AN EXAMINATION OF SOME PHYSIOLOGICAL AND INSTRUMENTAL PARAMETERS AFFECTING THE CONTRACTION OF CIRCULATED MAMMALIAN MUSCLE.

I hereby declare that the contents of this dissertation are my own work and that they have not been previously submitted as a dissertation or thesis for any degree.

L. B. Geffen.

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SECTION III :

DEVELOPMENT OF IMPROVED APPARATUS AND TECHNIQUE.

The rapid progress of the last decade has made possible the synthesis of the molar and molecular approaches to the mechanism of muscle contraction. It is now possible to offer a molecular explanation for many of the gross mechanical properties of muscle. As a result there is a necessity to re-examine the validity of the classical terminology used to describe these properties, and to define them more accurately.

Since Fick (1882), various "types" of contraction have been ascribed to muscle, according to the changes in length and tension of the activated muscle. These are dependent upon the load opposing the muscle. If the load is less than the force developed in the muscle, shortening occurs at a constant tension just exceeding the load. This process is termed isotonic contraction. If, on the other hand, the load is equal to the tension developed in the muscle, there is no overall change in length, although tension in the system rises. This constitutes an isometric contraction. Recently, studies of the ultra-structure and mechanical properties of muscle have revealed inherent difficulties in the classical terminology.

The sliding filament hypothesis of muscle contraction, evolved by A.F. and H.E. Huxley and their associates, suggests that at a molecular level, the mechanism of isotonic and isometric contractions is essentially the same. In both, shortening occurs as the result of two sets of filaments sliding past one another in an interdigitating fashion. In isotonic contraction external shortening results whereas in isometric contraction, the shortening is taken up in a series compliance so that no external length change occurs. (Huxley H.E. and Hanson, 1954; Huxley A.F. and Peachey, 1961).

The work of A.V. Hill and others on whole muscle has likewise established that the overall mechanical response of muscle is compounded of the interaction of contractile and elastic components. (Hill, 1949; Ritchie & Wilkie, 1958).

The mechanical conditions used to measure contraction will determine to what extent the elastic component will modify the response. Thus the terms, isotonic and isometric become functions of the recording system rather than "types" of contraction.

This emphasizes the importance of examining critically the recording method used to measure contraction, for it is necessary to avoid confusing the properties of the muscle with those of the recording system. No recording system measures purely isotonically or isometrically. Isotonic systems require some tension change to produce movement of the transducer and, conversely, all isometric transducers require some small length change in order to measure tension. In ideal apparatus these changes are minimised by reducing the inertia, friction and compliance of the system.

Furthermore, the mechanical properties of voluntary muscle have been almost exclusively studied in the isolate frog preparation. The study of mammalian muscle in situ introduces many more experimental variables, such as the significant effects of temperature and circulatory responses. The validity of extrapolating data derived from isolate studies in the frog to the properties of mammalian muscle in situ has been questioned by Rosenbleuth, Alanis and Rubio (1958 a & b). Working primarily on circulated cat muscle, they concluded that the length-tension diagrams of isometric and isotonic contractions are not superimposable and that there is no well defined optimal length for contraction within the physiological range. These findings are at variance with generally held concepts and have been ascribed to experimental error. (Fischer, 1961; Zierler, 1961; Wilkie, 1962).

In this dissertation some of these parameters, instrumental and physiological, which influence muscle contraction will be examined in detail. A standard controlled set of conditions will be established for the investigation of mammalian muscle in situ, and, in order to obviate some of the instrumental difficulties a new apparatus will be described, in which it is possible to measure with accuracy under controlled conditions, isometric and isotonic contractions of circulated rat muscle.

EXPERIMENTAL DESIGN.

In broad outline, the experimental design follows the principles of SYSTEM THEORY as enunciated by Trimmer (1950) and Stacy et al. (1955). They define a system as a group of interacting components of a physical mechanism which can be considered a functional unit. The properties of the system determine the relationship between the stimulus and the response. This relationship can be altered by variables affecting the stimulus, the biological system and the response recording system.

Since biological systems are invariably complex, the experimental derivation of their properties is often facilitated by the procedure of isolation of variables, whereby those components considered irrelevant are excluded. An example of such a design is the isolated muscle stimulated directly. However, such procedures entail a departure from physiological conditions which makes extrapolation to intact situations more difficult.

A compromise between these two requirements of design, the isolation of variables and the maintainence of a physiological situation, was attempted. The triceps surae of the rat was studied in situ. Isolation of stimulus variables was obtained by eliminating the effects of the central nervous system by interrupting connections with the spinal cord and stimulating the muscle through its motor nerve using electrical stimuli of known dimensions.

An attempt was made to strictly control rather than eliminate significant system variables such as anaesthesia, temperature, circulation and loading parameters. The first step toward controlling these variables was the measurement of their effects. They were therefore altered one at a time and the range of their effects noted. Thereafter they were maintained constant and reproducibility of results under standard conditions gave a measure of their control.

Finally it was necessary to isolate the response variables such as friction and inertia of the recording apparatus. In order to do this, it proved necessary to develop apparatus incorporating electronic rather than mechanical transducers.

In accordance with the above principles of experimental design this dissertation has three main sections:-

(i). SECTION I deals with the choice and development of a system; namely, the measurement by means of a mechanical lever of isotonic tetani of the circulated triceps surae of the rat, stimulated electrically through its motor nerve.

(ii) SECTION II concerns the isolation and control of system variables. A series of pilot studies of the physiological determinants of muscle performance in situ were conducted and a standard situation was evolved.

(iii) SECTION III is concerned with the development of electronic transducers and other apparatus, which reduced response variables by eliminating or diminishing the sources of error which became apparent in the use of the old mechanical lever system. In addition, an attempt was made to develop an accurate and reliable method of correlating the experimentally induced length changes with those occurring in the intact animal under physiological conditions.

The work described was done on a part-time basis over the period 1958 to 1963.

SECTION I

THE DEVELOPMENT AND STANDARDISATION OF A CIRCULATED MAMMALIAN MUSCLE PREPARATION FOR RECORDING ISOTONIC TETANIC CONTRACTIONS USING A MECHANICAL LEVER SYSTEM.

Experimental Animals.

Anaesthetic

Immobilisation.

Recording Apparatus.

Motor Nerve Stimulation.

Temperature Control.

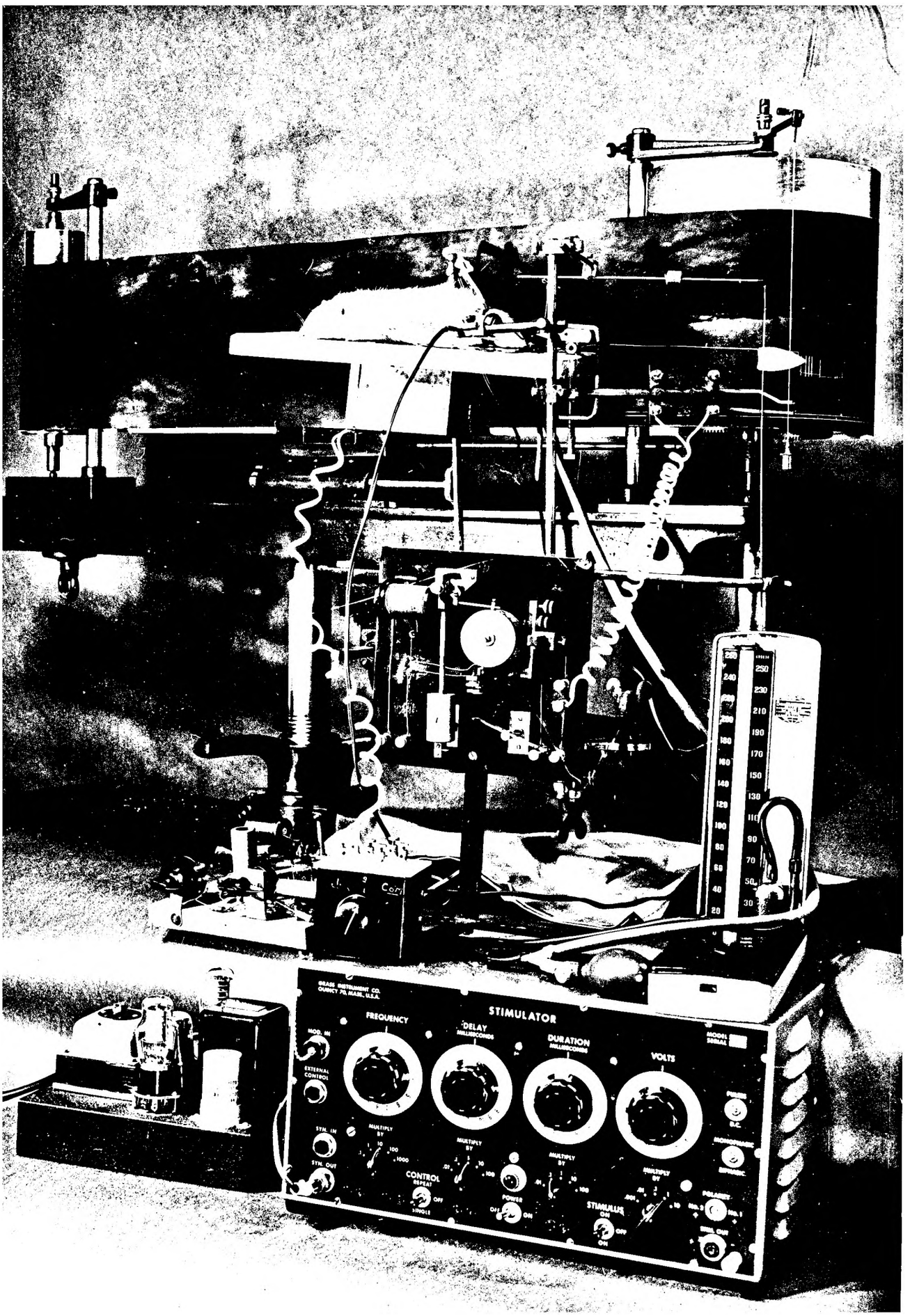
Occlusion Cuff.

Measurement of Mechanical Response

Measurement of Passive Length changes

Measurement of Active length changes.

Measurement of work output of Muscle.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QUINCY, MASS., U.S.A.
STIMULATOR
FREQUENCY
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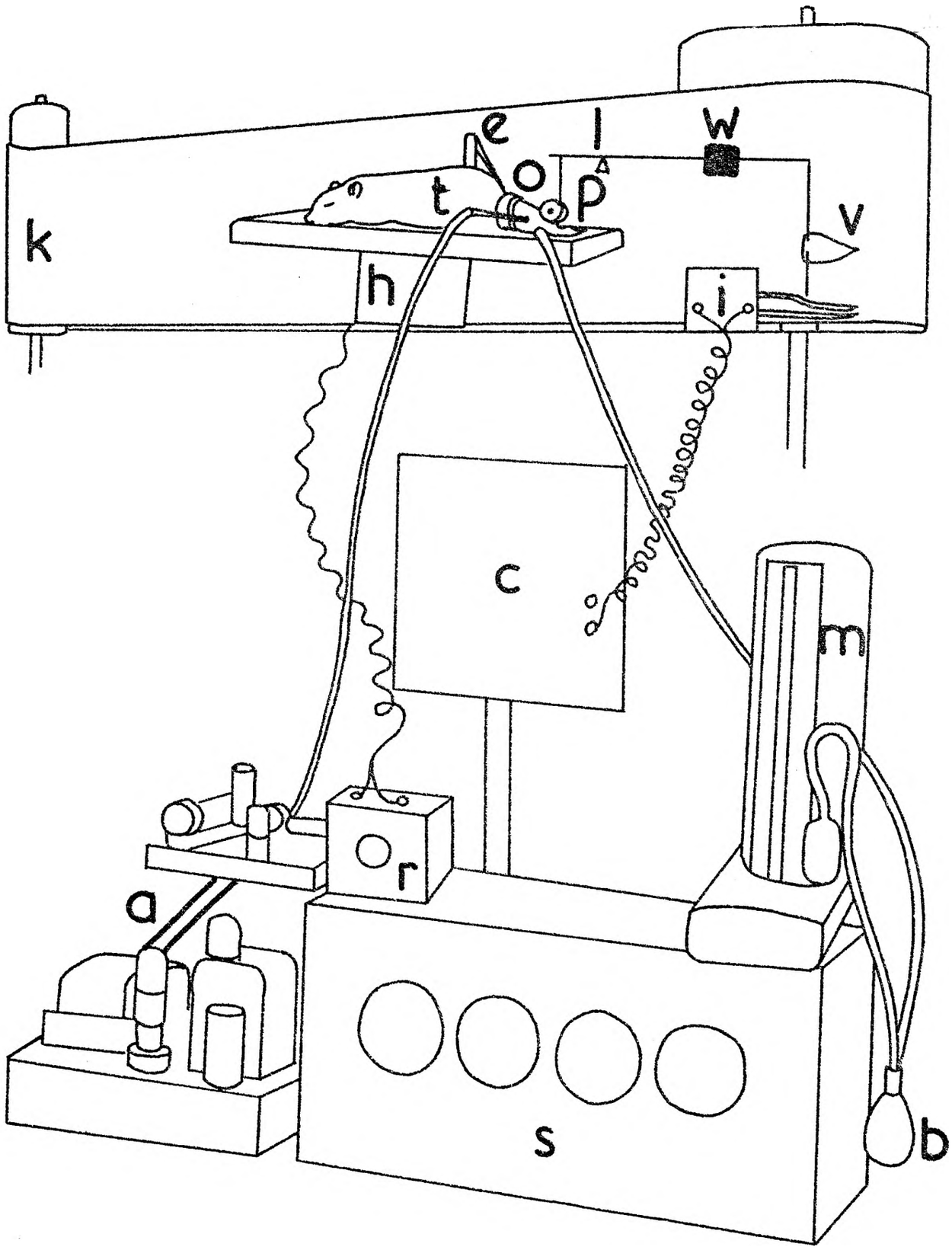


Fig I: General arrangement of earlier apparatus showing:-

(i) Stimulation with stimulator (s) and electrodes (e).
(ii) isotonic recording through pulley (p) with lever (l) weight (w) and vane (v) writing on Kymograph (k).
(iii) time base produced by mechanical clock (c) through marker (i)
(iv) body temperature controlled by heater (h) and rheostat (r)
(v) thermistor (t) measuring muscle temperature through amplifier (a).
(vi) occlusion cuff (o) connected to manometer (m) and hand-pump (b).

EXPERIMENTAL ANIMALS.

Over one hundred male albino rats, reared under standard laboratory conditions were used. They weighed between 250 and 350 gms. and were four to six months old.

ANAESTHETIC.

For the first few pilot experiments, "Sagatal" (pentobarbitone sodium B.Vet.C), supplemented by ether inhalation , was used in a dosage of 50 mgm/kilo body weight given subcutaneously or intraperitonealy. Its use was abandoned for three reasons.

(i) A small animal is very susceptible to overdose since the lethal dose is very little above that required to induce surgical anaesthesia.

(ii)The duration of anaesthesia is too short, being less than 90 minutes without supplementary doses, whereas the experimental procedure takes up to four hours. This necessitates giving frequent supplementary doses with the attendant risks of overdose and an inevitable fluctuation in the depth of anaesthesia.

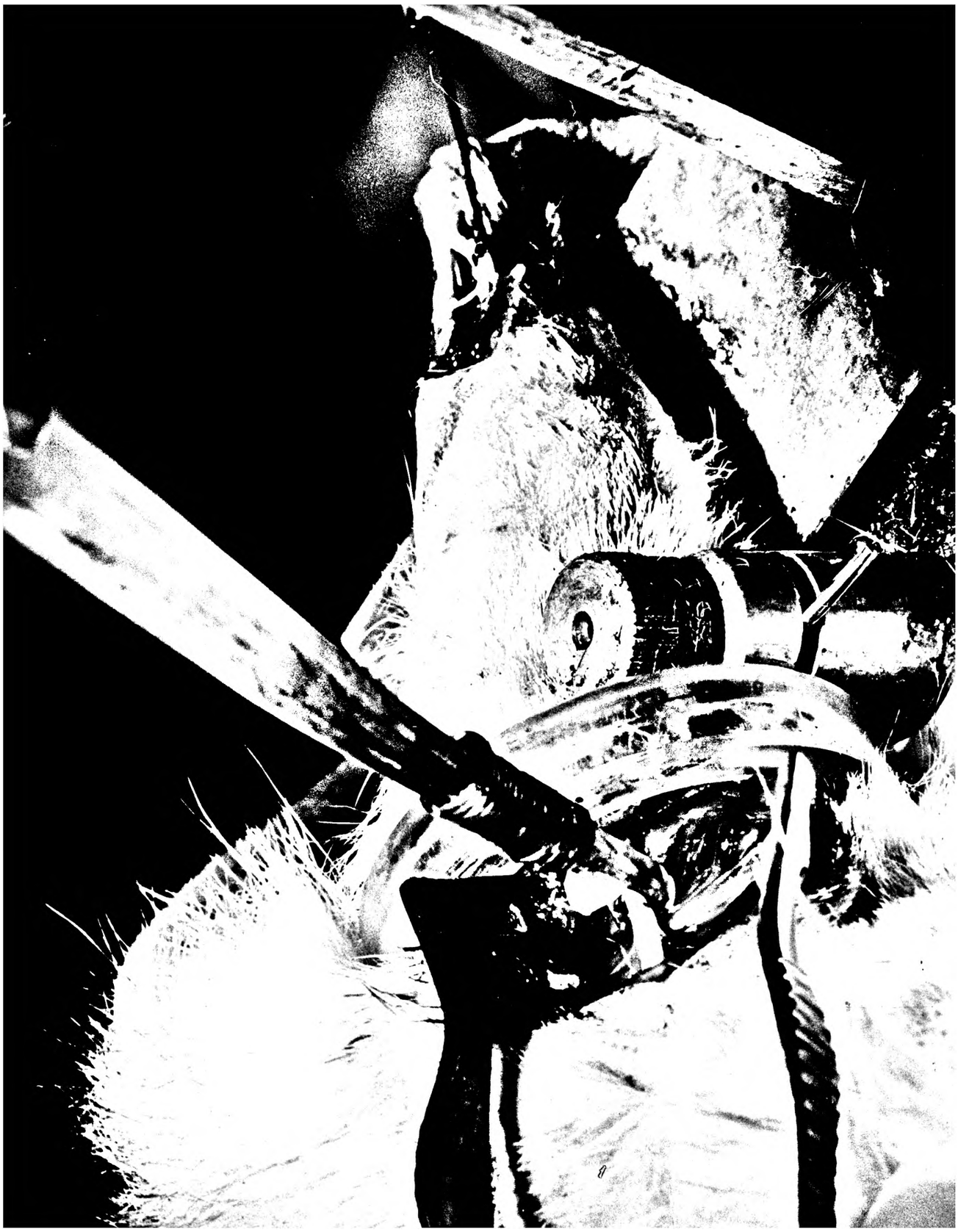
(iii) Barbiturates are known to affect the performance of muscle. Sirnes (1954) reports that low dosages potentiate twitches by a direct action on muscle. In intermediate dosages neuromuscular transmission block develops.. High dosages have a direct depressent action on muscle.

For all subsequent work, urethane (Ethyl carbamate. Merck.) was used in a dosage of 7 ccs/kilogram body weight of a 20% solution given intraperitoneal injection. This proved an ideal anaesthetic for the preparation since:-

(i) no fatalities ascr ble to overdose occurred in more than a hundred experiments.

(ii) it has a prolonged action of over 12 hours during which the level of anaesthesia, as assessed by reflexes and resporation remains constant.

(iii) No reports of a direct effect on muscle performance were found in the literature.



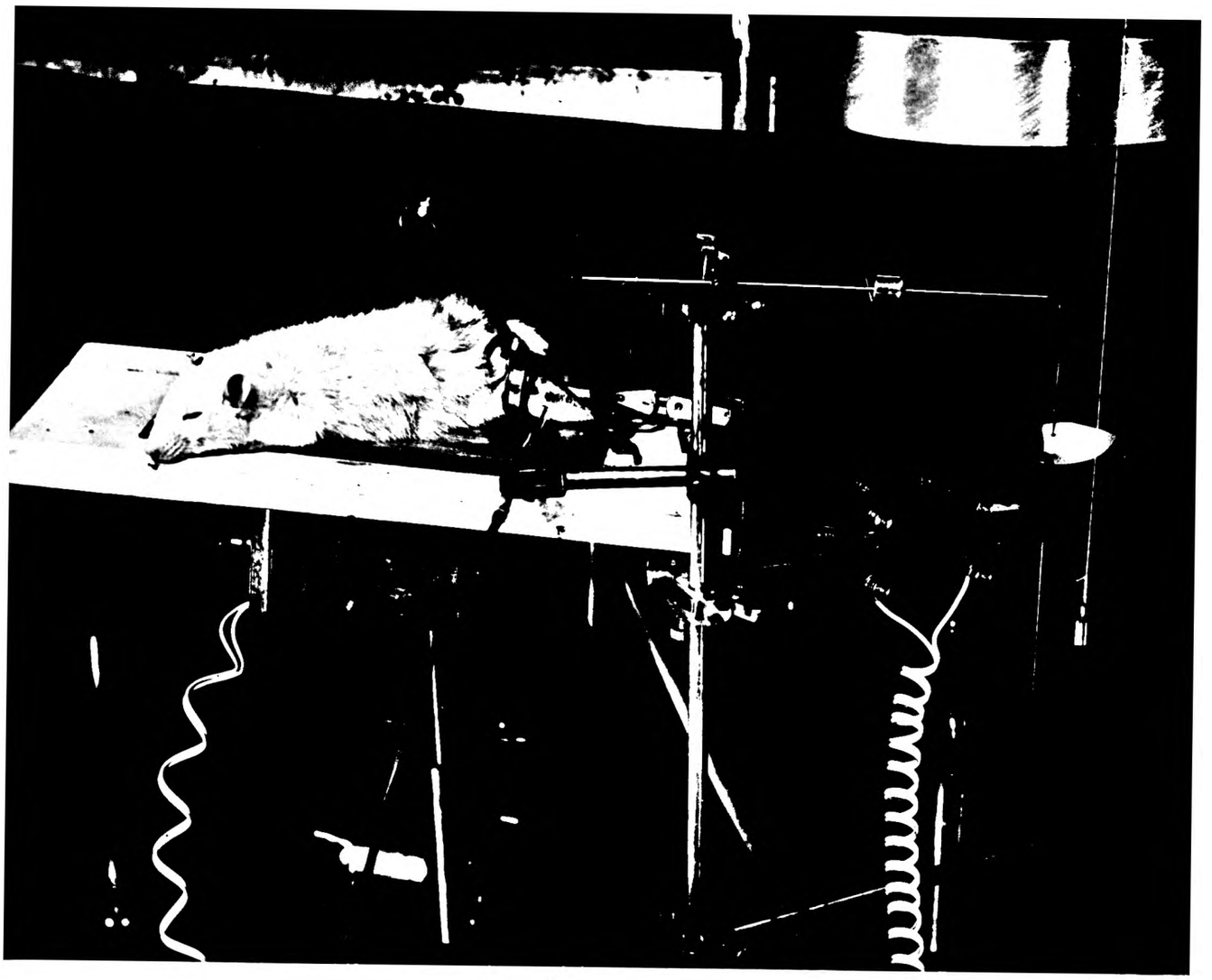
OPERATIVE PROCEDURE.

(Fig 2. opposite.)

After shaving the lower limb, the sciatic nerve was exposed by a small incision of less than one centimetre long into the thigh, parallel and posterior to the femoral shaft. The nerve was crushed proximally, thus abolishing reflex activity. The stimulating electrodes were then placed on the nerve distal to the block and the incision closed.

The Achilles tendon was exposed by a small skin incision over the posterior aspect of the ankle joint. In the rat the Achilles tendon, which is the conjoint tendon of the gastrocnemius and soleus muscles, is also intimately associated with the tendon of plantaris. (Parsons, 1894). All three muscles are innervated by branches of the sciatic nerve and assist in plantar flexion of the ankle joint. For convenience here, they will be referred to as triceps surae. The triceps tendons were tied firmly together with silk. The in situ length of muscle was measured, then the tendons were detached from their insertions and connected to the recording apparatus.

The whole operative procedure took about ten minutes and was practically bloodless. Only the detached tendon remains exteriorised and is kept covered with a wet saline swab.



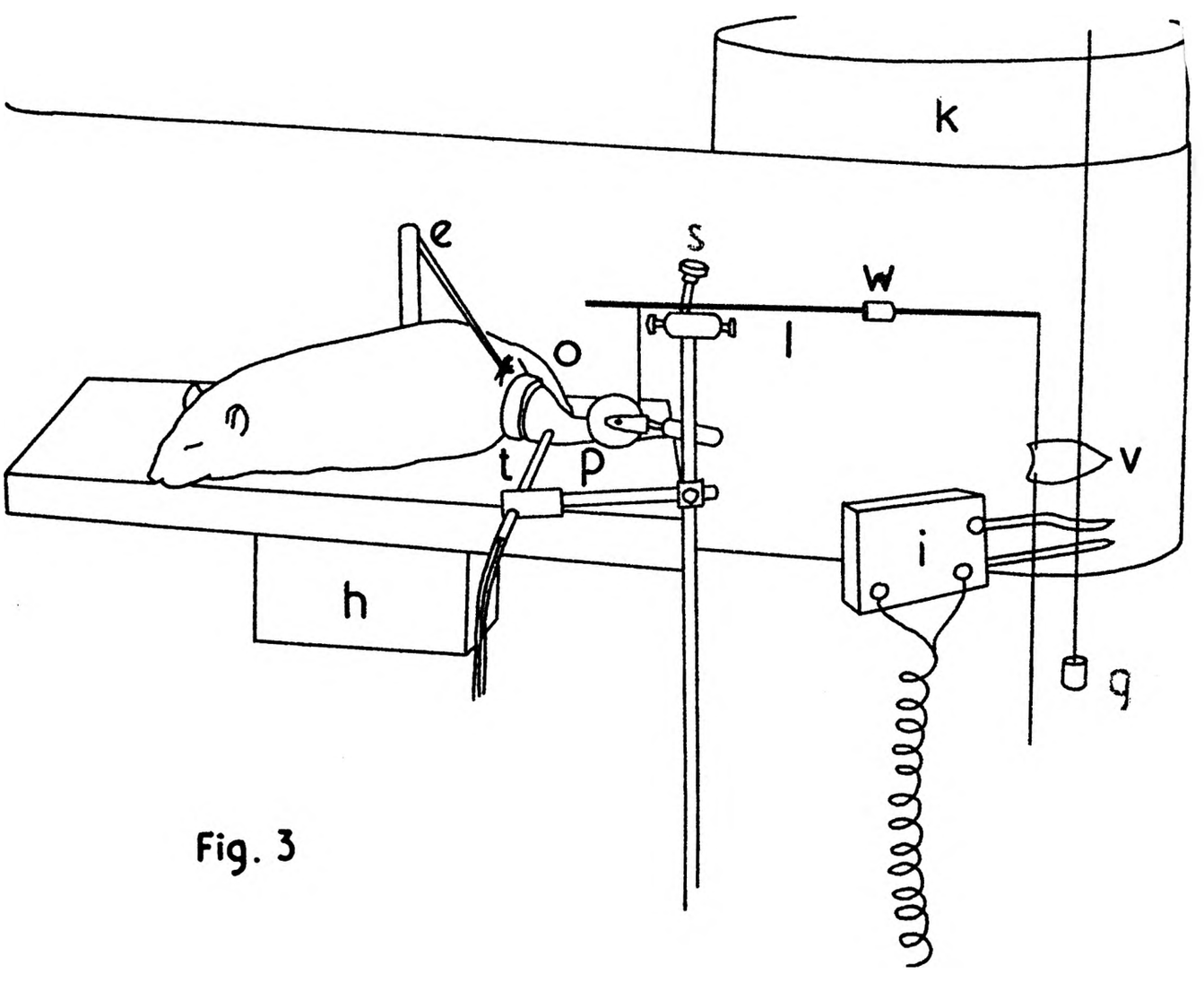


Fig. 3

ANIMAL BOARD. (Fig. 3).

On completion of the operative procedure the animal was transferred to the recording table on which the requisite limb was immobilised at two points. Proximally, the limb was held in position by a brass ring slipped over the thigh until it abutted on the pelvis. It was then firmly fixed to the board by means of a screw. The ring also formed part of a circulation-occlusion cuff described in a subsequent section. Distally, the limb was held firmly to the board by means of a small clamp over the lower end of the tibia.

The tendon was detached from the calcaneum, and tied firmly with strong thread. The thread passed around a pulley which converted the horizontal pull of the muscle to a vertical one.

It was found that at room temperature, the rectal temperature of the rat fell from 98°F to about 88°F, while the animal was under urethane anaesthesia. Therefore copperplate and heater were inlaid into the table. The animal's body temperature was controlled by taking routine observations of the rectal temperature and adjusting a thermostat on the heater accordingly.

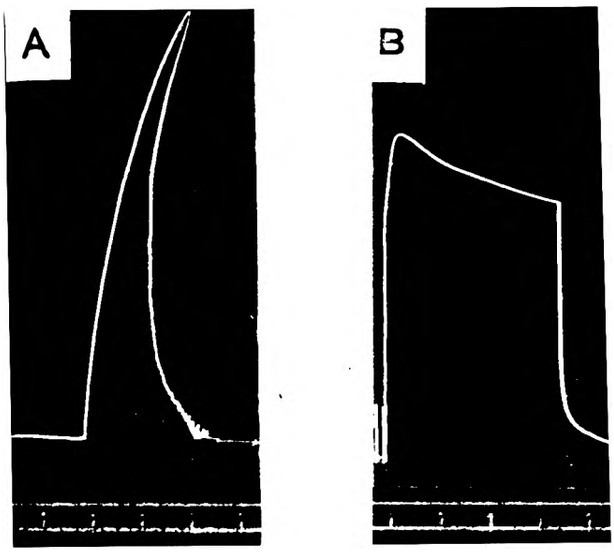


Fig 4. Elimination of curve distortion in recordings.

A: Distortion produced by standard lever.

B: Linear writing system employed.

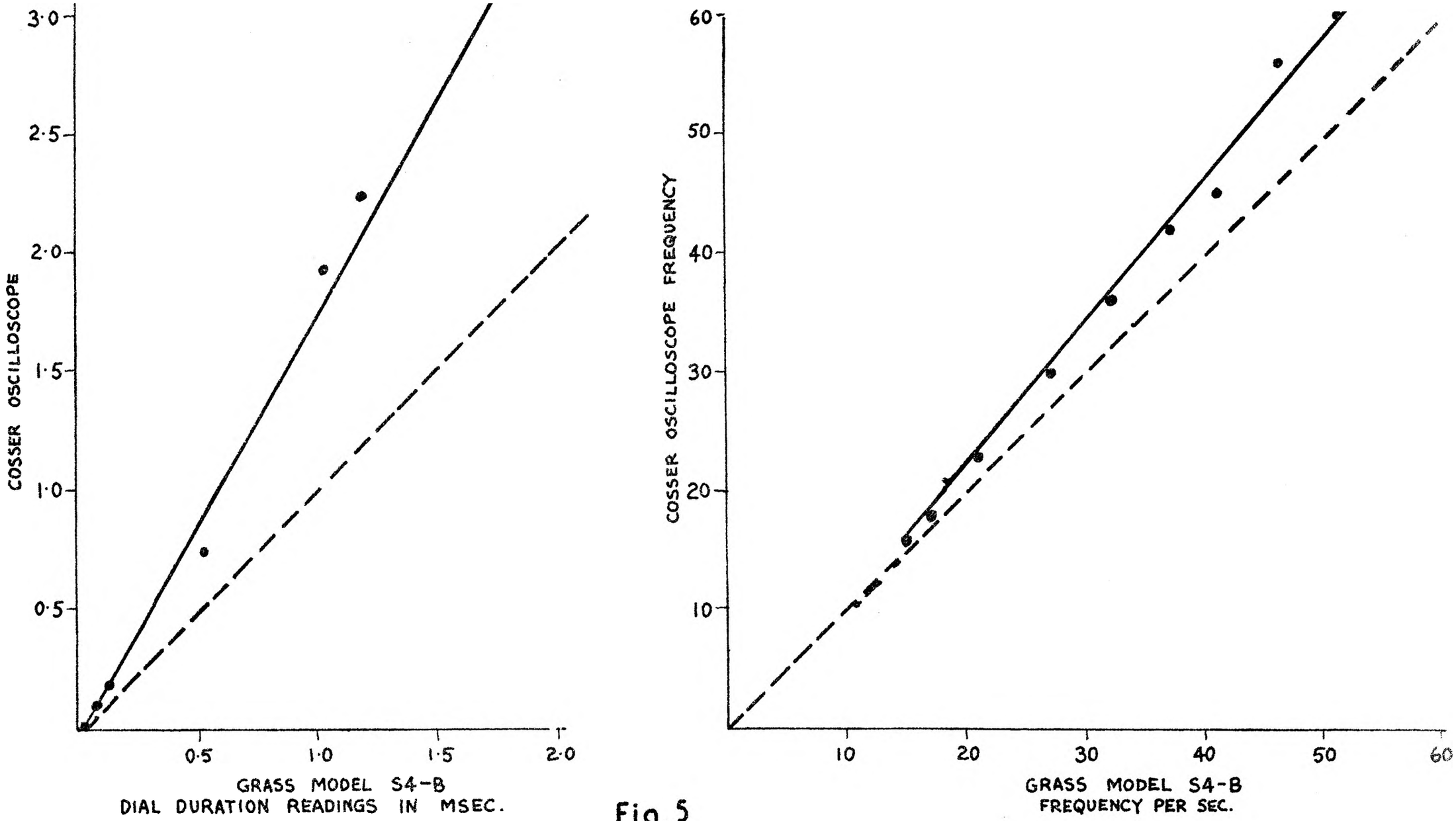
RECORDING APPARATUS. (See Figs. 1 and 3)

Since no isometric recording devices were available at the time the project began, the apparatus described in this section only permitted the recording of isotonic contractions. A lever system writing on smoked paper was used.

The use of the standard student laboratory lever was soon abandoned. Curve distortions in the recording made calculations difficult. There was no satisfactory way of increasing the sensitivity of the lever or of loading the muscle without introducing considerable friction and inertia. (Fig. 4).

In order to achieve a linear recording of sufficient magnitude, a magnifying and loading lever was introduced. The lever was made of aluminium and was very light (3 gms.), though not completely rigid. The writing arm was five times the length of the muscle arm. However, the difficulties of loading the muscle without greatly increasing inertia remained unresolved.The only practical method in a lever system is to keep the load as close to the fulcrum as possible. However, the loading system in the lever employed, consisted of a weight of 22.4 gms. which could be moved along the writing arm to produce increments in load. This inevitably led to increased inertia, a factor which will be considered in the evaluation of results. The magnifying lever moved a vertically writing vane. The system produced a five-fold magnification and a possible range of loads of 25 to 400 gms. The higher loads could, however, only be achieved with the production of considerable inertia. In addition there were numerous points of friction, chiefly at the pulley through which the muscle operated on the magnifying lever, and at the writing vane-smoked drum interface.

The kymograph consisted of two rollers, four feet apart, driven by a motor whose speed could be adjusted by three gears, and a rheostat. A time tracing of either 3 or 30 seconds was delivered by a battery-operated clock and an event marker was manually controlled with a tapping key.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GRASS MODEL S4-B
DIAL DURATION READINGS IN MSEC.
60
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GRASS MODEL S4-B
FREQUENCY PER SEC.

Fig. 5

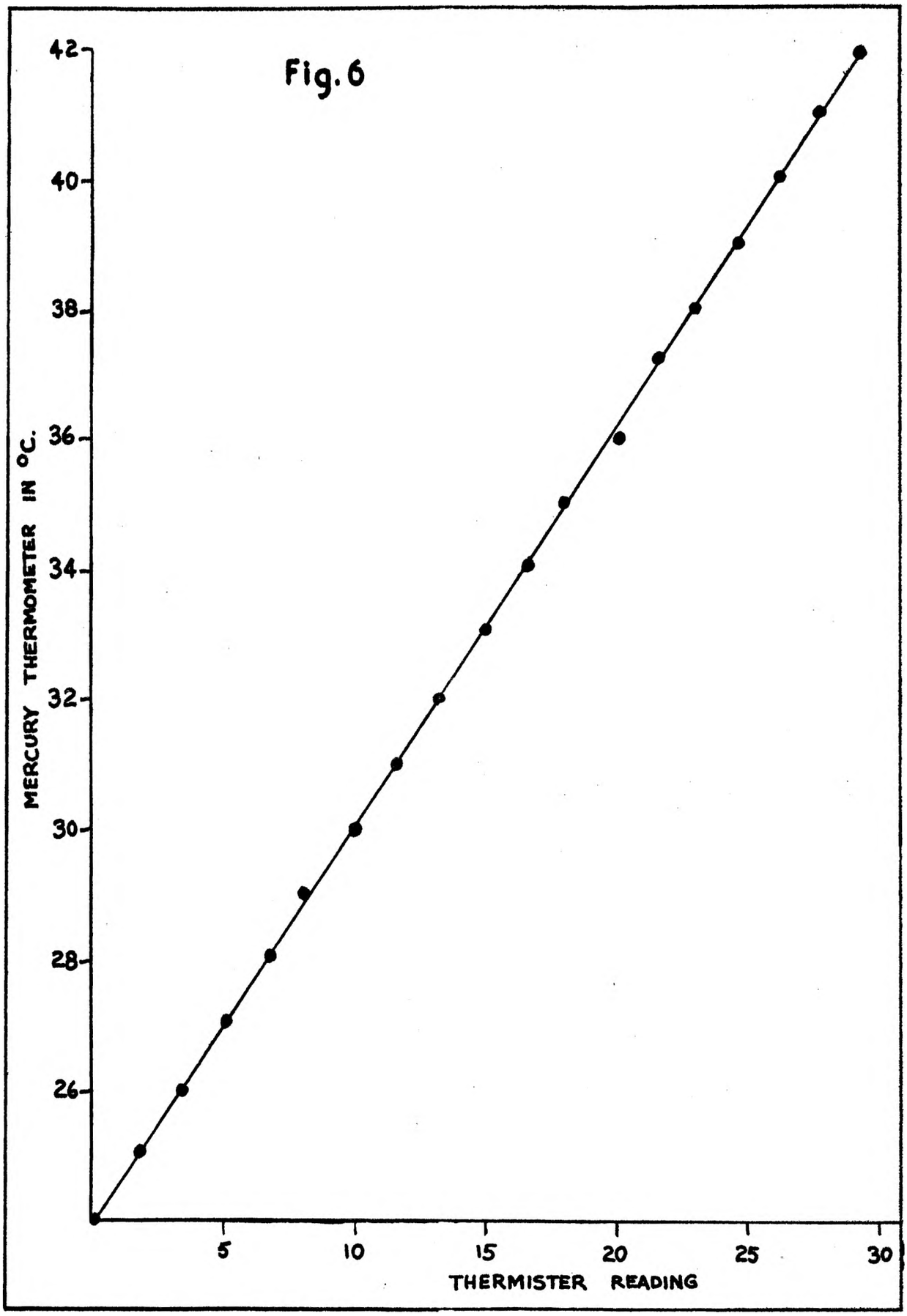
Fig. 6
42
40
38
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32
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26
MERCURY THERMOMETER IN °C.
5
10
15
20
25
30
THERMISTER READING

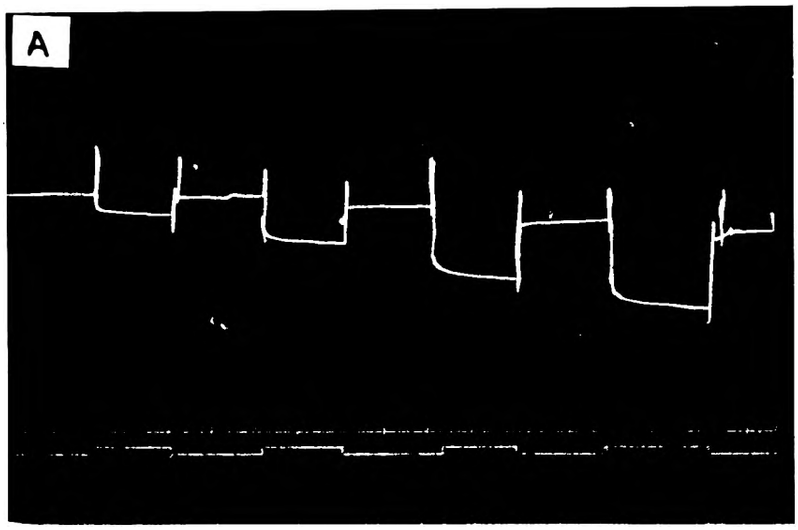
MOTOR NERVE STIMULATION.

The motor nerve supplying the muscle was stimulated by means of a Grass Model S-4b Electronic Square Wave Stimulator, with variable strength, duration, and frequency. (Fig. I). Display of the stimulator output on a Cosser Oscilloscope showed that, although the pulse form was satisfactorily rectangular, the dial readings were consistantly lower than the actual measurement made on the oscilloscope, but were reasonably linear within the range used. (Fig. 5).

The stimulating electrodes consisted of two steel wires embedded in a perspex cylinder, 5 mm. in diameter at its lower end, slotted to expose one surface of the electrodes. The nerve rested on the electrodes in this slot so that the other tissues were shielded. The electrodes were held in position by an adjustable clamp. (See Fig. 2).

MUSCLE TEMPERATURES.

The measuring device consisted of a Thermistor, (Standard Telephone and Cables Ltd. Temperature Probe F. 15) connected to a two stage directly coupled amplifier with a regulated power supply.(Fig. I). Readings were made on a sensitive galvonometer. Although the apparatus was capable of greater accuracy, temperatures were read to within $0.1^{0}C$. The calibration curve was linear in the range required.(Fig. 6)



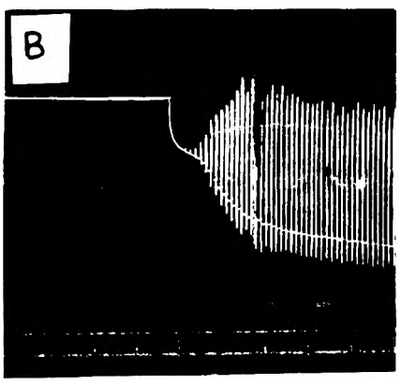


Fig. 7: Measurement of passive length.

A= Drop in base line produced by successively increasing loads applied during periods indicate by elevation of event marker trace.

B= Contracture at the end of a prolonged tetanus showing failure to relax completely and immediately. Recovery of muscle indicated by twitch size.

OCCLUSION CUFF. (See Figs. 1 and 3)

The blood flow to the limb was controlled by an occlusion cuff incorporated into the metal ring, previously described as immobilising the thigh. The ring was grooved inside, a piece of tubular rubber from the finger of a surgical glove was attached to it to form an elastic inner wall to the groove. The contained space was connected to a sphygnomanometer and hand pump by pressure tubing. When the pressure was raised, the elastic inner wall expanded and compressed the thigh to produce occlusion of blood vessels. The degree of occlusion was dependent on the pressure to which the cuff was pumped. However, no determinations of cuff pressure against blood flow were made.

MEASUREMENT OF THE MECHANICAL RESPONSE.

Measurement of passive length changes.

No satisfactory measurement of the absolute length of the muscle in situ could be obtained with the original method of recording. This only measured a change in length, when a particular load stretched a muscle, as a fall in the base-line of the recording. (Fig. 7a).

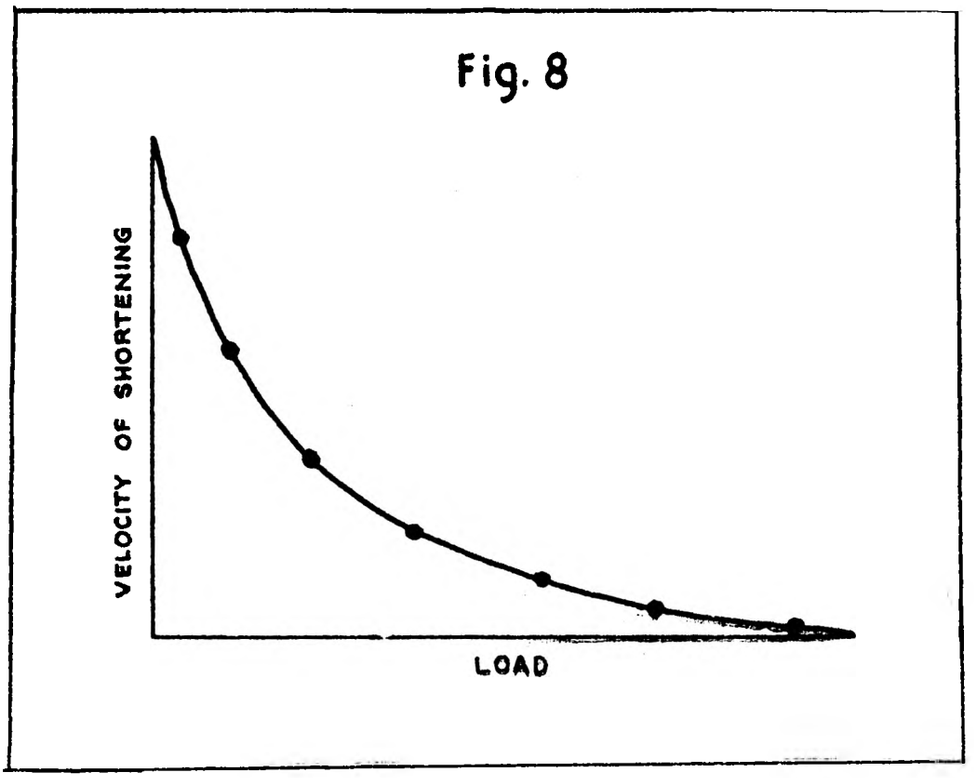
This method is open to criticism.

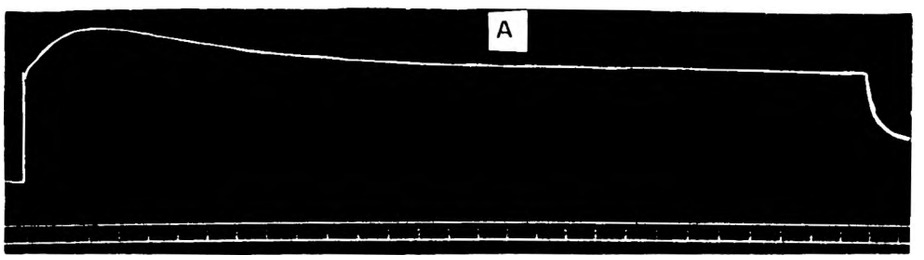
(i) It gives no correlation with the length changes occurring in the animal with its tendon insertion intact.

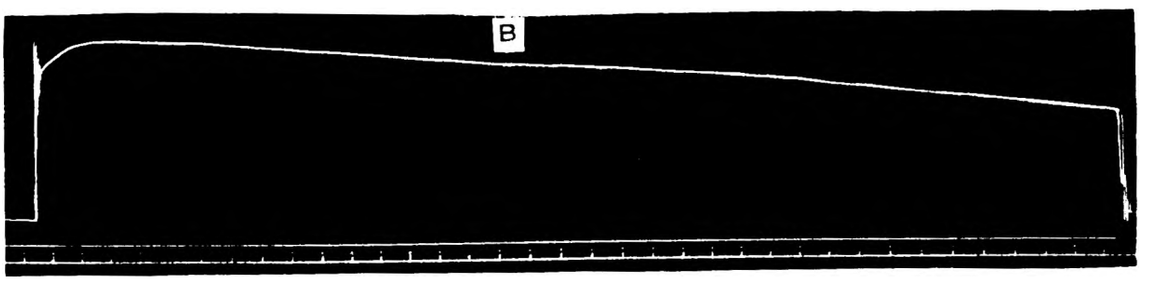
(ii) No comparison can be made between different experiments of the percentage length change for a given load.

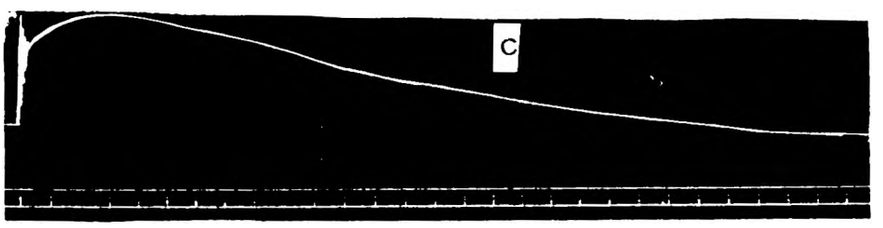
(iii) The drop in the base line is a measure of the compliance not only of the muscle but of the whole system including the lever and cotton connections.

(iv) The muscle often failed to return to its previous baseline following a prolonged tetanus. (Fig 7b) This is the well known phenomenon of contracture. The subject has been reviewed by Gasser (1930)who states that "the first contractures were observed on fatigue curves.... described in detail over half a century ago and they are annually recorded by the thousand....".... The presence of contracture indicates a dissociation between the propogated electrical and mechanical activity of muscle due to the accumulation of metabolites. It is reversible by rest.









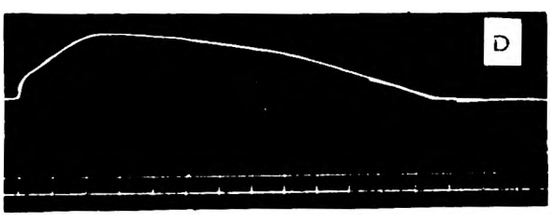


Fig. 9. Development of overshoot artefact.
A: load 25 gms. B: load 100 gms. C: load 115 gms
D: fatigued muscle, load 115 gms.
Note the increase in overshoot with larger loads except where the velocity of contraction has been decreased by fatigue.

Measurement of active length changes.

These were clearly recorded as a positive deflection with a magnification of five times. Because the lever system used possessed an inevitable inertia due to the method of loading, some of the records displayed an artefact due to overshoot. The momentum of the lever is the product of the mass of the lever and its velocity. Therefore, the two main experimental factors responsible for eliciting the artefact of overshoot would be increased loads on the lever and increased velocity of contraction.

There are two main determinants of velocity in the experimental situation, load and previous activity of the muscle. Velocity of contraction is related to the load (or force) by a hyperbolic function such that for a given state of muscle, increasing the load decreases the velocity of shortening. (Hill, 1938), (Fig. 8). This property of muscle would therefore tend to prevent significant changes in the momentum of the lever. The second factor which decreases velocity is fatigue consequent upon previous activity.

Therefore it would be expected on theoretical grounds that the overshoot would be most marked in the early tetani of a series, particularly where heavy loads were used. This was verified experimentally. (Fig. 9). The appearance of the artefact in the records was characteristic. It was not possible to eliminate it from all the records obtained with the lever system of recording.

Measurement of the work output of muscle.

The external work done by a muscle in the course of a tetanus can either be measured as the instantaneous work at any given moment (given by the product of the load and the height of the tetanus at that moment) or as the integrated work output over a given period (given by the product of the area enclosed by the tetanus and the load.)

The integrated work output was chosen because it is more reliable . The area enclosed by the tetanus can be measured in a number of ways. A planimeter was used because of its convenience. It was compared under standard conditions with two other methods, square counting and weighing, which did not give materially different results.

SECTION 11

AN INVESTIGATION OF SOME MAJOR PHYSIOLOGICAL DETERMINANTS OF IN SITU PERFORMANCE.

1. STIMULATION PARAMETERS.

(i) Strength & Duration.

Objective.
Results.
Discussion.

(ii) Frequency.

Objective
Results
Discussion.

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2. OCCLUSION OF CIRCULATION.

(i) Mechanical Response.

Objective
Results
Discussion

(ii) Temperature Response.

Objective.
Results.
Discussion.

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3. LOAD AND INITIAL LENGTH.

(i) Amplitude of contraction.

Objective
Results
Discussion.

(ii) Work Output.

Objective
Results.
Discussion.

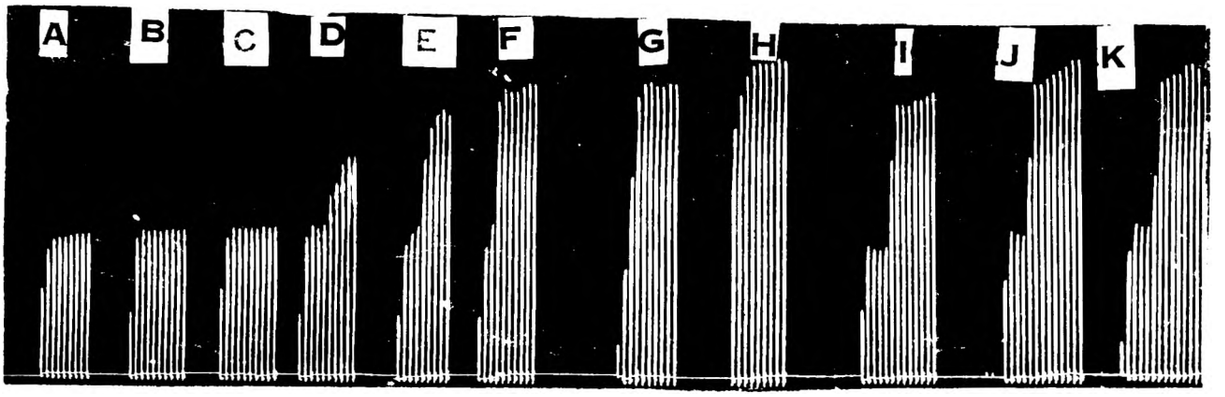


Fig. 10: Effect of varying the Strength and duration of single Motor Nerve Stimuli.

A - H : Voltage increased successively from 1-10 volts at the following durations (in msecs.)
A = 0.1, B= 0.4, C = 1.0, D = 1.25, E= 1.5, F= 1.75, G = 2.0, H=
I - K : Duration increased successively through (0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4, 4.5, 5.0, 8.0, 10.0 msecs.)
I : 3 volts. J: 6 volts. K: 10 volts.

Note the threshold, maximal and augmented twitches.

MOTOR NERVE STIMULATION PARAMETERS.

STRENGTH AND DURATION.

Objective:

In order to establish what parameters of nerve stimulation would produce a maximum reproducible response from the triceps surae of the rat, single twitches were first investigated to establish a stimulus of suitable strength and duration. However, it was found that the mechanical response to a single applied stimulus was complex. Three types of response could be elicited depending on the relative strength and duration of the square wave impulse. These were designated thus:- (Fig. 10)

(a) the first detectable response - "threshold" twitch.

(b) the maximum response beyond which increasing the parameters within limits has no further effect- "maximal" twitch.

(c) an increased response beyond the plateau of maximal twitches produced by long pulse durations - "augmented" twitch.

A similar phenomenon in the rabbit has been reported by Gjone (1955). He showed that the "threshold" twitch was due to excitation of the lowest threshold motor nerve fibres and that the "maximal" twitch constituted the mechanical response when all the motor units were activated. The "augmented" twitches were due to both the "make" and "break" of long pulses eliciting seperate action potentials. The mechanical response to the two action potentials fused to produce a summated response or "augmented twitch", Gjone (1955), points out that failure to take cognisance of this phenomenon can lead to anomalous experimental results where impulses of long duration are used. It was therefore necessary to establish standard stimulation parameters which did not introduce the summation complication.

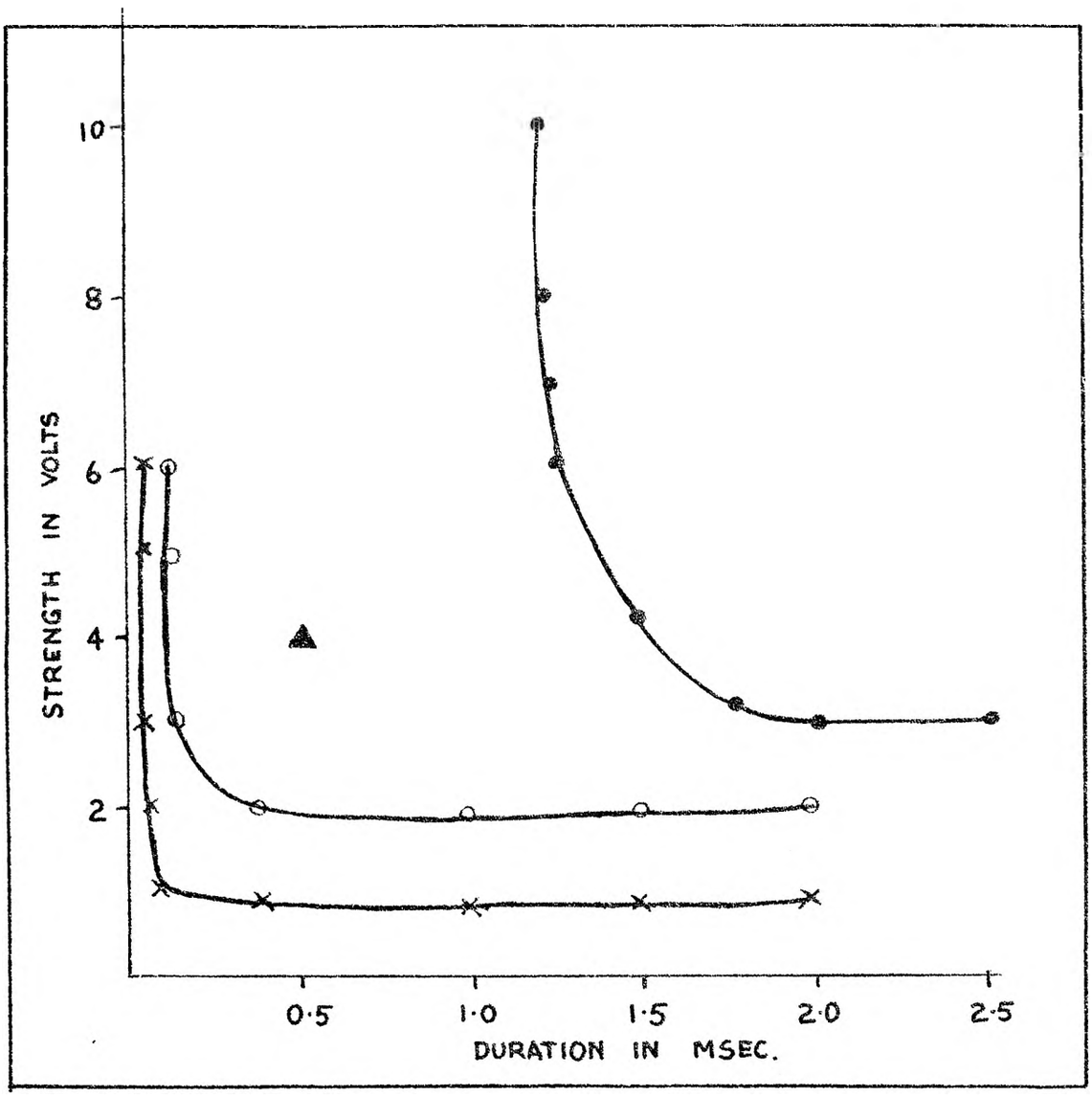


Fig. 11 Strength-duration relationship.

x - threshold response.

0 - maximal response.

• - augmented response.

▲ - standard maximum stimulus adopted.

Results.

Two methods were used in a series of ten experiments. In one, the strength was held constant and single pulses of gradually increasing duration were applied through a range of 0.1 to 10 msec. This procedure was then repeated holding the duration constant and increasing the strength stepwise from 1 to 10 volts. (Fig. 10).

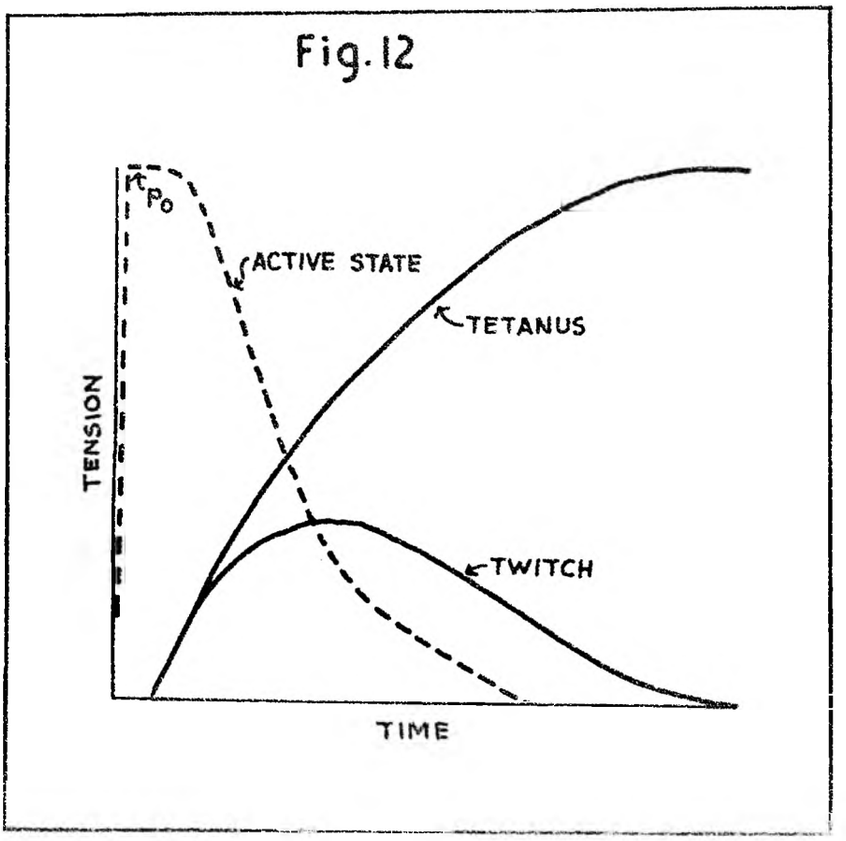
Strength duration curves were plotted for threshold, maximal and augmented responses (Fig. 11) These showed that augmented responses occur with pulses of over 1 msec. duration and that the strength of current required decreases with increasing pulse lengths.

Discussion.

The results obtained agree with the original work of Gjone (1955) with slight quantitative differences. The fact that a pulse duration of 1 msec. which is close to the refractory period of muscle can elicit two twitches requires an explanation. Graham and Lorente de No (1938) found that the rate of conduction of an action potentials may be halved by an immediately preceding one. Therefore action potential produced by the "break" current less than 1 msec after the "make" stimulus will arrive at the neuromuscular junction outside of the refractory period of muscle and be able to exert its summating effect. Recently Locke (1963) has shown that the absolute refractory period of the rat gastrocnemius motor unit varies from 0.6 to 2.3 msec., which also helps explain the "augmenting" effect of pulses whose duration is increased through this range. ~~As the duration is increased through this range~~. As the duration increases the two action potentials evoked by the "make" and "break" current will fall outside the refractory period of more and more motor units and hence the summated response will be augmented further. This can be seen in the experimental results obtained as an increasing rise above the plateau with longer pulse durations.

In all subsequent experiments the strength and duration were standardised, by choosing a combination which produced a single maximum twitch. On the basis of the strength duration curves established here the values selected were 0.5msec. duration and 3-6 volts.

Fig. 12



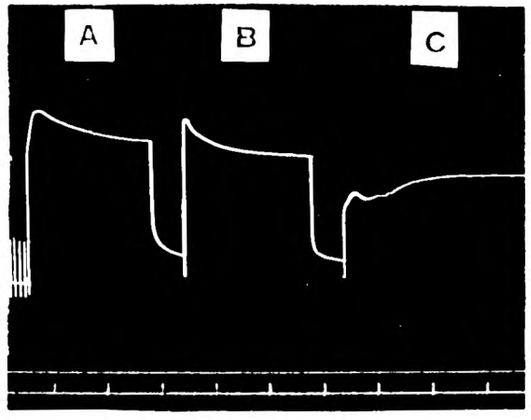
FREQUENCY OF STIMULATION.

Objective.

A criticism of many investigations on muscle is that the use of twitches does not permit a direct approach to the properties of the contractile component of muscle. During a twitch so much time is required to stretch the series elastic component that the active state has begun to decay at a time when the mechanical response of the whole muscle is just developing. (Hill, 1949). According to Fenn (1945) twitches measure the rate at which tension is developed rather than the amount. Tetani more closely approximate the active state whose decay is reduced by repetitive stimuli whatever mechanisms are responsible for its development.

The greater the frequency of stimulation the more closely will the mechanical response approximate the active state. (Fig. 12). However, there is a limit to this process which will be determined primarily by the rate at which the neuromuscular junction can continue to transmit the high frequency of stimulation.

It was therefore necessary to investigate at what frequency of stimulation a steady fused tetanus could be elicited without the development of transmission failure. It did not prove possible to monitor neuromuscular transmission performance either electromyographically or by micro-electrode techniques. Therefore more indirect evidence derived from the form of the mechanical response had to be relied upon in the interpretation of results. A stimulation frequency which produced a maximum and steady plateau response without immediately declining was sought.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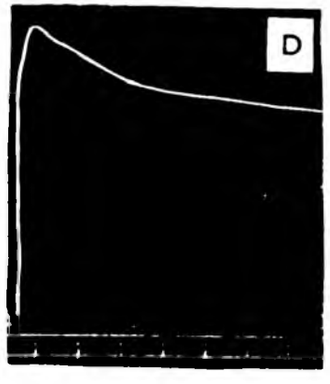
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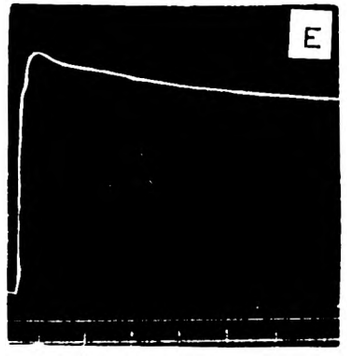
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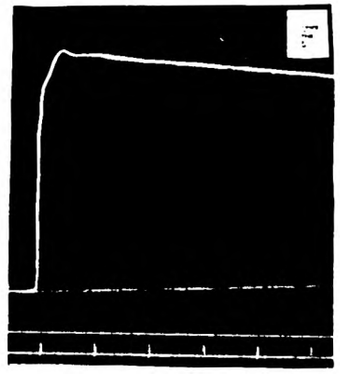
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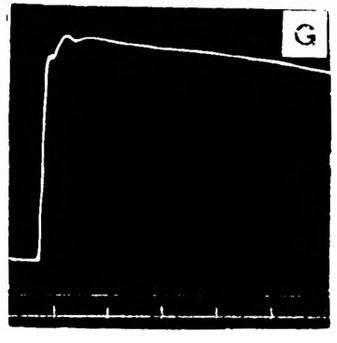
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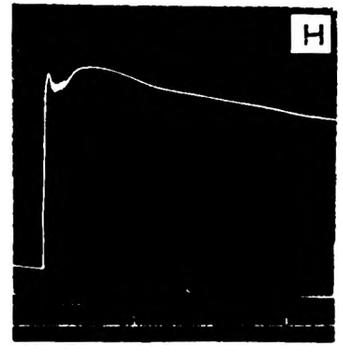

H

Results:

In a series of 15 experiments, the isotonic response of triceps surae to frequencies of nerve stimulation of 20, 35, 50 and 100 per second was investigated. (Fig. 13) (see opposite and below)

(i) At 100/sec. the graph rose vertically to a sharp peak and then declined rapidly until a steady level was reached.

(ii) 50/sec. the initial peak was less pronounced and the decline to a steady level less precipitous. The level finally attained at 50/sec. was approximately the same as at 100/sec.

(iii) At 35/sec. the initial peak was often smaller than the steady level which it finally achieved, usually by passing through a second intermediate peak. Again the final maintained level was approximately the same as at 50 and 100/sec.

(iv) At 20/sec. both the initial peak and the final level were significantly lower.

The time relations of the peaks were approximately as follows: Within the first second, the most rapid component had reached a maximum. The rising phase of the intermediate peak at 35/sec. was complete within 6 to 8 seconds and the final maintained height was achieved after 20 or more seconds.

← Fig. 13: The effect of different frequencies of stimulation on the initial form of the tetanus.

A= 50/sec. B= 100/sec. C = 20/sec.

D -H : 30/sec in different animals.

Note that the response at 30/sec is variable but is intermediate in shape between that at 50/sec. and at 20/second.

Discussion.

The two points of significance which emerge from these results are the relationship of frequency of nerve stimulation to the fluctuations in the initial response and to the final degree of maintained shortening.

The fluctuations in the initial degree of shortening are not easily explained on the basis of the above experiments. They are often seen in graphs reproduced in the literature though never explained. (Hoffman, 1903: Rosenbleuth and Morrison, 1937; Del Pozo, 1942). Del Pozo's only reference is to an "initial staircase" while Rosenbleuth & Morrison report that "stimulation of the nerve leads, as is well known, to an initial rise in tension succeeded by a slow fall.....".

Rosenbleuth & Luco (1939) and Rosenbleuth & Cannon (1940) report a complex series of rises and falls in the response. However, they used much higher frequencies of stimulation.

Although there is no direct evidence, it seems that the initial peak followed by the decline to a steady level at higher frequencies of stimulation could be explained by the development of neuromuscular block. Rosenbleuth & Morrison (1937), Naess& Storm-Mathison (1955), and Krnjevic & Miledi (1958), all report the development of neuromuscular block at frequencies above 30/sec. Del Pozo (1942) showed that in the circulated cat muscle preparation with stimulation frequencies of above 30/sec., transmission fatigue occurred, while below 30/sec. contraction fatigue eventually sets in. From this indirect evidence, the complex nature of the initial response at 30/sec could be ascribed to that being close to the critical frequency at which unimpaired neuromuscular transmission can still take place. Since the motor units of the rat gastrocnemius are neither structurally (Cole, 1951) nor functionally (Locke, 1963) homogeneous, some degree of "block" and "unblock"of the components of the motor unit pool contributing to the overall response could occur. This could result in the observed fluctuations in the tetanic response.

Hubbard & Schmidt (1963) have shown a variability in the excitability of motor nerve terminals during repetitive stimulation. Although these changes could be involved in the genesis of fluctuations in amplitude, the present study did not permit any such correlation.

At higher frequencies, the final steady level of the isotonic record becomes lower as the stimulus frequency gets greater (del Pozo, 1942). The decline to a steady level after an initial peak, a phenomenon often seen in the present experiments, could be explained by partial neuromuscular block developing so that only every alternate or third or fourth impulse is transmitted. The early workers in the field, Wedensky (1891) and Hoffman (1908), had shown that increasing the frequency during the steady state would further decrease the response, whereas slowing the frequency led to an increased response. This is further evidence of the development of neuromuscular transmission block at higher frequencies, and of the degree of this block determining the height of the steady state response.

It is now known that the frequencies of discharge of motor nerves in the intact mammal do not exceed a value variously reported as between 20 and 50/second (Smith, 1934; Denny-Brown, 1949; Bigland & Lippold, 1954). The purposes of the present study required a frequency of stimulation which would produce a maximum mechanical response without producing transmission failure.

On the basis of the results reported here and in the literature a frequency 30/second was therefore adopted as the standard.

OCCLUSION OF THE CIRCULATION TO THE LIMB.

EFFECT ON THE MECHANICAL RESPONSE.

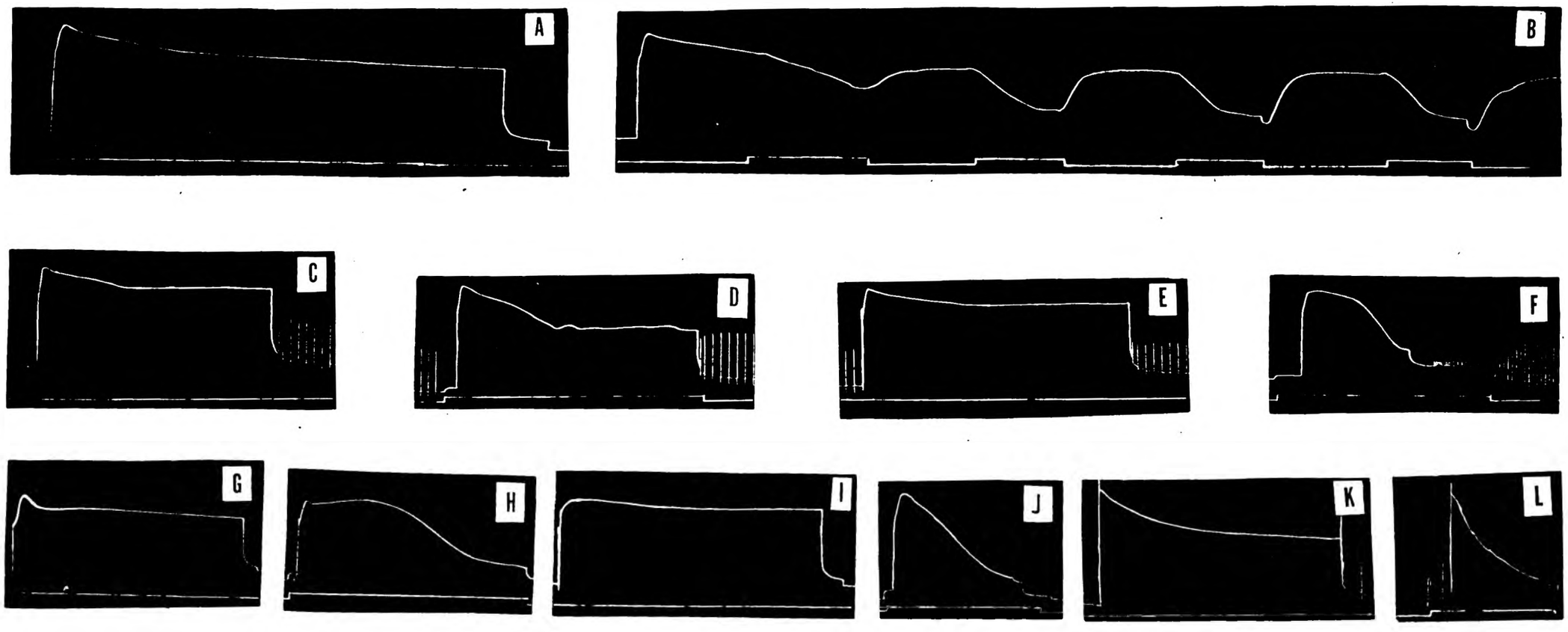
Objective:

Mammalian muscle differs from frog muscle in being acutely sensitive to deprivation of its blood supply. Schottelius & Senay (1957), found that ischaemic rat gastrocnemius developed less tension than controls under various isometric conditions. Merton (1954) demonstrated in humans that fatigue of maintained voluntary contractions occurs more rapidly under conditions of ischaemia and that no recovery takes place until the circulation is released. He concluded together with other authors (Naess & Storm-Mathison, 1955) that during a tetanic contraction, the muscle occludes its own blood supply and that the ischaemia thereby produced results in fatigue at some peripheral locus.

However, there are conflicting reports in the literature concerning the blood flow through muscle during a maintained tetanus. Bulbring & Burn (1939) found an increased flow except in the first five seconds, while Barcroft & Millen (1939) found that a voluntary contraction 20% of maximal occluded the circulation. These and other conflicting results have been ascribed to the differences in the muscle used in the loads and to whether, the contraction was isotonic or isometric. (Hemingway, 1956).

Humphreys & Lind (1963) investigated the blood flow through muscle at various isometric tensions. They found that in the human forearm, muscle blood flow increased steadily during each contraction at tensions 30 to 60% of maximum.

It was therefore necessary in the present preparation to find out whether under the standard conditions of stimulation, the muscle produced significant ischaemia by the mechanical compression of its own vessels, in spite of the physiological dilatation accompanying contraction. This was done in four experiments using the occlusion cuff already described.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Results. (Fig. 14). (see opposite & below)

A & B. During the steady maintained level of a tetanic contraction the circulation was arrested with various occlusion pressures. It was found that the response immediately began to decline steadily to a new steady state at a smaller degree of contraction. Release of the circulation resulted in a rapid return to the previous steady level. This phenomenon became more marked with increasing amounts of occlusion.

C- F. Occlusion before the onset of the tetanus resulted in a smaller initial height and a more rapid decline to a lower level as compared to controls. No recovery took place either of tetani or twitches until the circulation was released.

G- L. The effect of occlusion was related to the frequency of stimulation. The higher the frequency the more marked and rapid was the effect of ischaemia.

←Fig. 14:

Occlusion of circulation under different conditions.

Periods of occlusion indicated by marker on baseline.

A: Control. B: Increasing degrees of occlusion during tetanus.

C: control. D: Partial occlusion throughout tetanus.

E: control. F: Complete occlusion throughout tetanus.

G: control at 20/sec. H: Occlusion throughout at 20/sec.

I: control at 30/sec. J: Occlusion throughout at 30/sec.

K: control at 50/sec. L: Occlusion throughout at 50/sec.

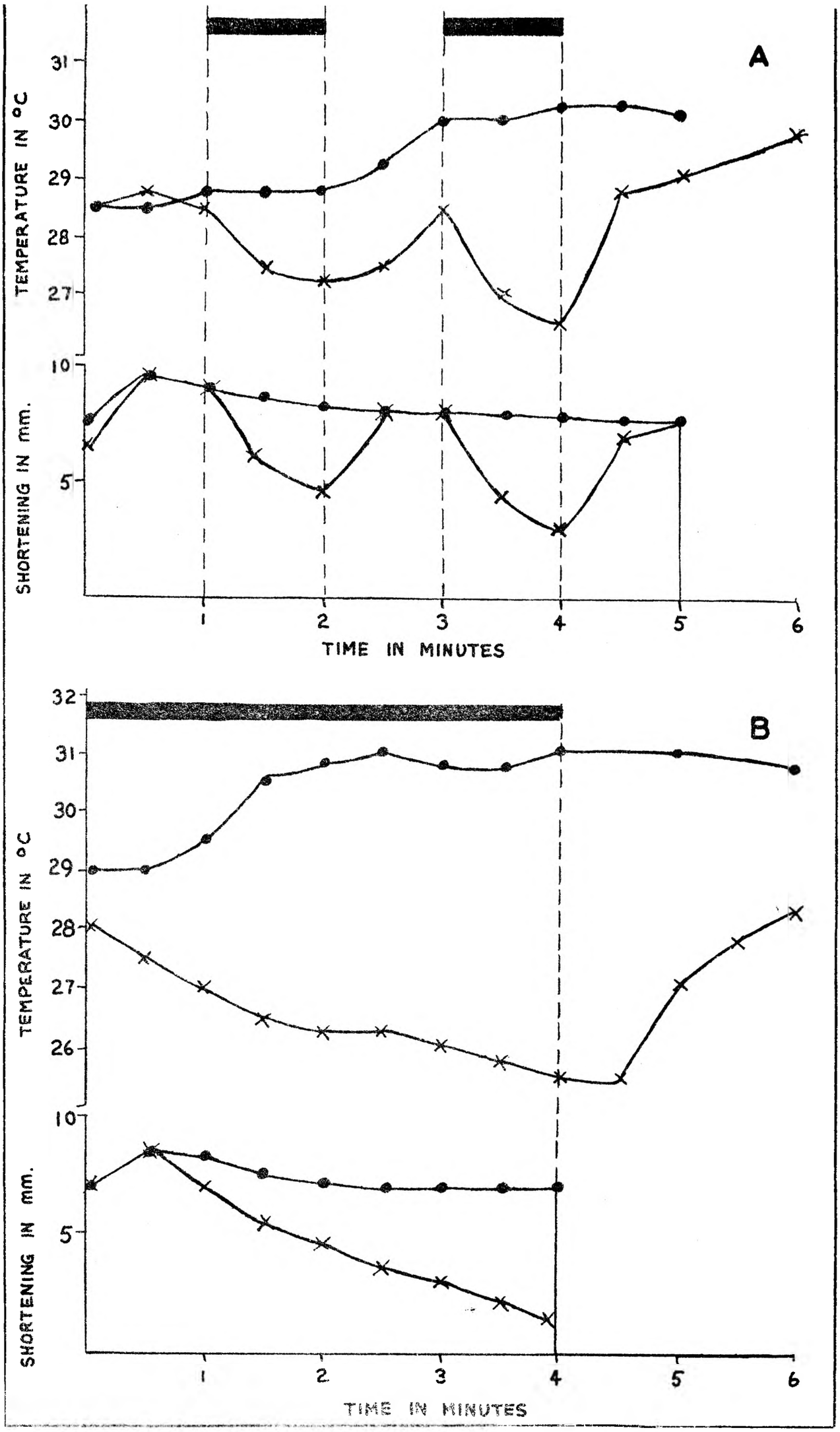
Discussion.

The decline in response produced by occlusion during a tetanus strongly suggests that, in this preparation, the tetanic response does not result in significant ischaemia. This is in accordance with the findings of Bulbring & Burn (1939) and Humphreys & Lind (1963). The graded effect on the mechanical response of different amounts of occlusion is compatible with the findings of Hiroven & Sonnenschein (1962) that the mechanical response is proportional to the blood flow, although no correlation can be given between values of occlusion pressure and absolute blood flow in the present experiments. Humphreys & Lind (1963) also found that the maintainence of contraction at all tensions that they examined was decreased by occlusion.

The finding of more rapid fatigue under ischaemic conditions and failure of recovery until the circulation is released is in agreement with Merton's (1954) results in man. However, since no electromyographic studies were done, it contributes little to the controversy concerning the site of fatigue, postulated by Merton to be within the muscle.

The question of whether the increased blood flow to active muscle is due primarily to local vasodilator factors as favoured by Hilton (1959) or central nervous system reflex activity as advocated by Humphreys & Lind (1963), remains unsettled.

Although the results obtained are in no sense quantitative they demonstrate the intimate dependence of the mechanical response of mammalian muscle upon its blood supply and confirm the necessity for studying such muscle in a circulated preparation.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TEMPERATURE IN °C
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SHORTENING IN mm.
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TIME IN MINUTES

Muscle temperature during tetanic contractions and occlusion.

Objective:

It is not always possible to record muscle blood flow plethysmographically during contraction, and so use has been made of muscle temperature as an indirect indication of blood flow. (Humphreys & Lind, 1963). Since plethysmographic methods were not available, it was considered of interest to investigate the effect of sustained contractions on intramuscular temperature and to compare this with the changes occurring during artificial occlusion of the circulation.

Results. (Fig. 15) (see opposite and below)

Results were obtained with two methods. In the one, after a control run, occlusion was produced during the steady state of a maintained tetanus. In the second experiment, the temperatures during an occluded and an unoccluded tetanus were compared.

(i) Controls: In both experiments the temperature of the muscle rose after 1-2 minutes contraction.

(ii) Occlusion: This results in a steady fall in temperature in parallel with the decline in mechanical response of the muscle.

(iii) On release of the occlusion and cessation of the contraction, there is a marked rise in temperature.

← Fig. 15:

The effect of occlusion on the intramuscular temperature and the mechanical response.

. control run.

X occlusion experiment

▬ period of occlusion.

shortening and temperature plotted on the ordinate with the same time base.

A: two periods of short occlusion during the tetanus.

B: occlusion throughout the tetanus.

Discussion.

The slight rise in temperature during a maintained tetanus indicates that the contraction itself does not significantly restrict the blood flow, for the moment the circulation is occluded, the temperature falls markedly. This supports the conclusions drawn in the previous section. Humphreys & Lind (1963) report very similar temperature changes in voluntarily sustained contractions in humans.

The rise in temperature during the latter half of the contraction and following cessation of contraction is ascribable to hyperaemia, probably induced by the metabolic products of contraction. (Clarke, Hellon & Lind, 1958). These authors found that not only do sustained contractions effect the intramuscular temperature but that temperature itself is a determinant of muscle performance. They found that in humans the duration of sustained contractions was optimal at 18°C while maximum tension occurred above this. In the present experiments muscle temperatures did not fall below 26°C. The reason for this is that while Clarke et al (1958) cooled the limbs in water baths, the present experiments were conducted at room temperature in an animal whose body temperature was controlled with a heater. No experiments were conducted in which the local temperature of the limb was altered alone.

It was concluded that the measurement of intramuscular temperature in the preparation gave no quantitative measure of local blood flow since account could not be taken of a number of factors including heat production due to muscle activity. Measurement of muscle temperature in subsequent experiments was consequently not performed.

EFFECT OF LOAD AND INITIAL LENGTH ON AMPLITUDE OF CONTRACTIONS.

Objective:

Under the section "Stimulation frequency", the fluctuations in the tetanic response to a 30/second motor nerve stimulus were discussed, and the characteristics of the response at other frequencies above and below this were noted. The possibility of changes in neuromuscular transmission being responsible for the form of the tetanus were considered.

In this section an attempt was made to examine whether mechanical factors, within both the muscle and the recording system were operative in producing the fluctuations.

Both the size and form of the peaks were therefore investigated with different loads under two sets of conditions. In the first, the load was permitted to stretch the muscle before contraction (free loading) and in the second, the muscle was kept at the same initial length by loading against a stop (or afterload screw).

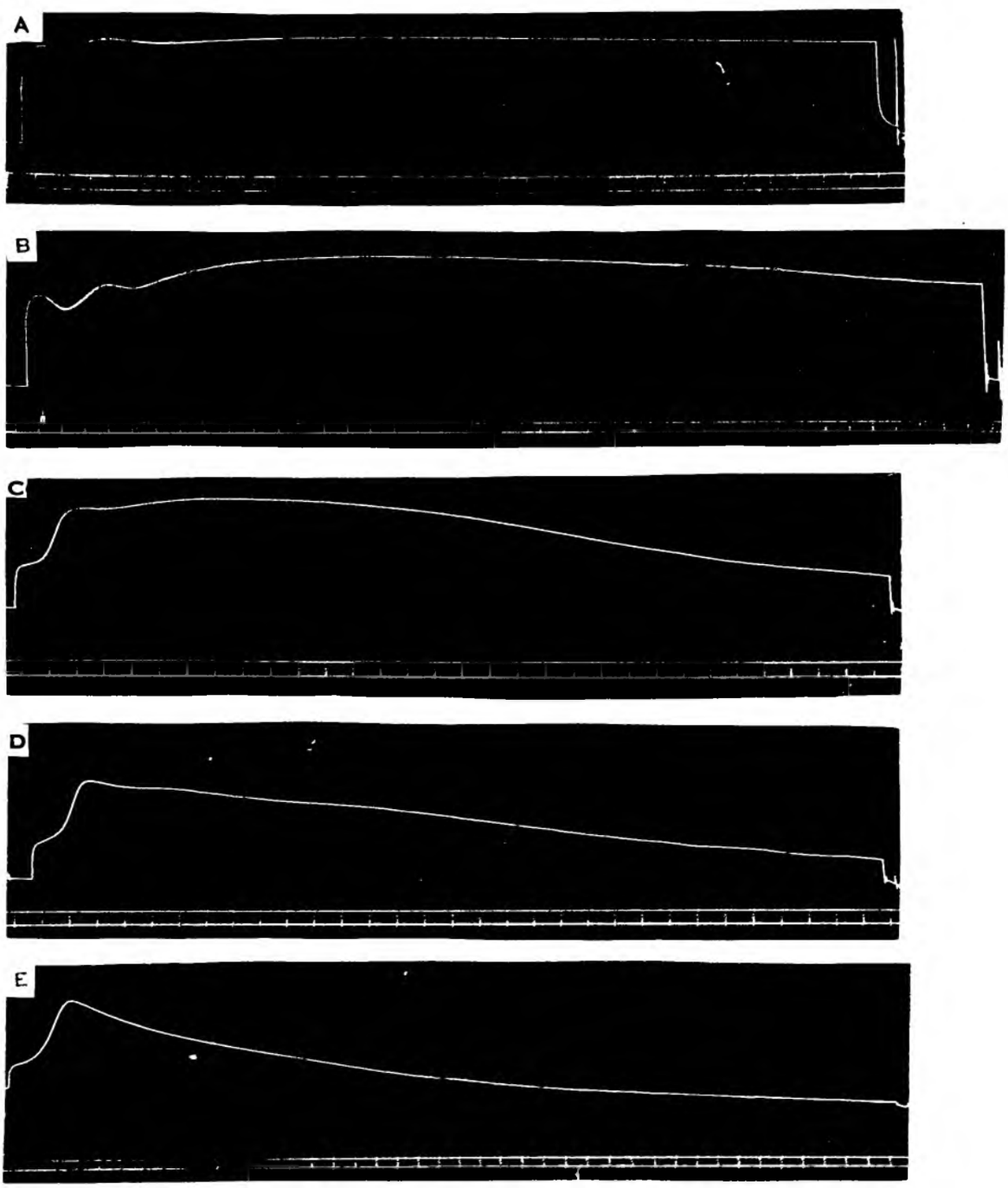


Fig. 16. Effect of increasing loads on free-loaded isotonic tetani of triceps surae.

Stimulation frequency 30/sec.

Time base = 3 secs.

Load: A = 25, B = 100, C = 215, D = 272, E = 362 gms.

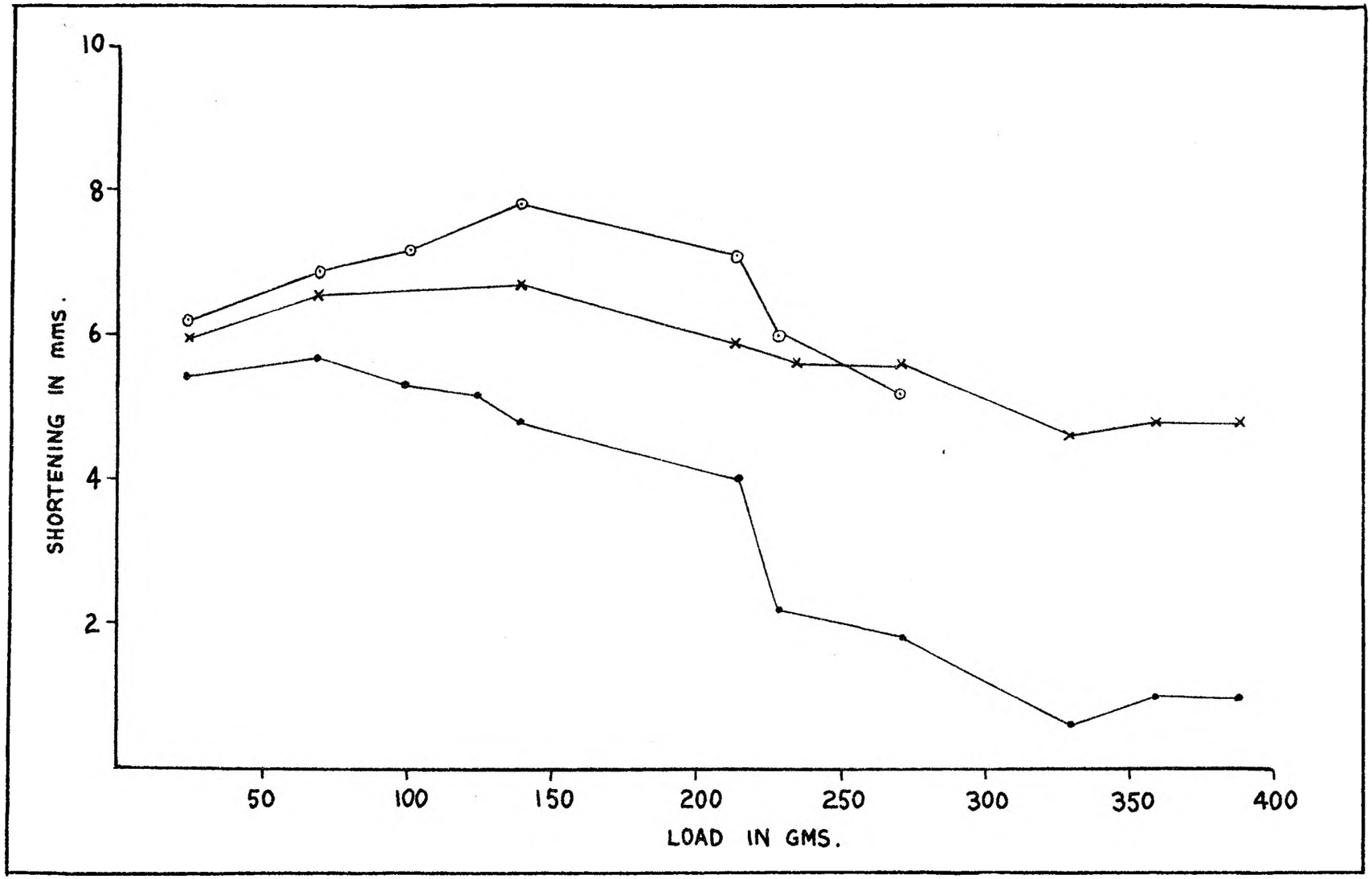


Fig. 17. The Effect of Load on the degree of Isotonic Shortening.

Note the decline in amplitude with higher loads of the various peaks in the response.

. = 1st. component.

x = 2nd. component.

● = 3rd. component.

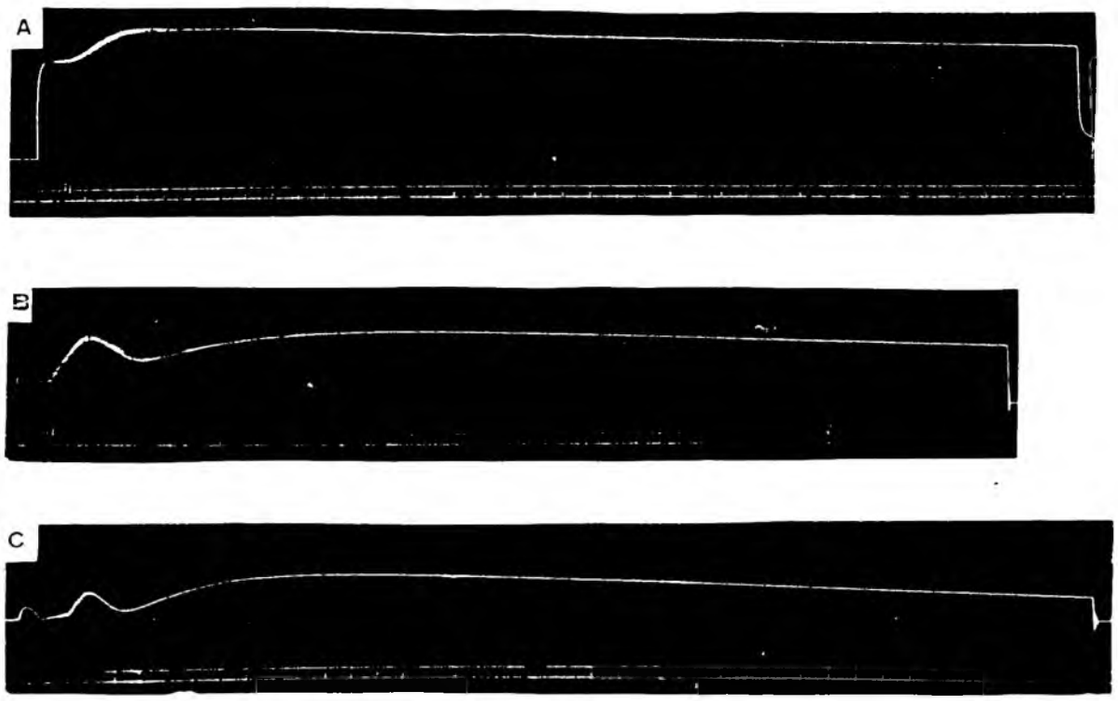


Fig. 18. Afterloading at the same initial length.
A = 25, B = 50, C = 115 gms.

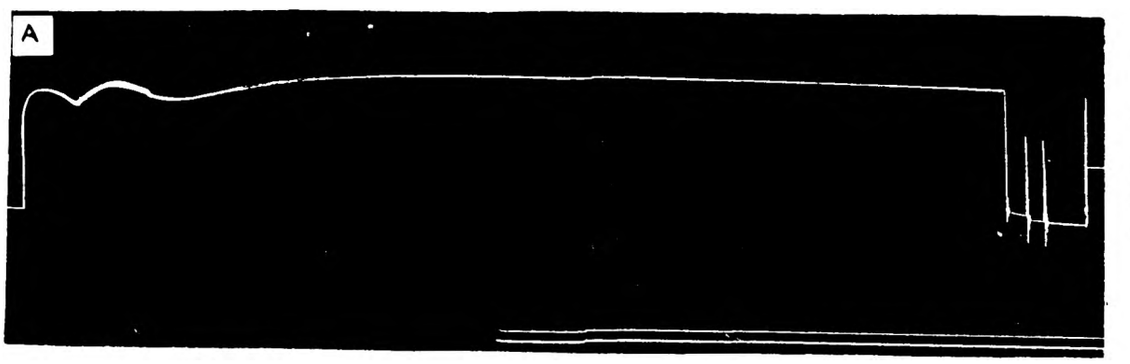


Fig. 19. Note the lack of fusion during the intermediate peak.

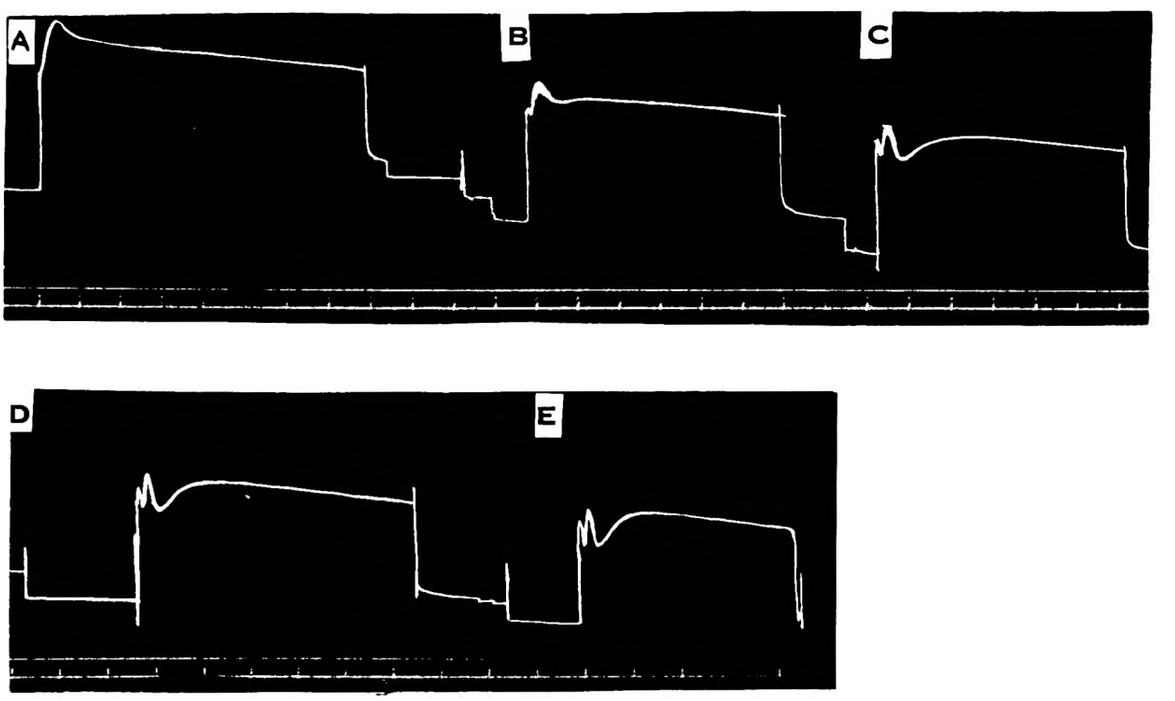


Fig. 20. <u>The effect of increasing free loads on the peaks</u>.
Note: time base slower (30 secs.) than previous recordin
to display effect on shape of peaks.

A = 25, B = 40, C = 55, D = 70, E = 110 gms.

Results:

The results obtained from eleven experiments were not clearcut principally because the preparation tended to deteriorate when large loads and stretches were applied to it. Nevertheless, the following changes in amplitude and shape of the components in the fluctuating response at 30/second were found.

Amplitude of the peaks.

Under freeloading, the height of each of the peaks remained unaffected until large loads were used. Then the amplitude declined. This occurred first in the initial peak. The intermediate and third components then merged and finally at the highest loads used, declined in amplitude. (Fig. 16 and 17).

Under afterloaded conditions, the amplitude of all three components decreased markedly but nevertheless all three components remained distinct. (Fig. 18).

Shape of the peaks.

It was noticed that the intermediate peak was associated with a lack of fusion of the contractions. This was present in most of the graphs, and did not appear to be affected by different loads or by the method of loading. (Fig. 19).

However, increased loads resulted in the peaks becoming sharper and more defined due to increased troughs between them. (Fig. 20).

Discussion.

These experiments did not, as was hoped, give much insight into the factors involved in the production of the peaks. By increasing the load, greater friction and inertia during the response was produced. Whether, however, the changes produced in the peaks were a consequence of these instrumental variables or of physiological mechanisms in the muscle was not clear from the results.

There is evidence from single fibre experiments that muscle under stretch does not contract uniformly. Huxley & Peachey1961), working with single fibres described fast and slow components during the first few seconds of a tetanus, which they ascribed to uneven stretching of sarcomeres during loading. Podolsky (1963) reported a "hunting" phenomenon in muscle subject to changes in tension during contraction. He found that the velocity of contraction, after a change in the load opposing it, fluctuates about a final steady level expected from the force-velocity relationships.

Both of these factors, uneven stretching of sarcomeres and alterations in the force opposing the muscle, which produced changes in the speed of contraction could be operative in the genesis of the fluctuations found in the preparation. Firstly it measures the response of a large number of fibres in three different muscles which are all differently stretched by the load as a result of their geometric disposition. Then when the muscle group contracts, it meets not a constant load which it accelerates, but a changing one as a result of the considerable inertia and friction in the present system.

However, an extrapolation from the results of experiments conducted on single fibres to the complex arrangement employed here has no justification. Little can therefore be concluded from these experiments about the nature of the peaks in the response of triceps surae under the given mechanical conditions. It therefore remained to investigate whether they were an artefact of the recording system, or a phenomenon arising from the use of three muscles in a group. This was done in Section III where each individual muscle was examined using apparatus designed to eliminate sources of artefact in the recording system.

THE EFFECT OF LOAD AND THE INITIAL LENGTH ON THE WORK OUTPUT OF ISOTONIC TETANI.

Objective.

It is well established property of muscle that it regulates its energy expenditure according to the mechanical conditions under which it contracts. (Hill, 1956). Hill (1938) demonstrated that the extra heat production associated with shortening is independent of the velocity of contraction and is proportional to the distance the muscle shortened. This laid the basis for the refutation of the viscoelastic theory of muscle contraction first propounded by E. Weber in 1846. This theory had held that the hyperbolic relation between force and velocity of contraction (previously discussed) was the consequence of the dissipation of a given amount of elastic energy as friction in a viscous medium, so that a greater velocity would incur more friction and hence develop less force and more heat. The thermodynamics of contracting muscle have been expressed by Hill (1949) in the following equation:

$$E = W + A + ax.$$

Where E is the energy flux

W is the mechanical work

A is a constant representing the heat resulting from the activation process.

ax is the shortening heat, where 'x' is the distance shortened and 'a' is a constant.

A muscle will therefore produce the same amount of heat when it lifts a large or a small load through the same distance, in spite of the difference in the work done. Thus muscle apparently possesses the remarkable property that "when a muscle shortening meets a load, it provides extra energy exactly equal to the work it has to do, without any effects on the heats A and ax" (Hill, 1956). Zierler (1961) remarks that it seems unlikely that muscle could actually perform this extra work with 100% efficiency and feels that a large body of theory is at the mercy of Hill's observations on excised amphibian muscle.

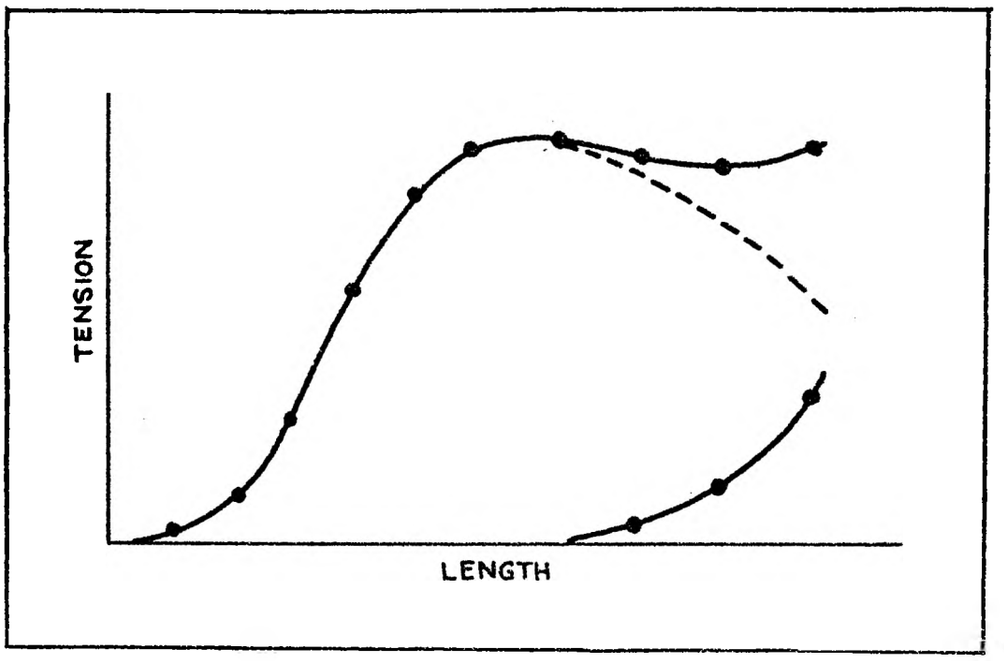


Fig. 21. Theoretical Length- tension diagram.
The upper continuous line = total tension.
lower continuous line = passive tension.
dotted line = active tension.

One important source of error which appears to have been overlooked in the work done to validate the Hill equation concerns the determination of the shortening 'x'. There is now good microscopic evidence to show that internal changes in length may occur without external shortening as a result of the contractile component stretching a series elastic component. (Huxley H.E. & Hanson, 1954) (Huxley A.F. & Peachey, 1961). As the load is increased therefore the amount of external shortening will decrease although it is likely that the amount of internal shortening of the contractile component will be the same provided sufficient time is allowed for the decreased velocity of contraction. Measurements of the external output of isotonic tetani under different loads are subject to the same criticism although it is probable that the loss of external shortening due to the series elastic component is small compared with the overall change of length during an isotonic tetanus.

The concept of an optimum length for maximum response of the muscle has been derived primarily from the investigation of the isometric length-tension diagram in isolate amphibian muscle or muscle fibres at 0°C. The active tension developed increases with stretch until an optimum is reached. Thereafter active tension declines approximately linearly. (Fig. 2). This optimum length is taken to correspond to that length the muscle has in the intact animal. (Hill, 1925; Ramsey & Street, 1940; Aubert et al. 1951; Jewell & Wilkie, 1958.)Huxley & Hanson, (1960) have attempted to explain this on the basis of the sliding filament hypothesis of muscle contraction. They postulate that force is generated in the area of overlap of two sets of contractile filaments and is therefore proportional to the degree of overlap, which in turn depends upon the degree of stretch placed on the muscle.

While authors such as Deleze (1961) accept that an optimum length for contraction exists at which the overlap between the myofilaments is maximal, and that this length

occurs at the maximum length which muscle has in the body, the ultrastructural evidence for this is incomplete, indirect and in some cases conflicting. (Huxley A.F & Niedergerke, 1954; Huxley H.E. & Hanson, 1954; Hanson, 1956; Sjostrand & Anderson-Cedergren, 1957; Huxley A.F. & Peachey, 1961; Carlsen, Knappies & Buchtal, 1961; Sjostrand, 1962; Huxley A.F. & Gordon, 1962; Spiro, Cottrell & Sonnenblick, 1963; Sonnenblick, Cottrell & Spiro, 1963.)

Nor is it yet clear whether this relationship applies to the isotonic work output of mammalian muscle in situ. Ralston et al. (1953), working on human amputees support the concept of an optimum length at which the isotonic work output is maximal. Rosenbleuth et al (1958b) reject the concept of an optimum length within physiological stretches, claiming that the decline at large loads is due to irreversible structural damage.

As a preliminary to a full scale investigation of this problem when the apparatus and technique have been satisfactorily developed, a pilot study was made of the effect of free loading on the external work output of isotonic tetani.

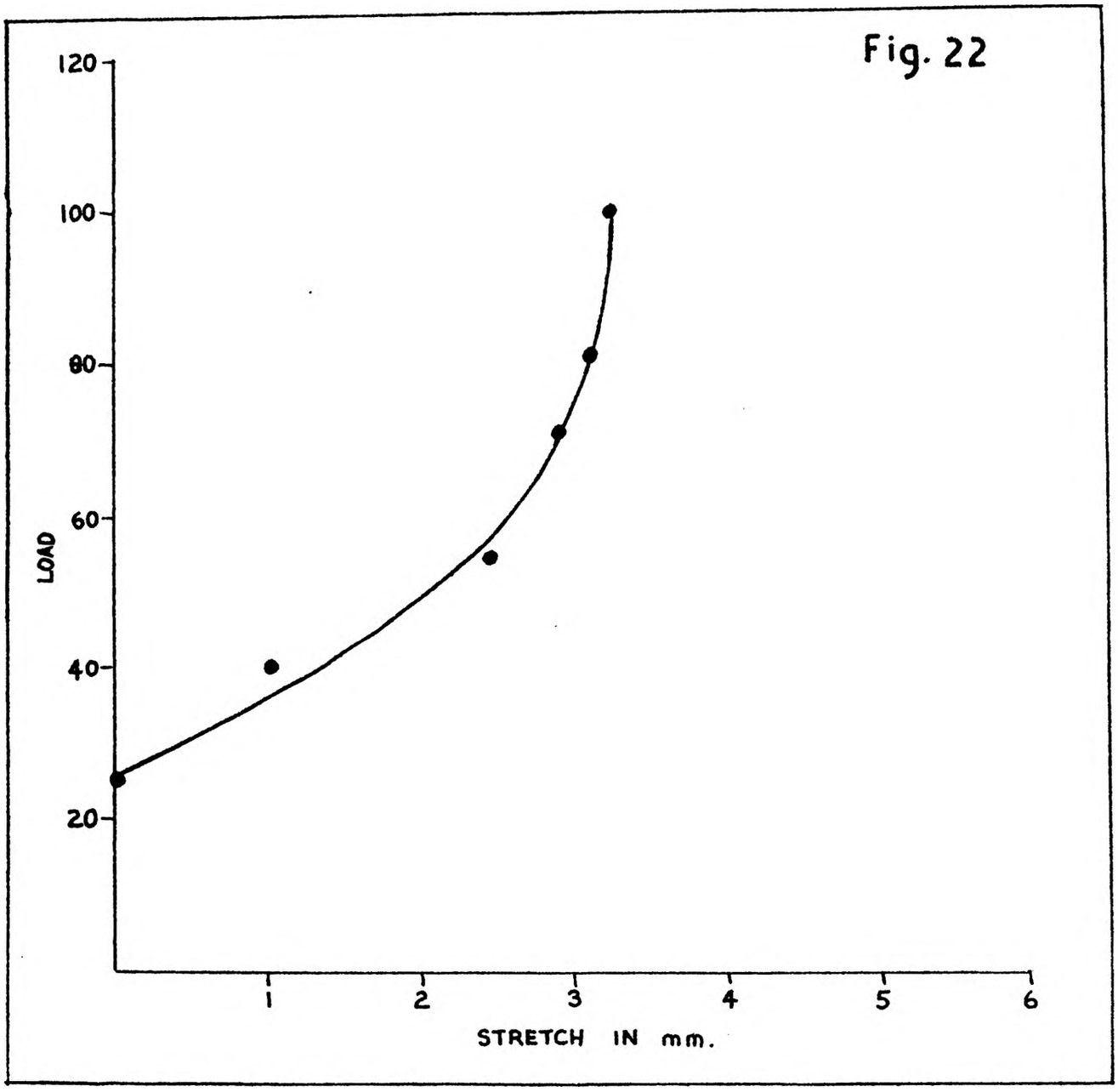


Fig. 22. Passive length-tension diagram of triceps surae. Note that all stretches are measured relative to the length at 25 gms. and that the shape of the curve suggests that at loads below 25 gms. the extensibility of the muscle is increasing.

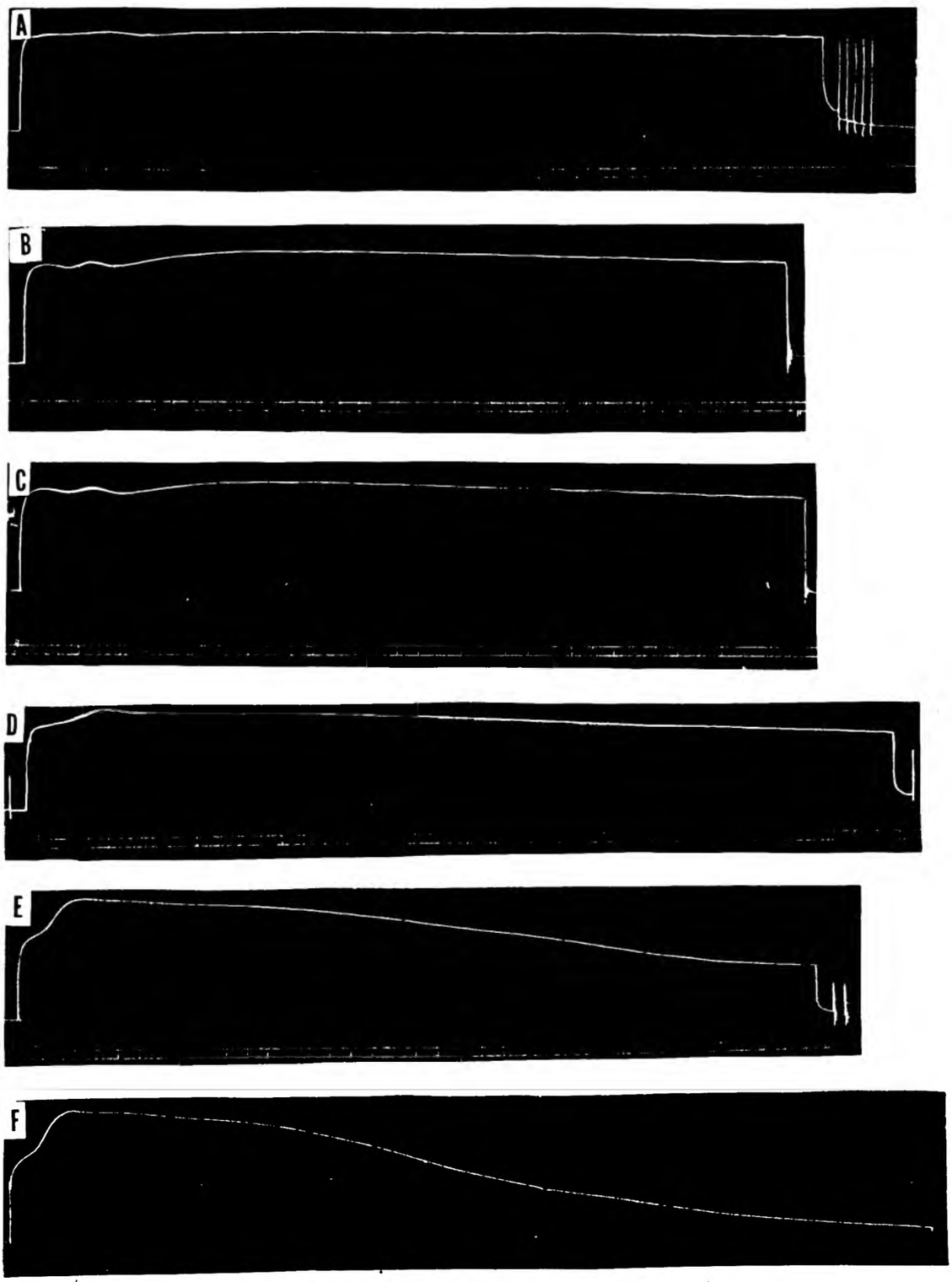


Fig. 23. The effect of free loading on work of isotonic tetani.
Load(in gms.) A = 25, B = 55, C = 100
D = 200, E = 272, F = 350.

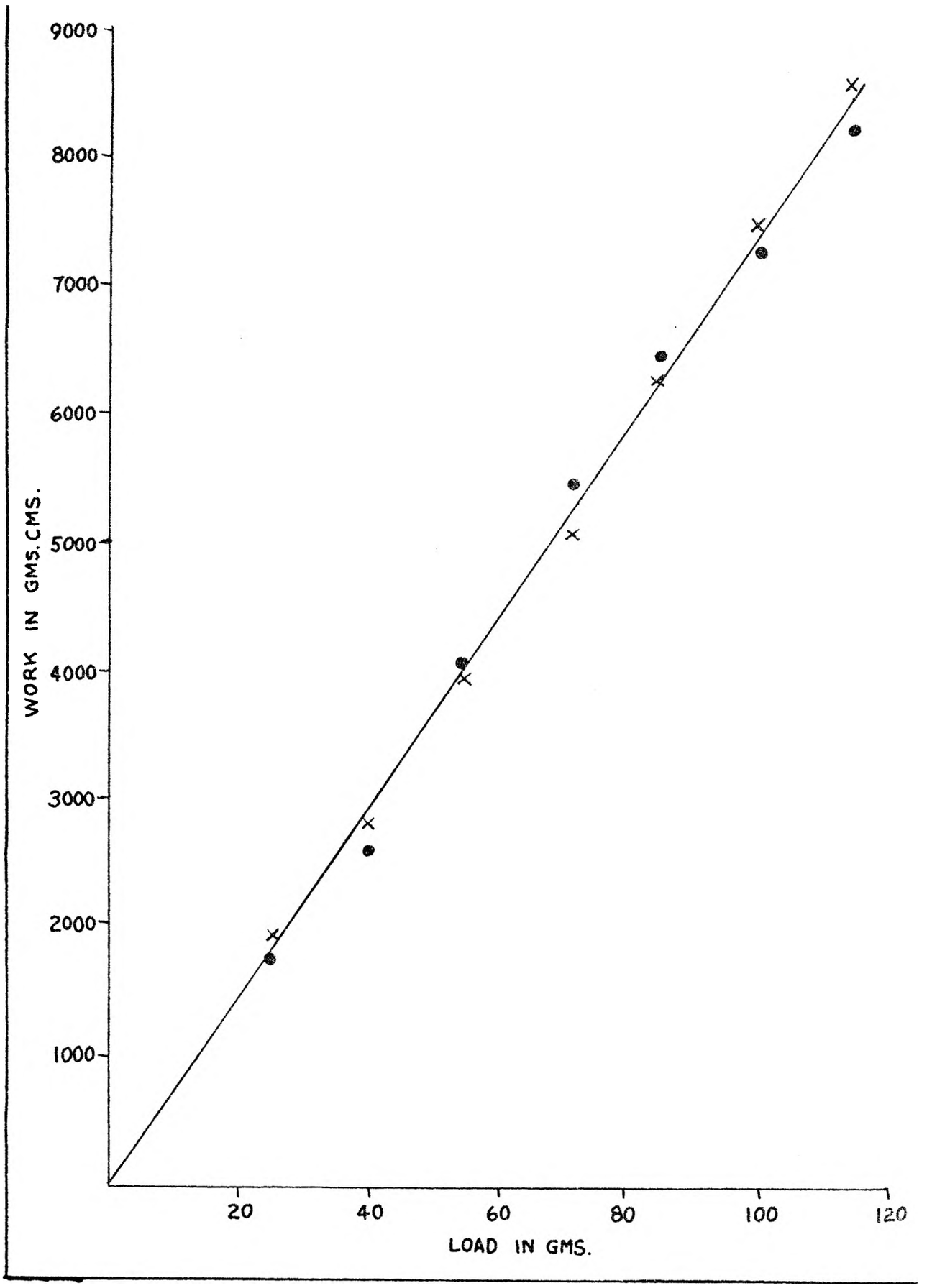


Fig. 24. The relationship between work output and freeloading in the lower range in two consecutive series on the same animal.

. - first series.
x - second series.

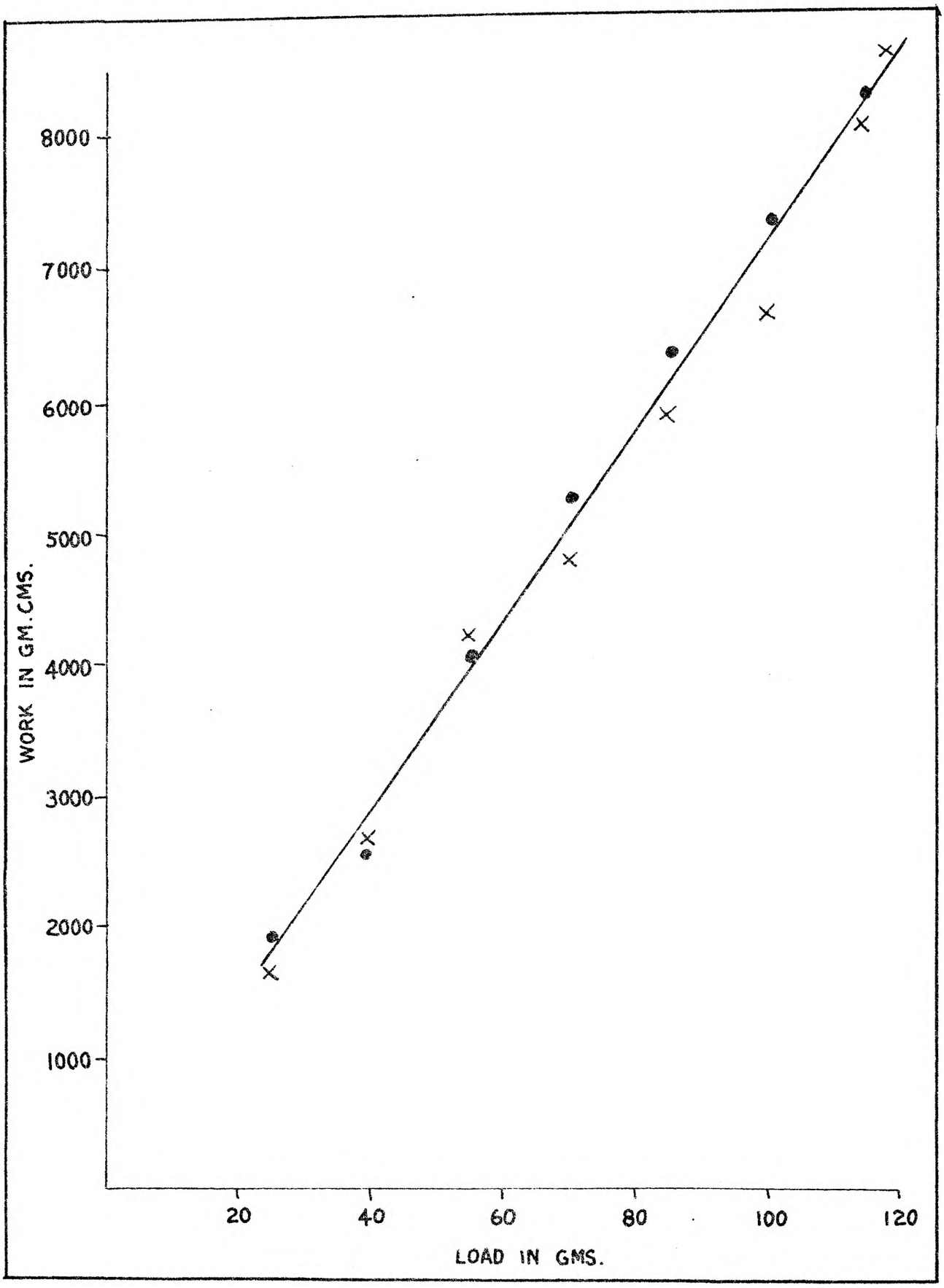


Fig. 25. The relationship between work output and free-load in two different experiments using rats of comparable weights.

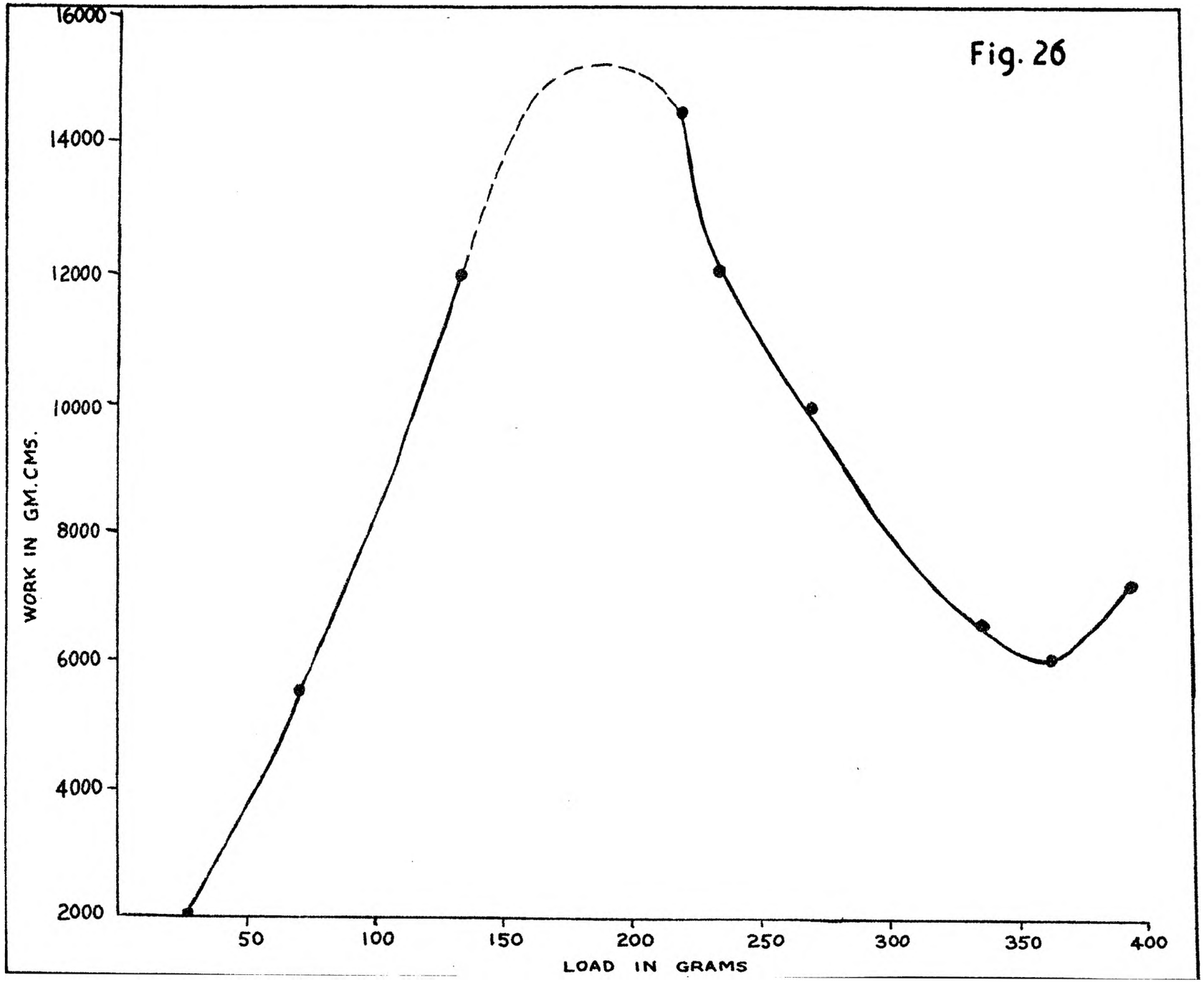


Fig. 26
16000
14000
12000
10000
8000
6000
4000
2000
WORK IN GM. CMS.
50
100
150
200
250
300
350
400
LOAD IN GRAMS

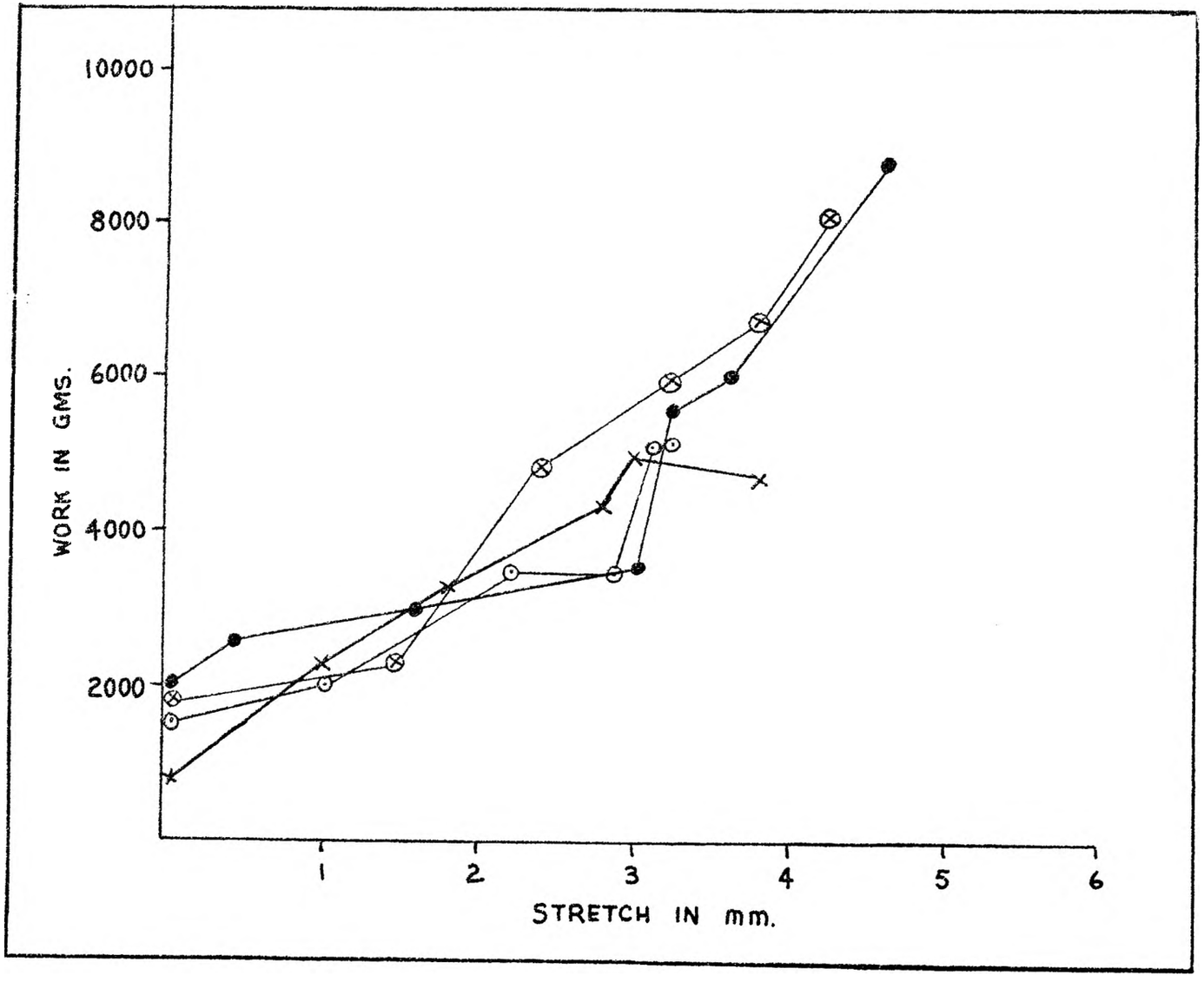


Fig. 27. The relationship between isotonic work output and stretch in four different animals. Note that in only one, (x) is there a suggestion of an optimum within the range of stretches used.

Results:-(see appendix for tables of actual measurements.)

(a) Passive length-tension diagram. (Fig 22).

Relaxed muscle was free-loaded in order to determine the passive length-tension diagram. It was found that comparatively small loads produced large increments in length but with increasing loads, the muscle became progressively more resistant to stretch. It was not possible to decrease the load below 25 gms. because of the mechanism of loading and because of the necessity for keeping the recording system taut. Hence the lower range of the length-tension diagram was not investigated.

Work output of active muscle. (Fig. 23).

(i) In seven experiments a loading range of up to 120 gms. was used. The work output of free loaded muscle shows an almost linear increase with increasing loads.

This relationship was comparable in two consecutive series on the same animal (Fig. 24) and in some cases for two different animals. (Fig. 25).

(ii) In four other experiments using a range of loads of up to 350 gms., it was found that the increase reached a maximum of about 200 to 250 gms. and thereafter declined. (Fig. 26).

At higher loads there was a more marked deterioration of the preparation with time. However, the sequence of loading was randomised so that the decline at high loads cannot be attributed primarily to this deterioration.

(iii) When the work output is plotted against stretch a similar relationship emerges. The work output increases with stretch, in a comparable fashion in four different experiments. (Fig. 27).

SECTION III.

AN IMPROVED TECHNIQUE FOR THE INVESTIGATION OF THE MECHANICAL PROPERTIES OF MAMMALIAN MUSCLE.

INTRODUCTION.

DEVELOPMENT OF APPARATUS.

Immobilisation.

Mechanical Response.

Recordings.

Length Measurements.

STANDARDISATION OF APPARATUS.

Calibration of Stimulator

Calibration of Displacement Transducer.

Calibration of Tension Transducer.

Frequency Response of Apparatus.

REPRODUCIBILITY OF RESPONSE.

ANALYSIS OF COMPONENTS IN RESPONSE.

Objective

Results.

Discussion.

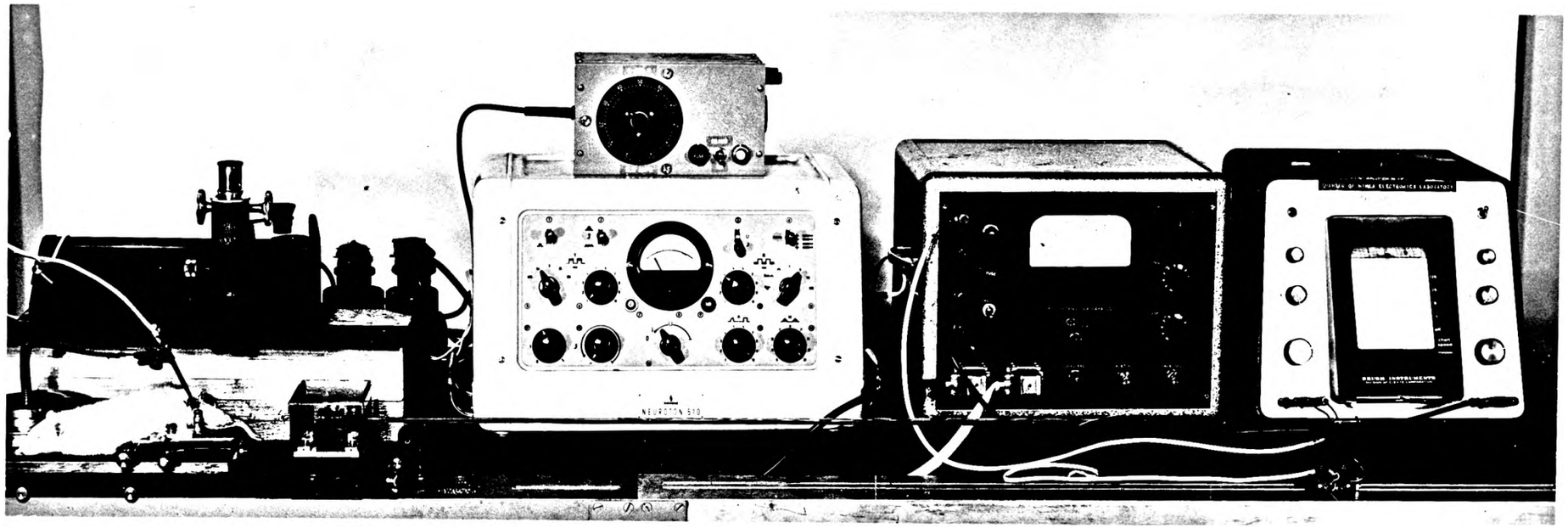
Discussion:

The limitations of the technique used became obvious during the course of these experiments. As a result of not being able to measure the stretches produced by loads below 25 gms., it was not possible to state whether the range of loads employed stretched the muscle beyond its physiological length. Since muscle is extremely extensible at lengths just greater than equilibrium length it is likely that a large proportion of the normal physiological range was not investigated.

Nevertheless in the range of loads investigated, the work output load curve obtained was similar to that found by Ralston et al (1953) in humans, in showing a well defined optimum load above and below which the work output fell approximately linearly with change in load. The results do not agree with the findings of Rosenbleuth et al (1958b), who described no optimum with the physiological range loads.

In order to obviate the criticism of Rosenbleuth et al (1958b) that the optima described in the literature where due to irreversible deterioration at the end of an ascending series of loads, a random sequence of loading was adopted. However, it was not possible to interpret the results obtained in terms of their criticism that optima could only be obtained outside of the physiological range of body lengths of the muscle.

As a result of the limitations and deficiencies of the mechanical apparatus revealed, it became necessary, building on the experience gained, to develop apparatus which would take into account the problems raised by these preliminary investigations before embarking on a thorough investigation of the length-tension relationship in circulated mammalian muscle.



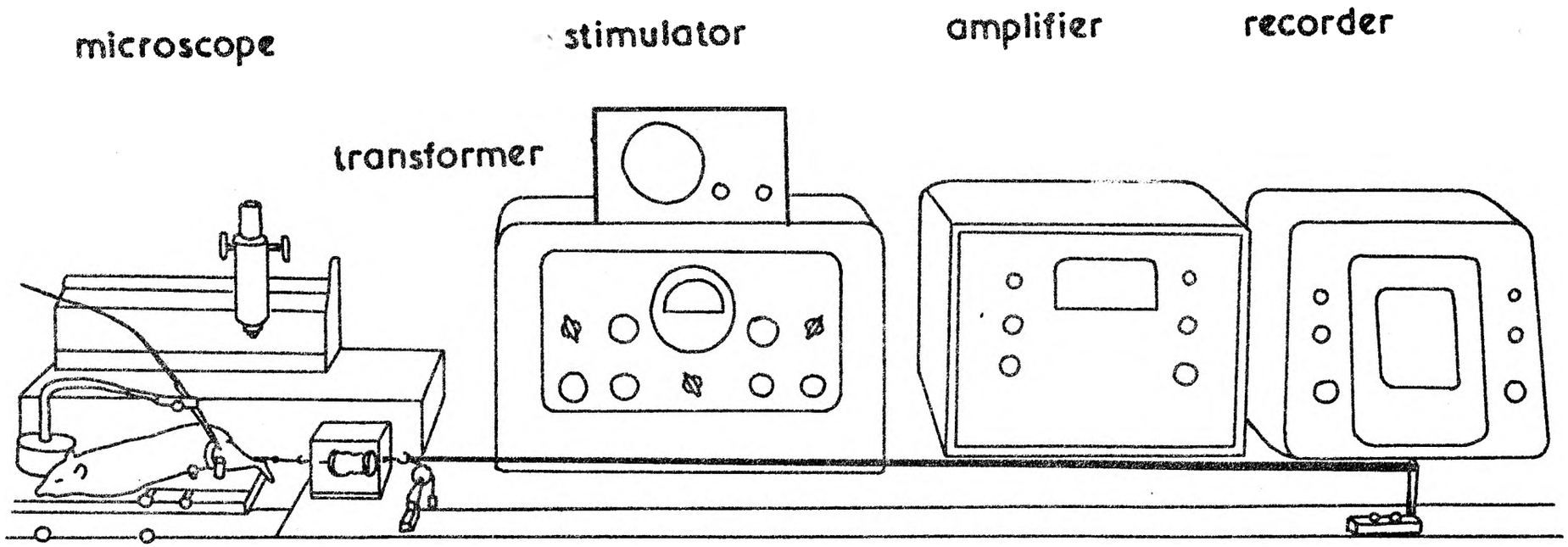

microscope
stimulator
amplifier
recorder
transformer
rat table
transducers
pulley & weights
metal rail
strain guage & spring

Fig. 29

INTRODUCTION.

Towards the end of 1962, through the offices of Professor C.H. Wyndham, the opportunity arose for developing apparatus specifically designed to eliminate many of the problems inherent in the mechanical apparatus reported and criticized in the preceding sections. This section of the thesis is devoted to a description of the development and standardisation of this apparatus. The principles used are based partly on the work of Rosenbleuth et al. (1958a). The design and construction of electronic transducers and circuitry capable of measuring changes in muscle length and tension was the work of Mr. A.R. Atkins, an electronics engineer from the Chamber of Mines Research Laboratories. His description of the electronic circuits is included as an appendix.

The apparatus consisted of the following:- (Fig. 29)

(1) An adjustable animal table.

(2) Isometric tension and isotonic shortening transducers.

(3) A rail on which is mounted the isotonic loading device consisting either of a pulley and weights or a long elastic band and strain guage.

(4) Stimulating electrodes.

(5) Travelling microscope.

(6) Variable Mains Transformer for the heater.

(7) Neuroton Electronic Stimulator.

(8) Amplifiers.

(9) Brush Mark II ink writing recorder.

The primary deficiencies of the apparatus used in Section I and the improvements introduced by the apparatus described in this section were as follows:-

(i) The immobilisation of the limb was inadequate and length changes could not be induced and measured with accuracy.

A new board for immobilising the animal was therefore constructed incorporating the following features:- (Fig. 30).

(i) An inlaid copper plate and heater were used to control the animal's body temperature, the heater being supplied through a Variable Mains Transformer.

(ii) Mounted on the copper plate were two brass pillars containing slots into which a steel pin, passing through the femoral condyles close to the insertion of the muscle fitted.

(iii) This did not prove quite satisfactory since the thigh of the animal tended to rotate around the pin as a fulcrum. Therefore a perspex cuff fixed to the base was used to hold the thigh. The efficacy of this method was demonstrated by the maintainence of tension under stretch without slipping.

(iv) The whole base was adjustable with a high degree of accuracy by mounting it on a microscope stand, which permitted vernier-controlled movement in two directions. The alignment of the muscle's pull and the length of the muscle could be adjusted with an accuracy of 0.1mm.

(v) The rat's foot was held by a nylon clamp in such a way that it did not interfere with the line of pull of the muscle.

(vi) The muscle was connected to the recording apparatus by means of a light but rigid steel wire attached to the tendon with braided nylon suture material.

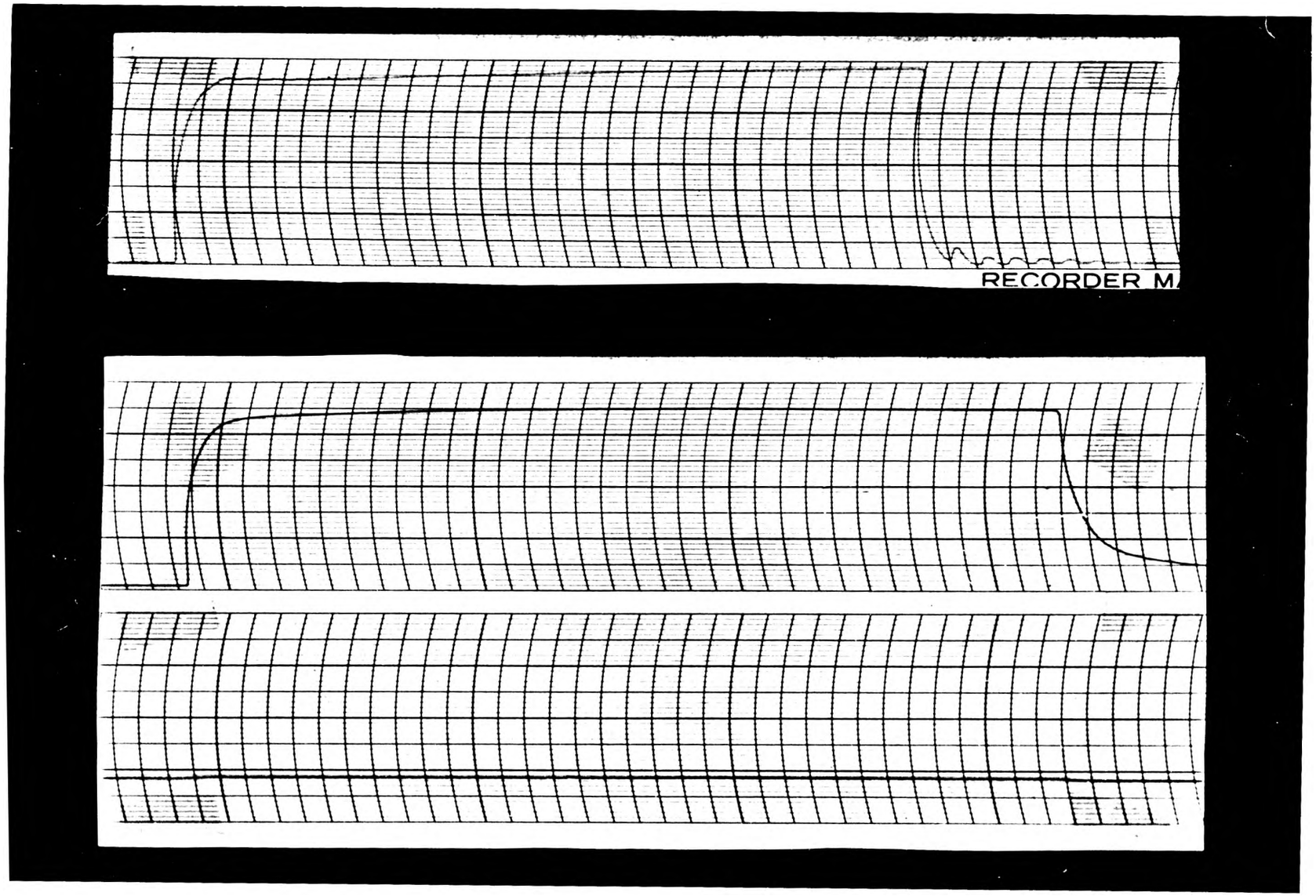


Fig. 31 Isotonic records to show the difference between loading with weights (Upper record and loading with a long elastic spring. (Lower record).

The artefact in the upper record both at the commencement and completion of the tetanus is produced by the friction and inertia of the system. In the lower record, this has been obviated as can be seen from the lowest trace, which is a record of tension changes in the muscle during contraction.

II. The use of pulleys, levers, weights and long lengths of cotton connections introduce distortion of the isotonic recordings as a result of friction, inertia and compliance inherent in such apparatus.

The lever system was therefore abandoned and use was made of electronic transducers for measuring length and tension changes.

The original apparatus described by Rosenbleuth, Alanis and Rubio (1958a) recorded isotonic shortening by the displacement of a light spot on photographic paper. The spot was produced by the movement of a light beam directed through a hole in a metal plate interposed between the muscle and the load. In the present apparatus this was replaced by a ferrox-cube rod acting as the core of a differential transformer. Movement of the rod produced an "out of balance" state and therefore resulted in an EMF. Up to 12 mm. displacement could be measured with an accuracy approaching 1%.

The ferrox-cube was held in the line of pull of the muscle by a set of small and light guiding pulleys and connected at its other end via a long elastic spring to a cantilever arm. Varying the length of the elastic band by moving the cantilever arm along a rail changed the load against which the muscle contracted. By making the length of the elastic band long, (up to 3 metres) the tension changes induced by the small length change occurring with contraction (up to 12 mm.) were rendered insignificant (less than 5% of the total tension.)Thus the inherent problems of mechanical levers, whose friction and inertia increase as the square of the distance of the load from the fulcrum, were overcome. (Fig. 31).

The load on the muscle depended on the amount of stretch in the elastic band and was measured directly by the cantilever arm which consisted of two 600 ohm. strain guages, one in tension and one in compression, capable of measuring tensions of up to 1000 grams. Rosenbleuth et al (1959b) had used a steel spring dynamometer for the measurement of load.

It has already been noted that muscle is highly extensible at lengths corresponding to the lower range of tension, and that it was not possible to investigate the length tension curve below 25 gms. with the mechanical lever system. Unfortunately, the same problem arose with the electronic transducer. The A.C. bridge used in the circuitry requires a small initial voltage of about 0.1 millivolts before tension is registered. This meant that with the strain guages employed, with a range of 1000 gms, it was not possible to record tensions of less than 20 gms. This could be remedied by the use of a D.C. bridge circuit, but the greatly increased expense involved did not permit this modification.

Instead, therefore, use was made of a pulley and weights for recording at less than 20 gms. Although this introduced some friction and inertia, these were not considered significant with such very low loads.

After-loading was achieved by the use of a metal stop attached to the displacement transducer.

Isometric tension changes were recorded on a second identical cantilever placed next to the differential transformer used for isotonic recordings.

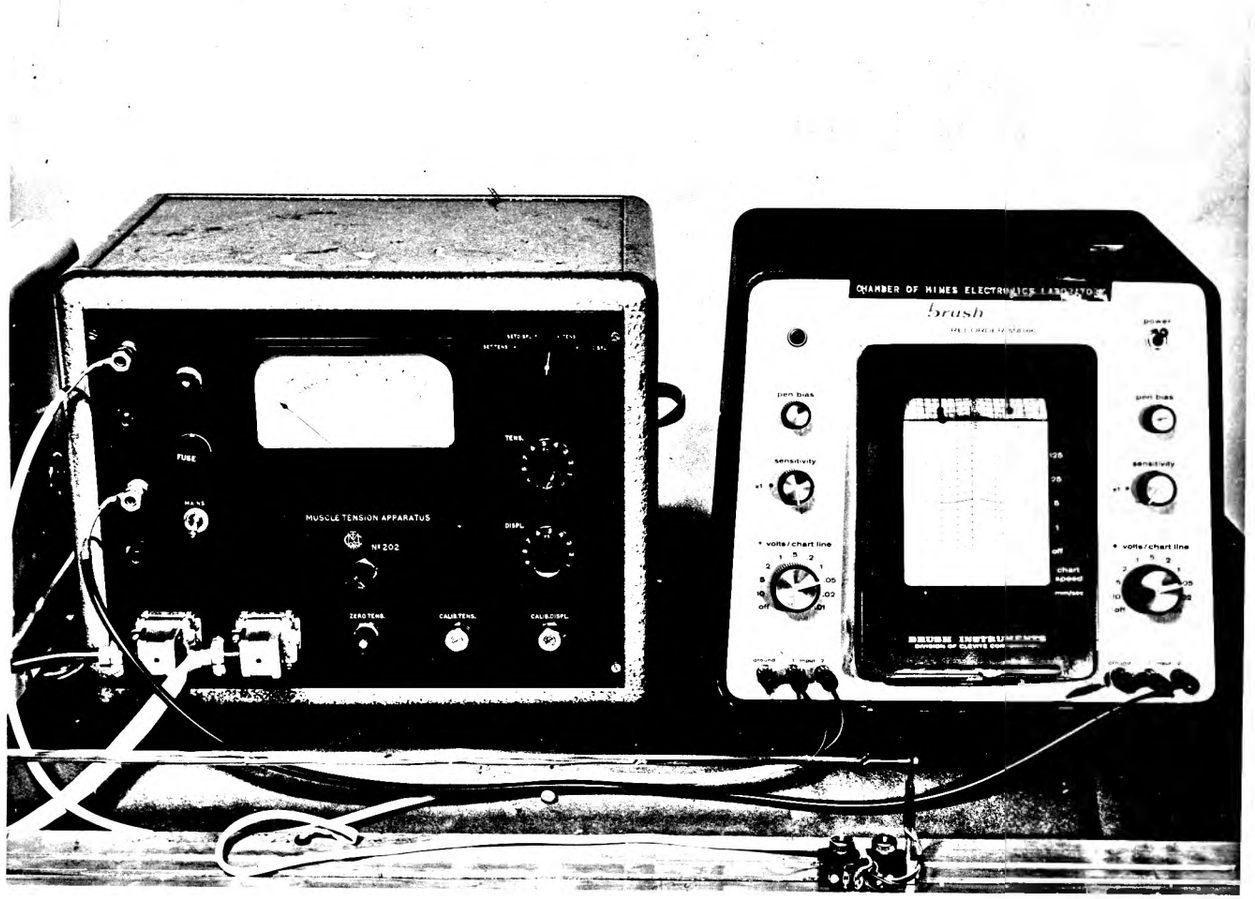


Fig. 32. Recording Apparatus

On the left is the amplifier showing the controls for calibration and varying the amplification of tension and displacement, which are recorded by the galvanometer needle. Permanent ink records are obtained by feeding the amplifier output into the Brush. Recorder (right) tension into one channel and displacement into the other. The recorder also has a variable amplification. In front is the isotonic strain gauge attached to the elastic band from the displacement transducer.

(iii) The smoked drum recordings were messy and time-consuming in preparation, and gave impermanent records which were difficult to manage.

The use of electronic transducers gave neat, permanent manageable graphs of variable magnitude, and at variable recording speeds on a Mark II Brush Recorder. (Fig. 32).

The strain guages were all connected through a Wheatstone bridge circuit to amplifiers which fed into a twin channel ink writing recorder. One channel monitored tension (either isometric or isotonic load), while the other recorded displacements. The amplifiers and electronic circuitry were so arranged to enable regular calibration and easy manipulation of sensitivity and magnification. (See Appendix).

In addition the responses could be monitored visually on a sensitive galvanometer.

(iv) The previous technique of measuring muscle lengths was not sufficiently accurate, nor did it enable direct comparisons with the in situ situation.

It has been pointed out that confusion exists in the literature on the correlation of experimentally induced length changes with those occurring in the body. The vast majority of studies have been technically inadequate in this respect. This confusion is added to by the various definitions given to common terms describing muscle lengths.

REST LENGTH, is variously described as:

(a) the unloaded resting length. (Rosenbleuth et al, 1958a)

(b) the length that muscle has in the body. (Deleze, 1961).

(c) the length at which tension development is maximal. (Ramsey, 1960).

In addition there are many modifications of this.

However, each of these is open to criticism:-

a) "the unloaded resting length" is a definition usually reserved for the term "equilibrium length".

b) "the length that muscle has in the body" is an embarrassingly vague definition because muscle is at a wide range of lengths in the functioning body.

c) "the length at which tension development is maximal" is an empirical usage which has often lent itself to a circular definition and in any event is of little use in the correlation with the body range.

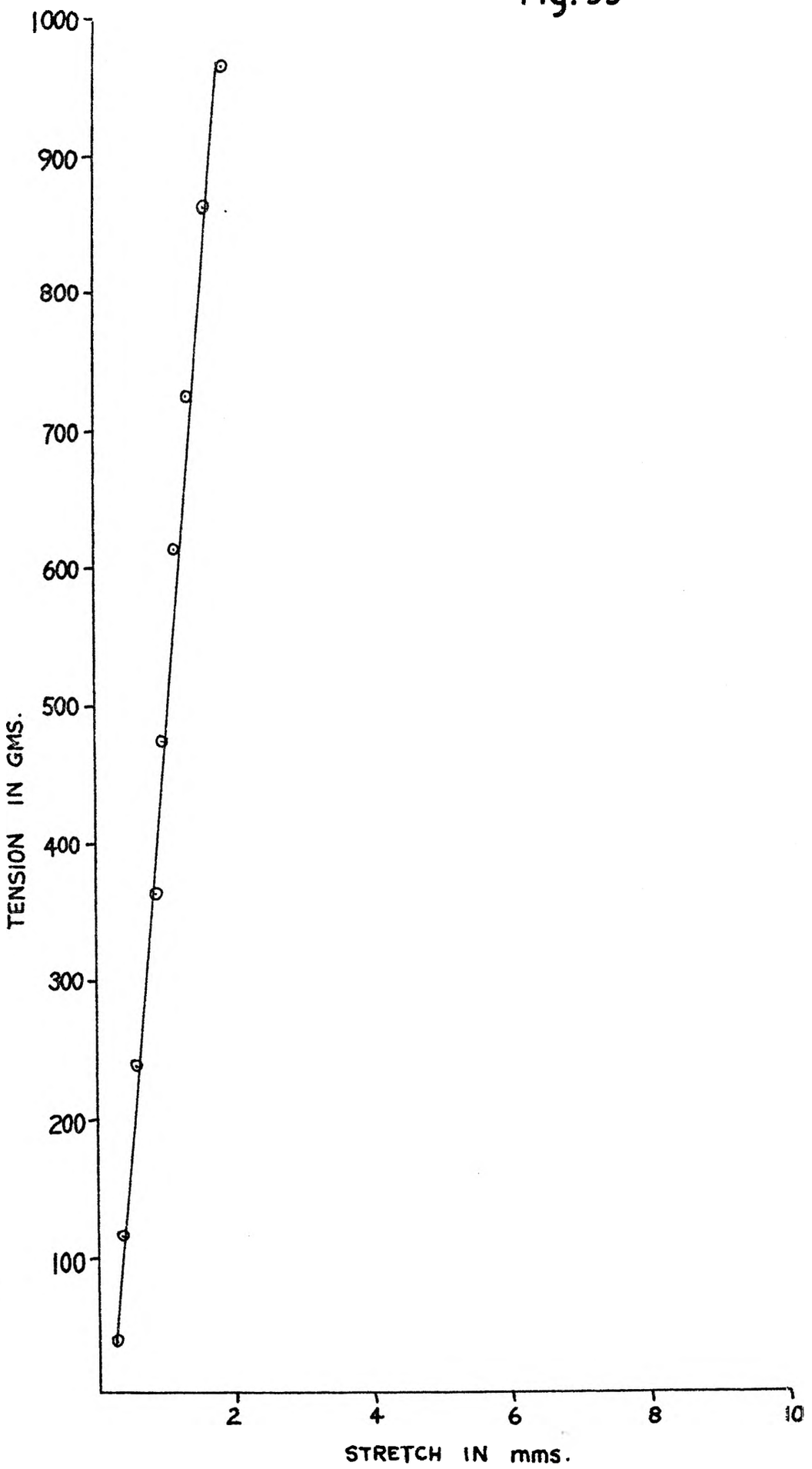
Therefore it was decided to adopt and measure three unambiguous in situ reference lengths:-

(a) Equilibrium length: defined as the length of the muscle with its tendon detached from its insertion. This was measured both in situ (ELi) and, at the end of the experiment, in isolation (ELo).

(b) Minimum body length: defined as the length of the muscle with its attachments intact when (in the case of an extensor), the joint about which it acts is fully extended. (MiBLi).

(c) Maximum Body length: defined as the length of the muscle with its attachments intact when the joint (in the case again of an extensor) about which it acts is fully flexed. (MaBLi).

Fig. 33


1000
900
800
700
600
500
400
300
200
100
TENSION IN GMS.
2
4
6
8
10
STRETCH IN mms.

Correction for compliance of apparatus (Fig. 32).

Correction of length measurements for the compliance of all factors in the apparatus other than the muscle was necessary. By performing a blank experiment, connecting the strain guage directly to the bone pin, it was found that a tension of 1000 gms. could be registered with less than 2 mm. of movement. This was not considered sufficiently small to be neglected and all readings were corrected for this compliance of the apparatus. (Fig. 33)

Measurements were made with a travelling microscope to an accuracy of 0.1 mm. In situ lengths (ELi, MiBLi and MaBLi) were measured as the distance between the bone pin inserted near the origin of the muscle and the tie at the myotendinous junction. However, it was not always possible to insert the bone pin at the origin of the muscle with an accuracy of within 0.1 mm. Therefore, in order to compare lengths in different rats, it was necessary to derive a correction factor.

Isolate lengths were therefore measured on the completion of the experiment by completely dissecting out the muscles, placing them on a glass slide, and measuring their equilibrium lengths outside of the body. (ELo). The difference between the in situ and the isolate equilibrium lengths (ELi - ELo) gave a factor corresponding to the distance from the insertion of the muscle to the bone pin, which could be used to correct all other lengths measured in situ to absolute lengths corrected for variations in the site of the bone pin.

Fig. 34

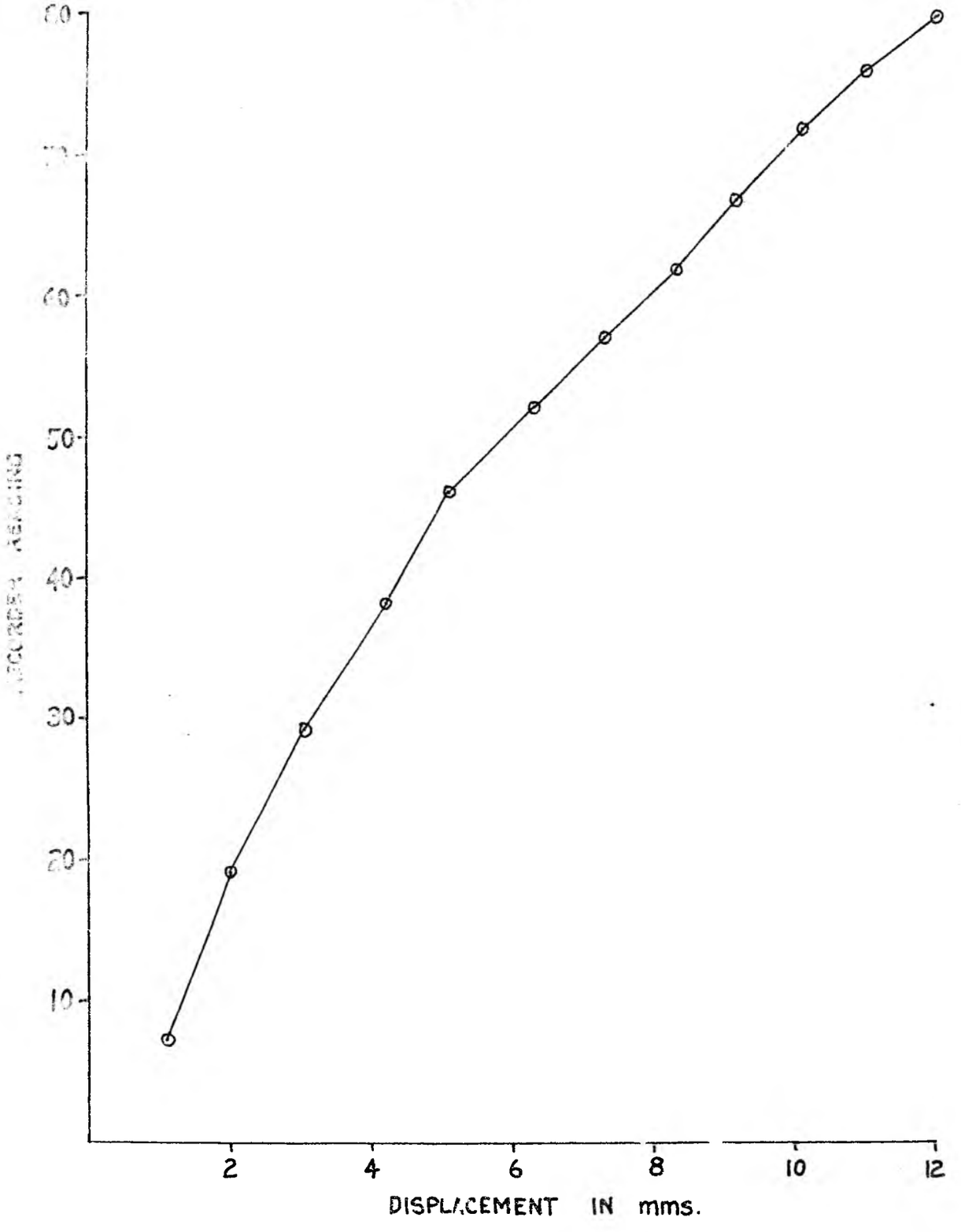
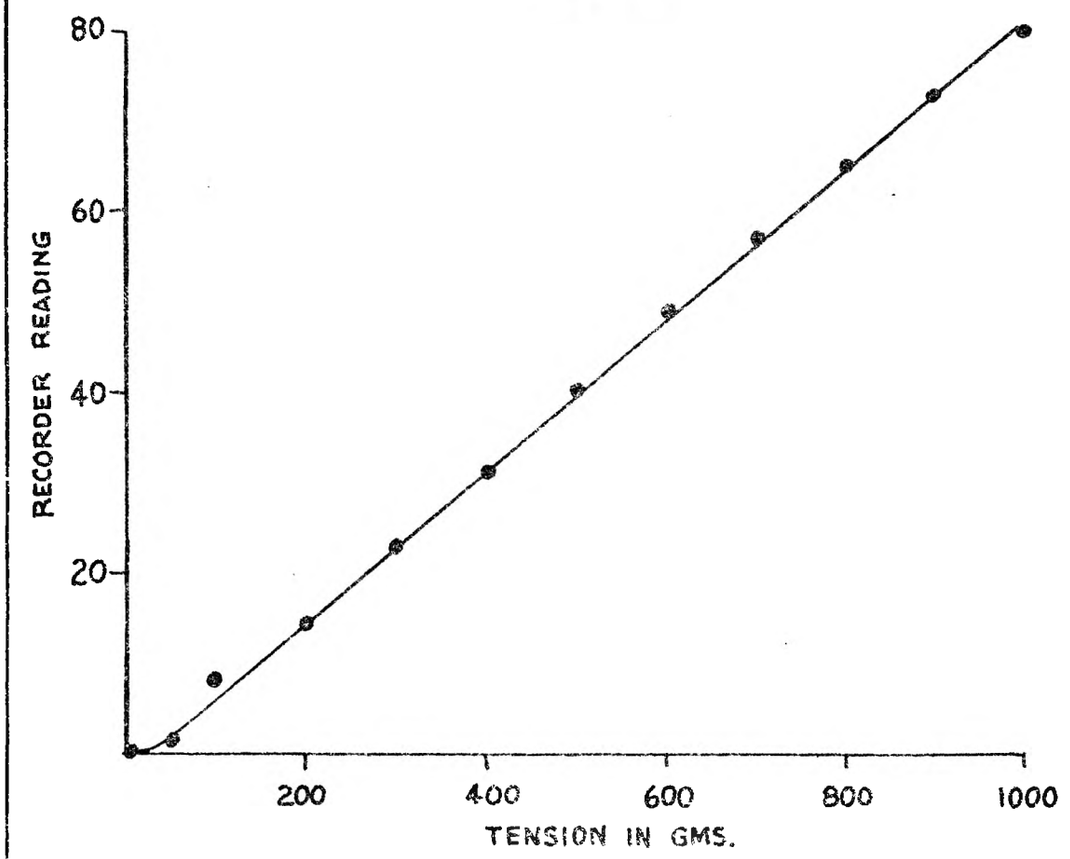
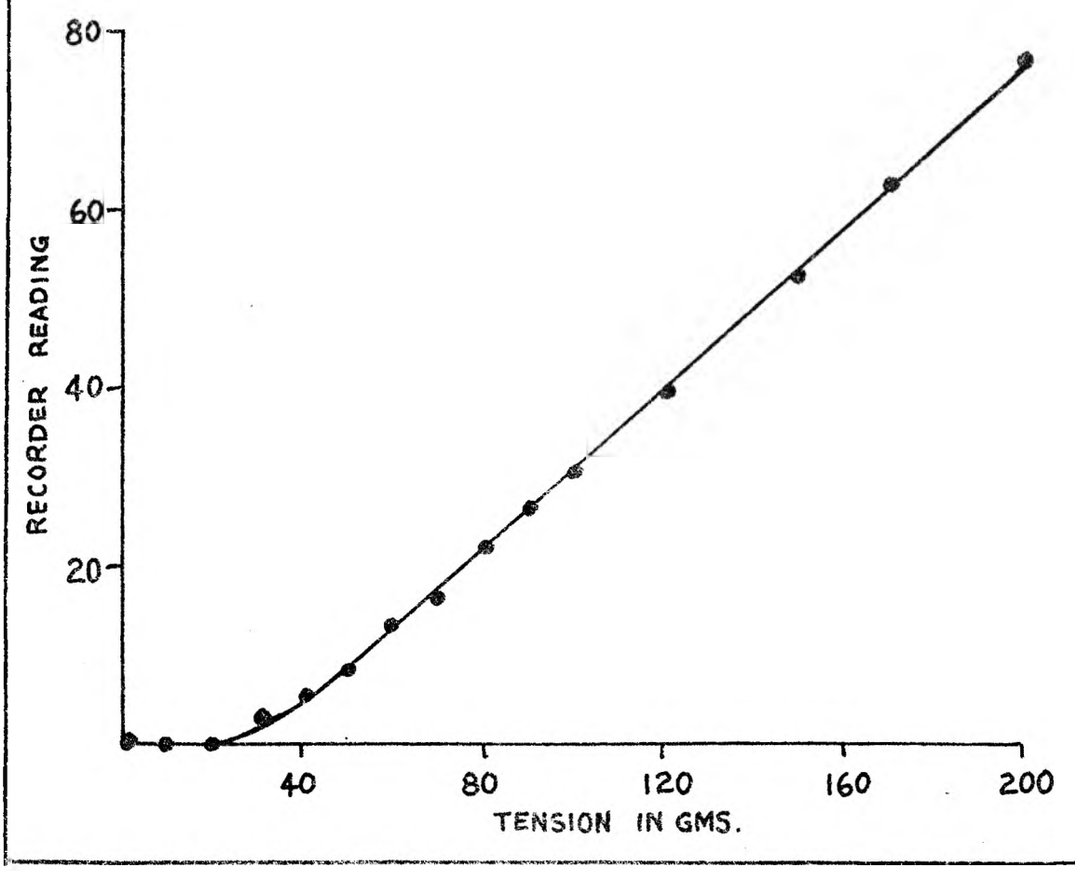


Fig. 35


80
60
40
20
RECORDER READING
200
400
600
800
1000
TENSION IN GMS.


80
60
40
20
RECORDER READING
40
80
120
160
200
TENSION IN GMS.

STANDARDISATION OF APPARATUS.

Calibration of Stimulator.

Since the Grass model S4-B used in the previous sections was not sufficiently accurate on calibration with an oscilloscope, a neuroton 510 Stimulator was used in the subsequent sections. It proved generally accurate to within 5% of its dial settings and a satisfactory square wave impulse of varying frequency, strength and duration could be produced.

Calibration of displacement transducer. (Fig. 34).

The displacement transducer was capable of recording length changes of up to 12 mm. to within 0.1 mm. However, its output was not completely linear and so all readings were taken from a calibration curve constructed for this purpose.

Calibration of tension transducer. Fig. (35).

The two strain guages for isometric tension and isotonic load were identical and give a fairly linear output from 20 to 1000 gms. By altering the sensitivity of the recorder, any range between 20 - 1000 gms. could be selected.

The guages were calibrated by hanging weights from them and measuring their response. All readings were made off calibration curves.

Frequency response of apparatus. (see appendix).

The recording apparatus had a frequency response of 25 cycles per second. (3 d.b. point).

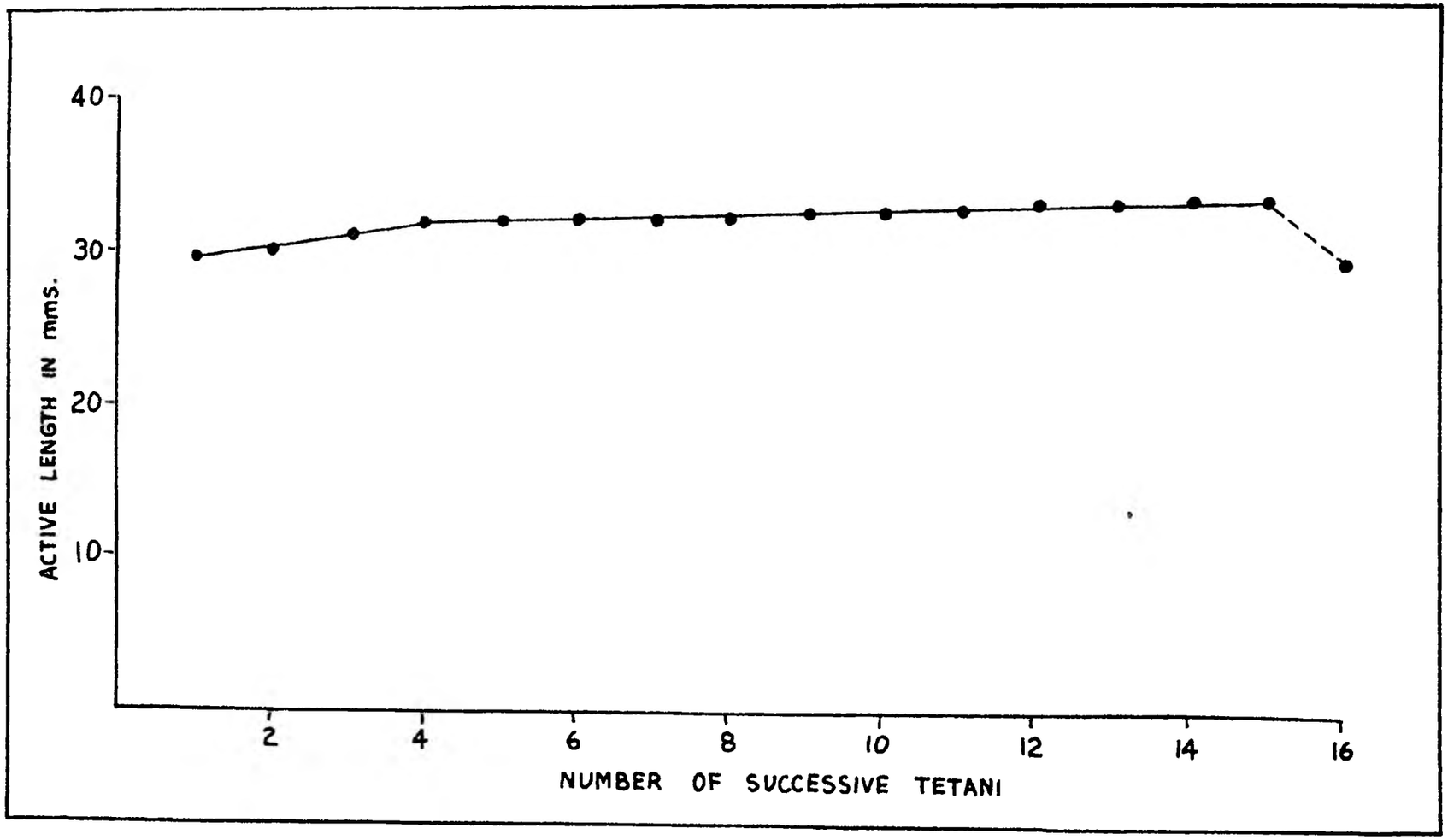


Fig. 36.
Reproducibility of isotonic tetani.
Note that 15 successive tetani 5 min. intervals show a small gradual change in the active length obtained after shortening and that this effect can be reversed after one hour's rest. (---)

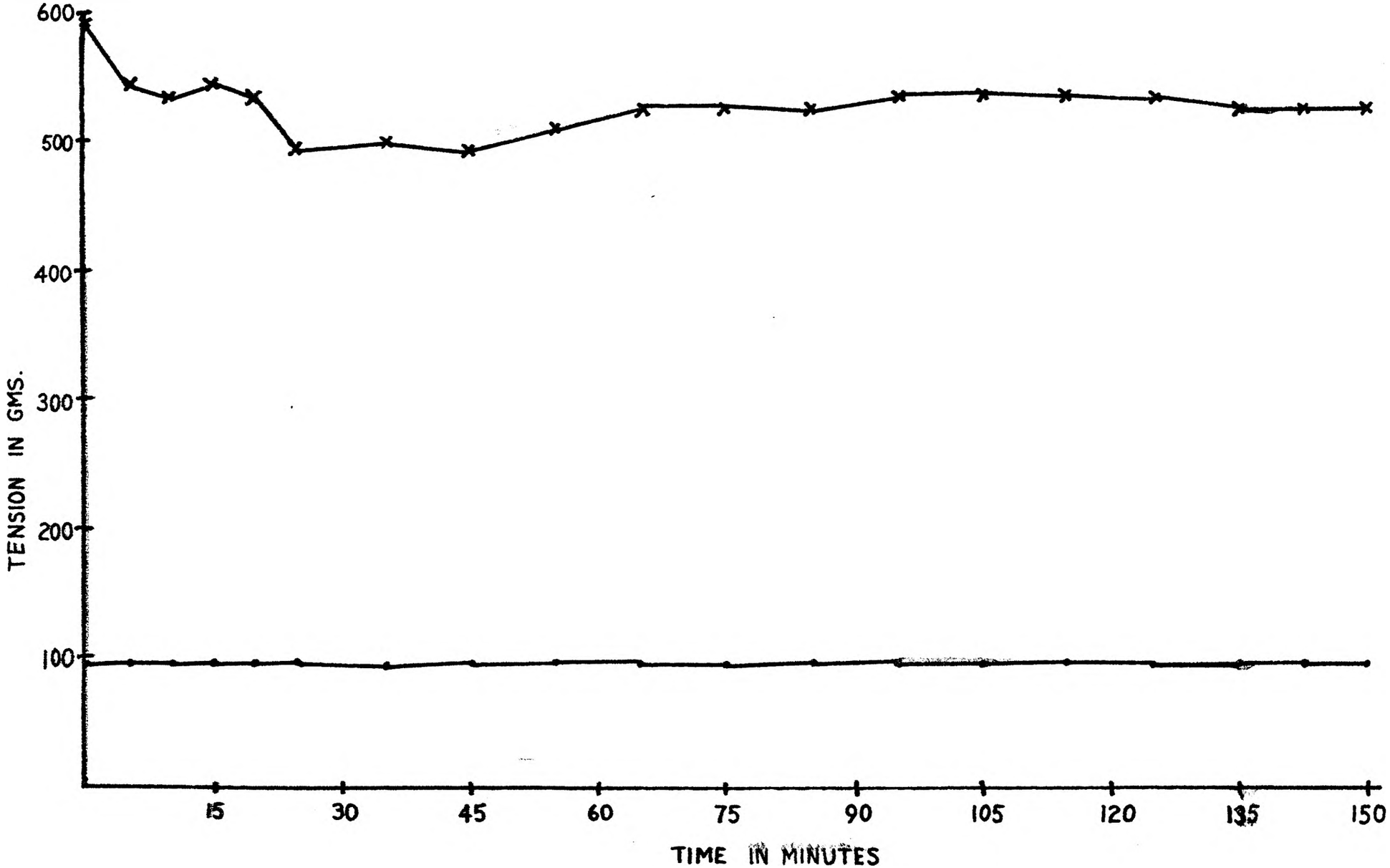


Fig 37. Reproducibility of isometric active tension (x) under constant passive tension (.) Note that with five minutes rest interval, as in the first six readings, the reproducibility is not as satisfactory as when ten minutes are allowed between tetani.

REPRODUCIBILITY OF RESPONSE.

The most satisfactory demonstration that adequate control had been achieved over all experimental variables was to establish that, given controlled conditions, the same response was evoked each time by an identical stimulus. Theoretically therefore, the only variables were time and previous activity.

The reproducibility of isotonic tetani was investigated in an experiment in which, under identical conditions, a series of fifteen successive tetani, each lasting six seconds with a five minute rest interval after each, was produced. From an initial shortening of 8 mm. there was a very slight steady decline of 0.2 mm. (average) per tetanus. This constitutes a 2.5% deterioration with time and previous activity, and proved completely reversible after one hour's rest. (Fig. 36).

The reproducibility of nineteen successive isometric tetani, of six seconds duration, at a passive tension of 95 gms. was investigated in an experiment in which the rest interval between successive tetani was either five minutes or ten minutes. It was found that the active tension produced in successive tetani was the same provided a ten minute interval was allowed between tetani. (Fig. 37 - see below)

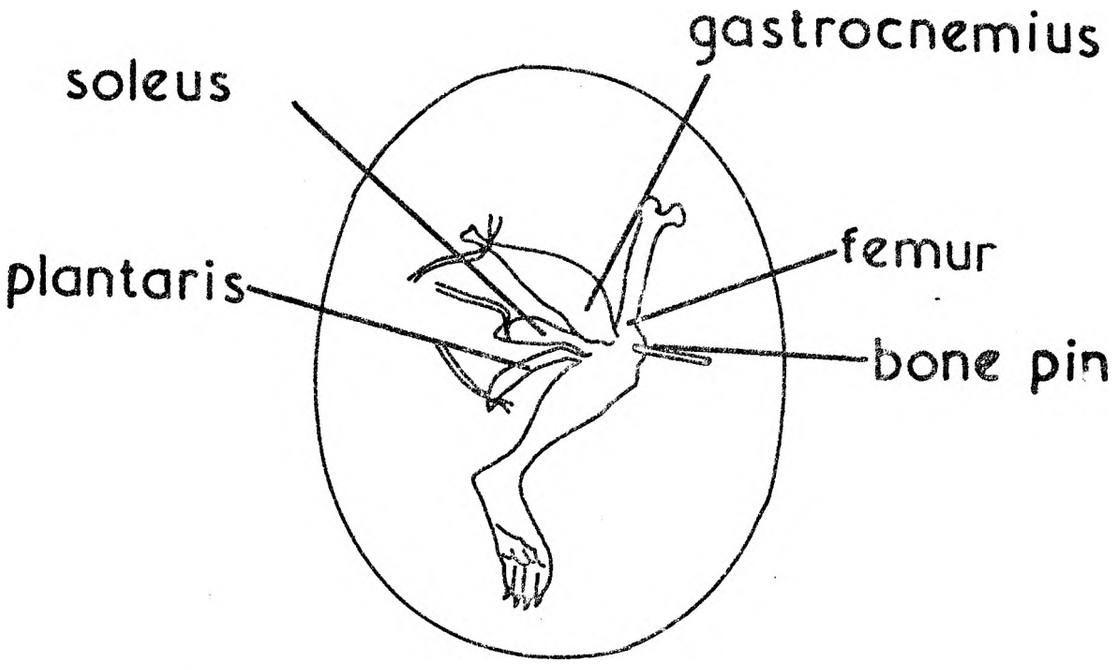
A standard rest interval of 10 minutes was therefore used between tetani of 6 seconds duration.

Fig 37. The Reproducibility of isometric tetani.

X = active tension
. = Passive tension.

Note that where 5 minutes are allowed between tetani as in the first six readings, the active tension produced is not satisfactorily reproducible for a given tension but when ten minutes are allowed between tetani, the active tension recovers to a steady and highly reproducible level.

Fig. 38



ANALYSIS OF THE COMPONENTS IN THE RESPONSE.

Objective.

In the previous section, it had been noted that the isotonic tetanic response to stimulation frequencies of about 30/second incorporated a number of fluctuations before a steady level was obtained. The pattern of the response was altered by the frequency of the stimulation, and by the load upon the muscles. The possibility of changes either in neuromuscular transmission, or of mechanical factors within the muscle and the apparatus being responsible for these fluctuations, has been considered, but on the evidence available hitherto neither could be eliminated. By examining this phenomenon with the new apparatus, in which friction and inertia had been reduced as much as possible, it was possible to assess the contribution of artefact due to recording and loading methods.

In addition, the previous section had dealt with the combined response of the three muscles constituting triceps surae. By dissecting the muscles apart and assessing their individual as well as combined contributions to the response, it was possible to determine whether the phenomenon was inherent in a single muscle, and, if so, which one. (Fig. 38).

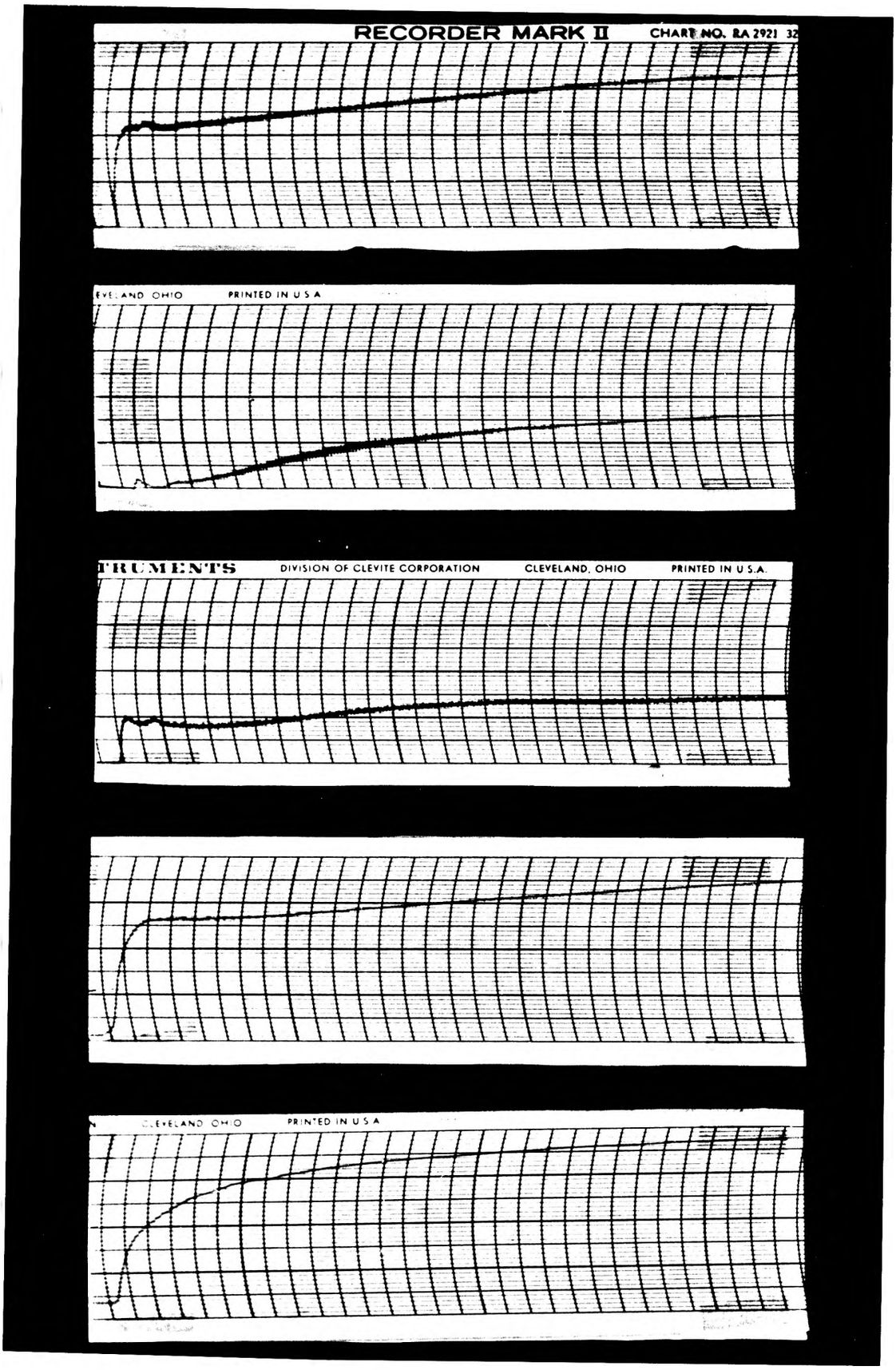


Fig. 39. Variations in the peaks of the tetanic response.

(a) triceps/surae, isotonic free load.

(b) triceps surae, isotonic after load.

(c) triceps surae, isometric.

(d) gastrocnemius, isotonic.

(e) soleus, isotonic.

Results. (Fig. 39)

(i) In nearly all records of triceps surae at a stimulation frequency of 30/sec. made on the electronic apparatus, fluctuations were noted in the initial response, but the peaks were generally less marked than with the mechanical apparatus and could often be resolved into two components - an initial rapid one, complete within 0.5 seconds followed by a steady and slower rise reaching a maximum within 6 seconds. There was usually, but not invariably, a dip between the two components of the curve. Occasionally a third peak would appear in place of the dip between the two components. Usually the most marked of these fluctuations were associated with a lack of fusion of the record.

(ii) The components were found in the isometric and after-loaded curves as well as the isotonic responses.

(iii) When the muscles are individually dissected off, it is found that soleus and plantaris both produce smooth curves whereas gastrocnemius alone still produces a fast and slow component.

DISCUSSION.

It is well known phenomenon that mammalian muscles have varying intrinsic speeds of contraction, a property which appears to stem from their motor innervation. (Buller, Eccles, & Eccles, 1960). As soleus is a slower muscle than gastrocnemius it might be expected that it would contribute more to the second component than the first. This was not found to be the case. Both soleus and plantaris alone produce smooth tetanic curves and therefore the fluctuations in contraction reported in the preceding sections could not have been due primarily to the contributions of these two muscles. From the results reported here it is apparent that there are two components in the gastrocnemius muscle of the rat, a fast and a slower one. Gastrocnemius consists of two bellies, a medial and a lateral with different dimensional and dynamic properties. (Thomson, 1961).

But these facts do not fully explain the two components found in the present graphs, for the time over which the peaks manifest is much larger than the reported differences in contraction time between the two bellies of the gastrocnemius muscle. Nevertheless, the double nature of gastrocnemius remains the most likely explanation. Acting together, as one contracts slightly quicker, it will relieve the other of the load, thereby increasing the latter's velocity according to the Hill (1938) force-velocity curve. In this way the muscle bellies could "hunt" against one another causing fluctuations in the response until a final plateau is achieved in a similar manner to the "hunting" phenomenon in single fibres contracting against a changing load reported by Podolsky (1963). The fact that the fluctuations were most marked when using the mechanical lever system could therefore be explained in terms of the inertia of such a system producing a changing load which in turn results in a changing velocity of shortening.

The inertia of the new apparatus is considerably less and the peaks, although present, are less accentuated. The role of neuromuscular transmission in the process has been discussed and cannot be eliminated as a contributory factor.

Therefore the most likely explanation for the fluctuations in the tetanic response is a mechanical one. The different contraction times of the muscle components modified by inertia of the apparatus could give rise to the phenomenon. The contribution of events at the neuromuscular junction is impossible to assess in the absence of recordings of electrical activity.

CONCLUDING DISCUSSION.

The advance in knowledge of the molecular mechanisms of muscle contraction coupled with the precise definition of the mechanical components in muscle have reinvoked interest in the mechanical properties of muscle. Recent studies on the ultrastructural basis of muscle contraction have emphasized that the terms isometric and isotonic do not imply different mechanisms of contractions. They merely denote the overall response of the whole muscle under certain conditions of recording. There is good experimental evidence that in isometric contraction some sarcomeres shorten, thereby stretching other sarcomeres or a series elastic component of undetermined anatomical location. Hence there is no change in muscle length.

(i) Hill (1953) found that in stretched muscle undergoing isometric contraction there is a redistribution of length in different regions of the muscle.

(ii) Hill and Howarth, (1957) inferred this from simultaneous measurements of heat production from several regions of a muscle undergoing isometric contraction.

(iii) Huxley A.F. & Peachey, (1961) showed by microscopy that in a stretched fibre undergoing isometric contraction "a high degree of contraction can occur at the ends of a fibre whose total length is held constant".

(iv) Huxley H.E. & Hanson, (1954) state that while the A and I bands do not change during isometric contraction, "a dark zone appears in the centre of the A band, as in isotonic contraction, as though some translation of material were taking place, presumably accompanied by stretch of a series elastic component".

(v) Podolsky (1961), states that in isometric contraction "the contractile mechanism shortens against the series compliance of the myofilaments, the tendons, the recording apparatus etc."

Abbot & Wilkie (1958), working on isolate frog muscle found that the tension in active muscle for a given length is the same irrespective of whether that active length was arrived at by means of an isotonic or an isometric contraction. Rosenbleuth et al (1958a), on the other hand, using a circulated cat muscle preparation, found that for a given length the tension developed isometrically is significantly greater than that developed isotonically. They also found that the tension developed increased with increasing initial length until the physiological range had been exceeded. The tension declined only when the muscle was irreversibly overstretched . This work conflicts with the concept of an optimum length within the body range established by such workers as Ramsey & Street (1940), Hill (1925), Abbot & Wilkie (1953), Deleze (1961). However, even amongst these workers there is dispute as to where the optimum falls in the body range.

In general, reviews have tended to dismiss the lack of satisfactory experimental verification of the equivalence of the length-tension diagram as being due to experimental error. (Zierler, 1961). This dissertation constituted an attempt to dissect out some of the sources of error inherent in the experimental situation. Particular attention has been given to the control of three categories of variables; those concerned with the stimulus, those affecting the performance of circulated muscle and those arising out of the deficiencies and limitations of the recording system.

Therefore in summary:-

(i) Stimulus parameters were investigated in order to select both a stimulus frequency, which would overcome the objections to the use of twitches and yet would not induce undue fatigue, and a strength duration combination which would elicit maximal and reproducible results. The parameters of 30 stimuli per second, 0.4 msec. in duration and 3 to 6 volts in strength were established as suitable for the preparation.

(ii) The effect of occlusion of the circulation on the performance and temperature of the muscle was noted. Care was subsequently taken not to interfere with the circulation during the experiment and the body temperature was carefully regulated.

(iii) The effect of loading on the amplitude of contraction and the work output of muscle was noted and the results critically examined in terms of the recording methods used.

(iv) An analysis was also attempted of certain fluctuations in the initial tetanic response at a stimulus frequency of 30/sec.

None of the experiments described constituted a thorough and complete analysis of a problem. Their purpose was rather to provide information about the preparation's parameters which could be used in the design and development of a satisfactory technique for investigating some properties of circulated rat muscle. This was finally achieved in the design, construction, standardisation and calibration of the new electronic apparatus described here.

Using this apparatus, the following was accomplished.

(i) The reproducibility of isometric and isotonic tetani under standard conditions was assessed and found to be satisfactory for the purposes of constructing a length-tension diagram.

(ii) The fluctuations in the tetanic response under isometric and isotonic conditions were examined in gastrocnemius, soleus, and plantaris muscles.

Apart from investigating the mechanical properties of muscle, another aspect of the technique evolved is its use as a neuromuscular preparation for the investigation of the pharmcology of the circulated neuromuscular junction, where factors affecting contraction other than the drug itself can be controlled. Some preliminary studies of a neuromuscular blocking agent have already been undertaken using the technique developed.

Finally the preparation could be used for neurological studies, particularly quantitative investigations on reflexly produced muscle contraction. Matthews (1959 a & b), has produced some excellent papers on the stretch reflex, relating the extension of the muscle to the reflex tension produced. He concludes that "quantitative analysis of the stretch reflex must await further quantitative studies on the behaviour of various parts of the reflex arc in isolation". It should be possible to do this with the technique described. It may even be desirable to repeat some of the stretch reflex experiments using the rat, for just as the frog has been an "ancient martyr" to muscle physiologists, so has the cat been used almost exclusively to study the properties of the stretch reflex.

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BIBLIOGRAPHY.

Aubert X., Roquet M.L., Van Der Elst J. (1951)
Tension-Length Diagram of Frogs Sartorius Muscle.
Arch. Internat. Physiol. 59: 239- 241.

Barcroft H., Millen J.L.E. (1939)
The Blood Flow Through Muscle During Sustained Contraction.
J. Physiol. 97: 11- 31.

Bulbring E., Burn J.H. (1939)
Blood Flow During Muscle Contraction and the Orbel Phenomenon in the Dog.
J. Physiol. 95: 203- 225.

Carlsen F., Knappeis G.G., Buchtal F, (1961).
Ultrastructure of the Resting and Contracted Striated Muscle Fibre at Different Degrees of Stretch.
J. Biophys. Biochem. Cytol. 11 95- 117.

Deleze J.B. (1961)
The Mechanical Properties of the Semitendinous Muscle at Lengths Greater than its Length in the Body.
J. Physiol. 158: 154-164.

Del Pozo E.L. (1942).
Transmission Fatigue and Contraction Fatigue.
Am. J. Physiol. 158: 154- 164.

Del Pozo E.L. (1942)
Transmission Fatigue and Contraction Fatigue.
Am. J. Physiol. 135: 763-771.

Fenn W.O. (1923)
Quantitative Comparison Between Energy Liberated and Work Performed by the Isolated Sartorius Muscle of the Frog.
J. Physiol. 58: 175.

Fenn W.O. (1945)
in "Physical Chemistry of Cells." . Ed. R. Hober, Blakiston Co.

Gasser H. S. (1930)
Contractures of Skeletal Muscle.
Physiol. Rev. 10: 35- 109

Gjone E. (1955)
The Reaction of Striated Mammalian Muscle to Variations of the Parameters of Electrical Stimuli applied to nerve and muscle Investigated with special Regard to Experimental Technique.
Acta. Physiol. Scandinav. 34(4): 311- 328.

Graham H.T. Lorente De No R. (1938)
Recovery of Blood-Perfused Mammalian Nerves.
Am. J. Physiol. 123: 326-340.

Hanson J. (1956)
Studies on the Cross-Striation of Indirect Flight Myofibrils of the Blow-Fly Calliphora.
J. Biophys. Biochem. Cytol. 2 (6): 691 -

Hemingway A. (1956)
in "The Control of the Circulation of the Blood". Ed McDowall.
Wm. Dawson & Sons. Ltd.

Hill A.V. (1938)
The Heat of shortening and the Dynamic constants of Muscle.
Proc. Roy. Soc. B. 126: 136- 195.

Hill A.V. (1925)
The Length of Muscle, and the Heat and Tension Developed in an Isometric Contraction.
J. Physiol. 60 (4) 237-268

Hill A.V. (1949)
Work and Heat in muscle twitch.
Proc. Roy. Soc., London, B. 136 :220 - 228

Hill A.V. (1956)
Thermodynamics of Muscle.
Brit. M. Bull. 12 :174 - 176

Hilton S.M. (1959)
A Peripheral Arterial Conducting Mechanism Underlying Dilatation of the Femoral Artery and concerned in Functional Vaso-dilatation in Skeletal Muscle. J. Physiol. 149 : 93 -111

Hiroven L., Sonnenschein R.R. (1962)
The Relation between Blood flow and Contraction Force in Active Skeletal Muscle.
Circulation Research. 10 : 94 103

Hoffman F. B. (1903).
Studien Uber Den Tetanus.
Pfluger Archiv. Fur Physiologie. 93 : 186 - 238

Hubbard J., Schmidt R.F. (1963)
An Electrophysiological Investigation of Mammalian Motor Nerve Terminals.
J. Physiol. 166 : 145

Humphreys P. W., Lind A. R. (1963)
The Blood Flow Through Active and Inactive Muscles of the Forearm during sustained Handgrip Contractions.
J. Physiol. 166 : 120 - 135

Huxley H.E., Hanson J. (1954)
Changes in Cross-Striations of Muscle during Contraction and Stretch and their Structural Interpretation.
Nature, London. 176 : 973 -976

Huxley A.F. Gordon A.M. (1962).
Striation Patterns in Active and Passive Shortening of Muscle.
Nature, London. 193 : 280: 1

Huxley A.F., Niedergerke R. (1954)
Structural Changes in Muscle During Contraction; Interference Microscopy of Living Muscle Fibres.
Nature, London. 173 : 971 - 973.

Jewell B.R., Wilkie D.R. (1958)
An Analysis of the Mechanical Components in Frog's Striated Muscle.
J. Physiol. 143 : 515 -540

Krnjewic K., Miledi R. (1958)
Failure of N euromuscular Propogation in Rats.
J. Physiol. 140 (3) : 440-461

Locke S. (1963)
The Response of the Motor Unit of the Rat to Paired Stimuli.
Am. J. Physiol. 204 (1) : 81-84

Merton P.A. (1954)
Interaction between Muscles Fibres in Twitch.
J. Physiol. 124 : 311 -324

Naess K., Storm-Mathisen A. (1955)
Fatigue of Sustained Tetanic Contractions.
Acta Phys. Scandinav. 34 (4) : 351 -366

Parsons F.G. (1894)
On the Morphology of the Tendino-Achilles.
J . Anat. and Phys. 28 : 414 -418

Podolsky R.J. (1963)
Isotonic Velocity Transients in Muscle and their Relation to the Contraction Mechanism.
Fed. Proc. 19 (1) : 258.

Ralston H.J. Inman V.J. Strait L.A, Shaffrath M.D. (1953)
Mechanics of Human isolated Voluntary Muscle.
Am. J. Physiol. 151 (2) : 612-620.

Ramsey R.W. , Street S.F. (1940)
Isometric Length-Tension Diagram of Isolated Skeletal Muscle Fibres of Frog.
J. Cell. & Comp. Physiol. 15: 11 - 34.

Rosenbleuth A., Alanis J., Rubio R., (1958a)
A comparative study of the Isometric & Isotonic Contractions of Striated Muscles.
Arch. Internat. Physiol. Biochem. 66: 330 -353

Rosenbleuth A. Alanis J., Rubio R. (1958b)
The Influence of initial Load and Length on Amplitude of Muscular Contractions.
Arch. Internat. Physiol. Biochem. 66: 354 - 372.

Rosenbleuth A., Cannon W.B. (1940)
Some features of early Stages of Neuromuscular Transmission.
Am. J. Physiol. 130: 205 - 218.

Rosenbleuth A., Luco J.V. (1937).
Study of Denervated Mammalian Skeletal Muscle.
Am. J. Physiol. 120: 781 - 797.

Rosenbleuth A. Morison R.S. (1937).
Electric Responses of Facial Muscles.
Am. J. Physiol. 120: 384 - 391.

Schottelius B.A., Senay L.C. (1957)
The effect of Ischaemia on the Length-Tension Diagram of Mammalian Skeletal Muscle.
Am. J. Physiol. 188(1) : 113 - 117.

Sirnes T.B. (1954)
Some effects of Barbituric Acid Derivatives on Function of Mammalian Skeletal Muscle.
Acta Pharmacol. et Toxicol (Supp. i) 10: 1 -170.

Sjostrand F.S. (1962)
The connections between A and I Band Filaments in Striated Frog Muscle.
J. Ultrastructure Research. 7: 225 -246.

Sjostrand F.S. , Anderson - Cedergren E. (1957)
The Ultrastructure of Skeletal Muscle Myofilaments at various states of Shortening.
J. Ultrastructure Research . 1 : 74.

Sonnenblick E.H. Cottrell J.S. Spiro D. (1963)
The Ultrastructure Basis of Contraction in Heart Muscle.
Fed. Proc. 22 (2) : 228.

Spiro D. Cottrell J.S. Sonnenblick E. H. (1963)
Comparison of Cardiac & Skeletal Muscle Ultrastructure Relative to Length-Tension Mechanics.
Fed. Proc. 22(2): 194.

Stacy R. W. , Williams D.T. Worden R. E., McMorris R.O. (1955)
in "Essentials of Biological & Medical Physics".
McGraw-Hill Book Co.

Trimmer J.D. (1950).
in "Response of Physical Systems".
John Wiley & Sons. Inc.

Weber E. (1846)
Quoted by Weber H.H.

Thomson J.D. (1961).
Dimensional & Dynamic Features of Mammalian Gastrocnemius Muscle.
Am. J. Physiol. 200 951 - 4.

Weber H.H. (1958)
"Motility of Muscle and Cells".
Harv. Univ. Press.

Wedensky (1891)
Quoted by Hoffmann (1903).

Zierler K.L. (1962)
in "Medical Physiology". Ed. Bard. C.V. Mosby & Co.

MUSCLE TENSION APPARATUS

INTRODUCTION

This instrument was developed for measuring the contraction of rat muscle under both isometric and isotonic conditions. Isometric means a negligible external shortening with tension development; isotonic means shortening under a constant load. The design is partly based on the original work of A. Rosenblueth, J. Alanis and R. Rubio[1].

The instrument requires an adjustable table upon which the rat is attached. The table has a built-in heater for warming the rat. A fine chord is tied to the muscle and to one of two transducers for measuring either the isotonic or isometric characteristics. The method is indicated in Figure 1.

(a) Isotonic Measurement

This requires a constant tension and variable displacement. The chord from the muscle is attached to a very long (3 M) elastic band so that when the muscle contracts by its maximum amount of one centimeter, the change in tension is relatively small. The tension produced by the elastic band is measured by a strain gauge stuck to the cantilever arm which supports the far end of the elastic band. The tension may be adjusted by sliding the cantilever up and down or adding additional elastic bands.

A small differential transformer is used for measuring the displacement. The core of the transformer is a small ferrox-cube rod (25 mm. x 4 mm. x 4 mm.) which is attached between the chord and the elastic band and passes through the transformer. A small displacement of this rod causes an out of balance state in the transformer and therefore an output emf. This transformer is capable of detecting a maximum displacement of 12 mm. Both ends of the rod are attached to short lengths of light metal tubing which run between small rollers so as to align the core correctly. It is important that the whole measuring system be as light as possible and have a minimum amount of friction.

The tension strain gauge and the displacement transformer are connected via amplifiers to a twin channel high speed recorder. One channel monitors the tension while the other records the displacement.

(b) Isometric Measurement

This requires the measurement of tension with negligible displacement. The muscle is connected by a short chord to a cantilever which has a strain

/gauge ....

gauge attached to it. There is only a small deflection of this cantilever (<0.5 mm.) for the full tension scale of 1,000 grams. The strain gauge is also connected to an amplifier and the recorder.

Constructional Details

The Figure 2 illustrates a block diagram of the instrument.

Each cantilever has two 600 ohm strain gauges, one in tension and the other in compression. The total of four are connected up as a Wheatstone bridge. Since only one cantilever is used at a time, it is useful to have one circuit for both isotonic and isometric tension measurements. The cantilevers and strain gauges are almost identical so the two tension measurements may be made very similar. An external variable resistor shunts one of the arms of the bridge to provide a nul balance. This is marked 'Zero Tens'. Since this is an A.C. bridge a capacitor trimmer is also required.

An oscillator (No. 1) supplies an alternating emf. of about nine volts to the bridge. This may be adjusted by a potentiometer marked 'Set Tens'. The frequency of the oscillator is about 500 c.p.s. A second oscillator supplies a similar emf. to the differential transformer (or displacement coil). The potentiometer marked 'Set Displ' adjusts the emf. to the transformer.

Two similar amplifiers are used for detecting the out of balance signal of the bridge or differential transformer. (The latter has a lower amplification). Before making a reading it is necessary to check the sensitivity of the bridge and amplifier, or transformer and amplifier 2. The first position of the switch S is marked 'Set Tension'. Part of the emf. supplied to the bridge is tapped off by the potentiometer marked 'Preset Calib' and applied via the switch to the amplifier. Similarly for the differential transformer in position 2 of the switch. By adjusting the oscillator supply for a given output of the amplifier, both bridge and amplifier sensitivity are adjusted or checked. The initial calibration is done by the preset calibration potentiometer. The positions 3 and 4 connect the amplifiers to the bridge and transformer for measurement. In position 3 the indicator is connected to the tension amplifier and in position 4 to the displacement amplifier. A twin channel recorder is connected to both amplifiers. The full scale is approximately two volts.

Note The two potentiometers preceding the amplifiers are merely a convenience for adjusting the gains of the amplifiers so as to obtain a suitable scale when calibrating. They appear as

/preset ....

preset controls on the front panel of the instrument and are incorrectly marked as 'Cal. Tens.' and 'Cal. Displ.'. The actual preset calibrating potentiometers are only accessible when the instrument is removed from the box by four screws.

(a) Bridge Oscillators

The circuit is given in Fig. 3. The first three transistors form the amplifier for a Wien Bridge oscillator. The output applied to the power amplifier is adjusted by the potentiometer marked 'Set Tens' or 'Set Displ'. The power amplifier is a pair of OC84 transistors operating under class B conditions. The 15K thermistor is used to stabilize the oscillator amplitude.

This circuit is taken from a Mullard publication of transistor circuits.

(b) Amplifiers

The circuit is given in Fig. 4.

The amplifiers are identical, with the exception of one resistor. This change is to reduce the gain of the displacement amplifier. The first four transistors form a D.C. amplifier with overall D.C. feedback for stability. The circuit is described by Chaplin and Owens[2]. The fifth transistor is to provide a low impedance output for the filter. A simple RC filter is used whose time constant is chosen to suit the desired time response of the apparatus. Assuming a high load impedance, then the transfer function of the filter is given by

$$2R\left(1 + s\,\frac{RC}{2}\right)$$

where the time constant $T = \frac{RC}{2} = \frac{10^{-3}}{2}$

$\therefore$ the cut off frequency $w_o = 1/T = 500$ rads./sec.

$$\text{or } f_o = \frac{1}{2\Pi T} = 80 \text{ c.p.s.}$$

The combined characteristics of the recorder and filter, which determines the response of the system is illustrated in Fig. 6. This was achieved by amplitude modulating the bridge oscillator at different frequencies and recording the modulated output of the amplifier.

/(c) ....

(c) Power Supplies

There are two power supplies for a negative and positive supply with respect to earth. The circuit is illustrated in Fig. 5.

(d) Indicator Circuit

Since only a 0 - 1 milliampere meter was available at the time, an emmitter follower circuit was constructed to provide a high input impedance. This is illustrated in Fig. 5. The two diodes provide the necessary bias so that with a zero input potential wrt. earth will give a zero indication on the meter. The 25 K ohm rheostat is for fine zero adjustment. This control is immediately below the meter on the front panel.

REFERENCES

(1) Rosenblueth, A. and Alanis, J. : 'A Comparative Study of the Isometric and Isotonic Contractions of Striated Muscles'. Archives Internationales de Physiologie et de Biochemie, 1958, V. 66, No. 3, p. 330.

(2) Chaplin, G. and Owens, A. : 'A Transistor High Gain Chopper Type D.C. Amplifier'. Proceedings I.E.E. Paper No. 2442 M, November 1957, Vol. 105 B, p. 258.

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25th July, 1963

ARA/GRF

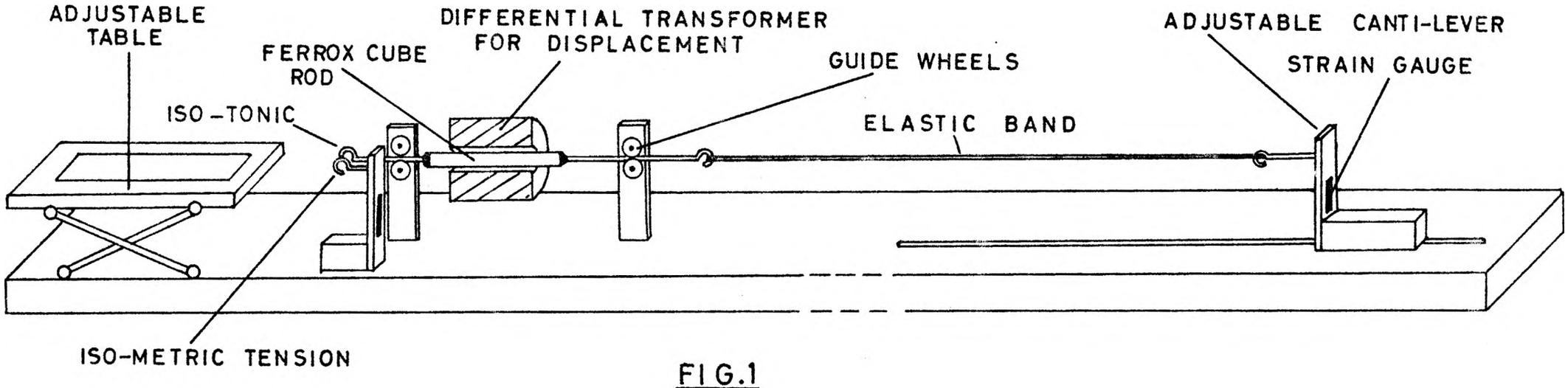


FIG.1
MUSCLE TENSION APPARATUS

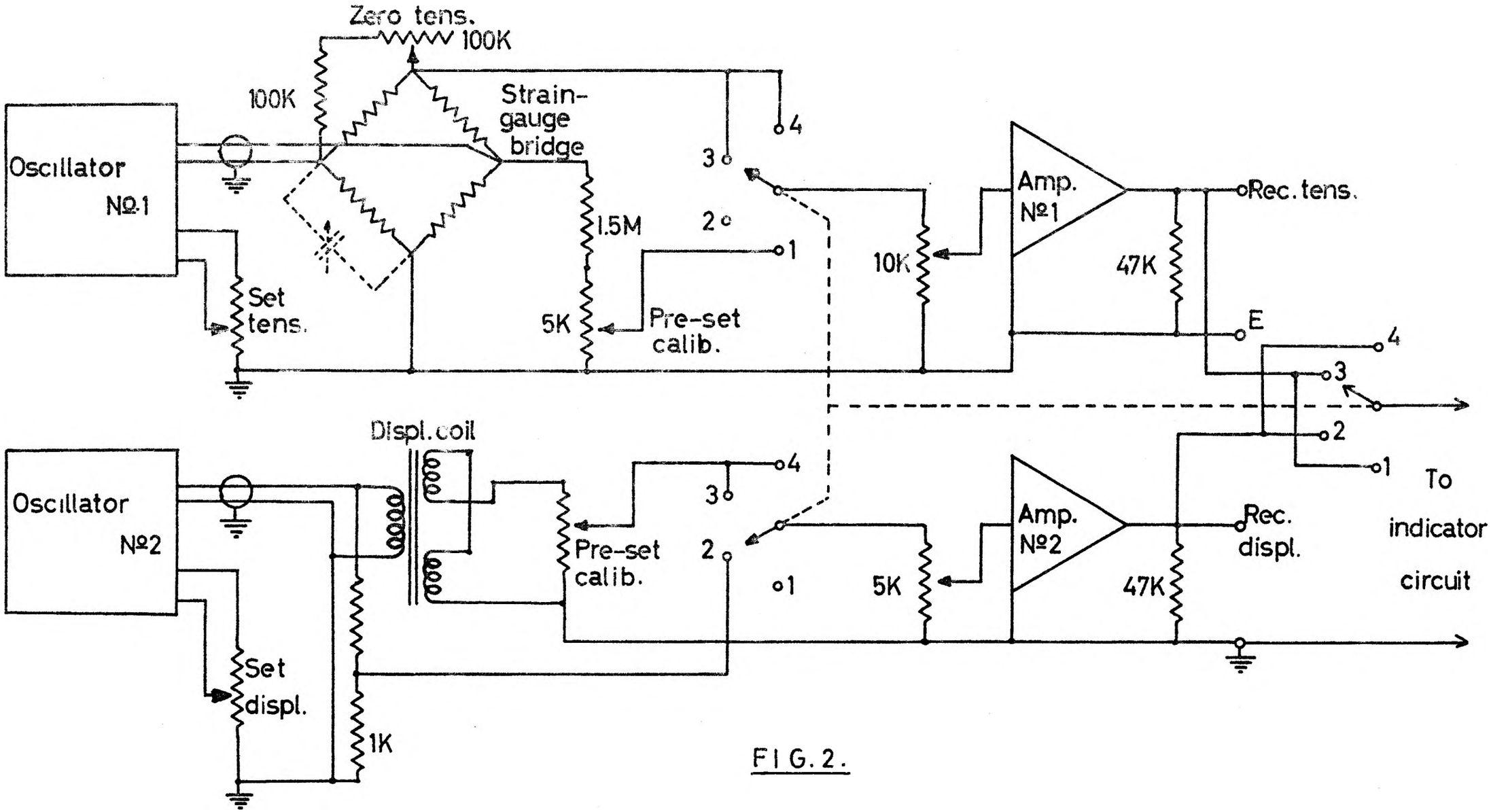

Zero tens.
100K
100K
Strain-
gauge
bridge
Oscillator
№1
Set
tens.
1.5M
5K
Pre-set
calib.
4
3
2
1
10K
Amp.
№1
47K
Rec. tens.
E
4
3
2
1
To
indicator
circuit
Displ. coil
Oscillator
№2
Set
displ.
1K
Pre-set
calib.
4
3
2
1
5K
Amp.
№2
47K
Rec.
displ.

FIG. 2.

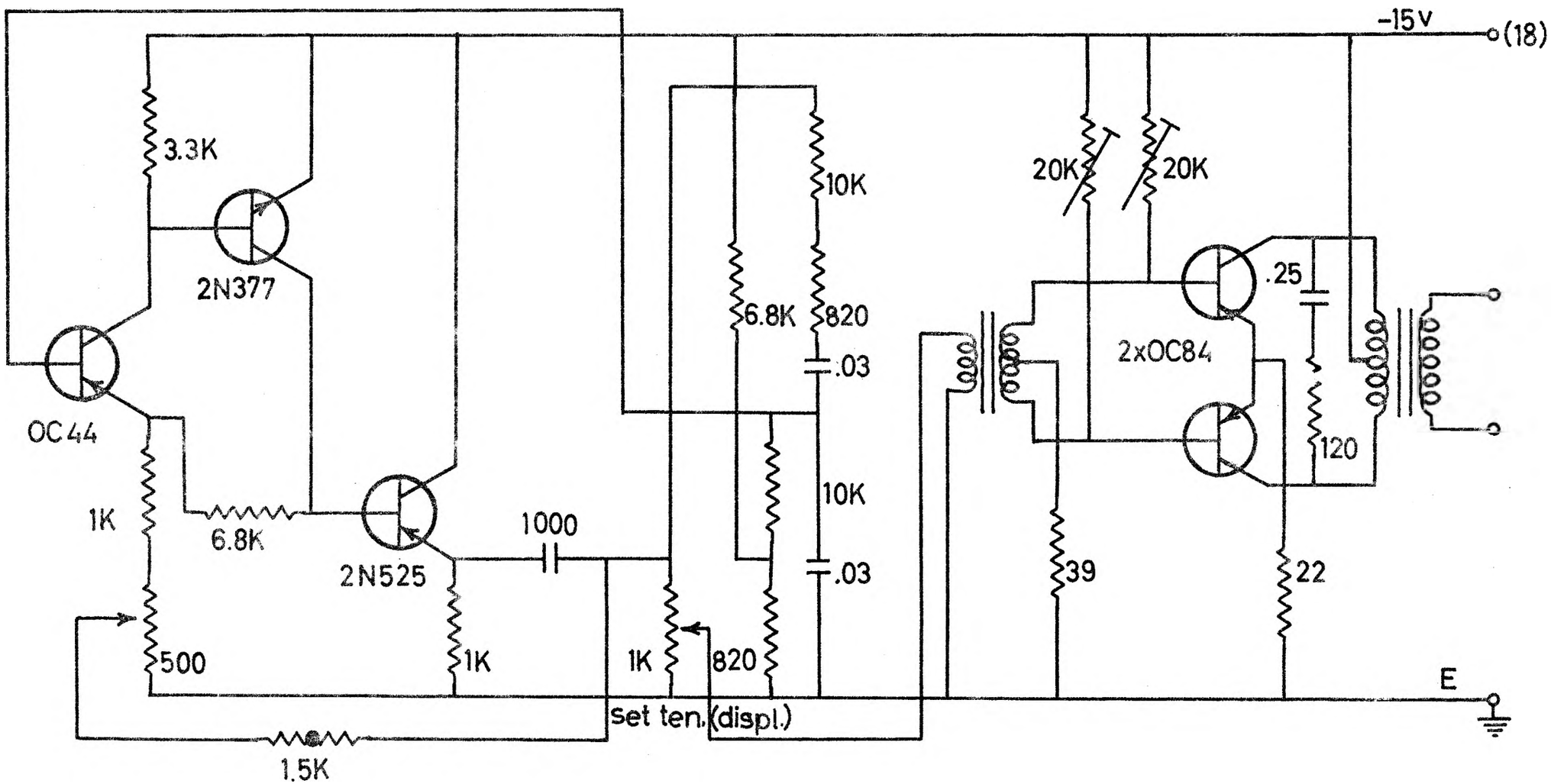


FIG.3.

BRIDGE OSCILLATOR (500cps)

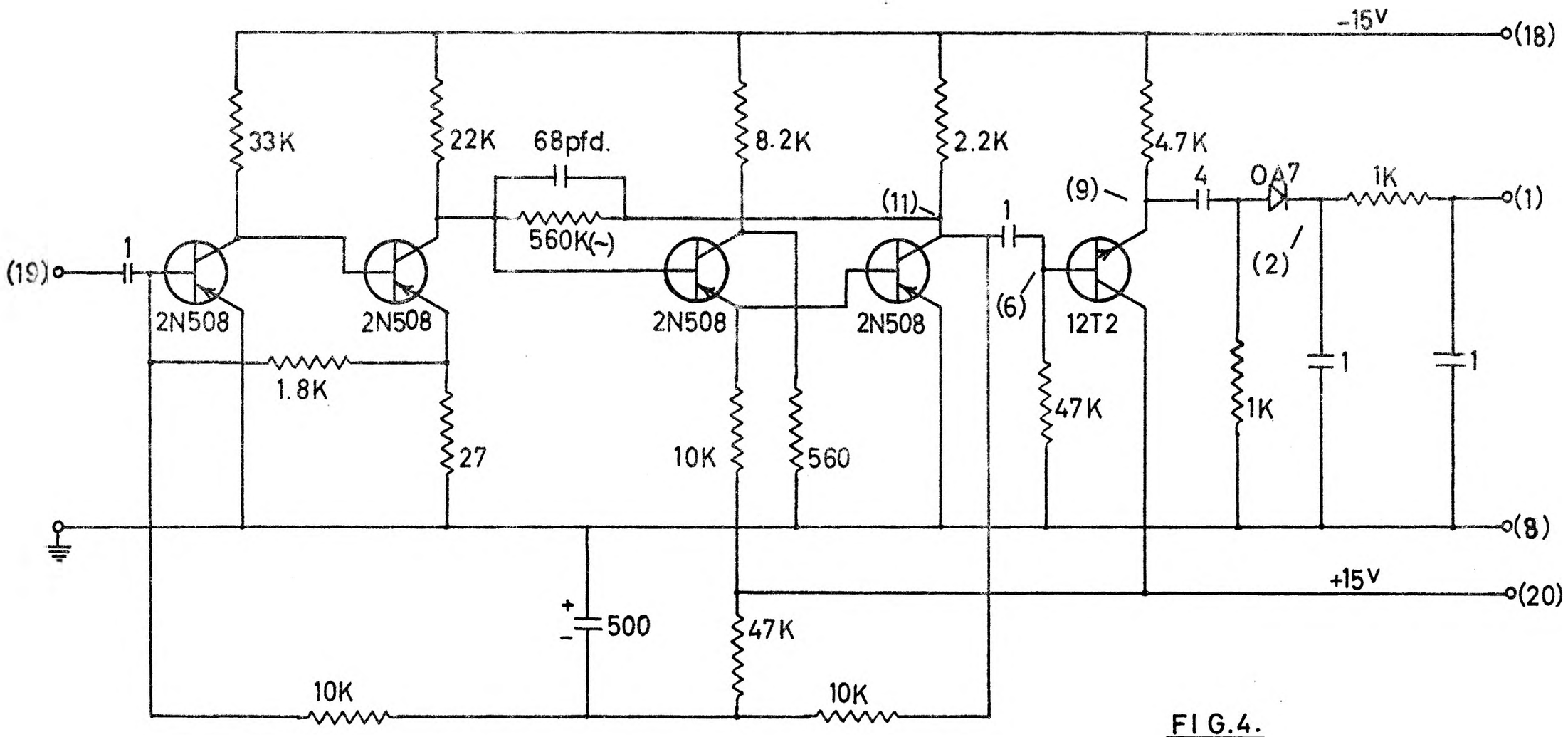
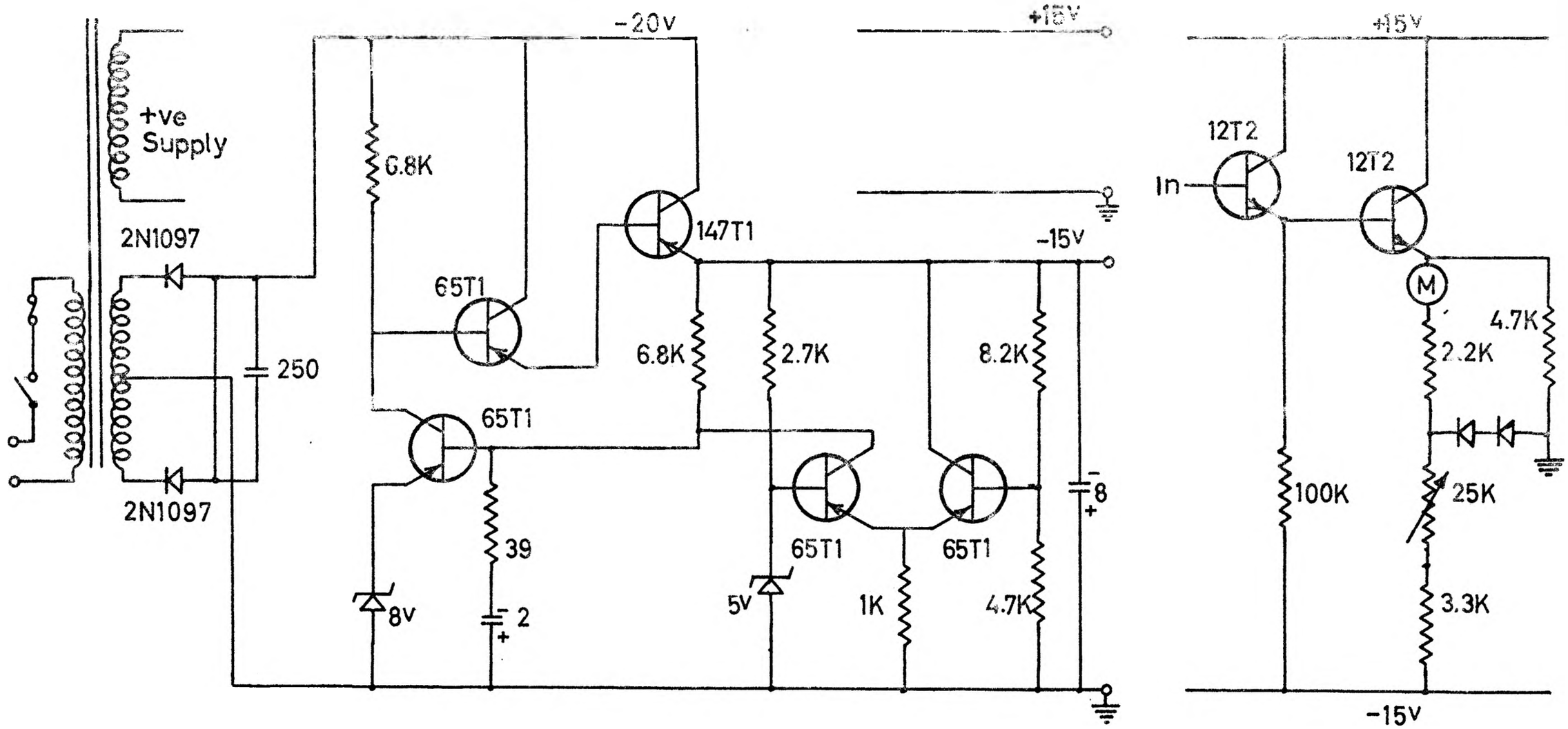


FIG.4.

A.C. AMPLIFIER (A = 4000)



POWER SUPPLY

FIG.5

INDICATOR

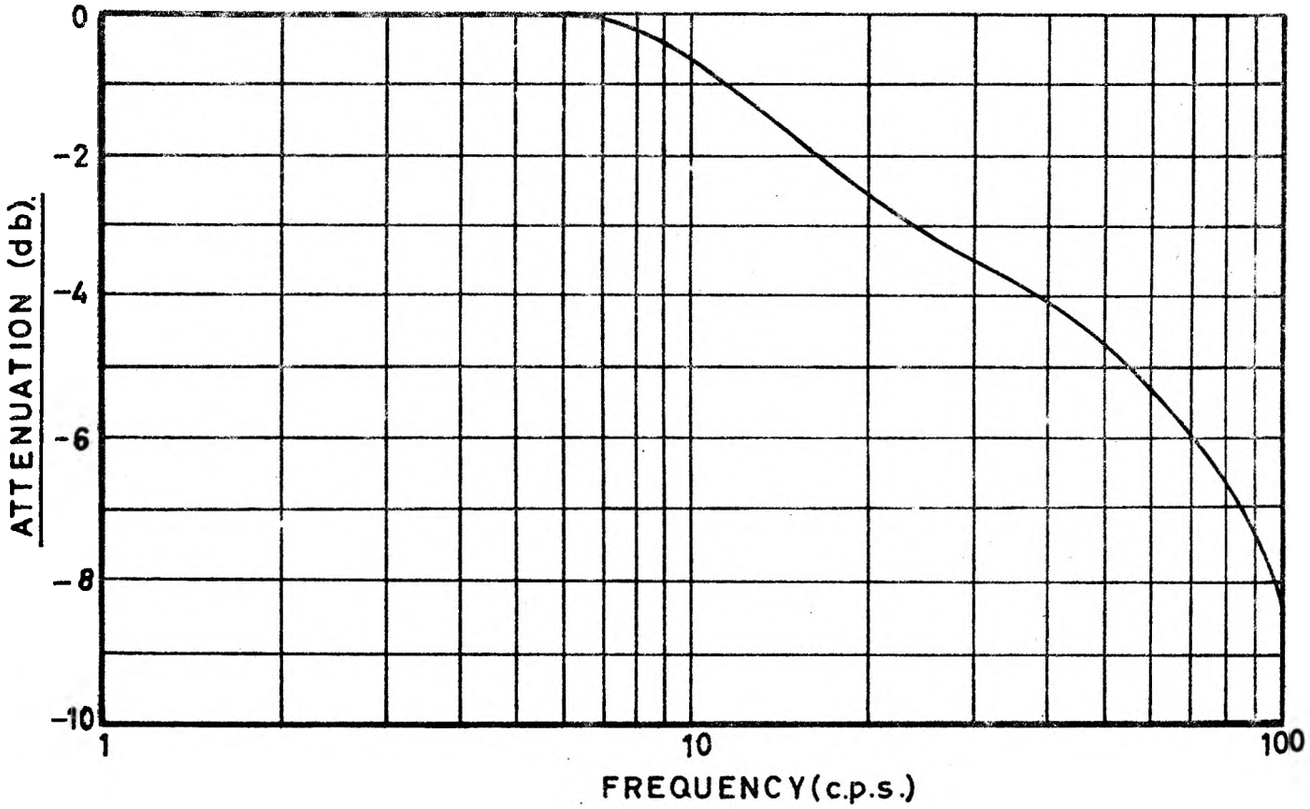


FIG. 6.

RESPONSE OF COMPLETE INSTRUMENT

APPENDIX

of

MEASUREMENTS OF

WORK OUTPUT OF

ISOTONIC TETANI.

(See Section II).

EXPT.	SUB-NO.	AMPLITUDES (in mm x 5) 1ST PEAK	2ND PEAK	3RD PEAK	STRETCH	(sq. cms.) AREA	(gms.) LOAD	(gm. cms.) WORK.
62	A	27	11		—	554	25	1385
	B	20	28		5	570	40	2280
	C	22	30		9	888	55	3230
	D	21	30		14	623	70	4361
	E	21	32		15	580	85	4940
	F	15	28		19	406	115	4640
63	A	15	24			465	25	1160
	B	—	3			90	115	104
	D	15	19			407	40	1630
	E	-	10			36	100	360
	F	6	16			118	55	650
	G	—	12			36	85	305
	H	3	15			76 ?	70	530
	I	17	26				25	
64	A	16	25			213	115	2450
	B	25	33			616	25	1540
	C	5	15			90	100	900
	D	13	19			311	85	2640
	E	17	22			328	70	
65.	A	23	39			827	25	2070
	B	24	33			686	25	1715
	C	17	25			496	40	1990
	D	16	23			468	40	1880
	E	11	18			297	55	1640
	F	10	16			226	55	1240
	G	6	10			117	70	840
	H	8	12			138	70	965
	I	6	10			127	85	1080
	J	2	5			37	100	370
	A	24	30		12	593	55	3240
	B	24	29		12	661	55	3640
	C	24	31		5	628	40	2540
	D	24	30		5	629	40	2530
	E	25	34		16	599	85	5100
	F	22	35		16	595	85	5050
	G	23	30		—	509	25	1270
	H	22	27		—	537	25	1340
	I	23	31		15	513	70	3600
	J	22	33		14	464	70	3250
	K	22	39		16	510	100	5100

Expt.	Sub-No.	Amplitudes. 1st Peak	2nd Peak	3rd Peak	Stretch	Area	Load	Work
66.	A	.7	31	–		531	70	3850
	B	18	28	–		485	70	3360
	C	.3	21	–		279	85	2370
	D	12	18	–		153	85	1300
	E	.7	21	22		455	55	2500
	F	.9	21	23		476	55	2620
	G	26	28	29		669	25	1450
	H	27	28	30		699	25	1430
	I	–	1	8		86	100	860
	J	1	1	9		89	100	890
	A	21	27	31	12	705	70	4930
	B	23	27	30	12	643	70	4500
	C	22	24	30	17	643	85	5720
	D	26	27	31	15	714	85	6080
	E	26	30	31	8	735	55	4050
	F	25	33	37	7	812	55	4460
	G	23	28	29	–	630	25	1580
	H	25	26	29	–	684	25	1720
	I	20	22	27	19	632	100	6320
	J	24	26	30	19	705	100	7050
	K	26	27	30	21	700	115	8050
	L	25	28	31	22	704	115	8150
67.	A	28	45	–			40	
	B	25	37	37			40	
	C	27	28	35			85	
	D	32	34	37			55	
	E	31	31	34			25	
	F	30	40	45			100	
68.	A	42	45	–			55	
	B	30	40	–			85	
	C	25	32	27			25	
	D	–	37	33			100	
	E	28	36	34			40	
	F	27	35	34			115	
	G	24	30	29			40	
69.	A	20	25	24		584	25	14675
	B	16	20	18		433	55	23815
	C	10	13	12		250	85	21250
	D	7	13	11		140	100	14000
	E	11	16	15		338	70	23660
	F	2	11	–		140	115	16100
	G	17	20	20		455	40	18200

EXPT.	SUB-NO.	AMPLITUDES				AREA	LOAD	WORK
		1ST PEAK	2ND PEAK	3RD PEAK	STRETCH			
'70.	A	28	43	—	20	945	100	9450
	B	29	36	35	14	833	70	5830
	C	25	36	40	28	880	140	12300
	D	23	34	40	39	939	215	15400
	E	26	34	38	24	802	125	10000
	F	27	30	36	—	416	25	1040
	G	25	28	31	21	777	100	7770
	H	28	30	36	14	739	70	5180
	I	33	31	34	28	825	140	11600
	J	17	26	38	?	680	215	14600
	K	11	28	33	40	526	230	12100
	L	3	23	30	54	200	232	6640
	M	9	28	—	52	368	272	10000
	N	6	24	26	63	186	390	7250
	O	5	24	—	61	140	300	6100
	P	21	31	30	—	615	25	1540
	Q	7	14	16	—	350	70	2450
	R	3	6	11	—	635	115	1560
	S	23	31	30	—	740	25	1860
'71.	A	32	51	—			100	
	B	10	26	21			100	
	C	24	43	—			215	
	D	2	—	—			215	
	E	21	28	—			25	
'73.	A	29	41	—	22	6865	115	76475
	B	—	30	—	—	628	125	15700
	C	—	19	—	—	139	115	15985
	D	—	—	—	—	—	248	
	E	16	35	—	52	268	278	74504
	F	19	26	25	5	648	55	26290
	G	19	28	22	—	500	55	24500
	H	—	—	—	—	—	355	
	I	12	23	—	48	106	355	37630
	J	18	28	28	11	405	85	34425
	K	13	19	18	—	245	85	23375
	L	20	25	24	—	525	25	13125
	M	10	19	20	—	220	200	44000
	N	—	—	—	—	—	200	
	O	9	20	—	34	100	278	27800
	P	—	—	—	—	—	278	
	Q	10	23	—	50	151	355	53605
	R	—	—	—	—	—	355	
	S	5	21	—	58	105	430	45150
	T	5	21	—	58	—	430	
	U	—	—	—	—	60	430	25800
	V	5	22	—	61	—	500	
	W	—	—	—	—	85	500	42500
	X	5	23	—	69	87	600	52200
	Y	—	—	—	—	—	600	
	Z	20	27	25	—	635	25	15875

EXPT.	SUB-NO.	AMPLITUDES. 1ST PEAK	2ND PEAK	3RD PEAK	STRETCH	AREA	LOAD	WORK
75.	A	20	34				100	
	B	20	32				100	
	C	20	24	26	—		25	
	D	20	24	25	23		115	
	E	14	23	24	3		55	
	F	18	21	23	10		85	
	G	14	22	24	8		90	
	H	14	19	22	18		115	
	A	14	18	18	—		25	
	B	10	13	12	—		25	
	C	18	20	20	—		25	
	A	12	—	14	—		25	
	B	15	16	14	—		25	
	C	18	20	18	—		25	
79.	A	13	29		14	525		5250
	B	15	32		34	500		10400
	C	14	26		43	346		9200
	D	14	24		14	351		4560
	E	10	25		56	198		5940
	F	8	21		56	142		5000
	G	18	23		9	278		1940
	H	18	21		—	345		865
80	A	1	32	—	21	645	100	6450
	B	14	41	—	42	735	208	15300
	C	13	22	22	45	428	265	11300
	D	22	22	25	—	615	25	1540
	E	8	16	—	55	200	350	7000
	F	5	14	—	62	93	436	4050
	G	16	18	24	18	420	130	5460
81.	A	32	42		30	625	208	3000
	B	25	29		?	594	100	5940
	C	23	34		40	340	265	9000
	D	23	24		—	530	25	1520
	E	12	27		40	185	350	6500
	F	14	21	25	14	300	130	3900