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THE EFFECT OF EXCESS VITAMIN A

ON

MUSCLE AND NERVE EXCITABILITY

by

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EXCESS VITAMIN A AND EXCITABILITY IN NERVE AND MUSCLE

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INTRODUCTION

Excitability is defined as the reciprocal of threshold for depolarization. Usually, it is stated in terms of a ratio of test excitability to excitability in a standard situation (Ruch and Fulton, 1959).

Neuromuscular irritability is dependent upon the concentration of ionic calcium as one of the factors in the relationship (Association for research in nervous and mental diseases, 1960):

$$\text{Irritability} \propto \frac{[K^+] + [Na^+]}{[Ca^{++}] + [Mg^{++}] + [H^+]}$$

So, diminution in the concentration of ionized calcium ion in the plasma results in increased "irritability" of motor nerves and muscles producing "tetany" (Association for research in nervous and mental diseases, 1960). Increase in calcium above 12mg/100ml, has an opposite effect and results in hypotonia and weakness, presumably due to decreased excitability of nerve and muscle.

More specifically, calcium modifies the excitation process by affecting the constrains imposed upon the movement of Na^+ and K^+, the principal carriers of the electric current across the cell membrane, which gives rise to the action current (Nickelson, 1960). The steady potential (E) across the cell membrane depends in large on the concentration and rate of flux of the three ions Na^+ , K^+

and Cl^- as we see in the equation:

$$E = \frac{RT}{F} \ln\left(\frac{P_{K^+}(K^+)_{in} + P_{Na^+}(Na^+)_{in} + P_{Cl^-}(Cl^-)_{out}}{P_{K^+}(K^+)_{out} + P_{Na^+}(Na^+)_{out} + P_{Cl^-}(Cl^-)_{in}} \right)$$ Giese, 1962a

where E is the steady potential across the cell membrane.

P_{K+} , P_{Na+} and P_{Cl-} refer to the permeability coefficients of K^+, Na^+ and Cl^- respectively.

R is the gas constant

T is the absolute temperature

F is the faraday (96,500 coulomb per gram equivalent).

The action current depends on both, the initial steady state and the movement of Na^+ and K^+ during excitation. The energy (W) required to move one mole of Na^+ from inside to outside of a two compartment system, and one mole of K^+ in the opposite direction is:

$$W = \frac{RT}{F} \left(\ln \frac{Na^+ \text{ out}}{Na^+ \text{ in}} + \ln \frac{K^+ \text{ in}}{K^+ \text{ out}} \right)$$ (Giese, 1962a)

So, it is clear now that Ca^{++} affects either the action potential or the energy required to produce it. It is found, however, that the rheobasic current decreases with a decrease in the Ca^{++} concentration in the exterior of the cell, (Nickelson, 1960).

It is worthwhile to recollect what Bailey stated in 1942. He wrote that "stimulation of the contractile material is connected with availability of activating Ca ions to the myosin adenosine triphosphatase fibrillar surface". "It is legitimate to assume", he continued, "that the living cell in the resting state can provide a mechanism for the separation of enzyme and activator, until they are brought together as the result of excitation." (Bailey, 1942).

This theory had at that time three evidences (Huxley, A.F., 1964):

1. Effect of Ca^{++} on the ATP-ase activity of myosin.
2. The need of calcium for excitation - contraction coupling of the heart.
3. The diffusion of muscle calcium after prolonged activity.

Recently, however, new evidences have accumulated in its favour. Some of these are:

1. Effect on separated fibrils (actomyosin system) (Giese, 1962a).
2. Uptake of calcium by components of the sarcoplasmic reticulum which contain the relaxing factor (Huxley, 1964).
3. Other studies on the whole muscle.

In the light of Bailey's theory and the new findings, Davies (1963) proposed the theory describing how the contractile forces arise. His theory proposes that the activation of muscle releases bound calcium (the author assumes that it should be the calcium which is bound with the relaxing factor) from the sarcolemma and sarcoplasmic reticulum. This calcium diffuses and forms chelating links between bound ATP at the end of an extended polypeptide of the cross bridges of the H-meromyosin and the bound ADP of the F-actin filaments. The calcium neutralizes the electric charge on the bound ATP of the polypeptide which spontaneously contracts to an α -helix by the energy of the hydrogen bond and hydrophobic bond formation. This contraction drags the actin filament along the myosin filament. This brings the ATP into the range of action of H-meromyosin ATP-ase, which cleaves off the terminal phosphate and breaks the link. On rephosphorylation of the ADP, the helix is pulled out to a largely extended chain by the repulsion of the negative charge on the ATP and a fixed charge on the H-meromyosin. This cycle is repeated during the active state, which ends when calcium is pumped back into the sarcolemma and sarcoplasmic reticulum.

Calcium and High Energy Bonds:-

The product of the concentration of calcium ions and phosphate ions is constant (Harper, 1961):

$$[PO_4^{\equiv}] \times [Ca^{++}] = 50$$

So, changes in the concentration of one of these ions are usually followed by inverse changes in the other. Most of the phosphate in muscles and nerves which sums up to 0.3% are found in the form of labile organic combinations, as nucleotides, phospholipids, phosphocreatine, and phosphorylated intermediates of metabolism (Mazia and Tyler, 1963).

The calcium ion functions as an uncoupling agent of phosphorylation from oxidation (Harper, 1961), possibly acting as an antagonist to magnesium ion, which is essential in oxidative phosphorylation.

The ATP-ase activity of both actomyosin and myosin is stimulated by Ca^{++} and inhibited by Mg^{++}, in contrast to the effects of these ions on the ATP-induced contraction of actomyosin. Contraction occurs only in the presence of K^{+} and Mg^{++} ions in concentrations similar to those in muscle cell fluids, and is inhibited by Ca^{++} ions (White et al, 1959). In media devoid of divalent cations but containing either K^{+} or $NH^{+}4$, hydrolysis of ATP is accelerated almost 100-fold (Harper, 1961).

Frog skeletal muscle uses approximately 10^{-4} moles of ATP/gram/minute when working maximally. There is only about 5×10^{-6} mole of ATP present per gram of resting muscle. This amount cannot meet the demands of the skeletal muscle for more than half a

second of intense activity (White et al, 1959).

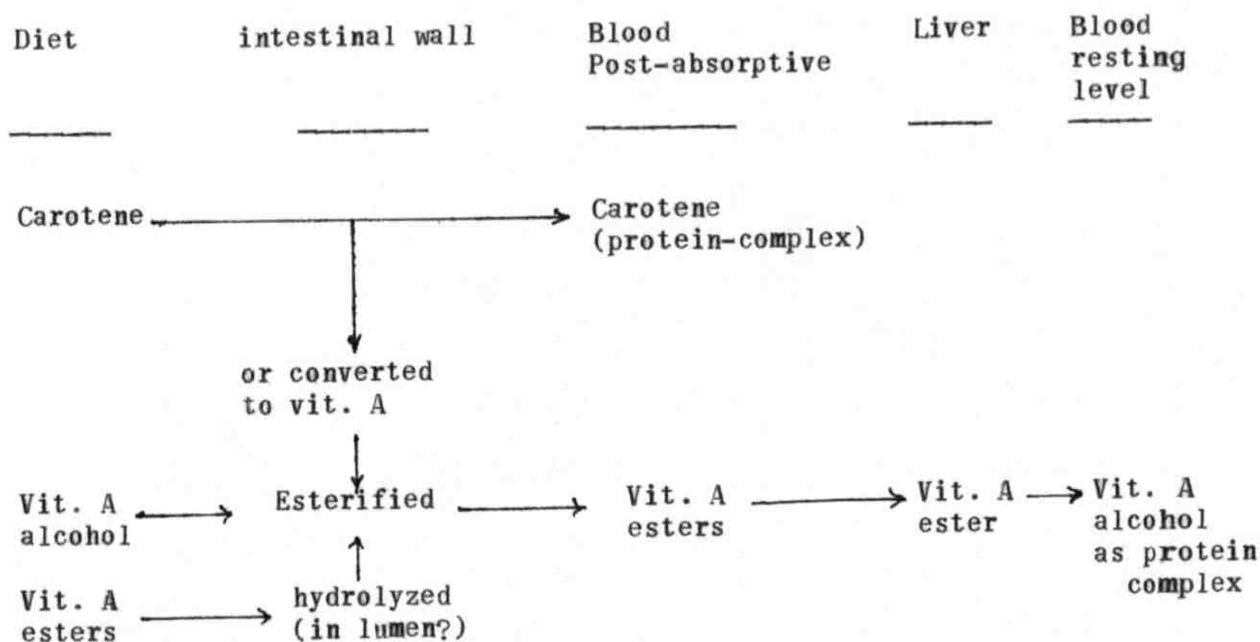
The Role of Vitamin A:-

Vitamin A is present only in animal material. It is mostly concentrated in the liver. Stores of this vitamin are generally greater in the female than in the male (Aykroyd, 1958). Strangely enough, kidney stores some of the vitamin and is greater in the male than in the female (Booth, 1952).

The complete picture of the mode of action of vitamin A in the retina was given by Wald (1958) and Morton (Moore, 1957). Thus we know that this vitamin is reversibly oxidized by an enzyme system to its aldehyde, which combines with protein to form the photo-sensitive pigment rhodopsin, or visual purple.

It is known that retinene can readily be reduced to vitamin A outside the eye. This reduction has been clearly demonstrated in the intestines. There is no evidence, however, that this mechanism is ever brought into action, except in the artificial circumstances of experimental dosing with retinene (Moore, 1957). Moore also assumes that the existence of such an enzyme system could provide protection for vitamin A, in regard to at least oxidation. Thus under any form of stress, vitamin A is oxidized to its aldehyde. Another system is present which can facilitate the reversion of the aldehyde form to alcohol.

The metabolic role of the vitamin in the liver is as yet unknown, but certain other information is available. Thus we know that the hepatic tissue containsthe vitamin predominantly in esterified form, and that by a delicate mechanism, a constant low level of the vitamin, in the form of the free alcohol, is maintained in the blood plasma (Moore, 1957). However, the main features of the transfer of vitamin A from the diet to the blood and liver is outlined below (after Moore, 1957).



Vitamin A, besides its action in the retina, encourages the formation of mucus-secreting cells, which synthesize gluco-proteins, as opposed to keratinised tissues. The general effect is to favour the formation of living secretory cells, at the expense of cells

which have stopped living (Moore, 1957). Disturbances in the vitamin A status, therefore leads to a lack of the correct balance between the living and dead components of the body.

It seems possible that vitamin A may be concerned at some definite point in the synthesis of mucins or gluco-proteins. Thus it may be necessary for the linkage between protein and glucosamine, or for the introduction of the sulfate group into the mucin molecule (Moore, 1957).

Wald and Brown (1952) opened a new horizon to follow the role of vitamin A in other tissues. They think that the major role of sulfhydryl (-SH) in the synthesis of rhodopsin in the retina, does suggest that this most reactive group known in biochemistry may be found in other tissues in combination with vitamin A or a derivative of it. In the retina the sulfhydryl groups of the protein "opsin" react with retinene, which is vitamin A aldehyde to form rhodopsin. Furthermore, retinene is converted into vitamin A by the sulfhydryl-containing enzyme, alcohol dehydrogenase which occurs in other tissues.

So far, no enzyme system is known which destroys vitamin A. Labelled material given to animals has resulted in the production of $C^{14}O_2$ in the exhaled air. So that "slowly but surely" vitamin A can be destroyed (Aykroyd, 1958). It is this fact which makes it a continuous indispensable nutrient.

Mobilization of Vitamin A by the Adrenal-Sympathetic System:

Chevallier, Malmejac and Choron studied the effect of nervous stimulation on the mobilization of vitamin A in dogs. They found that after stimulation of either the pneumogastric or the splanchnic nerve, the level of vitamin A in the blood was nearly doubled (Moore, 1957).

The French workers recognized that the adrenal glands of bovines are rich in carotene. They took this clue and designed experiments intended to decide whether nervous stimulation might exert its action in mobilizing vitamin A through the medium of the adrenals. Working on dogs, they got positive results (Moore, 1957).

Other factors were found to increase the vitamin A level in the blood. Physical excercise was found by James and El-Gindi (1953) to increase it by margins of 20 to 106%. Injection of adrenaline in human beings was found to increase the value by 25% above the resting level (Moore, 1957). The adrenaline effect on mobilization of vitamin A was confirmed by experiments on rabbits. Thus intravenous injections of about 1 mg of adrenaline caused the average vitamin A contents of the whole blood to increase from 80 i.u. per 100ml before injections to 160 i.u. thirty minutes after the injections (Moore, 1957). These results suggested that vitamin A might be controlled by the same sympathetic adrenal system which is responsible for the mobilization of sugar, and of certain plasma

proteins from the liver into the blood stream.

Effect of Vitamin A Deficiency on Muscle and Nerve:

Aberle and then Rao were in agreement in their reporting on incoordination in vitamin A deficient rats (Moore, 1957). Weaning rats were given a diet deficient in vitamin A. After 6-8 weeks, various graded stages of paralysis appeared. The first stage was indicated by the rats having their hind legs extended, rather than flexed, when they were held up by the neck. In the second stage there was incoordination of movement, and the feet slipped during walking. The third stage was reached when the feet were placed at a wide angle to the body during both standing and walking, and there was great weakness of the hind legs (Moore, 1957).

Another group of experimenters noticed that after 6-10 months from vitamin A deprivation, all their experimental pigs developed marked nervous disorders, characterized by blindness, incoordination and spasms. Histological studies revealed degeneration of the nerve bundles in the optic thalamus, in the optic, femoral and sciatic nerves, and in certain parts of the spinal cord (Moore, 1957).

Lecoq, Chauchard and Mazoue (Moore, 1957) found that when rats were restricred to a diet deficient in vitamin A, the chronaxie for the nerves began to fall after 15-17 days, while the chronaxie for the muscle rose after prolonged deprivation.

Another interesting thing about vitamin A deficiency, is that the animal seems to have an impaired ability to convert pyruvate to glycogen in the liver (Aykroyd, 1958). This important polysaccharide is indespensible for the muscle and cannot be synthesized in it.

Effect of Excess Vitamin A on Muscle and Nerve:-

The Japanese worker Suzuki, in 1920, reported that great excess of cod-liver oil was injurious to rats. He concluded, then, that the toxicity was due to the inferior quality and unpleasant smell of the material under investigation (Moore, 1957).

Linhard reported a poisoning accident among the members of an expidition to Novaya Zembla. He reported that a bear was shot which appeared to be healthy. On the following day, a stew was prepared from the livers, heart and kidneys. Although the hearts and kidneys of bears had often been eaten before, without ill effects, the nineteen men who ate from the stew on that occasion all became sick. The signs of distress occurred in two victims 2-4 hours after the meal, and most other became ill during the night. The symptoms described were drowsiness, sluggishness, "irritability" or an irresistible desire to sleep, and severe headache and vomiting (Moore, 1957).

Rodahl and Moore analyzed specimens obtained from Greenland and demonstrated the very high concentration of vitamin A which the bear's liver contained. Values of 13,000-18,000 i.u./gram were found by the antimony trichloride method. (Moore, 1957).

In 14 recorded cases of hypervitaminosis A of children over 6 months old, "irritability" was found in 12 of them. In all the 14 cases, Ca^{++} and P level in the blood were found to be normal. Failure to stand was found in 6 of these cases (Moore, 1957).

The first experimental evidence about excess vitamin A toxicity was put forward in 1925 by Takahashi and his colleagues. When daily doses 10,000 times greater than the minimum necessary for growth were given by mouth to rats or mice, death usually occurred after two weeks. The symptoms observed included paralysis of the hind legs. By substituting injections of the concentrates for oral doses, injuries were more rapidly produced. Paralysis of the hind legs was seen only 15 minutes after the concentrates had been injected, and was followed by cramp and death, sometimes within an hour (Moore, 1957).

Rat diaphragms from animals fed excess levels of vitamin A showed a decreased utilization of glucose and a decrease in glycogen synthesis (Sadhu, 1959).

Greber et al (1954) suggests that vitamin A in excess acts like the parathyroid hormone. Hyperparathyroidism can at times cause the calcium level in the plasma to rise to as high as 15-20 mg/100ml. Such an elevated calcium level causes depression to the excitability of the nerve and muscular weakness (Guyton, 1961).

Chevallier and Epsy noticed that the chronaxie of the motor nerves to the paws of guinea pigs and rats was related to their vitamin A reserves (Moore, 1957). The same people studied the chronaxie of flexor and extensor muscles of the guinea pig legs without measuring the reserves of vitamin A in the animal. They made similar observations on rats, pigeons and frogs. A further paper by Chevallier, Epsy and Choron (Moore, 1957) reported that in guinea pigs the vitamin A reserves tend to be higher in spring than in autumn, and that corresponding changes can be observed in chronaxie.

On the basis of the survival time and mortality rate of groups of rats after treatment with excessive amounts of vitamin A and D, it was shown that there is a kind of antagonistic action of vitamin A on the toxicity of vitamin D (Bassett and Clark, 1960). Since the role of vitamin D on Ca mobilization is well known, there is no reason at this stage to exclude it from the picture.

Aim of Work:-

The intention is to detect the immediate effect of vitamin A and its palmitate on the excitability of nerve and muscle before entering the metabolic pathways and the onset of other indirect factors. Such as the disturbance of Ca^{++} level due to the action of this vitamin on bones, or the keratinization of secretory cells and development of nerve lesions. So, we will see that the experiments are done within one hour after treatment of the preparation.

MATERIALS AND METHODS

I. Animals:-

Living frogs of the species Rana esculanta were collected from Antelias near Beirut and occasionally from Syria. They were kept in the laboratory in a cage with a slow running water stream for a period not exceeding a week after their arrival.

II. Solutions:-

The following preparations were used:

1. 1 ml ampoules of an oily solution of vitamin A having 300,000 i.u. (Dr. A. Wander S.A., Berne - Switzerland).
2. 5 mg water dispersible vitamin A palmitate synthetic type 7, crystals (Sigma Chemical Company) with a potency of 250,000 i.u./gram/ 1 c.c. of Ringer's solution.
3. Ringer's solution (Giese, 1962b):

$NaCl$	6.50 gms
KCl	0.14 gms
$CaCl_2, H_2O$	0.12 gms
$NaHCO_3$	0.20 gms
NaH_2PO_4	0.01 gms

Distilled water to make total of 1000.0 c.c.

III. Apparatus and accessories:-

1. Physiograph (E & M Instrument Co. Inc., Houston 21, Texas), with the following attachments and accessories:

a. Amplifier

b. Stimulator

c. Timer

d. Paper speed regulator

e. Myographs No. A-142, A-143
and No. C-89.

2. Dual beam neurophysiograph (E & M Instrument Co.) with a built-in stimulator and time calibrator.
3. Cathode ray oscilloscope (TEKTRONIX Type 514 AD).
4. Precision cathode ray oscilloscope (TEKTRONIX Type 317).
5. Stimulator (Grass Instrument Co.)
6. Stimulator (Built in the Physics Department under the supervision of Prof. Roger Douglass).
7. Separate Preamplifier MK III (E & M Instrument Co.).
8. Isolation Unit (Grass Instrument Co.)
9. Nerve conduction moist chamber (Harvard Apparatus Co.)
10. Special cables for the physiograph and neurophysiograph (E & M Instrument Co.)
11. Gastrocnemius muscle moist chamber, designed to be used with or without the nerve conduction moist chamber, illustrated in figure 1 (author's design made by the Physics Department).

12. Pin electrodes (E & M Instrument Co.)
13. Platinum electrodes (Harvard Apparatus Co.)
14. Myographic tension adjuster (E & M Instrument Co.)
15. "Myographic calibrator" (E & M Instrument Co.).

As four types of stimulators had been used, a reference in such a case was an obligation. The stimulator built by the Physics Department was not calibrated, so the output was connected to a precision oscilloscope, besides the stimulating electrodes, for reading the duration and voltage. The other stimulators, though they had their own calibrations, were calibrated against the calibrator of that precision oscilloscope which was considered as the reference in all the experiments.

IV. Preparations:-

As vitamin A reserves in females exceed that in males (Moore, 1957), all the muscle, nerve, and muscle-nerve preparations were taken from female frogs for consistency. In all the experiments, the control and experimental preparations were taken from the same animal.

A. Isolated Gastrocnemius Muscle Preparation:-

After pithing, the skin is removed from the legs of the frog by cutting a girdle completely around in the mid-

region and drawing the skin over the hind legs. With a blunt probe, the gastrocnemius is freed away from the other muscles of the leg and from the tibio-fibula. The tendon of Achilles is dissected to the calcaneum and then it is cut free to leave as much tendon with the muscle as possible. Tibio-fibula is cut just below the knee joint. Other muscles except the gastrocnemius are cut away from the distal end of the femur. The femur is cut in the middle of the thigh to serve as a handle for the muscle.

B. Isolated Nerve-Muscle Preparation*:-

After pithing the frog and skinning its legs, the abdomen is cut open and the viscera are removed. Then the animal is cut sagittally into two incomplete halves without injuring the sciatic nerves, starting from the anus up to the level of the first vertebra. The frog is turned over to have the dorsal surface up. With a glass rod, the biceps and semimembranosus muscles are separated by tearing the fascia in between them. Now, the white sciatic nerve and accompanying vessels can be seen in the sulcus between these two muscles. The vessels are avoided and the nerve is gently raised by a special glass hook without pinching or pulling it. The pyroformis and ilio-coccygeal muscles are severed.

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* This is a combination of the methods described by 1) Bures et al: "Electrophysiological Methods in Biological Research" p.202. 2) To the method of Mr. Antranig Tchelebian in demonstrating the isolation of the nerve, to whom the author is indebt. 3) And to the procedure of Pace and Riedesel: "Manual of Vertebrate Physiology."

The urostyle is raised gently to be dissected free without injuring the underlying plexus of nerves. Then the nerve is carefully freed up to the vertebral column where it is cut. The nerve is laid on the gastrocnemius, the thigh muscles are cut, the femur is laid bare and divided in the center. The tendon of Achilles is separated from the calcaneum and raised to free the gastrocnemius up to the knee-joint. Lastly, the tibio-fibula and the remaining muscles are severed below the knee-joint.¢

C. Isolated Sciatic Nerve Preparation:-

This is done just as described in the previous section, but as there is no need for the muscle, the nerve is cut just before joining it.

D. Intact Gastrocnemius Muscle Preparation:-

After pithing the animal and skinning its legs, the tendon of Achilles is cut free from the calcaneum and raised to free the gastrocnemius muscle up to the knee-joint.

E. Intact Nerve-Muscle Preparation:-

In addition to the previous procedure, the fascia between the biceps and semimembranosus is severed, and the sciatic nerve is raised by means of a glass hook from the sulcus.

V. Administration of the Vitamin:-

The experimental preparations were injected with 0.05 or 0.1 c.c. of vitamin A in cotton seed oil, and the controls were injected with an equal volume of cotton seed oil. The muscle preparations were injected with 0.1 c.c. and the nerve-muscle preparations were injected with 0.05 c.c.. A tuberculine syringe with needle No.25 was inserted near the tendon of Achilles and pushed forward horizontally toward the belly where the solution was deposited.

In the case of vitamin A palmitate, the experimental preparation was soaked into 5 mg vitamin A palmitate/1 c.c. Ringer's; the control was soaked into Ringer's solution only.

The period of treatment was always 45 minutes before the beginning of the stimulation, on the assumption that it is an adequate time for diffusion in the tissue.

VI. Stimulating and Recording Procedure:-

A. Chronaxie of the muscle:-

A control and an experimental preparations were placed in their proper muscle moist chamber, each was connected to a myograph (see illustration 2). The tension on both muscles was checked to be almost the same. The stimulating electrodes were placed always in the same region: the positive electrode was inserted into the belly of the gastrocnemius muscle near the blood vessel which runs there, and the negative electrode was pinned

into the tendon of Achilles (see illustration 3). The threshold of either control or experimental preparation for a pulse of 200 second duration was determined in the following way:

The duration was selected while the circuit to the two muscles was open. Then the voltage was increased slowly and progressively and the circuit was closed repeatedly by the key to give single shocks until the threshold was reached by either one of the preparations, indicated by the shortest contraction registered by the Physiograph. The electrodes stimulating the muscle reaching the threshold, were disconnected near the key, and the voltage was increased again, till the other preparation reached the threshold. The voltage reading was taken from the oscilloscope in each case. After that, the voltage output was set to zero, and the electrodes were reconnected.

The pulses were progressively shortened and for each new time interval, the threshold voltage was determined as before. The voltage was plotted against the duration, then the chronaxie was determined.

B. Threshold for Nerve-Muscle Preparation:-

Each muscle of the two preparations was placed into a muscle moist chamber, and connected as before to the myograph, the tension was checked. The nerve was passed through a hole into the nerve conduction moist chamber and laid on the platinum electrodes. The chamber was closed with a glass slide and the

junction between the two chambers was sealed with modeling clay (see illustration 4). The nerves were stimulated by the same procedure as above except that three durations were selected only.

C. Threshold for Nerve Excitation:-

Each nerve of the two preparations was placed into a nerve conduction moist chamber. Each at a time, was connected to the Grass stimulator through an isolation unit. The pick up electrodes were connected to a preamplifier feeding to the input of one beam of the oscilloscope (see illustration 5). The other beam was used as a timer by closing the oscillator circuit which was triggered with each sweep. Three durations were selected, 0.02, 0.2, and 50 milliseconds. After selecting the duration, the voltage was increased till the appearance of slight activity on the oscilloscope screen indicated the threshold. Once this was determined for a preparation for one duration, the stimulating electrodes were disconnected between the chamber and the isolation unit and the other chamber was connected instead. Also the wires between the first preparation and preamplifier were disconnected and the new preparation was connected instead. The threshold for this preparation was determined as before. And so on for the other two durations.

Ten experiments were performed in this group. In the last six, each preparation was used twice. The control preparation was treated with vitamin A palmitate solution, the experimental was washed out and the thresholds were determined as before.

D. Muscle Fatigue Recording:-

One muscle at a time was tied from the tendon of Achilles by a thread attached to a rigid myograph (isometric recording) or to a soft myograph (isotonic recording). The tension on the muscle was always checked by the myographic tension adjuster through the amplifier system. The muscle was stimulated directly or via its sciatic nerve. In the first case, the pin stimulating electrodes were placed as illustrated in figure 3. In the other, the platinum stimulating electrodes were placed underneath the sciatic nerve exposed in the femoral region.

To eliminate the time factor, as one preparation was stimulated while the other was quiescent, the recordings were taken alternatively. In one case the control was recorded first and in the other, the experimental.

The myograph-amplifier system was calibrated for tension change by weights, after each isometric recording; and for length change against a "myographic calibrator" in the case of isotonic recording.

RESULTS

I. Preliminary Studies:-

1. Chronaxie of Left and Right Muscles:-

The chronaxies for both left and right gastrocnemius muscles of the same frog stimulated directly were determined and found to be identical, 0.12 millisecond each, as seen from graphs 1a and 1b. One can observe that the situation changes when he compares the chronaxies of two muscles taken from different animals (see table 1).

2. Chronaxie of the Muscle Treated With Vitamin A:-

The chronaxie for the gastrocnemius muscle injected with 0.1 c.c. vitamin A and stimulated directly after 45 minutes is found to be 0.09 millisecond; and for its control which is injected with the same volume of cotton seed oil, is 0.08 milliseconds. So, the difference is 12.5% increase. The striking difference as seen in graph 2 is not in the chronaxie, but in the displacement of the experimental strength-duration curve to higher values along the voltage axis. That is the reason for choosing henceforth in the following experiments, few stimulus durations and comparing the threshold in volts for the experimental and control at each duration.

II. Threshold Studies:-

1. Excitability of Left and Right Nerve-Muscle Preparations Treated with Cotton Seed Oil:

Table 3 shows the threshold in volts, excitability, and index of excitability (to be presently defined) of both left and right nerve-muscle preparations after the injection of 0.05 c.c. cotton seed oil into the belly of each muscle. The excitability is calculated for each muscle at three durations, 0.01 msec., 0.16 msec., and 200 msec. Furthermore, in the third row for each table, the excitability value of the left muscle is divided by the excitability of the right muscle, and then multiplied by 100 to give a relative index of any change in the excitability. This is called the excitability index. As seen from the averages, there is a negligible difference. So, it may be said that both preparations have the same excitability.

2. Excitability of the Muscle Treated with Vitamin A:-

Table 4 shows that there is a reduction of 24%-84% in the index of excitability of the gastrocnemius muscle after the injection of 0.1 c.c. vitamin A and stimulating with 0.16 msec. pulse duration. The average reduction is 50% compared with the control from the same animal and injected with the same volume of cotton seed oil.

3. Excitability of the Muscle Treated with Vitamin A Palmitate:-

It is seen in table 5 that the reduction in the index of excitability of the gastrocnemius muscle is 35%-78% after treatment with vitamin A palmitate and stimulating with 0.01

msec. duration. The average reduction is 57.3%. Stimulating with 0.16 msec. durations, the reduction is 12%-89%, and the average reduction in the index of excitability is 50.2%. With stimuli of very long durations, there is no change in the excitability.

4. Excitability of Nerve-Muscle Preparation Treated with Vitamin A:-

When 0.05 c.c. vitamin A is injected into the muscle of the nerve-muscle preparation, and the nerve stimulated with pulses of 0.01 msec. duration, the index of excitability decreases 28%-87%, and the average reduction is 62%. With 0.16 msec. duration, the reduction is 25%-93% and the average reduction is 70%. With long durations, the reduction is 0%-97% and the average reduction is 39.5% as seen in table 6.

5. Excitability of Muscle-Nerve Preparation Treated with Vitamin A Palmitate:-

Table 7 shows the index of excitability of the muscle-nerve preparation after treatment for 45 minutes with vitamin A palmitate. The range of the reduction in the index of excitability is 0%-42% at 0.01 msec. duration, and the average in the reduction is 21%. With 0.16 msec. duration, the range of the reduction is 23%-49%, and the average reduction is 35%. Unlike the situation where the muscle itself was treated with vitamin A palmitate, there is a reduction in the excitability

with stimuli of long durations. The range of this reduction is 16%-40% and the average is 33%.

6. Excitability of the Nerve Treated with Vitamin A Palmitate:-

Table 8 shows the increase in the index of excitability of the nerve after treatment with vitamin A palmitate. The range of the increase at 0.02 msec. duration is 0%-36% averaging 14%, with the exception of experiment 6'. With 0.2 msec. durations, the range of the increase in the index of excitability is 0%-127%, with an average increase in the excitability of 34%. With 50 msec. duration, there is an inconsistency in the results. In some, there is an increase; and in the others, there is a decrease. The range of the change in the index of excitability is -37% to +48%. On the average, however, there is a reduction of 1%. In this group of experiments, for the first time, an attempt was made to observe the effect of the vitamin A palmitate after thorough washing of the preparation. The effect disappeared as you observe in the last six experiments (5'-10').

III. Continuous Stimulation Studies:-

1. Isometric Recording of a Vitamin A Treated Muscle:-

The isometric recording of a fatigue curve of a gastrocnemius muscle injected with 0.1 c.c. vitamin A and stimulated after 45 minutes with a repetitive square wave current of 25

volts, 0.2 msec. pulse duration and 2 stimuli/second is shown on plate 1 with its control. In this recording, the control was studied before the experimental. The following observations can be seen:-

1) The tension developed by each contraction at the beginning is higher than that of the control.
2) The manner in which the tension is dropping down with time in the experimental muscle is different from that in the control as seen by their respective slopes.
3) After 30 seconds from the beginning of the stimulation, both the experimental and the control muscles begin to develop a "tonic contractions" or a "sustained tension" besides the individual twitches. After about 90 seconds, the experimental muscle develops its highest "sustained tension" which is a little more than 100 gms gravity. Whereas the highest sustained tension which the control develops is about 80 gms gravity.

Plate 2 shows a recording as in plate 1, but in this, the experimental preparation is studied before the control. The following observations are noted.

1) The tension of each contraction at the beginning is higher in the experimental than that in the control.
2) From the beginning, the experimental preparation does not relax fully as compared to its control.

3) Again the highest "sustained tension" developed by the experimental is a little more than that of the control.

2. Isotonic Recording of Vitamin A Treated Muscle:-

Plate 3 shows isotonic recordings of a gastrocnemius muscle injected with 0.1 c.c. vitamin A and stimulated directly with a repetitive current of 25 volts, 0.16 msec. duration and 3 stimuli/second, with its control. Both are stimulated simultaneously. There is no striking difference. There is a sustained shortening "tonic contraction" in both, but in the experimental it lasted for 4 seconds more than in the control. At the beginning, both showed a kind of contracture and it lasted one second more in the experimental. The experimental lost the ability for contracting within 4 seconds earlier than the contol.

3. Isometric Recording of a Vitamin A Palmitate Treated Muscle:-

Plate 4 shows an isometric recording of a gastrocnemius muscle treated for 45 minutes with vitamin A palmitate and then stimulated with a continuous alternative current of 25 volts, 0.2 msec. duration and 2 stimuli/second. The experimental is stimulated before the control. It is observed that:

1) The initial contractions in the experimental develop tension as high as 160 gms gravity, compared to 140 gms

of the control.

2) After 70 seconds from the beginning of the stimulation, the highest sustained tension is developed in both, but in the experimental it is higher than 105 gms gravity, compared to less than 100 gms gravity in the control.

3) After 200 seconds, the tension of the control dropped to about 20 gms gravity, compared with the experimental which dropped to 50 gms only.

Plate 5 shows a recording of an experiment similar to that recorded on plate 4 except that the control is done before the experimental. The first two observations of plate 4 can also be noticed on this plate.

4. Isotonic Recording of a Vitamin A Palmitate Treated Muscle:

Plate 6 shows a recording of a fatigue curve for a gastrocnemius muscle treated with vitamin A palmitate for 45 minutes, and stimulated with a repetitive current of 25 volts, 0.2 msec. duration and 2 stimuli/second. The control is done before the experimental. The following observations are clear:-

1) At the beginning, the experimental muscle is not relaxing fully as the control.

2) After 50 seconds, the experimental muscle begins to develop a "tonic contraction" at a faster rate than the control and

it keeps on that till it is in a complete "tetany".

3) That "tetany" lasts in the experimental for about 300 seconds. The control doesn't show that "tetany".

4) After about 670 seconds, the experimental loses its ability for the tonic contracture and drops down to the initial baseline. The control, then, goes below the baseline.

Plate 7 shows a recording of an experiment similar to that recorded on plate 6 except that the experimental is done before the control. The same observations almost can be seen on this plate.

5. Isometric Recording of a Vitamin A Treated Nerve-Muscle Preparation:-

Plate 8 shows an isometric recording of a fatigue curve of a nerve-muscle preparation after injection of 0.05 c.c. vitamin A and stimulating after 45 minutes. It is observed that:

1) The experimental develops a tension in the period 25-70 seconds higher than 130 gms gravity, whereas the control doesn't exceed sometimes, during that period, a 120 gm gravity tension.

2) The highest "tonic tension" in the experimental is lower than that in the control, i.e. about 135 gms gravity in the experimental as compared to 150 gms gravity in the control.

Plate 9 shows again isometric recordings of fatique curves for a nerve-muscle preparation after injecting 0.05 c.c. vitamin A with its control. The control in this plate is stimulated before the experimental. The important observations are:-

1) The late low tonic tension developed by the experimental.
2) The low tension developed by the initial contractions of the experimental preparation.
3) The higher amplitude of the last contractions in the experimental, compared to the control.

6. Isotonic Recording of Vitamin A Treated Nerve-Muscle Preparation:-

Plate 10 shows isotonic recordings of a fatigue curve for an intact nerve-muscle preparation treated with 0.05 c.c. vitamin A and its control. It is observed that the experimental does not shorten as much as the control.

7. Isometric Recording of Vitamin A Palmitate Treated Nerve-Muscle Preparation:-

Plate 11 shows isometric recordings of fatigue curves for an intact nerve-muscle preparation after treatment with vitamin A palmitate, with its control. The calibration of the tension is indicated at the left of each recording by grams gravity. It is observed that:

1) The initial tension developed by each contraction is higher than 150 grams gravity, compared to less than 140 grams gravity in the control.

2) The initial sustained tonic tension from zero time is higher than 40 grams gravity in the experimental as compared to less than 30 grams gravity in the control.

3) The highest sustained tonic tension developed by the experimental is about 120 grams gravity, as compared to less than 70 grams gravity in the control.

4) The sustained tonic tension in the experimental, after 1400 seconds of continuous stimulation, is still higher than 45 grams gravity as compared to the control which is less than 30 gram gravity.

5) The diminished amplitude of the experimental contractions at the end, as compared to the control.

Plate 12 shows the same type of recording, except that the control is done before the experimental. The same observations almost can be noticed.

8. Isotonic Recording of a Vitamin A Palmitate Nerve-Muscle Preparation:-

Plate 13 shows isotonic recordings of fatigue curves for vitamin A palmitate treated preparation and stimulated before its control. The change in length can be associated

with the calibration scale on the left of each recording. The following observations can be noticed:

1) The initial change in length is more in the experimental than in the control.
2) At the end of the recording, the change in the experimental muscle length is less than that in the control.
3) A sustained contracture in the experimental by the end of the experiment, where as the control doesn't show that.

Plate 14 is a recording of the same kind of treatment and procedure as that of plate 13 except that the control is stimulated before the experimental and both preparations are stretched almost equally before stimulation. The following observations are noticed:-

1) The initial change in length is greater in the experimental than in the control as seen when the stylus begins to move within its limits.
2) The rate of the decrease in the amplitude of the experimental preparation is far more than that of its control.

DISCUSSION AND CONCLUSION

Chronaxie and the Index of Excitability:-

The preliminary studies aided the author to evaluate current ideas about chronaxie ranging from the consideration of "chronaxie as a poor measure of membrane properties" (Ramsey: Ch VI in Bourne: Muscle II, 1960), to its acceptance as a measure of excitability, (Chevallier and Epsy, 1936; Chevallier and Choron, 1936; Chauchard, 1946).

Mathematically, Ramsey followed the following logic: He started with the following formula given by Blaire (1932) to account for the strength-duration curve for a muscle,

$$R = V\ (1-e^{-kt})$$

where:

V = voltage (resistance constant)

k = constant whose value equals the rate of decay of excitation.

t = time

R = Rheobase

Ramsey (Bourne II, 1960) found it more convenient to put the equation in the following form:

$$\log_e \frac{V}{V-R} = kt$$

If 2R is substituted for V in this equation, the chronaxie value gets to be $\frac{1}{k} \log_e 2$. As $\frac{1}{k}$ is the time constant, chronaxie,

therefore, is a measure of the time constant of the excitation decay process.

Irrespective of the use of chronaxie as a measure of excitation or excitation decay, a more convenient way was to compare the threshold of depolarization of both experimental and control at a single duration (Ruch and Fulton, 1961) as suggested by Dr. Suhayl J. Jabbur.

Effect of Vitamin A and its Palmitate on the Muscle:-

The decrease in excitability upon use of vitamin A was probably due to increased affinity of the relaxing factor which is mentioned by Huxley (1964) to bind calcium. Consequently, it raised the amount of energy needed to release it again for the activation of the contractile fibrils.

The high tension developed by the initial contractions with vitamin A treatment and upon the continuous stimulation, might be explained by Davies theory (1963), if the above mentioned effect of the vitamin on the relaxing factor was true, as follows: The affinity of the relaxing factor for calcium was probably increased, so the amount of the bound calcium was expected to be more. Therefore, if the analogy "the amount of expenditure is directly proportional to the income" holds true for this case, then the amount of released calcium when the threshold was reached was expected to be more than normal. Hence, the activation process is stronger, there-

fore a higher tension developed.

If that was not completely possible, an alternative suggestion is present. Vitamin A may have increased the selective permeability of the membrane for Na^+ when it is stimulated with adequate stimuli, consequently an action potential with more duration developed which caused the release of more calcium ions.

It would be interesting to design an experiment showing the effect of vitamin A on the binding ability of the relaxing factor for calcium. And to design another intra-cellular recording experiment to detect if the vitamin affects the action potential of the myofibers.

Vitamin A, besides its probable effect on the relaxing factor in the twitch fibers, may have affected the slow fibers by increasing the rate of the rise of the "tonic contraction" and its magnitude. Huxley (1964) defined these slow fibers by their ability for long lasting contractures and the inability to respond to single shocks, but they give slow local contractions when stimulated repetitively. The gastrocnemius muscle of the frog has both slow and twitch fibers (Bourne II, 1960).

It would be interesting if one studies the effect of this vitamin on both twitch and slow fibers separately.

Denervated muscle treated with vitamin A palmitate, showed what Ruch and Fulton calls the "reaction of degeneration". This phenomenon was described as a relative loss of excitability to

"faradic" stimuli (pulse durations less than 1 millisecond), and as a retention of excitability to "galvanic" stimuli (current flows with long durations - about 300 milliseconds) (Ruch and Fulton, 1961).

Vitamin A palmitate, like vitamin A may have affected the slow fibers, and its effect lasted for a longer period. Probably, it affected the relaxing factor too as with vitamin A.

Effect of Vitamin A and its Palmitate on the Neuromuscular Junction:-

A nerve-muscle preparation treated with vitamin A showed a tremendous decrease in the excitability (table 6). Probably, the effect of the vitamin was exerted in one or more from the following possibilities, which was (or were) superimposed on the probable effect on the relaxing factor in the twitch fibers:

1) Inhibition to the release of acetylcholine from their packets (Granules of Kühne) of the sole feet.
2) Hinderance to the movement of the acetylcholine into the "synaptic cleft".
3) Decrease in the sensitivity of the palisade of the sarcolemma for the acetylcholine substance, or
4) Overactivity of the acetylcholinesterase.

Vitamin A probably affected the neuromuscular junction of the slow fibers too as noticed from the low sustained tonic contracture.

It would be interesting to study the effect of this vitamin and its palmitate on the EPP and on the "amplifying" efficiency for

the current in the neuromuscular junction.

Vitamin A - palmitate treated muscle -nerve preparation didn't show the "reaction of degeneration" as compared to the denervated muscle. Probably the effect was on the neuromuscular junction besides the symptoms of the "reaction of degeneration" in the muscle exclusively, evidenced by the decrease in excitability with stimuli of long durations.

The diminished amplitude of the contractions towards the end of the experiment in the nerve-muscle preparation treated with vitamin A and vitamin A palmitate might be due to what is called by Guyton (1961) the "fatigue of the neuromuscular junction".

Effect of Vitamin A Palmitate on the Nerve:-

The increased excitability of the vitamin A palmitate treated nerve was probably due to the increased permeability of the nerve membrane for sodium. With pulses of long durations, that increase in excitability didn't appear because the duration of the pulse was far more than the time course of the sodium permeability. The 1% decrease in the excitability at 200 milliseconds duration was insignificant.

SUMMARY

1. Comparison of the threshold of depolarization for experimental and control at a certain duration, was found more convenient than chronaxie for the study of excitability.
2. Excess vitamin A decreased the excitability of the denervated muscle, probably by increasing the affinity of the relaxing factor to bind calcium.
3. Excess vitamin A and its palmitate increased the tension of the initial contractions either by the greater amount of calcium released from the relaxing factor, or by increasing the duration of the membrane permeability for sodium when stimulated adequately.
4. Excess vitamin A and its palmitate increased the sustained tonic contracture of the denervated muscle by increasing the excitability of the slow fibers.
5. Excess vitamin A palmitate caused a decrease in the excitability of the denervated muscle with shocks of short durations and retention of it with single stimuli of long duration - "Reaction of degeneration".
6. Excess vitamin A and its palmitate caused the decrease in the excitability of the nerve-muscle preparation. The effect perhaps was exerted on the neuromuscular junction besides the effect on the relaxing factor.

7. Vitamin A treated nerve-muscle preparation developed low tonic contracture. The effect was probably on the neuromuscular junction of the slow fibers.
8. Vitamin A palmitate nerve-muscle preparation stimulated continuously, showed diminished amplitudes of contractions towards the end of the experiment - "Fatigue of the neuromuscular junction".
9. Excess vitamin A palmitate increased the excitability of the nerve, probably by increasing the permeability of the nerve membrane for sodium.

ILLUSTRATIONS

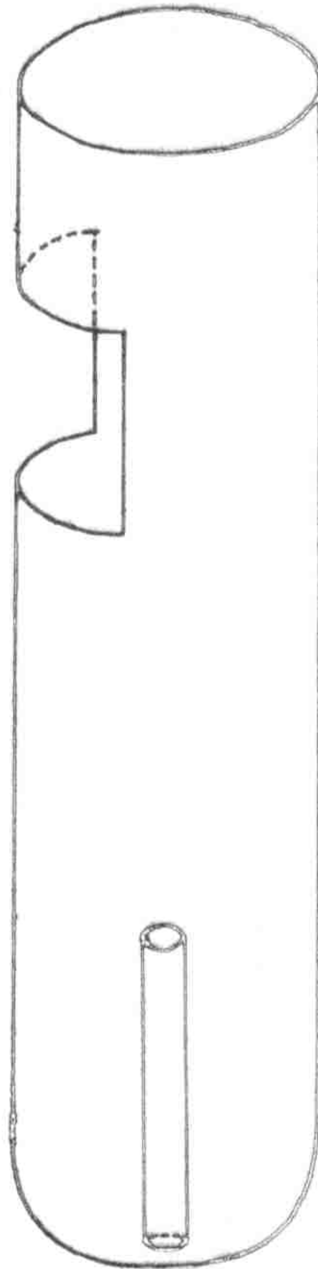


ILLUSTRATION I

Gastrocnemius Muscle Moist Chamber

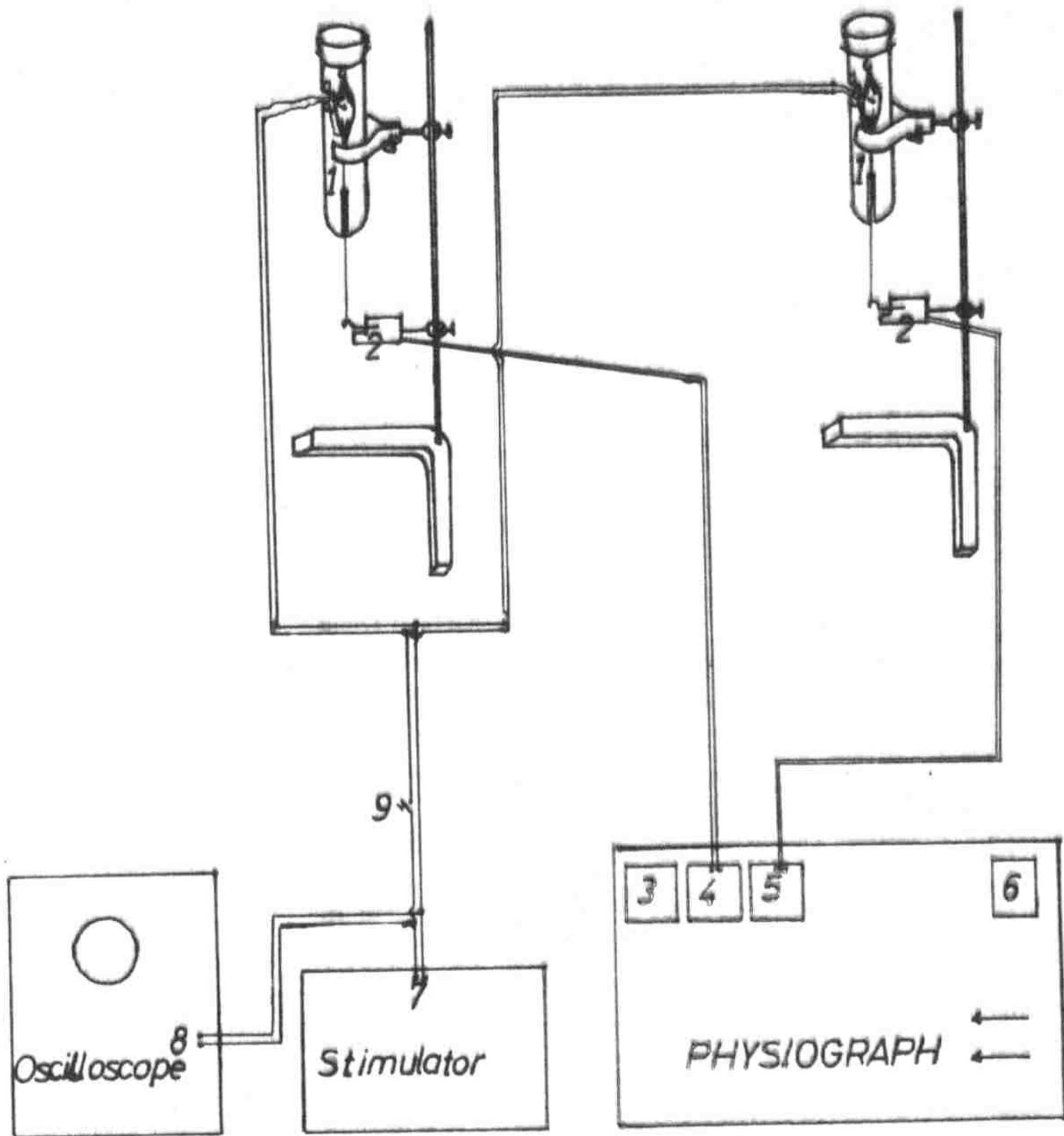


ILLUSTRATION 2

Set-up for the Isolated Muscle

1. Muscle inside the moist chamber
2. Myograph
3, 4 and 5: Amplifiers
6: Paper control
7: Output of Stimulator
8: Input of Oscilloscope
9: Key

ILLUSTRATION 3

Positions of Electrodes on the Muscle

1. Part of the femur bone
2. Positive Electrode
3. Negative Electrode

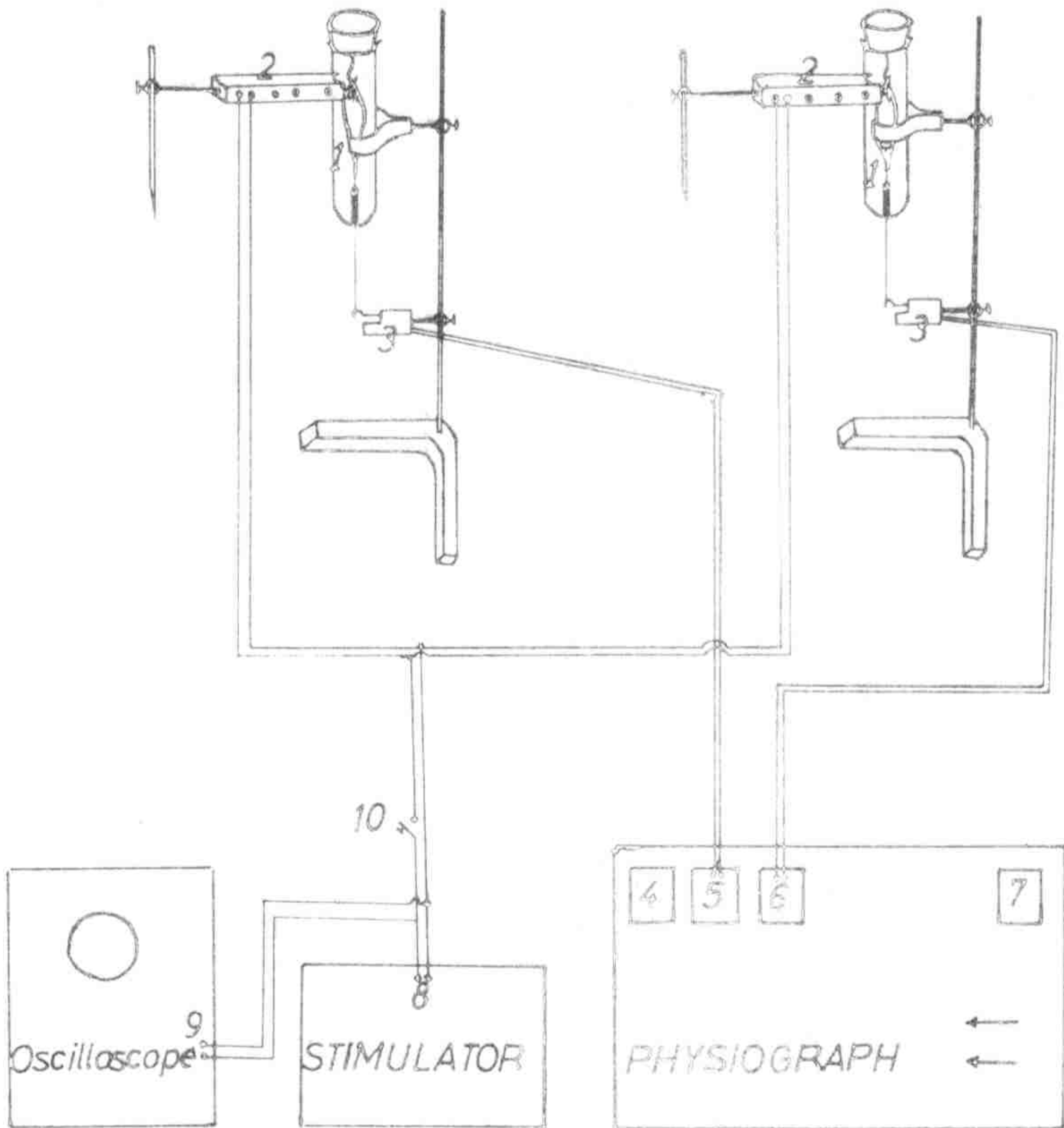


ILLUSTRATION 4

Set-up for the Isolated Nerve-Muscle Preparation

1. Muscle inside the moist chamber
2. Nerve inside its chamber
3. Myograph
4, 5 and 6: Amplifiers
7. Paper control
8. Output of stimulator
9. Input of oscilloscope
10. Key

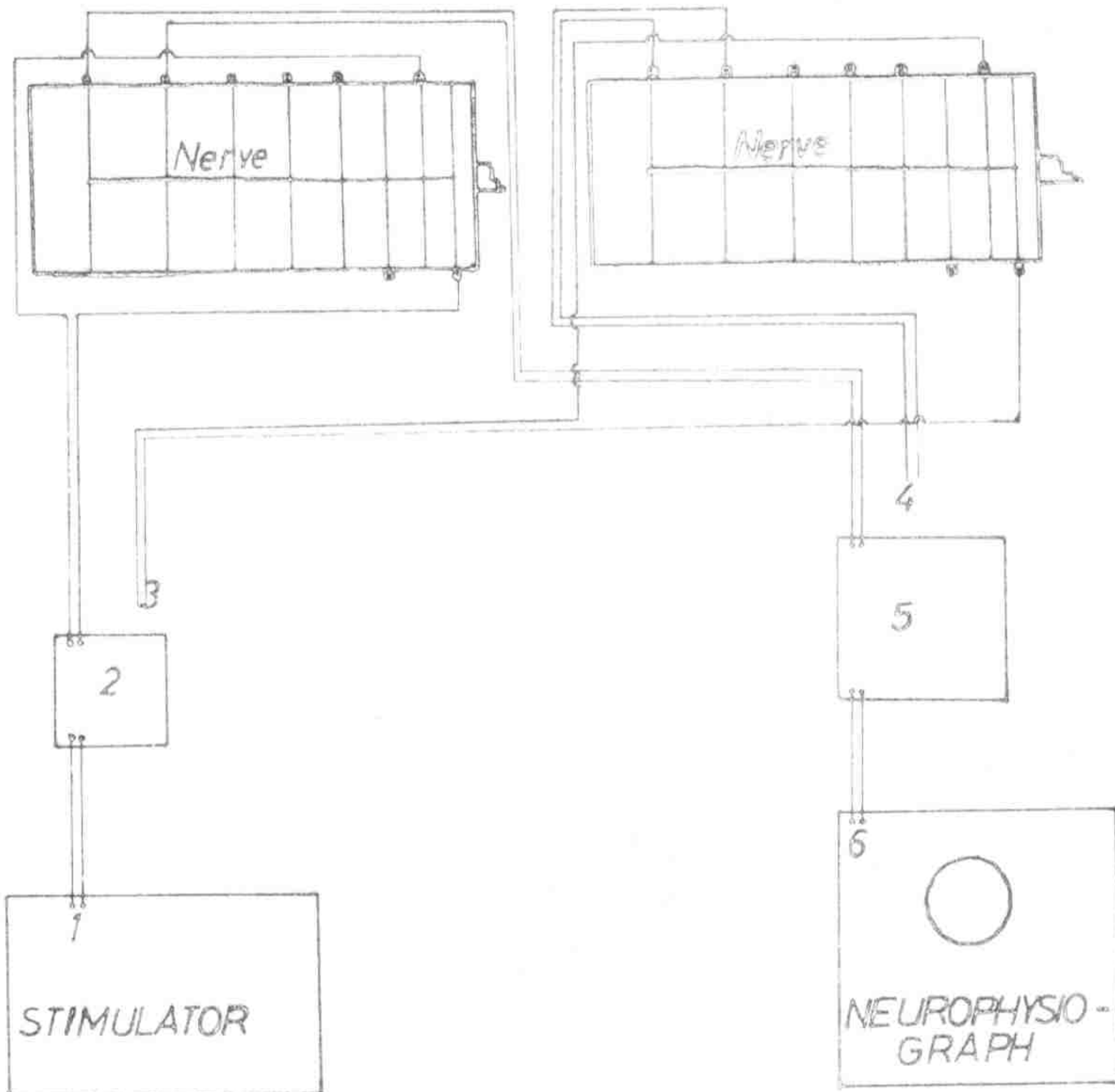


ILLUSTRATION 5

Set-up for the Isolated Nerve Preparation

1. Output of Stimulator
2. Isolation Unit
3. Wires to be connected to the isolation unit
4. Wires to be connected to the preamplifier
5. Preamplifier
6. Input of the neurophysiograph

TABLES

Table 1

The Thresholds for the Left and Right Gastrocnemius Muscles Without any Treatment and Stimulated Directly

Exp. No. / Dur. in msec.	Threshold in Volts											
	1		2		3		4		5		Average ± S.D.	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
0.01	8.2	8.2	3.5	3.5	5.0	3.8	4.7	4.2	4.0	4.4	5.1±1.84	4.8±1.92
0.02	3.2	3.2	1.75	1.75	3.5	2.5	3.2	3.0	2.2	2.3	2.4±0.82	2.6±0.58
0.04	2.4	2.4	0.8	0.8	2.2	1.7	2.0	1.9	1.1	1.1	1.7±0.71	1.6±0.64
0.08	1.55	1.70	0.55	0.55	1.35	0.95	1.45	1.40	0.65	0.65	1.1±0.50	1.1±0.49
0.16	0.95	0.95	0.48	0.48	0.70	0.57	0.85	0.78	0.45	0.45	0.68±0.22	0.64±0.20
0.32	0.85	0.88	0.43	0.43	0.65	0.55	0.70	0.68	0.42	0.42	0.61±0.18	0.60±0.18
0.64	0.85	0.85	0.42	0.42	0.55	0.46	0.62	0.55	0.40	0.40	0.57±0.17	0.54±0.18
1.28	0.85	0.85	0.42	0.42	0.50	0.41	0.55	0.50	0.40	0.40	0.54±0.17	0.51±0.19
200.0	0.62	0.78	0.35	0.42	0.32	0.30	0.40	0.35	0.35	0.40	0.41±0.12	0.45±0.19

Table 2

Thresholds for both experimental gastrocnemius muscle injected with o.1 c.c. Vitamin A and its control injected with o.1.c.c. cotton seed oil. Both stimulated directly after 45 minutes from the injection.

Exp. No.	1		2		3		4		5*		6		7*		8		9		10		Average ±S.D.	
Duration in milliseconds	Control	Experimen.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Experimen.
0.02	2.0	3.3	2.5	3.2	1.35	2.9	1.2	2.0	4.0	4.0	1.45	2.7	1.75	7.8	0.85	2.4	1.7	2.6	1.9	3.1	1.62∓0.52	2.77∓0.46
0.04	1.05	1.7	1.42	1.55	0.78	1.56	0.49	0.8	1.8	2.1	0.85	1.8	1.05	5.5	0.46	1.6	1.3	1.8	1.1	1.75	0.93∓0.34	1.56∓0.33
0.08	0.75	0.9	0.78	0.80	0.45	1.1	0.44	0.75	1.35	1.35	0.70	1.3	0.75	4.7	0.37	1.28	0.95	1.25	0.8	0.95	0.66∓0.21	1.04∓0.22
0.16	0.38	0.52	0.52	0.75	0.36	0.86	0.32	0.47	1.00	1.00	0.45	1.12	0.61	4.1	0.36	1.15	0.75	0.95	0.42	0.6	0.45∓0.13	0.80∓0.26
0.32	0.32	0.45	0.42	0.70	0.35	0.85	0.30	0.45	0.74	0.74	0.37	0.9	0.6	3.7	0.34	1.08	0.50	0.82	0.36	0.52	0.37∓0.06	0.72∓0.23
0.64	0.32	0.40	0.38	0.62	0.29	0.54	0.27	0.43	0.60	0.60	0.32	0.72	0.55	3.4	0.34	0.9	0.50	0.78	0.35	0.45	0.35∓0.07	0.61∓0.18
1.28	0.32	0.40	0.38	0.52	0.28	0.54	0.27	0.42	0.55	0.50	0.32	0.70	0.50	3.3	0.34	0.88	0.48	0.78	0.32	0.43	0.34∓0.07	0.58∓0.18
5.00	0.32	0.40	0.38	0.52	0.28	0.47	0.27	0.42	0.52	0.40	0.30	0.48	0.50	3.0	0.34	0.82	0.42	0.65	0.30	0.42	0.33∓0.05	0.52∓0.05
200.0	0.32	0.40	0.38	0.52	0.28	0.29	0.27	0.42	0.52	0.37	0.30	0.48	0.50	3.0	0.34	0.82	0.42	0.65	0.30	0.42	0.33∓0.05	0.50∓0.16

Threshold in volts

* These experiments deviate significantly, and they are omitted from calculations.

Table 3

Thresholds, Excitabilities and Indices of
Excitability of Left and Right Nerve-Muscle Preparations
Treated with 0.05 c.c. Cotton Seed Oil and Stimulated After 45 Minutes

	Duration in Milliseconds	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5		Average of thresholds ± S.D.	Average of excitabilities ± S.D.
		Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability		
Left Preparation	0.01	4.5	0.22	5.10	0.2	6.8	0.15	4.8	0.21	4.6	0.22	5.18±0.94	0.20±0.03
	0.16	1.9	0.53	2.70	0.37	4.2	0.24	3.1	0.32	2.0	0.50	2.78±0.93	0.39±0.12
	200	0.14	7.12	0.15	6.68	0.14	7.12	0.14	7.12	0.14	7.12	0.14±0.01	7.03±0.20
Right Preparation	0.01	4.5	0.22	5.0	0.20	6.8	0.15	4.9	0.2	4.6	0.22	5.16±0.93	0.20±0.03
	0.16	2.0	0.5	2.7	0.37	4.2	0.24	3.0	0.33	2.0	0.50	2.78±0.90	0.39±0.11
	200	0.14	7.12	0.15	6.68	0.14	7.12	0.15	6.6	0.14	7.12	0.14±0.01	6.93±0.26
$\frac{\text{Left exc.}}{\text{Right exc.}}$ X 100	0.01	100		100		100		105		100		101±2.2	
	0.16	106		100		100		97		100		101±3.2	
	200	100		100		100		108		100		102±3.6	

Table 4

Thresholds, Excitabilities and Indices of Excitability for Experimental Gastrocnemius Muscles Each Injected With 0.1 c.c. Vitamin A, With Their Respective Controls Injected with 0.1 c.c. Cotton Seed Oil. Stimulation With 0.16 msec. Durations After 45 Minutes From Injection.

	EXPERIMENT No.										Average $\pm$ S.D.
	1	2*	3	4	5	6	7	8	9	10	
Control threshold (Volts)	0.47	6.2	0.75	0.45	0.50	0.85	1.0	0.52	0.95	0.65	0.68 ± 0.21
Experimental threshold (Volts)	0.65	8.2	2.7	0.75	1.5	1.5	6.2	1.65	1.25	0.85	1.23 ± 1.86
Control Excitability	2.12	0.16	1.34	2.25	2.0	1.18	1.0	1.92	1.05	1.54	1.60 ± 0.50
Experimental Excitability	1.54	0.12	0.37	1.34	0.66	0.66	0.16	0.61	0.80	1.18	0.81 ± 0.44
$\frac{\text{Exp. Exc.}}{\text{Con. Exc.}} \times 100$	73	75	28	60	33	56	16	32	76	75	50 ± 23

* It deviates significantly, not considered in the average.

Table 5

Thresholds, excitabilities and indices of excitability of an experimental gastrocnemius muscle treated for 45 minutes with vitamin A palmitate with its control.

	Duration in Milliseconds	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5		Exp. 6		Exp. 7		Exp. 8		Exp. 9		Exp. 10		Average of thresholds ± S.D.	Average of Excitabilities ± S.D.
		Threshold (Volts)	Excitability	Threshold (volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability		
Control	0.01	2.30	0.44	3.9	0.26	3.20	0.31	4.00	0.25	2.00	0.50	2.50	0.40	2.10	0.48	3.00	0.33	3.20	0.31	3.50	0.29	2.97±0.75	0.36± 0.09
preparation	0.16	0.30	3.33	0.35	2.85	0.34	2.95	1.00	1.00	0.28	3.57	0.60	1.67	0.27	3.70	0.42	2.38	0.44	2.28	1.50	0.67	0.55±0.40	2.44± 1.18
	200	0.22	4.55	0.30	3.33	0.25	4.00	0.42	2.38	0.14	7.11	0.40	2.50	0.16	6.25	0.30	3.33	0.30	3.33	0.35	2.85	0.28±0.09	3.96± 1.58
Exp.	0.01	10.50	0.10	6.0	0.17	6.20	0.16	9.20	0.11	4.90	0.21	9.50	0.11	5.20	0.19	6.40	0.16	6.20	0.16	9.20	0.11	7.33±1.88	0.15± 0.04
prep.	0.16	2.60	0.38	0.43	2.32	1.00	1.00	2.40	0.42	0.32	3.15	2.10	0.48	0.35	2.85	1.50	0.67	0.82	1.22	2.80	0.36	1.43±0.98	1.29± 1.09
	200	0.22	4.55	0.30	3.33	0.25	4.00	0.42	2.38	0.14	7.11	0.40	2.50	0.16	6.25	0.30	3.33	0.30	3.33	0.35	2.85	0.28±0.09	3.96± 1.59
Exp.Exc. / con.Exc. XX100	0.01	22		65		53		42		41		26		40		49		52		39		42.7±12.7	
	0.16	11		81		34		42		88		29		77		28		54		54		49.8±25.6	
	200	100		100		100		100		100		100		100		100		100		100		100±00.0	

Table 6

Thresholds, excitabilities and indeces of excitability for an experimental nerve-muscle preparation treated with 0.05 c.c. Vitamin A for 45 minutes and its control.

	Duration in Milli-seconds	Exp. 1		Exp. 2 *		Exp. 3		Exp. 4		Exp. 5		Exp. 6		Exp. 7*		Exp. 8		Exp. 9		Exp. 10		Average of thresholds ± S.D.	Average of Excitabilities ± S.D.
		Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability		
Control	0.01	2.50	0.40	12.6	0.08	2.60	0.39	1.15	0.87	0.95	1.28	0.88	1.14	9.50	0.13	1.75	0.57	2.30	0.44	2.40	0.42	1.82∓0.74	0.69∓0.36
Preparation	0.16	0.52	1.92	2.30	0.44	1.00	1.00	0.14	7.15	0.14	7.15	0.18	5.55	3.00	0.33	0.21	4.75	0.40	2.50	0.25	4.00	0.36∓0.29	4.25∓2.35
	200	0.14	7.12	0.65	1.52	0.18	5.90	0.14	7.15	0.14	7.15	0.14	7.15	0.45	2.22	0.16	6.25	0.19	5.28	0.17	5.88	0.16∓0.02	6.48∓0.56
Experim.	0.01	9.00	0.16	18.00	0.06	8.50	0.12	2.70	0.37	6.20	0.16	1.95	0.51	No. Resp.	?	3.4	0.30	4.70	0.21	8.10	0.12	5.79∓2.6	0.22∓0.02
Prep.	0.16	0.70	1.43	4.50	0.22	2.70	0.37	0.20	0.50	1.20	0.83	0.35	2.85	No. Resp.	?	0.9	0.11	0.95	1.05	1.65	0.61	1.08∓0.59	0.84∓0.87
	200	0.28	3.56	1.80	0.56	0.72	1.39	0.14	7.15	0.24	4.15	0.16	6.25	105	0.01	0.21	4.75	0.31	3.22	0.62	1.62	0.34∓0.23	4.01∓1.55
Exp.exc. / can.exc. X100	0.01	40		72		31		43		13		45		?		52		48		29		38∓12.7	
	0.16	75		50		37		7		12		51		?		2		42		15		30∓25.5	
	200	50		36		24		100		58		87		3		76		61		28		60.5∓26.9	

* These are deviated significantly and omitted from calculations.

Table 7

Thresholds, excitabilities and indeces of excitability for an experimental nerve-muscle preparation treated with vitamin A palmitate for 45 minutes, and its control.

	Duration in Milli-seconds	Exp. 1*		Exp. 2		Exp. 3		Exp. 4		Exp. 5		Exp. 6		Exp. 7		Exp. 8		Exp. 9		Exp. 10		Average of thresholds ± S.D.	Average of excitability ± S.D.
		Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability	Threshold (Volts)	Excitability		
Control	0.01	3.5	0.29	2.05	0.49	2.5	0.40	2.15	0.47	2.1	0.48	2.6	0.39	2.5	0.40	2.1	0.48	3.0	0.33	2.7	0.37	2.41∓0.33	0.42∓0.06
preparation	0.16	2.0	0.50	0.19	5.28	0.19	5.28	0.20	5.00	0.19	5.28	0.2	5.00	0.19	5.28	0.2	5.00	1.8	0.56	0.21	4.75	0.37∓ 0.54	4.60∓1.55
	200	0.8	1.25	0.19	5.28	0.14	7.12	0.14	7.12	0.15	6.65	0.14	7.12	0.14	7.12	0.14	7.12	0.45	2.22	0.16	6.25	0.18∓0.10	6.22∓1.63
Experim.	0.01	No resp.	?	2.3	0.44	2.9	0.35	2.15	0.47	3.6	0.28	3.0	0.33	3.9	0.26	3.2	0.31	4.1	0.24	3.1	0.32	3.14∓0.66	0.33∓0.08
prep.	0.16	28.	0.04	0.27	3.70	0.32	3.12	0.23	4.35	0.37	2.70	0.32	3.12	0.34	2.94	0.32	3.12	2.4	0.42	0.28	3.56	0.54∓0.69	3.00∓1.08
	200	15	0.07	0.27	3.70	0.18	5.55	0.18	5.55	0.25	4.00	0.17	5.88	0.22	4.55	0.2	5.00	0.6	1.67	0.19	5.28	0.25∓0.14	4.57∓1.21
Exp.Exc. / Con.Exc. X 100	0.01	?		90		87		100		58		85		65		65		73		87		79 ∓ 14.1	
	0.16	7		70		59		87		51		62		56		62		75		75		65 ∓ 11.4	
	200	5		70		78		78		60		83		64		70		75		84		67 ∓ 10.8	

* It deviates significantly, and omitted from calculations.

Table 8

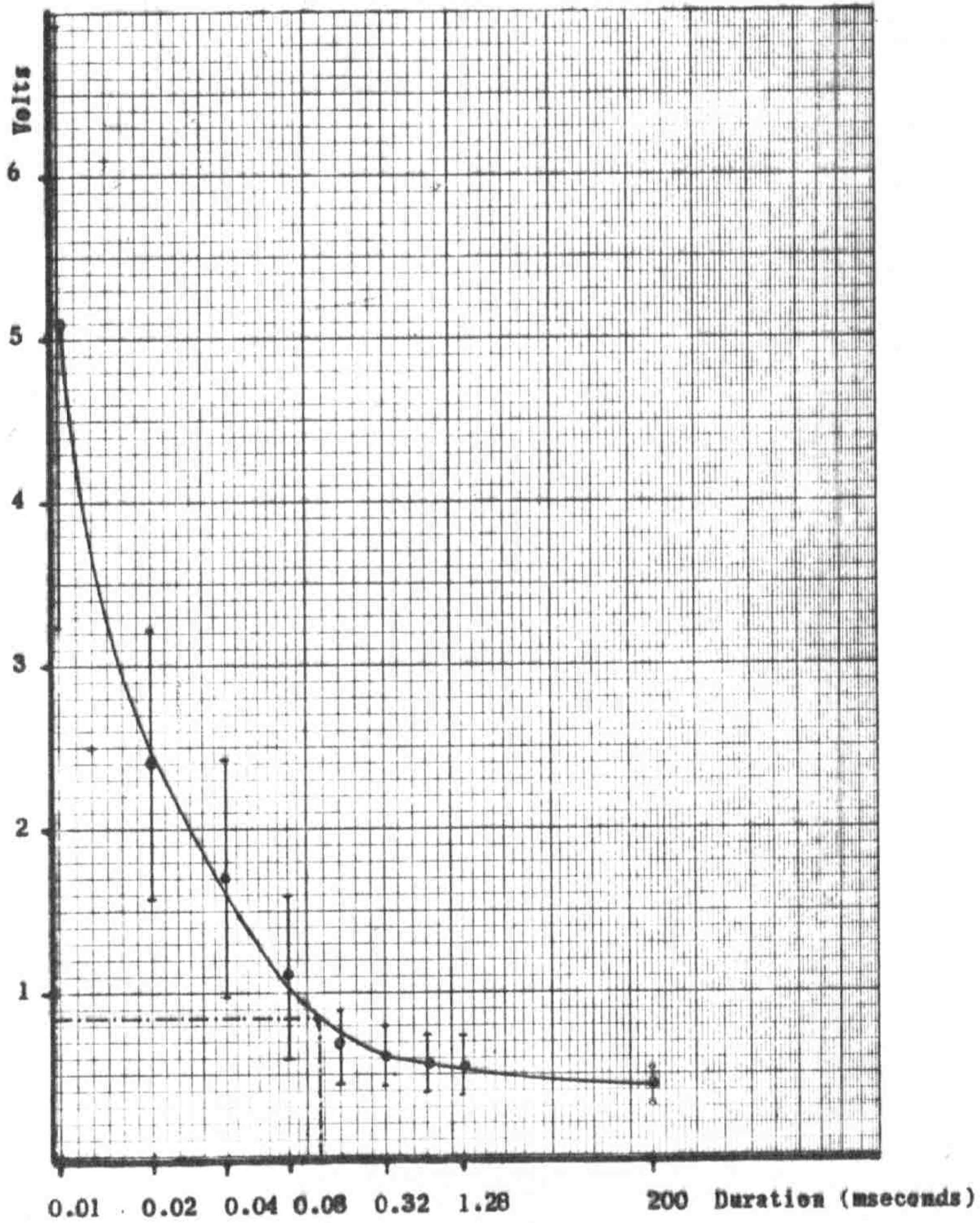
Thresholds, excitabilities, and indéces of excitability of the sciatic nerve treated with vitamin A palmitate for 45 minutes, and its control.

	Duration in milli-seconds	EXPERIMENT No.																Average ± S.D.
		1	2	3	4	5	6	7	8	9	10	5' *	6' *	7' *	8' *	9' *	10' *	
Control	0.02	0.80	3.00	1.15	1.25	0.95	1.05	0.95	1.02	1.25	0.95	0.95	1.00	0.98	1.00	1.35	0.95	1.17±0.54
threshold	0.20	0.11	0.16	0.20	0.14	0.105	0.25	0.125	0.20	0.18	0.10	0.11	0.135	0.115	0.22	0.18	0.95	0.21 ±0.20
(volts)	50.0	0.06	0.35	0.07	0.09	0.105	0.115	0.125	0.28	0.095	0.10	0.20	0.25	0.25	0.30	0.10	0.135	0.17±0.09
Experim.	0.02	0.65	1.10	0.85	1.25	0.75	0.90	0.95	0.90	1.25	0.72	0.80	1.05	0.92	0.95	1.25	0.80	0.96±0.19
threshold	0.20	0.09	0.10	0.115	0.12	0.085	0.11	0.105	0.11	0.14	0.08	0.10	0.135	0.108	0.125	0.16	0.085	0.11±0.02
(volts)	50.0	0.06	0.015	0.06	0.085	0.13	0.12	0.20	0.35	0.09	0.125	0.135	0.30	0.20	0.35	0.095	0.120	0.15±0.097
Control	0.02	1.25	0.33	0.87	0.80	1.05	0.95	1.05	0.98	0.80	1.05	1.05	1.00	1.02	1.00	0.74	1.05	0.94±0.20
Excitability	0.20	9.05	6.25	5.00	7.30	9.50	4.00	8.00	5.00	5.55	10.00	9.10	7.40	8.70	4.55	5.55	1.05	6.63±2.42
	50.0	16.60	2.85	14.30	11.05	9.50	8.70	8.00	3.57	10.50	10.00	5.00	4.00	4.00	3.33	10.00	7.40	8.50±4.10
Experim.	0.02	1.54	0.91	1.18	0.80	1.36	1.11	1.05	1.11	0.80	1.39	1.25	0.95	1.09	1.05	0.80	1.25	1.09±0.22
Excitability	0.20	11.05	10.00	8.68	8.30	11.88	9.10	9.50	9.10	7.12	12.50	10.00	7.40	9.25	8.00	6.25	11.88	9.37±1.77
	50.0	16.65	66.66	16.70	11.88	7.70	8.32	5.00	2.85	11.10	8.00	7.40	3.33	5.00	2.85	10.50	8.32	12.02±12.80
	0.02	124	275**	136	100	134	117	100	112	100	132	119	95	107	105	108	119	114±13.6
Exp.Exc. / Cont.Exc. X100	0.20	122	160**	174	114	125	227	119	182	128	125	110	100	103	176	112	113	134±27
	50.0	100	2320**	117	107	81	94	63	80	106	80	148	83	125	85	105	113	99 ±22

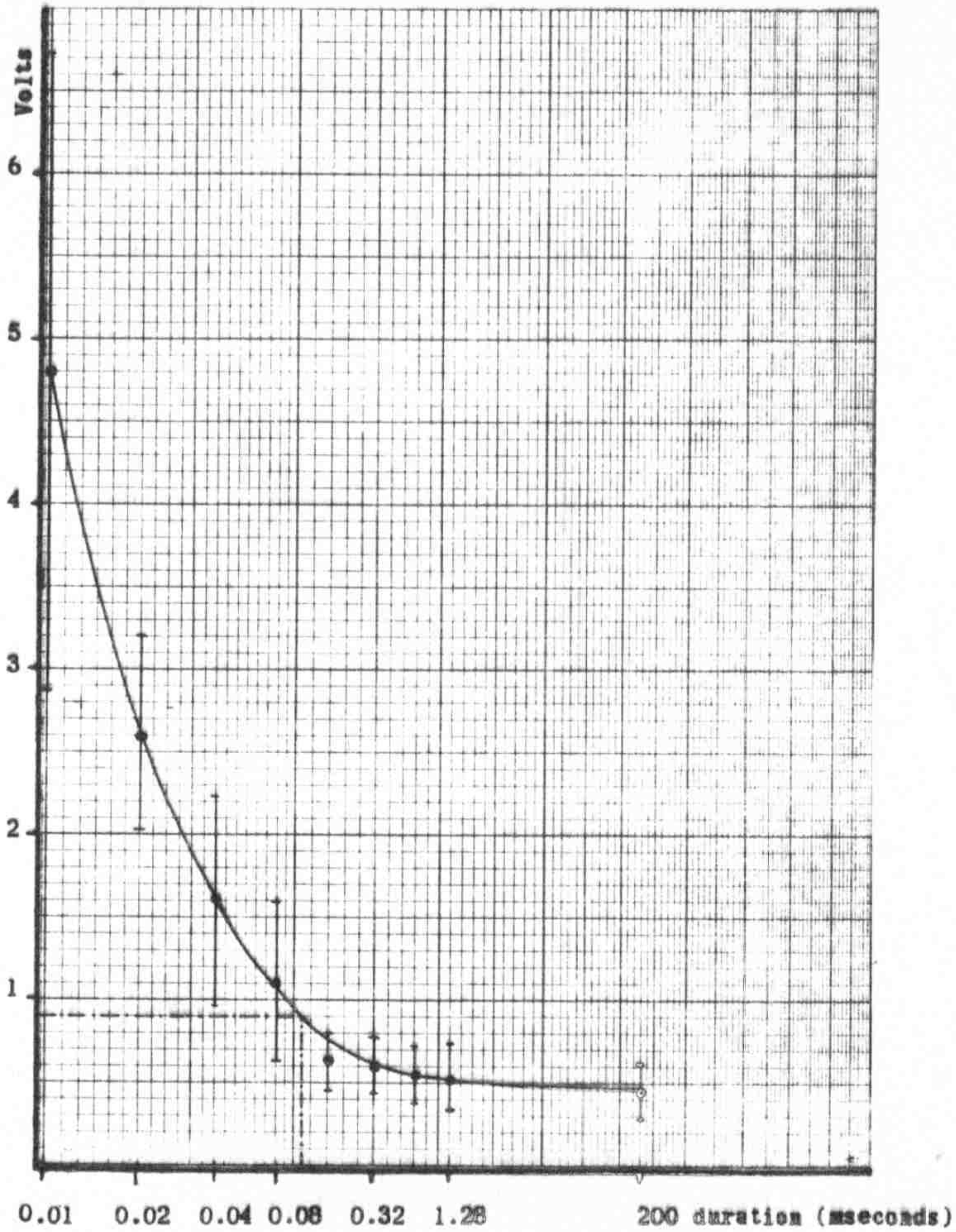
*The control of experiment 5' is the experimental of 5 after washing, and the experimental of 5' is the control of 5 after treatment. The same case with 6', 7', 8', 9' and 10' which correspond to 6,7,8,9 and 10 respectively.

** Excluded from average calculations for their obvious deviation.

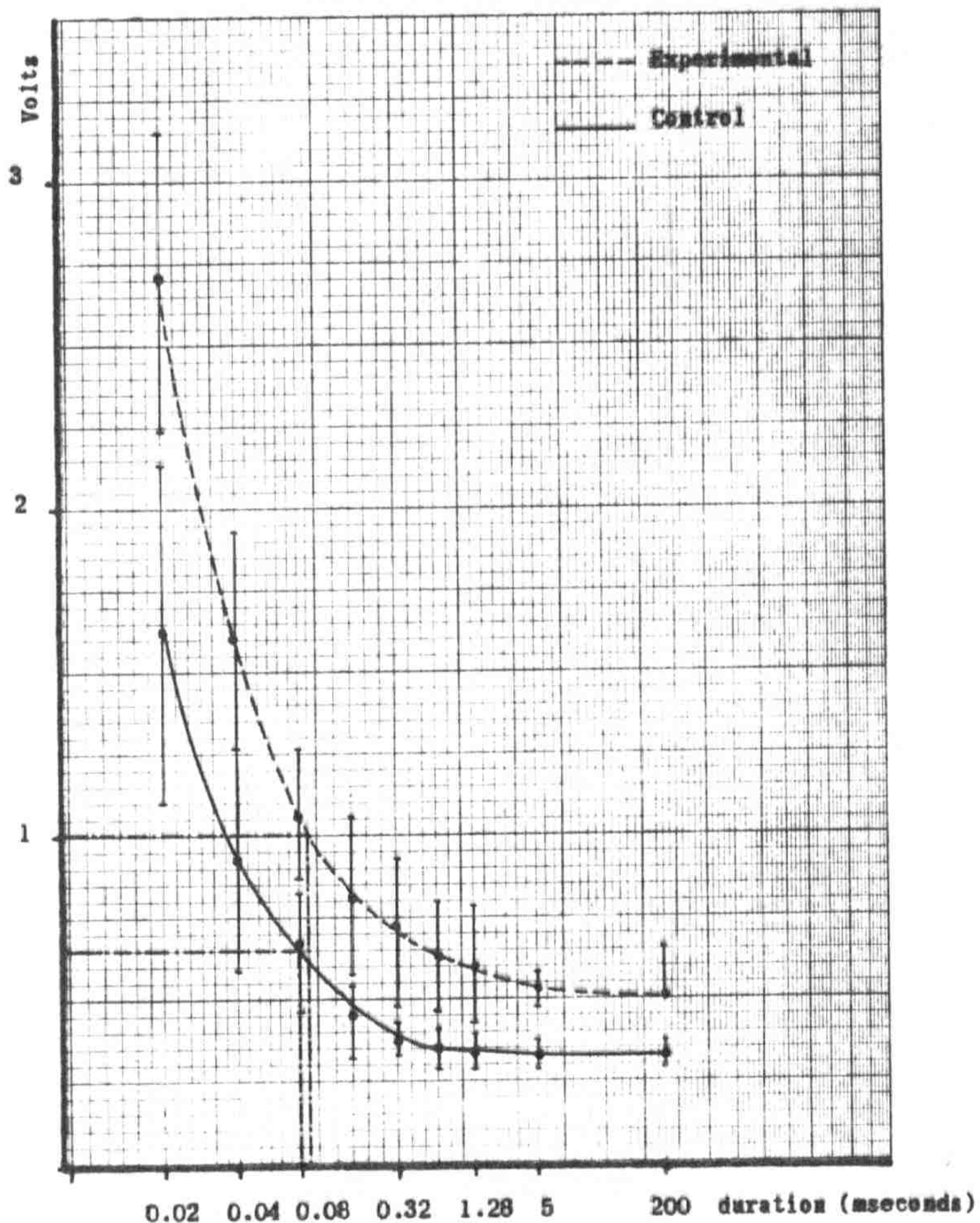
GRAPHS



Graph 1a: Strength-duration curve for the right gastrocnemius muscle stimulated directly.



Graph 1b: Strength-duration curve for the left gastrocnemius muscle stimulated directly.



Graph 2: Strength-duration curves for both experimental muscle treated with vitamin A and its control.

PLATES

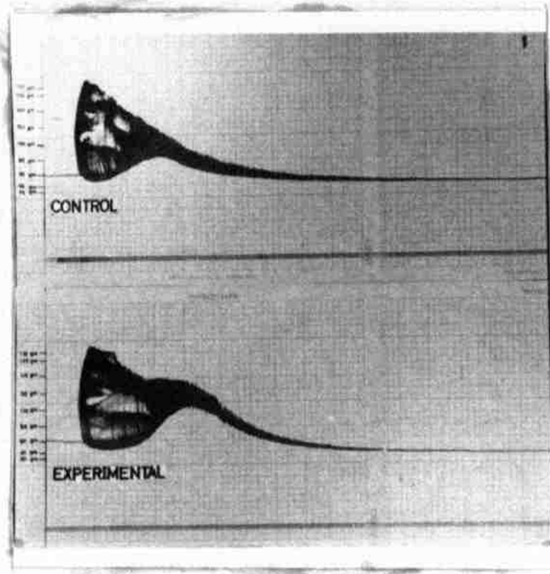


Plate 1: Isometric recording of a fatigue curve for an intact gastrocnemius muscle treated with vitamin A for 45 minutes. Control stimulated before experimental.

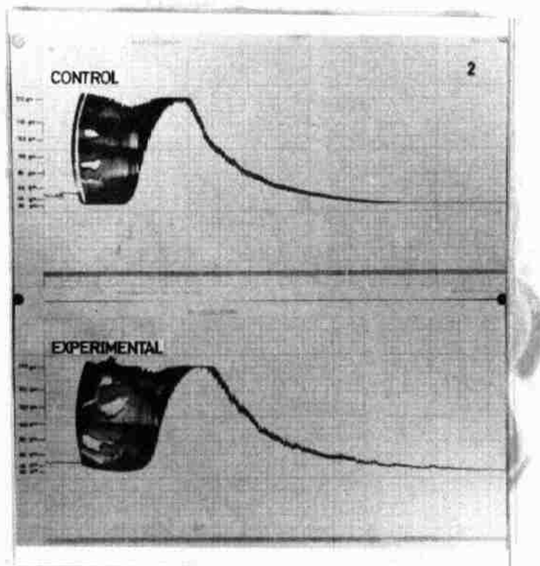


Plate 2: As in plate 1, but experimental stimulated before control.

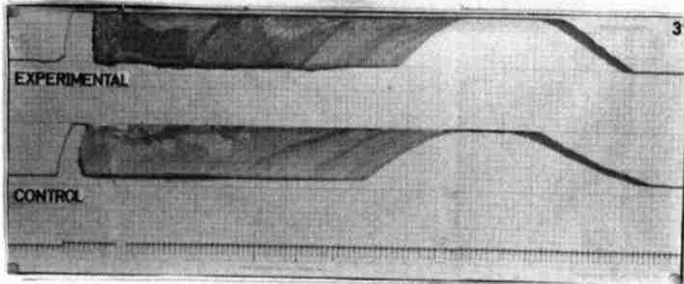


Plate 3: Isotonic recording of a fatigue curve for an intact gastrocnemius muscle treated with vitamin A. Experimental and control were stimulated directly.

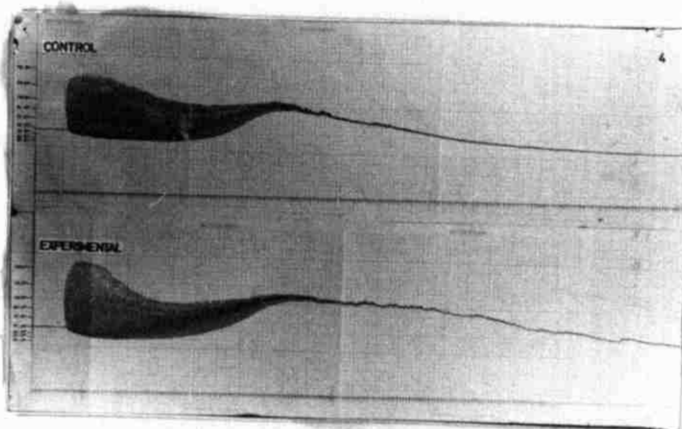


Plate 4: Isometric recording of a fatigue curve for an intact gastrocnemius muscle treated with vitamin A palmitate. Experimental was stimulated before control.

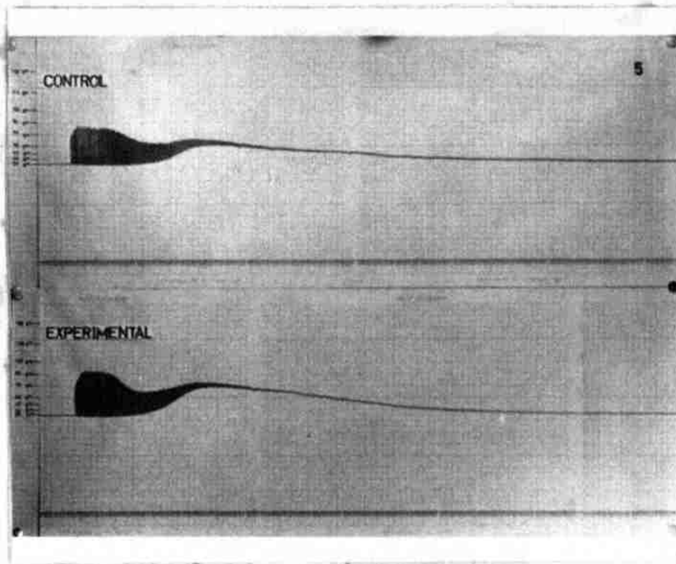


Plate 5: The same recording as in plate 4, except the control was stimulated before experimental.

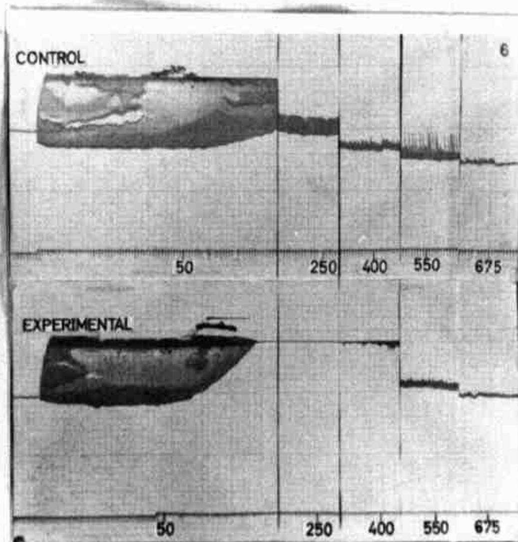


Plate 6: Isotonic recording of a fatigue curve for an intact gastrocnemius muscle treated with vitamin A palmitate. Control was stimulated before the experimental.

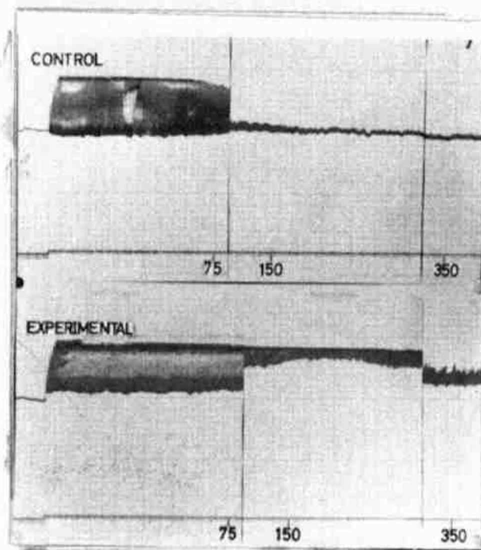


Plate 7: The same recording as in plate 6 except the experimental was stimulated before the control preparation.

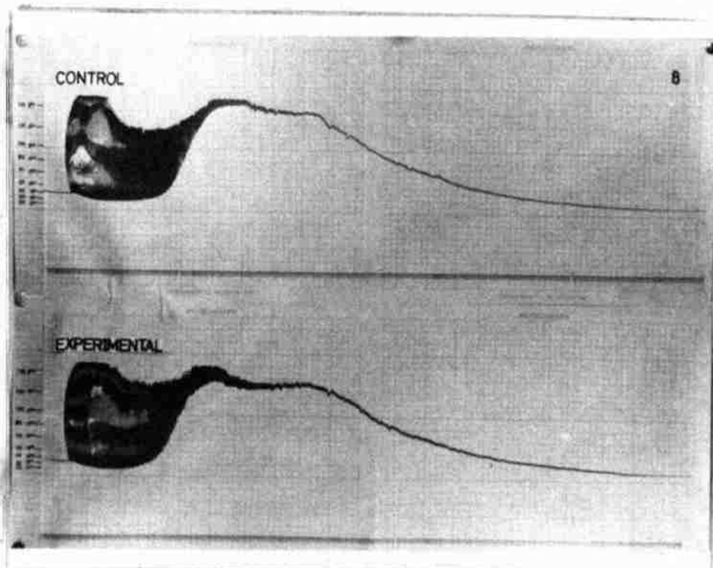


Plate 8: Isometric recording of a fatigue curve for an intact muscle-nerve preparation treated with vitamin A. Experimental was stimulated before control.

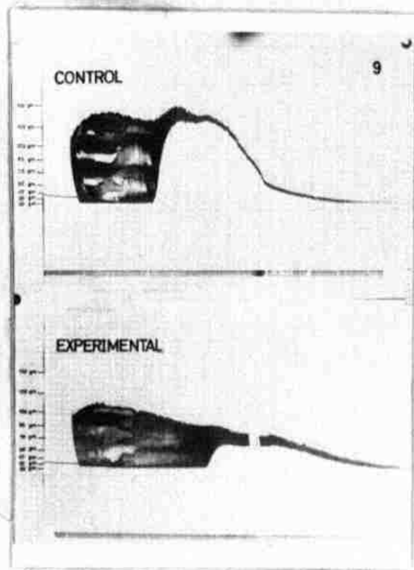


Plate 9: The same recording as in plate 8 except that the control was stimulated before the experimental.

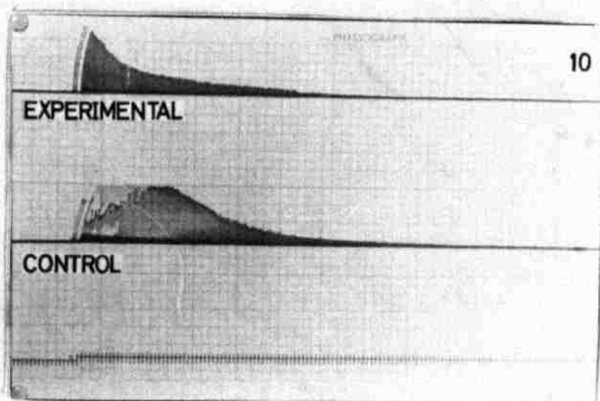


Plate 10: Isotonic recording of a fatigue curve for an intact nerve-muscle preparation treated with vitamin A. Experimental and control were stimulated simultaneously.

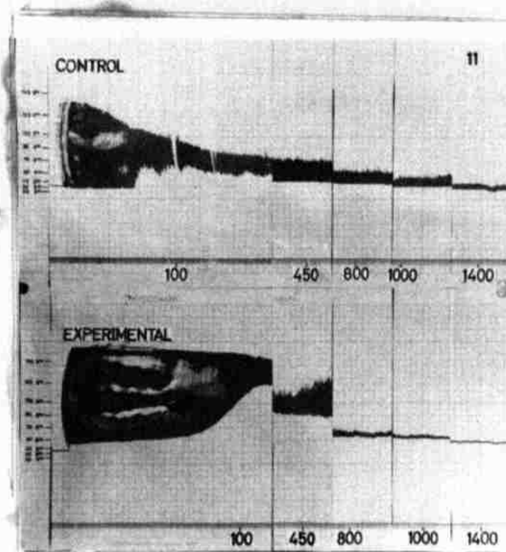


Plate 11: Isometric recording of a fatigue curve for an intact nerve-muscle preparation treated with vitamin A palmitate. Experimental was stimulated before the control.

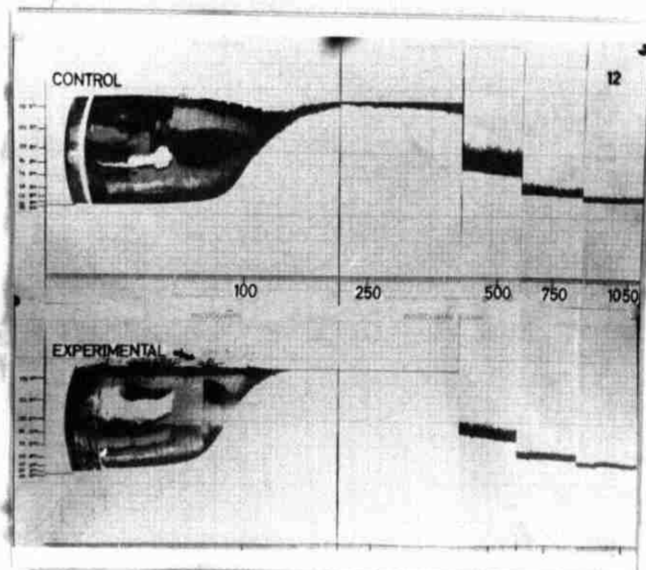


Plate 12: The same recording as in plate 11 except the control was stimulated before the experimental.

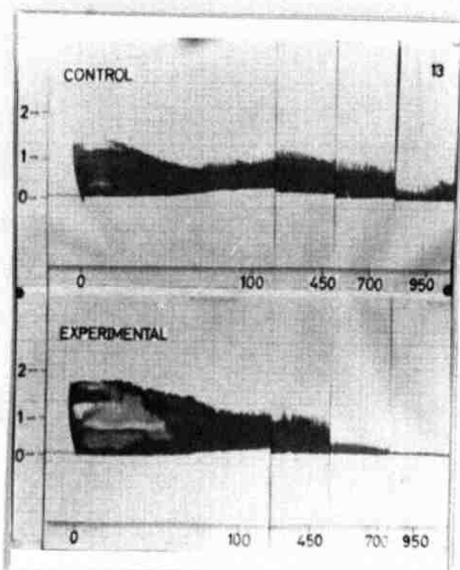


Plate 13: Isotonic recording of a fatigue curve for an intact nerve-muscle preparation treated with vitamin A palmitate. Experimental was stimulated before control.

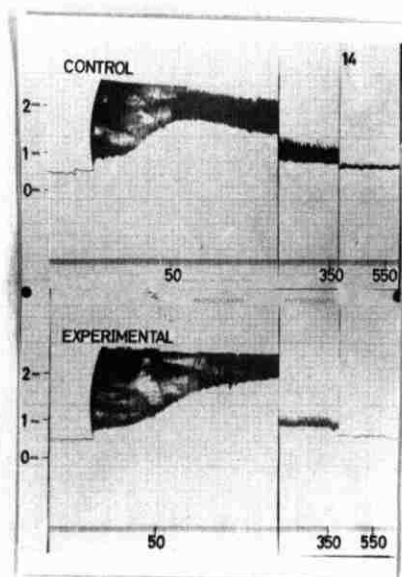


Plate 14: The same recording as in plate 13 except the control was stimulated before the experimental.

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