Results and discussion. Nearly 13fold purification was obtained by the method used and the results of purification are summarized in the table. The tissue trehalase from M. lamarrei showed optimum activity at pH 6.0 in 0.1 M phosphate buffer (figure 1). The pH optimum of whole body trehalase of insects ranged from 5.2 to 6.5; as for example Bombyx mori 5.2[11], Galleria mellonella 5.5[12], P. regina 5.8[13], B. discoidalis 6.0[5], Leucophaea maderae 6.0[14], H. cecropia 6.5[4], S. gregaria 6.0–6.5[7]. This value also resembles the pH optimum obtained for digestive gland trehalase of M. lamarrei (5.5)[15]. The purified preparation is highly specific for trahalose, as revealed by the substrate specificity tests using other α-linked substrates (sucrose, maltose, melezitose and p-nitrophenyl-α-D-glucoside).

A value for the Michaelis constant (K_m) of 2.85 mM was calculated from a Lineweaver-Burk plot (figure 2). K_m values obtained for the tissue trehalase from Blaberus 3.3 mM[5], Hyalophora 3.6 mM[4], Phormia 1.3 mM[13] and Schistocerca 3.8 mM[7]. The mol.wt of the enzyme was estimated to be around 120,000. This value is far less than the value obtained for the enzyme of Mandusa (250,000)[8] and Drosophila (200,000)[16] but it is higher than that reported for Blaberus (80,000)[5]. About 21.7% of the enzyme activity was inhibited in Tris-HCl buffer supporting the previous observations[7,17]. Repeated freezing and thawing of the 10,000 × g supernatent considerably increased the specific activity of the enzyme. The same results were found by earlier workers[4,5,7,14]. The enzyme was stable at all temperatures below 45 °C for at least 3 h, but at 65 °C it was completely destroyed. The enzyme could remain in deep freeze without any loss of activity for several weeks.

No reports are available so far on the properties of trehalase from a crustacean source. The present study reveals that the tissue trehalase of M. lamarrei has many features in common with the tissue trehalase of insects. The pH optimum falls within the same range, its activity is inhibited by Tris-HCl buffer and it has a K_m value of 2.85 mM. Hence it may be concluded that the trehalase of M. lamarrei is more or less similar in nature to that obtained from insect sources.

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Electrically-induced mechanical activity of the isolated guinea-pig sciatic nerve. Influences of calcium and anoxia[1]

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Summary. The electrically-induced motility of isolated segments of guinea-pig sciatic nerves is reported. This motility was characterized by waves of tensional variations. The removal of Ca^{2+} from the suspending solution, the addition of EDTA as well as anoxia, resulted in a decrement of tension accompanied by a prolongued duration of the cycles of mechanical activity.

One of the first reports of the existence of an axonal flow dates back to approximately 30 years ago[4,5]. Nevertheless, the mechanism by which axoplasmic components migrate along nerves are not presently known. The 2 theories which are more widely accepted[6] postulate: a) Periaxonal elements generate peristalic pressure waves which, by squeezing the axoplasm, can move its components distally. b) There are structural changes in axoplasmic macromolecules able to propel inner materials. Öchs has proposed the 'transport filament hypothesis' as an explanation for the axoplasmic transport. According to this theory, crossbridges existing between transport filament and neurofilaments or microtubules were considered to be involved in axonal movements, similarly to the situation described for the sliding filament model of muscle contraction[7].

Borisy and Taylor suggested a specific colchicine binding protein as the mayor component of many axonal microtubules[8]. Puskin and Berl have shown that the microtubular protein has actin-like properties[9] and lately an actomyosin-like protein has been isolated from the synaptosomal fraction of bovine and rat brain[10]. On the other hand, Weiss was able to show continuous peristaltic motions in isolated nerves of young mice using microcinematographic methods[11]. – Based on all these data, we considered it of interest to explore the possibility of recording distinct signs of mechanical activity in isolated nerve preparations electrically stimulated.

Methods. Sciatic nerves isolated from adult (500–700 g) guinea-pigs were used. After the animals were killed by decapitation, the distal part of the principal trunk of the sciatic nerve was dissected out. Segments of approximately 2 cm were removed, placed in a Petri dish con-

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taining Krebs-Ringer Bicarbonate (KRB) solution; gassed with a mixture of 95% O_2 5% CO_2. The substrate for this solution was glucose at 11.0 mM and its temperature and pH were maintained constant at 37 °C and 7.4, respectively. Nerve segments were then trimmed of extraneous tissue without stripping the perineural sheath and mounted for the recording of mechanical activity accordingly to a procedure; similar to that employed with other tissues[12,13]; employing a force transducer and an oscillographic recorder. After a resting tension of 500 mg was applied to the isolated nerves by means of a micrometric device; the preparations were allowed to equilibrate in the tissue bath for a 60-min-period; following which were stimulated with a conventional stimulator. The electrical stimuli (square waves of 3 msec duration, a frequency of 2–80 cps and an intensity of 2–80 V) were applied for 20 sec. The recording system allowed the determination of: a) tension magnitude and b) duration of tension waves. The amplitude of the tension developed by the tissue was expressed in mg and named as the Isometric Developed Tension (IDT); whereas the duration of the mechanical waves was measured in min.

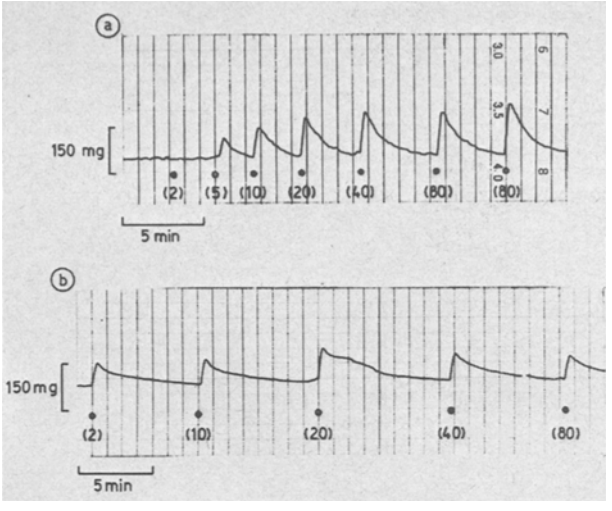

Fig. 1. Effects of different intensities and frequencies of stimulation upon the mechanical responses of isolated guinea-pig sciatic nerves. Black dots indicate the moment of stimulation. Panel a depicts responses obtained with increases voltages (figures between parentheses) at a constant frequency of 40 cps. Panel b shows responses following increasing frequencies (figures between parentheses indicating cps) at a constant intensity of 20 V.

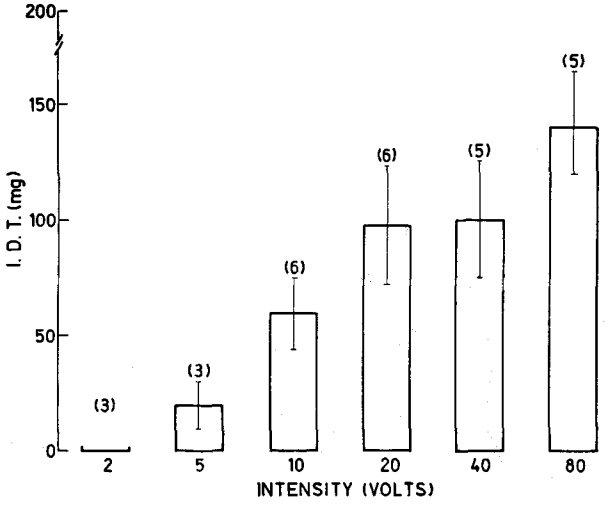

Fig. 2. Effects of increasing voltages of stimulation upon the magnitude of tension developed by isolated guinea-pig sciatic nerves. I. D. T. mean Isometric Developed Tension (in mg). Numbers below columns indicate the intensity of stimulation in volts (the frequency was kept constant at 40 cps). Figures between parentheses above columns represent the number of preparations explored. Vertical bars indicate the SEM.

3 different types of experiments were performed. 1. In one group of nerves, the stability with time of the electrically induced tensional changes were followed during a period of 60 min (the trains of impulses were delivered every 3–4 min). 2. In another set of preparations 4 trains of such impulses (each one also every 3–4 min) were applied and the responses obtained taken as controls. Forthwith, the tissue was washed 5 times with KRB-Ca^{2+}-free medium or with KRB-Ca^{2+}-free solution plus disodium ethylene diaminetetracetate (EDTA) at 1 mM (final concentration in the bath fluid). 60 min after imersion in one or the other medium, 4 new identical impulses were again delivered; the responses recorded and compared against controls. 3. A final group of nerves was explored for control activity (as already indicated) and then exposed for 5 min, to KRB-solution gassed with a mixture of 95% N_2 – 5% CO_2. Forthwith the preparations were again stimulated in the same fashion as before and the mechanical parameters of the control responses compared with those obtained during anoxia. In all cases, levels of significance between control and experimental values were compared employing the Student t-test. Differences between means were considered significant when $p=0.05$ or less.

Results. a) Effects of different intensities and frequencies of stimulation. Searching for 'optimal' conditions of electrical stimulation, stepwise increasing intensities of 2, 5, 10, 40 and 80 V (of 3 msec duration) were applied to several preparations maintaining the frequency of impulses at a constant value of 40 cps. As can be seen in figure 1 (panel a), and in figure 2; the threshold was attained with an intensity of 2–5 V. As the voltage aug-

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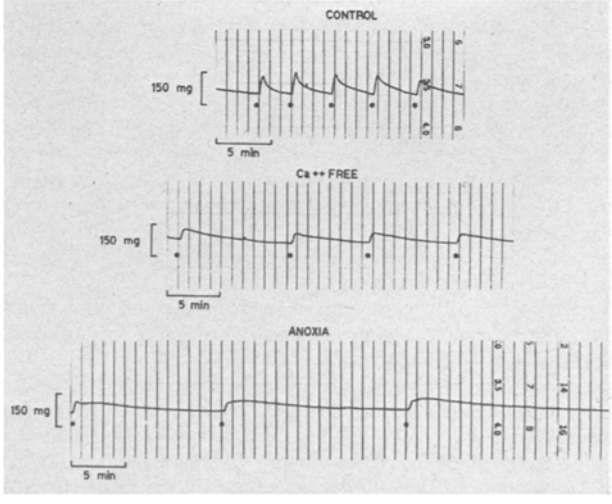

Fig. 3. Electrically-induced mechanical activity of isolated guinea-pig sciatic nerves. Black dots indicate the moment of stimulation.

Electrically-induced tensional changes of isolated guinea-pig sciatic nerves

Parameters	Experimental conditions* Normal KRB	KRB-Ca^{2+}-free	Normal KRB	KRB-Ca^{2+}-free + EDTA	Normal KRB	Normal KRB + N_2
IDT** (mg)	97.4 ± 17.0 (8)	64.3 ± 10.9 (8)	95.0 ± 11.6 (6)	48.6 ± 9.8 (6)	118.0 ± 16 (8)	56.0 ± 8.7
Duration***	4.2 ± 0.5 (8)	7.8 ± 0.9 (8)	4.0 ± 0.3 (6)	7.0 ± 1.4 (6)	4.4 ± 0.8 (8)	10.0 ± 1.5 (8)

* Mean values ± SEM. Figures in the parentheses refer to the number of preparations studied. KRB = Krebs-Ringer Bicarbonate solution. ** Isometric Developed Tension induced by the electrical stimulation. *** Duration of the tensional waves elicited by the electrical stimulation.

mented, nerve mechanical responses also increased. However, above 20 V and within a range of 20–80, the tension developed reached a plateau. In another group of nerves, the frequency of electrical impulses of 3 msec duration were incremented stepwise, whereas the output voltage remained constant at 20 V. As can be observed in figure 1 (panel b), we failed to detect significant variations in the responses within a wide range of frequencies (2, 10, 40 or 80 cps). In view of these findings, electrical stimuli of 20 V, 3 msec and 40 cps were selected to be employed in all further experiments. The electrically-induced mechanical responses to these trains (repeated every 3–4 min) were followed for a period of 1 h, in order to explore their characteristics and evolution with time. The results obtained were comparable during the whole experimental period, and no significant changes either in the magnitude of tension or in the duration of each mechanical wave, were detected.

b) Effects of Ca^{2+}-free medium; Ca^{2+}-free medium plus EDTA or anoxia. Following 60 min of equilibrium, the preparations were electrically stimulated with trains of impulses of 20 V, 40 cps, 3 msec duration, applied during 20 sec and repeated every 3–4 min. Results obtained are depicted in figure 3 (upper panel). After recording control responses, the procedure was repeated in KRB-Ca^{2+}-free solution. Figure 3 (middle panel) shows tracings of typical results. A quantitative analysis of the experimental findings are summarized in the table. A distinct reduction of IDT, as well as clear lengthening in the total duration of the mechanical cycles, were consistently detected. Comparable results were observed with preparations suspended in KRB-Ca^{2+}-free medium containing EDTA. Anoxia (following replacing O_2 for N_2 in the gas phase) also diminished the tension developed and resulted in an even greater prolongation of each mechanical wave (figure 3, low panel, and table).

Discussion. The in vitro existence of electrically-induced mechanical activity of sciatic nerves isolated from guinea-pigs was demonstrated for the first time. Even consisidering that several studies presented evidence about the presence of contractile-like proteins and a dynein-like ATPase in the central and peripheral nervous system of several species[7, 10, 14, 15], it was striking to observe measurable tensional changes in nerve segments subjected to an electrical stimulation. The fact that the lack of Ca^{2+} in the medium decreased significantly the magnitude of the induced isometric developed tension, suggests that a mechanism, similar to the one operating during the contraction of muscle cells, is also underlying the mechanical responses of nerve fibres. However, since the nerve activity in KRB-Ca^{2+}-free medium plus the addition of EDTA is not significantly different than in Ca^{2+}-free medium without EDTA, appears to indicate that the isolated nerve may have an important internal source of Ca^{2+}, or that Ca^{2+} is not such an important ion for its mechanical activity. Similar results have been obtained in some isolated blood vessels[16]. On the other hand, the substitution of O_2 by N_2 produced a distinct reduction in the magnitude of the developed tension, comparable to that observed in Ca^{2+}-free medium. Another point in common with the contractile reactivity of most muscle cells is the lengthening of relaxation during anoxia. It may be that mechanical responses to electrical stimuli of isolated nerves are somehow associated with the contractile proteins that are considered as related with the peristaltic movements observed in some nerves[11], as well as with the fast axoplasmic transport. However, in contradiction to this hypothesis, recent reports dissociated the excitability of the nerve membrane and the axoplasmic transport. Indeed, in completely depolarized nerve membranes (by replacing Na^+ with K^+ in the suspending solution) the rate of axoplasmic transport remained unaltered[17, 18]. In any event, the possible physiological role, if any, of the present findings, regarding the distinct mechanical activity of isolated nerves, deserves further studies.

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