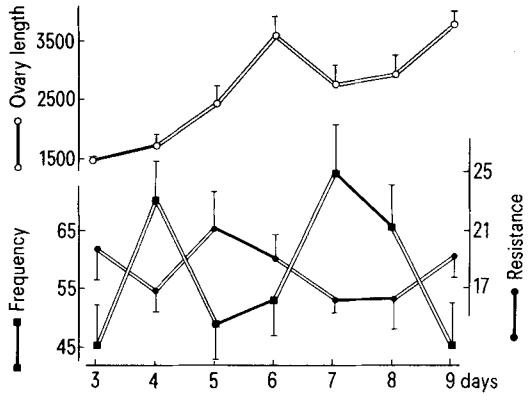

Figure 1. Firing frequency (imp/sec) and electrical resistance (MΩ) of labellar chemosensilla when tested with 0.5 M NaCl in protein-fed ('M') adult *Calliphora* females 3-9 day-old. Ovarian length (μm) of these insects is also reported. Yolk was present from day 4 to day 6 in the primary follicles, and from day 7 to day 9 in the secondary follicles. Experimental points are mean values ± SEM (vertical bars) of 40 (electrical resistance and firing frequency) and 10 (ovarian length) experiments. Experimental points joined by a double line are statistically different (Student's t-test; $p < 0.001$).

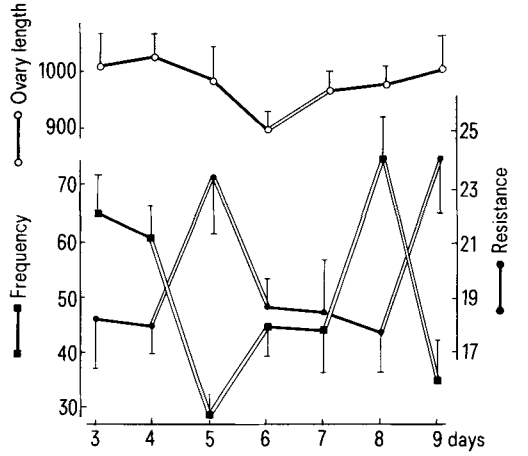

Figure 2. Firing frequency (ipp/sec) and electrical resistance (MΩ) of labellar chemosensilla when tested with 0.5 M NaCl in protein-deprived ('WM') adult *Calliphora* females 3-9-day-old. Ovarian length (μm) of these insects is also reported. Yolk was never present. Experimental points are mean values ± SEM (vertical bars) of 40 (electrical resistance and firing frequency) and 10 (ovarian length) experiments. Experimental points joined by a double line are statistically different (Student's t-test; $p < 0.001$).

provide direct information about the nature of this mechanism. Bearing in mind, however, that humoral control of chemosensory activity in other insect species[9-11] has already been demonstrated, it is likely to assume that a single endocrine mechanism controlling the ovarian function[12] also influences the sensitivity of taste chemosensilla in *Calliphora*.

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Alterations in the morphology of the neuromuscular junctions following experimental immobilization in cats

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Summary. Immobilization of the hindlimbs in cats, in the neutral position, by applying a plaster cast for 4 weeks, led to paler and larger neuromuscular junctions. Beyond 8 weeks, this procedure caused elaborately branched-out and paler junctions which were significantly larger in their diameters than those of the contralateral control limbs.

The neuromuscular junction, the important functional link between the nerve and the muscle, has been a subject of study for a long time. The morphology of the neuromuscular junction[2] and its enzyme histochemistry in the normal muscle[3] and in the functionally overloaded muscle[4,5] have been described in detail. Though the effects of immobilization on the morphology[6], physiology[7,8] and chemistry[9] of the skeletal muscle are very well known, detailed reports on the morphology of the neuromuscular junctions of an immobilized muscle are lacking.

Since it is known from tissue culture observations that the functional activity of a synapse influences its morphology[10], it was decided to immobilize the hindlimbs of cats and study in vivo the influence of function on the morphology of the neuromuscular junctions. Cole[11] attempted to study the morphology of the neuromuscular junctions after pinning the limb, and Vrbova[8] and Dias[12] studied them after tenotomy. The procedures of bone pinning and tenotomy, apart from causing immobilization, exert added strain on the muscle. Tenotomy produces contracture of the muscle and an increased excitability of the motoneurons, and later on connective tissue attachments are noticed between the tenotomized muscle and its bony attachment, resulting in increased tension[13,14]. The problem associated with the fixation of joints with bone pins is that an invasive object is implanted in the body, the effect of which on the muscular

and connective tissues are not too well known[15]. Hence it was decided to immobilize the limb for different periods by applying a plaster cast, as this method seems to be better, and also simulates the therapeutic measure adopted in orthopedic surgery on human beings.

Materials and methods. Adult cats *(Felis domestica)* of both sexes and weighing 2–3.8 kg were used. Under sodium pentothal anesthesia (30 mg/kg i.p.), the right or left knee and ankee were immobilized in the resting position by applying plaster of Paris. The paws and the terminal part of the foot were left uncovered. After allowing the limbs to be in the plaster for specific periods of 2, 4, 6, 8, 10 and 12 weeks, 2 animals in each group were anesthetized, the plaster removed and the gastrocnemius muscle dissected out. A strip of the medial head of the muscle where the nerve enters was excised, processed following a modification of Ranvier's gold chloride method[11], and then teased and mounted on a clean glass slide in a drop of glycerine with a cover slip pressed on it gently. The spread-out muscle fibers on the slides were scanned under a light microscope and the well-stained neuromuscular junctions, numbering 50 in each group, were observed and measured. The anteroposterior and transverse diameters of each junction were measured using the ocular micrometer and the mean value of these 2 was taken as the diameter of that particular junction. From these values the mean diameter of the neuromuscular junction for each group was calculated. Muscle strips cut from the medial head of the gastrocnemius in the contralateral hind limbs, processed and studied in the same manner, served as a control.

Results. When slides of normal muscle tissues were examined, bundles of nerve fibers were seen, which were scattered and ended on the surface of the muscle fibers. Each fiber terminated on the surface of the muscle fiber in a plate-like ending which is generally described as a 'termination-en-plaque'. The terminal part of the axon was seen to end in a well-stained bulblike structure before expanding into the end plate, and these disc shaped end plates were observed to have densely stained spherical branching structures distinctly outlined against the lightly stained muscle fibers. In general, each axon seemed to form only one end plate on a muscle fiber. Morphologically the appearance of the nerves and the neuromuscular junctions was more or less the same in both the control and the experimental muscle after 2 weeks of immobilization, except that these structures appeared paler in the latter group. As the periods of immobilization advanced to 4 and 6 weeks, thinning of the axons and irregular spacing of the nodes of Ranvier were the interesting features observed. At places, the axons showed swellings along their course. Though the terminal part of the axon was found to have stained dark, the neuromuscular junction was only lightly stained after about 6 weeks of immobilization and showed ramifications. After 8 weeks of immobilization, these ramifications became profuse, and the axons appeared to divide into two; in some cases both branches reached the same end plate, but in a few other fibers, the axon divided into two and formed separate end plates side by side on the same muscle fiber.

A further extension of the experimental period up to 10 and 12 weeks involved the end plates which appeared lightly stained and spread out, occupying a wider area with an irregular axon. Branching and anastomoses between these ramifications were also noticed.

The mean diameters of the neuromuscular junctions of the control muscle specimens and the experimental muscles are shown in the table. The differences between the mean diameters of the control and experimental neuromuscular junctions in animals immobilized for 8, 10 and 12 weeks were found to be statistically significant (table).

Discussion. The present study reveals that immobilization of a limb, besides leading to muscle atrophy, also alters the morphology of the neuromuscular junctions. Various factors could be held responsible for these changes. Immobilization of the limb interferes with the regular blood flow and tissue metabolism and this might have contributed to the morphological changes in the neuromuscular junctions to a minor extent. The possibility of damage to the peripheral nerve by compression cannot be ruled out although great care was taken, while applying the plaster cast, not to make it unduly tight. The marked changes noticed were the degeneration of the unmyelinated fibers followed by degenerative changes in the myelin sheath of some of the larger nerve fibers and thinning of the larger axons[19]. Though these changes apparently resemble those that result from direct compression of the nerve fibers[16], the earlier degeneration of the unmyelinated fibers, the absence of infiltration by the lymphocytes and macrophages and the fact that only some of the larger fibers showed myelin degeneration indicate that there may be some other important factors behind these morphological alterations.

The immobilized muscle has been shown to have increased sensitivity to acetylcholine[7] and reduced cholinesterase activity[17,18], which means that there is a fall in the acetylcholine release at the neuromuscular junctions of the immobilized muscle. This change could probably be responsible for the pale staining of these junctions as observed in this study. The reduced acetylcholine release may be associated with the reduction in diameter undergone by the axons as a result of immobilization[19]. Dias[20] observed a difference in the morphology of the neuromuscular junctions in normal slow and fast muscle fibers. A profusely branched-out, elaborate junction has been observed in the slow soleus muscle and a compact, darkly staining junction has been observed in the fast gastrocnemius muscle of the rabbit. Due to inactivity, the immobilized gastrocnemius muscle might have acquired the characteristics of a slow muscle and this explains the neuromuscular junctions being larger in the muscles which underwent prolonged immobilization. This is further supported by the findings of Tabary et al.[21] and Edgerton et al.[22] who reported that the contractile properties of the muscle undergo changes as a result of

Mean diameter of the neuromuscular junctions of control and immobilized muscle

Groups	No. of neuromuscular junctions studied	Control Mean diameter (±SEM/μm)	Experimental Period* in weeks	Mean diameter (±SEM/μm)	t-Values	p-Values
1	50	23.5±0.765	2	22.8±0.621	0.7198	<0.5
2	50	23.2±0.785	4	23.4±0.742	0.1711	<0.5
3	50	21.6±0.635	6	23.5±0.771	1.9015	<0.1
4	50	22.8±0.814	8	25.6±0.605	2.7184	<0.01**
5	50	23.4±0.761	10	26.2±0.764	2.4057	<0.01**
6	50	23.6±0.751	12	26.8±0.715	2.9718	<0.01**

*, Period of immobilization; p, probability; **, statistically significant.

immobilization, and the histochemical studies on the immobilized muscle also reveal changes in the properties of the muscle fiber types.

According to Price[23], the branched-out structure of the normal adult neuromuscular junction represents the postsynaptic membrane or the secondary synaptic clefts of the sarcolemmal membrane lined by an amorphous substance. Hence the profusely branched-out neuromuscular junction observed in this study might be due to changes in the sarcolemma and in fact Cooper[6], in his ultrastructural study on the immobilized muscle, observed an infolding of the sarcolemma.

Thus it is obvious that inactivity of a muscle for a longer period results in pale staining and profusely branched-out larger neuromuscular junctions. Cole[11] failed to observe any such alteration in the morphology of the junctions after 3 weeks of pinning the limbs, but in the light of the present experiment this seems to be rather too short a period for any marked changes to be produced. In fact the differences observed in the mean diameter between the control and experimental neuromuscular junctions were found to be statistically significant only after 8 or more weeks of immobilization, though they appeared paler and larger even in the earlier stages of immobilization.

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Quick plastic micropipettes and stainless steel microneedles for tissue manipulation

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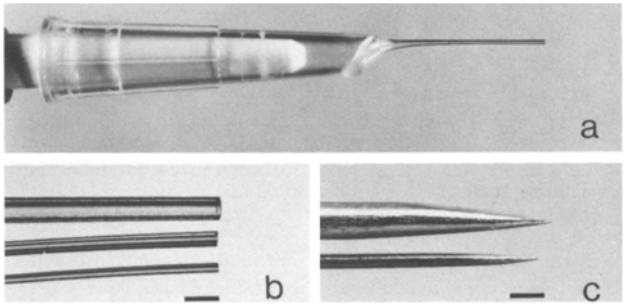
Summary. Simple methods for making fine, non-wettable plastic pipettes and for hand-sharpening stainless steel microneedles are outlined.

Small pieces of fixed or living tissue are usually destroyed if they happen to stick to the inside of non-siliconized glass micropipettes or to tungsten microneedles during preparation.

Mollenhauer[1] suggested using ordinary disposable plastic pipette tips to avoid tissue loss due to sticking, because these are non-wettable and hence not so sticky as glass pipettes. Such tips are too big for transferring minute tissue pieces, small organisms or single cells directly. However, they can be made into quite fine micropipettes by warming them locally and judiciously in a small flame, pulling them slowly as the plastic hardens and then cutting off the old tip with a sharp razor blade (fig., a). Before warming begins, a length of small diameter silicon rubber tubing is slipped over the original tip to provide a convenient finger hold away from the flame. Micropipettes with tip inside diameters down to about 50 µm can be made easily (fig., b). The piettes can be used directly with a 20 µl pipetter to transfer biological material in volumes as small as a few µl without much danger of loss due to sticking.

Stainless steel needles are more suitable for microdissection than tungsten ones, because they are less inclined to stick to tissue. Microneedles in stainless steel can be made by



a Plastic micropipette in place on a 20 µl pipetter, and *b* higher magnification views of 3 plastic micropipette tips. The smallest has an inside diameter of 50 µm. *c* Two hand-sharpened microneedle tips made from stainless steel wire. Bar: 0.5 mm.