

University of Stirling

Contributory studies to the development,
validation and field use of a telemetry
system to monitor ventilation and trophic
activity in wild Brown Trout.

by

Robert Leslie Oswald, B.Sc. (Newcastle)

Thesis submitted for the Degree of Doctor of Philosophy

September 1980

LIST OF CONTENTS

Quotations.	i
Declarations.	ii
Acknowledgements.	iv
Abstract.	vi
<u>Introduction</u>	
1. Review of Literature.	1
2. Review of Technology and Objectives of this investigation.	13
<u>Section I : Anatomy and Histology</u>	
<u>Introduction</u>	20
<u>Materials and Methods I.</u>	32
1.1 Fixation.	32
1.2 Processing.	32
1.3 Staining Methods.	33
1.4 Vascularisation of the Muscle.	33
<u>Results I.</u>	
1.5 Gross Anatomy.	34
1.6 Fibre Sizes.	35
1.7 Myofibrillar Array.	36
1.8 Nuclear Position.	37
1.9 Fibre shape.	37
1.10 Vascularisation.	37
1.11 Gross Anatomy of the V th Cranial Nerve.	39
1.12 Fibre Sizes.	40

Discussion I.

1.13 Gross Anatomy.	40
1.14 Fibre Sizes and Types.	43
1.15 The V th Cranial Nerve.	45
1.16 Innervation Pattern.	47

Section II : Muscle Physiology

<u>Introduction.</u>	49
----------------------	----

Materials and Methods II.

2.1 Animals	53
2.2 Anaesthesia and Surgical Methods.	53
2.3 Recording Equipment.	54
2.4 Investigations into the trout <u>adductor mandibulae</u> electro-myogram during ventilation.	55
2.5 Chronic EMG recordings in unanaesthetised trout.	57
2.6 Effects of Anaesthesia.	60
2.7 Frequency Spectrum Analysis of the EMG.	60
2.8 Indirectly stimulated <u>adductor mandibulae</u> preparations.	61
2.9 Innervation experiments.	64
2.10 Effects of drugs producing central or peripheral neuromuscular blockade upon the trout <u>adductor mandibulae</u> muscle.	65
2.11 Investigations into the pacesetter potential of the trout alimentary tract.	67

Results II

2.12 Depth probe experiments.	69
2.13 EMG mapping experiments.	71
2.14 Comparative EMG and other observations made upon Selachians and deepsea Teleosts.	72

2.15 Effect of Anaesthesia on the EMG.	74
2.16 Frequency spectrum analysis.	76
2.17 Indirectly stimulated preparation experiments.	80
2.18 Investigations into effect of nerve block on EMG.	82
2.19 Effects of Neuromuscular Blockading Drugs.	82
2.20 Electrical Activity of trout alimentary tract.	83
 <u>Discussion II.</u>	
2.21 EMG probe and mapping experiments upon Trout and Selachians.	85
2.22 Effects of anaesthesia on EMG.	89
2.23 Frequency Spectrum Analysis.	92
2.24 Indirectly stimulated preparations.	99
2.25 Nerve and Neuromuscular Blockade experiments.	101
2.26 Pacesetter potential investigations.	104
 <u>Section III : Biotelemetry</u>	
<u>Introduction.</u>	106
 <u>Materials and Methods III.</u>	
3.1 Ultrasonic Tracking Transmitter.	108
3.2 Receiving System.	109
3.3 The Study Area.	111
3.4 Tagging Procedures.	112
3.5 Interpretation of Records.	112
3.6 Data Analysis.	113
 <u>Results III</u>	
3.7 Laboratory tests of the transmitter.	115
3.8 Field Experiment Narratives.	117
3.9 Overall telemetry results.	120
3.10 Summary Statistics.	120

3.11 Comparisons between day and night results.	121
3.12 Comparisons of results between feeding peaks and the intervals between them.	121
3.13 Correlations between variables recorded.	122
3.14 Relationship between variables recorded and elapsed time.	123
3.15 Comparison of the Fish's movement patterns.	125
3.16 Relationship of movement patterns to underwater topography.	129
3.17 Circular Statistics.	129
 <u>Discussion III</u>	
3.18 Feeding criteria.	134
3.19 Cycles in Feeding and Ventilation.	137
3.20 Ventilation cycles.	140
3.21 Ventilation rate.	141
3.22 Relationship of feeding and ventilatory activity to photoperiod.	142
3.23 Relationship of Locomotor activity to photoperiod.	145
3.24 Relationship of Locomotor Activity to Feeding/ Ventilatory activity.	148
3.25 Relationship of Angle of Turn to Feeding Activity.	151
3.26 Relationship of Angle of Turn to Photoperiod/Time.	152
3.27 Relationship of Movement Pattern to Photoperiod and Feeding Activity.	152
3.28 Comparison of Step length data.	156
3.29 Relationship of Spatial position to Environment.	157
3.30 Relationship of Feeding Activity to Metabolic requirements.	160
3.31 Circular Statistics.	161

Plates		165
Appendix I	Copies of published papers in which work in this thesis has been incorporated.	179
Appendix II	Component lists for Electronic circuits	180
Appendix III	Microfiche tabulation of original data from each fish-tracking experiment.	181
References		182
Addendum to References		217

Quotations

The following lines are affectionately dedicated to all who have attempted to look into the 'private lives' of fishes, using telemetric means:

'O scaly, slippery, wet, swift, staring wights,
What is 't ye do? What life lead? Eh, dull goggles?
How do you vary your vile days and nights?
How pass your Sundays?'

James Henry Leigh Hunt

The Fish, the Man and the Spirit.

'No human being, however great, or powerful, was ever so free as a fish.'

John Ruskin

The Two Paths.

DECLARATION

Parts of the work included in this thesis have been embodied in two published papers which are bound into Appendix V.

OSWALD, R.L. (1978a) Injection Anaesthesia for Experimental Studies in Fish. *Comp. Biochem. Physiol.* 60C, 19-26

OSWALD, R.L. (1978b) The use of telemetry to study light synchronization with feeding and gill ventilation rates in Salmo trutta. *J. Fish Biol.* 13, 729-739.

DECLARATION

I wish to submit this thesis for examination in accordance with the Regulations for the degree of Doctor of Philosophy. I declare that the thesis embodies the results of my own research and that it has been composed by me. Where appropriate I have made acknowledgement to the work of others.

Signature R.L. Omer Date 19th September 1979

ACKNOWLEDGEMENTS

This project would not have been possible without the co-operation of several individuals. In particular, I would like to express my gratitude to the following people: Dr Peter Tytler for his supervision and direction of the project. Professor Fred Holliday upon whose grant part of the development work was carried out; Archie Young and John Wiewiorka of Shared Technical Services for the development, manufacture and enthusiastic participation in the use of the tracking equipment; Lindsay Ross and Monty Priede for advice, discussion and comradely help over the years; Jock Scott and Ian McGowan for assistance in fishing and tracking operations; Angus Annan, Richard Bambridge and Karl Gijbers of the Department of Psychology for the loan of equipment, Ron Roberts, Randolph Richards, Ian Macrae and Jim Buchanan of the Unit of Aquatic Pathobiology for the use of facilities and equipment; Dr J. Lenman, Reader in Neurology, Dundee University and Mr Mark Tulley, Medical Physics Department, Ninewells Hospital, Dundee for allowing me to use their frequency analyzer and for advice; Dr John Gordon of the S.M.B.A. Laboratory, Dunstaffnage for the privilege of having two sea trips aboard R.R.S. Challenger. Racal Limited, for their generous loan of the 'Store 7' instrumentation recorder; Jacek Wankowski for assistance with the high speed photography; Professors Hans Meidner and Bill Muntz in whose department the work was carried out; Paul Roberts, Rob Marshall and Alistair Scolley of the Computing Unit for patient and helpful programming advice and debugging; The Computer and R.J.E. operating staff, Glenda, Helen, Margaret, Wendy, Billy and Stephen for their friendly humour and cheerful service in the face of a multitude of

compile and run-time errors on my behalf; Graham Cameron for advice and plagiarism of one of his programs; to Margaret Buchanan, Secretary of the Computing Unit and Elizabeth Skinner of the Typing Pool, who typed the M.S. so excellently; to Bill Muntz who was given the unfortunate task of debugging this work.

Finally, I thank my parents for their forbearance and much appreciated support over the years.

R.L. Oswald was an N.E.R.C. Research Student.

ABSTRACT

This work was performed as part of a major research project into the evaluation of the ecology of lake dwelling Brown Trout, Salmo trutta L. using ultrasonic biotelemetry techniques. The supplementary research results leading up to and after the execution of a program of experiments involving the telemetry of feeding and ventilatory rhythms are described:

1. The presence of red (slow) fibres in the adductor mandibulae muscle of Brown Trout was confirmed to be as previously described in the Rainbow Trout, Salmo gairdneri Richardson and other salmonids.
2. By electromyographic (EMG) and pharmacological means, the red fibres in the a. mandibulae were shown to be active during ventilation and the mosaic fibres comprising the bulk of the muscle were recruited during more dynamic events such as feeding and coughing. Observations were made on the innervation of the red fibres.
3. Comparative investigations made at sea on large deep sea Squaloid and Galeoid sharks (which have a simple adductor muscle like the Trout) showed an identical functional differentiation as obtained in the Trout.
4. The presence of a migratory 'pace setter potential' was found for the first time in Fish. Its use as an indicator of feeding activity by telemetry was rejected on practical grounds.

5. An ultrasonic transmitter was developed to telemeter an analogue of the adductor mandibulae EMG from wild Brown Trout, using a novel electrode design. Four fish were so equipped and released into Airthrey Loch, University of Stirling and tracked for up to 24 hours (following a 24 hr allowance for post-anaesthetic recovery). Feeding and ventilatory periodicity, linear and angular movement patterns and photo-period were intercorrelated. Angle of turn and subsequent step length were positively correlated and feeding activity was marked by a preference for dextral turning. 'Area restricted searching' and 'area avoided searching' were the probable causes of the movement patterns seen in this and previous investigations at Airthrey Loch. A depth preference and orientation of the fish to topography was demonstrated. Following analysis of the angle of turn and step length data, it was concluded that the larger transmitter package and more severe surgery materially affected the fishes' behaviour relative to data previously obtained at Airthrey Loch using smaller transmitters.
6. Due to difficulties experienced in 5 above due to an unsuspected effect on the a.mandibulae EMG detectable up to 24 hrs post-anaesthesia, a frequency analysis was made of the a.mandibulae EMG of the Brown Trout and several other species. This disclosed that the EMG from red fibres has a frequency spectrum considerably lower than that of 'standard' mammalian muscle. The progressive failure of the EMG transmitter with time was due to a combination of the anaesthetic effect and the frequency spectrum relative to certain design features.

(vii)

In the light of these observations, subsequent designs of the EMG transmitter were able to take this into account.

INTRODUCTION

1. Review of Literature

Studies into the behaviour and physiology of fishes are extremely difficult to achieve in their natural surroundings. Their ease of mobility in an environment which limits man's visual range, can make direct observations of their behaviour in the wild difficult, if not frequently impossible. SCUBA equipment has not proved particularly useful for scientific work in this area because of limitations which are imposed upon the diver by his endurance, visual range and also by the timidity of many fish species. In shallow fresh water bodies, the situation is even worse than in the sea because stirred-up sediments, phytoplankton blooms etc., frequently impair vision so there have been few scientific studies made on fish in this habitat. Hasler and Bardach (1949) did, however, make some useful underwater studies on Perca flavescens and Wankowski (1977) has studied the feeding behaviour of juvenile Salmo salar in streams using diving techniques.

The solution adopted by most workers to this problem, has been to confine the fish in a cage in its natural habitat e.g. Hoar (1942), Swift (1962), or to use an artificial stream e.g. Jenkins (1969), Wankowski (1977), or in aquaria e.g. Siegemund (1969), Kleerekoper et.al. (1973). In general fish adapt well to captivity and many species commonly used in the laboratory, such as the Rainbow Trout (Salmo gairdineri Richardson) and the Goldfish (Carassius auratus L.) are usually artificially bred and have, therefore, been subjected to captivity and handling during their lives. Abel (1962), however, describes in captive marine fish a stress-induced condition which he terms "gefangenschaftsneurose" (Imprisonment neurosis). In such a case, therefore, the fish's behaviour and physiological status may not

not be truly representative of its species in the wild.

Wardle and Anthony (1973) believe that fish which have been tank adapted and also conditioned to a visual stimulus, may be fairly species-representative. They will tolerate experimental procedures better than non-conditioned individuals but only if they are replaced in their 'home' tank to ensure environmental continuity. Wardle (1974) also confirms that it is better to replace wild fish in their home area to minimise disturbance factors.

More recently, closed circuit television has been used to study fish behaviour and physiology e.g. Coates (1973), Wardle (1974). In some cases fixed underwater equipment has been used in fish studies e.g. Colin (1972). Needless to say, such systems are limited in the area they can cover in their tracking ability and they are very expensive. Since the late 1950's, with the development of solid state electronic devices such as the transistor and the integrated circuit, it has been possible to construct transmitters small enough to be carried by a fish either attached to its back, or placed inside its stomach or abdominal cavity. These transmitters allow the movements of a fish, so equipped, to be traced for extended periods. Because radio-frequency signals are readily absorbed by freshwater, and even more so by seawater, it has been generally necessary for such underwater transmitters to radiate their output sonically, via a suitable transducer. Liquids are superior conductors of sounds than air and McKay (1970) discusses the basic principles behind the requirements for and the uses of ultrasonic transmission in biotelemetric studies from aquatic animals. Transmitters so employed on fish, generally operate at frequencies well above the human hearing range (>20kHz) because below operating frequencies of 40kHz,

transducers are too large for most "normal" sizes of fish. Kanwisher et.al. (1974) did use a 20kHz transmitter with a range in seawater of 8km., but this was only suitable for use in large Tuna.

The exact movements of an ultrasonically tagged fish can be plotted by using either directional triangulation methods as in Young et.al. (1972) or by using differential propagational delay measurement as in Hawkins et.al. (1974), or alternatively using a transponding transmitter, by tracking the fish with sector scanning sonar (Mitson and Storeton-West 1971).

Stasko (1971) has reviewed the literature on fish orientation studies including ultrasonic tracking and Mitson and Young (1975), and Stasko and Pincock (1977) have reviewed the technical and biological considerations in the design of ultrasonic transmitters for use in small fish.

The chief virtue of telemetric methods lies in the fact that they do not appear to affect the fish's normal swimming or feeding behaviour, (Young et.al. 1972, McCleave and Stred 1975). Even fish of less than 20cm. in length can accommodate some of the smaller transmitters without detriment (Tytler and Thorpe pers.comm.) Fish are unlikely to be influenced by the output from such transmitters as Enger and Andersen (1967) showed that no measurable auditory responses could be obtained electrophysiologically from Cod (Gadus morrhua) above 1kHz though in the same species using cardiac conditioning methods, Chanman and Hawkins (1973) showed that the effective upper limit of hearing was less than 0.5 kHz. In practice, transmitter operating frequencies are much higher than this and Young et.al. (1972), showed that Brown Trout (Salmo trutta) were unaffected by pulses of ultrasound in the 250kHz range at a sound

pressure level some thirty times higher than that from the output of a standard tracking transmitter.

McKay (1970) has pointed out the fact that although an animal may not be able to hear directly the fundamental carrier frequency of a transmitter, due to any non linearity in its auditory system it may still be able to detect pulse or modulation envelopes superimposed upon the carrier frequency. So there is some uncertainty possible in fish with regard to the detection of pulse or modulated ultrasonic transmitter outputs. Recently, using cardiac conditioning methods, Facey et.al. (1977) were able to show no such detection of pulsed transmitters by Atlantic Salmon (Salmo salar) even though in some cases that the output envelope from a 75kHz transmitter was plainly audible to observers in air. More detailed work on the perception of modulated acoustic signals by fish is in progress at present at the Marine Laboratory, Aberdeen. (A.D. Hawkins pers.comm.).

Since 1969 there has existed at Airthrey Loch, University of Stirling, a static ultrasonic fish tracking facility, based upon directional triangulation positional fixing of transmitters operating in the 220-280 kHz ranges. The project was initiated by Professor F.G.T. Holliday and later directed by Dr. Peter Tytler. The primary objective of the work was to study the contribution of locomotor activity in the energy expenditure budget of free-swimming, wild Brown Trout (Salmo trutta). Later, the study was extended to include the analysis of movements within the home range and the homing behaviour of fish displaced from their territories. More recently, the work has included the telemetry of physiological parameters such as cardiac frequency (Priede and Young 1977).

To date, much information on activity levels and movements has been collected using the Airthrey Loch system and a stochastic model of Brown Trout movements has been developed for a hypothetical "Klaxon" shaped loch (i.e. like Airthrey Loch) using this accumulated data (Tytler et.al. 1977). An early attempt was made by Young et.al. (1972) to complement the movement data obtained from positional fixing, by developing a transmitter capable of telemetering the tail-beat frequency of a fish. Bainbridge (1958), Hunter and Zweifel (1971) and Hudson (1973) have all shown a fairly direct linear relationship between tail-beat frequency and the instantaneous swimming speed for a given length of fish. The transmitter employed was frequency modulated by the tail beat mechanically applying a deflection to a piezo-electric bimorph element. This transmitter design, unfortunately, was not very successful. Firstly, because of the fragility of the bimorph element and secondly by the heavy drain imposed upon the batteries by the continuous F.M. transmission mode. The records from this transmitter were also difficult to interpret (P. Tytler pers.comm.) and its use was subsequently abandoned.

With the evolution of pulsed tracking transmitters (which only draw appreciable current during each "on" period) the life expectancy of the standard Stirling tracking transmitter, suitable for employment in trout-sized fish, has been extended to 21 days. (Young et.al. 1976). The transmitter technology which has been developed at the University of Stirling has enabled the routine production of transmitters which are probably the smallest in use in the world at the present time, (Young and Wiewiorka 1975).

Much research has been devoted to the study of cyclical activities in fishes. In marine species in particular, this has often been provoked by the need to know of a species' daily activity habits in connection with commercial fishing operations. Often this has been achieved by serial trawling operations or by the periodic examination of set gill nets. In such cases the frequency of encountering a particular species is taken to be indicative of its locomotor activity pattern. Spoor and Schloemer (1939) showed in Catostomus and Ambloplites that there was a regular variation in the numbers of each species caught in set nets over a twenty-four hour cycle. Carlander and Cleary (1949) examined similar data for nine species of freshwater Teleosts and found considerable interspecific differences with regard to the timing of activity cycles and also with regard to the direction and depth of lateral migration. Hasler and Bardach (1949), found that shoals of Perca flavescens would show a regular daily migration to a specific site at which nets and later photoelectric detectors were fitted and that this timing was evidently related to sunset. Regular daily lateral migrations have also been described by Hobson (1973) in tropical Atherinid fishes.

Controlled field and laboratory studies upon fish activity cycles have been fairly widespread in both marine and freshwater species. In earlier days registration of the fishes movements was necessarily crude, Spencer (1939) used various species attached by threads to a Kymograph but nevertheless gained some fairly useful results. Hasler and Bardach (1949), utilised a crude form of light beam apparatus which had to be interpreted manually from a microammeter. More recently mechanical detectors, akin in principle to 'jiggle' cages and light or infra-red beams have been used.

With such apparatus, Wikgren (1955) has studied Lota lota, Kruuk (1963) Solea vulgaris, Swift (1962 & 1964) Salmo trutta, Davis (1964) Lepomis gibbosus and Micropterus salmoides, Davis and Bardach (1965) Fundulus sp., Verheijen and de Groot (1969) Pleuronectes platessa and Platichthys flesus, Chaston (1968 & 1969) Salmo trutta, Siegemund (1969) Perca fluviatilis, Tinca tinca, Scardinius erythrophthalmus, Olla et.al. (1972) Paralichthys dentatus, Gibson (1973) Pleuronectes platessa, Kleerekoper et.al. (1973) Carassius auratus and Ginglymostoma cirratum, Reynolds (1976) Lepomis macrocheirus.

The general finding of these investigations follows that of the early netting operations previously cited. Fish exhibit a cyclical activity pattern which may be either monophasic or polyphasic, the timing of which related to photoperiod being species-dependent. For example, Siegemund (1969), showed the Perch and the Tench to have bimodal activity peaks, those of the Tench being essentially nocturnal, those of the Perch being diurnal. The Rudd on the other hand had a monophasic pattern with its peak in the middle of the daylight hours. Marine species may exhibit tidal rhythms though Gibson (1973), using hatchery reared plaice which had never experienced tides, found that there was an inherent rhythm which was a bimodal Circadian and concluded in the wild, that this was cued into the tidal pattern by an unknown "Zeitgeber".

The results of the ultrasonic tracking programme at Airthrey Loch and in Loch Leven, Kinross (Young et.al. (1972) and Holliday et.al. (1973) have shown in Brown Trout that there is a clear bimodal activity

cycle, in this species, with dawn and dusk maxima although subsidiary peaks may occur at midday. They also noted that individual fish may show random minor peaks of activity not apparently linked to dawn and dusk. More recently, Kelso (1976) has shown by ultrasonic tracking in the Walleye, Stizostedion vitreum vitreum that it has daily activity cycles similar to the Brown Trout.

The major question raised by the foregoing is, what is the significance of these locomotor activities in the fishes' life? i.e. what is their adaptative significance? Holliday et.al. (1973), have noted that during the period of the year when the trout's daily activity increases (March/April), it coincides with a period of increased growth and feeding activity and, therefore, there may be a connection between locomotor and trophic activities.

Hoar (1942) showed in an experiment using caged Salmo salar and Salvelinus fontinalis parr, that they would normally only accept food offered to them in two daily peaks, post-sunrise and dusk but that feeding could occur during darkness. He also found strong light to depress feeding but he concluded that it was light and not the accompanying rise in temperature which was responsible for this depression because fish kept shaded would feed at the midday temperatures experienced.

Spencer (1939), found in Carassius auratus that following a regular feeding regime, an intense activity period persisted for about three hours after the introduction of food, despite the fact that they consumed all the food offered in the first few minutes. Morgan (1974), in his review, notes that in mammals that such instrumental responses

may persist even though the "final consummatory event" (Sherrington 1906) to which they were directed, has already been achieved.

Siegemund (1969), found that locomotor activity decreased on days during which Perch were given no food. One might reasonably expect the reverse to happen i.e. searching or appetitive behaviour to increase in the absence of food. Swift (1962 & 1964), on the other hand, showed that the presence of food was not the stimulus to locomotion because fish fed on different feeding schedules had equivalent activity cycles. Davis and Bardach (1965) concluded that in Fundulus that locomotor activity prior to being fed at dawn was an artifact of conditioning the animals to a feed-time which itself was cued by an exogenous cue i.e. light.

Rather different results have come from workers using demand-feeding or operant conditioning techniques. Rozin and Mayer (1961) found that the Goldfish would feed regularly throughout the day or night, whereas Landless (1976) working with Salmo gairdineri found that demand-fed Trout did feed in defined bouts which varied with time seasonally. Between July and September most of the food was taken between sunrise and sunset. In February, October and November much overnight feeding occurred and on occasions up to 40% of the daily total intake was taken in darkness. He interprets his data and that of Rozin and Mayers work in terms of positive feedback during feeding. The Trout, being predatory, would find an advantage in maximising spatially or temporally aggregated food sources e.g. Asellus might be only found on a stony bottom or an emergent Chironomid might only appear at a certain time of day. On the

other hand, positive feedback would be of no advantage to an omnivorous fish such as the Goldfish or Carp which tend to graze indiscriminately.

It has upto now been almost impossible to gather data on the feeding habits of individual fish in their natural environment. Although the ultrasonic tracking of fish gives us a measure of their activity cycles directly, it has been impossible to link this to feeding periodicity except by inference. Thorpe (1974,1977) has examined by serial netting the chronology of feeding in Brown Trout and Perch in Loch Leven. He showed that feeding periodicity varies seasonally which is a reflection of photoperiod and prey species availability. He notes that for the Brown Trout for example, in September there may be two clear peaks at midnight and around 1400 hours, whereas in July or August most of the food taken is immediately post dawn. One interesting point to emerge from his work was that feeding activity at night in wild fish may account for up to 20% of the daily ration in the month of September, for example. This is not altogether surprising since both Jenkins (1969) and Landless (1976), both concluded that Brown and Rainbow Trout were capable of feeding at very low levels of illumination. Jenkins, however, found that by using marked prey (ants) that the percentage taken in the dark was less than in similar trials by daylight. Elston and Bachen (1976) found, by serially netting the Mississippi Silverside, Menidia audens, that a post sunrise peak in feeding activity included prey sizes down to 2mm. in length. They interpret this as prey being visually identified by the fish. At

night, prey were still taken but the size ranges caught excluded the smaller ranges. This implies that they were still taking their prey by sight but that they needed a larger target in order to feed. At low levels of illumination their visual acuity would be lower in scotopic vision and the prey are probably seen as silhouettes against the lighter water surface. This was further borne out by the observation that the amount of feeding decreased on moonless nights. Jenkins (1969), on the other hand found no distinction in his fishes ability to feed by starlight or moonlight.

In the trout, night feeding activity is not apparent from the tracking records of the Airthrey Loch system. Typically, the hours of darkness are periods of low levels of locomotor activity. (Holliday et.al. (1973). However, the level of feeding activity at night is appreciably lower than in daylight (Thorpe (1974)), and it is also possible that the locomotor patterns produced during night feeding are different from those of the daytime. It is also not clear whether or not the increases in swimming activity revealed at dawn and dusk coincide with feeding bouts. It is well known in the feeding strategies of many animals ranging from insects to birds that successful location of prey by a predator modifies the locomotor activity pattern, e.g. Dixon (1959), Smith (1975). Typically the search path taken by the animal becomes convoluted and tortuous and the effort in searching is increased. Assuming that prey is aggregated, this would have the adaptative significance of retaining the predator within the general area of the prey aggregation (Tinbergen et.al. 1967).

Beukema (1968) and later Thomas (1974) show a parallel situation in the three-spined Stickleback, Gasterosteus aculeatus. They also noted that lack of success in food finding caused a direct move away from the search site and a reduction in the intensity of searching. Ware (1972) working with small Rainbow Trout, noted that the rate of predation could be stimulated by increasing the prey density over a given substrate type. He also noted that similar to the Stickleback, lack of success in finding food lead to a direct move away from the search site and also found that if a capture rate of one item per 17s was not exceeded, search intensity began to decline.

It is possible that the activity cycles recorded by the ultrasonic tracking represent these direct movements away from unsuccessful search sites. Normally the tracking schedule calls for a triangulation rate of one per five minutes and the accuracy of the fix at extreme range has been calculated by Young et. al. (1975) as being ± 2 m. Because of this low sampling rate and the resolution of the system, it is possible that periods of intensive searching of successful sites may be recorded as period of low activity. The experienced operator can, however, tell aurally when the fish is making small scale movements because when the transmitter transducer is rotated relative to the receiving hydrophone, a qualitative change in the sound occurs. This is due to factors such as non-linearity in the transmitting transducer (Mitson and Young (1975)), Doppler shift and also due to passive modulation of the signal caused by movements of the fishes tail and body. (Harden-Jones 1973; Stasko and Horra11 1976).

Normally the operator would not have time to record such fine detail and if the fish were making a series of tight turns during feeding it might still be classified as not making any movement, unless it shifted its position by a metre or so.

2. Review of Technology, and objectives of this Investigation

In the present study, it was decided that research should be directed towards the development of a transmitter small enough to be carried by a 30cm. length Brown Trout which would permit simultaneous tracking and monitoring of the fishes position and also the number of individual feeding acts performed.

Initially, consideration was given to using some sort of transducer which would suitably detect movements of the fishes jaws during the feeding act. Capra (1976) used a piezo-electric strain gauge to monitor respiratory movements in the free-swimming Port Jackson Shark, Heterodontus portjacksoni. Anemometry of buccal water flow using minute heated bead thermistors (e.g. the STC P23 bead) was another possible technique. Uglow (1973) used a form of impedance pneumography to monitor the activity of the scaphognathite in Carcinus and it was thought possible that the large changes in impedance across the opercular cavity during repiration might be monitored using a similar method. Rommel (1973) showed that simply by placing electrodes in a tank, the artifact created by opercular movements could be monitored and this might be of initial use in the laboratory. Another possibility was to use a transducer to record pressure changes or movements in the stomach or oesophagus. It is well known that both the mammalian (Code and Carlson 1968) and trout (Burnstock 1958) stomach exhibit regular contractions which are

subsequently modified by feeding and this might be used to determine the fishes feeding status.

Nearly all these methods had to be rejected because they require an energised bridge circuit to power the transducer and this imposes a heavy drain upon the transmitter's power supply. The disadvantages of bridge circuitry in ultrasonic transmitters are discussed in Stasko and Pinock (1977). Kanwisher et.al. (1974), did use a depth sensing transmitter with a bridge input circuit but this was only feasible because of the large batteries employed and the transmitter is too large to be used on the size of fish contemplated in this present study.

It was, therefore, decided to direct investigations into using the fish itself as a transducer i.e. use amplified biopotentials for the input signal. It is considerably easier to construct low current drain amplifiers than to use energised bridge circuitry with its high current drain. The electrocardiogram (ECG) of the fish was considered as a prime candidate for investigation because it is easy to record, is relatively large in amplitude and, therefore, the signal to noise ratio would be high. Cardioinhibitory reflexes, in response to external stimuli, have long been known in fish. Lyon (1926) noted that in Carcharias taurus, an atropine-antagonised cardioinhibitory reflex could be elicited from virtually any part of the animal, except its liver, by a range of mechanical, thermal, electrical and chemical stimuli. Lutz (1929) observed similar reflexes in the Carp and Dogfish as did Kisch (1950) in Acipenser sturio and Anguilla bosteniensis.

Hughes and Umezawa (1968) observed that passive opening and closure of the jaw of anaesthetized Callionymus lyra to evoke a cardioinhibitory reflex. Indeed, there have been several investigations, e.g. Shelton and Randall (1962), Satchell (1960), Weintraub and McKay (1975), which suggest that under certain conditions there may be an element of synchrony between respiratory and cardiac rhythms in fishes. This implies a possible reflex connection between the moving head parts and the heart. Priede (1973) noted in a free swimming trout that cardiac inhibition did occur at the seizure of a food pellet but noted that this was a non-specific occurrence which could be elicited by many different stimuli. He subsequently showed (Priede and Young 1977) that the ECG did not provide unequivocal evidence of a feeding event.

Owing to the fact that Priede was about to commence his study of trout heart rate in Airthrey Loch with an as yet undeveloped ultrasonic heart-rate transmitter, the use of the ECG to signal feeding events was set aside in preference to more direct methods.

Extracellular electromyography (EMG) was discovered as long ago as the 18th Century by Galvani, though it did not develop as a science in itself until about fifty years ago. Since then it has become a clinical diagnostic tool and has also been extensively used in the unravelling of the complex interactions of muscular functions in both vertebrate and invertebrate animals. The basic principles of the clinical uses are reviewed admirably in Lenman and Ritchie (1970)

and the Kinesiological approaches in Basmajian (1974).

Hughes and Ballintijn (1965 a, b), Ballintijn (1969), Hughes and Ballintijn (1968) and Hughes (1975) have used EMG's in several species of teleosts and elasmobranchs in order to evaluate the muscular basis of the ventilatory mechanism. These studies were all performed upon lightly anaesthetized animals although Bone (1966), Rayner and Keenan (1967), Roberts (1969), Hudson (1973) and Kaseda and Nomura (1973) have recorded EMG's from unrestrained or decerebrate fish, chiefly from the lateral swimming muscles.

Three outstanding studies have been performed upon fish cranial muscle function during feeding. Osse (1969) in Perca fluviatilis, Ballintijn et.al. (1972) in Cyprinus carpio and Elshoud-Oldenhove and Osse (1976) in Gymnocephalus cernua have made, using elegant techniques, direct multichannel recordings which were correlated with synchronized cine film recordings. Both studies showed that certain muscles may be differentially active during feeding and that peak EMG voltages produced thus may be four to ten times those of normal ventilation. Monitoring a suitable cranial muscle EMG seemed to be a fairly obvious way of telemetering the feeding act, and it was resolved that this was to be the main line of the research project. Kotchabhakdi (1973) had already telemetered EMG's from the cranial muscles of Mustelis canis and Kanwisher et.al. (1974) had telemetered EMG's from fish lateral muscles. Radio-telemetry has also been used to telemeter EMG's from the jaw muscles of cows in order to determine chewing rates (Devices Ltd.,

SNR102F literature, 1973). It also seemed feasible that the prototype ECG transmitter being developed by the N.E.R.C. Fish-Tracking Unit at the University of Stirling might be readily modified to accept EMG's as an input signal.

Because of the phenomenon of reciprocal antagonism (Basmajian 1974 q.v.) the EMG from fish head muscles occurs in discrete rhythmic bursts separated by periods of electrical silence. Alterations to the normal pumping rhythm would conceivably signal feeding events and also secondarily give a measure of the daily variations in ventilation frequency in a wild free swimming fish. Semi-quantitative analysis of the EMG by electronic integration (Bigland and Lippold, 1954; Bergstrom, 1959; Milner-Browne and Stein, 1975) might enable the semi-automated recognition of the feeding acts. Hughes and Ballintijn (1968), used such methods to quantify the relationship between environmental pO_2 and pCO_2 with the pumping effort, in the Dragonet, Callionymus lyra.

It was also thought desirable to have incorporated some measure of the depth at which the fish were feeding or even their attitude in the water. However, no small depth sensing transmitter has appeared yet and although an attitude sensing transmitter is easily made, (McPartland et.al. 1976) the constraints imposed by bridge circuitry and/or F.M. transmission mentioned earlier, tend to rule these out. Unfortunately, Airthrey Loch is generally too shallow (<4m) to allow indirect measurement by depth sensing hydrophone arrays such as used by Gardella and Stasko (1974). As an alternative

to physical measurement of depth altitude it was possible that there might be repertoires of feeding movements which might be classified in the laboratory e.g. EMG patterns from bottom feeding versus those from midwater or surface feeding. It might, therefore, be possible in the field to deduce where the fish was taking its prey from.

Yet another approach was to examine the activity of the oesophagus or stomach during or after feeding. Riley and Cook (1973), Lonsdale et.al. (1966) have devised transducers to monitor stomach pressures or movements though they require continuous F.M. transmission with its attendant high current drain. The smooth muscles of the stomach produce a characteristic electrical rhythm known as the "pacesetter potential" upon which faster-spiking EMGs concomitant with active contractions are superimposed. There is a vast amount of information on this subject in mammals and man, particularly with respect to clinical aspects of derangements. Bulbring (1962) and Prosser (1974 a, b) have reviewed the basic concepts in vertebrates. Knowledge of this in fish is almost non-existent. Ito and Kuriyama (1971) have examined the pharmacology of the stomach in Carassius auratus, including electrical events. They, however, worked in vitro using isolated segments of the alimentary canal and recorded electrical activity by the double sucrose gap method, which detects compound responses from large populations of cells (Wallis et.al. 1975). This method does not record faithfully migrating waves such as the pacesetter potential and is not representative of in vivo activity as the tissues are freed of extrinsic innervation.

It was felt that the recording of such activity and its modification by stomach filling might be a worthwhile secondary route to explore. Although the implantation of electrodes into the alimentary canal would require major surgery (laparotomy), there is much evidence in the literature (see Stasko and Pincock 1977 for review) to suggest that fish returned to the wild will tolerate such insult though Hart and Summerfelt (1975) took prophylactic measures by treating their fish with Oxytetracycline before release. The oesophagus contains a variable mixture of smooth and striated muscles and implantation of electrodes there could also signal the act of feeding although it would not provide a continuous signal which would enable the fish to be tracked though this might be overcome with a secondary tracking transmitter, operating on a different frequency. Doty and Bosma (1956) and Hellemans et.al. (1968) have reviewed the basic concepts of the oesophageal EMG during deglutition.

Section I : Anatomy and Histology

INTRODUCTION

It has long been known that at least two kinds of macroscopically distinct muscle are present in the lateral swimming musculature of Agnatha, Teleosts and Elasmobranchs. Lorenzini (in Bone 1966) first observed such a difference in 1678 in the Electric Ray, Torpedo. The two most obvious differences between the muscle types, when sufficiently aggregated, is that one type is pale and the other red in colour. This corresponds to twitch (fast or phasic) and tonic (slow) in physiological response and terminology, respectively. Since Lorenzini's day, there have been a large number of investigations into both the histological and physiological differences between the two muscle types in vertebrates. From these, it can be seen that there is fair agreement between the vertebrate groups, with respect to histological appearance.

General Histology

a) Fibre diameter

With the exceptions of mammals and man it can generally be said that white and red muscles can be segregated on the basis of their fibre diameter sizes. There is usually an overlap in the size ranges but simple statistical analysis will generally separate them clearly.

In the Amphibia, red fibres have been shown to occupy size ranges between 10-110 μm in diameter whereas the white fibres range between 60-150 μm . (Gray 1958, Lannergren and Smith 1966). In Elasmobranchs, (Bone 1966, Roberts 1969) red fibres have been shown to be in the range of 18 to 75 μm in diameter and white fibres in the 76 to

230 μ m in diameter ranges. There is much more information available on Teleost fishes, values for red fibres ranging from 10 μ m up to 50 μ m and White fibres from 30-160 μ m. (Boddeke et.al. 1959, Baretts 1961, George 1962, Nishiharra 1967, Greer-Walker 1970 and 1971, Johnston et.al. 1975, Patterson et.al. 1975). Unfortunately, amongst certain Teleost groups, there are complications, especially in the Salmonidae and Cyprinidae. In these, the white fibres have small diameter fibres intercalated amongst the larger diameter ones giving a pattern which has become known as mosaic (Boddeke et.al. 1959). Greene(1913) gives the size range for Oncorhynchus tshawytscha mosaic muscle as being from 25-250 μ m, whereas Johnston et.al. (1975) and Cust (1975) gave size ranges for Salmo gairdneri as 15-95 μ m and 27-121 μ m respectively. There is no data apparently available for Salmo trutta.

Many workers such as Baretts (1961), Bone (1966), Patterson et.al. (1975) and Kryvi and Totland (1977) have described aberrant or intermediate fibre types such as large and small "pink", in both Elasmobranchs and Teleosts. Such fibres, however, occupy only a small percentage when compared to fibres predominantly 'white' or predominantly 'red'.

In man and other mammals, most muscles are mosaics of white and red fibres called types I and II respectively. There is, however, in this case, no apparent size difference between type I and II fibres. In man, for this reason, all exsanguinated muscles are red in appearance. (Dale Smith and Giovacchini 1965). The soleus, however, is somewhat darker in appearance than its companion gastrocnemius.

Clearly in the case of mammals, the muscle systems are more complex than those of the fish in which functionally and anatomically separated fibre sizes occur, although in Salmonids and some Cyprinids there may be a wide variation of fibre size within the mosaic 'twitch' muscle. The functional significance of this, if any, is obscure at the present time.

b) Myofibrillar array

Again, there is concordance throughout most of the vertebrate groups between fibre size/type and the myofibrillar array present. Kruger and Gunther (1955) described from a range of mammals and Man, two kinds of myofibrillar array present in muscle fibres. In one kind, the fibrils are cylindrical, even sized and are packed together in large numbers into a regular pattern which has become known in the literature as 'Fibrillenstruktur' (fibrillar pattern). The other type has polygonal myofibrils but they are much fewer in number than in the former type. This is known as 'Felderstruktur' (field pattern) and takes its name from the irregular groupings into which the fibrils are arranged. As mentioned previously, most mammalian muscles are a mosaic of I + II fibres and this is borne out by the myofibrillar patterns also. In the extraocular muscles of mammals, however, it is known that separate bundles of muscle fibres of two different sizes occur. Hess and Pilar (1963) have shown that small diameter fibres with Felderstruktur are 'slow', whereas the larger diameter ones with Fibrillenstruktur are 'fast' in the physiological response.

Kruger and Gunther (1958) have also demonstrated in the Avian that in red muscles, such as the gastrocnemius externus, the muscle fibres primarily show Felderstruktur, whereas in a white muscle e.g. pectoralis major the fibres are primarily with Fibrillenstruktur.

In the Frog, Gray (1958) showed that 20% or less of the population of a mixed muscle (extensor longus digitorum) was composed of "areal" (= Felderstruktur) patterned fibres. Furthermore, he showed that in the sartorius, a muscle known physiologically to be devoid of slow fibres, that the muscle is solely composed of fibrillar-patterned fibres.

In fishes, Kruger (1950) noted that in the pelagic shark Lamna nasus that red fibres had Felderstruktur and white fibres, Fibrillenstruktur. Barets (1961) showed in Ameiurus that red fibres had, typically, Felderstruktur which he termed 'en ruban' (ribbon-like) and that the white fibres had not only Fibrillenstruktur but also a peripheral belt of radially arranged myofibrils which is somewhat different from the mammalian examples described by Kruger and Gunther (1955). Barets also described some fibres which he considered to be intermediate in fibrillar pattern i.e. tending to have attributes of both red and white fibres. Nishihara (1967) and Nakajima (1969), however, have noted that both red and white fibres in fish have ribbon-like peripheral myofibrils but that those of the white fibres are more pronounced.

c) Fibre shape

Greene (1914) stated that in the King Salmon, the small diameter red fibres appeared rounded in outline, whereas those of the mosaic muscle appeared angular. This criterion has been given little attention

by other workers although examination of Johnston et.al. (1975) and Boddeke et.al. (1961)'s text-figures does not suggest that this might be so, for Salmonids at least. Baret's (1961) and Nishihara's (1967) work in other species also suggests this though in other examples white fibres may appear rounded or even a mixture of both rounded and angular fibre-types. Undoubtedly, fixation methods have a great deal to do with this, especially as Watzka (1939) states that white muscle (avian and mammalian) shrinks at a differentially greater rate than red muscle after fixation.

d) Nuclear Pattern

Bone (1966) and Roberts (1969) have both observed that in elasmobranchs in particular, the nuclei of the muscle fibres are peripheral in red fibres but may be distributed in white fibres. Nishihara (1967) says that in the red fibres of the Goldfish the nuclei are peripheral but says nothing about the location of the nuclei in the corresponding white fibres.

Interestingly, in humans, the appearance of internal nuclei at a frequency greater than 3% is considered pathological and is indicative of myopathies such as dystrophia myotonica, (Bethlem 1970).

e) Vascularisation

There is absolute agreement upon this aspect throughout the vertebrates. Dale Smith and Giovacchini (1956) have examined and reviewed the vascularity of mammalian and human muscles. Red fibres are always better supplied with capillaries than white fibres.

George and Naik (1957 and 1968) showed in the Pigeon that this was also the case in Avians. In Elasmobranchs Bone (1966) and Roberts (1969) have both confirmed a similar difference in vascularity between red and white muscle fibres. This has also been seen in Teleosts by Boddeke et.al. (1959), Baretts (1961) and Nishihara (1967). Cameron and Cech (1970) have quantified the vascular differences between red and white muscles in the Striped Mullet, Mugil cephalus and showed that red muscles have approximately 2.25 times the number of capillaries /mm² compared to white muscles. More recently, Cameron (1975) has attempted to refine this further, using the radio-labelled microsphere method. In the unanaesthetized, normoxic Arctic Grayling (Thymallus arcticus) he showed that the blood flow in red muscle is ten times that of white muscle.

Innervation

a) Endplate type

In most of the vertebrates, nerve fibres supplying motor innervation to fast and slow muscles, have been shown to be related in diameter to the type of muscle they are innervating. Particularly from studies on the extraocular muscles of mammals and the locomotor muscles of Amphibia, in which slow and phasic fibres are physically separated, there is a generally accepted dichotomy of endplate type, according to the physiological type of muscle. In the mammalian extraocular muscles, Hess and Pilar 1963, Bach-y-Rita and Ito 1966, Harker 1972 and Browne 1976 have shown that the slow fibres typically receive a diffuse polyneural/multiterminal innervation terminating in an endplate known as "grape" or "en grappe". The phasic fibres, on the other hand, have a different ending usually described as "plate" or "en platte". The pattern in Amphibian muscle is likewise very clear,

following the above pattern (Gray 1957). Similar patterns are also described from Avians (Kruger and Gunther 1958) and Agnatha (Bone 1963 and 1964). In Elasmobranchs the pattern persists though the monofocal, myoseptal innervation of phasic fibres (Bone 1964, 1966 and Roberts 1969c) is slightly different, being basket like and giving rise to the term "en panier". In the Elasmobranchs, the slow fibres receive a distributed innervation of grape-type endplates though the actual location of these latter terminations depends on the species. Bone (1964), however, asserts that in elasmobranch slow fibres, the endings never occur in the myoseptal region. There are, described by Couteaux (1950), intermediate types of endplate type, just as there have been intermediate muscle fibre types described, based upon histology, histochemistry and development studies. However, both these "aberrant" fibre and endplate types are in the minority. In the case of the former, their functional significance has recently been questioned (Mosse and Hudson 1977), if so, then the significance of the latter might be small.

The endplate pattern in the Teleosts, is different and less coherent than those of the other vertebrate groups above. In all but a few Teleost species (Barets 1961) the phasic fibres are innervated by axons giving a distributed innervation with annular terminal regions on which each axon terminates. Therefore, each phasic fibre has a distributed pattern, shown by Hudson (1969) to be polyneural, in contrast to the monofocal pattern seen in the elasmobranchs and Agnatha. Certain Teleosts e.g. Ameiurus, may have a different pattern similar to that of the Elasmobranchs. In fact, the endplate pattern in both phasic and slow fibres may be outwardly similar i.e.

of a grape type (Nakajima 1969) but vary somewhat in the distribution of terminations along the length of the fibre. The endplate pattern of the slow fibres, however, is fairly uniform throughout the Teleosts.

Most observation on Teleost muscles have concentrated on either myotomal or fin muscles (probably because of practical problems associated with obtaining a discrete sample of one type of muscle). No observations appear to have been made on endplate types in Teleost cranial muscles save for Kordylewski (1973) who has shown that the extraocular muscles of the Gudgeon (Gobio gobio) are similar to those of the mammal. There are clearly separated bundles of small diameter fibres with grape endings and a larger mass of larger fibres with plate type axonal endings.

b) Fibre Size

It has long been known (Sherrington 1906) that efferent nerves to mammalian striated muscles may have segregated size classes of nerve fibres in their populations. Tasaki and Mizutani (1943) and Tasaki and Tsukagoshi (1943), showed in the Toad and Cat, that slow muscles were innervated by small diameter nerve fibres peaking at around $5\mu\text{m}$ in diameter. Fibres motor to phasic muscles were grouped around $11\mu\text{m}$ in diameter. In the Rabbit (Fernand and Young 1951) many of the limb muscles have bimodal size classes in their motor nerves, some of which are presumed to be afferent, others efferent belonging to the fusimotor system. Some muscles, particularly those of the head and neck e.g. Sternothyroid, thyrohyoid, recurrent laryngeal etc., have narrow unimodal distributions in the complement of their motor nerves.

Donaldson (1960) showed in the goat extraocular muscles, in which the sensory nerves are physically segregated from the motor nerves, that such nerves have a characteristically unimodal pattern. The extraocular nerves of the Rabbit and the Goat are both bimodal with peaks at 3 - 7 μ m and 12 - 16 μ m but where the former is devoid of muscle spindles (Browne 1976), the latter is rich in them (Donaldson 1960). Therefore, the small diameter fibres in the rabbit extraocular nerves cannot be γ efferents and must be either sensory or else motor to slow fibres.

The soleus, crureus, semitendinosus and quadratus femoris muscles of the rabbit are almost wholly slow muscles and although they may have bimodal motor nerve fibre distributions, the upper size ranges may be attenuated.

In the Amphibia, Kuffler, Laporte and Ransmeier (1947) and Kuffler and Vaughan Williams (1953 a + b) have shown that small diameter fibres can be stimulated independently of larger diameter fibres in the same nerve by a differential blocking stimulation method generally referred to as "anodal block" and which has now become quite sophisticated e.g. Accornero et.al. (1977). They demonstrated that small nerve stimulation activated slow fibres only, which was shown in the earlier work by Tasaki and his collaborators by the cruder, but expedient, method of teasing the nerve fibre to a muscle and selecting nerve fibre sizes for differential stimulation using visual discrimination.

In Elasmobranchs, Roberts (1969c) has shown by anodal block methods that the phasic fibres of the Dogfish (Scyliorhinus canicula) are activated by axons with a conduction velocity of such that it would infer axonal diameters of 10-14 μ m. Conversely he was able to show by reflex

activation of the red fibres, that their discharge was solely by groups of small diameter axons.

Hudson (1969) found that the axons innervating the phasic muscles of the Teleost Cottus scorpius have fast conduction velocities and are in the 10-14 μ m range which fits in with that of the amphibian fast motor axon, at the same temperature. Baretts (1961) also examined spinal nerves to myotomal muscles of various teleosts such as the Tench and Catfish (Ameiurus sp.), which have superficial lateral slow muscles, in contrast to those of the sea scorpion which has a very limited amount of red muscle, showed that the ventral roots have bimodal axonal size distributions, corresponding to the sizes known for fast and slow motor axons i.e. 8-14 μ m and 4-6 μ m respectively. Roberts (1969c), however, did not find a bimodal distribution in the dogfish motor nerve but considered that duality of the motor system in terms of axonal diameter might be obliterated by overlapping size ranges.

Histochemical differences

Much work has centred on histochemical differences between red and white muscles in fishes.

A brief synopsis of some of the major differences is tabulated below, based upon work by Bethlem 1970; Cust 1975; Patterson et. al. 1975; Johnston et. al. 1975; Kryvi and Totland 1977.

	<u>Glycogen</u>	<u>Lipid</u>	<u>Lipase</u>	<u>Motochondria</u>
R	++++	+++++	+++++	++++
W	+	+	+	+
	<u>Myoglobin</u>	<u>Fe⁺⁺</u>	<u>SDH</u>	<u>LDH</u>
R	++++	++++	+++++	++++
W	+	++	0	+
	<u>Phosphorylase</u>	<u>NADH</u>	<u>Myofibrillar ATP-ase</u>	
R	+	+++++	+	
W	+++++	0	+++++	

Key: relative abundance +(trace) to ++++(much)

0 = absent

The above is drawn from a variety of human, mammalian, avian, amphibian, selachian and teleost studies. It illustrates the basic biochemical differences which are generally held to mean that the red muscles of fish respire aerobically using fatty acids as a substrate and that white muscle respire anaerobically utilizing glycogen. However Bilinski (1974) in his review points out that "although pronounced quantitative differences in enzyme activities exist in fish between the red and white skeletal muscle, there is no evidence for a strict compartmentation between the two types with respect to the aerobic and anaerobic pathways or with respect to the utilization of fatty acids and carbohydrates".

Upon the foregoing hinges the Braekkan-Wittenberger school of thought in which the red muscle is seen in a supportive metabolic role to the white muscle (Braekkan 1956, Wittenberger 1967, Wittenberger et.al. 1969,

Wittenberger and Coprean 1977). To date, this aspect is still not proven either way.

Muscle differentiation in the cranial muscles of fish

As mentioned earlier, much of the research upon red and white muscles in fish has been carried out on the myotomal muscles because a) they are large and well differentiated, b) it is relatively easy to make fish do something i.e. swim, with them in an experimental situation. The other main lines of research have centred on the muscles of the fin ray mechanisms.

Only Hughes and Ballintijn (1965), Cameron and Cech (1970) and Kordylewski (1973) have paid any attention to the existence of separate red and white fibre tracts in the cranial muscles of teleosts. Cust (1975) investigated the histology and histochemistry of the adductor mandibulae muscle of Rainbow Trout. She demonstrated that the profound portion of the muscle is composed of small diameter fibres with the histological characteristics of red muscle whilst the remaining 80%+ of the muscle consists of typical salmonid mosaic. Ross and Tytler (pers.comm.) have also shown by Polyacrylamide gel electrophoresis, that the macroscopically distinguishable 'red' fibres of this muscle contain much myoglobin, which the mosaic portion does not significantly possess. As a necessary prerequisite to the EMG studies in Section II, a limited amount of histological investigation was undertaken in order to confirm that the structure of the Brown Trout adductor mandibulae was similar to that known to exist in that of the Rainbow Trout.

Section I : Anatomy and Histology

MATERIALS AND METHODS

1.1 Fixation

In most cases, samples of nerves and muscles were taken from fish which were fixed by a whole body perfusion method similar to that of Hinton (1975). The pericardium of the fish was opened under anaesthesia and a ligature passed around the bulbus arteriosus. The bulbus was severed from the ventricle and cannulated, the ligature being used to secure it in place. The cannula was immobilised with a suture and the fish exsanguinated by flushing out with 0.8% Sodium Chloride solution containing Heparin Calcium (Sigma) 10i.u./ml. delivered from a glass syringe. This was followed by saline containing 3% Procaine Hydrochloride (B.D.H.) which prevents arteriolar spasm when the fixative is added. The fixative employed was Sørensens Phosphate Buffered Formalin 10%. All solutions were cooled before use and after allowing time for fixation, the muscles and nerves were removed by dissection and stored in buffered formalin.

1.2 Processing

Teleost muscle is difficult to section and attempts to use a standard 24 hour tissue processing schedule on a 'Tissuemat' were unsatisfactory. Upon the advice of Mr I.H. Macrae, Unit of Aquatic Pathobiology, all muscle tissue was processed by a modified Peterfi's double embedding (Celloidin/Wax) which requires five days processing (and is, therefore, liable to cause a large degree of shrinkage of the tissue).

Nerves were processed on a normal 24 hour schedule and embedded in paraffin. T.S. of muscles were cut at 10 μ m and nerve at 8 μ m. L.S. of muscles were cut at 20-25 μ m.

1.3 Staining Methods

Transverse muscle sections were stained with Haematoxylin and Eosin, Toluidine Blue, Luxol Fast Blue/Cresyl Violet. Myelin sheaths were demonstrated with Luxol Fast Blue/Cresyl Violet.

Attempts were made to demonstrate the innervation of the muscles with the silver impregnation methods of Holmes (1947), Page (1971), Bone (1972) and Winkelman and Schmit (1957), but were generally unsuccessful (see Section 1.17).

Photomicrographs were taken using a Zeiss camera/microscope system. Fibre diameters were measured from enlargements with reference to a calibration slide.

1.4 Vascularisation of the muscle

This was visualized in the adductor mandibulae by exsanguinating a fish as described above. The head was severed and the dorsal aorta cannulated. Pigment containing a small addition of latex (Griffin George Ltd) was perfused (cephalad) using a glass syringe. The adductor mandibulae muscles were carefully dissected and fixed in cold formalin. Later it was dehydrated, cleared in Xylene and photographed using a Zeiss Tessovar system.

Section I : Anatomy and Histology

RESULTS

1.5 Gross Anatomy

The m.adductor mandibulae of the Brown Trout is, as might be expected from related species, very similar to that of the Rainbow Trout. It consists of two simple parts; a large cephalic portion, and a very much smaller mandibular portion which acts at right angles (with the mandible adducted) to the former. The fibres of the cephalic portion converge in a dorso-ventral direction into their insertion on the coronoid process of the mandible, via a broad, flat tendon. The fibres also converge in the lateral plane, giving an oval outline to the muscle. The thin mandibular portion inserts along the inner medial surface of the mandible, its fibres running in an antero-postero direction and terminating at the coronoid process. It is difficult to decide if they should be regarded as one digastric or two separate muscles.

Upon dissection, the cephalic portion is seen to have an obvious thin tract of red fibres on its inner medial surface, in contrast to the pale appearance of the muscle. The tendon is clearly visible at its lower extremity and originates about half-way along the length of the muscle, as it does in the Rainbow Trout.

It is difficult to distinguish much detail in the mandibular portion of the muscle as it is very thin, except that the fibres curve laterally outwards to meet their insertion onto the inner lateral aspect of the mandibular bone.

The muscles of the two species are, as previously stated, quite similar, i.e. there is no subdivision of the main cephalic portion into superficial and profound muscles as in the Cyprinidae, for example. The allometry is, however, different; the muscle of the

Fig. 1.1

Tracings made from T.S. of Brown Trout a. mandibulae muscle fibres at adjacent depths. Numbers indicate the depth of the tracing in mm. from the surface of the muscle.

There is a tendency for the numbers of large diameter fibres to decrease with depth until at 9 mm, the muscle solely consists of small diameter fibres. Scale bar = 100 μ m

Fig. 1.2

Frequency distribution histograms for muscle fibres, corresponding to the depths shown in Fig. 1.1. There is a clear difference between the mean fibre diameter at 9 mm depth and the remainder of the muscle.

FIG. 1-1

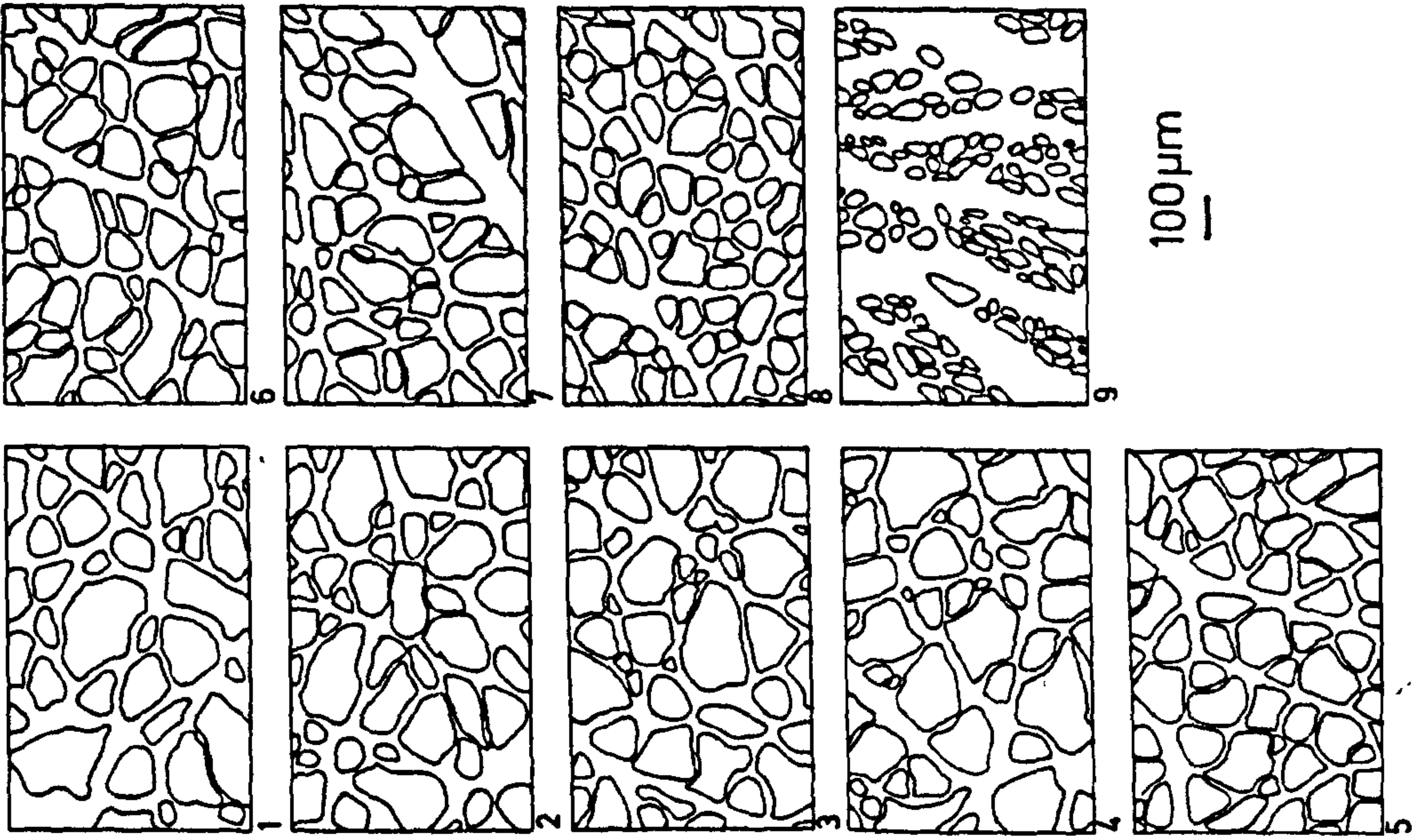
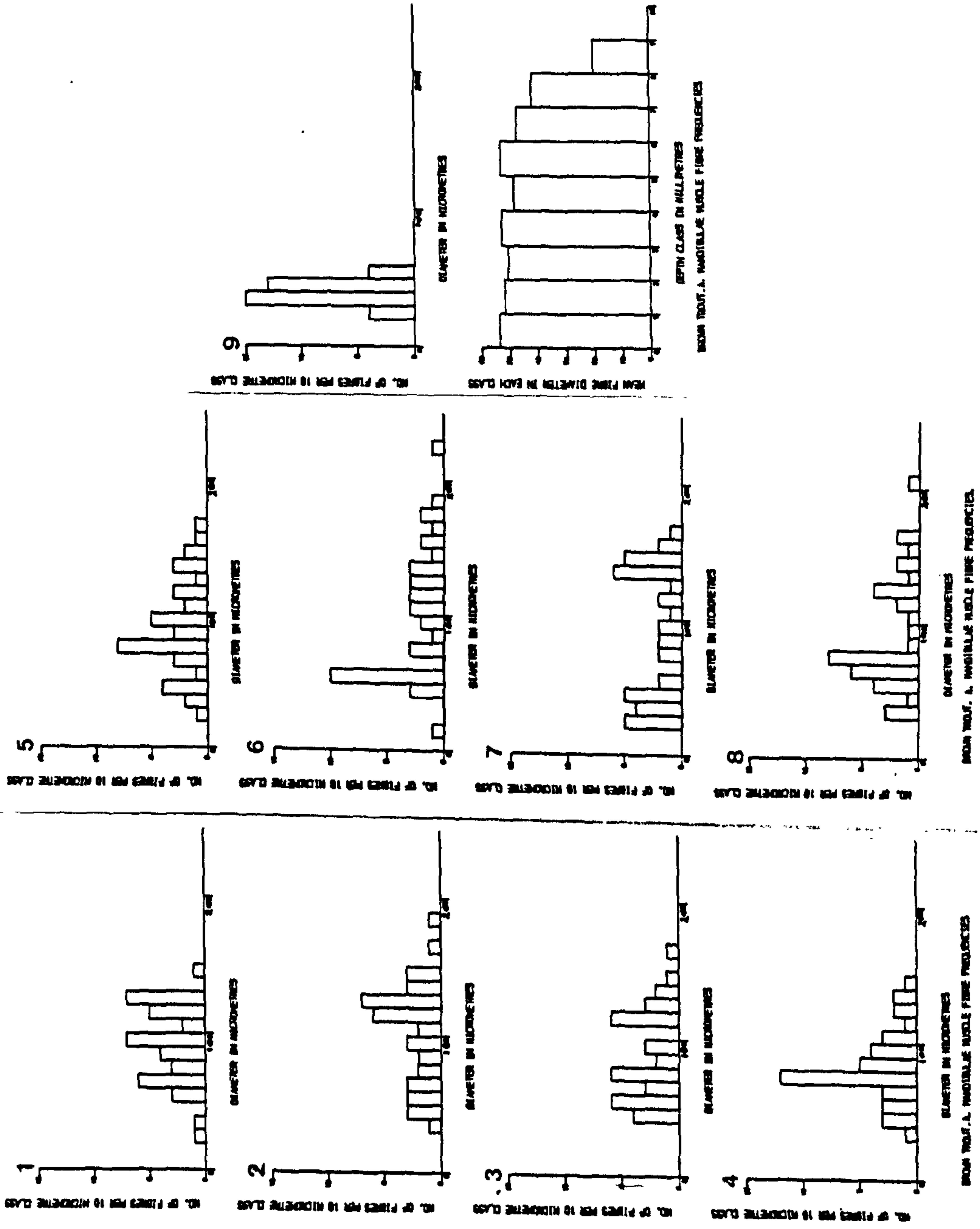


FIG 1-2



Brown is more elongated, broader and thinner than that of a Rainbow Trout of similar length.

1.6 Fibre sizes

The appearances of thin sections, through the Brown Trout m. adductor mandibulae is closely similar to that described in the Rainbow Trout by Cust (1975). The inner median portion, seen in transverse section, consists of small diameter fibres which differ markedly from the remainder of the muscle which is composed of typical salmonid 'mosaic' muscle fibres. In these there is a wide range of fibre sizes from small fibres up to quite large ones. The small fibre tract is arranged in flattened, narrow, fasciculi which are separated by vascular channels, giving this area a distinctive reticulate appearance. Plates (I.1 - .4). This is in contrast to the mosaic fibre area in which no obvious small-scale fasciculi are present

In order to correlate with the electrophysiological work in Section II, a series of adjoining photo-micrographs (Fig. 1.1),

each covering a rectangle of the muscle 1mm. wide, was examined in order to plot a transect line perpendicular to the midpoint of transverse sections of Brown Trout adductor mandibulae muscle. The level of section chosen was that just dorsal to the origin of the tendon in the muscle. This coincides with the favoured site for electrode implantation in both the laboratory and field studies in Sections II and III.

Fig. 1.2 shows the fibre diameters measured at each depth in two selected adductor mandibulae muscles from Brown Trout. Twenty fibres were measured at each depth, per muscle.

Table 1.1 shows the resultant summary of descriptive statistics.

Table 1.1 Mean Values, Range, Standard Deviation and Skewness for all the muscle fibres measured, at each depth.

	9mm.	8mm.	7mm.	6mm.	5mm.	4mm.	3mm.	2mm.	1mm.
mean(\bar{x})	20.02	42.03	47.35	52.93	48.48	52.60	50.35	51.70	53.52
min	11.00	15.00	21.00	20.00	15.00	18.00	16.00	10.00	15.00
max	28.00	72.00	100.00	102.00	81.00	112.00	90.00	112.00	91.00
S.D.	4.59	13.25	18.61	19.58	16.43	26.14	24.21	23.91	19.99
Skewness	-0.31	0.38	0.64	0.20	0.01	0.75	0.01	0.47	0.2

Table 1.2 T-test values for comparisons between the 9mm. depth and the remainder of the muscle.

	8mm.	7mm.	6mm.	5mm.	4mm.	3mm.	2mm.	1mm.
	10.01	9.08	10.53	11.25	8.06	7.70	8.67	10.7
	***	***	***	***	***	***	***	***
significance	***	p > 0.001,	**	p > 0.05	*	p > 0.1		

Table 1.3 T-test values between adjacent depth in the muscle

	9/8mm.	8/7mm.	7/6mm.	6/5mm.	5/4mm.	4/3mm.	3/2mm.	2/1mm.
	10.01	1.41	1.31	1.15	0.90	0.45	0.24	0.41

significance	***	p > 0.00	**	p > 0.05	*	p > 0.1		

Table 1.4 T-test values between the muscles examined, compared at each depth.

	9mm.	8mm.	7mm.	6mm.	5mm.	4mm.	3mm.	2mm.	1mm.
	1.50	1.52	0.25	0.25	0.83	2.78	0.22	1.34	0.53

Fibres measured in the 1 - 8mm. depth ranges have mean values ranging from 42.03 - 53.52 μ m whilst those at 9mm. depth have mean values of only 20.02 μ m. There is an obvious statistical difference in that the fibres at 9mm. depth have much smaller standard deviations. This contrasts the large standard deviations in the other depth groups which is a reflection of the mosaic nature of the latter.

Table 1.1 shows that the distribution curves for the 1 - 8mm. groups are left skewed which implies that the more extreme values are to the right (i.e. the larger fibre diameters).

Table 1.2 shows t-values computed between the fibres sampled at 9mm. and the other depths in the muscle. The mean value is significantly different in all cases. In addition the t-values between adjacent depths in the muscle are shown in Table 1.3. Only 9 and 8mm. depths, as expected, are significantly different.

In order to determine if any differences occur between the two muscles sampled due to processing, the t-values displayed in Table 1.4. were calculated. Only at 4mm. was any difference noted, due to a 50% larger mean value in Muscle 2.

Comparison with Cust's (1975) raw data on the fibre sizes of the Rainbow trout revealed no significant differences between the means for mosaic fibres though her red fibres were significantly larger.

1.7 Myofibrillar Array

In sections stained with Luxol Fast Blue/Cresyl Toluidine Blue, it can be seen that, in the small fibres at 9mm. in the muscle, there is a typical 'Felderstruktur' arrangement of myofibrils. The fibrils are packed irregularly in groups with separation between the groups. Sometimes a darkly staining outer border is present (Plates I.5 - .6). The fibres elsewhere in the

muscle have typical 'Fibrillenstruktur' in which the Fibrils are regularly spaced, close packed and cylindrical. Those of the periphery are elongated and flattened radially.

1.8 Nuclear position

In transverse sections of adductor mandibulae muscles examined, few nuclei were seen in a position other than peripheral. In sections stained with Cresyl Violet which stains only nucleii, the foregoing conclusions were confirmed. In contrast, sections of extraocular muscles commonly had distributed nucleii in the mosaic fibres but not in the small (red) fibres. Generally, these nucleii occupied a central position, a few being eccentrically located.

1.9 Fibre Shape

In the majority of Brown and Rainbow trout muscle sections there was a tendency for the small fibres to be rounded in outline whilst those of the mosaic muscle were polyhedral. It is obvious, however, from Fig.1.1 , that within the mosaic fibres, there may be areas of large diameter fibres which possess rounded outlines. In sections of extra-ocular muscles taken for comparison, both rounded and polyhedral mosaic fibres coexist in mosaic muscle but frequently they appear in separate zones. Similar observations were made in Cust's material.

1.10 Vascularisation

Because of the perfusion method used to fix most of the histological specimens in this investigation, it was difficult to distinguish the location of capillaries owing to the clearing out of the erythrocytes in this process. In a Rainbow Trout muscle, not

fixed by perfusion, some counts were made of the numbers of capillaries visible and the capillary/fibre ratio calculated as in Table 1.5 below.

Table 1.5 Capillary/Fibre ratios for Rainbow Trout Muscle.

Mosaic Muscle	Red Muscle
0.592	0.875
0.640	1.166
0.545	0.761
0.521	1.333
0.558	1.001
0.692	-
0.558	-
0.826	-
Mean = 0.616	Mean = 1.027
S.D. = 0.101	S.D. = 0.203

The computed t-value shows that the means of these two groups are significantly different ($t = 4.21$, $p < 0.01$). The capillary/fibre ratio in the red muscle is almost twice as great as that of the mosaic muscle.

It is also evident from the photographs and tracings of the muscle fibres (Plates I.1 - .4 , Fig . 1.1) that, because of the fascicular arrangement, the red fibres are in close proximity to the larger arteriolar blood vessels which separate them, implying that they are nearer to higher pressure blood than the mosaic fibres.

In the muscles which were injected with latex pigment and subsequently cleared, the very dense vascularisation of the red fibres is obvious. In Plates I.7 - .8 , many fine capillaries can be seen winding around the length of red fibres whereas those of the mosaic muscle have not such a dense supply.

1.11 Gross anatomy of the Vth Cranial nerve

The large common trunk comprising the Vth and VIIth nerves emerges from the cranium and traverses the postero-lateral margin of the orbit in a ventrad direction. (Plate I.9) It divides several times, branches of the Vth going anteriorly (maxillary branch), VIIth going laterally and anteriorly (external mandibular and inner buccal branches, respectively). The mandibular branch of the Vth passes ventrally beneath the orbital bones and courses down the posterior border of the m. adductor mandibulae (close to the surface), entering the muscle in the region of the tendon insertion, after turning anteriorly. It was thought by Cust (1975) and Tytler et al. (1975) that this was the motor nerve to the adductor mandibulae, solely. Sections of the nerve, however, taken proximally showed that the nerve there possessed distinct ensheathed bundles (Plates I.10 - 11) which are absent in distal sections. Careful dissection revealed that shortly after leaving the orbit, these nerve bundles leave the main trunk and enter the inner medial surface of the muscle (i.e. in the region containing red fibres). Arteries accompany these bundles and can clearly be seen in the specimens injected with latex (Plate I.12). Sections through the muscle at this level often show these branches (Plate I.13) and sections of distal main nerve trunk in which the surrounding connective tissue is preserved reveal that several small nerve trunks accompany the main trunk of the Vth nerve, but their destinations are not known.

1.12 Fibre sizes

It was obvious that owing to the inadequate condition of the micro-tome available, the sections of nerve produced fell a long way short of the quality of those produced by Hudson (1969) or Coggeshall et al.(1978). Because of the poor quality of the sections this aspect will not be considered in very great detail.

The Vth nerve taken peripherally consists of two or more divisions separated by internal septa. The main division contains mixed myelinated/unmyelinated axons with a wide range of axonal diameter (<4-20 μ m). The smaller divisions separated by septa contain larger myelinated axons of range 9-20 μ m.

Comparisons were effected between mean axonal diameters in the sections shown in plates I.10-11. T-tests were made between the mean axonal diameters in the main division, outlined above, and those of the smaller divisions and are displayed in Table 1.6 below.

Table 1.6 T-test between mean axonal diameters in μ m

(1) = Main division, (2) = Subsidiary divisions.

Nerve No.	Mean (1)	S.E. (1)	Mean (2)	S.E.(2)	t	Sig(t)
1	9.72	± 0.54	11.96	0.62	2.73	**
2	8.87	± 0.87	13.32	0.69	3.96	***

Thus it is seen that the smaller divisions have larger mean diameters than the main division.

Skewness and kurtosis values indicated that the distributions in both divisions are positive skewed and show various degrees of leptokurtosis for which the former may be due to excessive shrinkage during processing.

Section I : Anatomy and Histology

DISCUSSION

1.13 Gross Anatomy

There are no significant differences in the anatomy of the Brown Trout

(Salmo trutta) when compared with that of the Rainbow Trout (Salmo gairdnerii) (Cust 1975, Tytler et al. 1975) and the King Salmon (Oncorhynchus tshawytscha) (Greene and Green, 1914). The salmonid a. mandibulae is an uncomplicated muscle system. In most other Teleost species, the adductor mandibulae becomes subdivided with insertions onto e.g. the maxillary bones (Schaeffer and Rosen, 1961, Hughes and Ballintijn 1968, Osse 1969, Ballintijn 1969, Elshoud-Oldenhove and Osse, 1976). The success of the 'advanced' perciform fish in a variety of roles is due to the protrusible round-mouth apparatus (Alexander, 1974, Schaeffer and Rosen 1961). It is difficult to know if the salmonid jaw apparatus is a primitive or specialized structure; it might be considered specialized in that its gape exposes a long extent of teeth - bearing surfaces on the upper and lower jaws which is ideal for a predatory fish. I have not had the opportunity to examine the histology of a primitive species such as the herring, (Clupea harengus) or a very advanced one such as the mackerel (Scomber scombrus). This would be very instructive as these species like the salmonids are active predators with a facility for grazing on zoo-plankton. Schaeffer and Rosen (1961) suggest that in the advanced Carangidae for example, the secondary return to a non-protrusible mouth is an adaptation to a predatory life style. Clearly, it would be interesting to see if this is reflected in the muscle system. Ballintijn (1969) concluded the unitary adductor mandibulae of the trout was an adaptation to the seizure of mobile prey. Ballintijn and Hughes (1965) in their EMG studies of muscle coordination in the trout concluded in one line of the m. adductor mandibulae that: 'It shuts the mouth'. In the Selachii, in contrast to the Teleostii, all species possess a unitary adductor mandibulae. The mandibular apparatus is completely different from the Teleosts allowing protrusion of the whole apparatus (Alexander, 1974).

The Selachian adductor mandibulae might be considered an adaptation to the seizure and in particular, the shearing off, of prey.

The large size of the adductor mandibulae in the Selachii endows most of them with a bite-force unparalleled in the Teleosts. Snodgrass and Gilbert (1967) showed with their 'gnathodynamometer' that the forces between opposed teeth of Lemon Sharks (Negaprion brevirostris) could be as high as $30\text{kg}/\text{mm}^2$. There is common ground in comparing the selachian and salmonid mandibular structures to the perciform variety: in the former, practically all the contraction force produced by the adductor mandibulae can be converted into torque and there are fewer frictional surfaces involved. Because of the insertion of the muscle ahead of the fulcrum onto the coronoid process teleosts can gain leverage because this is in effect a 'bent lever'. Schaeffer and Rosen (1961) calculate that the progressive increase in the height of the coronoid process over the ancestral condition would give a geometric increase in torque available. Individuals of Sarotherodon mossambicus can remove several scales in one bite from one another yet a human equipped with forceps finds this difficult (own unpublished observations). This is an illustrative example of the previously mentioned mechanical advantage in Teleosts. A further interesting comparison might be made with Teleosts which can sever pieces from their prey e.g. Sphyraena and Serrasalmo.

The function of the small mandibular branch of the trout adductor mandibulae is not known and apart from Greene and Green (1914), who were admittedly working on large salmon in which it would be more obvious, its existence in the trout has largely been ignored. In perciform fish, a branch of the more complex adductor mandibulae (A_w) occurs in a homologous position to that of the salmonid mandibular branch (Osse, 1969; Elshoud-Oldenhove and Osse, 1976).

Osse (1969) only detected activity in this muscle during feeding and coughing. This suggests that the salmonid cephalic branch might be a 'spurt' muscle (Basmajian, 1974) and the mandibular branch might function as a 'shunt' muscle by whose co-contraction stabilization of the

quadrat-mandibular joint is achieved during high rotational forces.

1.14 Fibre sizes and types

The analysis of fibre sizes in the Brown Trout adductor mandibulae corroborates Cust's (1975) findings in the Rainbow Trout. In a muscle of some 9 mm. thickness, the fibres at 9 mm. depth have totally different characteristics from the remainder of the muscle. The distribution of fibre sizes in the mosaic portion parallels that found by Cust (1975) for adductor mandibulae and Johnston et al. (1975) for myotomal muscle in Rainbow Trout. There is a wide range of 10-100 μm in diameter with a left skewed distribution and mean diameter of around 50 μm . The slight shift in mean diameter to 40 μm at 8 mm. depth may indicate the presence of 'pink' fibres which appear to be intermediate in colour, position and diameter to the white or mosaic fibres (Bone 1966, Johnston et al. 1975, Patterson et al. 1975, Mosse and Hudson 1977).

It is a truism that, apart from unusual exceptions, in fish and other vertebrates, the presence of small diameter muscle fibres indicates a requirement for some form of sustained activity. Mosse and Hudson (1977) state that red fibres alone in the fish myotome are associated with the ability to swim continually, without fatigue.

Examples of exceptions include the Hippocampus fin muscle (Bergman, 1964) in which a rapid fanning action of the dorsal fin is required for its peculiar mode of locomotion; the bat cricothyroideus muscle (Revel, 1962) which is used for the mechanical production of the bat's ultrasonic echo-location system. These are examples of morphologically 'red' muscles with paradoxical 'fast' mechanical properties.

Although no histochemistry was carried out here, it is safe to assume that the red fibres seen in the Brown Trout adductor mandibulae are equivalent to those seen in the Rainbow Trout by Cust (1975) e.g. high SDH, lipid, glycogen etc. The high intra - and extracellular lipid content

(Greene 1912) is evident on dissection as it exudes from the transected ends of the red fibres. The high myoglobin content of the red portion of the adductor mandibulae has been mentioned previously (see Introduction, Section I).

The myofibrillar array types seen in the red and mosaic fibres would appear to be an acceptable criterion for distinguishing the two types in T.S. (Kruger, 1950, Kruger and Gunther, 1955, Baretts, 1961, Nakajima, 1969). The peripheral ribbon-like myofibrils (Nakajima, 1969) were also found in the mosaic but not the red fibres in the Brown Trout adductor mandibulae. The parameters of nuclear position and fibre shape were inconclusive and, in fish, little attention has been paid to either, save for Bone (1966), Roberts (1969) and Greene (1913). Further detailed study would be necessary to validate any premises in this context.

In the Selachian Scyliorhinus canicula, Hughes and Ballintijn (1965) noted the presence of a red fibre tract in the adductor mandibulae. They noted from EMG recordings functional division into portions active during respiration only, feeding only etc. Cust (1975) suggested that a similar functional differentiation might be present in the Rainbow Trout adductor mandibulae.

There is an obvious requirement that a Trout with its fairly rapid ventilation rate would need to sustain the ventilatory flow over its gills when not actively swimming. Cameron and Cech (1970) estimate that the energy cost of ventilation in the Mullet, Mugil cephalus is between 5 - 15% of the total metabolism at rest possibly decreasing with activity. This is due to the ram ventilation effect (Roberts, 1974). It is, therefore, possible that a fish requires a powerful pumping apparatus not for activity, but paradoxically, for the converse. There is a parallel situation in that the fish carries around some 70% of its body weight in 'Fast' muscle which it utilises only at the higher cruising speeds or in emergencies (Rayner and Keenan, 1962, Hudson, 1973). There is a similar ratio of mosaic fibres in

trout adductor mandibulae (Tytler et al. 1975) and it is concluded that the sustained effort for which the small amount of red fibres present are required must be that of active ventilation. Conversely the mosaic portion must be concerned with the non-sustained activities of feeding and coughing. In the mammal, it has been shown that the histological character of the masseter muscle depends on the feeding method (Hiraiwa, 1978). The masticatory muscles of the rat were phasic, those of the rabbit were mixed whilst those of the cow were wholly 'red'. The functional and adaptative significance of this should be obvious. It would be an interesting future project to study such differences in a wide range of ecological types of fish.

Finally, there has been shown to be a denser blood supply to the red fibres of the trout adductor mandibulae. The only worker to examine the blood flow in this muscle (Cameron, 1974) considered in the salmonid Thymallus arcticus, that the adductor mandibulae was a homogenous muscle. Consequently his results 'indicate a no greater than ordinary muscle flow'. In his previous work (Cameron and Cech, 1970) he did note the presence of red fibres in cranial muscles, other than the adductor mandibulae. The capillary density of red muscle was found to be about 2.5 times that of white muscle. In this investigation values of $< 2.0 \times$ were found although no allowance was made for differential shrinkage in processing (Watzka, 1939).

In the study on Thymallus using radio-labelled microspheres, Cameron (1974) found that the actual flow of blood was some ten times greater in somatic red muscle. Presumably this can be extrapolated to the red fibres of the trout adductor mandibulae.

1.16. The Vth cranial nerve.

Unfortunately, little is known of the comparative neurology of fishes. Most of the literature available dates from the turn of the century or even earlier, possibly resulting from the fact that zoological anatomy has become unfashionable. Saunders and Manton (1969) do not show the innervation of

the adductor mandibulae of the Brown Trout in any great detail. Luiten (1975) has carried out an intensive neurological study of the central projections of some cranial nerves in the Carp, Cyprinus carpio, using Wallerian methods. He was able to trace the course of the fibres into two main motor nuclei, rostral and caudal, situated in the ventral brainstem below the cerebellum. Also noted were neural connections between the Vth nerve and the lobus facialis which suggests that care should be exercised in stimulation studies.

Meijer (1975) showed in the Carp by indirect stimulation, that the Vth nerve is motor to the adductor mandibulae 3. (one of the main adductors of the mandible (Ballintijn et al. 1972)). Stimulation of the VIIth nerve had no effect on the adductor mandibulae. Unfortunately, Meijer did not pursue the detailed anatomical connections of the nerves.

The fibre sizes measured in the nerve sections may not be truly representative as Williams and Wendell-Smith (1960) have shown that relatively large errors may be introduced due to photographic and histological technique when measurement is contemplated.

In the peripheral spinal nerves of the Stingray, Dasyatis sabina, Coggeshall et al. (1978) found that the mean axonal diameter of fibres originating from the dorsal root (sensory) were 4.2 - 5µm in diameter whilst those of the ventral motor root were of 9.2 - 10.5µm mean diameter. Roberts (1969 b:) found that white muscles in the dogfish were innervated by axons in the 10-14µm class whilst in the Sea Scorpion, Cottus scorpius, Hudson (1969) found a similar picture for white muscles. It may be that the division into bundles seen in the Brown Trout nerve V corresponds to the dorsal and ventral roots of the nerve. In spinal nerves there is a septal division with the ventral (motor) portion containing mainly larger diameter axons, (Barets, 1961, Hudson, 1969, Roberts 1969b, Coggeshall et al. 1978). The fibres in group (1) in Table 1.6 have larger mean diameters than those in the literature for dorsal root axons; this may be due to the

poor histological practice making the smaller fibres indistinguishable and therefore weighting the sample mean in favour of the larger diameter ones.

There would not necessarily have to be large numbers of small diameter axons in the Vth nerve in order to innervate the red fibre portion of the adductor mandibulae. The latter is very thin and is not extensive and so the small number of red muscle fibres could easily be served by the pre-terminal branching of relatively few small-diameter motorneurons (Fernand and Young, 1951). Hudson (1969) shows in the ventral root fibres of the Sea Scorpion that discrete bundles of small diameter myelinated axons occur. This was not evident in the Brown Trout Vth nerve though it might be concealed by poor technique. There are, however, some bundles of larger diameter fibres in the, presumed, dorsal root fibres.

There is no doubt that branches formed by division of the perineurium do leave the Vth nerve and that these are probably the origin of the fibres seen entering the inner surface of the adductor mandibulae. At this stage it is only possible to state that these branches may contain the innervation of the red fibre portion but this would require electrophysiological evidence to confirm it. Certainly, sections of the Vth nerve taken more distally show no such subdivisions.

1.17 Innervation pattern

Unfortunately, none of the silver-based or methylene blue stains were successful despite numerous attempts. Other workers in this laboratory have found similar difficulties. Bone (1972) states that nerve histology is 'something of an art' and undoubtedly its successful execution is, like intracellular recording, easier if a local experienced 'practitioner' is available. The failure of the methylene blue method is interesting as it

is probably due to the purity of the dye used (I.H. McCrae, pers. comm.). Burnstock (1959) found that his successful results came from a crude dye of 1913 vintage.

The investigation of the innervation pattern of the trout adductor mandibulae would be a worthwhile future research project as little work has been carried out on non-myotomal muscles.

Section II : Muscle PhysiologyINTRODUCTION

As mentioned in Section I, Cust (1975) demonstrated the presence of red fibres in the trout adductor mandibulae. By indirect stimulation methods, she showed that the contractile properties of the red and mosaic portions were different. Separated red fibres produced tonic responses without fatigue whereas the mosaic portion produced twitch responses which fatigued readily. By consideration of twitch/tetanus ratios, she concluded that the red portion had the characteristics of 'slow' muscle and the mosaic portion that of 'fast' muscle. Tytler et al. (1975) also showed, using direct stimulation of the isolated whole adductor mandibulae, that by overstimulating the mosaic fibres until they fatigued out, the distinctive response of the red fibres could be separated. Anderson et al. (1967) observed a similar phenomenon in myotomal muscle of the Atlantic Hagfish, Myxine glutinosa. The theory has been advanced in ~~this thesis~~ that the red fibres of the adductor mandibulae in trout function during ventilation and the mosaic portion functions during feeding to supply the rapid closure during prey seizure.

Cameron and Cech (1970) and Roberts (1975) have remarked upon the presence of red fibres in the cranial muscles of Teleosts and Selachians, though they have never stated explicitly the functional significance of these observations. Roberts in particular, has shown that they occur, in the selachian adductor mandibulae, in a homologous position to that of the trout. Furthermore, as mentioned in Section I, Hughes and Ballintijn (1965) have also demonstrated differential EMG activity in parts of the adductor mandibulae of Scyliorhinus canicula.

In Fish, generally, red fibres have a different neuromuscular

excitation-contraction coupling from white fibres. In the former no propagated action potential results and the synaptic event is a junctional potential of some 100 mS duration (Takeuchi, 1959; Baretts, 1961; Hudson, 1969; Hidaka and Toida, 1969). In white muscles overshooting potentials of <10mS duration, which are propagated, result upon excitation, though some phylogenetic differences may occur. It is, therefore, highly probable that differences will exist between the EMGs of red and white muscles, in terms of the duration of their motor unit potentials.

Differences are to be expected, a priori, due to the volume conduction effect (Buchta et al. 1957; Kosarov and Gydikov, 1974,) and also due to the electrochemical effect at the electrode (Ferris and Stewart, 1974). It is likely that fine-wire electrodes will also have a substantially smaller recording territory (Basmajian, 1974) than the once-traditional concentric needle electrode (Dedo and Dunker, 1965). It was, therefore, intended to investigate differential EMG activity in the red and mosaic portions of the trout adductor mandibulae during voluntary and enforced activity, which, because of the requirements of the biotelemetry program, would include feeding. Furthermore, the EMG lends itself easily to frequency analysis techniques (Lenman and Ritchie, 1970) for which facilities were made available locally at Ninewells Hospital, Dundee courtesy of Dr J.A.R. Lenman. During the Biotelemetry program described in Section III it became obvious that anaesthesia altered the adductor mandibulae EMG and this was lowering the efficiency of the system. This and the frequency analysis were conveniently investigated by the same methods.

Apart from differences to be expected from (1) the number of fibres (2) temporal patterning of motorneuron discharge and (3) size of motorneurons (Basmajian, 1974; Hammond and Ridge, 1978), it is probable that the EMG produced by red

muscle fibres will have a larger percentage of long duration potentials and therefore a different frequency spectrum. Surprisingly, apart from the differences in motor unit potential duration resulting from (1) above, which must be recognised by the clinical electromyographer (Basmajian, 1974; Lenman and Ritchie, 1970), this seems to have been overlooked by previous workers. In addition the EMG response of a 'slow' muscle can be distinguished from that of 'fast' muscle. This has been shown in mammals by Bach-y-Rita and Ito (1966); Browne (1976), in Amphibia by Tasaki and Tsukagoshi (1943), Kuffler and Vaughan Williams (1953) and in Fish (Barets, 1961). This is useful as it has often been remarked that it is difficult to persuade Fish to use their white or mosaic muscles. There is a question to resolve about the motor innervation of the m. adductor mandibulae in the Trout; Cust (1975) assumed that the large branch of nerve V which penetrates the ventral aspect of the muscle is motor. Roberts (1975) has shown in Galeoid sharks that there is a motor branch of the Vth nerve to the inner medial portion of the muscle, homologous with that found in Section I here. The homologue of the large branch in the Trout, in Sharks is Maxillary and furthermore Roberts has shown that it is wholly sensory and that the branch to the adductor mandibulae is only 10% motor.

Finally, very little is known of the electrical activity of the alimentary canal of lower vertebrates (Berger and Dahl, 1974; Prosser, 1974). Burnstock (1958) and Ito and Kuriyama (1971) have described regular spontaneous mechanical and electrical activity in fish guts and given that the anatomy is similar but not absolutely equivalent to that of the mammal (Burnstock 1959), it would be reasonable to expect pacesetting potentials in the trout. These might be an alternative to the adductor mandibulae EMG as the input signal for the transmitter used in Section III. Wienbeck and

Janssen (1974) have shown in the cat that feeding rapidly causes propagated spiking activity in contrast to the resting slow-wave activity. This would signal groups, though not individual acts, of feeding activity.

Section II : Muscle PhysiologyMATERIALS AND METHODS2.1 Animals

Brown (Salmo trutta L.) or Rainbow Trout (Salmo gairdneri, Richardson) were obtained from Howietown and Northern Fisheries Ltd., Bannockburn, or from Loch Leven, Kinross by seine netting. They were held in either 2000 or 800 litre circular tanks in running non-toxic tap water which varied in temperature seasonally from 3⁰C to 20⁰C. The fish were fed daily on standard trout pellets (Edward Baker Ltd).

2.2 Anaesthesia and Surgical Methods

All experiments were conducted in a special room which contained a combined operating/anaesthetic table and was air conditioned to prevent thermal stress in fish held out of water for experiments. The average air temperature in the room was 10⁰C and it was also provided with non-toxic tap water from the main aquarium supply. (Fig. 2.1).

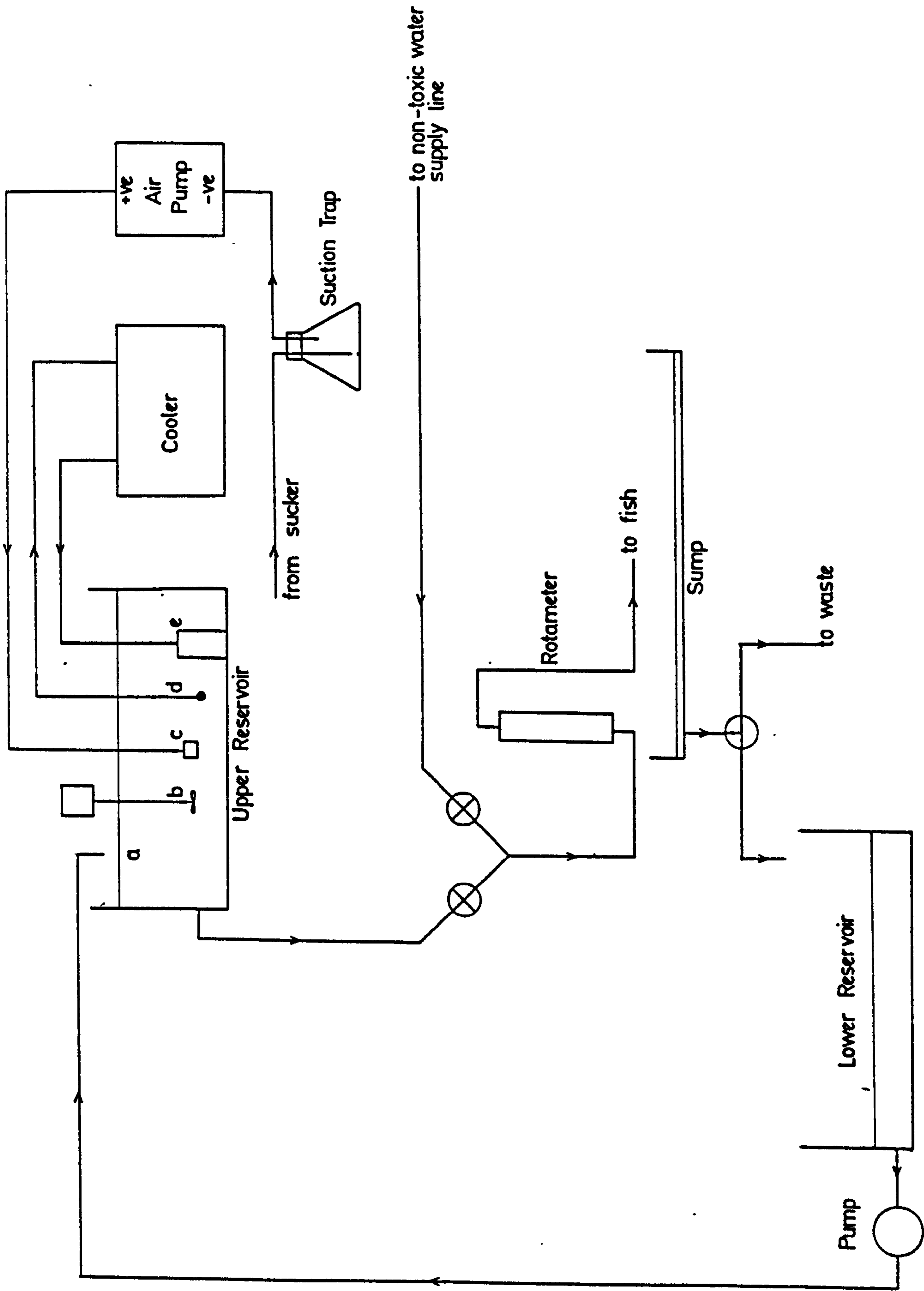
The anaesthetic table was similar to the design of Smith and Bell (1967). Anaesthetic solutions were stored in a 30 litre reservoir tank and were delivered to the fish by gravity feed. The solutions were continuously stirred by a propeller type stirrer (Grant Instruments Ltd), aerated with two airstones connected to a dual piston type air pump (Hyflo Pumps Ltd) and cooled with an immersion cooler (Frigidaire Ltd) which was thermostatically regulated by a contact thermometer (Heto Ltd) placed in an anaesthetic reservoir. This was set so that the temperature of the anesthetic was always maintained at the same temperature at which the fish were being held. This avoids the effects of sub-lethal heat stress (Wedermeyer 1973).

Fig. 2.1

Schematic diagram of the anaesthetic apparatus used in both the muscle physiology and biotelemetry work.

The upper reservoir contains (a) an inlet from the recirculation pump, (b) a stirrer, (c) an aerator, (d) a contact thermometer and (e) a diode cooler.

Note that during anaesthesia the taps are set so that spent anaesthetic is collected in the sump, and drained to the lower reservoir, from where it is pumped back into the upper reservoir. During recovery procedures, the fish is perfused with clean, non-toxic water from the copper-free supply line, the sump outflow being then directed to waste. The system is usually recharged with fresh anaesthetic after 2-3 days usage. Its capacity is 30 litres and the normal flow rate in use is 1.45 l/min^{-1} .



The fish were held in a sponge holder which was lined with a disposable cloth (Kimwipes - Bowater Scott), these do not disintegrate in water and do not damage the fishes delicate epidermis or mucus coats. The anaesthetic was perfused over the gills per os via a mouthpiece and the used solution collected in a sump which drained into a lower 30 litre reservoir tank from which it was periodically returned to the upper reservoir by a centrifugal pump (Charles Austen Ltd). The anaesthetic drugs are specified for each experiment, later. Alternatively, the fish could be supplied with non-toxic water from the aquarium supply lines. The flow rate of the water or anaesthetic was controlled by two taps and measured by a rotameter (G.A. Platen Ltd). A suction apparatus for the removal of blood and body fluids was also provided and was driven from the suction inlets of the aeration pump.

2.3 Recording Equipment

A variety of equipment was used in the experiments described below. Most of the experiments were performed using the facilities provided by a "CEPTU" unit (Epil Products Ltd). This provides two channels of differential or single ended preamplification, Oscilloscope display and audio output. In addition, a twin channel stimulator is included. Outputs taken from the preamplifiers were recorded on either George Washington 400 MD2 (Searle Bioscience) or Devices MX212 pen recorders. Occasionally, recordings were made directly using a Devices M2 pen recorder with DC8 and AC7 preamplifiers. In some cases signals were displayed on a twin beam storage oscilloscope (Telequipment DM53A) and recorded with a 35mm camera. Some experimental data was stored on a magnetic tape, for later analysis, using Thermionic T3000 or Racal "Store 7" F.M. instrumentation recorders.

Experimental Protocols

2.4 Investigations into the trout adductor mandibulae electromyogram during ventilation.

2.4.1 A series of experiments was performed upon both Brown and Rainbow Trout in order to determine the activity recorded in the muscle at different depths, during ventilation. The fish were anaesthetized with either MS-222 (Sandoz) 50mg/litre or Benzocaine 30mg/litre. Anaesthesia was maintained with the fish held firmly in the fish-holder. The skin overlying the m. adductor mandibulae was carefully removed and the surface of the exposed ~~fascial~~ sheath covered in paraffin to avoid dessication or contamination with anaesthetic solution. A stainless steel hypodermic needle inserted into the fish's tail served as the earth electrode. The recording probe consisted of a pair of 125 μ m diameter insulated stainless steel wires (Trimel-Johnson, Matthey Ltd), mounted in a brass tube with epoxy. The tube was carried in the holder of a manipulator (Prior Ltd) and the manipulator itself was earthed to a common earthing point. The tips of the electrode pair were bared for a distance of 1mm and electrolytically sharpened in 0.1N HCl with a 6V A.C. current. (Plate II)

Recordings were made at a ~~HF~~ top cut of 1kHz and AC coupled with a time constant of 50mS. By advancing the probe into the muscle in discrete steps, recordings of the EMG at varying depth were made until the probe reached maximum depth in the muscle as indicated by its touching the hyomandibula bone. In some cases the tension produced by the adductor mandibulae was monitored by passing a suture through the mandibular apex and attaching it to either George Washington D2 or Devices 4151 Isometric Strain Gauge Transducers.

2.4.2 In a second series of experiments, fish were prepared as in 1) above. A series of transects made with the probe and each insertion was noted on the manipulator scales, which in turn were related to landmarks around the muscle. This was to enable a map of the area containing active units to be built up.

2.4.3 A comparative experiment similar to 1) was attempted on board RRS "Challenger" in sea area Rockall during Cruise 6B April 1976.

The living fish brought up in deep trawls are chiefly squaloid sharks. These have unitary adductor mandibulae muscles unlike most teleost species in the UK. As such they form an interesting comparison and are technically easier to work upon because of their large size and their durability.

Dissections performed upon the adductor mandibulae muscles of Centrophorus squamosus Schneider, Centroscymnus crepidater Bocage and Capello, Centroscymnus coelolepis, Bocage and Capello, Deania calcea Lowe and Galeus melastomus Rafinesque showed a consistently similar pattern of morphology of red fibre tracts. In one Centroscymnus coelolepis (Portugese Shark) an attempt was made to repeat the experiment 2.4.1, carried out on trout. A ♀ shark of 43kg weight which had been swimming around fairly well in a holding tank and which responded well to cutaneous stimulation was used. The animal was anaesthetized with Thiopentone sodium (Pentothal-Abbott) injected intraperitoneally at a dose rate of 10mg/kg approximately. This was sufficient to inhibit swimming activity but not ventilation and Thiopentone only possesses weak curariform actions in contrast to other barbiturates (Kraatz & Gluckman 1954, Thesleff 1956, Proctor & Weakly 1974, Oswald 1978). The fish was transferred to a tank in

the ship's laboratory and its gills irrigated per os by a tube carrying non-toxic seawater lines provided on the ship. The skin overlying the adductor mandibulae was removed but only two depth probes were carried out: one into the area in which red fibres were noted in previous dissections, the other well away from the red fibres. This was due to heavy sea conditions prevailing. Secondly, recordings were made from the white fibres of the adductor mandibulae during depolarisation induced by the injection of 200mg of Suxamethonium Chloride (Anectine-Burroughs, Wellcome) into the red lateral muscles. It had been hoped to compare the discharges simultaneously with those of the red fibres but this was thwarted by a pen failure on the recorder. (Plates II, 2, 4).

2.5 Chronic EMG recordings made in unanaesthetized Trout

In developing the ultrasonic transmitter system to be described in Chapter III, it was necessary to have recordings made from free-swimming trout during ventilation and feeding. Initially, the fish were implanted with punctate electrodes, inserted into the m. adductor mandibulae similar to those used by Sutterlin (1969) and Priede (1973). Lightweight twin screened wire (R-S Components Ltd) was used to connect the fish to the recording apparatus, via a suspended swivel and plug assembly. Initially, the electrode leads were attached to the fish as per Priede (1973) using simple subcutaneous sutures. Both the electrode design and the method of attaching the leads left much to be desired, especially if chronic use was contemplated. Consequently, a new type of electrode was evolved, to allow a certain amount of stereotaxy in placement and also superior stability over long periods of time. This was designated as the 'T' - electrode. It is fabricated by soldering 125µm insulated

stainless steel wire (Trimel-Johnson, Matthew) to ordinary lightweight twin screened wire. This was achieved by using either o-Phosphoric Acid as a flux or more lately with 'Arax' acid cored solder (Multicore Solders Ltd). The joint is washed in warm water to avoid the corrosion of the lead wires by the flux residues and dried. A small amount of quick-setting epoxy resin is then smeared over the joint (Rapid Araldite - CIBA). A short length of heat shrink sleeving (Raychem Ltd or R-S Components) with a half slot cut longitudinally in it is slipped over the joint after having first bent the electrode wire to a right angle. On applying heat, the sleeving shrinks, squeezing out the epoxy resin until it seals all the openings. The surplus resin is trimmed off and the electrode is ready for use after fifteen minutes in a warm oven. (Fig. 2.2)

In use the electrodes are implanted in the following manner: a small incision is made horizontally in the skin overlying the midline of the muscle. Two simple sutures are passed on either side of the incision, at right angles to it, using 5/0 silk sutures (Geck and Davis Ltd) on No. 20 $\frac{1}{2}$ curved triangular needles (Vicarey Davidson Ltd). The sutures are then formed into two loops on either side of the incision. The 'T' - electrode is next tried for length and trimmed to size so that it will be exactly the depth of the hyomandibula from the skin surface. The two sutures are then tightened around the cross bar of the 'T' and this drives the electrode firmly into place, with minimal trauma to the muscle. The exact location of the electrode can be seen in the radiograph (Plate II. 5). Normally the last 1mm of the electrode was bared but sometimes 4-5mm was bared which would additionally detect potentials originating in the mosaic part of the muscle. Alternatively, the electrode can be

Fig. 2.2

Sectional drawing through a "T-electrode". It is formed from heat-shrink sleeving (1) which has been shrunk onto the end of a length of fine stranded insulated wire (3). The angled piece of 125 μ m insulated stainless steel wire (5) forming the electrode proper is butt-joint soldered to the conductor (2) at the point (4). The electrode protrudes through a slot cut in the heat-shrink sleeving and is waterproofed with quick-setting epoxy resin (6).

Fig. 2.3

Drawing of the construction of a standard anchoring plate. It is fabricated from 1.5mm thick glass fibre PCB (1), with two peripheral copper tracks etched (2). The recording (differential) leads (3,5) are connected to the latter whilst the earth lead (4) is connected to the 100 μ m diameter stainless steel suture wire, which anchors the plate to the fish, through the holes drilled into it (7).

The ends of the electrodes are connected to the points labelled (8).

Fig. 2.4

Diagram of sites used for infiltration of the v^{th} nerve with Xylocaine.

FIG. 22

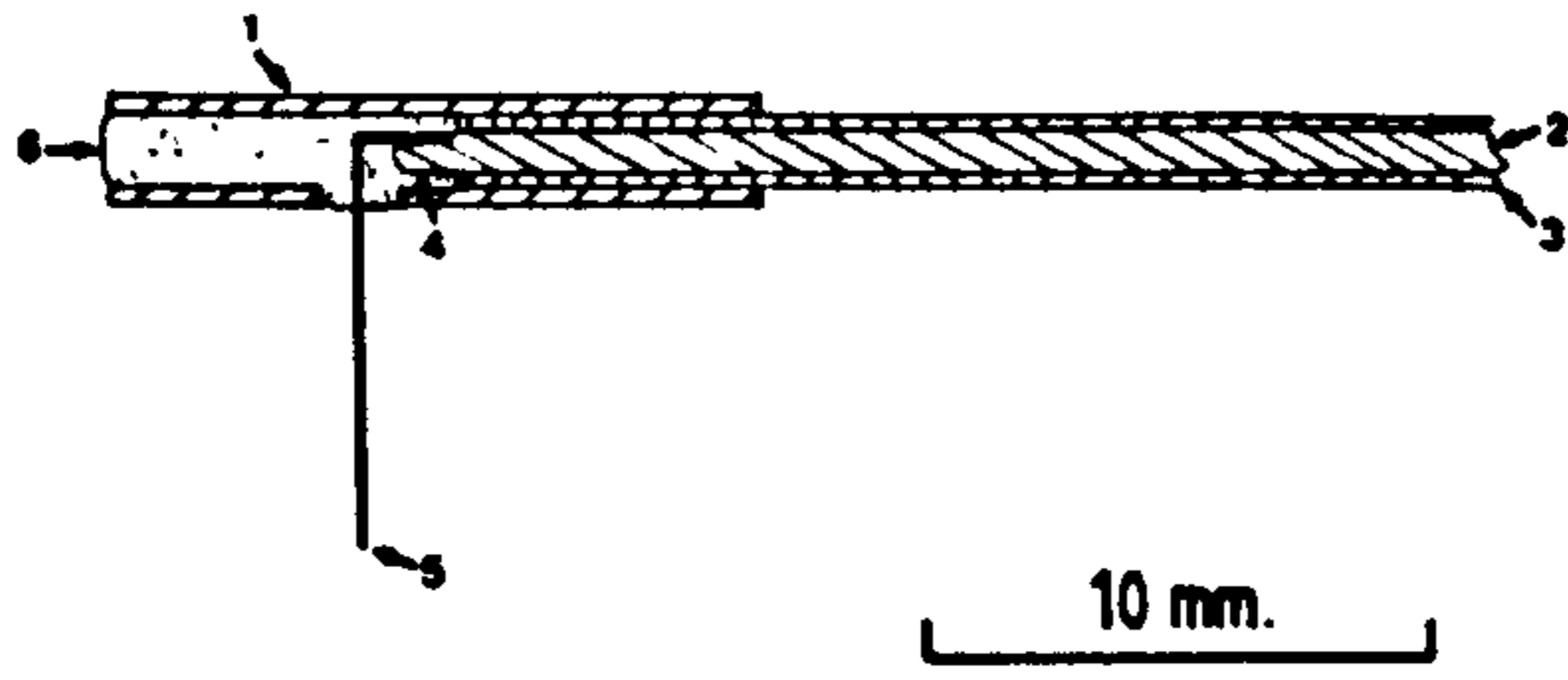


FIG 2.3

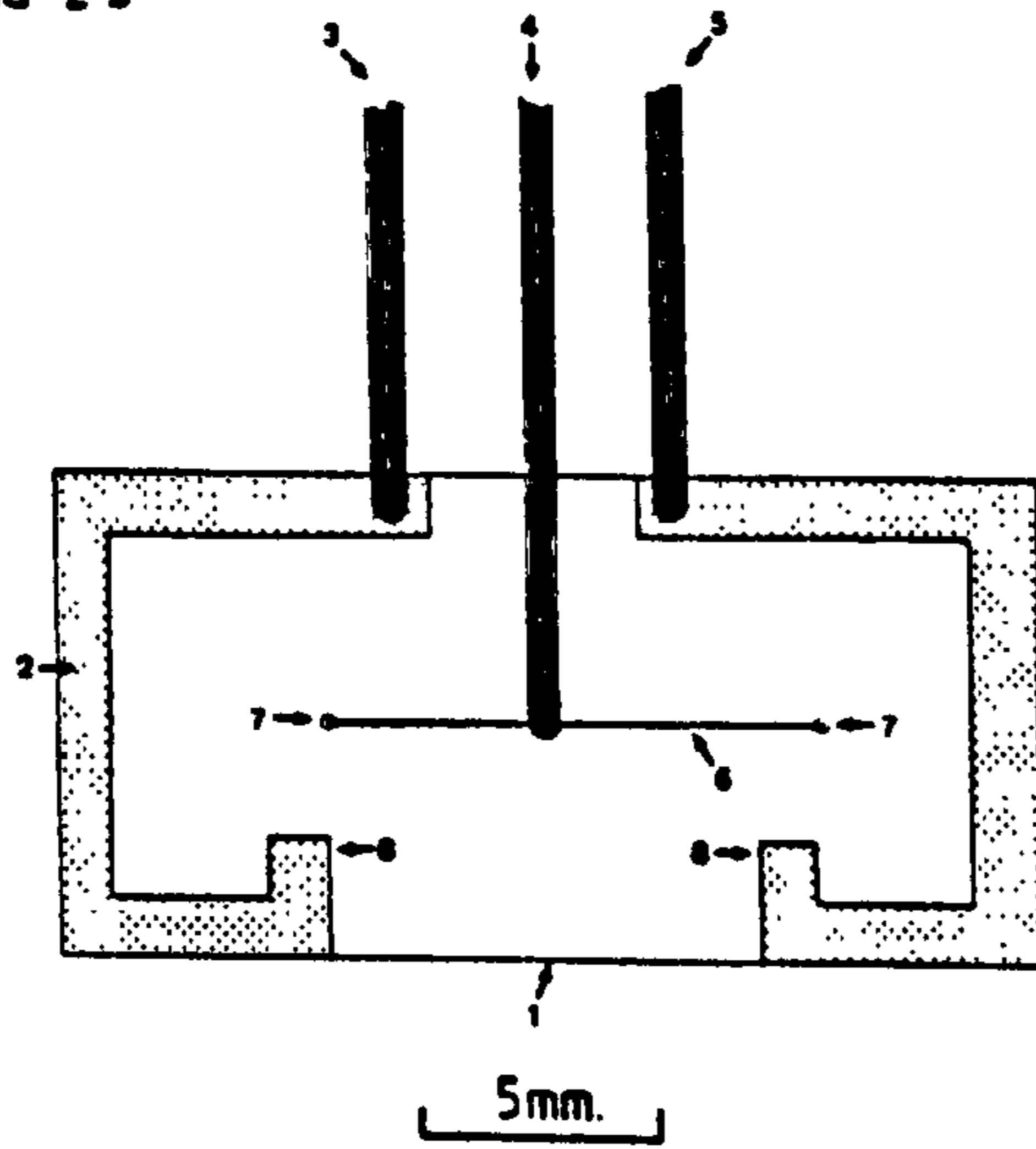
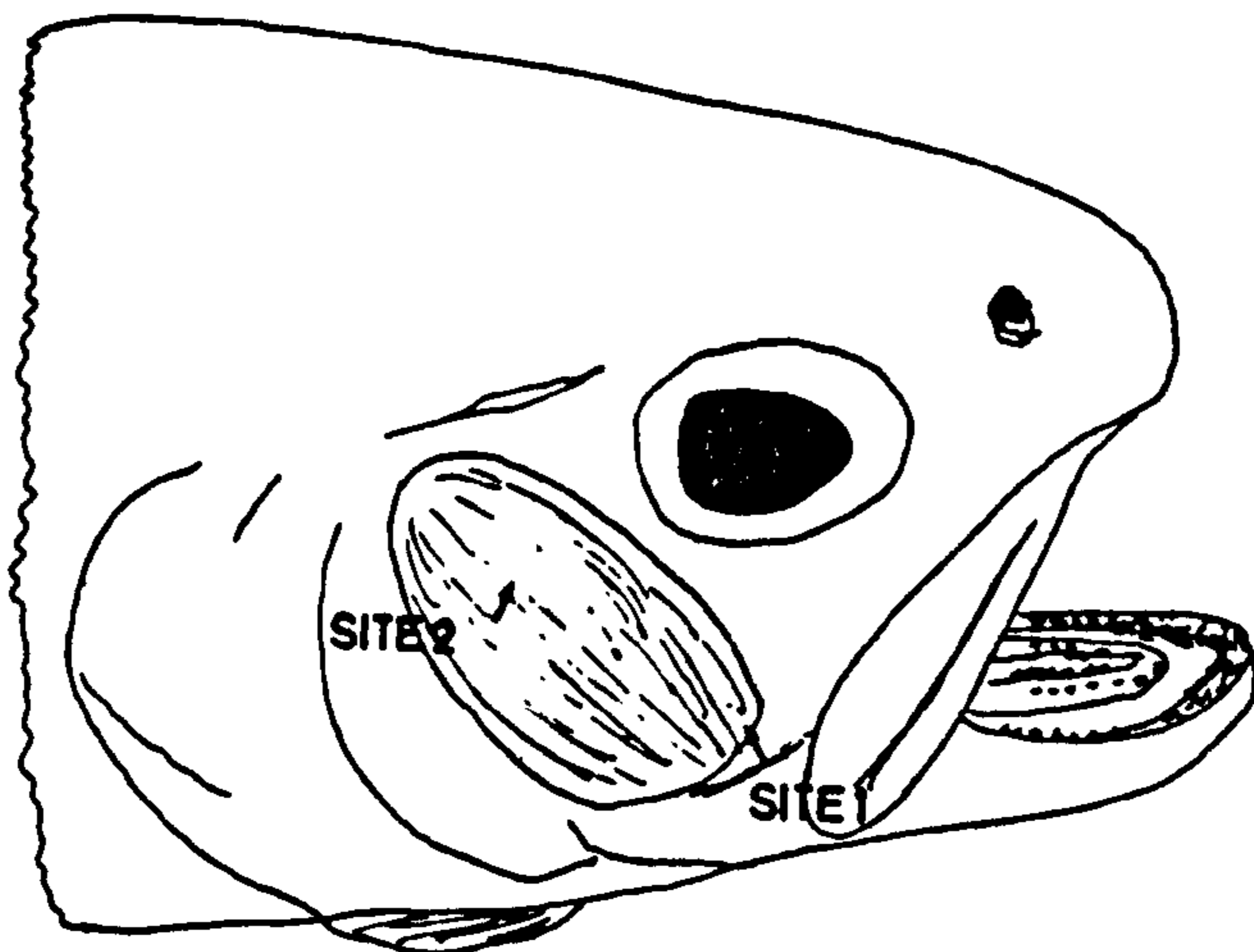


FIG 2.4



shortened to detect only potentials from the mosaic muscles. In some cases recordings were made using two electrode pairs in the adductor mandibulae. One pair being a 'T' electrode to record from the red fibres, the other a Basmajian and Stecko (1962) ~~standard~~ bipolar hook electrode, in order to record the mosaic fibres separately.

In all later experiments, the following method was adopted for the connection of the electrodes to the recording apparatus. A rectangular anchoring plate was made of glass-fibre printed circuit board, 1mm thick. As shown in Fig. 2.3, a pattern of conductive tracks is etched on board with Ferric Chloride. Stainless steel suture wire (100 μ m diameter, Johnson Matthey) is passed through two small holes in the middle of it and the earth wire to the recorder soldered to it, the amplifier input connections being soldered to the upper terminals. The proximal parts of the leads are coated in primer and the whole assembly encapsulated in either 'Impressil' quick setting silicone rubber (C.L. Attenborough Ltd) or in Dow Corning Marine Sealant. When hard, the lower pair of terminals were exposed with a scalpel. The entire assembly is affixed to the fish in an identical manner to Young et.al. (1972) except that 25 gauge needles are used and also because the backing plate on the opposite side of the fish is also encapsulated in silicone rubber, to make it more comfortable. The free ends of the suture wires were twisted together in the manner suggested by Kirk (1973) and it was found unnecessary to solder them. The free ends of the electrode leads were trimmed to size and soldered to the exposed terminals which were subsequently sealed with 'Impressil' before returning the fish to water.

This arrangement is very strong and the fish can be lifted out of the water with it without pulling out leads or the assembly. Later some experiments were performed using a miniaturised differential preamplifier similar to Basmajian and Hudson (1974) which is simply substituted for the bridle above. This gives a superior signal to noise ratio and avoids artifacts produced by the amplifier lead capacitances.

Fish "wired up" by the foregoing methods were allowed to recover in the 250 litre rectangular tanks before experimentation.

2.6 Effect of anaesthesia

Some fish prepared as above were kept anaesthetized for one hour to simulate the prolonged anaesthesia needed for affixing an ultrasonic transmitter. The EMG was recorded at intervals upto 24 hours.

2.7 Frequency Spectrum Analysis of the EMG

The frequency spectrum of the EMG from the Brown Trout m. adductor mandibulae, was studied both under conditions of light anaesthesia and under progressive recovery from anaesthesia. The experimental method was identical to that described in 25 above, except that fish were kept anaesthetized for a period of 60 minutes to simulate the normal period of time taken in preparing a fish for the ultrasonic transmitter. In addition, for comparative purposes recordings were made from lightly anaesthetized Pike (Esox lucius L.), Perch (Perca fluviatilis L.), Rainbow Trout (Salmo gairdneri, Richardson) and the marine Saithe or Coalfish (Pollachius virens L.). For the latter, it was of course necessary to use seawater in the anaesthetic system.

EMG's were recorded via the CEPTU preamplifiers and stored on a 'Store 7' F.M. Instrumentation Recorder (Racal Ltd) at a recording speed of 19cm/s). At this speed the frequency response of the recorder is substantially flat from DC-2.5kHz. The signal was processed by playing it through a 518C EEG Frequency Spectrum Analyser (University of Iowa, Bioengineering Research Facility). This has a normal band width of 0-30Hz in order to cover clinical EEG spectra. Its sampling rate is 128 samples/s and, therefore, it does not distort the lower frequencies. Duxbury et.al. (1975) point out that any frequency analyser must have a sample rate (F) at least twice as great as the highest frequency to be encountered. At too low a sample rate, the lower frequencies below the Nyquist frequency (F/2) are distorted by components of the upper frequencies. The fish EMG's were adapted to this instrument by playing it back at 0.93cm/s, giving an expansion factor of 8X and, therefore, effectively covering the band width of 0-240Hz. The analyser output was displayed on an SLE E18/16 EEG Recorder (Specialised Laboratory Equipment Ltd). The sample epoch adopted was 6s, at the end of which the analyser displays the frequency spectrum of the preceding one, as a histogram in 2Hz steps, representing voltage contribution. The information was extracted from the histograms manually as there were no facilities for automatic processing of the data available.

2.8 Indirectly stimulated adductor mandibulae preparations

In order to study, in vivo, the effects of stimulating the nerves motor to the trout adductor mandibulae, it was necessary to have a preparation which would have the required cardiovascular stability, but whose respiration would be so depressed that no efferent nerve traffic was detectable in the motor nerve. Using inhalational

anaesthesia with MS-222 (50mg/l), Benzocaine (30mg/l) or 2-Phenoxyethanol (385mg/l) it was difficult to obtain such conditions. Meijer (1975), working on indirectly stimulated preparations of Cyprinus carpio cranial muscles, found similar difficulties with MS-222 anaesthesia and obtained the best results by a combination of spinal section and cardiac puncture. He claimed that this method gave preparations viable in excess of three hours. Attempts to use this method on Rainbow Trout did not meet with success and other means were sought.

The most successful conditions were obtained using parenteral anaesthesia. Initial attempts using Pentobarbitone Sodium (Nembutal-Abbott) in a dose (48mg/kg I.P.) sufficient to inhibit spontaneous respiration, were largely unsuccessful. This was thought to be due to the peripheral neuromuscular blocking effects common to many of the barbiturates. These are well known in Thiopentone sodium (Kraatz and Gluckman 1954), Pentobarbitone Sodium (Seyama and Narahashi 1975) and Phenobarbitone (Proctor and Weakly 1976). The blood volume of fish is lower than that of most mammals, 3-5% of the bodyweight (Randall 1970), compared to 8% for the mammal (Hoar 1966). In a 250g trout injected with Pentobarbitone Sodium at 48mg/kg there would be an initial maximum concentration possible in the blood (assuming rapid partitioning into the blood) of 0.96mg/ml (5% blood volume assumed for trout, Stevens 1968). Wardle (1971) has shown the lymph volume of teleosts to be considerably larger than the blood so that the actual drug concentration in plasma must be much lower than this value. Even so, Thesleff (1956) has shown in the isolated frog sartorius muscle, that an external concentration (muscle-bath) of 200µg/ml could cause a 100% neuromuscular blockade. It is not, therefore, surprising that trout heavily anaesthetized with Pentobarbitone Sodium are unsuitable for experiments involving indirect stimulation of muscles.

The above difficulty was overcome by the use of the new synthetic steroidal anaesthetic combination of Alphaxalone/Alphadolone acetate (Saffan-Glaxo). This has no neuromuscular effects (R. Curtis pers.comm.) and has been shown to give excellent non-toxic anaesthesia in the trout for up to 6 hours (Oswald 1978 a)

In fish anaesthetized with Alphaxalone, they are kept alive by perfusing the gills with water alone. The drug exerts a marked vasodilatory action upon gill capillaries coupled with positive inotropic and chronotropic effects on the myocardium. This seems to ensure good oxygenation in contrast to the aminobenzoates and barbiturates which have vasoconstrictor and depressant actions (Oswald 1978 a)

In this series of experiments the Vth nerve was exposed by a different method. Following enucleation, haemostasis was effected with cotton wool pledgets soaked in Adrenaline (1:1000) solution. The nerve is carefully exposed as it crosses the postero-lateral border of the orbit. Rectangular stimuli were delivered to the nerve by a micro manipulator mounted pair of hook electrodes made of insulated stainless steel (200 μ m) with only the tips insulated. The orbit was filled with cold paraffin. Again, to prevent the effects of stimulating afferents, the nerve trunk was blocked at its entry to the orbit with Lignocaine Hydrochloride 2% (Xylocaine-Astra).

In all the foregoing methods the compound EMG from the muscle was recorded using differential D.C. coupled amplification. The potentials were detected either by two pairs of 125 μ m stainless steel electrodes, one pair implanted into the red fibres, the other pair implanted at a

depth of about 3mm into the mosaic fibres. Alternatively, by using a single pair of electrodes which had about 5mm of insulation removed and, therefore, capable of detecting red and mosaic potentials simultaneously though they would be separated temporally owing to differential conduction velocity in the nerves (Browne 1976). The tension produced by the muscle was monitored isometrically by attaching either George Washington D2 or Devices 4151 isometric strain gauge transducers by a suture passed through the strong connective tissue of the mandibular apex. (Plates II.6 - 7).

During these experiments, recordings were made of the compound EMG's and the tension records for different stimulus conditions. Some recordings were made by photographing the trace stored on a Telequipment DM53A Storage Oscilloscope. Where drugs were to be injected, the procedure described in section 2.10 was followed.

2.9 Innervation experiments

As described in section I, it was seen that the adductor mandibulae muscle of the trout has two anatomically distinct nerve supplies from nerve V. In order to elucidate which of these is responsible for innervating the red fibres, the following experiments were carried out. Brown and Rainbow Trout were lightly anaesthetized with Benzocaine 25mg/litre. Electrodes were implanted into the adductor mandibulae red fibres so that the ventilatory EMG was clearly distinguished. The jaw movement was monitored as previously. After recording control EMG's, the large superficial branch of the V nerve at the site indicated in fig. 2.4 with a 2% solution of Lignocaine Hydrochloride (Xylocaine-Astra). After allowing a minute or so for

any effects to occur, the profound nerve branch was infiltrated steriotaxically by injecting the drug after advancing a 25 gauge needle through the overlying muscle as indicated in fig.2.4 .

Some fish were prepared as ~~for~~ indirect stimulation. The nerve was stimulated and the effects of the section of the superficial nerve branch tried. Section of the profound nerve was difficult because of its inaccessibility and also because major arteries accompany the small nerve branch to the red fibres so blockage was effected with Xylocaine.

2.10 Effects of drugs producing central or peripheral neuromuscular blockade upon the trout adductor mandibulae muscle.

The effect of neuromuscular blockading drugs upon the activities of fish muscles is little understood. There is also some conflict in the literature as to exactly what is being recorded in the red fibre EMG. Bone (1966) thought that the potentials recorded were in fact extracellular action potentials from motor nerves. Roberts (1969), however, inclines to the view that they are junctional potentials because (a) d-Tubocurarine Chloride abolishes all potentials in elasmobranch preparations and (b) Suxamethonium Chloride which does not interrupt synaptic events does not abolish the small fibre potentials. It was decided to try the effects of such drugs on the trout adductor mandibulae muscle EMG.

Secondly, in mammals, Paton and Zaimis (1951), Zaimis (1953) and Jewell and Zaimis (1954) have shown differential sensitivity and antagonisms in red and white muscles between drugs of the curare group,

the methonium series and anticholinesterases such as Neostigmine. It was thought that it would be useful to examine these effects in the fish. Thirdly, the drug Guaiacol Glyceryl Ether (GGE) has been shown to give cessation of activity in tonic type muscles owing to the interruption of interneuronal transmission (Frey et.al. 1952, Westhues and Fritsch, 1965, Hall 1971). Clinically, it has been used in medical and veterinary anaesthesia to produce skeletal muscle relaxation without incurring the respiratory depression or apnoea produced by peripheral blockade with the customary drugs, d - Tubocurarine, Gallamine, Suxamethonium. This is due to the fact that in the larger mammals at least, the respiratory muscles have few red fibres and are, therefore, unaffected by interneuronal blockade. If, therefore, after administration of clinical doses of GGE, the EMG of the trout adductor mandibulae was affected by it, then this might indicate the tonic nature of the muscle.

Under the current Cruelty to Animals Act 1876, it is extremely difficult to obtain authorisation to administer a neuromuscular blocking drug to a conscious animal or to allow the effect of an anaesthetic to pass off from an animal given such a drug under anaesthesia. It was, therefore, necessary to carry out all experiments under light anaesthesia produced by Benzocaine (25mg/litre). Parenteral anaesthesia with Pentobarbitone sodium was precluded owing to the possible antagonism with curariform drugs. Fish were prepared as in 2.4.1 above and drugs were injected into the red lateral muscles from which uptake is rapid owing to the great vascularity and to the permeability of the capillaries. This was achieved without disturbing the fish during recording by clamping the syringe in a Palmer stand and delivering the drug via a needle tipped catheter implanted into the

muscles.

The drugs used in these experiments were; d - Tubocurarine Chloride (Tubarine - Calmic), Gallamine Triethiodide (Flaxedil - May and Baker), Suxamethonium Chloride (Anectine - Calmic), Decamethonium Bromide (Sigma), Guaiacol Glyceryl Ether (Sigma), Neostigmine Bromide (Sigma) and Atropine Sulphate (Sigma).

2.11 Investigations into the pacesetter potential of the trout alimentary tract

2.11.1 Acute Experiments

Rainbow Trout were anaesthetized in the initial few experiments with either 2 - Phenoxyethanol or Benzocaine. Later this was succeeded by parenteral anaesthesia with Pentobarbitone Sodium (Nembutal - Abbot) 40mg/kg I.P. A midline laparotomy was performed on the anterior abdominal cavity. The viscera exposed by the use of self-retaining retractors and haemostasis effected with haemostatic forceps or adrenaline soaked pledgets. The electrodes used were bipolar 125 μ m insulated stainless steel with sharpened points (1mm bared). They bore a small bead of epoxy resin about 4mm back from the tip to act as an anchoring point for sutures. Insertion was achieved by puncturing the serosa with a 25g needle tip, passing a 5/0 suture on a corneal needle anterior to the puncture, then the electrode is inserted and advanced subserosally. It is then retained by the suture. Two pairs of electrodes were used, one in the cardiac stomach, the other in the pyloric stomach. The free ends of the electrode were exteriorised through the intercostal muscles by threading them through with a 21 gauge needle. The laparotomy was closed with a Glovers suture and the leads connected to the recording apparatus.

The stomach had a cannula inserted, to record pressure changes via a Bell and Howell 4.422 Blood Pressure transducer. Recordings were made with a pair of pen recorders (Devices M2 & MX212) after the fish was paralysed with 2.0 mg of Gallamine Triethiodide Flaxedil - May & Baker). The ECG was continuously monitored on one recorder channel to confirm viability in the absence of vital signs.

(Plates II.8 - .14)

2.11.2 Chronic Experiments

The operative procedure above was followed except that only the electrodes were implanted and these were connected to an anchoring plate as in Section 2.5. This was specially constructed to accept two pairs of electrodes and was connected to the recording apparatus in an identical manner.

Section II : Muscle Physiology

RESULTS

2.12 Depth Probe Experiments

In small Rainbow Trout of about 100g weight, by implanting two pairs of electrodes, one pair at maximal depth (5mm.), the other at 1 mm. depth, into the adductor mandibulae, it was seen that EMG activity (Figs. 2.5, 2.6) was only properly detected at 5 mm. Driving the 1 mm electrode in to 5 mm depth resulted in the detection of EMG activity of a similar type. Conversely, retracting the 5 mm probe results in a rapid diminution of detectable activity and at 1 mm depth no activity is detected. In more controlled experiments, where the electrode pairs were advanced from the surface into the muscle in discrete steps, it was seen in every case that EMG activity was significantly greater in the last 1 - 2 mm. (see Figs 2.7, 2.8, 2.9). Small potentials recorded outside this zone observed on the oscilloscope are 'blurred' and probably result from volume conduction. Fig. 2.9 shows a 'close-up' at a faster recording speed. There is a particularly large change in EMG amplitude over this range (4 - 5 mm) and the potentials at 5 mm are noticeably 'sharper'. Note that the duration of these motor unit potentials (m.u.p.)^{*} are often 30 mS or greater.

It was noticed during the course of these experiments that if anaesthesia was allowed to progress to medullary collapse, so that the fish became apnoeic, continuous EMG activity was recorded from electrodes at maximum depth into the adductor mandibulae muscle. By chance, it was found that if the mouthpiece supplying the anaesthetic was removed and the jaw allowed to relax, this activity ceased. Fig. 2.10 shows the EMGs from such a fish with electrode pairs implanted at maximal depth in both left and right adductors. It will be noted that the activity begins and ceases with imposed mandibular abduction and adduction respectively and that the EMG amplitude is in increasing

* The use of 'm.u.p.' here and in subsequent pages does not imply equivalence to the same in the mammalian context as fish muscle is not organised into strict motor units.

Fig. 2.5

Recording made from free-swimming Brown Trout with two pairs of EMG electrodes implanted into a. mandibulae. No EMG activity is detected at 1mm depth whereas activity due to ventilation is found at 5mm depth.

Fig. 2.6

Lightly anaesthetized Rainbow Trout: EMG probe has been driven to full depth into the muscle to detect active red muscle fibres. Retracting electrodes causes diminution of EMG.

Fig. 2.7

Lightly anaesthetized Rainbow Trout: EMG recordings made at 0.5mm depth increments into a. mandibulae. There is a marked increase in the amplitude of the EMG detected in the final 1mm of the muscle, corresponding to the zone of active red muscle fibres.

FIG 2.5

1mm electrode

5 mm electrode



100 μ V |
1 S

FIG 2.6

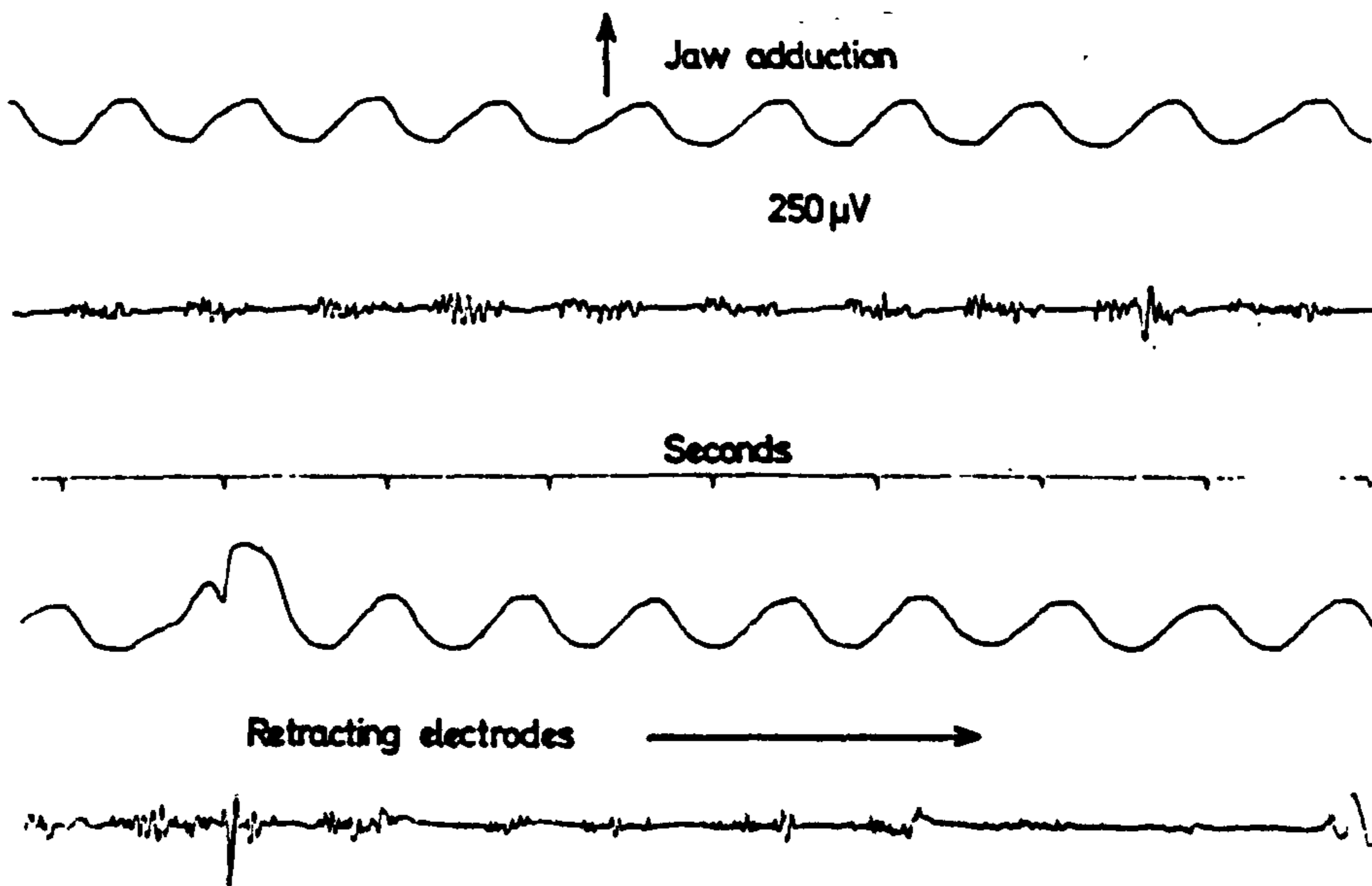


FIG 2.7

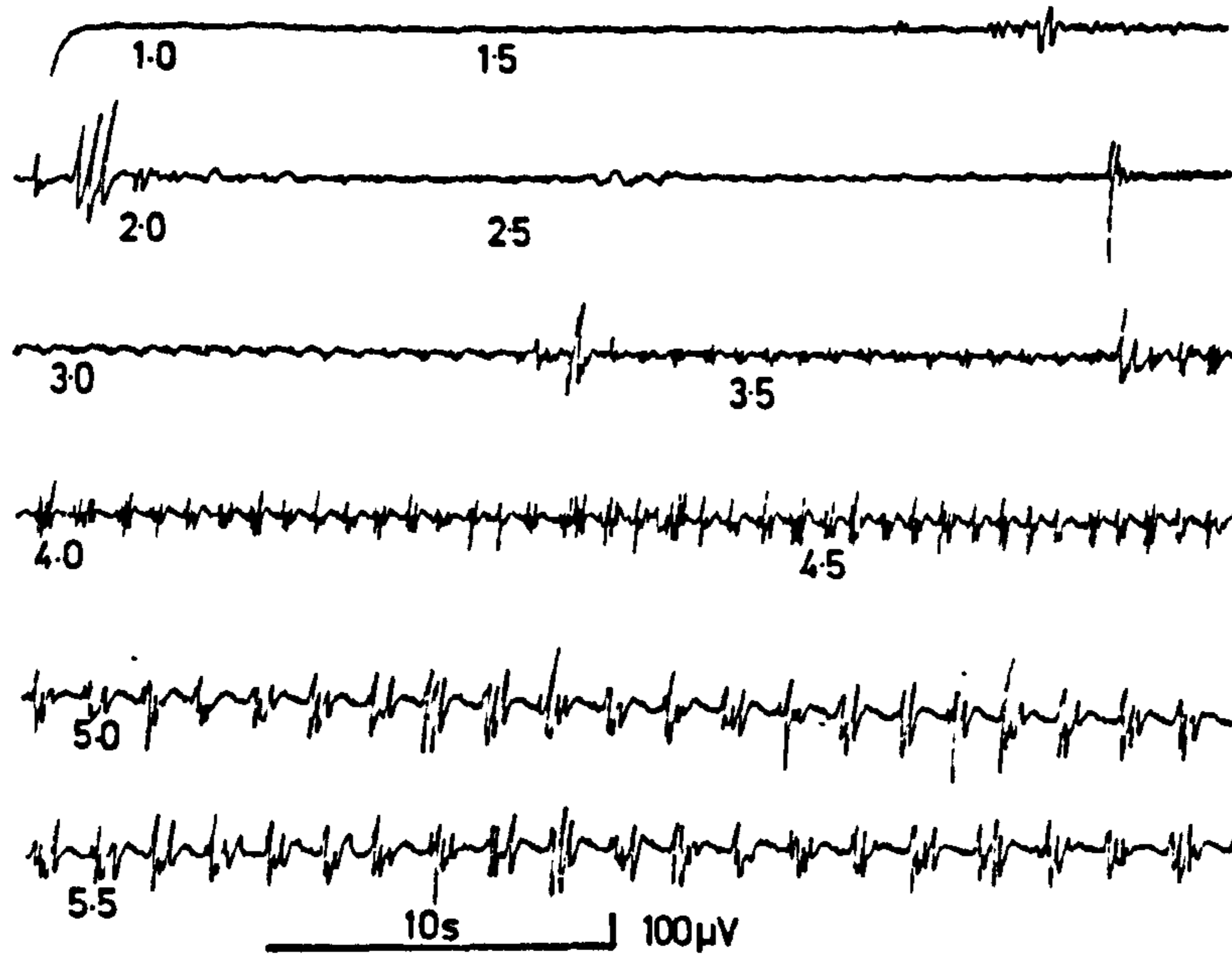


Fig. 2.8

(a) Lightly anaesthetized Brown Trout: Advancing EMG probe into a.mandibulae midline shows greatest amplitude of EMG over final millimetre depth.

(b) Advancing probe into a. mandibulae away from midline shows that no EMG activity is detectable at maximum depth (7mm) owing to lack of red muscle fibres.

Fig. 2.9

Lightly anaesthetized Rainbow Trout: EMGs recorded at high speed over final 1mm depth of a. mandibulae. Not only is there an increase in amplitude, but also the potentials at 4mm are "blurred" due to volume conduction.

Fig. 2.10

Deeply anaesthetized Rainbow Trout; EMG potentials evoked in both right and left adductor mandibulae red fibres due to stretch imposed by enforced abduction of mandible at arrows.

FIG 2:8

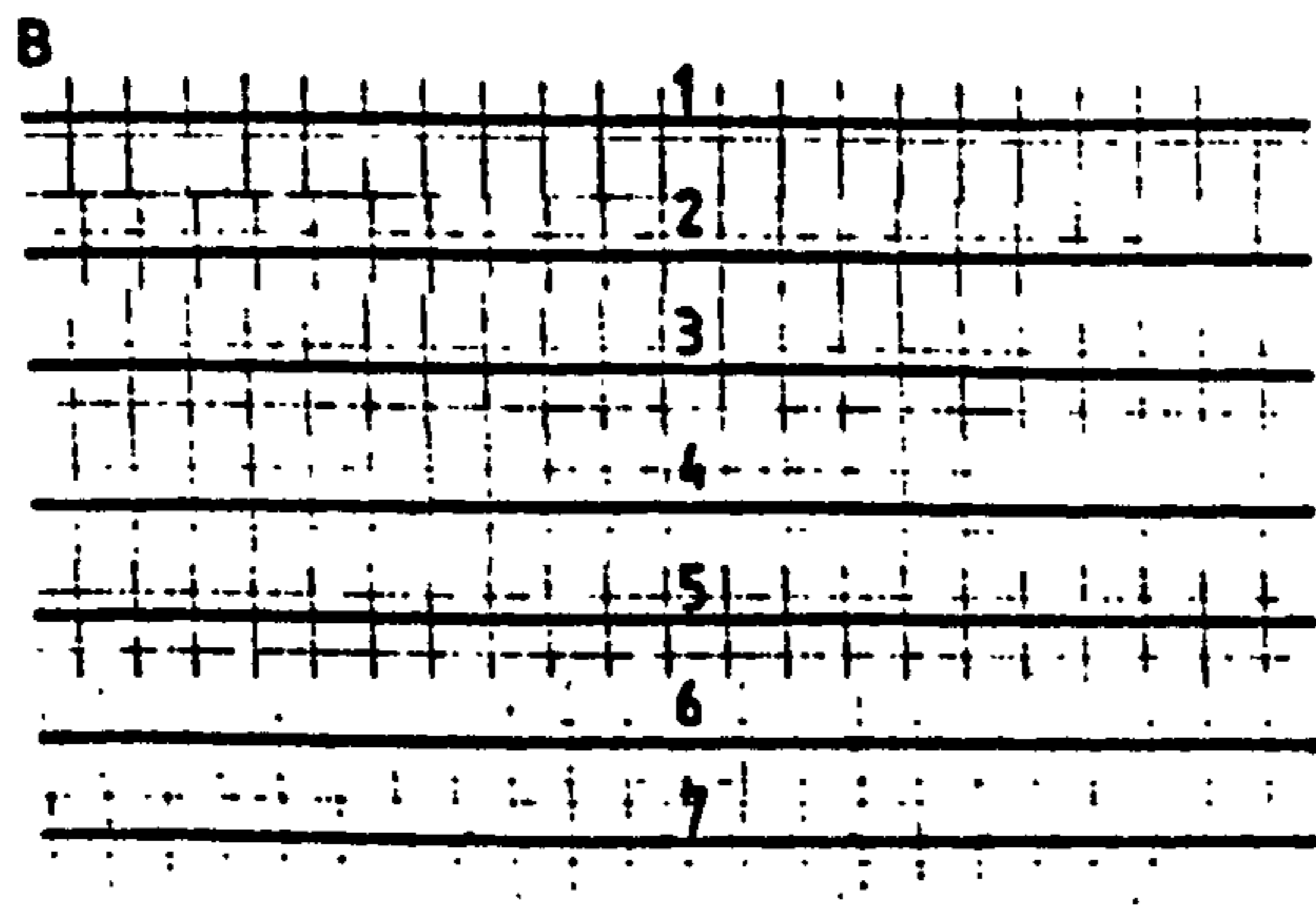
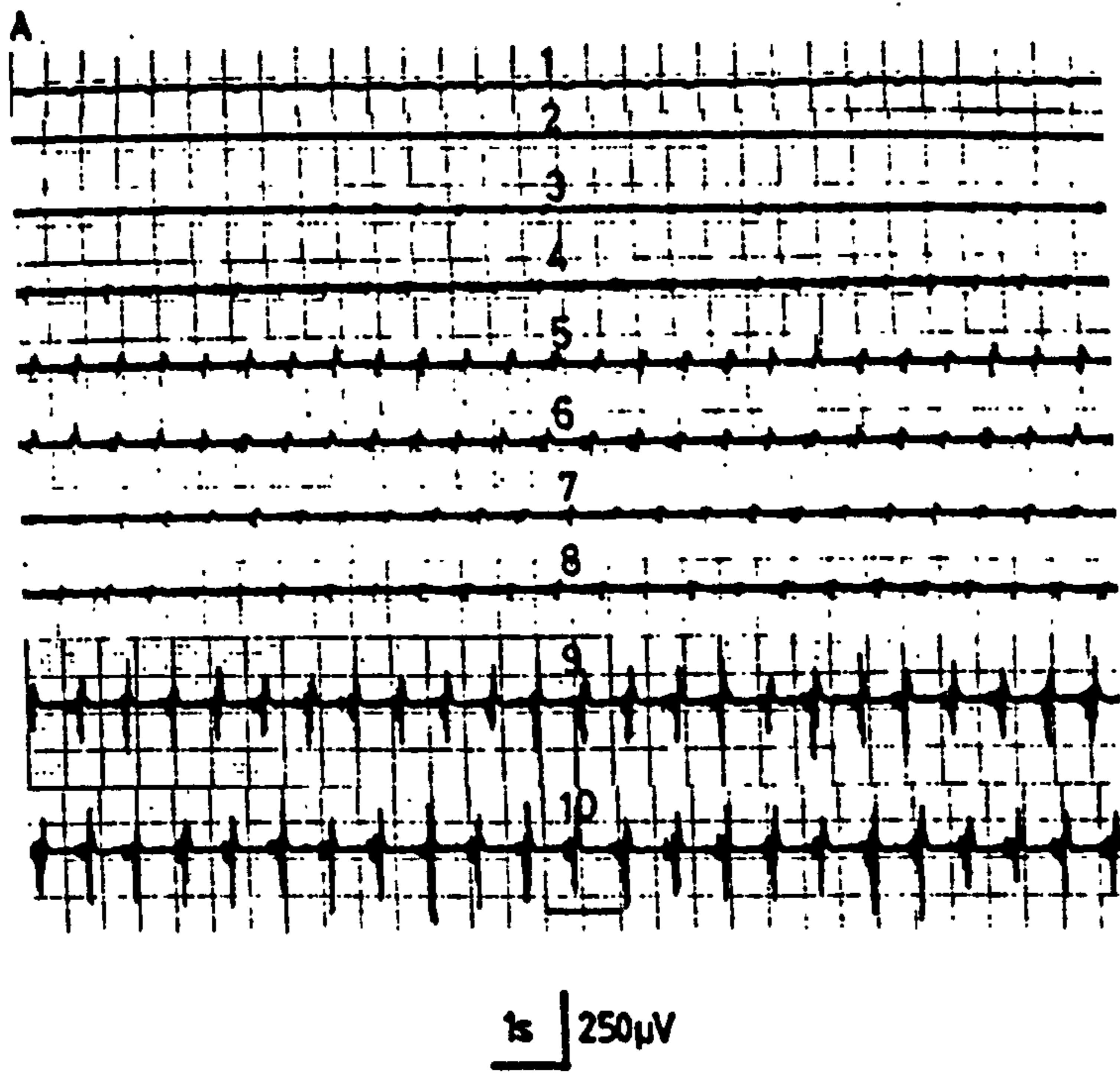


FIG 2.9

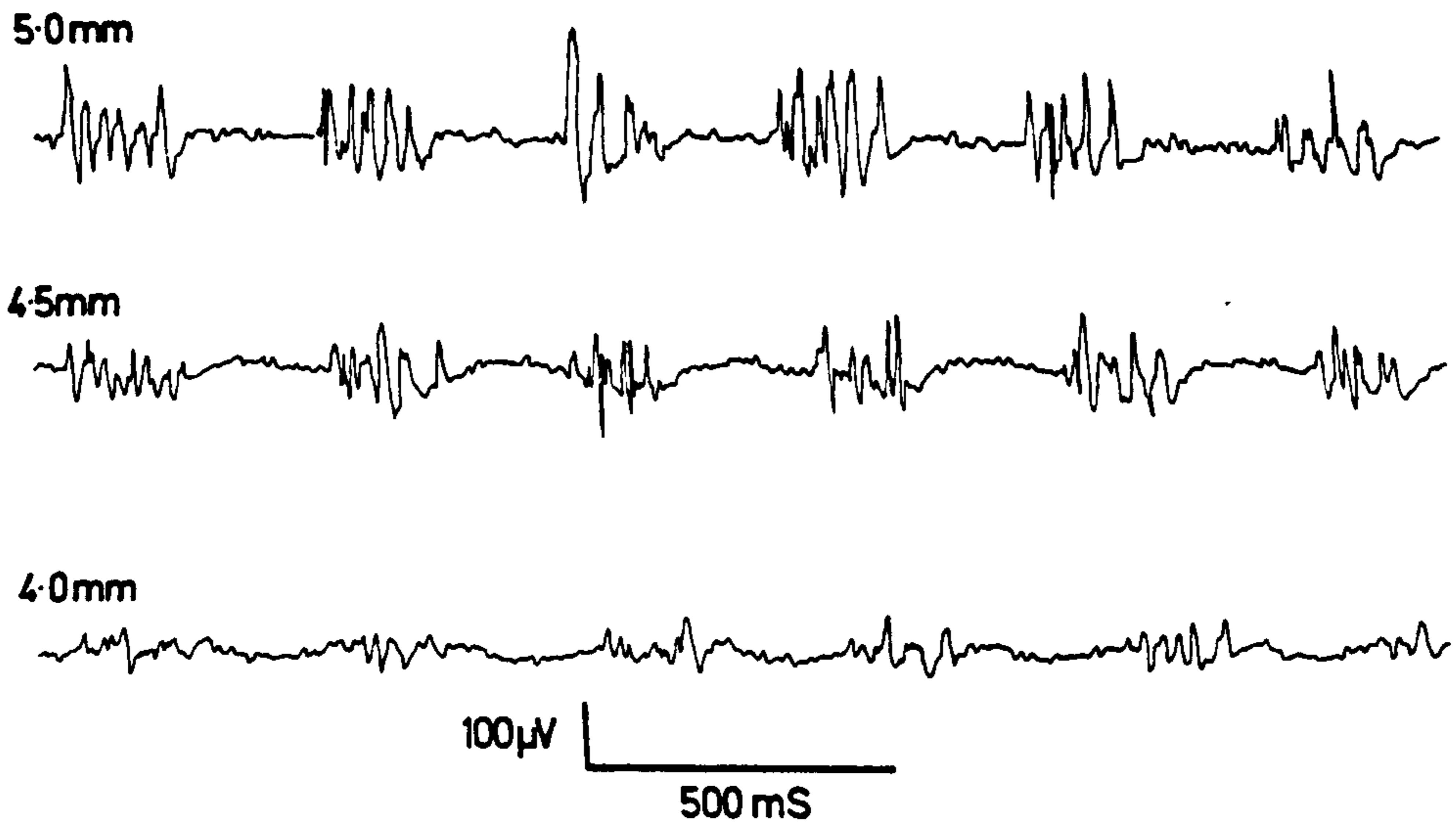
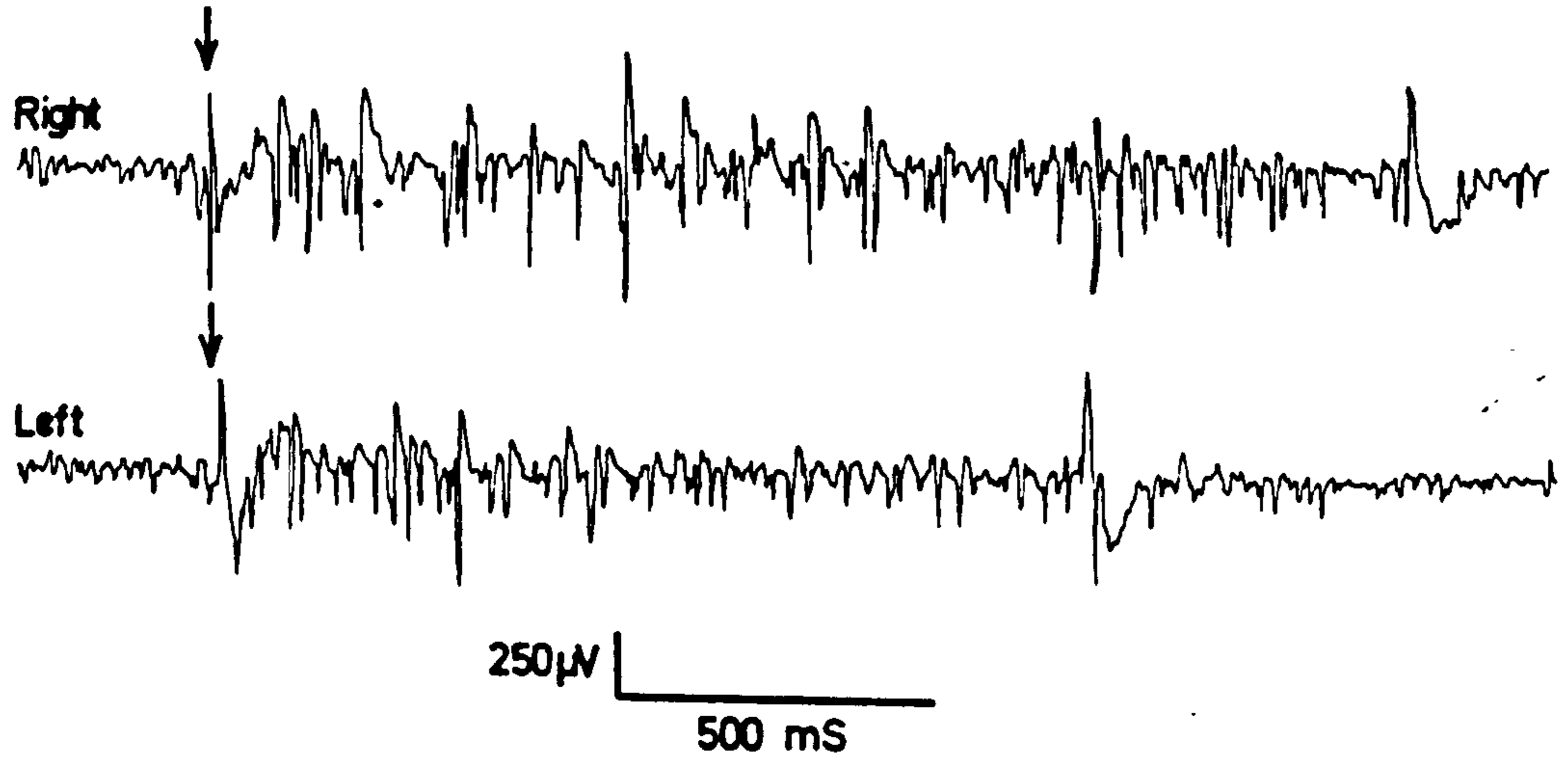


FIG 2.10



proportion to the degree of stretch imposed. This phenomenon was not recorded in mosaic fibres.

Finally, Fig. 2.11 shows the unusual result of EMG recordings from the adductor mandibulae of a Brown Trout anaesthetised with Thiopentone Sodium (Pentothal-Abbott) 30mg/kg I.P. and following the injection of d-Tubocurarine (Tubarine-Calmic) 0.5mg I.M. The fish, shortly after the injection of the latter, commenced performing an unusual 'coughing' type of manoeuvre which was periodically repeated. Note especially that the onset of each cough represents an increase in isometric tension to four times that in ventilation and also that the tension rise is rapidly developed, in contrast. The EMG at this point consists of high amplitude, fast-spiking potentials which the pen recorder is unable to reproduce faithfully but which were easily distinguished on the Audio Amplifier of the CEPTU unit. The occurrence of these EMG changes coincides with the rapid tension development. Unfortunately, it was not possible to employ two sets of electrodes simultaneously from the red and mosaic portions of the muscle in order to confirm the origin of these potentials. As an expedient, the probe was withdrawn and reimplanted into the mosaic fibres at 2mm depth. Fig. 2.12 shows the resulting EMG recording. Note that at this point in time, the red fibres have become paralysed but the high amplitude twitches persist and their activity is indeed accompanied by fast spiking activity in the mosaic muscle portion. In Rainbow Trout implanted with chronic 'T-electrodes' into the adductor mandibulae as in Section 2.5, when allowed to recover quietly from anaesthesia and surgery, the Fish would readily feed on their usual pellet diet (if they had first been allowed to accustom themselves to the tank previously). It is evident that, during feeding, a characteristic sequence of events would occur in the EMGs; this consisted of a series of very high amplitude ($> = 1mV$) m.u.p.s which are repeated at

Fig. 2.11

Brown Trout under Pentothal anaesthesia: Unusual 'coughing' manoeuvre following d-Tubocurarine injection. Large rises in tension are correlated with the appearance of fast potentials in the EMG recording.

Fig. 2.12

As Fig.2.11 except that electrodes have been moved into white (mosaic) muscle. Fast potentials are now detectable in white muscle, correlated with large rises in tension.

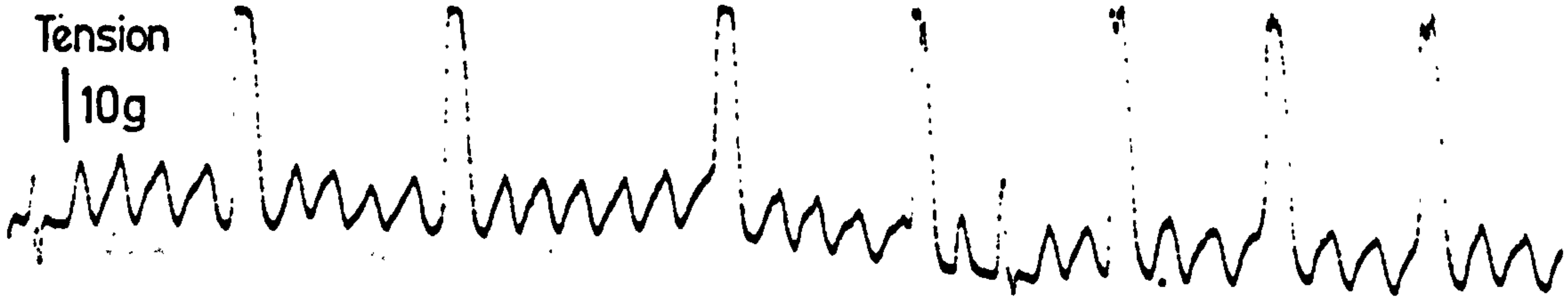
Fig. 2.13 (a - e)

EMG recordings from five different free-swimming Trout equipped with adductor mandibulae Electrodes, during feeding on pellets in laboratory. Note high amplitude, repetitive patterns produced. Arrows indicate point of taking pellet.

Fig. 2.14

Trout with adductor mandibulae EMG electrodes during antifungal treatment with Malachite Green: Note the appearance of high amplitude, fast potentials during rhythmic coughing.

FIG 2.11 Tension
| 10g



Red fibre EMG
| 250 μ V



FIG 2.12

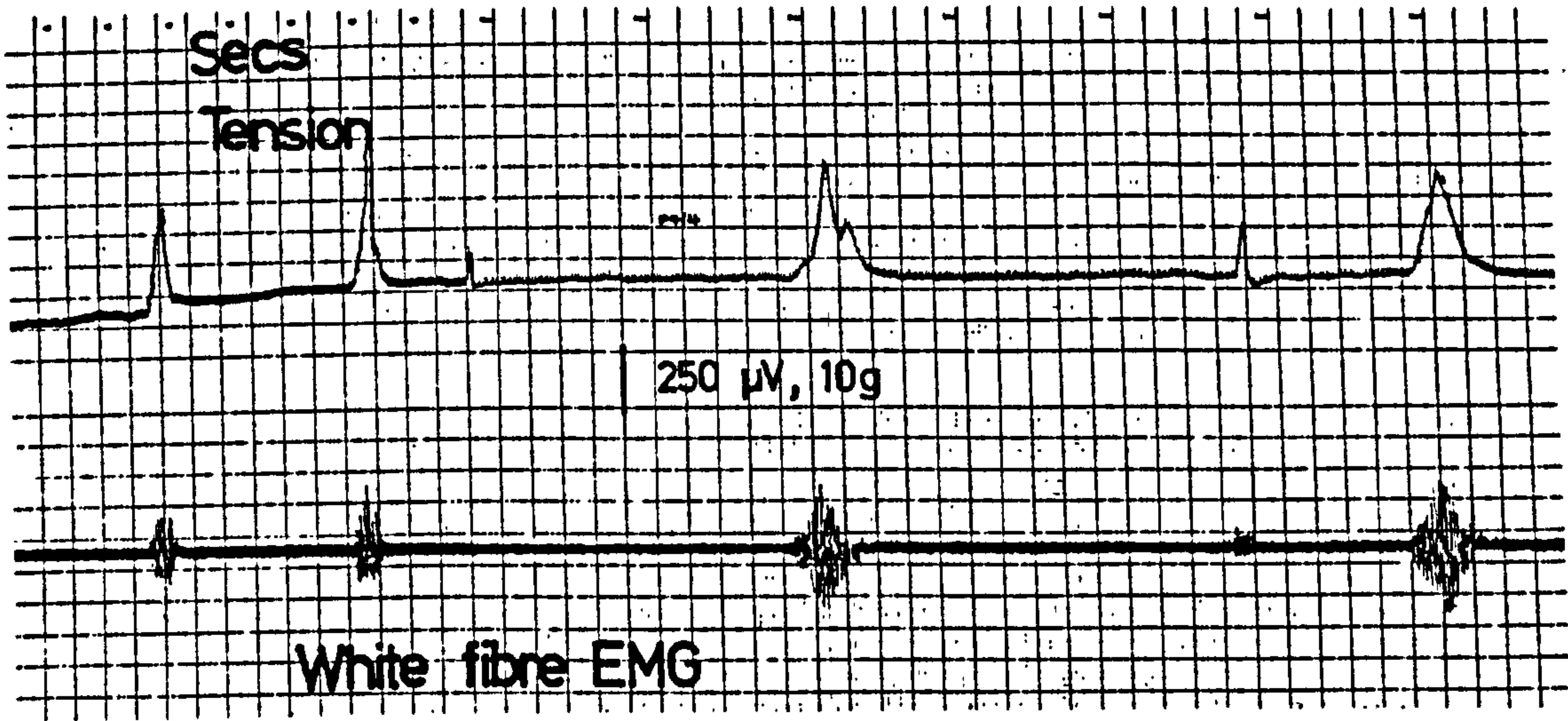


FIG 2.13

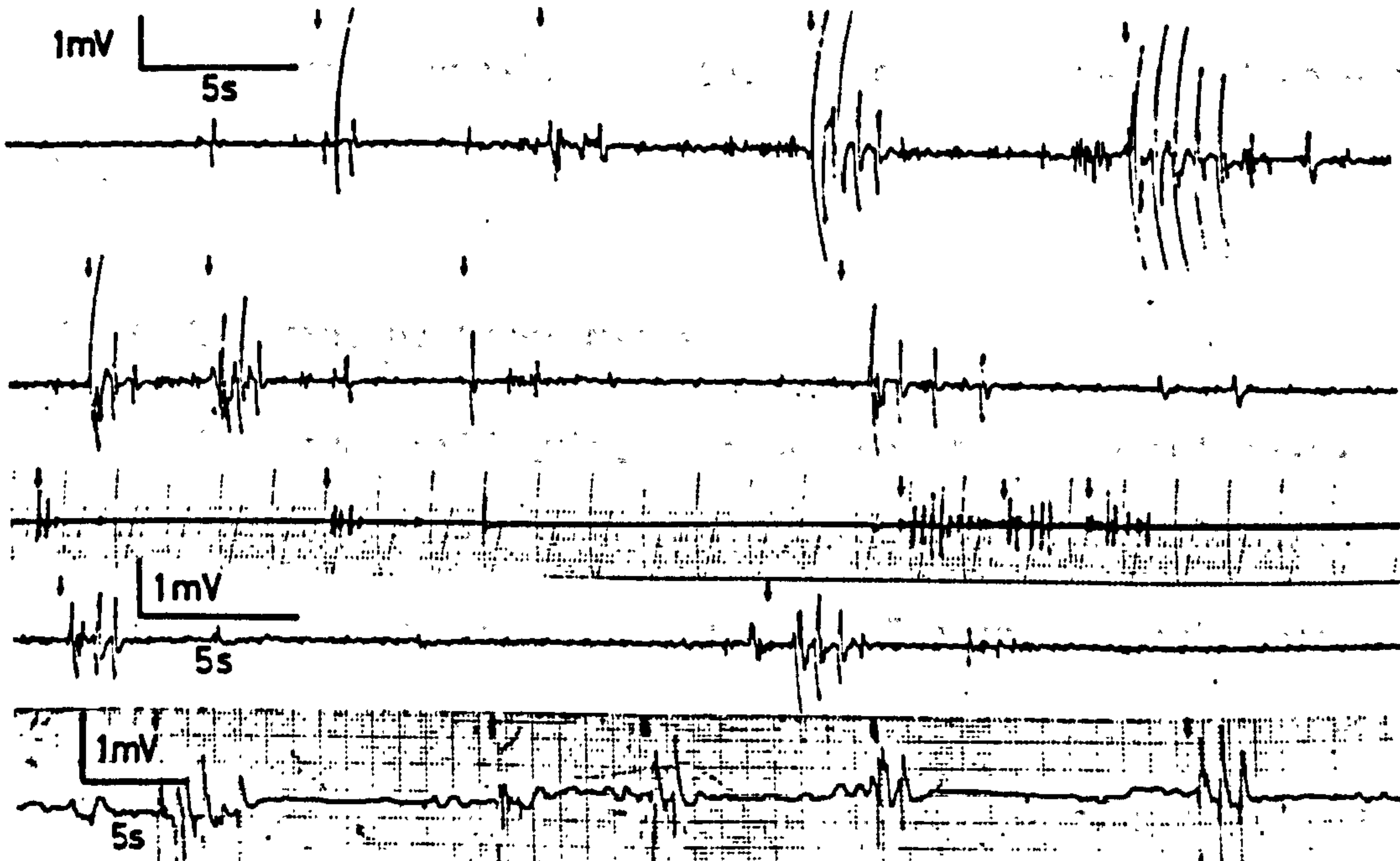
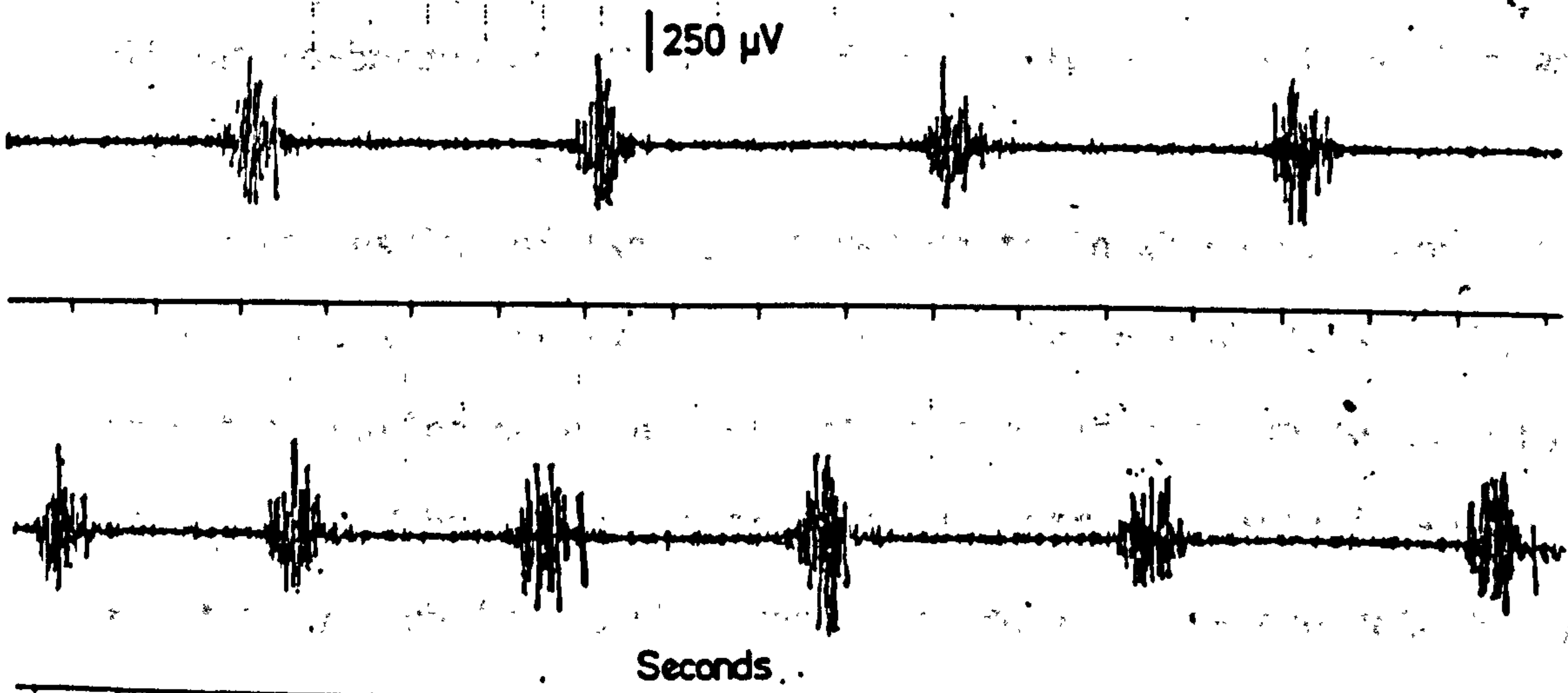


FIG 2.14



intervals over a period of 1 - 3S and which coincided with the rapid jaw movements seen during ingestion of a pellet. Often the m.u.ps would show a left to right decrement in amplitude (Fig. 2.13). In one Trout so equipped the treatment of a fungal infection caused an increase in the rate of spontaneous coughing. This was seen to be accompanied by the appearance of high amplitude fast-spiking m.u.ps in the EMG but the repetition pattern is totally different from that during feeding acts (Fig. 2.14).

In some animals attempts were made to record from the small mandibular branch of the adductor mandibulae during spontaneous ventilation, whilst the Trout was free-swimming. This is technically difficult to achieve owing to the thinness of the muscle but in three cases some EMG activity was detectable, always in synchrony with the EMG of the main cephalic branch. The m.u.ps recorded were generally small, $<100 \mu\text{V}$.

2.13 EMG mapping experiments

It proved difficult, in practice, to carry out these experiments because of the difficulty in maintaining a constant depth of anaesthesia over a period of time. The only reliable way, it was found to judge the level of the latter, was to observe the output from an isometric transducer attached to the mandible. It will be noted that if respiration becomes depressed (as it invariably does with amino-benzoates) then the EMG activity will decline also which will give misleading results.

The results of two such experiments in which even conditions were maintained, are displayed diagrammatically in Fig. 2.15. It will be seen that during spontaneous ventilation, at maximum depth into the adductor mandibulae muscle there is a restricted area in which relatively high ($>100 \mu\text{V}$) levels of EMG activity are detectable.

Fig. 2.15

Outline 'maps' of two Brown Trout adductor mandibulae muscles:
The figures indicate the maximum EMG voltage in uV recorded at that point. There is a distinct zone in the middle region in which EMGs can be recorded. This corresponds to the area occupied by the red muscle fibres.

Fig. 2.16

Portugese Shark (Centroscyrnus coeloepis):EMG recordings from medial adductor mandibulae at 5mm intervals. Strong EMG potentials appear in last 10mm, corresponding to red muscle fibres. Figures indicate depth in mm from surface.

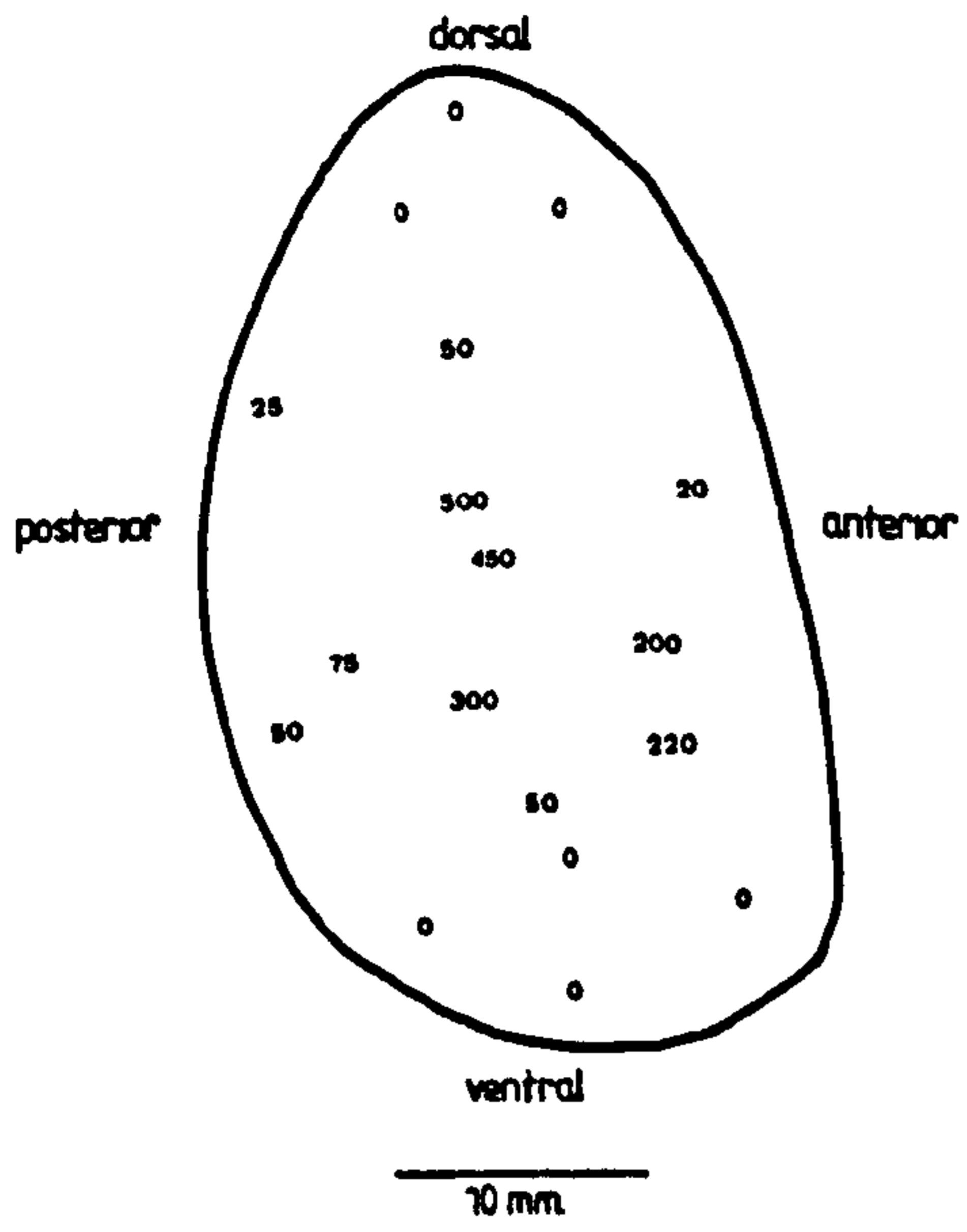
Fig. 2.17

As in Fig. 2.16 except that electrodes are advanced into area (periorbital) away from red fibre zone (total thickness 12mm). No EMG potentials are detectable.

Fig. 2.18

As in Fig. 2.17: following the injection into lateral muscles of Suxamethonium Chloride, unusual fast EMG potentials appear in the quiescent white muscle.

FIG 2.15
MUSCLE I



MUSCLE II

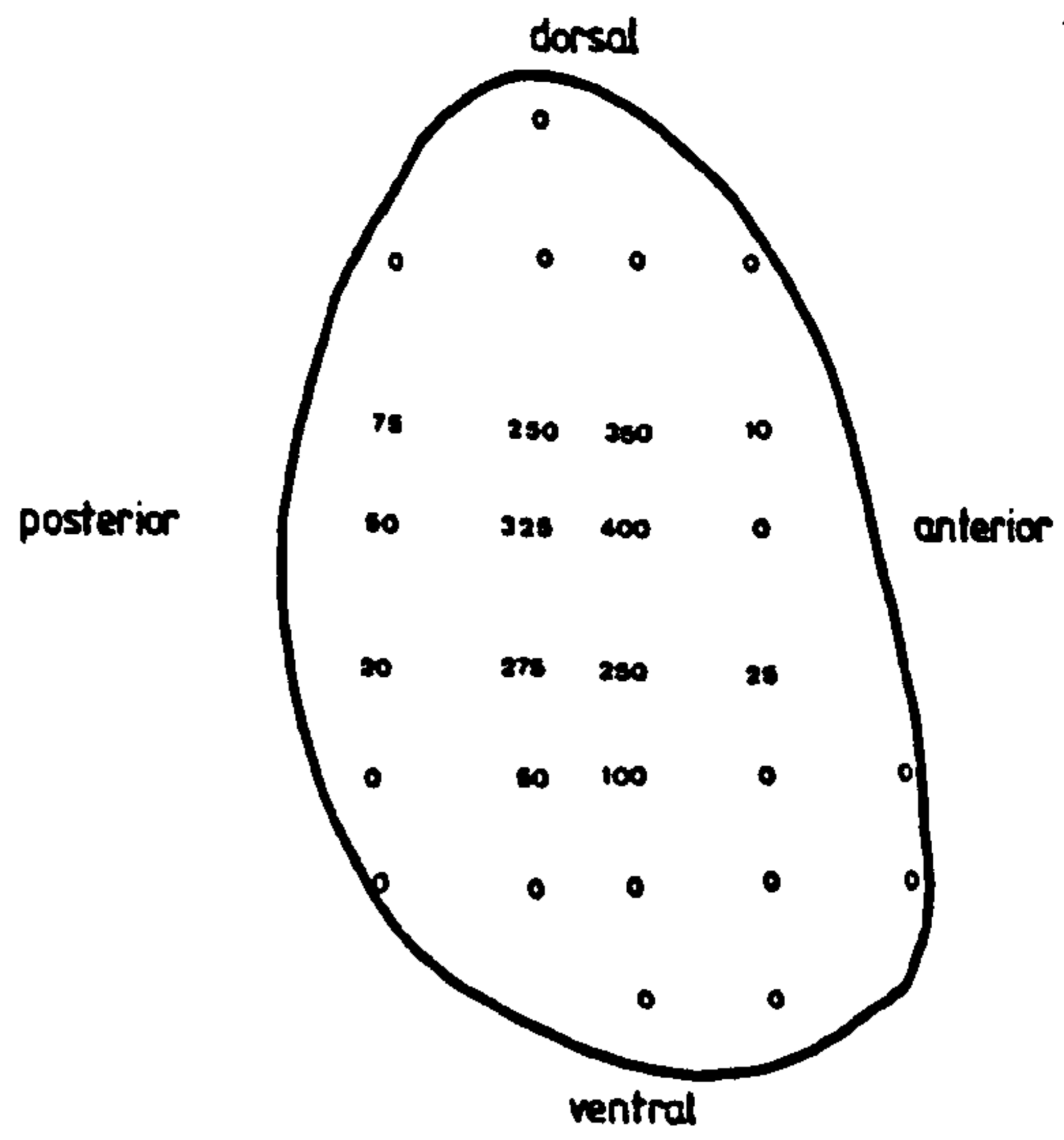


FIG 2.16

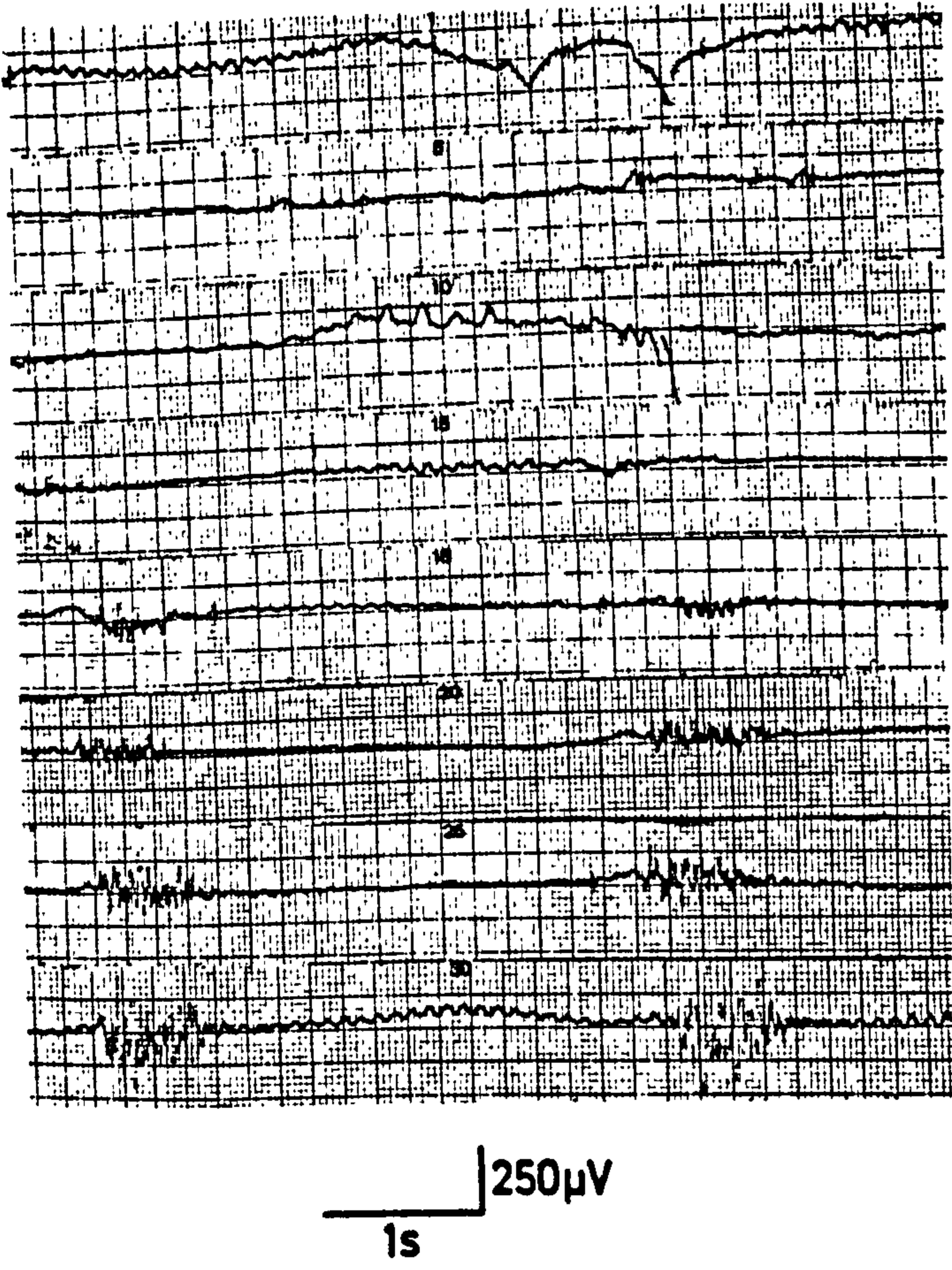


FIG 2.17

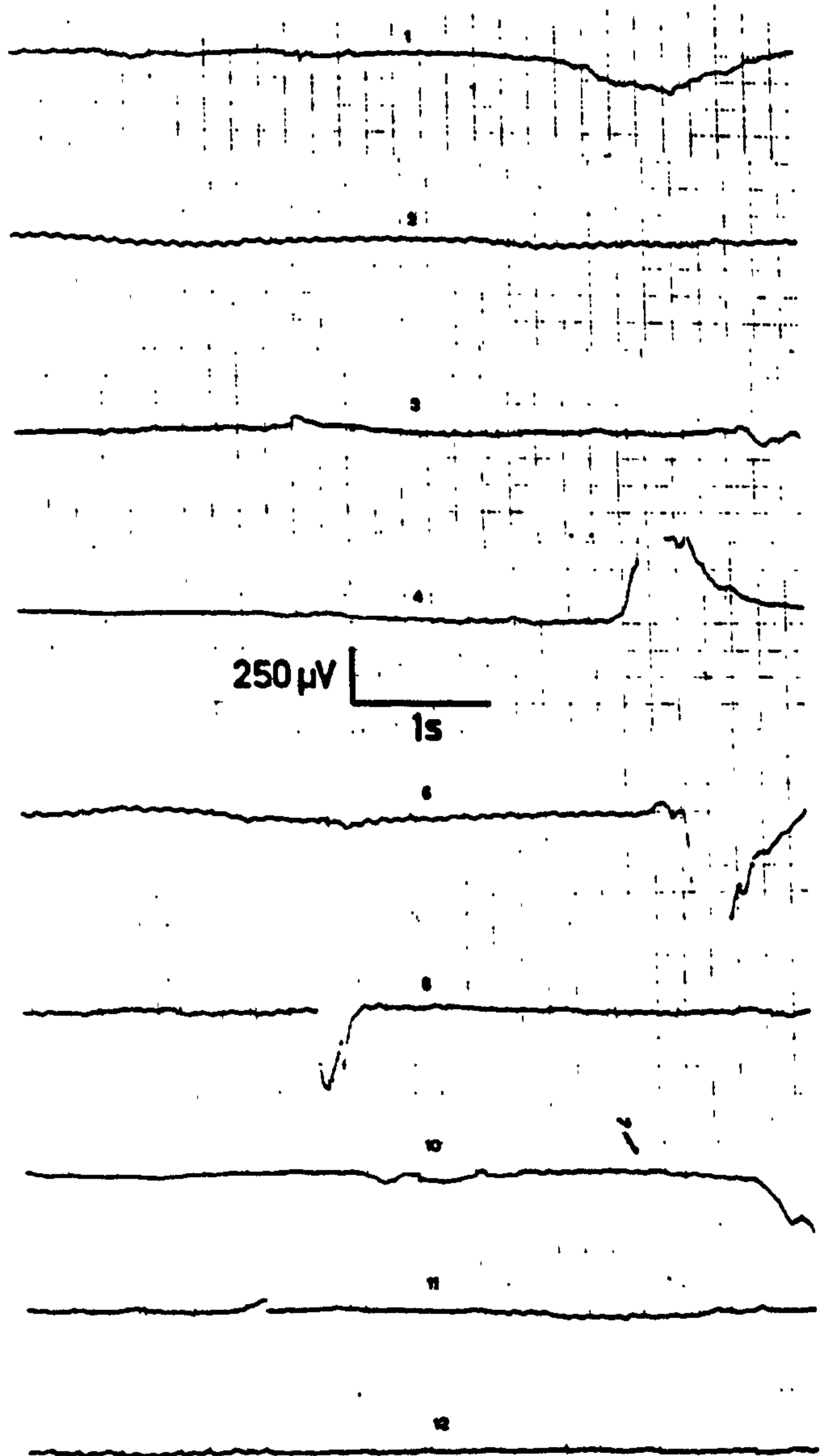
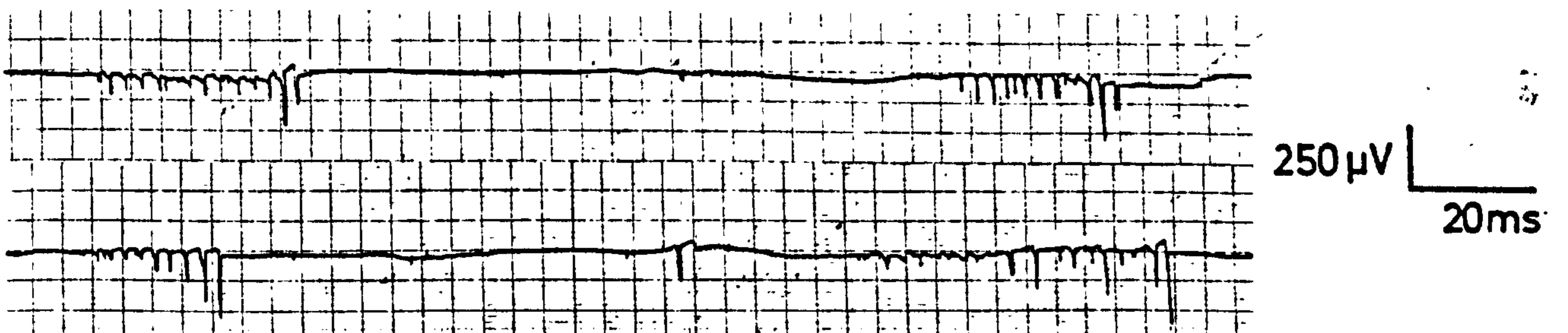


FIG 2.18



Note especially that little activity is detected around the lateral margins and none at all in the dorsal and ventral portions. In all cases, the greatest EMG voltages were observed at maximum depth into the muscle.

2.14 Comparative EMG and other observations made upon Selachians and other deep-sea Teleosts

From the deep water hauls aboard R.R.S. 'Challenger', dissections made of the adductor mandibulae muscles of a wide range of Selachians (as listed in Section 2.4.3) revealed very large discrete tracts of red fibres in the muscle, directly comparable in anatomical position to those seen in the Trout. In the Portugese Shark, Centroscyms coelolepis, the thickness of the red portion was about 10mm, compared with a maximum of 30mm for the whole muscle. In all cases, because of the relatively large size of the muscle and the animals themselves, the red fibres were macroscopically very obvious by their colour and the width of the red fibre portion may exceed 15mm in large specimens. The red fibres inserted onto Meckel's cartilage by a broad, flat tendon, as in the Trout.

Observations upon the deep sea Salmoniform Alepocephalus bairdii Goode & Bean, (Smoothhead), showed an almost identical morphology to that of the Trout except that the muscle was thicker and angled at 45° caudad presumably because of the huge eyes and the opercular bone structure. The distribution of red fibres showed an exact parallel with that of the Trout. A brief examination using a field staining method for thick muscle slices using Formalin and Toluidine Blue showed that the red fibres had a range of 10 - 40 um whereas the remainder was mosaic with a range of 20 - 120 um. All the fibres had a peculiar vacuolated appearance, presumably due to the reduction in protein content (i.e. in the myofibrils) common in deep-sea fish as an aid to specific gravity reduction.

Dissection of the Chimaeroids Hydrolagus mirabilis Collett and Hydrolagus affinis Capello showed that they possess a very large adductor mandibulae apparently almost wholly white muscle save for the inner aspect of the ventral anterior part which has a thin, sheet like tract of red fibres present.

In the preparation of a Portugese Shark, Centroscymnus coelolepis (Plate II.4), insertion and retraction of the electrode probe demonstrated true EMG activity at depths of 15-18mm from the surface; when driven into the area of the red fibres. EMGs of up to 500 μ V were found to be correlated with the slow mandibular adduction during ventilation (Fig. 2.16). When the probe was inserted into the inferior periorbital area from which, in previous dissections, it was seen that overt red fibres were absent, no EMG activity was detectable at any depth (Fig. 2.17). It will be noticed that the maximum thickness at this point is only 12 mm., whereas in the belly of the muscle it was 30mm. Following the injection of Suxamethonium Chloride (Anectine-Calmic), 200mg \equiv 4.65 mg/kg into the red lateral muscle at the level of the dorsal fin, ~~motor~~ potentials were observed in the white muscle portion of the adductor mandibulae very shortly afterwards (Fig. 2.18). Note that the 'spikes' appear in rhythmic groups corresponding to the arterial pulse. These spikes had a very fast rise time and short duration. A further injection of Suxamethonium Chloride, 20mg, directly into the adductor mandibulae, provoked a powerful contracture which bent the recording electrodes. Nevertheless, there could be seen to be many fast spikes during this phase before relaxation occurred.

2.15 Effects of Anaesthesia on the a.mandibulae EMG

Four experiments, as detailed in Section 2.6, were carried out upon two Brown and two Rainbow Trout. Some selected examples to illustrate the results are shown in Figs 2.19 to 2.20.

After anaesthesia (1:30,000 Benzocaine), the appearance of continuous, small motor unit potentials (m.u.p's) is the first sign of emergence from apnoea: The m.u.p's are of around 50-100 μ V in amplitude, often of short duration with little evidence of interference with adjacent m.u.p's. At + 5 mins from recovery, an exaggerated ventilation cycle is set up with vigorous movements of all the cranial muscles and head parts concerned. The ventilation frequency is extremely high, 120c.p.m., and the burst length of the EMG train is 200mS with the interval between bursts equal to this. There are, characteristically, large m.u.p's >500 μ V at the commencement of the train, giving a triangular appearance to the EMG envelope, i.e. there is a rapid decay in amplitude from left to right. There are generally <20 m.u.p's in the train and there are a majority of potentials in the high frequency <10mS duration classes.

As recovery proceeds there is an obvious pattern of change in the EMG recorded. At +10 and +20 mins low frequency (<10Hz) motion artifact may become more obvious. Two motions are evident; the first occurs about 100mS from the start of the EMG train, corresponding to the actual contraction of the adductor mandibulae during the buccal phase of ventilation. The second occurs immediately after the cessation of activity in the a. mandibulae, this corresponds to the opercular phase and results from the combination of contractions by the sternohyoideus, dilator operculi etc. At + 30 minutes, the peak amplitude is >600 μ V and there are now large numbers of m.u.p's of this size. There is still a tendency for a left to right decrement in m.u.p. size. The ventilation

Fig. 2.19

Adductor mandibulae EMGs from Brown Trout over period following 1 hours Benzocaine anaesthesia in order to simulate effect of tagging a fish with an ultrasonic transmitter. Changes in amplitude, frequency and length of EMG bursts are evident.

Fig. 2.20

As in Fig. 2.19 but at higher recording speed:

a) + 5mins b) + 1 h c) + 5h d) + 24h

In d) the EMG potentials are so temporally dispersed that little or no summation occurs compared to b) in which summation is increasing the overall amplitude.

Fig. 2.21

Summary of EMG changes during recovery from Benzocaine anaesthesia.

Vr: ventilation rate (cpm); mv: peak amplitude (uV); bl: burst length (mS); sb: spike potentials/time ratio (nos./mS).

Burst length has intermediate low phase then lengthens; all others tend to decline with time.

FIG 2.19

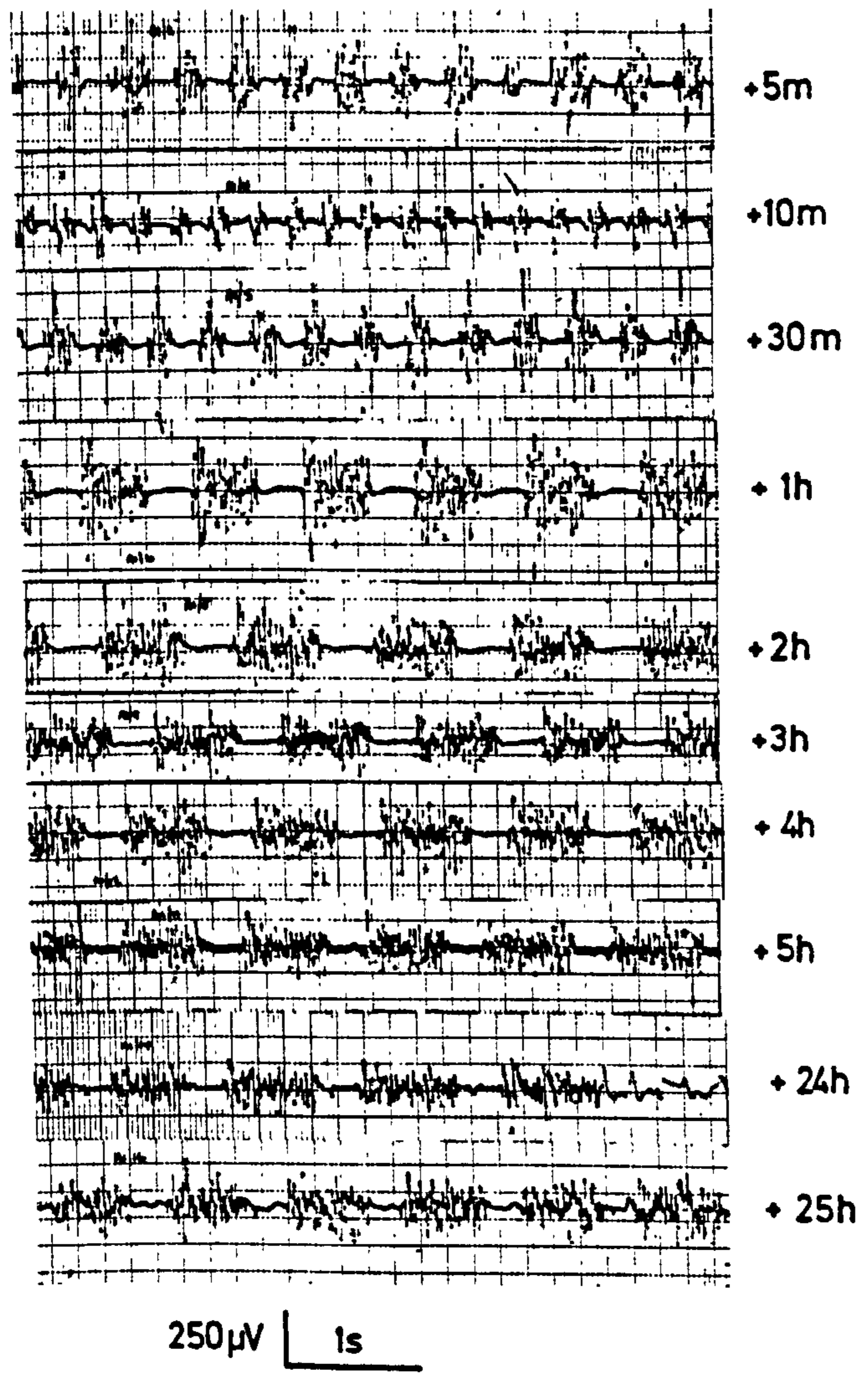


FIG 2.20

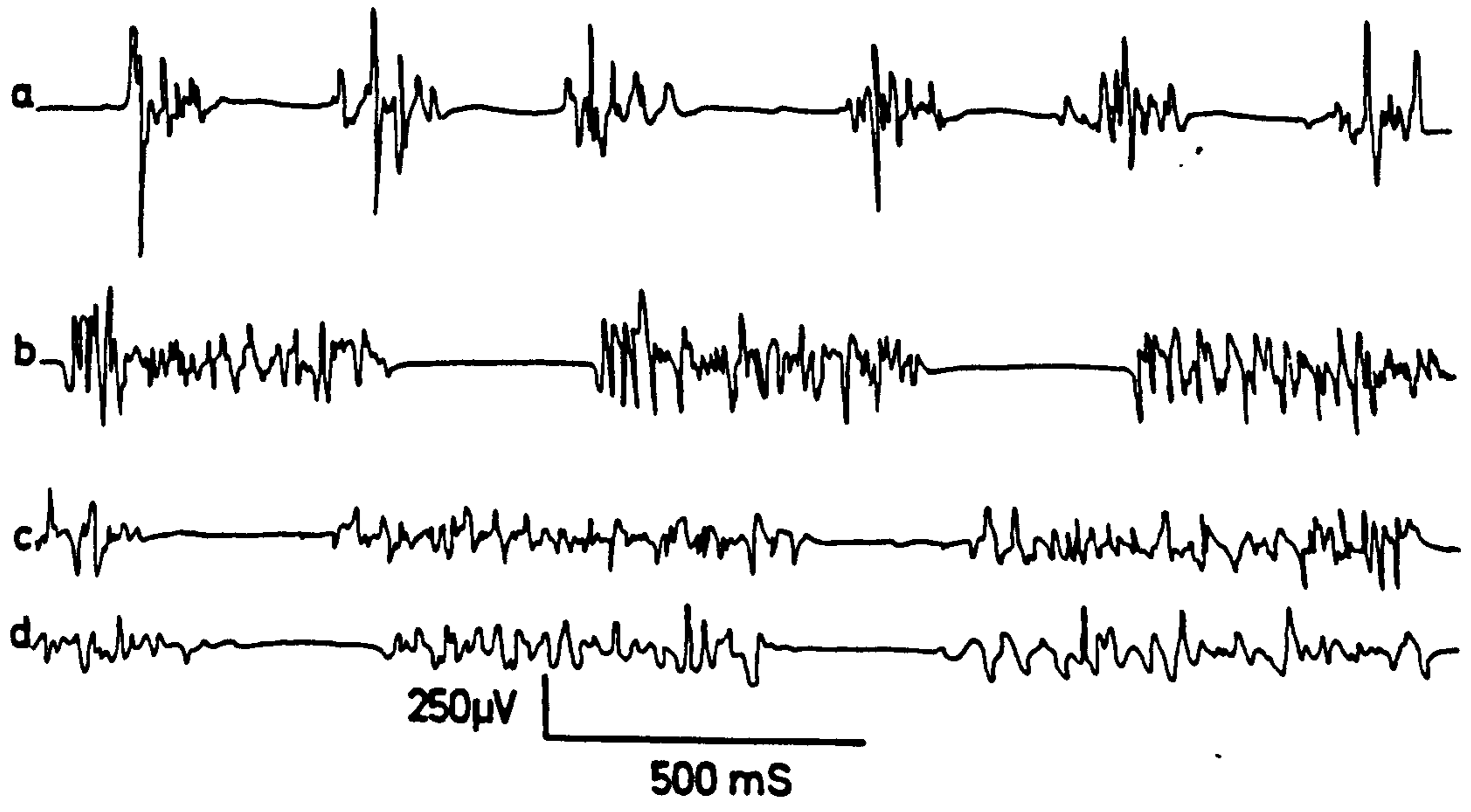
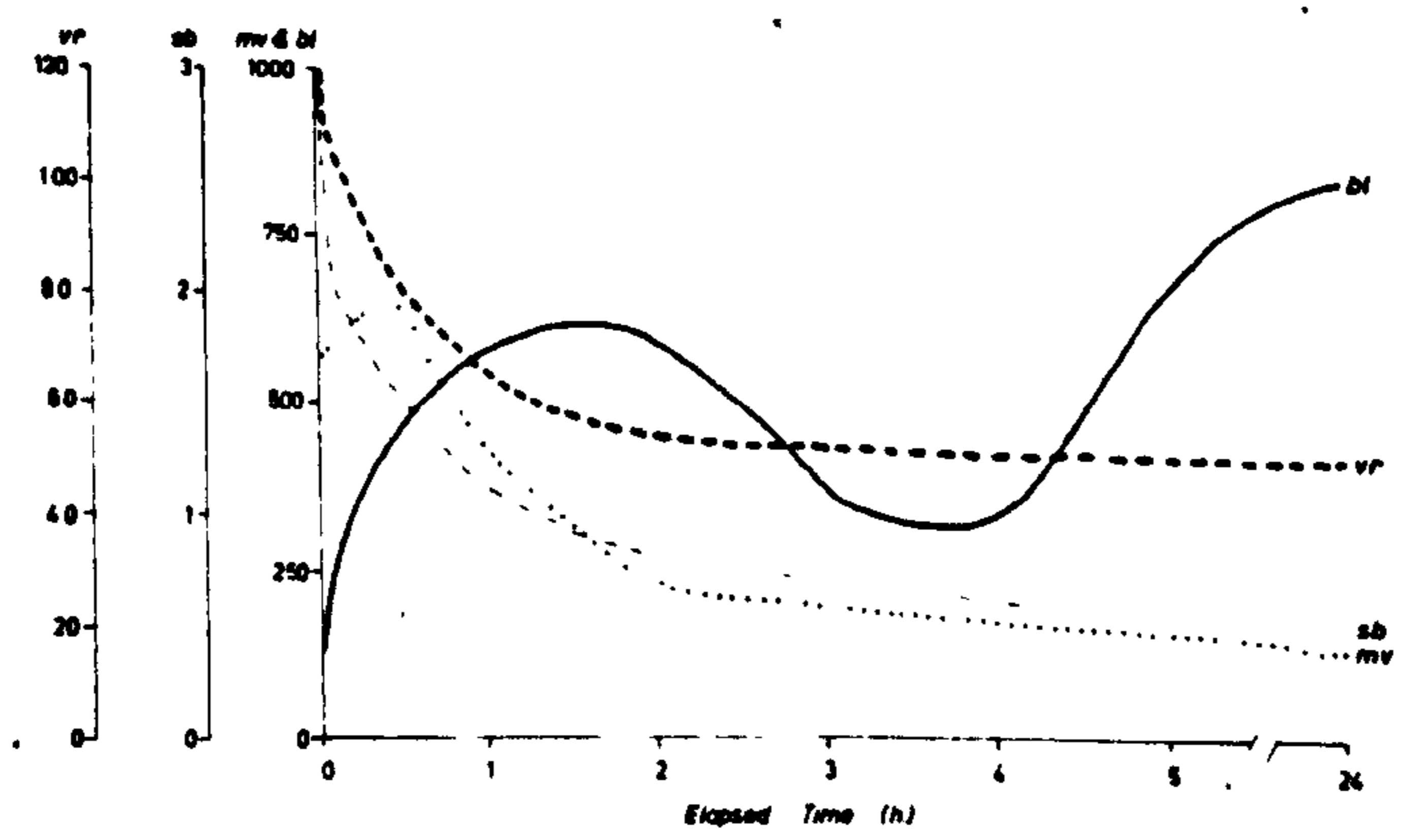


FIG 2.21



frequency has declined to 75-80c.p.m. and the EMG train is extended to 500mS. Many more m.u.p's are visible now, generally >60 per burst. At + 60 mins, the burst length is increased to around 600mS though the ventilation frequency is reduced to 60c.p.m. The mean peak amplitude has also decreased to <450 μ V and there is a change in the shape of the EMG train envelope; it has become rectangular in outline and there is a tendency for m.u.p's in the middle of the burst train to be smaller than elsewhere.

At +120 minutes the train length is now 680mS with the ventilation rate steady at 60cpm. The mean peak amplitude has declined to <250 μ V. Examination of the higher speed traces shows that the number of m.u.p's has altered and also that their form has changed. There are now generally <55 m.u.p's per burst and there is a predominance of longer duration m.u.p's, some of which are as long as 30mS contrasting with the majority of 10mS earlier.

At +240 minutes the ventilation frequency is again lowered to 50c.p.m. and a slight reduction in burst length to 600mS noted. The peak amplitude is consistently <250 μ V but a considerable reduction in the number of m.u.ps to <40. They are predominantly of long duration potentials.

In the sequence of experiments made for undertaking a frequency analysis, measurements were made at +24 hours. A low ventilation frequency of 50 c.p.m. was noted but the EMG trains are now 850mS long composed of <40m.u.ps, which are obviously widely spaced, and are of long duration with a consistently uniform amplitude of \approx 200 μ V.

The foregoing pattern was consistently found in all the experiments carried out.

Fig. 2.21 shows diagrammatically the relationship between ventilation rate, m.u.ps/mS, EMG burst length and peak amplitude of the EMG. Note that apart from an initial plateau phase in the amplitude curve, there was a common tendency to logarithmic decay. There was a contrasting

pattern in the curve for the EMG burst length. The latter tended to increase at first to a plateau, decrease, then stabilise at a higher (longer) value. There is an obvious levelling out of the other three variables (which are more important in the reliable function of the EMG transmitter) by +120 minutes and apart from the burst length, stability is, for practical purposes, achieved at about +300 minutes.

2.16 Effect of Anaesthesia and Recovery on the EMG Frequency Spectrum

The results of the frequency analysis in terms of the mean voltage contribution in per 10Hz bandwidth are given as cumulative curves in Figs. 2.22 and 2.23

Key to results

1. Brown Trout under light Benzocaine anaesthesia.
2. " " after 10 mins recovery
3. " " " 12 " "
4. " " " 15 " "
5. " " " 30 " "
6. " " " 60 " "
7. " " " 120 " "
8. " " " 180 " "
9. " " " 240 " "
10. " " " 300 " "
11. " " " 24 hours "
12. Perch (Perca fluviatilis) under light Benzocaine anaesthesia
13. Rainbow Trout (Salmo gairdnerii)
under " " "
14. Pike (Esox lucius) " " " "
15. Saithe (Pollachius virens) " " " "

From inspection of Fig. 2.22 it can be seen that there are no obvious differences between the six curves displayed. The curve (line 1) for

Fig. 2.22

Brown Trout Frequency Spectra from a. mandibulae EMG.

Recordings made at intervals following anaesthesia of one hour. There is a tendency for the spectrum to 'sag' during the later recordings.

Fig. 2.23

EMG Frequency Spectra from five Teleost species under light anaesthesia. Apart from the Saithe, all species show remarkably similar spectra.

Figs. 2.24 - 2.26

Linear regression plots of pooled EMG spectra

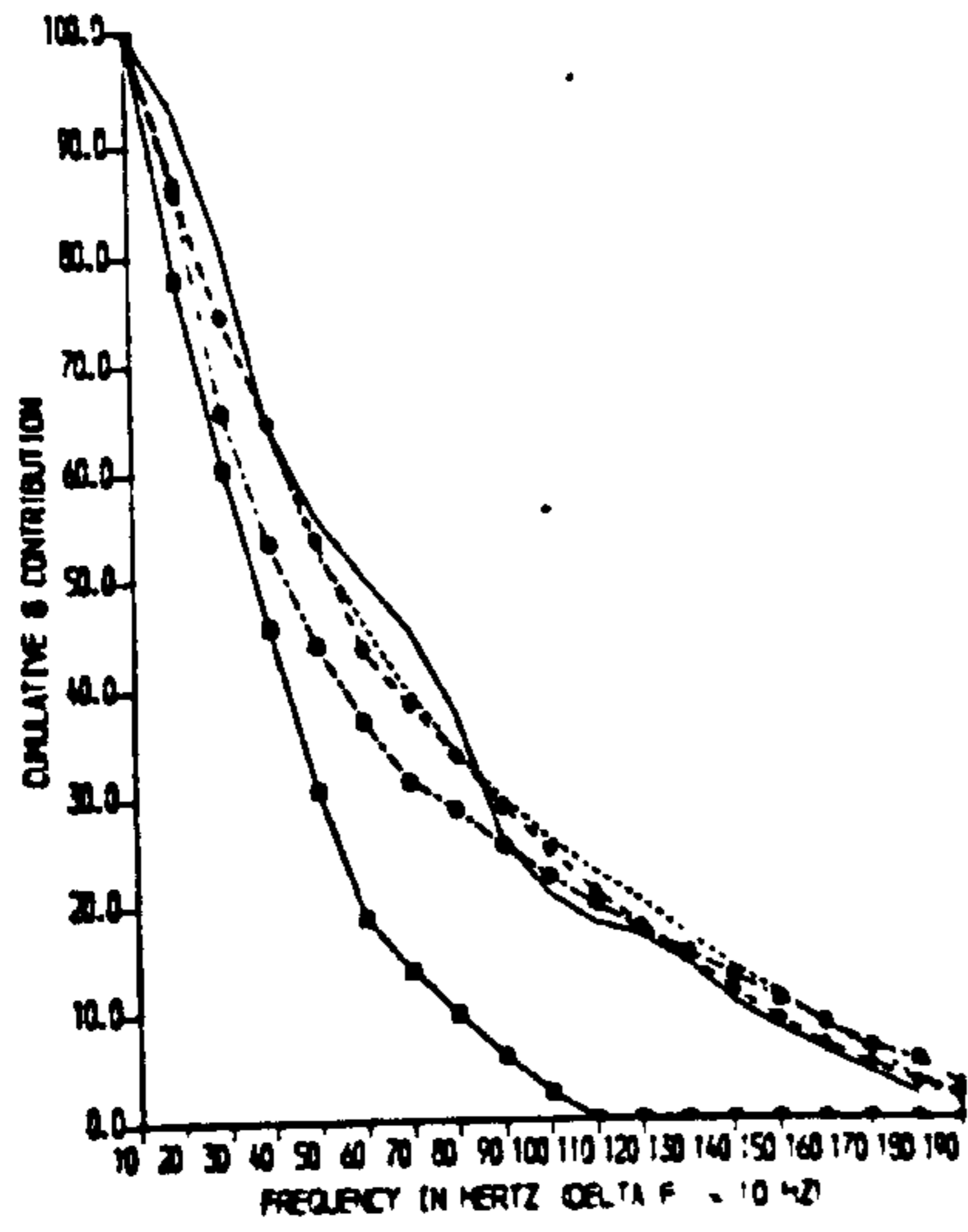
2.24 raw data

2.25 log 10y transform

2.26 In y transform

Although significant none of these regressions are a good fit to the data.

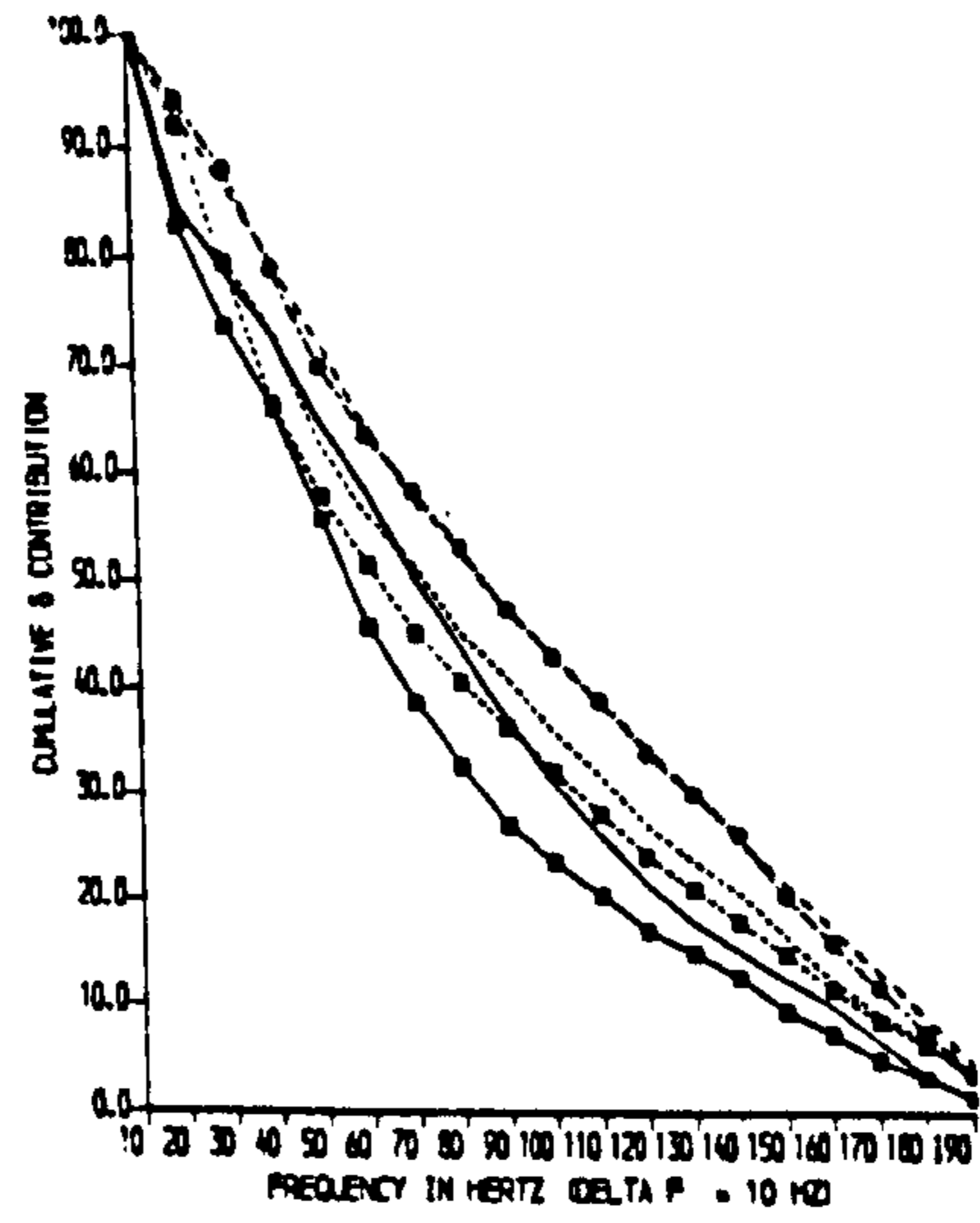
FIG 2.23



COMPARISON OF THE FREQUENCY SPECTRUM OF THE ADDUCTOR MANDIBULAR ENG FROM 5 SPECIES OF TELEOST FISH, UNDER LIGHT BROMOCALINE ANAESTHESIA (30 NEGATIVE) FREQUENCIES EXPRESSED AS A CUMULATIVE % CONTRIBUTION

— LINE 1 BROWN TROUT
 - - - LINE 2 PERCH
 ···· LINE 3 RAINBOW TROUT
 ○··· LINE 4 PIKE
 ●··· LINE 5 SALMON

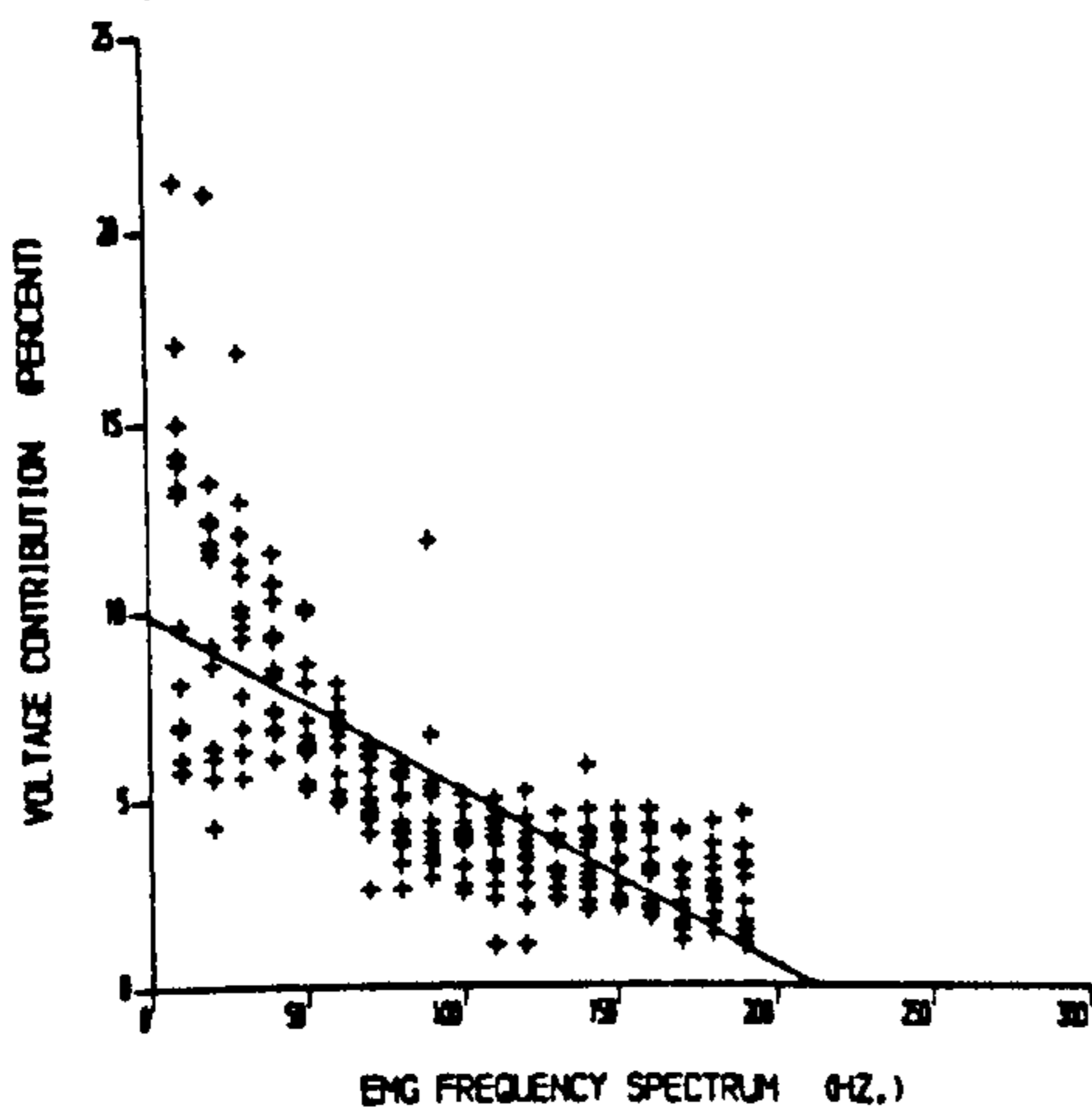
FIG.2 22



BROWN TROUT, EFFECT OF BROMOCALINE ANAESTHESIA UPON THE FREQUENCY SPECTRUM OF THE ADDUCTOR MANDIBULAR ENG. TIMES STATED ARE FROM THE BEGINNING OF THE RECORDING PERIOD FOLLOWING ONE HOUR ANAESTHESIA

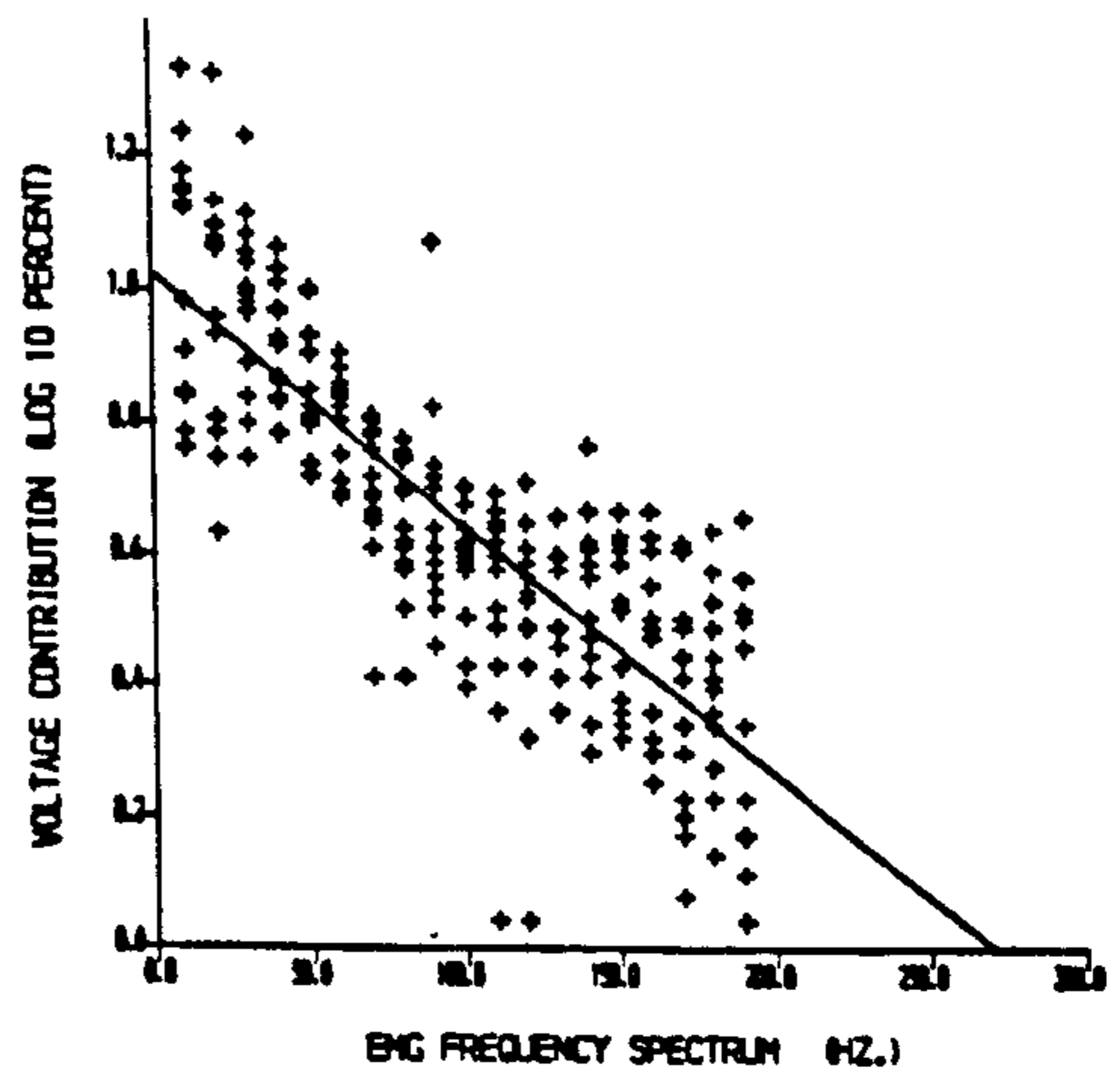
— LINE 1 -10 MINS
 - - - LINE 2 -30 MINS
 - - - LINE 3 -60 MINS
 ○··· LINE 4 -120 MINS
 ●··· LINE 5 -300 MINS
 ●··· LINE 6 -24 HRS

FIG 2.24



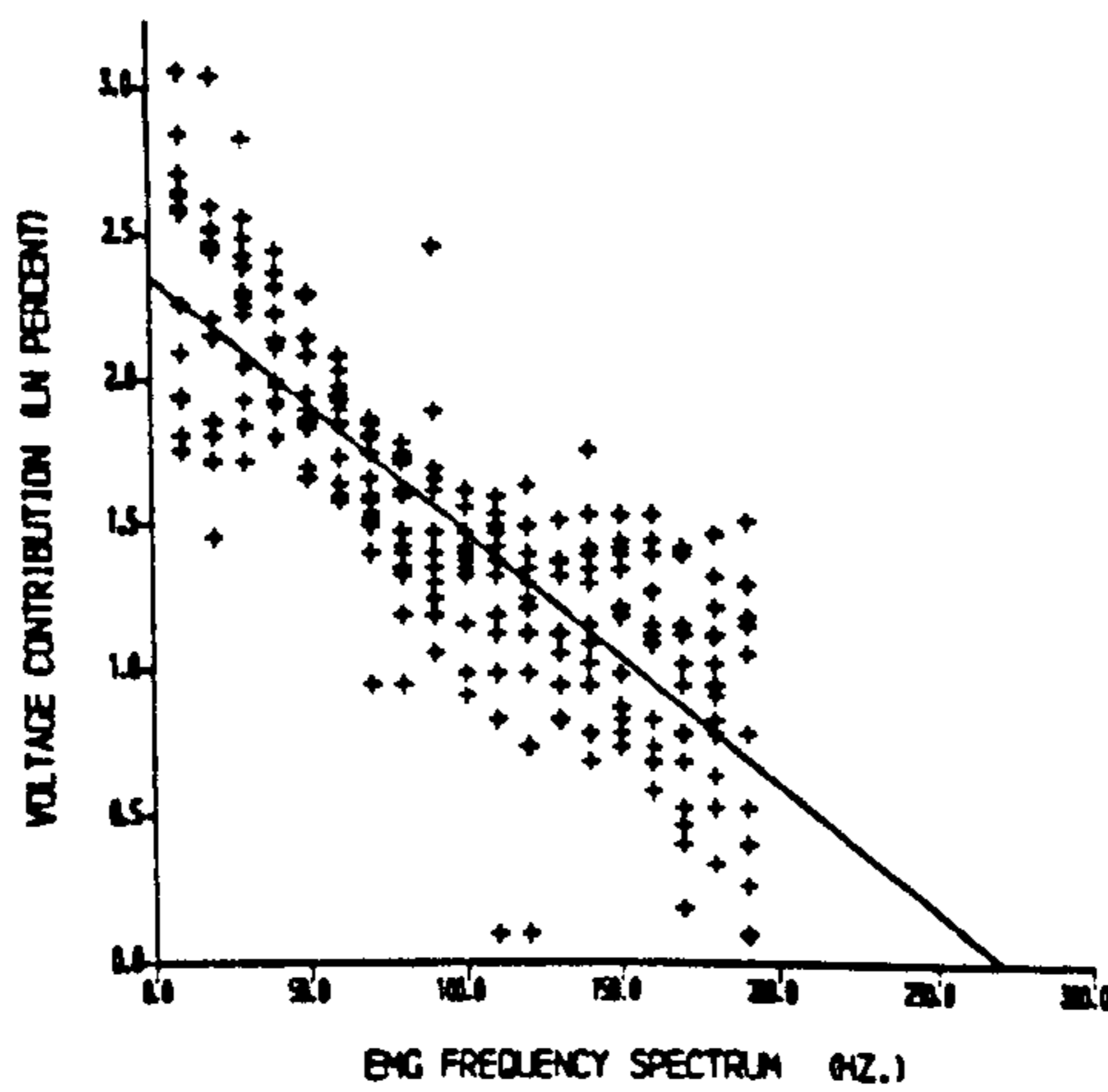
LINEAR REGRESSION ANALYSIS OF E.M.G. FREQUENCY SPECTRUM.
 SCATTERPLOT AND BEST FIT LINE.

FIG 2.25



LINEAR REGRESSION ANALYSIS OF E.M.G. FREQUENCY SPECTRUM.
 SCATTERPLOT AND BEST FIT LINE.

FIG 2.26



LINEAR REGRESSION ANALYSIS OF E.M.G. FREQUENCY SPECTRUM.
 SCATTERPLOT AND BEST FIT LINE.

10 minutes after recovery is approximately median whilst some of the later curves (+300 mins. +24 hours (lines 5 and 6) show a "sag" between 50-100Hz although the curve for 120 mins (line 4) is almost indistinguishable for that at 60 minutes (line 3).

Reference to Fig. 2.2² shows that although not significantly different, the curves for 1,5,6 are shifted to the left of curves 2,3,4. This would indicate that the tendency is for the spectra in anaesthesia and recovery to contain a larger proportion of lower frequencies.

Linear regression analysis of the pooled frequency spectrum data was carried out using program "REGRI". A highly significant negative correlation coefficient was obtained between frequency and EMG voltage contribution. Details are set out in Table 2.1 below and the regression plot displayed in Fig.2.2₄.

Table 2.1

$$r = 0.760 \quad a = 9.910 \pm 0.280 \quad b = 0.047 \pm 0.002$$

$$t_{(n-2)}(r) = 19.00 \quad F \text{ (for ANOVA)} = 361.174$$

$$\text{sig}(r) = *** \quad \text{sig}(F) = ***$$

Transformation of the data using the log or ln transform facilities of "REGRI" gave a more highly significant correlation of which the most significant was by transforming $y := \log_{10}(y)$. The transformed regression plot is displayed in Fig. 2.2₅ and the regression statistics shown below in Table 2.2.

Table 2.2

$$r = -0.821 \quad a = 1.024 \pm 0.019 \quad b = -0.004 \pm 0$$

$$t_{(n-2)}(r) = 23.365 \quad F(\text{For ANOVA}) = 544.064$$

$$\text{sig}(r) = *** \quad \text{sig}(F) = ***$$

The significance of the difference between the two regressions above was tested using program "REGCOMP", results given below in Table 2.3.

Table 2.3

$$F(a) = 20.613$$

$$t(b) = 11.373$$

$$\text{sig}(F) = ***$$

$$\text{sig}(t) = ***$$

The two regressions are significantly different, therefore.

It was, however, noted that much of the correlation was due to clustering of y-values with increasing frequency. (Reference to Table 2.4 will show that the Standard Error tends to decrease with increasing frequency). Secondly, the shapes of the cumulative frequency curves (Figs. 2.22-23) suggest that the distribution might better be described by a curvilinear relationship of the form $y = a.x^b$

To simplify display, the data was formed into three groups:=

Group 1 comprising Brown Trout 0 to +60 mins.

Group 2 comprising Brown Trout +120 mins. to +24 hours.

Group 3 comprising the other species

Values for a and b are tabulated below:=

Table 2.5 Calculated values of a and b coefficients for groups 1 - 3.

	a	b
Group 1	83.392	-0.663
Group 2	33.024	-0.489
Group 3	179.239	-0.862

These values were read by a short FORTRAN program which calculated y values at 1Hz intervals. The curves so formed were plotted in Figs. 2.27 - 2.29 along with the relevant observed data giving a fairly good fit. Fig. 2.30 shows the three curves compared. It will be noted that from about 50Hz upwards, they are essentially parallel and similarly from about 40Hz downwards. The break of slope appears at their intersection at the 6% level giving values at this point for

$$\text{Group 1} = 53\text{Hz},$$

$$\text{Group 2} = 43\text{Hz},$$

$$\text{Group 3} = 52\text{Hz}$$

Examination of the curves fitted with the data shows that it may be

Figs 2.27 - 2.29

Curve-fitting of EMG spectra to power function

2.27 Group I

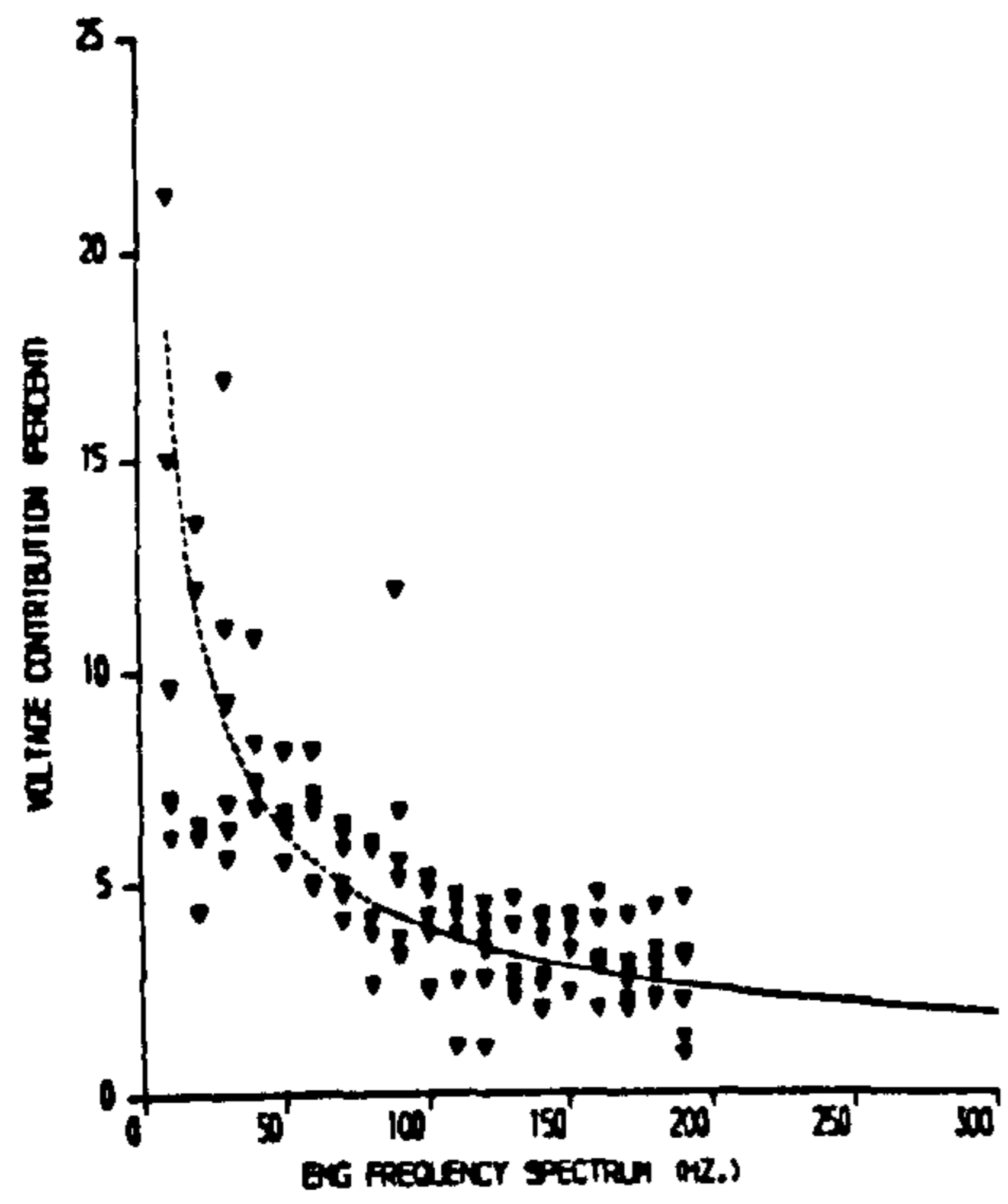
2.28 Group II

2.29 Group III

Fig. 2.30

The three fitted curves from Figs. 2.27 - 2.29 extracted and compared. Note that the break of slope is around 60Hz in all cases and that the bulk of the EMG spectra lies to the left of this point.

FIG 2.27



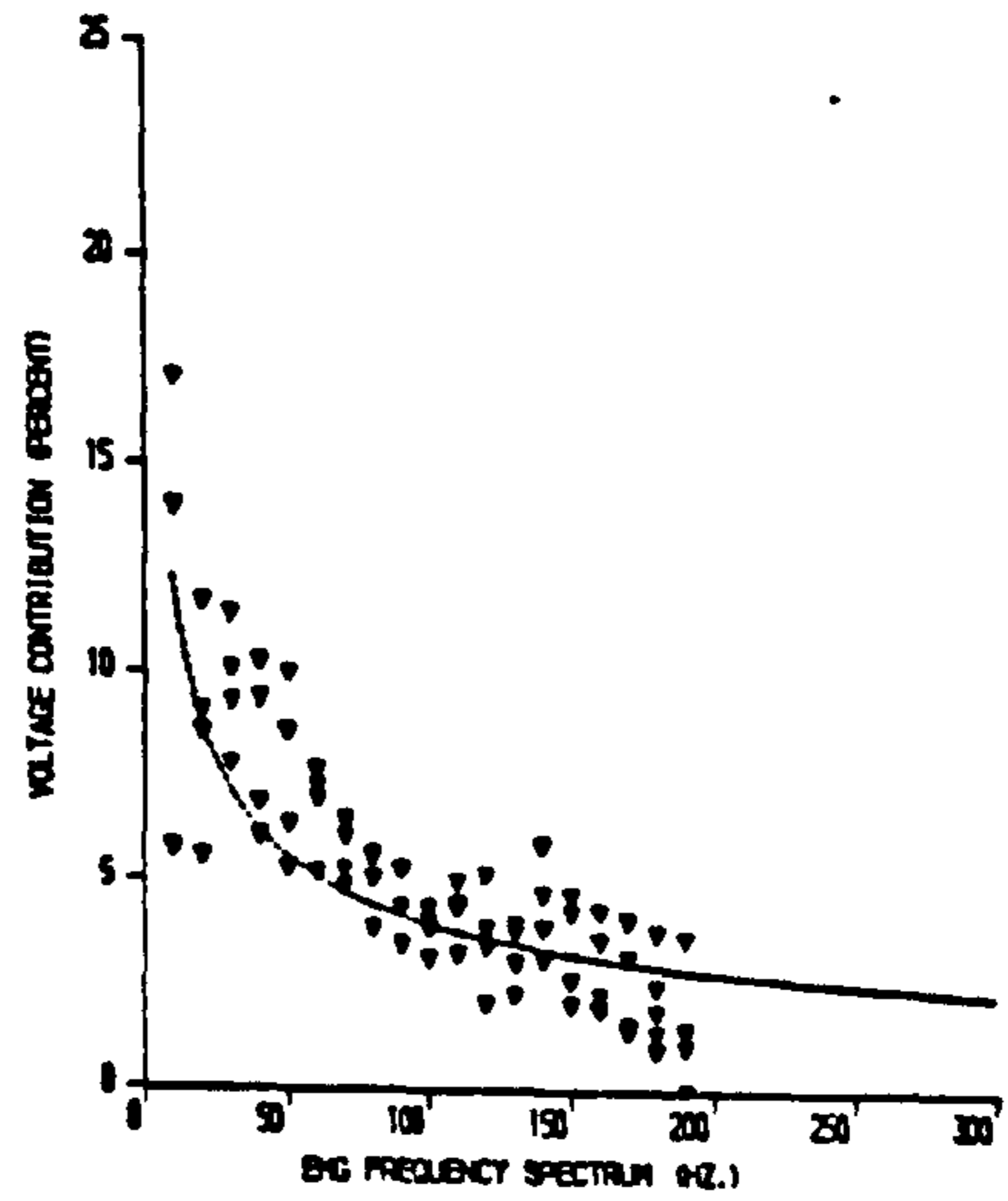
CURVE FIT OF ENG DATA TO $Y=Ae^{-Bx}$

◆ LINE 1 DATA
 - - - LINE 2 CURVE

GROUP 1

1 $A=83.392$ $B=0.663$

FIG 2.28



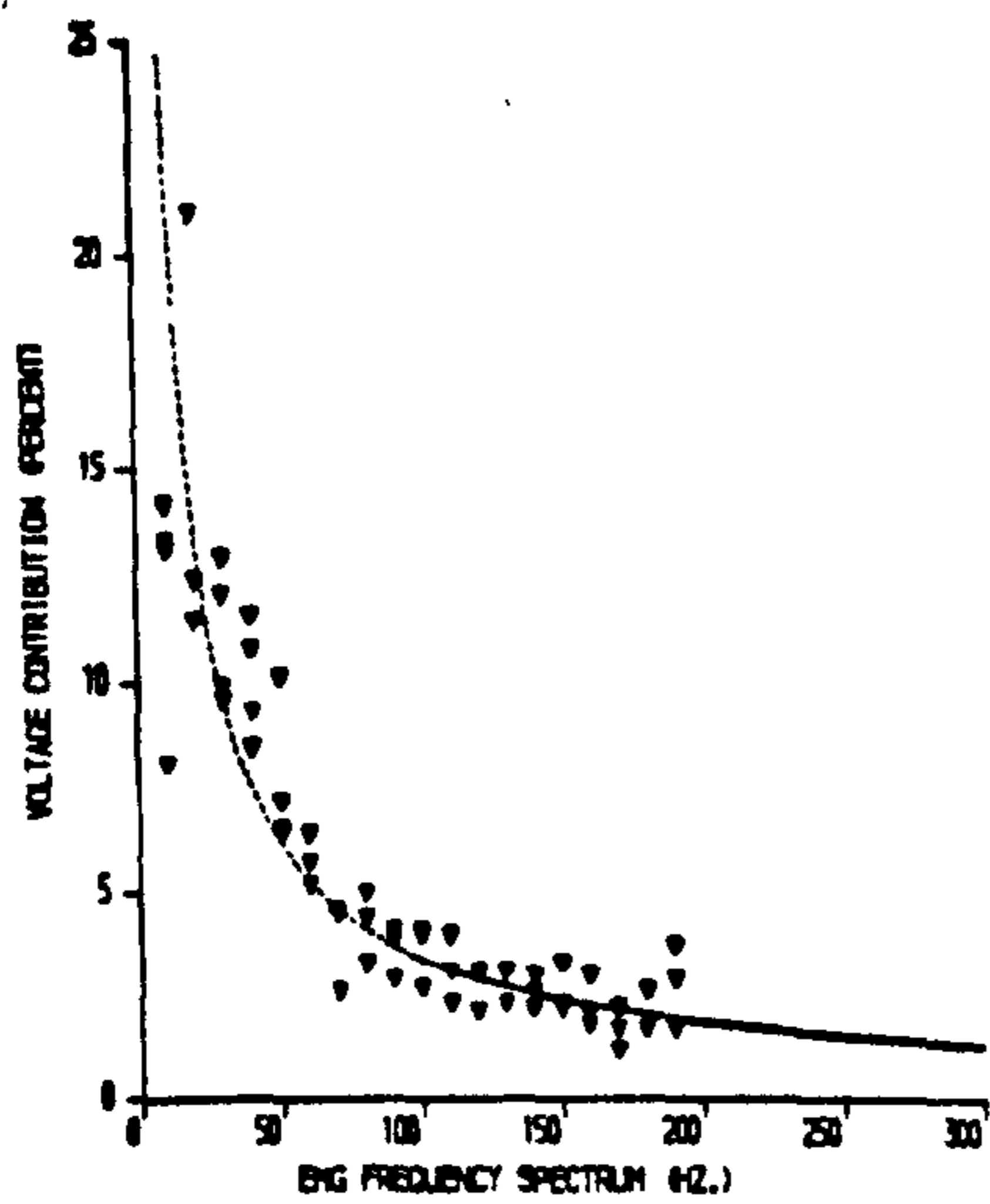
CURVE FIT OF ENG DATA TO $Y=Ae^{-Bx}$

◆ LINE 1 DATA
 - - - LINE 2 CURVE

GROUP 2

2 $A=38.024$ $B=0.489$

FIG 2.29



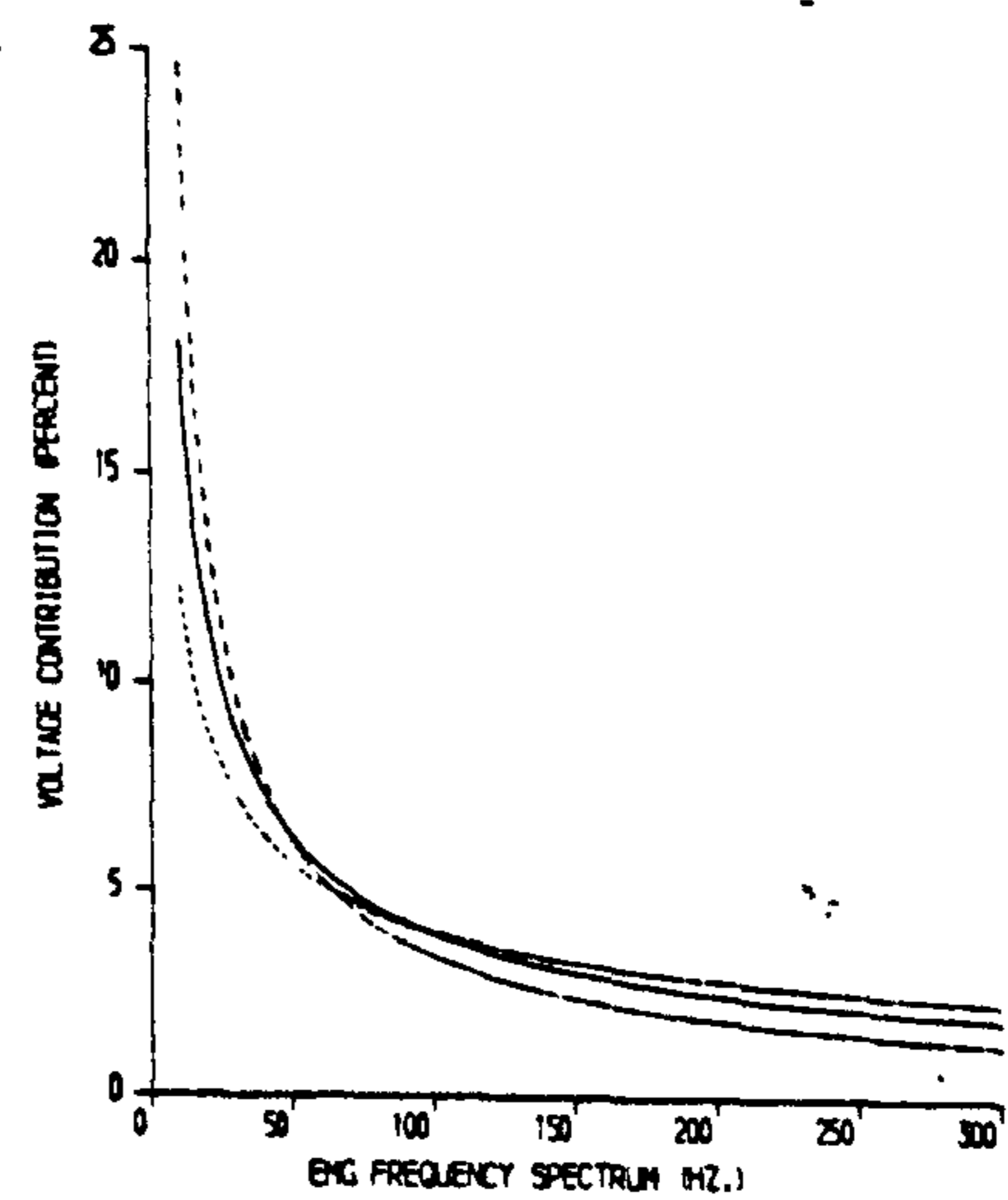
CURVE FIT OF ENG DATA TO $Y=Ae^{-Bx}$

◆ LINE 1 DATA
 - - - LINE 2 CURVE

GROUP 3

3 $A=179.239$ $B=0.862$

FIG 2.30



CURVE FIT OF ENG DATA TO $Y=Ae^{-Bx}$

— LINE 1 GROUP 1
 - - - LINE 2 GROUP 2
 - - - LINE 3 GROUP 3

1 $A=83.392$ $B=0.663$

2 $A=38.024$ $B=0.489$

3 $A=179.239$ $B=0.862$

said with confidence that above 100 Hz, a particular frequency band will contribute <5% to the total EMG voltage.

Examination of Fig. 2.23 which compares the Frequency spectra of the other species with that of B the anaesthetized Brown Trout as a baseline, shows that a large measure of variation is introduced due to the data from the Saithe (line 5).

2.17 Indirectly stimulated EMG preparations

During this series of experiments it became obvious that the preparation was unsuitable for studies involving tetanic stimulation because couplings between moving head parts (Ballintijn and Hughes, 1965) tended to shift the head relative to the fine stimulating electrodes even when clamped and this made it difficult to maintain a constant stimulus. Fig. 2.31 shows an example of tetanic stimulus of a perfused preparation, with intra cranial stimulation of the nerve. It can be seen in this case that mosaic twitch units fatigue rapidly but overall tension is maintained by red units. Only responses to single shocks will be considered here, therefore.

In most cases, recordings from electrodes in both red and mosaic fibres separately, or in single electrodes spanning both showed that typically there was a large amplitude biphasic excursion followed by a smaller and slower monophasic wave. The latter is not due to capacitance in the preamplifier as DC coupling was used throughout, (Fig. 2.32). Recordings over threshold always gave a very large spike EMG from the mosaic portion. (Fig. 2.33)

In the absence of a high voltage isolated stimulator, it was found that by stimulating at a pulse width of 50 μ S and varying the intensity around threshold for the preparation, some differences in the EMG response were evident. At threshold (Fig. 2.32) in both red and mosaic portions, no 'spikes' are present. Increasing the stimulus to 2 x threshold causes the appearance of such 'spikes', of 10 - 15mS duration, though the monophasic potential seen at threshold is still present. Fig. 2.34 shows the result of recording the EMG from red and mosaic fibres simultaneously with the tension record: At threshold a monophasic potential is associated with a slow rising tension increase. At 2 x threshold, a high amplitude EMG

Fig. 2.31

Brown Trout prepared for indirect stimulation of adductor mandibulae. Repetitive stimulation at 5Hz, 20V leads to rapid fatigue of the mosaic fibres as evidenced by the decline in twitch response. Overall tension above baseline is due to red muscle fibres.

Fig. 2.32

Brown Trout prepared for indirect stimulation:

T = red muscle EMG electrode

M = Mosaic muscle electrode

a) at threshold stimulation

b) at twice threshold stimulation

The monophasic potential present in the red muscle EMG is temporally preceded by the faster spike of the mosaic fibres.

Fig. 2.33

Brown Trout prepared for indirect stimulation:

Upper trace : EMG from both red and mosaic muscle

Lower trace : EMG from mosaic muscle only

Stimulation at twice threshold produces a monophasic potential in the red muscle EMG preceded by a fast spike correlated temporally with the mosaic muscle EMG. This implies that the red muscle is innervated by slower nerve axons.

FIG 2.31

50g Brown trout: adductor mandibulae indirect stimulation

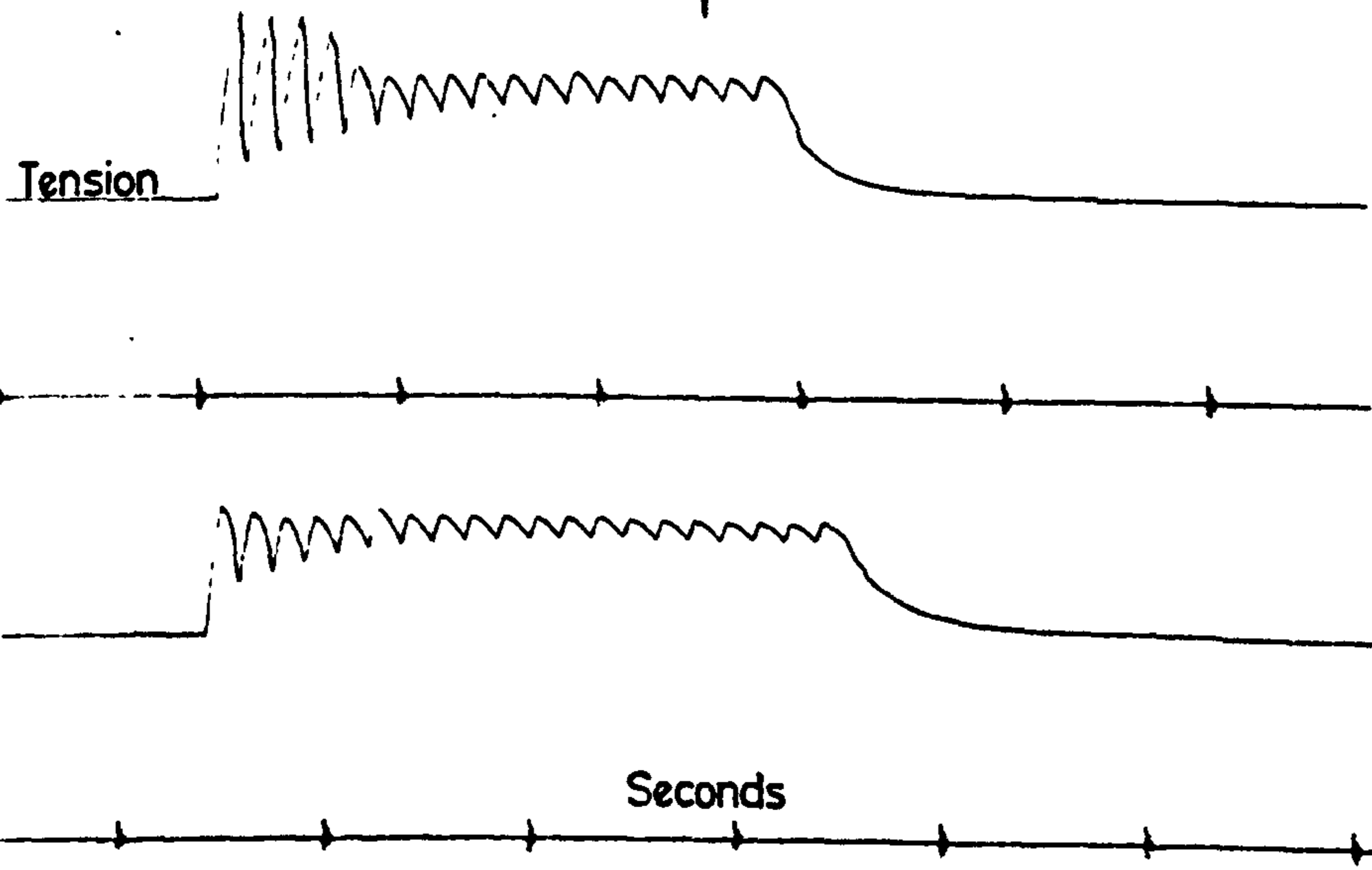


FIG 2.32

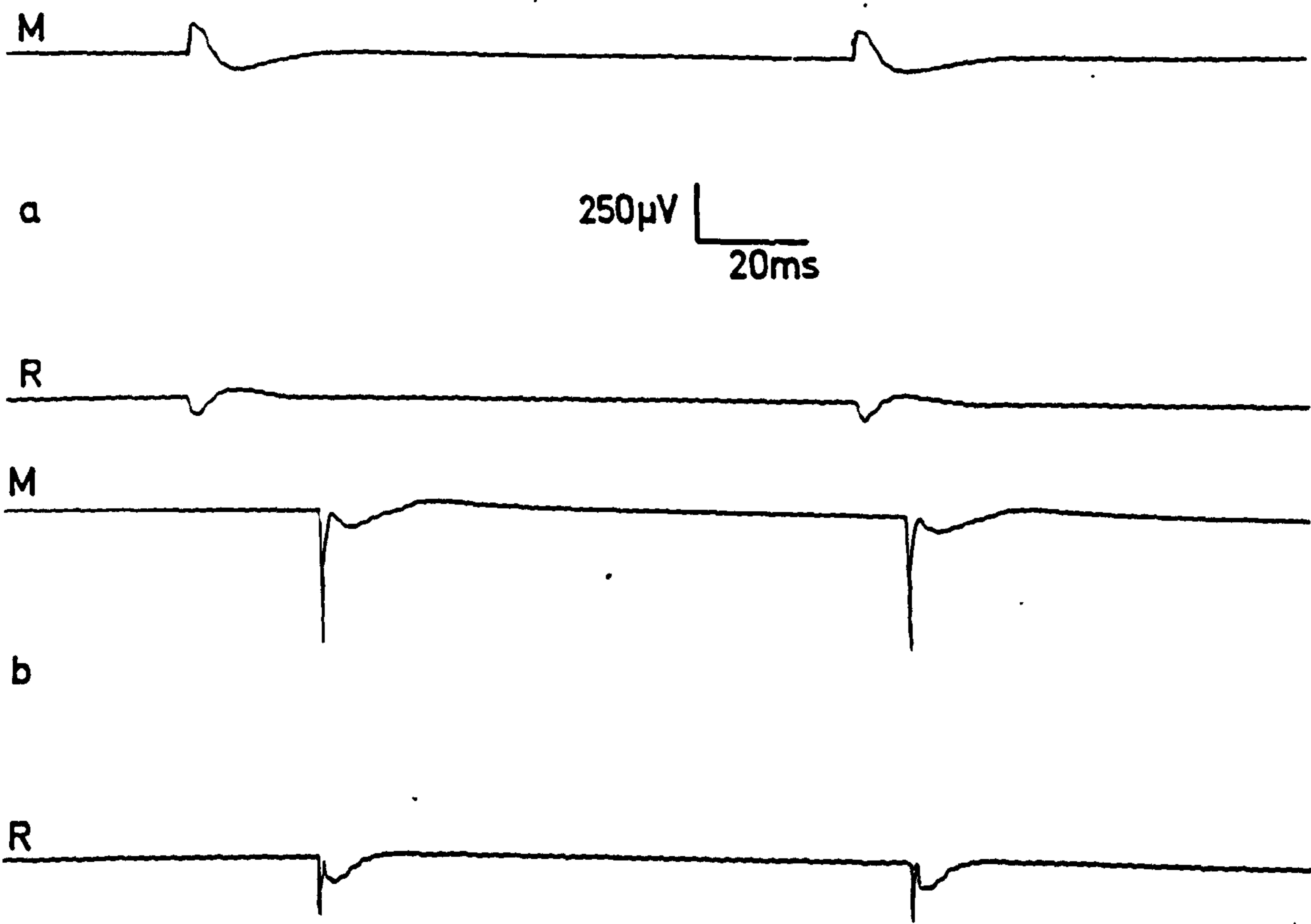
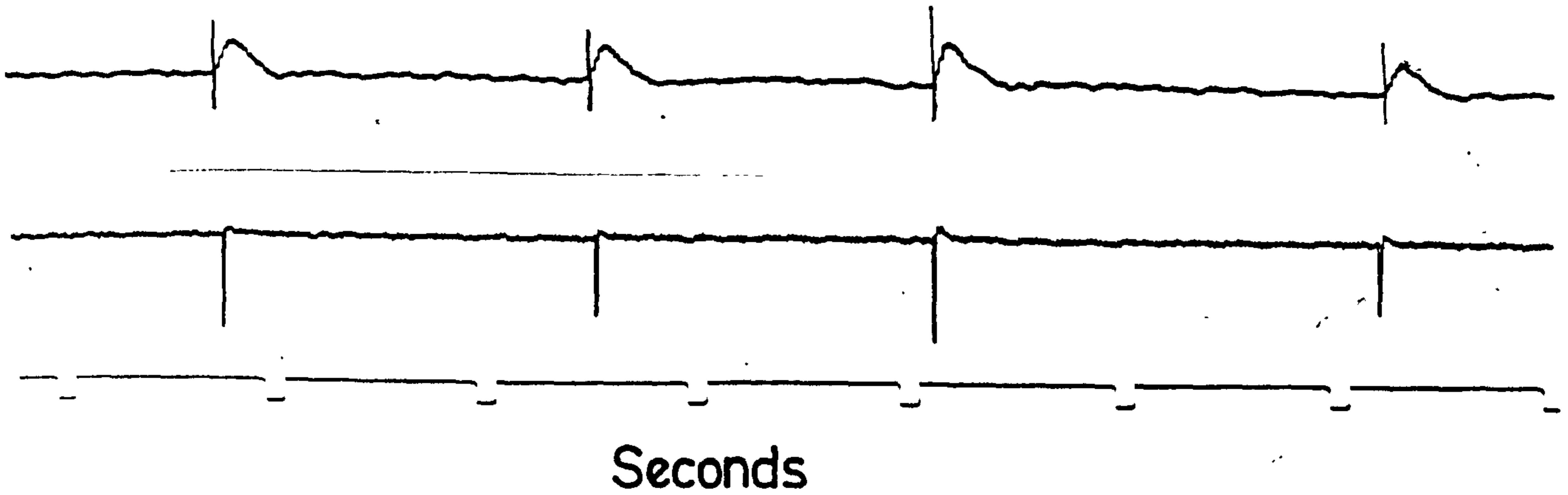


FIG 2.33



is associated with a fast, and large rise in the twitch tension. Plate II.4 shows a recording from an oscilloscope: at threshold a monophasic potential is correlated with the small rise in tension. Increasing to 2 x threshold gives the large biphasic spike and a fast tension rise. Plate II.5 shows the effect of stimulating the preparation in 0.5V steps from below threshold: the first three steps over threshold (3.0 - 4.5V) cause the slow rises of tension. Increasing the stimulus further causes a jump in twitch amplitude and rise time. Plate II. 6 shows the responses over this transition at faster recording speed; there is a noticeable shift in the latency of the tension development. The jump in twitch tension is also seen in Fig. 2.35 where the stimulus intensity was increased slightly between each pulse; at 7.5V there was a two-fold rise in twitch tension and a corresponding change in the EMG.

In two preparations the effect of Decamethonium blockade was tried. Following the onset of a block by 200 μ g Decamethonium, 0.1 mg of Neostigmine Sulphate was successful in restoring the block for a short while though it faded afterwards (Fig. 2.36). In the second experiment the application of a 100 Hz tetanus restored transmission and the twitch to almost 2/3 of the pre-block level. In this case this dose of Decamethonium was only 100 μ g.

Fig. 2.34

Rainbow Trout prepared for indirect stimulation :

M = Mosaic Muscle EMG

R = Red muscle EMG

a) at threshold stimulation

b) at superthreshold stimulation

in b) the rise time of the red muscle EMG is never as rapid as that of the mosaic muscle which is additionally polyphasic.

Fig. 2.35

Brown Trout prepared for indirect stimulation :

upper trace : EMG

lower trace : tension

At 1Hz stimulation Frequency, the stimulus intensity is gradually increased until at 7V (arrowed), there is a sudden jump to maximal twitch tension produced. This shows the recruitment of mosaic phasic fibres due to the stimulation of larger-diameter motor nerve fibres.

Fig. 2.36

Rainbow Trout prepared for indirect stimulation.

In both a) and b) Decamethonium Iodide (500 $\mu\text{g}/\text{kg}$) has been administered I.M.

a) Upper trace : EMG

b) Lower trace : Tension

Neuromuscular block has caused the decline of the EMG and twitch response. Injection of Neostigmine Sulphate at arrow causes restoration of transmission due to AChE inactivation.

b) Tension trace only. At arrow, a 100 Hz tetanus which produces an excess of ACh is successful in restoring the twitch response.

FIG 2.34

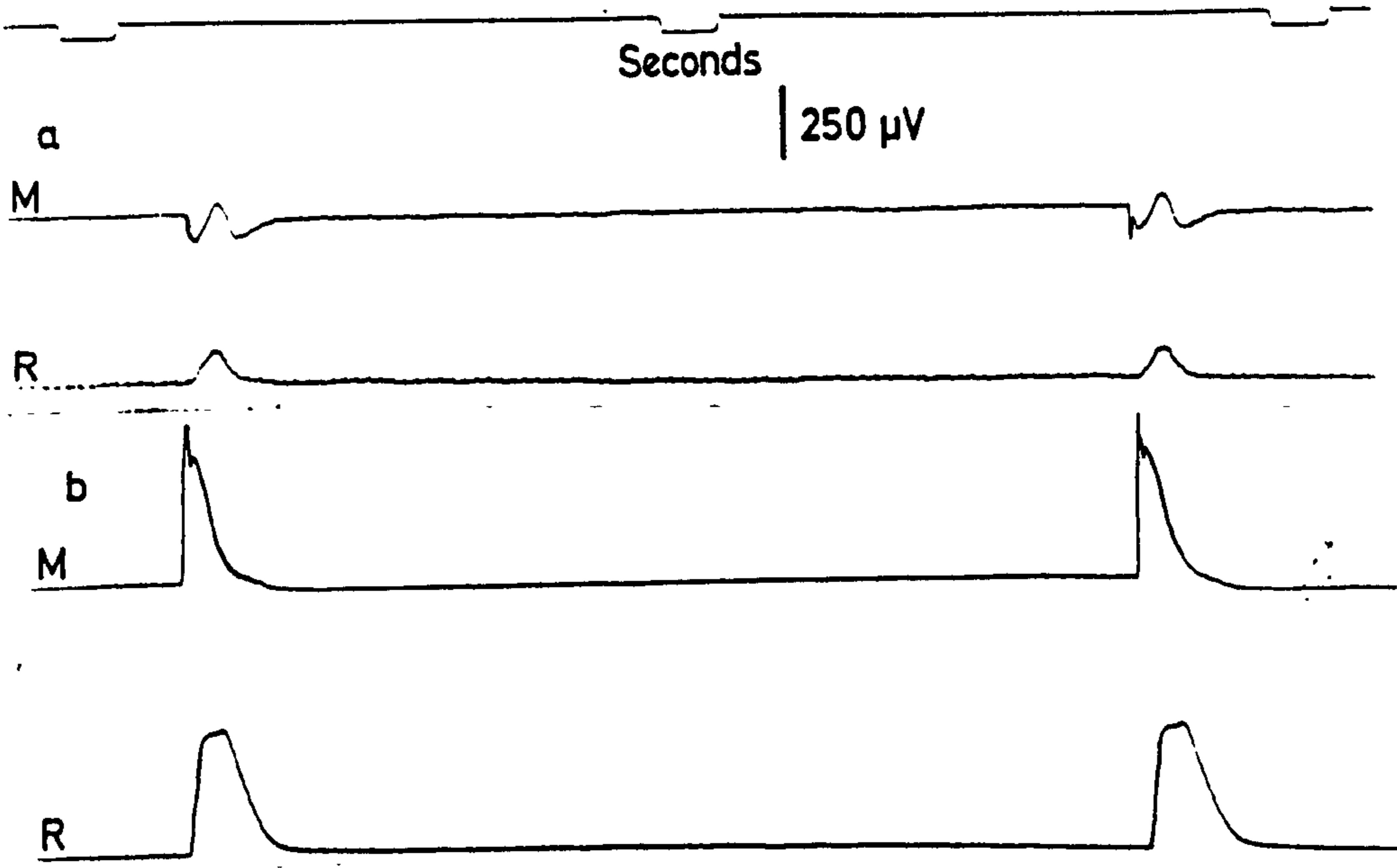


FIG 2.35

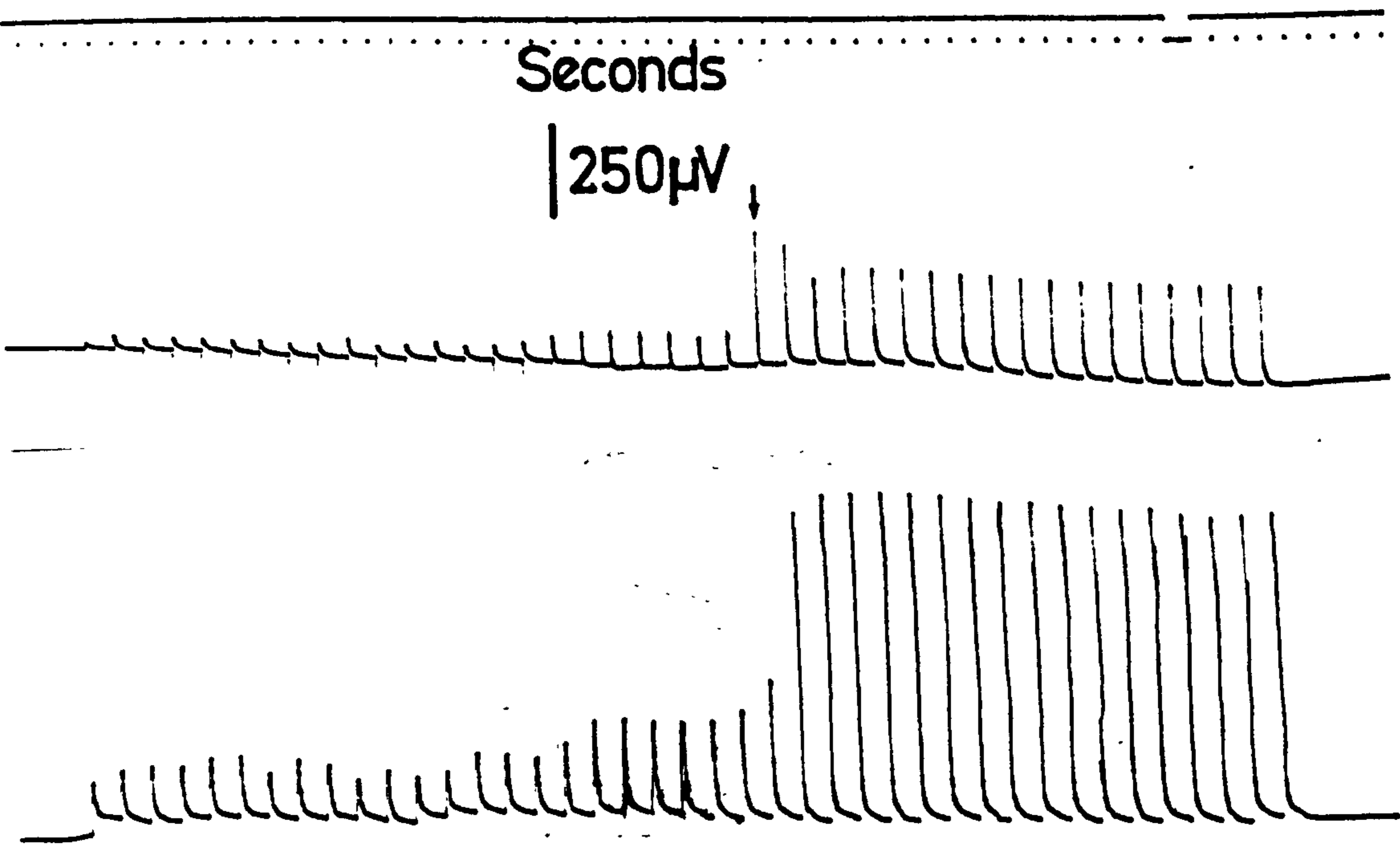
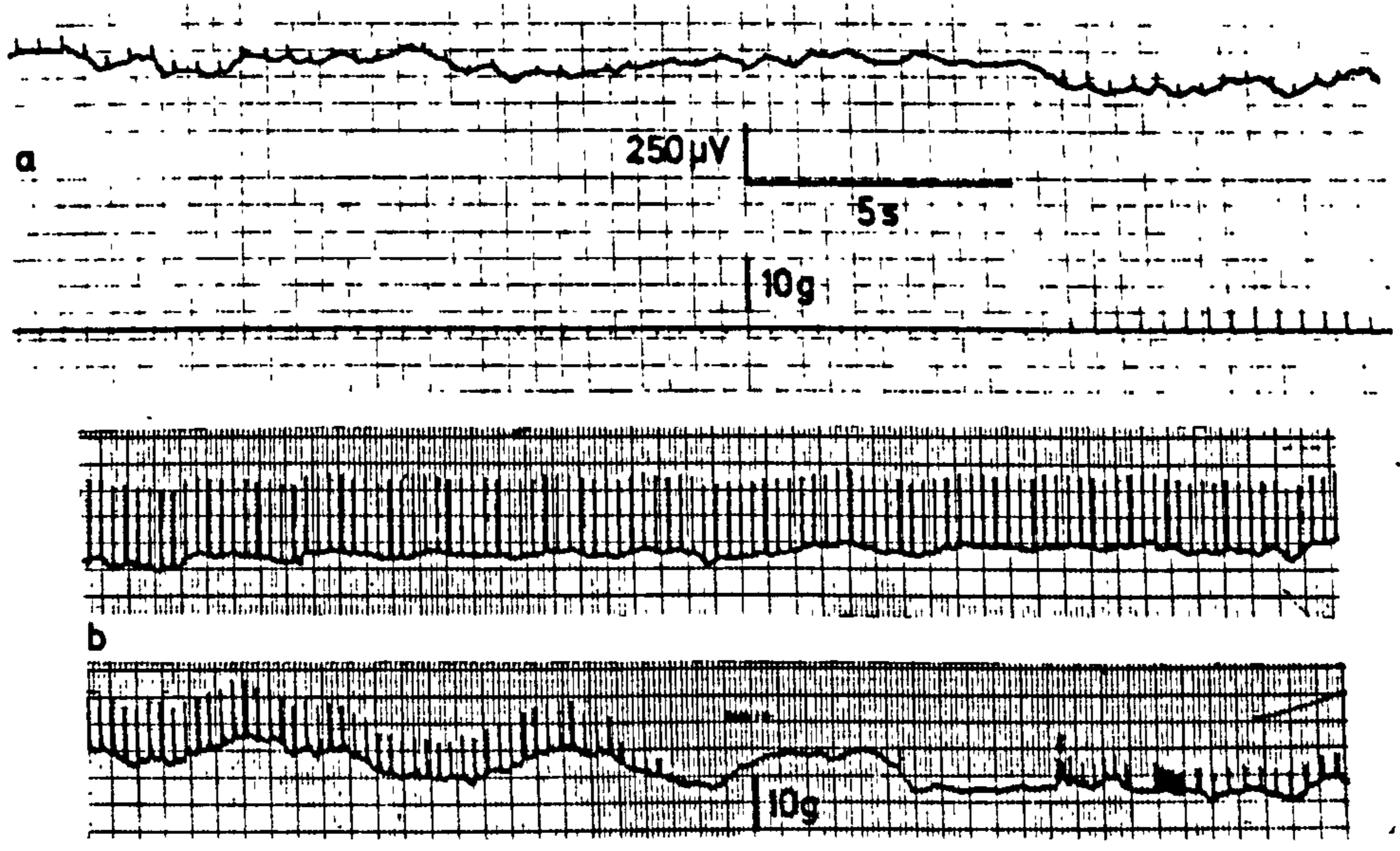


FIG 2.36



2.18 Investigations into effect of nerve block on EMG

In spontaneously ventilating fish from which EMG recordings were made, in no case was any significant difference noted subsequent to the infiltration of the large branch of the Vth nerve with Xylocaine, as it courses down the anterior margin of the adductor mandibulae (Fig. 2.37) Following infiltration at site 2 (Fig. 2.4) there is a very rapid diminution in the amplitude of the EMG and also in the number of M.U.Ps present (Figs. 2.38 - 2.39) Note that in Fig. 2.39 a slight decrease in the contralateral EMG has occurred.

In fish anaesthetized with Alphaxalone/Alphadolone Acetate, 36mg/kg, section or infiltration with Xylocaine of the nerve branch at site 1 had no effect on the twitch tension or compound EMG (Fig. 2.40) produced by indirect stimulation. The stereotaxic infiltration of site 2 (Fig. 2.41) caused an immediate decline in both.

2.19 Effects of Neuromuscular Blocking Drugs

In order to validate that potentials recorded as 'motor unit potentials' did originate from cholinergic muscle fibres and not, as has been suggested elsewhere (Bone, 1966), from the extracellular detection of end plate potentials and secondly, in order to establish a base line with which to compare the somewhat novel experiments with Guaiacol Glyceryl Ether (GGE), a series of experiments involving the non-depolarising neuromuscular blocking agent Gallamine Triethiodide (Flaxedil - May & Baker) were performed.

In spontaneously ventilating fish, following the injection of Gallamine, 3 mg/kg into the red lateral muscle, there is a rapid decline in the amplitude of M.U.Ps recorded from the adductor mandibulae. By + 8 mins after injection relaxation is complete (Fig. 2.42). This contrasts with the effects of a depolarising blocking agent such as Suxamethonium Chloride, of which an example

Fig. 2.37

EMG recording from adductor mandibulae red muscle (Rainbow Trout)
Upper trace : At arrow, the large nerve trunk is infiltrated at Site 1 with Xylocaine. (fig.2.4)
Lower trace : one minute later there is no appreciable effect upon the red muscle EMG.

Fig. 2.38

As fig. 2.37 : At arrow the muscle is infiltrated with Xylocaine at Site 2 (fig.2.4) leading to a rapid abolition in EMG activity in the red muscle fibre. Thus, the red fibres must be innervated by the small nerve trunks entering the inner medial surface of the muscle.

Fig.2.39

Simultaneous bilateral recording from Brown Trout adductor mandibulae red muscle fibres. At arrow, left adductor is infiltrated with Xylocaine at Site 2 causing rapid abolition of EMG activity. Right adductor continues normally demonstrating their effect is local and not due to central anaesthetic effect.

FIG 2.37

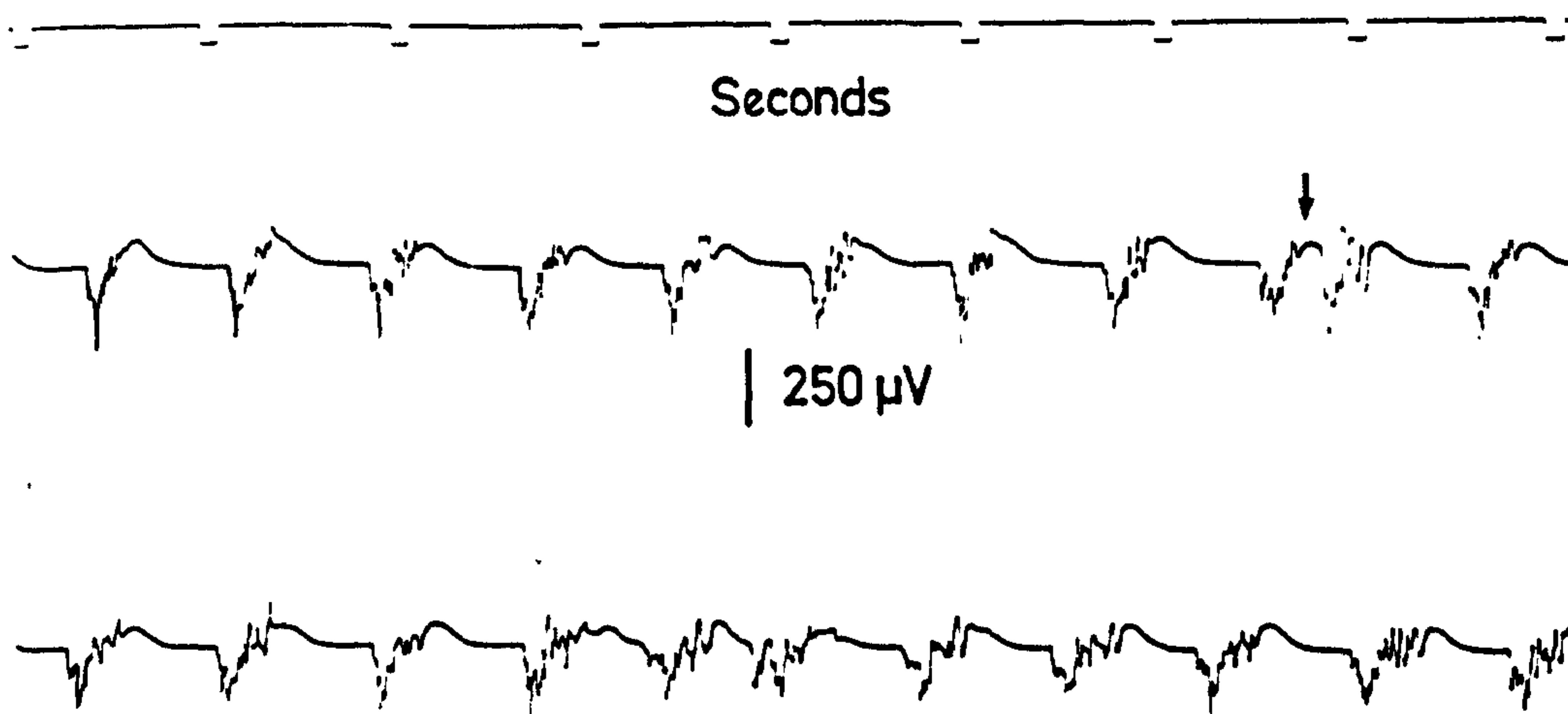


FIG 2.38

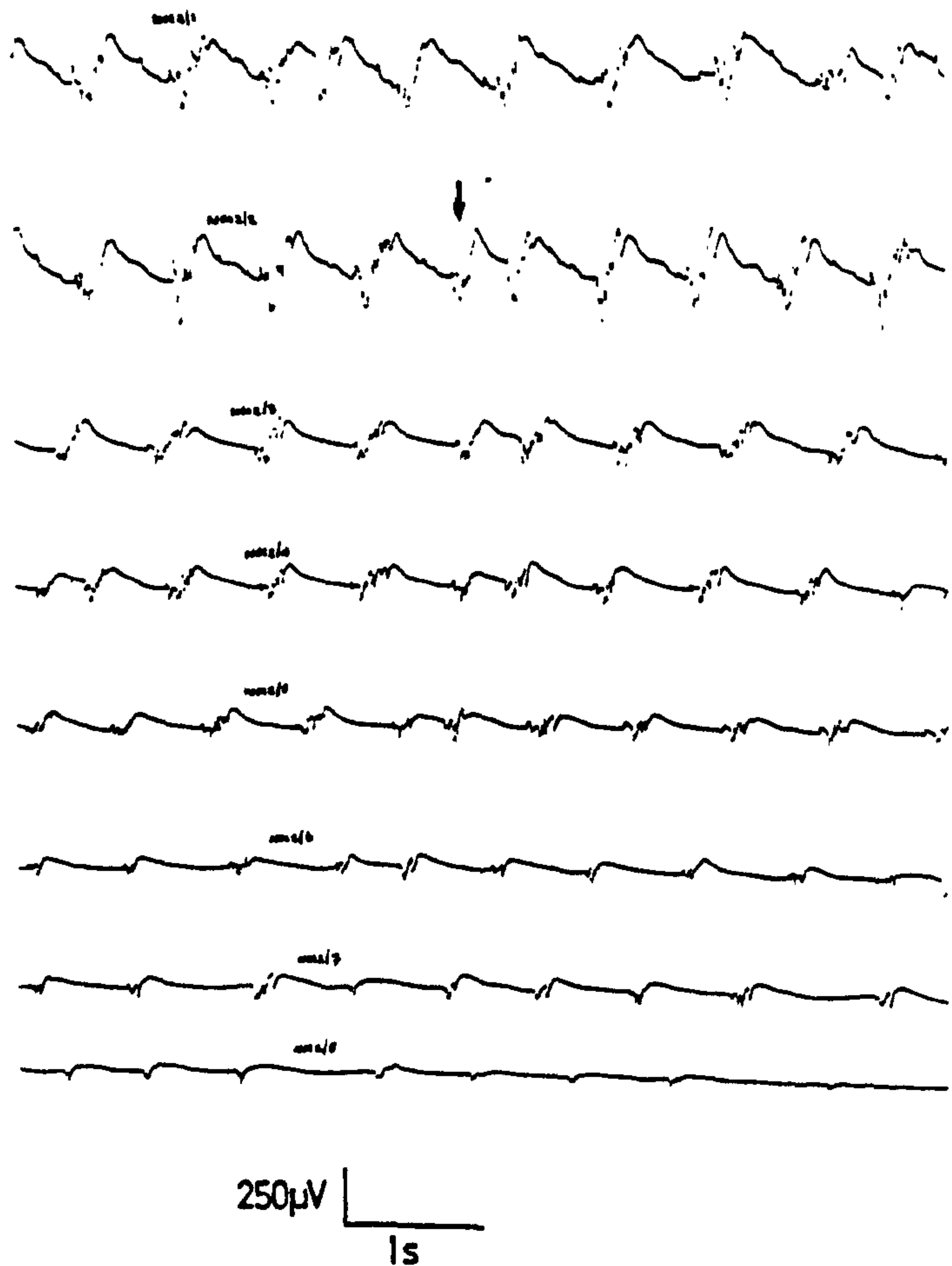


FIG 2.39

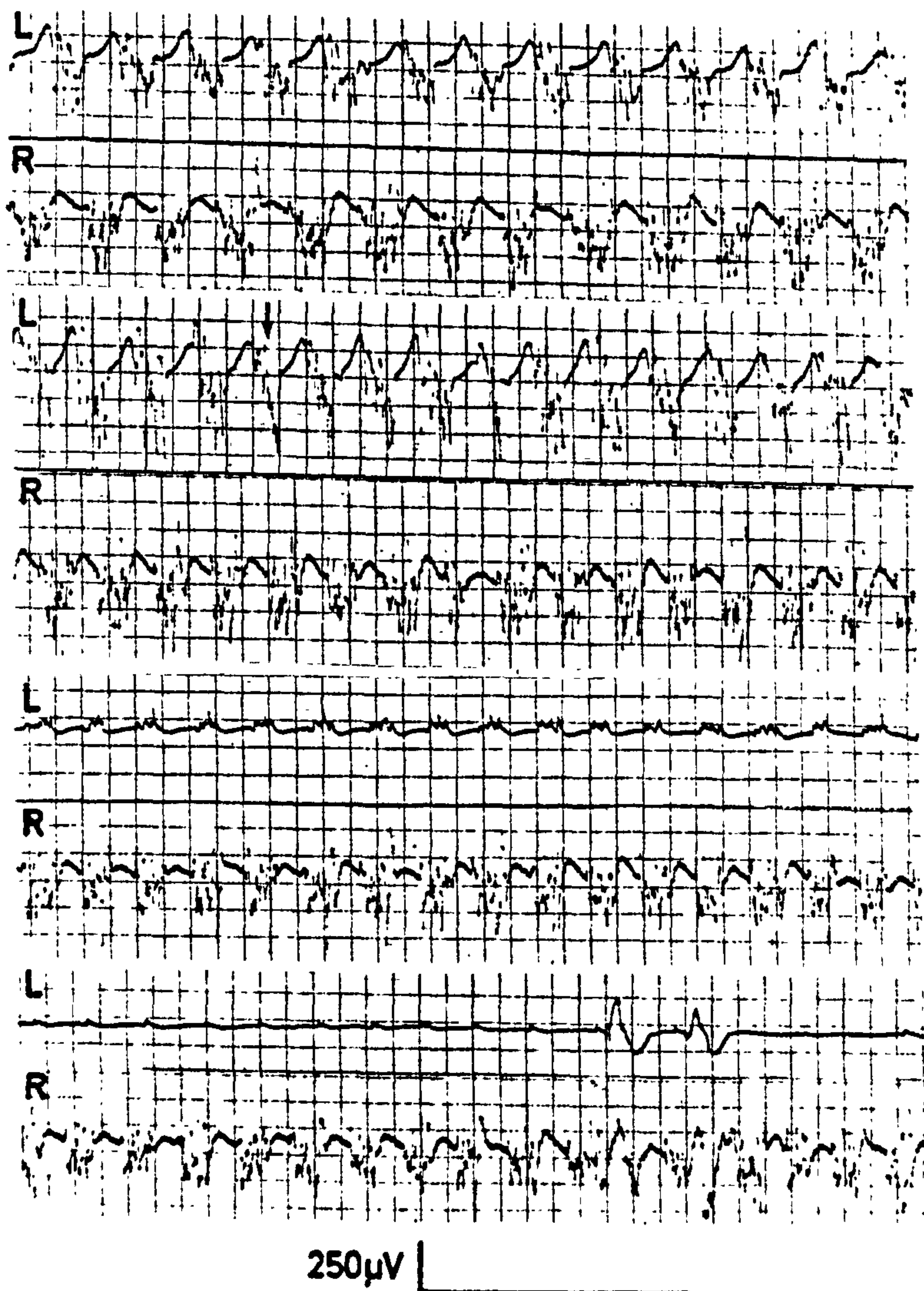


Fig. 2.40

Brown Trout prepared for indirect stimulation of
addictor mandibulae :

Upper trace : EMG

Lower trace : Tension

Stimulation at 0.5Hz frequency, suprathreshold.

At a) the main nerve is infiltrated with Xylocaine and at
b) it is sectioned. Neither has any appreciable effect on
the twitch tension.

Fig. 2.41

Conditions as in fig. 2.40 :

At arrow the adductor is infiltrated at Site 2 with Xylocaine
and there is a rapid abolition of EMG and twitch tension.

FIG 2.40

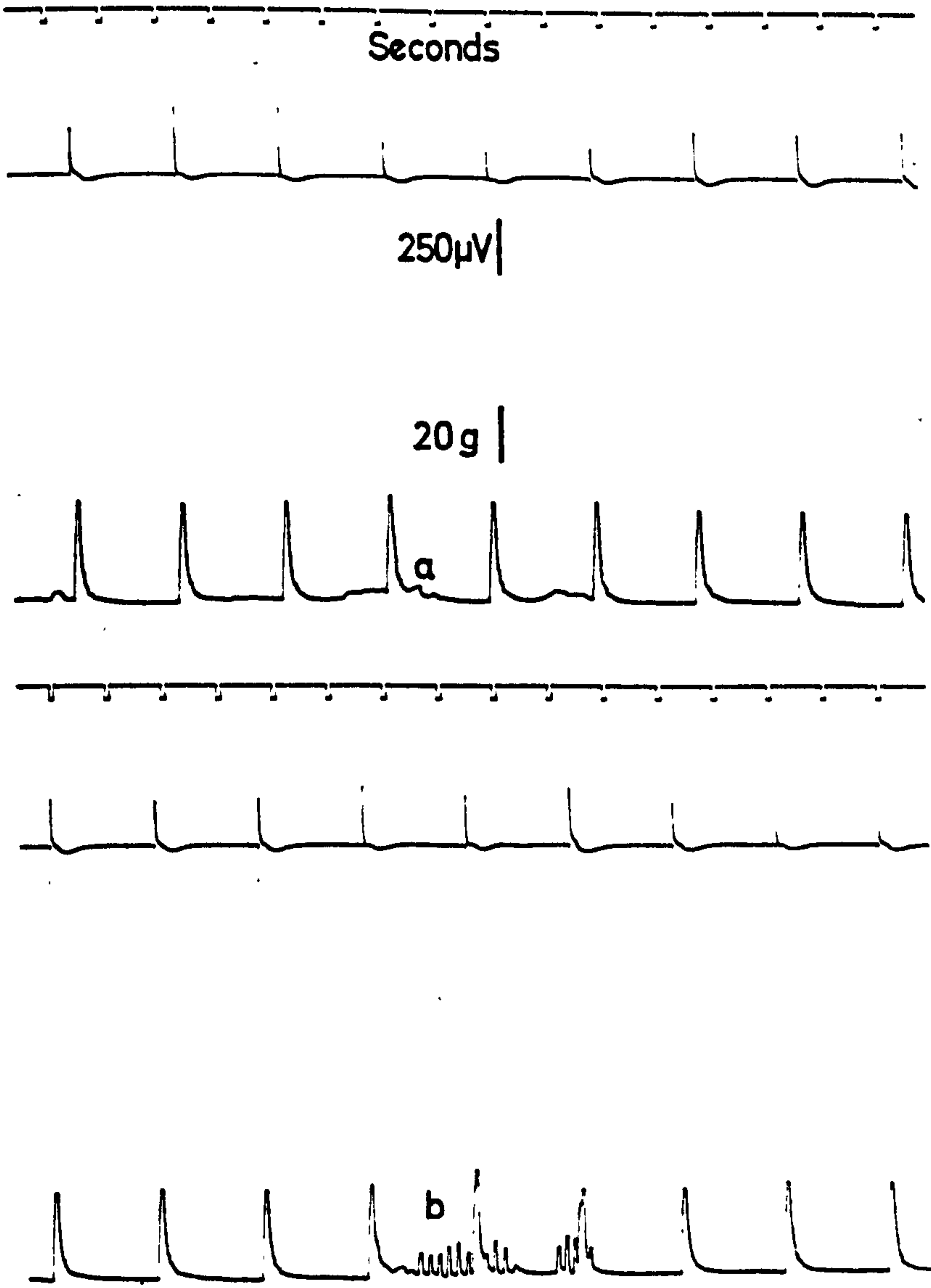


FIG 2.41

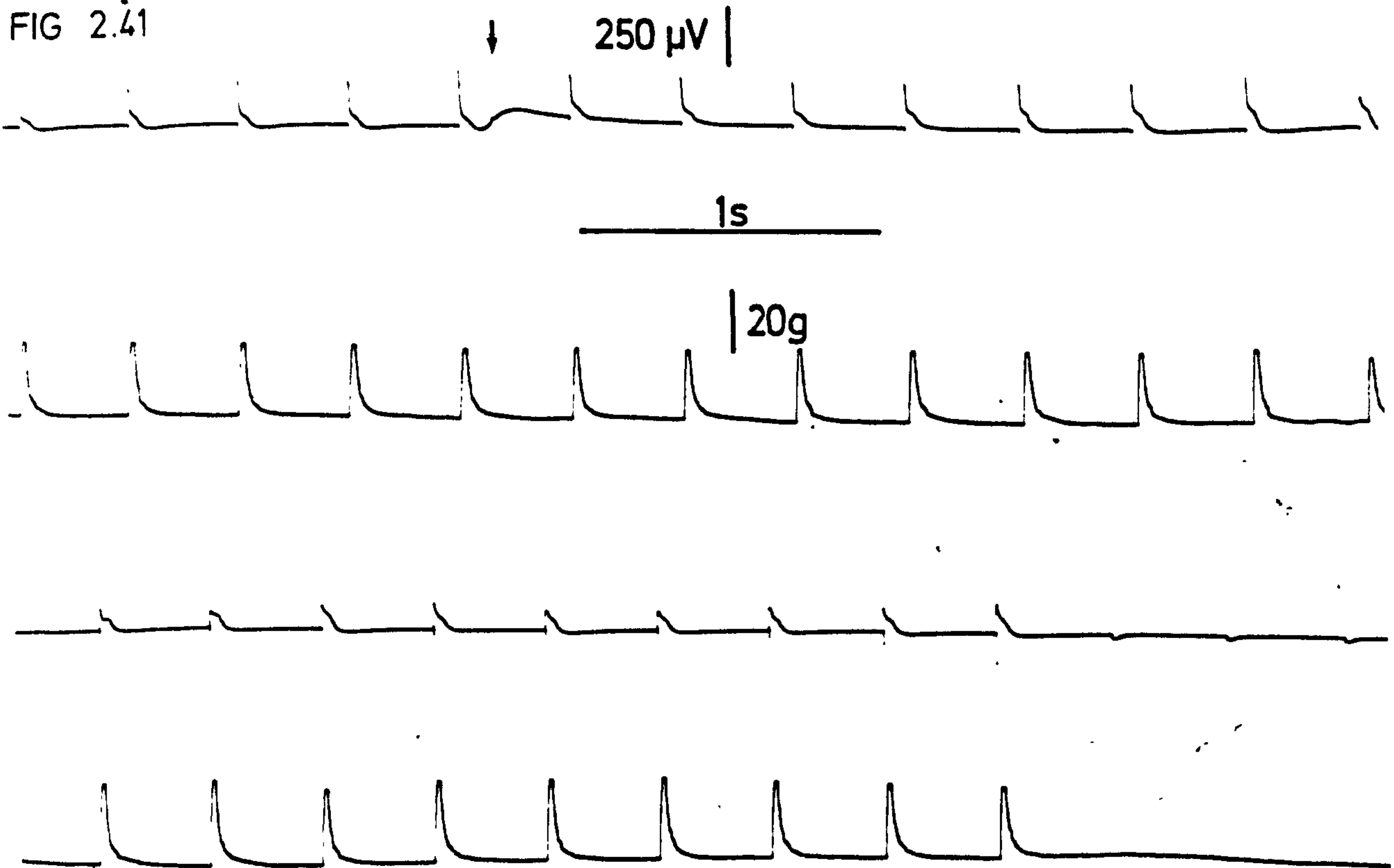


Fig. 2.42

Rainbow Trout adductor mandibulae EMG :

At arrow fish is injected with Gallamine (1 mg IM).

Subsequent decline in EMG activity is typical of non-depolarising agent in that amplitude of EMG potentials is gradually reduced without facilitation.

Fig. 2.43

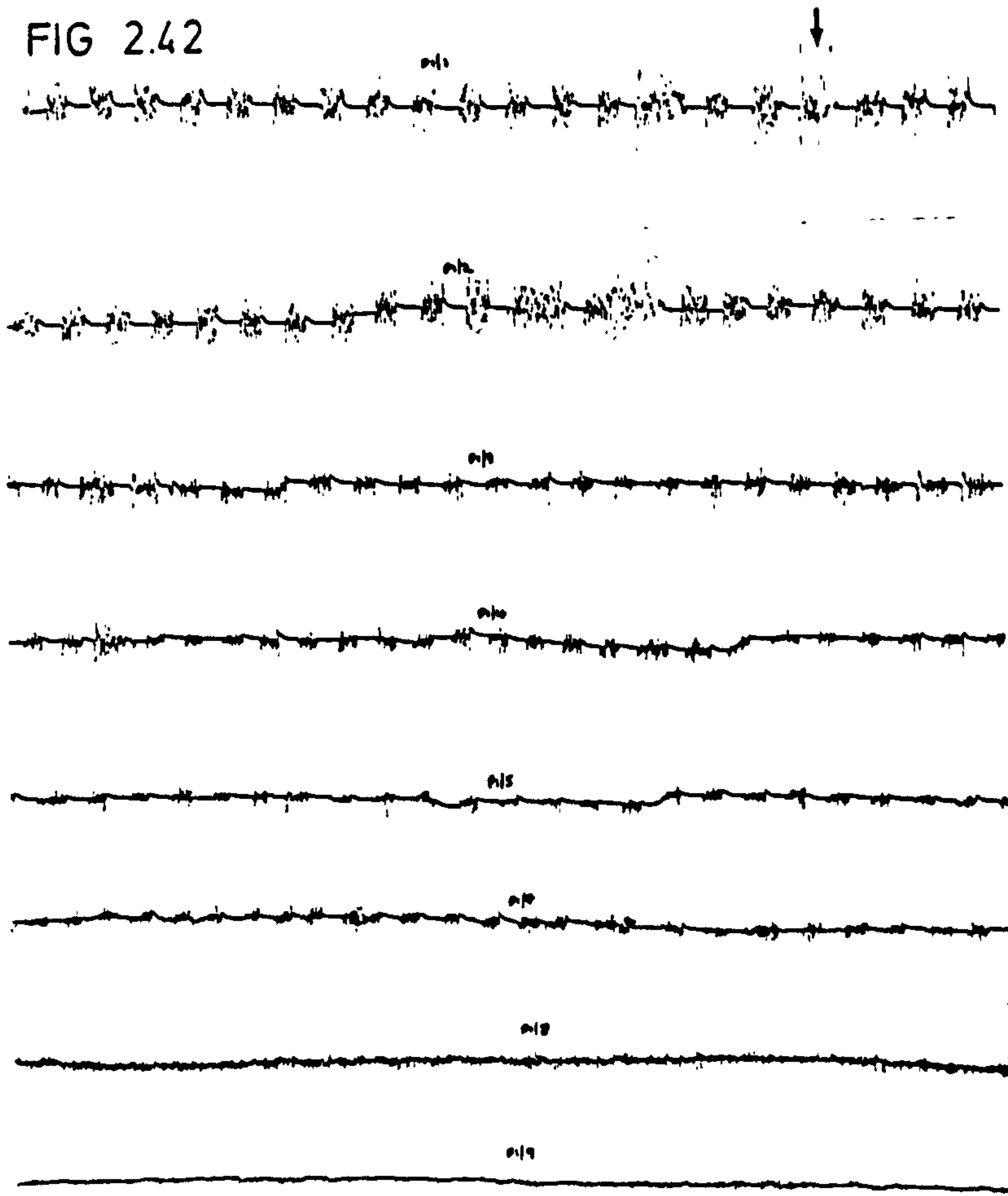
Adductor mandibulae EMGs from red and mosaic muscle of

Brown Trout :

At arrow 500 ug of Suxamethonium is injected 1.M

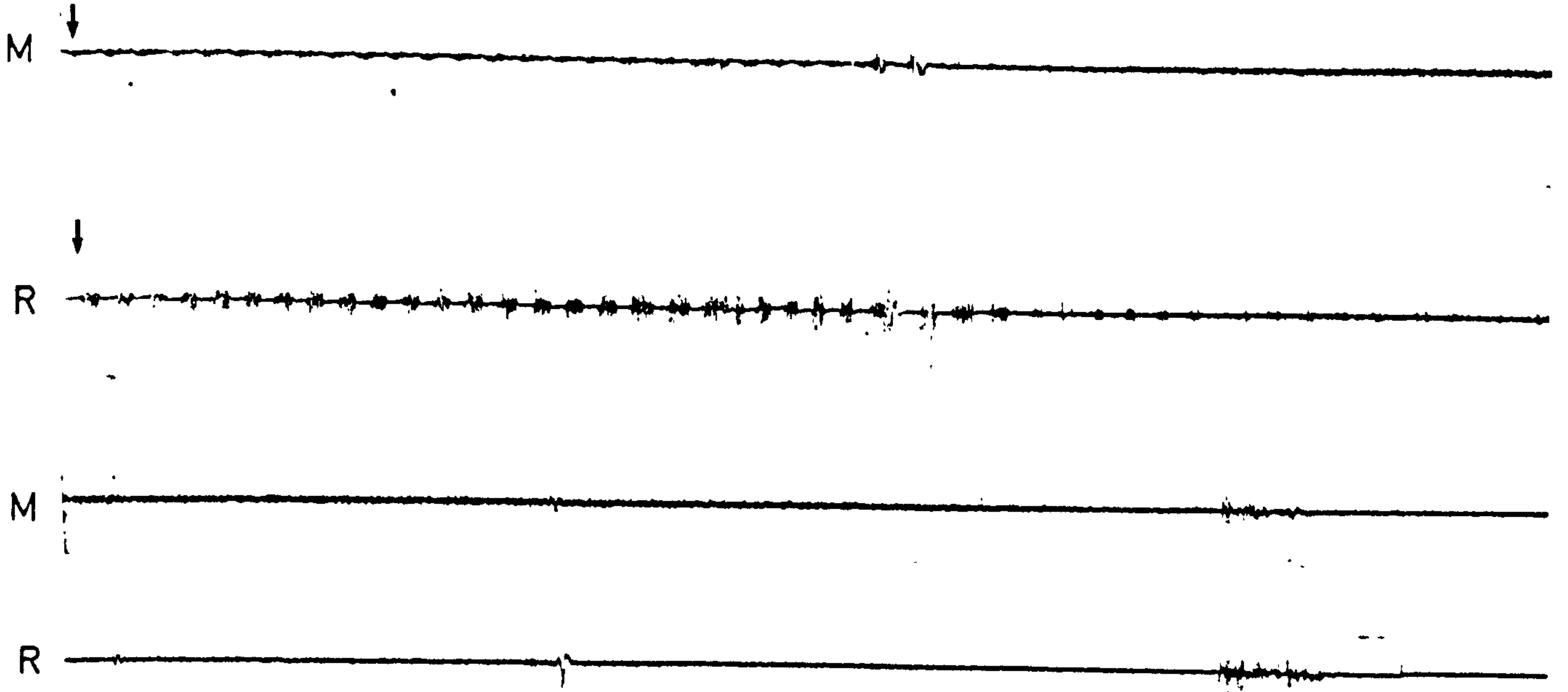
There is evidence of facilitation and the generation of fascicular contractions, typical of a depolarising blocking agent.

FIG 2.42



250 μ V |
5s

FIG 2.43



250 μ V |
5s

Fig. 2.44

Conditions as in fig. 2.43 :

Injection of Decamethonium (1 mg I.M) causes similar decline of EMG as Suxamethonium.

Fig. 2.45

Rainbow Trout adductor mandibulae EMG, upper trace; tension record, lower trace.

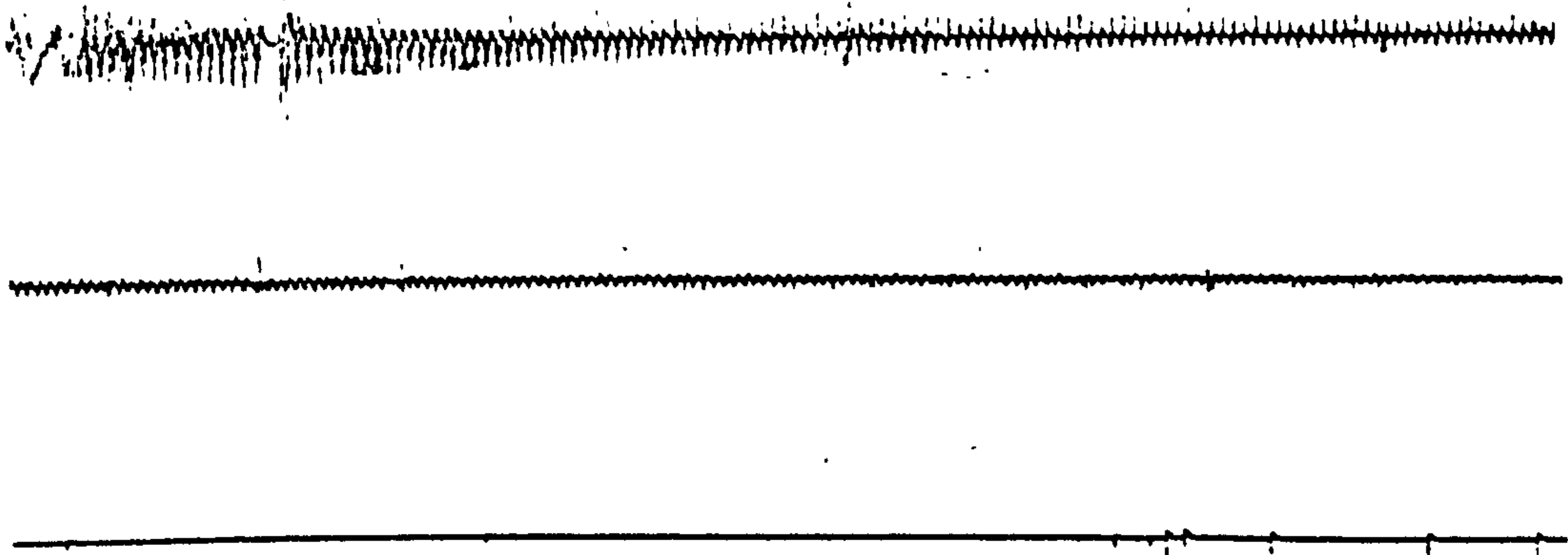
Injection of GGE (100 mg/Kg IP) exerts a peculiar effect upon the EMG, suppressing all but the largest motor potentials whilst decreasing the tension amplitude.

Fig. 2.46

Brown Trout adductor mandibulae EMG :

A decamethonium induced block occurring over the first three traces is partially antagonised by an injection of Neostigmine Sulphate, given between the third and fourth traces.

FIG 2.44 ↓



250 μ V |
5s

FIG 2.45

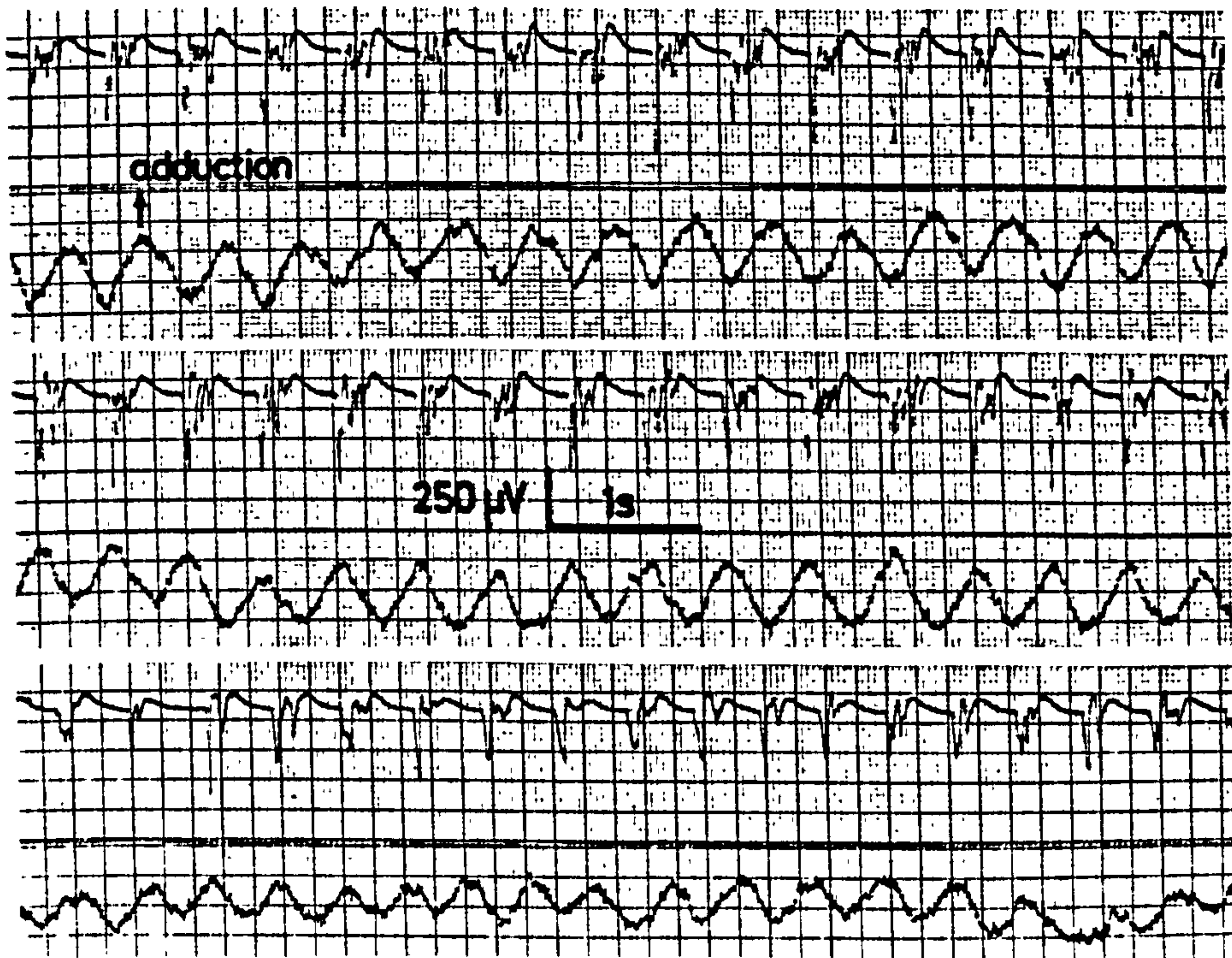
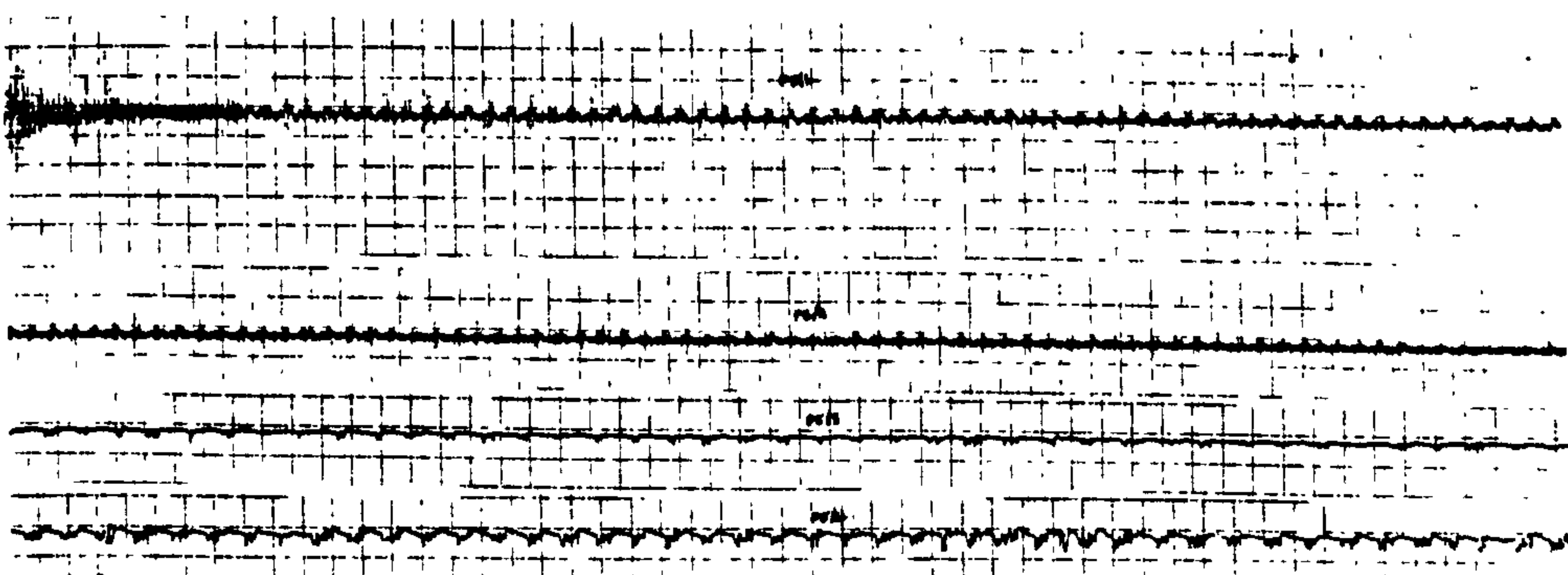


FIG 2.46



250 μ V |
10 s

is shown in Fig. 2.43 . In a few seconds after injection of 500 μ g into the red lateral muscles there is evidence of facilitation or stimulation in both traces followed by a very rapid decline in the amplitude and number of M.U.Ps. This was also seen with Decamethonium Iodide (Fig. 2.44). GGE, 100 mg I.P., had a completely different effect on the adductor mandibulae EMG. There was a progressive decline in the number of M.U.Ps recorded ultimately leaving the largest one with unaltered amplitude but not duration. Eventually the muscle is relaxed though it is still indirectly excitable (Fig 2.45).

In Trout injected with Decamethonium, 500 μ g I.M., antagonism of the blockade with Neostigmine Sulphate, 0.5 mg I.M., was attempted. In one case, (Fig. 2.46) a definite restoration of EMG and a low level of ventilatory movement was achieved shortly after the administration of Neostigmine. In the case of the EMG trace from a Rainbow Trout almost totally paralysed by 4 mg Gallamine , the injection of 0.5 mg Neostigmine Sulphate (+ 0.5 mg Atropine Sulphate to prevent Muscarinic effects) caused the incomplete restoration of transmission. In another Brown Trout paralysed with 1 mg d-Tubocurarine, the injection of 1.0 mg Neostigmine caused the rapid restoration, without warning, of transmission in the skeletal muscles so that the fish was able to jump off the operating table.

2.20 Electrical Activity of the trout alimentary tract.

In trout equipped with gastric EMG electrodes as acute preparations, cyclical electrical activity was observed. Characteristically, this took the form of slow spike potentials of 5 - 10 S duration with amplitude varying from 100 μ V to 2 mV (Figs. 2.47 to 2.48). Typically the slow spikes were biphasic though a strong negative bias was often found. They were often

accompanied by slow waves of 20 - 25S duration. The periodicity of the slow spikes was never very regular but at the ambient temperature (10°C) varied between 1 per 1.5 - 3.0 minutes.

Intraluminal pressure recordings showed cyclical changes in pressure (Fig. 2.49), 5 - 20mm Hg in range. In some cases (Fig. 2.50) when recording conditions were good, correlations were seen with the electrical records. It was noted that changes in the pressure recordings were paralleled by changes in the slow spike amplitude. Some attempts were made to alter the rhythm pharmacologically: injection of Physostigmine Sulphate, 200 ug I.P. caused a large increase in the frequency of the gastric EMG. Injection of the morphinomimetic drug Etorphine HCl, 500 ug I.P. also caused an increase in the amplitude and regularity of slow spiking activity (Figs. 2.51 - 2.52). There was some evidence for propagation of the slow spikes, aborally and only in one case were bursts of fast spiking activity seen (Fig. 2.53).

In free-swimming trout equipped with chronic electrodes, it was observed that the slow spikes occurred at a faster rate of 1 per 23 - 25S. Unfortunately, these fish could not be persuaded to feed readily. Although the adductor mandibulae EMG was eventually chosen as the input signal for the biotelemetry the pacesetter potentials of the trout were interesting in their own right and therefore briefly described above.

Fig. 2.47

Rainbow Trout Gastric EMG :

Slightly irregular 'pacesetter' potentials in a free swimming fish. Note that the duration of the potentials are long, in the order of seconds.

Fig. 2.48

Conditions as in fig. 2.47 : Pacesetter potentials at higher recording speed.

There is a regularly repeated waveform present unlike somatic muscle EMGs.

Fig. 2.49

Anaesthetized Rainbow Trout with implanted gastric pressure-sensing cannula :

Regularly repeated pressure increases, which vary in amplitude are correlated with peristaltic contractions of the stomach.

FIG 2.47

Minutes



500 μV



FIG 2.48

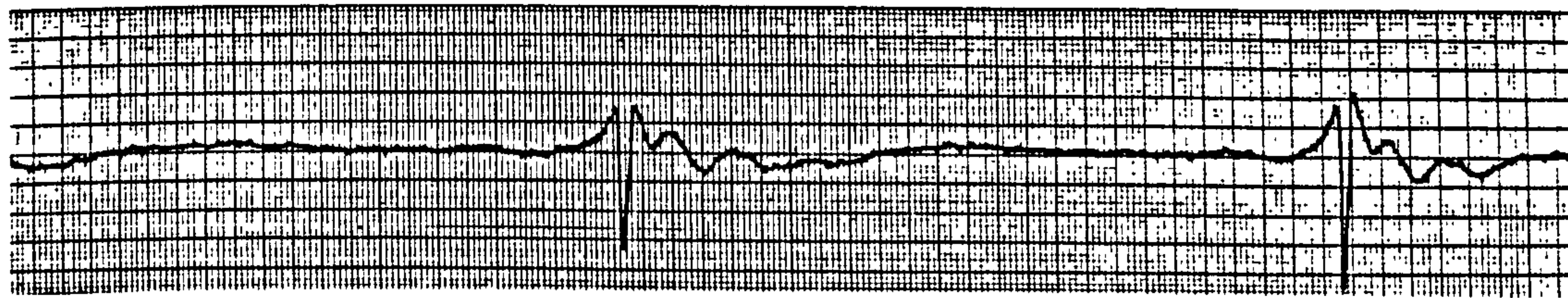
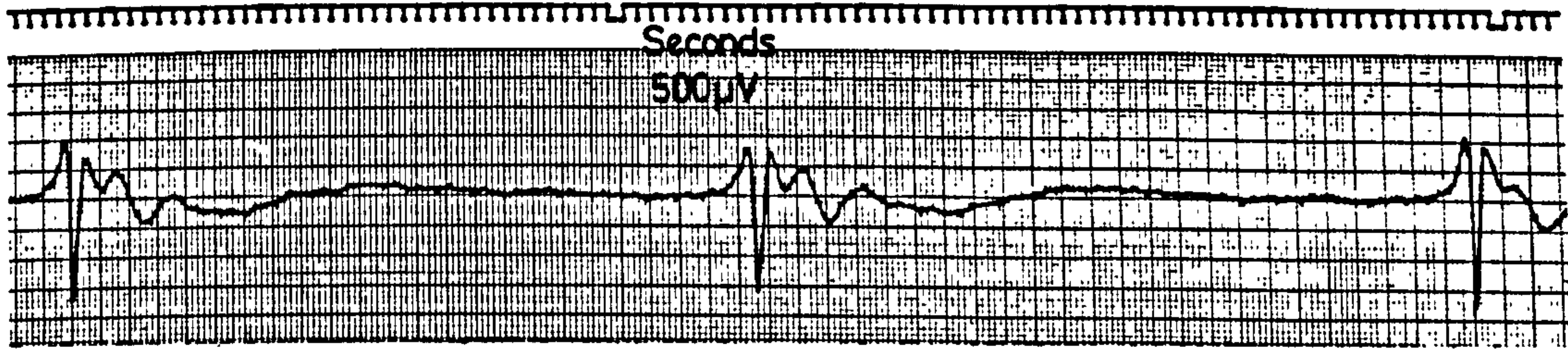
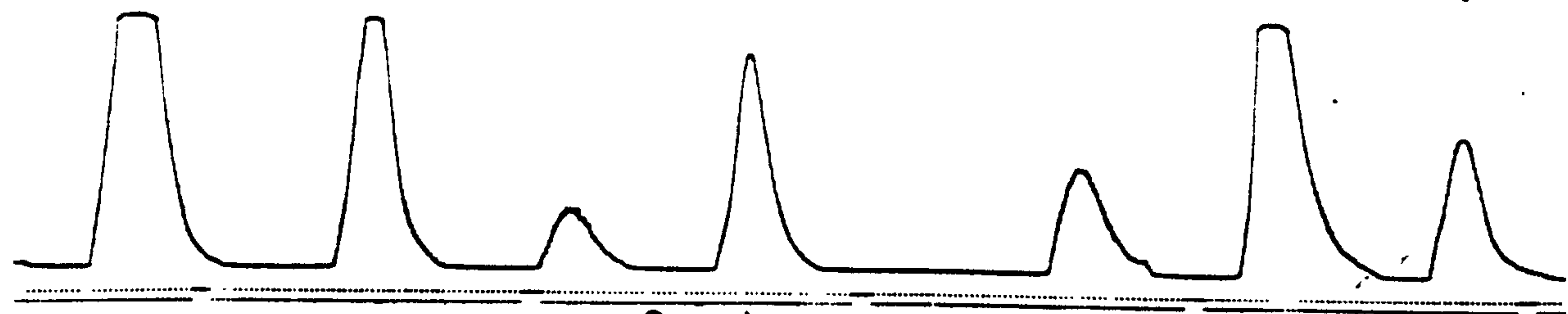
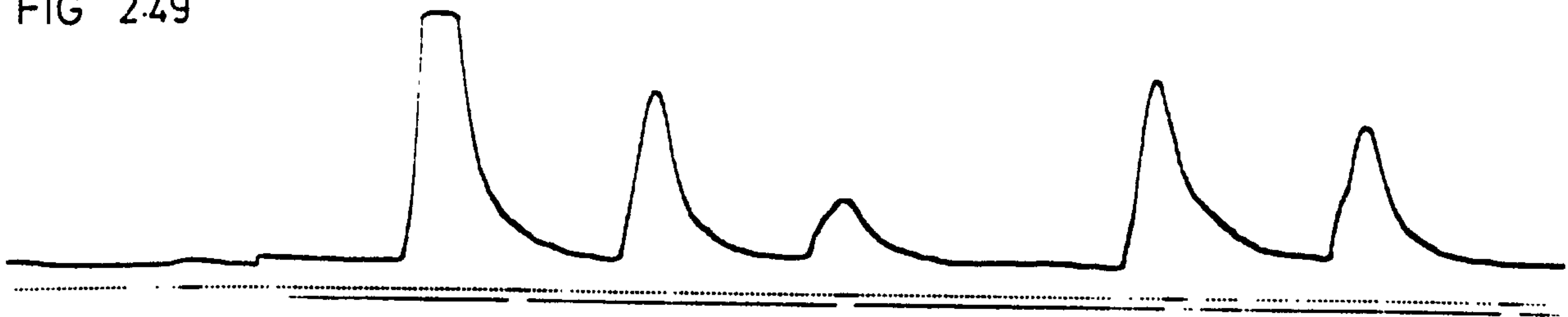


FIG 2.49



Seconds

5mm.Hg.

Fig. 2.50

Anaesthetized Brown Trout :

Top two traces : anterior and posterior gastric EMG

Lower trace : intragastric pressure

Pressure rises, previously correlated with peristalsis are seen here to be linked to the 'pacesetter' potentials.

Fig. 2.51

Rainbow Trout gastric EMG : the pacesetter potential in the upper trace has been increased in frequency following the injection of Physostigmine Sulphate. (lower trace)

Fig. 2.52

Conditions as in fig. 2.51 : The irregular, low amplitude pacesetter potential visible in the upper trace, has been shown to be susceptible to a morphinomimetic agent, Etorphine HCl by an evidence of the increase in rate, amplitude and regularity shown in the lower trace.

Fig. 2.53

Periodic burst activity sometimes recorded from Trout gastric EMG. Although superficially like a somatic EMG, the time scale is considerably longer.

FIG 2.50

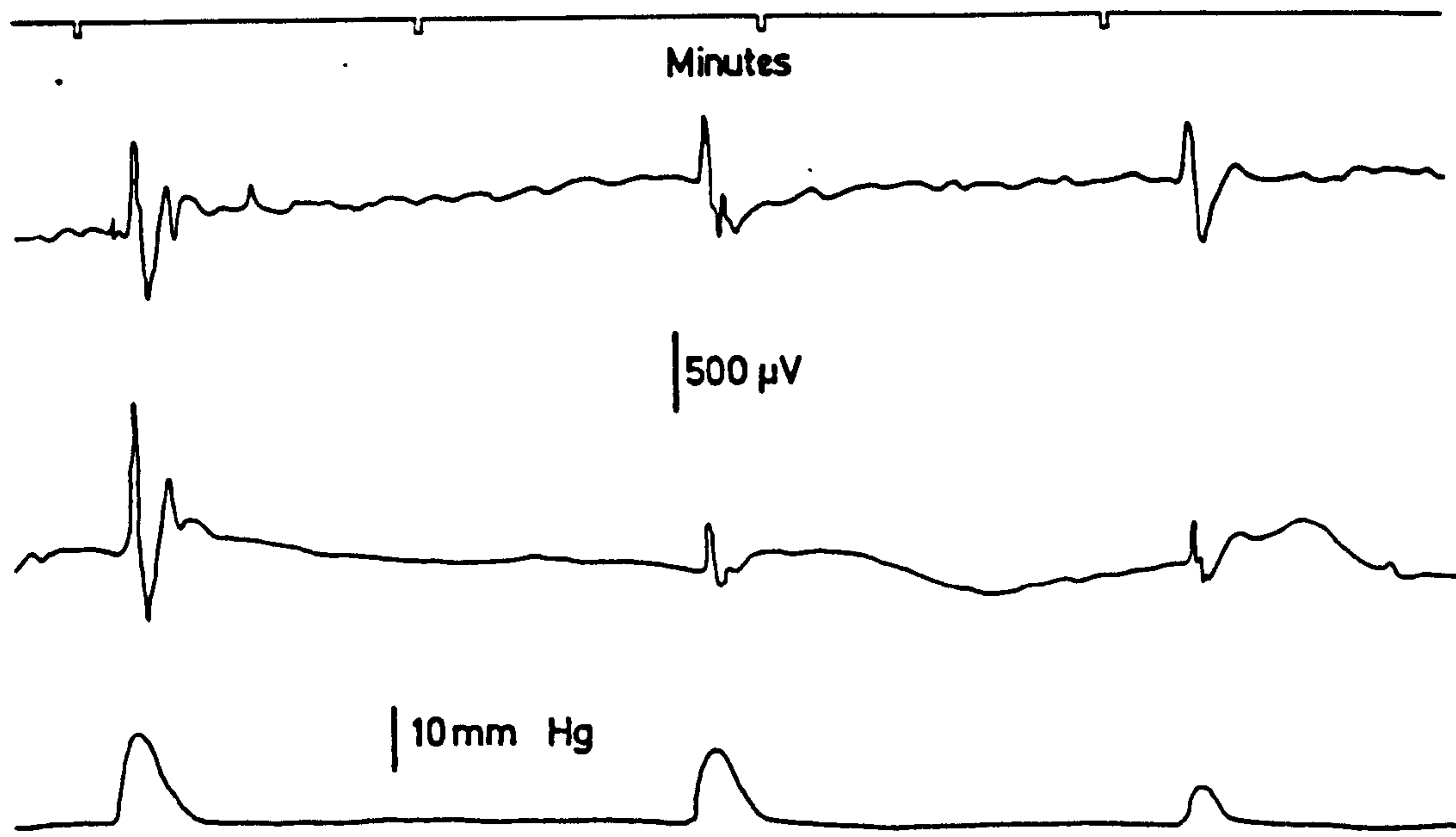


FIG 2.51

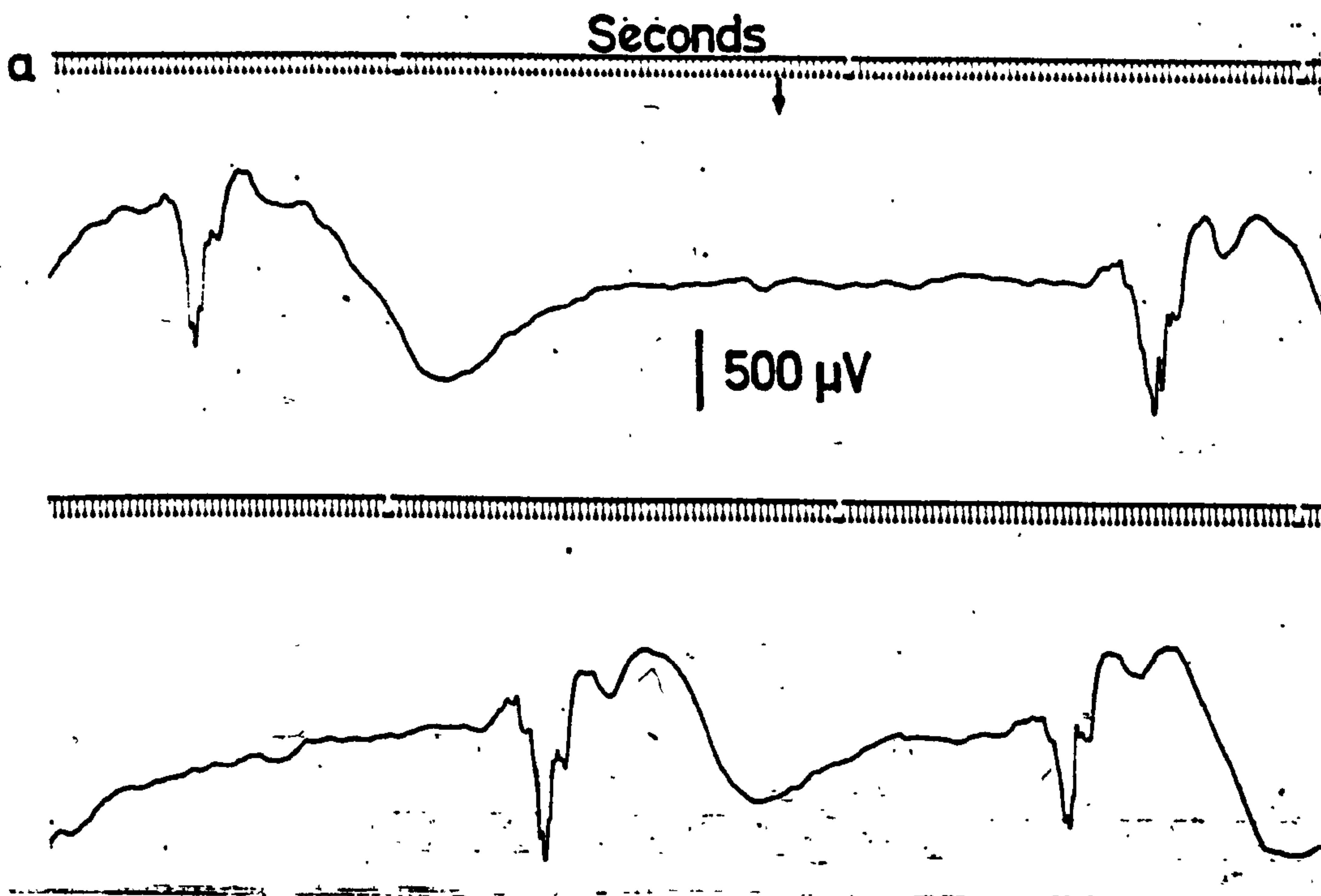


FIG 2.52

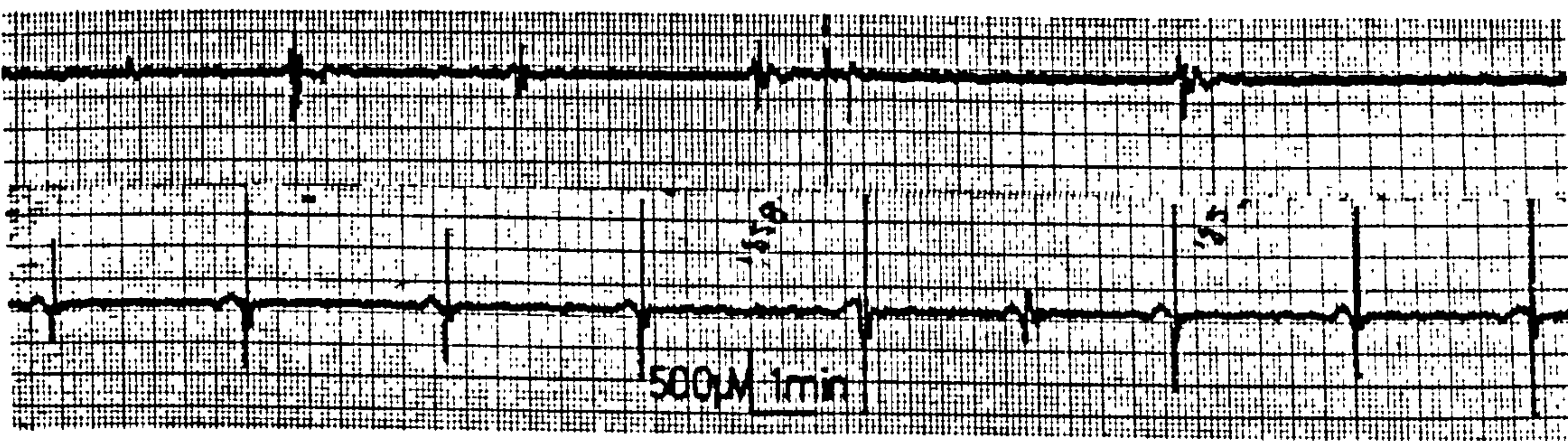


FIG 2.53

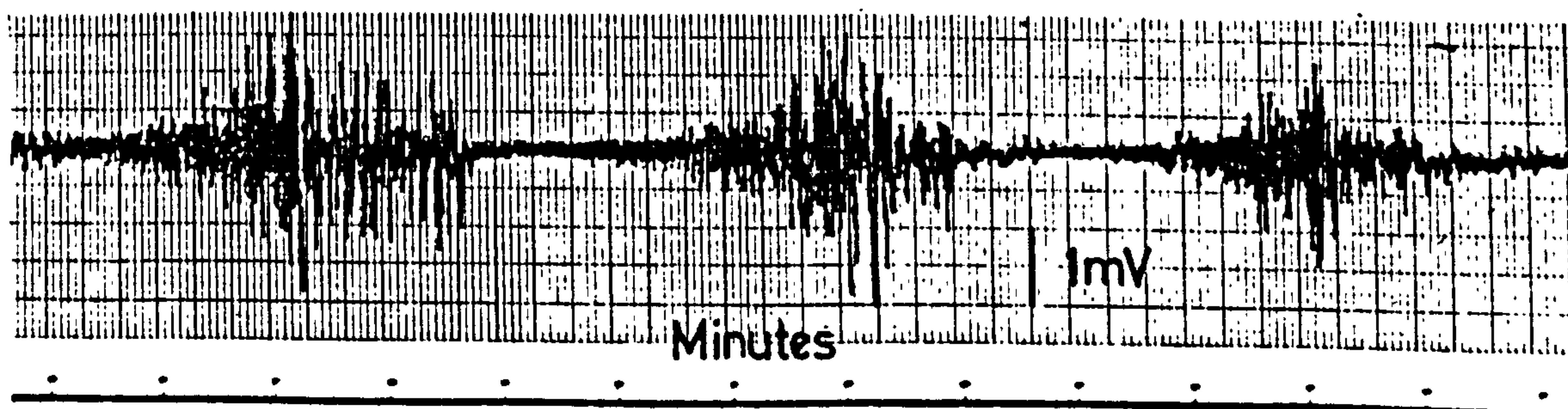


Fig. 2.54

Brown Trout pacesetter potentials:

Upper trace : Cardiac Stomach EMG

Lower trace : Pyloric Stomach EMG

Phase differences between the two recording points suggest that the pacesetter potential is migratory.

Fig. 2.55

Brown Trout : EMG from epaxial mosaic muscle during enforced swimming. The trace has been stretched with an FM tape recorder. Note the brief duration of the EMG potentials in contrast to those seen previously from red muscles.

FIG 2.54

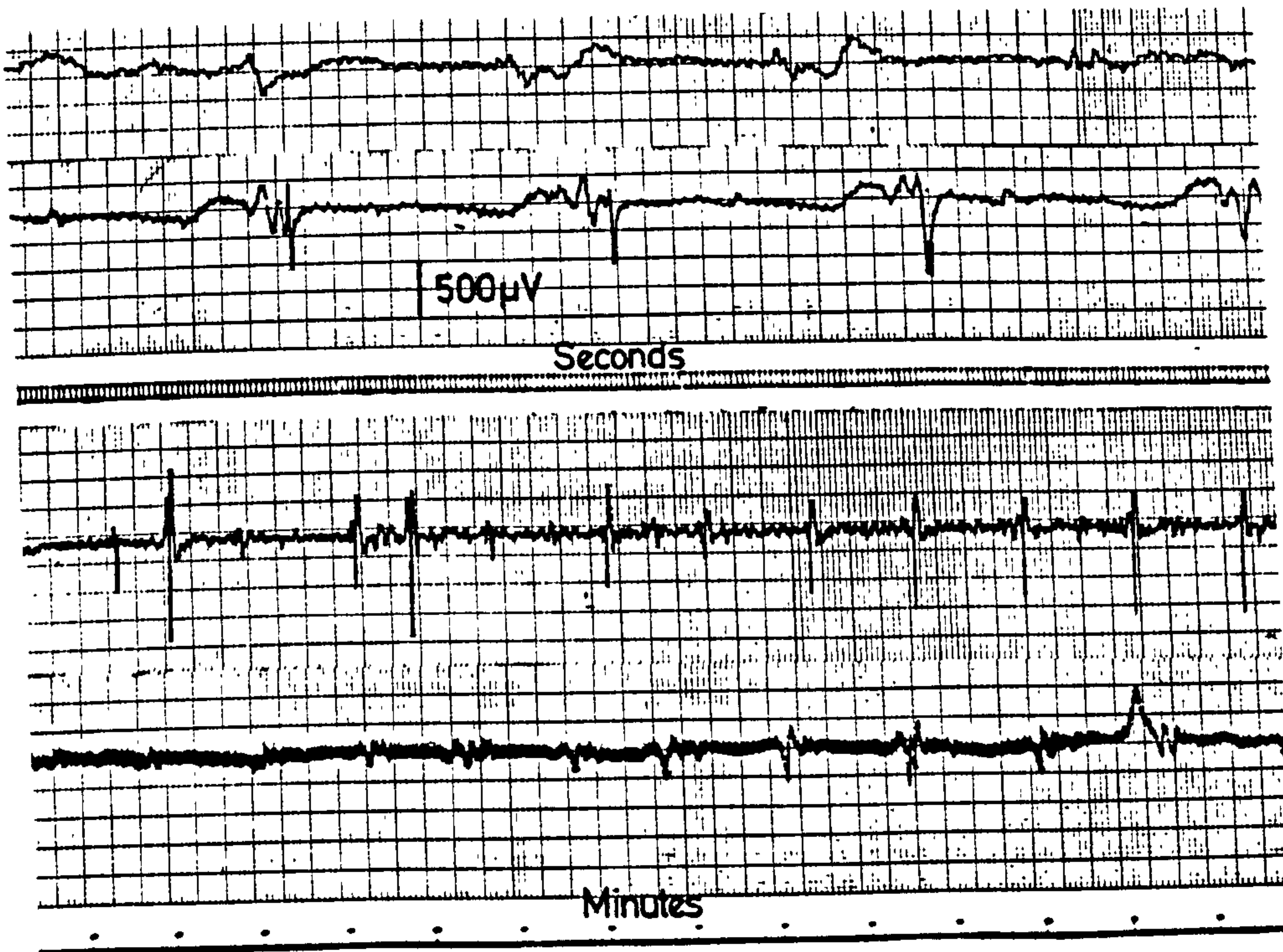
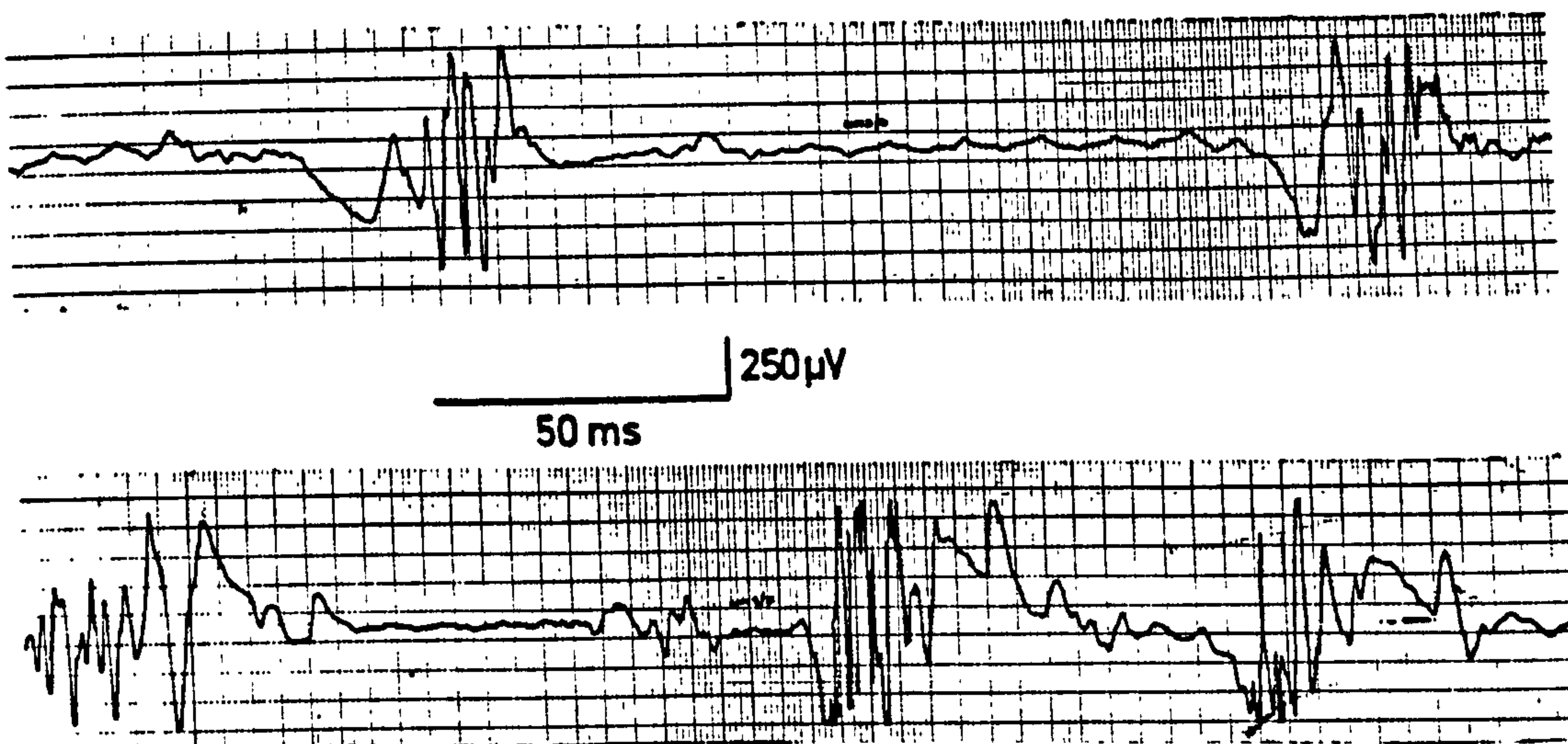


FIG 2.55



DISCUSSION.2.21. EMG probe and mapping experiments upon Trout and Selachians

It is now clear from the EMG experiments in the present work, on Trout and Selachians, that a previously undescribed functional division exists within the m. adductor mandibulae. In normal ventilation, in both anaesthetized and free-swimming fish, only the red portion was active. The functional significance of this is fairly obvious; apart from fast pelagic species, e.g. Carangidae, Scombridae, Thunnidae which continuously utilise the ram-jet effect (Roberts, 1975), most fish will spend all their lives pumping water with only brief interruptions due to feeding, coughing etc. For this purpose red muscle is ideally suited as it does not fatigue and does not respire anaerobically to produce quantities of the limiting factor lactic acid. (Alexander, 1974; Bilinski, 1974). In all situations where slow, sustained or repetitive contractions are required, red muscle will usually be found, often in association with white muscle e.g. Selachian fins Roberts (1969c); Goldfish pectoral fins, Yamamoto, 1972; Gudgeon extraocular muscles, Kordylewski, 1973. In terrestrial animals the functional difference between red and white muscles is due to the need for antigravity postural muscles; Smith et al. (1977) have shown in the cat soleus (a red muscle) that it is continually active and shows little increase in EMG activity during exercise e.g. jumping. They concluded that the companion gastrocnemius (a white muscle) is required for 'dynamic movements which require greater torques about the ankle joint'. In contrast, the Striped Skunk, Mephitis mephitis has a different locomotor pattern to the cat in that it is a nocturnal animal which covers large distances (> 3.6km) in a night but is incapable of short-term burst activity. The primary ankle extensors are all in this case slow and well suited to its way of life (Van de Graaff et al. 1977). In the Amphibia, Kuffler and Vaughan Williams (1953)

and Lannergren and Smith (1966) have demonstrated red muscles e.g. the iliofibularis which are, like the trout or shark adductor mandibulae, formed as discrete patches on larger white muscles.

The slow contraction of red muscle probably offers a fine degree of control if this is necessary but note that white muscle systems too, are also capable of graded response (Roberts 1969c; Hudson, 1969). The oxygen consumption of red muscle is about 50% lower than that of white muscle (Cameron and Cech, 1970). Following exercise glycogen (the main substrate) is rapidly depleted from white fibres whereas the main substrate in red fibres (lipid) is only moderately depleted by sustained activity (Bone, 1966; Bilinski, 1974). In the context of the fish ventilation system, red muscles are therefore more efficient. This is consistent with the observations (Bone, 1966; Rayner and Keenan, 1967; Hudson, 1974) that red lateral muscles are used for sustained swimming whereas the white or mosaic muscles are used for burst speed swimming (which fish do not often do).

The duration of the m.u.ps recorded from the red fibres is similar to those figured in EMG recordings from red muscle by Ballintijn and Hughes (1965), Roberts (1969c) and Hudson (1974). There are few good examples in the literature of white muscle EMGs, possibly due to their fast rise time and discharge frequency being too fast for most pen recorders. Bone (1966) shows some EMGs from dogfish white muscle which have fast spiking activity of the graded type. Fig. 2.55 shows a recording made from the lateral mosaic muscle of a Brown Trout during enforced swimming. The recording was made on the T3000 instrumentation recorder and played back at 8X slower speed. It will be noticed that the m.u.p.s are of brief duration (1.5 - 10mS) in contrast to those recorded from the trout/shark adductor mandibulae red fibres. They also have a considerably

faster rise time than the latter.

With regard to the stretch activation of the red fibres in deeply anaesthetised Trout: this has been recorded in Amphibia by Tasaki and Tsukagoshi (1943) and in Selachians by Roberts (1969c). In mammals, neuromuscular facilitation occurs on stretching a muscle (Hutter and Trautwein, 1956) and is a property located at the end-plate itself. It may be that in the trout, stretching facilitates the action of motorneuron impulses which would otherwise be sub-threshold or as is more probable, it is of central origin and is mediated by proprioception. Under normal conditions it is probably masked by the ventilatory rhythm. The subject of proprioception is a vexed one in Teleosts: no piscine equivalent of a muscle spindle or tendon organ has been demonstrated. Roberts (1969b) has shown in Selachians that proprioception of body movement occurs in the subcutaneous Wunderer corpuscles and that afferent sensory discharges in dorsal roots can be recorded in response to muscle activity. Roberts and Witkovski (1975) state that no receptor organs are present in the dogfish adductor mandibulae. Ballintijn (1972) has categorically proven the existence of the direct proprioceptive control of ventilation but as yet no specific receptor is implicated. Most authors now conclude that in Teleosts, proprioceptors must be located in some sort of undistinguished nerve ending.

The functional significance of the stretch activation may be in facilitating the rapid closure of the jaw during feeding/coughing/spitting. Adduction of the suspensoria in Teleosts is largely passive due to elastic elements (Osse, 1969) and in Trout paralysed with Flaxedil (own unpublished observations) the neutral position of the mandible is almost adducted. The trout, therefore, does not need to invoke much muscle activity in the adductor mandibulae during ventilation except for the positive pressure phase. When it feeds,

however, it generally does so from maximal gape (Wankowski, 1977). Because it is not sucking in its prey as in the Perch, Ruff or Carp, it would be more efficient to have as rapid a jaw closure as possible. Osse (1969) stresses that in the trout 'the biting function is the most important'. During feeding and coughing the EMGs recorded from the adductor mandibulae of the trout were suggestive of mosaic fibres. Reference to the figures of Osse (1969) and Ballintijn et al. (1972) suggests that white fibres are involved in the Perch and Carp during these manouevres also. The attempts to map the area of the active red fibre units closely paralleled the zone of red fibres demonstrated by Cust (1975). Note that potentials were absent from the ventral area where in Section I it will be remembered, the tendon was shown to replace the red fibres.

Browne (1976) has said that Suxamethonium will only produce a depolarising contracture in multiple innervated slow fibres. In the Shark, EMG evidence of a contracture ~~was~~ induced with Suxamethonium but this was not clear in the trout. Possibly the response of the Selachian or Teleost to the drug is different from the mammal, in which considerable species variation occurs (Westhues and Fritsch, 1965; Hall, 1971).

2.22 Effects of Anaesthesia Upon the Form of the adductor mandibulae EMG

Houston et al. (1973) described changes in cardio-respiratory parameters e.g. heartrate, ventilation rate, gill flow rate, etc., resulting from anaesthesia, handling and the surgical procedures necessary to gain the information. The commonest change in the Brook Trout (Salvelinus fontinalis) was of a parabolic form. In this investigation similar shaped changes were seen in ventilation rate, EMG amplitude and spike/burst. Regressions of log y against time gave significant correlation coefficients in all three cases. The curve for EMG burst length (b1) displayed in Fig. 2.21 shows a pattern with an intermediate minimum. This was also seen in some parameters of Houston et al.'s work. Parallel changes were noted in Perch cranial muscles by Osse (1969). These changes clearly represent an undefined 'hangover' effect of benzocaine anaesthesia, which persists for a considerable length of time after the fish has 'recovered'. Stabilisation times are of the same order of magnitude as those for the Brook Trout i.e. 2 - 3 hours. This suggests that the changes induced by benzocaine are comparable to MS-222 for which it is an isomer. It is very interesting to note that the time course of the EMG changes correlate with the known drug clearance rates in arterial blood. Houston and Woods (1972) estimated the $t_{\frac{1}{2}}$ value for MS-222 in Salvelinus as 20 minutes whereas Hunn and Allen give a $t_{\frac{1}{2}}$ value for the Catfish as around 6 minutes. There is a noticeable stabilisation of blood drug levels at +6 hours which is not significantly different from that obtained at +24 hours, 4.1mg/l which is about 1/30th of the blood level at surgical anaesthesia.

Because of the absence of a suitable in vivo biopsy technique for fish, most investigations have relied on chronic blood sampling to obtain time course data. Houston et al. (1972) have, however, noted that both muscle and brain anaesthetic levels may continue to increase after the

blood has reached equilibrium.

It may be, therefore, that the muscles and other lipid rich tissues may form a depot from which a persistent blood level of the drug is maintained during the 'hangover' period. This is the mechanism by which the short-acting thiobarbiturates e.g. Thiopentone Na, Thiamylal Na operate (Westhues and Fritsch 1965, Hall 1971). Salmonids have a large amount of red lateral muscle (Boddeke et al. 1959) which, in contrast to the mosaic muscle, has a high intracellular lipid content. (Johnston et al. 1975). The aminobenzoic acid esters are highly lipid soluble (Hunn and Allen 1974) and so might be expected to be retained in the fish for extended periods.

During the recovery period there are short term changes in blood parameters (Houston et al. 1971 a + b). In particular, changes occur in the muscle and blood content of the ions important in muscle contraction e.g. K^+ Mg^{++} Ca^{++} .

In humans, a syndrome known as Periodic Paralysis exists where sudden falls in serum K^+ occur leading to a decrease of excitability of muscle. The changes seen after anaesthesia are complicated by translocation of ions and it is impossible to postulate if any EMG changes can be ascribed to the latter. Houston et al. 1973 point out that the regulation of respiratory activity in the fish is largely determined by pO_2 and pCO_2 levels. It is now wellknown that the aminobenzoates, in contrast to some other anaesthetic agents used in fish, even with enforced irrigation of the gills generally produce hypoxia. (Oswald 1978a; Houston et al. 1973; Soivio et al. 1977). This is due to a succession of contributory factors: decrease in respiration; decrease in heart rate; decrease in cardiac output; decrease in gill flow; swelling of erythrocytes. Soivio et al. (1977) show, however, that the time course of recovery from the hypoxia is very short compared to that of the

ventilatory parameters.

One can therefore presume that the changes in the EMG are from either central nervous system effects or else of peripheral actions. The latter are discussed in section 2.24. This conclusion is achieved because the time course of the changes seems to parallel the time course of the anaesthetic clearance in the blood, (a similar conclusion was reached in an elasmobranch, Squalus acanthias, Hunn and Allen 1974).

It is well established that changes in the amplitude and numbers of m.u.p's in an EMG reflect changes in isometric tension or isotonic shortening velocity (Inman et al; 1952, Bergstrom 1959; Bigland and Lippold 1954; Close et al. 1960; Milner-Brown and Stein 1975). Referring therefore, to the EMG changes in this investigation; there is an elevation of cranial muscle activity on recovery, which rapidly declines. The fish is obviously placing less effort into gill irrigation (Houston et al. 1973). At +24 hours the EMG amplitude, ventilation frequency and the m.u.ps/mS are not significantly different from those of +5 hours, there is a subtle difference in that the EMG burst length is so much longer in duration. This implies that a more gentle ventilation occurs at +24 hours with a slower development of tension in the a.mandibulae hence the velocity of gill water flow must be considerably decreased.

The foregoing allows the visualisation of some of the problems which were experienced with the EMG transmitter system described in Section III.

The entity called an EMG is a complex structure which no two are identical (Hudson 1974). Attempts have been made to define stochastic models to describe their generation (Brody et al. 1974; Agarwal and Gottlieb 1975; Basmajian 1974) though these are highly complex, requiring digital simulation techniques. The complex interference pattern is due to the interaction of temporally and spatially dispersed

m.u.p's in the vicinity of the recording electrodes. If there are a large number of m.u.p's/mS then the probability of summation in either electrical direction must be greater. This would appear to fit the observation that the amplitude curve follows the ~~spikes~~ /mS curve. If the situation arises that individual m.u.p's are so temporally dispersed so that no interaction is possible then the peak amplitude of the EMG will be no larger than that of its largest motor unit, generally $<200\mu\text{V}$. The EMG transmitter, it will be seen later, is a gated device which requires an input threshold voltage to be exceeded in order to function. Because of the decline in summation experienced between +5 and +24 hours then the output of the transmitter will be curtailed by the ~~deficit~~ of supra-threshold m.u.ps.

Close examination of expanded records of EMGs from the adductor mandibulae shows that there are possibly two components; one is the typical m.u.p. and the other is a slower more rounded potential. The latter may be resulting from distant motor units subject to 'blurring' (Basmajian 1974) but my personal theory is that they may be artifacts induced by the 'microvibrations' described by Basmajian. If so, then because they contribute to interaction, one might expect even greater summation effects as muscle activity is increase.

There is a further consideration for the field use of the EMG signal; as the burst length stabilises at 850mS (+24 hours) then the interburst interval would be 150mS. Because of the lack of summation many m.u.ps would fail to trigger the transmitter output stage. Owing to this the telemetered ventilation trains would become garbled making identification of information difficult both aurally and visually.

2.23 EMG Frequency Spectrum Analysis

There is an outstanding result from the adductor mandibulae EMG frequency spectrum analysis; the spectral content is totally different

from that of the 'standard' mammalian muscle EMG. The reasons for undertaking this analysis in the first place were directed by the development needs of the EMG transmitter. In the design of the EMG transmitter it was postulated that the expected frequency spectral range of the a. mandibulae muscle, would be similar to that of normal mammalian muscle. The lower and upper frequency cut-off points were therefore limited by CR networks with calculated 6dB points of 159.0Hz and 3.3kHz respectively. Part of the rationale behind this stems from the large amount of development work which had to be carried out in electrically 'noisy' aquarium conditions and also from the fact that fish were more conveniently kept for testing transmitters with 'hard' wiring already completed. This meant that the transmitter could be made in 'breadboard' fashion, making experimental modifications to the circuit easy. This meant that capacitance artifact (high frequency) and movement artifact (low frequency) were a common nuisance. For field use, it should have been obvious that both 50Hz interference and cable artifacts would be absent.

Reference to the fitted curves Figs. 2.27 to 2.29

shows that the observed data is heavily left-skewed and that the dominant frequencies are considerably lower than in the mammalian examples.

Table 2.7 shows a selection of f_p values from the literature; as expected, it is mainly drawn from human studies owing to the interest therein for the electrodiagnosis of myopathic diseases. There is an alarming range of f_p values given though for similar electrodes, and muscles differences appear to be a function of the analysis method. In earlier papers, motor unit duration was used but all later works (as in this investigation) use spectral analysers. As this seems to be the most up to date, then one can therefore define the 'average' mammalian muscle as having an f_p value of 200-400Hz though specialised muscles may go higher than this.

Table 2.7

EMG frequency spectra in the literature

<u>Mean fP</u> (Hz)	<u>Range</u> (kHz)	<u>Muscle</u>	<u>Source</u>
311.0	0 - 1.0	Human <u>masseter</u>	Duxbury <u>et al.</u> (1975)
20 - 110.0	0 - 1.0	Monkey <u>anterior temporalis</u> (<u>Macaca</u>)	Miller (1978)
125.0	0.038 - 0.25	Human <u>biceps brachii</u>	Kaiser and Petersen (1965)
116.5	0.05 - 1.0	Human <u>biceps brachii</u>	Buchtal, Guld and Rosenfalck (1955)
130.2	0.05 - 1.0	Human <u>interosseus</u>	Petersen and Kugelberg (1949)
132.3	0.05 - 1.0	Human <u>biceps brachii</u>	Petersen and Kugelberg (1949)
438.6	0.05 - 1.0	Human <u>facial</u>	Petersen and Kugelberg (1949)
72.0	0.02 - 0.8	Human <u>rectus femoris</u> (Children)	Vomi and Viitasalo (1976)
114.9	-	Human <u>biceps brachii</u>	Buchtal, Guld and Rosenfalck (1957)
185.0	0.077 - 1.0	Human <u>biceps brachii</u>	Basmajian and Cross (1971)
160.0	0.077 - 1.0	Human <u>interosseus</u>	Basmajian and Cross (1971)
187.0	0.077 - 1.0	Human <u>vastus lateralis</u>	Basmajian and Cross (1971)
202.8	0.077 - 1.0	Human <u>anterior tibialis</u>	Basmajian and Cross (1971)
388.0	0.0 - 2.0	Human <u>biceps brachii</u>	McLeod <u>et al.</u> (1976)
200.0	0.150 - 0.989	Human <u>quadriceps</u>	Lenman and Ritchie (1970)

* In frequency spectral analysis, fP is defined as the principal frequency present.

In contrast, from the Trout a.mandibulae muscle, it is obvious that its f_p value is considerably lower than any other muscle quoted; 10-30Hz. Duxbury et al. (1976) gives the 85% bound as including all frequencies <600Hz and the 50% bound is <210Hz. From Fig. 2.23 it can be seen that the 85% level here is 130-140Hz and the 50% level is between 55-65Hz (Saithe data not included).

The published EMG spectra tend to have long tails into the upper frequencies as does the fitted curve in this investigation. Substituting the calculated a and b values for each of the three groups in the equation $y = ax^b$ gives values at 600Hz of 1.2, 1.6, 0.72% and at 1000Hz of 0.85, 1.2, 0.46% respectively.

This is slightly higher than values found by Duxbury et al. (1976) in the human masseter muscle, but not markedly so. In any case the analyser used here could only cover a band width of 0-240Hz and as the instrument was purely graphical it was not possible to measure accurately the small voltages at the higher frequencies from the charts.

Although a curvilinear relationship was approximated for the frequency spectrum here other investigations have usually shown skewed normal distributions. Kaiser and Petersen (1965) were able to demonstrate a Poisson distribution but this did not hold for frequencies below 100Hz. The distribution of the data in this investigation all exhibited very strong left skew ($g_1 > 1.4$) and were additionally strongly leptokurtic ($g_2 > 2.0$).

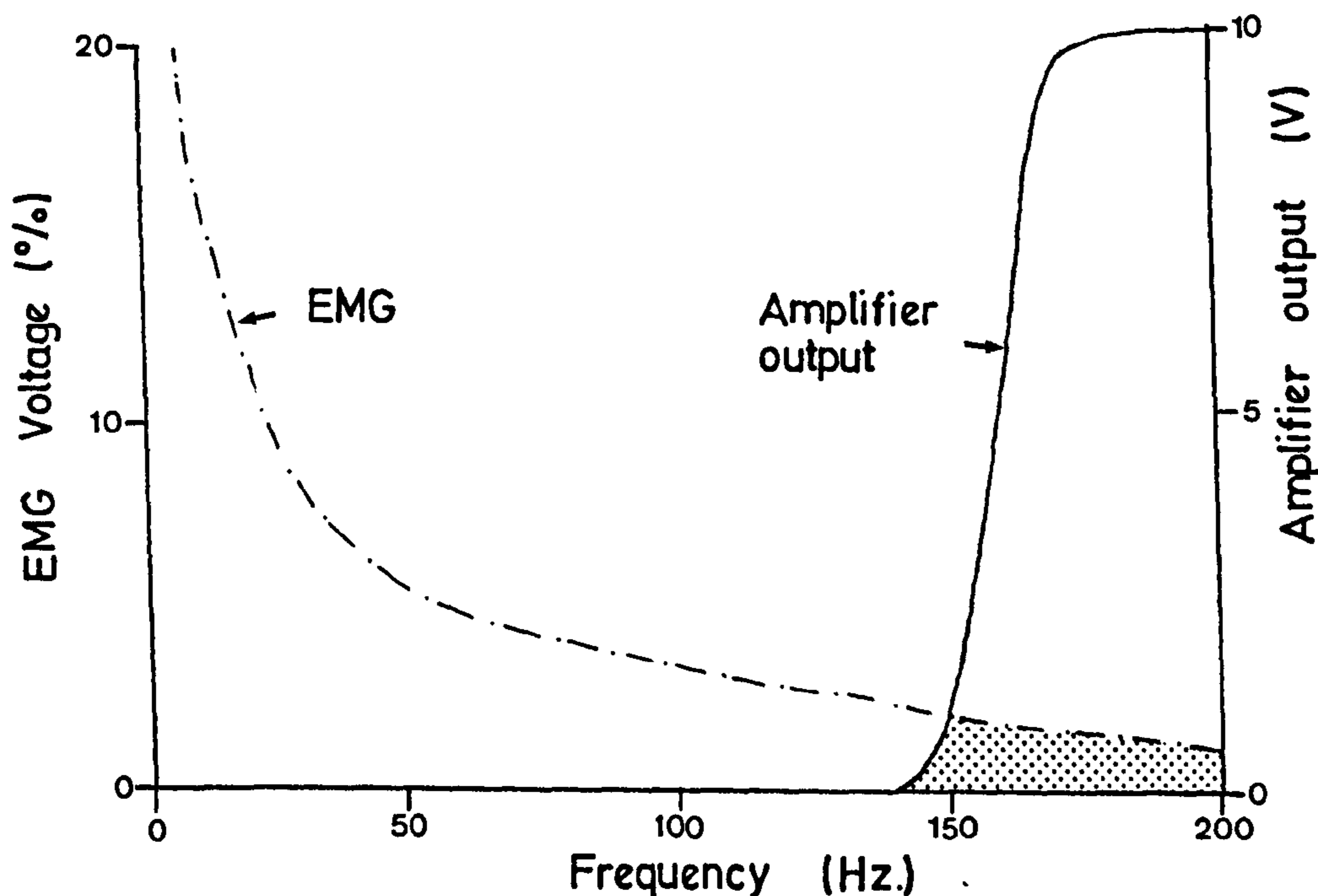
In terms of the transmitter design, this has an extremely important bearing upon its function and some of the practical consequences are enumerated in section III.

The full-power bandwidths of most operational amplifiers are generally flat from 5Hz > 100kHz. The frequency cut-off networks impose a peaked response upon this which, over the range of frequencies present

in the Trout a.mandibulae EMG spectrum, can be thought of as in

Fig.2.56 below :

Fig. 2.56 The relationship between EMG input voltage and the theoretical output of the amplifier of the EMG transmitter.



It would, therefore, appear that the amplifier (and transmitter) response curve shares only a limited overlap with the fitted EMG curve (which is, of course, the best approximation available). Excluding temperature shifts, the amplifier response is fixed but a small shift in the EMG signal would alter the useful output by reducing the working area. This might well be significant to the transmitter even though it was not statistically significant relative to the remainder of the spectrum. This leads to a consideration of the effects of the prolonged anaesthesia usually required to fit an EMG transmitter to a fish.

In Fig. 2.22 the plotted cumulative percentage curves for the effects of anaesthesia show no clear pattern save that the curves for the period between 30 - 120 mins have substantially higher frequency values at the 85% points compared to the curves for +10, +300 and the curve for under

anaesthesia (line 1 on Fig. 2.22). There is an upward shift which implies that the area under the curve contains a greater proportion of higher frequencies. Table 2.4 shows that there are no significant differences between the mean value for each frequency band, taking the first hour after anaesthesia (1 - 6) and the remaining period (7 - 11). Inspection of Table 2.4, however, suggested that groups 4 - 8 (15 - 180 mins) might constitute a grouping with differing mean values. T-tests were performed between the latter and the remaining groups belonging to the Brown Trout (1 - 3, 9 - 11). Highly significant differences were obtained $p < 0.05$ for all frequencies above 110Hz and also below 30Hz. This means that in this period after recovery there is a demonstrable shift in the frequency spectrum with a significantly greater portion of the total EMG voltage in the higher frequencies. There is also a significant decrease in the contribution to the lower frequencies. (Table 2.8)

One very interesting point is that there is no significant difference between the curves for all the freshwater fish in Fig. 2.23 (under anaesthesia). Table 2.6 shows some significant differences when compared to the Brown Trout and this undoubtedly results from the inclusion of the somewhat aberrant Saithe data. Removal of the latter removes all significant differences.

Unfortunately, there is very little work in the literature with which to compare these effects. Drugs such as the Aminobenzoates, Xylocaine, Procaine, Propanidid etc., which can be systemically anaesthetic or analgesic in various species, may also exert a direct effect on cardiac muscle which is characterised by a reduced excitability and contractility sometimes called 'endoanaesthesia' (Westhues and Fritsch (1964), Burgen and Mitchell (1972), Rollason (1975)). Propanidid has been shown to also possess some direct depolarising

effects on skeletal muscle (Ellis (1968)).

The common link is that all the aforementioned drugs are all potent local anaesthetics which exert the latter effect by changing the permeability of nerve membranes (at the relatively high concentrations experienced following infiltration). The effects on cardiac muscle occur at considerably lower organ concentrations than the latter. It is not known if benzocaine, the anaesthetic drug of choice here, can exert any effects, peripherally, on skeletal muscle in fish. It can and does exert marked effects on the ECG of the Brown and Rainbow Trout (Oswald and Richards in preparation). There is no real incentive for research to be carried out on the effects of local anaesthetics on muscle but for obvious reasons much work has been carried out upon the effects of CNS depressants used in surgical anaesthesia e.g. the barbiturates and thiobarbiturates, upon skeletal muscle. (See Oswald 1978 a for brief review). Their effect is focal, acting principally at the myoneural junction (Thesleff 1956, Proctor and Weakly 1976) as opposed to a generalised effect as in Propanidid.

Local anaesthetics employed systemically in man are used in very low doses ($1.4\text{mg/kg} \equiv 0.02\text{mg/ml}$ blood) whereas in the fish levels seven times greater than this occur routinely in anaesthesia ($0.12 - 0.14 \text{ mg/ml}$ blood) (Hunn and Allen 1974, Houston and Woods 1972). In addition, the blood volume of a fish in relation to the body weight is only 3 - 5% compared to 8% for Man (Green 1977, Randall 1970) and therefore the concentration at a specific membrane will be higher in the fish. It is known (Mrose and Ritchie, 1977) that in nerves, Xylocaine (an amide) and Benzocaine (an aminobenzoate) may act on different sites. They concluded that 'benzocaine may be acting in the same way as the alcohols and volatile general anaesthetics'. This fits in neatly with the observation (own unpublished) that Xylocaine is ineffective as a fish

anaesthetic whilst benzocaine is very potent in this role. In the absence of contrary data it is probable, therefore, that changes in the frequency spectrum, after benzocaine, are of central origin.

Miller (1978) has carried out the only parallel study, in the rhesus monkey: Using the EMG from the anterior temporalis muscle (a muscle of equivalent function to the Trout adductor mandibulae). He has made spectral analyses of the EMG at intervals following the administration of Ketamine HCl, a dissociative anaesthetic which has pharmacologic actions unlike any other anaesthetic. Following Ketamine, a significant shift in the spectrum occurred, enhancing the lower frequencies especially in the 20 - 40Hz range and after one hour, a decline in the number of active motor units. In the fish, there was a definite biphasic response with enhancement of the higher frequencies during the middle of the recovery period.

It is difficult though, to derive parallels with such widely differing drugs and animals, particularly as the Ketamine acts on structures in the mammal which the fish does not possess. (See Oswald 1978 a , McCarthy et al. 1965 and Massopust et al. 1972 for basic pharmacologic effects).

2.24 Indirectly stimulated adductor mandibulae preparations

Whilst not intended to be definitive, this series of experiments shows that consistent differences in the evoked EMG of the Trout adductor mandibulae to indirect stimulation occur.

The temporal separation of the spike and monophasic EMG potentials is similar to that seen by Browne (1976) in the sheep extraocular muscles although the time scale is different, presumably due to the higher temperature in the sheep. Tasaki and Tsukagoshi (1943) have shown monophasic EMGs in Amphibian slow fibres and biphasic EMGs in fast fibres, recorded extracellularly. Similarly, Bach-y-Rita and Ito (1966) have demonstrated the same in Cat extraocular muscles. Some authors, using EMGs, have found that monophasic potentials may occur in indirectly stimulated fast muscles depending on electrode placement (Kuffler and Vaughan Williams, 1953; Cuypers and Fessard, 1954). In mammals (Bowen, 1974) the normal evoked potential from red and white muscles is biphasic.

The temporal separation of the two types of evoked potential is due to differences in the conduction velocity of their respective motorneuron. It is generally accepted that fast motor units will have fast conducting axons (Hudson, 1969; Hammond and Ridge, 1978), which will be of large diameter (Gasser and Erlanger, 1927). The extremely rapid block of the red fibre EMG by Xylocaine supports the assumption that they are innervated by small diameter nerve fibres. The susceptibility of nerve fibres to local anaesthetic blockade depends on their size (Gasser and Erlanger, 1929). Small fibres block readily and have lower stimulation thresholds.

The sudden rises in tension seen in Plates II. 4. - 5 are, therefore, probably due to recruitment of mosaic fibres i.e. their threshold to short durational stimuli is higher than in red fibres.

Finally, some more substantial evidence of the 'dual block' response to Decamethonium has been found though it is not possible to say if the block is antagonised in red, mosaic or both fibre types.

Little is known of neuromuscular pharmacology in fishes and there is clearly much scope for research in this field especially as there are probably phylogenetic differences in muscle structure between Teleost Groups (and Elasmobranches) (Bone 1966; Hidaka and Toida, 1969).

2.25 Nerve and Neuromuscular Blockade Experiments

In the experiments involving the local anaesthetic block of the y^{th} nerve during spontaneous ventilation or indirect stimulation, it is evident that further direct electrophysiological evidence is required in order to determine the function of the large branch of the nerve which enters the central aspect of the m. adductor mandibulae. It is possible that this may be largely sensory which could be resolved by nerve recordings. It is not the motor supply to the red portion of this muscle and it is suggested that the nerve branches seen on the inner surface of the muscle in Section I contain the motor supply. Roberts and Witkovsky (1975) have shown a monosynaptic reflex activation of the adductor mandibulae in the Dogfish by mechanical stimulation of the perioral area and that there is a predominance of sensory neurons in the mandibular branch of the y^{th} nerve which may point to a homologue in the Trout.

The experiments into the effects of neuromuscular blockade of the adductor mandibulae during spontaneous ventilation, were somewhat inconclusive: The drug Decamethonium produces in red muscle a 'dual block' which has the characteristics of non-depolarising agents such as d-Tubocurarine, Gallamine, Pancuronium and those of an overt depolarising agent such as Suxamethonium. In a red muscle such as the soleus, a Decamethonium induced block can be antagonised by Neostigmine and increased by d-Tubocurarine in contrast to white muscle in which the block is unaffected by Neostigmine and antagonised by D-Tubocurarine (Paton and Zaimis, 1951; Zaimis, 1953; Jewell and Zaimis, 1953). Although the use of these drugs has never been reported as a 'diagnostic' method in muscle physiology, it was worthwhile to try their effects on the trout adductor mandibulae. Ballintijn (1965) has shown that Suxamethonium-induced paralysis is

irreversible in the Carp. The use of Gallamine in the Trout gives a paralysis of approximately three times the duration of that in the mammal (own unpublished observations; Westhues and Fritsch, 1965). This is interesting as Suxamethonium is used clinically for its brief action of 5 minutes or so. Species differences are encountered due to the variability in the levels and activity of the enzyme plasma pseudocholinesterase which normally hydrolyses Suxamethonium to choline and succinic acid, thus rapidly terminating the blockade (Westhues and Fritsch, 1965; Hall, 1971; Burgen and Mitchell, 1972). The attempts at opposing the block induced by Gallamine and d-Tubocurarine were only partially successful, although the reversal of a putative 'dual block' with Neostigmine and tetanic contraction were slightly more successful. It is, therefore, possible that dual block does not occur in Teleosts or else the effect of Decamethonium may be biased toward depolarisation. Interneuronal blockade by GGE produced interesting results, particularly in the unusual alteration of the EMG. The ventilatory EMG and contractile activity of the adductor mandibulae red fibres were depressed in the trout but differently from the 'classic' agents. Westhues and Fritsch (1965) state that GGE only blocks red muscles via interneurons and if so, then as the red fibres were affected this points to the tonic nature of the red fibres. GGE, however, has central effects (Marcus and Lobermeyer, 1955; Frey et al. 1952) which include respiratory and cortical depression at higher doses. The doses employed here were in the range used clinically in the domestic animals, though. It would have been interesting to have tried the effects of the more modern interneuronal blocking ataractics such as Diazepam (Valium-Roche) and Xylazine (Rompun-Bayer). The latter has been shown to produce apnoea in effective doses in Trout (Oswald, 1978a) but as the dose rates were much greater than in mammals it is not known if

the effect is due to interneuronal blockade or else to general CNS depression.

2.26 Electrical activity of the trout alimentary tract

EMG recordings from the trout stomach indicated that they were probably not suitable as transmitter input signals. Although the slow spike activity was of relatively high amplitude and therefore the signal to noise ratio is high, the periodicity is too long to allow tracking of the fish. The periodicity resembled those of gastric circular muscle described by Burnstock (1958, 1959). Considerably faster slow wave activity in the Carp was demonstrated by Ito and Kuriyama (1971), although the temperature was elevated (17°C) and contraction rate is known to follow the Arrhenius Equation.

At temperatures $> 18^{\circ}\text{C}$ Burnstock (1958) has demonstrated a reversible inactivation of peristalsis. In the wild, temperatures greater than this may be found in summer and this would presumably affect gastric evacuation and hence feeding periodicity.

The EMG waveforms did not resemble those recorded from Amphibian stomachs (Berger and Dahl, 1974; Prosser 1974) but rather more resembled those seen in dog intestine (Daniel, 1968). The work does show, at least, that the contractile system of the fish gut has electrical features comparable to other vertebrates.

Some evidence for an aborad migration of a pacesetter complex has been found, similar to other species. This is not true propagation (Christensen, 1974; in Daniel 1974), but is a migratory spread of excitation in three dimensions from active pacemaker regions via cellular nexuses (Oki and Daniel, 1974).

The rhythms have been shown to be susceptible to agents which modulate the controlling nerve plexi.

This is an unexplored field of research with an already established technology in the human field from which to draw upon.

Unfortunately, none of the fish in this work would feed after surgery and because of the short life of the EMG transmitter (which depends on interrogation rate) this limits the usefulness of the technique. Investigations in the mammal have generally allowed long recovery periods to allow the growth of tissues around the electrodes, which would not be desirable for field telemetry. If a simpler method such as Stevens and Worrall's (1974) EGG or Implant of fine wire electrodes per os and exteriorised like Wardle and Kanwisher (1974), or even miniature strain gauges (Kelly, 1974) affixed to the fishes abdomen externally to record girth changes with feeding could be implemented then it is probable that a more successful attempt could be made. Analysis of data might be difficult though there is much software e.g. Linkens and Cannel (1974), available from the human field.

Section III : Biotelemetry

Introduction

As previously stated, the object of the present study was to develop and prove, for field use, a telemetry system to permit the simultaneous monitoring of the fish's spatial position, trophic and ventilatory activities.

A decade ago, such a project would have been considered technically unfeasible, but progress in microelectronic applications has continued to advance at a staggering rate since then. As pointed out by Stasko and Pincock (1977) it is a case of a technology in search of suitable problems. In the case of aquatic animal biotelemetry, however, the technical problems are greater, and the amount of skill and finance available is usually much reduced, compared to biomedical applications. It is, therefore, important to realise that work as the present study, which is carried out at the frontiers of available technology, is seldom productive in terms of experimental quantity. A survey of twenty published papers on fish telemetry reveals that 50% contain the reports of four or fewer experiments. The largest number used over a long period has been 26 fish (Hasler et al. 1969) but this was using simple tracking transmitters only.

Apart from the work carried out using a computer monitoring system (Kleerekoper et al. 1970, 1974) there is very little information on the relationships between a fish's movement parameters and other behavioural variables. The only baseline which with the present study can be compared with is that of the preceding work on the Airthrey Loch system e.g. Tytler et al. 1976, Priede and Young 1976. This is because it is the only work in which any real quantitative approach has been used. It was, therefore, intended that the maximum amount of information be extracted from the costly and

difficult to obtain data in order to gain the maximum usefulness out of the study. It is natural that techniques such as the comparison of detailed movement patterns, qualitatively and quantitatively be used in order to facilitate comparison with the earlier work at Airthrey Loch and that carried out by Kleerekoper's group in Texas.

Section III : BiotelemetryMATERIALS AND METHODS3.1. Ultrasonic Tracking Transmitter

The transmitter first employed in tests to telemeter EMGs from trout in the laboratory was essentially the same as that described by Young and Wiewiorka (1975) and used by Priede and Young (1977) to telemeter cardiac rhythms from Brown Trout in Airthrey Loch. This was rejected because of instability problems in the circuit design and subsequently an improved version was designed. This differed in that the third operational amplifier (A3) in the package is no longer connected as a comparator but is simply connected as another amplifier. In addition, RC combinations (C4/R7, C7/R8) now introduce upper and lower frequency cut-off points to avoid triggering the device by mains hum or spurious high frequency noise. The supply voltage is increased to 3.05V using three RM312 cells (Mallory Ltd) instead of two. The overall gain of the amplifier has been increased to accommodate the low signal levels experienced in EMG work. Amplifier A1 is deliberately kept at a low gain in order to maintain a high input impedance to the electrodes. (Fig. 3.1, Plates III. 1-2).

The transmitter functions in a similar manner as the ECG transmitter, incoming spike potentials from the muscle are amplified and if large enough, the output from A3 will turn on the base of TR1 for a period of 10mS. Thus via the feedback coil L1, TR1 oscillates and the resulting RF burst is applied to the PZT transducer XT1 via L2/L3 which forms a step-up transformer to excite XT1 at a peak to peak voltage of about 30V. The arrival of a second pulse at TR1 is rejected if it arrives at a time after a preceding one within a time constant set by $0.7 R9/C5 = 47.6mS$.

Fig. 3.1

Schematic diagram of the circuit of the final
version of the EMG transmitter

Fig. 3.2

Schematic diagram of the recording equipment
used in the field experiments

Fig. 3.3

Schematic diagrams of the pulse former circuit
used to process the telemetry receiver output.

Fig. 3.4

Schematic diagram of the Timer/Oscillator used
in the field telemetry experiments

(Component values are given in Appendix II)

FIG 3.1

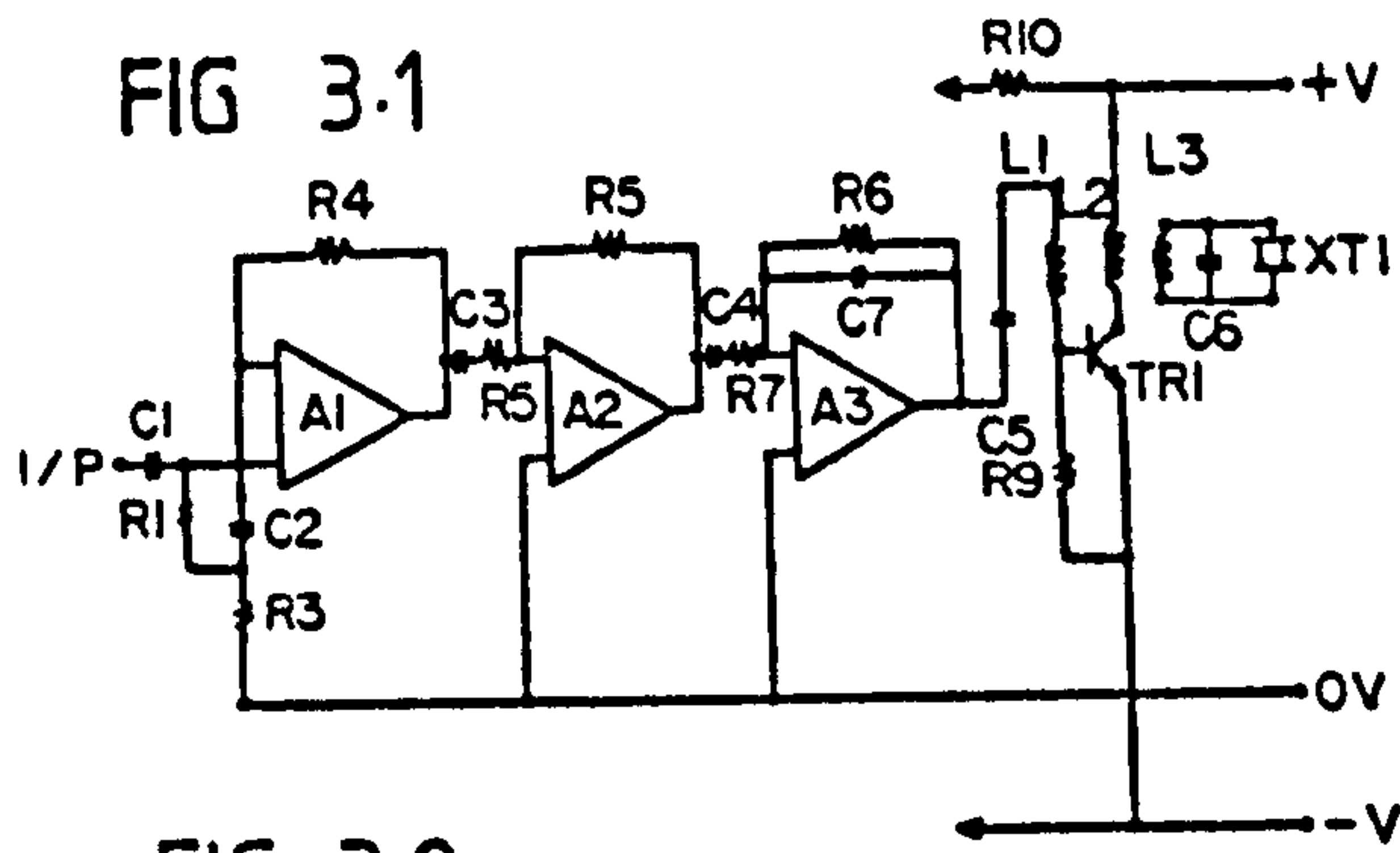


FIG 3.2

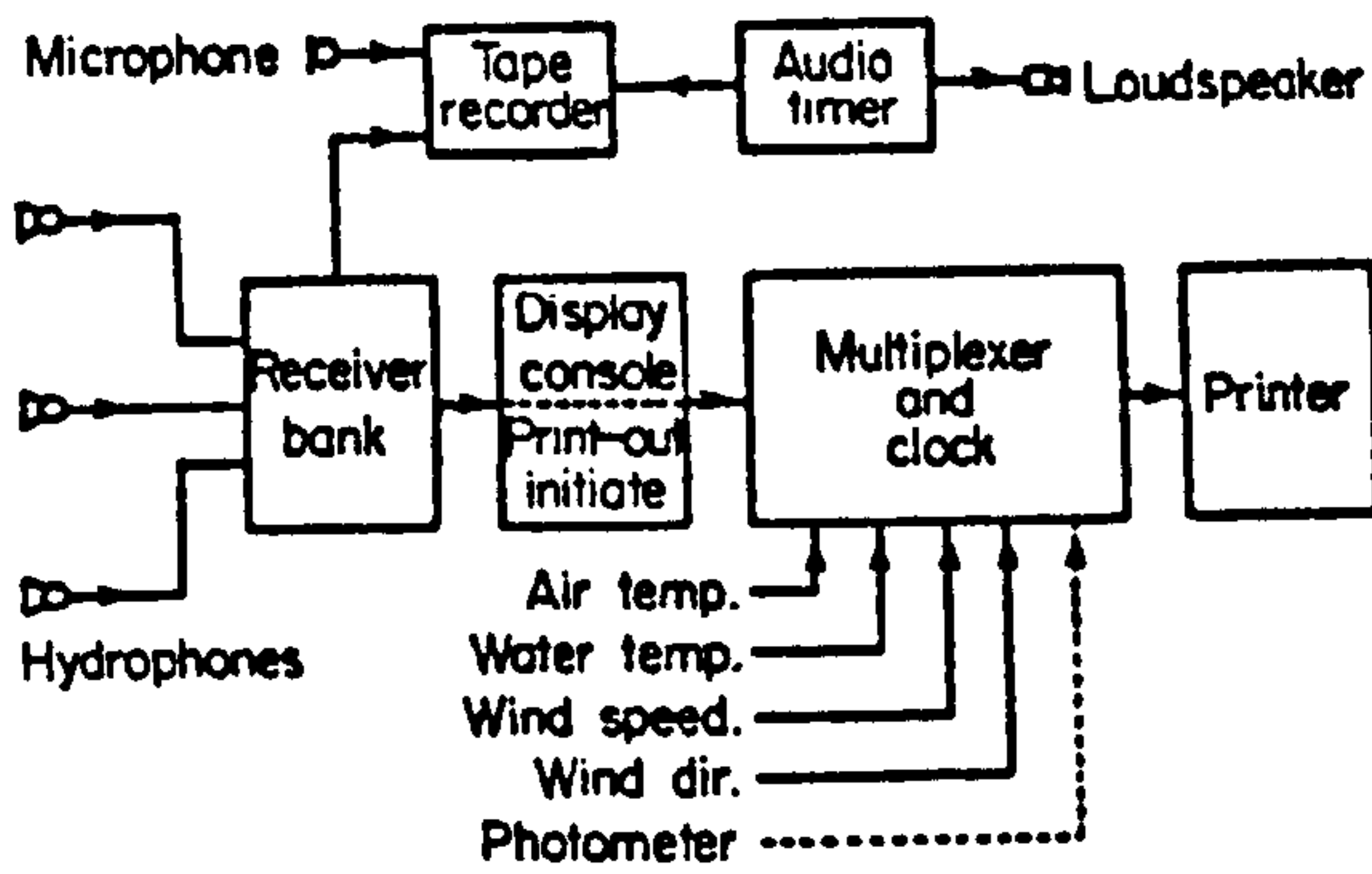


FIG 3.3

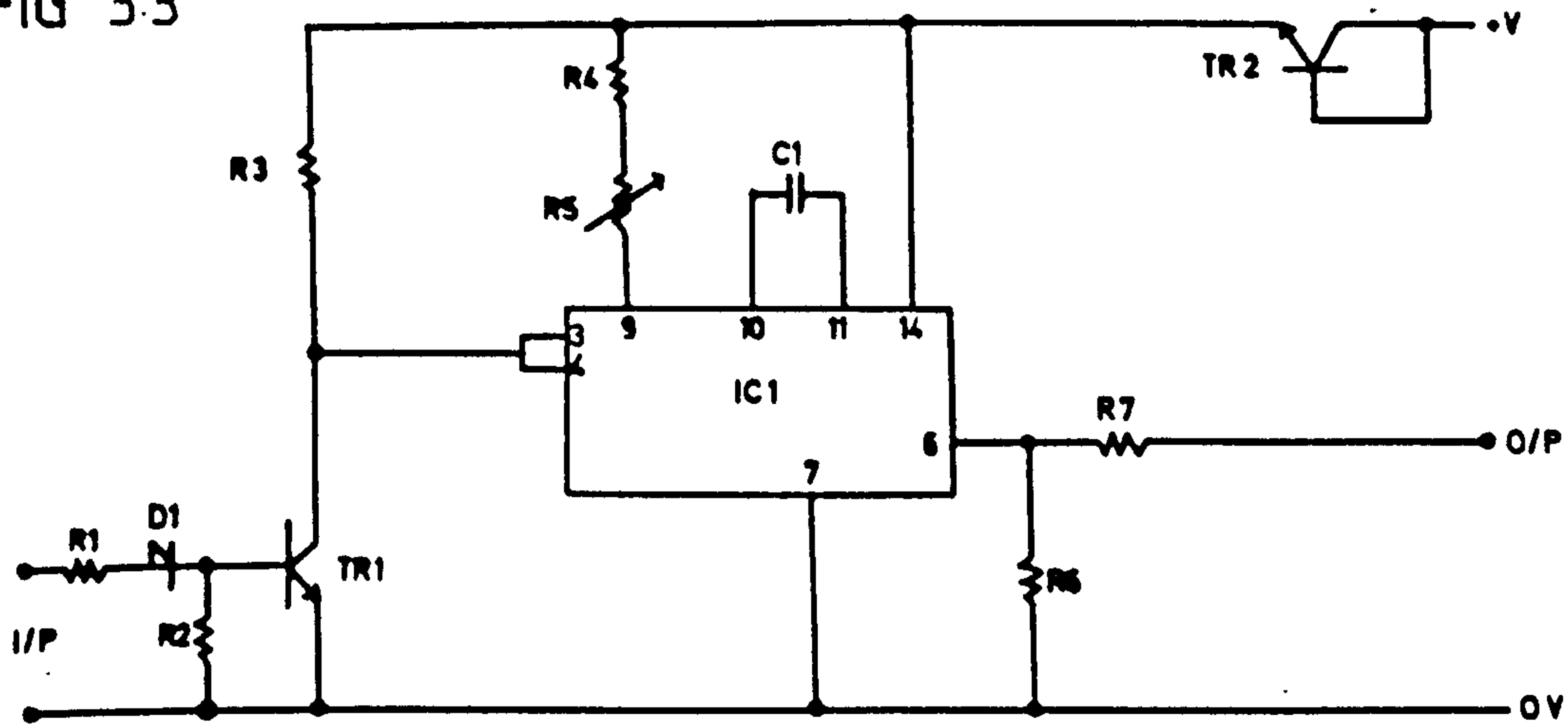
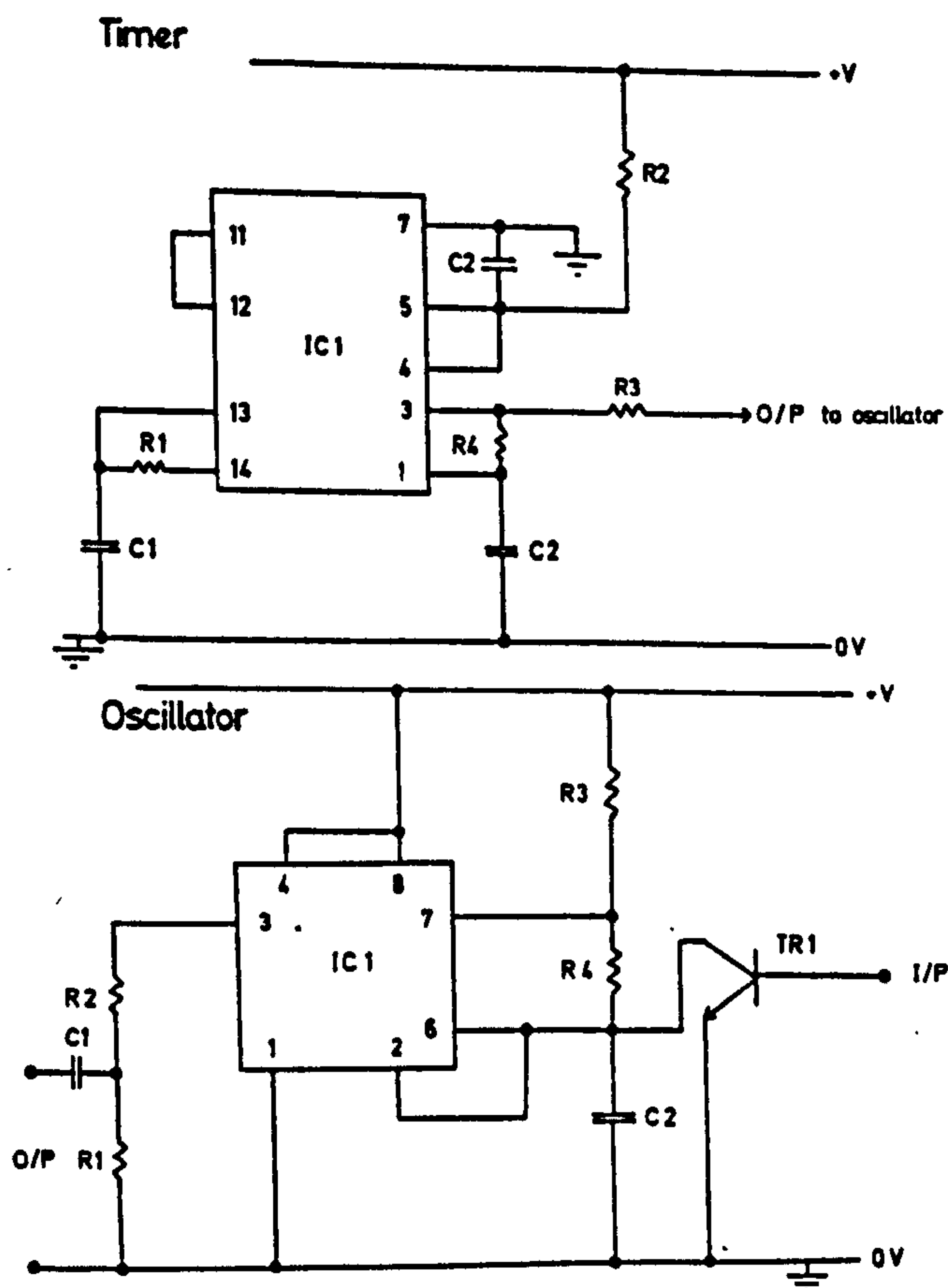


FIG 3.4



Initially, the transmitter was encapsulated in the same manner as Priede and Young using silicone rubber lightened with microspheres. Later this was abandoned in favour of the following method. The transmitter is fitted into a cutdown 5ml polypropylene syringe barrel (Plastipak-Becton, Dickson) which is itself positively buoyant. The input terminals are produced through a rubber bung which also acts as an injection septum for the filling of the tube with light paraffin (S.G. 0.85). This ensures incompressible insulation and also good acoustic transfer efficiency as the transducer is no longer in contact with the water. This reduces the apparent weight in water to 2.5g, a reduction of 0.7g over Priede and Young's transmitter, despite the addition of another cell weighing 0.64g. The length is increased slightly to 44mm. (Plate III.2).

The transmitter is attached to the fish by the two silver wires visible in Plate III.2. A backing plate on the opposite side of the fish bears the transmitter serial number, in case of recovery.

3.2 Receiving System

The Airthrey Loch tracking system is as described by Young et.al. (1976) and Priede and Young (1977). The position of the fish is determined manually by triangulation with three precision-rotatable directional hydrophones. These are now controlled from a console which incorporates an illuminated visual display of the loch with three projected light beams which represent the hydrophone bearings. The hydrophones are rotated by rocker bar controls which incorporate a touch control switching system so that the appropriate hydrophone audio output is automatically selected during scanning. Digital display of the

hydrophone bearing is also switched by this means. The speed of rotation of the hydrophones can be selected by the operator using a variable control.

Each hydrophone station has a secondary omnidirectional hydrophone for the continuous detection of the telemetry signal without the necessity of tracking. Each directional hydrophone has an individual receiver in the receiver bank (Lafayette HA600A) but the signal from the omnidirectional hydrophone selected is routed through a fourth receiver for continuous monitoring. Outputs from the console were fed to a data transfer unit (multiplexer) (Solartron, Schlumberger Ltd) which switches the time, hydrophone bearings and then up to sixteen additional channels of information in turn, as required. The sequential read-out of information is fed to a numeric printer (Addo Ltd) and a print-out is initiated either by the operator pressing a button on the console or by clock command from the multiplexer clock unit. In the last field experiment (Fish 4), the environmental parameters indicated in Fig.3.2 were also fed into the multiplexer from a Heathkit Electronic Weather Station. The photometer was not connected at this stage but would, however, be so in the future. (fig. 3.2)

The telemetry signal was processed by a pulse former circuit which (Fig. 3.3) converted the varying spike signals to rectangular pulses, 4V amplitude, 8mS duration. Initially, this was recorded on either Devices M2 or Mx212 high speed pen recorders, but owing to frequent pen damage because of the fast rise time of the signal and the high cost of recording paper, this was abandoned in favour of magnetic recording on a domestic four-track tape recorder (Ultra Ltd). Timing marks were superimposed on the tape records by a Timer/Oscillator

(Fig. 3.4) which was synchronized with the multiplexer clock using a stopwatch. This device produced at exact five minute intervals, a 1kHz square wave signal of 5S duration. This was also fed into a loudspeaker to pace the operator.

In the laboratory, tests of transmitters were generally achieved by the convenient method of detecting the near field R.F. output from the transmitter, using a conventional A.M. portable radio.

3.3 The Study Area

Airthrey Loch is a shallow eutrophic loch of artificial origin. It is of 90300m² area but except for certain experiments (Tytler et.al. 1977) all tracking operations are conducted in the west pool of the loch (44,400m²). The mean depth of the loch is 1.5m and a contour map derived from echo sounding (Ferrograph Ltd) is shown in Fig. 3.5. The level of the water may be nearly one metre higher than those shown in the map. The loch varies in temperature from around 4°C in winter to over 26°C in summer (B. Finlay - pers. comm.). The pH of the water varies widely in summer but is never lower than pH7. Dense algae blooms are a feature of summer and are usually due to Cyanophytes. The loch is annually restocked by the University Angling Club as immigration into the loch is impossible and spawning fish are usually trapped during the winter spawning run as they attempt to ascend the burn. (Plate III.4)

Other fish species present include vast numbers of Sticklebacks (Gasterosteus aculeatus L.) and a few Minnows (Phoxinus phoxinus L.). No predators such as Pike are present.

Fig. 3.5 a

Computer produced contour map of the West Pool
Airthrey Loch, derived from echo-sounding transects.
Contours in metres relative to shoreline.
Note the prominent 'shelf' opposite the west shore.

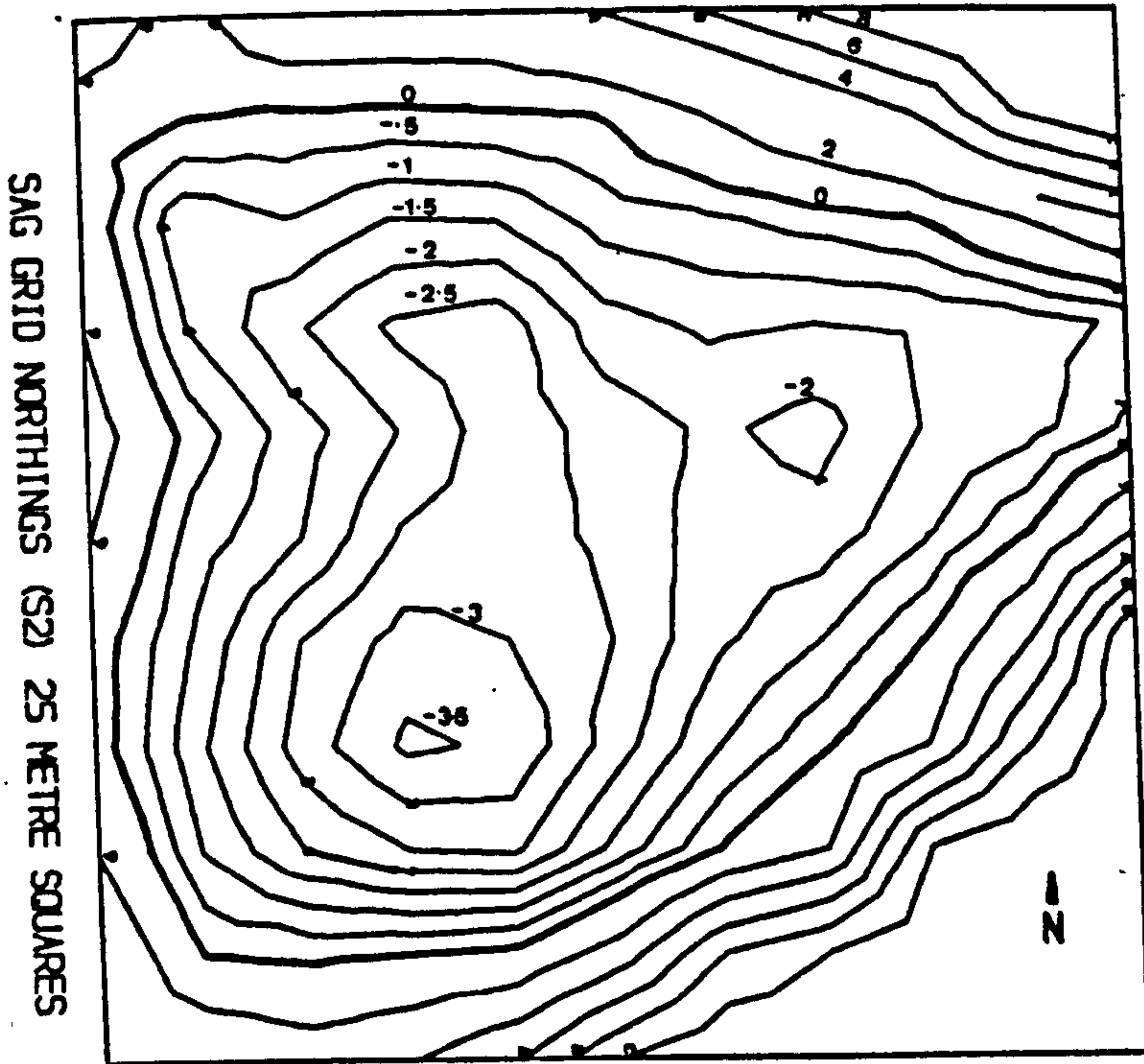
Fig. 3.5b

The locations of the hydrophones, the bearing system and
the location of the Standard Airthrey Grid. Each grid
square is 25 metres square.

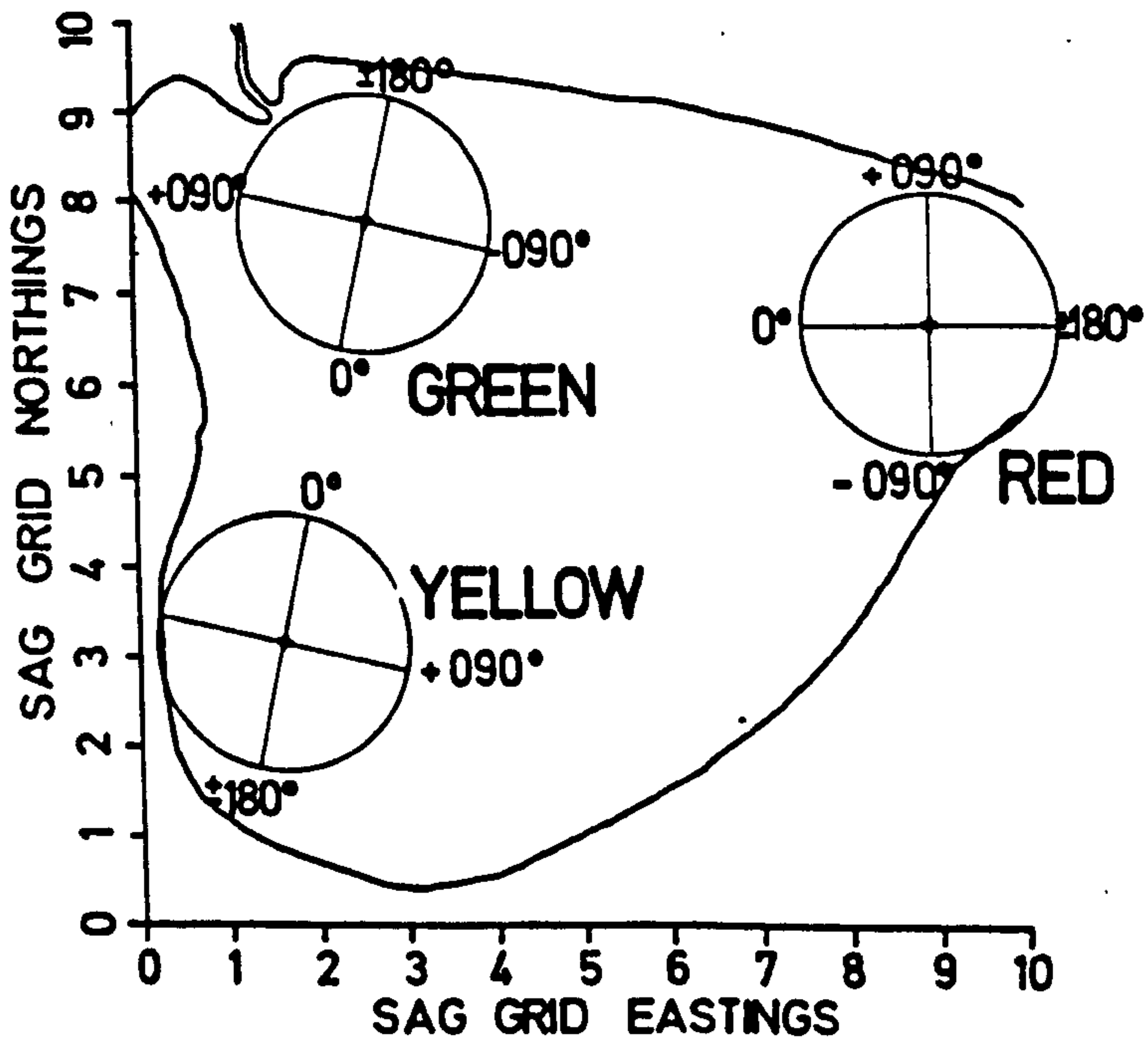
FIG 3.5

a

SAG GRID EASTINGS (S1) 25 METRE SQUARES



b



HYDROPHONE BEARING CONVENTIONS.

3.4 Tagging Procedures

Fish were caught on rod and line by trolling a lure from a boat equipped with an electric motor. After capture, the fish were held in a rectangular 'Netlon' cage moored in the loch near the caravan. Tagging operations were carried out using the anaesthetic facility described in Section 2.2. The transmitter was affixed to the fish in an identical manner to Holliday et. al. 1973. The electrodes were of the 'T-pattern' and affixed as in Section 2.5. Following evaluation of the EMG on the CEPTU unit, the electrodes were connected to the transmitter terminal, the ends of the leads and the transmitter painted in fast drying primer. The connections are then waterproofed with fast setting silicone rubber (Impressil) which is rendered less conspicuous with a brown colouring additive. To obviate any irritation to cutaneous afferents caused by the mounting wires, the punctures were subcutaneously infiltrated with a long acting local anaesthetic made up to the composition of the discontinued preparation 'Proctocaine' (Allen & Hanbury). After recovery the fish is transferred to a tank to assess its suitability for release. Fish to be released were transported in polythene bags and liberated from a boat, as near to their capture point as possible.

3.5 Interpretation of Records

Attempts were made to use mean voltage processing of the signal, to aid in the identification of events. This was done by a four diode bridge rectifier, smoothed by three switched RC filters giving time constants of 72, 33 and 14 ms respectively. (Inman et. al. 1952, Bigland and Lippold, 1954). The counting circuit used by Priede and Young was unsuitable for use with EMGs because of the variability in the number of spikes in the EMG and secondly because the transmitter

itself performs a certain amount of processing due to the fact that it only triggers an output pulse for input spikes above a threshold level. Because the development of a computer program, to decode the highly variable EMG recordings automatically, was liable to be both difficult and time-consuming, the simpler (though tedious) but effective method of decoding them aurally was adopted. It was easy to recognise feeding events by simply listening to the tapes and noting the time of the events relative to each timing pulse. (See Section 3.7).

3.6 Data Analysis

The tracking data from the printer output and the information on feeding and ventilation, derived from magnetic tape, were punched on card separately. Using the University of Stirling ICL 4130 Computer, the angular data was processed by an ALGOL program "FISHTK" which was modified version of the program used by Tytler et. al. (1977) for which acknowledgement is made to D. Machin and the late P. Guyatt for the original version. This program reads the tracking data and calculates subsequent positional fixes in terms of the Airthrey Grid System (Fig. 3.5b), the distance between successive fixes and the angle of turn made by the fish. Should the fix resolve into a triangle of error, then the mean centre of the triangle is calculated as the fix. If the bearings read suggest an impossible position, then various warning messages are output to the lineprinter. Should only two bearings be obtainable (a common occurrence in practice) then a missing value is indicated and the fix is then calculated on the two bearings only.

Graphical output of tracking movements and special statistics were obtained using specially written FORTRAN programs. Statistical analysis of the data was carried out using the implementation of SPSS for the CDC 7600 computer (Nie et. al. 1974).

Statistical significances are shown as either:=

- a) the absolute level of significance or probability, or
- b) a series of asterisks indicating the following levels:

* = 5% ** = 1% *** = 0.1%

Data tabulations of the tracking results are included as a microfiche in Appendix III.

Mnemonic names were assigned to recorded and derived variables and are listed below:=

- REF : is the line number of the data file.
- TIME : is the time in hours and minutes BST.
- TE : is the time elapsed since tracking commenced in decimal hours BST.
- M1 : is the total number of feeding events directed, at the end of each sampling period.
- M2 : is the cumulative total number of feeding events, at the end of each sampling period.
- M3 : is the total number of feeding events (M1) expressed as a percentage of the total recorded.
- V1 : is the ventilation rate, in cycles per minute, at half hourly intervals.
- D1 : is the distance travelled in metres, since the preceding positional fix at five minute intervals.
- D2 : is the cumulative distance travelled, in metres, since the last positional fix.
- D3 : is the mean specific swimming speed in BL/s^{-1} since the last positional fix.
- AN1 : is the angle of turn expressed in degrees $0-360^{\circ}$ clockwise from the preceding course.
- S1 : is the Airthrey Grid Co-ordinate Easting.
- S2 : is the Airthrey Grid Co-ordinate Northing.
- D4 : is \log_{10} step length (D1).

Section III : Biotelemetry

RESULTS

3.7 Laboratory tests of the transmitter

From laboratory tests of trout equipped with electromyographic electrodes, it was seen that during feeding there was a characteristic series of large (often $>1\text{mV}$), fast-spiking EMGs which was unlike the regular rhythmic bursts due to ventilatory activity. It was decided that although ideally the transmitter should be silent until the fish actually fed, it was desirable to have a regular signal in order to track the fish. The gain of the transmitter was therefore increased to be sensitive enough to display the ventilatory EMG.

In its latest form the transmitter was very sensitive. Fig. 3.6 (a) and (b) shows a simultaneous recording of the EMG and the transmitter pulse output. Note that even small spikes of around $50\mu\text{V}$ are telemetered and the pulse trains indicate the ventilatory cycle.

It was found relatively easy to distinguish between 'coughs', 'yawns' etc. and feeding acts. During a yawn the adductor mandibulae was inactive and there was typically a silent period from it. The 'cough', as described by Hughes (1975), Hughes and Adeney (1977), was readily distinguished from the telemetered EMG signal. It is seen that there is an extended burst followed by a short burst; this corresponds to the holding closed of the jaw followed by a rapid opening and shutting. Fig. 3.6 (c) shows the telemetry record from a trout in which coughs occurred. The cough cycle is short, generally under 1 s and the normal ventilatory rhythm is soon restored. Fig. 3.6 (d) shows the EMG record from a trout during a cough and there is evidence at the start of the second burst to suggest fast (mosaic) fibre involvement. The high discharge frequency of the mosaic fibres gives a distinct change of timbre to the telemetry recording during a cough. Similarly, when

Fig. 3.6

Simultaneous recording of transmitter output pulses (a) and ventilatory EMG input (b). Note that the $50\mu\text{V}$ spike arrowed in (b) is telemetered in (a). (c) Transmitter output envelopes from free-swimming Brown Trout in the laboratory. Two coughs are arrowed. Note time course of cough and the extended burst length preceding it. (d) Adductor mandibulae EMG during a cough. The fast spikes arrowed correspond to the rapid closure phase of the jaw. The remainder of the EMG is composed of motor potentials derived from junctional potentials, hence their relatively low frequency. (e) Groups of electromyograms recorded from a free-swimming Trout during feeding. Note high amplitude and repetition pattern of the EMG.

Fig. 3.7

Telemetered EMG trains recorded from Brown Trout during feeding acts; arrows indicate the onset of the feeding act, time of day is given below each figure. (a) Fish 2 (b) Fish 3 (c) Fish 4 (d) Fish in laboratory tank.

Fig. 3.8

A successful example of automatic identification of feeding events by RMS integration:

Arrowed are feeding events, timer pulses identified by spots. In the lower trace, at a high recording speed, it can be seen that the baseline is shifted during a feeding event. The large pulse on the right is a timer pulse. Data from Fish 3.2.

FIG 3.6

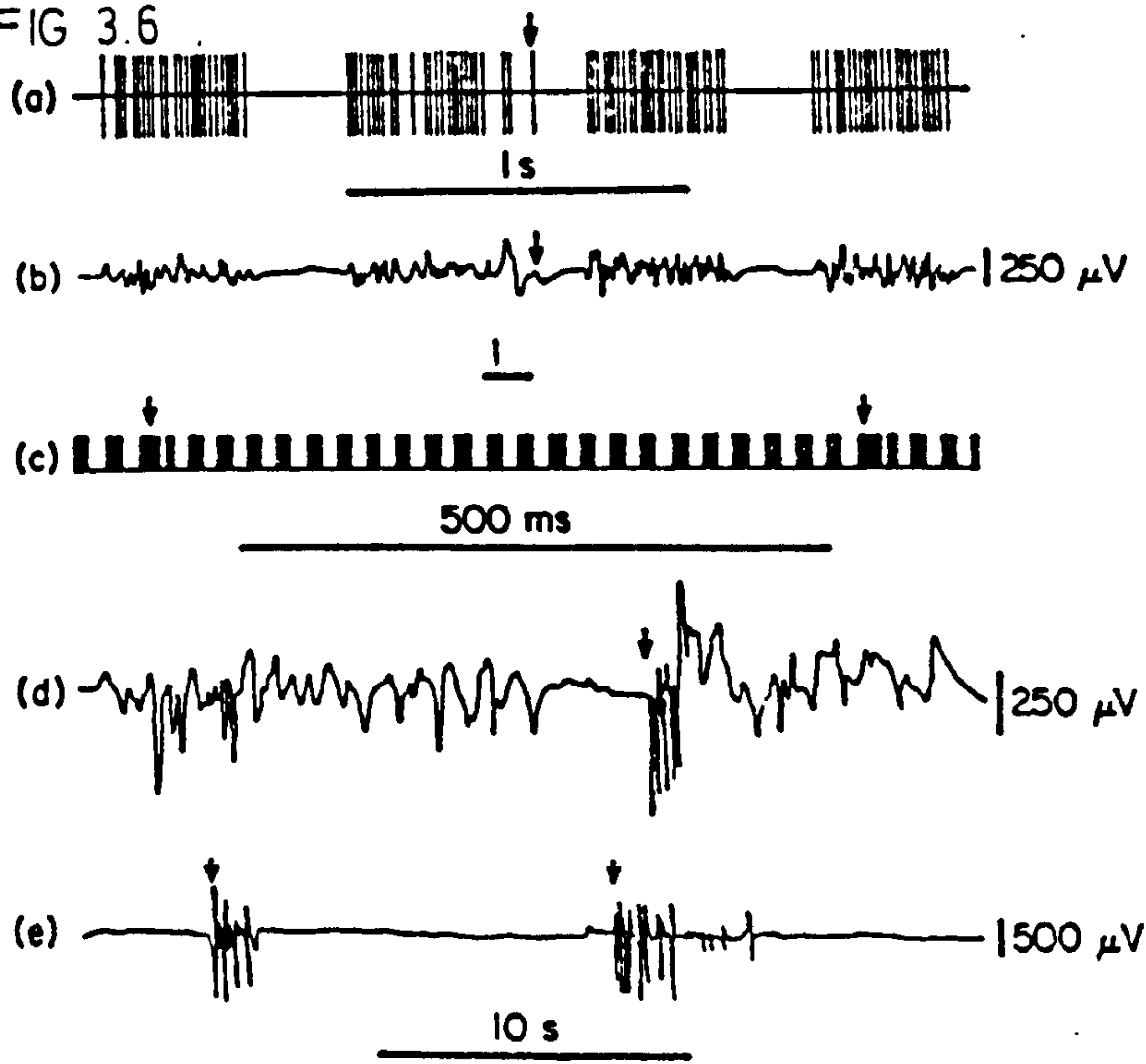


FIG 3.7

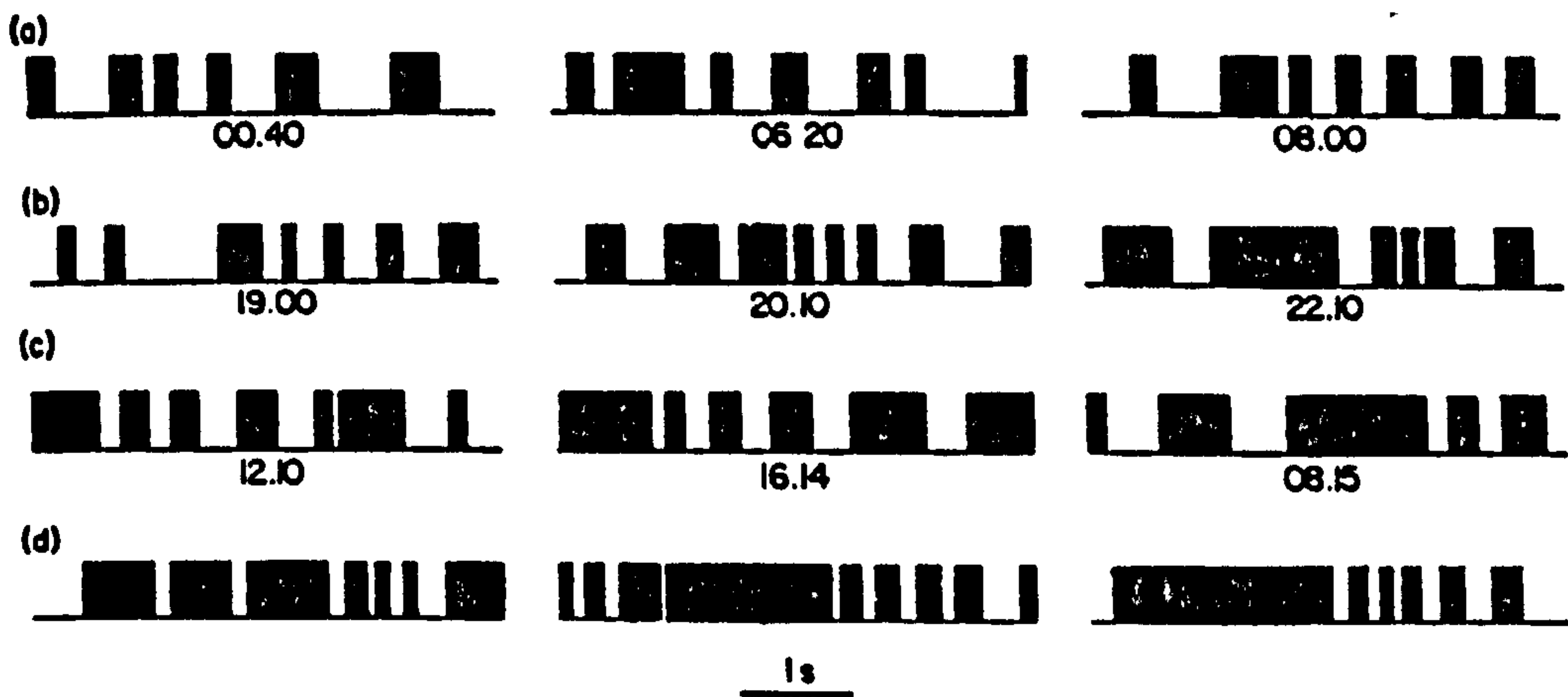
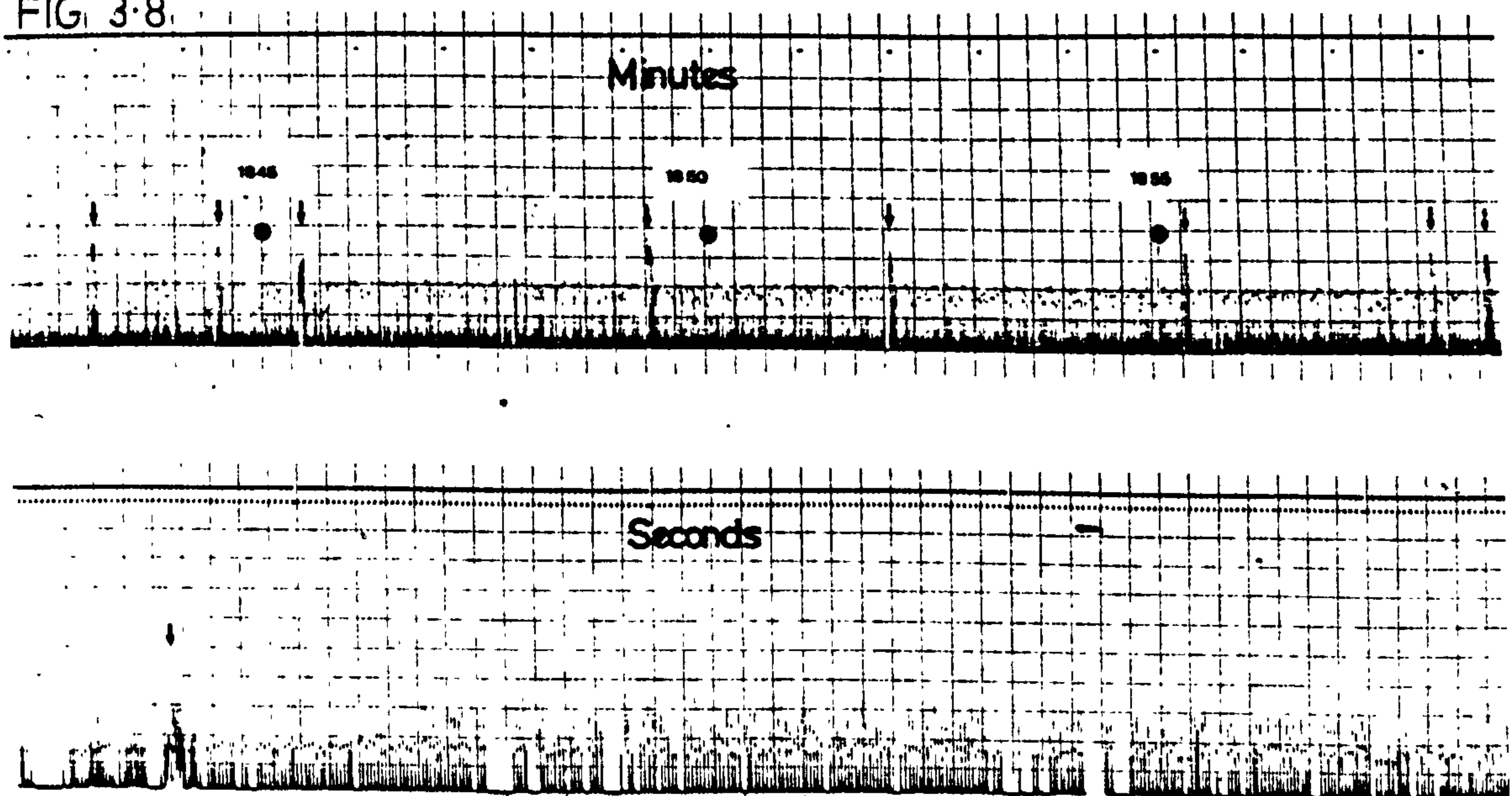


FIG 3.8



the mosaic fibres are recruited during feeding, there is a similar tonal change. This, coupled with the typical decreasing repetition rate following food capture allows confidence in the positive identification of the feeding event from the telemetry records.

The transmitter did not appear to affect the fishes ability to feed. A 550 g Brown trout was the dominant fish in a hierarchy of six; it readily fed on pellets whilst carrying one of these transmitters. This fish almost invariably managed to take single introduced pellets before its subdominants thus implying that its foraging ability was not impaired. There was little evidence to suggest that the fish's swimming performance was affected as retrieval of fish equipped with transmitters from large tanks was as difficult as usual.

Fig. 3.7 shows EMG envelopes traced from a storage oscilloscope screen using data stored on magnetic tape. Examples from the field and the laboratory are shown. It was found easiest to identify feeding events by listening to the magnetic tape recordings though in some cases success was achieved in using an R.M.S. rectifier-integrator circuit (Fig. 3.8).

It proved extremely difficult to persuade single trout, equipped with the EMG transmitter, to feed in the special laboratory. Results did not appear until very much later in the project when it was realised that due to the lack of social interaction (Uematsu, 1971, Stirling, 1977) and disturbance caused by unfamiliar surroundings (Wardle 1974), anorexia was being caused. Replacement of the tagged fish in their 'home' tank with their companions generally solved this problem.

3.8 Field Experiment Narratives

Four fish have been equipped with the EMG transmitter with adductor mandibulae electrodes and released into Airthrey Loch, University of Stirling. The relevant biometrics are given below in Table 3.1. A fifth fish was equipped with EMG electrodes to monitor tailbeat (which gives a large reliable signal) and was released on 2/12/75. This was intended as a system test and demonstration and no results are presented for it.

Table 3.1 Biometrics of Fish released in Airthrey Loch

Fish No.	Date	Time	Sex	Weight g	Length cm	K*-value	Tag Life days	Water Temp. °C
1	10/9/75	15.50	♀	487	35.6	1.08	9.0	12.0
2	5/11/75	20.00	♂	560	36.5	1.15	1.6	8.5
3	7/6/76	21.00	♂	460	32.5	1.34	2.9	20.4
4	23/9/76	16.23	♂	480	34.0	1.22	3.7	11.0

* Fulton's Condition factor ($K = \frac{W * 100}{L^3}$)

a) Fish 1

Fish 1 was released on 10/9/75 at 15.50. This was the initial field test of the adductor mandibulae EMG transmitter and served to 'debug' the design of the transmitter and the operating procedures. Problems with recording the telemetry signal on Devices recorders were quickly evident and magnetic tape recording was substituted in later experiments.

It was noticed after a few hours that the EMG signal was becoming less clear until eventually, the regularity of the signal was lacking. When the fish made a more vigorous excursion or when it was clearly feeding, the signal returned to its former quality and remained so for a short while until it decayed again. This suggested that some biological change was happening to the fish during the period after

release and that there was no fault with the transmitter.

Because of the time and cost involved, it became imperative that the cause of these changes was elucidated. The most likely causes included post-anaesthetic effects (Houston et. al. 1973) and incompatibility between the inherent filtration of the transmitter and the frequency spectral content of the adductor mandibulae EMG.

The results of the investigations were described in Section II and were important factors in the success of this project and its successors.

Because of the low interrogation rate, the transmitter continued to function for nine days though the poor signal quality remained unchanged. No results are therefore presented for Fish 1.

b) Fish 2

Fish 2 was released on 5/11/75 at 20.00 hours and was tracked from 21.00 hours on 6/11/75 to 12.25 hours on 7/11/75.

Good signals were obtained up to 12.25 hours on 7/11/75. The fish then made a direct excursion out of the West Pool into the inner part of the middle pool, out of range of all three hydrophones. Although its position was monitored on portable hydrophones, the transmitter failed at 16.30 hours.

c) Fish 3.1

Tracking was commenced at 22.00 hours on 8/6/76; the transmitter output was excellent. Tracking continued into the evening of 9/6/76 although difficulties were experienced with poor reception, noise and broadcast radio break-through on some directional hydrophones. In addition the omni-directional hydrophones were ineffective due to a phytoplankton bloom in the loch. Contact with the fish was lost at 22.00, radio breakthrough becoming particularly overpowering and so

the experiment was temporarily abandoned at 00.30 hours.

Because of the break in the middle of the records for this fish it was considered more convenient from the computational point of view if the two parts were regarded as separate experiments. Therefore, the first part up to the break on 22.20 hours on 9/6/76 has been designated as Fish 3.1 and the remaining part as Fish 3.2.

d) Fish 3.2

Contact was regained with the fish near the yellow hydrophone at 09.00 hours on 10/6/76 and the transmitter appeared to be working well. Tracking was continued from 09.10 hours through the night. At 08.00 hours on 11/6/76, it was found that the transmitter was not functioning correctly probably because of an electrode having worked loose.

e) Fish 4

The fish was released into the loch at 16.23 hours on 23/9/76. Tracking began at 11.10 on 24/9/76. This run employed the full data input set up shown schematically in Fig. 3.2. The transmitter worked well although difficulty was experienced with the hydrophones. During the later part of the tracking period, a gale gusting up to 70km/hr occurred.

At 06.35 on 25/9/76 the fish exited the west pool returning at 06.50 hours. The wind, by 10.00 hours was threatening exposed equipment due to flying broken branches of trees and so the run was terminated and the equipment retrieved.

On 27/9/76 a brief hydrophone search found the fish still in the West pool and the transmitter still functional but there was no financial provision for tapes etc., in order to extend the tracking on Fish 4.

3.9 Overall Telemetry Results

The gross results from the three field studies are shown graphically in Figs. 3.9 and 3.10. The former shows the number of feeding events (M1) and the ventilation rate (V1) recorded at half-hourly intervals. The latter shows the gross activity patterns recorded as the mean hourly swimming speed in BL/s^{-1} .

It can be seen from Fig. 3.9 that, particularly in the cases of Fish 3.1 and Fish 4, there are peaks of feeding activity present. Using the method of Landless (1976) for defining feeding peaks, the histogram blocks were grouped into peaks and intervals for the purposes of statistical computation. The ventilation rates displayed in Fig. 3.9 do not show any overt cyclical activity though some linear trends may be present. The activity cycle data displayed in Fig. 3.10 would indicate some differences between the three fish :

Fish 2 and 3.1/3.2 are dark-active whereas

Fish 4 is probably a day/crepuscular-active individual.

3.10 Summary Statistics

Summary descriptive statistics for each experiment are shown in Table 3.2 (a-d).

In all cases the mean number of feeding events (M1) is low, which is to be anticipated in a cyclical situation. The mean step length varies from 17.84 metres in Fish 4 to 32.31 metres in Fish 2. The largest single step length (D1) recorded was 184.3 metres (Fish 3.2) is also the fastest swimming speed recorded (D3). This is equivalent to a mean speed of 1.89 BL/s^{-1} , easily sustainable by Brown Trout. Mean swimming speed (D3) varied from $.17 \text{ BL/s}^{-1}$ in Fish 4 to $.3 \text{ BL/s}^{-1}$ in Fish 2.

The greatest total distance covered (D2) was surprisingly large,

Fig. 3.9

Feeding activity (histograms) and ventilation rate (VR) (dots) for Fish 2(a), Fish 3.1(b), Fish 3.2 (c) and Fish 4(d).

The period of darkness is indicated as black bars above each diagram.

Fig. 3.10

Mean hourly swimming speed for the experimental fish.

The dark period is indicated as black bars above the graph.

FIG 3.9

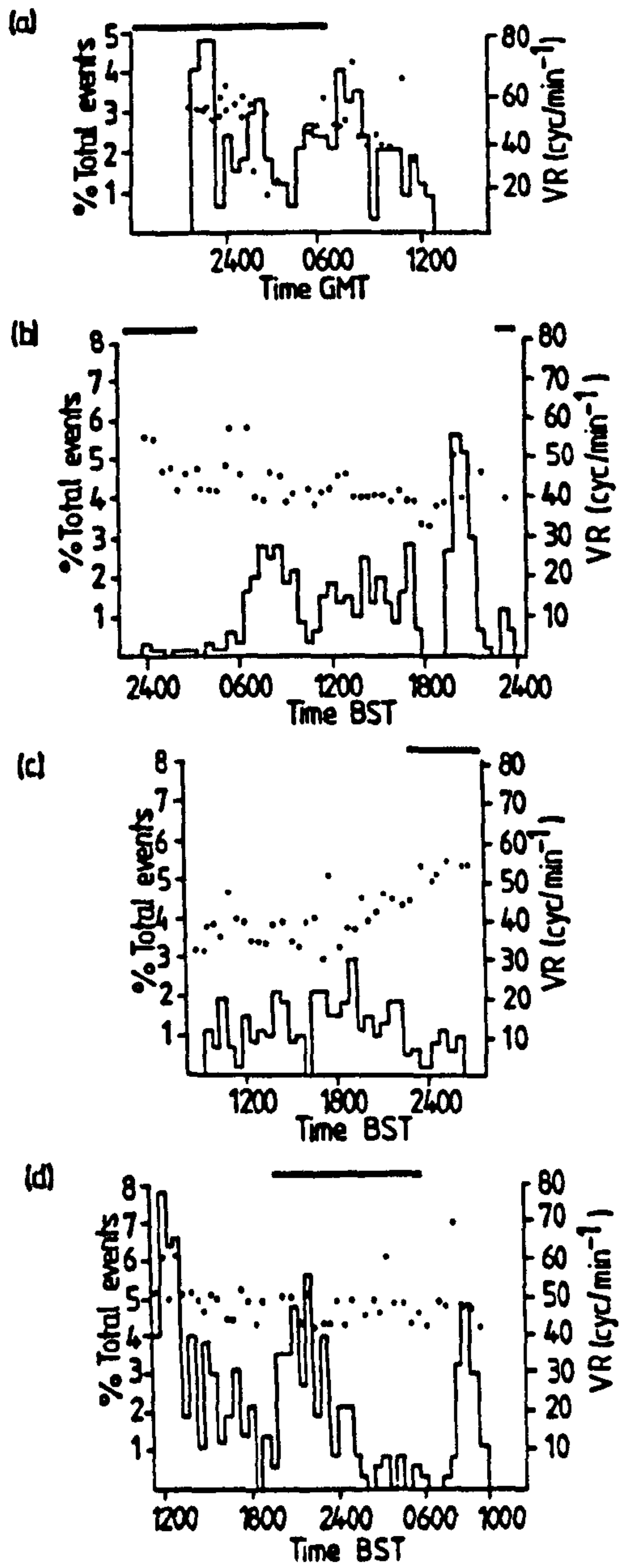


FIG 3.10

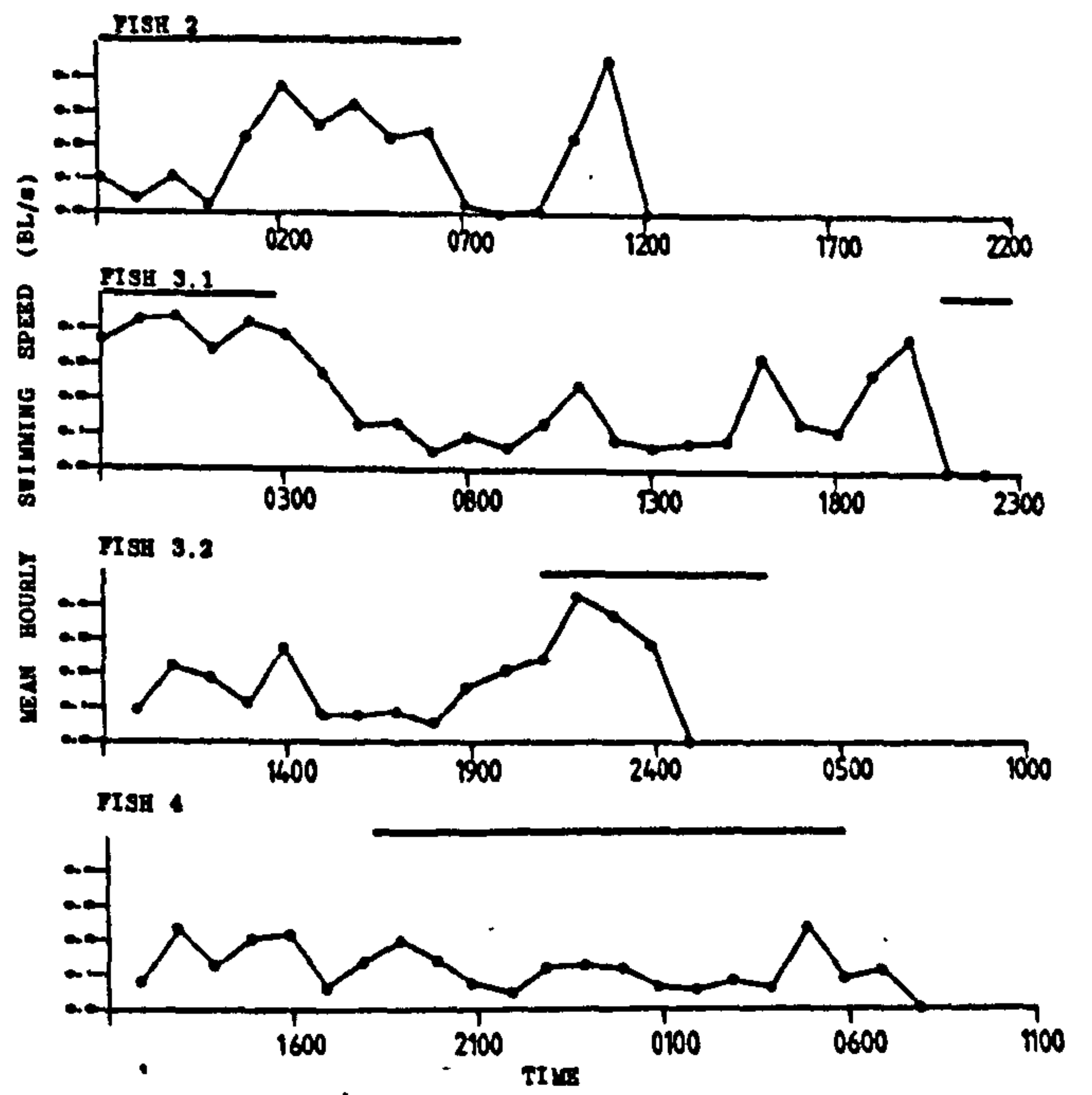


TABLE 3.2 a

SUMMARY DESCRIPTIVE STATISTICS FOR FISH 2

	M1	M2	M3	D1	D2	D3	AN1	V1	TE
MEAN	2.76	#####	0.54	32.31	#####	0.30	181.93	45.76	#####
S.E.	0.21	#####	0.06	3.46	#####	0.03	11.50	2.71	#####
MINIMUM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.00	0.00
MAXIMUM	9.00	249.00	3.61	171.10	3823.0	1.56	359.90	50.00	15.50
SKEWNESS	0.72	#####	1.50	1.01	#####	1.40	-0.17	-0.25	#####
KURTOSIS	0.32	#####	1.69	0.82	#####	0.83	-1.26	0.06	#####

TABLE 3.2 b

SUMMARY DESCRIPTIVE STATISTICS FOR FISH 3.1

	M1	M2	M3	D1	D2	D3	AN1	V1	TE
MEAN	2.61	#####	0.34	28.73	#####	0.29	212.50	55.90	#####
S.E.	0.27	#####	0.04	2.67	#####	0.03	8.49	1.13	#####
MINIMUM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.00	0.00
MAXIMUM	17.00	347.00	4.90	161.80	5974.5	1.66	359.60	74.00	24.42
SKEWNESS	1.62	#####	2.75	1.52	#####	1.52	-0.37	1.11	#####
KURTOSIS	3.34	#####	3.34	1.04	#####	1.04	-1.17	0.78	#####

TABLE 3.2 c

SUMMARY DESCRIPTIVE STATISTICS FOR FISH 3.2

	M1	M2	M3	D1	D2	D3	AN1	V1	TE
MEAN	2.46	#####	0.49	19.71	#####	0.20	185.54	42.23	#####
S.E.	0.22	#####	0.06	2.35	#####	0.02	8.25	1.18	#####
MINIMUM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.00	0.00
MAXIMUM	10.00	246.00	4.07	184.30	3468.5	1.09	359.60	66.00	10.33
SKEWNESS	0.98	#####	1.63	2.92	#####	2.92	-0.07	0.45	#####
KURTOSIS	0.74	#####	3.11	7.89	#####	7.09	-1.61	-0.17	#####

TABLE 3.2 d

SUMMARY DESCRIPTIVE STATISTICS FOR FISH 4

	M1	M2	M3	D1	D2	D3	AN1	V1	TE
MEAN	1.41	#####	0.35	17.84	#####	0.17	181.02	48.56	#####
S.E.	0.12	#####	0.03	1.41	#####	0.01	7.76	0.91	#####
MINIMUM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	42.00	0.00
MAXIMUM	10.00	372.00	2.62	125.00	3424.5	1.23	359.80	70.00	22.92
SKEWNESS	1.78	#####	1.83	2.51	#####	2.50	-0.02	1.63	#####
KURTOSIS	3.34	#####	3.34	7.79	#####	7.75	-1.22	2.94	#####

5.9km in 24 hours (Fish 3.1). This must be considered an underestimate due to missing observations.

The mean angle of turn values (ANI) were almost universally around 180° except for Fish 3.1. Mean ventilation rates were of the order of between 45.7 to 55.9 but the individual ranges were great, varying from 17 to 74 cycles per minute⁻¹.

3.11 Comparisons between day and night results

Table 3.3 shows the result of t-test comparisons between the means of the variables between night and day. For Fish 2, it will be noted that no significant differences were evident between the day and night periods for any of the variables. In Fish 3.1, however, highly significant differences exist between the means for all the variables. Step length is over three times greater by night and the feed total data is greater by day. Angle of turn is also greatly increased by night as is, apparently, the ventilation rate. Significantly lower mean values were found for step length, angle of turn and ventilation rate by day whilst the feed total data mean was increased by day, in the case of Fish 3.2. Finally, in Fish 4, significant differences were noted for all variables except angle of turn. These results imply that the means for the day were greater than those for the night, for all variables except angle of turn.

3.12 Comparisons of results between feeding peaks and intervals between them

T-tests between means of feeding peaks and intervening intervals are shown in Table 3.4. In Fish 2, there are no significant differences revealed between the feeding peaks and the intervals between except for the feed total data ($p = 0.021$). The angle of turn means are significantly different between peaks and intervals ($p = 0.05$) suggesting

TABLE 3.3 a T - TEST COMPARISON DAY/NIGHT FISH 2
 GROUP 1 = DAY, GROUP 2 = NIGHT

	D1	D3	M1	M3	V1	AN1
MEAN (1)	39.72	2.36	2.38	0.47	47.09	193.03
S.E. (1)	6.63	0.06	0.38	0.09	4.21	19.86
MEAN (2)	29.51	0.26	3.00	0.57	44.71	177.60
S.E. (2)	4.03	0.03	0.25	0.07	3.66	13.29
T - VALUE	1.32	1.31	1.36	0.78	0.43	0.65
SIG (T)	0.0485	0.0485	0.0445	0.1090	0.1685	0.1300
F - VALUE	1.02	1.02	1.32	1.35	1.04	1.18
SIG (F)	0.2280	0.2280	0.0905	0.3425	0.2330	0.1540

TABLE 3.3 b T - TEST COMPARISON DAY/NIGHT FISH 3.1
 GROUP 1 = DAY, GROUP 2 = NIGHT

	D1	D3	M1	M3	V1	AN1
MEAN (1)	18.03	0.19	3.93	0.49	53.85	189.42
S.E. (1)	2.31	0.03	0.34	0.06	1.21	9.33
MEAN (2)	66.39	0.68	0.27	0.04	59.85	293.79
S.E. (2)	6.40	0.06	0.08	0.01	1.98	14.58
T - VALUE	8.79	7.29	10.36	7.55	2.59	6.03
SIG (T)	0.0000	0.0000	0.0000	0.0000	0.0040	0.0000
F - VALUE	2.18	2.18	26.75	26.75	1.45	1.44
SIG (F)	0.0000	0.0000	0.0000	0.0000	0.1025	0.0380

TABLE 3.3 c T - TEST COMPARISON DAY/NIGHT FISH 3.2
 GROUP 1 = DAY, GROUP 2 = NIGHT

	D1	D3	M1	M3	V1	AN1
MEAN (1)	15.08	0.15	2.66	0.54	47.38	167.53
S.E. (1)	1.58	0.04	0.23	0.07	1.29	8.58
MEAN (2)	37.10	0.38	1.79	0.35	54.63	253.03
S.E. (2)	8.98	0.09	0.33	0.08	1.58	15.63
T - VALUE	3.98	3.99	2.07	1.00	3.57	4.56
SIG (T)	0.0000	0.0000	0.0105	0.0185	0.0005	0.0000
F - VALUE	8.56	8.55	2.12	2.12	1.72	1.12
SIG (F)	0.0000	0.0000	0.0125	0.0125	0.0975	0.1755

TABLE 3.3 d T - TEST COMPARISON DAY/NIGHT FISH 4
 GROUP 1 = DAY, GROUP 2 = NIGHT

	D1	D3	M1	M3	V1	AN1
MEAN (1)	21.97	0.22	1.64	0.41	49.65	189.29
S.E. (1)	2.28	0.02	0.17	0.04	1.40	10.59
MEAN (2)	13.16	0.13	1.13	0.29	17.09	171.64
S.E. (2)	1.41	0.01	0.16	0.04	1.08	11.40
T - VALUE	3.19	3.18	2.17	2.15	1.62	1.13
SIG (T)	0.0005	0.0005	0.0360	0.0360	0.0280	0.0645
F - VALUE	2.94	2.96	1.13	1.13	1.77	1.02
SIG (F)	0.0000	0.0000	0.0350	0.0350	0.0510	0.2285

TABLE 3.4 a T - TEST COMPARISON PEAKS/INTERVALS FISH 2
 GROUP 1 = PEAKS, GROUP 2 = INTERVALS

	D1	D3	M1	M3	V1	AN1
MEAN (1)	30.96	0.28	2.97	0.58	44.19	174.38
S.E. (1)	3.56	0.03	0.26	0.07	2.84	12.10
MEAN (2)	39.51	0.36	2.09	0.41	54.00	221.14
S.E. (2)	22.01	0.10	0.33	0.09	7.76	25.82
T - VALUE	0.76	0.74	2.10	2.10	1.19	1.64
SIG (T)	0.1170	0.1170	0.0105	0.0105	0.0750	0.0250
F - VALUE	1.81	1.62	1.98	1.98	1.43	1.16
SIG (F)	0.0190	0.0190	0.0225	0.0225	0.1320	0.1710

TABLE 3.4 b T - TEST COMPARISON PEAKS/INTERVALS FISH 3.1
 GROUP 1 = PEAKS, GROUP 2 = INTERVALS

	D1	D3	M1	M3	V1	AN1
MEAN (1)	15.83	0.26	4.68	0.64	53.00	174.52
S.E. (1)	2.32	0.02	0.37	0.07	1.17	10.56
MEAN (2)	48.14	0.49	0.50	0.33	58.62	269.71
S.E. (2)	5.03	0.05	0.12	0.02	1.70	11.62
T - VALUE	6.49	6.55	10.65	7.41	2.73	6.06
SIG (T)	0.0000	0.0000	0.0000	0.0000	0.0025	0.0000
F - VALUE	3.14	3.14	9.74	9.74	2.34	1.24
SIG (F)	0.0000	0.0000	0.0000	0.0000	0.0185	0.0730

TABLE 3.4 c T - TEST COMPARISON PEAKS/INTERVALS FISH 3.2
 GROUP 1 = PEAKS, GROUP 2 = INTERVALS

	D1	D3	M1	M3	V1	AN1
MEAN (1)	18.14	0.19	3.23	0.67	48.45	178.34
S.E. (1)	1.95	0.02	0.31	0.08	1.45	10.58
MEAN (2)	21.98	0.23	1.40	0.27	50.31	195.95
S.E. (2)	5.01	0.05	0.23	0.05	1.99	12.30
T - VALUE	0.80	0.80	4.78	3.91	3.76	1.08
SIG (T)	0.1075	0.1075	0.0000	0.0000	0.1140	0.0700
F - VALUE	4.58	4.58	2.53	2.53	1.35	1.07
SIG (F)	0.0000	0.0000	0.0005	0.0005	0.1385	0.1430

TABLE 3.4 d T - TEST COMPARISON PEAKS/INTERVALS FISH 4
 GROUP 1 = PEAKS, GROUP 2 = INTERVALS

	D1	D3	M1	M3	V1	AN1
MEAN (1)	17.74	0.17	2.25	0.55	49.55	180.16
S.E. (1)	2.08	0.02	0.20	0.05	1.57	11.51
MEAN (2)	17.92	0.18	0.71	0.18	46.60	181.73
S.E. (2)	1.95	0.02	0.11	0.03	0.99	10.58
T - VALUE	0.06	0.05	6.48	6.24	1.10	0.10
SIG (T)	0.2375	0.2400	0.0000	0.0000	0.0700	0.2300
F - VALUE	1.04	1.05	3.12	3.18	2.18	1.02
SIG (F)	0.2175	0.2085	0.0000	0.0000	0.0200	0.2315

that there are smaller angles of turn during feeding peaks (dextral). In contrast, for Fish 3.1, highly significant differences exist all around. Means for step length, ventilation rate and angle of turn being significantly larger during the intervals, whilst the feed total data is significantly larger during the defined feeding peaks. In Fish 3.2 and Fish 4, however, only feed total means were significantly different.

3.13 Correlations between variables recorded

Tables 3.5 to 3.8 show correlation matrices of Pearson zero-order correlation coefficients between relevant variables. For Fish 2, there is a particularly strong positive correlation between step length and the angle of turn ($p = 0.001$) and also a strong positive correlation between step length and elapsed time. No correlation appears to exist between the feed total data and the step length or angle of turn data but a significant ($p = 0.001$) negative correlation exists between feed total and time. For Fish 3.1, a significant negative correlation exists between step length and feed total data ($p = 0.035$) whilst there is a very strong correlation between angle of turn and step length ($p = 0.001$). Note that the step length significantly declines with time ($p = 0.001$).

Feed Total is negatively correlated with angle of turn ($p = 0.001$) and also shows a significant negative correlation with time ($p = 0.001$). Both angle of turn and ventilation rate show strong negative correlations with time.

In Fish 3.2, step length again shows a heavy positive correlation with angle of turn ($p=0.001$) and also with time. The feed total data shows no correlations at all. Ventilation rate is positively correlated with time, as is angle of turn.

Table 3.5

Correlation matrix of Pearson Zero-order Coefficients for Fish 2

	D1	D3	D4	M1	M3	VI	AN1	TC
D1	NC	NC	NC	-0.14 (0.324)	-0.13 (0.321)	-0.61 (0.022)	0.38 (0.001)	0.26 (0.005)
D3	NC	NC	NC	-0.14 (0.325)	-0.13 (0.322)	-0.61 (0.021)	0.38 (0.001)	0.26 (0.006)
D4	NC	NC	NC	-0.17 (0.224)	-0.17 (0.224)	-0.58 (0.033)	0.41 (0.001)	0.26 (0.005)
M1	-0.14 (0.324)	-0.13 (0.325)	-0.17 (0.224)	NC	NC	0.04 (0.839)	-0.04 (0.724)	-0.34 (0.001)
M3	-0.13 (0.321)	-0.13 (0.322)	-0.17 (0.221)	NC	NC	0.04 (0.837)	-0.04 (0.724)	-0.34 (0.001)
VI	-0.61 (0.022)	-0.61 (0.021)	-0.57 (0.033)	0.04 (0.839)	0.04 (0.837)	NC	-0.49 (0.074)	-0.09 (0.659)
AN1	0.38 (0.001)	0.38 (0.001)	0.40 (0.001)	-0.05 (0.724)	-0.05 (0.724)	-0.50 (0.074)	NC	0.11 (0.268)
TC	0.26 (0.005)	0.26 (0.006)	0.26 (0.005)	-0.34 (0.001)	0.34 (0.001)	-0.09 (0.659)	0.11 (0.268)	NC

Table 3.6

Correlation matrix of Pearson Zero-order Coefficients for Fish 3.1

	D1	D3	D4	M1	M3	VI	AN1	TE
D1	NC	NC	NC	-0.22 (0.035)	-0.22 (0.035)	0.44 (0.014)	0.48 (0.001)	-0.28 (0.001)
D3	NC	NC	NC	-0.22 (0.035)	-0.22 (0.035)	0.44 (0.014)	0.48 (0.001)	-0.28 (0.001)
D4	NC	NC	NC	-0.24 (0.021)	-0.24 (0.021)	0.40 (0.022)	0.48 (0.001)	-0.28 (0.001)
M1	-0.22 (0.035)	-0.22 (0.034)	-0.24 (0.021)	NC	NC	-0.19 (0.234)	-0.33 (0.001)	0.54 (0.001)
M3	-0.22 (0.035)	-0.22 (0.035)	-0.24 (0.021)	NC	NC	-0.19 (0.233)	-0.33 (0.001)	0.54 (0.001)
VI	0.43 (0.014)	0.43 (0.014)	0.40 (0.022)	0.19 (0.234)	0.19 (0.234)	NC	0.22 (0.227)	-0.45 (0.004)
AN1	0.48 (0.001)	0.48 (0.001)	0.48 (0.001)	-0.33 (0.001)	-0.33 (0.001)	0.22 (0.227)	NC	-0.27 (0.001)
TE	-0.28 (0.001)	-0.28 (0.001)	-0.25 (0.001)	0.54 (0.001)	0.54 (0.001)	0.45 (0.004)	-0.28 (0.001)	NC

Table 3.7

Correlation matrix of Pearson Zero-Order Coefficients for Fish 3.2

	D1	D3	D4	M1	M3	V1	AN1	TE		
D1	NC	NC	NC	-0.04	-0.04	0.10	0.32	0.20		
				(0.692)	(0.692)	(0.627)	(0.001)	(0.008)		
D3	NC	NC	NC	-0.04	-0.04	0.10	0.32	0.20		
				(0.689)	(0.692)	(0.619)	(0.001)	(0.008)		
D4	NC	NC	NC	0.10	0.10	0.27	0.33	0.11		
				(0.374)	(0.374)	(0.188)	(0.001)	(0.178)		
M1	-0.04	-0.04	0.09	NC	NC	-0.04	0.07	-0.01		
				(0.692)	(0.689)	(0.374)	(0.828)	(0.472)	(0.961)	
M3	-0.04	-0.04	0.09	NC	NC	-0.04	0.07	-0.01		
				(0.692)	(0.689)	(0.374)	(0.828)	(0.472)	(0.960)	
V1	0.10	0.10	0.27	-0.04	-0.04	NC	0.26	0.49		
				(0.627)	(0.627)	(0.188)	(0.828)	(0.828)	(0.203)	(0.005)
AN1	0.32	0.32	0.33	0.07	0.07	0.26	NC	0.19		
				(0.001)	(0.001)	(0.001)	(0.472)	(0.472)	(0.203)	(0.005)
TE	0.20	0.20	0.11	-0.01	-0.01	0.49	0.19	NC		
				(0.008)	(0.008)	(0.178)	(0.961)	(0.960)	(0.005)	(0.005)

Table 3.8

Correlation matrix of Pearson zero order Coefficients for Fish 4.

	D1	D3	D4	M1	M3	V1	AN1	TE		
D1	NC	NC	NC	0.01	0.01	0.18	0.23	-0.07		
				(0.902)	(0.904)	(0.316)	(0.001)	(0.302)		
D3	NC	NC	NC	0.01	0.01	0.18	0.23	-0.07		
				(0.902)	(0.904)	(0.316)	(0.001)	(0.302)		
D4	NC	NC	NC	0.00	0.00	0.26	0.41	0.01		
				(0.994)	(0.994)	(0.123)	(0.001)	(0.911)		
M1	0.01	0.01	0.00	NC	NC	0.22	-0.08	-0.30		
				(0.902)	(0.902)	(0.994)	(0.167)	(0.239)	(0.001)	
M3	0.01	0.01	0.00	NC	NC	0.22	-0.08	-0.30		
				(0.902)	(0.904)	(0.994)	(0.167)	(0.239)	(0.001)	
V1	0.18	0.18	0.26	0.22	0.22	NC	-0.30	-0.14		
				(0.316)	(0.316)	(0.123)	(0.167)	(0.167)	(0.079)	(0.363)
AN1	0.23	0.23	0.41	-0.07	-0.07	0.30	NC	-0.07		
				(0.001)	(0.001)	(0.001)	(0.241)	(0.241)	(0.079)	(0.299)
TE	-0.07	-0.07	0.01	-0.30	-0.30	-0.14	-0.07	NC		
				(0.302)	(0.302)	(0.911)	(0.001)	(0.001)	(0.363)	(0.299)

3.14 Relationship between variables recorded and elapsed time

Temporal variations in feeding activity, step length and angle of turn are shown in Figs. 3.11 to 3.14.

In Fish 2 there is an especially noticeable increase in feeding activity immediately at dawn. (Fig. 3.11a). The cumulation rate histogram (3.11b) is especially rapid at first then progresses less steeply with portions of increased rate corresponding to the feeding peaks. It will be noticed that at first the step lengths are small and the distance travelled low, most of the steps being <25m. Around 0400 hours, however, there is an apparent increase in step length, tending to decrease towards dawn. Unfortunately, a large part of the data is missing post-dawn owing to a technical failure. From 0925 onwards there is an apparent increase in step length towards midday, the steepening of the slope in the cumulative curve is marked; at this point the fish escaped from the west pool and operations were suspended. Fig. 3.11e shows the successive angles of turn of the fish. It is extremely difficult to visualize any linear patterns in this series. By superimposing diagrams it is easy to see corresponding patterns between step length and angle of turn. There may, however, be short-term cyclical grouping of angles of turn not revealed by conventional analysis methods. For example, there would appear to be short phases when the angles of turn may build up to a peak and decrease again, e.g. 0155, 0515 onwards.

From Fig. 3.12 a and b, it can be seen that in Fish 3.1 the rate of food intake is low until dawn 06.10, after which it rises more steeply then proceeds in a series of steps. Before dusk (21.10) there is a sudden steepening of the cumulation curve.

The changes in step length are interesting; up to one hour after dawn, the fish tends to make large step lengths in the 50-100 metres

Figs. 3.11 to 3.14

Histograms showing the change with time for feeding activity, step length and angle of turn for each of the experimental fish.

a) Raw feeding activity (M1) b) Cumulative feeding activity (M2)

c) Raw step length (D1) d) Cumulative step length (D2)

d) Raw angle of turn (AN1)

FIG 3.11

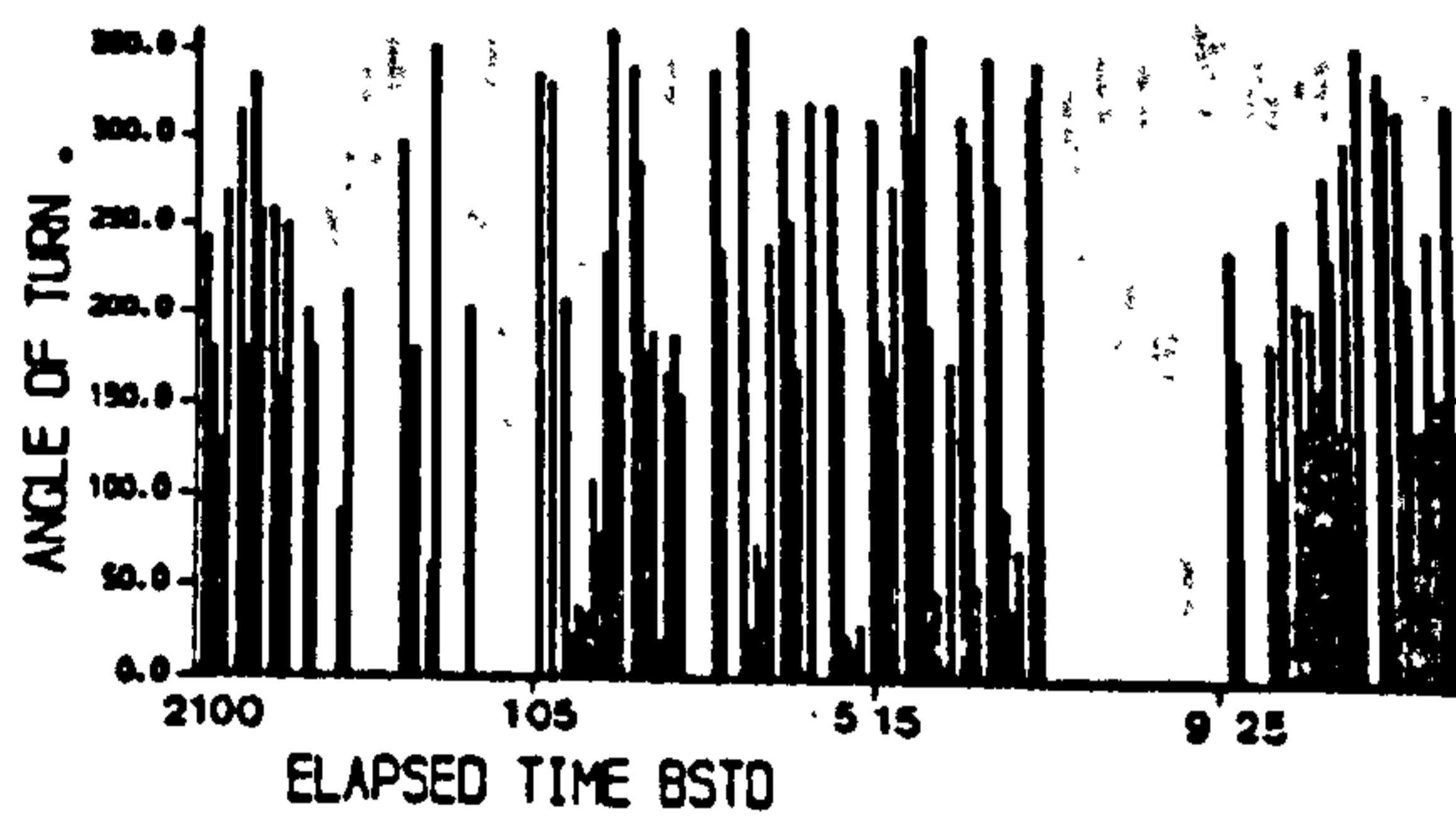
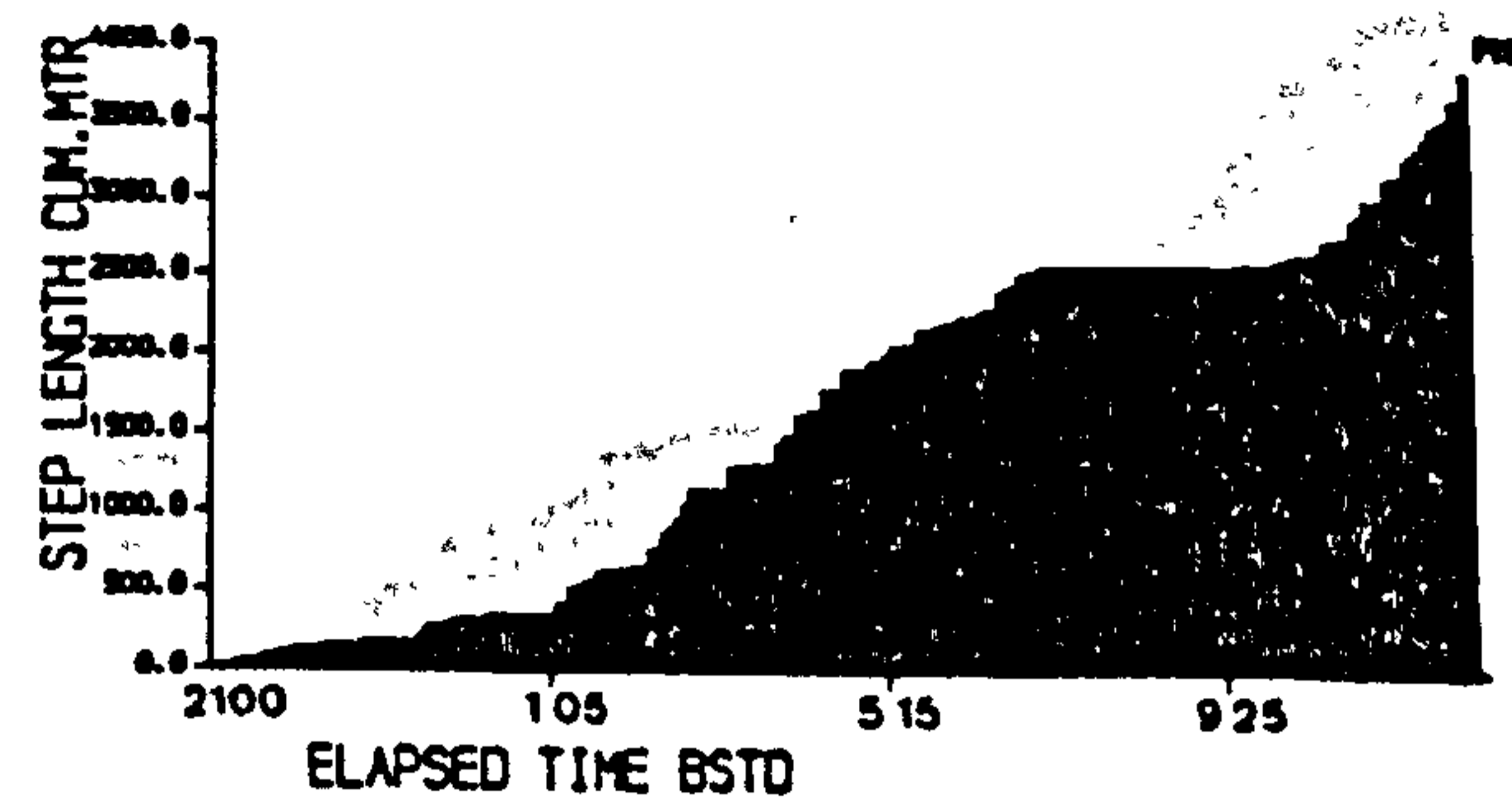
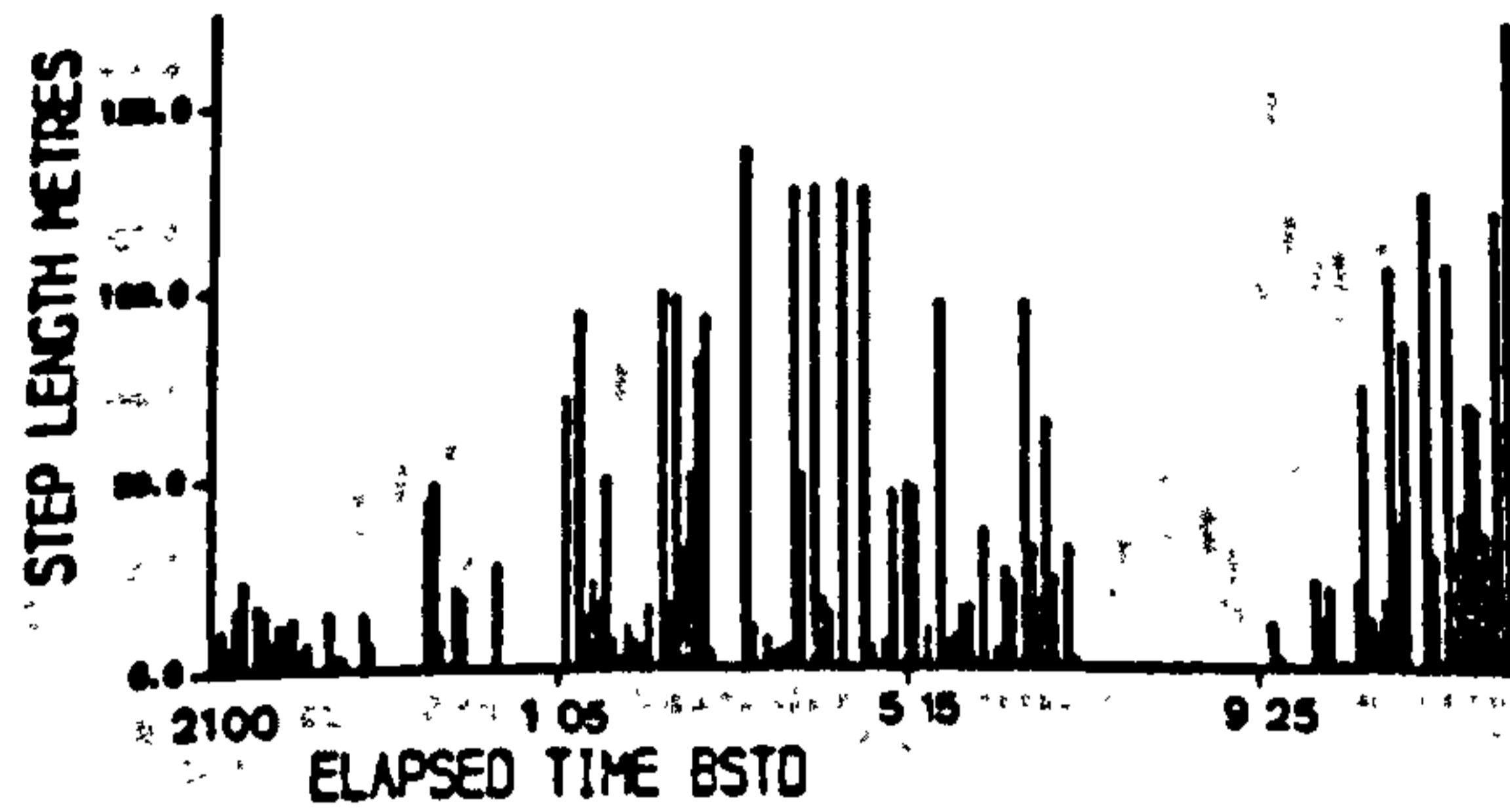
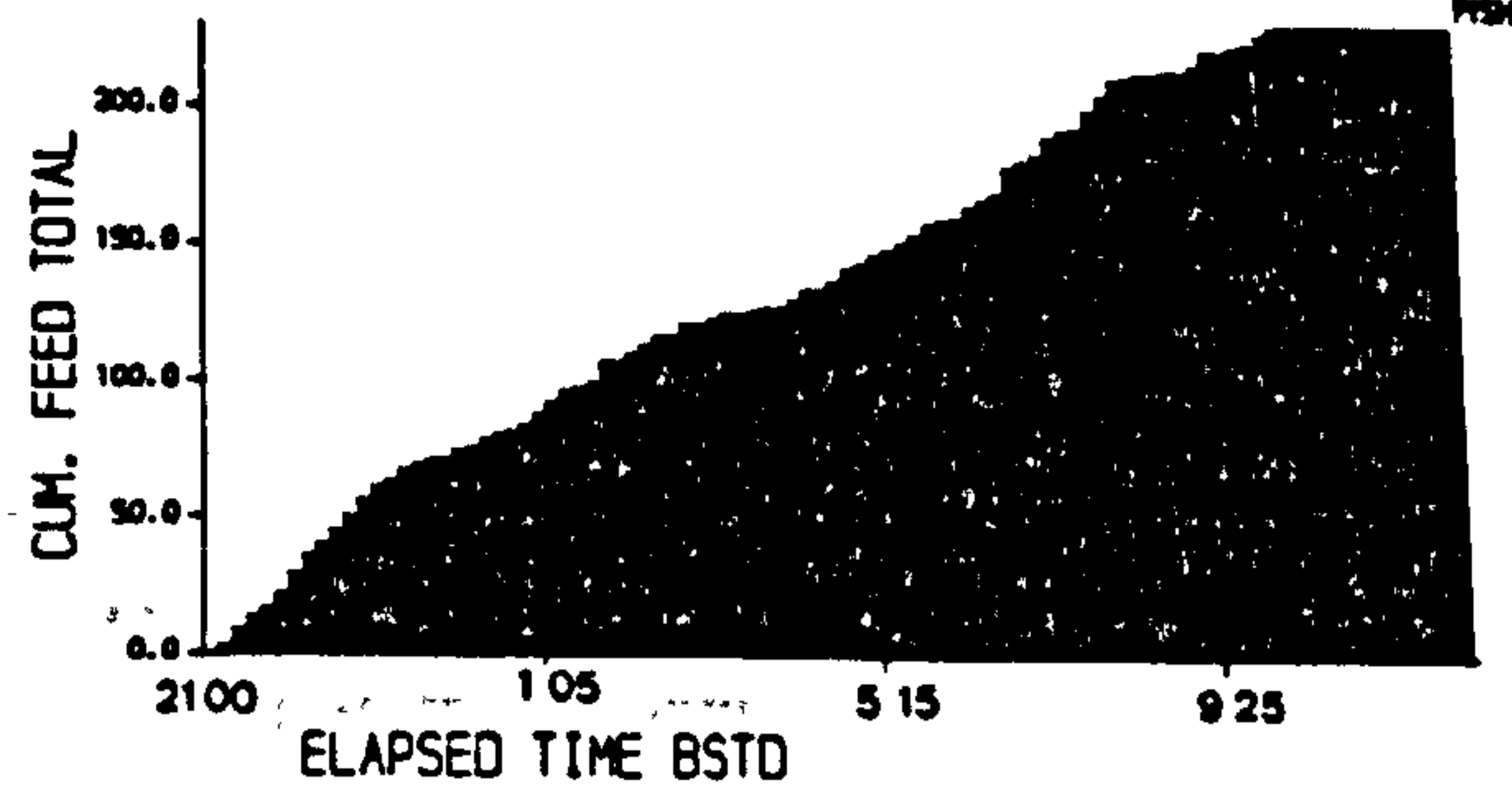
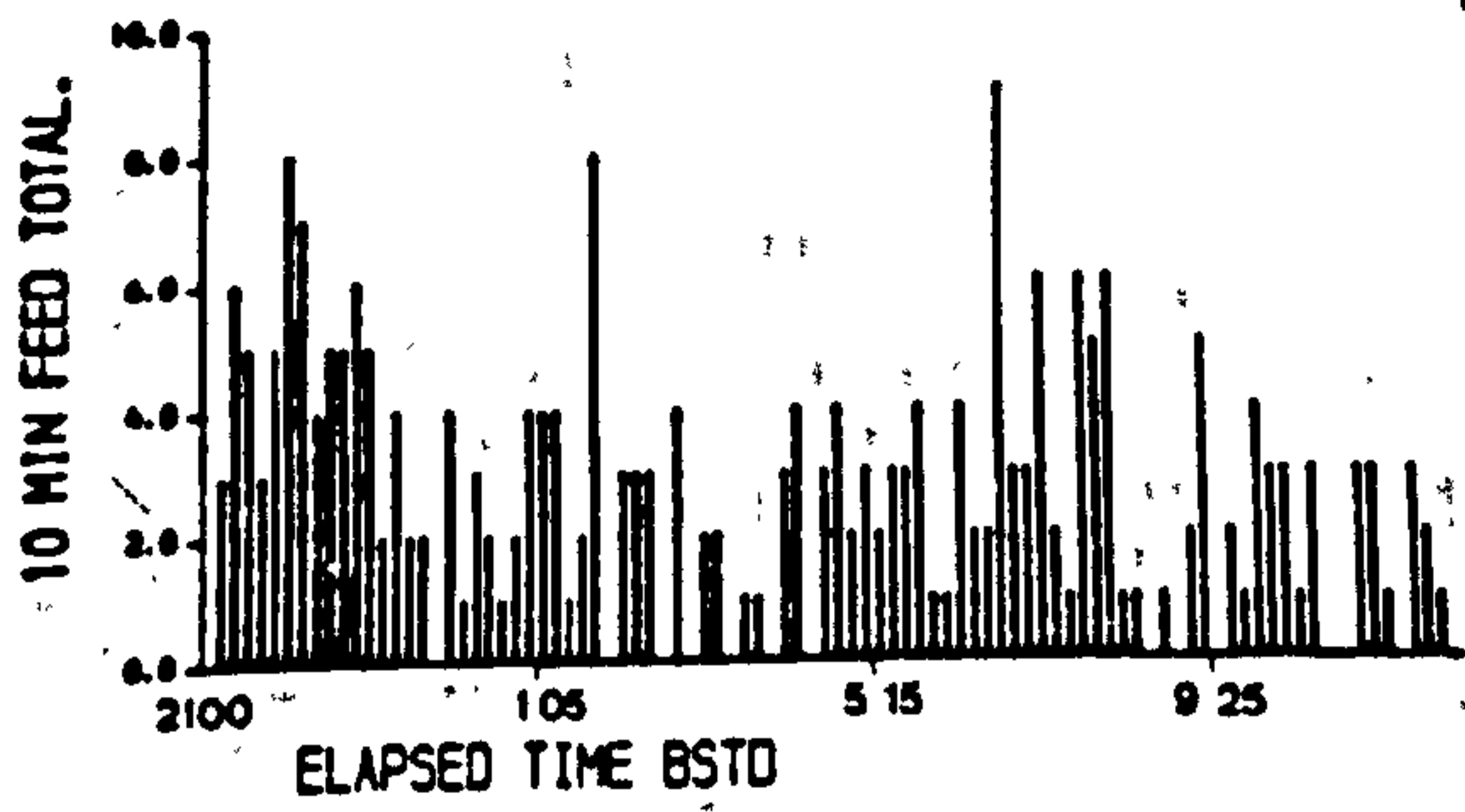


FIG 3.12

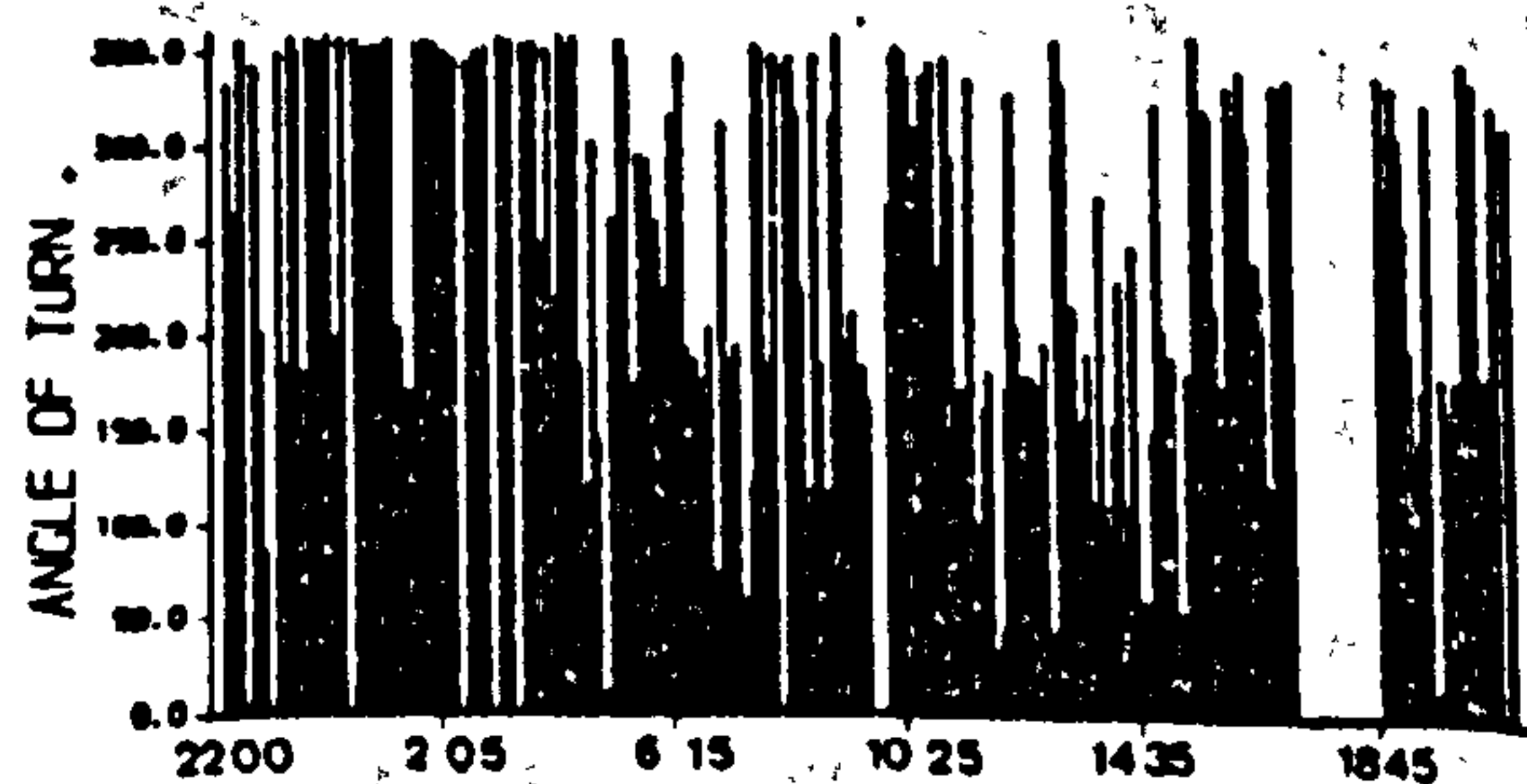
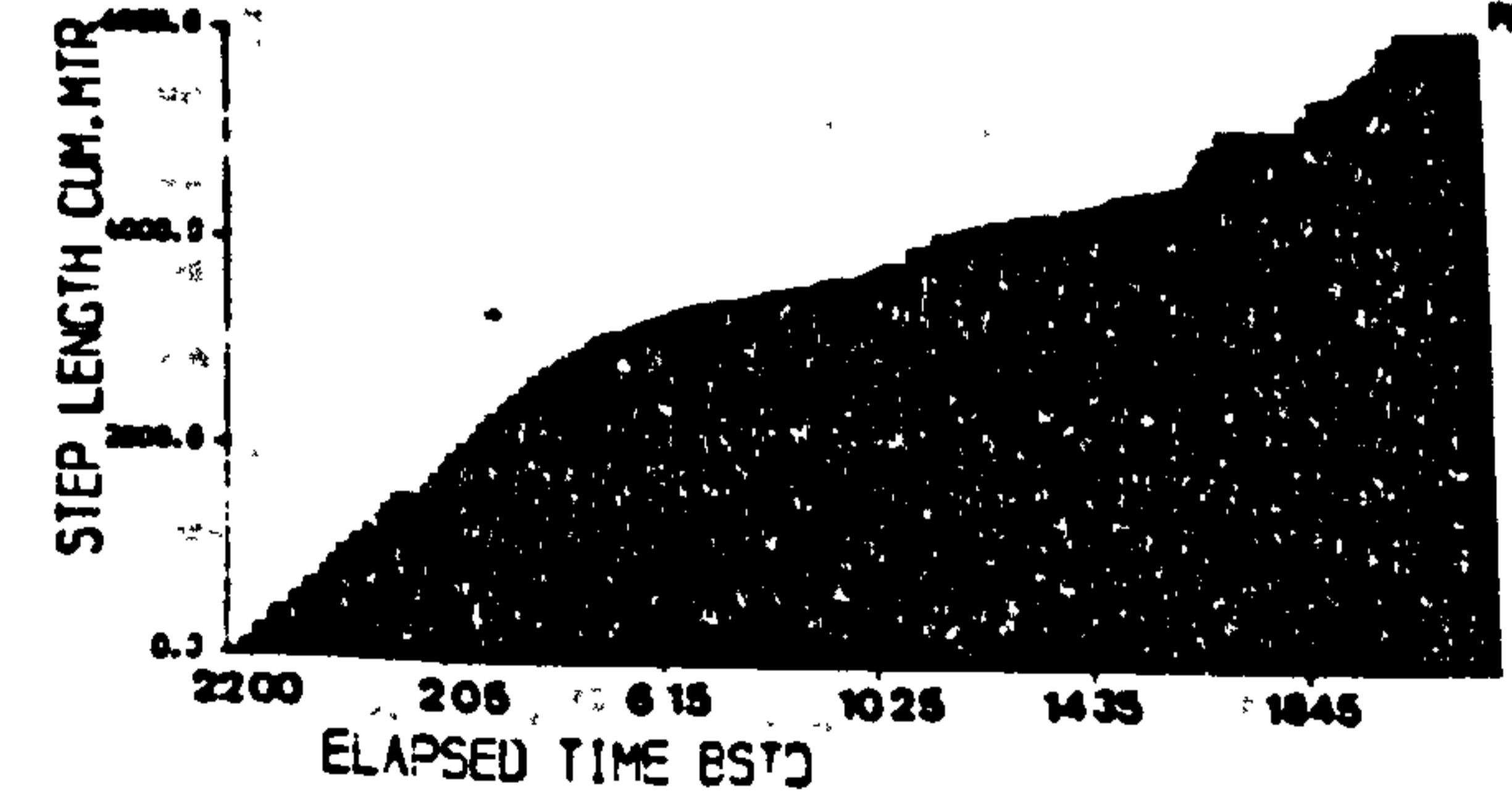
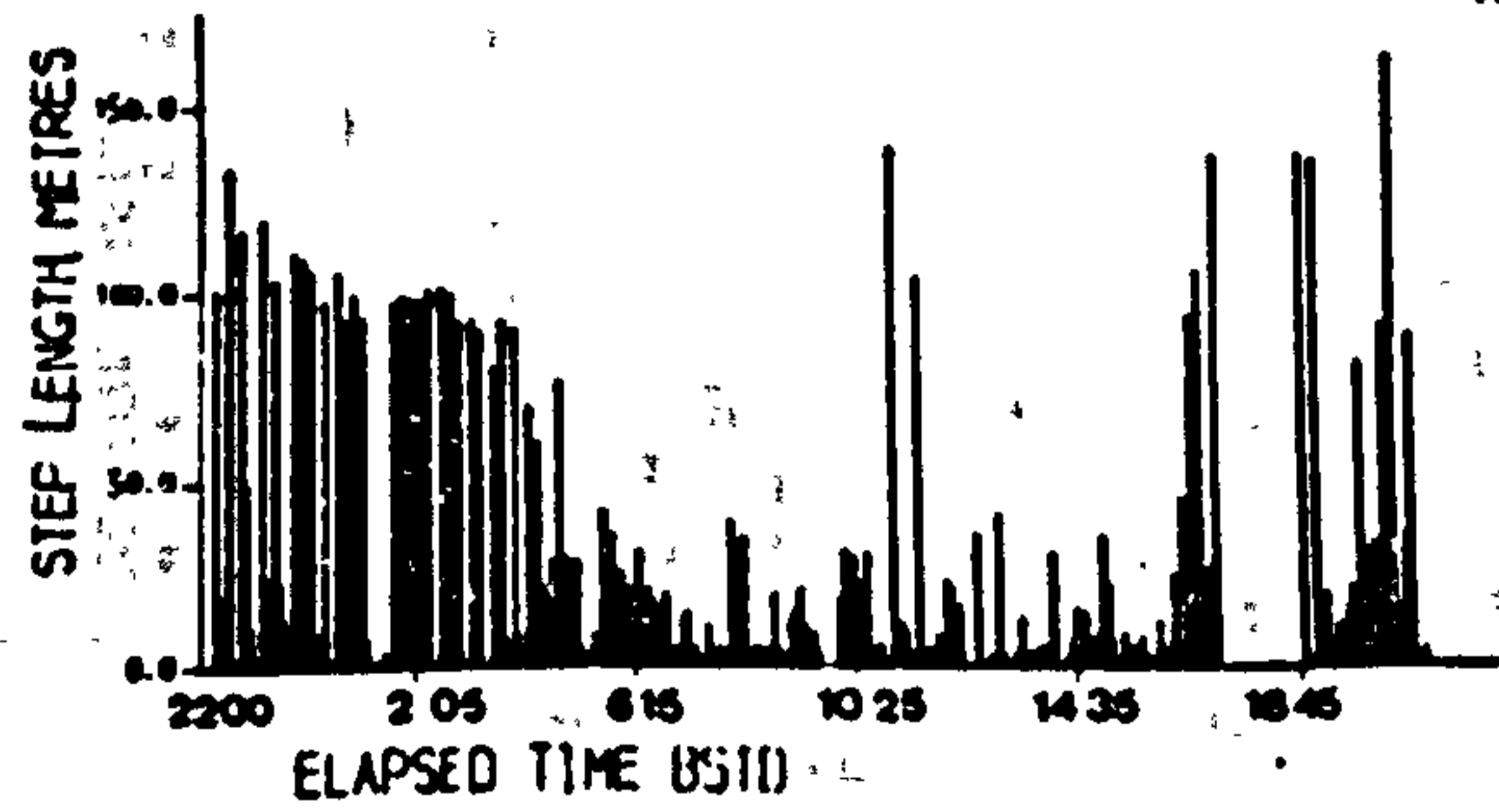
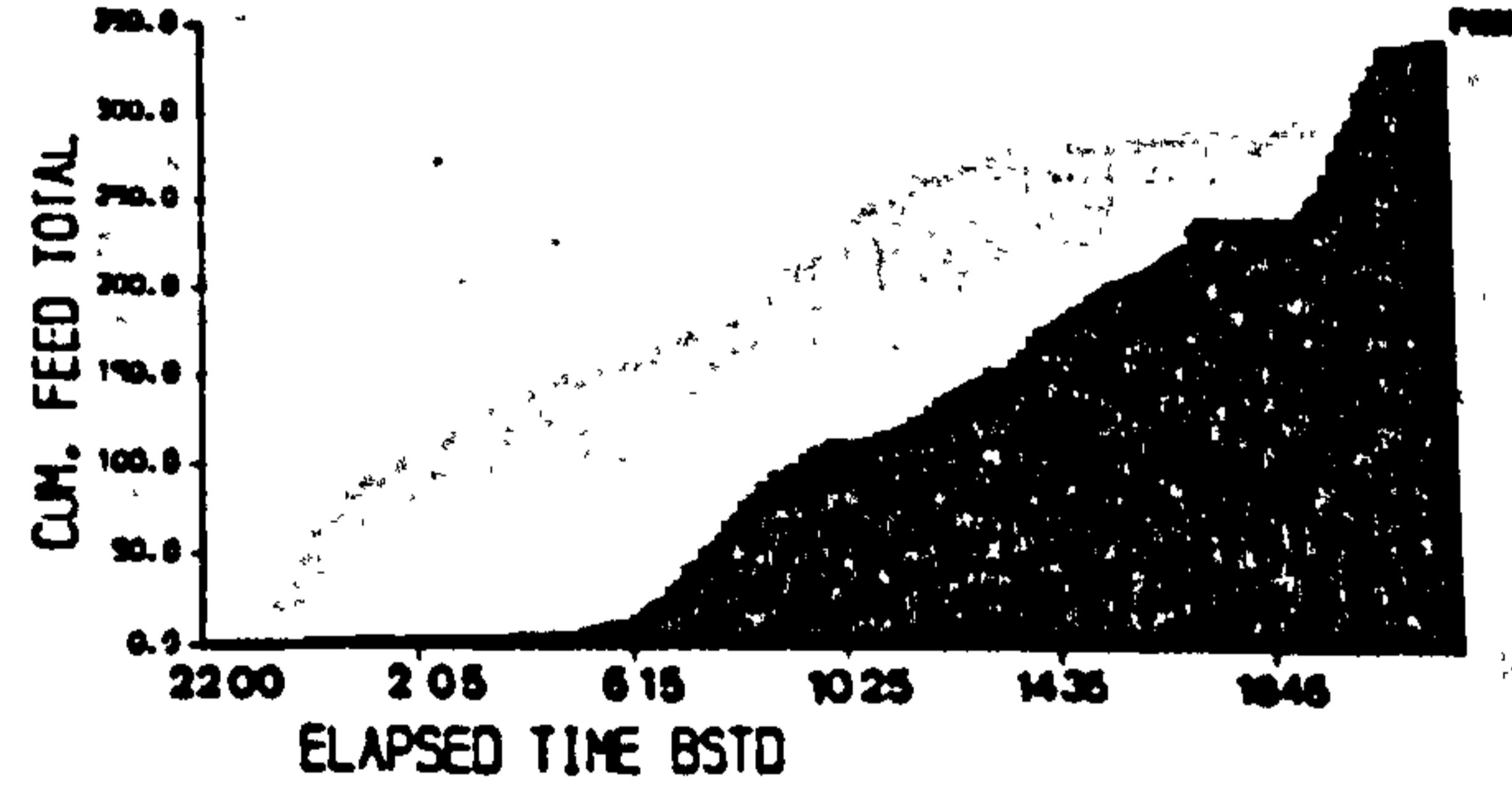
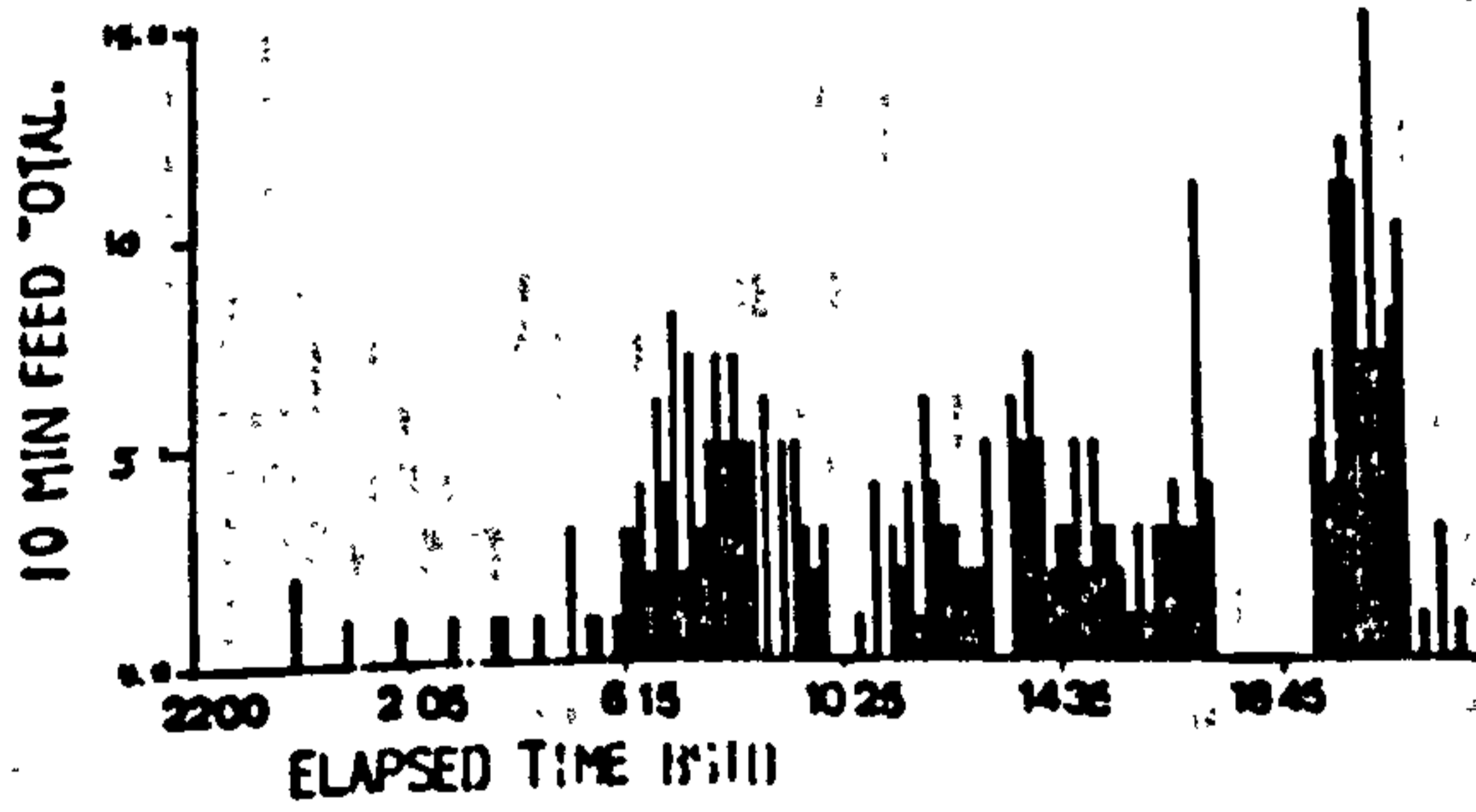


FIG 3.13

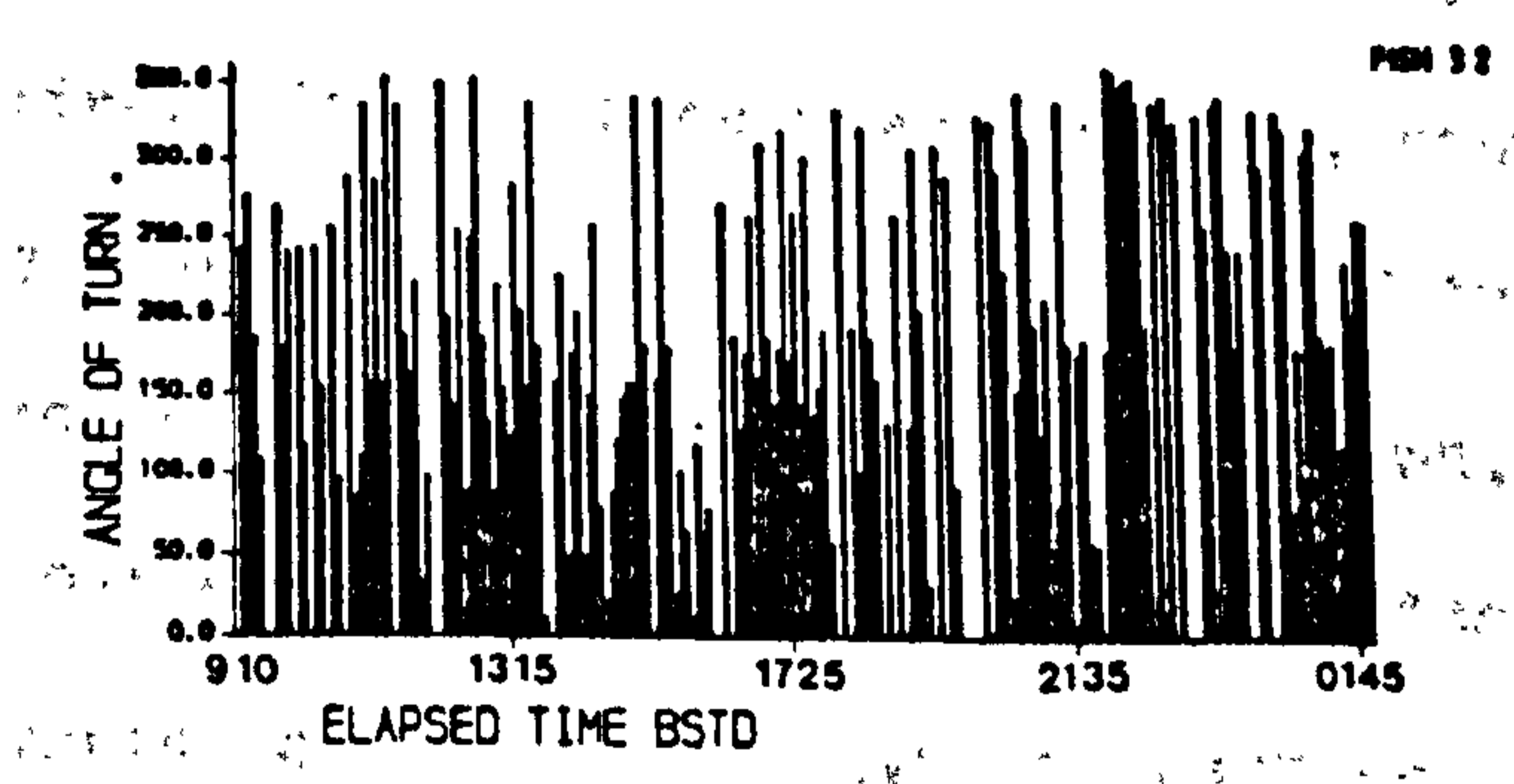
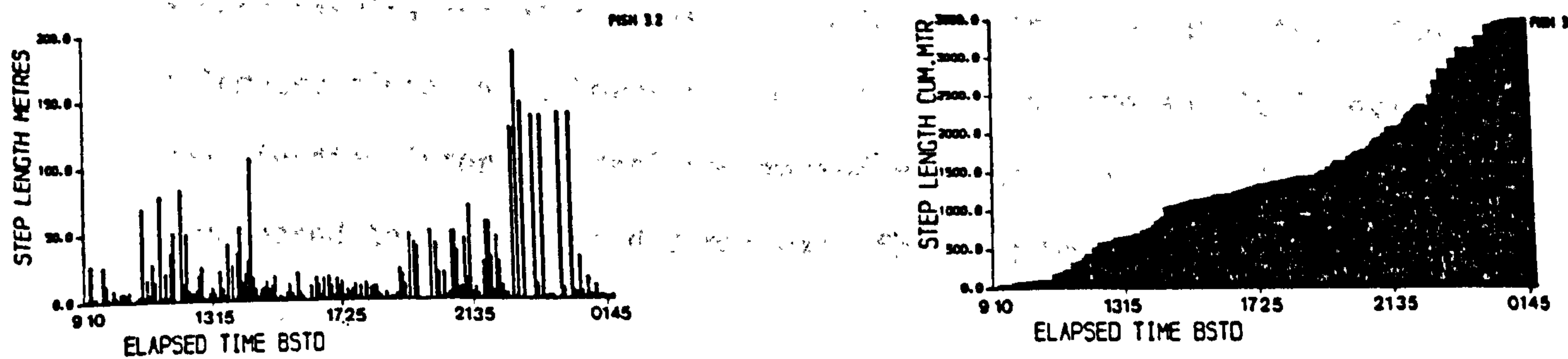
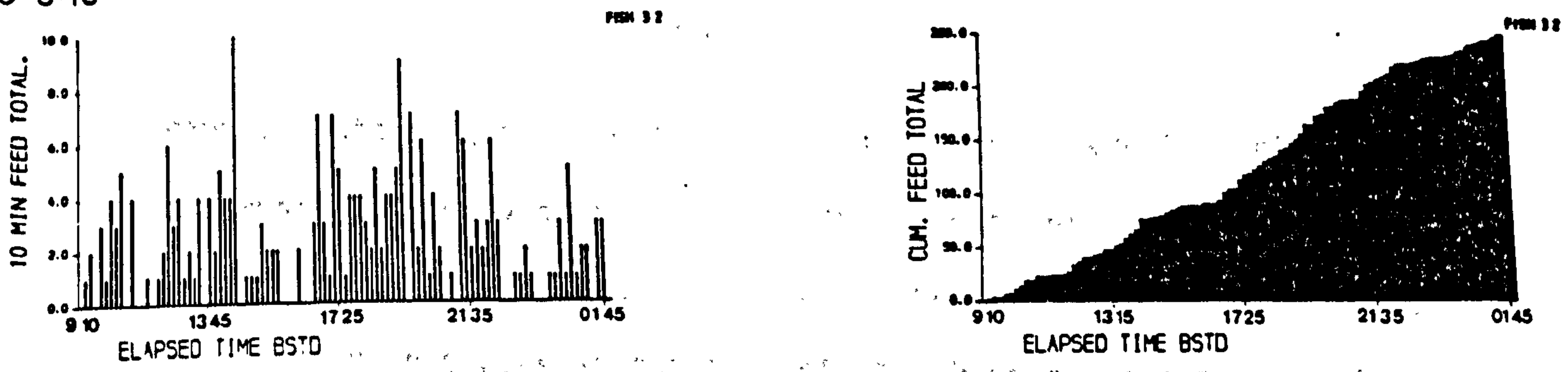
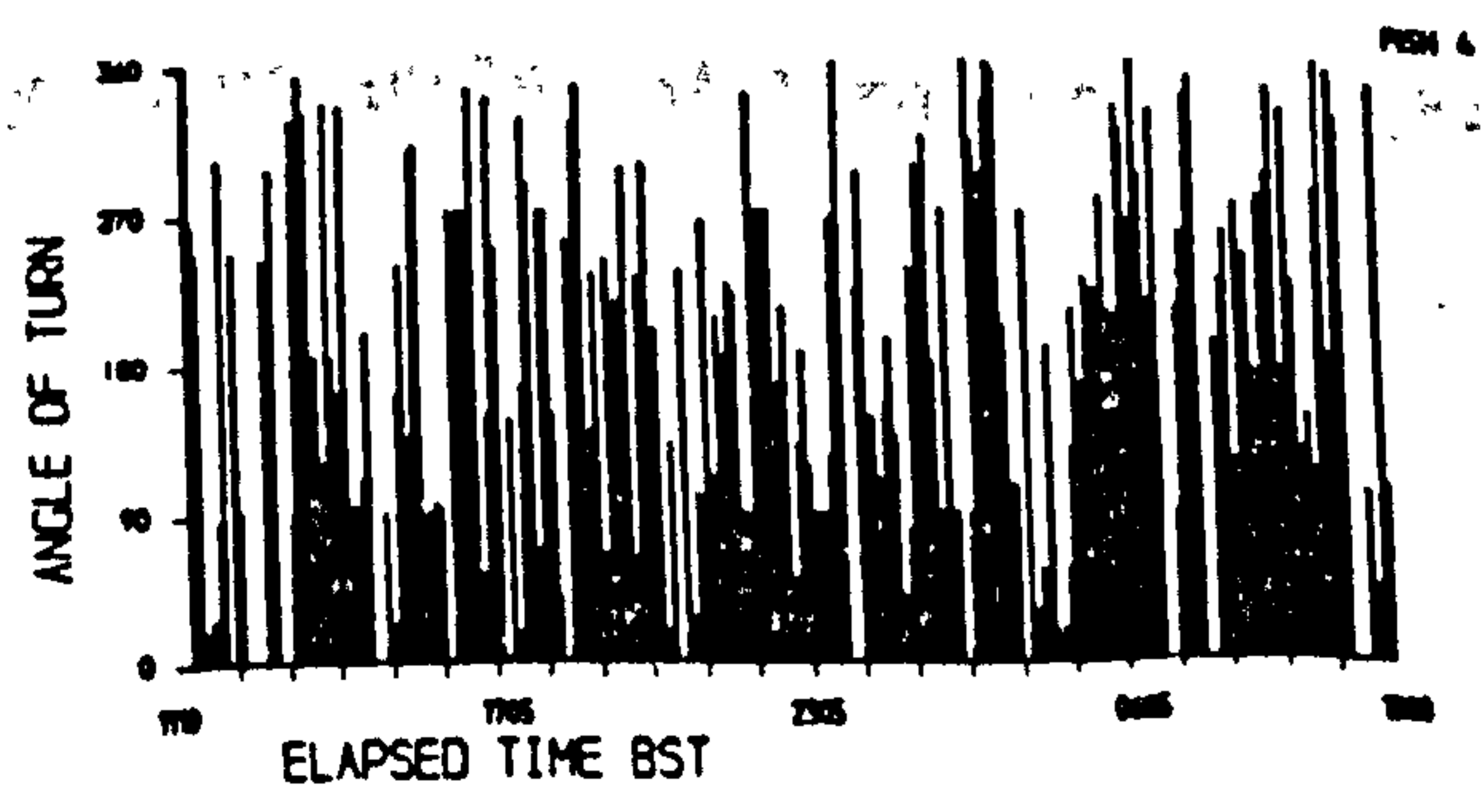
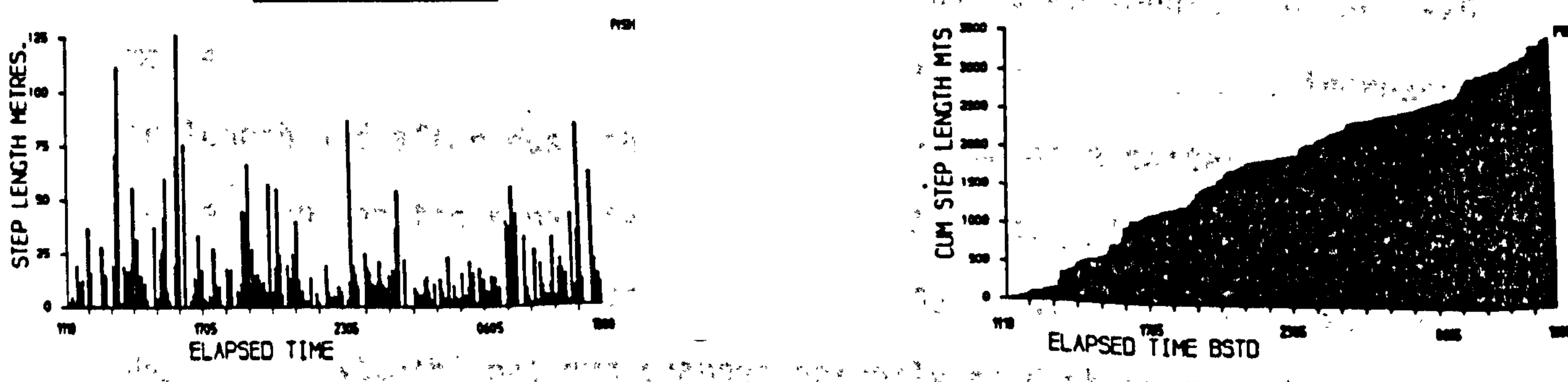
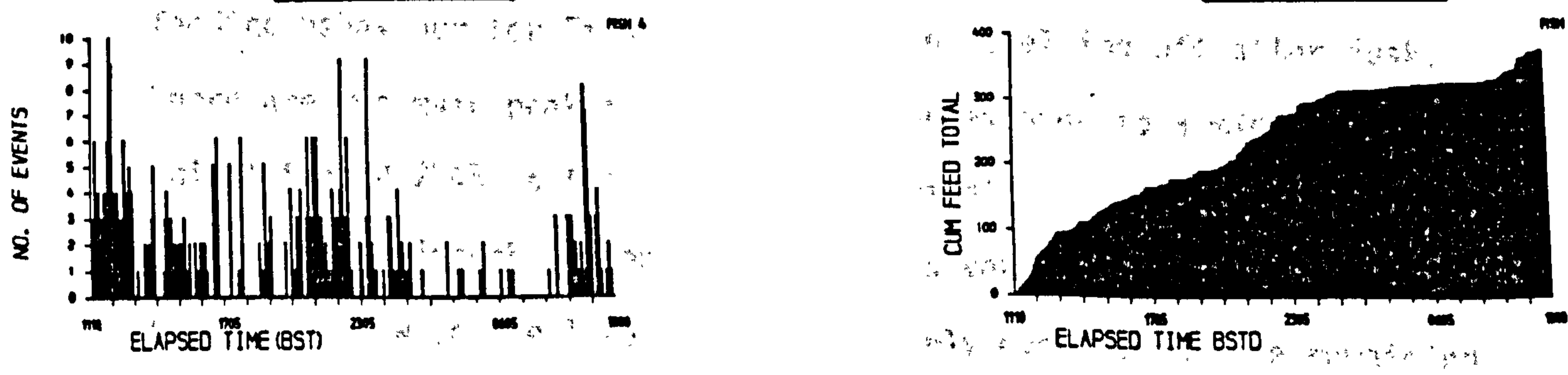


FIG 3.14



range. After this it tends to make a series of small (0-40 metres) movements with only an occasional larger movement. This coincides with the beginning of the first feeding peak. A noticeable increase in step length occurs at the time of the third feeding peak. The abrupt termination at dusk is due to loss of tracking ability. The telemetry signal was, however, not lost at this time and it is evident that feeding intensity declined markedly at this time. (It will be remembered that the fish did not exit from the West Pool until 22.30 hours).

As in Fish 2, the angle of turn histogram (Fig. 3.12e) is quite bewildering at first sight. Closer examination, however, reveals some interesting changes. At first, the angle of turn tends to alternate between zero and near-maximal then, around dawn, (at which point the step length pattern changes) it follows a "sawtooth" pattern until dusk when it reverts to the initial type of pattern.

Fig. 3.13a and b discloses a less well-developed pattern of feeding behaviour for Fish 3.2. Note the levelling off after dusk. There are two main peaks; that at 1315 corresponds to a midday 'rise' whilst that at 2045 is the late evening 'rise'.

The step length pattern Fig. 3.13c is very interesting; there is a difference between the late afternoon - early evening and the remaining period. This pattern consists of many small step lengths in the 0-25 metre class. Towards late evening the step lengths tend to increase in length and after dusk there is a repetition of a pattern of very large step lengths separated by much small ones. If this is compared with the angle of turn histogram Fig. 3.13e it will be seen that the daytime 'sawtooth' pattern changes abruptly, at dusk to a pattern in which predominantly large angles of turn occur in the 300.0° class.

Fig. 3.14 a shows the feeding pattern clearly for Fish 4. There is an initial peak at the time of commencing tracking (11.10) terminating around 14.05 hours. After dusk there is another peak which terminates around 0105. After dawn, there is another peak commencing at 08.05 and which continues up till the enforced end of the tracking period. These changes show up well on the cumulative curve Fig. 3.14b. There are some significant changes in the step length pattern Fig. 3.14c and d; before dusk, there is a pattern of 'sawtooth' - type consisting of small step lengths with several larger ones intercalated. After dusk the pattern changes, with fewer large step lengths. From line 02.05 to dawn the pattern is exclusively composed of step lengths less than 25 metres. Immediately at dawn there is a distinct group of larger step lengths, followed by resumption of the previous daylight activity pattern. Finally in Fig. 3.14e the angle of turn pattern is again difficult to interpret; during the night, for example, there are many more larger angles. Close examination shows that certain identifiable groups of step lengths are correlated with small angles of turn.

3.15 Comparison of the Fish's movement patterns

Computer-produced plots of fish movements are shown in Figs. 3.15 to 3.18.

Fish 2 (Fig. 3.15) During daytime the longer mean step length is reflected in the pattern of movement.

There are a number of rather dispersed positions near to the yellow hydrophone station. The fish appears to confine itself to the southern half of the loch.

The night-time pattern is considerably more confusing though the period is longer and the mean step length is lower so, therefore, one might expect more complicated track diagrams. The fish frequents a

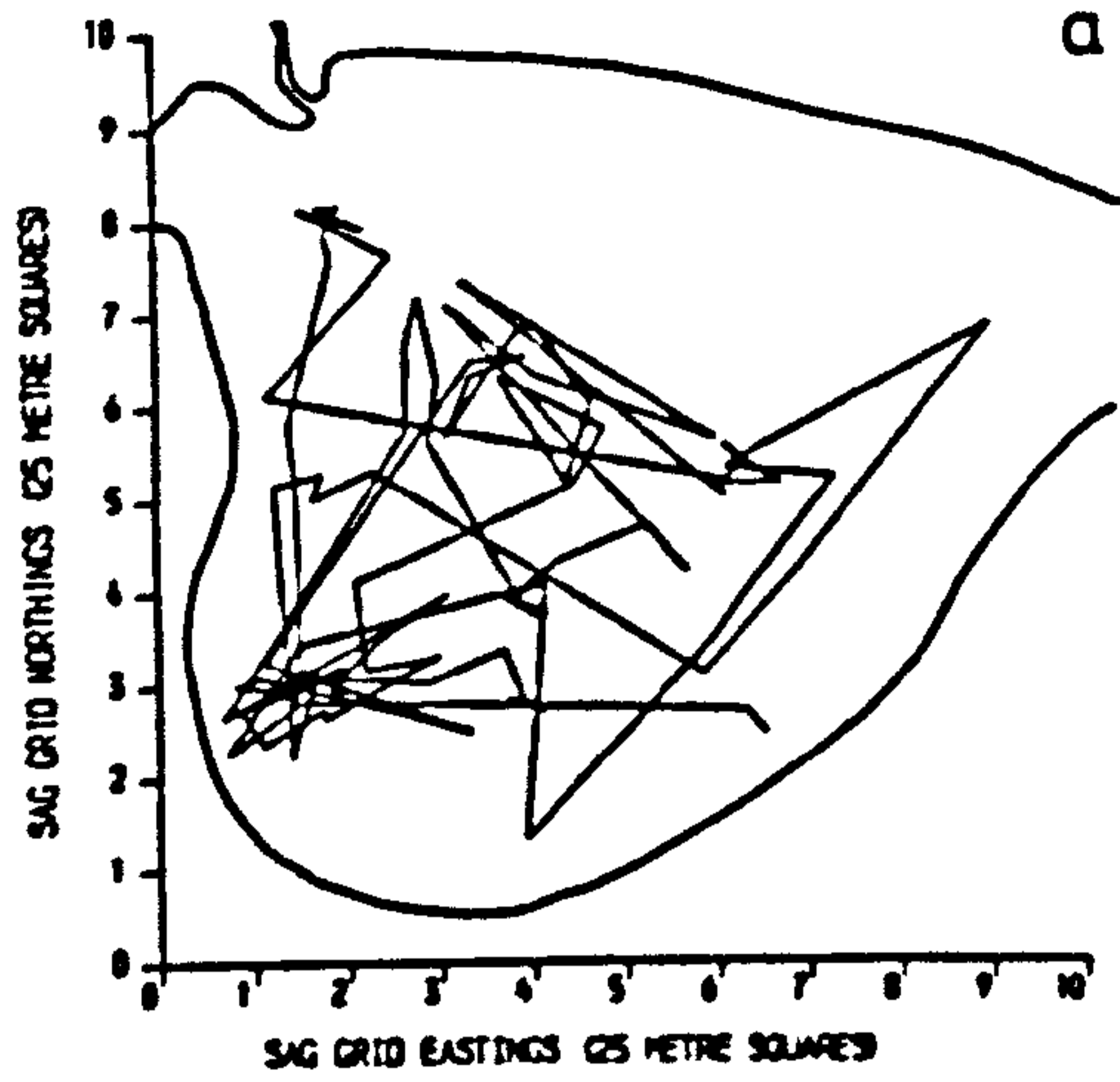
Fig.3.15

Computer-generated plots of the movement pattern
for Fish 2.

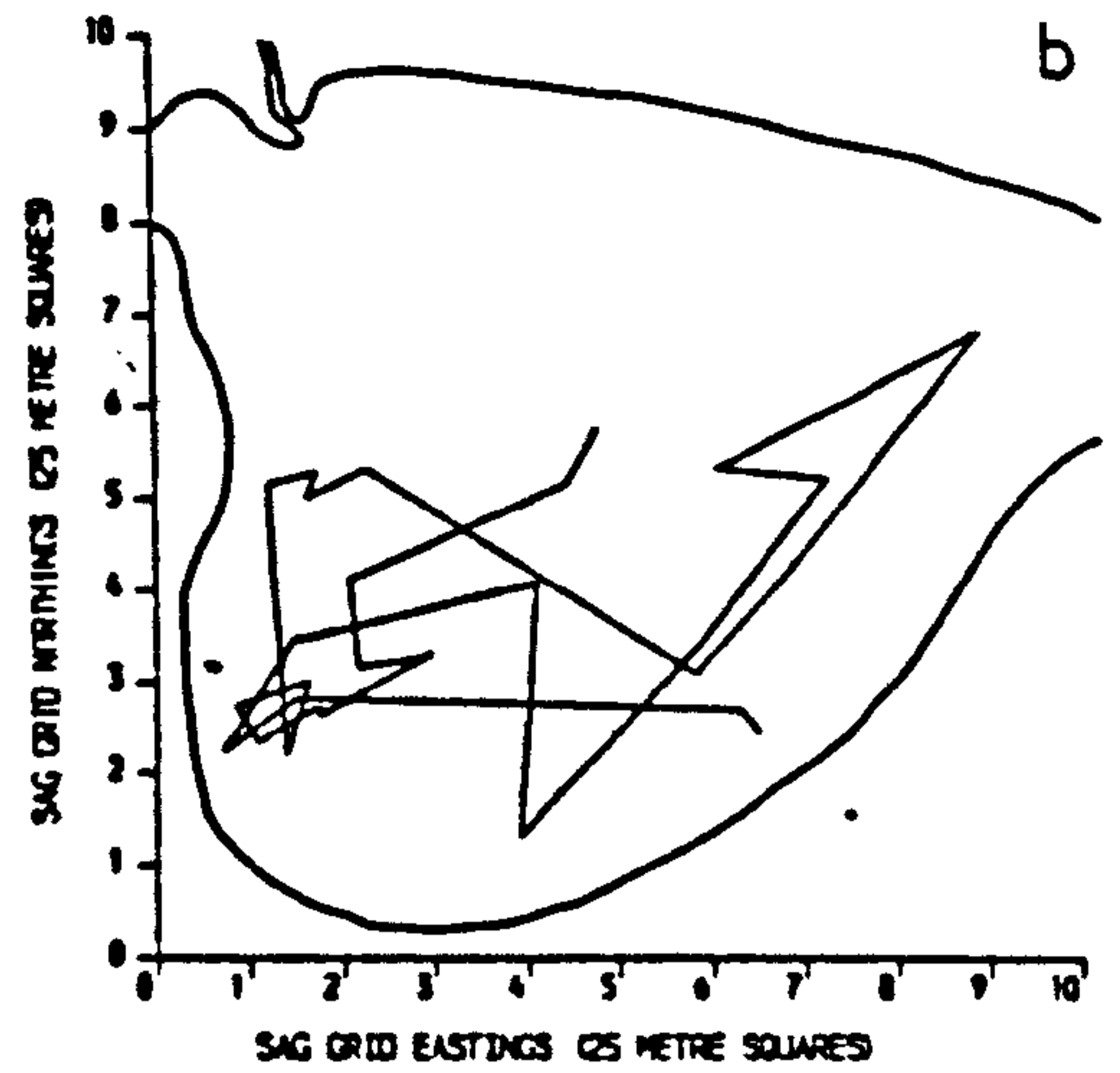
- a) Complete pattern during whole experiment.
- b) During daylight.
- c) During the night-time
- d) During the feeding peaks.

The fish shows a distinct preference for the area around
the yellow hydrophone station and an avoidance of the north
and east parts of the loch.

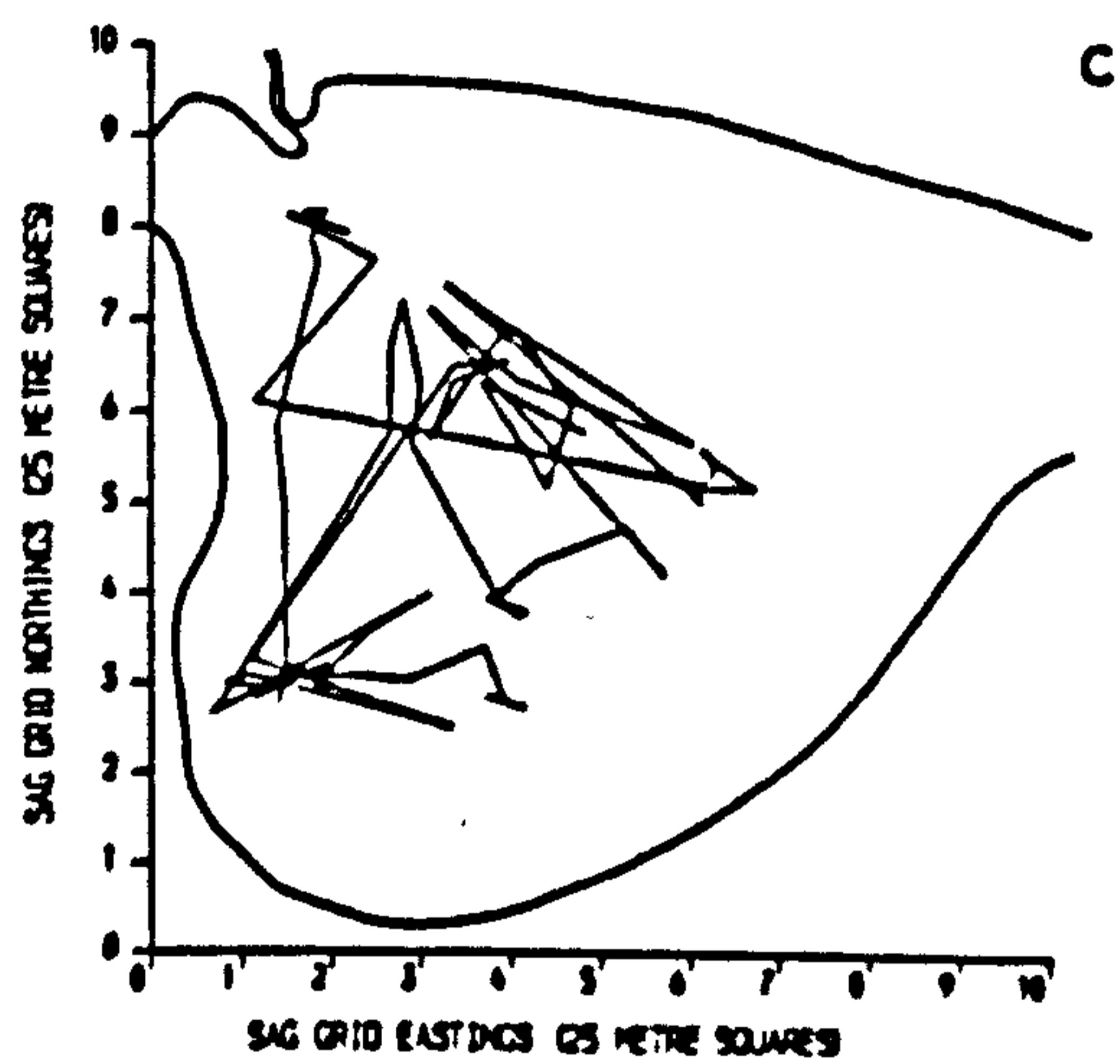
Fig. 3.15



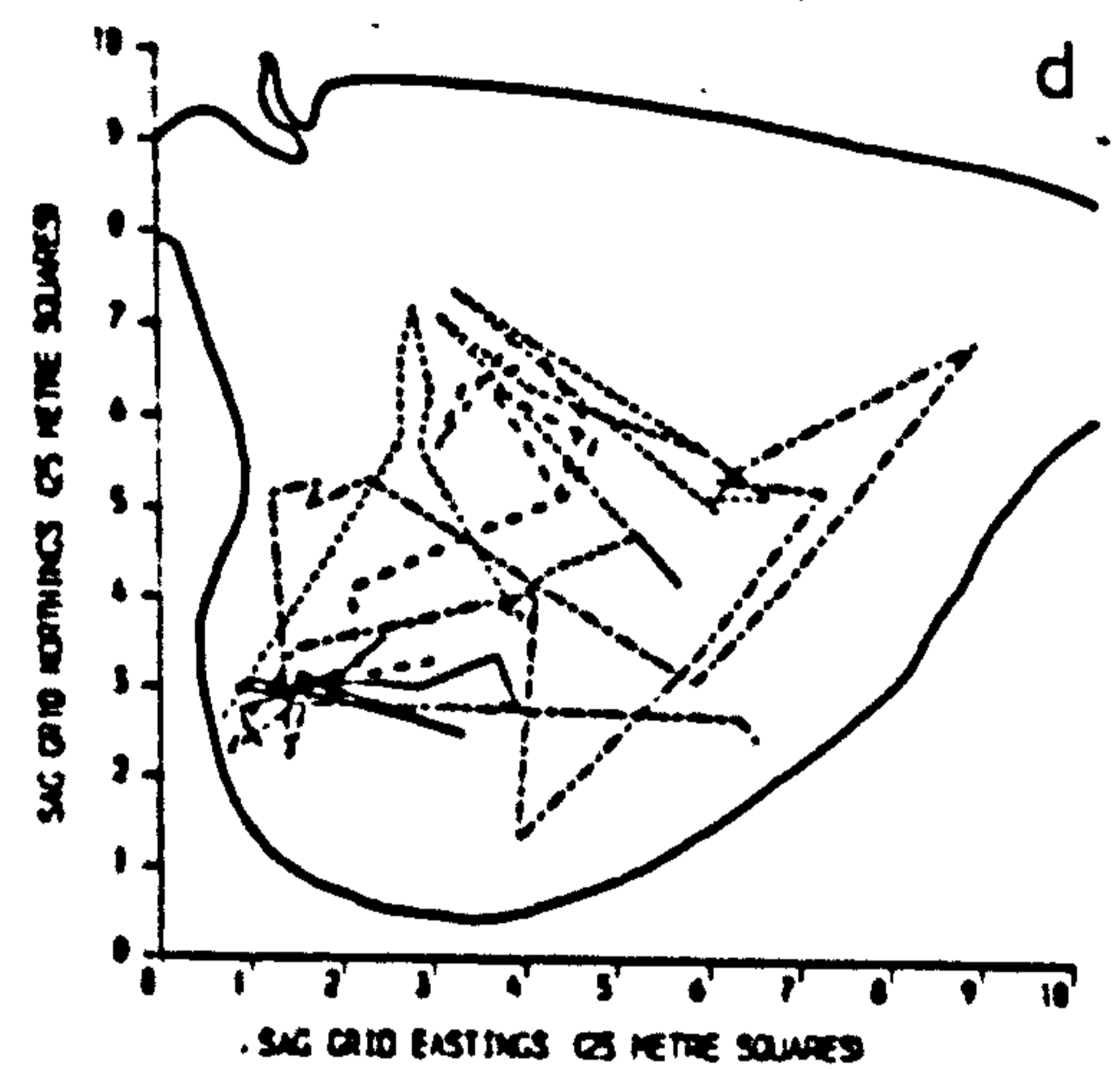
FISH 2 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 FISH
THE TRACKING PERIOD 6-7/11/75.



FISH 2 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 0645-1225
THE DAYLIGHT PERIOD 0645-1225.



FISH 2 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 2100-0645
PERIOD 2100 ON 6/11/75 TO 0645 ON 7/11/
75. NIGHT-TIME PERIOD.



FISH 2 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 2100-2350
THE FEEDING PEAKS 2100-2350, 0015-0355
, 0550-0815 AND 1000-1225 ON 6-7/75. --- LINE 2 0015-0355
- - - LINE 3 0550-0815
- - - LINE 4 1000-1225

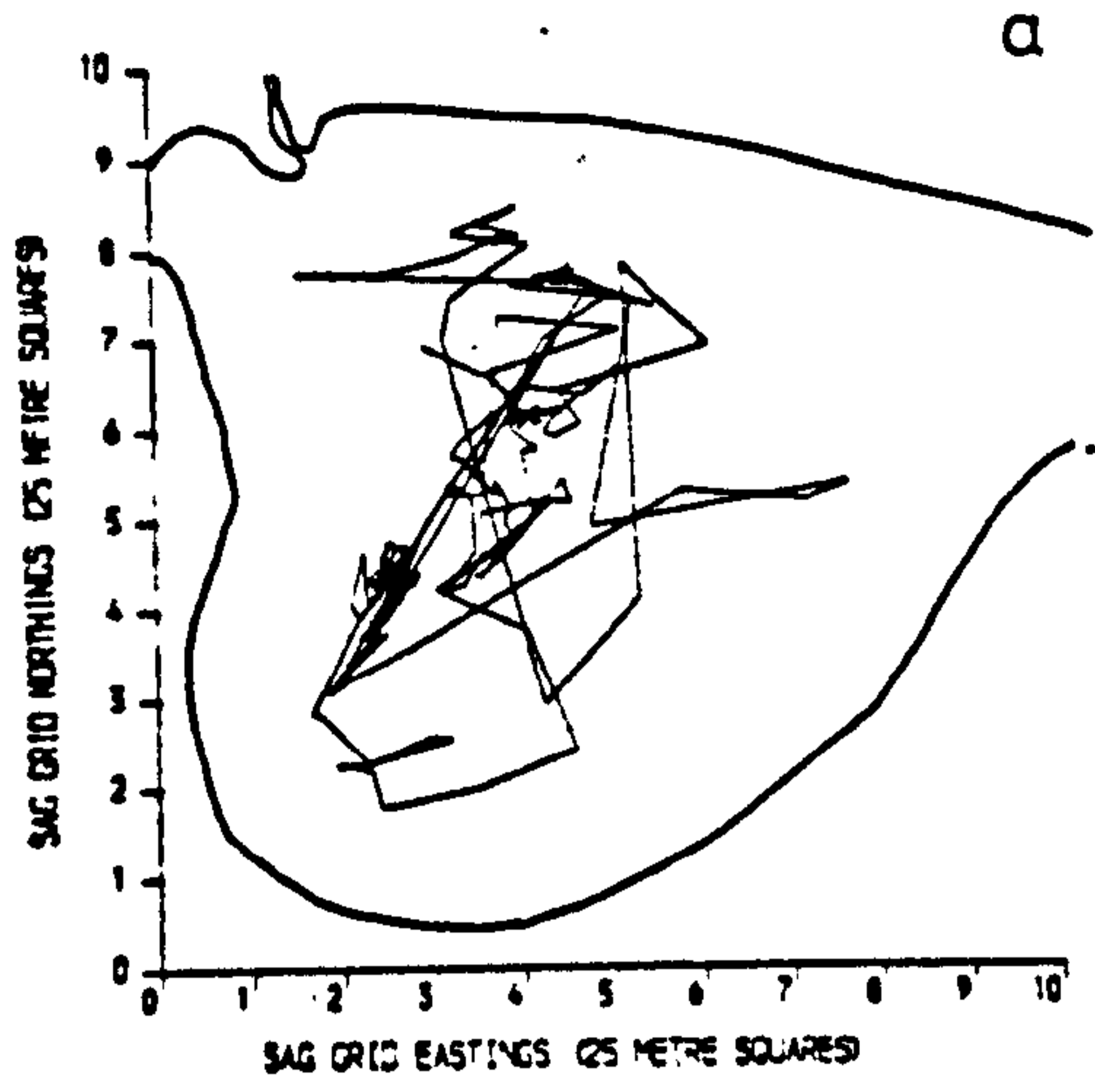
Fig. 3.16

Movement pattern for Fish 3.1, arranged as in Fig. 3.15 :

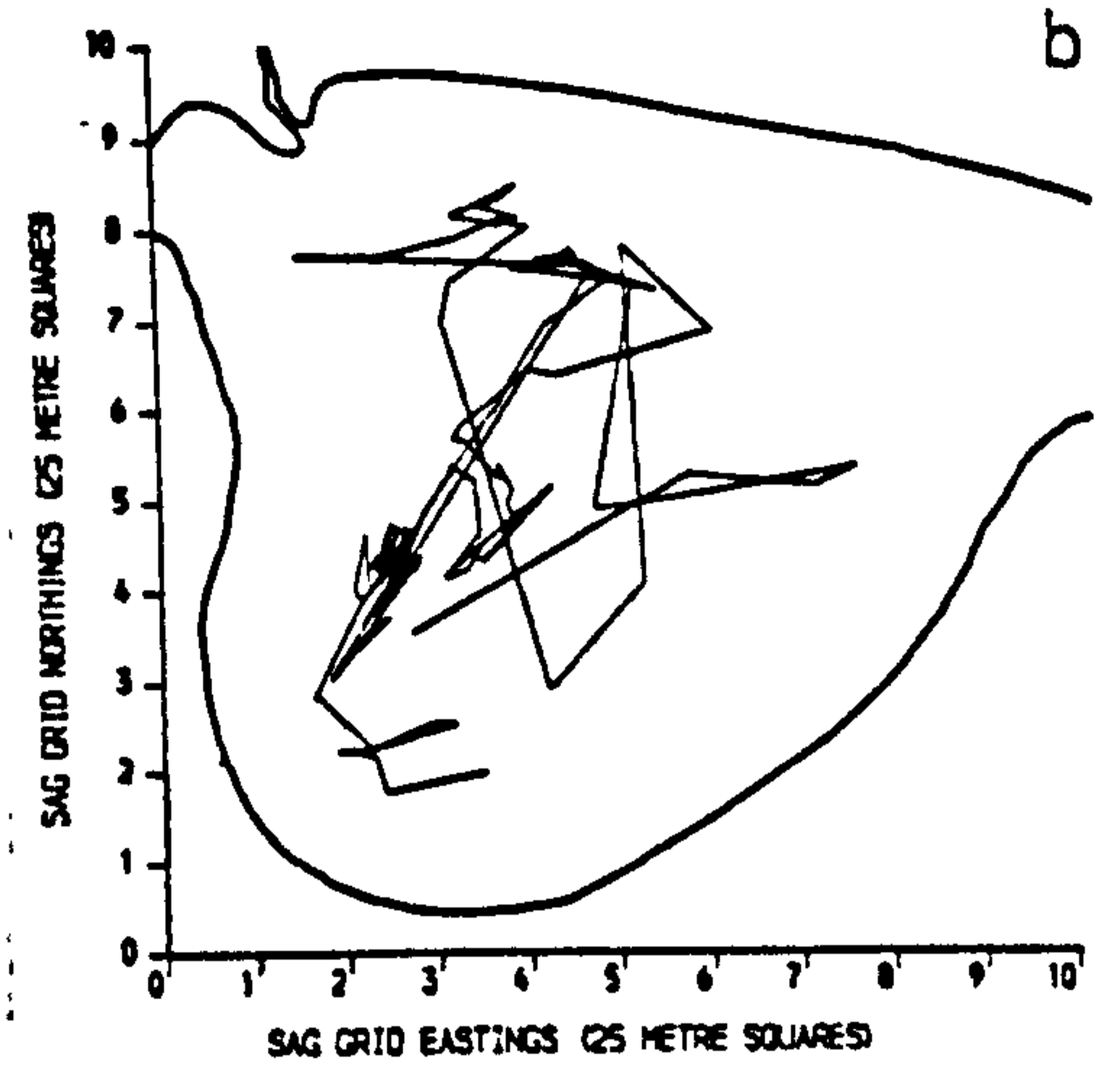
This fish tends to frequent a narrow zone, parallel to the west shore of the loch. There is an obvious correlation between movement pattern and hydrography (see Fig. 3.5).

During the morning feeding peak, there is a sequence of intricate small scale movements, located to the north of the yellow hydrophone.

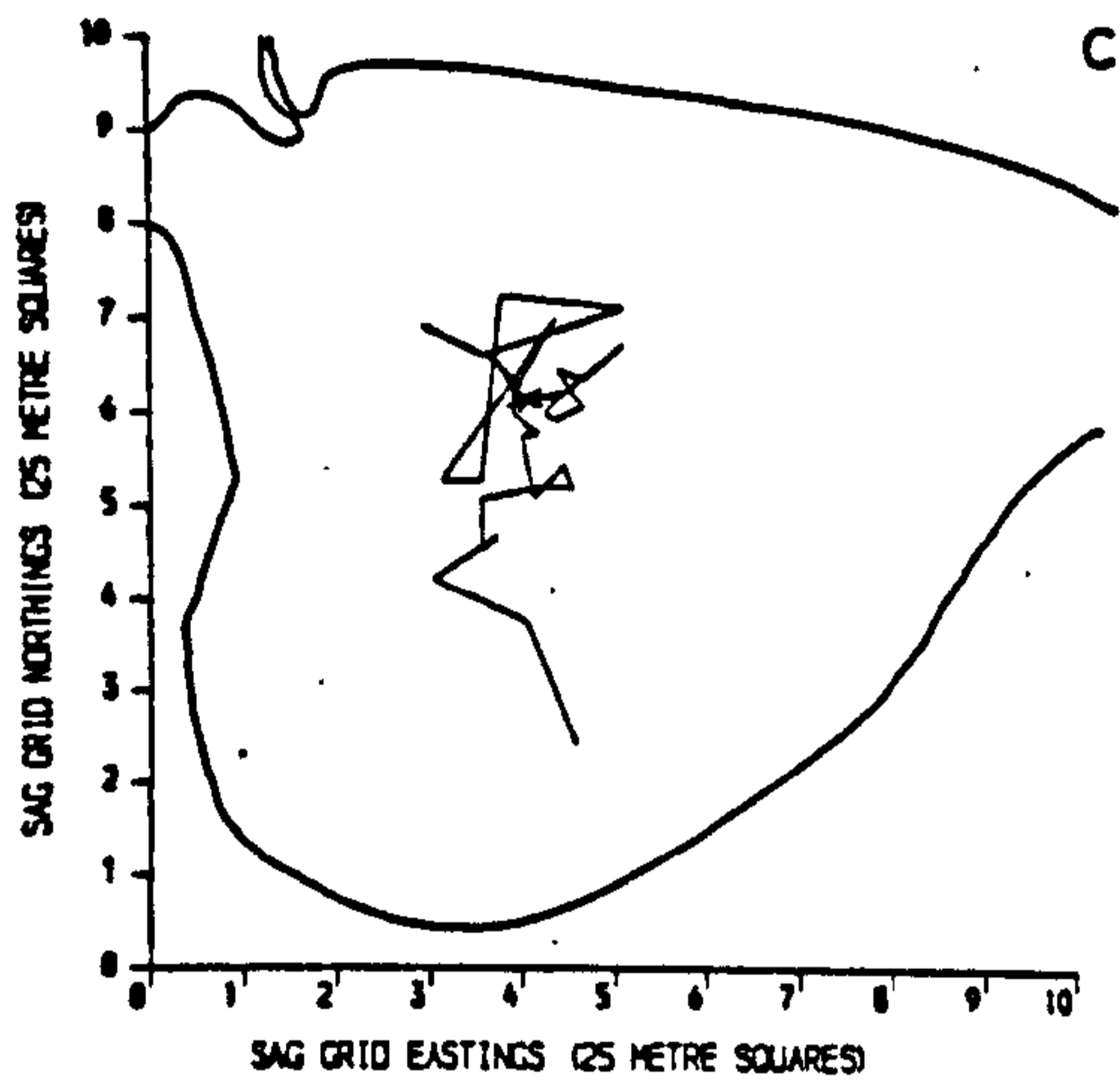
Fig. 3.16



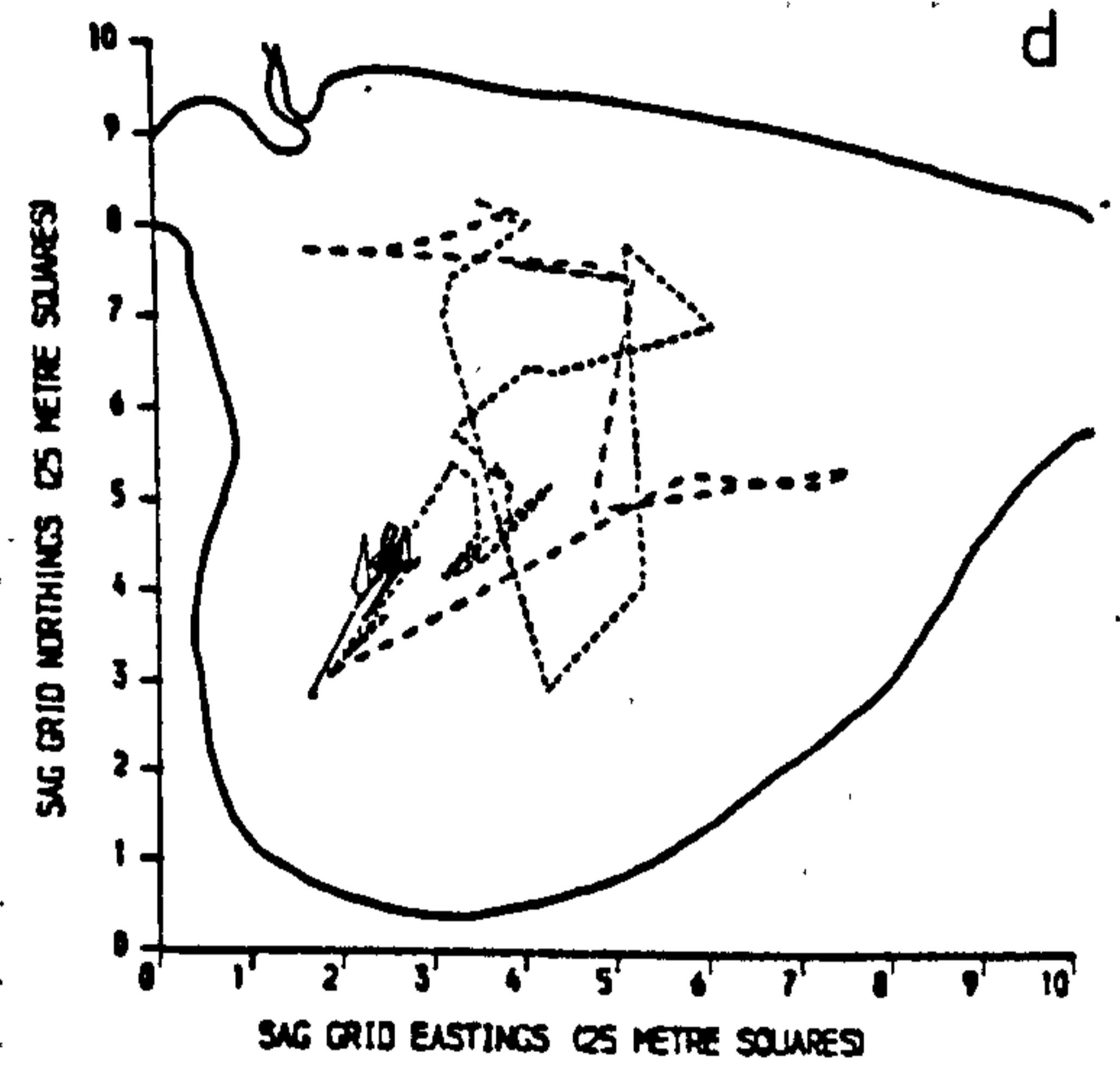
FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 2200-2220
THE TRACKING PERIOD 8-9/6/76 (3.11)



FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 0455-2100
PERIOD 0455 TO 2100 HOURS BST, DAYLIGHT
8-9/6/76.



FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 2200-0450
NIGHT-TIME PERIOD, (2200-0450 ON 8-9/6/76 — LINE 2 2110-
AND 2110- ON 8-10/6/76.



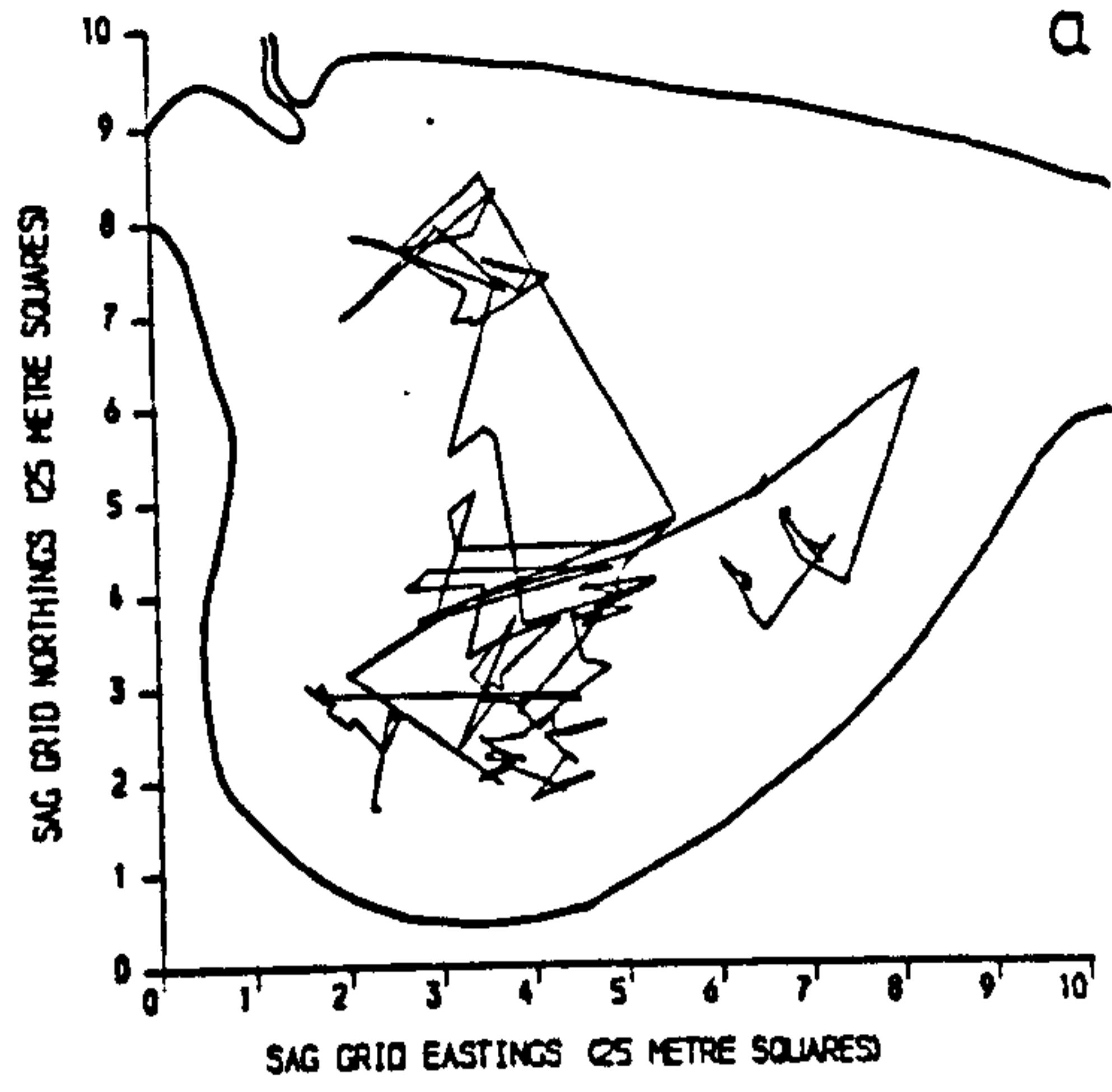
FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 0610-1005
FEEDING PEAKS 0610 - 1005, 1200 - 1725, — LINE 2 1200-1725
1920 - 2130 HOURS BST ON 9/6/76 --- LINE 3 1920-2130

Fig. 3.17

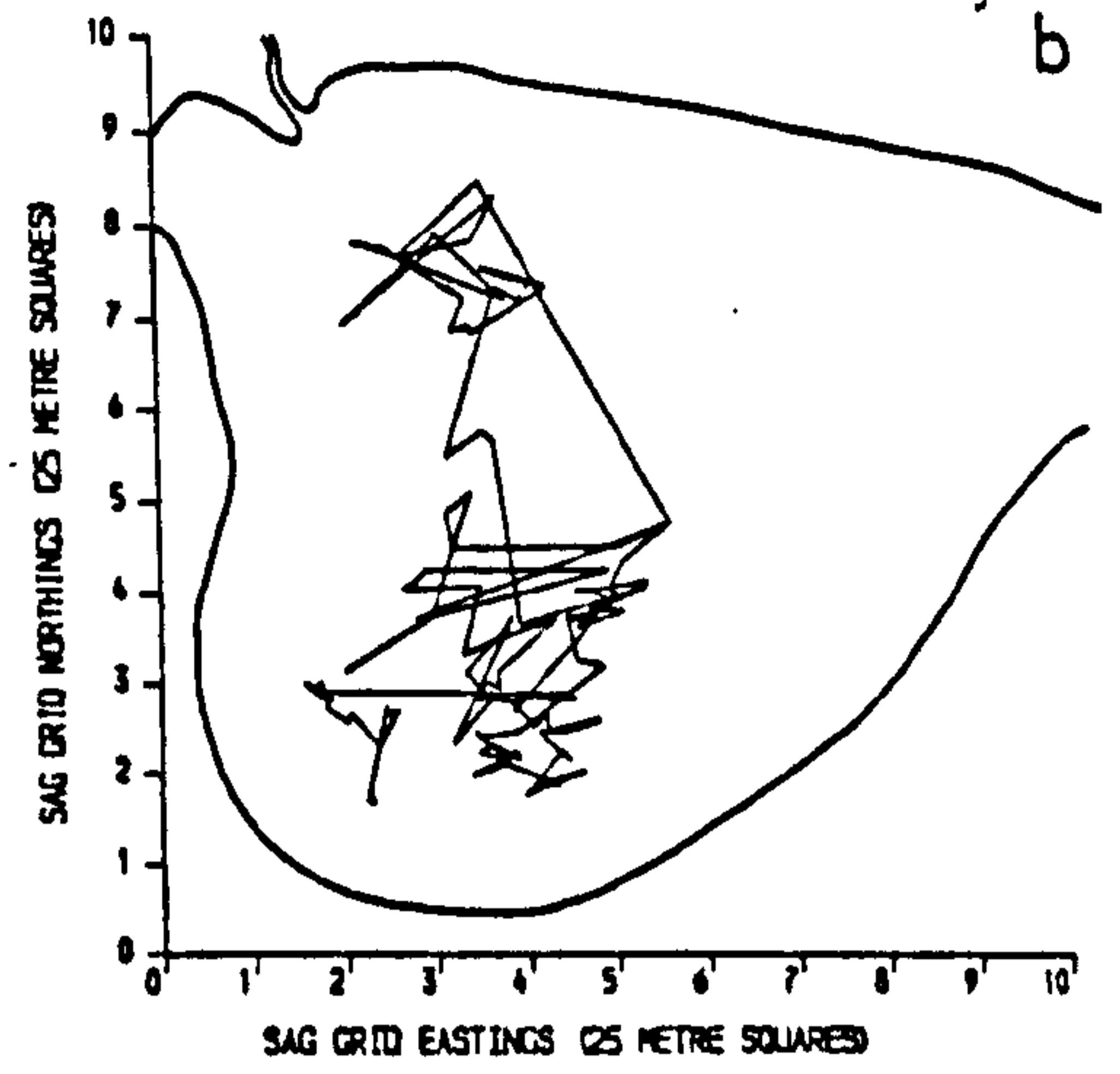
Movement pattern for Fish 3.2: arranged as preceding figures :

The fish continues to frequent the same zone of the loch as previously, parallel to the west shore of the loch. Both the feeding peaks are periods of intricate small-scale backtracking.

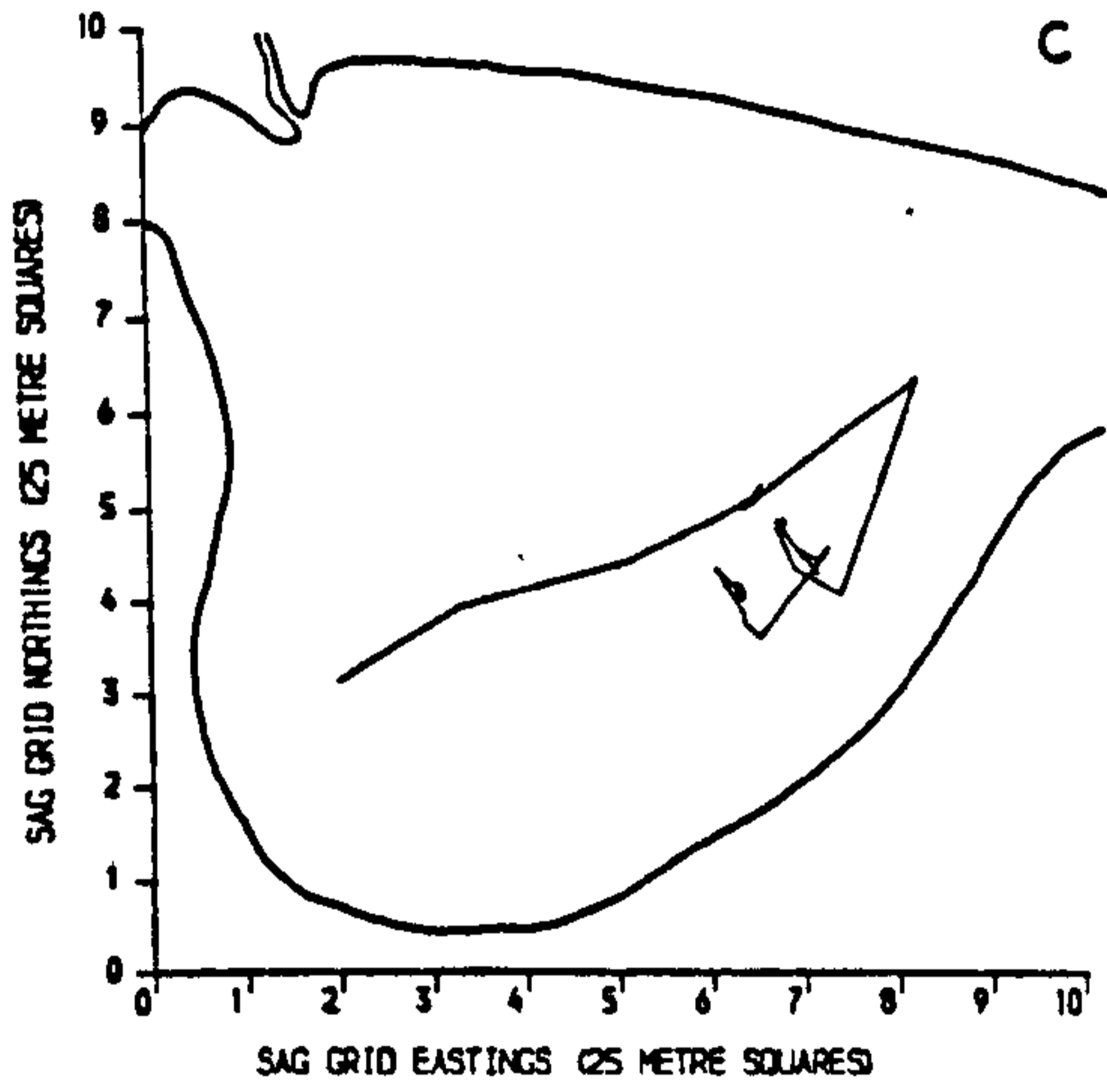
Fig. 3.17



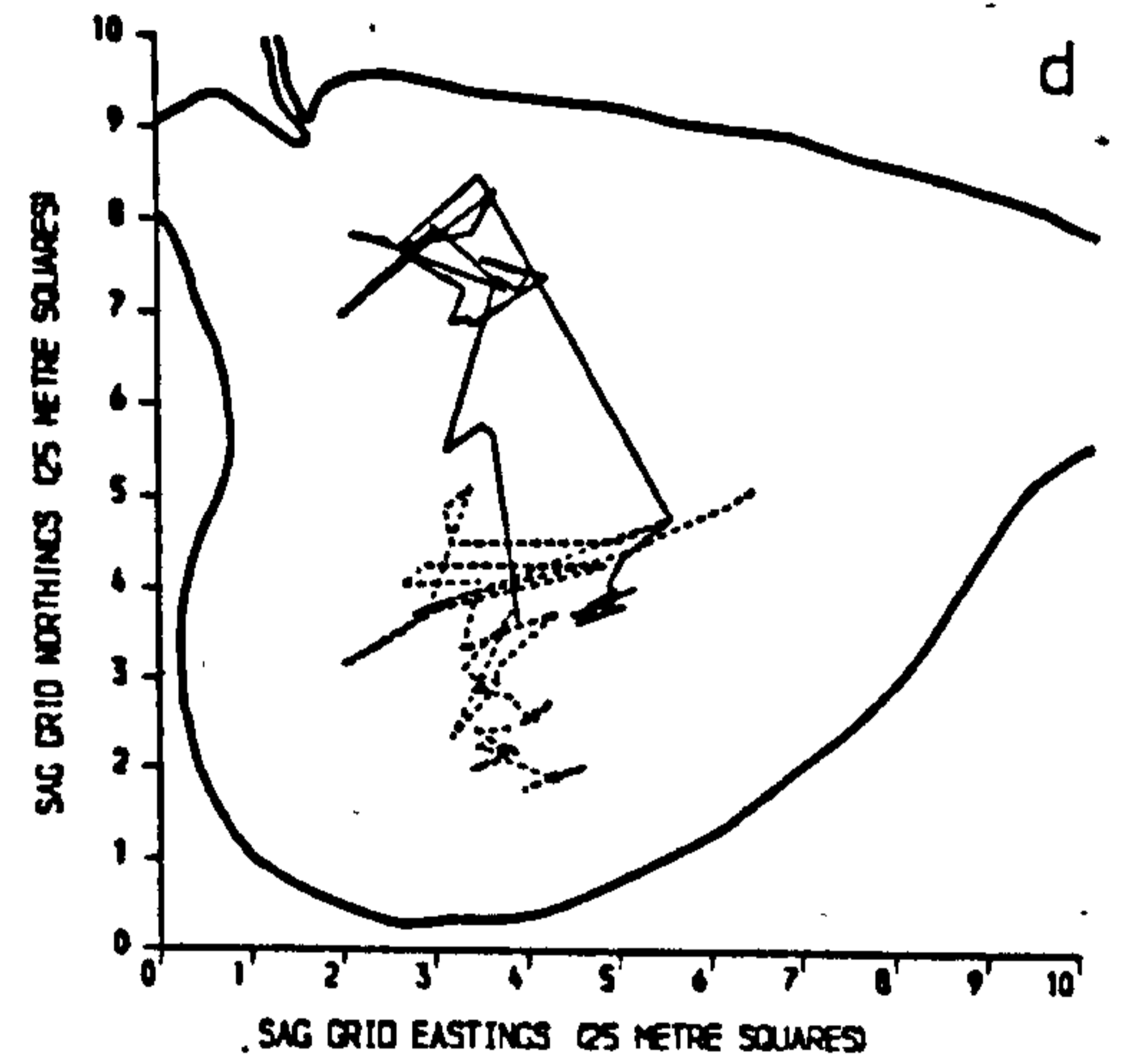
FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 0910-0155
WHOLE TRACKING PERIOD FROM 0910 ON 9/6/
76 TO 0155 ON 10/6/76.



FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 0910-2200
THE DAYLIGHT PERIOD 0910-2200 ON 9/6/76.



FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 2200-0155
THE PERIOD 2200 ON 9/6/76 TO 0155 ON 10/
6/76. NIGHT-TIME.



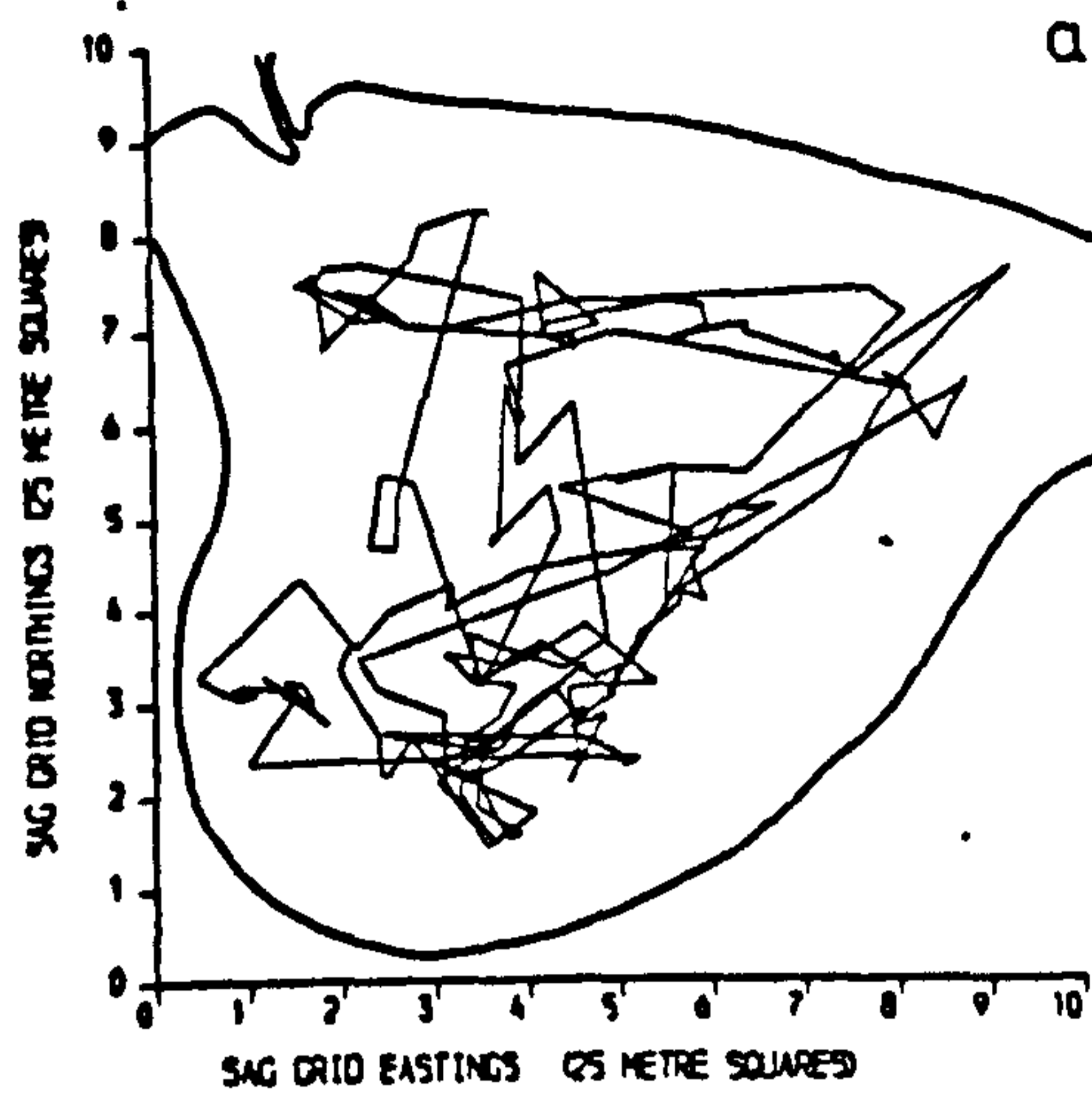
FISH 3 TRACKING PLOT OF MOVEMENTS DURING — LINE 1 1155-1530
THE FEEDING PEAKS 1155-1530 AND 1645- — DOTTED LINE 2 1645-2230
2230 HOURS.

Fig. 3.18

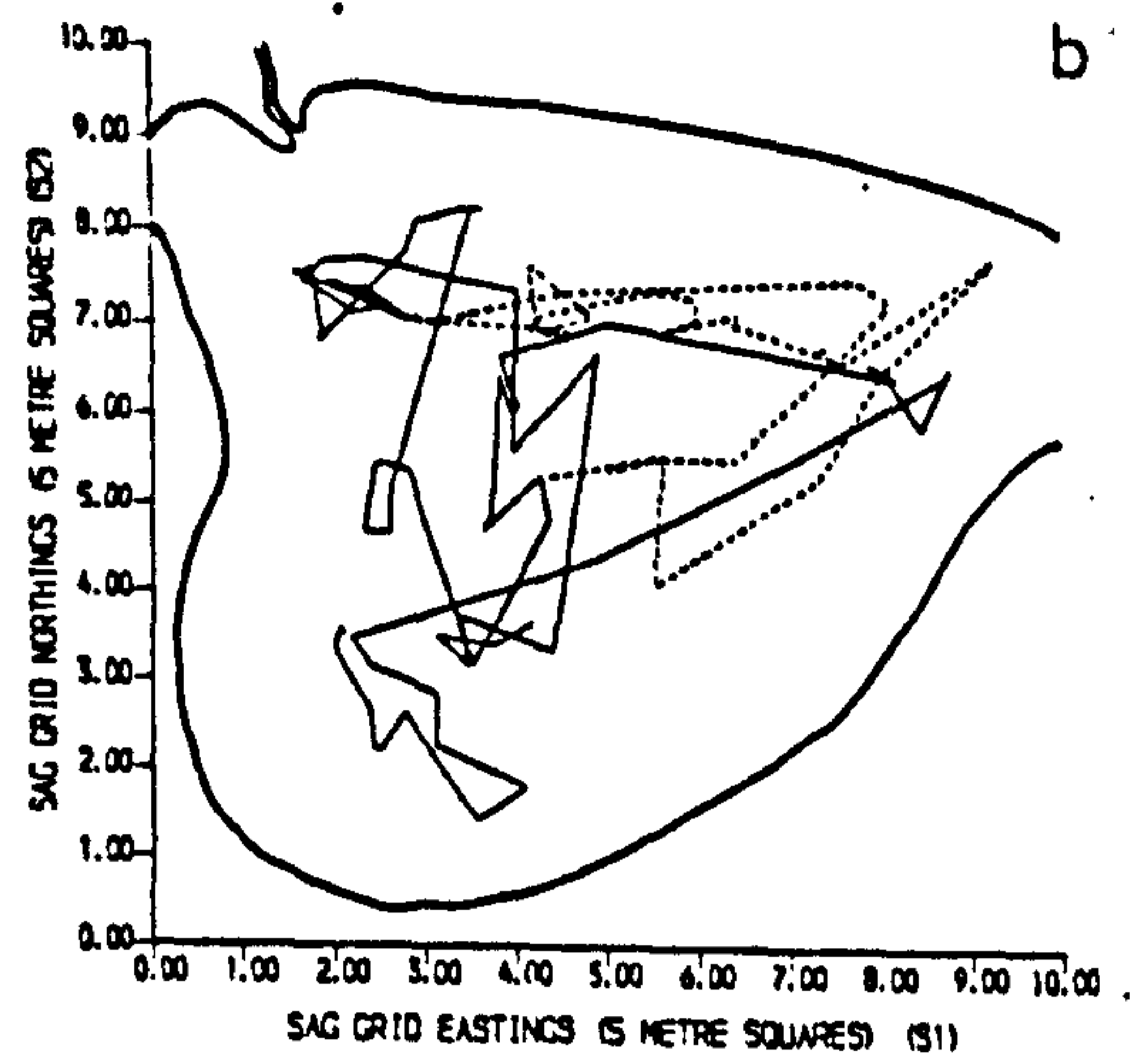
Movement pattern for Fish 4 arranged as preceding diagram.

This fish also shows a tendency to be directed by the hydrography in its movement pattern. The west shore 'shelf' is conspicuously avoided and there is a preferred area to the east of the yellow hydrophone station.

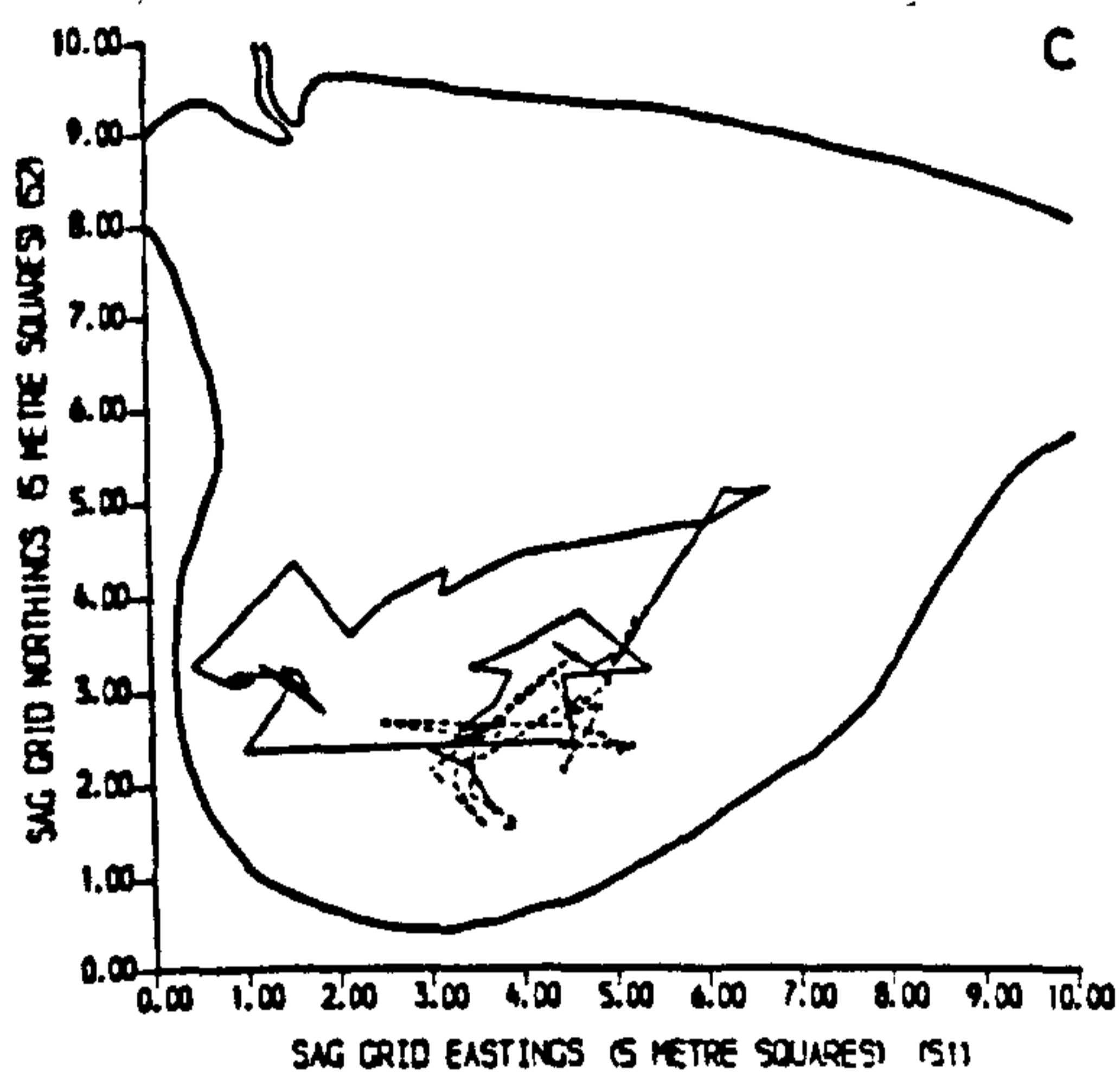
FIG. 3.18



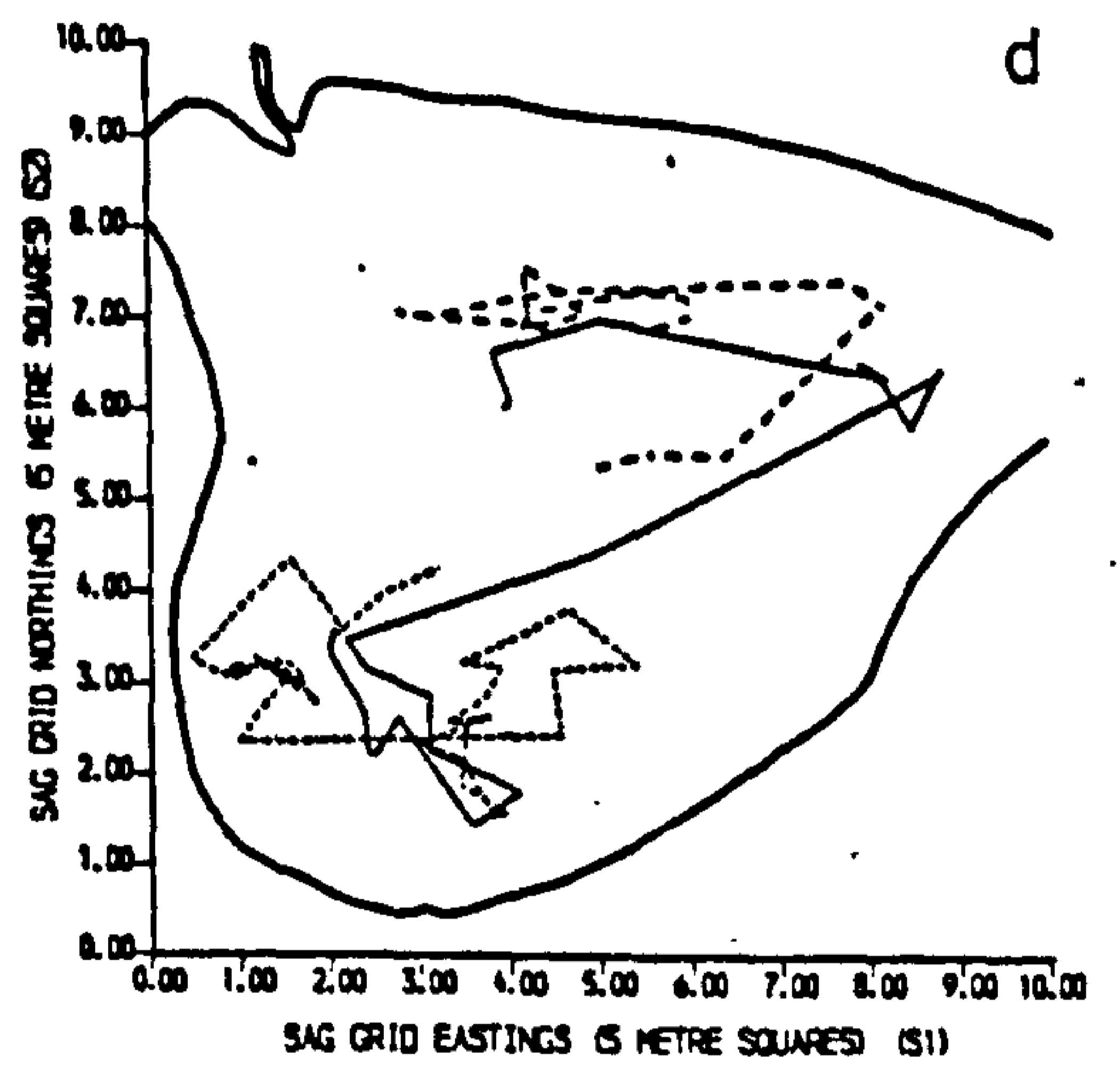
FISH 4 TRACKING PLOT OF POSITIONAL FIXES — LINE 1 FISH 4 DURING THE WHOLE TRACKING PERIOD.



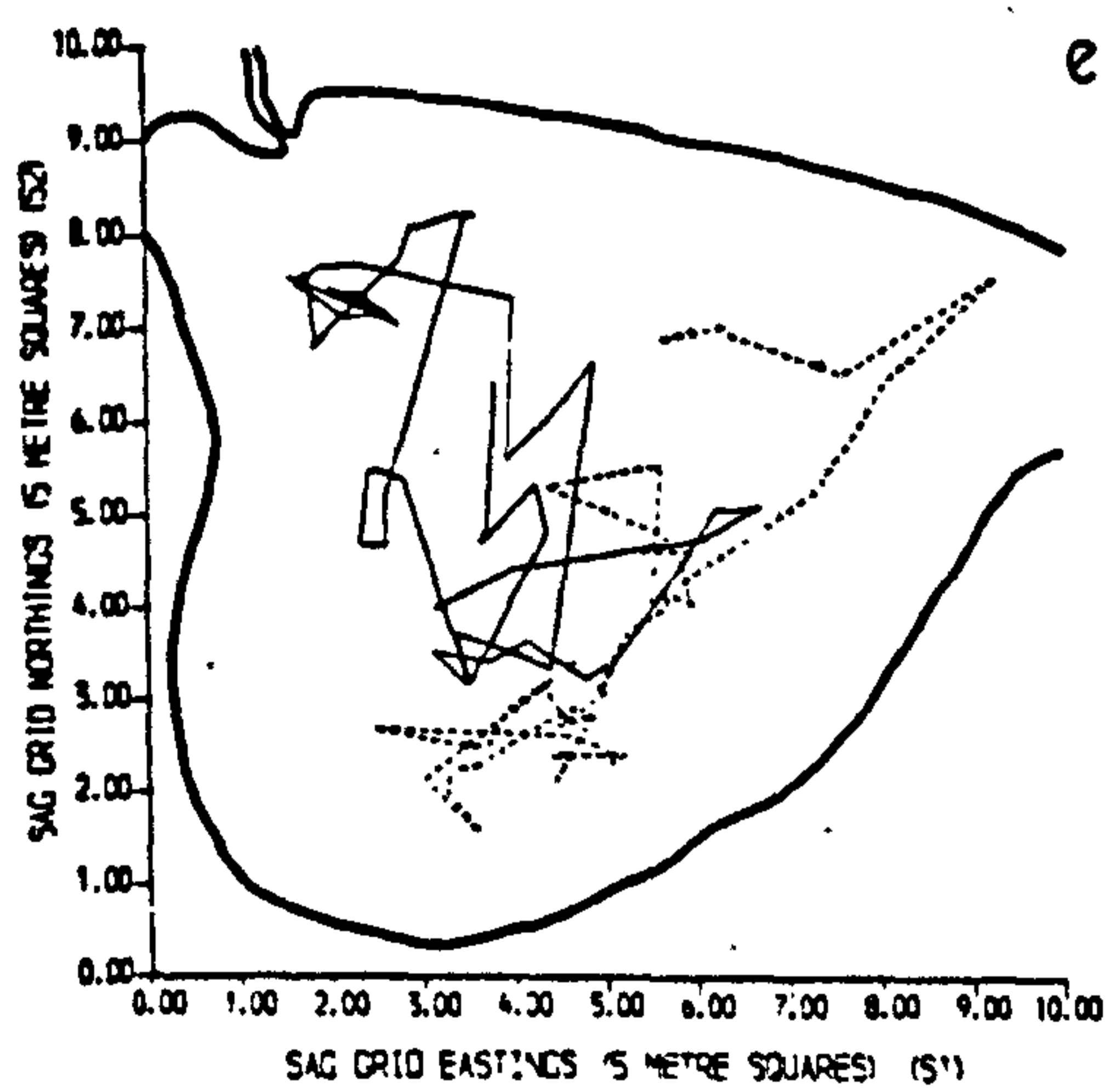
FISH 4 24-25/09/76. TRACKING PLOT OF — LINE 1 1100-1935 MOVEMENTS FOR EXPERIMENT BETWEEN 1100 TO 1935 HOURS BST ON 24/9/76, AND 0600 TO 1000 HOURS BST ON 25/9/76. THIS CORRESPONDS TO THE DAYLIGHT HOURS FOR THE PERIOD OF THE EXPERIMENT.



FISH 4 24-25/09/76. TRACKING PLOT OF — LINE 1 1940-0100 MOVEMENTS FOR EXPERIMENT BETWEEN 1940 HOURS BST ON 24/09/76 TO 0600 HOURS BST ON 25/09/76. THIS CORRESPONDS TO THE DUSK TO DAWN PERIOD.



FISH 4 24-25/09/76. TRACKING PLOT OF — LINE 1 PEAK 1 MOVEMENTS DURING THE THREE FEEDING PEAKS — LINE 2 PEAK 2 PEAK 1 1000-1430 HOURS BST ON 24/9/76 — LINE 3 PEAK 3 PEAK 2 2030-0120 HOURS ON 24-25/9/76 PEAK 3 0730-1000 HOURS BST 25/9/76.



FISH 4 PLOT OF MOVEMENTS FOR PERIOD 1435 TO 2025 HRS ON 24/9/76 AND 0125 TO 0735 HRS ON 25/9/76. THIS CORRESPONDS TO THE INTERVALS BETWEEN THE THREE MAJOR FEEDING PEAKS.

trapezoidal area of the loch near the yellow hydrophone. There is an apparent element of reciprocal courses in the night-time period, causing zig-zag track diagrams.

There are some differences in the pattern of movements; the first peak pattern is very restricted in area as it is composed of small step lengths; compare this with that from the daylight hours which features considerably more dispersion of the fixes.

Finally, the tracking plots for the whole tracking period show, overall, a distinct preference for the area around the yellow hydrophone. Nearly all the movements are within a 150 metre radius of this location, save for one visit out to the red hydrophone. This is clearly a 'home range' from which the fish appears to make excursions.

Fish 3.1 (Fig. 3.16). The daytime plot shows a highly complex track diagram with strong evidence of preferred areas. There is a very intense concentration around the yellow hydrophone which accounts for the succession of small step lengths. Later on, much larger step lengths occur towards dusk until the termination.

The night plot on the other hand is a period of larger mean step length though this is not particularly obvious from the track diagram. Nevertheless, the night mean is some three and a half times that of the day period, owing to the influence of the intense periods of short step length activity during the day.

The first feeding peak is largely composed of small step lengths, whilst the next peak has two tightly nucleated areas. There are, however, some large excursions north and south.

The tracking plot for the whole period is quite instructive. This consolidates the theory that the fish has a preferred area from which nearly all the plotted fixes are less than 150 metres distant.

Fish 3.2 (Fig. 3.17) The tracking plot for daytime is highly complicated owing to the long period and the short mean step length. The fish moves north to a new centre of activity from which it returns southwards around 14.30 hours. The fish's movements are restricted to a narrow arcuate zone in an area similar to that occupied by it in the previous day.

The night-time plot was unfortunately terminated after only four hours owing to the transmitter hardware failure. It can, however, be seen that an interesting movement pattern is developing. At dusk, the fish makes a large directed north-easterly movement from its daytime centre of activity then appears to set up a new centre of activity. The movement pattern is then composed of very much smaller step lengths. This accounts for the pattern visible in the step length pattern.

During the first feeding peak, the fish moves northward to around the green hydrophone. This appears on the step length pattern as a series of step lengths more than 50 metres followed by a sequence of small intersecting movements in the new home range. There is a directed movement back to the old home range at 14.30, characterised by small intersecting movements over a small area.

During the second peak, the fishes activity centres on the 'old' home range. It makes a complex series of small movements, progressing due northwards. At 19.15 the fish changes into a pattern of greater step length and it tends to cross and recross a narrow zone parallel to the S.E. shore of the loch.

When the fish is making groups of insubstantial movements, large amounts of feeding may occur and conversely, large movements may be associated with periods of low feeding activity.

Fish 4 (Fig. 3.18) During the first daytime plot, the fish frequents three sites; it commences around the yellow hydrophone and

then moves out to the red hydrophone. It next moves to the green hydrophone, returning to the initial area.

There is a tendency to frequent a narrow zone parallel to the eastern shore of the loch. In the second day-time period, which is only of four hours duration, the fish's activity appears to be centred more northward parallel to the north shore of the loch. There are a series of large step lengths and, as in the first day-light period, a rather dispersed pattern of movement. At 06.05 the fish makes a movement northward from which it returns at 0910. The night-time period is of very short mean step length and it can be seen that at first there are periods of short movements punctuated by larger movements but later there are more small scale movements.

For Fish 4 only, plots were produced of the positions and movements during the intervals between feeding peaks. It can be seen that the interval pattern is similar to that of the peaks, consisting of large step lengths interspersed with groups of smaller scale movements. In the first interval, the fish covers a narrow area parallel to the west shore of the loch and there is a confined intersection of small scale movements off the green hydrophone. The second interval plot shows that the fish covers a narrow area, parallel to the south east shore of the loch from which the fish subsequently moves northwards towards the red hydrophone station.

The tracking plot for the first feeding peak is interesting; as first the movements are of short step length. The large step lengths which move the fish out towards the red hydrophone at 13.25 coincide with a low feeding rate.

The second peak is confined to the southern half of the loch and the tendency is for large amounts of feeding to be occurring at times of small scale movements.

The final peak, which was terminated by the enforced close down of the tracking equipment, was centred in the northern part of the loch and features some wide-ranging movements of which some of the larger appear to be associated with low feeding activity.

3.16 Relationship of movement patterns to underwater topography

In the case of Fish 2 there is a tendency for it to favour water greater than 1 metre in depth although it does make some excursions into the marginal shallows. The v-shaped distribution appears to be influenced by the shelf which protrudes into deeper water off the control caravan. Reference to the loch hydrography maps (Fig. 3.5) shows this shelf clearly.

Fish 3.1 has a distinct preference for water greater than 2.0 metres in depth, whilst at night the fixes are in water over 2.5 metres. The distribution of the fish's position in relation to the previously mentioned 'shelf' is evident.

Later (Fish 3.2) the fish occupies a restricted area in the centre of the loch with a clear preference for water greater than 2.0 metres in depth. Once again the influence of the 'shelf' is obvious. The fishes plotted positions are all less than 150 metres from the major centre of activity.

Fish 4 has a pattern of movements which is both confusing and highly complicated. The fish covers a wide range in the loch, in contrast to previous experiments. Once again there is a preference for deeper water greater than 1.5 metres in depth and the avoidance of the west shore 'shelf' is again evident.

3.17 Circular Statistics

Over the recent years a new statistics has grown up with the development of interest in animal orientation and biorhythmometry in general (see Batschelet, 1965, for introduction).

Although such methods involving angular variables have been

applied to fish in the laboratory (Kleerekoper et al. 1970, 1974), the only application to field studies of fish, using telemetric methods, has been Tytler et al. (1976).

In the latter study, one of the techniques mentioned briefly was that of correlation when one variable is circular and the other is linear. Using Mardia's (1976) formula, the linear-circular correlation coefficients were calculated using program 'LINCIRC' for Angle of turn/Step length and Angle of turn/Feed total (Table 3.9). The significances of the values were assessed according to the formula for calculating t_{n-2} given by Meddis (1975).

It will be seen that, except in the case of Fish 4, significant correlations were obtained between angle of turn/step length though significant correlations for angle of turn/feed total were obtained for Fish 3.1 and Fish 4.

A serious problem in dealing with circular variables is outlined by Batschelet (1965); arithmetic means of directional angles are usually meaningless, depending on how the zero reference is defined. Completely contradictory mean directions can result from this. The same applies to other descriptive statistics.

In the case of angle of turn as defined in this investigation; program 'FISHTK' calculates the angle, in a clockwise direction through which the fish turns, relative to its prior course. The zero reference direction is, therefore, continuously updated and the result is a measure not an actual compass direction. It may be, therefore, that in this case continuous descriptive statistics are meaningful. To make compatible reference with Tytler et al.'s (1976) work, however, the program 'CIRCULAR' was used to calculate circular descriptive statistics. The mean direction is in this case assessed by calculating the centre of mass of the angles. If the centre of mass is not zero then there is at least one

Table 3.9

Linear-Circular Correlation Coefficients

Fish 2	ANI v D1		Fish 3.1	ANI v D1	
	$R1_{\theta} = 0.278$	d.f. = 104		$R1_{\theta} = 0.366$	d.f. = 192
	$t_{n-2} = 2.949$	Sig(t) = **		$t_{n-2} = 5.449$	Sig(t) = ***
	ANI v M1			ANI v M1	
	$R1_{\theta} = 0.138$	d.f. = 50		$R1_{\theta} = 0.269$	d.f. = 89
	$t_{n-2} = 0.979$	Sig(t) = N.S.		$t_{n-2} = 2.635$	Sig(t) = **
Fish 3.2	ANI v D1		Fish 4	ANI v D1	
	$R1_{\theta} = 0.305$	d.f. = 163		$R1_{\theta} = 0.049$	d.f. = 199
	$t_{n-2} = 4.087$	Sig(t) = ***		$t_{n-2} = 0.694$	Sig(t) = N.S.
	ANI v M1			ANI v M1	
	$R1_{\theta} = 0.039$	d.f. = 81		$R1_{\theta} = 0.145$	d.f. = 197
	$t_{n-2} = 0.347$	Sig(t) = N.S.		$t_{n-2} = 2.062$	Sig(t) = *

Table 3.10

Descriptive Circular Statistics

Fish 2	χ_0	r	Fish 3.1	χ_0	r
	283.333	0.135		330.598	.184
	$S_0 = 0.884$	$S_0 = 119.003^{\circ}$		$S_0 = 0.816$	$S_0 = 105.481^{\circ}$
	$g_1 = 0.081$	$g_2 = -0.225$		$g_1 = 0.466$	$g_2 = 0.459$
	$z = 1.910$	Sig(Z) = <5%		$z = 6.557$	Sig(Z) = >1%
	$\kappa = 0.271$	$I_0(\kappa) = 1.109$		$\kappa = 0.374$	$I_0(\kappa) = 1.035$
Fish 3.2	χ_0	r	Fish 4	χ_0	r
	195.376	0.097		348.353	0.032
	$S_0 = 0.902$	$S_0 = 123.529^{\circ}$		$S_0 = 0.968$	$S_0 = 150.287$
	$g_1 = 0.229$	$g_2 = 0.079$		$g_1 = 0.011$	$g_2 = -0.001$
	$z = 1.519$	Sig(Z) = < 5%		$z = 0.272$	Sig(Z) = N.S.
	$\kappa = 0.197$	$I_0(\kappa) = 1.009$		$\kappa = 0.064$	$I_0(\kappa) = 1.001$

Table 3.11

χ^2 Tests on the Angular Data taken by 40° Groups

Fish	0-40°	40-80°	80-120°	120-160°	160-200°	200-240°	240-280°	280-320°	320-360°	Total χ^2 and D.F.
2	5.679	0.520	4.546	3.423	2.361	0.016	0.520	0.016	1.573	18.650 8
3.1	2.693	6.347	8.616	7.438	12.342	3.592	5.342	3.592	83.347	133.300 8
3.2	0.016	2.919	0.646	2.124	2.834	4.669	0.016	1.578	4.561	19.364 8
4	0.925	3.974	1.375	0.093	0.093	0.093	1.376	2.469	0.291	10.688 8

Mean directions	$\bar{\chi}_0$	Sig χ^2	Z	Sig(Z)
Fish 2	283.333	*	1.910	**
Fish 3.1	330.598	***	6.557	***
Fish 3.2	195.376	*	1.519	*
Fish 4	348.353	N.S.	0.272	N.S.

preferred direction for which a vector can be calculated. By converting the vector into polar coordinates, the true mean direction can be found. The value r , the 'length' of the vector, is a measure of concentration about the mean direction. With increasing concentration, r approaches unity.

Circular variance, unlike its linear counterpart, when the directions about the mean are dispersed will be nearly unity but if clustered it will be nearly zero. (Values in radians).

To test the significance of the concentration around the mean vector, the Rayleigh Test of Uniformity is applied to the angular data using program 'RAYLEIGH'. The Rayleigh Test statistic z is designed to test the null hypothesis that the angular distribution is uniform. The significance of z is assessed from tables. A calculated value of z greater than the tabulated value, therefore, rejects the null hypothesis. Some pitfalls, however, may arise with the Rayleigh test and errors may occur if the distribution should in fact be multimodal. In such a case a goodness of fit test such as χ^2 can be used. Both are applied here. Tytler et al. (1976) drew some interesting conclusions about their experimental fishes' behaviour from studying the distribution of the angles of turn. The circular normal distribution is known as the Von Mises distribution. The fit of the data to this distribution was calculated by program 'VONMISES'. When the parameter $k=0$ and $I_0(k)=1$ then the distribution is that of the circular uniform distribution.

Tables 3.10 shows the statistics produced by 'RAYLEIGH', 'CIRCULAR' and 'VONMISES'; in most cases, the mean direction differs from that given by the arithmetic mean. In all cases, the circular variance is large, tending towards 1 and the resulting circular standard deviations are greater than 100.0° , indicative of dispersion about the mean. Significant z values were only obtained for Fish 3.1 and it can be seen that most of the k values

Fig.3.19

Fit of the angle of turn data (histograms : 40° interval) to the theoretical von Mises curve (solid line). There is no good fit of the theoretical curve to the observed data as was observed by Tytler et al. (1976).

Fig. 3.20

Polar diagrams showing the relationship between angle of turn and step length for Fish 2 - 4.

The circle represents a distance equivalent to 175 metres. Except in the case of Fish 4, there appears to be a tendency for left hand turns in the $330-360^{\circ}$ class to be associated with the longer step lengths.

Fig. 3.21

Polar diagrams for Fish 2 - 4 showing the raw distribution of the angles of turn recorded. The tendencies to circular uniform distributions are evident.

FIG. 3.19

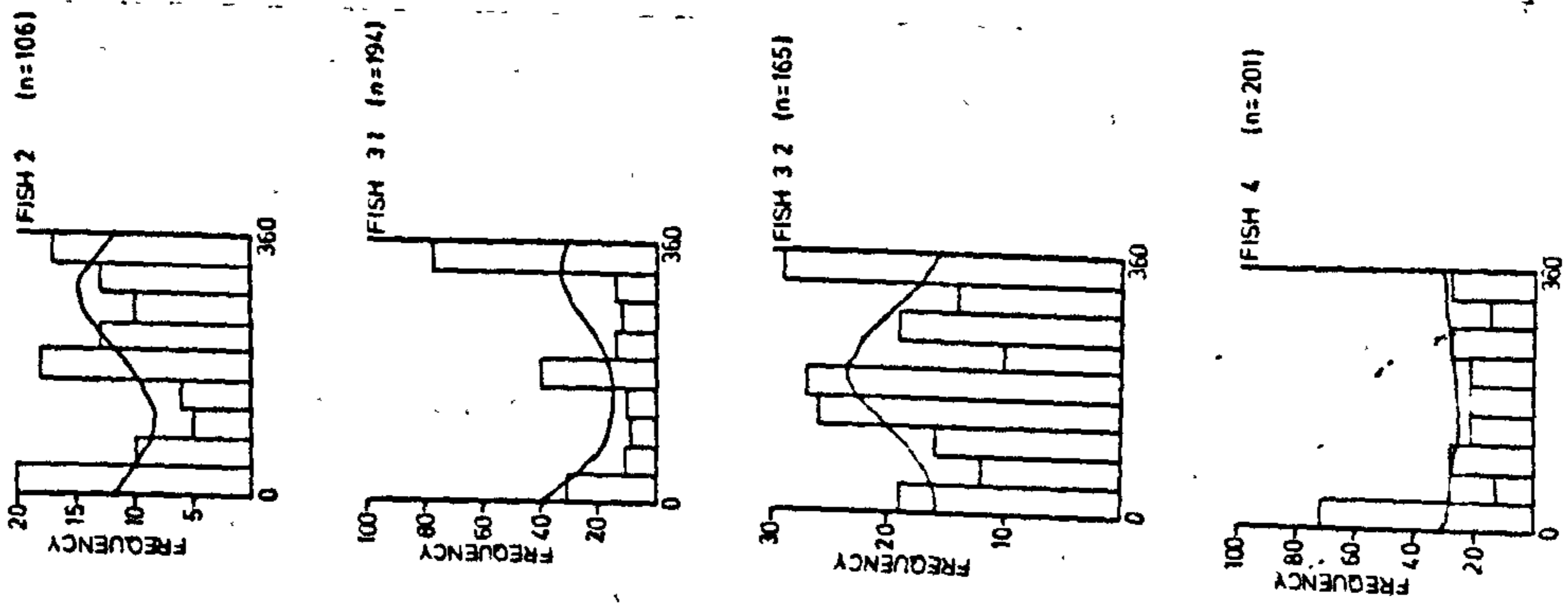


FIG. 3.20

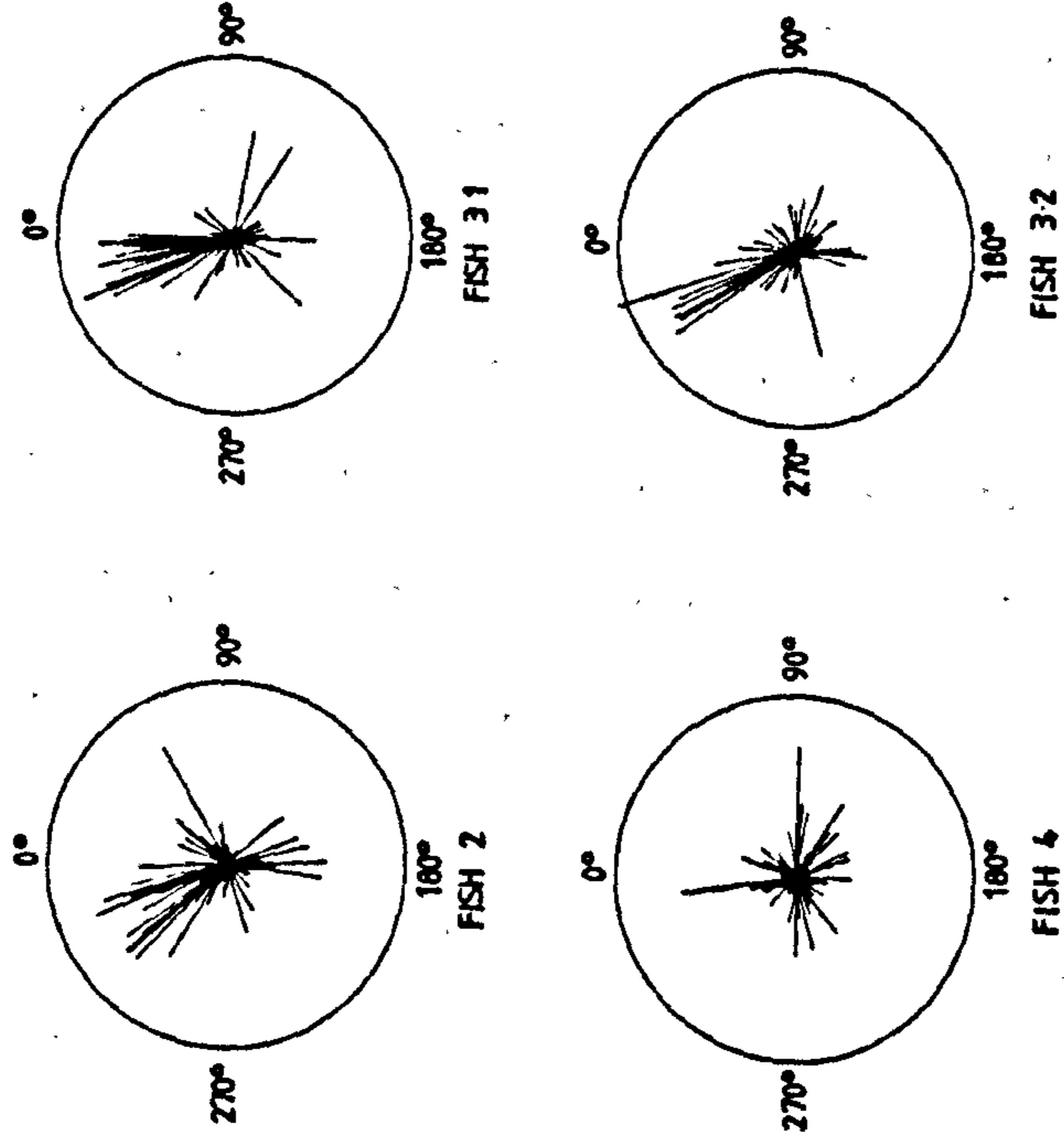
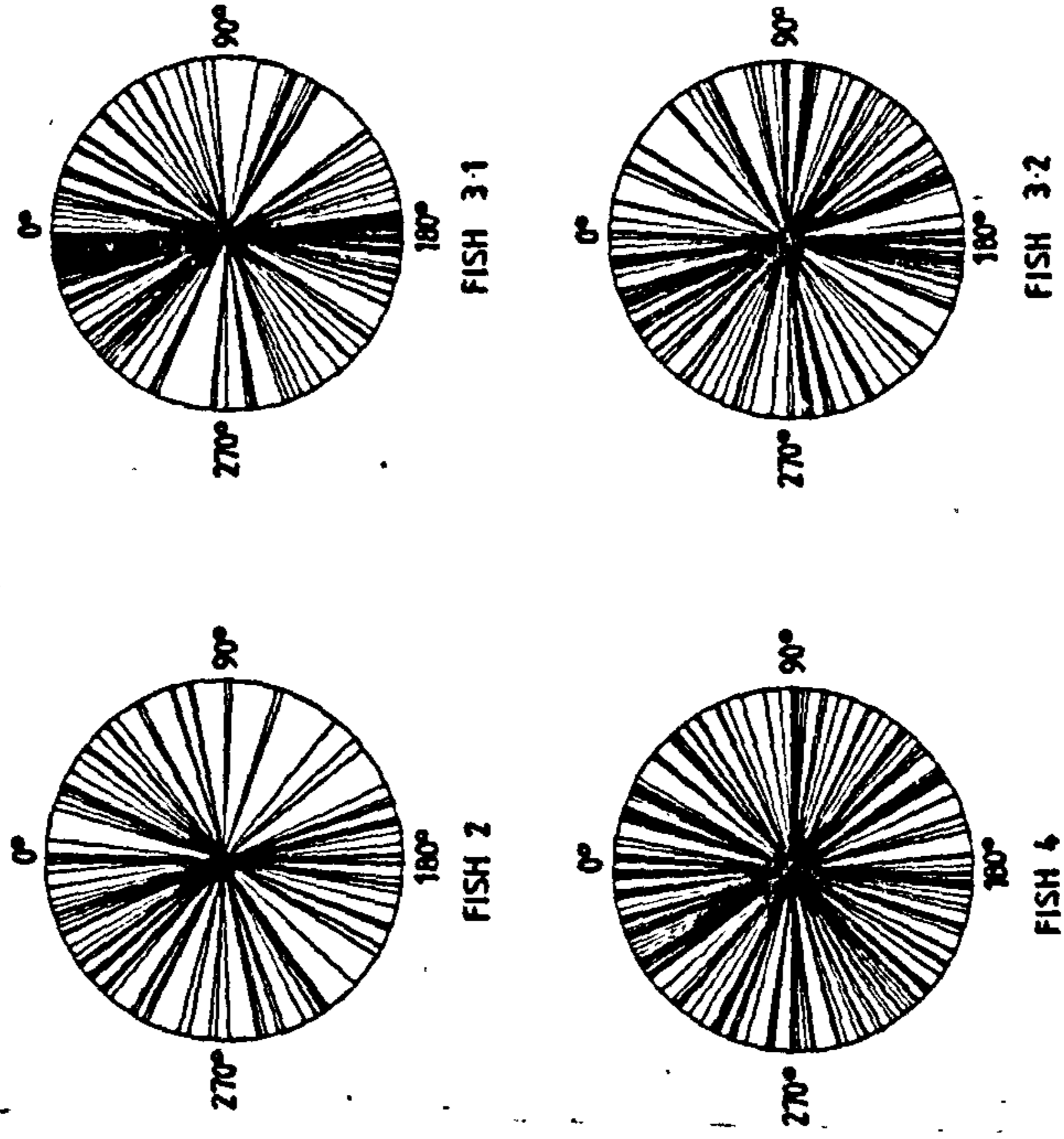


FIG. 3.21



approach 0 and I (k) approaches 1. This strongly indicates the lack of a fit, of the data to the von Mises distribution, except for Fish 3.1. Fig.3.19 shows the von Mises curve fitted to the data in 40° intervals.

Table 3.11 displays the results of a χ^2 test on the angular data, using the same 40° groupings as in the von Mises fit above. It can be seen that the mean direction of 330.5° for Fish 3.1 is upheld but those for Fish 3.2 and 2 were (just) accepted. An insignificant χ^2 value was obtained for Fish 4. Inspection of the table; indicates a general lack of significant modality except for Fish 3.1.

Figs. 3.20 and 3.21 show polar plots produced by program 'CIRCLES'. In the first set, the angles are displayed with line lengths proportional to step length and the defining circle is at a radius of 175.0 metres.

In Fish 2 there seems to be a tendency for the angles of turn between $310-345^{\circ}$ and also between 140° and 185° to be associated with larger step length whilst in other angular classes there is a lack of large step lengths. For Fish 3.1 there is a very strong tendency of angles between $325^{\circ}-360^{\circ}$ to contain large step lengths with certain sectors, e.g. $0-90^{\circ}$, only featuring small step lengths. Yet again, in Fish 3.2, the same pattern is repeated with $325^{\circ}-350^{\circ}$ being the sector with the largest step lengths. Examination of the plot for Fish 4 show a completely different picture; there is no apparent tendency to associate large step lengths with a particular angular grouping. In this case many sectors appear to be associated with rather small step lengths, e.g. $0-20^{\circ}$, $180-230^{\circ}$.

In the second set of polar diagrams, (Fig.3.21) the angles have no implied length component.

For Fish 2, the pattern is somewhat dispersed but reference to Table 3.11 makes it clear that certain sectors contribute more to the

total χ^2 and this is because they contain lower frequencies than expected, e.g. $0^\circ - 40^\circ$, $80^\circ - 120^\circ$, $120^\circ - 160^\circ$. For Fish 3.1 there is an obvious concentration into two groups; one around the mean vector and the other around 180° . Again there are several 'bare' areas hence the large χ^2 value and the significant z value (which may be erroneous because of the multimodality). Again some sectors show significantly low frequencies, e.g. $200^\circ - 240^\circ$, $40^\circ - 80^\circ$ and there is some evidence of clustering around 195° (the mean direction). The polar diagram for Fish 4 correlates with the insignificant χ^2 value, showing an almost continuous uniform distribution without any significant 'bare' areas, except for, possibly, $40^\circ - 80^\circ$.

Tytler et al. (1976) also made significant inferences about their fishes behaviour from examination of the distribution of the step length data. An attempt was, therefore, made to fit the observed data to the log-normal distribution found by their investigation. Program 'LOGNORM' was used to achieve this and the results are displayed in Fig. 3.22. The data ($\log_{10} l$) is shown as a histogram in Fig. 3.23 in 0.1 logunit steps. There is no good fit of the data to these curves at all. Reference to the figures shows that the fitted curve is several orders of magnitude more than the observed data.

Fig. 3.22

Theoretical log-normal curve fitted to step length data
for Fish 2-4.

Fig. 3.23

Observed log 10 step length data in 0.1 log units.

If this is compared with fig. 3.22, it will be seen
that there is no good fit of the observed data to
the fitted curve.

FIG. 3.22

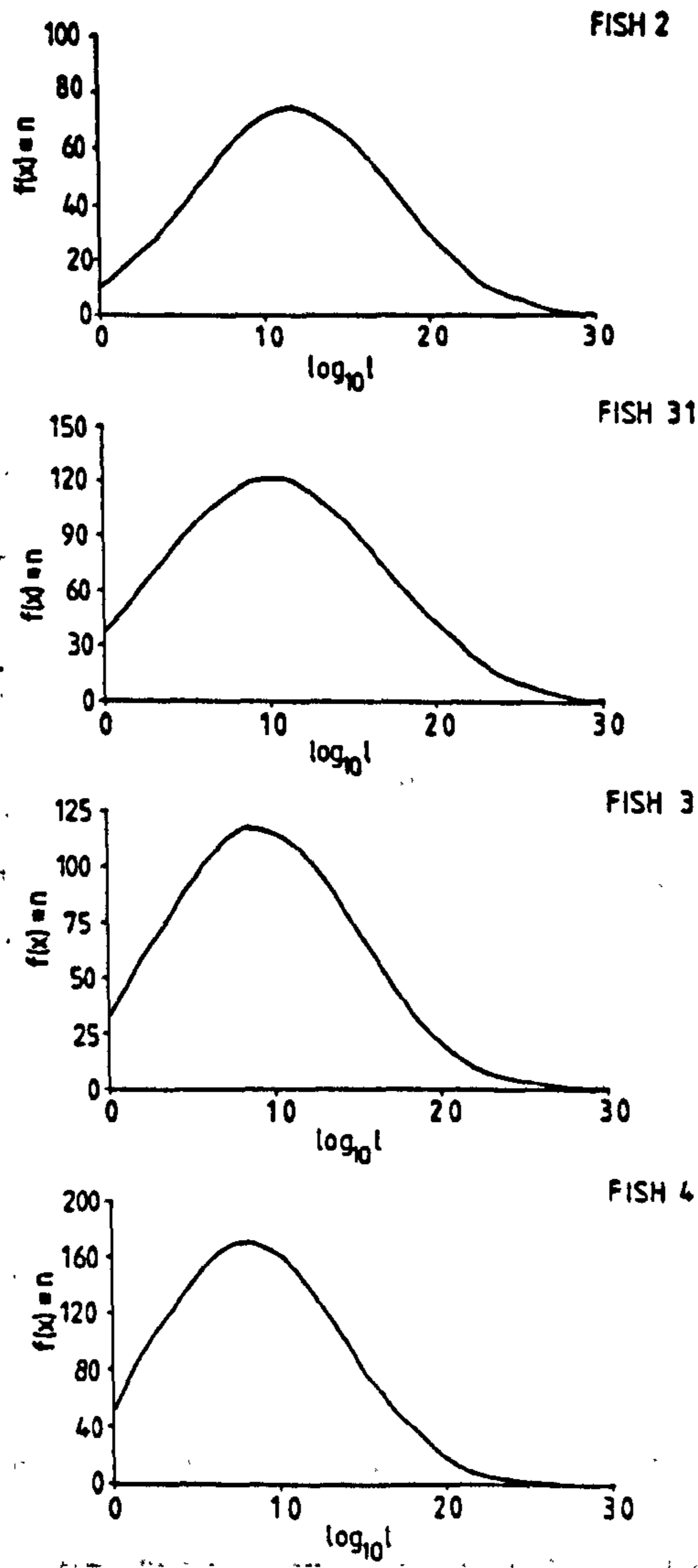
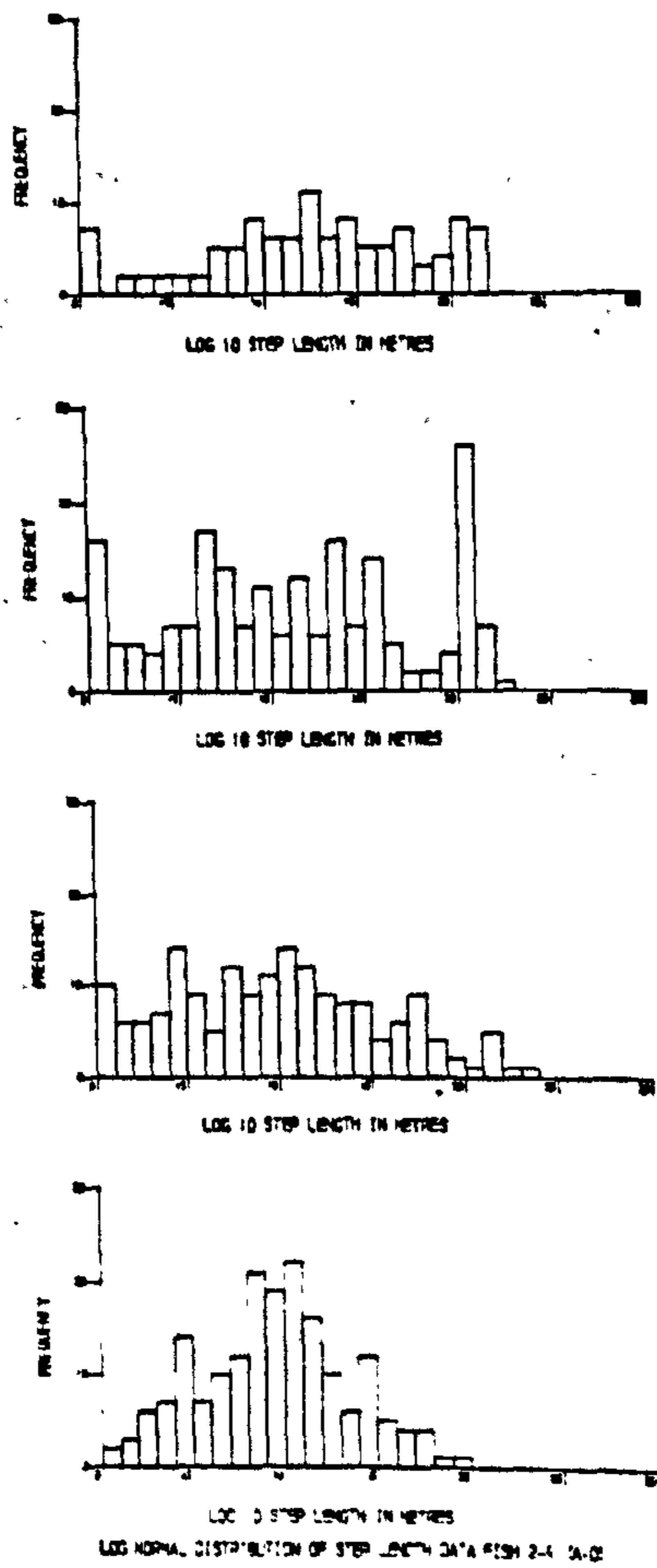


FIG. 3.23



Section III : BiotelemetryDISCUSSION III3.18 Feeding criteria

From section 3.23 it can be seen how well the ventilatory EMG could be telemetered in an analogue fashion by the latest make of transmitter. In the laboratory, the visual correlation of feeding acts with the telemetry signal was perfect. The characteristic signal pattern occurred after prey seizure and corresponds to Wankowski's (1977) manipulative phase in which a series of rapid mandibular, hyoid, opercular and branchial arch movements occur. This is the hyoid/palatine ratchet system described in other species by Western (1968) and McNeil Alexander (1974). In the trout, however, it is not strictly comparable because the higher Teleosts generally have pharyngeal teeth systems in which a certain amount of trituration is performed before deglutition (McNeil Alexander 1974). Wankowski notes that the movements after ingestion were of a lower amplitude than the initial capture manoeuvre and that the period of each movement was short and several repetitions could be dispersed over a period of 1 - 3 seconds. This occurrence was obvious from both the field and laboratory studies. In collaboration with Wankowski, some high speed photographs of Rainbow Trout feeding on pellets were taken (and subsequently published in his thesis). These confirmed the validity of the pattern herein described.

The laboratory work confirmed that the 'cough' cycle would not be confused with the feeding manoeuvre as their time courses were different. The periodic coughing described by Hughes and Adeney (1977) is said to occur in the Trout at a low frequency of less than one per minute although nothing is known of its occurrence in the wild. Hughes and Adeney note that coughing may be absent under certain forms of stress but increased by others (Hughes 1975). By listening to the tape recordings of EMG transmitters the human

observer is able to discriminate the cough quite readily. The sound of the transmitter output can be compared to listening to a railway carriage on old-style rails; coughs can be compared to a set of points whereas a feeding event is rather more like a junction.

Unfortunately, the trout has another respiratory manoeuvre akin to coughing, in which prey is rejected by 'spitting'. In the trout, but not the salmon, this is usually followed by one or more rejection-reingestion sequences (Wankowski, 1977). Priede (1977) shows the effect of this on his ECG recordings, but wrongly ascribes it to the deglutitive phase.

It is unknown how the feeding manoeuvres will vary in the wild with natural prey. Most of the previously cited references have used pelleted diets for their tests. It is possible that because of their shape, size and /or texture, their breakup in water, they may contribute to unnatural regimes of coughing or rejection. My own unpublished observations on Sarotherodon mossambicus shows that given two similar sized pellets, one hard and the other semi-moist, the fish seldom rejected the latter although the former was invariably rejected. Flake diets were invariably ingested in one attempt.

Although it is possible that feeding event data telemetered by the transmitter might give elevated numbers of events due to rejection - reingestion sequences, it was hoped that natural food would be more palatable to the fish. In any case, no allowance could be made for this in the analysis of the data tapes as it would prove extremely costly in time.

It proved impossible to categorise types of feeding by their EMG patterns. Elshoude-Oldenhove and Osse (1976) could only distinguish two types of feeding behaviour from EMG and cine recordings. Gorniak (1977) was able to distinguish different

feeding patterns, e.g. gnawing on pellet, chewing sunflower seed from the EMG patterns of the hamster. One can expect rather more larger repertoires from the mammal than the fish, though.

There is also the possibility that feeding patterns common in the wild are not covered by the admittedly small range of prey type provided by pelleted diets. For example, the ram feeding seen in the scombroid Euthynnus affinis might occur in trout should this be the method (as yet indetermined) by which large trout can assimilate quantities of Cladocera (Thorpe 1973). Roberts (1975) shows that Rainbow Trout do have the capability of ram-ventilation at least. If so, then the expected EMG would be composed of large silent periods from the a. mandibulae owing to the persistent mandibular abduction required.

An attempt was made to carry out a series of experiments similar to those of McNeil Alexander (1970) because some of the high speed photographs suggested that an element of suction might be present during feeding by trout. This conflicts with the statement by McNeil Alexander (1974) that fish without protrusible premaxillae (like the trout) are not adapted to sucking in their prey. Steelhead Trout (Salmo gairdneri gairdneri) were presented with a pellet containing a fine catheter connected to a pressure transducer. On feeding repetitive pressure changes similar to the EMG pattern were observed. Because of the pressure artefact introduced by bending of the fine, fluid-filled catheter (McNeil Alexander, 1970), the validity of the experiment was dubious and no further work was carried out on this aspect.

3.19 Cycles in Feeding and Ventilation.

The results presented in Fig. 3.9 clearly demonstrate the existence of cyclical feeding activity in the free-swimming Brown Trout. The sharp delimitation into peaks was most marked in Fish 4 and 3.1. In all cases, statistically significant greater feeding activity was found for the chosen peaks.

Landless (1976) found that demand-fed Rainbow Trout fed in bouts during which the individual feeds were taken at 4-8 minute intervals. He also found that the rate of feeding was not uniform within a bout but tended to accelerate at first then decline as satiation opposed it. Reference to the histograms showing the cumulative feeding intake for each fish shows that the segments during feeding peaks are often of this form, with a pronounced levelling off of the upper end. Much psychologically orientated work has been carried out in mammals upon the form of this satiation curve. McCleery (1977) found by fitting various curves to experimental data that the cumulative curve of intake against time is best fitted to a negative exponential curve: $\gamma_t = c - a \exp^{-kt}$, where c , a and k are constants. Many of the feeding peak cumulative curves are like this and sometimes show the initial 'warm-up' inflexion noted by McCleery. Little work of this sort appears to have been carried out on Fish. Brett (1971) and Ware (1972) illustrate this form of curve in their studies of Salmon and Trout satiation times. Elliott (1973) has predicted the satiation time according to a size dependent formula viz. $S_t = cW^d$ where W = the fishes weight and c and d are constants. For Trout of the sizes used in this investigation Elliott would indicate satiation times of circa 100 minutes at 10°C and ca. 180 + minutes at the highest temperature encountered in the field. The feeding peaks telemetered by the EMG transmitter were of considerably longer duration, generally >180 minutes. Landless (1976) notes that the satiation time is longer in demand fed Trout than when hand-fed; i.e. when the rate of food availability is entirely dependent on the

motivation of the fish. More will be said about this later, in Section 3.24

The relationships of the feeding peaks to each other is interesting as they tend to be between 6-8 hours apart. Adron et al. (1973) and Landless (1976) noted that 8 hour cycles of feeding were common in Rainbow Trout and they both suggest that these are related to stomach filling/evacuation rates. Brett and Higgs (1970), Elliott (1973), Windell et al. (1976), Thorpe (1977) and Persson (1979) have described exponential functions for evacuation rates in Freshwater fish. Tyler (1977) shows that, at least, in the Cod the gastric evacuation is not affected by swimming activity. There is, however, considerable variation in rates for various natural (Elliott, 1972) and artificial foods (Windell and Norris, 1969). An effect, well known in vertebrates, is the delay in gastric emptying induced by lipid intake. Insects, for example, vary in their fat body content and the same is true of the fat content of pelleted diets. Unfortunately, the diet of tagged fish is, and always will be, indeterminate. It would have been instructive to have sampled the stomach contents of the Airthrey Loch fish population in order to infer the most probable food items at the time of tracking. This was not possible due to political reasons and in any case, Bryan and Larkin (1972) have shown that individual fish may show a marked food specialization. The major items in Airthrey Loch for Trout are Planorbid and Limnaeid gastropods, Amphipod and Isopod Crustacea, Chironomidae. The only vertebrate prey are Sticklebacks (Gasterosteus aculeatus) and Toads. For most of these items over the temperature range 10-20°C, the gastric 50% evacuation time is between 8-12 hours for Brown Trout (Elliott, 1972).

These parameters may help explain the control of the feeding cycles detected in the telemetry experiments. Most of the laboratory based experiments in the literature have presented the food from a dispenser or else by hand. This somewhat simplifies the field situation in which additional degrees of difficulty in sighting, evaluating and acquiring prey

are introduced due to the prey behaviour and the amount of cover provided by its chosen habitation. Ware (1972) found that the rate of predation upon a prey species by Trout was inversely proportional to the complexity of the substrate. Ware also found dependence upon prey, size and density. In the wild one might expect satiation to take longer than in the laboratory because prey might be temporally or spatially 'difficult' for the Fish to acquire. In this context then, prey density is not equivalent to prey availability.

Published satiation times must therefore be regarded as tending towards maximal. The feeding pattern recorded by the EMG transmitter would appear to fit this hypothesis although there is apparently a large amount of random feeding between 'meals' occurring. Landless (1976) interprets feeding rhythms in terms of a positive feedback system released by food availability which is opposed by an antagonistic negative feedback due to the sensory input from gastric stretch receptors for which, surprisingly, no physiological correlates are available in Fish. Priede (1973) found that bilaterally vagotomised Trout cease feeding. In sectioning the cardiac branch he was also isolating the visceral branch which contains the main motor and sensory pathways to the stomach in fish with a defined morphological stomach (Nicol (1952), Barrington, (1957), Campbell (1973)). Paintal (1949) found that in the cat, afferent nerve traffic was proportional to gastric distension. Paintal's experiments, however, were rather naive in terms of present knowledge of nervous systems. Woods (1975) has shown that many different types of neuron may be seen in Auerbach's plexus of the intestine, based upon their discharge pattern. Vagotomy may have a very complex effect on the central interoception of satiation status particularly if coding is dependent on combinations of neuronal activity such as 'bursters', mechano-sensitive neurons, tonic type neurons, etc. Woods (1975).

Rozin and Mayer (1961 and 1964) show by operant conditioning methods in the Goldfish, feeding tends to be continuous with no clear clustering into bouts. Landless (1976) points out that the Goldfish is omnivorous and is also without a true storage stomach so that it is of no advantage for it to feed in bouts. This continuous, herbivore-like grazing is the reason why the carps have such an inordinate amount of red muscle in the myotome, in order to cope with the required sustained activity (Boddeke et al. 1959).

3.20 Ventilation Cycles

The data for ventilation rate does not show any evidence of cyclical change in contrast to the only telemetric work on this subject (Eriksson and Ulveland 1977) which demonstrated a diel rhythm of ventilatory activity, in the Pike Esox lucius. It is known that changes in the instantaneous ventilation rate of the trout are correlated with changes in swimming speed (Sutterlin, 1969). Marvin and Heath (1968) showed in the trout that ventilation rates were affected by environmental hypoxia. Down to 50% sat. ventilation rate increased then at lower PO_2 values, declined. Hughes and Umezawa (1968) showed in the Dragonet Callionymus lyra a simple tachypnoea in response to environmental hypoxia. It might have been expected in the field studies, that a correlation between swimming speed and ventilation would exist. The recovery from exercise induced tachypnoea in trout (and some other species) is quite rapid. To detect such short-term changes it would be necessary to employ a more frequent sampling rate preferably continuous counting as was used by Priede and Young (1977) for ECG rate.

No evidence of any diurnal changes in ventilation rate as described by Sparks et al. (1972), in the Bluegill Lepomis sp., were seen in the EMG records. From the tables of regression equations, it is evident that in Fish 3.1 and 4 there is a significant negative correlation of ventilation

rate with elapsed time though in Fish 3.2 an upward trend was seen. Such long term changes are reflections of arousal state (Laming and Savage 1978) and may simply reflect the recovery from the stress of handling, anaesthesia and surgery.

There are no truly significant correlations of ventilation rate with step length/swimming speed, angle of turn or feeding activity although some correlations were suggested, at low significance levels. Conclusions drawn from these may not be particularly valid owing to the low sample rate.

3.21 Ventilation rate

Apart from the possibility of missing acute occurrences of extreme values due to the sampling rate, the ventilation rates seen here seemed to confirm the generally low levels of metabolic expenditure now known to occur in lake-dwelling trout. From their study of heartrate telemetry of Brown Trout in Airthrey Loch, Priede and Young (1977) concluded that the time spent at heart rates = to a metabolic rate of 1.5 times resting was .20%. Because of the low levels of energy expenditure in movement they note that oxygen consumption over and above resting is mainly due to specific dynamic action. This does not necessarily imply (and was not evident from the field studies) that ventilation rate would increase after a large intake of food because the fish can modify its oxygen consumption by a variety of routes without necessarily increasing ventilation rate greatly. If a transmitter with continuous F.M. transmission had been available e.g. similar to Wardle and Kanwisher (1974), it would have been possible to draw some conclusions on ventilation stroke volume by direct integration as did Hughes and Ballintijn (1968) in the anaesthetized Dragonet.

The ventilation rates recorded in the field studies were at or below the values quoted for the resting Brown Trout (Sutterlin, 1969) of 45/min

at 8°C. There is an interesting species difference in that the ventilation rates quoted for the Rainbow Trout at normal ambient temperatures are usually 100/min. (Marvin and Heath 1968, Nomura et al. 1969, Ballintijn and Hughes 1965, Hughes 1975, Hughes and Adeney 1977).

The highest value recorded in the field was 70/min in Fish 4. Ventilation rates of this magnitude were only recorded in the laboratory from Brown Trout during anaesthetic recovery. They were, however, commonly seen in recovered Rainbow Trout.

3.22 Relationship of feeding and ventilatory activity to photoperiod.

In the telemetry work previously carried out on Airthrey Loch (Young et al. 1972, Holliday et al. 1973) a pattern of swimming activity with crepuscular peaks was typical but other, subsidiary peaks occurred at times dissociated from LD transitions. In the relationship of the feeding peaks to photoperiod, there is a similar pattern. All the EMG fish show peaks at or just after dawn and additionally around midday.

Because the seasonal rise in feeding rate and growth is seen to be paralleled by a seasonal increase in mean swimming speed (Holliday et al. 1973), it has always been assumed that the increased activity detected by 'conventional' tracking transmitters was related to the former. If so, then it would not be surprising to discover feeding activity during darkness because tagged fish are never absolutely still at night. From the field experiments it is seen that a considerable amount of feeding does occur at night. In all cases, except for Fish 2, a significantly higher proportion of food was taken in each five minute period during daylight though feeding was always detected during the night-time period. This was especially true of Fish 2 and 4. Traditionally, the Brown Trout has been considered as a diurnal animal; this undoubtedly stems from the angler's view of trout, although any competent poacher knows differently!

Elliott (1965) and Frost and Brown (1967) note the occurrence of night

feeding, especially in the hours immediately after sunset, from stomach contents and observation.

From experiments, Hoar (1942), whilst convinced that Salmon and Brook Trout were diurnal in feeding habit, nevertheless noted the capability to feed in darkness. Ali (1959) concluded that in Salmon fingerlings light had no appreciable effect on feeding rate and feeding persisted in DD conditions. Jenkins (1969) found by using marked prey (ants) that Brown and Rainbow Trout were capable of feeding at very low levels (starlight) of natural illumination. In both demand fed and natural feeding pattern analyses (Landless 1976, Thorpe 1973) there is a consistent occurrence of some 40% of the daily intake being taken in darkness. Landless found in his fish that the commencement of the major daily feeding peak was linked to sunset; he concludes that 'trout were potentially able to feed for 24 hours per day'. This would clearly be advantageous for a carnivore which has a geographical range from 70-30°N (latitude), (Frost & Brown, 1967). The same is true for the genera Thymallus, Salvelinus and Oncorhynchus. Jenkins (1969) found that the capture efficiency of trout declined at night and in the non-salmonid Menidia audens Elston & Bachen (1976) have shown in conditions requiring scotopic vision, a larger target size is taken in night feeding, possibly by silhouette recognition. Whilst generally considered as visual feeders, Brown and Rainbow Trout blinded by Diplostomulum infections can still feed adequately though x-radiographs of such fish often reveal stones etc in the stomach, indicating a lack of selectivity (own unpublished observations). Hoar (1942) observed that strong light would depress the midday feeding peak but his experiments took place in shallow waters. In the deeper, eutrophic conditions of Airthrey Loch, light at midday would be much attenuated. In Fish 4, for example, a vigorous feeding peak occurred at the time of highest light intensity measured on the photometer viz. 1300-1400 hours, which would imply that the fish was not deterred by the light. A

foraging trout will of necessity be searching near the substrate because 'the greater part of the trout's diet consists of animals which live on the bottom of stream, river or lake' (Frost and Brown 1967). This means that the fish will tend to remain at a near maximal depth in minimal light conditions, unless it is involved in zooplankton or emergent insect feeding. A factor not taken account of in previous work at Airthrey Loch is that the loch is always illuminated at night by powerful quartz-halogen security lighting. This may provide a measure of assistance to the fish particularly if they are taking prey by silhouette recognition. Indeed, trout are often observed 'rising' during the night on the loch although never in very great numbers.

With respect to ventilation; in some cases (Fish 3.1 and 3.2) the mean ventilation rates were, surprisingly, marginally higher at night. This may simply reflect the long term changes due to anaesthesia and thus the day/night differences may be spurious and dependent on time of release, anaesthesia. In any case, a more frequent sampling rate and the application of harmonic analysis technique might well reveal the presence of short-term ventilation rhythms superimposed upon the more obvious longer term ones. Such work has been fruitful for example, in Cancer and Carcinus heart rate studies (A. Bottoms, pers. comm.).

3.23 Relationship of Locomotor activity to photoperiod.

Unlike the feeding activity data, the locomotor activity pattern does not seem to fall into distinct groupings except in the case of Fish 2, in which there is some evidence of cyclical activity. Unfortunately, a large part of the record between 0720 - 1110 is missing and the total recording time is only 15.5 hours. This makes it difficult to draw conclusions about cyclical activity in the absence of extended recording periods.

Table 3.3 a to d, which displays the results of T-tests taken between day and night shows a significant diurnal pattern for Fish 2 and 4 with mean swimming speeds of 0.22 and 0.36 BL/S⁻¹ respectively. Whereas in Fish 3.1 and 3.2, this individual was distinctly nocturnal with mean swimming speeds at night of 0.68 and 0.38 BL/S⁻¹.

Over the last 20 years, a considerable amount of interest has evolved in the relatively new science of Biorhythmometry. Although certain long term rhythms e.g. seasonal variation in cardiac rate (Siegmund and Vogel, 1977) may be readily fitted to a mathematical function (in the latter case, a cosine function), in most cases the activity rhythms are nearly always circadian in period (circa = about, dian = day) apart from such obvious exceptions as tidally entrained rhythms (Gibson 1978). There are methods for analysing rhythms called periodogram analysis (Enright, 1965). Unfortunately they are quite complex and no ready made software is available. In this work, therefore, only overt differences between day and night will be considered.

Most of the studies into circadian rhythms in fish are laboratory based using light/infra-red/ultrasonic/magnetic/mechanical activity sensors. There is consequently a difficulty in relating such studies to the field situation because of the unnatural experimental conditions used. E.g. often 'open fields' are used when it is known that the presence of shelter can completely alter the activity pattern

(Edel, 1975). For convenience sake most workers tend to use small juvenile fish (in salmonids at least) and it is known (Byrne, 1971) that ontogenetic changes in activity patterns can occur. In fact, the only work on activity cycles in adult trout has been that of Chaston (1968 & 1969), Swift (1962, 1964) and the telemetry work from Airthrey Loch.

Eriksson (1978) points out that it is hazardous to assess whether a species, per se, is nocturnal or diurnal. In some cases this is clear-cut; e.g. the Sole Solea vulgaris (Kruuk, 1963) is a definitely dark-active animal. The salmonids are especially confusing. A selection of reports on activity patterns is given in Table 3.12 below.

Table 3.12 Reports of diurnal/nocturnal activity in Salmonid Fish.

Species	Diurnal	Nocturnal	Author
S. trutta		X	Chaston (1968, 1969)
S. trutta	X		Swift (1962, 1964)
S. salar	X	X	Richardson & McCleave (1974)
† S. trutta	X		Priede and Young (1977)
S. salar	X		Hirata (1973)
S. salar	X		Ali (1959)
S. salar		X	Varanelli and McCleave (1974)
O. kisutch	X		Byrne (1971)

There is a dualistic capability in the activity patterns of most salmonids (Eriksson, 1978) in their response to photoperiod. This is an individualistic property i.e. a sample of fish will show individuals phased one way or another. Richardson and McCleave (1974) were able to classify their fish into diurnal, nocturnal, crepuscular and aperiodic individuals. This is also reflected in seasonal changes in activity patterns (Swift, 1962, 1964; Chaston, 1968, Eriksson, 1978, Holliday et al. 1974). It has also been noted that marked crepuscular activity can result from the stress of displacement (Tytler et al. 1976).

It is concluded that the nocturnal/diurnal patterns of activity

observed in this investigation are representative insofar as they show the individual variation expected. Holliday et al. (1974) concluded from a series of 15 experiments in Airthrey Loch, that the activity patterns were not consistent which is to be expected considering that they used 15 individual trout over a complete seasonal range.

One fairly consistent conclusion derived from the literature has been that although dark or light may not be so important to the fish in terms of its activity cycles, the transition, particularly from light to dark seems to be especially important as a synchronizer or 'Zeitgeber'. Priede and Young (1977) analysed the transition period of \pm 90 minutes around dusk and dawn. They found a significant correlation between heart rate with elapsed time across the twilight periods and suggested a predawn anticipation by the fish of one hour. T-tests, Table 3.13 a-d and regressions (not displayed) were almost universally insignificant for all variables in this investigation. It is probable, therefore, that heart rate is a sensitive indicator of behavioural arousal, though not, as Priede and Young point out, a good indicator of feeding acts. It is known that certain drugs and chemicals e.g. D_2O have the property of perturbing circadian cycles (Palmer, 1976). It would have been interesting to have investigated how the effect of aminobenzoate anaesthesia might upset the established rhythm in the Trout. Interestingly, the reverse is also true; Yuwiler and Samuel (1974) have shown in Poecilia formosa that there is a circadian rhythm of susceptibility to MS-222 anaesthesia. This has also been shown for Pentobarbitone Sodium in the Rat (Simmons et al. 1974).

It can at least be said that the fish in this investigation demonstrated circadian activity cycles, be it nocturnal or diurnal. Priede (1978) in his reassessment of his 1977 data found that the best approximation to describe the diurnal changes in heart rate was that of a step function. This was not attempted in the case of the step length data

TABLE 3.13 a T - TESTS FOR DD TRANSITION FISH 2
 GROUP 1 = DAWN/DUSK +/- 90 MINS , GROUP 2 = REMAINDER.

	D1	D3	M1	M3	V1	AN1
MEAN (1)	28.84	0.26	3.50	1.40	47.80	214.55
S.E. (1)	6.08	0.06	0.52	0.21	2.11	26.39
MEAN (2)	35.83	0.33	2.58	1.04	45.25	188.72
S.E. (2)	4.23	0.04	0.23	0.09	3.33	11.79
T - VALUE	0.94	0.94	1.63	1.63	0.64	0.89
SIG (T)	0.1750	0.1765	0.0580	0.0585	0.2640	0.1895
F - VALUE	1.95	1.95	1.23	1.23	13.21	1.24
SIG (F)	0.0460	0.0445	0.2655	0.2650	0.0180	0.2465

TABLE 3.13 b T - TESTS FOR DD TRANSITION FISH 3.1
 GROUP 1 = DAWN/DUSK +/- 90 MINS , GROUP 2 = REMAINDER.

	D1	D3	M1	M3	V1	AN1
MEAN (1)	44.35	0.46	2.28	0.75	62.20	240.24
S.E. (1)	5.88	0.06	0.64	0.19	3.10	16.04
MEAN (2)	26.04	0.27	2.62	0.75	53.86	222.11
S.E. (2)	3.09	0.03	0.25	0.07	0.83	9.36
T - VALUE	2.76	2.75	0.07	0.07	3.67	0.98
SIG (T)	0.0035	0.0035	0.4720	0.4735	0.0005	0.1660
F - VALUE	1.22	1.22	3.10	3.10	4.64	1.02
SIG (F)	0.1880	0.1890	0.0000	0.0000	0.0005	0.4900

TABLE 3.13 c T - TESTS FOR DD TRANSITION FISH 3.2
 GROUP 1 = DAWN/DUSK +/- 90 MINS , GROUP 2 = REMAINDER.

	D1	D3	M1	M3	V1	AN1
MEAN (1)	36.34	0.37	2.39	0.97	52.33	220.78
S.E. (1)	7.92	0.08	0.51	0.21	3.37	21.92
MEAN (2)	17.34	0.18	2.47	1.00	48.48	171.10
S.E. (2)	2.33	0.02	0.25	0.10	1.21	7.98
T - VALUE	3.09	3.10	0.15	0.15	1.08	1.27
SIG (T)	0.0010	0.0010	0.4400	0.4395	0.1620	0.1665
F - VALUE	2.65	2.65	1.04	1.04	1.87	1.73
SIG (F)	0.0000	0.0000	0.4900	0.4905	0.1375	0.0185

TABLE 3.13 d T - TESTS FOR DD TRANSITION FISH 4
 GROUP 1 = DAWN/DUSK +/- 90 MINS , GROUP 2 = REMAINDER.

	D1	D3	M1	M3	V1	AN1
MEAN (1)	18.92	0.18	0.91	0.24	47.73	186.33
S.E. (1)	2.09	0.02	0.20	0.05	1.55	13.52
MEAN (2)	17.33	0.17	1.58	0.41	48.81	178.77
S.E. (2)	1.81	0.02	0.14	0.04	1.09	9.48
T - VALUE	0.56	0.54	2.73	2.73	0.59	0.46
SIG (T)	0.2885	0.2945	0.0035	0.0035	0.2810	0.3240
F - VALUE	1.77	1.77	1.56	1.57	1.00	1.16
SIG (F)	0.0085	0.0085	0.0175	0.0170	0.2235	0.2620

because of the lack of extended recording periods in this investigation.

The relationship of step length to other parameters will be discussed later.

3.24 Relationship of Locomotor Activity to Feeding/Ventilatory activity

Overall, no case in this work showed a correlation between locomotor and feeding activity, except for fish 3.1 which was later demonstrated by partial correlation to be a spurious relationship. Mean step lengths during feeding peaks were not significantly different from those of the intervals except in fish 3.1. In this case it was attributed to the unusual pattern of long step lengths during the night period when little feeding activity was occurring.

As mentioned in the main introduction, there is a classic accepted pattern in the food searching strategy of predatory animals. This is the concept of 'area restricted searching' (Beukema, 1968; Thomas, 1974; Zach and Falls 1977). This implies a decrease in step length between turns and an increase in the frequency of turning leading to an increase of tortuosness for which Thomas was able to compute a tortuosity index. The adaptative significance of this manoeuvre is that it maintains the predator's position in the vicinity of the prey aggregation. In the Airthrey Loch system there is a lack of precision in measuring small changes in position because of a) the inherent accuracy truncation of $\pm 2m$ (Young et al. 1975) and b) the five minute sampling routine introduces another degree of coarseness. I consider that an attempt to make a direct comparison with the work of Beukema, Thomas etc. might be misleading. The former studies were laboratory based in small scale aquaria. Thus the fish tracking experiments in the field represent both a temporal and spatial scale many orders of magnitude greater than those of Beukema (1968), Ware (1973) and Thomas (1974) who were working in units of centimetres and seconds. The resolution and the reaction time of the

Airthrey Loch system could never approach this level of efficiency.

By measuring the track of the fish at 600s intervals it is introducing a large component of uncertainty into the data; for example, in such a period, a 30cm. Brown Trout swimming at 2 BL/S^{-1} could make a maximum excursion of 400 metres. If the fish does not swim in a straight line then a considerable underestimate of activity will always result. Even with a fully automated system using hyperbolic navigation (LORAN) principles, it is unlikely, especially in shallow water like Airthrey Loch, that a position fixing accuracy of $\pm .2\text{m}$. will ever be achieved (Hawkins et al. 1974). Where such a system scores over the Airthrey Loch system is in that sample rates of 1 per second can be achieved, which would give more realistic data when contemplating this kind of study.

I believe that locomotor activity detected in the EMG experiments, in relation to feeding activity, would better be regarded as 'strategy' whilst the small scale patterns seen in the laboratory studies are therefore 'tactics'. There is certainly no evidence anywhere to suggest that the movements of a Brown Trout in a 250 x 250 metre loch are simply 'scaled-up' versions of those seen in a 5 x 5 metre tank by Goldfish.

Because of the constraints imposed by the tracking system it would be more prudent to consider the differences in gross movement pattern during feeding peaks. It is known, however, that fish do not move about at random but have an organised, internally motivated, exploratory behaviour, even in an open field (Kleerekoper et al. 1974). In the laboratory it is possible to observe extra behavioural indicators of motivation such as the 'nose down attitude' of Thomas (1974) or aggressive intentions (Chizar et al. 1975) and so activity patterns can be subdivided. In the field telemetry situation, however, it is not possible to distinguish food-orientated searching behaviour from that of non-specific exploratory activity.

In Birds and Fish, food searching is nearly always accompanied by a decrease in speed in order to increase the search scan effectiveness (Zach and Falls 1977; Thomas, 1974). Tytler et al. (1976) concluded that the fish sensory system could not cope with searching speeds of over 0.5 BL/S^{-1} . In the field the mean swimming speed is in any case considerably lower than this and owing to the time scale and the sampling period adopted, the decrease in mean speed might not be easily detected by the telemetry system. The median swimming speed is considerably lower than the mean and is in fact probably a better measure of central tendency because of the skewed distributions experienced here. Taking a median swimming velocity of $.1 \text{ BL/S}^{-1}$ for a hypothetical fish; if it reduced its speed to 0.05 BL/S^{-1} then the difference between the two distances covered is less than the system resolution of $\pm 2\text{m}$.

In any case, Ware (1972) and Thomas (1974) give the time constant for the decline of food-orientated exploratory behaviour following a prey rejection, to be in the order of $20\text{-}30\text{S}^{-1}$. In the wild, particularly if the fish has a specific search image, where prey may not always be exposed, unsuccessful captures would tend to be frequent and given the time scale involved the switch from food-seeking to other behaviour, as expressed in terms of locomotor activity, might well be outside the handling time of the tracking system.

It is now evident, with the benefit of hindsight, that the kind of study attempted here is ~~is much more~~ ineffective if automatic data storage or even on-line processing of frequently obtained data are used. This has always been intended at Airthrey Loch although cost is, as always, a barrier. It is probable that for the moment at least, that the most fruitful studies will come from telemetry of physical parameters e.g. preferred temperature, light, depth, etc. which are much easier to encode than biological signals.

With respect to ventilatory behaviour; significant correlations

between step length were obtained for Fish 2 and 3.1 however because of the infrequent sampling of ventilation rate, these values are probably spurious and again instantaneous rate measurement is desirable. Only in the case of Fish 3.1 were significantly different (smaller in feeding peaks) ventilation rates found. No partial correlations were calculated but it is probable that they share a common variation with time. The time course of the return of ventilation rate back to resting levels following vigorous swimming gives a $t_{1/2}$ of 5-10 mins (data from Sutterlin 1969) and therefore would easily be missed on a 30 min. sampling schedule.

3.25 Relationship of Angle of Turn to Feeding Activity

In the Loch-based experiments described in this work, the angle of turn is open to some similar criticisms to those for the step length. In all the previously mentioned laboratory based studies the angle of turn measured, is that actually taken by the animal at a time of its own volition. In the EMG experiments in Airthrey Loch the angle of turn was that between successive positional fixes which themselves were determined at arbitrary intervals decided by the experimenter. Consequently no inferences can be made about the rate of turning which is often affected following prey location.

The angle of turn related to feeding activity was (except for Fish 3.1) not correlated and this is obvious from the regression plots. The T-test tables, 3.4a, 3.4b, 3.4c and 3.4d show that in all cases, the angle of turn is significantly smaller during feeding peaks suggesting a preference for right-handed turns. Such behaviour has been noted in insects and Fish (Banks 1957, cited in Thomas 1974; Kleerekoper et al. 1970). Kleerekoper et al. (1973) have described in Goldfish and Sharks, a movement pattern classified as a logarithmic spiral which occurs in a variety of experimental conditions. This implies a preference for a turn bias though, again, the scale of the spiral was less than 5 metres across.

More will be said later of the angle of turn in relation to feeding activity, when the circular statistics are reviewed.

3.26 Relationship of Angle of Turn to Photoperiod/Time

In the case of Fish 3.1/3.2 significant differences between the angle of turn during day and night occurred. This is interesting because it has been shown in all cases that step length and angle of turn are strongly positive correlated (see regression plots and statistics). In Fish 2 and 4 the increased step length by day is not mirrored by a significantly larger angle of turn. If the former correlation is universal then from previous work at Airthrey Loch (Young et al. 1972, Holliday et al. 1972) it would be fairly safe to assume larger mean angles of turn by day. Unfortunately Tytler et al. (1976) were not interested in diurnal changes in angle of turn and so there is no published work with which to compare this aspect.

In a larger water body (L. Mendota), Hasler et al. (1969) related course changes in ultrasonically tagged White Bass (Roccus chrysops) to current, wind and sun directions. No attempt has been made to process the meteorological data resulting from Fish 4, though it may be completed elsewhere. The original intention was to have an on-line range of physical sensors including wave amplitude, thermocline measurements etc. Regretfully this has not materialised.

3.27 Relationship of Movement Pattern to Photoperiod and Feeding Activity

Tytler et al. (1976) have noted that when applied to the movements of Fish in small bodies of water, track diagrams can become confusing and they considered in this case that statistical analytic methods were superior. This is true, but for example, in the case of the analysis of step length pattern and angle of turn, this will not always be a good measure of linear displacement. If it was stated that for a period the

mean step length was significantly larger than it might be assumed that the fish was covering large ground distances. If the angle of turn was around 180° then the fish would be covering only a narrow area of the Loch. Conversely, depending on the angle of turn pattern, large linear displacements could result from the cumulation of many small step lengths. In this context then, both statistics and intuitive pattern recognition are both valid tools in fish-tracking.

Examination of the plot sets for the various experiments reveals certain tendencies not evident from the statistics. It must be borne in mind that at the position of Airthrey Loch ($56^{\circ}09' N$, $3^{\circ} 55' W$) daylight can be prolonged in midsummer and in June there may be five hours of darkness. Fish 2 shows distinctly different movement patterns by day; the pattern is mainly of wide ranging movements showing dispersion over the southern half of the Loch. Unfortunately part of the record between 0720-0930 is missing but the remainder is fairly complete. By night, a less 'open' pattern occurs and the mean angle of turn is lower indicating a preference for right-handed turns. Noticeable are the near-reciprocal courses, quite often extending over long distances. In Fish 4 there is a similar pattern with again a greater mean step length by day and less of the crossing and back-tracking seen at night. Fish 3.1 on the other hand had a nocturnal activity pattern with considerably larger mean step lengths by night despite the appearance of the track diagram which shows some apparently large steps which are due to loss of data between fixes. Even so, one can see a tendency for a greater linear displacement by night. Again, this tendency repeats itself in Fish 3.2 although the night-time period is truncated by some 90 minutes owing to loss of signal. The day-time mean step length is smaller and the fish had made one large directed movement parallel to the south shore of the Loch and had apparently started

to return when tracking was terminated.

During feeding peaks, there is a common pattern of movement in all the experiments. There are sites around which complicated intersecting movements occur from which the fish eventually moves away from, often in a direct line. This generally fits in with the theory of 'area restricted searching' and the notion that a predator improves his overall efficiency by minimising its losses i.e. moving away from unprofitable areas. Thomas (1974) noted that in the Stickleback, there was a great reluctance by the fish to revisit areas previously rejected. Kleerekoper et al. (1970) on the other hand in examining non-specific movement in the Goldfish, found a tendency to revisit a site. Possibly the EMG Fish may show elements of both types. During feeding peaks there is a distinct change in the angle of turn with a preference for right-handed turns. This might be taken to be the large-scale manifestation of the increase in small-scale tortuosity predicted by Ware's (1972) model and the observations of Beukema (1968) and Thomas (1974) on the Stickleback. It will be interesting to see if the logarithmic spiral model can be fitted to the data from the EMG fish.

Because of the lack of extended recordings, only in the case of Fish 4 was it possible to consider the fishes behaviour in the intervals between feeding peaks, as a control. In this case there are some movement patterns which are similar to those of the feeding peaks. This might suggest that they are in fact part of the general exploratory behaviour of the Trout. Reference to a plot showing the distribution of fixes for which the preceding feeding activity was 1+ showed that, in many cases, these groupings were arbitrarily excluded by the choice of 'peaks'. For example, in Fish 4 there is a cluster of points at 2,7 [SAG] during the interval 14.35-2025; this contains a fair number of feeding acts. (See Appendix III) Clearly, in the wild the natural difficulty in finding food may mean that the Trout may not always be able to satiate

itself, in true predator-style, in one session. Elliott (1973) found that intermittent feeding often continued after the main bout had ended, particularly at the higher temperatures. Landless (1976) has shown in demand-fed Rainbow Trout that there is a preference to feed at 4-8 minute intervals which conflicts with Ware's (1972) maximum attack rate of $0.67/S^{-1}$. The maximum rate recorded in this study was equivalent to an attack rate of $0.056/S^{-1}$, which is almost exactly at the level at which Ware noted a decline in food-orientated search behaviour. Brett (1971) has indicated that 'appetite' of Sockeye Salmon is increased by deprivation and Ware's fish had been starved for 48 hours prior to use. The fish in the EMG experiments, by being released 24 hours previously, would have had time to feed after the deprivation induced by captivity. Tytler (pers. comm.) has noted that caged fish soon learn to feed on invertebrate drift, etc. and the author has personally observed feeding behaviour in such a caged fish.

In contemplating further biotelemetric studies of this nature, it is obvious that the temporal distribution of individually detected feeding events needs to be recorded if this is to be correlated with other parameters. It would be necessary to give careful thought to exactly how the data is to be acquired and managed and also to investigate correlations with physical functions.

The limiting factors on telemetry for small fish are the transmitter size/weight and battery capacity (which are of course related). Hopefully electronic technology will continue to progress and it might become possible to record directly, small movements of fish using techniques already used by Ferrel et al. (1973) in large Blue Sharks, Prionace glauca. Possibilities include multichannel coded telemetry using miniature compasses with Hall-effect sensors or a drum of graded photographic strip used to modulate the light from a 'Betelight' source onto a photo-diode which would then be used to telemeter the fishes' compass direction. McKay (1974) has already made a telemetering

dynamometer, small enough to be affixed to the back of a cockroach so this is not outwith the bounds of possibility for fish. Thus by applying the principles of inertial navigation it should be possible to build up a more detailed picture of the fishes behaviour, including the measurement of accelerations in three planes, than hitherto possible by traditional position fixing.

3.28 Comparison of Step Length data

The distribution of the step length data for the four experiments is very interesting. They are all strongly positive-skewed indicating a preference for short step lengths. In such a situation, the median value is often more reliable than the mean (Meddis, 1975). As expected, the median step lengths are all < 20 metres. Other workers have noted the same; Kleerekoper et al. (1970) and Tytler et al. (1976) both show similar skewed distributions for step lengths though their scales are different by a factor of X1000. Winter et al. have shown by telemetry in the field that in Largemouth Bass, (Micropterus salmoides), the mean activity radii from the geometric activity centre form a positively skewed distribution with a mean of 30 metres and a mode of 20 metres. The mean step lengths from the EMG fish exhibit rather low values when compared with Tytler et al.'s work from the same loch. It will be noted that the values are of the same range as their displaced fish (Table 3.14). It was also not possible to obtain a good fit of the data to the log-normal distribution (see : Figs. 3.22-23). It is concluded, and also supported by the angular data distribution, that the EMG fish show symptoms of being 'displaced'. Because the fish were carefully returned to their home area, the reason probably lies in the more severe surgery/anaesthesia and/or the size of the transmitter. This might result in larger than normal step lengths which would tend to shift the mean to the right. The phenomenon of post-release hyperactivity is wellknown (Holliday et al. 1974); possibly the EMG

Table 3.14

Step Length Data compared

	\underline{n}	\underline{l}	$\underline{\delta l}$	$\underline{\log_{10}(l)}$	$\underline{\delta \log_{10}(l)}$
Fish 2	113	32.315	36.774	1.184	0.607
Fish 3.1	208	28.725	38.506	1.1014	0.686
Fish 3.2	176	19.705	31.137	0.910	0.595
Fish 4	192	17.836	19.557	1.046	0.432
Pooled 2 - 4	659	25.067	32.758	1.071	0.563

transmitter and procedures prolong this further than the 'standard' 24 hour recovery period. The previous work at Airthrey Loch must be considered the best baseline for telemetric behavioural studies on Trout. It is unlikely that an effective tracking transmitter can be made any smaller without compromising transmitter life. It is not economic (and may never be) to construct integrated circuits for biotelemetry (Mitson, 1978). (Interestingly, all the currently available VHF-FM radio biotelemetry transmitters available commercially for mammalian and human studies are fabricated from 'potted' discrete components).

The Stirling Mk III transmitter is probably the smallest in the world capable of giving 14 days life. A 'half-size' version (Mk V) was made but life was reduced to 3 days. Because of the apparent derangement of the step length pattern previously mentioned, it is suggested that any future work with relatively large transmitters in small fish should have sufficient longevity to be able to detect recovery effects and that movement pattern parameters e.g. step length are carefully monitored to this end.

3.29 Relationship of Spatial Position to Environment.

The fish in the EMG experiment show signs of orientation to the underwater topography. In all cases there is a preference for water over 1 metre in depth. The frequent occurrence of a Trout in water of a particular depth may be taken to infer a preference for that depth because lake-dwelling Trout tend to move just above the substrate. The design of a sufficiently small depth-sensing transmitter has yet to be achieved so that no directly measured results are available for comparison. It is known in small Sockeye Salmon (Oncorhynchus nerka) that diel vertical migratory movements occur, related to a circadian shift in temperature preference from 6°C by day to 16°C by night (McDonald, 1973). In the lake dwelling fish vertical migration of prey

e.g. Zooplankton may lead to apparent vertical migration. In the parallel situation in the sea, vertically migrating demersal and pelagic fish (Blaxter, 1970) may be controlled by light intensity with preference for remaining in 'isolumes' (Blaxter, 1976). Fish have been shown to exhibit (experimentally) behavioural thermoregulation which is a diel rhythm in the Goldfish (Reynolds et al., 1978). The preference for a particular temperature regime was shown to be at a time which maximised gonadal and somatic growth. Activity was never related to temperature. Another factor which has never been considered before is that fish have also been shown to show behavioural pyrexia following inoculation even with killed pathogenic bacteria (Reynolds et al. 1975). It is known that opportunist invasion of tagging lesions by bacteria and mycotic fungi occurs very soon after the surgery (Roberts et al. 1973). There is always the possibility that the Trout's behaviour subsequent to tagging is affected by this. The pelagic Albacore, Thunnus alalunga, (Lauris et al. 1977) has been shown, using ultrasonic tracking, to have a distinct thermal preference. In Airthrey Loch there may be thermal preference (or avoidance) occurring. The loch is too shallow to stratify but the shallow margins can become 10°C or more warmer than the deeper water on hot days. Fish 2 and 4 were more frequent visitors to the shallows possibly because of the cooler temperatures generally $\leq 10^{\circ}$ experienced in September/November (see Table 3.1).

Carlander and Cleary (1949) found differential activity at different depths in a wide range of freshwater Teleosts. Siegmund (1969) has shown in Perch and Tench that during 'rest' periods they are positioned or near to the substrate and therefore in these species no vertical migration occurs though 'sleeping' on the bottom might be a preference for cooler water.

It is unlikely that the handling of the experimental Fish has

disrupted any presumed thermobehavioural activity because great care was taken to avoid heat shock. Beitinger (1974) has shown in the Bluegill that sublethal thermal shock had no significant behavioural effects although the physiological effects (Wedermayer, 1973) may be more prolonged.

Yet another factor which may influence the movement pattern is the presence of boundaries, physical and territorial. The latter are impossible to detect by telemetry but Kleerekoper et al. (1970) found that the effect of the non-tactile perception of tank walls by Goldfish was to decrease the frequency of turning leading to a tendency to move parallel, but some distance away from the boundary. Thomas (1974) also noted the effect of a physical barrier in the movement pattern of his Sticklebacks. From Fig. 3.5 it will be seen that if one considers Airthrey Loch as a large fish tank, the 'walls' are sharply defined. In all the experimental fish movements tend to be made parallel to one or more of the shores and where the west shore 'shelf' juts out, a significant effect is seen in the avoidance of this area. Tytler et al. (1976) also noted this phenomenon in other tagged Brown Trout. The shallower water may have different benthic macrophytes which the fish either perceives as a boundary or has a preference for those of the deeper water. Examination of the data of Young et al. (1972) and Holliday et al. (1974) discloses that the Fish used in their experiments (N= 17) which were of a similar size class to the EMG fish had depth preferenda similar to the fish in this work. Holt et al. (1977) found in Walleye, Stizostedion vitreum and also Johnsen and Hasler (1977) working on Carp, Cyprinus carpio, found using radio and ultrasonic telemetry that both species, in shallow water, had movement patterns largely governed by the underwater topography. Also using telemetry, Winter et al. (1977) found that in the Largemouth Bass, Micropterus salmoides home ranges were in shallow waters and were elongated parallel

to a shoreline. The Trout is a visual animal and has been noted to orientate by features of the environment. These need not necessarily be underwater owing to the 'window' caused by refraction at the air/water interface (Frost and Brown 1967).

The hydrophone stations themselves provide convenient 'landmarks' and the subjective impression is that Trout may often use these as navigational turning points. They may also form a rich feeding area with benthos on the supports and on the slight mound of mud which builds up around each station. During ordinary tracking experiments the Fish would frequently have the annoying habit of sitting directly at one of the stations, rendering position fixing impossible because of swamping by the near field effect.

No investigation has been made here of the relationship of movement to sun compass orientation or sub-surface light waves (Hasler et al. 1969; Blaxter, 1970) as this will be done elsewhere.

3.30 Relationship of Feeding Activity to Metabolic Requirements

There is a generally accepted maintenance energy requirement for a standard 0.5 kg Trout of 40 kJ/day (Morgan, 1974) which may increase seasonally to 50 kJ/day (Thorpe, 1974). Priede and Young (1977) have calculated the number of individuals, of average weight for the species, necessary to make up a 40kJ diet using the commonest food items in Airthrey Loch. The EMG fish were all of around 0.5kg weight and the levels of feeding detected were of the order of magnitude required for prey species such as Limnaea sp., Gammarus sp., Asellus sp. It must be remembered, however, that only in Fish 4 was continuous monitoring of feeding activity undertaken and so the actual total recorded must be much greater in Fish 2/3 because of the alternate sampling. The level of feeding activity does not suggest that Sticklebacks are the prey because relatively few would be required to satisfy the maintenance

ration. Elliott (1973) concludes that Brown Trout are capable of consuming their daily maintenance ration in one meal. He also noted the tendency to regulate intake calorifically around the maintenance level. This has also been noted in Goldfish by Rozin and Mayer (1961). However, Elliott's work (and that of Ware (1972)) was based on a relatively uncomplicated substrate and the ability of prey species to conceal themselves under stones (e.g. Asellus) undoubtedly prolongs the satiation time in the wild.

3.31 Circular Statistics

Tytler et al. (1976) at the time of completing their paper had only just commenced the investigation of the linear-circular correlation coefficient (Their results : $R_{l,\theta} = 0.23$ to 33 d.f. for which I calculate $t_{n-2} = 1.32$ which is not significant). In the present experiments, with the exception of Fish 4 a significant linear-circular correlation was obtained between angle of turn and step length. This is interesting as all the other forms of correlation and regression gave highly significant results for Fish 4, for which I am unable at present, to offer an explanation. Similarly, the $R_{l,\theta}$ values for Feed Total/Angle of Turn were only significant in Fish 3.1 and 4 whereas the common zero-order coefficients were only significant for Fish 3.1.

Because the angle of turn as obtained here is a relative measure this implies that the larger step lengths are associated with turns to the left. Because the g_1^0 values are close to zero and most of the angular distributions appear to be uniform then mean (\bar{X}) values are probably fairly representative.

During feeding peaks, the mean angle of turn was always less than 180.0°

(See Table 3.4 a to d for data). This means that feeding peaks are associated with right-handed turning behaviour in all cases. Kleereker et al. (1973) found that in a sample of normal Goldfish had equal numbers of dextral/sinistral individuals although when the left nares were occluded, 70% became dextral. Kleereker et al. (1970) note in Goldfish that angular bias or 'handedness' is common but was constant whereas in some other species temporal changes occurred. With reference to Table 3.11; apart from Fish 3.1 which has some unusual behaviour initially, the net interpretation is that there is not a great deal of departure from the circular uniform distribution. Thus any angular bias in time must be of biological significance. Table 3.15 shows that, excepting Fish 3.1, over the whole of each tracking period, the numbers of right or left turns are not significantly different, though the left/right ratio was always > 1 . The feeding peaks are significantly dextral or reciprocal in angular movement (Fish 4 $\equiv 180.0^{\circ}$). The functional significance of such behaviour may lie in leading to 'area restricted searching'. If it is of the log spiral form as per Kleereker et al. (1973) then it would by definition lead to an infinitely decreasing search area, until broken off. This type of pattern is nicely described in terms of a mathematical model of a homing missile:= 'Any target-seeking device which approaches the target in a direct pathway which, at all times, deviates with a constant angle from a straight line connecting the vehicle with the target, will describe a logarithmic spiral around the target'.

The only systems presently available for monitoring the movements of adult trout-sized freshwater fish in a reasonable area to a high degree of precision are those of Kleereker in Texas, U.S.A., and the Airthrey Loch System. LKB of Sweden market a sophisticated apparatus known as ANIMEX which is capable of measuring very fine movement patterns of up to human sized animals but has not yet to my knowledge been used

Table 3.15

Analysis of Turning Behaviour on a Right/Left Basis

	Fish 2	Fish 3.1	Fish 3.2	Fish 4
No. of left turns	59	128	89	98
No. of right turns	47	67	76	93
χ^2 right/left	1.36	18.99	1.02	0.14
Sig. (χ^2)	N.S.	0.001	N.S.	N.S.
Right/left ratio	0.79	0.52	0.85	0.95
Left/right ratio	1.26	1.91	1.17	1.06
Median angle	200.00	223.00	186.00	183.75
Mean angle	194.24	227.00	196.80	181.04

in fish studies, it is very expensive and is really designed for the behavioural testing of CNS-active drugs in small mammals.

Nothing is known, therefore, of the angular behaviour during food searching by trout; Kleerekoper et al. have chosen to concentrate on the Goldfish and some Selachians both of which locate food by olfaction initially. The Trout is a visual feeder (Ware, 1972; Wankowski, 1977) and its turning behaviour would not necessarily be similar to the Goldfish or Shark. Unfortunately, although working on a visual feeder, the Stickleback (Gasterosteus aculeatus) Thomas (1974) chose to measure linear displacement rather than angular transition.

The mean angles of turn \bar{X}_0 found here were of the same range as found by Tytler et al. (1976). Their native fish had means \bar{X}_0 of around 180° and there was also a close fit to the vonMises distribution. In the present work this was not so and the fish all had angular distributions bearing a striking resemblance to those of Tytler's control displaced fish but not to those of his anosmic displaced fish (see Fig. 3.19). This contrasts with Kleerekoper et al. (1970) who found modes in the $0-10^\circ$ classes (on a left-right turn basis). I believe this to be a function of the very high resolution of their system, which is evident from the detail shown in their tracking plots. In Airthrey Loch we are really approximating the general cumulative direction formed by many angles of turn which interact to form a result. Kleerekoper has observed an angle compensation system in Fish (q.v.) in which the expression $(\Sigma_\theta \text{ left} - \Sigma_\theta \text{ right})$ is approximately zero. This is evident in the trout data. The close similarity of the angular distributions of the EMG fish to Tytler's displaced fish strongly suggests that the locomotor behaviour of the former is not normal, possibly due to the tagging procedures. Priede and Young (1977) and Priede (1978) have taken the 24 hour recovery period as 'standard' whereas Holliday et al. (1974) thought that effects might be detectable up to 48 hours

post-recovery. The exponential clearance of anaesthetic from adipose tissue may possibly be causing atypical locomotor behaviour, which is supported by the regressions of ventilation rate with time. Also, the EMG transmitter is larger cross-sectional area (but of lower apparent weight) than the previously used version, though its size is unlikely to seriously affect swimming performance at the low speeds seen in the field (Priede and Young, 1977). McCleave and Stred (1975) noted a significant decline in swimming performance with a rather less streamlined transmitter than used in the present work. They did, however, work on smaller fish (Salmon smolts) and in fact recommended the type of transmitter attachment favoured at Stirling.

It will be recalled that Fish in this study had their punctures infiltrated with a long acting local anaesthetic. This was done because in the laboratory, large Rainbow Trout equipped with the EMG transmitter were shown, using CCTV, to have a preference for right turns i.e. on the opposite side from the transmitter. In the laboratory, the 'Proctocaine' surrogate abolished this tendency though the long-term uptake and clearance of the drug are not known. The Procaine HCl component would not have any appreciable CNS depressant effects but the effects of the butyl-p-aminobenzoate and benzyl alcohol components are unknown. My view is that all might contribute but consider that the greatest contribution comes the larger and undoubtedly less comfortable transmitter encapsulation which must exert considerably more pressure on the fishes skin. No such effects were noted with the original 'soft', but less reliable packaging.

PLATES SECTION I : HISTOLOGY & ANATOMY.

Plate I.1

T.S. of Brown Trout m. adductor mandibulae. Zone of red fibres is tinted yellow. 0 = outer edge of muscle.

Scale bar = 1 mm

Plate I.2

T.S. of Brown Trout m. adductor mandibulae taken near the mandible to contrast with the previous section. Note that at this level the muscle is wholly mosaic, the remainder being largely tendon (1) and connective tissue (2). Scale bar = 500 μ m.

0 = outside edge of muscle.

Plate I.3

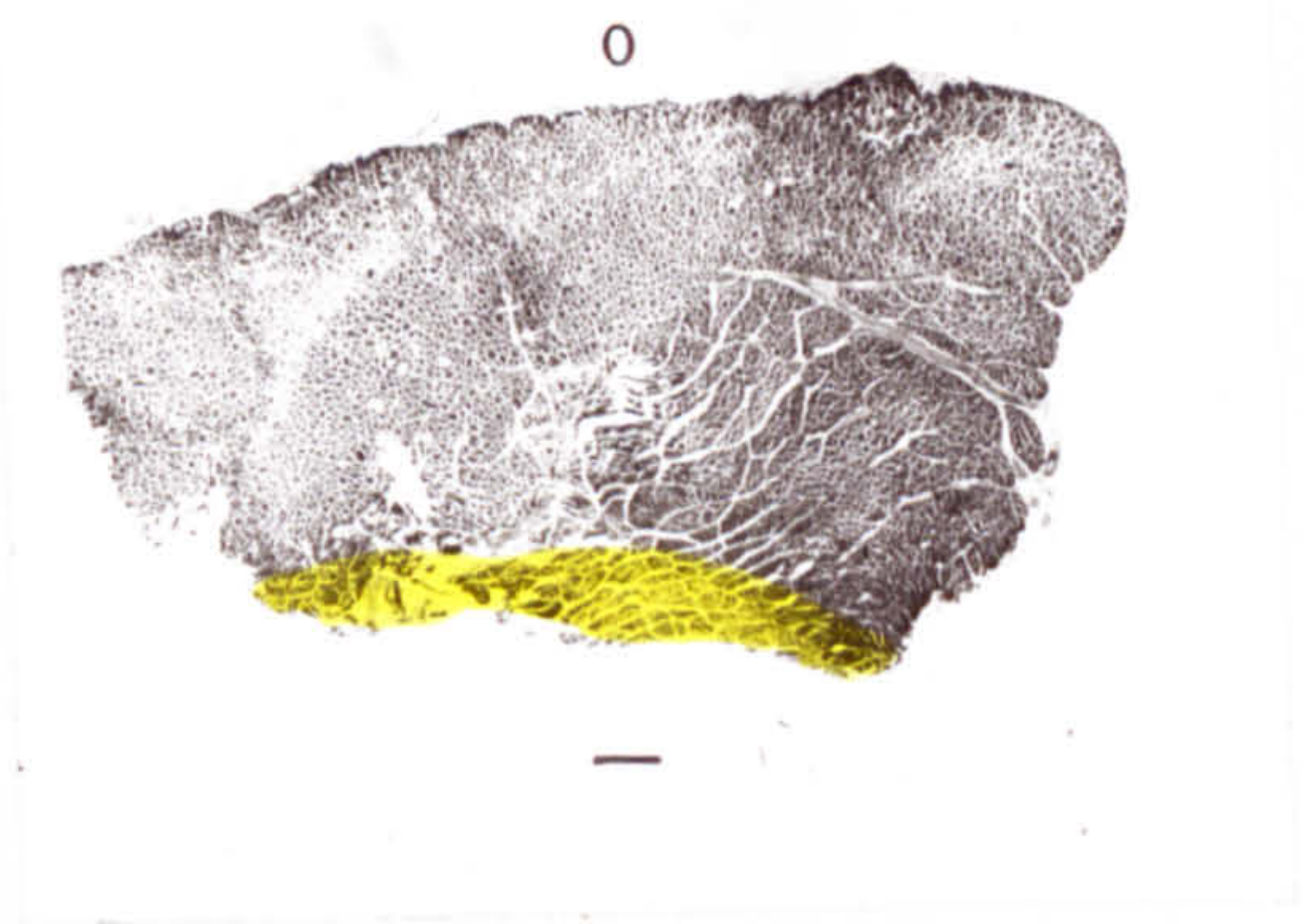
T.S. of Rainbow Trout for comparison. The tendon is clearly visible (1) and the zone of red fibres tinted yellow and arrowed.

0 = outside edge of muscle. Scale bar = 500 μ m.

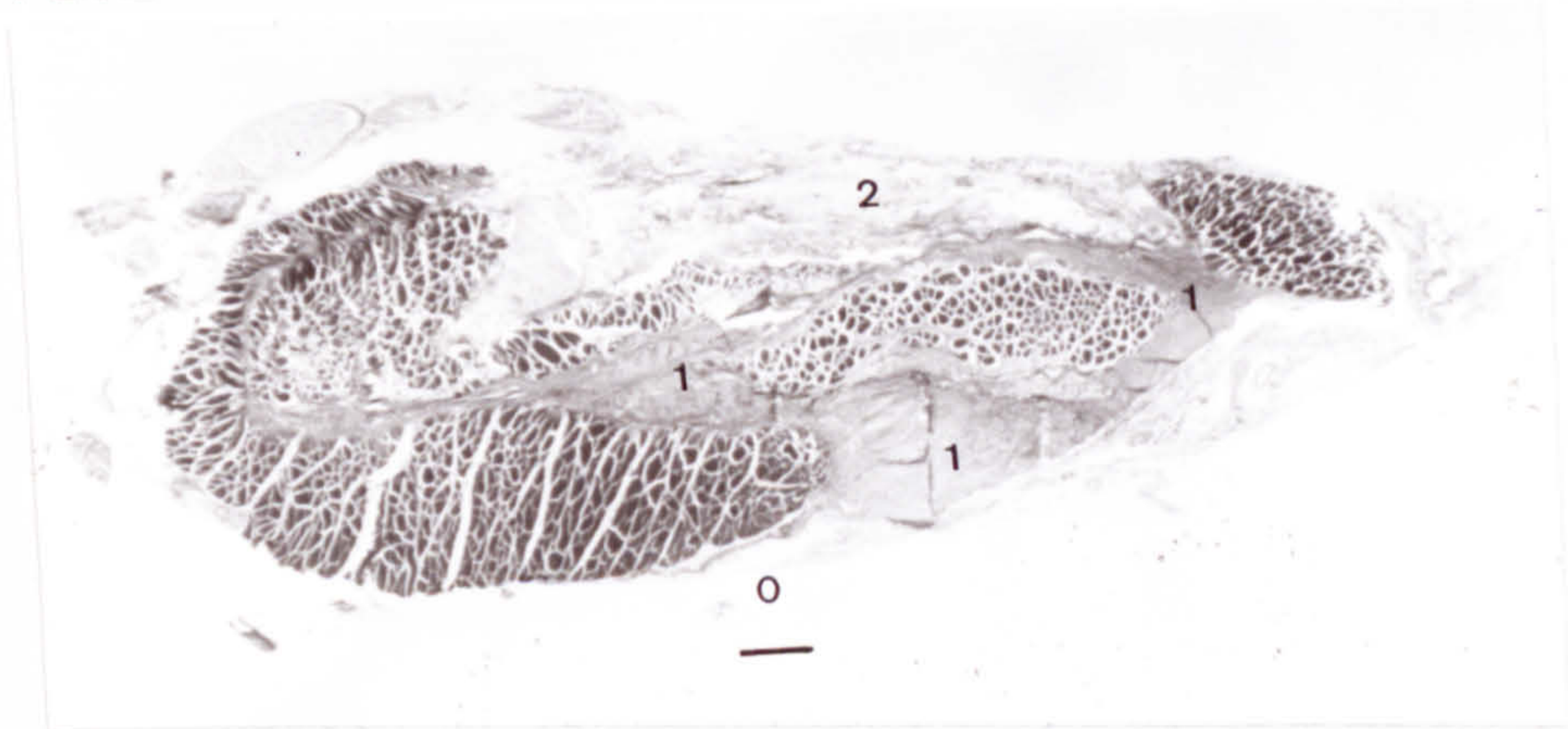
Plate I.4

T.S. of Brown Trout m. adductor mandibulae to show the fascicular arrangement in the red fibre zone. Scale bar = 100 μ m.

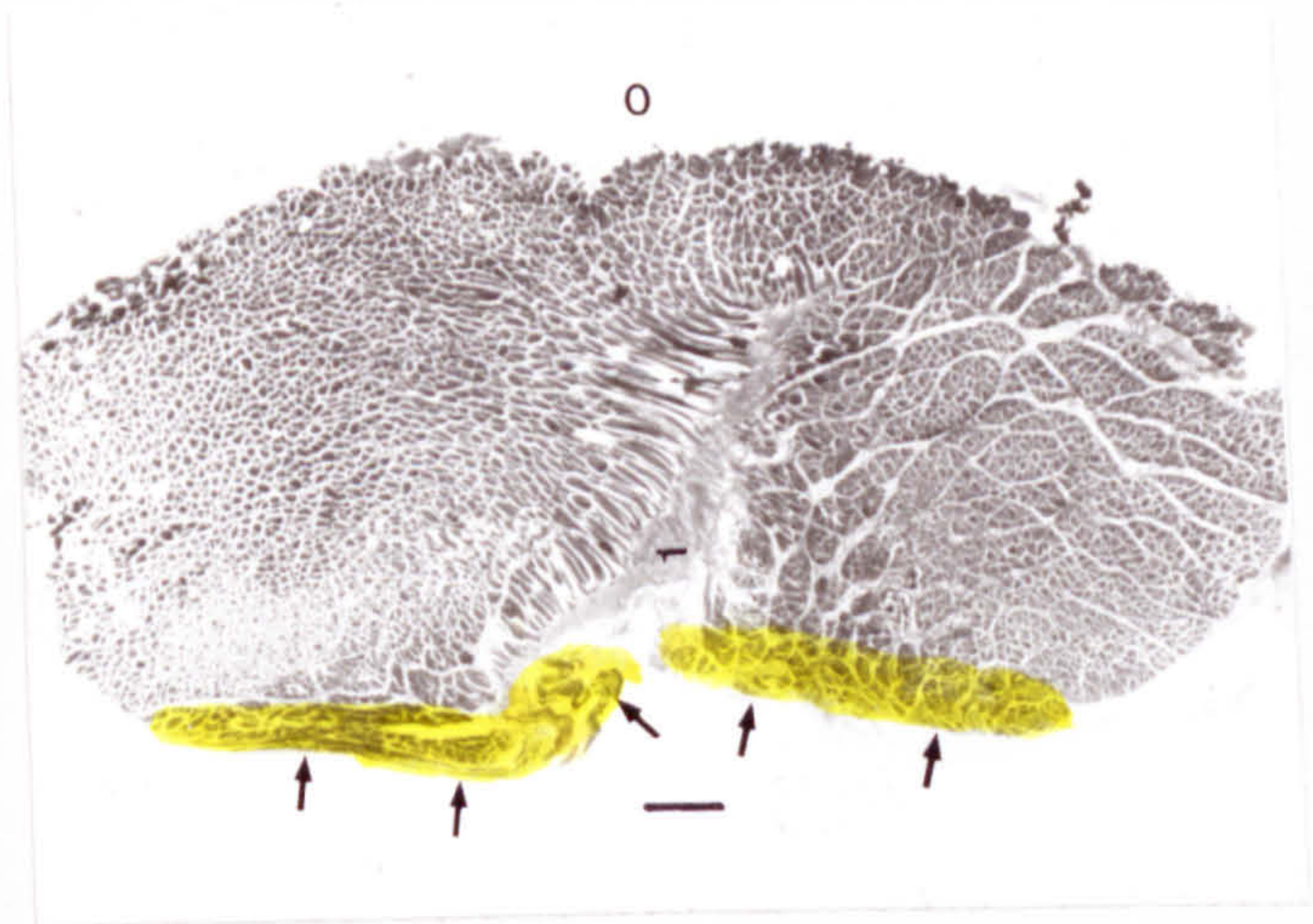
PL. I.1



PL. I.2



PL. I.3



PL. I.4

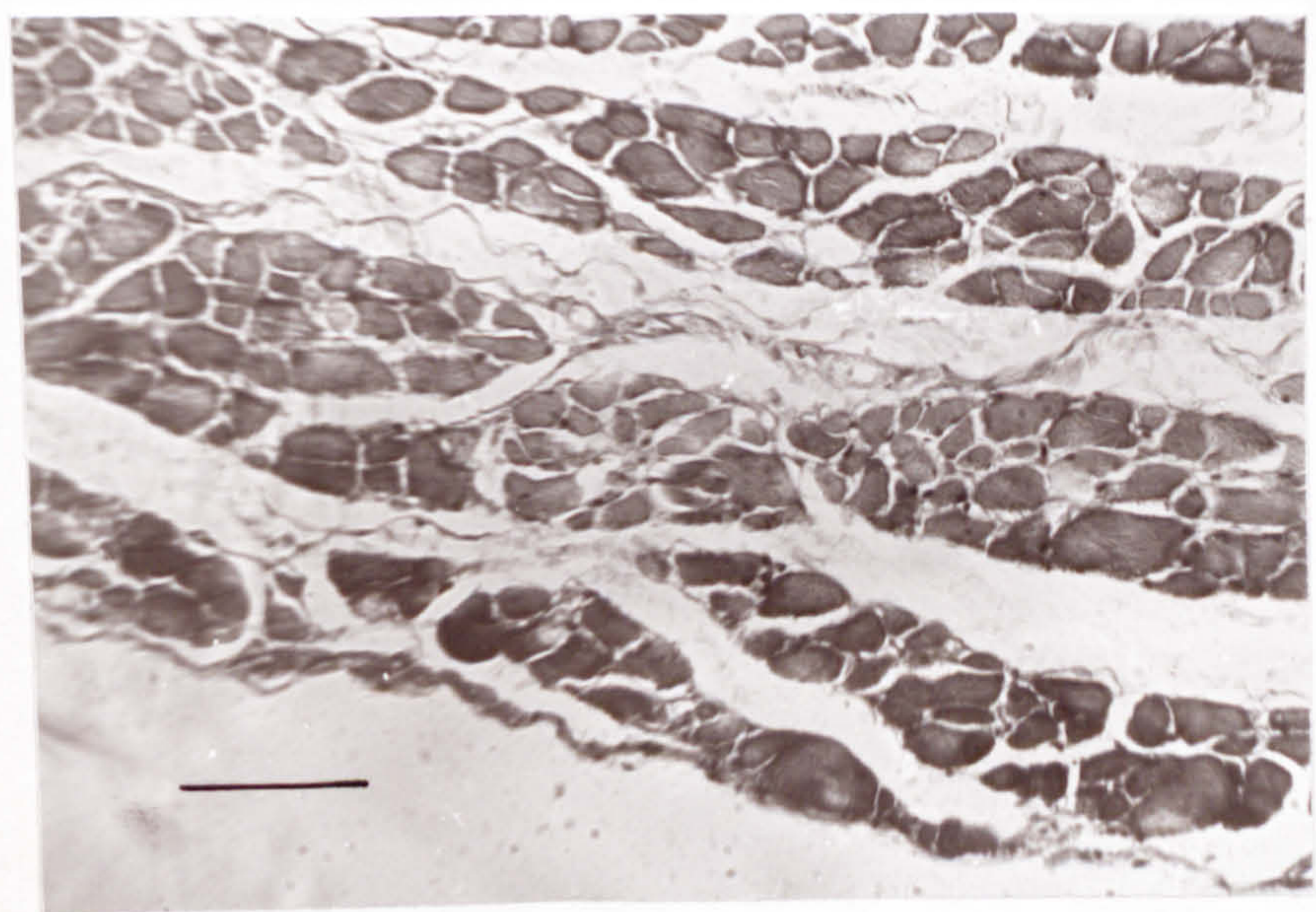


Plate I.5

T.S. of Brown Trout m.adductor mandibulae taken in middle region of the muscle, in the mosaic fibre portion. Note the different appearance of the muscle compared to Plate I.4

Scale bar = 100 um.

Plate I.6

High power view of mosaic fibre to show 'Fibrillenstruktur' arrangement of myofibrils. Scale bar = 100 um.

Plate I.7

High power view of red muscle fibre showing 'Felderstruktur' arrangement of myofibrils (1).

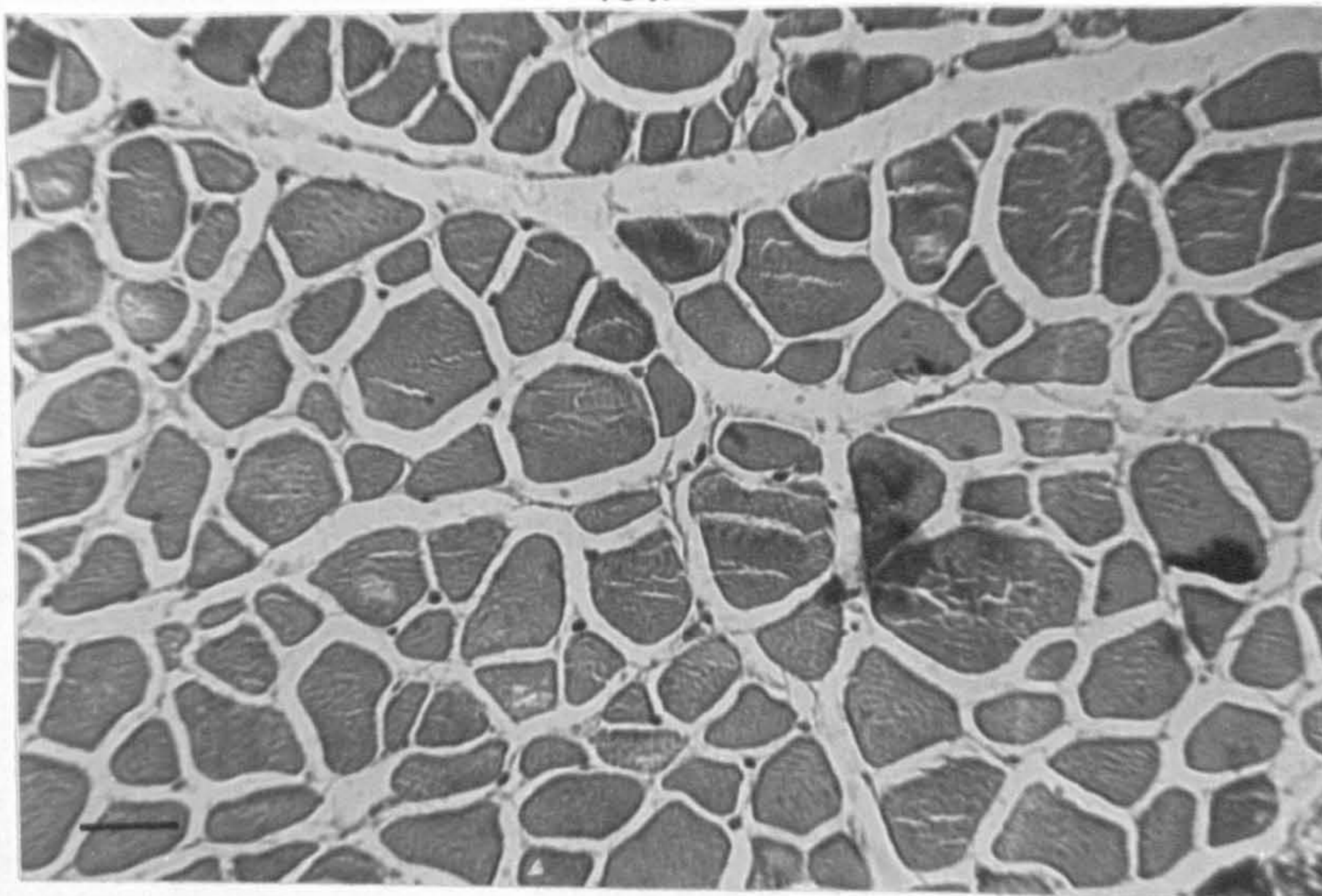
Scale bar = 20 um.

Plate I.8

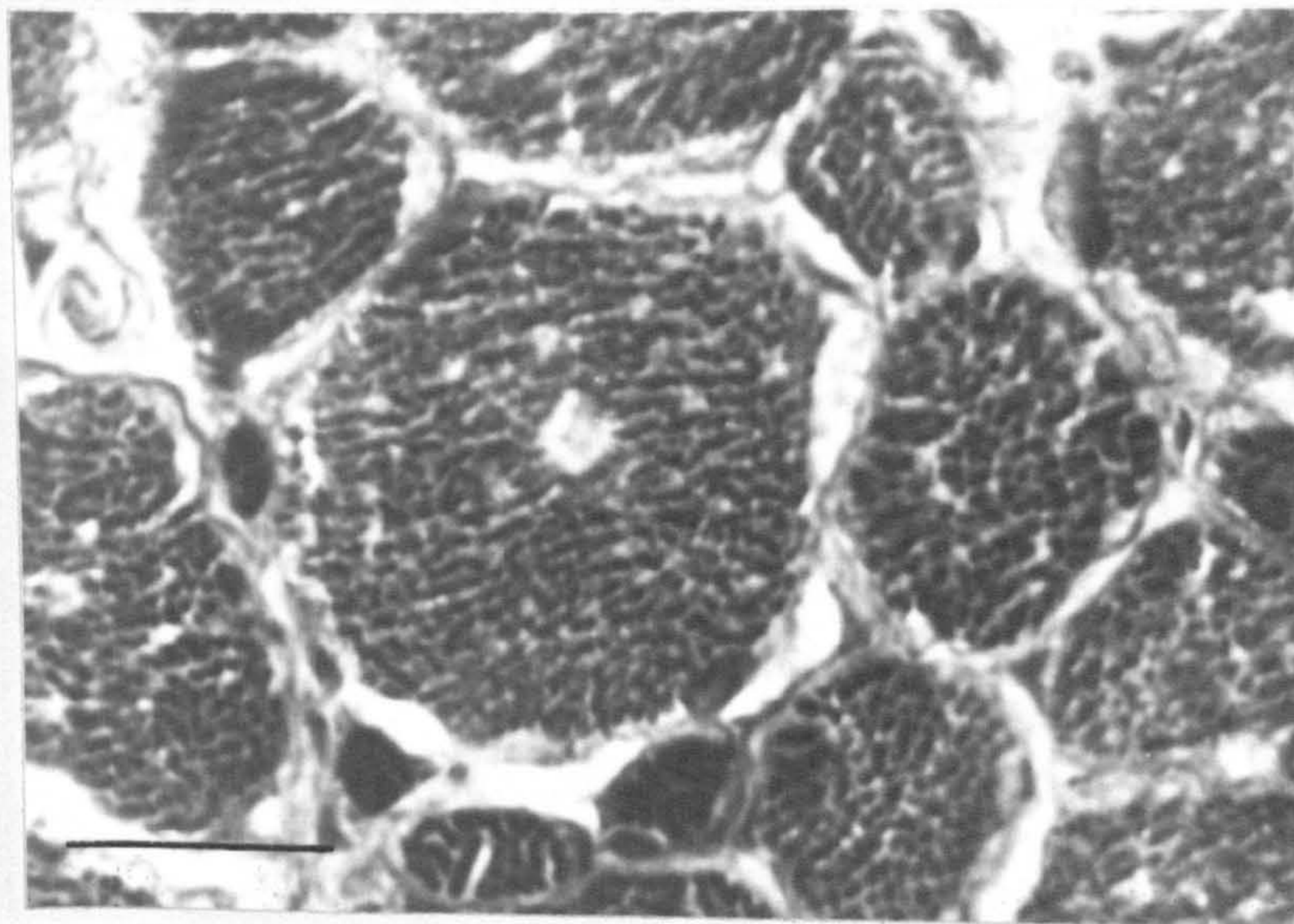
Close-up of inner surface of Brown Trout adductor mandibulae, injected with latex and cleared in Xylene. The density and complexity of the capillaries winding around these red muscle fibres is evident. An arteriole is present at (1).

Scale bar = 1 mm.

PL. I.5



PL. I.6



PL. I.7



PL. I.8

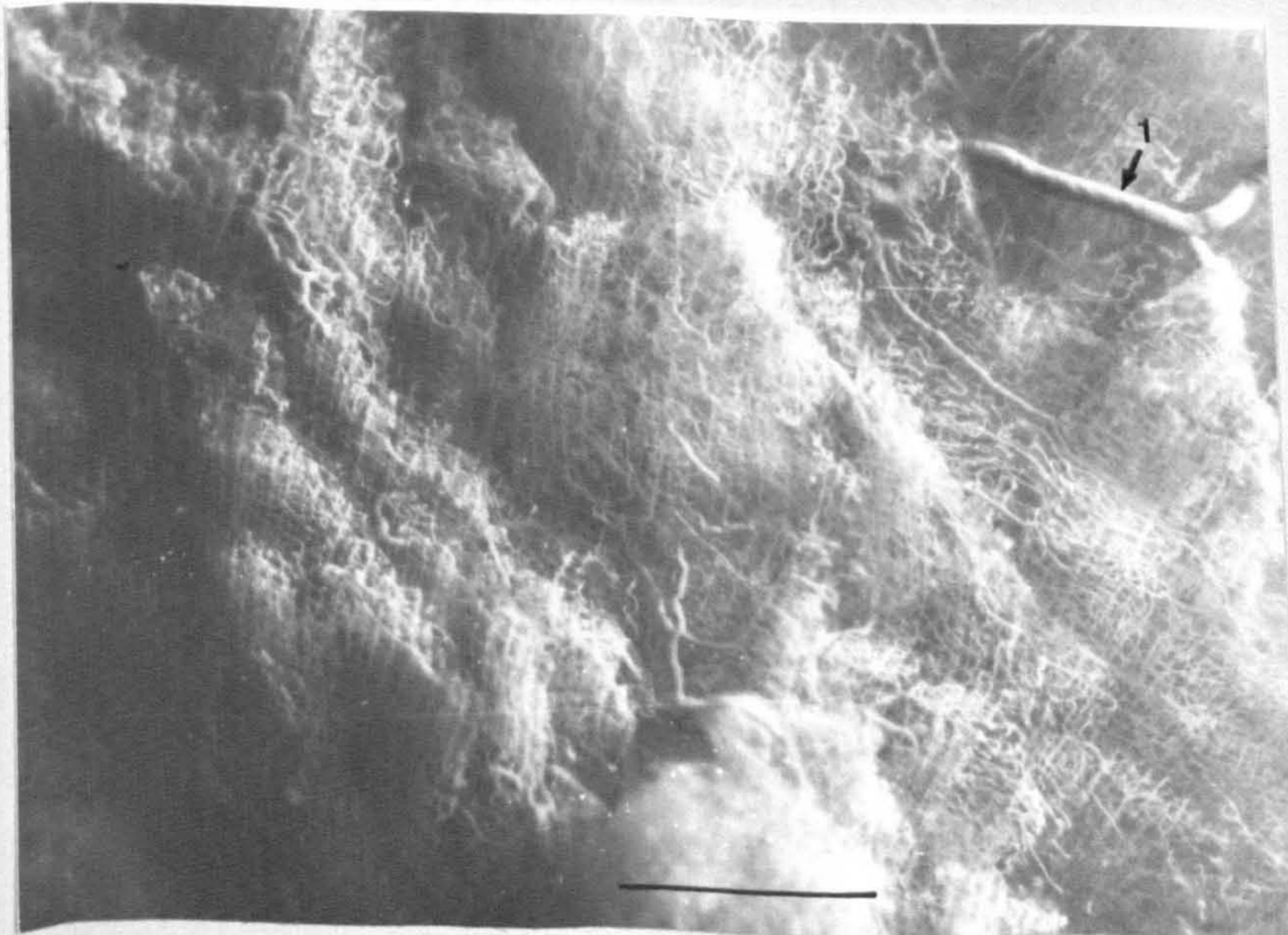


Plate I.9

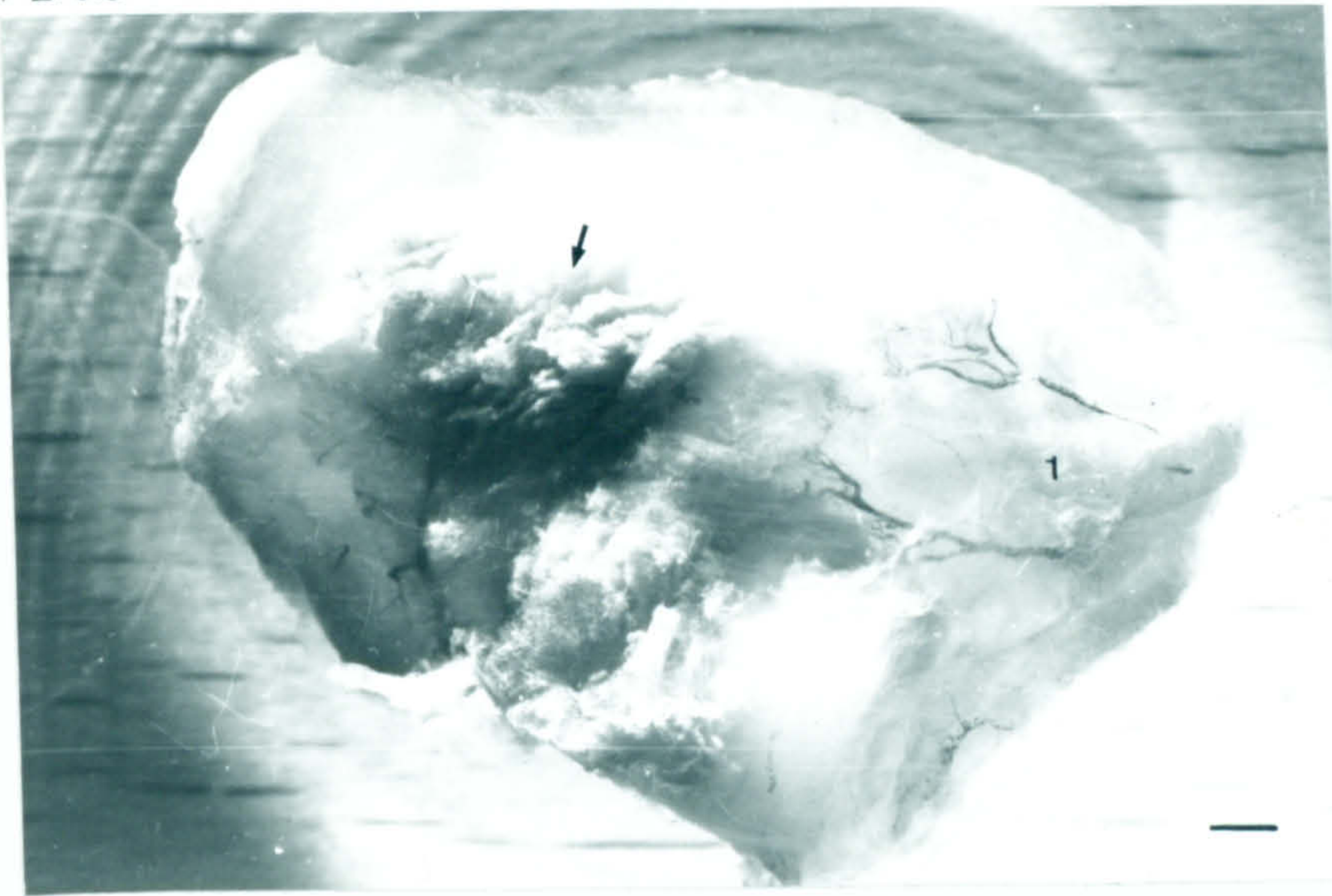
Inner surface of Brown Trout m. adductor mandibulae partially injected with latex and cleared in Xylene. The red fibre area (arrowed) and appears dark because of its vascularity. Two arteries are seen at (1).
Scale bar = 1 mm

Plate I.10

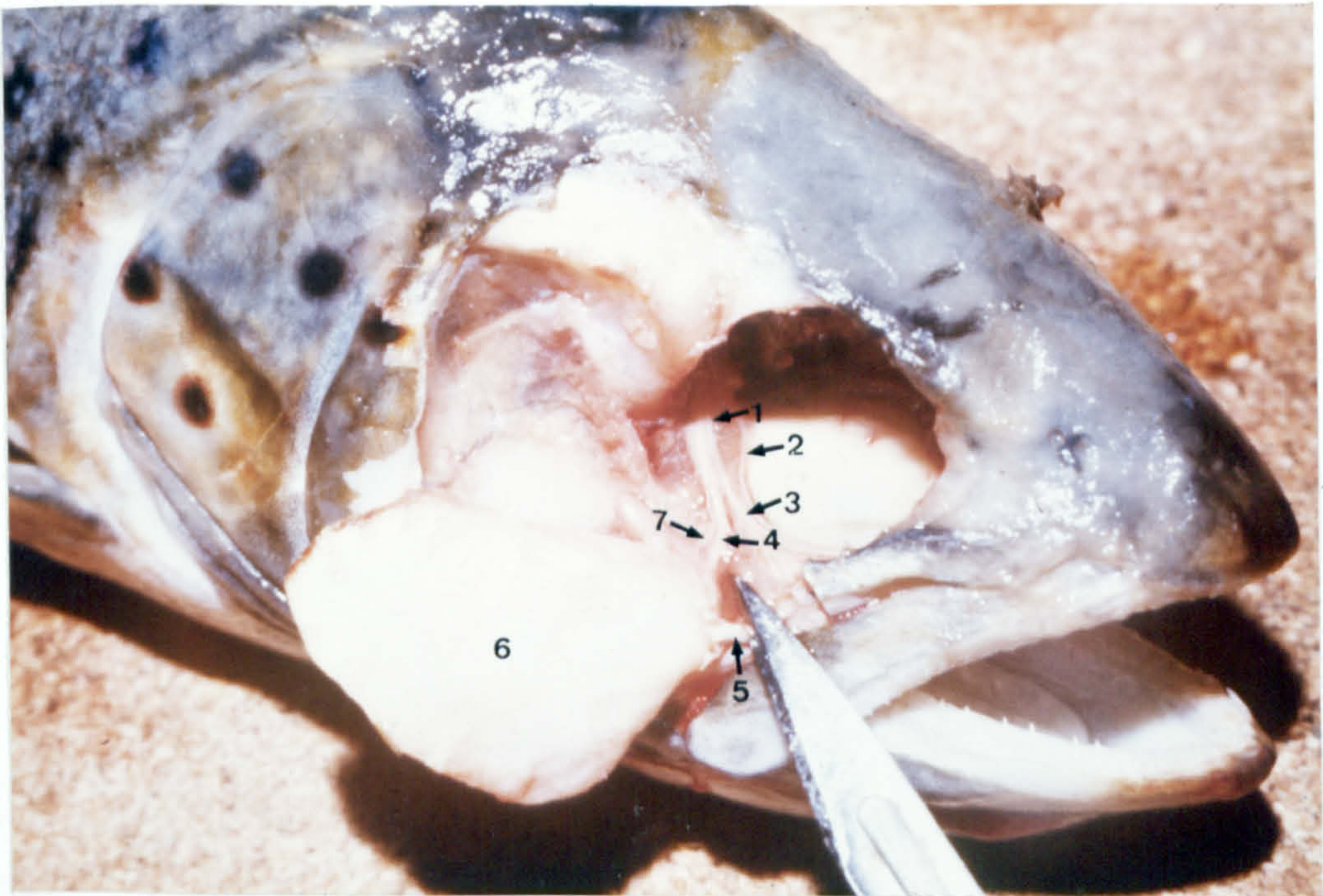
Partial dissection of preserved Brown Trout head. The adductor mandibulae is freed laterally (6). Nerves are (1) combined Vth and VIIth; (2) Inner buccal; (3) Maxillary; (4) Vth Mandibular. There is a small branch accompanying (4). Note the major trunk of the Vth nerve (5) entering at the level of the tendon.

Plate I.11

Section through the main Vth nerve trunk taken proximally. Note the subdivision (1,2) formed by division of the perineurium.
Scale bar = 100 μ m.



PL. I.10



PL. I.11

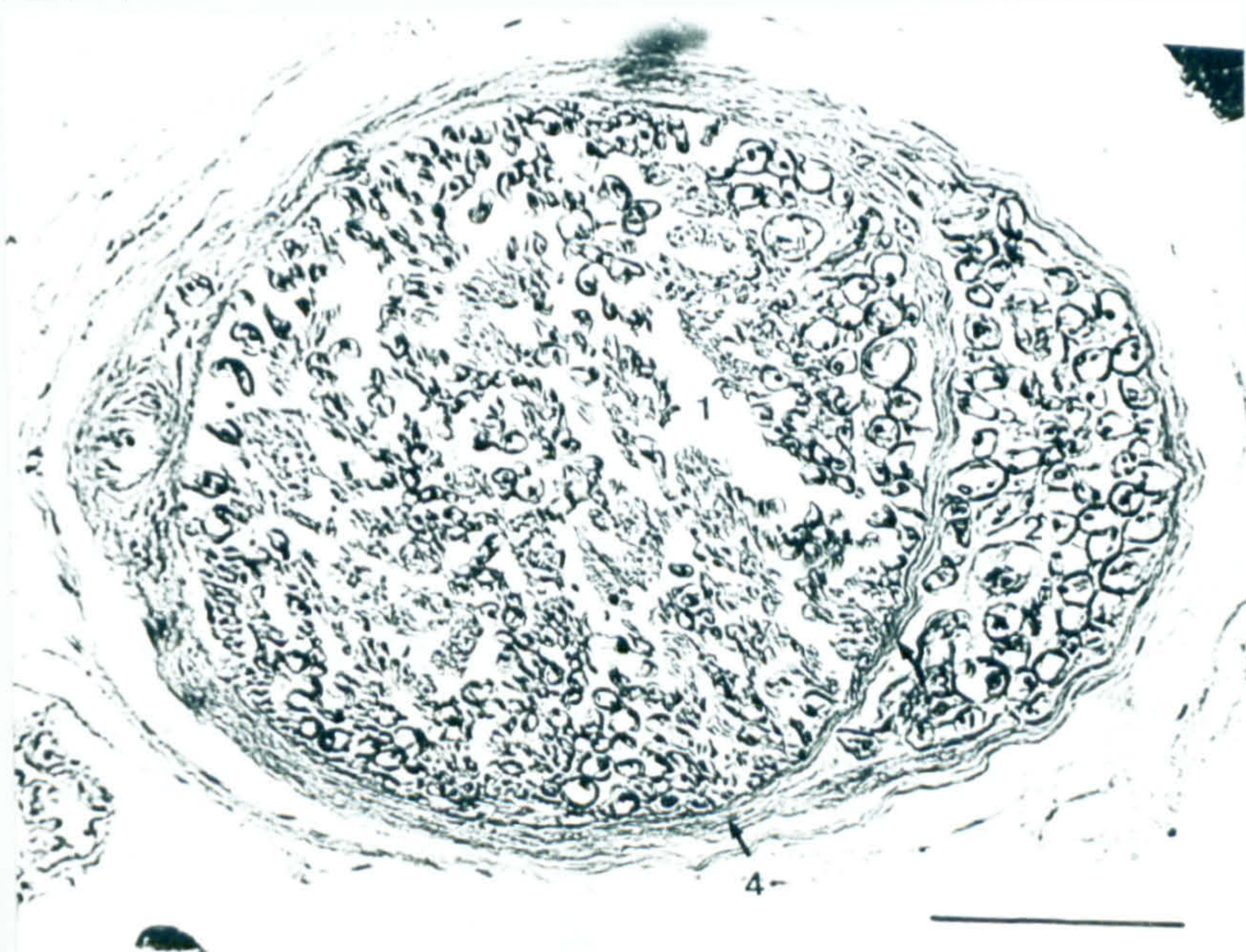


Plate I.12

T.S. of Vth nerve taken more proximally than Plate I.11.

Note division into three branches (1,2,3) and greater thickness of perineum (6) which divides into two secondary sheaths (4,5)

Scale bar indicates 100 μ m

Plate I.13

Cleared, latex-injected m. adductor mandibulae of Brown

Trout viewed from inner lateral aspect. The major trunk of the Vth nerve (1) with associated arteries is seen dividing into

branches (2,3,4,5). Scale bar = 1 mm

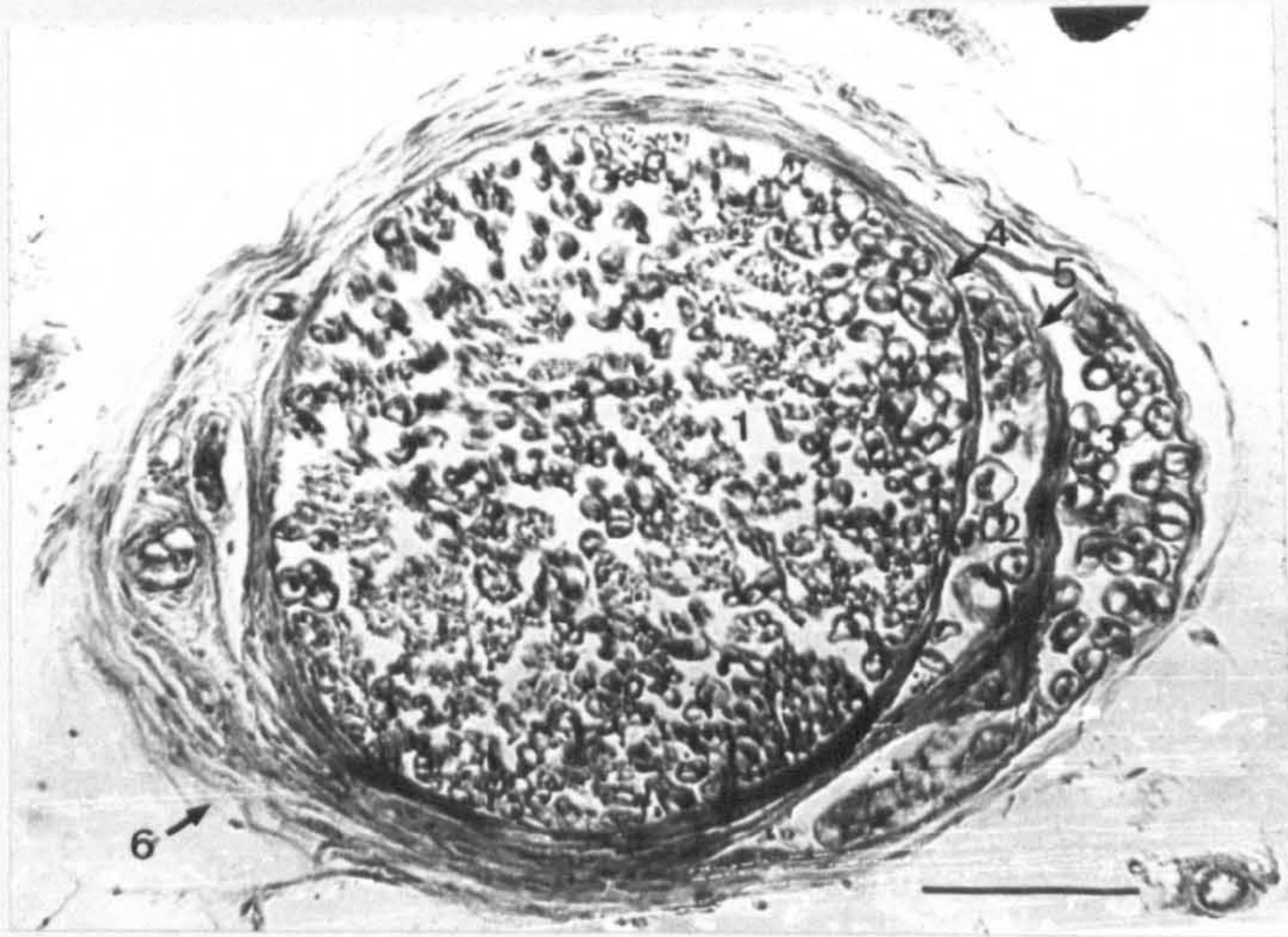
Plate I.14

Three nerve branches entering inner medial surface of Brown

Trout m. adductor mandibulae. Note association with blood vessels

(4,5) and Adipose tissue (6). Scale bar indicates 100 μ m

PL.I.12

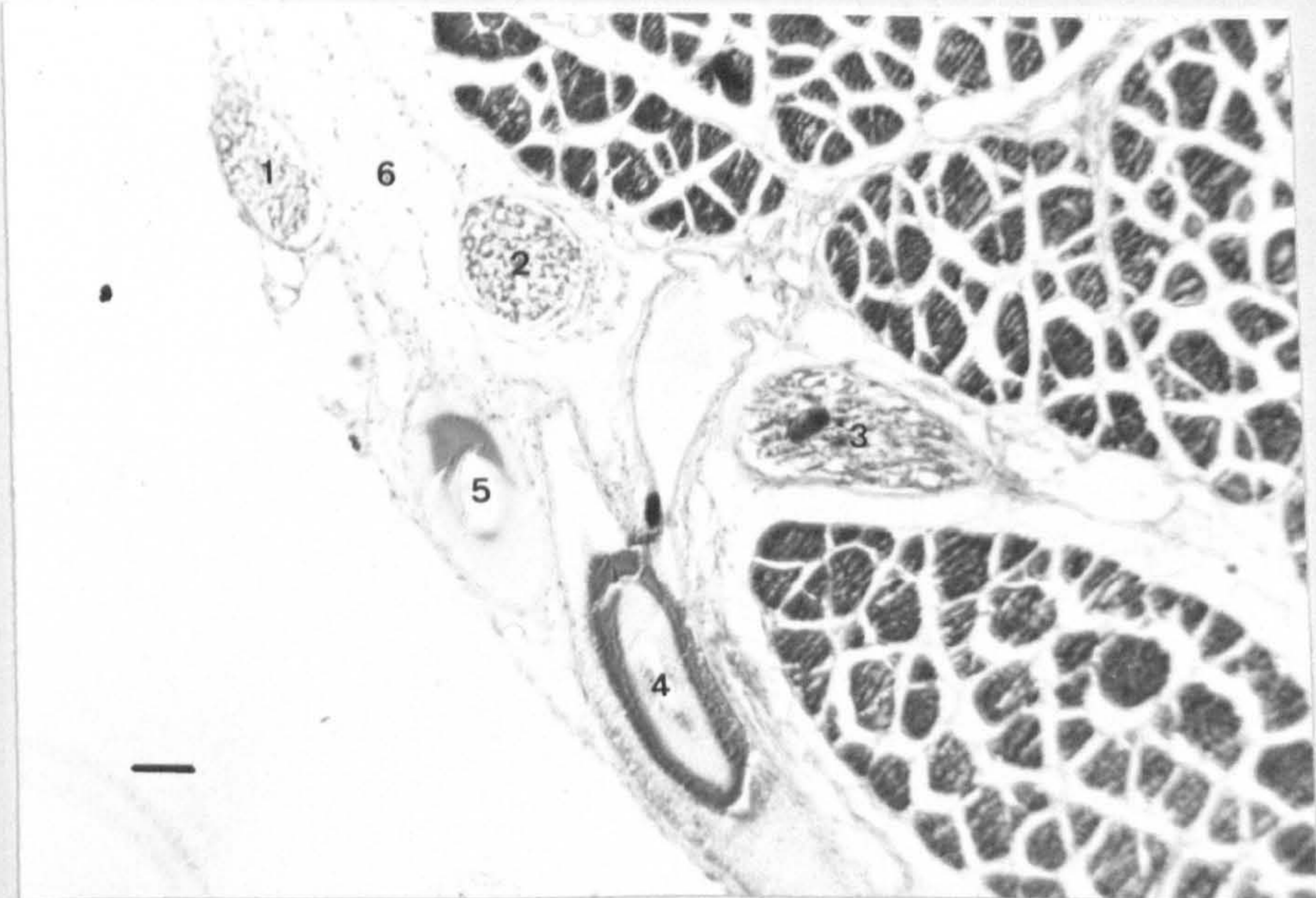


PLATES SECTION II MUSCLE PHYSIOLOGY

PL.I.13



PL. I.14



PLATES SECTION II : MUSCLE PHYSIOLOGY.

Plate II.1

Depth probe EMG experiment on Rainbow Trout under light Benzocaine anaesthesia. The skin has been removed from over the m. adductor mandibulae (1) and the bipolar probe electrode (2) is being driven into the muscle by the manipulator (3). The other electrodes (4) were for an unconnected experiment. The fish is earthed by the needle electrode near the tail (5).

Plate II.2

Specimen of Portugese Shark (Centrosymnus coelolenis) under Thiopentone anaesthesia. The right m. adductor mandibulae has been freed from its insertions into the hyomandibula/quadrata complex and reflected anteriorly by the haemostat (2). Clearly visible running in a dorso-ventral direction is a band of red muscle fibres on the inner medial surface. The pipe (3) is carrying non-toxic seawater to respire the preparation.

Plate II.3

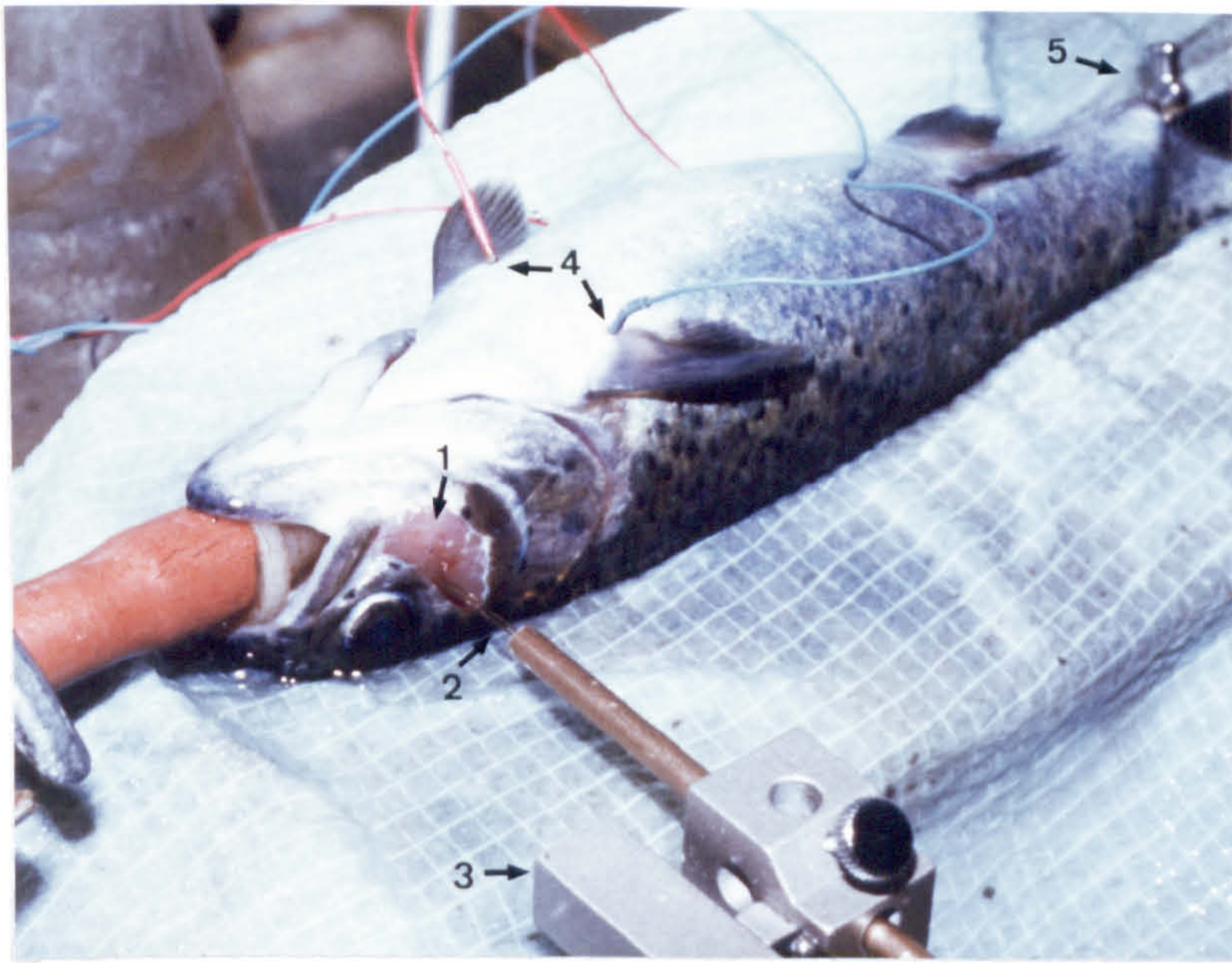
Dissected m. adductor mandibulae from the related Centrosymnus crepidater. The band of red muscle (1) contrasts markedly in colour with the remainder of the muscle (2). Scale in cms.

Plate II.4

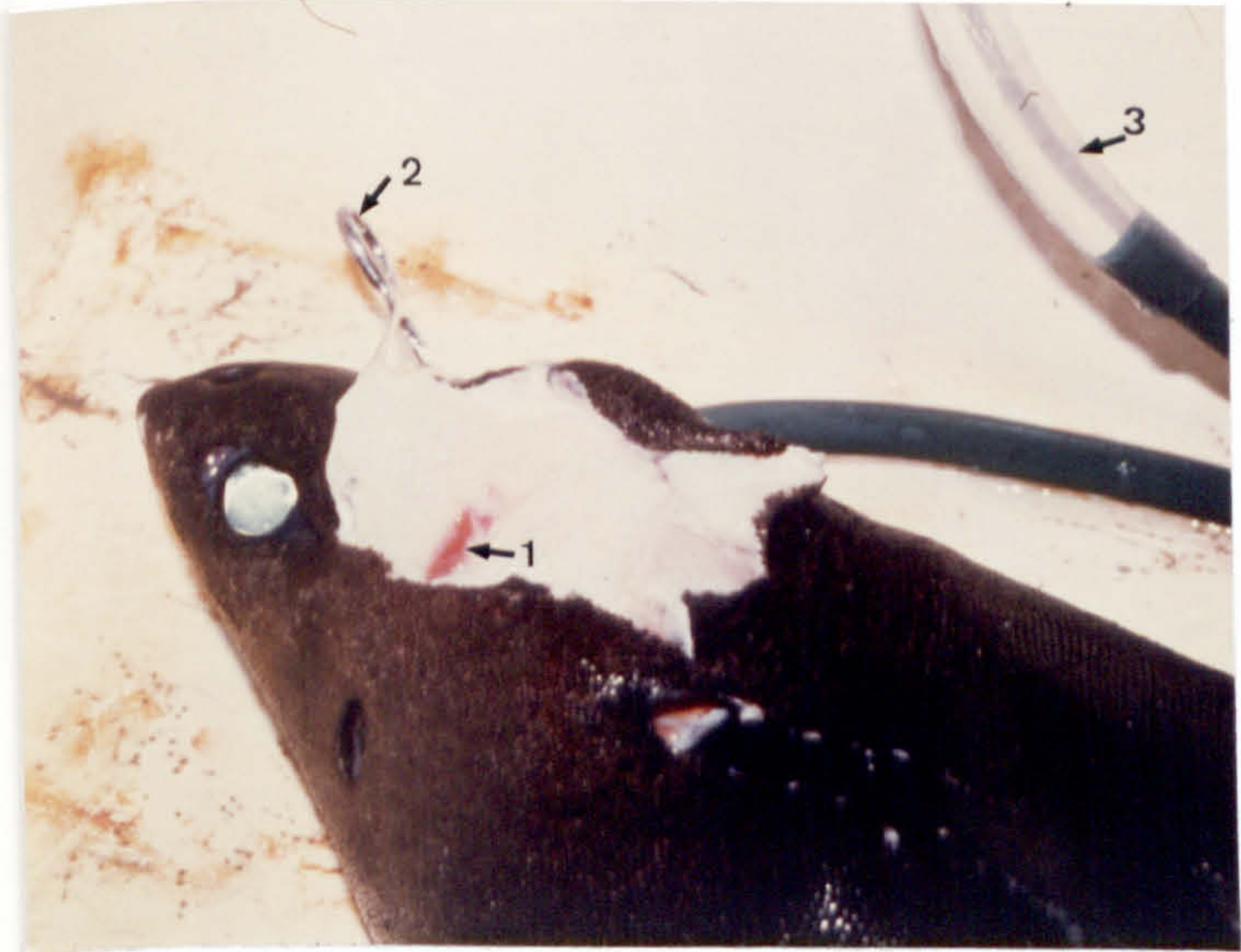
Depth probe experiment on Portugese Shark (Centrosymnus coelolepis) under Thiopentone anaesthesia. The m. adductor mandibulae (1) has been exposed and the EMG bipolar probe (2) is driven into various sites using the manipulator (3). The fishes head is prevented from shifting due to the ship's motion by the wooden holder (4).

PL. II.1

171.



PL II.2



PL. II.3



PL. II.4

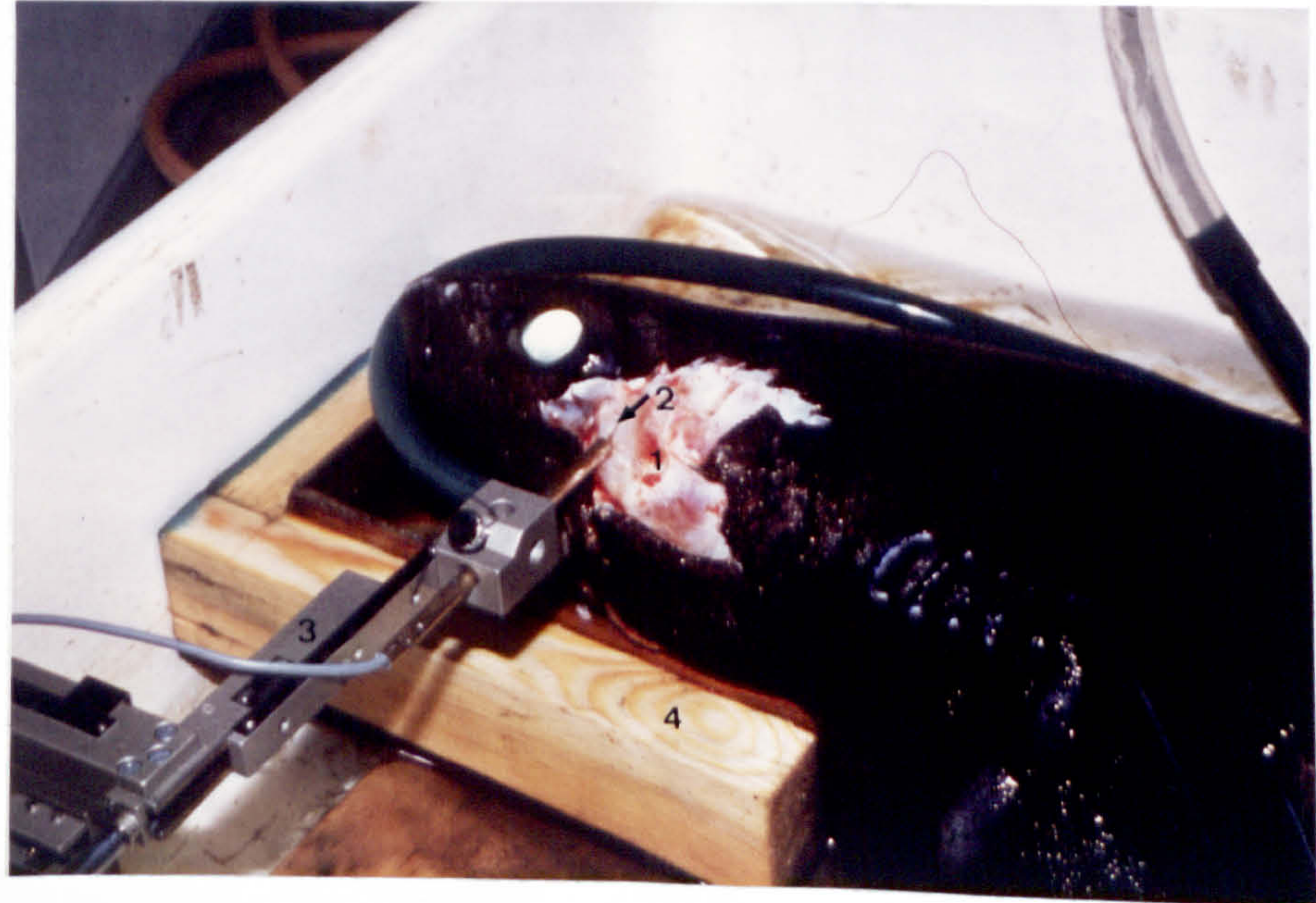


Plate II.5

Enlargement of Radiograph (slightly oblique) of the anterior part of a 30cm Brown Trout equipped with a pair of "T-electrodes". This X-ray was taken five days after the initial implantation. The thickness of the m. adductor mandibulae can be seen (1) and the stability of the implant is obvious. The electrode tips can be seen in place at (2) in order to record from the red fibre portion of the muscle. Enlarged about times from a direct X-ray.

Plate II.6

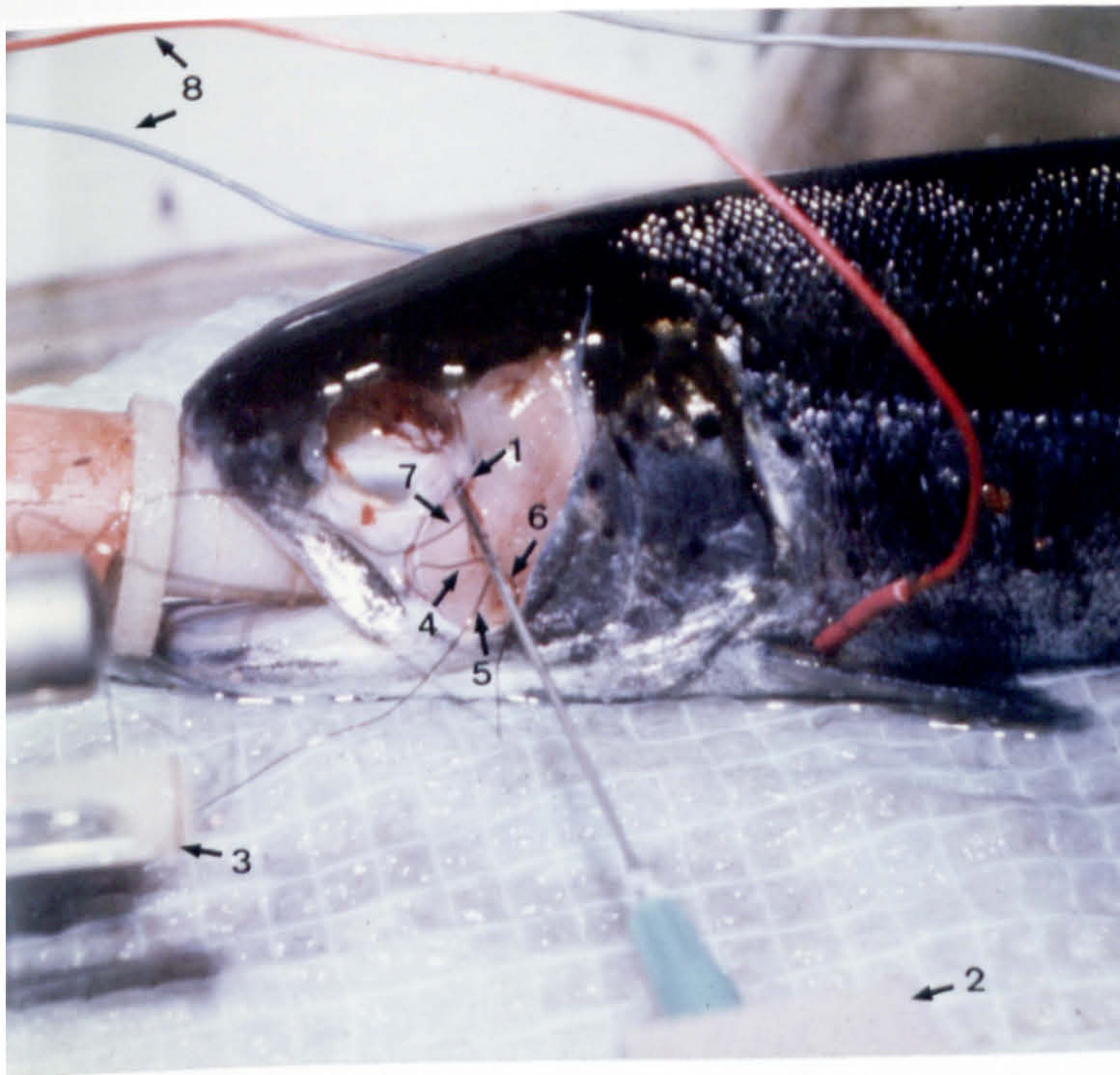
In this experiment a bipolar earthed needle electrode (1) mounted on a manipulator (2) is used to stimulate the Vth nerve. EMG's from the adductor mandibulae are recorded by the electrodes 4, 5, 6, 7 and as neuromuscular blocking drugs were to be used, the ECG was monitored on a separate oscilloscope to confirm the viability of the preparation.

Plate II.7

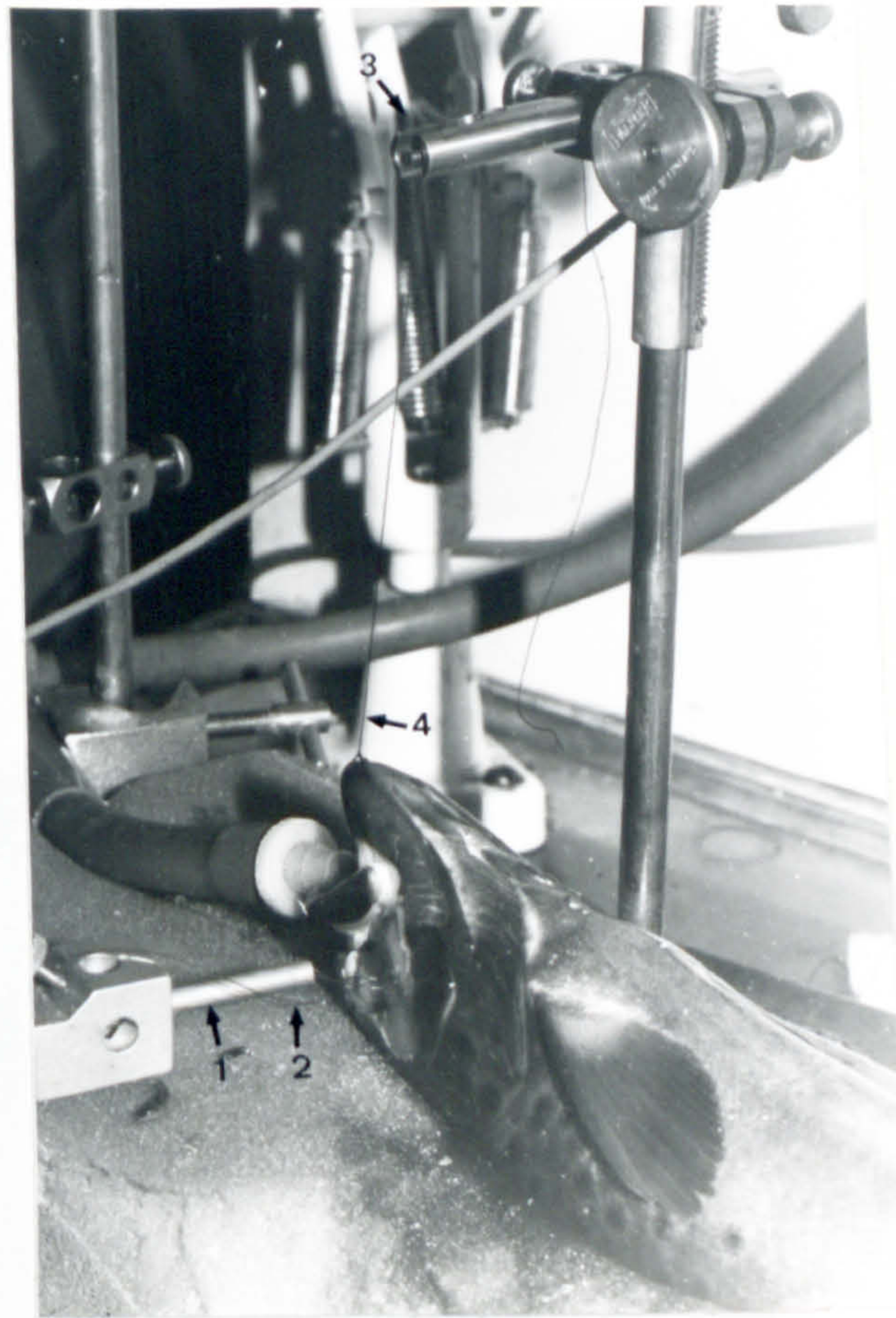
Alternative preparation. The Vth nerve is stimulated with a hook electrode (1) and EMG's recorded with one pair of electrodes (2). The tension produced is monitored by the Devices Isometric Transducer (3) connected to the mandibular apex by a silk suture (4).



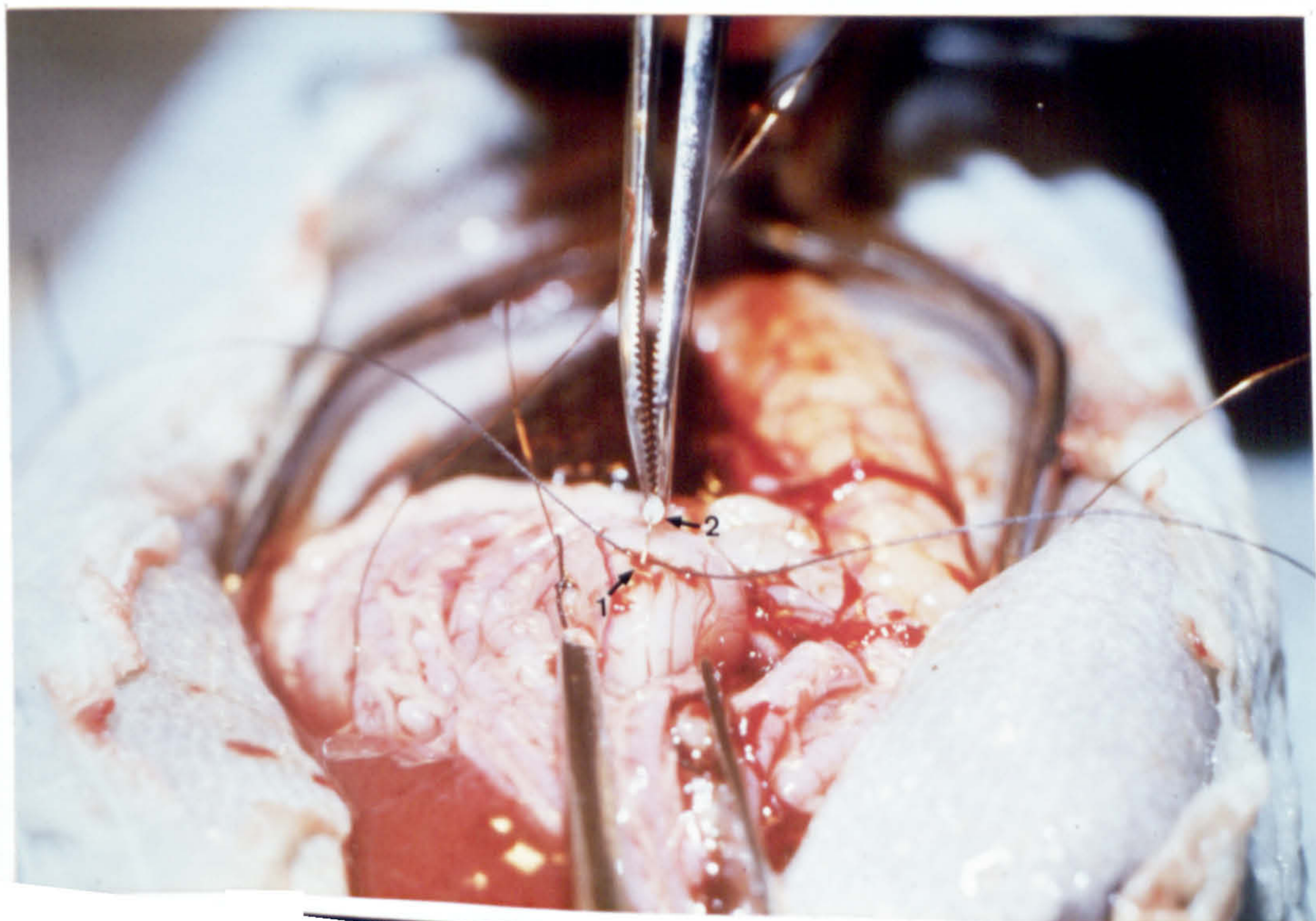
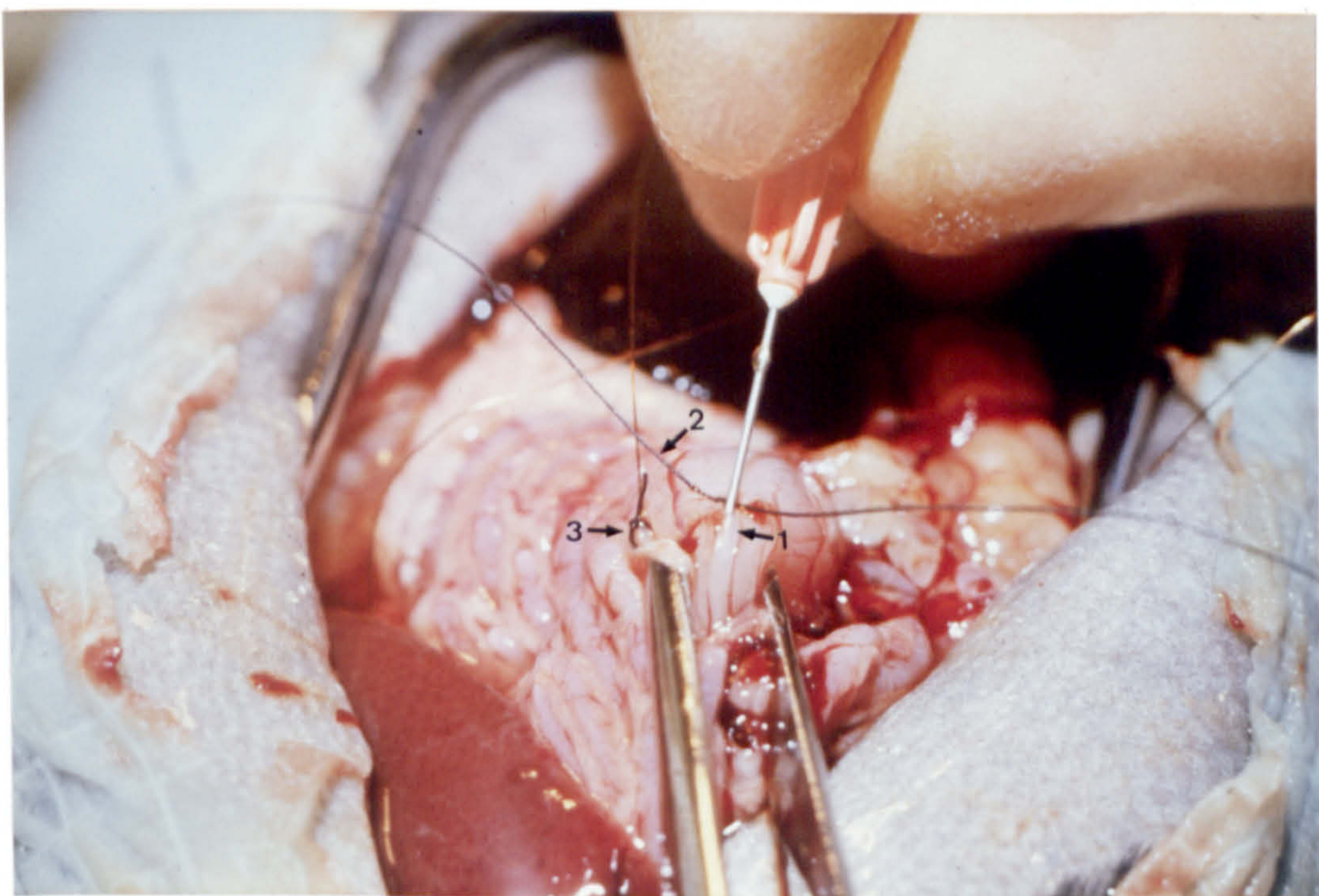
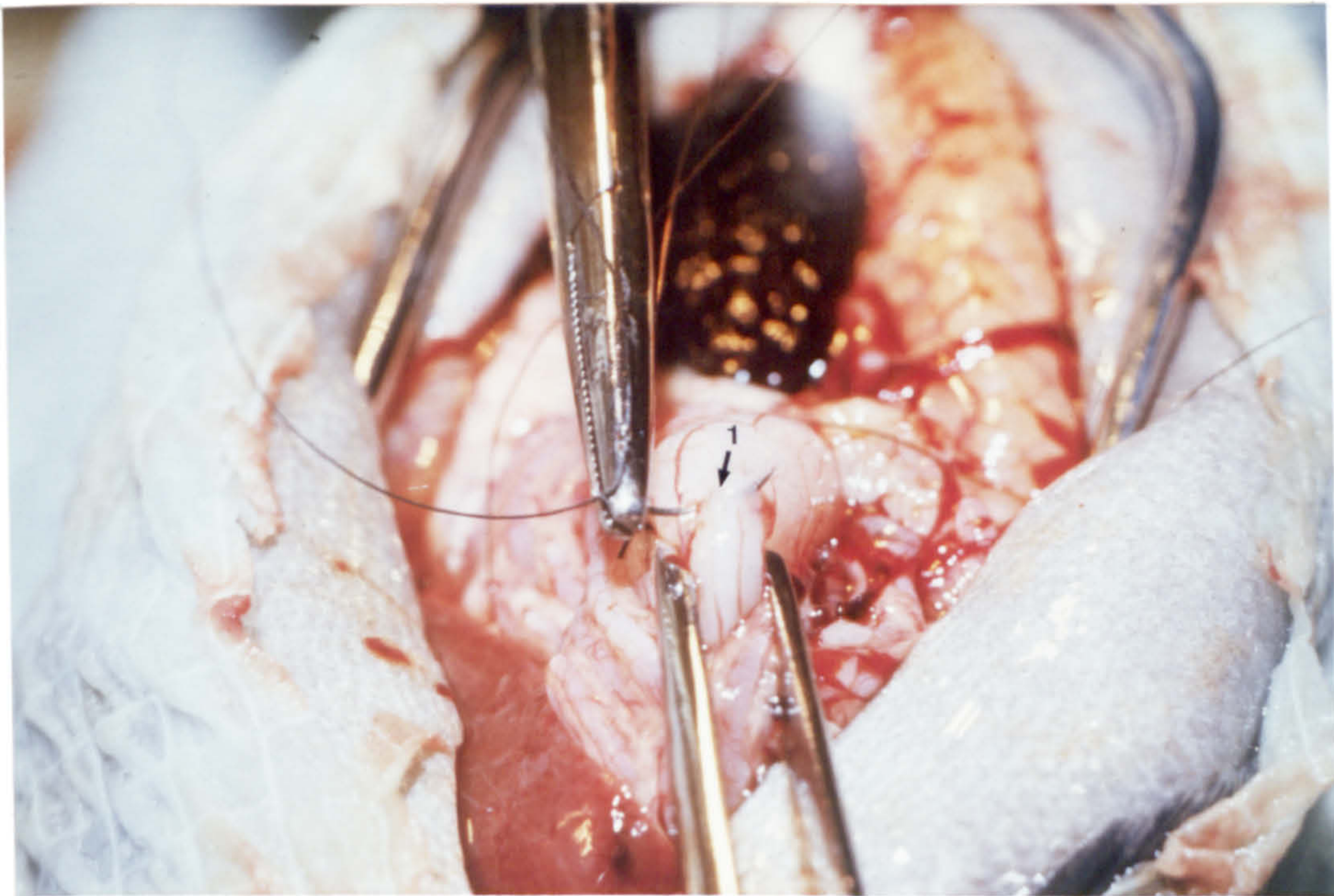
PL. II. 6



PL. II. 7



- Plate II. 8 Stages in the implantation of sub-serosal electrodes into the trout stomach (a). A laparotomy has been performed and the incision retracted. (In this example, the incision was considerably larger than necessary, for demonstration purposes). A fine suture is being passed through the muscle layers (1).
- Plate II. 9 (b) The serosa is perforated (1) anterior to the suture (2), using a 25 gauge needle. The other electrode of the pair has already been implanted and is seen at (3).
- Plate II. 10 (c) The punctate electrode is inserted in the puncture created by the needle (1). Note the bead of epoxy resin (2) which serves to anchor the electrode.



- Plate II. 11 (d) Two electrodes have been implanted into the cardiac stomach. Their two anchoring beads and sutures can be seen at (1), whilst their leads are seen at (2).
- Plate II. 12 (e) The electrode leads are threaded down the barrel of a 21 gauge hypodermic needle (1) inserted through the intercostal muscles (2). On withdrawing the needle, the leads follow and are thus produced to the exterior.
- Plate II. 13 (f) Completed preparation. This example shows an acute type of preparation. Two catheters have been introduced into the stomach and anchored (1). One is to measure pressure and the other to enable X-ray opaquecontrast material to be injected. The laparotomy incision has been closed by a continuous suture (2) and the electrode leads are seen at (3). For a chronic, free swimming implant, the catheters are omitted and the electrode leads connected to an anchoring plate as per the adductor mandibulae implants.

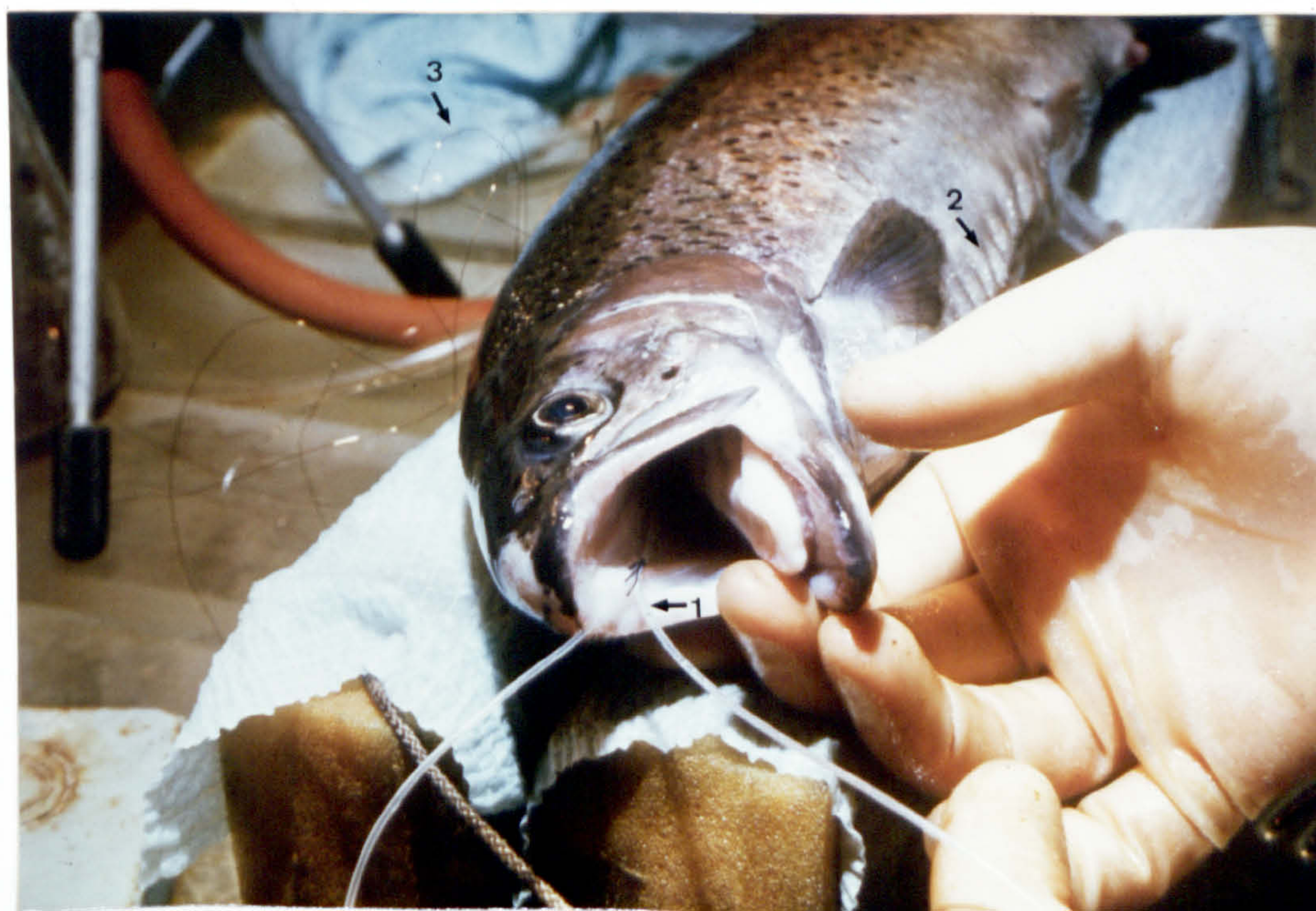
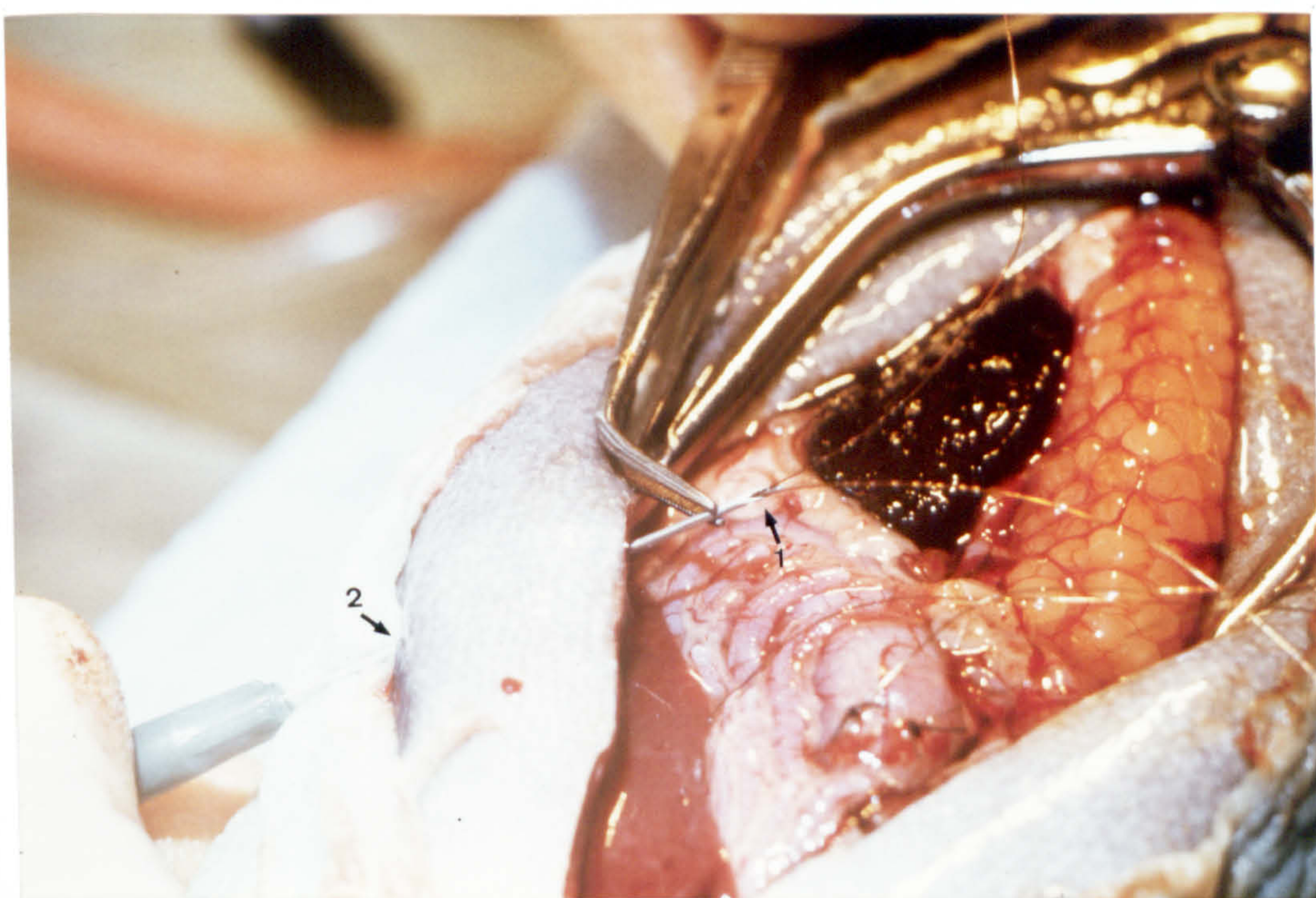
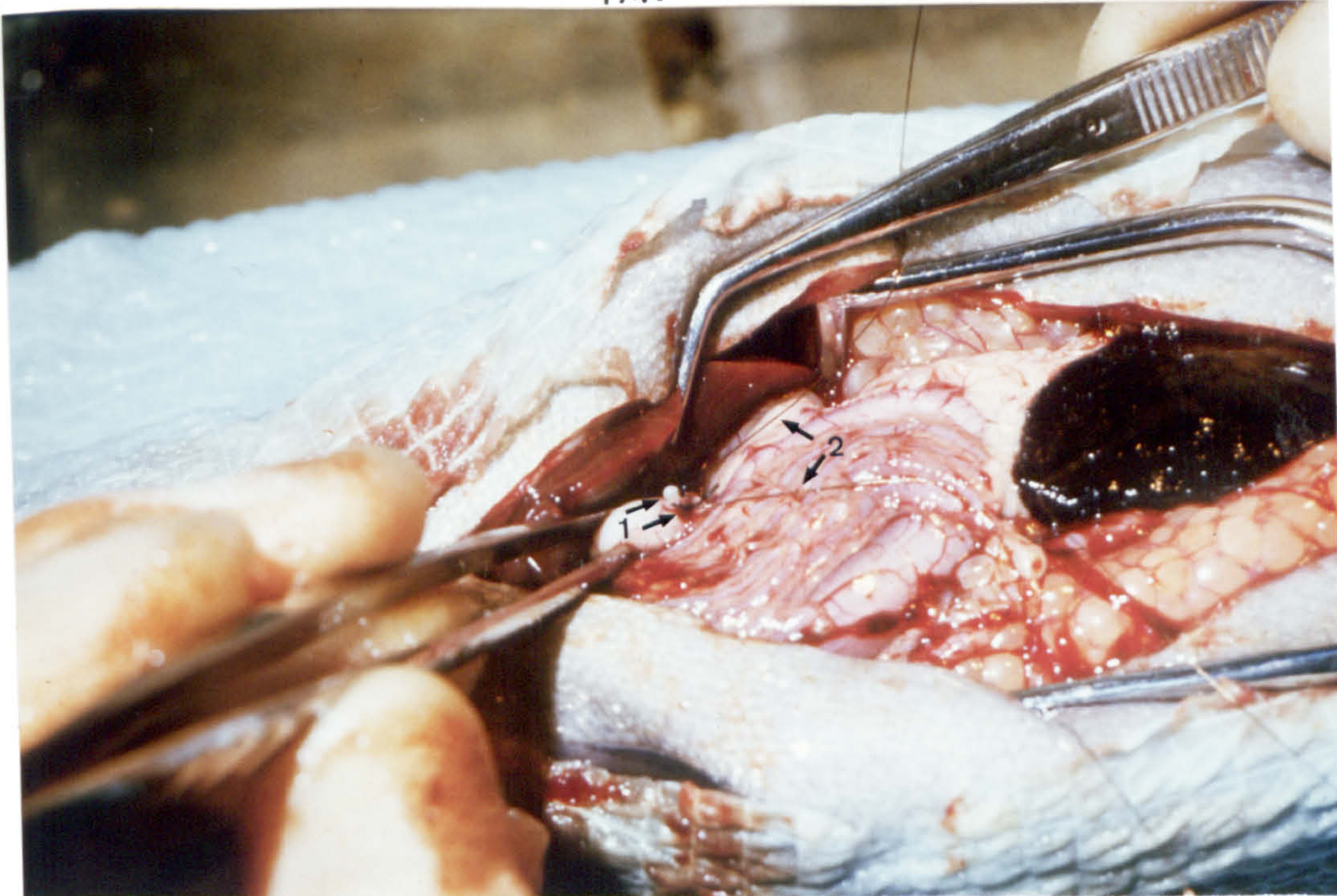


Plate II.14

Brown Trout adductor mandibulae

Upper trace : EMG

Lower trace : tension

Effect of stimulating nerve V at threshold and 2 x threshold. Large tension rise and biphasic EMG is associated with mosaic fibre recruitment.

Plate II.15

Brown Trout adductor mandibulae

Upper trace : EMG

Lower trace : tension

Effect of stimulating nerve V at 0.5V increments 50 μ S pulse width from below threshold. Note abrupt jump in twitch response due to the involvement of mosaic fibres.

Plate II.16

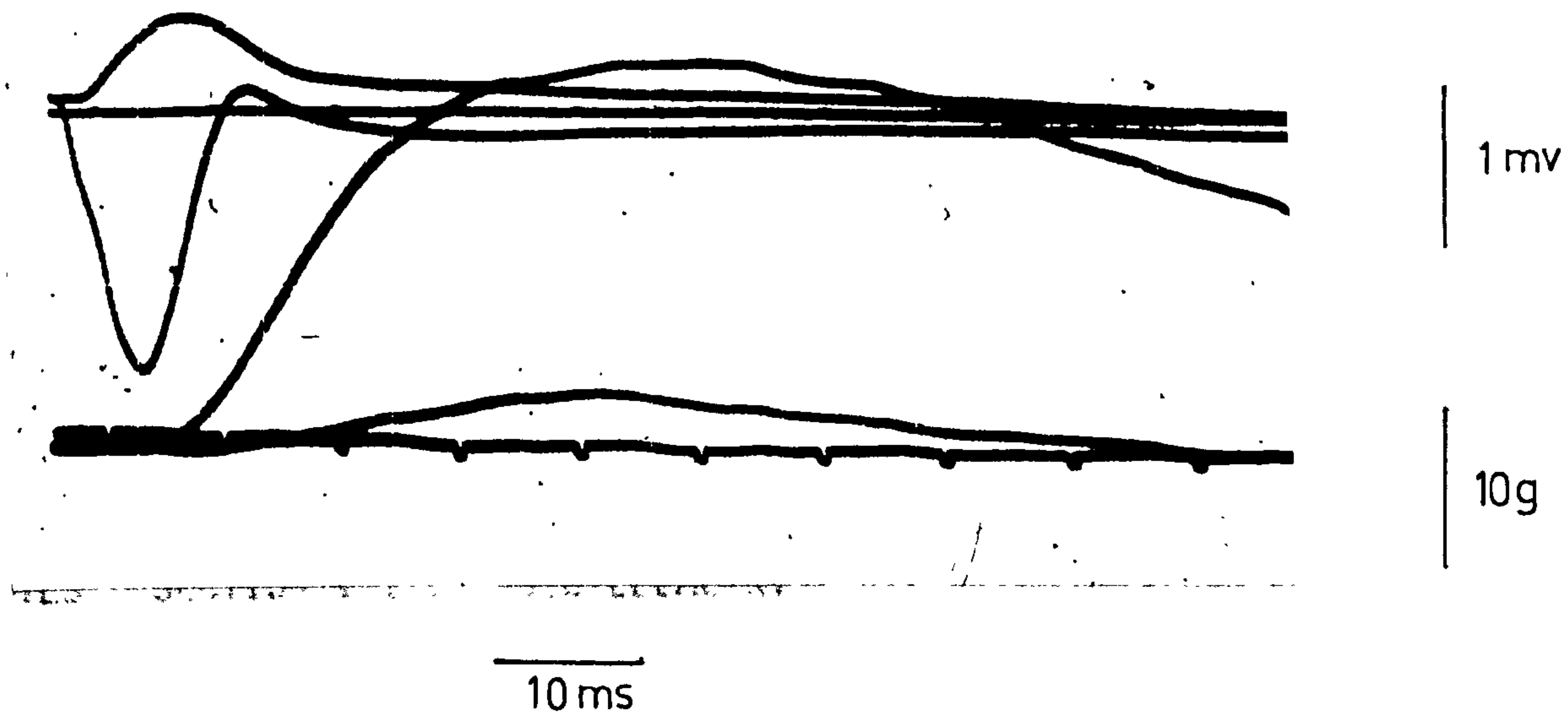
Brown Trout adductor mandibulae

Upper trace : EMG

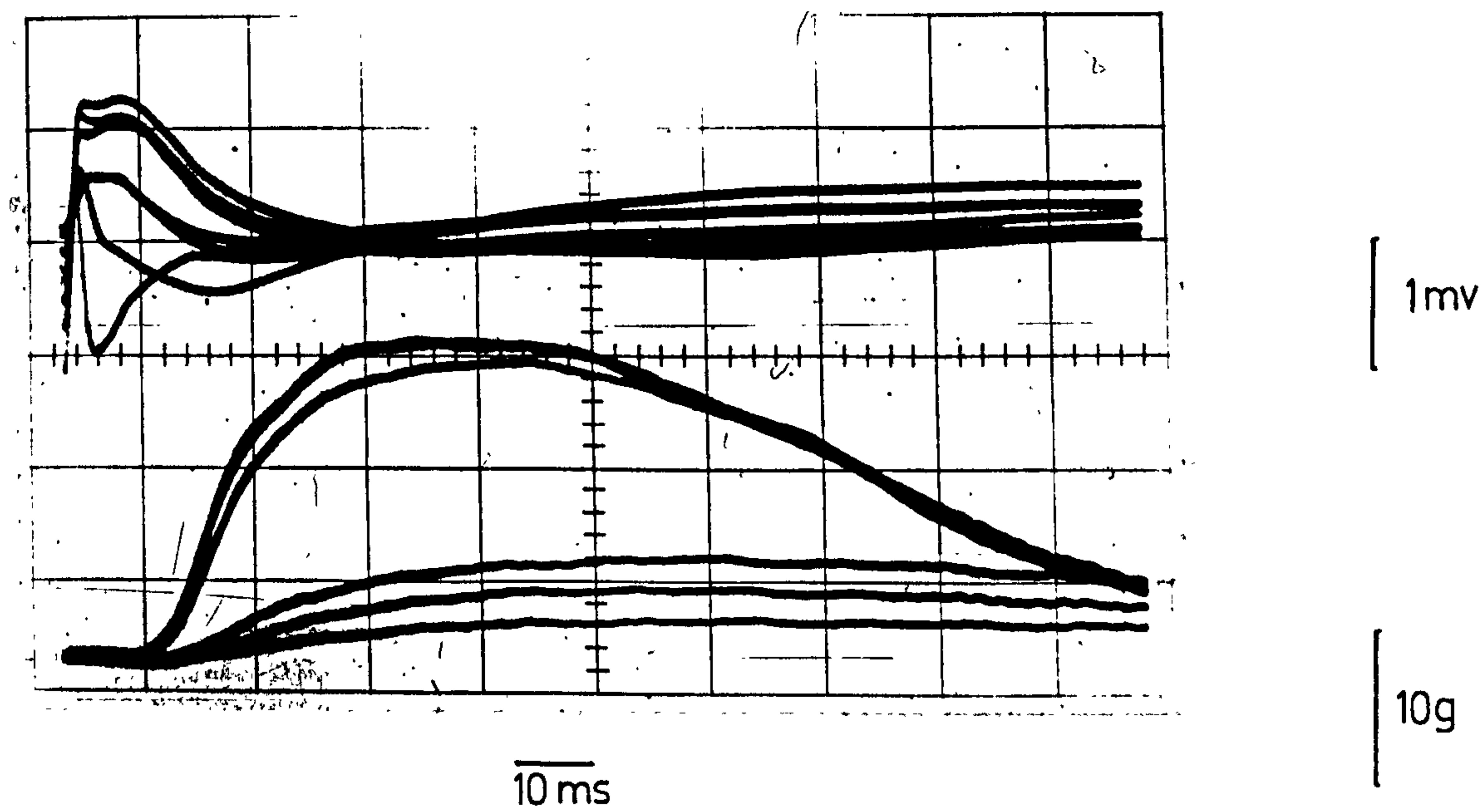
Lower trace : tension

Effect of stimulating Vth nerve at 3.5, 5.0 and 7.5V, 50 μ S. Note change in latency of twitch as mosaic fibres are recruited.

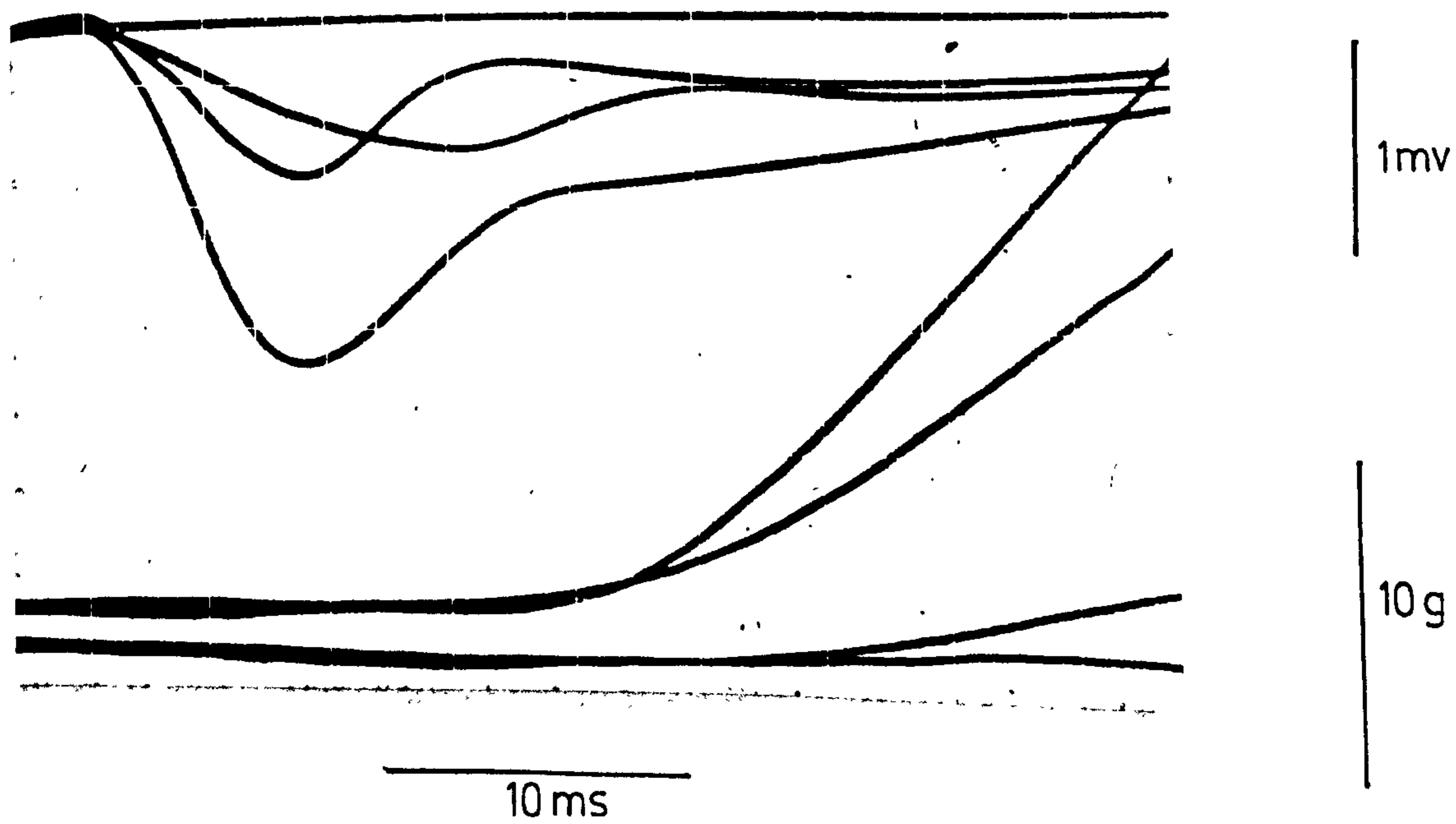
PL. 2.14



PL. 2.15



PL. 2.16



PLATES SECTION III : BIOTELEMETRY.

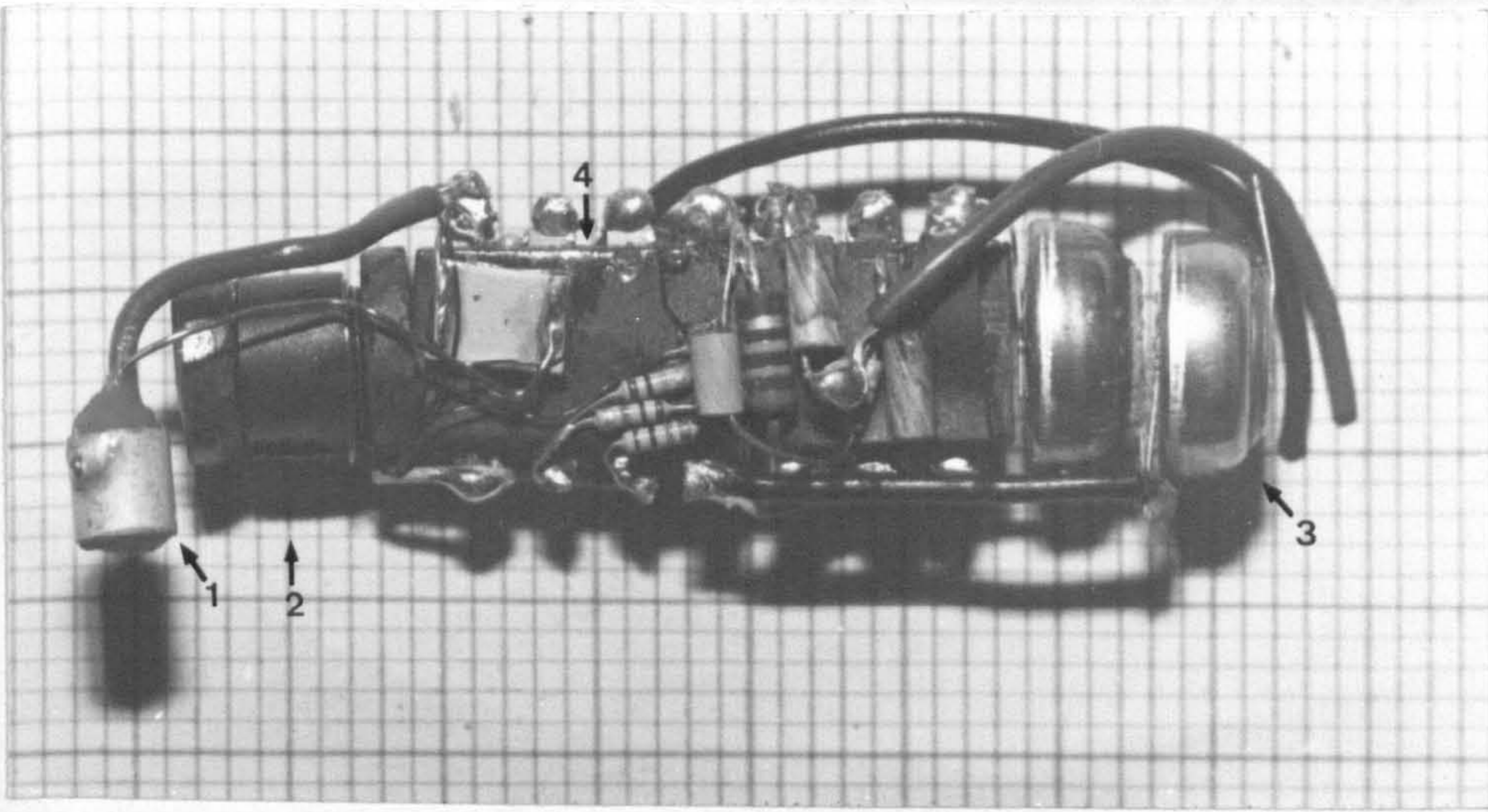
Plate III.1 View of unencapsulated EMG transmitter to show the main components, scale in mm.

1. Lead-Zirconium Titanate Transducer.
2. Ferrite Core Transformer.
3. Mallory RM312 Mercury Cell.
4. L144CJ Triple operational amplifier.

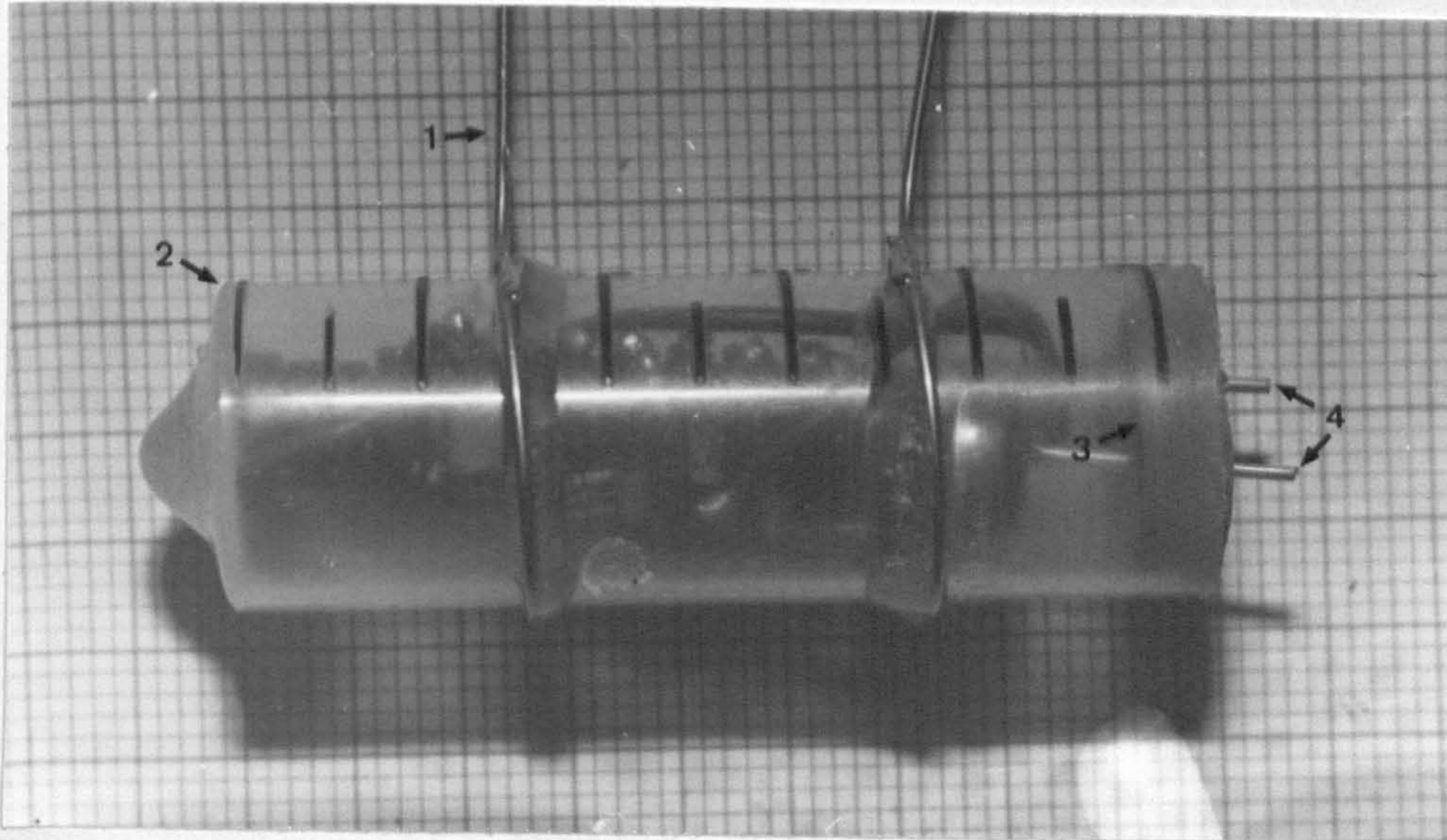
Plate III.2 View of encapsulated transmitter.

The silver wires used to anchor the transmitter to the fish are seen at (1). The transmitter is encapsulated in a cut-down 5 ml polypropylene syringe barrel (2) and filled with light paraffin through the rubber bung with a hypodermic needle. The input connections are produced through the bung (3), to the terminals (4). Scale in mm.

Plate III.3 View of Rainbow Trout equipped with early version of EMG transmitter (in silicone rubber encapsulation) and "T" electrodes implanted into red fibres of the m. adductor mandibulae.



PL III. 2



PL III. 3

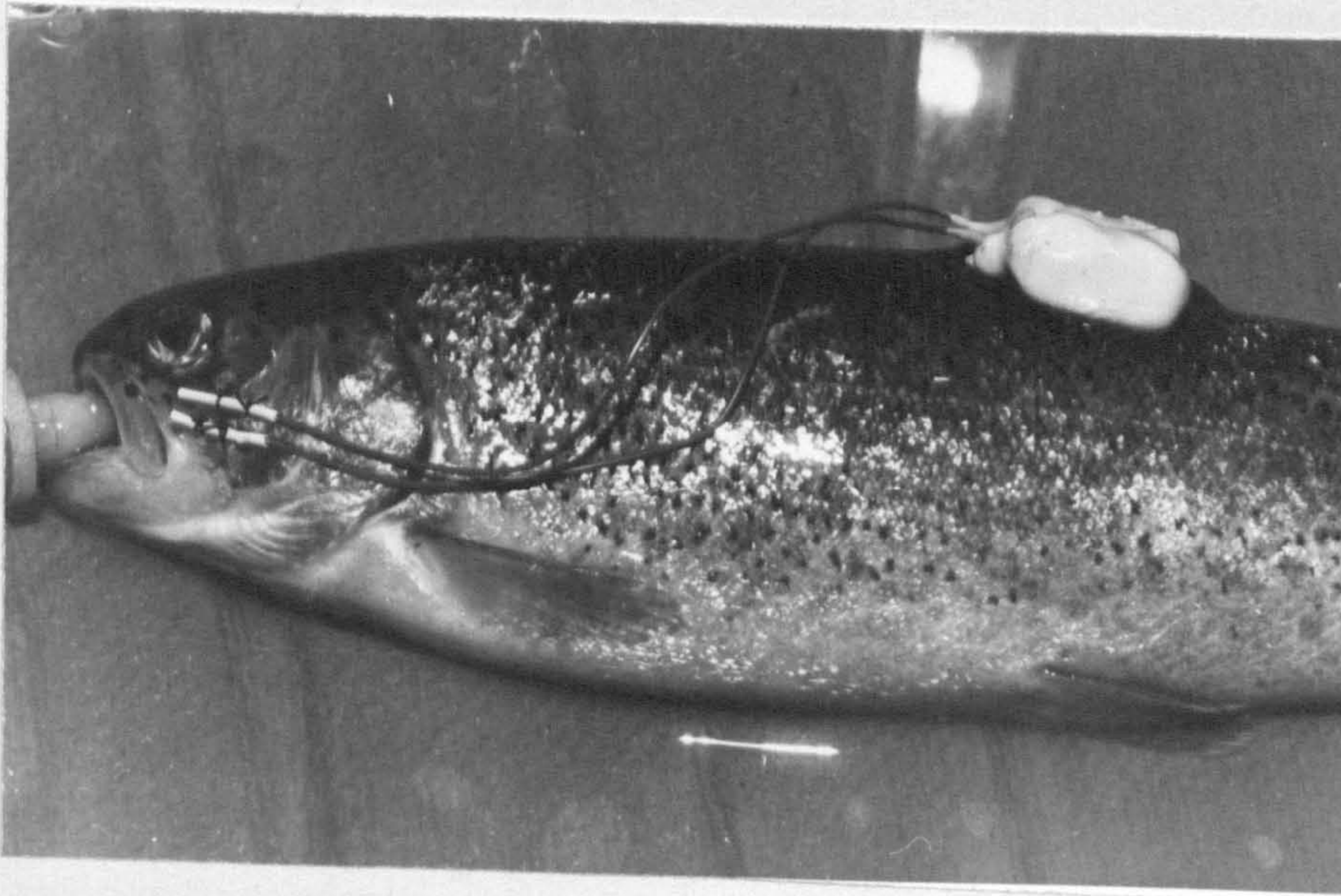
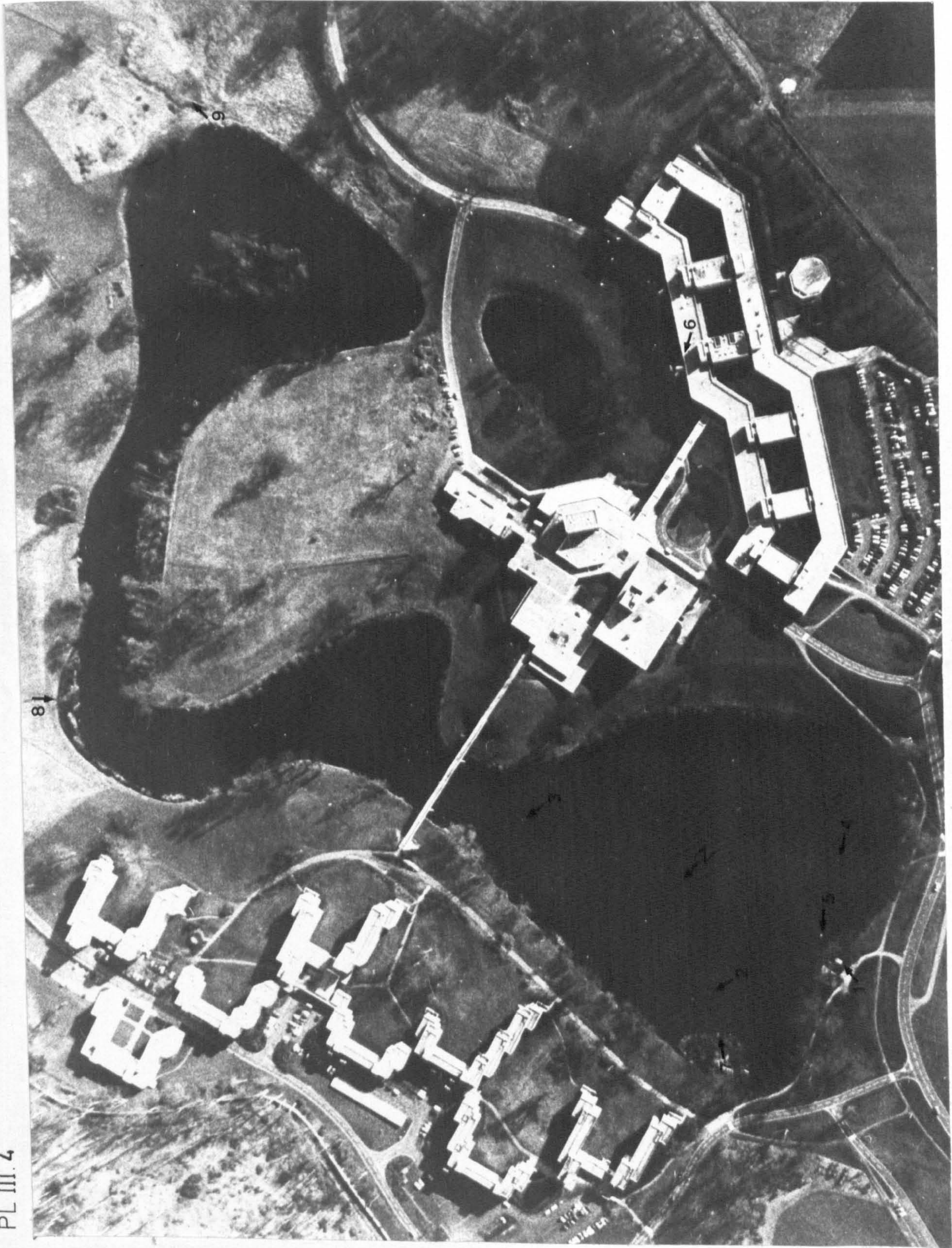


Plate III.4 Aerial view of Airthrey Loch and the University Campus. Tracking experiments are confined to the West Pool. The control caravan is located at (1) and underwater cables control and receive signals running out to the Green (2), Red (3) and Yellow (4) hydrophone stations. The cage used to hold specimens prior to tagging is at (5) whilst the laboratory used to implant ultrasonic EMG transmitters is at (6). The Loch has its main source of water entering at (7) with a smaller and less reliable inflow at (8). Drainage is entirely by permeation or else by the culverted stream (9).



PL III. 4

APPENDIX I

Copies of Published papers in which work in this
thesis has been incorporated.

INJECTION ANAESTHESIA FOR EXPERIMENTAL STUDIES IN FISH

R. L. OSWALD

Department of Biology, University of Stirling, Scotland

(Received 18 July 1977)

Abstract—1. Eight anaesthetic drugs were tested by intramuscular or intraperitoneal injection in trout.

2. The alphaxalone/alphadolone acetate mixture (Saffan) produced superior long-duration anaesthesia though assisted ventilation was sometimes required.

3. Propanidid (Epontol) was considered to be the best short-acting agent tested.

4. The neuroleptanalgesic combination of etorphine HCl/acetylpromazine maleate (Immobilon) gave, qualitatively, the best anaesthesia of all the drugs tested and was immediately reversible by injecting the antagonist diprenorphine HCl (Revivon).

5. Spinal anaesthesia with lignocaine HCl (Xylocaine) was found to be another effective means of immobilizing fish.

INTRODUCTION

Fish have become increasingly popular as subjects for physiological experiments in recent years. Virtually any experimental procedure demands some form of restraint, chemical or physical, owing to the problems associated with the aquatic habitat of the animal. The most popular method of producing anaesthesia is by immersing the fish in a solution of the drug. The thin gill membranes act as the site of absorption of the drug, which then passes directly into arterial blood. This is analogous to inhalational anaesthesia in the mammal. On being returned to or irrigated with clean water, the fish excretes the drug back across the gills and recovers rapidly (Hunn & Allen, 1974).

For experimental work, however, long periods of anaesthesia are often desirable. This can be achieved by using a recirculating anaesthetic system to provide continuous inhalational anaesthesia. It is desirable to include cooling, re-aeration and stirring in such reservoir systems and so they are expensive to construct. The drugs commonly used have certain disadvantages. MS-222 (ethyl-*m*-aminobenzoate methane sulphate) is irritant to fish and sometimes to humans. It is also quite expensive. Benzocaine, (ethyl-*p*-aminobenzoate) is infinitely cheaper, more potent and less stressful for the fish (Laird & Oswald, 1975). 2-Phenoxyethanol produces respiratory depression, yet has poor analgesic properties. Thus it is unsuitable if assisted ventilation is not available and pain reflexes are often evident upon incision (Oswald, unpublished observations).

Barbiturates such as amylobarbitone and pentobarbitone may also be used in solution, but their relatively high cost is disadvantageous, though Durve (1975) has shown several to be of value in the transport of fish fry. Quinaldine has been used by many workers but recently has been shown to induce cataracts in salmonid fish (R. H. Richards, personal communication). The most potent fish anaesthetic is propoxate (Janssen) (Thienpont, 1965). This is seldom used owing to the extremely high cost of the drug.

In most laboratories, ships and aquaria it is gener-

ally easy to supply fish with a stream of freshwater at their living temperature. In a recirculatory anaesthetic system, unless cooling is provided, the anaesthetic will equilibrate with the ambient temperature which may cause heat stress. Most drugs degrade rapidly on standing and have to be discarded; benzocaine is particularly susceptible to this and the degradation process is generally accelerated by elevated temperatures.

The alternative is to use injectable anaesthesia. This has been used by Levy (1928), Keys & Wells (1930), Oets (1950) and Young (1971), and was achieved with various barbiturates. In the last ten years several new medical and veterinary agents have become used routinely in human and animal surgery, though they do not appear to have been seriously tried in fish. This investigation has set out to evaluate a range of injectable anaesthetics which may have great practical value to experimental workers.

MATERIALS AND METHODS

Brown and Rainbow trout (*Salmo trutta*, L.) and (*Salmo gairdneri*, Richardson) were used in the tests. Fish ranging in weight from 100–500 g were held in 800 l. circular tanks for several months before experimentation. Before injection, the fish were immersed in a solution of benzocaine (50 mg/l). Within 30 sec the fish were sufficiently sedated to permit weighing and the subsequent injection of the calculated anaesthetic dose. The injection route was usually intraperitoneal (i.p.), though some injections were intramuscular (i.m.) or a combination of i.p. and i.m. methods.

Following injection the fish were immediately replaced in their tank to recover from the brief benzocaine sedation. The subsequent induction with the injected drug usually proceeded within 5–10 min of this. Control fish were sham-injected with sterile 0.8% sodium chloride solution.

In experiments where apnoea ensued, artificial ventilation was instituted by placing the fish in the holder of the anaesthetic table and passing a stream of freshwater over the gills until spontaneous respiration returned. The water was at the fish's holding-temperature (10°C) thus avoiding sub-lethal heat stress (Wedermeier, 1973).

The drugs tested in this investigation were ketamine hydrochloride (Vetalar Parke, Davis), etorphine hydrochloride/acetylpromazine maleate (Large Animal Immobilon Reckitts), propanidid (Epontol Bayer), xylazine hydrochloride (Rompun Bayer), alphaxalone/alphadolone acetate (Saffan Glaxo), chlorpromazine hydrochloride (Largactil-May and Baker), pentobarbitone sodium (Nembutal-Abbott) and benzocaine (Aldrich Chemical Co.).

In many cases the ECG was recorded using subcutaneous stainless-steel electrodes in the axillary position and the potentials were amplified, displayed on a "CEPTU" unit (Epil Products Ltd) and recorded on a high-speed pen recorder (Devices MX212).

In two small Rainbow trout (120 and 150 g in weight), spinal anaesthesia was attempted under light benzocaine sedation. A 25 s.w.g. needle was introduced vertically downwards from the dorsal midline surface, at a point about 1 cm posterior to the occipital process. The needle tip was moved until a gap between the vertebrae was found, after which it was gently advanced until a twitch indicated contact with the spinal cord. 0.1 ml of lignocaine hydrochloride 2% solution (Xylocaine-Astra) was deposited, and the fish returned to water.

One of the drugs tested (Saffan) proved so useful that it was employed practically in a number of surgically severe experiments. The experimental protocol and results are briefly described to illustrate the kind of work in which it can be successfully employed.

In five Brown or Rainbow trout alphaxalone was given at a dose rate of 36 mg/kg. The fifth cranial nerve was exposed after enucleation and stimulated proximally to its point of entry to the orbit. The evoked potentials from the red and white portions of the m. adductor mandibulae (Oswald, 1977, in preparation) were led off using a bipolar pair of 125 μ m insulated stainless-steel electrodes (Trimel-Johnson Mathey). The twitch tension was measured by an isometric transducer (Devices 4151) attached by a suture to the mandibular apex. In these experiments the results were recorded via the CEPTU unit using differential d.c. amplification and were photographed from the screen of a storage oscilloscope (Telequipment DM53A), in addition to the pen recorder.

In collaboration with my colleague, L. G. Ross, three trout were used in pilot experiments to determine regional blood flow in various organs using the Sephadex Microsphere method (Pharmacia A/S). Under alphaxalone anaesthesia at 36 mg/kg, the tail was severed and the dorsal aorta cannulated as a reference organ. The pericardium was opened and 100 μ l of ^{58}Co -labelled microsphere suspension was injected intraventricularly. After allowing for the circulation time, the animal was sacrificed and the relevant organs removed for scintillation counting. The ECG was continuously monitored.

RESULTS

All the drugs tested possessed anaesthetic activity upon parenteral administration, with the exception of benzocaine and chlorpromazine.

Ketamine hydrochloride (Vetalar)

Six fish were injected with ketamine at doses of 130 and 150 mg/kg. At 130 mg/kg, anaesthesia only

persisted for 20 min but at 150 mg/kg, it lasted between 50 and 80 min. The veterinary product concentration is 100 mg/ml so intramuscular injection was used. (In fish, owing to the skin rigidity, intramuscular injections larger than 0.2–0.3 ml are not recommended owing to reflux out of the puncture.)

In two fish, apnoea occurred and the fish required assistance. In spontaneously ventilating fish, loss of tone in the adductor mandibulae muscle caused inefficient reciprocatory ventilation as evidenced by dye injected into the buccal cavity.

The recovery period was prolonged, taking up to 90 min before completion, and was characterized by excitement and ataxia. The ECG from a ketamine-anaesthetized trout is shown in Fig. 1 and is normal for the size of fish.

Propanidid (Epontol)

Seven trout were injected with propanidid at dose rates of 80–500 mg/kg. The optimal dosage lies between 300 and 350 mg/kg. At 300 mg/kg, the sleep time was variable, lasting from 30 to 120 min. 325 mg/kg, however, reliably produced a sleep time of 2½ hr.

The outstanding feature of propanidid was its minimal respiratory depressant effects. Even at 325 mg/kg, no fish were apnoeic though ventilation was reciprocatory. In one case at this dosage, Cheynes-Stokes ventilation was noted.

In one fish injected with 500 mg/kg, apnoea ensued and anaesthesia lasted 180 min, from which the fish made an excellent recovery.

Recovery from propanidid was very good. The initial return of sensitivity to vibrational stimuli was succeeded by a transitory ataxic phase. The fish then regained equilibrium and remained in a sedated condition on the tank bottom. Within 1 hr it had recovered, as evidenced by its reaction to an unconditioned visual stimulus.

The ECG with propanidid is normal for the species (Fig. 2).

Alphaxalone (Saffan)

Twelve fish were injected by the i.p. or a combination of $\frac{2}{3}$ i.p. $\frac{1}{3}$ i.m. routes with alphaxalone at doses ranging from 12 to 36 mg/kg.

Doses of 18 mg/kg and over produced anaesthesia, but lower doses produced sedation only. Sleep times ranged from 1 to 3 hr at 24 mg/kg to 4–6 hr at 36 mg/kg. Doses of 24 mg/kg and over always provoked apnoea and in spontaneously ventilating fish at lower doses, ventilation was reciprocatory.

Recovery from anaesthesia was marked by a slight phase of excitement, quickly succeeded by sedation. In most cases, recovery was complete within 2 hr.

The *in vivo* neuromuscular preparation showed no evidence of blockade of transmission. The prep-

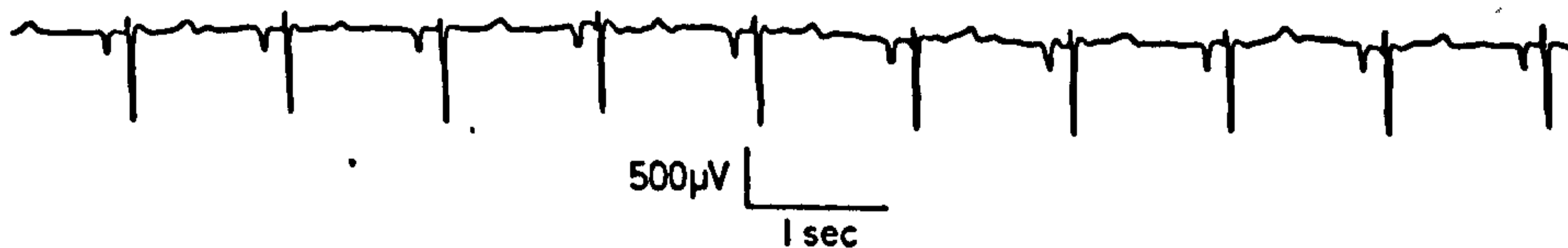


Fig. 1. ECG recorded from a Rainbow trout anaesthetized with ketamine HCl (150 mg/kg). Inversion of P and QRS waves is normal, due to small size of fish used. Heart rate: 53.5 beats/min.



Fig. 2. ECG recorded from Brown trout anaesthetized with propanidid (325 mg/kg). Heart rate: 42.2 beats/min.

arations lasted many hours and gave excellent, consistent results, an example of which is shown in Fig. 3.

In the experiments to determine blood flows, it was noted that if it was desired to calculate cardiac output and stroke volume, the heart rate should be constant.

Under alphaxalone anaesthesia it was seen that the ECG was extremely regular, showing neither the beat-to-beat variation demonstrated by Priede (1974) in the unanaesthetized fish, nor the dysrhythmias which can occur under prolonged anaesthesia with the aminobenzoates.

Cardiac inhibitory reflexes were still preserved under this anaesthetic; holding the operculae shut (Priede *op. cit.*) or virtually any manipulation of the fish caused cardiac inhibition and transient dysrhythmia (Fig. 4).

This was corrected by injection of 0.5 mg atropine sulphate (Sigma) into the red lateral muscles. This is a valuable technique as it is as rapid as intravenous

injection in the mammal (Oswald, unpublished observations). This reduced the amplitude of the QRS complex (Fig. 5). The gills of alphaxalone anaesthetized trout are a very bright red colour, the heart rate is fast for the temperature (Priede *op. cit.*) and the systolic contractions are extremely forceful, being clearly visible from the exterior of the fish.

Figure 6 shows that cholinergic blockage successfully regulates the heart rhythm upon test. Results of the regional flow experiments gave relative distributions of blood flow similar to those recorded by Cameron (1974) in the unanaesthetized Arctic grayling (*Thymallus arcticus*) under normoxia.

Xylazine hydrochloride (Rompun)

Xylazine was injected into five fish at dose rates of 40–125 mg/kg. The minimal anaesthetic dose was found to be 100 mg/kg and this consistently produced apnoea. Induction and recovery were marred by the

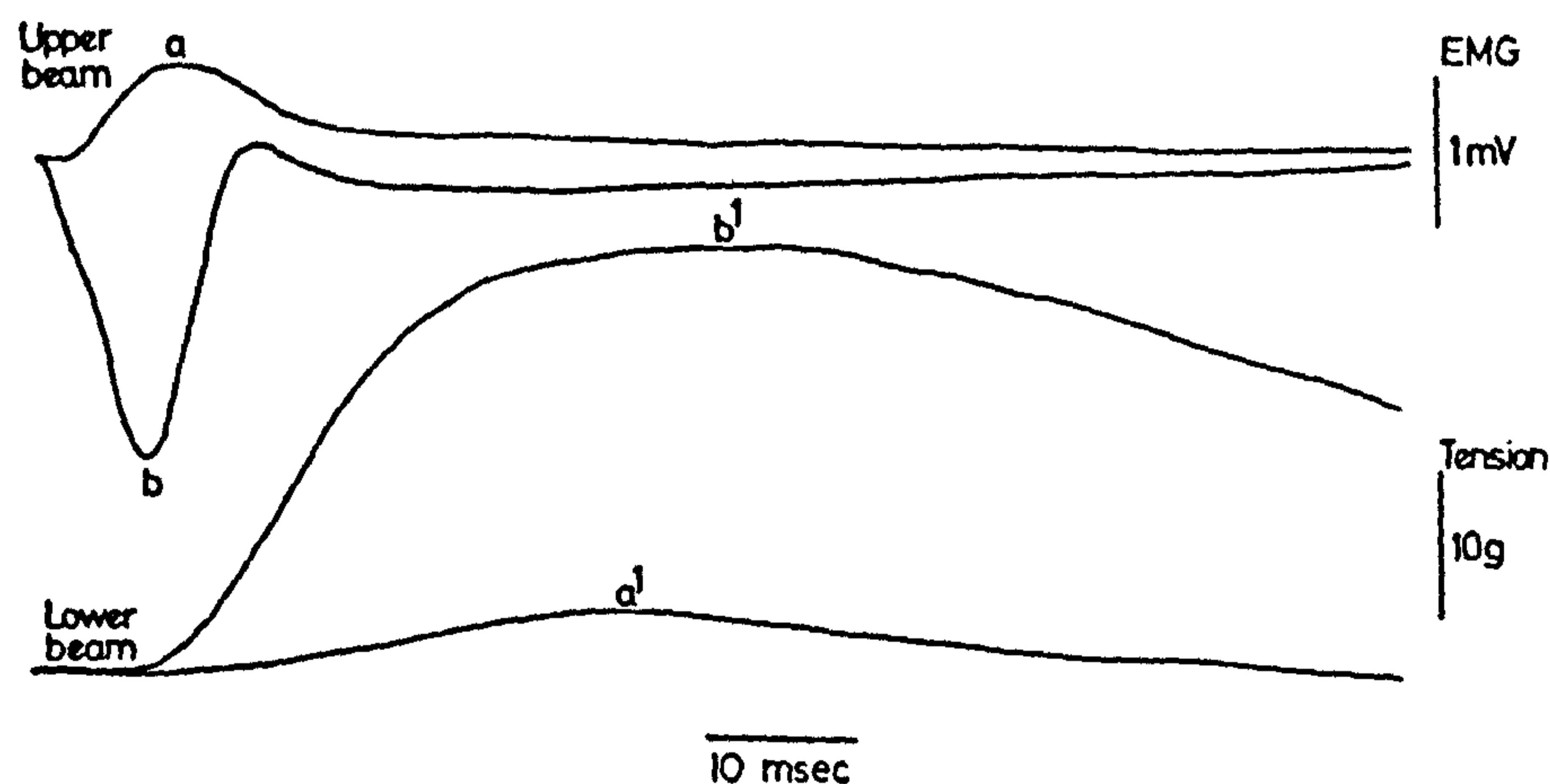


Fig. 3. Neuromuscular experiment; recorded from adductor mandibulae of Brown trout anaesthetized with alphaxalone/alphadolone, 36 mg/kg. Upper beam: EMG. Lower beam: Isometric tension. Motor nerve is stimulated with 50 μ sec rectangular pulses. At a and a' (threshold) monophasic potential and low tension rise are due to red fibre activation. At b and b' ($2 \times$ threshold) the mosaic fibres are recruited, giving large tension rise and large diphasic EMG potential.

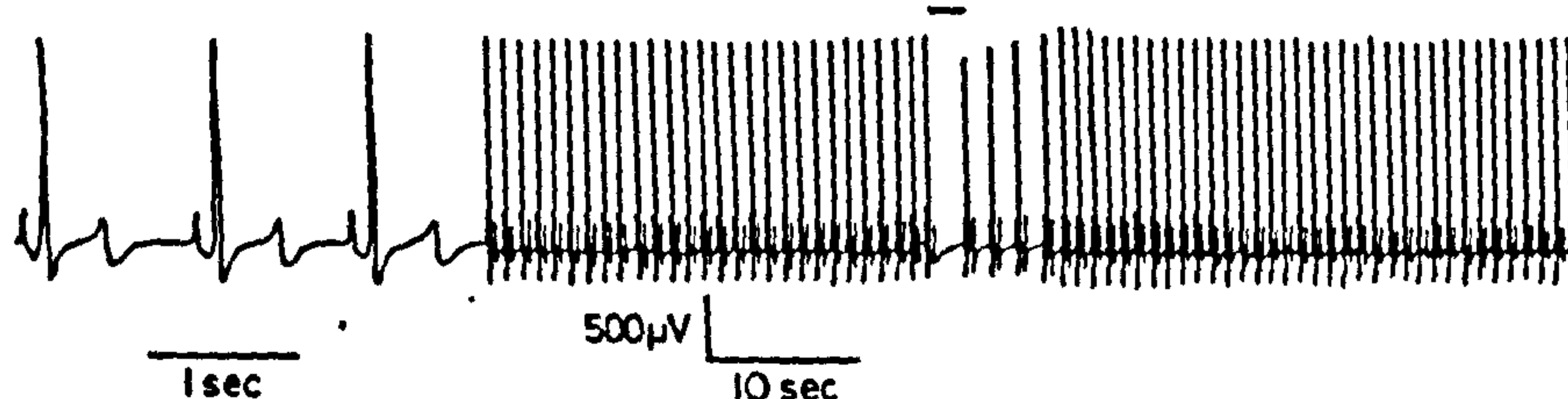


Fig. 4. ECG recording from Brown trout anaesthetized with alphaxalone/alphadolone acetate (36 mg/kg). Operculae were held shut for the duration of the black bar. Note cardiac inhibition followed by bradycardia, and also change of time scale. Heart rate: 56.6 beats/min.

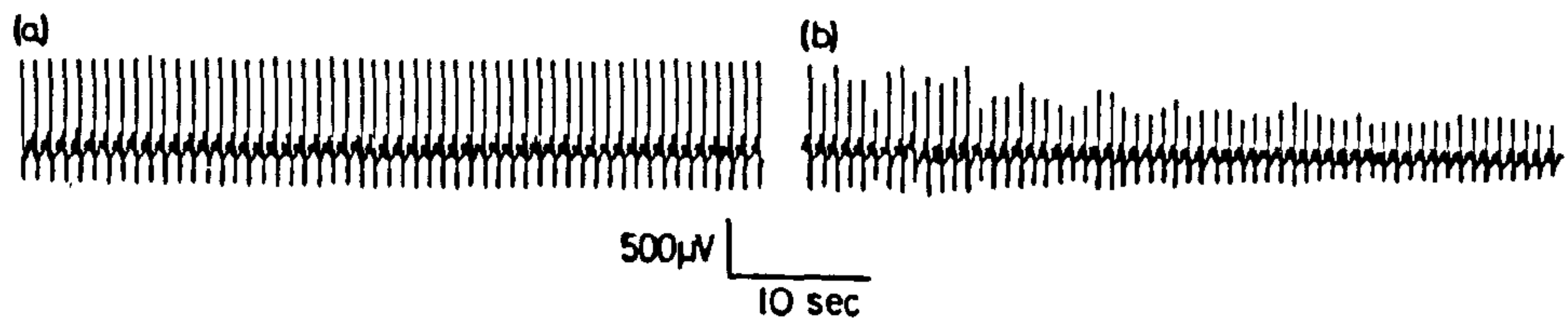


Fig. 5. ECG recording from Brown trout anaesthetized with alphaxalone/alphadolone acetate (36 mg/kg) (a) before and (b) after atropine sulphate injection. Note diminution of QRS complex.

appearance of convulsant activity. It was difficult to ensure artificial ventilation because the clonic convulsions frequently dislodged the water supply of the fish. Co-ordinated spontaneous ventilation did not reappear until much later, so fish would only survive if given an additional injection of pentobarbitone, 48 mg/kg, which has long-duration anticonvulsant activity.

Xylazine also caused gross ECG disturbances, including changes in duration of P, QRS and T waves (Fig. 7a). Atropine, 0.5 mg, partially corrected this (Fig. 7b) and the ECG became normal during recovery when convulsions re-appeared (Fig. 7c).

Pentobarbitone sodium (Nembutal)

Pentobarbitone was given to four fish in doses ranging from 30 mg/kg to 72 mg/kg by the i.p. route. Doses of 30 mg/kg produced sedation only, without loss of equilibrium. At 48 mg/kg the fish were apnoeic for nearly 6 hr and one fish given 72 mg/kg was anaesthetized until the following day, yet subsequently recovered. Recovery from pentobarbitone was very pro-

longed and characterized by persistent ataxia. Sedation was evident the following day in fish given 48 mg/kg and, at 72 mg/kg, effects were noticeable 2 days later.

The ECG in one fish showed a marked bradycardia (Fig. 8).

Etorphine/acetylpromazine (Large Animal Immobilon)

Initial injections based on mammalian potency were without effects. Following the very high dosages employed by Wallach & Hoessle (1970) in frogs, doses of 1.25–10 mg/kg were tried. Rates of 8–10 mg/kg were found to be effective. Three fish were immobilized by this neuroleptanalgesic method which produces effects slightly different from conventional anaesthetics. The fish lie immobile and cutaneous pain sensation is abolished, but the eye still performs saccadic movements. The gill ventilation flow is unidirectional as shown by dye injection.

Neuroleptanalgesia was allowed to continue for 30 min in two fish and 1 hr in a third. It was then abolished by injection of an equal volume of the

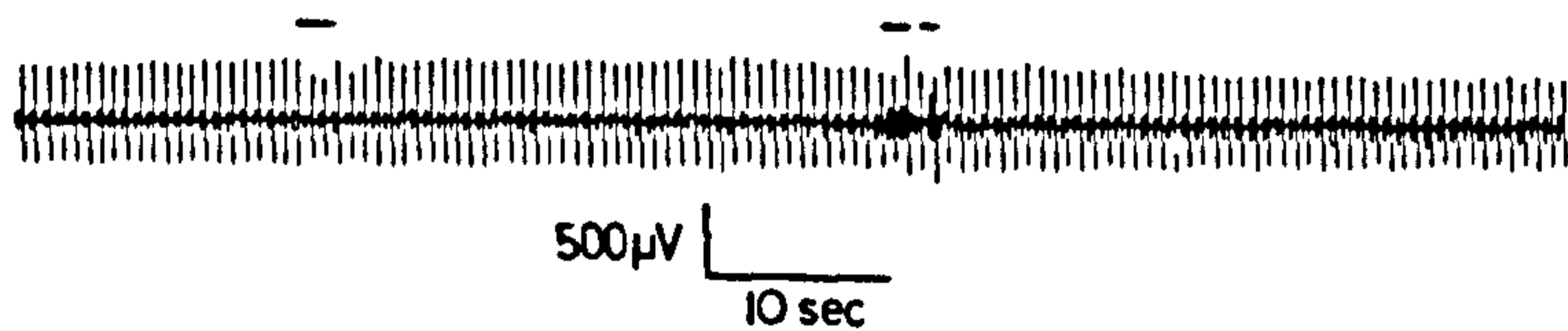


Fig. 6. ECG recording as in Fig. 6 (b). For the duration of the black bars the operculae were held shut. Cholinergic blockade abolishes the cardiac inhibitory reflex associated with this manoeuvre.

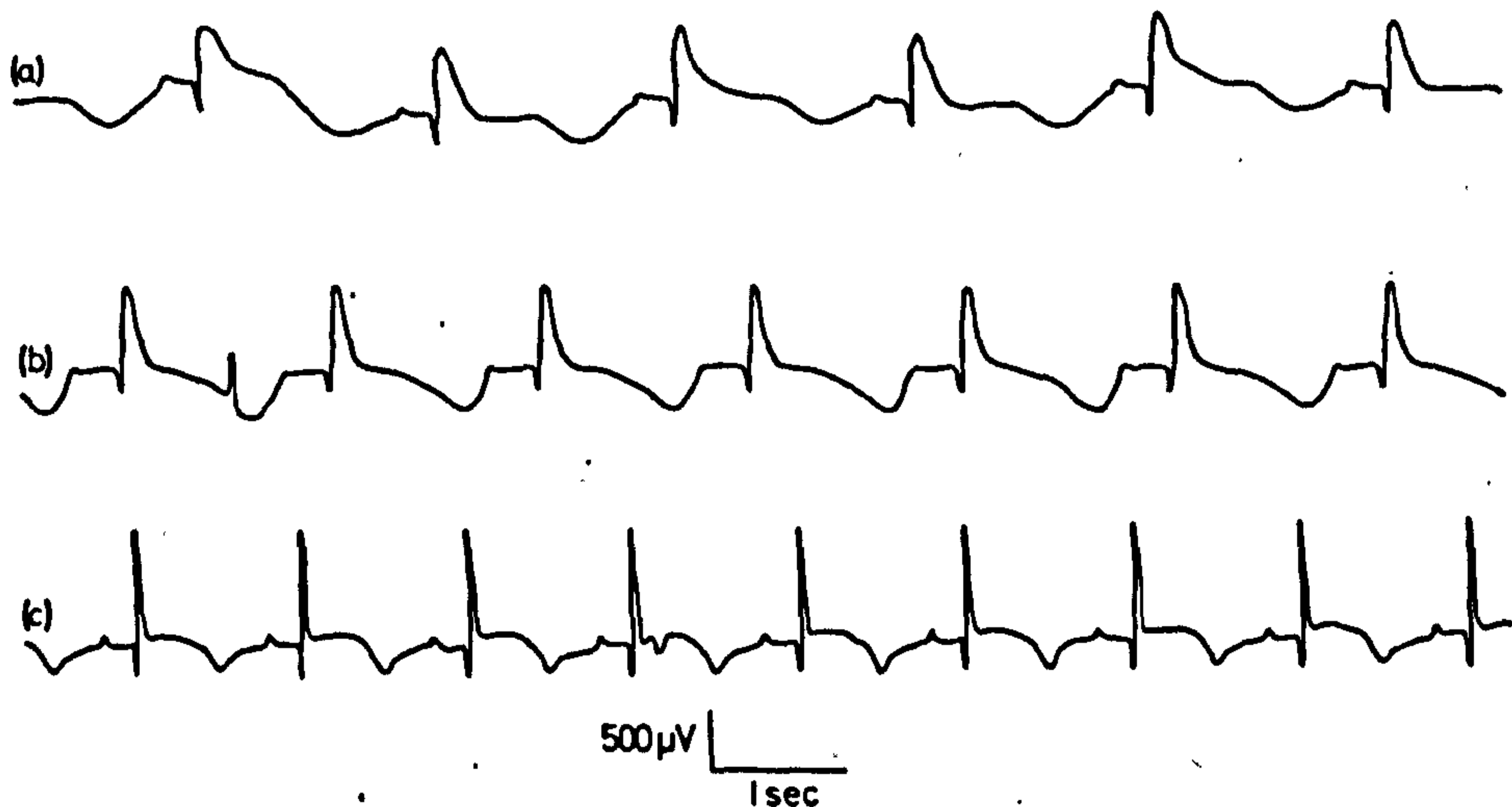


Fig. 7. ECG recordings from Rainbow trout anaesthetized with xylazine HCl (100 mg/kg) (a) 30 min after injection. (b) After 0.5 mg atropine, 5 min after (a). (c) At beginning of recovery (1 hr). Note exaggerated duration of P, QRS and T waves in (a), partial correction in (b) and return to normal in (c). Heart rate in (a): 33.3 beats/min, in (b): 47.6 beats/min.

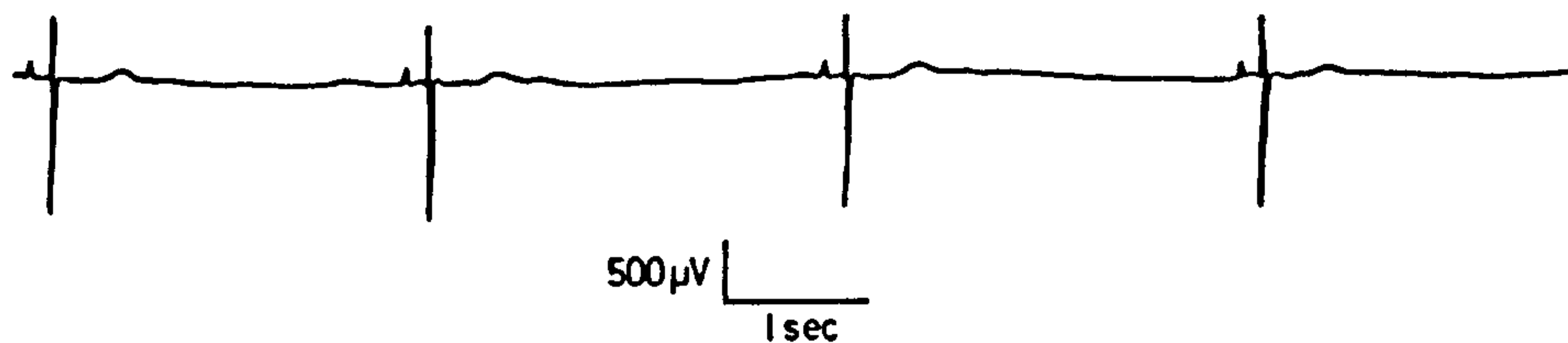


Fig. 8. ECG recorded from Brown trout anaesthetized with pentobarbitone sodium (48 mg/kg). Note characteristic intense bradycardia. Heart rate: 20.7 beats/min.

specific antagonist, diprenorphine hydrochloride (Large Animal Revivon, Reckitt) given by deep i.m. injection in two divided doses.

Within 5 min of diprenorphine administration, the fish suddenly regained equilibrium. This was followed by a transitory ataxic phase and within 6 min the fish were swimming normally. They showed no signs of sedation and were immediately responsive to an unconditioned visual stimulus.

The ECG was reasonably normal, though "tenting" of P and T waves was noted. (Fig. 9).

Lignocaine hydrochloride (Xylocaine)

Spinal anaesthesia was successfully achieved in both fish by 0.1 ml Lignocaine injection. The onset of anaesthesia was very rapid, immobilization occurring in 5 min. The extraocular muscles performed saccadic movements and ventilation was normal and efficient. Recovery took place rapidly between 45 and 50 min after injection, the first signs being the return of motor activity in the pectoral fin muscles. 10–15 min after this, the fish were swimming normally and would avoid an unconditioned visual stimulus. There was no apparent "hangover" type sedation. The ECG was not measured in these cases.

Other drugs

Benzocaine, dissolved in propylene glycol, injected i.p. at 120 mg/kg, had no effect in one fish. Chlorpromazine hydrochloride injected i.p. into two fish at 50 and 100 mg/kg was similarly ineffective.

Control fish

Four control fish recovered from the benzocaine sedation weighing and sham injection within 3 min, the fifth recovered in 5 min and all were slightly sedated, but this passed off in a few minutes.

DISCUSSION

Four of the drugs tested may be particularly useful in experimental studies of fish.

Alphaxalone (Saffan)

Alphaxalone always gave long sleep-times. It was difficult to give a dose which simultaneously abolished locomotion yet preserved normal ventilation. For long-duration, acute experiments, with or without recovery, it is the drug of choice. Its advantage lies in its chronotropic and inotropic stimulatory effects on the heart coupled with a vasodilatory action upon the gill capillaries. This seems to ensure adequate oxygenation of the blood, in contrast to the aminobenzoates which cause vasoconstriction and generally produce hypoxia (Soivio *et al.*, 1977).

Recovery from alphaxalone was excellent, leaving none of the prolonged hangover effects of the barbiturates. An added advantage over the barbiturates is the lack of any peripheral neuromuscular blocking effects. These are well-known in pentobarbitone, (Seyama & Narahashi, 1975), phenobarbitone (Proctor & Weakly, 1976), thiopentone (Kraatz & Gluckman, 1954). Alphaxalone has been shown to possess no such effects in the cat anterior tibialis preparation (R. Curtis, personal communication). This was also borne out in the trout adductor mandibulae experiment. Previously this experiment was unsuccessful because pentobarbitone sodium was used in a dose (48 mg/kg) sufficient to inhibit efferent traffic in the 5th cranial nerve. The blood volume of fish is low—3–5% of body weight compared to 8% for mammals. In a 250 g trout there would be 12 mg of pentobarbitone distributed in 12.5 ml blood, giving a concentration of 0.96 mg/ml for the blood. The lymph volume is larger than the blood (Wardle, 1971) and so the drug may be more dilute than this theoretical maximum.

Thesleff (1956) showed that 100% block could be caused in the isolated frog sartorius by 0.2 mg/ml pentobarbitone, so it is not surprising that fish heavily anaesthetized with pentobarbitone are unsuitable for *in vivo* neuromuscular preparations.

It is suggested that alphaxalone anaesthesia could replace the paralysis by curariform drugs used by many workers in fish neurophysiology. This is generally done to immobilize the fish, yet leave afferent nerve traffic intact. The objection to the aminoben-

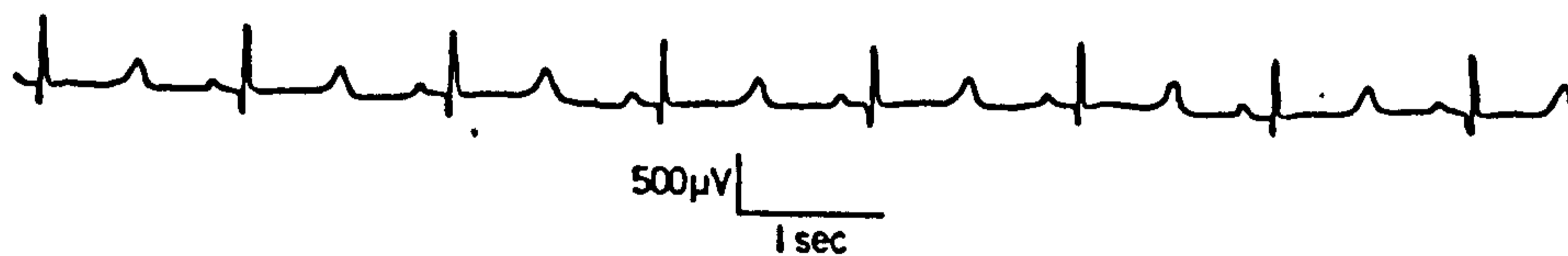


Fig. 9. ECG recording from Brown trout anaesthetized with etorphine HCl/acepromazine maleate (8 mg/kg). Note the rather "tenting" T waves. Heart rate: 45.4 beats/min.

zoates may be that they are local anaesthetics and may therefore alter sensory processes in organs like the olfactory rosette. It would, of course, be more humane to use anaesthesia rather than neuromuscular blockade, especially in cases where severe surgery is contemplated. Afferent traffic in the lateral line nerve of the saithe *Pollachius virens* L. was found to be preserved under alphaxalone anaesthesia and preparations could be kept viable for extended periods in contrast to decerebrate preparations. (P. Tytler, personal communication). Extension of the sleep-time could be effected by using higher or repeated doses, as alphaxalone has a high therapeutic index (30) and does not produce cumulative dosage effects (Child *et al.*, 1971).

Propanidid (Epontol)

Propanidid was a less potent anaesthetic in the trout than alphaxalone, but it may be a useful drug for short procedures, particularly where a fairly rapid recovery is desired. Clinically, propanidid's chief virtue is that the drug is rapidly deactivated by plasma esterases into two inert metabolites. This means that it is of ultra-short action (3–4 min) and possesses no persistent after-effects in contrast to the barbiturates (Clarke & Dundee, 1966).

The period of action in fish was much longer than in the mammal. This could be due to several factors but, like all the drugs tested here, the mode of inactivation in fish is unknown and needs investigation.

Effective mammalian doses are in the 8–9 mg/kg range (Bayer Ltd, packing literature) whereas effective doses in trout were much higher (325 mg/kg). If inactivated like the inhalational agents, such as the amino-benzoates, the gills are the site of excretion. Clearance of such drugs is usually rapid, with plasma half-lives of about 6 min (Hunn & Allen, 1974). Using intraperitoneal injections creates a depot of drug which must prolong the plasma half-life, in contrast to the inhalational agents which only have to be cleared from plasma, fat, brain, etc. If, on the other hand, plasma esterases are responsible, the rate of inactivation would be very much slower owing to the ambient temperature. Although pseudocholinesterases may not be the enzymes responsible for propanidid inactivation in the mammal (Clarke *et al.*, 1967), fish are noted for the fact that the drugs suxamethonium (Ballintijn, 1969) and decamethonium (Oswald, unpublished observations) are irreversible, indicating low pseudocholinesterase activity.

Though propanidid has no marked actions of its own, it does potentiate neuromuscular blocking drugs. Ellis (1968) showed that this was probably due to generalized muscle cell partial depolarization. This should be borne in mind if contemplating its use in neuromuscular experiments.

Etorphine/acetylpromazine (Immobilon)

This drug combination was very interesting as there is little evidence of morphinomimetic drugs being tried on fishes. McFarland (1959) found morphine sulphate to be ineffective in the mosquito-fish *Gambusia*.

There is probably little effect due to the phenothiazine component as other neuroleptics of this group

are ineffective (McFarland, *op cit.* Jolley *et al.*, 1972). The narcosis is reversed dramatically after the administration of the specific antagonist, diprenorphine, which has no effect on the phenothiazines. No other drug tested in this work offers such a rapid return to "normality" as etorphine. All others have a variable period of sedation following the regaining of equilibrium, during which the fish is unresponsive to visual stimuli.

The advantage of the neuroleptanalgesic technique in the mammal is that the drugs used are so potent that the minimal amounts necessary cause much less metabolic stress, compared to conventional anaesthesia. Whilst the amounts used in the fish are relatively small (8–10 mg/kg) they would be fatal if accidentally injected into a human. Because of this danger it cannot be recommended for routine use in fish except by workers familiar with the usage and treatment of accidental injections of such very potent drugs.

Ketamine hydrochloride (Vetalar)

Although it could be useful as a short-duration anaesthetic, and also had the advantage of being a concentrated solution permitting intramuscular injection, the main disadvantage with ketamine was its long recovery period with excitement. The related cyclohexylamine drug phencyclidine (Sernylan—Parke, Davis) was not tested. In mammals it is more potent and longer acting than ketamine. Chen *et al.* (1959) found phencyclidine to be effective by inhalation in *Betta splendens* at 50 mg/l, though Jolley *et al.* (1972) found no such effect at 20 mg/l.

Xylazine hydrochloride (Rompun)

Although effective as a fish anaesthetic, xylazine cannot be recommended for use owing to its convulsant activity during induction and recovery.

Pentobarbitone sodium (Nembutal)

This drug has been in use for fifty years in mammalian research despite its many disadvantages which include cardiovascular changes (Goldberg *et al.*, 1968) and the marked curariform action mentioned previously. In fish it may be of use for long-duration experiments but it seems to be a cardiac depressant. Long recovery and sedation limit its usefulness for recovery experiments. The short-acting barbiturates in common use, thiopentone sodium (Pentothal) and methohexitone sodium (Brietal) are normally used by intravenous injection. Thiopentone is very irritant and causes necrosis at the injection site if given by the intramuscular route. In *Perca fluviatilis* the author's limited experience with thiopentone given by the intraperitoneal route gives an anaesthesia of approx 1 hr, with a recovery period rather like that of pentobarbitone. This is not altogether surprising as the short action of the thiobarbiturates is due to their rapid uptake by fat depots (Hall, 1971) which the perch would not possess to any great extent. Methohexitone may be a worthwhile drug to explore as a short-acting fish anaesthetic as its duration of action is governed by its rate of uptake by muscle

(Lilly & Co. Ltd. Literature). Green (1975) notes that it can be given by the intraperitoneal route in small animals.

Lignocaine hydrochloride (Xylocaine)

Spinal anaesthesia with Xylocaine was a very effective method of immobilizing fish, though it was only attempted on two small individuals in this investigation. The head is not affected by the block but the body caudal to it could be operated upon. The duration of the drug could be increased by using the preparation with adrenaline as a vasoconstrictor. Alternatively, the newer local anaesthetics could be tried, e.g. bupivacaine hydrochloride (Marcain B.D.H.) which is effective for up to approx 12 hr in humans (Martindale, 1972). The technique adopted here minimized the passage of drug along the extradural space to avoid respiratory depression and other central effects. Kisch (1950), however, used intracranial injections of procaine to anaesthetize a range of elasmobranchs and teleosts for up to an hour with recovery afterwards, yet preserving circulation and ventilation. Clearly this technique and spinal anaesthesia warrant further investigation as experimental tools, though the latter may be quite stressful to the fish as it will still perceive sensory stimulation from its semicircular canals, eyes, and possibly from its lateral lines, whilst immobilized.

This preliminary study will, the author hopes, stimulate further research into the actual physiological effects of these drugs, because their usefulness is limited by the amount of such data which is available to the experimenter. The work of Houston *et al.* (1971 a, b; 1973), Houston & Woods (1972) and, more recently, Soivio *et al.* (1977) has shown that the effects of anaesthetic methods cannot be ignored or taken for granted.

Acknowledgements—The author gratefully acknowledges the gifts of the drugs used in this study from Parke-Davis Ltd, Reckitt & Colman Pharmaceuticals, Bayer (U.K.) Ltd and Glaxo Ltd. Thanks are also due to Mr L. G. Ross for the microsphere experiments, Mr J. Hambrey for reading the Ms and finally Professor H. Meidner, in whose Department the work was carried out.

REFERENCES

- BALLINTUN C. M. (1969) The influence of proprioception upon respiratory neurons in the medulla oblongata of fishes. *Acta physiol. pharmac. néerl.* 15, 25–27.
- CAMERON J. N. (1974) Blood flow distribution as indicated by tracer microspheres in resting and hypoxic Arctic Grayling (*Thymallus arcticus*). *Comp. Biochem. Physiol.* 52A, 441–444.
- CHEN G., ENSOR C. R., RUSSELL D. & BOHNER B. (1959) The pharmacology of 1-(1-Phenylcyclohexyl) Piperidine. HCl. *J. Pharmac. exp. Ther.* 127, 241–250.
- CHILD K. J., CURRIE J. P., DODDS M. G., PEARSE D. R. & TWISSELL, D. J. (1971) The pharmacological properties in animals of CT 1341—a new steroid anaesthetic. *Br. J. Anaesth.* 43, 2–13.
- CLARKE R. S. J. & DUNDEE J. W. (1966) Survey of experimental and clinical pharmacology of propanidid. *Anesth. Analg. curr. Res.* 45, 250–262.
- CLARKE R. S. J., DUNDEE J. W. & HAMILTON R. C. (1967) Interactions between induction agents and muscle relaxants. *Anaesthesia* 22, 235–248.
- DURVE V. S. (1975) Anaesthetics in the transport of mullet seed. *Aquaculture* 5, 53–63.
- ELLIS F. R. (1968) The neuromuscular interaction of Propanidid with Suxamethonium and Tubocurarine. *Br. J. Anaesth.* 40, 818–824.
- GOLDBERG S. J., LINDE L. M., GAAL P. G., MOMMA K., TAKAHASHI M. & SARNA G. (1968) The effects of barbiturates on pulmonary and systemic haemodynamics. *Cardiovasc. Res.* 2, 136–142.
- GREEN C. J. (1975) Neuroleptanalgesic drug combinations in the anaesthetic management of small laboratory animals. *Lab. Anim.* 9, 161–178.
- HALL L. W. (1971) *Wright's Veterinary Anaesthesia and Analgesia*, 7th edn. Balliere & Tindall, London.
- HOUSTON A. H. & WOODS R. J. (1972) Blood concentrations of Tricaine Methane Sulphonate in Brook Trout, *Salvelinus fontinalis* during anaesthesia, branchial irrigation and recovery. *J. Fish. Res. Bd Can.* 29, 1344–1346.
- HOUSTON A. H., CZERWINSKI C. L. & WOODS R. J. (1973) Cardiovascular-respiratory activity during recovery from anaesthesia and surgery in Brook Trout (*Salvelinus fontinalis*) and Carp (*Cyprinus carpio*). *J. Fish. Res. Bd Can.* 30, 1705–1712.
- HOUSTON A. H., MADDEN J. A., WOODS R. J. & MILES H. M. (1971a) Some physiological effects of handling and Tricaine Methane Sulphonate anaesthesia upon the Brook Trout, *Salvelinus fontinalis*. *J. Fish. Res. Bd Can.* 28, 625–633.
- HOUSTON A. H., MADDEN J. A., WOODS R. J. & MILES H. M. (1971b) Variations in the blood and tissue chemistry of Brook Trout subsequent to handling, anaesthesia and surgery. *J. Fish. Res. Bd Can.* 28, 635–642.
- HUNN J. B. & ALLEN J. L. (1974) Movement of drugs across the gills of fishes. *A. Rev. Pharmac.* 14, 47–55.
- JOLLY D. W., MAWDSLEY-THOMAS L. E. & BUCKE D. (1972) Anaesthesia of fish. *Vet. Rec.* 91, 424–426.
- KEYS A. B. & WELLS N. A. (1930) Amytal anaesthesia in fish. *J. Pharmac. exp. Ther.* 40, 115–128.
- KISCH B. (1950) Electrographic investigations of the heart of fish. *Expl Med. Surg.* 6, 31–62.
- KRAATZ C. P. & GLUCKMAN M. I. (1954) The actions of barbiturates on the contractions of voluntary muscle. *J. Pharmac. exp. Ther.* 111, 120–129.
- LAIRD L. M. & OSWALD R. L. (1975) A note on the use of Benzocaine (Ethyl-*p*-aminobenzoate) as a fish anaesthetic. *J. Inst. Fish. Mgt* 6, 92–94.
- LEVY J. (1928) Hypnose et accoutumance des poissons du genre *Gobius* sous l'influence de quelques hynotiques de la serie barbiturique. *Cr. Soc. Biol.* 99, 1325–1326.
- MARTINDALE W. (1972) *The Extra Pharmacopeia*, 26th edn (Edited by BLACOW N. W.). Pharmaceutical Press, London.
- McFARLAND W. N. (1959) A study of the effects of anaesthetics on the behaviour and physiology of fishes. *Publ Inst. mar. Sci. Univ. Tex.* 6, 24–55.
- OETS J. (1950) Electrocardiograms of fishes. *Physiologia comp. Oecol.* 2, 181–186.
- PRIEDE I. G. (1974) The effect of swimming activity and section of the vagus nerves on heart rate in Rainbow Trout. *J. exp. Biol.* 60, 305–319.
- PROCTOR W. R. & WEAKLY J. N. (1976) A comparison of the presynaptic and postsynaptic actions of Pentobarbitone and Phenobarbitone in the neuromuscular junction of the Frog. *J. Physiol., Lond.* 258, 257–268.
- SEYAMA I. & NARAHASHI T. (1975) Mechanism of blockade of neuromuscular transmission by Pentobarbital. *J. Pharmac. exp. Ther.* 192, 95–104.
- SOIVIO A., NYHOLM K. & HUHTI M. (1977) Effects of anaesthesia with MS 222, neutralised MS 222, and Benzocaine

on the blood constituents of Rainbow Trout, *Salmo gairdneri*. *J. Fish Biol.* **10**, 91-101.

THESLIFF S. (1956) The effect of anaesthetic agents on skeletal muscle membrane. *Acta physiol. scand.* **37**, 335-349.

THIENPONT D. (1965) Immobilisation et anesthésie des Poissons. *Bull. Soc. r. Zool. Anvers* **35**, 11-18.

WALLACH J. D. & HOLSSIE C. (1970) M99 as an immobilising agent in poikilotherms. *Vet. Med* **65**, 163-167.

WARDLE C. S. (1971) New observations on the lymph sys-

tem of the plaice (*Pleuronectes platessa*) and other teleosts. *J. mar. biol. Ass. U.K.* **51**, 977-990.

WIDERMAYER G. (1973) Some physiological aspects of sublethal heat stress in the juvenile Steelhead Trout (*Salmo gairdneri*) and Coho Salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd Can.* **30**, 831-834.

YOUNG S. (1971) EMG activity in tench (*Tinca tinca*) gill lamellae and its association with coughing. *J. Physiol., Lond.* **215**, 37-39P.

The use of telemetry to study light synchronization with feeding and gill ventilation rates in *Salmo trutta*

R. L. OSWALD

Department of Biology, University of Stirling, Scotland

(Accepted 11 May 1978)

A technique is described whereby the electromyogram of the *m. adductor mandibulae* of brown trout is detected by implanted extracellular electrodes and used as the input signal for an ultrasonic transmitter attached externally to the fish. The periodic electrical activity of the muscle during ventilation is relayed by the transmitter using an analogue pulse system.

As the ventilatory electromyogram occurs in discrete rhythmic trains, it follows that alterations to this rhythm can be used to telemeter, instantaneously, single feeding events from a free swimming fish. Laboratory tests have shown that the feeding act is unequivocally distinct electromyographically from other manoeuvres such as 'coughing'.

Four adult brown trout have been equipped with this transmitter system and released in Airthrey Loch, Stirling. Using a tracking facility, feeding activity and ventilatory rhythms have been recorded for extended periods.

The results indicate the presence of three daily peaks of feeding activity, which is discussed in relation to changes in light levels. The telemetry records indicate that night feeding is a common occurrence in brown trout. In addition, ventilatory rates were found to be at or near resting levels.

I. INTRODUCTION

Since 1969 a static ultrasonic fish-tracking facility at Airthrey Loch, University of Stirling, has been employed to trace the movements of brown trout, *Salmo trutta* L. The system utilises directional position fixing on fish equipped with small ultrasonic transmitters (Young & Wiewiorka, 1975; Mitson & Young, 1975; Young *et al.*, 1976).

Much data have been obtained, using this system, on the activity and movement patterns of free-ranging trout and has been published in Young *et al.* (1972), Holliday *et al.* (1973) and Tytler *et al.* (1977). Two of the main findings were that trout in lochs exhibit very low mean hourly swimming speeds (<0.2 B.L. s^{-1}) and that they often show peaks of increased swimming activity, apparently related to dawn and dusk. Secondary peaks also occurred at mid-day and in individual fish, at times dissociated from solar cues. Such diel activity rhythms are well known from actographic studies on freshwater and marine fishes. (Spencer (1939), Hasler & Bardach (1949), Wikgren (1955), Swift (1962), Kruuk (1963), Davis (1964), Davis & Bardach (1965), Verheijen & de Groot (1967), Chaston (1968), Siegmund (1969), Gibson (1973), Reynolds (1976)).

Hoar (1942), found in young *Salmo salar* and *Salvelinus fontinalis*, that feeding frequently followed a diel rhythm with dawn and dusk peaks. It is tempting to ascribe the activity peaks revealed by the ultrasonic tracking, to feeding orientated movements. However in laboratory studies of activity cycles there is some conflicting data. Spencer (1939) *op. cit.* showed that an intense activity period in goldfish *Carassius auratus*, linked to their feed-time, continued for three hours despite the fact that they consumed

all their food in the first fifteen minutes. Swift (1964) *op. cit.* concluded from trout, that the presence of food was not the stimulus to locomotory activity. If the activity period was somehow linked to food searching one might expect such appetitive behaviour to be continuous in the absence of a consummatory response. Ware (1972), however, found in *Salmo gairdneri* that below a capture rate of approximately one item per 20s, food searching motivation declined. Foraging activity is therefore controlled by success in finding food. Landless (1976) believes that a positive feedback mechanism in Rainbow trout exists so that the initial capture of food increases the capture rate until antagonism by satiation sets in. In this case one would therefore expect locomotor activity to be increased by food finding and Siegmund (1969) *op. cit.* shows in the perch, *Perca fluviatilis* that locomotor activity is relatively depressed on days in which food is withheld.

To date no method has been devised which will telemeter feeding events from a free swimming fish. Priede & Young (1977), using the precursor of the transmitter described in this paper, attempted to relate cardioinhibitory reflexes, in Brown trout, to feeding events. They concluded that possibly due to inadequate recording methods, this was not a reliable indicator.

The method employed in this investigation to signal feeding events depends on telemetering the electromyogram from red fibres present in the parietal portion of the *m. adductor mandibulae* which is responsible for closing the mouth (Ballintijn & Hughes, 1965). It has been shown in elasmobranchs and teleosts (Oswald, 1978 *ms in prep.*) that only a small part of each cranial muscle is active during ventilation, corresponding to activity in discrete tracts of red (slow) muscle fibres. During feeding, the remaining white or mosaic (twitch) muscle is recruited to supply the acceleration needed during prey seizure. Osse (1969), Ballintijn *et al.* (1972) and Elshoud (see ref. list) Oldenhave & Osse (1976), have recorded E.M.G.'s from the cranial muscles of perch, *Perca fluviatilis*, carp *Cyprinus carpio* and ruff *Gymnocephalus cernua*. They have demonstrated an augmentation of electrical activity in certain muscles during feeding acts and it was intended that similar changes in the E.M.G. from the trout *adductor mandibulae* would serve as an indicator. The signal would be used to trigger analogue ultrasonic transmitters similar to those of Priede & Young (1977) *op. cit.* On the basis of work by Hughes & Ballintijn (1965), Ballintijn (1969), Hughes (1975) and Hughes & Adeney (1977) it was thought that respiratory manoeuvres not connected with feeding, such as the 'cough', would not be confused with feeding acts.

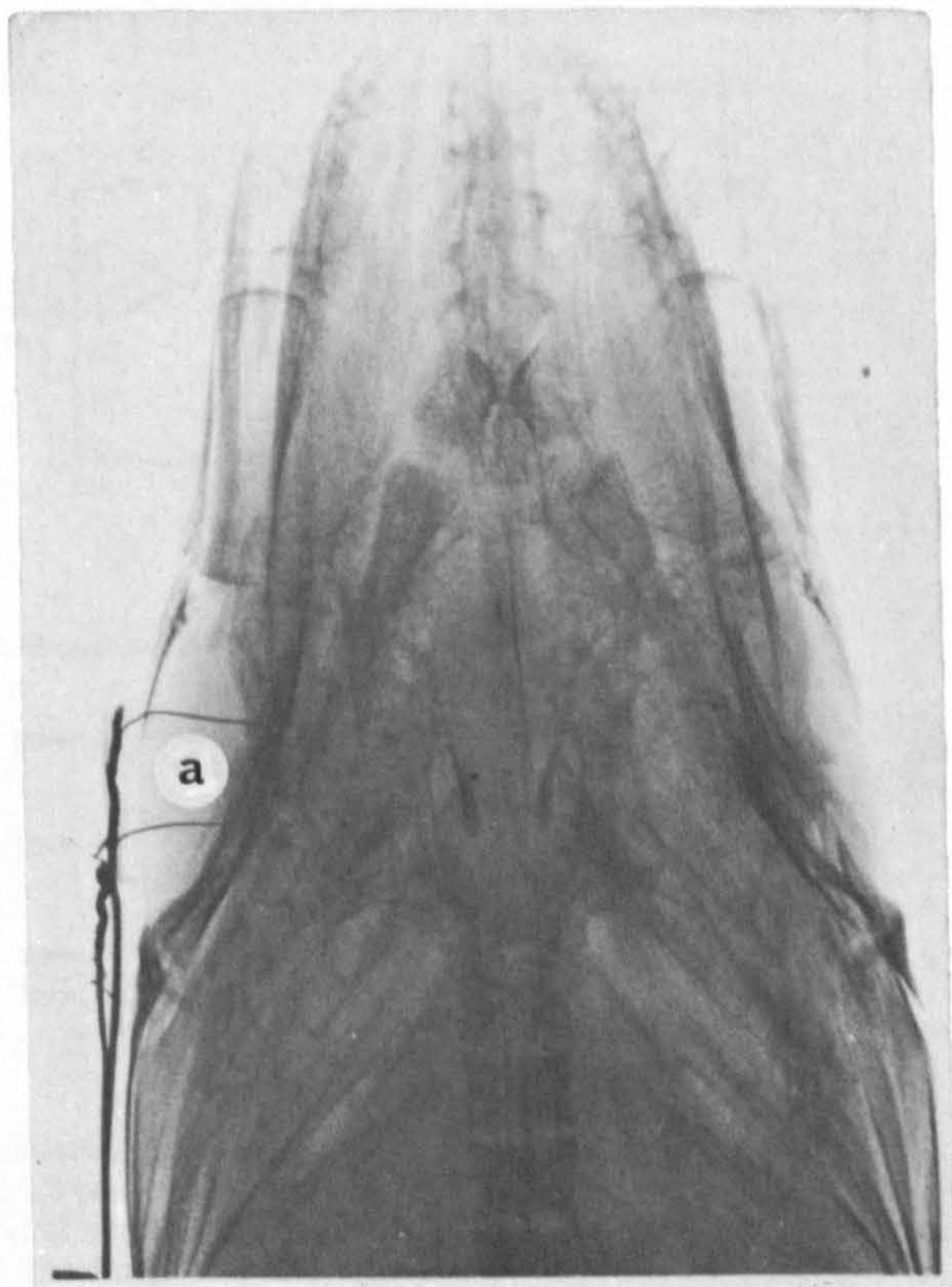
II. MATERIALS AND METHODS

ULTRASONIC TRANSMITTER

The transmitter is essentially the same as that described by Priede & Young (1977) for ECG telemetry. The circuit has been modified to overcome the original instability problems and the third operational amplifier in the package is connected as an amplifier instead of as a comparator [Fig. 1(a)]. This allows an overall high gain but maintains a high impedance input. Instead of silicone rubber encapsulation, the transmitter is fitted into a cutdown 5 ml polypropylene syringe barrel filled with light paraffin which gives an apparent weight in water of 2.5 g.

THE RECORDING SYSTEM

The system is mainly as described by Priede & Young except that in the last experiment the equipment was set up as in Fig. 1(b). The display console gives the operator both a visual and digital display of the three hydrophone bearings and on pressing a button, initiates a



1 cm

PLATE I. Radiograph of brown trout head (dorso-ventral, slightly oblique) to show correct location of microelectrodes abutting the hyomandibula. (enlarged, scale indicated on plate.) *a*, *m. adductor mandibulae*.

digital data print-out. The multiplexer switches the time, hydrophone bearings and the environmental parameters (except the photometer which had to be read manually at this stage) to give a sequential print-out of the data each time. The signal from the fish, detected by separate omnidirectional hydrophones is recorded on a domestic tape recorder (Ultra Ltd.) and five minute timing marks were superimposed by a separate 1kHz audio timer which was also played into a loudspeaker to pace the operator. Owing to the high cost of recording paper and frequent pen damage due to the fast rise time of the signal, recording on Devices M2 or MX212 recorders was abandoned in favour of the former method.

Laboratory tests of transmitters were generally achieved by detecting the R.F. output from the transmitter, which extends for a metre or so, using a conventional portable radio. Electromyograms were recorded by direct wiring in the laboratory using similar methods to Sutterlin (1969).

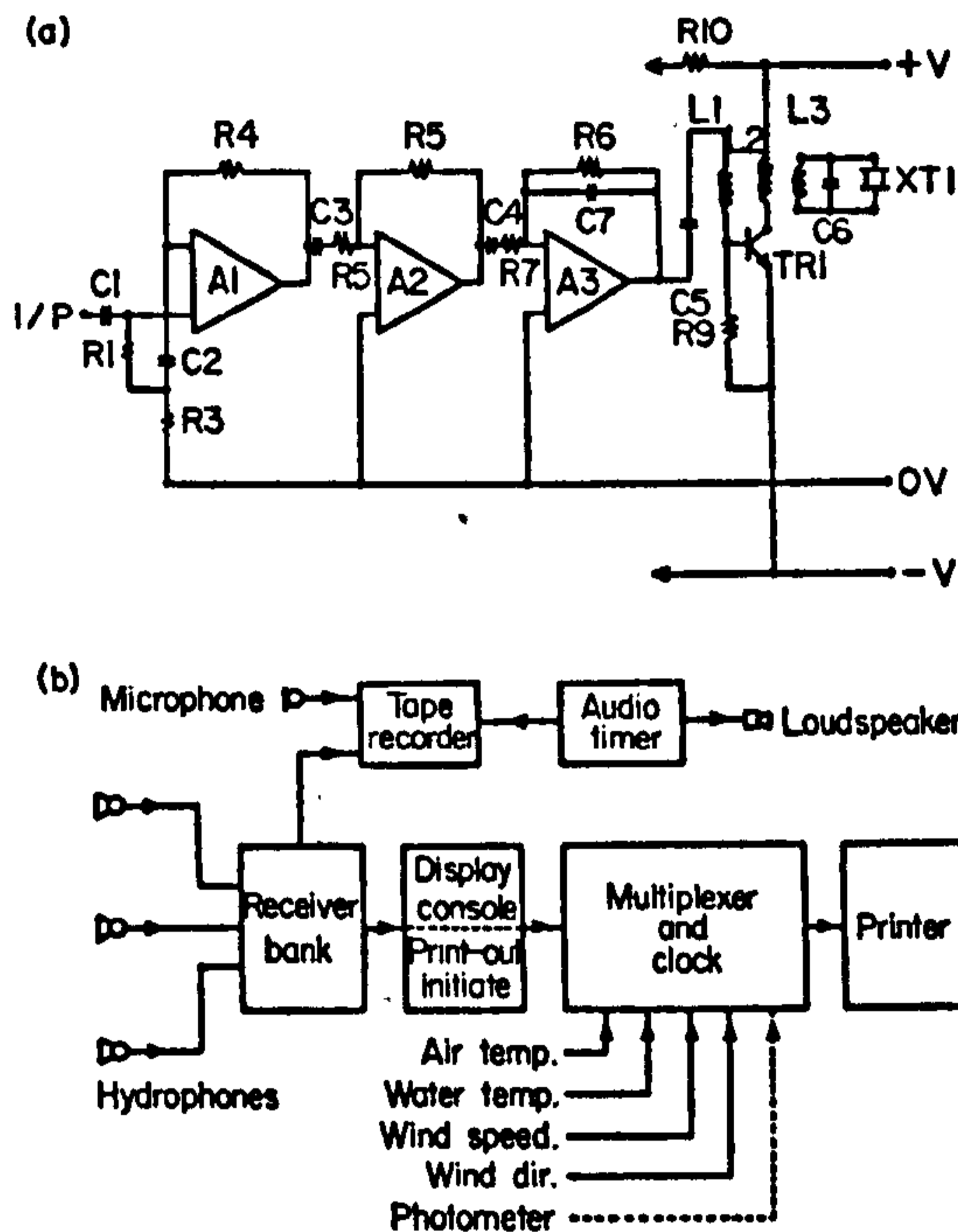


FIG. 1. (a) Schematic transmitter circuit: Amplifier A3 is now connected as an amplifier instead of as a comparator. C4 and C7 set the low and high frequency cut-off points, respectively. (b) Block diagram of the receiving system; the photometer was not connected to the multiplexer input during the course of this study.

TAGGING PROCEDURES

Brown trout were caught on rod and line in the West Pool of Airthrey Loch. They were kept, generally for less than 24 h, in a 'Netlon' cage moored near the tracking caravan. All tagging operations were carried out in the nearby laboratory because extended anaesthesia was often required and also because modifications to the transmitters were sometimes made during the tagging procedure. Fish were anaesthetized with benzocaine, 30 mg l⁻¹, (Laird & Oswald, 1975) and operations conducted in air using an anaesthetic apparatus similar to Smith & Bell (1967), incorporating thermal regulation. Attachment of the transmitter was similar to that described by Holliday *et al.* (1973). A special electrode was devised which would allow a certain amount of stereotaxy in placement into the chosen muscle. It is 'T-shaped' and was affixed in the following manner. An incision was made horizontally in the skin overlying the mid-line of the muscle. Two sutures were passed at right angles forming two loops. The 'T-electrode' was checked for length, trimmed and placed in the incision and the two sutures tightened around the cross bar of the 'T' refer to the radiograph (Plate I). The electrode leads were connected to the transmitter and waterproofed using quick setting silicone rubber

(Impressil—C. L. Attenborough Ltd.). The fish was then revived from the anaesthesia and transferred to a holding tank pending a decision on its release in the Loch. Successfully tagged fish were transported in polythene bags and released from a boat, near to their capture point.

INTERPRETATION OF RECORDS

Attempts to use R.M.S. integration of the signal (Inman *et al.*, 1952; Bigland & Lippold, 1954; Bergstrom, 1959) were not reliable in the automatic identification of feeding events. The human ear was more successful in discriminating between feeding events and spurious noise, and was used throughout.

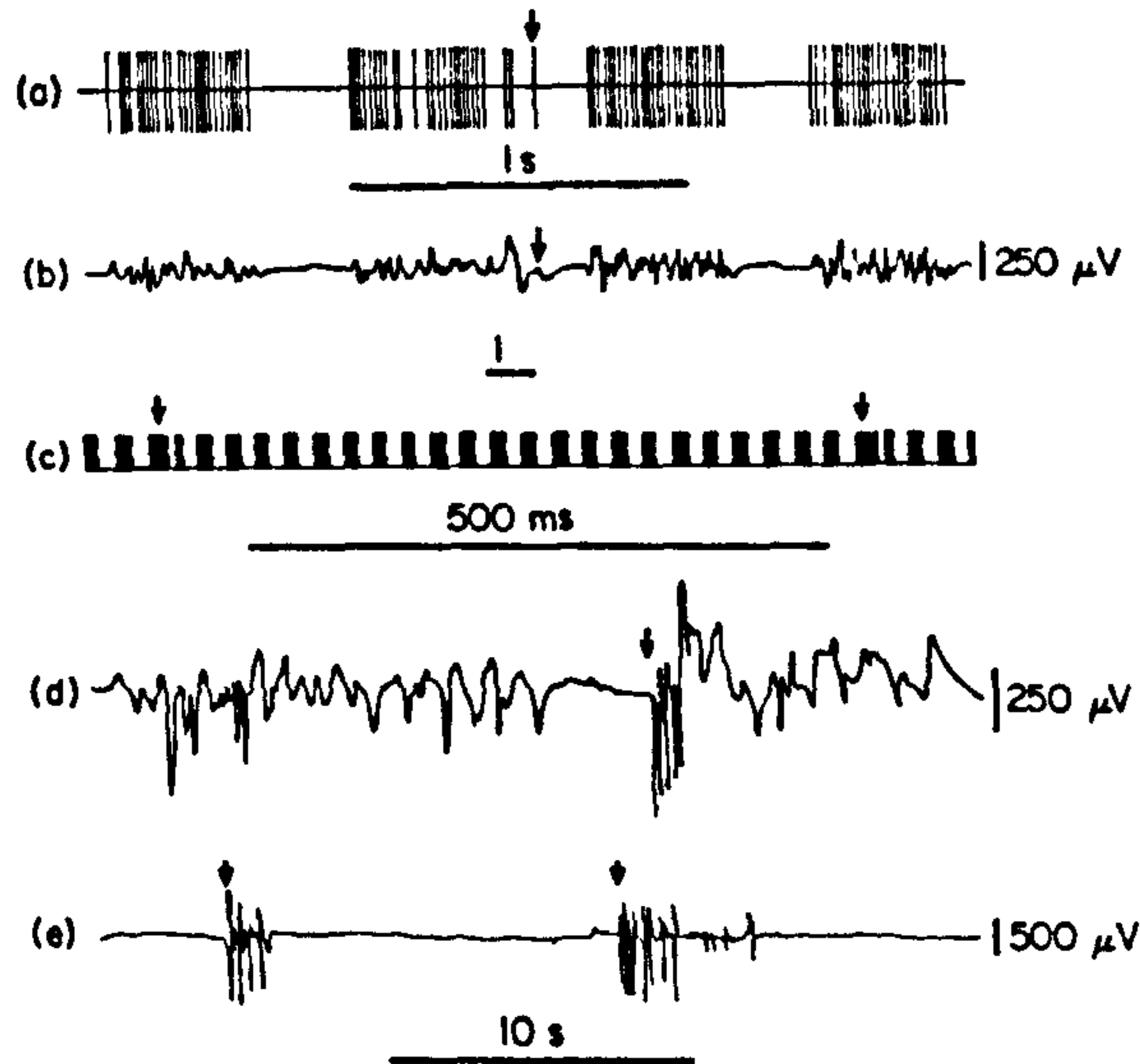


FIG. 2. Simultaneous recording of transmitter output pulses (a) and ventilatory E.M.G. input (b). Note that the $50 \mu\text{V}$ spike arrowed in (b) is telemetered in (a). (c) Transmitter output envelopes from free-swimming brown trout in the laboratory. Two coughs are arrowed. Note time course of cough and the extended burst length preceding it. (d) *Adductor mandibulae* E.M.G. during a cough. The fast spikes arrowed correspond to the rapid closure phase of the jaw. The remainder of the E.M.G. is composed of motor potentials derived from junctional potentials, hence their relatively low frequency. (e) Groups of electromyograms recorded from a free-swimming Trout during feeding. Note high amplitude and repetition pattern of the E.M.G.

III. RESULTS

LABORATORY TESTS OF THE TRANSMITTER

From laboratory tests of trout equipped with electromyographic electrodes, it was seen that during feeding there was a characteristic series of large (often $> 1 \text{ mV}$), fast-spiking EMGs which was unlike the regular rhythmic bursts due to ventilatory activity. It was decided that although ideally the transmitter should be silent until the fish actually fed, it was desirable to have a regular signal in order to track the fish. The gain of the transmitter was therefore increased to be sensitive enough to display the ventilatory EMG.

In its latest form the transmitter was very sensitive. Figure 2(a) and (b) shows a simultaneous recording of the EMG and the transmitter pulse output. Note that even small spikes of around $50 \mu\text{V}$ are telemetered and the pulse trains indicate the ventilatory cycle.

It was found relatively easy to distinguish between 'coughs', 'yawns' etc. and feeding acts. During a yawn the *adductor mandibulae* was inactive and there was

typically a silent period from it. The 'cough' in trout has been described; there is an extended burst followed by a short burst. This corresponds to the holding closed of the jaw followed by a rapid opening and shutting. Figure 2(c) shows the telemetry record from a trout in which coughs occurred. The cough cycle is short, generally under 1 s and the normal ventilatory rhythm is soon restored. Figure 2(d) shows the EMG record from a trout during a cough and there is evidence at the start of the second burst to suggest fast (mosaic) fibre involvement. The high discharge frequency of the mosaic fibres gives a distinct change of timbre to the telemetry recording during a cough. Similarly, when the mosaic fibres are recruited during feeding, there is a similar tonal change. This, coupled with the typical decreasing repetition rate following food capture allows confidence in the positive identification of the feeding event from the telemetry records. [Fig. 2(e)].

The transmitter did not appear to affect the fishes ability to feed. A 550 g ♂ Brown trout which was the dominant fish in a hierarchy of six, readily fed on pellets whilst carrying one of these transmitters. This fish almost invariably managed to take singly introduced pellets before its subdominants thus implying that its foraging ability was not impaired.

FIELD EXPERIMENTS

Four brown trout were tagged with the EMG transmitter and released.

TABLE 1

	Weight (g)	Date of Release	Tag life (days)	Temp (°C)
Fish 1	485	10.9.75	9	12.0
Fish 2	560	5.11.75	1.6	8.5
Fish 3	460	8.6.76	2.9	20.4
Fish 4	480	23.9.76	3.7	11.0

FEEDING ACTS

Feeding acts were readily discerned from the audible signals on magnetic tape and chart records though the aural method was easier. Some examples of feeding events telemetered from the field and in the laboratory are shown in Fig.3. The feeding act

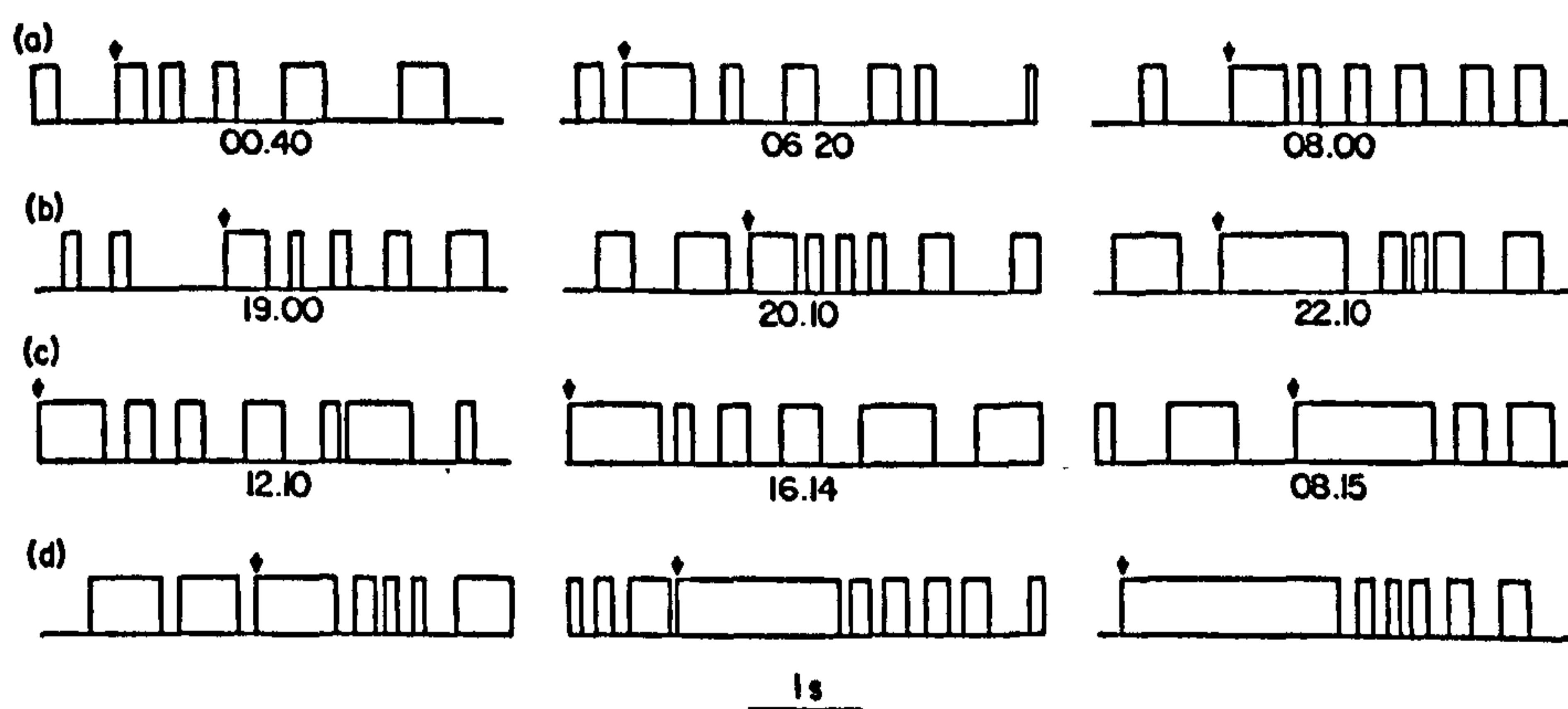


FIG. 3. Telemetered E.M.G. trains recorded from brown trout during feeding acts; arrows indicate the onset of the feeding act, time of day is given below each figure. (a) Fish 2 (b) Fish 3 (c) Fish 4 (d) Fish in laboratory tank.

was often preceded by a silent period which is even more obvious when the tape recordings are heard.

FEEDING RATE

The feeding rate for fishes 2, 3 and 4 are presented in Fig. 4 as a % of the total number recorded. It had been hoped to complete this as a % of the daily total but this was not feasible owing to the gaps in the records. There is an obvious grouping of feeding activity into peaks. In Fish 2 only a small portion of the record is shown as most of the first day was not continuously recorded. There is a peak between 2200 and 2300 h, which is halfway through the night at these latitudes, and another peak post dawn (07.30).

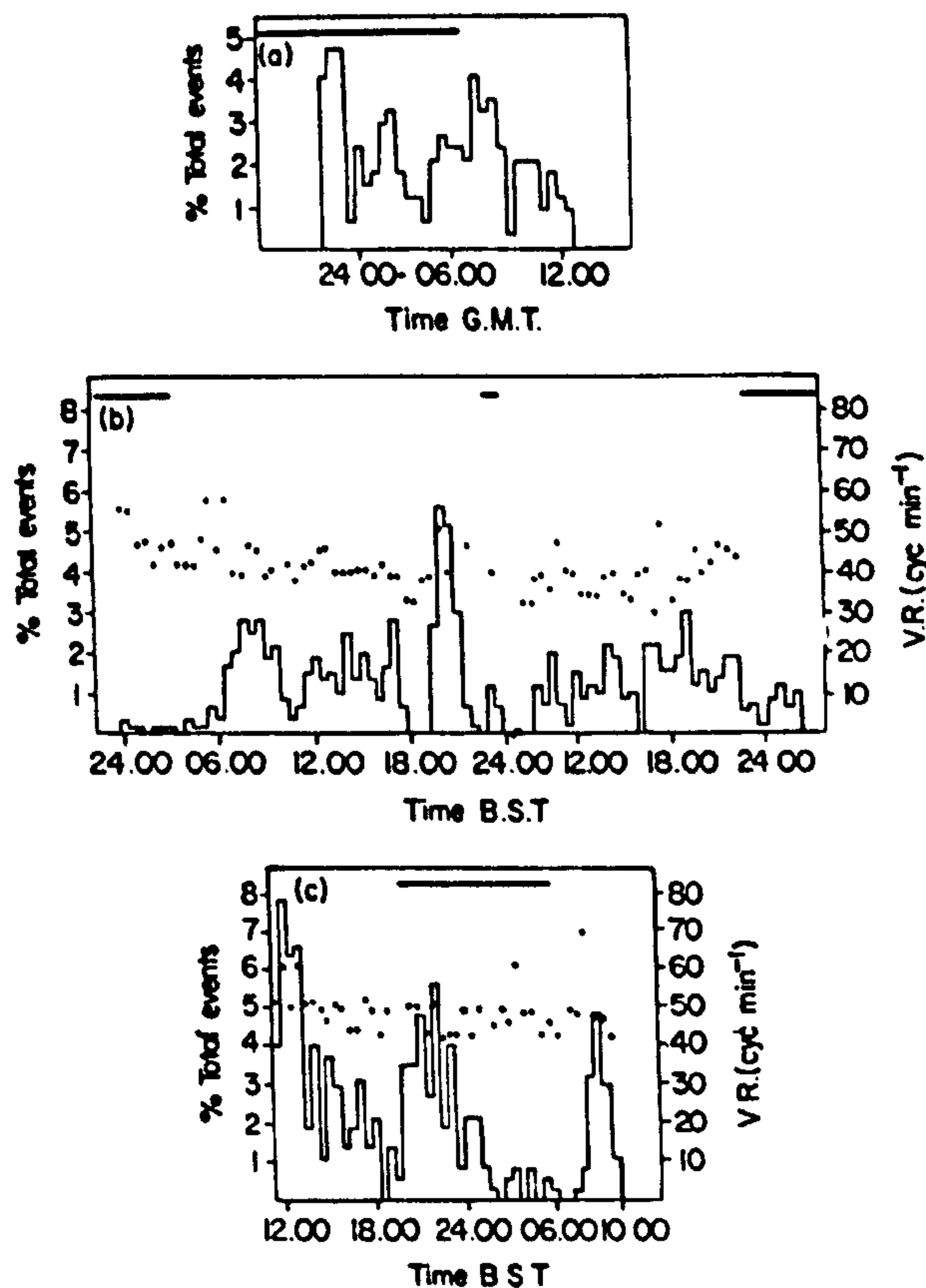


FIG. 4. Feeding periodicities of Fish 2, 3 and 4. Black bars indicate night. (a) Feeding rate of Fish 2 is expressed as a % of the total number. (b) Feeding rate (histograms) and ventilation rate (VR), (dots), in cycles min^{-1} for Fish 3. (c) Feeding rate and ventilation rate for Fish 4, legend as in (b).

If the records for the first 24 h of Fish 3 are compared with those for Fish 4 it will be seen that there are three main feeding peaks. In Fish 3 at 07.30–09.30, 12.00–17.00, and 20.00–22.00 h; in Fish 4 at 12.00–14.30, 20.00–23.30 and 08.00–10.00. There are differences between the three histograms in that in Fish 3 the crepuscular peaks begin after dawn and before dusk whereas in Fish 4 they are immediately at dawn and at dusk, continuing into darkness. In Fish 2 there was also an immediate post dawn peak of feeding activity. In Fish 3 the dawn period was missed due to technical faults on

10.6.76 but there is a mid-afternoon peak followed by an early evening peak before dusk, although the transmitter started to become unreliable after dusk of this day. Mid-afternoon peaks were common to all three fish.

VENTILATION RATE

Figure 4 shows the rates recorded at half-hour intervals although this was not always possible. It can, however, be noted in Fish 4 that the ventilation rate did not vary unduly, the maximum being a 56% increase over the lowest recorded rate and similarly in Fish 3 a maximum increase of 52% was apparent. The levels of ventilatory activity are generally near the resting levels given by workers from laboratory studies.

IV. DISCUSSION

TRANSMITTER SYSTEM

The transmitter system in its final form was satisfactory although it did not perform as well in the loch as in the laboratory. This was because the development work was performed on lightly or recently anaesthetized fish which exhibit an anaesthetic induced increment in the peak EMG voltages produced by the *adductor mandibulae*. Another consideration is that the red fibre EMG has a different frequency spectrum from 'normal' (mammalian) muscle. McLeod *et al.* (1976) have shown for the type of electrode used in this study, the normal human *biceps brachii* muscle has a frequency peak of around 400 Hz but extends to 2 kHz. Our frequency analysis of the trout *adductor mandibulae* muscle EMG shows that 78% of the EMG is below 100 Hz. This is because the red muscle fibres do not exhibit propagated action potentials (Kuffler & Vaughan Williams, 1953; Hidaka & Toida, 1969). The transmitter was designed to have a bandwidth 159 Hz to 3.3 kHz to eliminate 50 Hz artifact. It is now realised that this was eliminating 80% of the useful input signal which explains the irregularities of the signal in some cases once the fish had recovered from the anaesthesia. A new design of transmitter has been made to obviate this defect.

FEEDING CRITERIA

The electromyographic signal provided an unequivocal indication of a feeding event. The characteristic signal was produced by the fish moving its food with the hyoid/palatine ratchet system (McNeil Alexander, 1974; Western, 1968), for which it has to release its grasp by relaxing the jaws thus accounting for the repetitive EMG signals. This action has also been confirmed in young Salmon (*Salmo salar*) using high speed photography (J. Wankowski 1977 ms.) who also showed that these pre-swallowing movements may be fast or alternatively, extend over several seconds. This occurrence was obvious from both the laboratory and field telemetry studies.

FEEDING CYCLES

The results of this work are in keeping with the activity cycle work at Airthrey Loch (Young *et al.*, 1972; Holliday *et al.*, 1973). Feeding was generally grouped into distinct bouts, which is to be expected for a carnivore such as the trout (Landless, 1976) but not for an omnivore such as the Goldfish (Rozin & Mayer, 1971). The timing of the bouts parallels the locomotor patterns recorded by Young *et al.* though as was observed by Holliday *et al.* there may be subsidiary peaks of activity apparently unconnected to photoperiod which also occurred in the feeding results. The exact timing of the feeding peaks varied between the three fish. In Fish 2 and 4 the crepuscular peaks occurred

shortly after dawn and directly at sunset. In Fish 3, which was recorded during the month with the longest daylength (19.5 h), its peaks were well after sunrise and before sunset. Priede & Young (1977) *op. cit.* found that in some cases crepuscular peaks of cardiac activity may occur before visible solar cues but did not imply an inherent 'clock' mechanism. Landless *op. cit.* who also demonstrated crepuscular feeding patterns in the laboratory, suggests that stomach filling and evacuation rates in fish may act as a controller of feeding bouts. Brett (1971), Elliott (1973), Windell *et al.* (1976) have all shown a temperature dependent evacuation rate in salmonids which gives a semi-logarithmic model of percentage evacuation against time. There is, however, variation in the rate with different foods, Windell *et al.* (1969). Elliott gives the 50% evacuation time for trout fed on aquatic animals at 20° C as 2–3 h, whereas at 11° C it is between 4–7 h. This therefore may explain the short term control of feeding rate variations but it does not account for the larger groupings. It is, however, known that gastric emptying is unaffected by exercise (Tyler, 1977).

The tendency for a mid-day peak is interesting and although a mid-day rise is known to anglers, Hoar (1942) suggested that feeding was inhibited by the mid-day light intensity. In the last experiment (Fish 4) where a photometer was employed, the highest light intensities were recorded between 13.00–14.00 h which was during an intense feeding bout. Hoar however, had his fish in relatively shallow water but in deeper, algal-filled water with sediment often stirred by wave action as obtained in Airthrey Loch, the mid-day light would be much attenuated.

From the records it is clear that night feeding is a common occurrence in Brown trout. Landless *op. cit.* has shown that demand fed trout may take 40% of their daily ration by night. Jenkins (1969) has shown brown and rainbow trout to be capable, at taking food at low levels of illumination. Elston & Bachen (1976) have also observed *Menidia audens* to night feed, though requiring a larger organism size presumably to overcome the lower visual acuity in scotopic vision, perhaps by silhouette recognition. Unfortunately Airthrey Loch is always illuminated by security flood-lighting and although at night no light was detectable on the photometer, the results from Airthrey Loch may be affected by this factor.

VENTILATION RATE

Since it was only possible to make half hourly estimates, the data only serves to give an additional measure of the low levels of metabolism which the telemetry studies here have indicated. Priede & Young (1977) and Holliday *et al.* (1973) have shown the cardiac and swimming rates for loch-living trout to be low and out of character with their capabilities known from laboratory studies. The mean ventilatory rates for Fish 3 and 4 were 53 and 48 per minute respectively. Sutterlin (1969) gives the resting rate for Brown trout at 8° C as around 45 per min. whereas Marvin & Heath (1968) give values nearly double this for rainbow trout. This illustrates the relatively low levels of energy expenditure by lake dwelling trout. Priede & Young (1977) noted periods of intense tachycardia, when the fish were making little movements. It may be that such periods correspond to feeding bouts and result from the 'area restricted searching' proposed by Thomas (1974).

I am indebted to Dr P. Tytler and Professor F. G. T. Holliday, under whose N.E.R.C. grants and direction this work was carried out. To A. H. Young and J. Wiewiorka for the development and manufacture of the electronics systems; to Messrs. J. Scott, L. G. Ross, I. McGowan, I. G. Priede for assistance in catching and tagging of fish and finally to the volunteers who

assisted with the actual tracking and recording operations. R. L. Oswald was an N.E.R.C. Research Student.

References

- Alexander, R. McN. (1974). *Functional design in fishes*, 160 pp. London: Hutchinson.
- Ballintijn, C. M. & Hughes, G. M. (1965). The muscular basis of the respiratory pumps in the trout. *J. exp. Biol.* **43**, 349–362.
- Ballintijn, C. M. (1969). Muscle co-ordination of the respiratory pump of the Carp (*Cyprinus carpio*, L.) *J. exp. Biol.* **50**, 569–591.
- Ballintijn, C. M., Van den Burg, A. & Egberink, B. P. (1972). An electromyographic study of the adductor mandibulae complex of a free-swimming carp (*Cyprinus carpio* L.) during feeding. *J. exp. Biol.* **57**, 261–283.
- Bergstrom, R. M. (1959). The relation between the number of impulses and the integrated electric activity in Electromyogram. *Acta Physiol. Scand.* **45**, 97–101.
- Bigland, B. & Lippold, O. C. J. (1954). The relation between force, velocity, and integrated electrical activity in human muscles. *J. Physiol.* **123**, 214–224.
- Brett, J. R. & Higgs, D. A. (1970). Effect of temperature on the rate of gastric digestion of fingerling sockeye salmon (*Oncorhynchus nerka*) *J. Fish. Res. Bd Can.* **27**, 1767–1779.
- Chaston, I. (1968). Influence of light on activity of Brown Trout (*Salmo trutta*) *J. Fish. Res. Bd Can.* **25**, 1285–1289.
- Davis, R. E. (1964). Daily pre-dawn peak of locomotion in fish. *Anim. Beh.* **12**, 272–283.
- Davis, R. E. & Bardach, J. (1965). Time co-ordinated pre-feeding activity in fish. *Anim. Beh.* **13**, 154–162.
- Elliott, J. M. (1972). Rates of gastric evacuation in brown trout *Salmo trutta* L. *Freshwat. Biol.* **2**, 1–18.
- Elshoud-Oldenhave, M. J. W. & Osse, J. W. M. (1976). Functional morphology of the feeding system in the ruff *Gymnocephalus cernua* *J. Morphol.* **146**, 399–422.
- Elston, R. & Bachen, B. (1976). Diel feeding cycle and some effects of light on feeding intensity of the Mississippi silverside *Menidia audens* in Clear Lake, California. *Trans. Am. Fish. Soc.* **105**, 84–88.
- Gibson, R. N. (1973). Tidal and circadian activity rhythms in juvenile plaice, *Pleuronectes platessa* *Mar. Biol.* **22**, 379–386.
- Hasler, A. D. & Bardach, J. E. (1949). Daily migrations of Perch in Lake Mendota, Wisconsin. *J. Wildlife Mgt.* **13**, 40–51.
- Hidaka, T. & Toida, N. (1969). Neuromuscular transmission and excitation-contraction coupling in fish red muscle. *Jap. J. Physiol.* **19**, 130–142.
- Hoar, W. S. (1942). Diurnal variations in feeding activity of young Salmon and Trout. *J. Fish. Res. Bd Can.* **6**, 90–101.
- Holliday, F. G. T., Tytler, P. & Young, A. H. (1973). Activity levels of trout (*Salmo trutta*) in Airthrey Loch, Stirling and Loch Leven, Kinross. *Proc. R. Soc. Edinb. B.* **74**, 315–331.
- Hughes, G. M. (1975). Coughing in the rainbow trout (*Salmo gairdneri*) and the influence of pollutants. *Rev. suisse Zool.* **82**, 47–64.
- Hughes, G. M. & Ballintijn, C. M. (1965). The muscular basis of the respiratory pumps in the dogfish (*Scyliorhinus canicula*) *J. exp. Biol.* **43**, 363–383.
- Hughes, G. M. & Adeney, R. J. (1977). Variations in the pattern of coughing in Rainbow Trout. *J. exp. Biol.* **68**, 109–122.
- Inman, V. T., Ralston, H. J., Saunders, J. B. C. M., Feinstein, B. & Wright, E. W. (1952). The relation of human EMG to muscular tension. *EEG. Clin. Neurophysiol.* **4**, 187–194.
- Kruuk, H. (1963). Diurnal periodicity in the activity of the common sole, *Solea vulgaris* Quensel. *Neths. J. Sea Res.* **2**, 1–28.
- Kuffler, S. W. & Vaughan Williams, E. M. (1953). Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they innervate. *J. Physiol.* **121**, 289–317.
- Laird, L. M. & Oswald, R. L. (1975). A note on the use of benzocaine (Ethyl-p-aminobenzoate) as a fish anaesthetic. *J. Inst. Fish Mgt.* **6**, 92–94.

- Landless, P. (1976). Demand-feeding behaviour of Rainbow Trout. *Aquaculture*, 7, 11-25.
- McLeod, W. D., Nunally, H. N. & Cantrell, P. E. (1976). Dependence of EMG Power spectra on Electrode type. *I.E.E.E. Trans. Biomed. Eng.* 23, 172-175.
- Marvin, D. E. & Heath, A. G. (1968). Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of fresh-water fish. *Comp. Biochem. Physiol.* 27, 349-355.
- Mitson, R. B. & Young, A. H. (1975). A survey of the Engineering problems of developing small acoustic fish tags. *Proceedings of the conference on Instrumentation in Oceanography, Bangor*, 163-174.
- Osse, J. W. M. (1969). Functional morphology of the head of the Perch: an electromyographic study. *Neth. J. Zool.* 19, 289-392.
- Priede, I. G. & Young, A. H. (1977). The ultrasonic telemetry of cardiac rhythms of wild brown trout (*Salmo trutta* L.) as an indicator of bio-energetics and behaviour. *J. Fish Biol.* 10, 299-318.
- Reynolds, W. W. (1976). Locomotor activity rhythms in the Bluegill sunfish, *Lepomis macrochirus*. *Am. Midl. Nat.* 96, 221-225.
- Rozin, P. & Mayer, J. (1961). Regulation of food intake in the Goldfish. *Am. J. Physiol.* 201, 968-974.
- Siegmund, R. (1969). Lokomotorische Aktivität und Ruheverhalten bei einheimische Susswasserfischen. (Pisces: Percidae, Cyprinidae) *Biol. Zbl.* 88, 295-312.
- Smith, A. W. M. & Code, C. F. (1958). The effect of an ordinary and of an excessively fatty breakfast on gastric motility. *Gastroenterology*, 35, 398-405.
- Smith, L. S. & Bell, G. R. (1967). Anaesthetic and surgical techniques for Pacific Salmon. *J. Fish. Res. Bd Can.* 24, 1579-1588.
- Spencer, W. P. (1939). Diurnal activity rhythms in freshwater fishes. *Ohio J. Sci.* 39, 119-132.
- Sutterlin, A. M. (1969). Effects of exercise on cardiac and ventilation frequency in three species of freshwater teleosts. *Physiol. Zool.* 42, 36-52.
- Swift, D. R. (1962). Activity cycles in the Brown Trout. 1. Fish feeding naturally. *Hydrobiologia* 20, 241-247.
- Swift, D. R. (1964). Activity cycles in the Brown Trout. 2. Fish artificially fed. *J. Fish. Res. Bd Can.* 21, 133-138.
- Thomas, G. (1974). The influences of encountering a food object on subsequent searching behaviour in *Gasterosteus aculeatus* L. *Anim. Beh.* 22, 941-952.
- Tyler, A. V. (1977). Evidence for the assumption of independence between gastric emptying rate and swimming activity using Atlantic Cod, *Gadus morhua*. *J. Fish. Res. Bd Can.* 34, 2411-2413.
- Tytler, P., Machin, D., Holliday, F. G. T. & Priede, I. G. (1977). A comparison of the patterns of movement between indigenous and displaced Brown Trout (*Salmo trutta* L.) in a small shallow loch. *Proc. R. Soc. Edinb. B.* 76, 1-24.
- Verheijen, F. J. & de Groot, S. J. (1967). Diurnal activity pattern of plaice and flounder (Pleuronectidae) in aquaria. *Neths. J. Sea Res.* 3, 383-390.
- Wankowski, J. W. J. (1977). The role of prey size in the feeding behaviour and growth of juvenile Atlantic Salmon (*Salmo salar* L.). Unpublished Ph.D. Thesis, University of Stirling.
- Ware, D. M. (1972). Predation by Rainbow trout (*Salmo gairdneri*): the influence of hunger, prey density and prey size. *J. Fish. Res. Bd Can.*, 29, 1193-1201.
- Western, J. R. H. (1969). Studies on the diet, feeding mechanism and alimentary tract in two closely related teleosts, the freshwater *Cottus gobio* L. and the marine *Parenophrys bubalis* Euphrasen. *Acta Zoologica* 50, 185-205.
- Wikgren, B. J. (1955). Daily activity pattern of the burbot. *Mem. Soc. Fauna. Flora Fenn.* 31, 91-97.
- Windell, J. T., Noriss, D. O., Kitchell, J. F. & Norris, J. S. (1969). Digestive responses of Rainbow Trout *Salmo gairdneri* to pellet diets. *J. Fish. Res. Bd Can.* 26, 1801-1812.
- Windell, J. T., Kitchell, J. F., Norris, D. O., Norris, J. S. & Foltz, J. W. (1976). Temperature and rate of gastric evacuation by Rainbow Trout, (*Salmo gairdneri*) *Trans Am. Fish. Soc.* 105, 712-717.

- Young, A. H. & Wiewiorka, J. (1975). Ultrasonic tracking and telemetering system for use on small fish. *Underwater Telemetry Newsletter* 5, 10-13.
- Young, A. H., Tytler, P., Holliday, F. G. T., & MacFarlane, A. (1972). A small sonic tag for measurement of locomotor activity in fish. *J. Fish Biol.* 4, 57-65.
- Young, A. H., Tytler, P. & Holliday, F. G. T. (1976). New developments in ultrasonic fish tracking *Proc. R. Soc. Edinb. B.* 75, 145-155.

APPENDIX II

Component Lists for Electronic circuits

a) Component list for EMG Transmitter

TRI	BCW32R - Ferranti Limited	R5, R7	1K
A1, A2, A3	L144CJ - Siliconix Limited	R9	68K
C1, C2, C3, C4	1 mfd. Type 2105 - Waycom Ltd.	R10	220K
C5, C7	0.1 mfd. Type 0474 - Waycom Ltd	XT1	Type 2-2025-5 - Vrtniyton
C6	Select to suit, typically 400-500pF	T1	25T 34SWG
R1	82K	T2	10T 40SWG
R3, R6	10k	T3	120T 40SWG

b) Component list for Timer/Oscillator

Timer

ICI	ZN1034E
R1	110k
R2	390 ohms
R3	10K
R4	10K
C1	1 μ F
C2	0.1 μ F
C3	10 μ F

Oscillator

ICI	555
R1	1K
R2	100K
R3	820 ohms
R4	4.7K
C1	0.1 μ F
C2	0.1 μ F
TR1	BC 109
Vs	12V

c) Component list for Pulse Former

ICI	74121	R4	1K
TR1	BC107	R5	10K
TR2	BFY51	R6	1K
D1	1GP10	R7	470 ohms
R1	10 ohms	C1	1 μ F
R2	1K	Vs	6V
R3	4.7K		

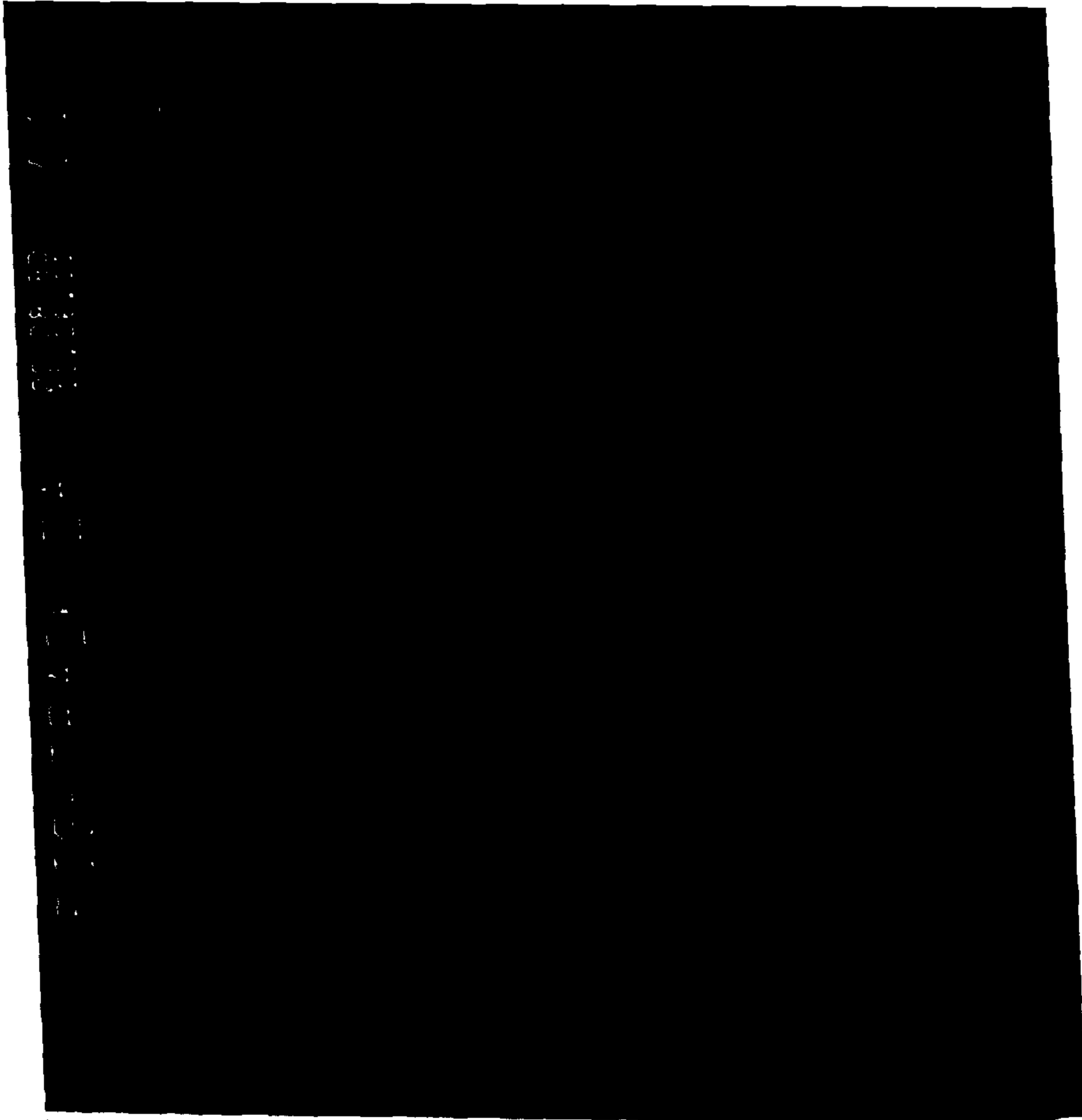
BEST COPY

AVAILABLE

APPENDIX III

Microfiche tabulation of original data from each fish-tracking experiment.

(Magnification = 48X)



REFERENCES

- ABEL, E.F. (1962). Freiwasserbeobachtungen an Fishen Im Golf von Neapel als Beitrag zur Kenntnis ihrer Ökologie und ihres Verhaltens. *Int. Rev. ges. Hydrobiol. Hydrogr.* 47, 219 - 290
- ACCORNERO, N., BINI, G., LENZI, G.L. and MANFREDI, M. (1979). Selective activation of peripheral nerve fibre groups of different diameter by triangular shaped stimulus pulses. *J. Physiol.* (1977), 273, 539 - 560
- ADRON, J., GRANT, P.T. and COWEY, C.B. (1973). A system for the quantitative study of the learning capacity of Rainbow Trout and its application to the study of food preferences and behaviour. *J. Fish Biol.* 5, 625 - 636
- AGARWAL, G.C. and GOTTLIEB, G.L. (1975). An analysis of the electromyogram by Fourier, Simulation and experimental techniques. *I.E.E.E. Trans. Biomed. Eng.* 22, 225 - 229
- ALEXANDER, R.McN. (1970). Mechanism of the feeding action of various species of teleost fishes. *J. Zool.* 162, 145 - 156
- ALEXANDER, R.McN. (1974). Functional design in fishes. Hutchinson, London. 160 pp.
- ALI, M.A. (1964). Diurnal rhythm in the rates of oxygen consumption, locomotor and feeding activity of yearling Atlantic Salmon (Salmo salar) under various light conditions. *Proc. Ind. Acad. Sci.* 60, 249 - 263
- ANDERSEN, P., JANSEN, J.K.S. and LØYNING, Y. (1963). Slow and fast muscle fibres in the Atlantic Hagfish (Myxine glutinosa). *Acta. Physiol. Scand.* 57, 167 - 179
- BACH, L.M.N. (1948). Conversion of red muscle to pale muscle. *Proc. Soc. Exp. Biol. Med.* 67, 268 - 269

- BACH-Y-RITA, P. and ITO, F. (1966). In vivo studies on fast and slow muscle fibres in cat extraocular muscles. *J. Gen. Physiol.* 49, 1177 - 1198
- BAILEY, N.T.J. (1976). *Statistical methods in Biology*. Hodder and Stoughton, London. 198 pp.
- BAINBRIDGE, R. (1958). The speed of swimming of fish as related to size and frequency and amplitude of the tail beat. *J. Exp. Biol.* 35, 109 - 133
- BALLINTIJJN, C.M. (1969). Functional anatomy of carp respiratory pump. *J. Exp. Biol.* 50, 547 - 567
- BALLINTIJJN, C.M. (1969). The influence of proprioception upon respiratory neurons in the medulla oblongata of fishes. *Acta. Physiol. Pharmacol. Neerl.* 15, 25 - 27
- BALLINTIJJN, C.M. (1972). Efficiency, mechanics and motor control of fish respiration. *Resp. Physiol.* 14, 125 - 141
- BALLINTIJJN, C.M. and BAMFORD, O.S. (1975). Proprioceptive motor control in fish respiration. *J. Exp. Biol.* 62, 99 - 114
- BALLINTIJJN, C.M. and HUGHES, G.M. (1965). The muscular basis of the respiratory pumps in the Trout. *J. Exp. Biol.* 43, 349 - 362
- BALLINTIJJN, C.M., VAN DEN BURG, A. and EGBERINK, B.P. (1972). An electromyographic study of the adductor mandibulae complex of a free-swimming Carp (Cyprinus carpio L.) during feeding. *J. Exp. Biol.* 57, 261 - 283
- BARETS, A. (1961). Contribution à l'étude des systèmes moteurs "lent" et "rapide" du muscle latéral des téléostéens. *Arch. Anat. Micros. Morphol. Experimentale.* 50, 91 - 187
- BARRINGTON, E.J.W. (1957). The alimentary canal and digestion. In: *The Physiology of Fishes*. Vol. 1, 109 - 161. Brown, M.E. (Ed). Academic Press, New York

- BASMAJIAN, J.V. (1974). Muscles Alive: Their functions revealed by electromyography. 3rd Edition. Williams and Wilkins, New York. 525 pp.
- BASMAJIAN, J.V. and HUDSON, J.E. (1974). Miniature source attached differential amplifier for electromyography. Am. J. Phys. Med. 53, 234 - 236
- BASMAJIAN, J.V. and LEE CROSS. (1971). Duration of motor unit potentials from fine-wire electrodes. Am. J. Phys. Med. 50, 144 - 148
- BASMAJIAN, J.V. and STECKO, G. (1962). A new bipolar indwelling electrode for electromyography. J. Appl. Physiol. 17, 849
- BATSCHELET, E. (1965). Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. A.I.B.S. monograph, Am. Inst. Biol. Sci., Washington
- BEITINGER, T.L. (1974). Thermoregulatory behaviour and diel activity patterns of Bluegill, Lepomis macrocheirus, following thermal shock. Fish. Bull. 72, 1087 - 1093
- BERGER, W.K. and DAHL, G.P. (1974). Pacemaker activity in the circular smooth muscle of Frog stomach. Pflugers Arch. ges. Physiol. 351, 133 - 145
- BERGMAN, R.A. (1964). Mechanical properties of the dorsal fin musculature of the marine teleost Hippocampus hudsonia. Bull. John Hopkins Hosp. 114, 344 - 353
- BERGSTROM, R.M. (1959). The relation between the number of impulses and the integrated electrical activity in electromyogram. Acta. Physiol. Scand. 45, 97 - 101
- BERKOWITZ, E.C. (1956). Functional properties of spinal pathways in the Carp, Cyprinus carpio L. J. Comp. Neurol. 106, 269 - 289
- BETHLEM, J. (1970). Muscle Pathology: Introduction and atlas. North-Holland. 132 pp.

- BEUKEMA, J.J. (1968). Predation by the three-spined Stickleback (Gasterosteus aculeatus L.). Behaviour. 33, 1 - 126
- BIGLAND, B. and LIPPOLD, O.C.J. (1954). The relation between force velocity and integrated electrical activity in human muscles. J. Physiol. 123, 214 - 224
- BILINSKI, E. (1974). Biochemical aspects of fish swimming. In: Biochemical and Biophysical Perspectives in Marine Biology. Vol. 1. Malins, D.C. and Sargent, J.R. (Eds). Academic Press, London. 343 pp.
- BLAND, R., LLOYD-JONES, S. and MCGOLDRICK, J. (1976). The Statistical Package for the Social Sciences. University of Stirling.
- BLAXTER, J.H.S. (1970). 2. Light. 2.3. Animals. 2.32. Fishes. In: Marine Ecology. Vol. 1. Pt. 1., 213 - 320. Kinne, O. (Ed). Wiley, London
- BLAXTER, J.H.S. (1976). The role of light in the vertical migration of fish - a review. In: Light as an ecological factor: II., 189 - 210. Evans, G.C., Bainbridge, R. and Rackham, O. (Eds). Blackwell Scientific Publications, Oxford
- BODDEKE, R., SLIPJER, E.J. and VAN DER STELT, A. (1959). Histological characteristics of the body musculature of fishes in connexion with their mode of life. Proc. K. ned. Akad. Wet. C. 62, 576 - 588
- BONE, Q. (1963). Some observations upon the peripheral nervous system of the Hagfish, Myxine glutinosa. J. Mar. Biol. Ass. U.K. 43, 31 - 47
- BONE, Q. (1964). Patterns of muscular innervation in the lower Chordates. Int. Rev. Neurobiol. 6, 99 - 147
- BONE, Q. (1966). On the function of two types of myotomal muscle fibre in elasmobranch fish. J. Mar. Biol. Assoc. U.K. 46, 321 - 350

- BONE, Q. (1972). Some notes on histological methods for peripheral nerves. *Medical Laboratory Technology*. 29, 319 - 324
- BOWEN, J.M. (1974). Electromyographic analysis of evoked potentials of canine muscle motor points. *J. Am. Vet. Med. Assoc.* 164, 509 - 512
- BRADBEER, R. and MARSHALL, G. (1977). *Casio Programmable Scientific Calculators, Program Manual*. Casio Electronics, London. 171 pp.
- BRAEKKAN, O.R. (1956). Function of the red muscle in fish. *Nature (London)*. 178, 747 - 748
- BRETT, J.R. (1971). Satiation, appetite and maximum food intake of Sockeye Salmon. *J. Fish. Res. Bd. Can.* 28, 409 - 415
- BRETT, J.R. and HIGGS, D.A. (1970). Effect of temperature on the rate of gastric digestion of fingerling Sockeye Salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd. Can.* 27, 1767 - 1779
- BRODY, G., SCOTT, R.N. and BALASUBRAMANIAN, R. (1974). A model for myoelectric signal generation. *Med. Biol. Eng.* 12, 29 - 41
- BROWNE, J.S. (1976). The contractile properties of slow muscle fibres in sheep extraocular muscle. *J. Physiol.* 254, 535 - 550
- BRYAN, J.E. and LARKIN, P.A. (1972). Food specialization by individual Trout. *J. Fish. Res. Bd. Can.* 29, 1615 - 1624
- BUCHTAL, F., GULD, C. and ROSENFALK, P. (1954). Action potential parameters in normal human muscle and their dependence on physical variables. *Acta. Physiol. Scand.* 32, 200 - 218
- BUCHTAL, F., GULD, C. and ROSENFALK, P. (1957). Volume conduction of the spike of the motor unit potential investigated with a new type of multi-electrode. *Acta. Physiol. Scand.* 38, 331 - 354
- BUCHTAL, F. and ROSENFALK, P. (1955). Action potential parameters in different human muscles. *Acta. Physiol. Scand.* 30, 125 - 131

- BÜLBRING, E. (1962). Electrical activity in intestinal smooth muscle. J. Physiol. Rev. Suppl. 5. 42, 160 - 174
- BURGEN, A.S.V. and MITCHELL, J.F. (1972). Gaddum's Pharmacology. 7th Edition. O.U.P., Oxford. 251 pp.
- BURNSTOCK, G. (1958). The effect of drugs on spontaneous motility and on response to stimulation of the extrinsic nerves of the gut of a teleostean fish. Brit. J. Pharmacol. 13, 216 - 226
- BURNSTOCK, G. (1958). Reversible inactivation of nervous activity in a fish gut. J. Physiol. 141, 35 - 45
- BURNSTOCK, G. (1959). The innervation of the gut of the Brown Trout (Salmo trutta). Q.J.M.S. 100, 199 - 219
- BYRNE, J.E. (1971). Photoperiodic activity changes in juvenile Sockeye Salmon (Oncorhynchus nerka). Can. J. Zool. 49, 1155 - 1158
- CAMERON, J.N. (1974). Blood flow distribution as indicated by tracer microspheres in resting and hypoxic Arctic Grayling (Thymallus thymallus). Comp. Biochem. Physiol. 52A, 441 - 444
- CAMERON, J.N. and CECH, J.J. (1970). Notes on the energy cost of gill ventilation in Teleosts. Comp. Biochem. Physiol. 34, 447 - 455
- CAMPBELL, G. (1969). Autonomic nervous systems. In: Fish Physiology. Vol. 11, 109 - 133. Hoar, W.S. and Randall, D.J. (Eds). Academic Press, New York.
- CAPRA, M.F. (1976). A technique for recording the respiratory movements of unrestrained elasmobranch fish. J. Fish Biol. 4, 1 - 2
- CARLANDER, K.D. and CLEARY, R.E. (1949). The daily activity patterns of some freshwater fishes. Am. Midl. Nat. 41, 447 - 452
- CARLSON, F.D. and WILKIE, D.R. (1974). Muscle Physiology. Prentice-Hall, New Jersey. 170 pp.

- CHAPMAN, C.J. and HAWKINS, A.D. (1973). A field study of hearing in the Cod. *J. Comp. Physiol.* 85, 147 - 167
- CHASTON, I. (1968). Influence of light on activity of Brown Trout (Salmo trutta). *J. Fish. Res. Bd. Can.* 25, 1285 - 1289
- CHASTON, I. (1969). Seasonal activity and feeding pattern of Brown Trout (Salmo trutta) in a Dartmoor stream in relation to availability of food. *J. Fish. Res. Bd. Can.* 26, 2165 - 2171
- CHIARANDINI, D.J. (1976). Activation of two types of fibres in rat extraocular muscles. *J. Physiol.* 259, 199 - 212
- CHISZAR, D., DRAKE, R.W. and WINDELL, J.T. (1975). Aggressive behaviour in Rainbow Trout (Salmo gairdneri Richardson) of two ages. *Behav. Biol.* 13, 425 - 431
- CLARIDGE, P.N., POTTER, I.C. and HUGHES, G.M. (1973). Circadian rhythms of activity, ventilatory frequency and heart rate in the adult River Lamprey, Lampetra fluviatilis. *J. Zool.* 171, 239 - 250
- CLOSE, J.R., NICKEL, E.D. and TODD, F.N. (1960). The significance of motor unit action potential counts. *J. Bone and Joint. Surg.* 42, 1207 - 1222
- COATES, D.J. (1973). Social behaviour in Pomacentrid reef fishes. Unpublished undergraduate thesis. University of Newcastle upon Tyne
- CODE, C.F. and CARLSSON, H.C. (1968). Motor activity of the stomach. *Handbook of Physiology Sect. 6. Pt. 4. 1903 - American Physiological Society*
- COGGESHAL, R.E., LEONARD, R.B., APPLEBAUM, M.L. and WILLIS, W.D. (1978). Organisation of peripheral nerves and spinal roots of the Atlantic Stingray, Dasyatis sabina. *J. Neurophysiol.* 41, 97 - 107

- COLIN, P.L. (1972). Daily activity patterns and effects of environmental conditions on the behaviour of the yellowheaded jawfish Opisthognathus aurifrons with notes on its ecology. *Zoologica (N.Y.)*. 57, 137 - 169
- COUTEAUX, R. (1950). Sur l'origine de l'appareil nerveux terminal des organes électriques. *C.r. Ass. Anat.* 37, 80 - 84
- CRAGG, B.G. and THOMAS, P.K. (1957). The relationship between conduction velocity and the diameter and internodal length of peripheral nerve fibres. *J. Physiol.* 136, 606 - 614
- CUST, L.A. (1975). The anatomy and physiology of the adductor mandibulae muscle of Rainbow Trout. Unpublished undergraduate thesis. University of Stirling
- CUYPERS, A. and FESSARD, A. (1954). Etude electrophysiologique des potentiels d'action des muscles à double innervation myoseptale du Poisson-Chat. *J. Physiol. (Paris)*. 46, 322 - 325
- DALE SMITH, R. and GIOVACCHINI, R. (1956). The vascularity of some red and white muscles of the rabbit. *Acta. Anat.* 28, 342 - 358
- DANIEL, E.E. (1968). The electrical activity of the alimentary tract. *Am. J. Dig. Dis.* 13, 297 - 319
- DANIEL, E.E. (1974). (Ed). *Proc. 4th Int. Symp. G - I Motility*, Vancouver
- DAVIS, R.E. (1964). Daily predawn peak of locomotion in fish. *Anim. Behav.* 12, 272 - 283
- DAVIS, R.E. and BARDACH, J. (1965). Time co-ordinated pre-feeding activity in fish. *Anim. Behav.* 13, 154 - 162
- DEDO, H.H. and DUNKER, E. (1965). The recording area of concentric needle electrodes. *Pflugers Arch. ges. Physiol.* 283, 40 - 41
- DIAMOND, J. (1971). The Mauthner Cell. In: *Fish Physiology*. Vol. 5. Chap. 9, 265 - 345. Hoar, W.S. and Randall, D.J. (Eds). Academic Press, New York

- DIXON, A.F.G. (1959). An experimental study of the searching behaviour of the predatory Coccinellid Beetle Adalia decempunctata (L). J. An. Ecol. 28, 259 - 281
- DONALDSON, G.W.K. (1960). The diameters to the extrinsic eye muscles of the Goat. Q. J. Exp. Physiol. 45, 25 - 34
- DOTY, R.W. and BOSMA, J.F. (1956). An electromyographic analysis of reflex deglutition. J. Neurophysiol. 19, 44 - 60
- DUXBURY, A.J., HUGHES, D.F., CLARK, D.E. and SHARPE, T.C. (1975). The bandwidth of masseter electromyograms from bipolar surface electrodes. Electromyogr. Clin. Neurophysiol. 15, 209 - 221
- EDEL, K. (1975). The effect of shelter availability on the activity of male silver eels. Helgolander wiss. Meeresunters. 27, 167 - 174
- ELLIOTT, J.M. (1965). Daily fluctuations of drift invertebrates in a Dartmoor stream. Nature. 205, 1127 - 1129
- ELLIOTT, J.M. (1972). Rates of gastric evacuation in Brown Trout. (Salmo trutta L.). Freshwat. Biol. 2, 1 - 18
- ELLIOTT, J.M. (1975). Weight of food and time required to satiate Brown Trout Salmo trutta L. Freshwat. Biol. 5, 51 - 64
- ELLIS, F.R. (1968). The neuromuscular interaction of Propanidid with Suxamethonium and Tubocurarine. Brit. J. Anaesth. 40, 818 - 824
- ELSHOUD-OLDENHAVE, M.J.W. and OSSE, J.W.M. (1976). Functional morphology of the feeding system in the Ruff (Gymnocephalus cernua). J. Morphol. 146, 399 - 422
- ELSTON, R. and BACHEN, R. (1976). Diel feeding cycle and some effects of light on feeding intensity of the Mississippi Silverside, Menidia audens in Clear Lake, California. Trans. Am. Fish. Soc. 105, 84 - 88

- ENGER, P.S. and ANDERSEN, R. (1967). An electrophysiological field study of hearing in Fish. *Comp. Biochem. Physiol.* 22, 517 - 525
- ENRIGHT, J.T. (1965). Accurate geophysical rhythms and frequency analysis. In: *Circadian Clocks*, 31 - 42. Aschoff, J. (Ed). North-Holland Publishing Co., Amsterdam.
- ERIKSSON, L-O. (1978). In: Thorpe (1978)
- ERIKSSON, L-O. and ULVELAND, S. (1977). Long-term telemetric system for gillstrokes of fish. *Aquilo. Ser. Zool.* 17, 61 - 64
- FACEY, D.F., MCCLEAVE, J.D. and DOYON, G.E. (1977). Responses of Atlantic Salmon Parr to output of Pulsed Ultrasonic Transmitters. *Trans. Am. Fish. Soc.* 106, 480 - 487
- FERNAND, V.S.V. and YOUNG, J.Z. (1951). The sizes of the nerve fibres of muscle nerves. *Proc. Roy. Soc. B.* 139, 38 - 58
- FERREL, D.W., NELSON, D.R., SCIAROTTA, T.C., STANDORA, E.A. and CARTER, H.C. (1973). A multichannel ultrasonic marine biotelemetry system for monitoring marine animal behaviour at sea. *Instrumentation in the Aerospace Industry.* 19, 71 - 84
- FERRIS, C.D. and STEWART, L.R. (1974). Electrode produced signal distortion in electrophysiological recording systems. *I.E.E.E. Trans. Biomed. Eng.* 21, 318 - 327
- FRANK, T.H. (1968). Telemetering the electrocardiogram of free swimming Salmo Irideus. *I.E.E.E. Trans. Biomed. Eng.* 15, 111 - 114
- FREY, R., GÖPFERR, H. and RAULE, W. (1952). Vergleichende Untersuchungen der Wirkungen muskelerschlaffender Mittel auf das Atemzentrum. *Der Anaesthetist.* 1, 33 - 44
- FROST, W.E. and BROWN, M.E. (1967). *The Trout*. Fontana, London. 316 pp.

- GARDELLA, E.S. and STASKO, A.B. (1974). A linear array hydrophone for determining swimming depth of fish fitted with ultrasonic transmitters. *Trans. Am. Fish. Soc.* 103, 635 - 636
- GASSER, H.S. and ERLANGER, J. (1927). The role played by the sizes of the constituent fibers of a nerve trunk in determining the form of its action potential wave. *Am. J. Physiol.* 80, 522 - 547
- GASSER, H.S. and ERLANGER, J. (1929). The role of fiber size in the establishment of nerve block by pressure or cocaine. *Am. J. Physiol.* 88, 581 - 591
- GEORGE, J.C. (1962). A histophysiological study of the red and white muscles of the mackerel. *Am. Mid. Nat.* 68, 487 - 494
- GIBSON, R.N. (1973). Tidal and circadian activity rhythms in juvenile Plaice *Pleuronectes platessa*. *Mar. Biol.* 22, 379 - 386
- GIBSON, R.N. (1978). In: Thorpe (1978)
- GORNIAK, G.C. (1977). Feeding in Golden Hamsters, *Mesocricetus auratus*. *J. Morph.* 154, 427 - 458
- GRAY, E.G. (1958). The structures of fast and slow muscle fibres in the frog. *J. Anat.* 92, 559 - 563
- GREEN, J.H. (1977). An introduction to Human Physiology. Oxford University Press, Oxford
- GREENE, C.W. (1912). A new type of fat storing muscle in the Salmon, *Oncorhynchus tshawytscha*. *Am. J. Anat.* 13, 175 - 181
- GREENE, C.W. (1913). An undescribed longitudinal differentiation of the Great Lateral Muscle of the King Salmon. *Anat. Rec.* 7, 99 - 101
- GREENE, C.W. and GREENE, C.H. (1913). The skeletal Musculature of the King Salmon. *Bull. Bur. Fish.* 33, 25 - 59

- GREER-WALKER, M. (1970). Growth and development of the skeletal muscle fibres of the Cod (Gadus morhua L.). J. Cons. Int. Explor. Mer. 33, 228 - 244
- GREER-WALKER, M. (1971). Effect of starvation and exercise on the skeletal muscle fibres of the Cod (Gadus morhua L.) and the Coalfish (Gadus virens L.) respectively. J. Cons. Int. Explor. Mer. 33, 421 - 426
- HALL, L.W. (1971). Wright's Veterinary Anaesthesia and Analgesia. 7th Edition. Balliere and Tindall, London
- HAMMOND, G.R. and RIDGE, RM.A.P. (1978). Properties of twitch motor units in snake costocutaneous muscle. J. Physiol. 276, 525 - 533
- HARDEN JONES, F.R. (1973). Tail beat frequency, amplitude and swimming speed of a shark tracked by sector scanning sonar. J. Cons. Int. Explor. Mer. 35, 95 - 97
- HARKER, D.W. (1972). The structure and innervation of sheep superior rectus and levator palpebrae muscles. I. Extrafusul Muscle fibres. Investve. Opth. 11, 956 - 969
- HARNESS, D., SEKELES, E. and CHACO, J. (1974). The double motor Innervation of the opponens pollicis muscles: an electromyographic study. J. Anat. 117, 329 - 331
- HART, L.G. and SUMMERFELT, R.C. (1975). Surgical procedures for Implanting ultrasonic transmitters Into Flathead Catfish (Pylodictis olivavis). Trans. Am. Fish. Soc. 104, 56 - 59
- HASLER, A.D. and BARDACH, J.E. (1949). Daily migrations of Perch in Lake Mendota, Wisconsin. J. Wildl. Mgt. 13, 40 - 51
- HASLER, A.D., GARDELLA, E.S., HERRALL, R.M. and HENDERSON, H.F. (1969). Open-water orientation of White Bass, Roccus chrysops, as determined by ultrasonic tracking methods. J. Fish. Res. Bd. Can. 26, 2173 - 2192

- HAWKINS, A.D., MCLENNAN, D.N., URQUART, G.G. and ROBB, C. (1974). Tracking Cod Gadus morhua L. in a Scottish sea loch. J. Fish Biol. 6, 225 - 236
- HELLEMANS, J., VANTRAPPEN, G., VALEMBOS, P., JANSSENS, J. and VANDENBROUCKE, J. (1968). Electrical activity of striated and smooth muscle of the esophagus. Am. J. Dig. Dis. 13, 320 - 335
- HESS, A. and PILAR, G. (1963). Slow fibres in the extraocular muscle of the cat. J. Physiol. 169, 780 - 798
- HIDAKA, T. and TOIDA, N. (1969). Neuromuscular transmission and excitation-contraction coupling in fish red muscle. Jap. J. Physiol. 19, 130 - 142
- HINTON, D.E. (1975). Perfusion fixation of whole fish for electron microscopy. J. Fish. Res. Bd. Can. 32, 416 - 422
- HIRAIWA, T. (1978). Histochemical properties of masticatory muscles of growing rat and of matured mammals. Comp. Biochem. Physiol. 59A, 231 - 238
- HIRATA, H. (1957). Diurnal rhythm of the feeding activity of Goldfish in winter and early spring. Bull. Fac. Fish. Hokkaido Univ. 8, 96 - 107
- HIRATA, H. (1973). Diurnal rhythm of metabolic and activity rates in juvenile Atlantic Salmon, Salmo salar Linnaeus. Mem. Fac. Fish. Kagoshima Univ. 22, 73 - 93
- HOAR, W.S. (1942). Diurnal variations in feeding activity of young Salmon and Trout. J. Fish. Res. Bd. Can. 6, 90 - 101
- HOBSON, E.S. (1963). Feeding behaviour in three species of sharks. Pacific Sci. 17, 171 - 194
- HOBSON, E.S. (1973). Diel feeding migrations in tropical reef fishes. Helgol. Wiss. Meers. 24, 361 - 370

- HOBSON, E.S. and CHESS, J.R. (1973). Feeding oriented movements of Atherinid Fish Pranesus pinguis at Majuro Atoll, Marshall Is. Fishery Bull. 71, 777 - 786
- HOLMES, W. (1943). Silver staining of nerve axons in paraffin sections. Anat. Rec. 86, 157
- HOLLIDAY, F.G.T., TYTLER, P. and YOUNG, A.H. (1973). Activity levels of Trout (Salmo trutta) in Airthrey Loch and Loch Leven, Kinross. Proc. Roy. Soc. Edinb. B. 74, 315 - 331
- HOLT, C.S., GRANT, G.D.S., OBERSTAR, G.P., OAKES, C.C. and BRADT, D.W. (1977). Movement of Walleye, Stizostedion vitreum, in Lake Bemidji, Minnesota, as determined by radio-biotelemetry. Trans. Am. Fish. Soc. 106, 163 - 169
- HORTON, B.J., TURLEY, S.D. and WEST, C.E. (1974). Diurnal variation in the feeding pattern of rabbits. Life Sciences. 15, 1895 - 1908
- HOUSTON, A.H., MADDEN, J.A., WOODS, R.J. and MILES, H.M. (1971a). Some physiological effects of handling and Tricaine Methane Sulphonate Anaesthesia upon the Brook Trout, Salvelinus fontinalis. J. Fish. Res. Bd. Can. 28, 625 - 633
- HOUSTON, A.H., MADDEN, J.A., WOODS, R.J. and MILES, H.M. (1971b). Variations in the blood and tissue chemistry of Brook Trout subsequent to handling, anaesthesia and surgery. J. Fish. Res. Bd. Can. 28, 635 - 642
- HOUSTON, A.H. and WOODS, R.J. (1972). Blood concentrations of Tricaine Methane Sulphonate in Brook Trout, Salvelinus fontinalis during anaesthesia, branchial irrigation and recovery. J. Fish. Res. Bd. Can. 29, 1344 - 1346
- HOUSTON, A.H., CZERWINSKI, C.L. and WOODS, R.J. (1973). Cardiovascular-respiratory activity during recovery from anaesthesia and surgery in Brook Trout (Salvelinus fontinalis) and Carp (Cyprinus carpio). J. Fish. Res. Bd. Can. 30, 1705 - 1712

- HUDSON, R.C.L. (1969). Polyneural Innervation of the fast muscles of the marine Teleost Cottus scorpius L. J. Exp. Biol. 50, 47 - 67
- HUDSON, R.C.L. (1973). On the function of the white muscles in teleosts at intermediate swimming speeds. J. Exp. Biol. 58, 509 - 522
- HUGHES, G.M. (1975). Coughing in the Rainbow Trout (Salmo gairdneri) and the Influence of pollutants. Rev. Suisse Zool. 82, 47 - 64
- HUGHES, G.M. and ADENEY, R.J. (1977). Variations in the pattern of coughing in Rainbow Trout. J. Exp. Biol. 68, 109 - 122
- HUGHES, G.M. and BALLINTIJN, C.M. (1965). The muscular basis of the respiratory pumps in the Dogfish (Scyliorhinus canicula). J. Exp. Biol. 43, 363 - 383
- HUGHES, G.M. and BALLINTIJN, C.M. (1968). Electromyography of the respiratory muscles and gill water flow in the Dragonet. J. Exp. Biol. 49, 583 - 602
- HUGHES, G.M. and ROBERTS, J.L. (1970). A study of the effects of temperature on the respiratory pumps of the Rainbow Trout. J. Exp. Biol. 52, 177 - 192
- HUGHES, G.M. and UMEZAWA, S-I. (1968). On respiration in the Dragonet (Callionymus lyra). J. Exp. Biol. 49, 565 - 582
- HUNN, J.B. and ALLEN, J.L. (1974). Movement of drugs across the gills of fishes. Ann. Rev. Pharmacol. 14, 47 - 55
- HUNT, C.C. (1951). The reflex activity of mammalian small-nerve fibres. J. Physiol. 115, 456 - 469
- HUNTER, J.R. and ZWEIFEL, J.R. (1971). Swimming speed, tailbeat frequency and size in Jack Mackerel Trachurus symmetricus and other fishes. Fish. Bull. 69, 253 - 266
- HURSCH, J.B. (1939). Conduction velocity and diameter of nerve fibers. Am. J. Physiol. 127, 131 - 139

- HUTTER, O.F. and TRAUTWEIN, W. (1956). Neuromuscular facilitation by stretch of motor nerve-endings. *J. Physiol.* 133, 610 - 625
- INMAN, V.T., RALSTON, H.J., SAUNDERS, J.B. de C.M., FEINSTEIN, B. and WRIGHT, E.W. (1952). Relation of human E.M.G. to muscular tension. *E.E.G. Clin. Neurophysiol.* 4, 187 - 194
- ISHIWATA, N. (1968). Ecological studies on the feeding of fishes. VI. External factors affecting satiation amount. *Bull. Jap. Soc. Sci. Fish.* 34, 785 - 791
- ITO, Y. and KURIYAMA, H. (1971). Nervous control of the motility of the alimentary canal of the Silver Carp. *J. Exp. Biol.* 55, 469 - 487
- JENKINS, T.M. (1969). Night feeding of Brown and Rainbow Trout in an experimental stream channel. *J. Fish. Res. Bd. Can.* 26, 3275 - 3278
- JEWELL, P. and ZAIMIS, E. (1953). A differentiation between red and white muscle in the cat based on responses to neuromuscular substances. *J. Physiol.* 120, 47 - 48
- JEWELL, P.A. and ZAIMIS, E.J. (1954). A differentiation between red and white muscle in the cat based on responses to neuromuscular blocking agents. *J. Physiol.* 124, 417 - 428
- JOHNSON, P. and HASLER, A.D. (1977). Winter aggregations of Carp (Cyprinus carpio) as revealed by Ultrasonic Tracking. *Trans. Am. Fish. Soc.* 106, 556 - 559
- JOHNSTON, I.A., WARD, P.S. and GOLDSPIK, G. (1975). Studies on the swimming musculature of the rainbow trout. I. Fibre types. *J. Fish Biol.* 7, 451 - 458
- KAISER, E. and PETERSEN, I. (1965). Muscle action potentials studied by frequency analysis and duration measurement. *Acta. Neurol. Scand. Suppl.* 13, 213 - 236
- KANWISHER, J., LAWSON, K. and SUNDNES, G. (1974). Acoustic telemetry from fish. *Fishery Bulletin.* 72, 251 - 255

- KASEDA, Y. and NOMURA, S-I. (1973). Electromyographical studies on the swimming movement of Carp. *Jap. J. Vet. Sci.* 35, 335 - 342
- KATZ, R.L. (1973). Electromyographic and mechanical effects of Suxamethonium and Tubocurarine on twitch, tetanic and post-tetanic response. *Brit. J. Anaesth.* 45, 849 - 859
- KELLY, M.A. (1974). In: Daniel (1974)
- KELSO, J.R.M. (1976). Diel movements of Walleye Stizostedion vitreum vitreum in West Blue Lake, Manitoba as determined by ultrasonic tracking. *J. Fish. Res. Bd. Can.* 33, 2070 - 2072
- KIRK, R.M. (1973). *Basic Surgical Techniques*. Churchill Livingstone, London. 168 pp.
- KISCH, B. (1950). Reflex cardiac inhibition in the ganoid Acipenser sturio. *Am. J. Physiol.* 160, 552 - 555 .
- KLEEREKOPER, H., TIMMS, A.M., WESTLAKE, G.F., DAVY, F.B., MALAR, T. and ANDERSON, V.M. (1970). An analysis of locomotor behaviour of Goldfish (Carassius auratus). *Anim. Beh.* 18, 317 - 330
- KLEEREKOPER, H., ANDERSON, V.M. and TIMMS, A.M. (1973). Logarithmic spiral components in locomotor patterns of fish. *Can. J. Zool.* 51, 397 - 400
- KLEEREKOPER, H., MATIS, J., GENSLER, P. and MAYNARD, P. (1974). Exploratory behaviour of Goldfish (Carassius auratus). *Anim. Behav.* 22, 124 - 132
- KNEIS, P. and SIEGMUND, R. (1976). Heart rate and locomotor activity in Fish: Correlation and circadian and circannual differences in Cyprinus carpio L. *Experientia.* 32, 474 - 476
- KOMI, P.V. and VIITASALO, J.H.T. (1976). Signal characteristics of EMG at different levels of muscle tension. *Acta. Physiol. Scand.* 96, 267 - 276

- KORDYLEWSKI, L. (1973). Motor nerve endings of extraocular muscles of Gobio gobio L. Folia. Histochem. Cytochem. 11, 323 - 333
- KOSAROV, D. and GYDIKOV, A. (1975). The influence of the volume conduction on the shape of the action potentials recorded by various types of needle electrodes in normal human muscle. E.M.G., Clin. Neurophysiol. 15, 319 - 335
- KOTCHABHAKDI, N., KANWISHER, J. and PROSSER, C.L. (1973). Acoustic telemetry of electrical activity from brain of free swimming dogfish. Underwater Telemetry Newsletter. 3, 7 - 8
- KRAATZ, C.P. and GLUCKMAN, M.I. (1954). The actions of barbiturates on the contractions of voluntary muscle. J. Pharmacol. Exp. Ther. 111, 120 - 129
- KRÜGER, P. (1950). Über das Vorkommen von zweierlei Fasern in der Muskulatur von Haien. Z. Naturforschung. 56, 218 - 220
- KRÜGER, P. and GÜNTHER, P.G. (1955). Fasern mit "Fibrillenstruktur" und Fasern mit "Felderstruktur" in der quergestreiften Skelettmuskulatur der Säuger und des Menschen. Z. ges. Anat. i.z. Anat. Entn-Gesch. 118, 313 - 323
- KRÜGER, P. and GÜNTHER, P.G. (1958). Innervation und Pharmakologisches Verhalten des m.gastrocnemius und m.pectoralis maior der vögel. Acta. Anat. 33, 325 - 338
- KRUUK, H. (1963). Diurnal periodicity in the activity of the Common Sole. Neth. J. Sea Res. 2. 1 - 28
- KRYVI, H. and TOTLAND, G.K. (1977). Histochemical studies with microphotometric analysis of the lateral muscles in the sharks Etmopterus spinax and Galeus melanostomus. J. Mar. Biol. Assoc. U.K. 57, 261 - 271
- KUFFLER, S.W., LAPORTE, Y. and RANSMEIER, R.E. (1947). The function of the frog's small-nerve motor system. J. Neurophysiol. 10, 395 - 408

- KUFFLER, S.W. and VAUGHAN WILLIAMS, E.M. (1953). Properties of the "slow" skeletal muscle fibres of the frog. *J. Physiol.* 121, 318 - 340
- KUFFLER, S.W. and VAUGHAN WILLIAMS, E.M. (1953). The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they innervate. *J. Physiol.* 121, 289 - 317
- KYNASTON THOMAS, P. and YOUNG, J.Z. (1949). Internode lengths in the nerves of fishes. *J. Anat.* 4, 336 - 351
- LAIRD, L.M. and OSWALD, R.L. (1975). A note on the use of benzocaine (Ethyl-p-aminobenzate) as a fish anaesthetic. *J. Inst. Fish. Mgt.* 6, 92 - 94
- LAMING, P.R. and SAVAGE, G.E. (1978). Flow changes in visceral blood vessels of the chub (Leuciscus cephalis) during behavioural arousal. *Comp. Biochem. Physiol.* 59A, 291 - 293
- LANDLESS, P.J. (1976). Demand feeding behaviour of Rainbow Trout. *Aquaculture.* 7, 11 - 25
- LANNERGREN, J. and SMITH, R.J. (1966). Types of muscle fibres in toad skeletal muscle. *Acta. Physiol. Scand.* 68, 263 - 274
- LAURS, R.M., YUEN, H.S.H. and JOHNSON, J.H. (1977). Small-scale movements of Albacore, Thunnus alalunga, in relation to ocean features as indicated by ultrasonic tracking and oceanographic sampling. *Fish. Bull.* 75, 347 - 355
- LAWLER, G.H. (1969). Activity periods of some fishes in Hemming Lake, Canada. *J. Fish. Res. Bd. Can.* 26, 3266 - 3267
- LAWSON, K. and KANWISHER, J. (1973). Acoustic telemetry in studies of fish behaviour and physiology. *Underwater Telemetry Newsletter.* 3, 5 - 7
- LEHOULTER, J. and CHATELAIN, A. (1974). Analysis of electrical responses of newt skeletal muscle fibres in response to direct and indirect stimulation. *J. Physiol. (Paris).* 68, 615 - 633

- LENMAN, J.A.R. and RITCHIE, A.E. (1970). Clinical Electromyography. Pitman, London. 175 pp.
- LINKENS, D.A. and CANNELL, A.E. (1974). Interactive graphics analysis of gastro intestinal electrical signals. I.E.E.E. Trans. Biomed. Eng. 21, 335 - 339
- LONSDALE, E.M., STEADMAN, J.W. and PANCOE, W.L. (1966). A telemetring system for securing data on the motility of internal organs. I.E.E.E. Trans. Biomed. Eng. 13, 153 - 159
- LUITEN, P.G.M. (1975). The central projections of the trigeminal, facial and anterior lateral line nerves in Carp. J. Comp. Neurol. 160, 399 - 418
- LUTZ, B.R. (1929). Reflex cardiac and respiratory inhibition in the elasmobranch fish, Scyllium canicula. Am. J. Physiol. 90, 493
- LUTZ, B.R. (1930). Reflex cardiac and respiratory inhibition in the elasmobranch, Scyllium canicula. Biol. Bull. Woods Hole. 59, 170 - 178
- LYNCH, P.G. (1974). Vital staining of nerve fibres and endings. J. Anat. 118, 405 - 409
- LYON, E.P. (1926). A study of the circulation, blood pressure and respiration of sharks. J. Gen. Physiol. 8, 279 - 287
- MARDIA, K.V. (1976). Linear circular correlation coefficients and rhythmometry. Biometrika. 63, 403 - 405
- MCCARTHY, D.A., CHEN, G., KAUMP, D.H. and ENSOR, C. (1965). General anaesthetic and other pharmacological properties of 2-(O-Chlorophenyl)-2-Methylamino Cyclohexanone HCL (CI-581). J. New Drugs. 5, 32 - 33
- MCLEAVE, J.D. and STRED, K.A. (1975). Effect of dummy telemetry transmitters on stamina of Atlantic Salmon (Salmo salar) smolts. J. Fish. Res. Bd. Can. 32, 559 - 563

- MCCLEERY, R.H. (1977). On satiation curves. *Anim. Behav.* 25, 1005 - 1015
- MCCUTCHEON, F.H. (1970). Yawning in teleosts. *Comp. Biochem. Physiol.* 34, 339 - 344
- MCDONALD, J. (1973). Diel vertical movements and feeding habits of underyearling Sockeye Salmon (*O. nerka*) at Babine Lake, B.C. Tech. Rep. Fish. Res. Bd. Can. No. 378, 1 - 55
- MCLEOD, W.D., NUNALLY, H.N. and CANTRELL, P.E. (1976). Dependence of EMG power spectra on electrode type. *I.E.E.E. Trans. Biomed. Eng.* 23, 172 - 175
- MACPARTLAND, R.J., GORDON FOSTER, F., KUPFER, D.J. and WEISS, B.L. (1976). Activity sensors for use in psychiatric evaluation. *I.E.E.E. Trans. Biomed. Eng.* 23, 175 - 176
- MARCUS, G.H. and LOBERMEYER, G. (1955). Über eine bisher nicht beschriebene Wirkung eines α -substituierten Glycerin-Athers. *Der Anaesthetist.* 4, 167 - 169
- MARVIN, D.E. and HEATH, A.G. (1968). Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of freshwater fish. *Comp. Biochem. Physiol.* 27, 349 - 355
- MASSOPUST, L.C., WOLIN, L.R. and ALBIN, M.S. (1972). Electrophysiologic and behavioural responses to ketamine hydrochloride in the Rhesus monkey. *Anesth. Analg. Curr. Res.* 51, 329 - 341
- MATIS, J.H., KLEEREKOPER, H. and GENSLER, D. (1974). Non-random oscillatory changes in orientation of the Goldfish (*Carassius auratus*) in an open field. *Anim. Behav.* 22, 110 - 117
- MEDDIS, R. (1975). A statistical handbook for non-statisticians. McGraw-Hill, London. 162 pp.
- MEIJER, N.W. (1975). Cranial motor nerves innervating superficial respiratory muscles in Carp. *Neths. J. Zool.* 25, 103 - 113

- MILLER, A.J. and BALDWIN, J.P. (1974). Divergent synaptic influences affecting patterning of genioglossus motor units. *Brain Res.* 78, 179 - 191
- MILLER, A.J. (1978). Spectral analysis of the electromyogram of the temporal muscle in the Rhesus monkey (Macaca mulatta). *EEG. Clin. Neurophysiol.* 44, 317 - 327
- MILNER-BROWN, H.S. and STEIN, R.B. (1975). The relation between the surface electromyogram and muscular force. *J. Physiol.* 246, 549 - 569
- MITSON, R.B. (1978). In: Thorpe (1978)
- MITSON, R.B. and STORETON-WEST, (1971). A transponding acoustic fish tag. *Rad. and Electron. Eng.* 41, 483 - 489
- MITSON, R.B. and YOUNG, A.H. (1975). A survey of the problems of developing small acoustic fish tags. *Proceedings of the Conference on Instrumentation in Oceanography, Bangor*, 163 - 174
- MORGAN, M.J. (1974). Resistance to satiation. *Anim. Behav.* 22, 449 - 466
- MORGAN, R.I.G. (1974). The energy requirements of Trout and Perch populations in Loch Leven, Kinross. *Proc. Roy. Soc. Edin.* 74B, 334 - 345
- MOSSE, R.L. and HUDSON, R.C.L. (1977). The functional roles of different muscle fibre types identified in the myotomes of marine teleosts: a behavioural, anatomical and histochemical study. *J. Fish Biol.* 11, 417 - 430
- MROSE, H.E. and RITCHIE, J.M. (1978). Local anesthetics: do Benzocaine and Lidocaine act at the same single site. *J. Gen. Physiol.* 71, 223 - 225
- MURPHY, R.A. and BEARDSLEY, A.C. (1974). Mechanical properties of the cat soleus muscle in situ. *Am. J. Physiol.* 227, 1008 - 1014

- NAG, A.C. (1972). Structural organization and histochemical properties of red and white muscle fibers of the trunk region of the rainbow trout. *Am. Zool.* 12, 427
- NAG, A.C. and NURSALL, J.R. (1972). Histogenesis of white and red muscle fibres of trunk muscles of a fish, Salmo gairdneri. *Cytobios.* 6, 227 - 246
- NAKAJIMA, Y. (1969). Fine structure of red and white muscle fibres and their neuromuscular junctions in the Snake Fish (Ophiocephalus argus). *Tissue and Cell*, 1, 229 - 246
- NICOL, J.A.C. (1952). Autonomic nervous systems in lower chordates. *Biol. Rev.* 27, 1 - 49
- NIE, N.H., HULL, C.H., JENKINS, J.G., STEINBRENNER, K. and BENT, D.H. (1975). *Statistical Package for the Social Sciences*. McGraw-Hill, New York. 675 pp.
- NISHIHARA, H. (1967). Studies on the fine structure of red and white fin muscles in the fish (Carassius auratus). *Arch. Histol. Jap.* 28, 425 - 447
- NOMURA, S-I., IBARAKI, T., HIROSE, H. and SHIRIHATA, S. (1972). Applications of back-pack radiotelemeter for fishes. I. Heart rate and cardiac reflex during unrestrained swimming. *Bull. Jap. Soc. Sci. Fish.* 38, 1105 - 1117
- NORRIS, J.S., NORRIS, D.O. and WINDELL, J.T. (1973). Effect of simulated meal size on gastric acid and pepsin secretory rates in Bluegill (Lepomis). *J. Fish. Res. Bd. Can.* 30, 201 - 204
- OKI, M. and DANIEL, E.E. (1974). In: Daniel (1974)
- OLLA, B.L., SAMET, C.E. and STUDHOLME, A.L. (1972). Activity and feeding behaviour of the Summer Flounder (Paralichthys dentatus) under controlled laboratory conditions. *Fish. Bull.* 70, 1127 - 1136

- OSSE, J.W.M. (1969). Functional morphology of the head of the Perch; an electromyographic study. *Neth. J. Zool.* 19, 289 - 392
- OSSE, J.W.M., OLDENHAVE, M. and VAN SCHIE, B. (1972). A new method for insertion of wire electrodes in electromyography. *Electromyography.* 12, 59 - 62
- OSWALD, R.L. (1977a). Injection anaesthesia for experimental studies in Fish. *Comp. Biochem. Physiol. C.* 60C, 19 - 26
- PAGE, K.M. (1971). Histological methods for peripheral nerves - Part II. *Medical Laboratory Technology.* 28, 44
- PAINTAL, A.S. (1954). A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. *J. Physiol.* 126, 255 - 270
- PALMER, J.D. (1976). *An Introduction to Biological Rhythms.* Academic Press, New York. 375 pp.
- PATON, W.D.M. and ZAIMIS, E.J. (1951). The action of D-Tubocurarine and of Decamethonium on respiratory and other muscles in the cat. *J. Physiol.* 112, 311 - 331
- PATTERSON, S., JOHNSTON, I.A. and GOLDSPINK, G. (1975). A histochemical study of the lateral muscles of five species of teleost fish. *J. Fish Biol.* 7, 159 - 168
- PERSSON, L. (1979). The effects of temperature and different food organisms on the rate of gastric evacuation in Perch (Perca fluviatilis). *Freshwat. Biol.* 9, 99 - 104
- PETERSEN, I. and KUGELBERG, (1949). Duration and form of action potential in the normal human muscle. *J. Neurol. Neurosurg. Psychiat.* 12, 124 - 128
- PRIEDE, I.G. (1973). The physiology of circulation during swimming activity in the Rainbow Trout. Unpublished Ph.D. thesis, University of Stirling

- PRIEDE, I.G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in Rainbow Trout. *J. Exp. Biol.* 60, 305 - 319
- PRIEDE, I.G. (1978). In: Thorpe (1978)
- PRIEDE, I.G. and YOUNG, A.H. (1977). The ultrasonic telemetry of cardiac rhythms of Wild Brown Trout (Salmo trutta L.) as an indicator of bio-energetics and behaviour. *J. Fish Biol.* 10, 299 - 318
- PROCTOR, W.R. and WEAKLY, J.N. (1976). A comparison of the presynaptic actions of Pentobarbitone and Phenobarbitone in the neuromuscular junction of the frog. *J. Physiol.* 258, 257 - 268
- PROSSER, C.L. (1974a). Smooth muscle. *Ann. Rev. Physiol.* 36, 503 - 537
- PROSSER, C.L. (1974b). In: Daniel (1974)
- RALIS, H.M., BEESLEY, R.A. and RALIS, Z.A. (1973). *Techniques in Neurohistology*. Butterworth, London. 162 pp.
- RANDALL, D.J. (1971). The circulatory system. In: *Fish Physiology*. vol. 4. Chap. 4. Hoar, W.S. and Randall, D.J. (Eds). Academic Press, New York
- RAYNER, M.D. and KEENAN, M.J. (1967). Role of the red and white muscles in the swimming of the Skipjack Tuna. *Nature (London)*. 214, 392 - 393
- REVEL, P. (1962). The sarcoplasmic reticulum of the bat cricothyroid muscle. *J. Cell. Biol.* 12, 571 - 588
- REYNOLDS, W.W. and CASTERLIN, M.E. (1976). Locomotor rhythms in the Bluegill Sunfish, Lepomis macrochirus. *Am. Midl. Nat.* 96, 221 - 225
- REYNOLDS, W.W., CASTERLIN, M.E. and COVERT, J.B. (1976). Behavioural fever in teleost fishes. *Nature*. 259, 41 - 42

- REYNOLDS, W.W., CASTERLIN, M.E., MATTHEY, J.K., MILLINGTON, S.T. and OSTROWSKI, A.C. (1978). Diel patterns of preferred temperature and locomotor activity in the Goldfish Carassius auratus. Comp. Biochem. Physiol. 59A, 225 - 227
- RICHARDSON, N.E. and MCCLEAVE, J.D. (1974). Locomotor activity rhythms of Juvenile Atlantic Salmon (Salmo salar) in various light conditions. Biol. Bull. 147, 422 - 432
- RILEY, J.L. and COOK, H.M. (1974). A small inexpensive radio-telemetering capsule for measuring rumen motility. The Cornell Veterinarian. 64, 225 - 232
- ROBERTS, B.L. (1969a). The coordination of the rhythmical fin movements of dogfish. J. Mar. Biol. Assoc. U.K. 49, 357 - 378
- ROBERTS, B.L. (1969b). Spontaneous rhythms in the motorneurons of spinal dogfish (Scylliorhinus canicula). J. Mar. Biol. Assoc. U.K. 49, 33 - 49
- ROBERTS, B.L. (1969c). The spinal nerves of the dogfish (Scylliorhinus) J. Mar. Biol. Assoc. U.K. 49, 51 - 75
- ROBERTS, J.L. (1975). Active branchial and ram ventilation in fishes. Biol. Bull. 148, 85 - 105
- ROBERTS, B.L. and WITKOVSKY, P. (1975). A functional analysis of the mesencephalic nucleus of the fifth nerve in the selachian brain. Proc. Roy. Soc. Lond. B. 190, 473 - 495
- ROBERTS, M.G., WRIGHT, D.E. and SAVAGE, G.E. (1973). A technique for obtaining the electrocardiogram of fish. Comp. Biochem. Physiol. 44, 665 - 668
- ROBERTS, R.J., MCQUEEN, A., SHEARER, W.M. and YOUNG, H. (1973). The histopathology of salmon tagging. I. The tagging lesion in newly tagged parr. J. Fish Biol. 5, 497 - 503
- ROLLASON, W.L. (1975). Electrocardiography for the anaesthetist. 3rd Edition. Blackwell, Oxford. 182 pp.

- ROMMEL, S.A. (1973). A simple method for recording fish heart and opercular beats without the use of implanted electrodes. *J. Fish. Res. Bd. Can.* 30, 693
- ROZIN, P. and MAYER, I. (1961). Regulation of food intake in the Goldfish. *Am. J. Physiol.* 201, 968 - 974
- ROZIN, P.N. and MAYER, I. (1964). Some factors influencing short term food intake in the Goldfish. *Am. J. Physiol.* 206, 1430 - 1436
- SATCHELL, G.H. (1960). Reflex co-ordination of the heart beat with respiration in the Dogfish. *J. Exp. Biol.* 32, 719 - 731
- SAUNDERS, J.T. and MANTON, S.M. (1969). A manual of practical vertebrate morphology. Clarendon Press, Oxford.
- SCHUMANN, D. and PIIPER, J. (1966). Der Sauerstoffbedarf der Atmung bei Fischen nach Messungen an der narkotisierten Schleie (Tinca tinca). *Pflugers Arch. ges. Physiol.* 288, 15 - 26
- SCOTT, R.N. (1969). An improved bipolar wire electrode for electromyography. *Med. Biol. Eng.* 7, 677 - 678
- SEYAMA, I. and NARAHASHI, T. (1975). Mechanism of blockade of neuromuscular transmission by Pentobarbital. *J. Pharmacol. Exp. Ther.* 192, 95 - 104
- SHAEFFER, B. and ROSEN, E.F. (1964). Major adaptive levels in the evolution of the actinopterygian feeding mechanism. *Am. Zool.* 1, 187 - 204
- SHELTON, G. and RANDALL, D.J. (1962). The relationship between heartbeat and respiration in teleost fish. *Comp. Biochem. Physiol.* 7, 237 - 250
- SHERRINGTON, C.S. (1906). The integrative action of the nervous system. Arno Press, New York. 433 pp.

- SIEGMUND, R. (1969). Lokomotorische Aktivität und Ruheverhalten bei einheimischen Süßwasserfischen. (Pisces; Percidae, Cyprinidae). Biol. Zbl. 88, 295 - 312
- SIEGMUND, R. and VOGEL, J. (1977). The dependence of heart rate and locomotor activity in the Carp (Cyprinus carpio L.). Experientia. 33, 1607 - 1609
- SIMMONS, D.J., LESKER, P.A. and SHERMAN, N.E. (1974). Induction of Sodium Pentobarbital anaesthesia: A circadian rhythm. J. Interdis. Cyc. Res. 5, 71 - 77
- SMITH, A.W.M. and CODE, C.F. (1958). The effect of an ordinary and of an excessively fatty breakfast on gastric motility. Gastroenterology. 35, 398 - 405
- SMITH, J.L., EDGERTON, R., BETTS, B. and COLLATOS, T.C. (1977). EMG of slow and fast ankle extensors of cat during posture, locomotion and jumping. J. Neurophysiol. 40, 503 - 513
- SMITH, J.N.M. (1975). The food searching behaviour of two European Thrushes. II. The adaptiveness of the search pattern. Behaviour. 49, 1 - 61
- SMITH, L.S. and BELL, G.R. (1967). Anaesthetic and surgical techniques for Pacific Salmon. J. Fish. Res. Bd. Can. 24, 1579 - 1588
- SNODGRASS, J.M. and GILBERT, P.W. (1967). Sharks, Skates and Rays. The John Hopkins Press
- SOIVIO, A., NYHOLM, K. and HUHTI, M. (1977). Effects of anaesthesia with MS222, neutralised MS222, and Benzocaine on the blood constituents of Rainbow Trout Salmo gairdneri. J. Fish Biol. 10, 91 - 101
- SPARKS, R.L., CAIRNS, J. and HEATH, A.G. (1972). The use of Bluegill breathing rates to detect zinc. Water Res. 6, 895 - 911

- SPENCER, W.D. (1939). Diurnal activity rhythms in freshwater fishes. *Ohio J. Sci.* 39, 119 - 132
- SPOOR, W.A. and SCHLOEMER, C.L. (1939). Diurnal activity of the Common Sucker Catostomus commersoni (Lacépède) and the Rock Bass Ambloplites rupestris (Rafinesque) in Muskellunge Lake. *Trans. Am. Fish. Soc.* 68, 211 - 220
- STEVENS, E.D. (1968). The effect of exercise on the distribution of blood to various organs in Rainbow Trout. *Comp. Biochem. Physiol.* 25, 615 - 625
- STEVENS, J.K. and WORRAL, N. (1974). External recording of gastric activity: the electrogastrogram. *Physiol. Psychol.* 2, 175 - 180
- STASKO, A.B. (1971). Review of field studies of fish orientation. *Ann. N.Y. Acad. Sci.* 188, 12 - 19
- STASKO, A.B. and HERRALL, R.M. (1976). Method of counting tailbeats of free-swimming fish by ultrasonic telemetry techniques. *J. Fish. Res. Bd. Can.* 33, 2596 - 2598
- STASKO, A.B. and PINCOCK, D.G. (1977). Review of underwater biotelemetry, with emphasis on ultrasonic techniques. *J. Fish. Res. Bd. Can.* 34, 1261 - 1285
- STASKO, A.B. and ROMMEL, S.A. (1974). Swimming depth of adult American Eels (Anguilla rostrata) in a saltwater bay as determined by ultrasonic tracking. *J. Fish. Res. Bd. Can.* 31, 1148 - 1150
- STIRLING, H.P. (1977). Growth, food utilization and effect of social interaction in the European Bass Dicentrarchus labrax. *Mar. Biol.* 40, 173 - 184
- SUTTERLIN, A.M. (1969). Effects of exercise on cardiac and ventilation frequency in three species of freshwater teleosts. *Physiol. Zool.* 42, 36 - 52

- SWENSON, W. and SMITH, L.L. (1973). Gastric digestion, food consumption, feeding periodicity and conversion efficiency in Walleye Stizostedion vitreum. J. Fish. Res. Bd. Can. 30, 1327 - 1336
- SWIFT, D.R. (1962). Activity cycles in the Brown Trout. 1. Fish feeding naturally. Hydrobiologia. 20, 241 - 247
- SWIFT, D.R. (1964). Activity cycles in the Brown Trout. 2. Fish artificially fed. J. Fish. Res. Bd. Can. 21, 133 - 138
- TAKEUCHI, A. (1959). Neuromuscular transmission of fish skeletal muscles investigated with intracellular microelectrode. J. Cell. Comp. Physiol. 54, 211 - 220
- TASAKI, I. and MIZUTANI, K. (1943). Comparative studies on the activity of the muscle evoked by two kinds of motor nerve. Part I. Myographic. Jap. J. Med. Sci. III. 10, 237 - 248
- TASAKI, I. and TSUKAGOSHI, M. (1943). Comparative studies on the activities of the muscle evoked by two kinds of motor nerve fibres. Part II. The Electromyogram. Jap. J. Med. Sci. III. 10, 245 - 255
- TAVOLGA, W.N. (1971). Sound production and detection. In: Fish Physiology. Vol. 5, 135 - 205. Hoar, W.S. and Randall, D.J. (Eds). Academic Press, New York
- THESLEFF, S. (1956). The effect of anaesthetic agents on skeletal muscle membrane. Acta. Physiol. Scand. 37, 335 - 349
- THOMAS, G. (1974). The influences of encountering a food object on subsequent searching behaviour in Gasterosteus aculeatus L. Anim. Behav. 22, 941 - 952
- THORPE, J.E. (1973). Trout and Perch populations at Loch Leven, Kinross. Proc. Roy. Soc. Edin.B. 74, 295 - 313
- THORPE, J.E. (1977). Daily ration of adult perch Perca fluviatilis L. during summer in Loch Leven, Scotland. J. Fish Biol. 11, 55 - 68

- THORPE, J.E. (Ed). (1978). Rhythmic activity of fishes. Academic Press, London. 312 pp.
- TINBERGEN, N., IMPEKOREN, M. and FRANK, D. (1967). An experiment on spacing out as a defence against predation. Behaviour. 28, 307 - 321
- TYLER, A.V. (1977). Evidence for the assumption of independence between gastric emptying rate and swimming activity using Atlantic Cod, Gadus morhua. J. Fish. Res. Bd. Can. 34, 2411 - 2413
- TYLER, A.V. (1970). Rates of gastric emptying in young cod. J. Fish. Res. Bd. Can. 27, 1177 - 1189
- TYTLER, P. CUST, L.A. and FERGUSON, H. (1975). The structure and mechanical properties of the adductor mandibulae muscle of Rainbow Trout. Society for Experimental Biology: Stirling Meeting, 1975 (Unpublished)
- TYTLER, P., MACHIN, D., HOLLIDAY, F.G.T. and PRIEDE, I.G. (1976). A comparison of the patterns of movement between indigenous and displaced brown trout (Salmo trutta L.) in a small shallow water loch. Proc. Roy. Soc. Edinb. 76B, 1 - 24
- UEMATSU, T. (1971). Social facilitation in feeding behaviour of the guppy III. Jap. J. Ecol. 21, 96 - 103
- UGLOW, R.F. (1973). Some effects of acute oxygen changes on the heart and scaphognathite activity in some Portunid crabs. Neths. J. Sea Res. 7, 447 - 454
- VAN DE GRAAFF, K.M., FREDERICK, E.C., WILLIAMSON, R.G. and GOSLOW, G.E. (1977). Motor units and fiber types of primary ankle extensors of the Skunk (Mephitis mephitis). J. Neurophysiol.
- VARANELLI, C.C. and MCCLEAVE, J.D. (1974). Locomotor activity of Atlantic Salmon parr (Salmo salar L.) in various light conditions and in weak magnetic fields. Anim. Behav. 22, 178 - 186

- VERHEIJEN, F.J. and DE GROOT, S.J. (1969). Diurnal activity pattern of Plaice and Flounder in aquaria. *Neth. J. Sea Res.* 3, 383 - 390
- WALLIS, D.I., LEES, G.M. and KOSTEVLITZ, H.W. (1975). Recording resting and acting potentials by the sucrose gap method. *Comp. Biochem. Physiol.* 50C, 199 - 216
- WALTERS, V. (1966). On the dynamics of filter feeding by the Wavyback Skipjack (Euthynnus affinis). *Bull. Mar. Sci.* 16, 209 - 221
- WANKOWSKI, J.W.J. (1977). The role of prey size in the feeding behaviour and growth of juvenile Atlantic Salmon (Salmo salar L.) Unpublished Ph.D. thesis. University of Stirling. 157 pp.
- WARDLE, C.S. and ANTHONY, P.D. (1973). Experimental methods used for the study of fish behaviour in large tanks. I.C.E.S. C.M. Gear and Behav. Cttee. B22
- WARDLE, C.S. (1971). New observations on the lymph system of the Plaice (Pleuronectes platessa) and other teleosts. *J. Mar. Biol. Ass. U.K.* 51, 977 - 990
- WARDLE, C.S. (1974). A new form of barbed attachment device for short-term spear tagging of fish by divers at sea. I.C.E.S. C.M. Gear and Behav. Cttee. B12
- WARDLE, C.S. and KANWISHER, J.W. (1974). The significance of heart rate in free-swimming Cod Gadus morhua: Some observations with ultrasonic tags. *Mar. Behav. Physiol.* 2, 311 - 324
- WARE, D.M. (1972). Predation by Rainbow Trout: The influence of hunger, prey density and prey size. *J. Fish. Res. Bd. Can.* 29, 1193 - 1201
- WARE, D.M. (1973). Risk of epibenthic prey to predation by Rainbow Trout. *J. Fish. Res. Bd. Can.* 30, 787 - 797
- WATZKA, M. (1939). "Weisse" and "rote" Muskeln. *Z. mikr.-anat. Forsch.* 45, 668 - 678

- WEINTRAUB, M.J. and MACKAY, R.S. (1975). Respiratory and heartbeat synchrony studied by telemetry in the Trout (Salmo gairdneri) Copela. 1975, 78 - 85
- WESTERN, J.R.H. (1969). Studies on diet, feeding mechanism and alimentary tract in two closely related Teleosts, the freshwater Cottus gobio L. and the marine Paranophrys bubalis Euphrasen. Acta. Zool. 50, 185 - 205
- WESTHUES, M. and FRITSCH, R. (1964). Animal Anaesthesia. Vol. 1. Local Anaesthesia. Oliver and Boyd, Edinburgh. 223 pp.
- WESTHUES, M. and FRITSCH, R. (1965). Animal Anaesthesia. Vol. 2. General Anaesthesia. Oliver and Boyd, Edinburgh. 458 pp.
- WIENBECK, M. and JANSSEN, H. (1974). In: Daniel (1974)
- WIKGREN, B.J. (1950). Daily activity pattern of the Burbot. Mem. Soc. Fauna. Flora. Fenn. 31, 91 - 97
- WILKIE, D.R. (1956). The mechanical properties of muscle. Brit. Med. Bull. 12, 177 - 182
- WILLIAMS, P.L. and WENDELL-SMITH, C.P. (1960). The use of fixed and stained sections in quantitative studies of peripheral nerve. Q.J. Micr. Soc. 101, 43 - 54
- WINDELL, J.T., KITCHELL, J.F., NORRIS, D.O., NORRIS, D.J. and FOLTZ, J.W. (1976). Temperature and rate of gastric evacuation by Rainbow Trout (Salmo gairdneri). Trans. Am. Fish. Soc. 105, 712 - 717
- WINDELL, J.T., NORRIS, D.O., KITCHELL, J.F. and NORRIS, J.S. (1969). Digestive response of Rainbow Trout Salmo gairdneri to pellet diets. J. Fish. Res. Bd. Can. 26, 1801 - 1812
- WINKELMANN, R.K. and SCHMITT, R.W. (1957). A simple method for nerve axoplasm. Proc. Staff Meeting, Mayo Clinic. 32, 217

- WINTER, J.D. (1977). Summer home range movements and habitat use by four Largemouth Bass in Mary Lake, Minnesota. *Trans. Am. Fish. Soc.* 106, 323 - 330
- WITTENBERGER, C. (1967). On the function of the lateral red muscle of Teleost fishes. *Rev. Roum. Biol.* 12, 139 - 144
- WITTENBERGER, C. and COPREAN, D. (1977). Some effects of denervation upon white and red muscles in Carp. *Comp. Biochem. Physiol.* 56A, 307 - 312
- WITTENBERGER, C. CORO, A., SUAREZ, G. and PORTILLA, N. (1969). Composition and bioelectrical activity of the lateral muscles in Harengula humeralis. *Mar. Biol.* 3, 24 - 27
- WOLF, K. (1963). Physiological salines for freshwater teleosts. *Prog. Fish. Cult.* 25, 135 - 140
- WOOD, J.D. (1975). Neurophysiology of Auerbach's Plexus and Control of Intestinal Motility. *Physiol. Rev.* 55, 307 - 324
- YAMAMOTO, T. (1972). Electrical and mechanical properties of the the red and white muscles in the Silver Carp. *J. Exp. Biol.* 57, 551 - 567
- YOUNG, A.H., TYTLER, P. and HOLLIDAY, F.G.T. (1976). New developments ultrasonic fish tracking. *Proc. Roy. Soc. Edinb. B.* 75, 145 - 155
- YOUNG, A.H., TYTLER, P., HOLLIDAY, F.G.T. and MACFARLANE, A. (1972). A small sonic tag for measurement of locomotor behaviour in fish. *J. Fish. Biol.* 4, 57 - 65
- YOUNG, A.H. and WIEWIORKA, J. (1975). Ultrasonic tracking and telemetering system for use on small fish. *Underwater Telemetry Newsletter.* 5, 10 - 13
- YOUNG, S. (1971). EMG activity in Tench (Tinca tinca L.) gill lamellae and its association with coughing. *J. Physiol.* 215, 37 - 38

- YOUNG, S. (1973). Basic Electronics In the Life Sciences.
Macmillán, London. 198 pp.
- YOUNG, S. (1974). Electromyographic activity during respiration and coughing in the Tench. J. Physiol. 227, 18 - 19
- YUWILER, A. and SAMUEL, D. (1974). Ethyl-m-aminobenzoate (MS-222) anaesthesia in the Newt - effect of D₂O, pH and time of day. Experientia. 30, 1058 - 1060
- ZACH, R. and FALLS, J.B. (1977). Influence of capturing a prey on subsequent search in the Ovenbird (Aves: Parulidae). Can. J. Zool. 55, 1958 - 1969
- ZAIMIS, E.J. (1953). Motor endplate differences as a determining factor in the mode of action of neuro-muscular blocking substances. J. Physiol. 122, 238 - 251

ADDENDUM TO REFERENCES

Proceedings of Symposia not fully listed in references :

1. In : DANIEL, E.E. (1974) (Ed.) Proc. 4th Int. Symp. G - I Motility,

Vancouver.

KELLY , M.A. (1974) The use of miniaturised Mercury Strain Gauges to record gastric motility. pp 323 - 329.

PROSSER, C.L. (1974) Diversity of electrical activity in gastrointestinal muscles. pp 21 - 37.

WIENBECK, M. AND JANSSEN, H. (1974) Electrical control mechanisms at the ileo-colic junction. pp 97 - 107.

2. In : THORPE, J.E. (1978) (Ed.) Rhythmic activity of Fishes.

Academic Press, London.

ERIKSSON, L - O. (1978) Nocturnalism versus diurnalism - dualism within fish individuals. pp 69 - 89.

GIBSON, R.N. (1978) Lunar and tidal rhythms in fish. pp 201 - 213.

MITSON, R.B. (1978) A review of Biotelemetry techniques using acoustic tags. pp 269 - 283.

PRIEDE, I.G. (1978) Behavioural and Physiological rhythms of fish in their natural environment, as indicated by ultrasonic telemetry of heart rate. pp 153 - 168.