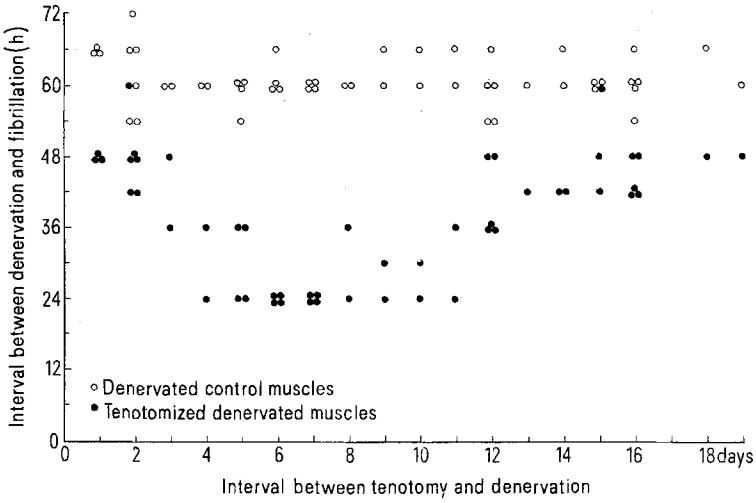

Fig. 2. Onset time of fibrillation in tenotomized denervated muscles, as a function of the interval between tentomy and denervation. Individual values are presented from 55 animals. Control data obtained from the contralateral, simply denervated muscles, are also reported.

taken 12–18 h after denervation, and afterwards every 6–12 h, until fibrillation had fully developed.

Results. In nearly all the muscles denervated without any other experimental procedure, the first fibrillation potentials could be detected only 60–66 h after denervation (see control data of Figure 2).

In the muscles denervated after cord section, limb immobilization or tenotomy, fibrillation appeared consistently earlier.

As shown in Figure 1, the average lag in the onset of fibrillation gradually shortened as the interval between cord section, or limb immobilization, and denervation was prolonged, reaching a minimum of 24 h when the interval was 5 days long; thereafter, the lag leveled off on this minimal value, even for intervals as long as 15 days. There was no difference between the effects of immobilization in flexion and in extension.

The minimal lag of 24 h was reached also in all the muscles denervated 6–7 days after tenotomy (Figure 2). When the intervals between tenotomy and denervation were longer than 6–7 days, fibrillation set in, on an average, less precociously: for intervals 13–19 days long, the lag ranged between 42 and 48 h. The latter result was obtained also when the scar tissue between the proximal and the distal stumps of the tendon had been cut 3–7 days before denervation. So it appeared to be independent of the re-establishment of the tendon, although some resumption of functional activity could not be excluded, because new adhesions rapidly developed between the tenotomized muscles and the surrounding tissues.

Discussion. The present results clearly indicate that some muscular change, not due to the lack of a trophic nervous influence, has an important role in the genesis of fibrillation.

The agreement between the data obtained in animals treated with different procedures, makes it likely that the change, or the changes, favouring the onset of fibrillation were mainly related to disuse, which in the 3 groups of experiments was the common feature preceding denervation.

It seems reasonable to assume that the alterations from disuse occur in denervated muscle as well, possibly at a faster rate and to a higher degree than in muscle put into disuse without a complete suppression of motor nerve activity. If this is the case, the longer interval between denervation and the onset of fibrillation in non pre-treated muscles as compared to the pre-treated ones, could reflect the time required for these alterations to develop[8].

Riassunto. Nei muscoli soleo-gastrocnemio di ratto la denervazione ha determinato insorgenza della fibrillazione dopo 60–66 h. Se eseguita dopo qualche giorno di disuso muscolare (conseguente a tenotomia, sezione del midollo spinale, immobilizzazione dell'arto) la denervazione ha determinato insorgenza della fibrillazione molto più precocemente, con un intervallo minimo di 24 h. Viene suggerito che nella genesi della fibrillazione dopo denervazione, il disuso muscolare abbia un ruolo importante.

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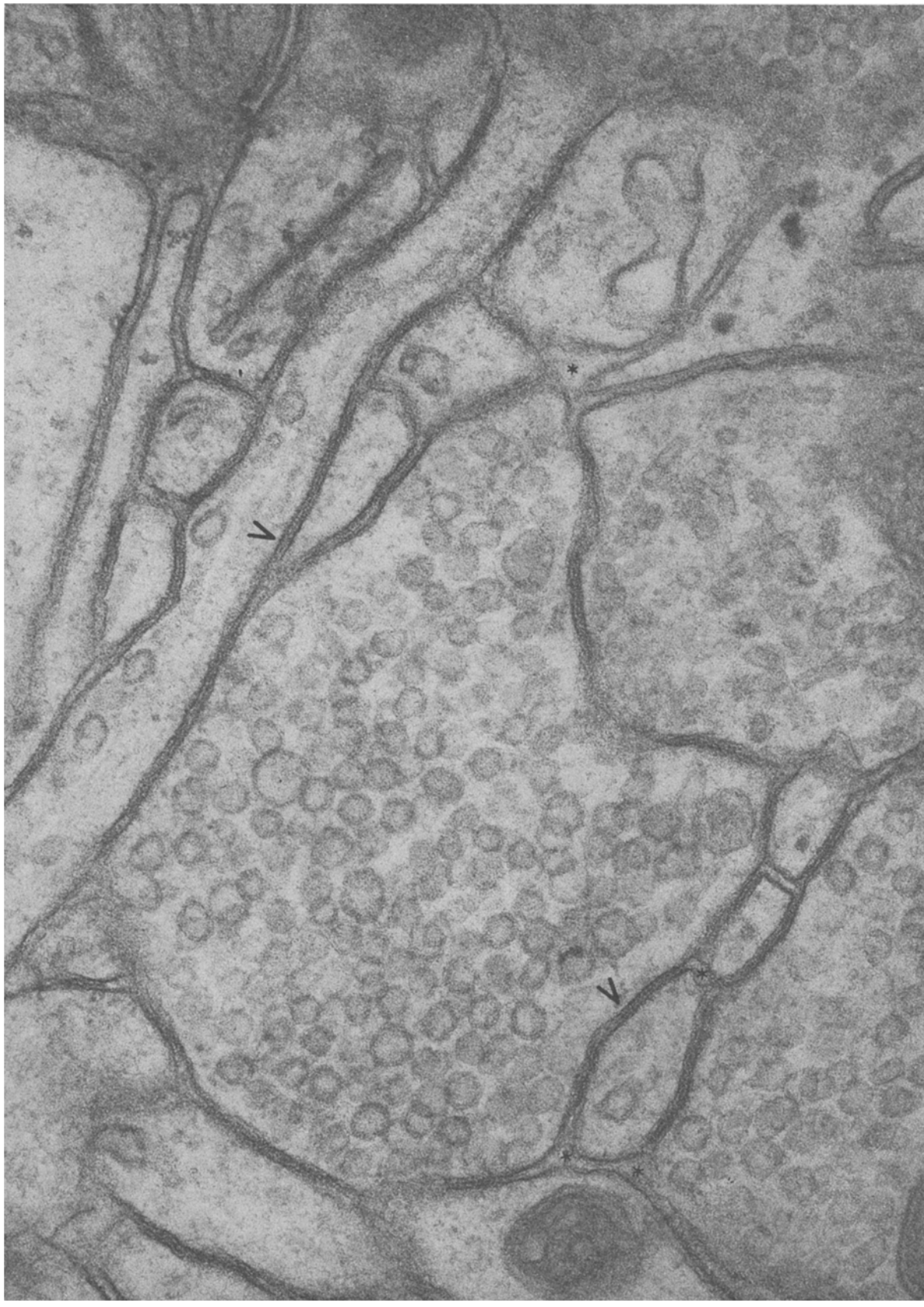
Neuron-Neuronal Attachments in the Parietal Cortex of the Rat

The parietal cortex of the rat fixed by perfusion of 100% formaline (12 *M* formaldehyde) shows abundant closely apposed synaptic boutons[1]. Because the method of perfusion fixation employed had been specially designed to minimize hypoxia[2] no significant hypoxic reduction of the extracellular space (ECS)[3] was feared. As for the fixative, it was found to be so hypertonic that if perfused for more than 3 min the brain looked 'like a walnut in its shell', and the ECS was coarsely enlarged[1]. However, almost simultaneously, Brightman and Reese[4] reported failing in detecting interneuronal close appositions in mice, other than those related to hypotonicity and or poor preservation of different origins. These spurious entities they called 'labile appostions' and were described as 'five layered structures resembling tight junctions'... 'showing a middle laminae as dense as but wider than an individual outer leaflet of adjacent cell membranes'... 'their overall width was approximately twice that of an individual cell membrane'. These appositions lacked 'any associated cytoplasmic fuzz'. Lately, we have confirmed that,



Despite the hypertonicity of both the washing solution and the fixative (note the enlarged ECS at asterisks), frequent interneuronal tigh-junctions appear. None of them shows the thicker middle line which is characteristic of labile appositions. Moreover, the middle line is often thinner (arrows). Two closely apposed synaptic boutons (one of which contains flatened type synaptic vesicles[6,7], suggest intercellular communication; however, this could be the result of oblique sectioning.

indeed, asphyxia, ischemia and or autolisis, do produce this type of appositions in the brain of the rat[5]. Moreover, these 'labile appositions' can be demonstrated despite the fixative hypertonicity, which indicates that, had the interneuronal appositions we had described[1] developed during the blood-washing period preceding the perfusion of the hypertonic fixative, they should not be expected to reverse because of the fixative hypertonicity. It was therefore important to find out whether or not some interneuronal tight junctions would persist after the perfusion of both a hypertonic washing solution and a hypertonic fixative. Thus, the 12 *M* formaldehyde method of fixation[1] has been applied to young adult normal albino rats, but, in addition to the usual components, the washing solution contained 1.8% NaCl.

At the electron microscope, the ECS ranged from moderately to extremely enlarged, but bouton-bouton attachments were present in all instances. Moreover, it was possible to detect neuron-neuronal quintilinear complexes in which the middle line was thinner than the outer ones (Figure). Such images indicate that neuron-neuronal quintilinear complexes do not necessarily result from mere apposition of contiguous outer leaflets belonging to independent unit membranes.

No hypothesis about the possible functional significance of these axo-axonic close appositions shall be formulated at this moment. It will be, however, mentioned that, considering the conspicuous synaptic vesicles which appear at these complexes, the implication of electrical synapses is not favored.

Zusammenfassung. Eine quintilineare neuronale Verbindung, die nicht durch hypertonische Spül- und Fixationsmittel zu sprengen ist, wird beschrieben.

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Feeding Elicited by Injections of Ca^{++} and Mg^{++} into the Third Ventricle of Sheep

The ionic composition of cerebrospinal fluid plays an important role in the function of central mechanisms involved in behavior[1]. While Ca^{++} and Mg^{++} decrease the excitability of neurons, Na^{+} and K^{+} increase it[1–3], thus regulating neuroconductivity. Ca^{++} injections into the lateral ventricles of rats[4] and perfusions of the ventromedial hypothalamus of cats[5] elicit feeding and often ataxia and a sleep-like conditon. Perfusions of the posterior hypothalamus of cats with excess Na^{+} produce arousal and hyperexcitability[3]. A specific role has been attributed to the Ca^{++}/Na^{+} ratio in the hypothalamus for temperature[6] and energy balance regulation[4] in cats, monkeys and rats. An increased ratio results in hypothermia and feeding, while a decreased ratio results in hyperthermia[6]. Although these responses might be predicted from the known effects of Ca^{++} and Na^{+} on neuro-excitability and the effect on feeding and temperature of CNS active drugs, e.g. barbiturates[7–10], neither Mg^{++} nor K^{+} were effective in these tests, in spite of their similar neural effects[4–6].

In the present experiments our objective was to determine if: 1) 'sated' sheep eat following injections of Ca^{++} into the cerebrospinal fluid (CSF), 2) Mg^{++}, which also decreases neuro-conductivity, elicits feeding, and 3) body temperature changes as a result of injecting Ca^{++} and Mg^{++} into CSF.

Ten sheep (40–45 kg wethers) were surgically implanted with third ventricular guides[11]. For temperature probes, a silastic tube closed at the proximal end was located near the dorsal portion of the liver and held in place with a dacron mesh skirt sewn subcutaneously. The animals were fed ad libitium, the daily ration given 1 h before the injection. Water was available at all times. One half ml of

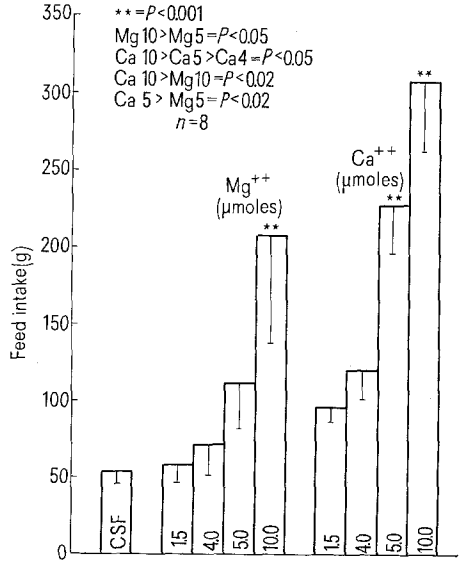

Fig. 1. Average 60 min feed intake of sheep as affected by injections of Ca^{++} and Mg^{++} into the third ventricle. xx = Denotes that those treatments were different from the control.

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