EFFECT OF TEMPERATURE ON NERVOUS THRESHOLD

by

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Abstract

The effect of temperature on threshold of excitation is investigated in frog nerve by the methods of Newman and Raymond (1971). In a nerve depressed by past activity threshold has a negative temperature coefficient. When temperature is changed very rapidly the corresponding change in threshold is seen to consist of two components. The second, slow component is shown to bear resemblance to the accumulation or decay of depression as defined by Newman and Raymond (1971).

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Acknowledgements

I must acknowledge a great personal debt to Dr. J.Y. Lettvin for telling me how to be a scientist. This work would not have been possible without the assistance of America's batrachian masses and the staff of R.L.E.

Lastly I dedicate this thesis to my advisor and friend, Dr. S.A. Raymond, whose empathic tendencies have caused him to suffer greatly during my many misguided efforts.

I. INTRODUCTION

> Nerve physiologists have been so concerned with mechanisms of transmission that they have paid little attention to events which persist longer than 100 milliseconds. Yet the most interesting nervous impressions are those which last for seconds, minutes, lifetimes. Systematic modifications of behavior, called conditioning, have been produced... Perhaps a comparison of persistent changes in excitability in nerve centers of varying degrees of organization may point the way to understanding this most intriguing property of nervous systems.
>
> (Prosser 1952)

The mechanisms of nervous transmission mentioned above are the fast nervous phenomena which directly give rise to the action potential. Neurophysiologists have been very successful describing these fast processes (Hodgkin and Huxley 1952, Fitzhugh 1969) in large measure because the processes seem to persist when the nerve is in a nonphysiological state. The durability of the nerve to conduct under adverse circumstances has allowed investigators to reduce all manner of electrical artifact.

The study and theory of slow electrical processes has not evolved as rapidly or successfully. Because the

slow processes are closely related to the nerve's metabolic activity or to the nerve's intimate association with other tissues, investigators have not been able to reduce electrical artifact without introducing severe physiological artifact.

A. Slow Phenomena

Gotch and Burch (1898) were the first to notice the slowly declining negative aftercurrent that follows an action current, and Forbes and Thatcher (1920) found that it might last for seconds in winter frogs. With frog nerve Boruttau and Frolich (1904) and Amberson and Downing (1929) found respectively that condition of the nerve and previous activity affected the aftercurrents. Aftercurrents were measured in these studies across a dead-live junction, and the 1920's were a time of debate as to the propriety of making measurements this way. In particular there was confusion as to the very existence of the positive afterpotential corresponding to the positive aftercurrent (Woronzow 1924). In 1930 Gasser and Erlanger by differential application of drugs and temperature changes to the nerve at the recording electrodes proved that the positive afterpotential following

a single impulse was an artifact of the dead-live junction. By 1973 Gasser was able to publish a review of after-potentials and it was perfectly clear that different nerves, even different fiber types in the same nerve had markedly different properties. The positive afterpotential was reinstated as a phenomenon of type C fibers following an impulse and of type A fibers following a tetanus. Following tetanus a positive afterpotential might last for many minutes.

B. Correlations

It was natural that physiologists should attempt to correlate afterpotentials with other measurable signs of nervous activity. In 1933 Hill showed that heat production of the nerve occurred in two phases, and that there was a temporal correspondence of these two phases to the negative and positive afterpotentials. Schmitt and Gasser (1933) poisoned a nerve with carbon monoxide, reversed the action with bright light, and concluded that the negative afterpotential is a sign of metabolic activity. This was in opposition to Levin's (1929) conclusion that the negative potential was a sign

of accumulated catabolites and was reduced by metabolic activity. Shanes (1951) and Frankenhauser and Hodgkin (1956) showed that accumulation of potassium played a role in the production of afterpotentials. It seems reasonable to conclude from this history that the afterpotentials are the net result of several oscillating processes (Gasser 1937, Shanes 1958).

C. Threshold as a Correlate of Afterpotential

By far the most striking correlate of afterpotentials has been threshold of excitation. In 1912 Adrian and Lucas gave the first lucid description of the supernormal phase of nervous excitability. Gasser (1931) showed that afternegativity and supernormal phase are both affected similarly in a variety of experimental circumstances. Graham (1935) showed under what conditions a subnormal phase of excitability might arise, and that these same conditions generally accentuated the positive afterpotential. Lorente de No (1947) gave the best description to date of the complex interrelationship of membrane potential to threshold. All other membrane parameters held equal, the excitability is a relatively simple function of membrane potential; however, the membrane

potential usually varies as a function of some other parameter of which excitability is also a function. Despite the difficulties in relating membrane potential to threshold Gasser and Grundfest (1936) felt that rather than compromise the integrity of their preparation by recording potential directly, they would infer the potentials from a study of the threshold oscillations. Recently Zucker (1973) used the same reasoning in his determination of afterpotentials by measuring threshold oscillation in crayfish motor-nerve terminals.

D. Threshold for Invadabilities' Sake

Raymond (1969) and Bittner (1968) both offered interesting evidence of the role played by a branch point in an axon. Chung et al (1970) described a system whereby an axonal arborization might function as a time domain filter. The arbor in this model does its filtering at branch points which operate by means of threshold-mediated invasion. Slow threshold oscillations are crucial in this model if the time domain filter is to operate over any significant period of time. Newman and Raymond (1971) through a novel measurement technique obtained the clearest description to date of threshold

changes strong enough to mediate invasion of axons. These changes were shown to last tens of minutes. Curiosity about what happens at a point of low safety factor when the temperature changes (as it might in any poikilothermic animal) has motivated this investigation of threshold.

E. Acclimatization and Nervous Activity

Hering found in 1883 that the reflection of nerve impulses back from the cut end of a frog nerve was successful only in winter frogs. Garten and Sulze (1913) investigating the effects of temperature on nervous activity found that previous acclimatization of the frog before sacrifice would affect the results of the experiments. Gasser & Erlanger (1930) found that the unusual afterpotentials of the winter frogs reported by Forbes and Thatcher (1920) could be normalized by holding the frog at laboratory temperatures for a week before measurement. Interestingly this property is not exclusive to poikilothermic animals. Chatfield et al (1948) found a difference in the ability of excised nerves from hibernating and nonhibernating mammals to conduct at extremes of temperature. Chatfield et al (1953) investi-

gated the feet of the Herring Gull which in the winter are often naturally at near freezing temperatures. They found that the excised peroneal nerve from a winter gull had interesting properties: at temperatures suitable for conduction in the proximal portion the distal portion was heat blocked, and at temperatures suitable for conduction into the distal portion the proximal section was cold blocked. If the gull was allowed to acclimate to room temperature for a week before the experiment the nerve would behave in a more uniform manner.

It is not altogether surprising to find that a nervous system subject to temperature changes might have evolved some mechanism to cope with these changes, and this report will attempt to shed some further light on the subject.

F. Temperature and Nervous Activity

Despite contradictory results by Gasser (1931), Tasaki and Fujita (1948), and others the literature seems to have descended quite firmly on the notion that action potential latency, duration, and peak amplitude increase with decrease in temperature. The notion is

well supported in both theory and practice (Hodgkin and Katz 1949, Schoepfle and Erlanger 1941, Huxley 1959). Maruhashi* was able to explain the results of Tasaki and Fujita as an artifact of recording in series with a substantial amount of axoplasmic resistivity which has a temperature coefficient of its own. Tasaki and Spyropoulos (1957) point out on theoretical grounds that the membrane current during an action potential is likely to be reduced by cold. Fitzhugh and Cole (1964) predict otherwise. In any case it is certain that the total net charge passed during an action potential is increased by cold, and this is well supported by nonelectrical measurement (Shanes 1954).

G. Temperature and Excitability

Whether or not a zone of low safety factor will block invasion depends upon at least these two factors: the stimulus strength applied to the zone (by means of the approaching action potential) and the threshold of the membrane beyond the zone. As mentioned above, cold increases the stimulus but there is no conclusive theory or data for the effect of temperature upon threshold. Both Tasaki and Spyropoulos (1957) and

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*as reported in Tasaki and Spyropoulos 1957

Fitzhugh (1966) emphasize that temperature-threshold relations are dependent upon stimulus form, and draw sharp distinctions between the expected temperature influence on Q_o* and rheobase. However, their predictions are at variance with each other; Tasaki's theoretical emphasis is based upon cable constants, Fitzhugh's emphasis is upon the Hodgkin-Huxley rate constants. Sjoden and Mullins (1958), Guttman (1962, 1966), Tasaki and Fujita (1948), and Tasaki (1949) have all investigated temperature-threshold relations. The results do not fall into a pattern; e.g. Tasaki** was plagued by an hysteresis phenomenon, and all of the techniques are subject to serious criticims (e.g. the sucrose gap technique causes hyperpolarization of the membrane under test). It is the intent of the author to investigate the effects of temperature changes upon threshold using primarily the methods of Newman and Raymond (1971).

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*minimum excitory charge

**discussed further on page 34

II. METHODS

The frogs used in this research varied at the discretion of the supplier. It is unfortunate that both northern and southern varieties were provided. All frogs were kept for at least a week at 18-19° C before sacrifice.

The sciatic nerve was excised with great care to prevent any damage to the epineurium, particularly in the proximal portion which was to be stimulated. The nerve was mounted in the chamber as illustrated in Figures 1 and 2. The proximal portion of the nerve was tied off with ordinary cotton sewing thread, and the thread was pulled taut until the nerve was just about to slip through the vaseline seal.

The silver block was chloridized on its internal surface and a silver-silver chloride electrode was mounted upon a micrometer head for precise control of position. The nerve was always positioned such that the stimulating electrode intersected the nerve at least 2 1/2 centimeters from the cut end. The stimulus isolation unit provided cathodal (with reference to the silver block)current pulses (normally used in the range .05 - .05 milliamps) of duration controlled by Dr. S. Raymond's* hunter circuit.

*described in Newman and Raymond (1971)

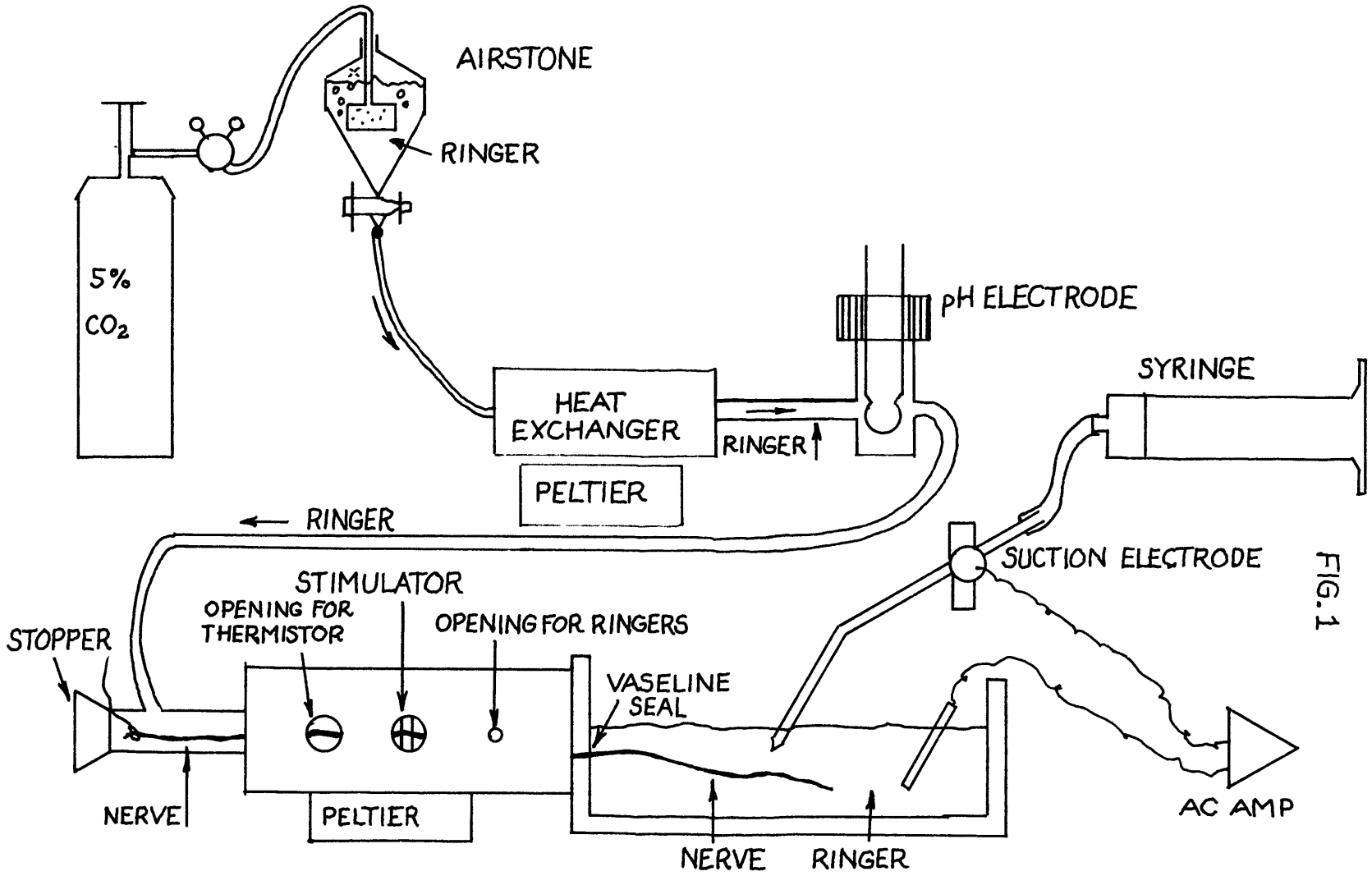
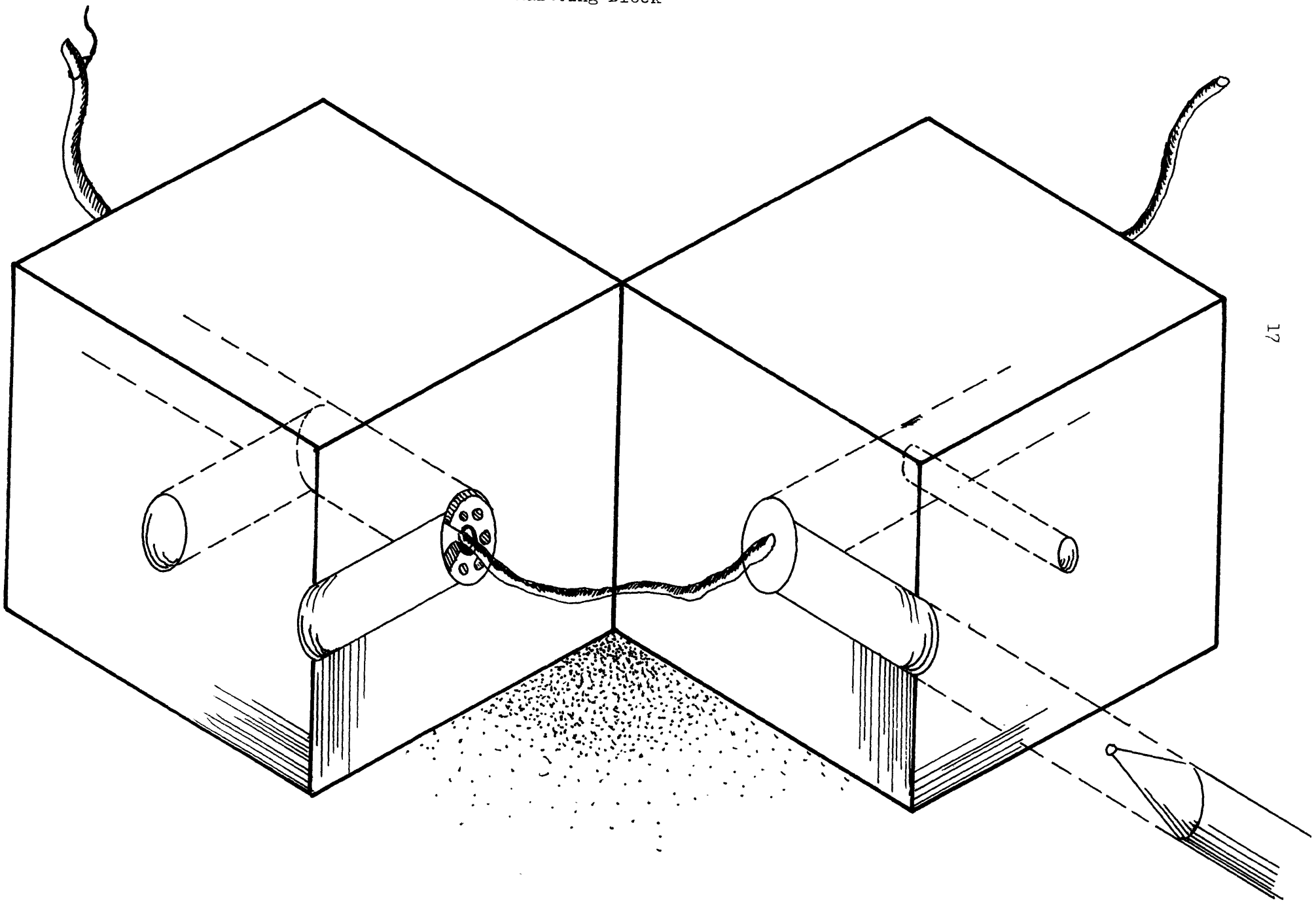

AIRSTONE
RINGER
5%
CO_2
pH ELECTRODE
SYRINGE
HEAT
EXCHANGER
RINGER
PELTIER
RINGER
SUCTION ELECTRODE
STIMULATOR
OPENING FOR
THERMISTOR
OPENING FOR RINGERS
STOPPER
VASELINE
SEAL
NERVE
PELTIER
AC AMP
NERVE
RINGER

FIG. 1

Figure 2

Silver Stimulating Block



The silver block was mounted upon a Cambion Thermionic Devices Peltier device. A thermistor mounted inside the silver block in conjunction with a temperature control circuit was capable of holding the silver block at any temperature between 12 and 25° C with fluctuations less than 0.3°C. When changing temperatures the system would reach a new set point at a rate of 4° C per minute.

Both the silver block and the recording dish had their own ringer supplies. That ringer which perfused the silver block first passed through a preheater (precooler) also operated by the temperature control circuit. Following this step it was led past a glass pH electrode and then into the silver block. It emerged from the block and was pumped back up to the aerating chamber from which it would recirculate under the force of gravity.

The ringer was that used by Newman and Raymond (1971) but it is reprinted here because of a typographical error in their publication:

Salt	Millimolarity	Salt	Millimolarity
NaCl	80.5	Na_2SO_4	0.66
KCl	1.99	Na_2HPO_4	2.55
$NaHCO_3$	25.01	KH_2PO_4	0.52
$CaCl_2$	1.70	$C_6H_{12}O_6$	3.3
$MgSO_4$	1.17		

The ringer was aerated by a humidified 5% CO_2 in air mixture which held the pH at about 7.4. The pH never varied by more than .02 units during the course of an experiment. Although the nerve might remain functional for up to 72 hours, all experiments were done within 12 hours of pithing the frog.

After loading the nerve in place, and with the stimulating electrode advanced to within close proximity of the nerve, the stimulator would be set to deliver a pulse every 2.5 seconds. A small group of fibers was teased away from the nerve in the recording chamber and aspirated into a suction electrode. The fibers were cut or crushed one by one until only one action potential remained upon the monitoring oscilliscope. In order to adjust the position of the stimulating electrode the circuit was set to hunt the threshold of this single unit. If the threshold plot was unsteady as in the left hand portion of Figure 3 the electrode would be advanced very slightly (corresponding to time "A" in Figure 3). Moving the electrode closer to the nerve would make the stimulus more effective and thus the hunter would hunt a lower threshold. The unsteadiness of the trace in the left hand portion of Figure 3 is probably due to motion of the nerve as the ringer flowed past. After time "A" the electrode was

3-26-75

Fig 3

probably in contact with the nerve.

The stimulus durations used in this report varied from 50 to 250 microseconds. This range falls upon the hyperbolic portion of a frog strength-duration curve (Verveen 1961).

Because a gross nerve is being stimulated it is important to be sure that the threshold of the fiber that is being recorded is not affected through ephaptic contact with its neighbors. Pecher's (1939) demonstration of the independence of two similar fibers in an axon bundle can be invoked as long as resting threshold is being measured. To be certain that threshold oscillations, such as the supernormal period, are not ephaptic artifacts Newman and Raymond (1971) devised a control experiment and showed the absence of ephaptic-like interaction.

III. RESULTS

A. Resting axon

Most axons are considered to be in their resting state when they conduct impulses less often than once per two seconds. When in this rested state on axon would maintain stability (as in figure 3 after time "A") for several hours with less than 3/4 centimeter of drift. This corresponds to a 5% variation in stimulus strength.

1. Slow temperature changes

When changed slowly in the range 10° to 24°, temperature had no effect upon threshold in most fibers tested. The threshold of occasional fibers would increase as temperature was lowered below lab temperature (18°C), however these fibers could be shown to be depressed* at the temperatures below 18°.

2. Fast temperature changes

Figure 4 displays the result of an experiment on a rested axon when the temperature was changed very rapidly. The threshold is measured two seconds after a

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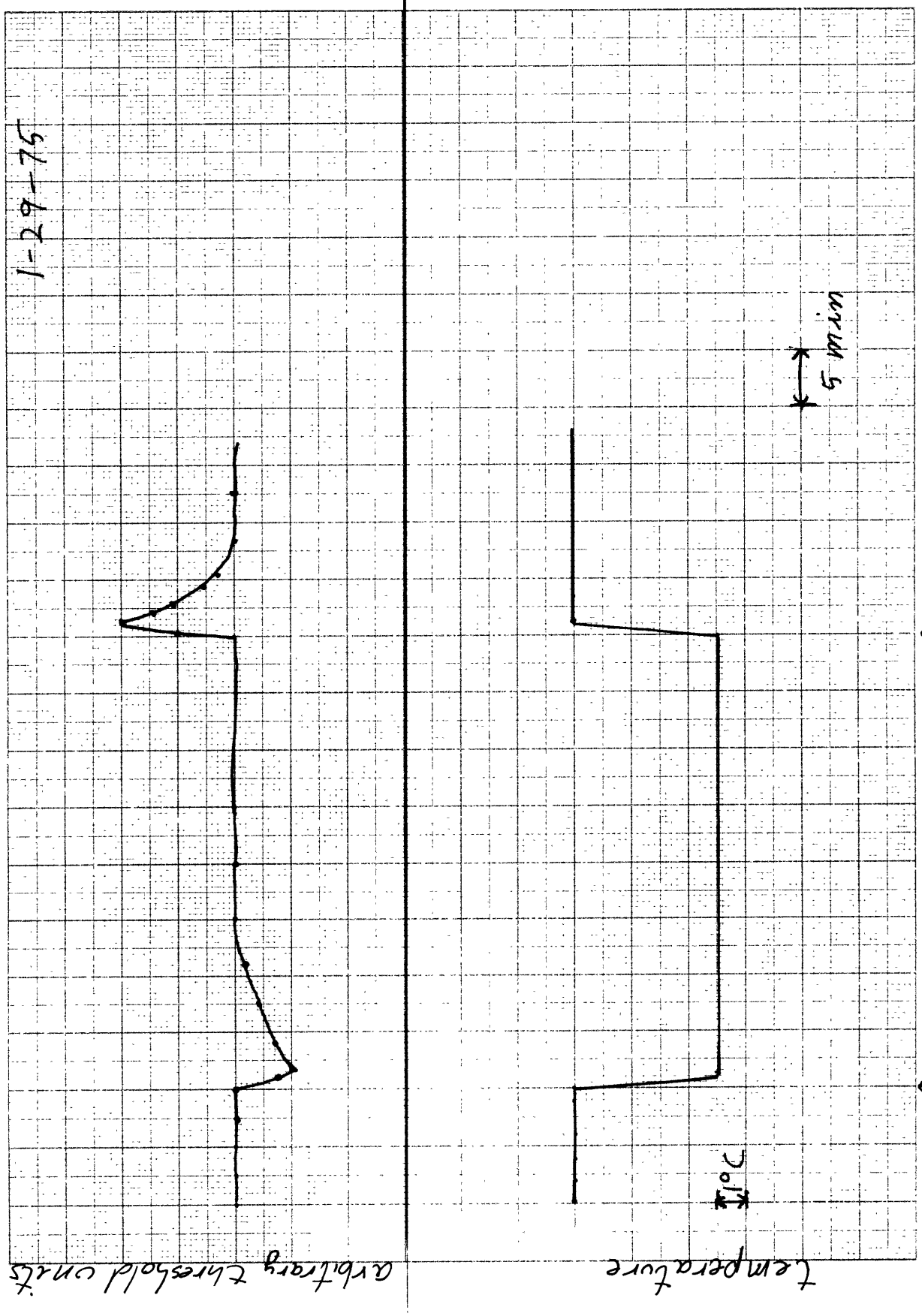
*depression as defined in Newman & Raymond (1971)

Fig 4


Temperature
Arbitrary threshold units
1°C
5 min
A
B
Time
1-29-75

conditioning impulse which is delivered to the nerve every four seconds. At time "A" the temperature was dropped from 18° to 13° and at time "B" raised back to 18°. As in the experiment with slow temperature change there was no net difference in threshold after changing temperature, but the striking result of changing temperature rapidly was that threshold temporarily changed. When the nerve was cooled(heated) rapidly the threshold quickly (1 minute) decreased (increased) and then slowly (5-15 minutes in figure 4) recovered its initial value.

There was some variation of this "recovery" among different axons, and within the same axon as its age increased. In particular, the "recovery" would often take as long as 30 minutes, rarely exceeding 45 minutes.

In all axons the initial rapid deflection of threshold was greater when temperature was raised than when lowered. In those axons which became slightly depressed at lower temperatures, the time course of threshold to a sudden temperature drop was similar to the threshold change shown in figure 4, with the exception that the "recovery" overshot its original position.

3. Threshold Oscillation

Newman and Raymond (1971) showed that supernormality

in a rested axon lasted about a second. The 18° curve of figure 5 is in excellent agreement with their results. Upon cooling, the relative refractory period and the slightly decreased peak of supernormality were delayed. This is in excellent agreement with the results of Gasser and Erlanger (1930) although their supernormality only lasted about 200 milliseconds.

4. Sudden temperature change in the supernormal period

Figure 4 is a graph of threshold vs time where the threshold is taken at a fixed delay of two seconds after conditioning. If the fixed delay was set in the range 15-1000 milliseconds (supernormality) figure 4 would remain a good representation of a sudden temperature change; however the initial and final values of threshold would be as given in figure 5.

B. Depressed axon

The initial segment of figure 6 shows the threshold of an axon measured 3 seconds after a single conditioning pulse which is repeated every four seconds. The axon is in its resting state. At time "A" the conditioning pulse was made a conditioning volley (10 impulses at 30 impulses

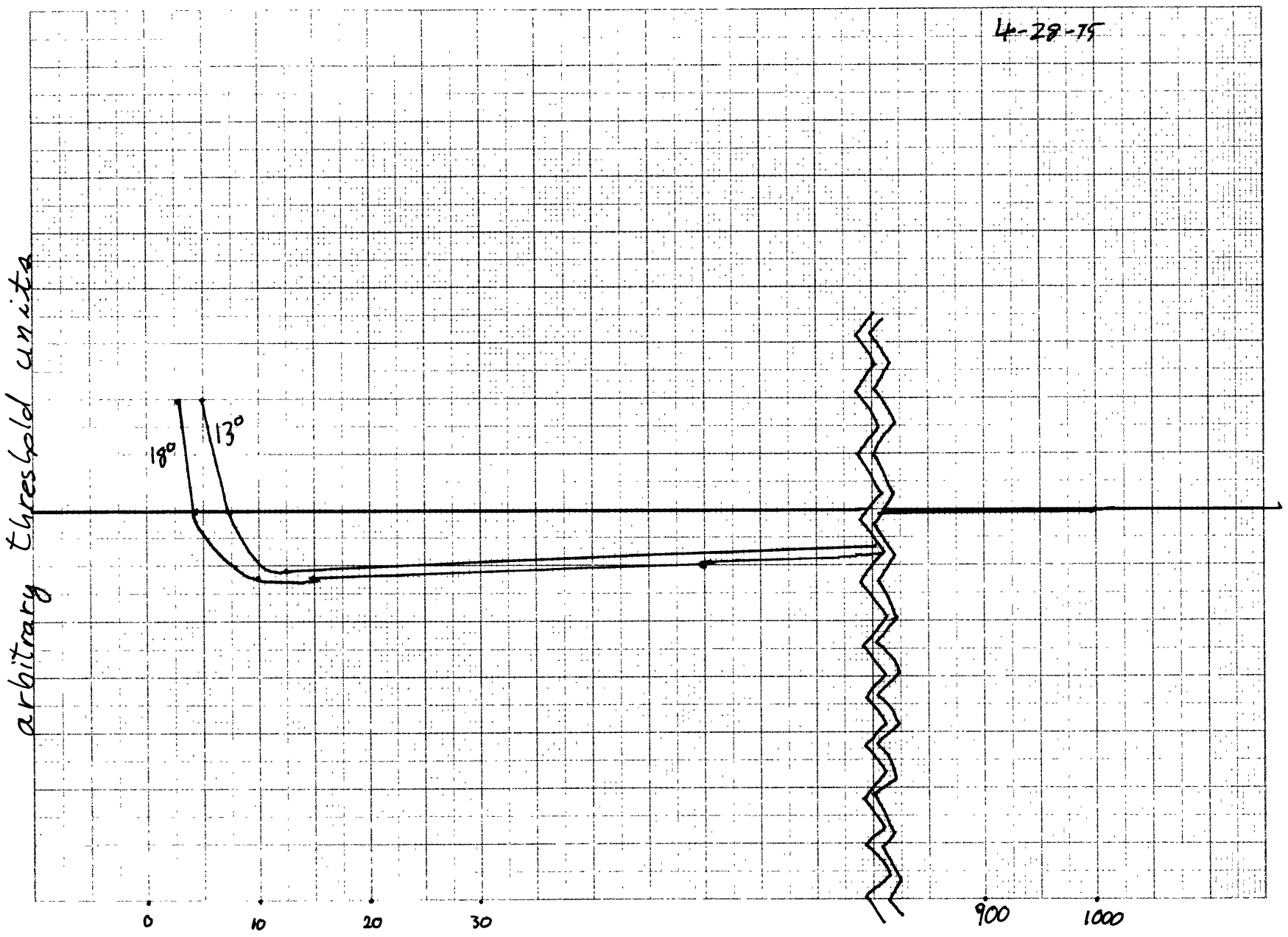

4-28-75
arbitrary threshold units
18°
13°
0
10
20
30
900
1000
Delay after single conditioning stimulus in milliseconds

Fig 5

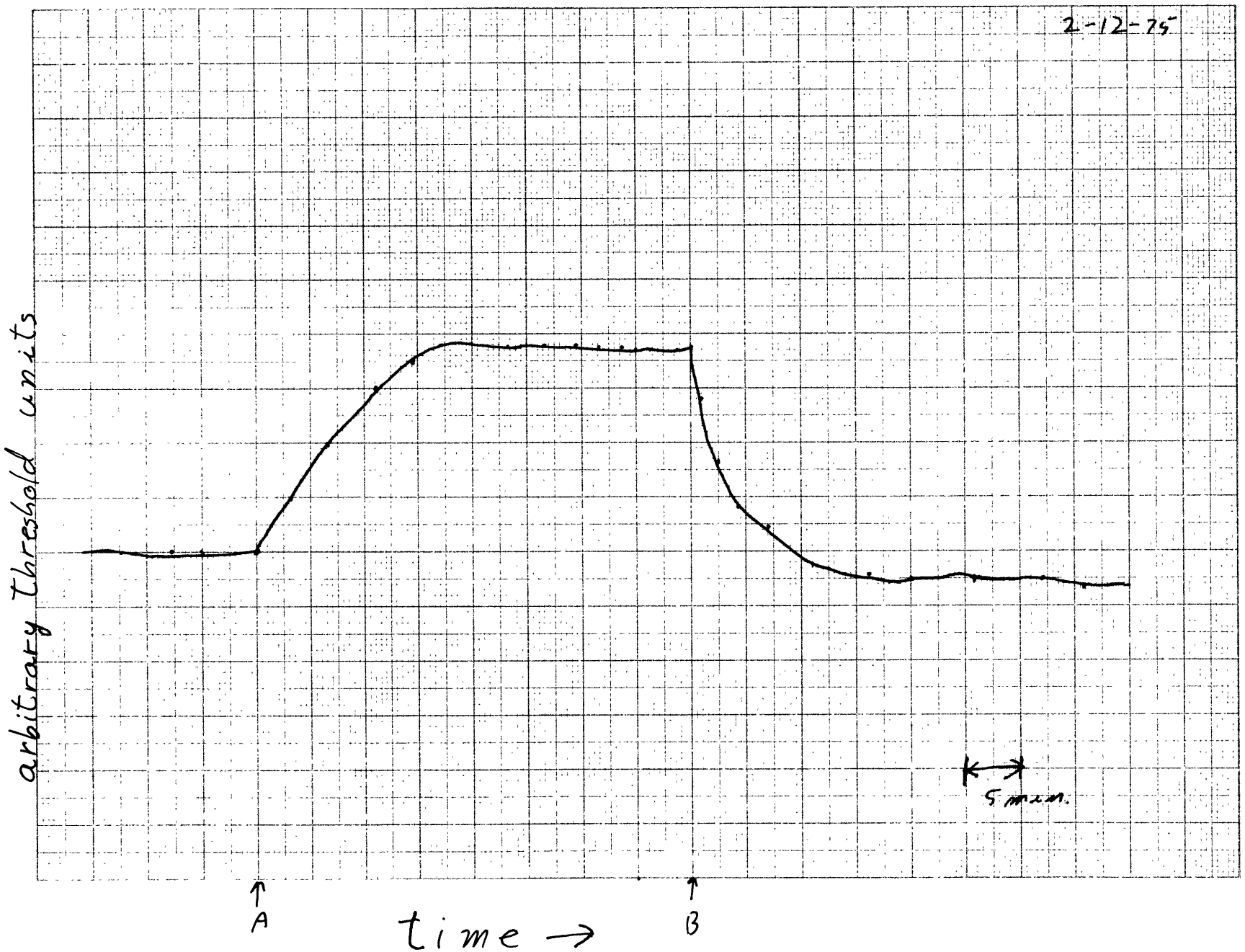


Fig. 9

per second) and at time "B" the initial conditions were reinstituted. The total accumulation of depression was quite variable from fiber to fiber, and in the same fiber as the fiber aged. The rates of accumulation and decay were also quite variable, depression often taking up to an hour to accumulate or decay. Newman and Raymond (1971) did not report such long time periods.

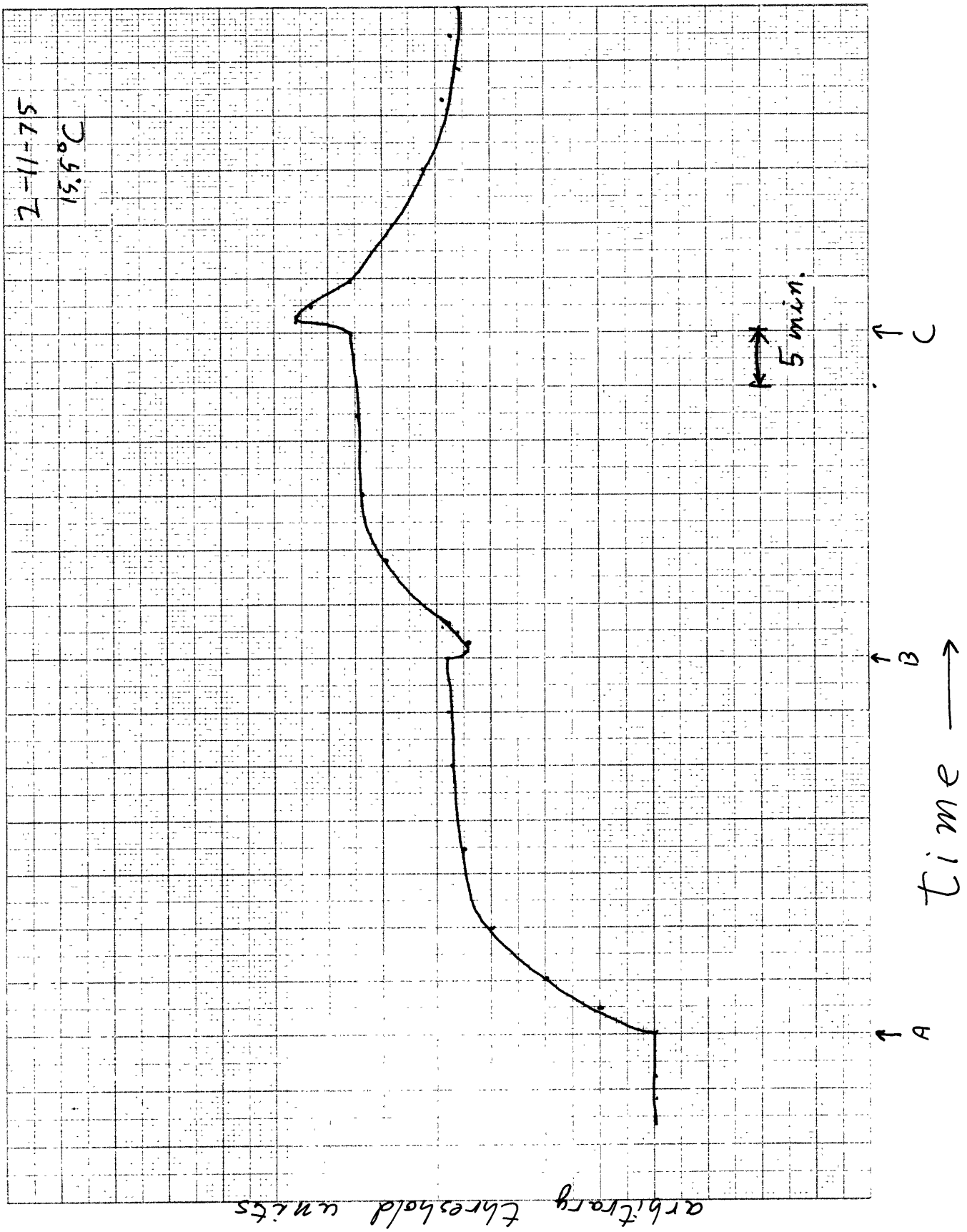
1. Temperature and depression

An experiment designed to show the effect of temperature upon rate of accumulation (or decay) of depression could not be done conclusively because the experiment would have to last about 5 hours during which time drift and ageing would cloud the results. The amount of depression in a fiber was always decreased with increase in temperature. This is illustrated in Figure 7 which is recorded in the same manner as figure 6, but at time "B" the temperature is suddenly dropped from its initial value (17.5°) to 14.5°, and at time "C" raised back to 17.5°.

2. Threshold Oscillatons

Figures 8 and 9 both show the time course of threshold, measured at variable time after the last stimulus delivered in the conditioning volley (10 impulses

Fig. 7



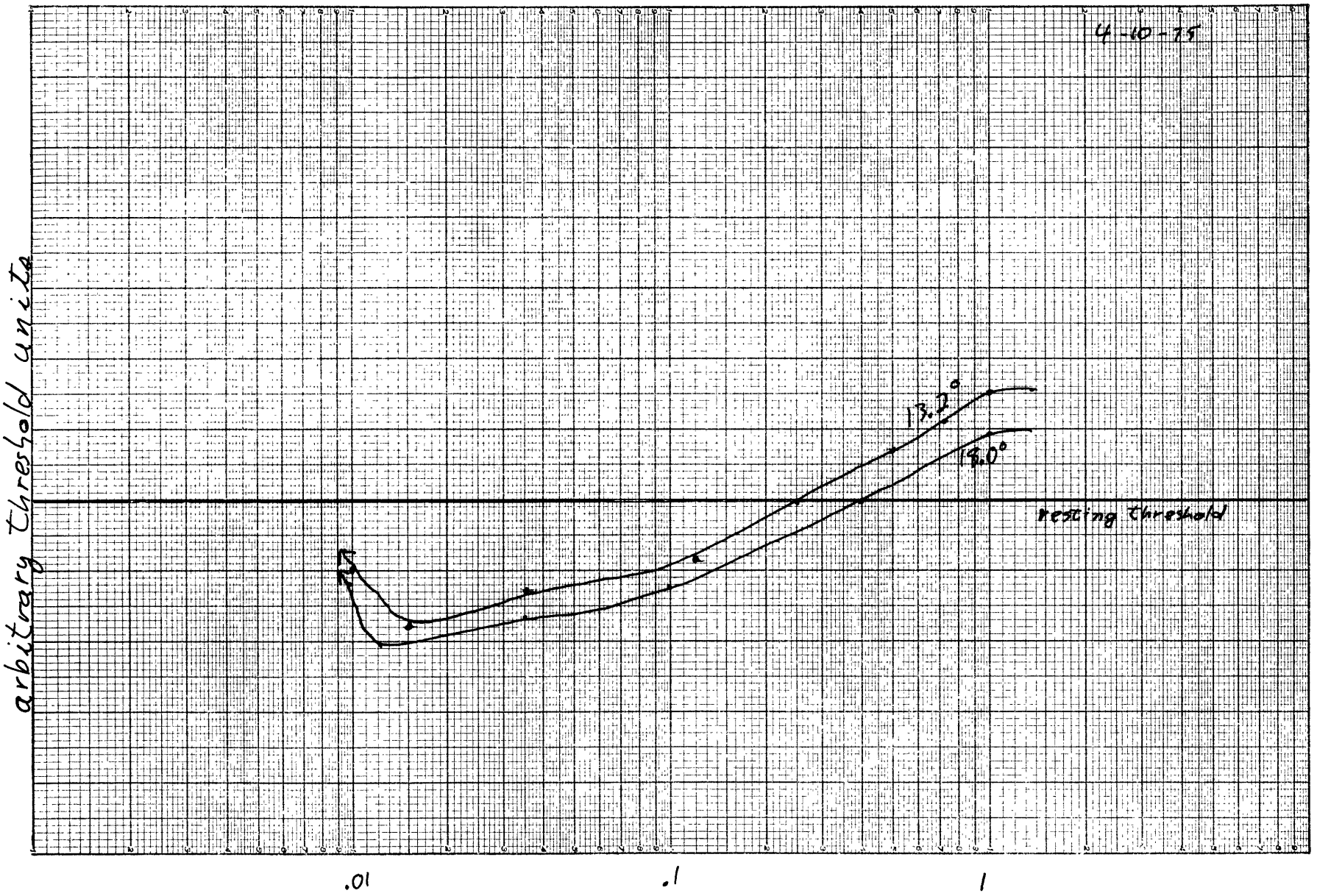


Fig. 8

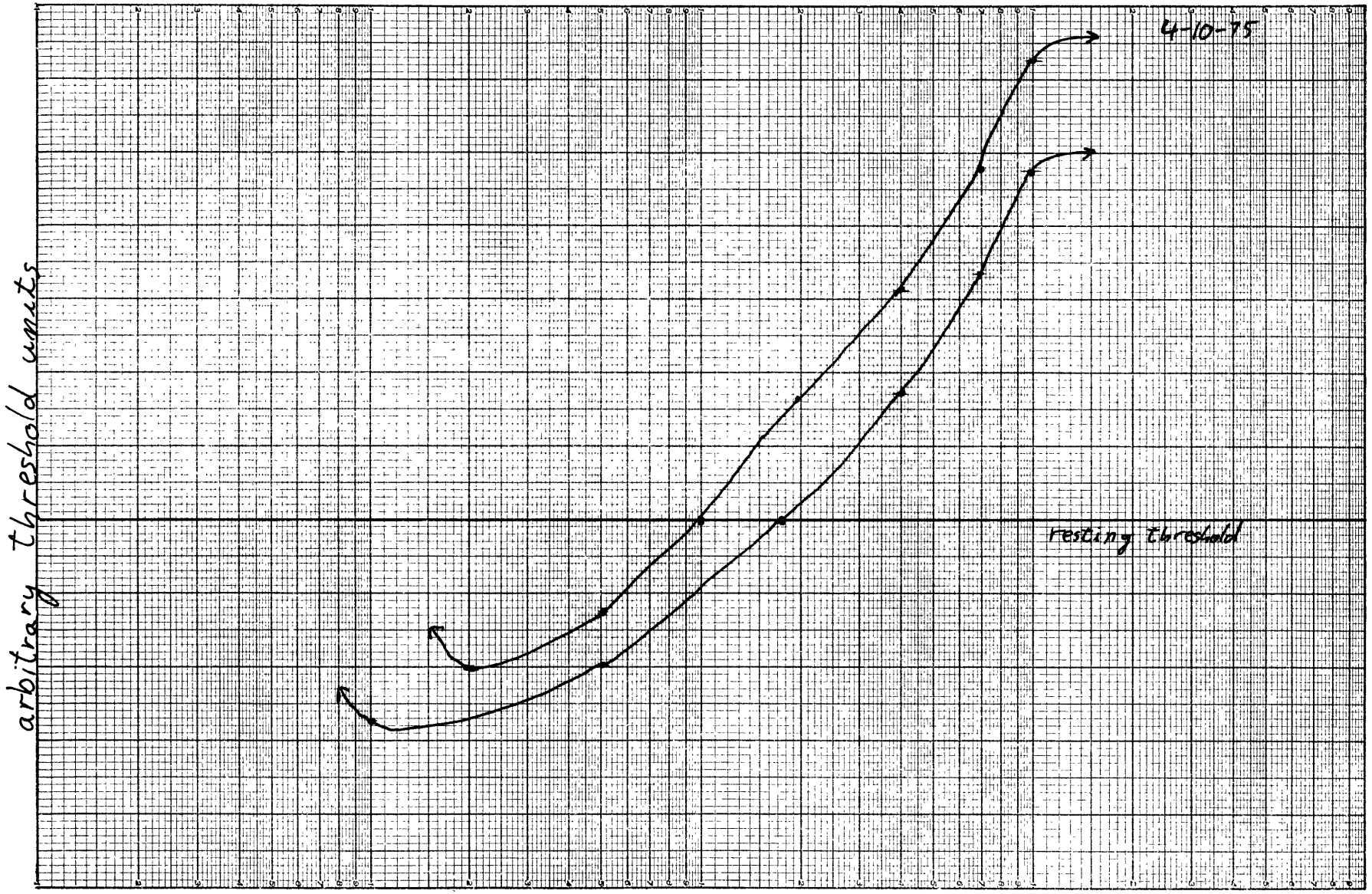


Fig. 9

at 30 impulses per second repeated every 5 seconds). The fiber recorded from in figure 8 was slightly depressed by this proceedure and reached peak depression in about 1.5 seconds. The highly depressed fiber of figure 9 reached peak depression in about 2 seconds. Newman and Raymond found that in all depressed axons peak depression was reached in 1-3 seconds.

3. Sudden temperature change in supernormal or depressed phase

If a threshold vs time graph (such as figure 7) was plotted with any fixed delay of 20 milliseconds or more after the conditioning volley, the threshold would undergo a transition qualitatively similar to figure 7 but with initial and final threshold values consistent with the data plotted in a graph such as figure 8 or 9.

IV. DISCUSSION

A. Methods

1. Circulation

Liesse (1938) and Parrack (1940) pointed out that there are small differences in the excitation characteristics of nerves which are excised with respect to nerves in situ, and that the reason for this is the lack of natural circulation in the excised tissue. Feng et al (1950) and others have shown that brisk perfusion of a nerve by ringers solution can, in many ways, negate the need for a natural circulation.

The nerves used in this study are suspended in a 1/4 inch diameter canal (through the silver block) through which ringers solution briskly flows. The point made by Liesse and Parrack is well taken, however the stability of the threshold over long periods and the longevity of the nerves used in this report are convincing evidence that the nerves were in physiologically sound condition.

2. Ageing and deterioration

Ageing and deterioration of the nerve was manifest through a tendency for the value of threshold to drift

slowly, especially when the nerve was depressed or heated above room temperature.

When data such as figure 8 was recorded, at least 45 minutes elapsed after the first trace (18°) before the second trace (13°) was taken. The 45 minute time interval was necessary to allow the threshold to stabilize at its new value. After another 45 minutes a trace was taken again under the initial conditions. If there was any significant difference between the two initial (18°) curves the data was not used. Due to the increased aging and deterioration of the nerve at warm temperatures, it was most difficult to obtain data above 18°.

B. The Time Course

There has been no mention in the literature of any threshold response to sudden temperature change similar to the data presented in this report. Tasaki (1949) has reported a hysteresis associated with threshold measurements in his attempt to describe excitability in terms of temperature.Because it is sometimes necessary to wait 45 minutes between taking threshold measurements at two different temperatures, waiting a lesser time could give the illusion of a hysteresis.

The time course that threshold follows after a sudden change in temperature is not unique in the neurophysiological literature; an initial deflection followed by a slow recovery has been reported for membrane potential and spike height under certain conditions.

Lorente de No (1947) reported that a frog nerve in air if exposed to CO_2 would quickly hyperpolarize and than slowly depolarize. Crescitelli (1957) reports that in a nerve which has been sodium blocked, application of sodium and potassium will quickly relieve the sodium block. but that slowly a potassium block would be set up. The action potential in this case would increase and decrease in much the same manner as threshold in response to sudden heating. The mechanisms behind these processes have not been elucidated although Shanes (1958) has suggested that the action of CO_2 causes a sudden decrease in P_{Na} followed by either slow movement of potassium out of the cell or a slow decrease in P_K. The phenomena discussed in this section have been presented only because their interesting temporal resemblence to some of the phenomena presented in the results section.

C. Speculations

What is to be presented in this section is pure

speculation. It is useful in that it suggests meaningful experiments that may be done to aid in the elucidation of a mechanism for the action of temperature upon threshold.

1. The fast threshold deflection

There is compelling theoretical evidence (Fitzhugh 1966) that a sudden change in temperature should cause a sudden change in threshold. As Fitzhugh points out it is extraordinarily difficult to actually predict magnitude or direction of the effect in a real system.

A speculative explanation for the quick threshold deflection is that increase (decrease) of temperature increases (decreases) the current through the sodium pump which hyperpolarizes (depolarizes) the membrane and therefore raises (lowers) the threshold.

2. The "recovery" speculation

All other things being equal an exchange of extracellular sodium with intracellular potassium will raise threshold (assuming that the concentration of extracellular potassium is not changed). This is because increasing the intracellular sodium concentration

will have an effect upon the sodium potential. When the potential is reduced there is less driving force in the sodium activation process necessary for activation.

The concentration of intracellular sodium is increased by nervous activity and by membrane leakage. It is reduced by the action of the sodium pump. The "recovery" that follows a temperature change is actually a readjustment of the intracellular sodium concentration, as is the accumulation and the decay of depression.

Newman and Raymond have shown clearly that the accumulation of depression is related to how often the nerve is stimulated. After a sudden temperature change it would not be surprising to find that the rate of "recovery" is function of how often the axon is tested for threshold. Cold is known to reduce membrane leakage and at the same time increase the ionic debt associated with the conduction of each impulse; "recovery" would than be hastened (hindered) after cooling (heating) by testing for threshold.

The rate of recovery (after heating) is increased by higher temperatures because the pumping capacity of the nerve is increased with temperature. This effect would be opposed by the increased membrane leakage at

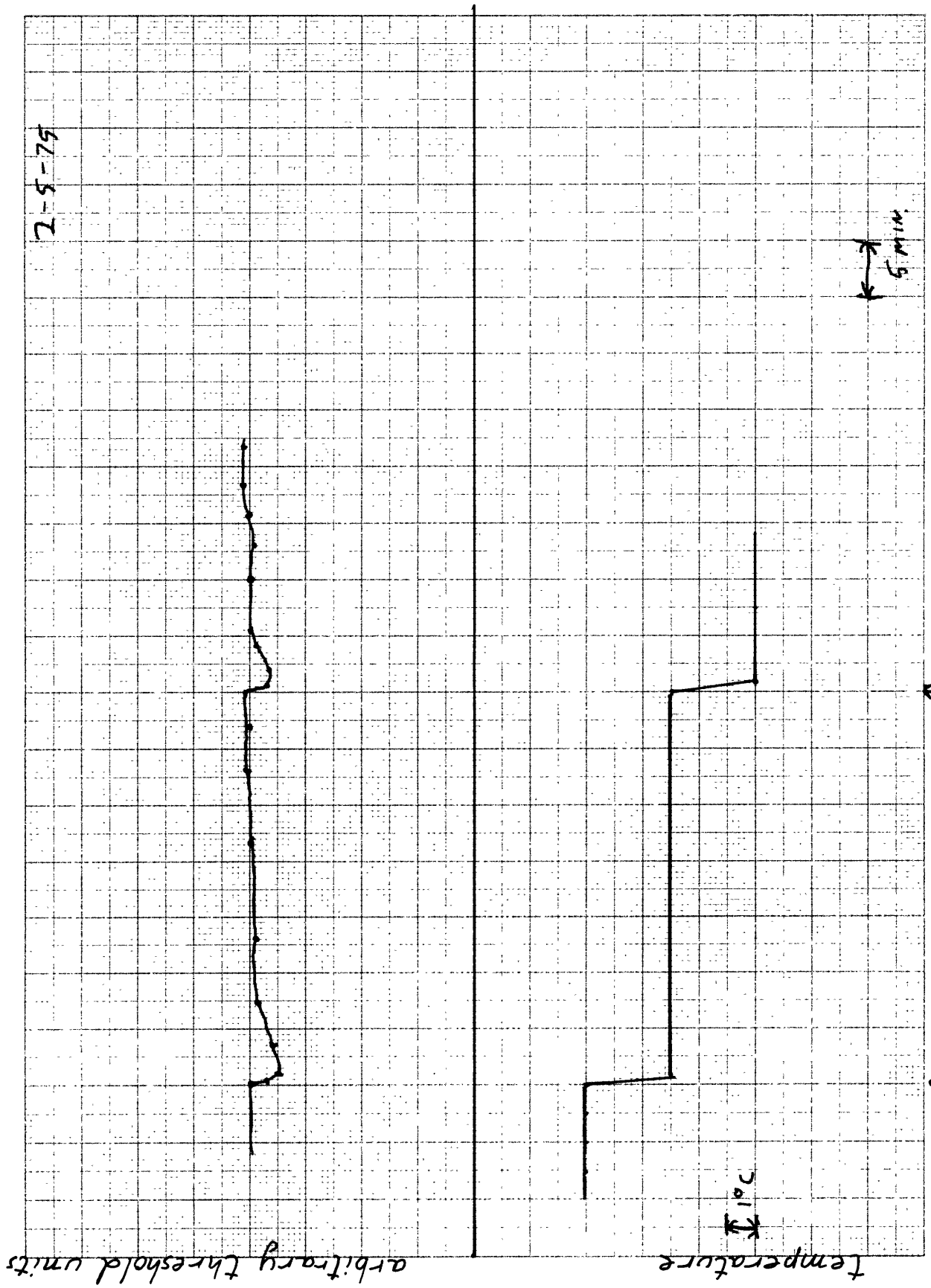
higher temperatures; however Shanes (1958) points out that the cell may expend energy to exclude (as opposed to transport) sodium, thereby effectively reducing its leakage permeability.

The rate of "recovery" after cooling is increased by lower temperatures, since the "recovery" is actually an accumulating sodium debt. This is seen in figure 10 where the temperature is lowered at times "A" and "B". The experimental use of sodium pump inhibiters should shed some light upon the soundness of these speculations.

D. Invadability

A nerve fiber active at physiological frequencies would normally be somewhat depressed. If depression is the result of a pumping debt, than surface to volume considerations predict that smaller axons or axon branches (which conduct impulses at lower frequencies in vivo) would be depressed at lower frequencies. It is expected then, that increase of temperature will lower threshold for most axons and their branches in vivo. Since action potential strength (as a stimulus to a zone of low safety factor) is also reduced by increased temperature, it is possible to speculate that the invadability of a zone of low safety factor is not influenced by temperature.

Fig. 10


7-5-75
5 min.
1°C
Temperature
arbitrary threshold units
time →
A
B

This could easily be tested through methods already established by Bittner 1968 or Pomas 1972, although neither of them has actually investigated the effects of temperature in their studies of differential invadability of crustacean branch points.

V. Conclusion

In an axon conducting impulses at physiological frequencies threshold has a negative temperature coefficient. When the temperature of an axon was changed suddenly, the corresponding threshold change was broken into two components. The second slow component bears resemblance to accumulation or decay of depression.

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