NEWS AND VIEWS

Communication between Nerve and Muscle

THE transmission of excitation across the neuromuscular synapse reflects only one aspect of the complex relationship between nerve and muscle. Many of the normal properties of muscle are dependent on an intact supply of motor nerves, interruption of which evokes a series of morphological, physiological and biochemical changes in the muscle which are known as denervation atrophy. Features of the vertebrate skeletal muscle fibre that are known to be dependent on such "trophic" influences include the stability of membrane and intracellular constituents, the acetylcholine sensitivity of the fibre membrane, the cholinesterase activity of the muscle, the speed of contraction of the muscle, the rejection of any further innervation of the fibre, and certain enzymatic activities differentially associated with red and white muscle.

The nature of the trophic information passed from nerve to muscle is controversial. It is not, of course, necessary that the same molecular moiety be responsible for all the trophic phenomena. One viewpoint is that the normal neuromuscular transmitter, acetylcholine, is involved. Another attributes the trophic effects to an alternative, unidentified, substance, and a third group claims that the "atrophic" changes can be prevented by muscle activity, implying that the atrophy following nerve section is secondary to the resultant muscle inactivity.

The failure, in the past decade, to differentiate adequately between these opposing views on the basis of *in vivo* experiments presumably reflects the number and complexity of the variables involved. The more artificial but also, it is hoped, more controlled *in vitro* experiments may thus help to elucidate the molecular events involved in these trophic interactions. The first observation that contacts could form between tissue cultures of nerve and muscle was made more than sixty years ago, but it is only in the past few years that unequivocal evidence has been obtained that the formation of synapses may occur in tissue culture. On page 296 of this issue, Harris, Heinemann, Schubert and Tarakis report an elegant tissue culture study on the effect of contact between a neural cell and a developing muscle cell on the distribution of acetylcholine sensitivity over the muscle cell membrane.

In the normal muscle there is an area of very high sensitivity to acetylcholine in the region of the motor endplate whereas the rest of the muscle membrane seems insensitive to acetylcholine. In denervated muscle or in developing muscle before the onset of innervation, the membrane displays a uniformly high sensitivity to acetylcholine throughout its extent. This region of high sensitivity is progressively restricted to the site of innervation on innervation or re-innervation ; this restriction is thus one of the trophic effects of nerve on muscle. Harris and his colleagues decided to observe the effect on cultured rat skeletal muscle myotubes (a stage in the development from primitive myoblasts to mature striated muscle fibre) of contact by a process from cultured mouse neuroblastoma cells. The sensitivity of the membrane to acetylcholine was measured by inserting a microelectrode into the muscle cell and recording the change in membrane potential in response to very small amounts of acetylcholine iontophoretically applied at various sites along the membrane of the muscle fibre. Harris *et al.* found that in the non-innervated fibre there was a uniform sensitivity to acetylcholine but that in those fibres contacted by a process from a neuroblastoma cell there was usually a very high sensitivity at the site of contact, associated with a considerable lowering of the sensitivity over the remainder of the fibre. Close contact between the neuroblastoma cell process and the developing muscle fibre seemed sufficient to mediate this trophic effect because the authors were unable to demonstrate that a true synapse had formed or that synaptic transmission had occurred.

Harris *et al.* are modest in their conclusions and merely comment that their system may well be a promising one by which to study the mechanisms involved in synapse formation and the trophic effects of nerve on muscle. In fact their observations already raise some intriguing questions. The trophic effect they demonstrate is exerted by the processes of neuroblastoma cells which are derived essentially not from motor neurones but from the sympathetic system, and the enzymatic composition of which is thus characteristic of sympathetic neurones. It is possible therefore that some of the trophic effects of nerve on muscle can be produced as effectively by sympathetic nerves as by motor nerves. If further studies indicate that nerve fibres which do not release acetylcholine from their endings can produce the trophic effects, this would be powerful evidence against the acetylcholine hypothesis.

Harris *et al.* also report that the more differentiated of their muscle cells spontaneously propagate action potentials of a frequency of 1 s^{-1}, and these cells also contract spontaneously. If these non-innervated but active cells were found to possess a uniformly high sensitivity to acetylcholine over their entire surface this would be damaging to the view that such sensitivity is merely a reflexion of muscle inactivity.

Most work on the nerve–muscle relationship emphasizes the dependence of muscle on nerve and it is sometimes overlooked that the relationship is reciprocal. Developing motor neurones which fail to establish contact with muscle die. Separation of a motor neurone from its muscle fibre or contact between a motor axon and denervated muscle both evoke marked changes in neuronal metabolism. The selectivity with which neurones form connexions only with the appropriate muscles during normal development argues for refined intercellular recognition mechanisms, the nature of which is unknown, of a much higher degree of specificity than those normally included in the trophic range.

The interdependence of nerve and muscle thus requires the extensive exchange of information. Harris *et al.*'s work represents a significant advance in the techniques available for investigating the language with which cells communicate. Some further light on the matter has just been provided by Robbins and Yonezawa (*Science*, **172**, 395 ; 1971) who describe the onset of chemical transmission in rat neuromuscular synapses formed in tissue culture.