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Effect of Calcium Chloride, Acid, and Alkali on Irritability and Conductivity of Nerves Locally Cooled

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EFFECT OF CALCIUM CHLORIDE, ACID, AND ALKALI ON IRRITABILITY AND CONDUCTIVITY OF NERVES LOCALLY COOLED

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Vita

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INTRODUCTION

In search for more clues which might lead to knowledge of the intimate nature of the nerve impulse a great deal of work has been done in trying to correlate the different phenomena displayed by the nerves with the fundamental properties of conduction and irritability. One of the recent aspects of this work has been the effect of temperature change upon these properties of nerves. Dworkin and Florkin (6) in 1930 found that the chronaxie and the recovery period of nerves were distinctly prolonged with a decrease in temperature. In working with the electrical phenomenon accompanying the nerve impulse Gasser (8) in 1931 noted a progressive decrease in the spike potential of the action current, the rate of this decrease becoming greater as the lower temperature values were approached. He also noticed that the frogs used in the hot summer climate were less resistent to the action of cold. Bremer and Titeca (3) in 1933 also working on the effect of temperature changes upon nerve potentials reported that the greatest amplitude in the nerve action potentials occurred in a temperature range of 15 to 22 C., slight variations being found in different animals. On either side of this optimum range there was a regular decrease of the amplitude of the action potential. This decrease being more rapid in the case of cooling than in the case of warming the nerve. Similar effects were seen in the amplitude of the demarcation current of the nerves.

In 1933 Boyd and Ets (2) reported two types of cold blocks in nerves. They found that a nerve that is locally cooled may lose conduction in two ways: by a freezing or by a non-freezing type of cold block. In the freezing type of cold block there is an actual formation of ice about the nerve; the nerve shows little or no tendency toward recovery when warmed to room temperature; and the temperature at which the block occurrs may be at zero or anywhere below this temperature value. The non-freezing type of block allows recovery of conduction almost immediately upon warming to room temperature; this block may occur at any temperature above ice formation. Quite low temperatures are obtained in cases where there is a great degree of supercooling.

Continuing the work on the cold block Ets and Boyd (7) treated sciatic nerves from the frog with Ringer's solution containing an excess of potassium. They found that a four fold or more increase of potassium in normal Ringer's solution produced a non-freezing type of cold block in nerves. The temperature at which the block occurrs gradually increasing with increased time of immersion in the solution. Normal Ringer's solution made the nerve relatively resistant so that the nerve blocked by freezing only. They also reported that storing the frogs in a cold environment for a short time previous to their use made the nerves more resistant to the action of the potassium ion and produced a freezing type of block.

Now there may be some correlation between the type of cold block produced in a nerve and the effect of different ions upon the action potential. Graham (10) found that potassium decreases the after-potential of the nerve's action potential and that calcium has an opposite effect that is, it increases the after-potential of the action potential. Gasser and Erlanger (9) in 1930 reported that increased hydrogen ion concentration resembles calcium in its effect upon the after-potential while alkali produces a decreased after-potential. A group of workers, Hober (12), Woronzow (14), and others also reported that alkalis have the same action as potassium, while acids behave as calcium in respect to the stability or permeability of the nerve membrane.

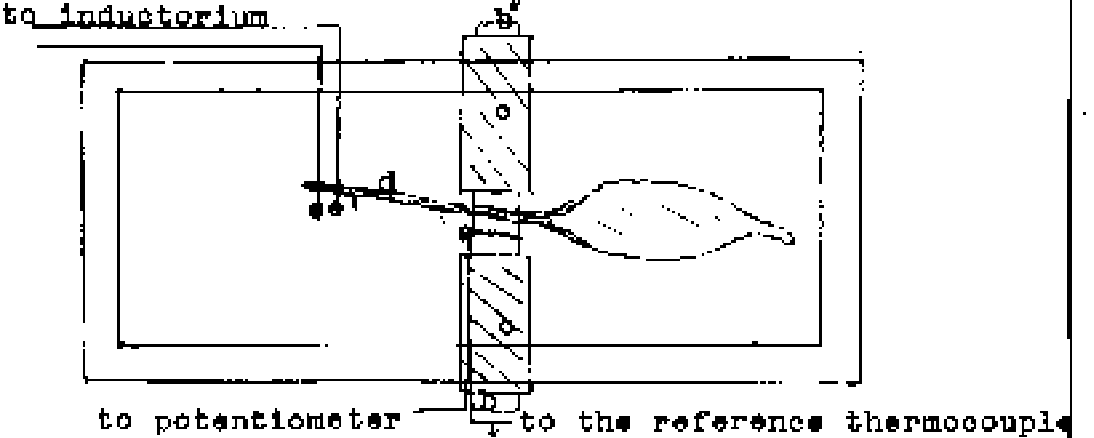
With this data in mind an attempt was made to study the effect of calcium and of Ringer's solution of different hydrogen ion concentration upon the cold block.

That calcium produces non-irritability of muscle in isotonic solutions is a well known fact, Overton (13). Calcium in such concentrations has a narcotizing effect or produces non-irritability by rendering the muscle more resistant to stimulation. Likewise nerves immersed in an isotonic solution of calcium chloride become non-irritable. A series of experiments were carried out in which the threshold stimulus was determined after varying periods of immersion to note whether this gradual increased resistance to stimulation occurred in nerves also to compare this effect with that produced by changes in hydrogen ion concentration.

METHODS AND MATERIALS

In our experiments medium sized frogs, Rana pipiens, were used. Frogs designated as cold stored are those that had been kept in a shallow pan of water in a refrigerator for 24 to 48 hours at a temperature of 5° to 8° centigrade. The warm stored frogs were animals which were placed in a shallow pan of water in an incubator for 24 to 48 hours at 25° centigrade. The sciatic-gastrocnemius preparations were dissected out without the use of Ringer's solution. Mated preparations were used, one was immediately placed in the solution under investigation, care being taken to prevent drying and contact of the muscle with the solution. The other preparation, served as a control, was mounted in a paraffin moist chamber immediately after dissection and blocked by cooling. The method used to determine the cold block was described by Boyd and Ets (2) in 1934. The following figure I shows the arrangement of the apparatus in the moist chamber.

Figure I

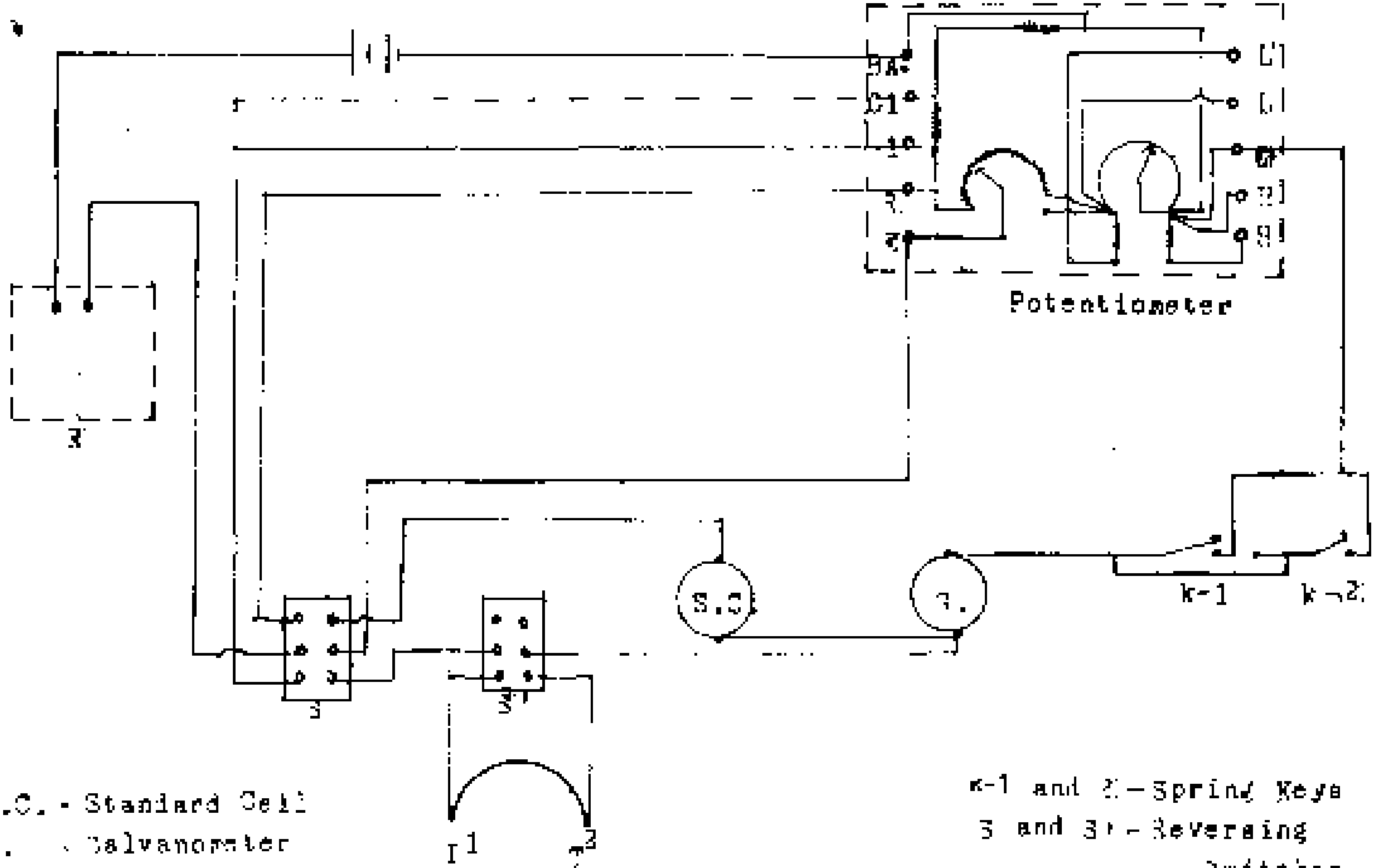


As one sees in the figure the nerve (d) is placed upon the silver tube (b) through which a cooled salt solution was syphoned; the rate of cooling is controlled by regulating the flow of the salt solution through the tube. The silver tube was insulated from the atmospheric moisture by rubber tubing (c) except for a small area to allow room for the thermocouple (a) which was sodered to the tube and also room for the nerve. This insulation was found to allow a greater degree of supercooling by preventing condensation of water on the tube. The nerves were stimulated by single induced shocks of minimal break strength from an inductorium at the electrodes (e and e'), e' being the cathode. The strength of the minimal break shock varied with the type of treatment of the nerve. The muscle twitch was used as the index of conduction.

The temperature at which conduction failed was determined by means of copper-constantine thermocouples, as shown in the figure II. One thermocouple (T^1) lay upon the silver tube immediately adjacent to the portion of the nerve to be blocked as shown at (a) in figure I. This thermocouple was connected with a similar one (T^2) in figure II which served as the reference thermocouple--the reference thermocouple was kept in an ice-water mixture, in a thermos bottle and its temperature determined by an accurate thermometer. The difference in electrical potential between the two couples produced by

Figure II

Apparatus for Temperature Measurement



S.C. - Standard Cell
G. - Galvanometer
R. - 4 dial Resistance Box

K-1 and 2 - Spring Keys
S and S' - Reversing Switches
T^1 and T^2 - Thermocouples

differences of their temperature was measured by means of the galvanometer and the potentiometer. The thermocouples were callibrated against a standard thermometer and their constant determined. In making the determination, the potential difference (P. D.) in millivolts was read from the potentiometer and the temperature of the reference thermocouple (T^2) read from the thermometer. By using the following equation the temperature of the silver tube thermocouple (T^1) was calculated.

$$\frac{P.D.}{k} - T^2 = T^1$$

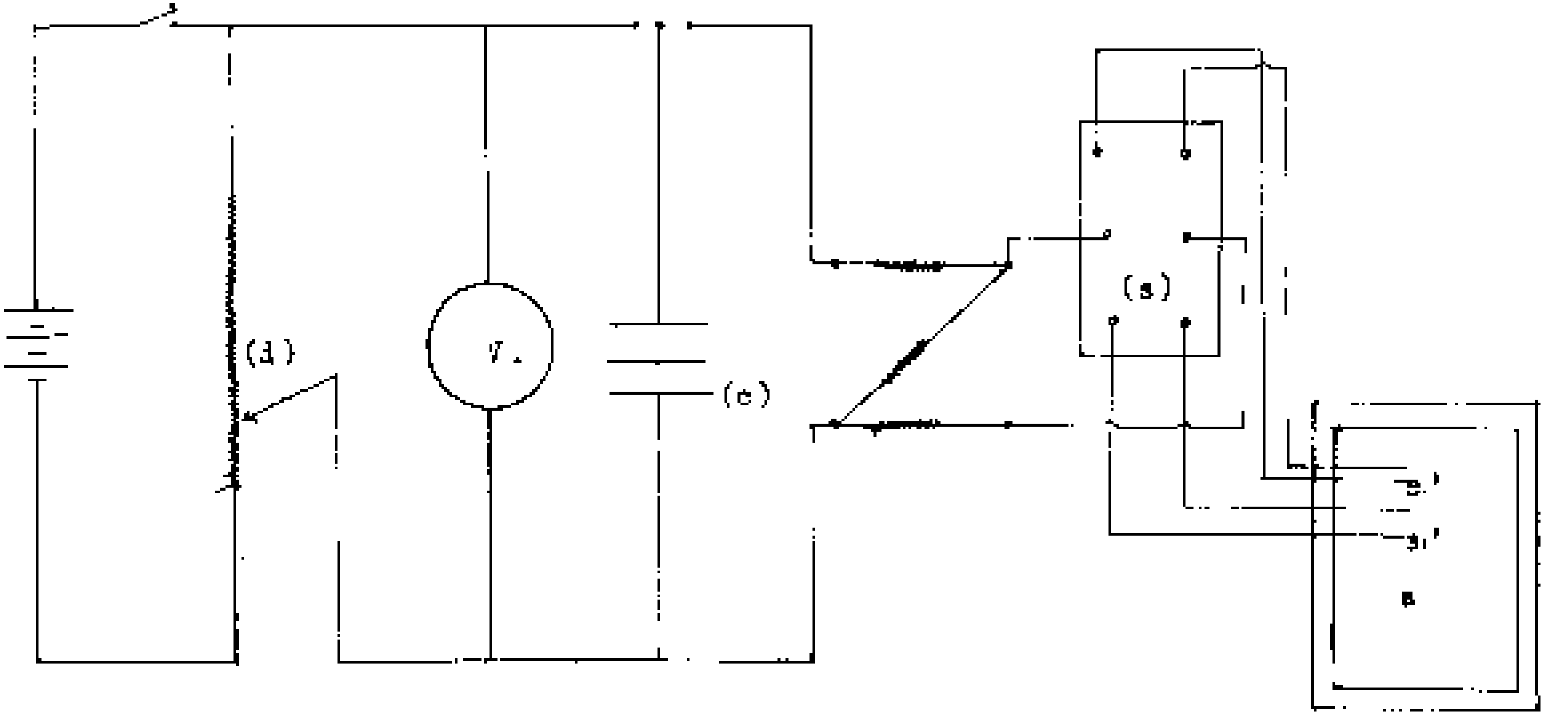
k is the constant determined by callibration of each set of reference thermocouples used.

The solutions used were made with distilled water and chemically pure reagents. The calcium chloride solution was a 0.98 per cent solution which is isotonic with frogs blood. The acid-Ringer's solution was made up with unbuffered Ringer's solution containing 0.65 per cent sodium chloride, 0.014 per cent potassium chloride, and 0.012 per cent calcium chloride to which was added enough hydrochloric acid to bring the pH to 1.8. The alkaline-Ringer's solution likewise was unbuffered Ringer's solution to which was added enough 0.1 normal sodium hydroxide to make the pH approximately 12.5. The Hydrogen ion concentration was determined colorimetrically. Due to the buffering action of nervous tissue these solutions were changed at fifteen minute intervals during immersion in an attempt to maintain an approximately constant hydrogen ion concentration. The four potassium-four calcium solution was made by in- creasing the concentration of the potassium to 0.056 per cent and that of the calcium to 0.048 per cent with a subsequent decrease in the sodium chloride content to maintain the original isotonicity.

In determining the blocking temperature the minimal break stimuli were roughly measured. An inductorium was used as the source of stimulus and the position of the secondary was recorded. The inductorium was callibrated in terms of current strength for various positions of the secondary coil. Sudden and marked changes of the threshold stimulus were noted with some of the solutions and it seemed desirable to further study these changes. In the rheobase measurements the nerves were put through the same preliminary treatment as those used for the cold block. The nerves were dissected without the use of Ringer's solution, placed in a paraffin moist chamber, (a) figure III, in which the solution studied could be drained off at will without disturbing to any great extent the position of the nerve on the electrodes (e' being the cathode). The rheobase was determined by the use of a 3 microfarad condenser discharge (c). As one notices from the figure III the position of the electrodes is such that measurements may be made at two positions of the nerve. The strength of the current at the electrodes was varied by modifying the voltage in the circuit going to the condenser by means of a rheostat (d). The values

Figure III

Apparatus for Rheobase Measurement



V. Voltmeter

(d) Rheostat

(c) Condenser

(s) Reversing Switch

(e') Cathode Electrode

of the rheobase were read from the voltmeter (v) which was graduated to read directly in decivolts or in in univelts. The stimulating shock was changed from one set of electrodes to the other by means of a reversible switch (s); at both pairs of electordes the cathode (e') was kept nearest the muscle.

Table I.

Effect of Isotonic Calcium Chloride Solution on Cold Block

Immersion Time-min.	Cold Stored Frogs F.T.	Cold Stored Frogs N.F.T.	Cold Stored Frogs T.S.	Warm Stored Frogs F.T.	Warm Stored Frogs N.F.T.	Warm Stored Frogs T.S.
10	-5.6		100	-7.9		100
15	-9.0		2000			
15	-6.4		1400			
20	-5.6		660	-7.7		360
30	-9.1		2000	-7.3		660
40	-9.0		660		N.I.	
50	-9.1		660			
60	-5.8		4080			
70	-9.8		3500			
80	-12.1		5000			
90		-0.9	6130			
100	-7.8		4080			
110	-6.6		4080			
120	-4.4		6130			
125		-2.4	4080			
130		N.I.				
135	-6.3		6130			
140	-9.3		2520			
140	-9.0		2000			
150		N.I.				
150		N.I.				

N.I.-non-irritable at room temperature

T.S.-relative threshold stimulus

F.T.-freezing type of cold block

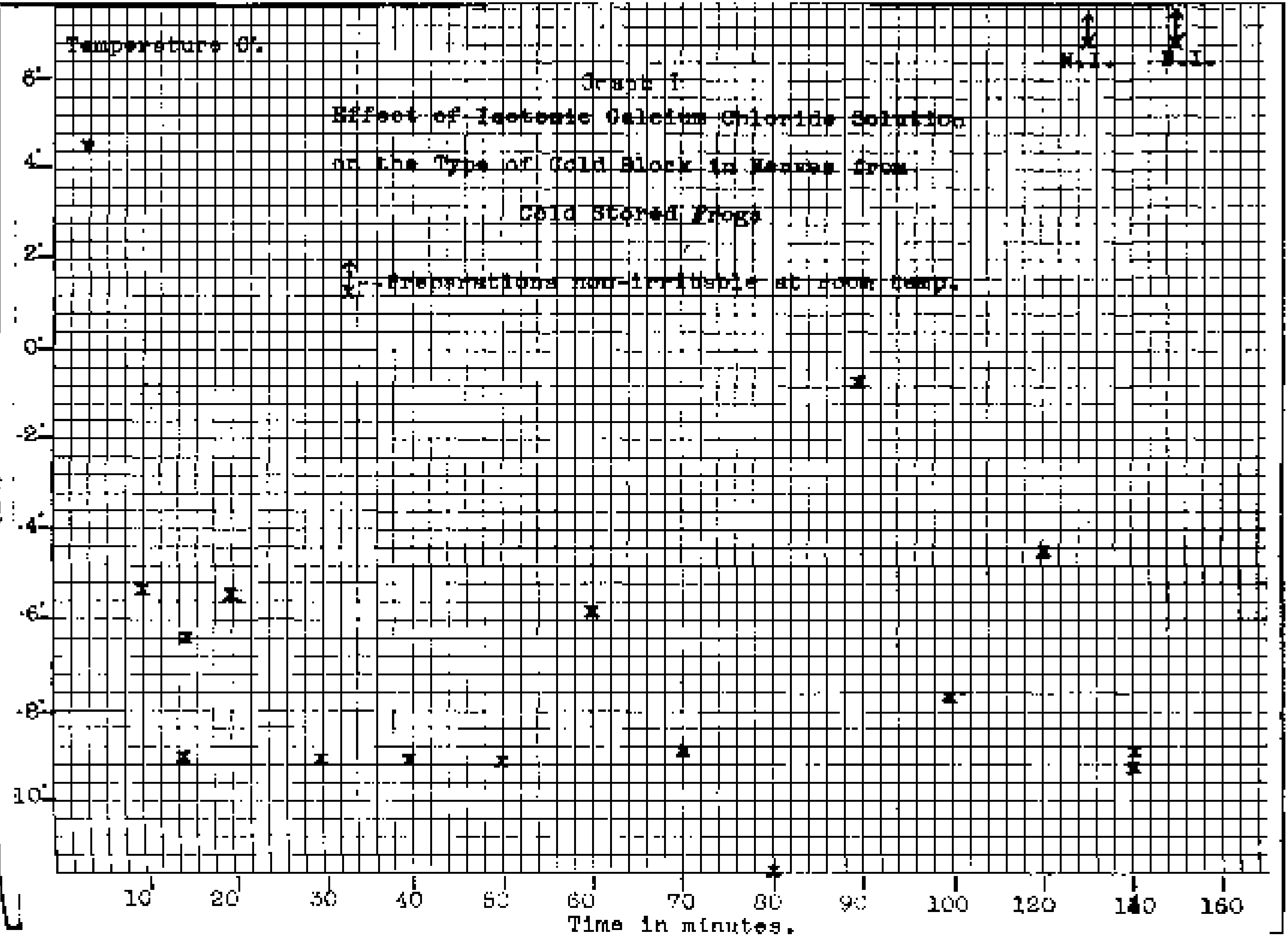
N.F.T.-non-freezing type of cold block

RESULTS

I. Type of Cold Block

(a) Calcium Chloride; Nerves immersed in an isotonic calcium chloride solution tend to block only by the actual formation of ice about the nerve. Table I shows the effect of the solution upon the nerves. Using cold stored preparations one sees from the table that of 21 preparations used 16 of them blocked by freezing, and two of them show the non-freezing block, the others being non-irritable. The temperature at which the blocks occurred ranging from -4 to -12 degrees centigrade. The calcium chloride solution produced non-irritability in nerves after they had been immersed in it from 130 to 150 minutes. The relative threshold stimulus rose rather irregularly in the cold stored preparations but reached a very high value before being non-irritable. All the mated preparations ran as controls blocked by the formation of ice. The nerves which blocked by freezing showed little or no tendency to recover upon being placed in Ringer's solution, corroborating the work of Boyd and Ets (1) in 1933. Graph I shows the trend of the calcium chloride cold blocks to remain at freezing temperatures.

To eliminate any conflicting or abnormal results which might be due to the method of preliminary treatment, a series of animals were used which were placed in warm storage previous to their use. It was found that the type of cold block was not changed by this treatment although acclimitization of frogs


Temperature C.
Graph I.
Effect of Isotonic Calcium Chloride Solution
on the Type of Cold Block in Nerves from
Cold Stored Frogs
Preparations non-irritable at room temp.
N.I.
N.I.
6°
4°
2°
0°
-2°
-4°
-6°
-8°
10°
10
20
30
40
50
60
70
80
90
100
120
140
160
Time in minutes.

to a temperature of 25 C. seems to make them more susceptible to the calcium. Refering again to table I it is seen, that the warm stored preparations become non-irritable after being in contact with the calcium solution within a period much shorter than that required by preparations from cold stored frogs. The controls in the warm stored frogs varied, both types of cold blocks were obtained. The relative threshold stimulus of the warm stored preparations showed a gradual rise but became non-irritable rather suddenly.

(b) Four Potassium-Four Calcium Ringer's Solution; A series of experiments were carried out in which the immersing solution was a Ringer's solution modified to contain four times the normal concentration of potassium and of calcium. The results obtained varied; in some the potassium effect was dominant especially in the longer periods. The irregular distribution of the blocking temperatures may be noted from Graph II. Seven nerves showed a non-freezing type of cold block while the remaining eleven nerves blocked only after ice formation. The nerves became non-irritable after being immersed in the solution for a period of 300 minutes. The relative threshold stimulus showed very little change up to the period when the nerve became non-irritable.

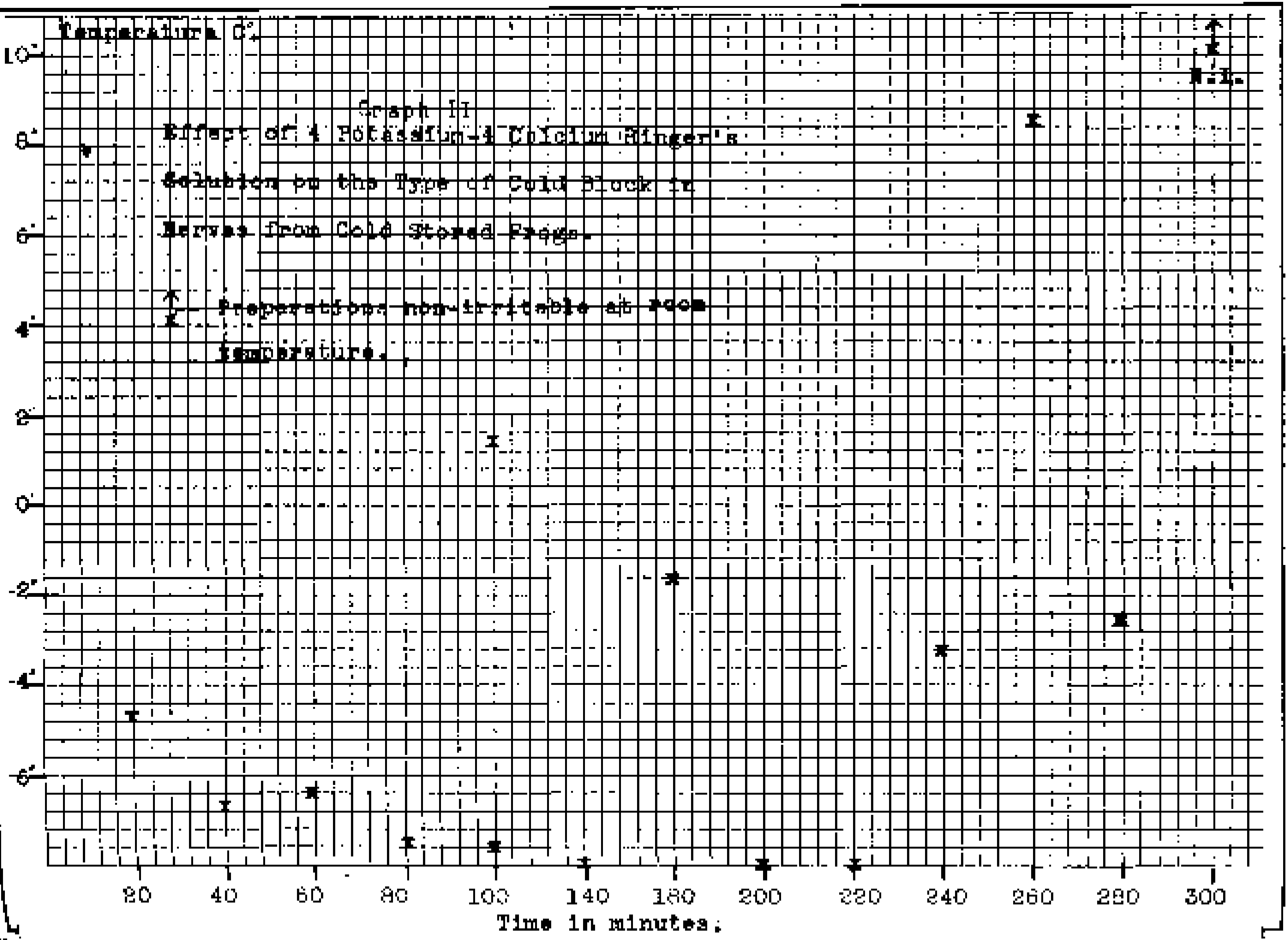

Temperature C°.
Graph II
Effect of 4 Potassium-4 Calcium Ringer's
Solution on the Type of Cold Block in
Nerves from Cold Stored Frogs.
Preparations non-irritable at room
temperature.
N.I.
10°
8°
6°
4°
2°
0°
-2°
-4°
-6°
20
40
60
80
100
140
180
200
220
240
260
280
300
Time in minutes.

Table II

Effect of Alkaline-Ringer's Solution Ph 12.5 on Cold Block

Immersion Time-min.	Cold Stored Frogs			Warm Stored Frogs		
	F.T.	N.F.T.	T.S.	F.T.	N.F.T.	T.S.
10	-6.9		40	-4.5		40
15	-7.4		60			
20	-5.0		40	-5.0		40
20	-6.1		40			
30	-7.9		660	-3.6		40
30	-6.2		2000			
40		+3.9	660		N.I.	
40	-7.6		7100			
40	-7.5		2000			
50	-8.0		40			
50		-2.6	10000			
60		N.I.				
70		N.I.				
70	-6.3		360			
80		+17.7	10000			
80	-7.7		4000			
90		N.I.				
90		N.I.				

N.I.-non-irritable at room temperature

T.S.-relative threshold value

F.T.-freezing type of cold block

N.F.T.-non-freezing type of cold block

(c) Sodium Hydroxide-Ringer's Solution; Immersing nerves in an alkaline-Ringer's solutionwith a pH of 10, resulted in the cold blocks of the freezing type. The immersion was carried out to a period of four hours at which point it was thought prudent to increase the alkalinity rather than to continue increasing the time of immersion. The effects of an alkaline Ringer's solution with a pH of 12.5 may be seen in table II and Graph III.

According to the tabulations the nerves are made non-irritable by immersion in the alkaline-Ringer's for a period varying from sixty to eighty minutes. Immersions of less than sixty minutes duration seem to make the nerves resistant to cold so that the majority of them block only by freezing. By comparing the warm and the cold stored preparations it may be noted that the cold stored nerves are more resistant to the action of alkali than the nerves of frogs placed in an incubator; although the type of cold block that persists is the same whether the preparation is treated with warm or cold storage. The relative threshold stimulus showed a very irregular rise in the case of the cold stored nerves while the warm stored nerves maintained the initial threshold value up to the period when irritability is lost.

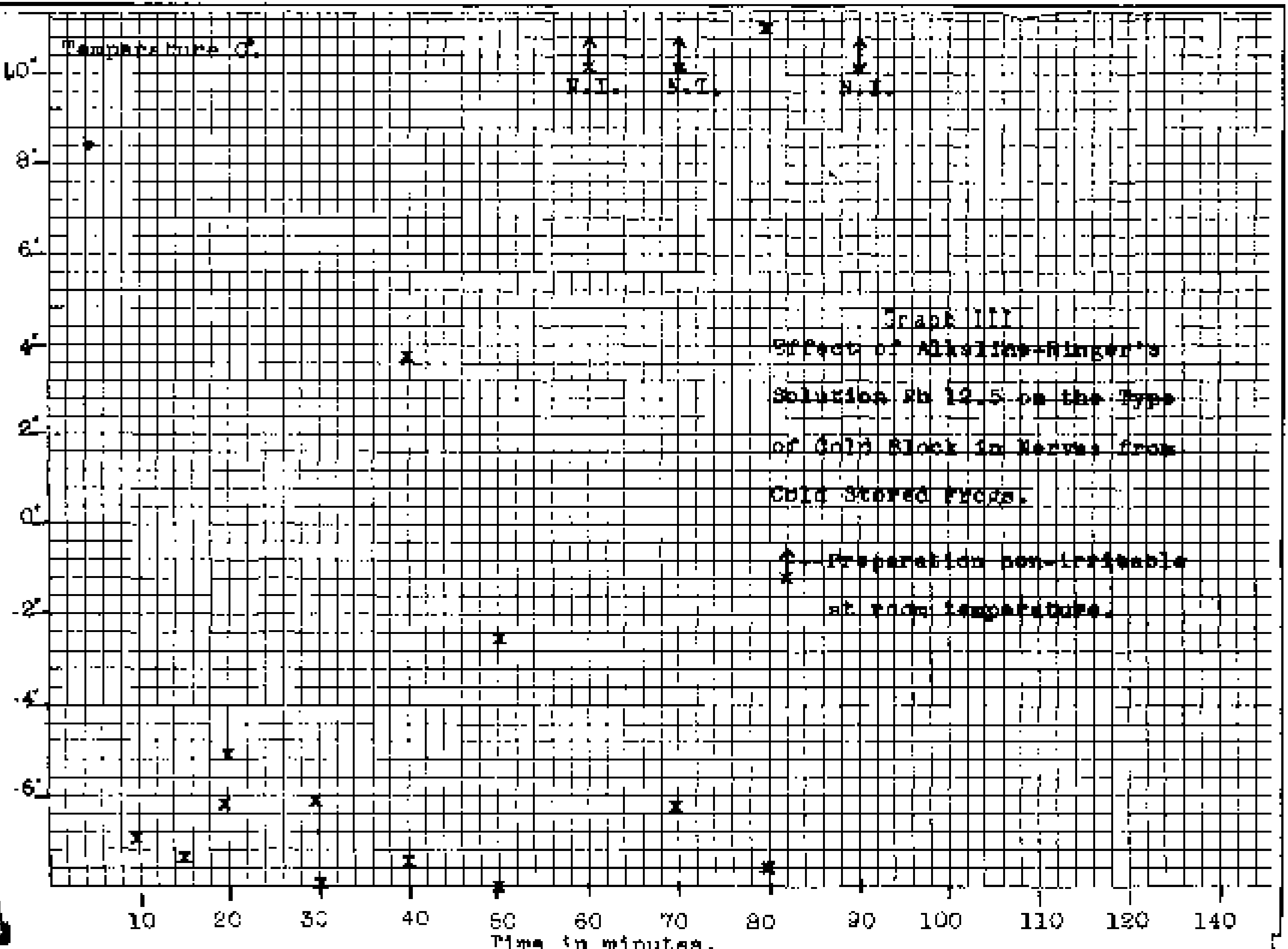

Temperature C.
10°
8°
6°
4°
2°
0°
-2°
-4°
-6°
N.I.
N.I.
N.I.
Graph III
Effect of Alkaline-Ringer's
Solution Ph 12.5 on the Type
of Cold Block in Nerves from
Cold Stored Frogs.
Preparation non-irritable
at room temperature.
10
20
30
40
50
60
70
80
90
100
110
120
140
Time in minutes.

Table III

Effect of Acid-Ringer's Solution Ph 1.8 on the Cold Block

Immersion Time-min.	Cold Stored Frogs			Warm Stored Frogs		
	F.T.	N.F.T.	T.S.	F.T.	N.F.T.	T.S.
10		-6.0	40	-5.6		1400
15		+0.4	40			
15		+1.9	40			
20		-1.7	1560		+7.6	2000
20		-2.2	40			
30		+3.9	3120		+0.7	7100
35		+2.2	10000			
40	-7.7		10000		N.I.	
45	-9.5		10000			
50		N.I.				
60		N.I.				

N.I.-non-irritable at room temperature

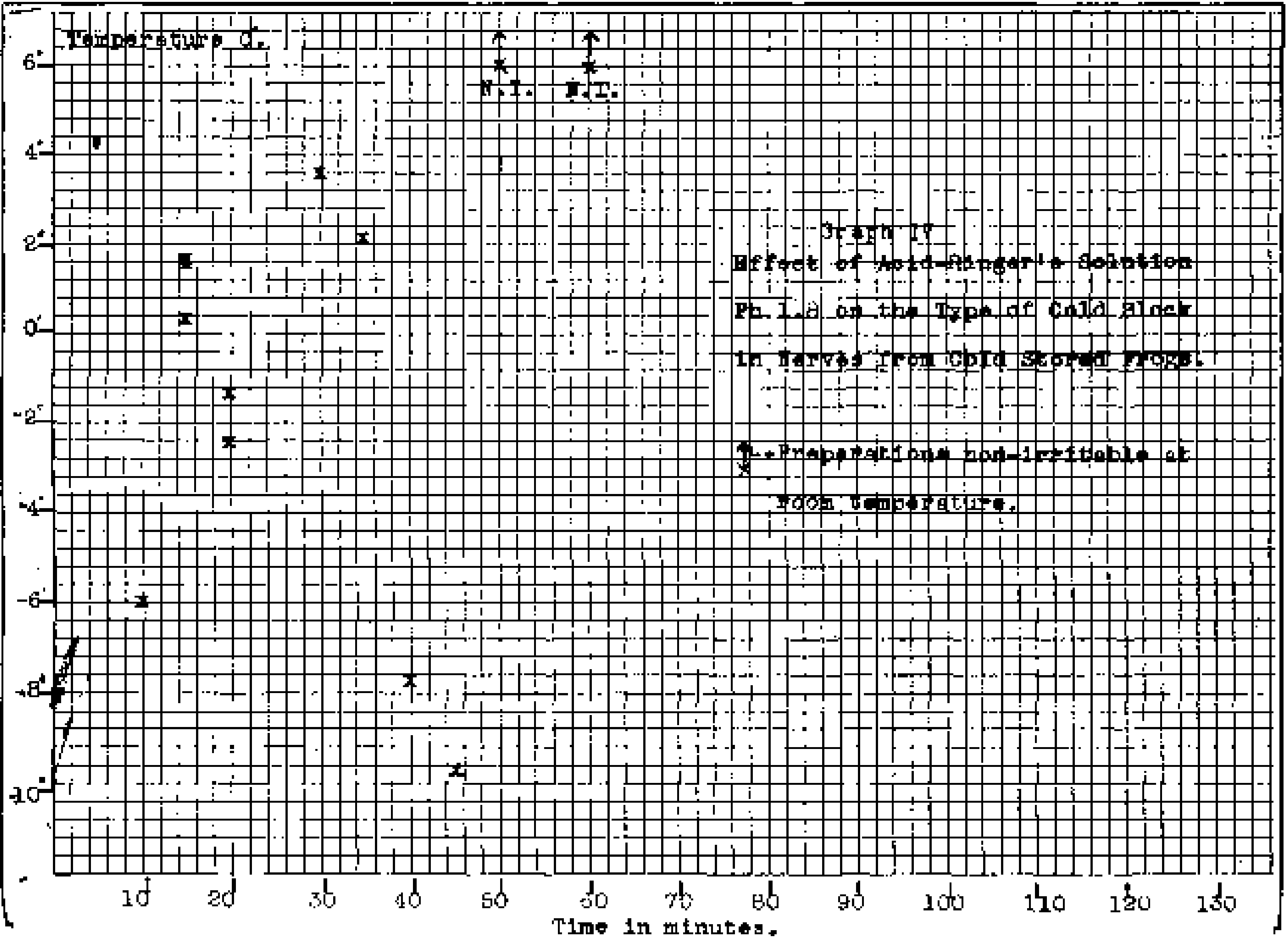
T.S.-relative threshold value

F.T.-freezing type of cold block

N.F.T.-non-freezing type of cold block

(d) Hydrochloric Acid-Ringer's Solution; Acid solution with a pH of 3 seems to have no specific effect upon the type of cold block. Preparations immersed for three hours in this solution blocked only by freezing. However after increasing the hydrogen ion concentration so that the pH was 1.8 a change was produced in the nerve shown by the development of the non-freezing type of block with increased time of immersion. Results obtained are shown in table III and in Graph IV. The non-freezing type of cold block persists up to immersions of forty minutes or shortly before the nerve becomes non-irritable; there occurred two exceptions. One may also notice from table III that the warm stored frogs are more susceptible than the cold stored frogs. The relative threshold stimulus shows a sudden increase after a short period of immersion in both the warm and the cold stored preparations.

Preparations that become non-irritable at room temperature from the action of the isotonic calcium chloride solution and from the Ringer's solution containing an excess of potassium and of calcium showed a tendency to rapid recovery when placed in normal Ringer's solution. This recovery does not occur in nerves treated with either the acid or the alkaline-Ringer's or occurrs only to a very small degree even after being kept in normal Ringer's solution for a long time. All non-irritable preparations before being placed in Ringer's were tested by direct stimulation of the muscle to remove any results that might be due to a non-irritable muscle.


Temperature C.
6°
4°
2°
0°
-2°
-4°
-6°
-8°
-10°
N.I.
N.I.
Graph IV
Effect of Acid-Ringer's Solution
Ph 1.8 on the Type of Cold Block
in Nerves from Cold Stored Frogs.
↑ - Preparations non-irritable at
room temperature.
10
20
30
40
50
60
70
80
90
100
110
120
130
Time in minutes.

II. Rheobase Measurements

A series of experiments were carried out in which a more accurate measurement of the threshold stimulus could be obtained. Values were obtained at two points on the nerve; (A) being the point proximal to the muscle and (B) the point distal to the muscle.

Table IV

(a) Calcium Chloride Isotonic Solution

Immersion Time-min.	A. volts	B. volts	A. volts	B. volts
Initial	0.37	0.16	0.16	0.22
10	1.0	2.0	1.1	1.1
20	1.1	2.4	1.4	1.9
30	1.1	2.4	1.1	1.9
40	1.8	3.2	1.2	2.9
50	1.8	3.4	1.4	3.8
80	1.8	N.I.	2.2	4.5
90	1.8	N.I.	2.1	N.I.
100	2.4	N.I.	2.1	N.I.
110	1.4	N.I.	N.I.	N.I.
120	1.5	N.I.		
130	1.7	N.I.		
140	2.1	N.I.		
150	N.I.	N.I.		

(b) 4K-4Ca Ringer's Solution

Immersion Time-min.	A. volts	B. volts	A. volts	B. volts
Initial	0.29	0.24	0.59	0.10
10	.19	.27	.71	.47
20	.55	.27	.62	.49
30	.49	.55	.52	.54
40	.55	.40	.53	.65
50	.52	.62	.53	.65
60	.52	.65	.60	.75
70	.52	.50	.52	.80
80	.55	.70	.53	.75
90	.52	.51	.50	.50
100	.55	.75	.60	.61
110	.65	.62	.57	.62
120	.50	.65	.53	.62
130	.47	.65	.56	.80
145	.54	.65	.66	.68
165	.57	.68	.67	.68
200	.55	.68	.68	.68
230	.51	.68	.60	.62
245	.50	.71	.57	.70
275	.50	.83	.57	.72
305	.48	.63	N.I.	N.I.
335	.54	.80		
365	N.I.	N.I.		

(c) Alkaline-Ringer's Solution Ph 12.5

Immersion Time-min.	A. volts	B. volts	A. volts	B. volts
Initial	0.26	0.06	0.31	0.15
10	.22	.21	.11	.34
20	.13	.20	.12	.50
30	.18	.48	.18	.64
45	.23	.21	.22	2.1
60	.15	.22	.49	4.5
75	.20	.48	.58	N.I.
95	1.5	N.I.	.40	N.I.
105	2.0	N.I.	.40	N.I.
115	N.I.	N.I.	N.I.	N.I.

(d) Acid-Ringer's Solution Ph 1.8

Immersion Time-min.	A. volts	B. volts	A. volts	B. volts
Initial	0.45	0.43	0.42	0.36
10	.31	.80	.29	N.I.
20	1.8	N.I.	1.0	N.I.
26	N.I.	N.I.	N.I.	N.I.

Although the initial value at the sectioned end is lower than that at the electrodes most proximal to the muscle, this soon changes so the rheobase at the distal electrodes is higher than the value at the proximal ones. The greatest relative change in the threshold value seems to occur in the first ten minutes of immersion from then on the rate of rise depends upon the solution in which the nerve is immersed. In the case of the calcium and the acid-Ringer's solution the nerve definitely loses irritability at the sectioned end before losing it at the proximal end. In the 4K-4Ca Ringer's solution the threshold stimulus seems to maintain a rather uniform value until a very short time before the whole nerve becomes non-irritable.

DISCUSSION

In working with a calcium chloride solution we took advantage of the well known fact that an excess of calcium will block conduction in nerves, shown by Overton (13) and others. Using an isotonic solution of calcium chloride we found the nerves failed to respond to stimuli after a relatively short period of immersion. From the time when the nerves were placed in the calcium chloride solution there was a persistent increase in the minimal stimulus required to produce a response, these results compare with those obtained by Graham (11) in her work on calcium.

Adrian (1) reported a loss of conduction to single stimuli in nerves after being immersed in an acid-Ringer's solution pH of 2.8 for eighty minutes; with a Ringer's solution pH of 2.1 the nerves failed to respond to single stimuli afternan immersion of thirty-nine minutes. Our results seem to fall in with these findings, since we found that using an acid-Ringer's solution with a pH of 1.8 that nerves lost irritability within twenty to thirty minutes. Like calcium the acid-Ringer's solution increased the threshold stimulus with increased periods of immersion. Such a decrease in irritability due to acid-Ringer's has been reported by several workers, Greisheimer (12), Delius (6), and others.

On the other side of neutrality we find similar changes produced upon the irritability of the nerves. An alkaline-Ringer's solution pH of 12.5 increased the threshold stimulus until after immersions of sixty to one hundred minutes the nerve failed to respond even to stimuli of considerable strength. The nerves of Adrian (1) seem to have been less resistant to the action of alkali, since he found that a solution pH of 11.8 produced non-irritability in nerves in twenty-three minutes.

In explaining the action of calcium, acid, and alkali upon the nerve to produce this decreased irritability itmay be well to turn to the well known membrane theory of nerve conduction. Adrian (1) gives the theory of Lillie in which the decline of electric response is said to be caused by a reduction in the permeability of the surface membrane, and the return of excitability is due to a return of the membrane from a stable to a more unstable condition. So that the ability of the nerve to conduct an impulse will depend very intimately upon the condition of the nerve membrane. Results reported from workers seem to imply that calcium renders the membrane of the conducting element of the nerves more stable than normally thus making it too resistant to change. Increased hydrogen ion concentration is thought to have the same effect upon the membrane as calcium; while a decrease in the hydrogen ion concentration acts oppositely to increase the permeability of the membrane thus destroying its integrity.

As shown by the results of this investigation a marked increase in the threshold value of some of the nerves occurred

Kato (15) in 1926 indicated that stimuli which were apparently not excessively strong may take effect as far as thirty mm. from the electrodes. Unfortunately Kato expressed his stimuli merely as coil distance so that the exact evaluation of the spread is impossible. He found that a stimulus ten times the maximal took effect nineteen mm. from the electrode. Cooper (5) slightly later found that fluid electrodes decrease the spread of current quite markedly nevertheless stimuli could still be made to take effect a short distance from the electrodes.

From our results on the cold block it may be noted that the stimuli at times were of such a caliber that might warrant an investigation as to the irritability of the nerve at the point of contact with the electrodes. In many cases we had to increase the stimulus as much as two hundred times the initial threshold stimulus to obtain a response. In our rheobase determinations the apparatus did not permit such an enormous increase in the stimuli applied to the nerves, so that perhaps the rheobase values may give a more accurate picture of the changes that were occurring in the irritability of the nerve. Since the position of the electrodes in the cold blocking moist chamber corresponds more nearly to that of the distal electrodes in the rheobase investigations, the values obtained at this latter point should be the logical ones to consider in correlating the changes in irritability with the type of cold block which develops.

Considering the changes in the threshold value of the calcium chloride solution treated nerves we find from table I that after fifty minutes of immersion the stimulus must be increased markedly over the initial stimulus to produce a response. This corresponds to the period, table IV (a), in which the nerve begins to show signs of non-irritability. The condition of non-irritability in the rheobase determinations is specific for stimuli of twenty to twenty-five times the initial stimulus; if one increased the strength of the stimulus still more a response occurred. Similar conditions were found in nerves after varying periods of immersion in the acid-Ringer's solution pH of 1.8 and also in the alkaline-Ringers solution pH of 12.5. Therefore we thought it justifiable to set an arbitrary limit as to the strength of the stimuli used. This limiting stimulus was set as twenty times the initial threshold value, so that any preparation or results derived from it may be disregarded if the stimulus required to produce a response is beyond this limiting value.

In coming to this conclusion it seems that a way has been opened by which many of the irregularities in our results may be removed, for instance, in the calcium treated preparations the non-freezing type of block occurred after the stimulus had increased beyond our limit. However in the results from the cold block experiments it may be noticed that using excessively strong stimuli either type of cold block may result; just why this happens is not known.

Another occurence which might increase the possibility of a spreading of the stimuli along the nerve is the fact that the nerves subjected to the action of the calcium and acid solution began to lose irritability at the sectioned end, this state of non-irritability gradually progressing toward the muscle. Cooper (5) reported similar results in her work with nerve narcotics. This loss of irritability at the distal end first is not manifested in the nerves treated with a Ringer's solution containing four times the normal concentration of potassium and of calcium. Likewise the alkali treated nerves showed variable results, no portion of the nerve demonstrating any particular susceptibility to the alkali.

SUMMARY

The preliminary treatment of frogs by placing them in cold storage makes their nerves more resistant to the action of calcium, acid, and alkali than preparations from warm storage.

Frog nerves treated with an isotonic calcium chloride solution when locally cooled block by freezing.

Frog nerves treated with an acid-Ringer's solution pH of 1.8 when locally cooled block by a non-freezing type of block.

An alkaline-Ringer's solution pH of 12.5 produces variable types of cold block--some nerves block by freezing, other block by a non-freezing type of block.

The rheobase value was followed through the treatment of nerves with an isotonic calcium chloride solution, acid and alkaline-Ringer's solution and with Ringer's solution containing four times the normal concentration of potassium and calcium.

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