intentionally very deep (prolonged exposure to high concentration of agents) the early potentials were reversed in polarity (peroneal distally negative, tibial distally positive). Within 5 min the gradients fell to zero and following this the normal polarities appeared. The amplitudes gradually increased as recovery from anæsthesia occurred, highest values being found after respiratory movements returned and before co-ordinated escape movements occurred. The remarkable consistency with which the differential polarization of the two branches was noted appeared to indicate some basic difference between the two rather than some artefact of dissection or measurement. Since each branch is a mixed nerve, an estimate of the percentage of motor versus sensory fibres in each was desirable. Fibre-size spectrum determinations were made (myelinated fibres only) since there is generally believed to be some correlation between fibre-size and function[2]. The tibial branch was found to contain a preponderance of large (12–18μ outside diameter) fibres while the peroneal branch revealed a preponderance of small (3–8μ outside diameter) fibres. It was then decided to measure gradients along spinal nerves of predominantly motor or sensory function. A second series of 12 frogs was then evaluated using the branch of the femoral nerve to the sartorius as the motor nerve and the nerves running between the dorsal body wall and the skin over the back of the animal as predominantly sensory nerves. Fibre-size determinations were made of these nerves and they were found to contain much greater percentages of large and small fibres, respectively, than did either the tibial or peroneal. In each animal of this series, one sciatic nerve and both of its branches were first exposed and their direct-current gradients measured. In all 12 animals the peroneal and tibial branches showed the same differential polarization as in the first series (that is, peroneal distally-positive, tibial distally-negative). A total of 33 predominantly sensory nerves were measured, with the gradients in each case distally positive and ranging in magnitude from 0·5 mV/cm to 6 mV/cm. Ten predominantly motor nerves were examined, with the gradient in each case distally negative and ranging in magnitude from 1 mV/cm to 4 mV/cm.

A final series of 8 frogs were obtained in the 'hibernating' state and kept at temperatures between 40° and 45° F until used. The same polarities of the direct-current gradients were obtained, but the magnitudes were considerably reduced and the

changes with anæsthesia prolonged. The normal polarities (peroneal positive, tibial negative) were seen only late in recovery from anæsthesia and the reversed gradients (peroneal negative, tibial positive) persisted in each case until after the corneal reflex had returned. Tissue fragility appeared to be increased in these winter frogs and 3 of the 8 had to be discarded because of bleeding or nerve damage. For this reason, measurements on the motor nerve to the sartorius muscle were unsatisfactory. It was possible, however, to measure gradients along the dorsal dermal branches. Like the sciatic branches, these also showed a polarity reversal, being distally negative until late in recovery from anæsthesia when they became positive, remaining so up to the point when escape movements began. In no case, however, did these gradients exceed 1 mV/cm.

The differential polarization of the motor and sensory nerves suggests that both halves of the postulated electrical circuit are within neural tissue, with charge carrier movement in one direction in motor fibres and in the opposite direction in sensory fibres. It would be desirable to relate this, if possible, to some general neuronal mechanism. The possibility of longitudinal current flow within neural structures has been given little attention. However, in 1947 Lorente de No[3] postulated the presence of a longitudinally polarizable structure in the axoplasm, and recently Müller[4] has presented experimental evidence for this in isolated segments of peripheral axons. In intact neural structures Gerard and Libet[5,6] have reported a longitudinal or 'axo-dendritic' direct-current potential along the cerebral neurones of the frog. Gradients up to 10 mV in magnitude were measured and a relationship noted between the magnitude and polarity of the gradient and the activity of the neurones. Goldring *et al.*[7–9] afterwards extended these observations and advanced the thesis that the longitudinal direct-current gradient determined the level of irritability of these neurones. In all these studies, measurements were made between the pial and ventricular surfaces of the brain, that is, from the terminal dendritic arborizations across the cell bodies to a point on the outgoing axons. Anatomical considerations prevented us from measuring across the cell bodies of the spinal neurones. However, if an axono-dendritic direct-current gradient were present in these neurones, then our method of electrode placement would indicate a direct-current gradient of one polarity on motor nerves and a gradient of opposite polarity on sensory nerves. Other studies[10,11] have indicated that in the spinal, as in the cerebral, neurones the longitudinal direct-current gradient determines in part the level of irritability of these neurones. While the status and significance of the neural direct-current gradients in the intact, unanæsthetized frog can only be inferred at this time, it appears possible that the concept of a longitudinal direct-current polarization gradient may be extended to include the spinal as well as the cerebral neurones. The significance of the differential polarization as a means of circuit completion for current flow requires further experimentation.

Detailed technique. Large, adult bullfrogs (*R. catesbeiana*) were used as the experimental animal, and atraumatic surgical approaches were devised for exposure of the internal structures of the posterior thigh, including the sciatic nerve. Electrical measurements were taken only of intact structures, and cut tissue edges (particularly skin) were carefully retracted from any contact with either the electrodes or the structure to be measured. Damage to a structure was either visually apparent or evidenced by injury potentials and such preparations were discarded. Particular care was exercised to avoid open bleeding into the area because of the resultant shunting effect and electrode contamination. Electrical isolation was secured by slipping a thin sheet of polyethylene beneath the structure to be measured. Electrodes were silver–silver chloride, saline-agar filled with thin terminal cotton wicks. Electrode pairs were chosen for stability, resistance to polarization, and low inter-electrode potentials ($< 100\ \mu V$). Measurements were made over successive 1-cm segments of the structures with the reference electrode always placed proximally and the recording electrode distally. Potentials were measured with either a Kiethley 600*A* electrometer (input impedance 10^{14} ohms) or a Hewlett–Packard 425*A* chopper amplifier (input impedance 10^{6} ohms). Comparison measurements showed no difference between measured potential with either instrument. (If the variable input impedance of the 600*A* was utilized, potentials were maintained with little drop in magnitude over the range 10^{14}–10^{6} ohms, a 10 per cent drop was noted at 10^{5}, and 10^{4} ohms effectively shorted the potential.) All measurements were taken within a double-walled, grounded Faraday cage. The preparations were ungrounded although comparison measurements revealed no changes in recorded potentials if some remote portion of the preparation was connected through a similar electrode to circuit (not chassis) ground. Anæsthesia was obtained by titration with 'Tricaine' (Sandoz) and complete anæsthesia was arbitrarily designated as loss of corneal reflex, respiratory movements, and response to trauma. Following anæsthetization, the animals were rinsed in de-chlorinated tap water, drained and placed on 4-in. thick blocks of polyethylene for dissection and measurements. Potential readings were taken at intervals of approximately 5 min during subsequent recovery from anæsthesia. Small pledgets of saline-moistened surgical cotton were used to cover the incision when measurements were not being made, care being taken not to contact the nerve. The functional integrity of the sciatic, its branches, and the motor nerve to the sartorius was determined by their ability to transmit stimuli and produce a muscle contraction at the end of each procedure.

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