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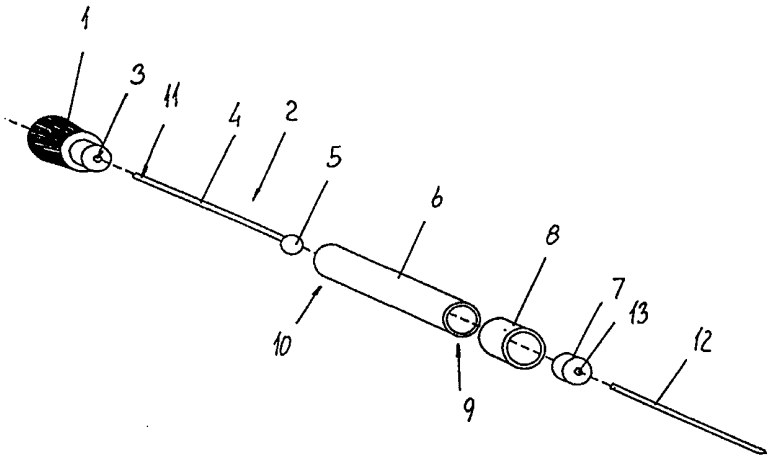

(57) Abstract

An electrode holder for use with research, medical and agricultural applications, involving the measurement of membrane potentials and of electrochemical impulses in moving, vibrating or contracting tissues is capable of both allowing insertion of needle electrodes or pulled glass electrodes. The design includes a silver rod (2) which is able to move freely within a glass cylinder (6). The glass cylinder (6) is connected to the recording electrode (12), while the silver rod (2) is attached to a suitable amplification system. The glass cylinder (6) is filled with electrolyte so that electrochemical impulses sensed by the electrode (12) can be transmitted to the silver rod (2) and thus to the amplifier and onwards. The overall design enables measurement of stable EMG signals from tissues that are prone to move, vibrate or contract during the period of investigation.

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Background of the Invention

ELECTRODE PERMITTING MEASUREMENT DURING TISSUE MOVEMENT

The present invention relates to a method of measurement of electrochemical impulses during movements in tissue, the electrochemical impulses being causative of the movements in the tissue, and which method comprises use of a sensor with an electrode intended for insertion into the tissue, whereby the electrode of the sensor is exposed to the electrochemical impulses in the immediate vicinity of a tip of the electrode. The invention also relates to an apparatus to be used by the method.

The present invention relates to research and medical instrumentation usable in the field of membrane potentials and of electromyography (ElectroMyoGraphics (EMG); M-waves; Compound Action Potentials), i.e. for the sensing and recording of stable electrochemical impulses as transmitted through the nervous systems and muscles of humans and animals. Specifically, the invention allows recordings of membrane potentials and electrochemical impulses to be recorded via mono and bipolar electrodes, from tissues that are prone to move or contract during the period of observation.

It is well known that in man and animals nerve impulses, transmitted electrochemically through the nervous system, are used to control tissues such as smooth and skeletal muscle.

M-waves, which are the sum of single fibre action potentials, have often been used in fatigue experiments where they provide information about the net effects of ion fluxes, Na^+-K^+ pump activity and neuromuscular transmission in skeletal muscles. A recent review discussed the potential of M-wave analysis as a non invasive predictor of muscle force.

Experiments involving M-wave measurements in man indicate that repetitive voluntary or stimulated contractions of muscles lead to a decline in peak contractile force ultimately causing M-waves to decline in amplitude and broaden. This agrees with observations that high-frequency stimulation of muscles leads to;

1) an increase in intracellular Na^+

2) a substantial loss of intracellular K^+ , and

3) an ensuing reduction in excitability.

While the ultimate fate of M-waves recorded in muscles exposed to repetitive contraction, is a decrease in M-wave amplitude, others report a transient increase in M-wave amplitude shortly after the onset of contraction. This transient potentiation of M-wave amplitude has been attributed to an electrogenic effect of increased Na^+, K^+ pumping, since following short periods of stimulated contraction in rat soleus muscle, Na^+, K^+ pumping increased, raising membrane potential and facilitating excitation of Na^+-channels. These studies, along with many others only examined M-wave amplitude, and obtained M-wave recordings percutaneously or from the surface fibres of exposed muscles. It is well known, however, that fibre type proportions differ between the superficial and deep layers of a muscle, and it might be expected that loss of excitability arising from K^+ accumulation in the extracellular water space during contraction, will be more severe in central than peripheral fibres due to problems associated with restricted diffusion. Thus assessment of M-wave properties from surface fibres alone may not be totally representative of the whole muscle.

To date the majority of systems capable of recording electrical signals in nerves and muscles employ medical electrodes applied to the surface of isolated nerves or muscles, or in the case of human subjects, to the skin. Alternatively, some investigations are undertaken using electrodes that penetrate the skin of the subject referred to as needle electrodes. The electrical signals detected by any one of these means can be suitably amplified and either displayed on an oscilloscope, recorded on a chart recorder, recorded as audio signals or collected by computer software for graphical presentation and manipulation. Since the electrical impulses recorded percutaneously often represent a mixing of electrical signals from an undesirably large area, it is often preferable to use subcutaneous needle electrodes to obtain not only signals from a specific location, but also from deep within tissues as opposed to just the surface. However, problems encountered in the use of currently available EMG recording equipment include the following.

It is a disadvantage of conventional surface EMG equipment, in which the sensor electrodes are rigidly wired to the associated amplification and recording/display equipment that the wiring involved not only restricts the range of motion of the subject under investigation, but may also lead to movement of the electrode with relation to the tissue causing a loss or change in the recorded signal. This limitation may require the investigator to forego certain movements of interest.

In the case of needle EMG, used to assess the electrochemical impulses deep within tissues, it is well established that the shape of motor unit potentials may show considerable changes with slight needle movement, contributing to uncertainty when quantifying EMG recordings as described in Staalberg, Journal of Biomedical Enginering, Vol. 2, October 1980. Even during isometric contractions where the length of the contracted muscle is prevented from changing, there are slight movements of the fascia, the skin and the electrode as mentioned in De Luca, Methods in Clinical Neurophysiology, Vol. 4, No. 1, March 1993. It has often been reported that these movements can cause signal instability. This is typically overcome by the investigator holding the needle during periods of muscle stimulation or voluntary contraction.

It is the object of the present invention to reduce or eliminate some or all of the disadvantages of prior electrode holder assemblies as discussed above by avoiding exterior movements or electrical signals from interfering with the measurements taken.

This object is obtained by a first method that is characterised in that the electrical signals are transmitted from the electrode to an electrolyte, that the electrical signals from the electrode are converted by the electrolyte into electrochemical signals, that the electrochemical signals are transmitted from the electrolyte to a sensor head, that the electrochemical signals in the electrolyte are converted by the sensor head into electrical signals, and that the electrical signals are transmitted from the sensor head to a receiver.

The object is also obtained by a second method that is characterised in that the electrochemical reactions are converted into electrochemical signals in the electrode, that

the electrochemical signals are transmitted from the electrode to an electrolyte, that the electrochemical signals from the electrode are converted by the electrolyte into other electrochemical signals, that the other electrochemical signals are transmitted from the electrolyte to a sensor head, that the other electrochemical signals in the electrolyte are converted by the sensor head into electrical signals, and that the electrical signals are transmitted from the sensor head to a receiver.

In the latter second method the electrolyte functions both as a conductive agent facilitating transmission of electrochemical impulses in a cell or between cells to be passed directly to a sensor head, said sensor being capable of movement within the electrolyte. Thereby a housing for the electrode serves as both electrode itself, being directly inserted into a cell or being inserted between cells, whilst at the same time serving to act as a reservoir for the electrolyte used to enable electrical contact between signals within or between cells and the sensor head.

An apparatus according to the invention is characterised in that a sensor has a reservoir, that the reservoir is located between the electrode and a sensor head made from metal, that an electrolyte is contained in the reservoir, that the electrode is fastened to the reservoir, and that the sensor head is displaceable inside the reservoir.

The present invention provides for uses of electromyographic equipment, whether in the fields of research, medicine or athletics, to measure stable membrane potentials and electrochemical pulses in cells of moving tissues. Different sizes of the equipment allow the user to select a holder that is most appropriate to their requirements. By using an apparatus according to the invention it is possible to make accurate measurements that are almost independent of exterior handling, vibrations or other movements. In addition, use of the present invention in conjunction with a micromanipulator enables recordings of membrane potential to be made in consecutive cells within a tissue, providing a profile of the tissue under investigation.

A preferred embodiment of an apparatus according to the invention is characterised in that the sensor comprises an abutment surface, that the reservoir is mounted on to the

abutment surface, that the electrode is mounted to the reservoir, that a holder is mounted to the abutment surface, and that the sensorhead is mounted to the holder.

Application of an abutment surface such as a sucking disk ensures that the electrode will stay in place in the correct and same position inside the tissue regardless of possible movement of the surface of the tissue. Furthermore the reservoir and the sensorhead are attached to the abutment surface and the electrical leads from the sensor are attached to a holder independent of the attachment of the reservoir and the sensorhead. This means that possible movement of the leads will not affect the measurements.

The present invention can be used in the field of research to measure the membrane potentials and electrochemical impulses of either exposed tissues or isolated tissues, such as nerves, smooth muscles, skeletal muscles and blood vessels.

The present invention can be used in the field of medicine to measure, relatively non-invasively, the membrane potential and electrochemical impulses of tissues that are diseased, or damaged or to follow the post-operative recovery of the function of various tissues.

The present invention can be used in the field of agriculture to measure the electrochemical properties of muscles in animals prior to and after slaughter. In such a way, livestock producers and meat producers alike will be able to assess the quality of the product more precisely.

The present invention can be used in the field of sports training to measure the electrochemical impulses of muscles used by different athletes. Information concerning EMGs from various muscle groups can be used during training sessions to improve the performance of athletes. Currently available equipment involving surface EMG recordings is currently employed in improving the swings of professional Golfers (see for example Nihon Koden & BioResearch Centre Co. 1997.

The present invention may also be used in conjunction with other appropriate devices, for instance the device described and detailed in US Patent US 5,579,781. Thus the present invention, combined with a wireless, self-contained and self-poared, transmitter, can enable the remote recording of electrical signals from for instance muscles of both human subjects and animals during both ambulatory and exercise associated movements. The present invention allows various diameters, lengths, and types of both commercial and home-made needle and pulled glass electrodes to be used within a standard electrode holder. The present invention can also be used to record stable membrane potentials in surface and in deep muscle fibres of isolated muscles both at rest and during periods of contraction.

Furthermore the apparatus according to the invention has shown properties in measurering membrane potentials which are not hitherto seen. Until now, it has only been possible to measure the membrane potential of muscle fibres at the surface of the muscle. This is due to the fact that stimulation and/or movement of the muscle leads to either breakage of a recording electrode inserted into the muscle or to loss of signal because the recording electrode jumps out of the muscle fibre that the electorde is inserted into. However, measuring at the surface cannot with certainty show how the membrane potential in muscle fibres deep within the muscle will change when the muscle is stimulated or performs a movement. Furthermore the timing of measurements is confined to before and after a period of contraction, giving rise to uncertainty as to whether measurements have been performed on the one and same muscle fibre.

With the apparatus according to the invention it is now possible to measure membrane potentials of both surface fibres as well as deep fibres in a muscle during contraction and at the same time be sure that the measurements are made in the one and same muscle fibre during the whole period of contraction. Thus, measurements of changes in the concentration of various cytosolic ions such as calcium, potassium and hydrogen can be performed during periods of contraction and in the same single muscle fibre.

These and other features, advantages and unique characteristics of a membrane potential and EMG needle holder capable of performing in tissues prone to move, vibrate or contract, will be apparent to those skilled in the art of electromyography. Hereafter the invention will be described in more detail with reference to the accompanying drawings.

Description of the Drawings

fig. 1 is an exploded view of the preferred embodiment of the EMG electrode holder for use in research with isolated tissues,

fig. 2 is a photograph of a preferred embodiment of the EMG electrode holder, for use in research applications, in its assembled form,

fig. 3 is a photograph of a preferred embodiment of the EMG electrode holder for use in the medical and athletic fields.

fig. 4 is a photograph of a miniaturised embodiment of the EMG electrode holder suitable for use with small tissues,

fig. 5 is a sketch of preferred equipment to be used when performing measurements with the sensor according to the invention

fig. 6 is a diagram showing a general measurement of M-wave propertes during stimulation

fig. 7 is a diagram showing a first simultaneous measurement of force and deep M-wave properties during tetanic force stimulation

fig. 8 is a diagram showing an second simultaneous measurement of force and deep M-wave properties during tetanic force stimulation

fig. 9 is a diagram showing an third simultaneous measurement of force, deep M-wave and surface M-wave properties during tetanic stimulation

fig. 10 is a diagram showing an first simultaneous measurement of force, deep M-wave and surface M-wave properties during twitch force stimulation

fig. 11 is a diagram showing a second simultaneous measurement of force, deep M-wave and surface M-wave properties during twitch force stimulation

fig. 12 is a diagram showing an alternative simultaneous measurement of force, deep M-wave and surface M-wave properties during tetanic force stimulation

fig. 13 is a first diagram showing external and internal measurements of M-waves in a muscle in relation to the tension of the muscle,

fig. 14 is a second diagram showing external and internal measurements of M-waves in a muscle in relation to the tension of the muscle,

fig. 15 is a first diagram showing measurement of membrane potential in a muscle with a sensor according to the invention,

fig. 16 is a second diagram showing measurement of membrane potential in a muscle with a sensor according to the invention

fig. 17 is a photograph showing the location among fibres in a muscle of a tip of a glass electrode forming part of a sensor according to the invention,

fig. 18 is a diagram showing recordings of membrane potentials in consecutive muscle fibers made from the surface inwards, and

fig. 19 is a diagram showing the recording of membrane potentials made from the surface inwards in up to 19 consecutive muscle fibers in one isolated muscle.

Fig. 1 shows an embodiment of an EMG electrode holder assembly according to the present invention. The EMG electrode holder assembly includes an electrical connector that is configured to connect to monitoring or recording equipment, namely EMG monitoring equipment. Examples of such electrical connectors are shown in Fig. 2 and in Fig. 4.

The EMG electrode holder includes a cylindrical stopper 1, which serves to hold a rod 2 in such a fashion that the rod is able to slide freely through a hole 3 in the centre of the cylindrical stopper 1. The rod 2 has a shaft 4 and bulbous recording head 5 and at least the recording head 5 is preferably made of silver or of gold. The rod 2 is inserted into a cylindrical glass tube 6, which is filled with an electrolyte solution, in such a fashion that the bulbous recording head 5 is able to move freely within the cylindrical glass tube 6. As an alternative to the cylindrical stopper 1 the cylindrical glass tube may have a collar provided in a backwards end 10 of the glass tube, the collar being provided with a hole through which the rod 2 is able to move freely. This alternative embodiment is especially advantageous when inserting very brittle electrodes of the sensor such as glass electrodes into tissue, because friction between the rod 2 and the

collar is much less than friction between the cylindrical stopper 1 and the rod 2. Thereby the risk of accidentally applying a momentum to the electrode via the rod and the glass tube upon insertion, is reduced.

In the embodiment shown the bulbous recording head 6 is shown to be a spherical ball with a smooth surface. However, the surface may have a pattern making the surface of the spherical ball non-smooth. Also other shapes may be used for the bulbous recording head. Thus the bulbous recording head may be cylindrical such as circular cylindrical or it may be conical with linear generatrices or with curved generatrices.

To prevent electrolyte leaving the cylindrical glass tube 6, a rubber stopper 7 is held in place by a rubber sleeve 8 at a forward end 9, and the cylindrical stopper 1 performs a similar job at an opposite backwards end 10 of the cylindrical glass tube 6. An end 11 opposite the bulbous recording head 5 of the rod 2 is intended for protruding out of the hole 3 in the cylindrical stopper. The shaft 4 of the rod 2 is subsequently coupled to an electrical connector (not shown, see fig. 2 and fig. 4) appropriate for the type of amplifier preferred.

The rubber stopper 7 is designed to releasably and reusably accept different electrodes 12 which are inserted through a small hole 13 in the rubber stopper 7. In such a way, metal needle electrodes obtain electrical contact with the rod 2 via the electrolyte solution held within the cylindrical glass tube 6. In the case of the electrode being made of a pulled glass electrode, which are by nature hollow and can therefore be filled with electrolyte, electrical impulses in the tissue are transmitted through the electrolyte within the pulled glass electrode, to the electrolyte in the cylindrical glass tube 6 and on to the rod 2 where they are sent on to the electrical connector (see fig. 2 and fig. 4) and through electrical leads to the monitoring equipment.

Furthermore, the invention may be enclosed or mounted in conjunction with other apparatus in order to facilitate its use in other diverse situations. One such situation being the measurement of EMG in muscles of human patients. Fig. 3 shows a possible design for just such an application. The EMG electrode holder is linked to a deep re-

cording needle electrode via an electrolyte contained within a semi-circular vessel. Electrical impulses recorded by the needle electrode can be transmitted to the silver rod of the EMG electrode holder whilst at the same time preventing any movement of either the recording needle electrode or the leads attached to the amplifier, to affect the recorded signal.

Alternatively, it may be desirable to reduce the overall size of the EMG electrode holder to facilitate recordings with very small or delicate tissues, such as nerves and the smooth muscle of blood vessels, or to enable multiple recordings of close proximity within one tissue. In such a case a miniature EMG electrode holder similar to that shown in Fig. 4 may be preferred.

Further alternatively, a preferred embodiment which is smaller than the embodiment shown in fig. 3 weighs only about 1.0 g when not filled with electrolyte. The preferred embodiment has new features compared to the features of the sensor shown in fig. 3.

The preferred embodiment of the sensor is mounted at one end to a hemispherical reservoir. The sensor is also connected at an opposite end to a connector made of gold. A sensor may also be made of gold primarily for clinical use. The connector is mounted in an arm made of plastic. The plastic arm allows for some degree of movement of the sensor in the reservoir as a result of possible movements of the cables leading from the sensor to the measuring equipment. The recording electrode is inserted through a rubber socket which is mounted in the abutment plate. The recording electrode is inserted into the hemispherical reservoir thus allowing for movement of the electrode as a result of tissue movement.

While a specific embodiment of the invention has been illustrated and the preferred embodiment of the invention has been described, it will be appreciated that, within the scope-of the invention, various changes can be made therein without departing from the spirit and scope of the invention. If the electrode is made of glass with a hole penetrating the electrode from the tip to the base of the electrode along a longitudinal axis of the electrode it will be possible to avoid using a reservoir such as a glass tube

and instead place the sensor head and the rod in the hole of the glass electrode. The sensor head and the rod may in such an embodiment be very small compared to the sensor head and the rod shown in the figures. Thereby the size of the sensor is limited. As an option the abutment plate may have an adhesive covering for fixation to the skin of patients.

Equipment

Fig. 5 is a sketch of equipment generally used when measuring M-wave properties. Isolated rat muscles are mounted vertically in thermostatically controlled chambers, stimulated directly with supramaximal pulses and force development and M-wave properties can be measured and recorded. The thermostatically controlled chambers are made of perspex and have an internal depth and diameter of 5.5 and 3.2 cm, respectively, holding approximately 44 ml of Krebs Ringer buffer. The mounting/stimulation block is also made of perspex 8 cm tall, 1.5 cm wide and 1 cm deep. Into this perspex block are inserted two steel pins to hold the isolated muscle, two silver stimulating electrodes (0.88 mm in diameter) and 2 silver recording electrodes (1.0 mm in diameter), fashioned out of jewelry grade silver (Dansk Hollandsk Ædelmetal A/S, Copenhagen, Denmark). The silver electrodes are covered with heat shrink plastic coating and set in the perspex block in such a way to allow movement towards or away from the suspended muscle.

The stimulator used, is a Master 8 Programmable Pulse Generator (A.M.P.I., Jerusalem, Israel) and is linked to a stimulus isolator (Isolator-10, Axon Instruments Inc., Foster City, CA). Tetani are evoked through two silver electrodes mounted on the mounting/stimulation block by supramaximal field stimulation at either 30 or 90 Hz, 10-100 mA and 1 ms pulse duration, whereas twitches are evoked by supramaximal constant current field stimulation at 2 Hz, 10-100 mA and 0.2-ms pulse duration. Deep M-waves are collected from stimulated muscles by either a glass or tungsten microelectrode, inserted into the centre of the muscle, which is linked to a home built high impedance differential amplifier. Surface M-waves are collected from muscles by one of two flat silver recording electrodes placed adjacent to the muscle which send sig-

nals to a DAM 70 Differential Amplifier, mounted with a low noise headstage (World Precision Instruments Inc., Sarasota, FL). Differential recordings of M-waves can be made from the two surface recording electrodes facilitating the calculation of the speed of M-wave transmission in muscles. Twitch and tetanic contractions are measured using a FIO03 force displacement transducer (Grass Instrument Co., West Warwick, RI.) connected to a CP122 AC/DC strain gauge amplifier (Grass Instrument Co.). Contractile force measurements are sent directly to a chart recorder (Servograph Pen Drive REA 3 1 0, Radiometer, Copenhagen, Denmark), and via an A/D converter (TL-1 DMA Interface, Axon Instruments Inc.) to both an IBM Compatible 386 and Mac computer hard disk. Likewise, both differential amplifiers send measurements of M-waves via the same A/D Converter to both a IBM Compatible and a Macintosh computer. M-wave and contractile force measurements are made to the PC by means of pClamp software (Axon Instruments Inc.), whereas measurements are made to a Macintosh 8200/120 via a Maclab 4s running Maclab Chart software (ADInstruments Ltd., Hastings, U.K.). The pClamp software supports triggering of the stimulator directly from the IBM Compatible computer, a facility that is often used with paradigms involving regular repeated stimulation of isolated muscles. The home built high impedance differential amplifier can be set to make DC recordings via a glass microelectrode, providing a means of measuring field potential.

Animals, Muscles and Force Development

Wistar rats, typically of between 60 and 70 g (4 weeks of age), are used. They are fed a standard rat pellet (Altromin Nr. 1314 Spezialfutterwerke, Lage, Germany) ad libitum, water is freely available, environmental temperature is maintained at 21°C, and lighting is on a 12:12-hour light-dark cycle. Rats are killed by a blow to the head, followed by cervical disslocation in accordance with local and national guidelines, and with permission of the Animal Welfare Officer, University of Aarhus, Denmark.

Soleus muscles, which predominantly comprise slow-twitch Type I fibres in rats, lie deep to the gastrocnemius in the lower hind-limb. They originate upon the fibula and insert together with the gastrocnemius upon the calcaneus by means of the Achilles

tendon. Soleus muscles are dissected intact with both the tendon attached to a portion of the fibula, to facilitate anchoring, and a portion of the Achilles tendon at the opposite end of the muscle. The portion of fibula is placed between two metal pins and secured in place by a piece of thread. The section of Achilles tendon is impaled on a steel rod which is subsequently fastened to a force transducer, with the result that the muscle lie both between two silver stimulating electrodes, and on top of two silver recording electrodes. Suspended isolated muscles are placed into the thermostatically controlled chambers which are filled with a standard incubation medium, Krebs-Ringer bicarbonate buffer, containing (in mM) 120.1 NACl, 25 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.3 $CaCl_2$, and 5.0 D-glucose. The buffer is kept at 30°C and equilibrated continuously with a mixture of 95% O_2 and 5% CO_2 (pH 7.3). Soleus muscles of 4 week old rats weigh 20.7 ± 0.6 mg wet weight and have an isometric force of 16.2 ± 0.4 N/g wet weight, and a twitch to tetanus ratio of 1:5. Adjustments to muscle length are made to ensure that maximal isometric force is achieved.

M-wave Recordings

Surface M-wave recordings are via one of two silver field electrodes placed adjacent to the muscle surface, whereas intracellular M-wave recordings (deep Mwaves) are either via a glass microelectrode (5 µm tip), or via a tungsten microelectrode TM33B01 (World Precision Instruments Inc.), positioned in the centre of the muscle but directly over the surface recording electrode. In order to prevent distortion of M-wave recordings, which results from movement of the electrode tip within contracting muscles, a sensor according to the present invention capable of absorbing movement, is used. In this way compound action potentials can be transferred from the microelectrode positioned deep within the muscle to the differential amplifier, while at the same time preventing any contraction-associated movement of the microeletrode tip.

Fig. 6 shows M-wave area and amplitude measurements. Conduction velocity, expressed in m/s, is calculated by dividing the time from the beginning of the stimulus-artifact to the action potential peak, by the distance between the stimulating and recording electrodes, a distance of 6.5 mm.

RESULTS

M-wave Parameters and Tetanic Force

Measurements of M-wave conduction velocity showed that low frequency stimulation at a frequency of 2 Hz induced an M-wave, with an average (surface and deep) conduction velocity of 2.6 ± 0.1 m/s (n = 7), followed by a twitch in isolated soleus muscles.

Fig. 7 shows measurements during continuous direct stimulation (30 Hz, 10 mA, 1 ms pulse duration) applied to isolated rat soleus muscles for 25 sec. Tetanic force remains unaltered over this period of time. There is a rapid and significant increase in M-wave area however, which occurred over the first 5-30 sec whereas a significant potentiation of M-wave amplitude is measured over only the first 5-15 sec of stimulation.

Fig. 8 shows measurements during a higher stimulation frequency (90 Hz, 10 mA, 1 ms pulse duration), and a different pattern is observed. A significant decline in contractile force is measured after only 15 sec of continuous direct stimulation (approximately a 20% decline in peak tetanic force), coinciding with a decline in deep M-wave amplitude ($r = 0.978$; $n = 5$; $P<0.004$), which is significantly different from the control value after only 10 sec of stimulation. Measurement of deep M-wave area, however, revealed no significant effect of stimulation before muscles had been stimulated for a period of 25 sec.

Effects of Inhibition of Na^+-K^+ Pump Capacity on M-wave Parameters

Fig. 9 shows measurements for muscles incubated for 30 min with ouabain at a concentration of 10^{-6} M, that are stimulated at a frequency of 90 Hz for 40 sec, after which recovery of both tetanic force, shown with white dots, and both M-wave area, shown with white triangles, and amplitude, shown with black dots, are recorded 30 and 60 sec after cessation of stimulation. Use is made of the cardiac glycoside ouabain, at a

concentration of 10^{-6} M, to reduce Na^+-K^+ pump capacity and thereby impair maintenance of membrane excitability. A 9 % reduction in tetanic force is noted after 5 sec of continuous stimulation at 90 Hz (88.7 % ± 1.6 and 97.8 % ± 2.1; n = 6; P<0.01) in ouabain incubated and control muscles, respectively. More impotantly, however, this figure shows how closely deep M-wave parameters follow changes in tetanic force, thus plots of tetanic force against deep M-wave area and amplitude revealed very close correlations (r = 0.96; n = 6; P<0.003), and (r = 0.94; n = 6; P<0.005), respectively.

Since it is difficult to see any effect of ouabain on M-wave parameters during the early period of high frequency stimlation, the time course of the effects of 10^{-6} M ouabain on twitch force and M-wave parameters were investigated

Fig. 10 and fig. 11 show that incubation of muscles with this low concentration of ouabain over a period of 30 min has no significant effect on either twitch force or area. Indeed no significant effect of ouabain is measured on deep M-wave area or amplitude either. Ouabain at a concentration of 10^{-6} M does, however, reduce the area of surface M-waves by 11% after 30 min (n = 5; P<0.05), although a more striking effect of ouabain is noted on surface M-wave amplitude (Fig. 11). After 10 and 30 min incubation with ouabain, a decrease of 16% (n = *5;* P<0.05) and 37% (n = 5; P<0.01) in surface M-wave amplitude is measured, respectively. Removal of ouabain from the incubation buffer results in a recovery of surface M-wave amplitude, to such an extent that 20 min after washout surface M-waves are no longer significantly different in terms of area or amplitude from control values.

DISCUSSION

The results in which contractions of both tetani and twitches have been related for the first time to both deep and surface M-wave parameters, show that contractility is closely dependent upon M-wave properties. Results highlight the importance of both measurement of M-wave area as an index of contractile force, and the simultaneous measurement of surface and deep M-wave recordings when investigating the effects of pharmacological agents on muscle function.

M-wave Conduction Velocity

In the present study, conduction velocities for isolated soleus muscles of 4 week old rats compare favourably with those reported by others; soleus muscles of a mouse (2.9 ± 0.4 m/sec), and soleus muscles of adult rats (2.7 ± 0.4 m/sec). Increasing the stimulating current in 1 mA steps from between 1 to 10 mA leads to both a step-wise increase in M-wave amplitude and area, and twitch force. A close correlation has previously been reported between the area of M-waves, calculated from surface recordings, and the twitch force of isolated soleus muscles.

M-waves and Tetanic Force

The initial increase in deep M-wave amplitude during direct stimulation of isolated muscles at 30 Hz compares favourably with former results for surface M-waves. Former results reported a gradual increase in M-wave amplitude (15-20%) which peaked after 30 sec of percutaneous 20 Hz stimulation of tibialis anterior muscles of human subjects. The present study also serves to extend the work by revealing an initial increase in deep M-wave area during direct stimulation of isolated muscles. Both the increase in M-wave amplitude and area are likely to be responsible for the fact that tetanic force did not decrease during the initial period of continuous direct stimulation. At the higher, and unphysiological frequency of 90 Hz, which is used to ensure activation of all functional Na^+-K^+ pumps, M-wave amplitude decreased in a linear fashion with the commencement of stimulation and in conjunction with the decline in tetanic force. The results indicate that the phenomenon of initial potentiation of M-wave amplitude, and area during periods of continuous stimulation, is restricted to lower and more physiological frequencies.

Na^+-K^+ Pump Capacity and M-waves

It has previously been shown that after 30 min incubation of isolated muscles in ouabain at a concentration of 10^{-6} M, approximatley 80% of ouabain binding sites are

unoccupied. Interestingly, a comparison of fig. 8 and fig. 9 shows a reduction in pumping capacity of approximately 20% had no significant effect on deep M-wave amplitude or area which are not significantly different from those of untreated muscles over the first 20 sec of stimulation at 90 Hz. These results indicate that Na^+-K^+ pumping may not be entirely responsible for the initial potentiation of M-wave amplitude and area seen during a period of sustained contraction.

Investigation of the time course of the effects of ouabain (10^{-6} M) on twitch and both deep and surface M-wave properties revealed that ouabain, at this concentration, is regionally located in isolated muscles. Ouabain incubation specifically decreased the M-wave amplitude and to some extent the area of the surface fibres without having any significant effect on the properties of deep M-waves or twitches. Furthermore ouabain significantly and specifically reduced the conduction velocity of the surface fibres, without affecting the conduction velocity of deep fibres. These observations clearly have great importance for the interpretation of M-waves as an index of muscle force. First, when using pharmacological agents or indeed ions, it is essential that both surface and deep recording electrodes are used to monitor the diffusional effects on M-wave properties. Second, in terms of assessment of contractile force and muscle excitability it is important to measure the area of M-waves, since our results show that a 30% reduction in the amplitude of surface fibres has no significant effect on twitch force in isolated soleus muscles of 4 week old rats. Third, these results give a greater insight into the effects of ouabain on muscle performance. The difference between fig. 10 and fig. 11 is likely to be due to a reduction in the conduction velocity of M-waves in some fibres treated with ouabain contributing to a decrease in M-wave amplitude since it will result in a gradual widening of the action potentials of some of the recorded fibres leading to greater temporal overlap and signal cancellation of the recorded M-wave. The results for surface M-wave area presented in Fig. 10 support this conclusion, since M-wave area remains unaffected by incubation with ouabain during the first 20 min, at a time when the amplitude of the surface M-wave decreased by 28%.

Physiological Perspectives:

This study is the first to combine measurements of both surface and deep M-waves of an isolated muscles during periods of tetanic and twitch contraction. It shows that during periods of sustained low, but not high frequency stimulation deep M-wave amplitude and area potentiate initially in isolated muscles, and that changes in tetanic and twitch force correlate closely with changes in M-wave area and amplitude. Furthermore investigation of the time course of the effects of the cardiac glycoside, ouabain, on muscle performance show that a decrease in surface M-wave amplitude, area and conduction velocity can occur in the absence of any change in deep M-wave properties. This study indicates the need for simultaneous recordings of both surface and deep M-waves in experiments designed to investigate the electrical and contractile properties of muscles, and highlights the risk associated with predicting contractile performance based on surface M-wave parameters alone.

Other properties

Fig. 12 shows simultaneous measurements of twitch force, and both deep M-waves and surface M-waves in percentage form during a period of 30 sec stimulation at 30 Hz. The measurements are made with and without use of aconitine (10^{-6} M), the white and the black dots, respectively. Aconitine is a plant alkaloid which causes sodium channels to remain open twice as long as normal, thereby causing muscles treated with aconitine to fatigue at a faster rate.

The measurement of twitch force shows a fatigue during the period resulting in a decrease of the twitch force down to about 90% of the initial strength of the muscle in a muscle without aconitine, and to about 79 % in a muscle with aconitine. The deep M-wave measurement shows a decrease to about 88 % and about 77 % of the muscle without and with aconitine, respectively. However, the surface M-wave measurement shows a decrease to about 82 % and about 49 % of the muscle without and with aconitine, respectively. Thus, deep M-wave measurement with a sensor according to the

invention is much more accurate in showing what is really taking place in the muscle compared to known measurements of surface M-waves. It can now be seen that the deep fibres are not as influenced by aconitine as are the surface fibres.

Fig. 13 shows another measurement of twitch force, which in the diagram is denoted Tension, compared to measurements of deep M-waves and surface M-waves, which in the diagrams are denoted Int. M-wave and Ext. M-wave, respectively. The three panels show that both deep and surface recordings of M-waves can be made simultaneously in conjunction with measurements of contractile force.

Fig. 14 shows an exploded view of the first 50 ms of the data presented in fig. 13. A much clearer view of the deep and surface M-waves is presented along with the preceeding stimulus artifact. Furthermore, the temporal delay that exists between the M-wave and the onset of contraction can be readily discerned.

Fig. 15 shows the change in membrane potential as recorded by a glass microelectrode, filled with 2 M potassium acetate and 0.1 M potassium chloride (pH 7.4) upon insertion into a single fibre of an isolated muscle from a 6 week old rat. The glass microelectrode was combined with a sensor according to the invention. In this instance, a resting membrane potential of approximately -50 mV was recorded in the fiber (lower panel) at the same time that twitch force, denoted Tension, was measured (upper panel).

Fig. 16 shows in greater detail measurements of membrane potential and twitch force as presented in fig. 15. The diagram shows that the membrane potential of a single muscle fibre is in this case approximately -50 mV. When the muscle is subjected to electronic stimulation (2 Hz, 10 mA) a stimulus artifact is first recorded by the sensor, denoted by a change in the membrane potential value from -50 mV to +5 mV and back to about -50 mV (first peak). This is followed by the propagation of an action potential in the muscle fibre from -50 mV to -10 mV and back to -50 mV, thus an order of magnitude of approximately 40 mV (second peak). After the action potential from the single muscle fibre has been measured the membrane potential, as mentioned, returns

to its resting value of approximately -50 mV. Subsequent to the action potential, a twitch force, denoted Tension, is recorded (upper panel).

The figure shows that the measurement is taking place in only one muscle fibre and that the measurement is taking place in the one and same muscle fibre during the period of contraction. Thus, the electrode stays in the one and same single fibre during contraction and is neither broken nor pushed out of the single fibre. This is shown by the discrete measurements of the electronic stimulation and the action potential and by the membrane potential returning to the same resting potential as before stimulation of the single muscle fibre.

Fig. 17 shows measurements with a glass microelectrode used for DC recordings of M-wave parameters injected in a single fibre of a muscle of a 4 week old rat. Injection is made with toluene blue and inserted into soleus muscles attached to a force transducer. Muscles are briefly stimulated at 2 Hz (10 mA and 0.2-ms pulse duration) in order to determine whether stable M-waves are being recorded by the glass electrode. Muscle length is noted before muscles are removed, placed on a plastic rack, stretched to their previously noted length and rapidly frozen in liquid nitrogen. Frozen muscles are sectioned longitudinally (10 μm) at -14°C in a cryostat, mounted on microscope slides and examined under a microscope for toluene blue staining. The toluene blue is shown as dark grey, almost black, "stains" in the left side of the photograph. The results show that the tip of deep recording electrodes are indeed located in the medial region of the muscle and that there is no appreciable movement of the tip with periods of sustained contraction.

Fig. 18 shows measurement of membrane potential made with a glass microelectrode combined with a sensor according to the invention. The four panels represent individual, isolated soleus muscles from separate animals, namely 10 week old Wistar rats. The filled columns within each panel represent the membrane potentials of individual consecutively recorded fibres from the first fiber at the surface into the sixth fiber from the surface of the muscle. The results show that with use of the sensor according

to the invention, consecutive recordings can be obtained from adjacent fibres within a muscle giving rise to a profile of membrane potential for different muscles.

Fig. 19 shows measurements of membrane potential made with a glass microelectrode combined with a sensor according to the invention. Results of membrane potentials for fibres from one isolated soleus muscle form a 10 week old Wistar rat are presented in order of recording from the first fiber at the surface to the 19th fiber from the surface of the muscle. These result, made in conjunction with a sensor according to the invention facilitate the construction of a profile of membrane potentials for fibres throughout a muscle and also enable assessment of the degree of penetration into isolated muscles and the effect on membrane potential of externally applied substances or compounds.

CLAIMS

1. Method of measuring electrochemical impulses during movements in tissue, the electrochemical impulses being causative of the movements in the tissue, and which method comprises use of a sensor with an electrode intended for insertion into the tissue, whereby the electrode of the sensor is exposed to the electrochemical impulses in the immediate vicinity of a tip of the electrode, and whereby the electrochemical impulses are converted into electrical signals in the electrode, characterised in that the electrical signals are transmitted from the electrode to an electrolyte, that the electrical signals from the electrode are converted by the electrolyte into electrochemical signals, that the electrochemical signals are transmitted from the electrolyte to a sensor head, that the electrochemical signals in the electrolyte are converted by the sensor head into electrical signals, and that the electrical signals are transmitted from the sensor head to a receiver.

2. Method of measuring electrochemical impulses during movements in tissue, the electrochemical impulses being causative of the movements in the tissue, and which method comprises use of a sensor with an electrode intended for insertion into the tissue, whereby the electrode of the sensor is exposed to the electrochemical impulses in the immediate vicinity of a tip of the electrode, and, characterised in that the electrode contains an electrolyte, that the electrochemical impulses are transmitted to the electrolyte in the electrode, that the electrochemical impulses are transmitted from the electrode to a sensor head, that the electrochemical impulses in the electrolyte are converted by the sensor head into electrical signals, and that the electrical signals are transmitted from the sensor head to a receiver.

3. Method according to claim 2, characterised in that the electrochemical impulses are transmitted to the electrolyte in the electrode, that the electrochemical impulses are converted by the electrolyte into electrochemical signals, that the electrochemical signals are transmitted from the electrolyte to the sensor head, that the electrochemical signals in the electrolyte are converted by the sensor head into electrical

signals, and that the electrical signals are transmitted from the sensor head to a receiver.

4. Sensor for use by the method according to claim 1, which sensor has an electrode intended for insertion into tissue, which electrode is made of metal, c h a r a c t e r i s e d in that the sensor has a reservoir, that the reservoir is located between the electrode and a sensor head, that an electrolyte is contained in the reservoir, that the electrode is fastened to the reservoir, and that the sensor head is displaceable inside the reservoir.

5. Sensor for use by the method according to claim 2 or claim 3, c h a r a c t e r i s e d in that the electrode is made of glass or plastic, that the electrode has a hole penetrating from the tip of the electrode to a base of the electrode, that the hole in the electrode is intended for containing an electrolyte and that a sensor head is displaceable in relation to the electrode.

6. Sensor according to claim 5, c h a r a c t e r i s e d in that the sensor has a reservoir, that the reservoir is located between the electrode and the sensor head, that an electrolyte is contained in the reservoir, that the electrode is fastened to the reservoir, and that the sensor head is displaceable inside the reservoir.

7. Sensor according to any of the claims 4-6, c h a r a c t e r i s e d in that the sensor head at least has a surface made of metal, preferably of platinum, more preferably of gold, most preferably of silver, and that the electrolyte preferably is potassium chloride, alternatively is potassium acetate, alternatively is sodium chloride.

8. Sensor according to any of the claims 4-7, c h a r a c t e r i s e d in that the reservoir is a tube with a cylindrical hole, preferably a tube made of glass, that the sensor head is a body shaped as a rod, and which is intended for extending inside the hole, and that the sensor head preferably is provided with a bulb in an outer end of the sensor head.

9. Sensor according to any of the claims 4-7, c h a r a c t e r i s e d in that the sensor comprises an abutment surface, that the reservoir is mounted on to the abutment surface, that the electrode is mounted to the reservoir, that a holder is mounted to the abutment surface, and that the sensor head is mounted to the holder.

10. Sensor according to claim 9, c h a r a c t e r i s e d in that the reservoir consists of a first reservoir extending from the abutment surface upwards on one side of the abutment surface in relation to the electrode extending downwards on the other side of the abutment surface, and a second reservoir extending form the first reservoir sideways in relation to the upwards extension of the first reservoir, and that the sensor head is extending inside the second reservoir.

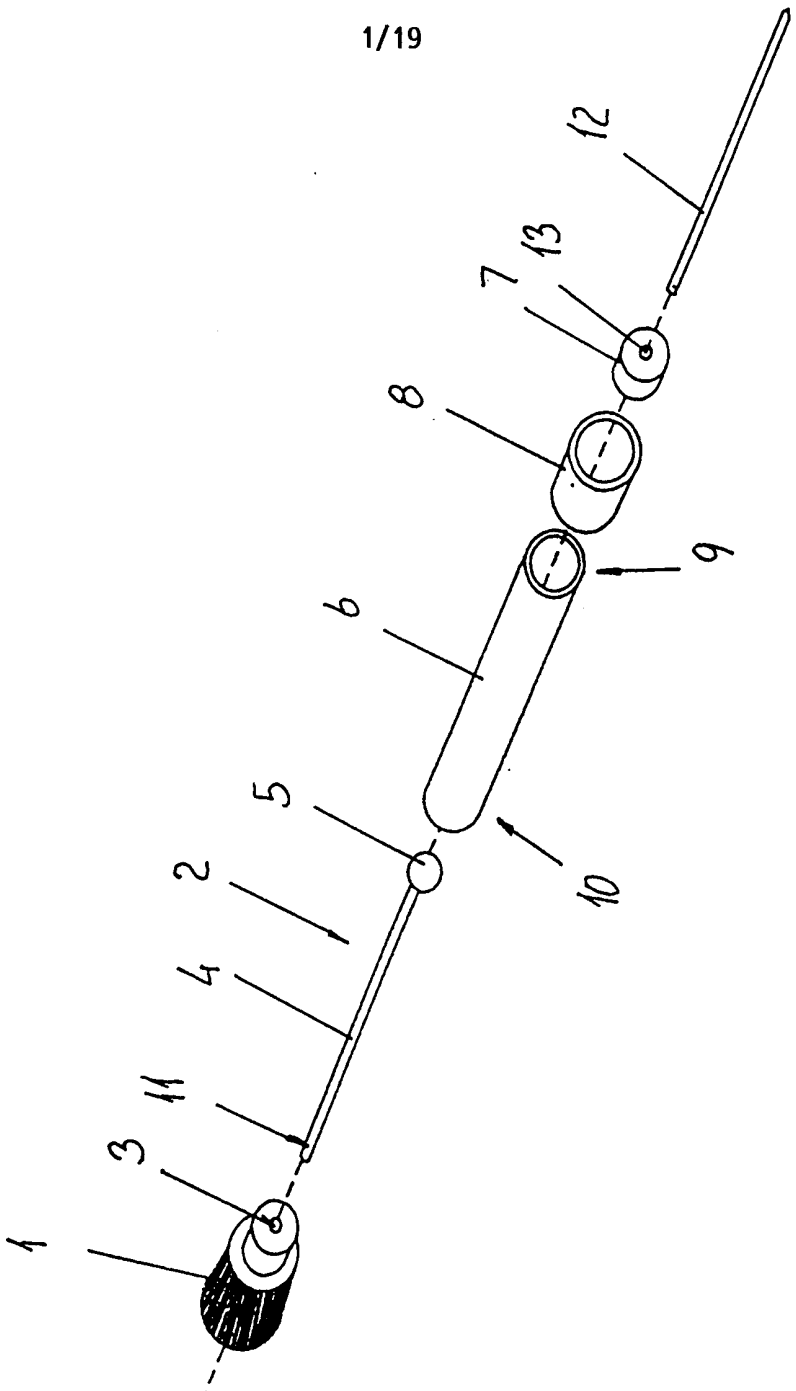


FIG. 1

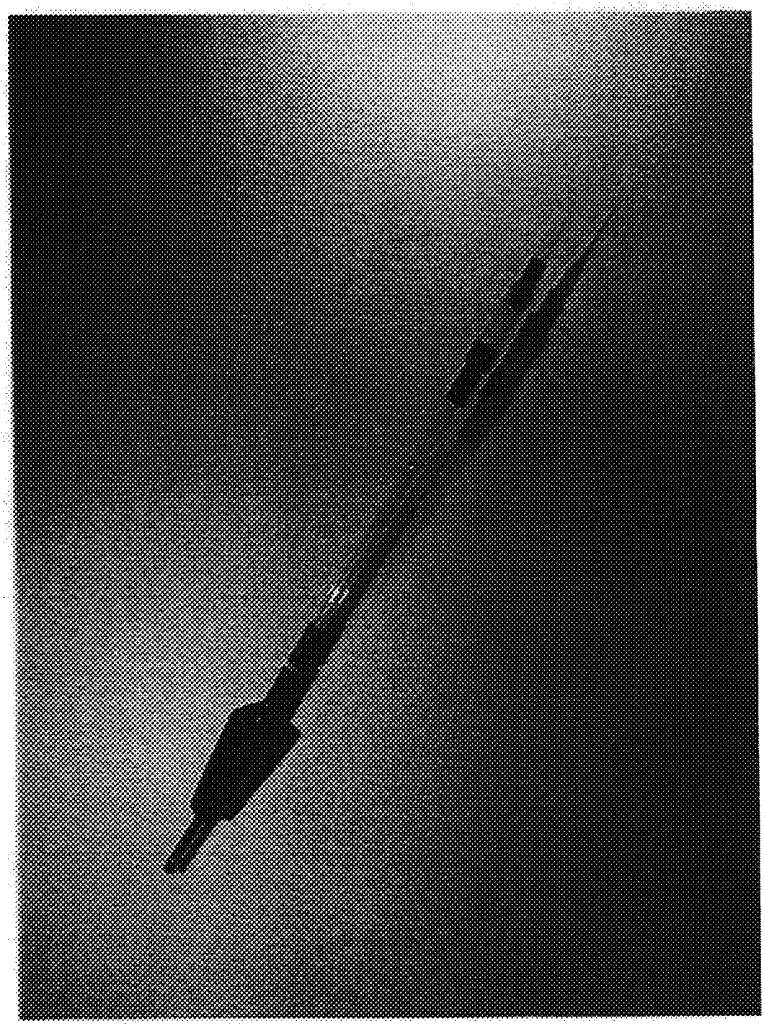
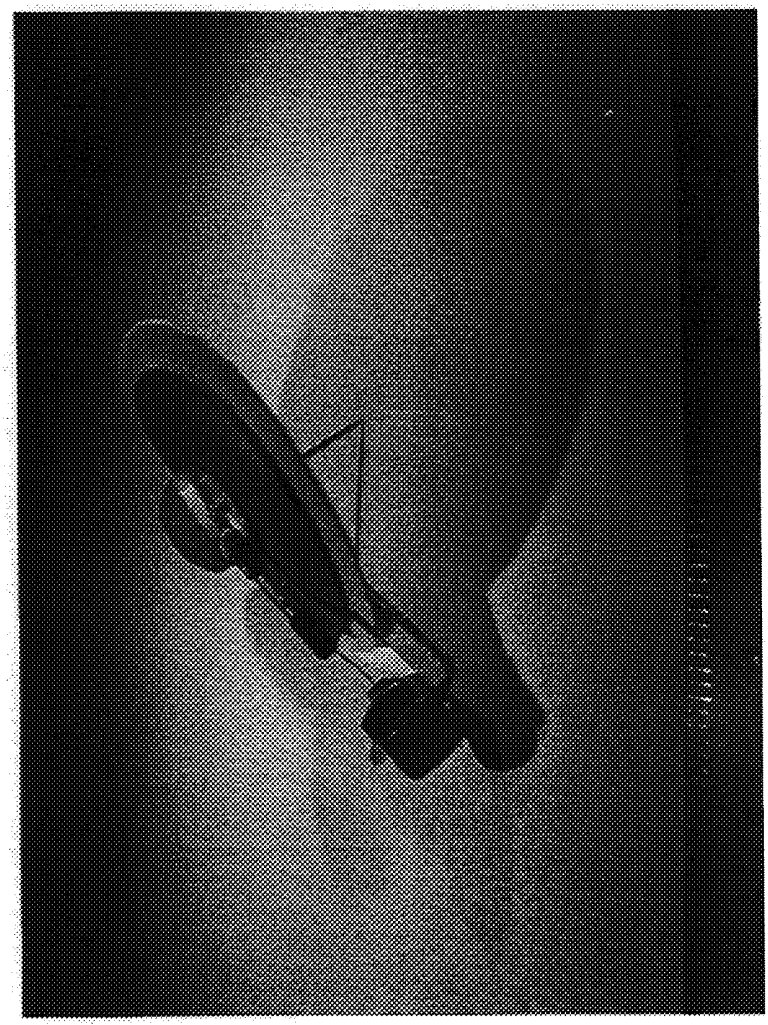


Fig. 2

Fig. 3



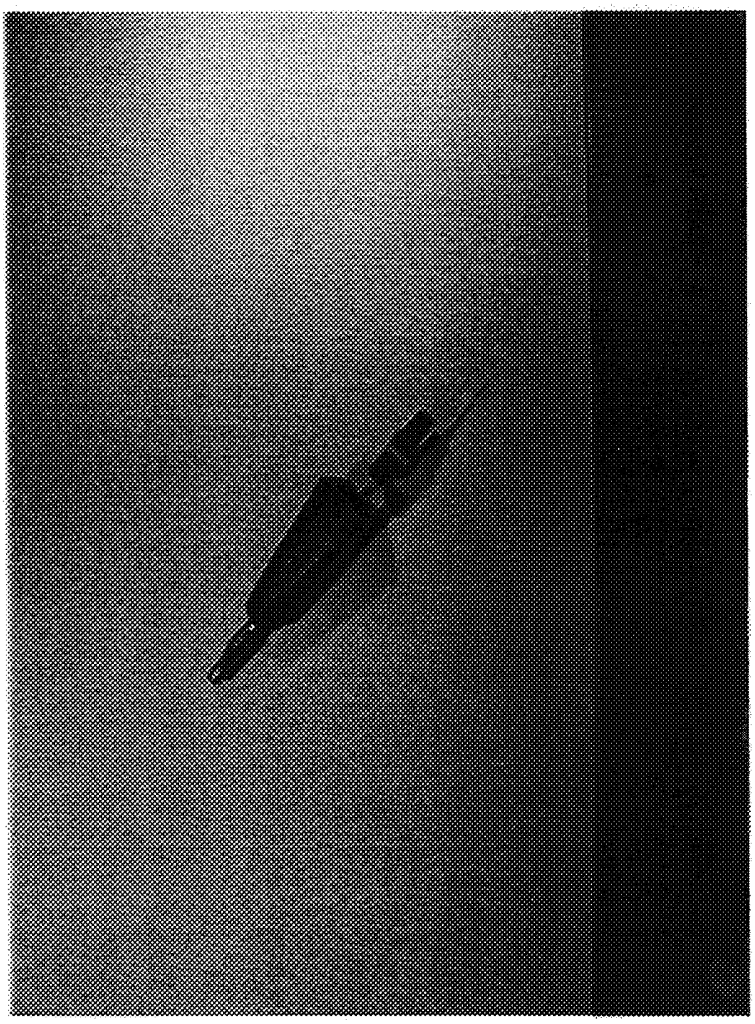


FIG. 4

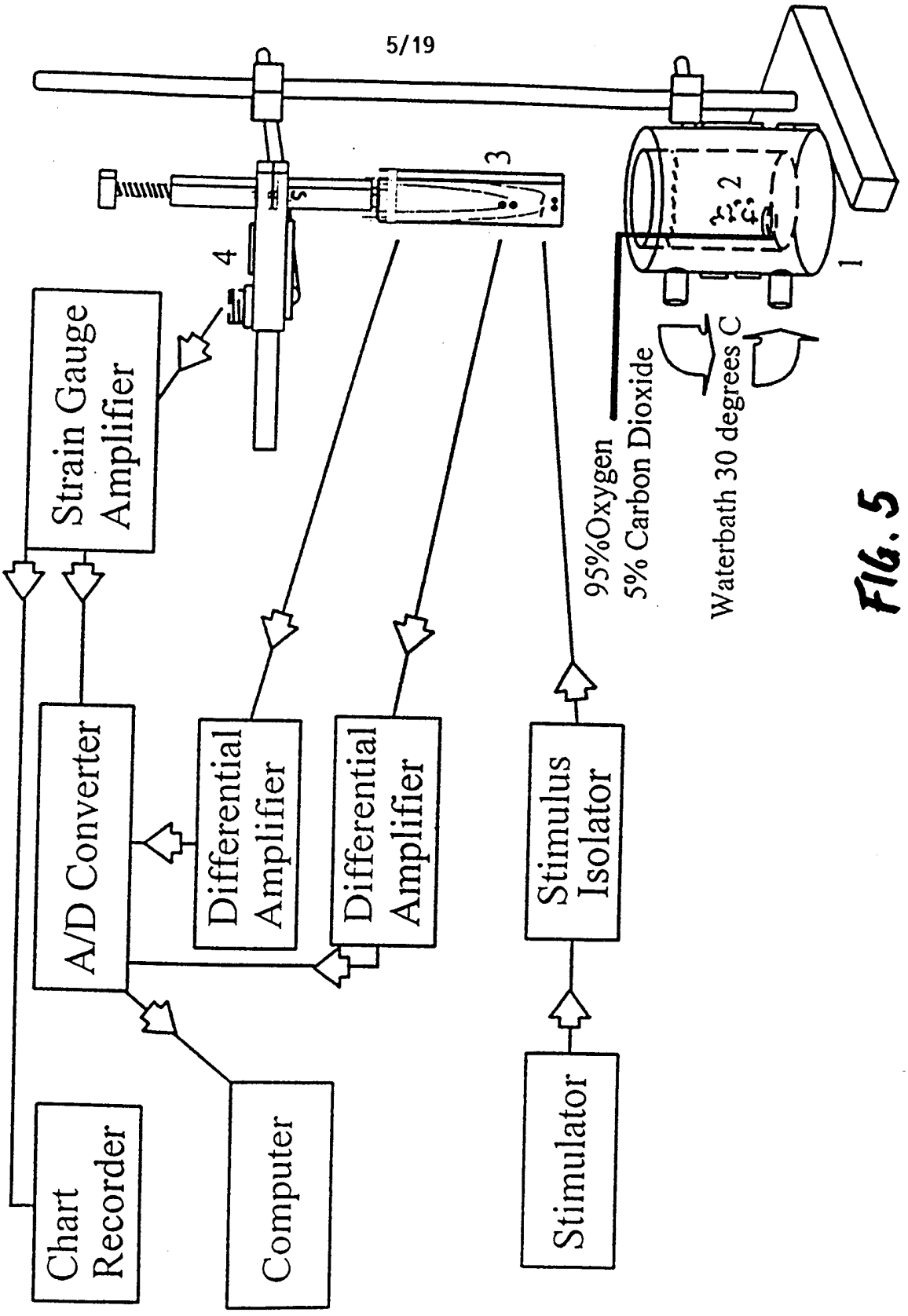

Strain Gauge Amplifier
A/D Converter
Differential Amplifier
Differential Amplifier
Stimulus Isolator
Chart Recorder
Computer
Stimulator
95%Oxygen
5% Carbon Dioxide
Waterbath 30 degrees C
1
2
3
4
5

FIG. 5

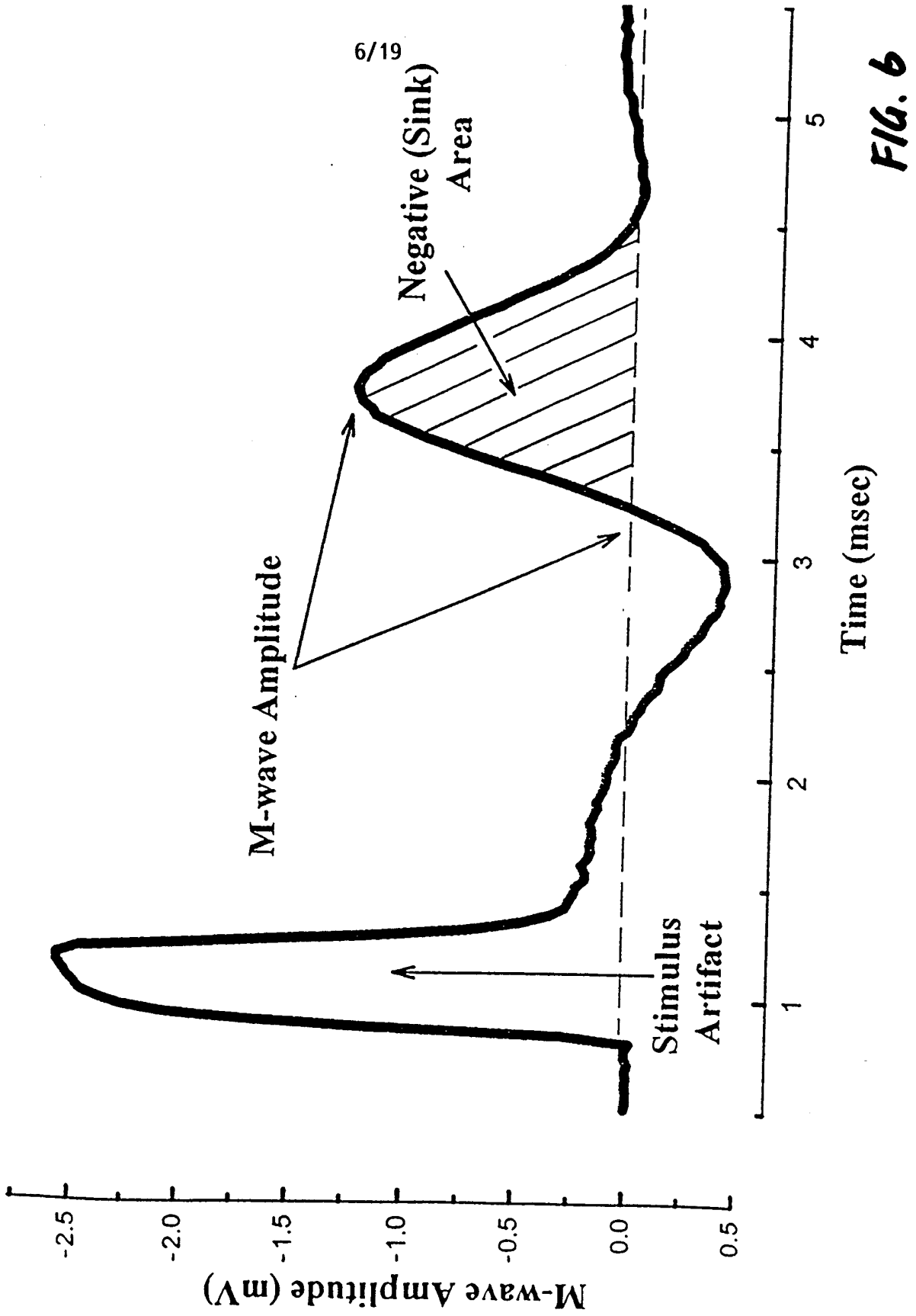

M-wave Amplitude (mV)
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
Time (msec)
1
2
3
4
5
M-wave Amplitude
Negative (Sink) Area
Stimulus Artifact

FIG. 6

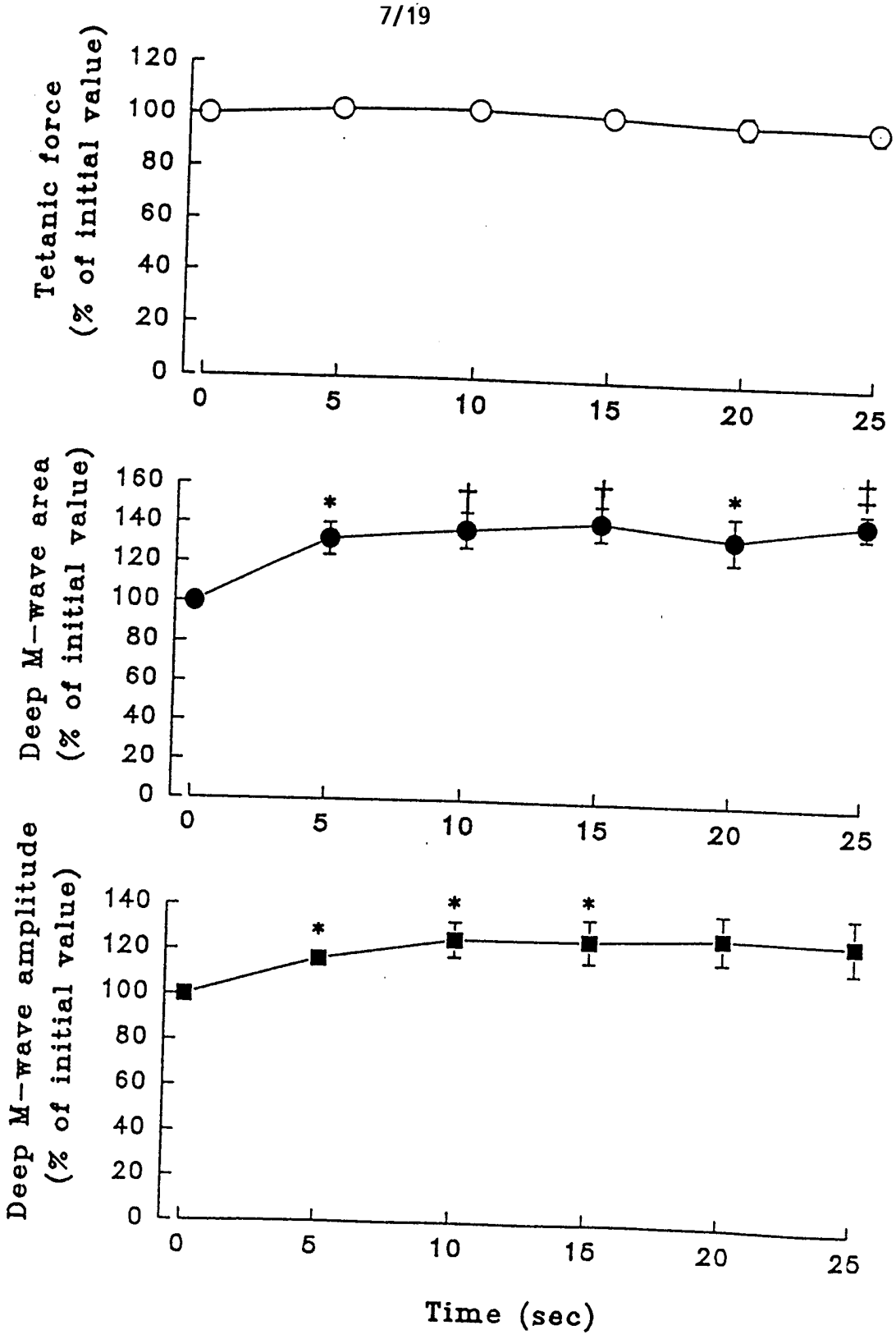

Tetanic force
(% of initial value)
120
100
80
60
40
20
0
0
5
10
15
20
25
Deep M-wave area
(% of initial value)
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
*
Deep M-wave amplitude
(% of initial value)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
*
Time (sec)

FIG. 7

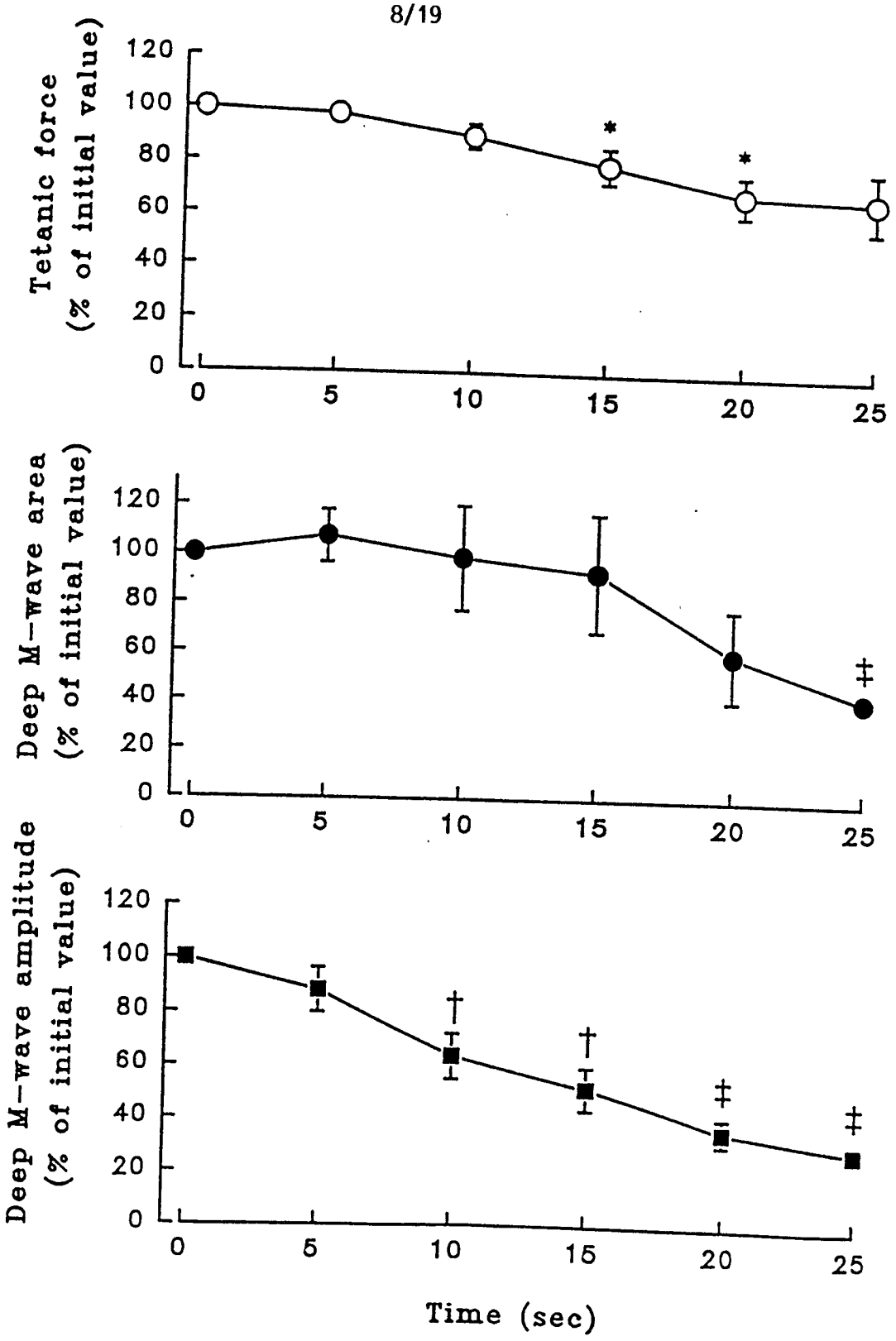

Tetanic force
(% of initial value)
120
100
80
60
40
20
0
0
5
10
15
20
25
*
*
Deep M-wave area
(% of initial value)
120
100
80
60
40
20
0
0
5
10
15
20
25
‡
Deep M-wave amplitude
(% of initial value)
120
100
80
60
40
20
0
0
5
10
15
20
25
†
†
‡
‡
Time (sec)

FIG. 8

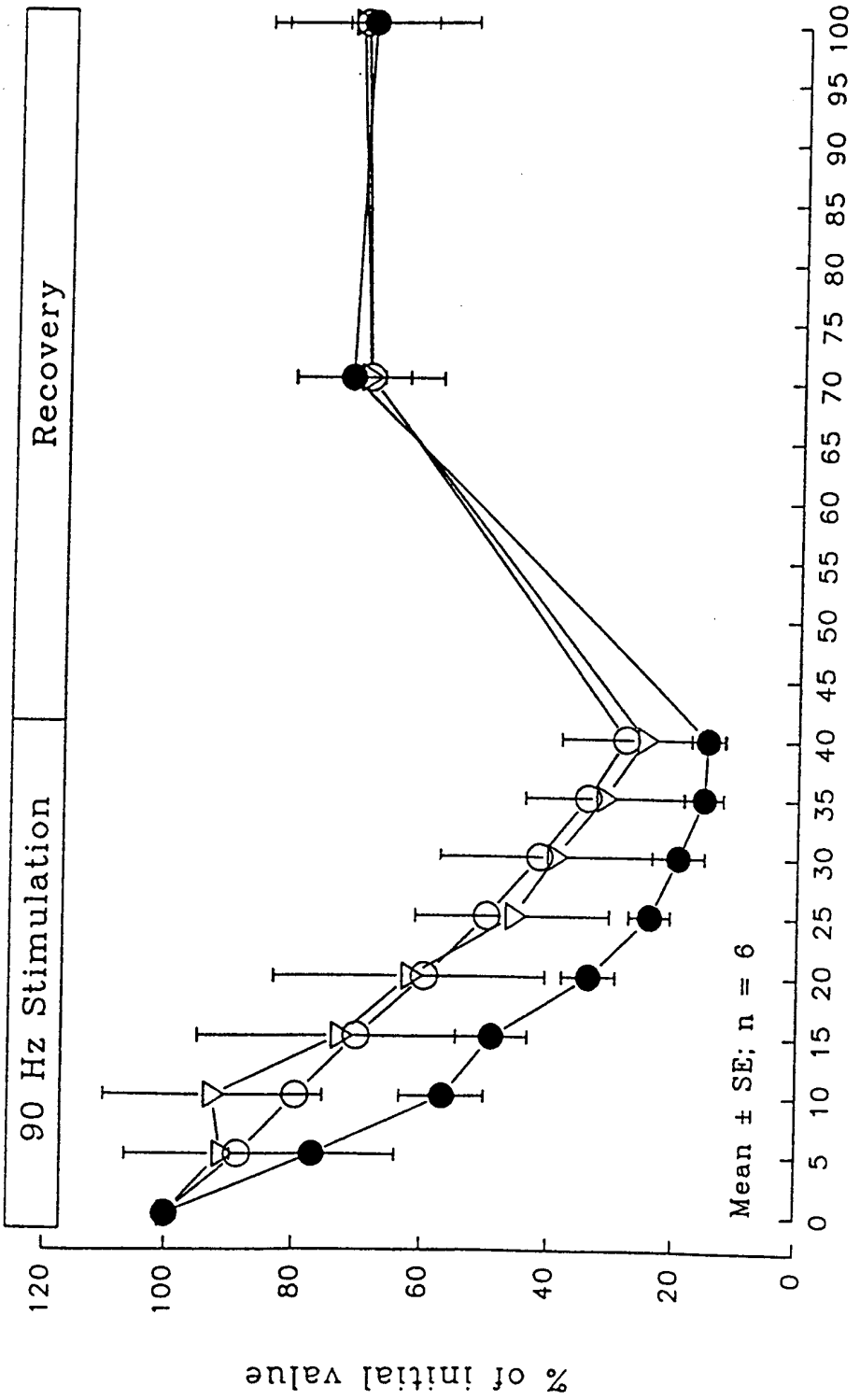

90 Hz Stimulation
Recovery
% of initial value
120
100
80
60
40
20
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Time (sec)
Mean ± SE; n = 6

FIG. 9

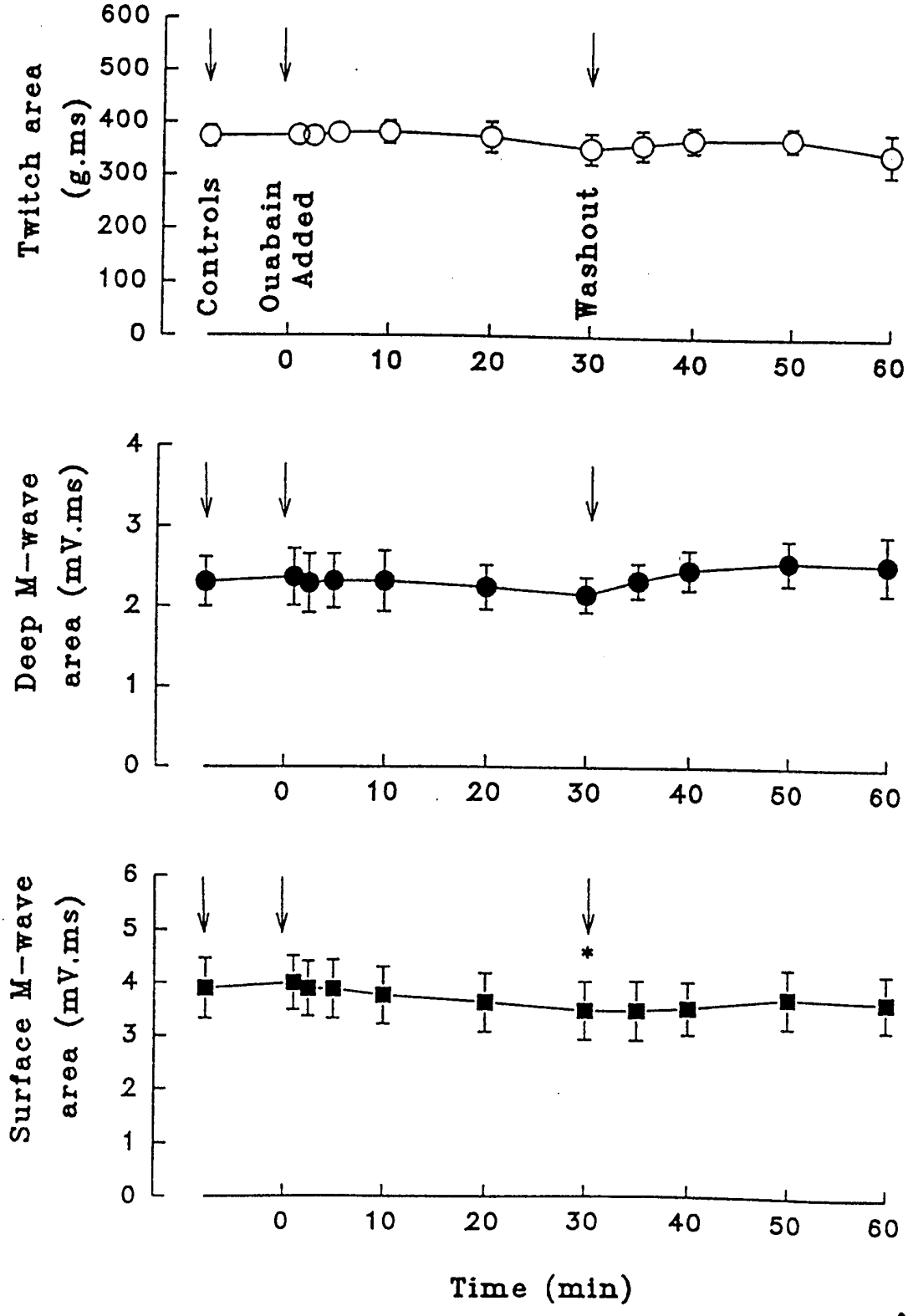

Twitch area (g.ms)
600
500
400
300
200
100
0
Controls
Ouabain Added
Washout
0
10
20
30
40
50
60
Deep M-wave area (mV.ms)
4
3
2
1
0
Surface M-wave area (mV.ms)
6
5
4
3
2
1
0
*
Time (min)

FIG. 10

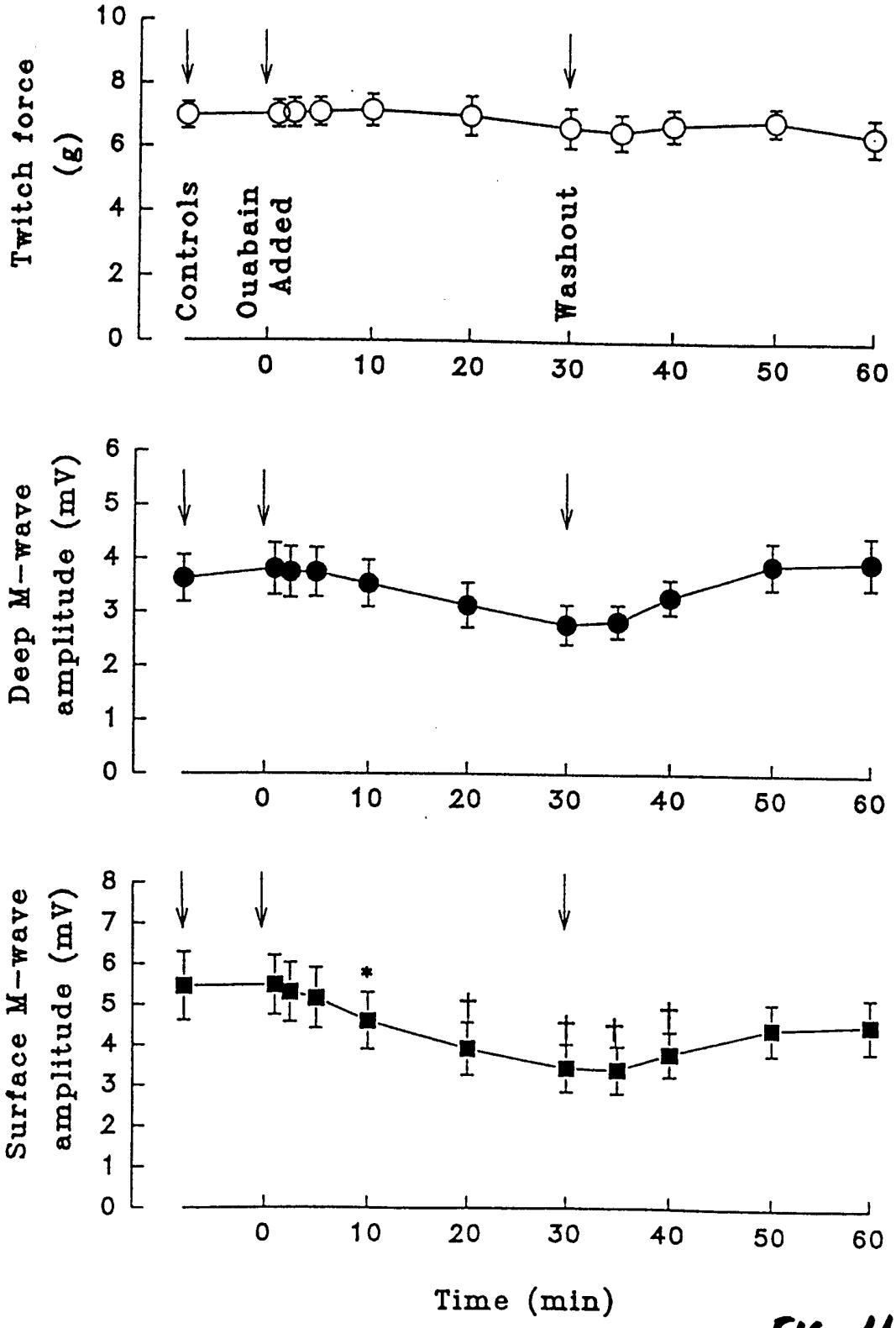

Twitch force (g)
Controls
Ouabain Added
Washout
Deep M-wave amplitude (mV)
Surface M-wave amplitude (mV)
*
Time (min)

FIG. 11

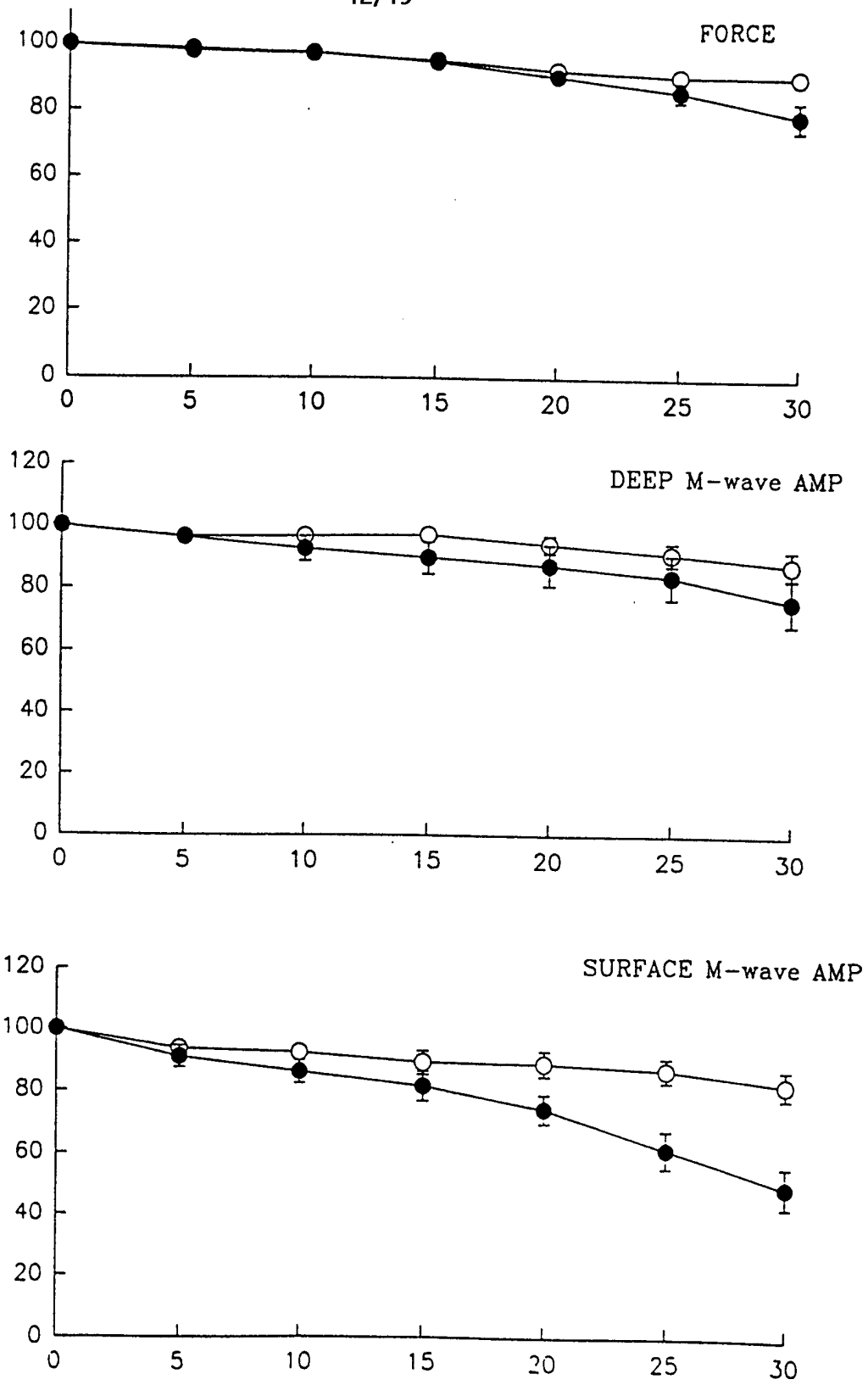

FORCE
100
80
60
40
20
0
0
5
10
15
20
25
30
DEEP M-wave AMP
120
100
80
60
40
20
0
0
5
10
15
20
25
30
SURFACE M-wave AMP
120
100
80
60
40
20
0
0
5
10
15
20
25
30

FIG. 12

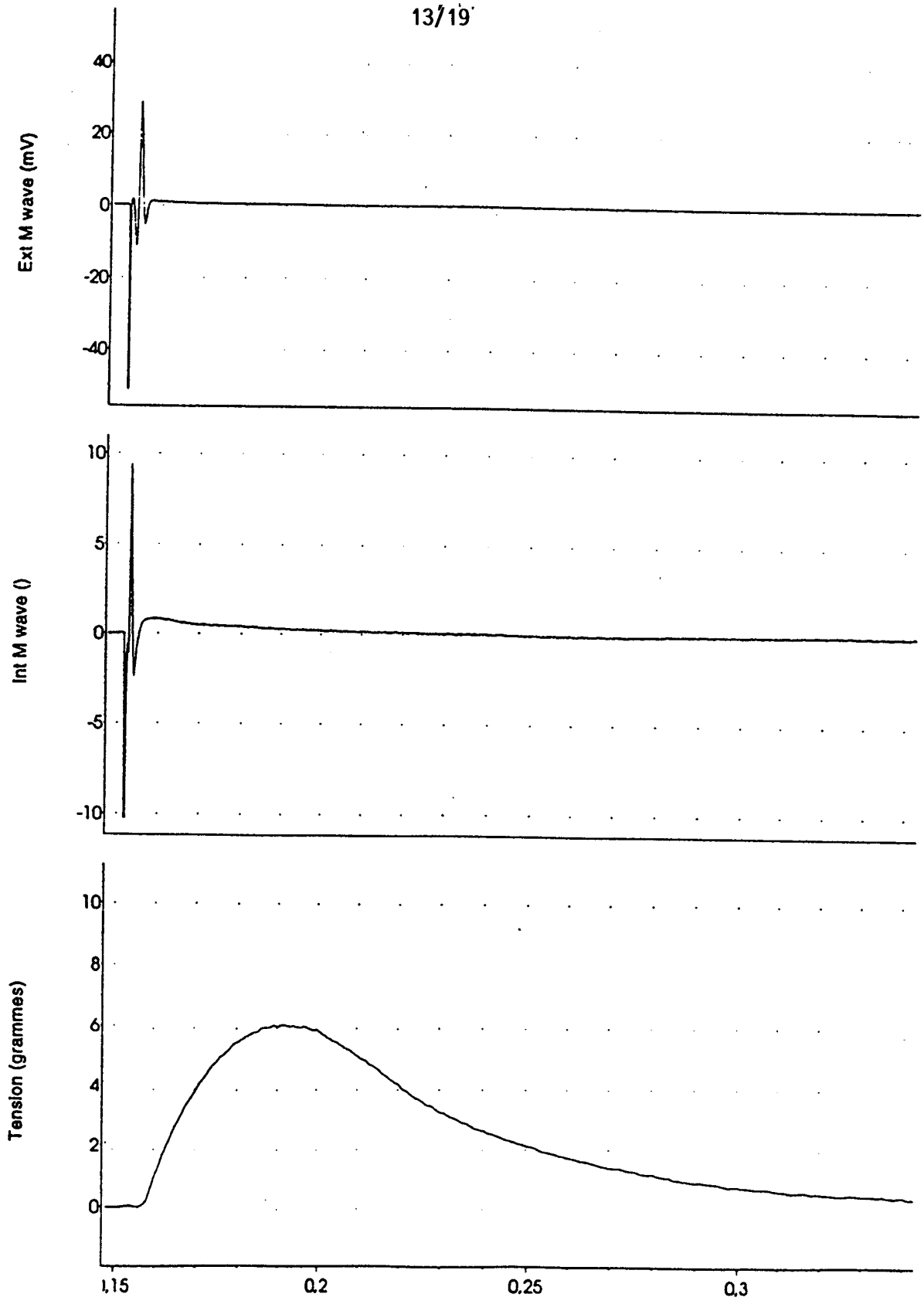

Ext M wave (mV)
40
20
0
-20
-40
Int M wave ()
10
5
0
-5
-10
Tension (grammes)
10
8
6
4
2
0
1,15
0.2
0.25
0,3

FIG. 13

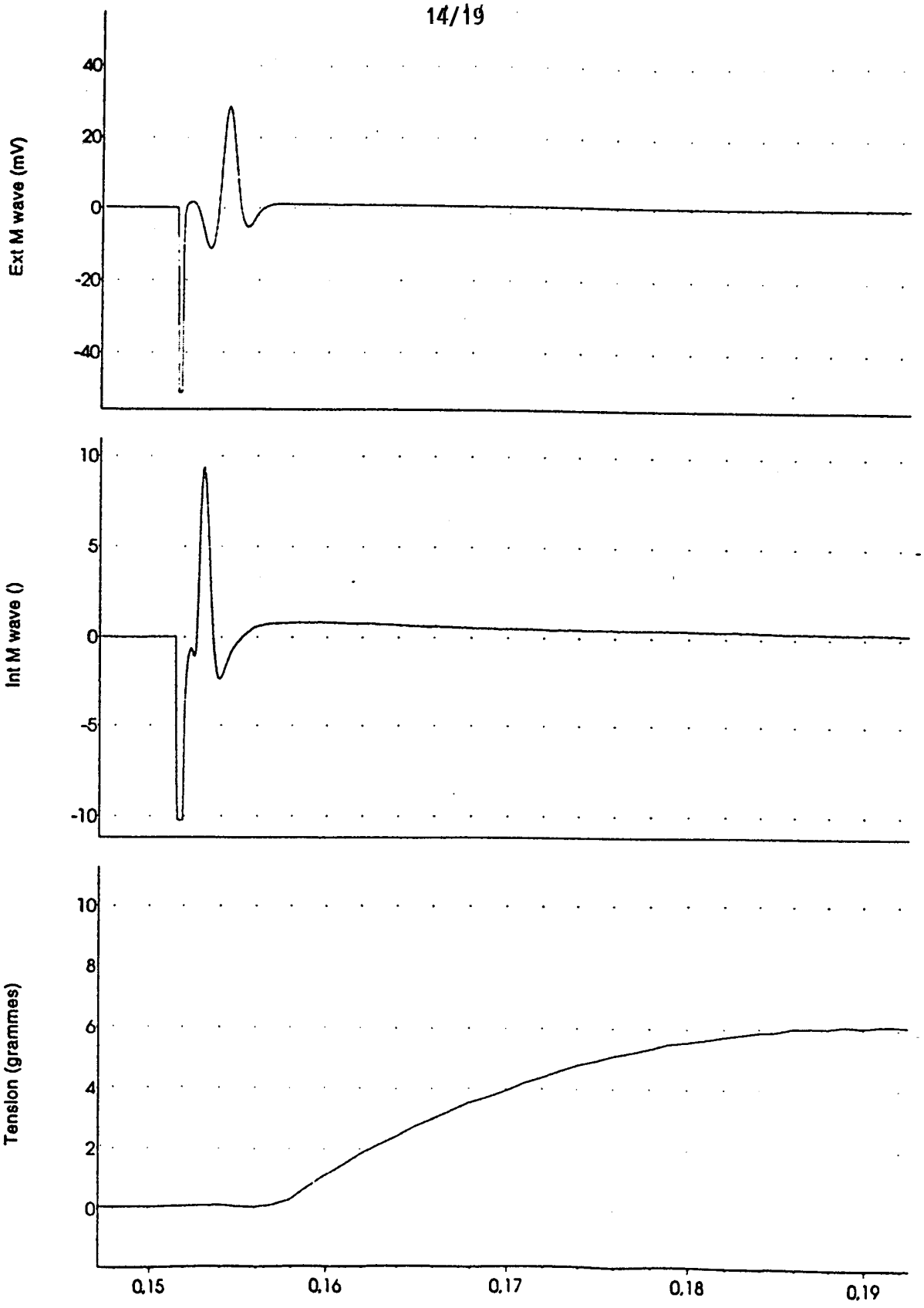

Ext M wave (mV)
40
20
0
-20
-40
Int M wave ()
10
5
0
-5
-10
Tension (grammes)
10
8
6
4
2
0
0.15
0.16
0.17
0.18
0.19

FIG. 14

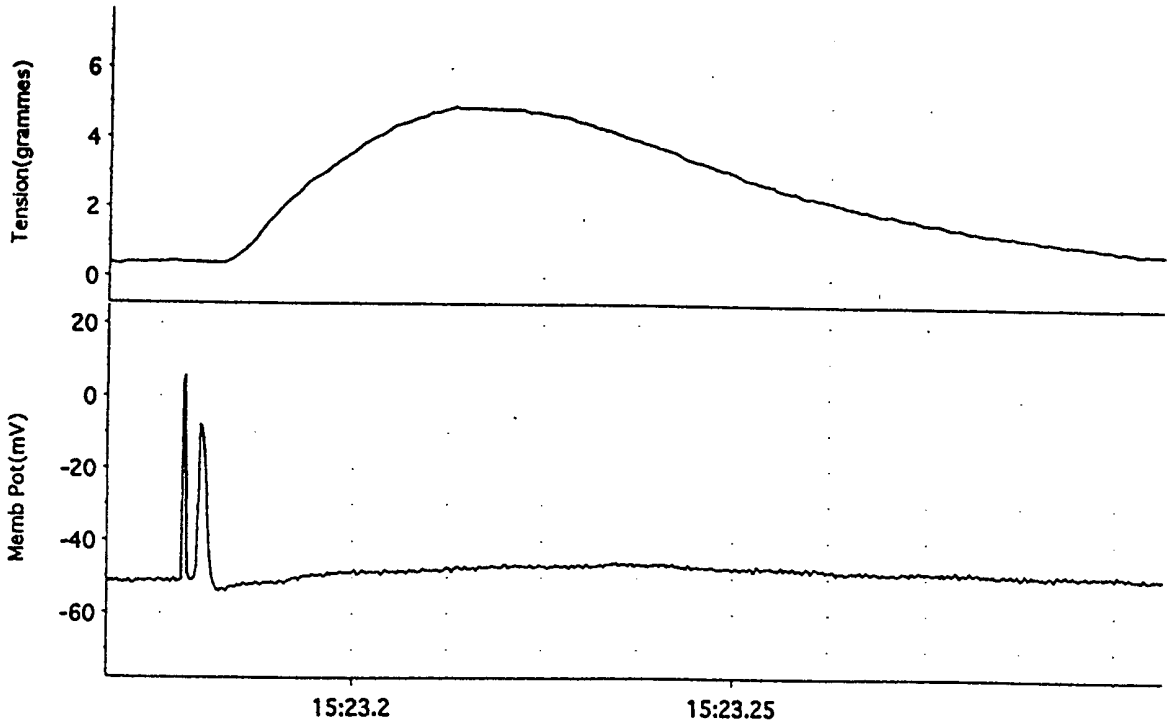


FIG. 15

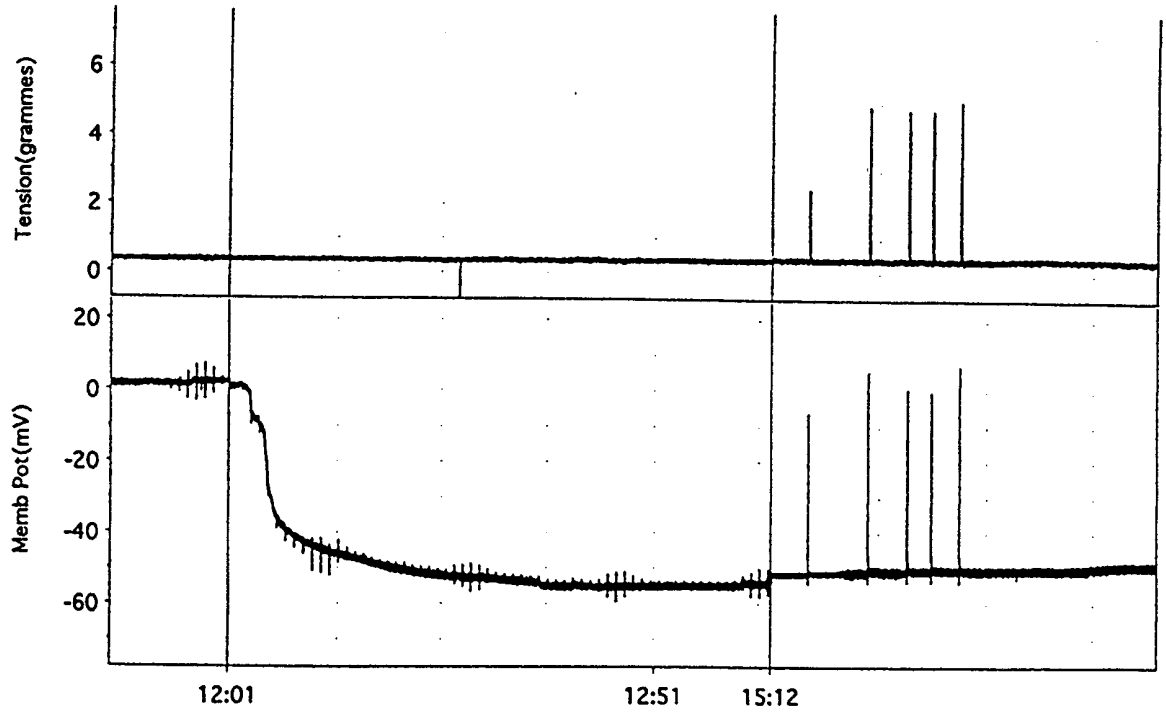
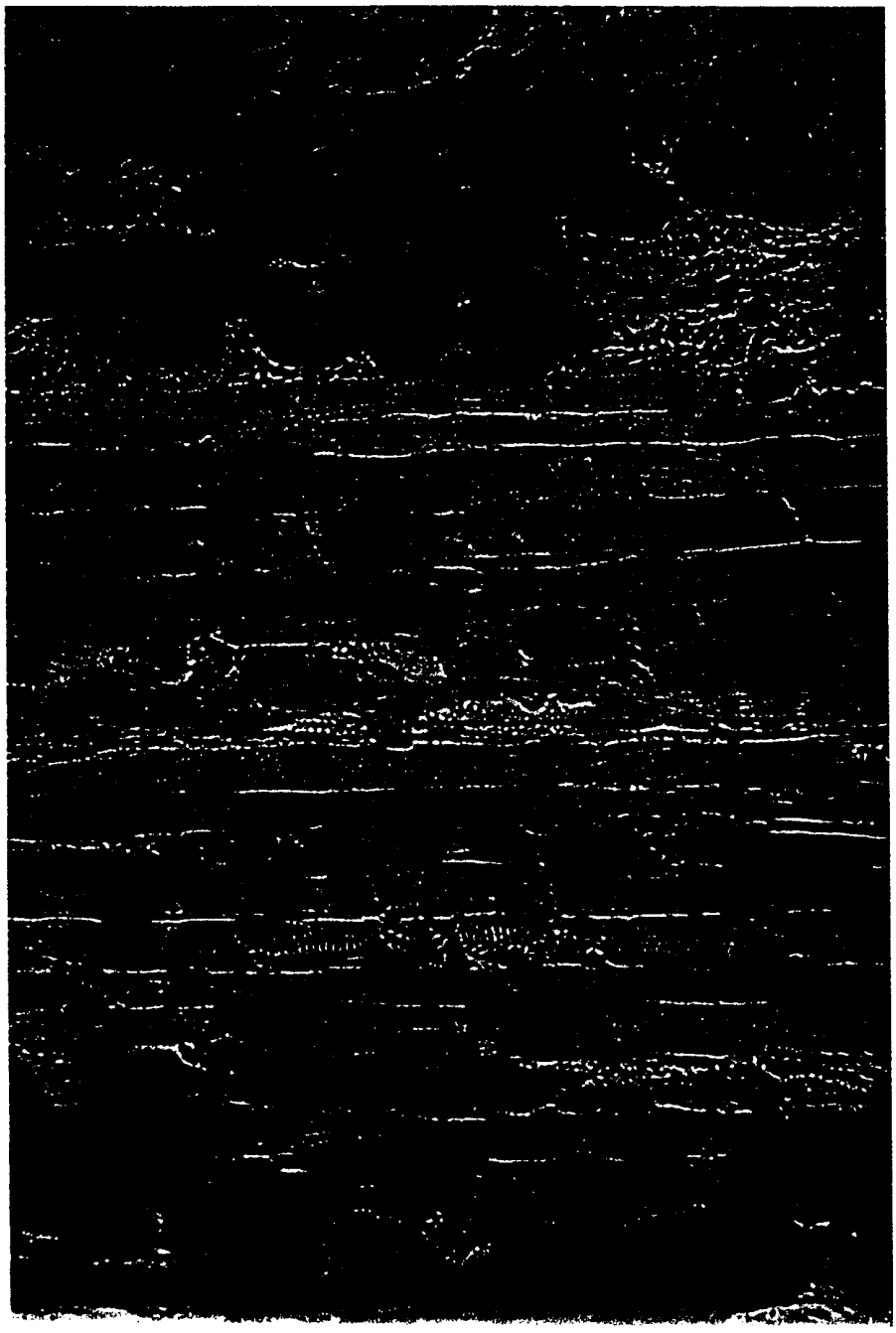


FIG. 1b

FIG. 17



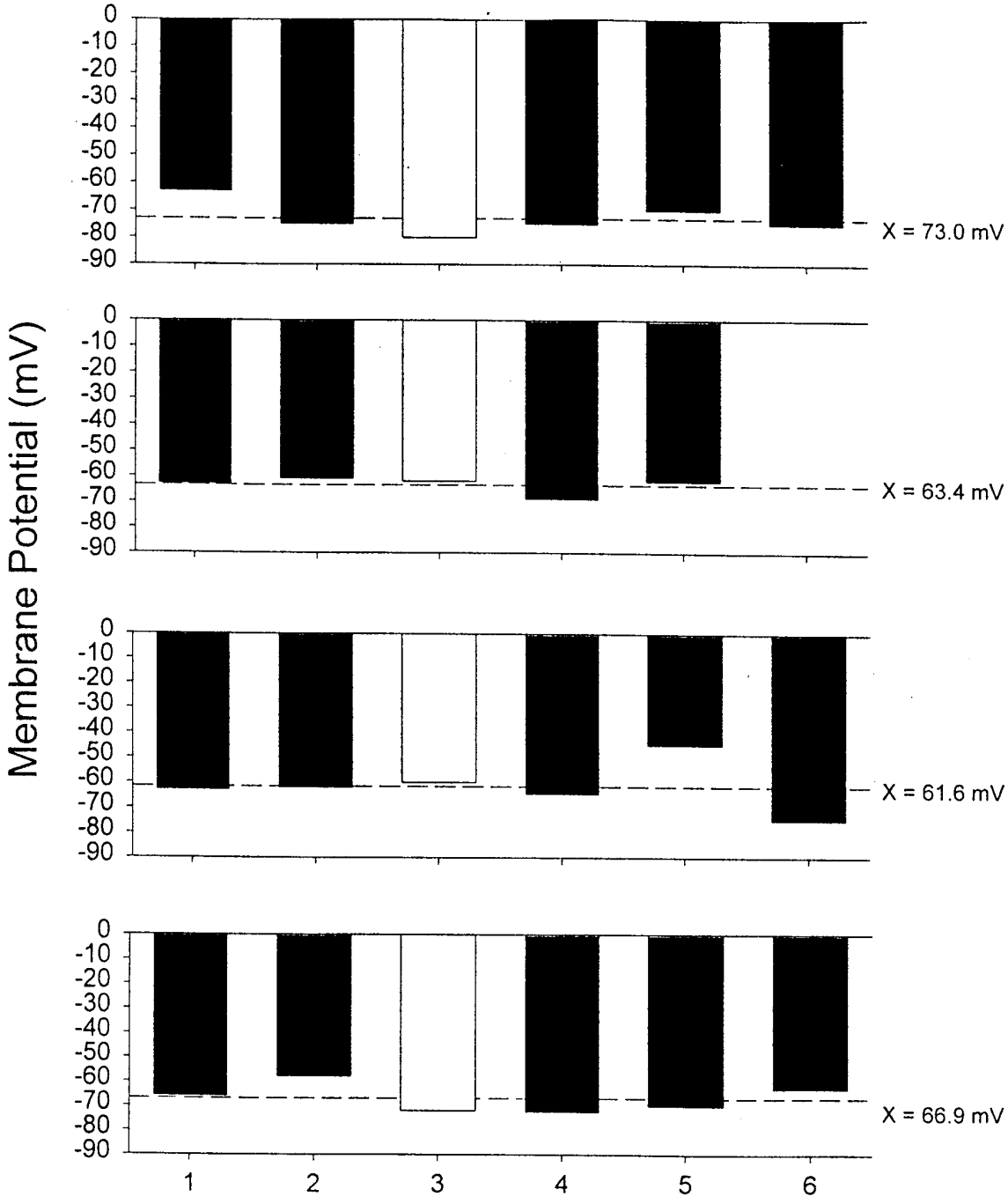
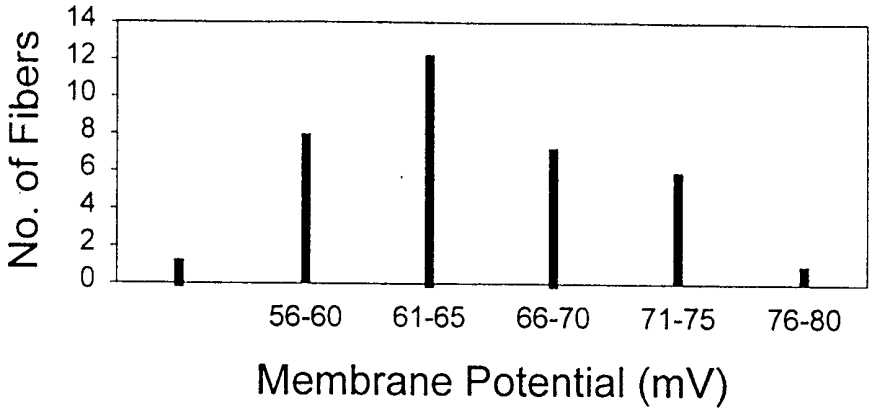

Membrane Potential (mV)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
X = 73.0 mV
X = 63.4 mV
X = 61.6 mV
X = 66.9 mV
1
2
3
4
5
6

FIG. 18


No. of Fibers
14
12
10
8
6
4
2
0
56-60
61-65
66-70
71-75
76-80
Membrane Potential (mV)

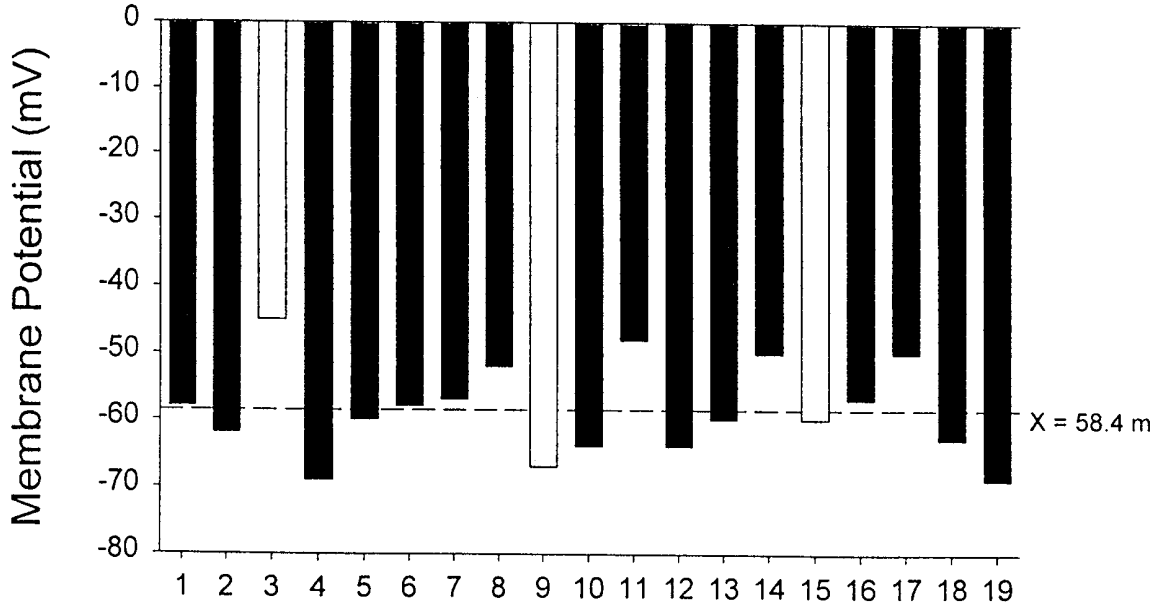

Membrane Potential (mV)
0
-10
-20
-30
-40
-50
-60
-70
-80
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
X = 58.4 m

FIG. 19

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/DK 99/00432

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B5/0492 A61B5/11 A61B5/0402

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 30 38 885 A (E. FRESENIUS) 27 May 1982 (1982-05-27)	1-3,5,6
X	page 7, line 13 -page 10, line 2	9
A	page 12, line 3 - line 19; claims 1,6,12	4,7
X	GB 2 274 396 A (BRITISH AEROSPACE) 27 July 1994 (1994-07-27)	1-3
A	page 2, line 11 -page 4, line 19 page 5, line 13 -page 8, line 2	4-9
X	O.C.J. LIPPOLD ET AL.: "A method of mounting micro-electrodes for intracellular recording from contracting muscle" PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY, vol. 147, 20 - 21 March 1959, pages 3P-4P, XP000856300 page 3P -page 4P; figure 1	1-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1999	03/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rieb, K.D.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 99/00432

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. AKIYAMA ET AL.: "Intracellular recording from beating heart in situ using a special micropipette holder" JOURNAL OF THE AMERICAN PHYSIOLOGICAL SOCIETY, vol. 237, no. 3, 1979, pages 392-394, XP000856299 page 392 -page 394; figure 1	1-6

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK 99/00432

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3038885 A	27-05-1982	NONE	
GB 2274396 A	27-07-1994	NONE	