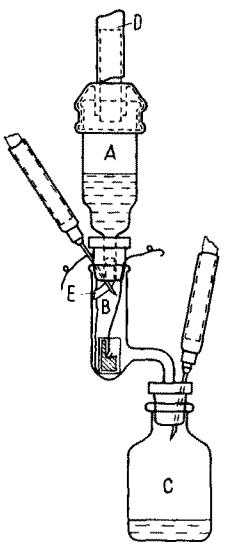
This has the beneficial effect of helping to wash away blood or breakdown products resulting from the explantation. The needle air-vents with cotton bacterial filters in the culture chambe B and receiving bottle C allow free exit for gases to the external air and promote free flow from B to C. The piece of cellulose sponge E inserted into the lower end of the Buchner funnel and touching the side of the culture chamber serves to conduct the supernatant to the side of the culture tube, smooth out the flow and prevent traumatisation of the culture by drops of supernatant. A piece of chemically clean braided fiberglass sleeving in the outflow arm of the culture tube facilitates the exit of supernatant from the culture tube and thus helps to prevent changes in the level of supernatant in the culture chamber. Bacteria are filtered out of the air during its passage through the dialysing membrane thus preservin gthe sterility of the supernatant.



Schematic diagram of apparatus

Zusammenfassung. In der Arbeit wird eine Einrichtung für kontinuierliche Flüssigkeitszufuhr beschrieben, wie sie für Gewebskulturen wertvoll sein kann. Durch eine feinporöse Membran kann die Menge der Flüssigkeit reguliert werden.

A. W. B. CUNNINGHAM and N. O. LUNELL[3]

Tissue Dynamics Laboratory, Pathology Department, University of Texas Medical Branch, Galveston (Texas), November 21, 1960.

COGITATIONES

Spontaneous Miniature Activity and Gradation of Transmission at the Neuromuscular Junction

The discovery of the randomized spontaneous miniature activity at the neuromuscular junction by FATT and KATZ[1,2] opened new perspectives for the interpretation of facilitation of synaptic transmission[3-5]. Specifically, it was shown in frog- and mammalian nerve-muscle preparations that the neurally evoked endplate potential (epp) results from a statistical coincidence of quantal units of identical magnitude (i.e. the min epp's)[6-8]; that a conditioning nerve impulse enhances the probability of occurrence of spontaneous miniature activity as well as increases the quantum content of response to a subsequent nerve volley[9,10]; and that the quantum content of a neurally evoked response increases in the course of tetanic stimulation[9,10], while marked augmentation of spontaneous miniature activity persists for several minutes in the post-tetanic period[8,11].

However, DEL CASTILLO and KATZ ([12], see there Table I) also pointed out that rate of spontaneous miniature discharges and quantum content of evoked epp are not necessarily covariant: e.g. Mg ions effect a decrease of quantum content of epp, while leaving the frequency of spontaneous miniature activity unaltered. Furthermore, a decrease of quantum content of epp after prolonged tetanic stimulation is accompanied by increase of min epp-frequency[9]. Weak anodic polarization of terminal motor nerve branches augments the evoked epp, but the spontaneous discharge rate remains unaltered; cathodic polarization, on the other hand, depresses transmission and augments spontaneous discharge rate[12,13]. An additional instance of dissociation between spontaneous and evoked activity is provided by the fact that spontaneous discharges continue in the absence of Na-ions and in preparations depolarized by K_2SO_4[14], wherefrom it is apparent that spontaneous miniature activity 'does not depend upon the occurrence of electrical activity of the action potential type in any structural unit within the nerve terminal'[5].

To these instances of dissociation between direction of change of spontaneous and evoked junctional activity may now be added the finding of KRAATZ and TRAUTWEIN[15] that 2,4-dinitrophenol, while greatly augmenting spontaneous random discharge rate, diminishes the epp-amplitude. Hydrazinium-ions[16], and tetrodotoxin in the presence of NH_4^+-ions[17] block transmission without affecting the discharge rate of min epp's. Conversely, guanidine apparently augments the evoked epp[18], while spontaneous miniature activity does not change[17]; however, spontaneous giant potentials appear with an amplitude smaller than that of evoked epp's, but larger than that of min epp's. Botulinus toxin, on the other hand, depresses frequency of spontaneous discharges and reduces the amplitude of the evoked epp as well[11].

In summary, then, there is ample evidence that the mechanism for spontaneous quantal discharges can operate in autonomy from nervous control; and that the frequency of spontaneous activity does not uniquely define the potential of the presynaptic apparatus to transmit incoming nerve volleys to the postsynaptic site. The differences between spontaneous and evoked junctional activity, to which DEL CASTILLO and KATZ[3] directed attention, can, however, be ascribed to the responsiveness of the motor nerve endings to invasion by a nerve impulse (see: factor 'N' in [12]). This, then, would constitute an additional limiting step in the chain of events of transmission, and would attribute to spontaneous miniature activity a permissive, rather than a determining role in junctional transmission and facilitation of transmission.

It then becomes pertinent to inquire of what nature this limiting neural event is, and whether it is subject to gradation by physiologic and pharmacologic parameters.

In the spinal cord, posttetanic potentiation of the monosynaptic reflex is paralleled by augmentation of the pre-

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synaptic action potential amplitude[20]. This increase of action potential height is attributed to posttetanic hyperpolarization of presynaptic nerve fibres[20–22]. Since terminal anelectrotonus must also result in hyperpolarization of presynaptic neural structures, DEL CASTILLO and KATZ[12] suggested that anelectronic facilitation of neuromuscular transmission is, likewise, related to increase of amplitude (and possibly duration) of the action potential in terminals (see also [23]). Facilitation of neuromuscular transmission by conditioning nerve stimulation can, accordingly, be attributed to invasion of motor nerve terminals by the test volley during the positive afterpotential left by the preceding nerve impulses.

Posttetanic facilitation of transmission at the mammalian neuromuscular junction is accompanied by the occurrence of retrograde motor nerve activity: specifically, tetanic stimulation with ensuing transmission failure (presumably due to presynaptic conduction block[24,25]) is followed by a period during which single nerve impulses elicit repetitive activity in muscle fibres, and antidromically conducted trains of impulses in corresponding motor nerve fibres[26,27]. These posttetanic effects are identical with the effects obtained on single (or double) volley stimulation after administration of agents classed 'facilitators' of neuromuscular transmission: a single orthodromic volley is, then, also seen to elicit repetitive activity in muscle fibres, and to set up a train of repetitive antidromic impulses in the motor axon which carried the primary efferent volley[28–33]. When these repetitive antidromic responses are genuine (i.e.: originating in the motor unit whose axon conducted the primary efferent volley[34]), they are followed by a characteristic recovery cycle[27]: a second efferent motor nerve impulse following the conditioning stimulus within 1 to about 4 msec greatly augments repetitive muscle- and antidromic nerve activity, while a test stimulus at 50 to about 100 msec does not elicit repetitive activity in either muscle or nerve. This time course of augmentation and depression of repetitiveness coincides with that of the sequence of negative and positive afterpotentials in nerve fibres with slow conduction velocity; the early phase of augmentation corresponding to negative, and the late phase of depression corresponding to positive afterpotentials. The generation of antidromic repetitive activity was attributed to a negative afterpotential in motor nerve terminals[27]. A prolongation of the negative afterpotential in frog motor nerve terminals in the presence of tetraethylammonium ions was observed by KOKETSU[35]. The possibility that the negative afterpotential in motor nerve terminals constitutes *per se* a transmitter controlling agency, was explored by KUFFLER[36]: since veratrine prolongs nerve afterpotentials without prolonging the active phase of the transmitter process, this possibility can be excluded[37].

On the basis of these considerations, it appears more appropriate to view the magnitude of the terminal membrane breakdown on orthodromic invasion as the critical event in the neural control of transmission[20,22]. Provided augmentation of afterpotentials reflects hyperpolarization (equivalent to an anelectronic state) of the terminal nerve membrane[38], an increase of the terminal action potential can be inferred from events which reflect augmentation of the terminal afterpotentials, i.e. post-activity motor nerve repetition[27].

It then follows that this hyperpolarized state of motor nerve terminals can be brought about by tetanic stimulation alone, and by single or repetitive nerve shocks in the presence of a variety of quaternary ammonium ions with, and without[33], cholinesterase inhibiting activity; agents whose minimal structural requirements coincide with those of agents which increase the polarizability of nerve membranes ([39]; for review, see[40]). Furthermore, it becomes permissible to identify the phasic changes of augmented afterpotentials with the oscillatory changes of nerve membrane potential constituting the 'nerve reaction' described by LORENTE DE NÓ[41]. It is, therefore, proposed that genuine postactivity motor nerve repetition whose initiation and recovery cycle can be attributed to negative and positive afterpotentials in nerve terminals, reflects an anelectronic state of these terminals and, accordingly, indicates their facilitator potentiality[42].

From the context of these findings, it emerges that transmission at the neuromuscular junction, while operating through the quantal mechanism, is subject to gradation by the terminal electrical event whose absolute magnitude depends on the membrane characteristics of motor nerve terminals. Conversely, the transmitter- and facilitator potentiality of a junction is not uniquely defined by the frequency of spontaneous miniature activity; nor can a neural mechanism of facilitating (or depressant) action be excluded for agents which do not alter the spontaneous discharge rate of min epp's.

Zusammenfassung. Aus den bisher veröffentlichten experimentellen Befunden über presynaptische, für die neuromuskuläre Erregungsübertragung massgeblich erscheinende Vorgänge wird geschlossen, dass die Förderung der Erregungsübertragung in direkter Beziehung zur Grösse des Aktionspotentials in den motorischen Nervenendigungen steht, während die Frequenz der spontanen Miniaturentladungen an sich kein eindeutiges Mass für die Intensität des Übertragungsvorganges von Nervimpulsen zum Muskel darstellt.

G. WERNER

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[42] To designate presynaptic events expressed in graded variation of transmission and facilitation in neuron pools, LLOYD, HUNT and MCINTYRE[19] introduced for the monosynaptic spinal cord reflex system the terms 'transmitter potentiality' and 'facilitator potentiality', indicating, thereby, two aspects of synaptic function, not necessarily covariant. These terms are here employed to designate the ability of the individual neuromuscular junction exhibiting spontaneous miniature activity, to respond to a nerve volley with transmission and facilitation of transmission.