

ELECTRICAL *VERSUS* CHEMICAL THEORY OF NEUROMUSCULAR TRANSMISSION

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UNSTRIATED muscle has some interesting properties that the mechanical response produced by electric current differs in some respects from that produced by a chemical substance. These differences are most striking in *Mytilus* muscle (Singh, 1938 *a*). Substances, which produce tonic contraction, decrease the excitability to electric current and increase that to chemical stimulation, such as by potassium, acetylcholine, sodium, barium, etc. These substances are monovalent cations, lithium, sodium, ammonium, potassium and hydrogen-ions; monovalent anions, bromide, nitrate, iodide, thiocyanate; divalent cations, calcium, strontium and barium; drugs, adrenaline, acetylcholine, veratrine, caffeine, strychnine, curare, nicotine, novocaine, ephedrine, ether, chloral hydrate, chloroform.

The above factor therefore affects the excitability to electrical and chemical stimulation in opposite directions. There is another factor that affects the two excitabilities in the same direction. This factor is that of accommodation (Singh, 1938 *b*). Substances, which diminish accommodation, increase the excitability; the above substances, which produce tonic contraction, have this effect in small concentrations; if the muscle is not much sensitive to chemical stimulation, then larger concentrations increase the excitability to both chemical and electrical stimulation. Calcium increases accommodation, hence decreases excitability to both when increased beyond a certain minimum concentration, but by virtue of its tone-producing properties, it may affect the two excitabilities in the opposite directions; the minimum concentration of calcium referred to being presumably required to preserve the membrane and other structures of the muscle. Substances, which antagonise the action of calcium, will thus decrease accommodation. Large concentrations of ammonium and potassium antagonise the action of calcium, though small concentrations produce similar effects. Sudden increase in osmotic pressure of the saline by addition of sucrose or sodium chloride produces contraction (Singh, 1942), but in excess of ammonium or potassium, slow increase of osmotic pressure will produce contraction (Singh, 1939; Singh and Acharya, 1957). Toxic concentrations

decrease the excitability both to electrical as well as chemical stimulation.

In frog's stomach muscle, most of the substances mentioned above affect the two excitabilities in the same direction (Singh, 1939), but in dog's stomach muscle, though most of the substances affect the two excitabilities similarly, some of them such as excess of calcium and hydrogen-ions, which increase tone, affect the two excitabilities in opposite directions (Singh, 1940). These substances increase the response to chemical stimulation, so long as they are in the process of producing tonic contraction, that is, so long as they act as subliminal stimuli capable of potentiating and stimulating action of other tone-producing substances, but once the tonic contraction has developed and the base line raised, then the response to all forms of stimulation decreases. This is presumably due to altered properties of the contractile mechanism. It is probable that when one kind of tone develops, then the folded polypeptide chains are held together in that condition by development of cross-linkages, which then retard contraction as well as relaxation (Singh and Singh, 1954).

The above differences between the responses to chemical and electrical stimulation can, therefore, be used to decide whether nerve stimulates the muscle by virtue of its action potential or by secreting a chemical substance such as acetylcholine (Singh, 1957). Experimental findings suggest that neuromuscular transmission in unstriated muscle is an electrochemical event, the primary response being produced by the action potential, and this is tonically maintained by the secretion of acetylcholine (Singh and Singh, 1947).

In the present research, the effect of some substances which can be used to differentiate between the two excitabilities, has been studied on vagus-muscle preparation from dog's stomach.

METHODS

Nerve-muscle preparations from dog's stomach were employed by cutting out a piece of the muscle along with the vagus nerve attached (Narayana and Singh, 1944). Simultaneously two pieces from the same stomach were stimulated by alternating current (12 volts for 10 sec.), acetylcholine (1 in 10^5) or potassium (20 p.c. of the sodium of the saline being replaced with potassium). At 37° C. many substances produce such marked increase of tone, that the response to all forms of stimulation is diminished, hence to prevent this increase of tone, these experiments were performed at 18–20° C.

Mammalian saline (Singh, 1940) was used. A difficulty in these experiments is that a long time, 3 to 6 hours, might be required to get a constant response. This time was least at pH 8, using borate buffer. Hence at this pH, borate buffer was used. Response is maximum at pH 8 and at pH 6.5. Hence to test the effect of excess of calcium and hydrogen-ions, the saline was buffered with phosphate at pH 6.5. To increase the concentration of hydrogen-ions further, salines buffered with phosphate at pH 6, 5.5 and 5.2 were used. The experiments with excess of calcium were also performed at pH 8. All other experiments were performed at pH 8.

RESULTS

Effect of anions

Effect of bromide.—Replacement of the chloride of the saline with bromide produces a tonic contraction, which however is slight at 18 to 20° C., the temperature at which these experiments were performed, and hence it does not interfere with the responses of the muscle to electric current, nervous stimulation, acetylcholine or potassium. Such replacement decreases the response to alternating current (Fig. 1), and increases that to acetylcholine

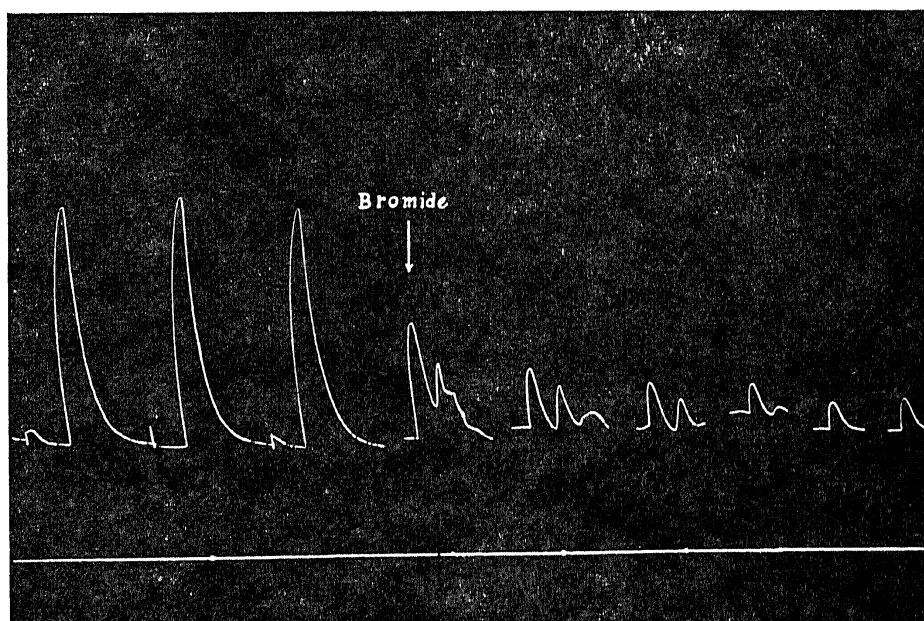


FIG. 1. Dog's stomach muscle. Effect of replacement of the chloride of the saline with bromide on the response to alternating current (12 volts/10 sec.). Response every 15 min.

(Fig. 2) and to potassium which produces responses similar to those produced by acetylcholine (Fig. 3). The response to nervous stimulation is decreased (Fig. 4). Small concentrations of bromide, about 5 per cent,

replacement of the chloride, may increase the response to alternating current. Complete replacement of the chloride of the saline with bromide shows that the response to nervous stimulation resembles that produced by electric current and not those produced by acetylcholine or potassium.

Effect of nitrate.—Replacement of the chloride of the saline with nitrate causes tonic contraction. Nitrate increases the response to acetylcholine and potassium and decreases the response to nervous stimulation (Fig. 4).

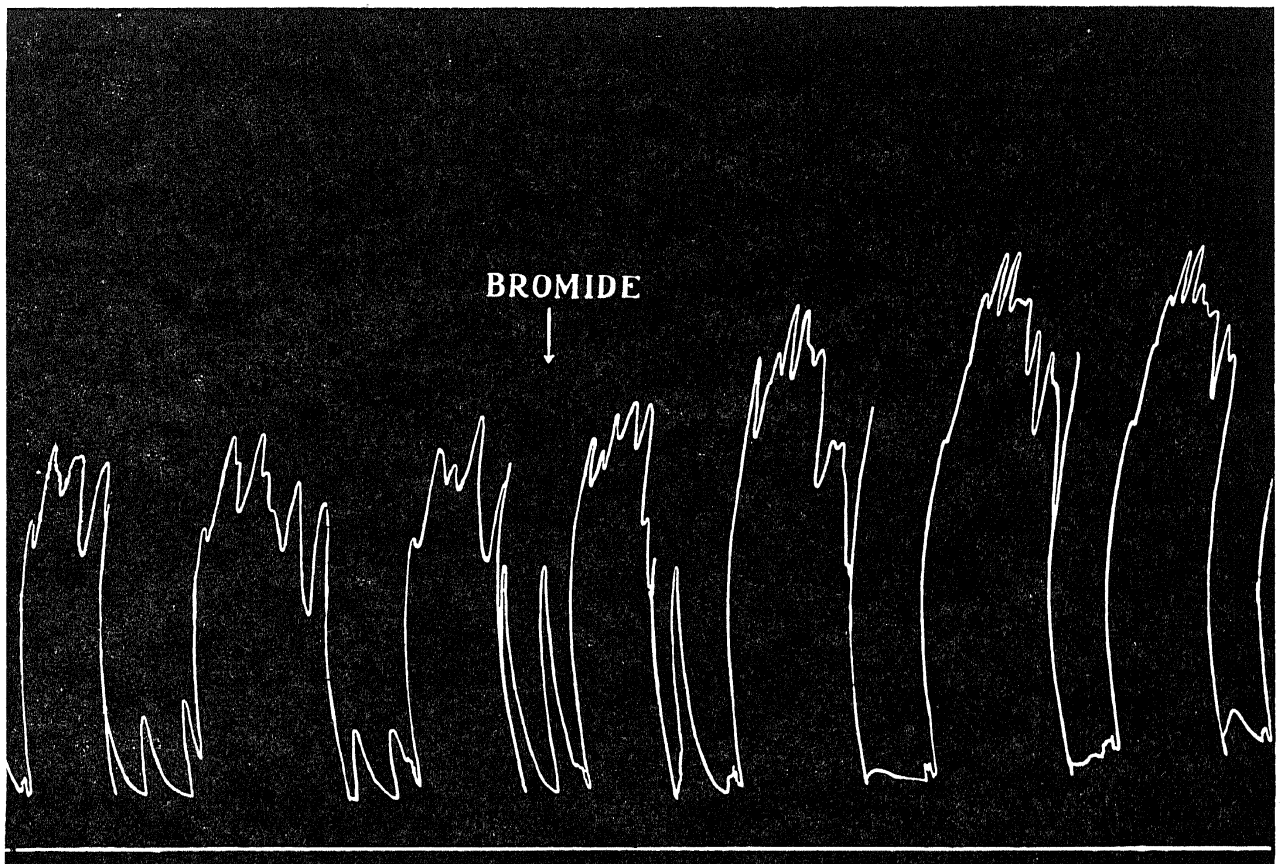


FIG. 2. Dog's stomach muscle. Effect of replacement of the chloride of the saline with bromide on the response to acetylcholine (1 in 10^6). Response every 15 min.

The response to electric current is variable, it may increase or decrease, or increase at first and then decrease. The response to nervous stimulation thus resembles more that to electric current than to acetylcholine or potassium.

Effect of iodide.—Replacement of the chloride of the saline with iodide causes tonic contraction. Iodide increases the response to acetylcholine (Fig. 5), potassium (Fig. 6), and decreases that to nervous stimulation (Fig. 4).

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The response to electric current decreases from the outset or it may first increase and then decrease (Fig. 7). The response to nervous stimulation therefore resembles that produced by electric current.

Effect of thiocyanate.—Replacement of the chloride of the saline with thiocyanate produces tonic contraction like other anions. Thiocyanate

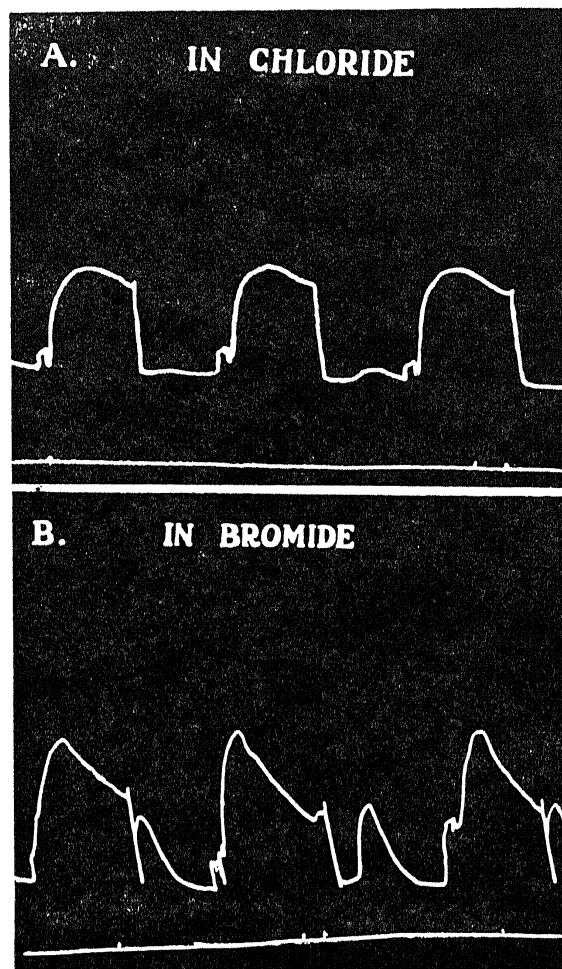


FIG. 3. Dog's stomach muscle. Effect of replacement of the chloride of the saline with bromide on the response to potassium. Response every 15 min. A. Responses in chloride. B. Responses in bromide.

decreases the response to nervous stimulation (Fig. 4). The response to electric current is at first increased and then decreased (Fig. 8). The response to potassium and acetylcholine is increased (Fig. 9). The response to nervous stimulation thus resembles that to electric current and not that produced by acetylcholine or potassium.

Effect of cations

Effect of calcium.—In dog's stomach muscle, excess of calcium ions produce tonic contraction, which may be marked in alkaline solutions at pH 8; this is greatly reduced if the temperature is kept at 18–20° C. The

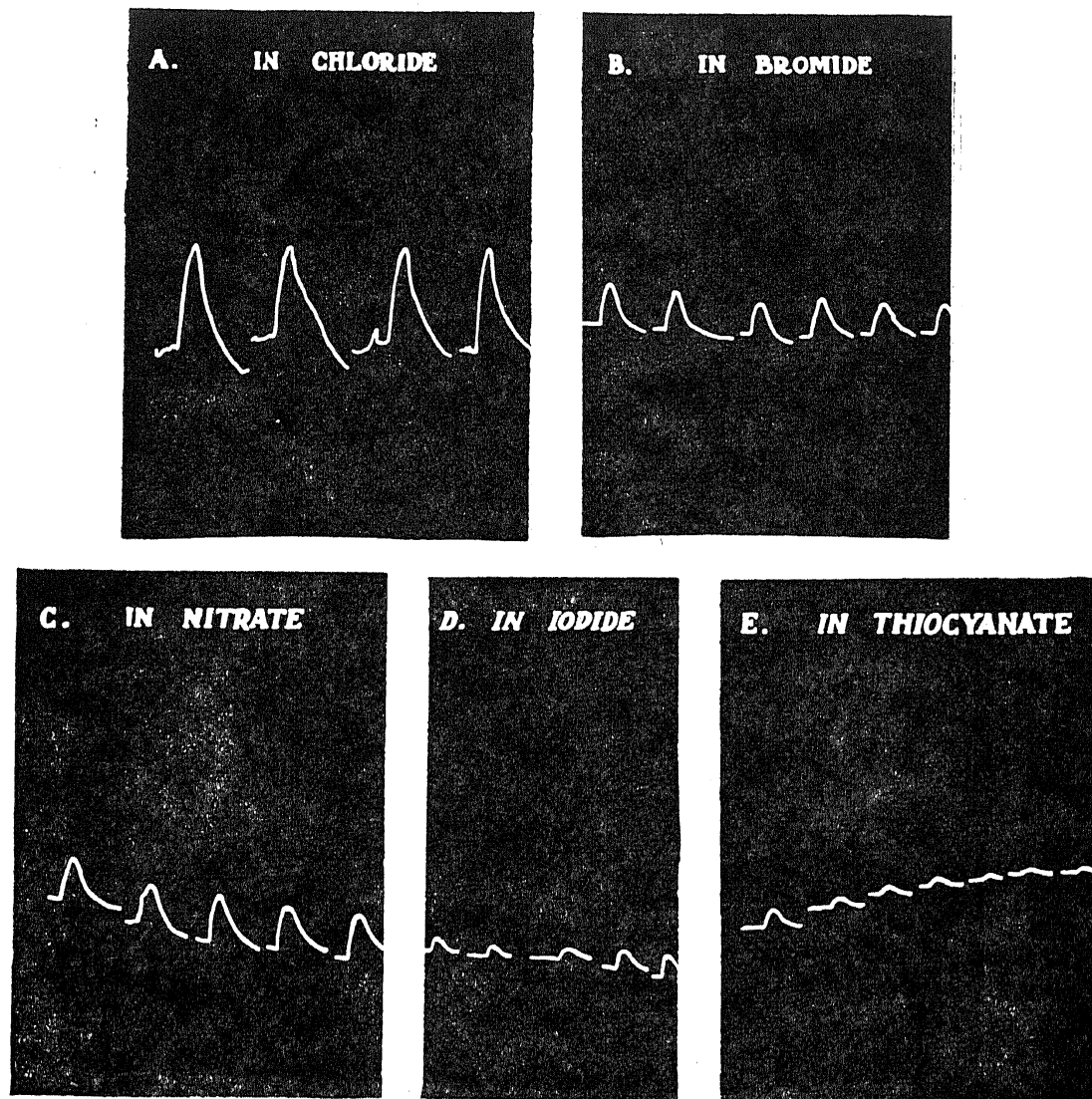


FIG. 4. Dog's stomach muscle. Effect of replacement of the chloride of the saline with other anions on the responses to nervous stimulation for 15 sec. every 15 min. A. Responses in chloride. B. Responses in bromide. C. Responses in nitrate. D. Responses in iodide. E. Responses in thiocyanate.

effect of calcium ions was tested both in alkaline as well as acid solutions (pH 7–6.5).

In acid solutions, excess of calcium ions potentiate the response to potassium, but antagonise that to electric current (Singh, 1940). The optimum

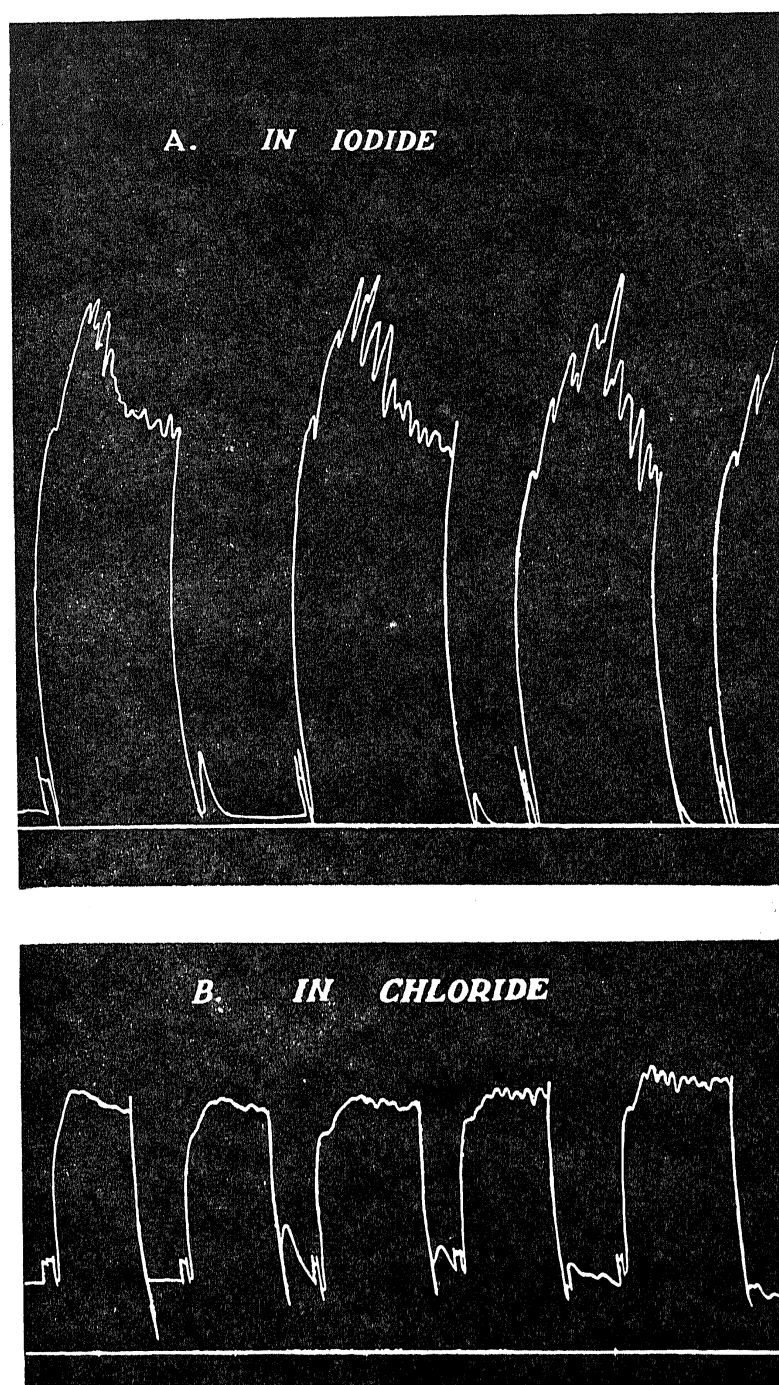


FIG. 5. Dog's stomach muscle. Effect of replacement of the chloride of the saline with iodide on the response to acetylcholine (1 in 10^5). Response every 15 min. A. Responses in iodide. B. Responses in chloride.

concentration of calcium for the response to electric current in acid solutions is $0.004 M$; beyond this the response decreases (Fig. 10). The response

to acetylcholine increases in excess of calcium. It gives a marked response in pure solutions of calcium chloride (Fig. 11). As the concentration of calcium is increased beyond 0.004 M, the response to acetylcholine may

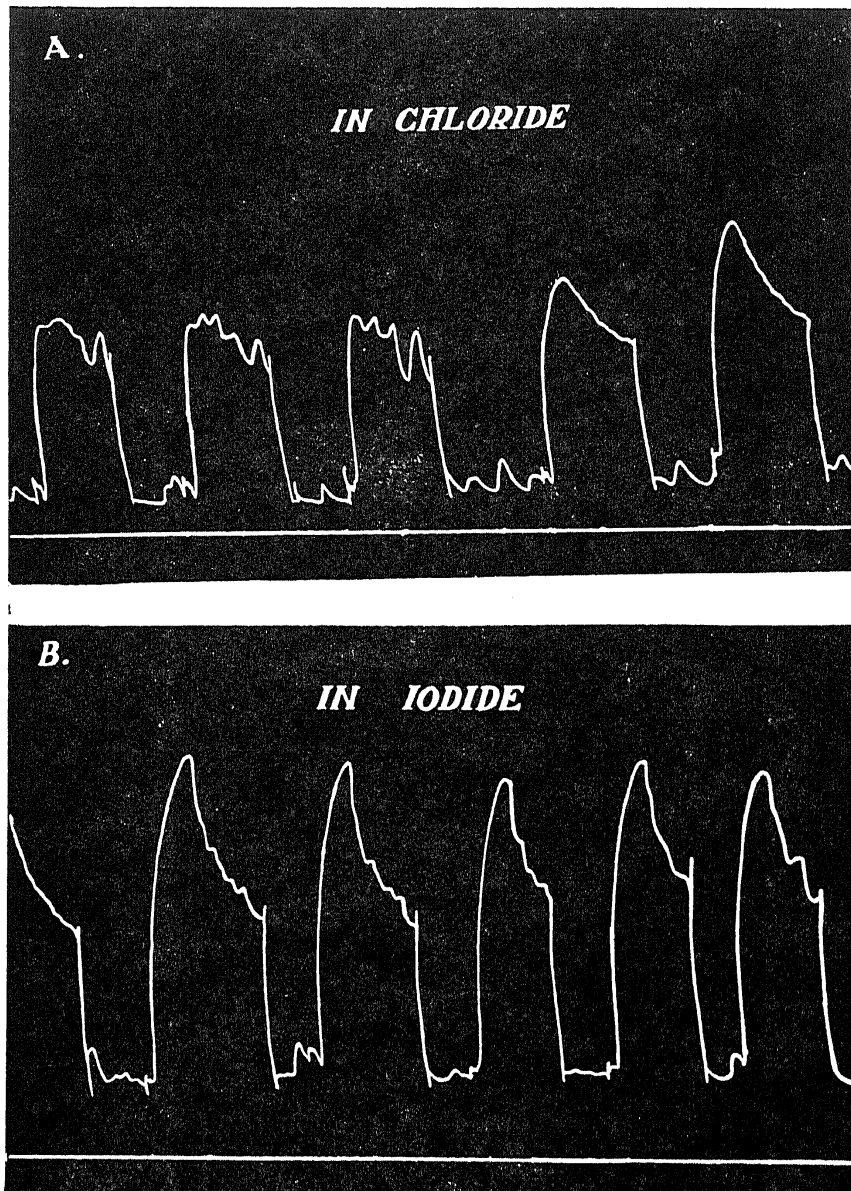


FIG. 6. Dog's stomach muscle. Effect of replacement of the chloride of the saline with iodide on the response to potassium. Response every 15. min. A. Responses in chloride and iodide. B. Responses in iodide.

slightly increase after a preliminary increase, and then increase again. This slight decrease is presumably due to increased accommodation produced by calcium.

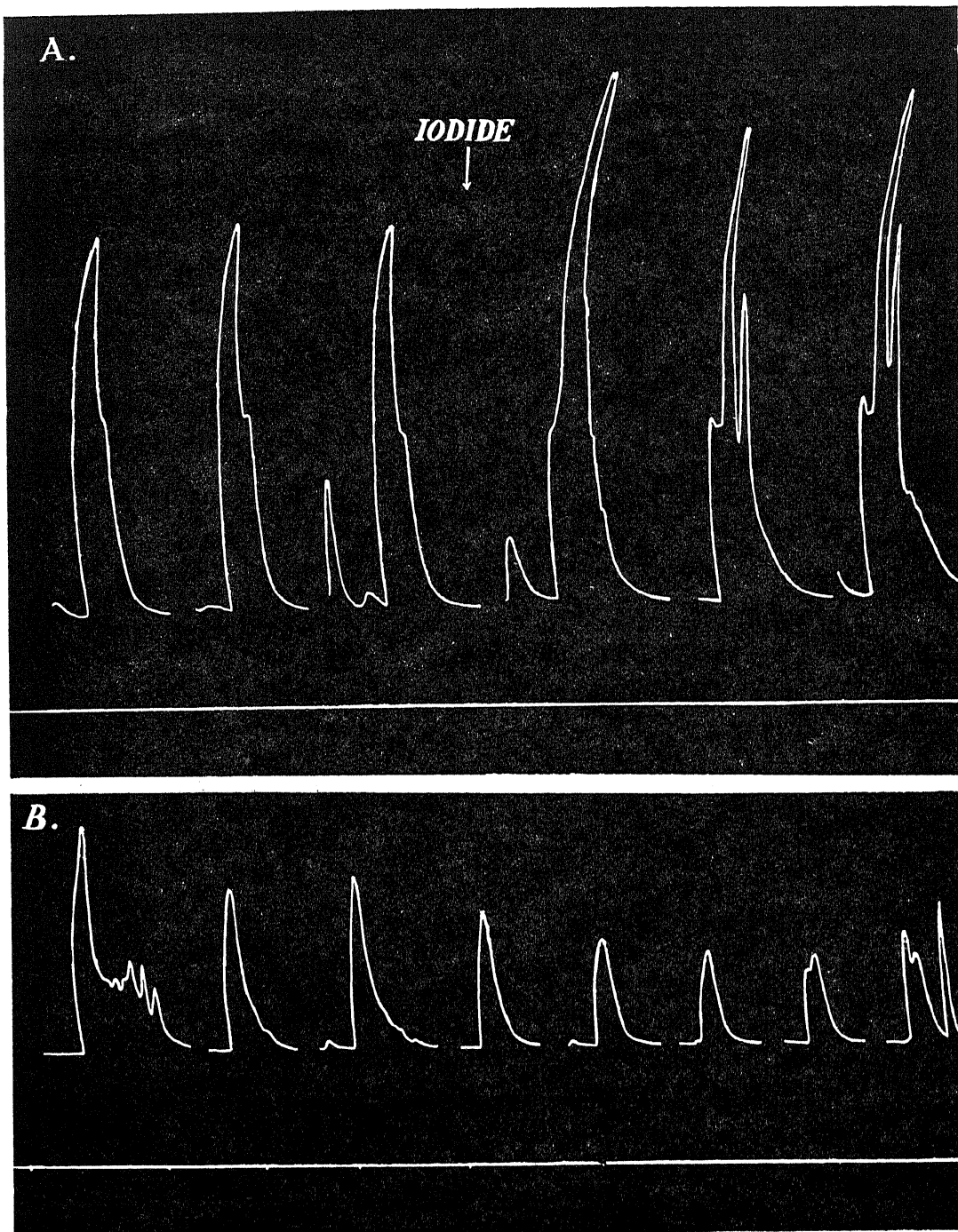


FIG. 7. Dog's stomach muscle. Effect of replacement of the chloride of the saline with iodide on the response to alternating current (12 volts/10 sec.). Response every 15 min. A. Responses in chloride and iodide. B. Responses in iodide.

In acid solutions the response to nervous stimulation increases up to $0.008-0.01 M$, and then decreases, but may be obtained in excess of calcium ions up to $0.06 M$ (Fig. 12). There is no doubt, therefore, that excess of calcium ions potentiate the response to acetylcholine, potassium and nervous stimulation, but decrease that to electric current. The final suppression of the response to nervous stimulation in excess of calcium is presumably due to interference with neuromuscular transmission.

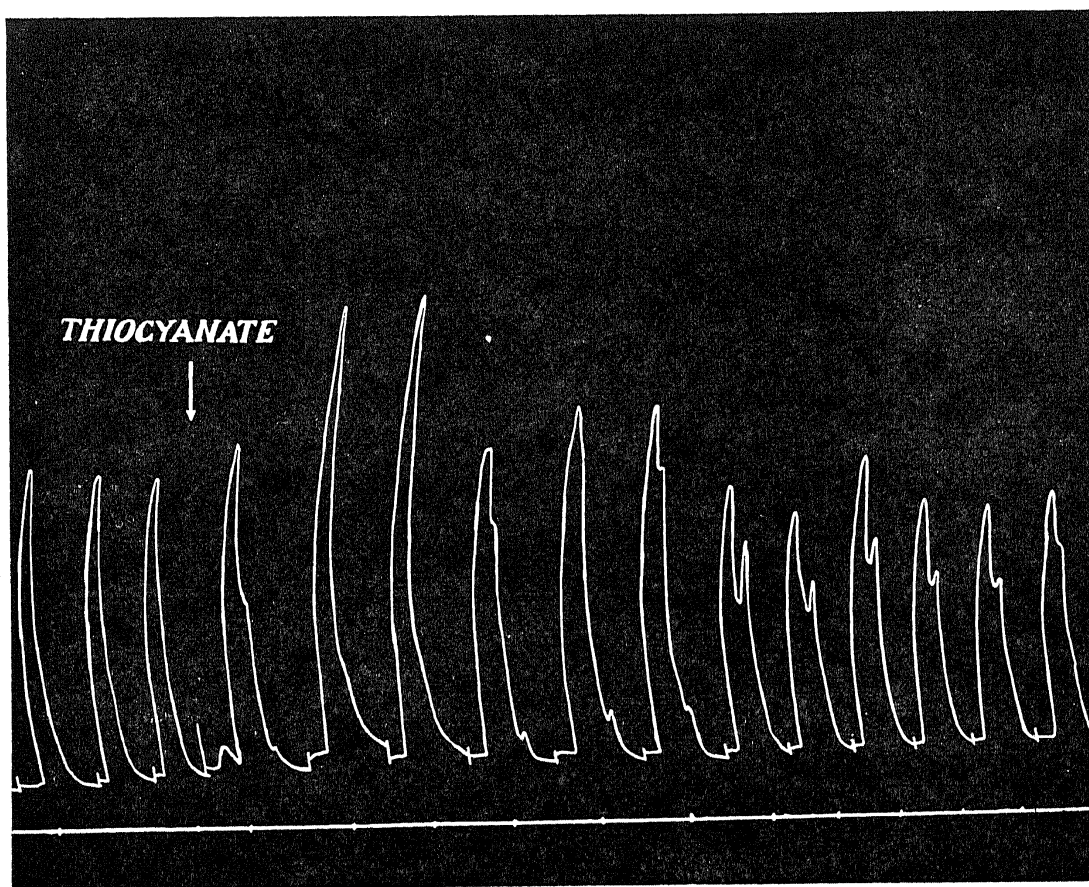


FIG. 8. Dog's stomach muscle. Effect of replacement of the chloride of the saline with thiocyanate on the response to alternating current (12 volts/10 sec.). Response every 15 min.

In alkaline solutions, pH 8, excess of calcium produces a tonic contraction, which however decreases as the concentration of calcium is increased. Calcium is antagonistic to tonic contraction, hence when in excess, decreases its own response. The response to alternating current sharply decreases, if the concentration of calcium exceeds $0.002 M$ (Fig. 13). The response to acetylcholine is increased in excess of calcium, up to a certain value (Fig. 14). The response to nervous stimulation also increases in

excess of calcium, though it may decline at first owing to increase of tone (Fig. 15).

Effect of hydrogen-ions.—If the concentration of hydrogen-ions is increased beyond 6.5, the response to electric current, acetylcholine and nervous stimulation decreases, hence hydrogen-ions are not useful in differentiating these responses.

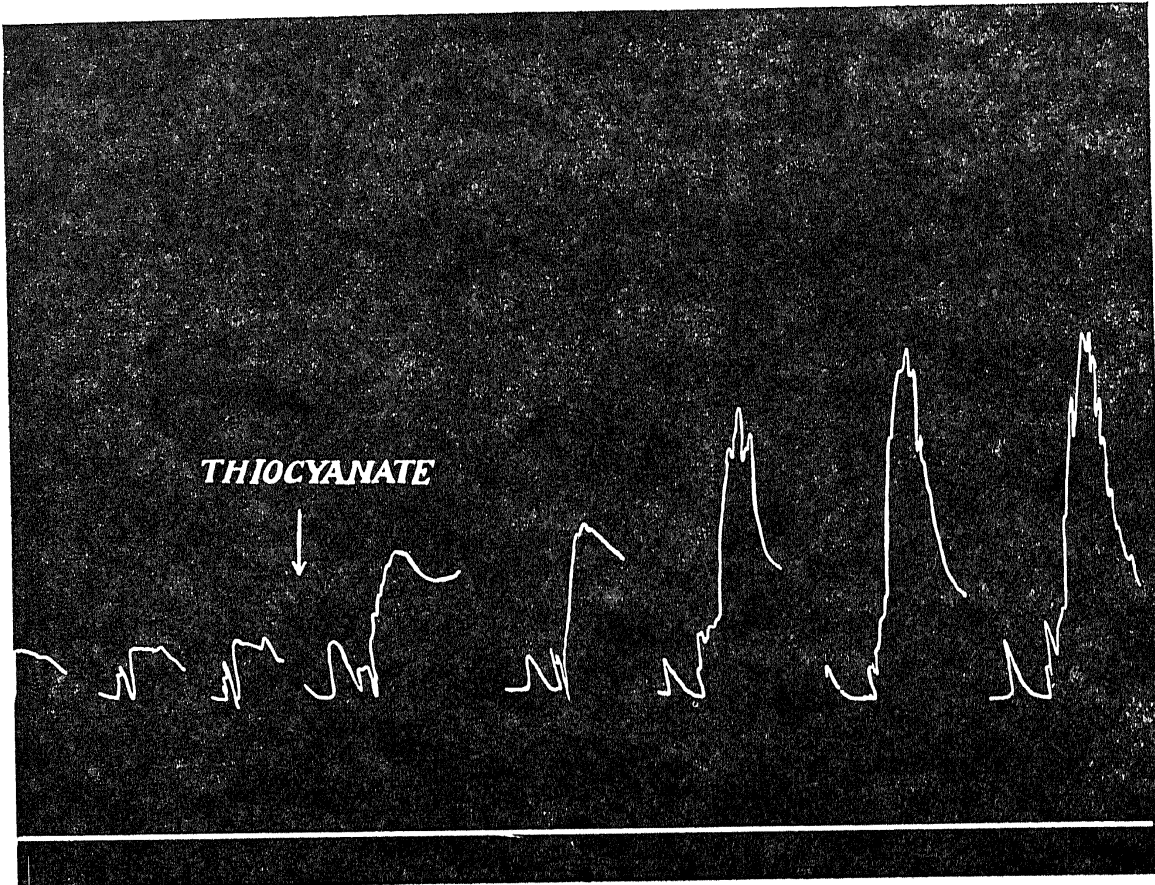


FIG. 9. Dog's stomach muscle. Effect of replacement of the chloride of the saline with thiocyanate on the response to acetylcholine (1 in 10^5). Response every 15 min.

DISCUSSION

Neuromuscular transmission is in its simplest form in unstriated muscle as there are no intervening motor end plates as in skeletal muscle, or complicated synapses as in the central nervous system. The nerve fibres appear to end directly in relation to the muscle fibres.

The anions produce a tonic contraction, hence they antagonise the response to electric current (Singh, 1938 *a*), but potentiate the response to other

tone-producing substances, such as acetylcholine and potassium. They antagonise the response to nervous stimulation, which shows that it is produced by electric current, and this must be the action potential of nerve.

The effect of excess calcium shows that nervous stimulation results in liberation of some tone-producing substance, which is presumably acetylcholine. The initial action potential therefore produces a phasic response,

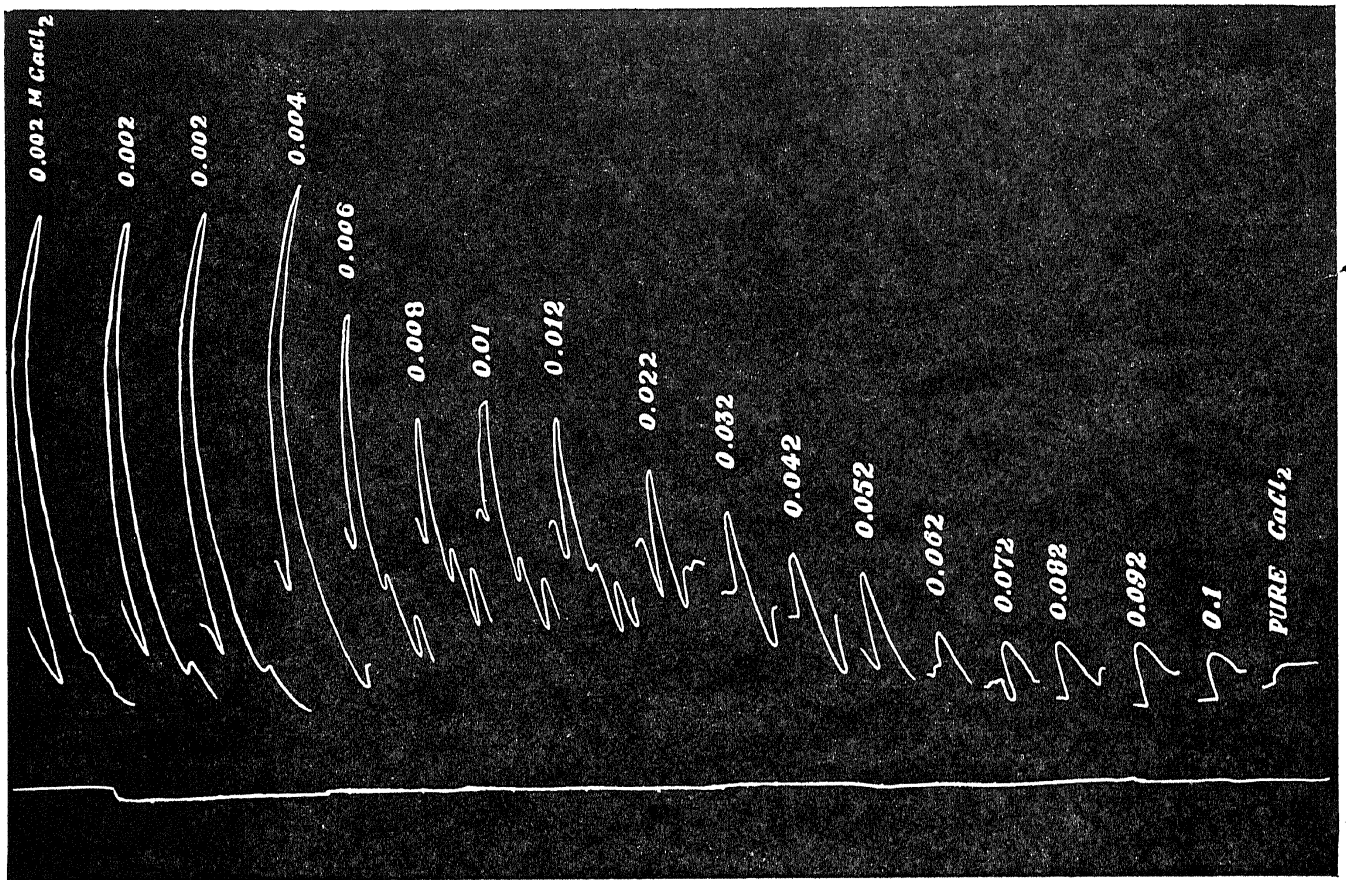


FIG. 10. Dog's stomach muscle. Effect of excess of calcium on the response to alternating current (12 volts/10 sec.). Response every 15 min. pH 6.6, phosphate.

which however is sustained by the subsequent liberation of acetylcholine. This process resembles the sympathicoadrenal response, the sympathetic producing its effects by nervous stimulation, which are sustained by the hormones from the adrenal medulla.

These experiments show that there are two components in transmission, one electrical and the other chemical, one of which may be suppressed. Thus transmission in various parts of the body may be electrical, chemical or electro-chemical. If nervous stimulation is abolished by atropine, then the

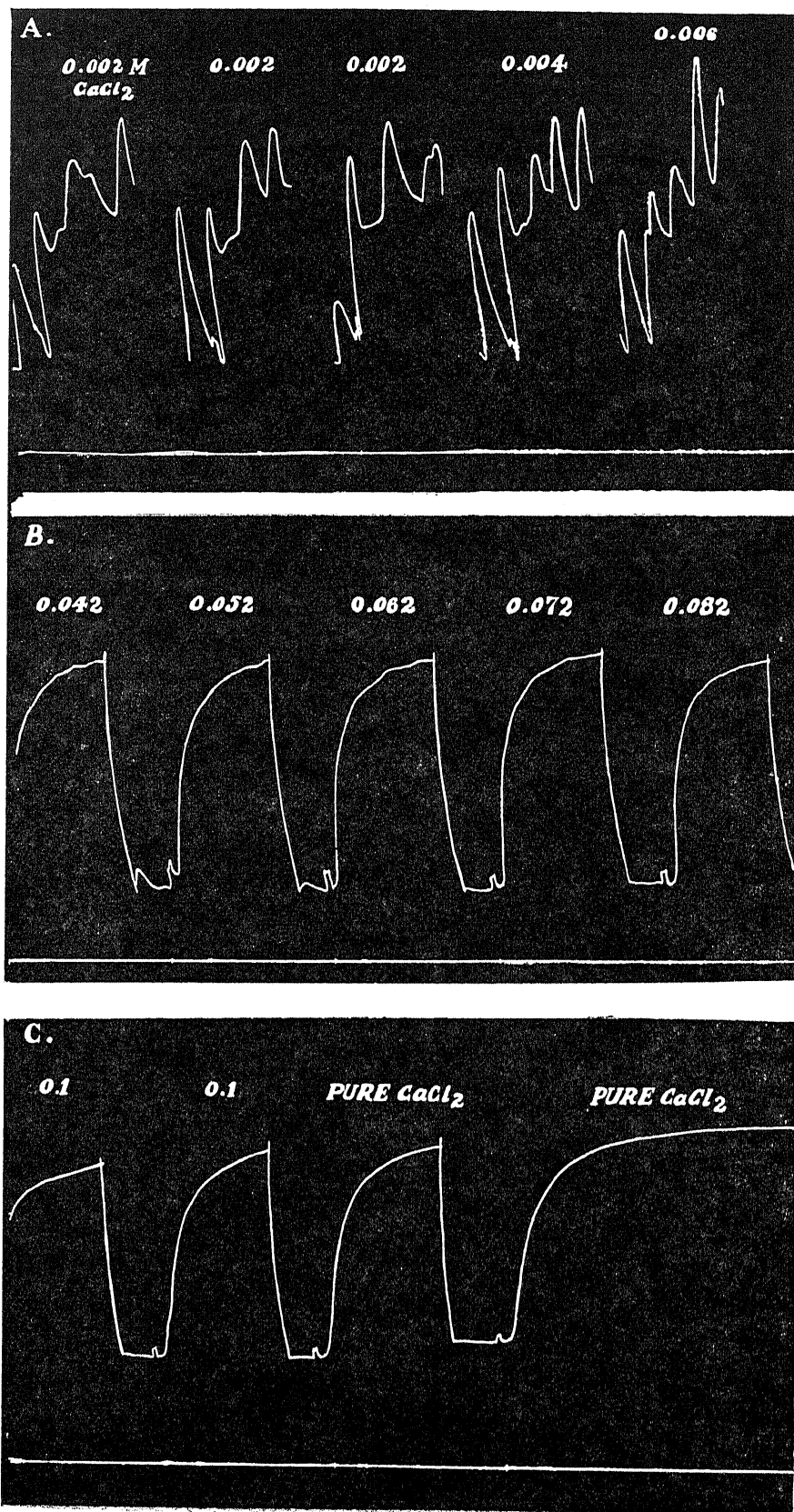


FIG. 11. Dog's stomach muscle. Effect of excess calcium on the response to acetylcholine (1 in 10⁵). Response every 15 min. pH 6.6, phosphate,

transmission is presumably electrical. If it is not abolished by atropine, then it presumably electro-chemical, as no substance has been found which suppresses the response to electric current so completely as atropine suppresses that produced by acetylcholine in dog's stomach muscle, leaving other excitabilities unaffected.

That the response to nervous stimulation is not purely due to the liberation of acetylcholine is shown by the fact that the responses to nervous and

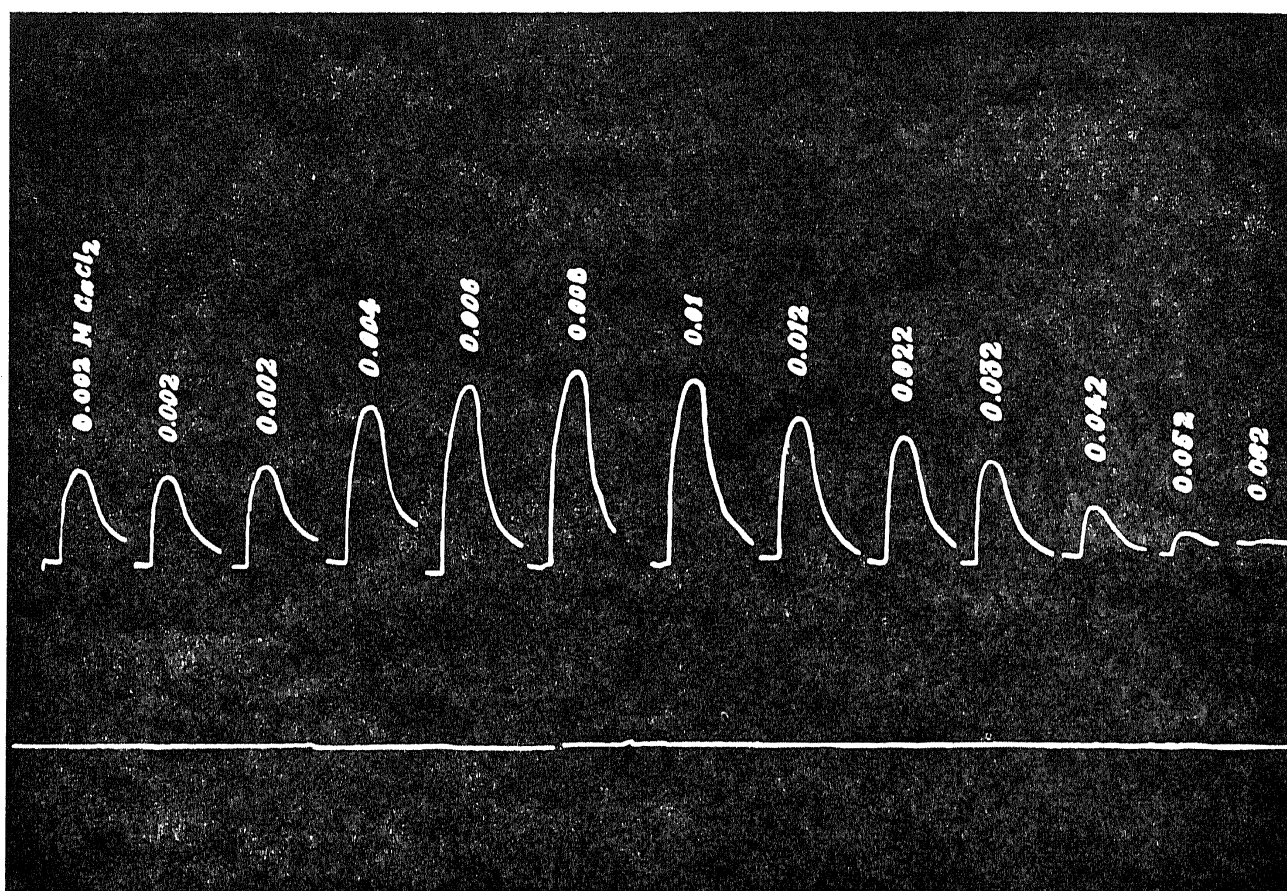


FIG. 12. Dog's stomach muscle. Effect of excess calcium on the response to nervous stimulation for 15 sec. every 15 min. pH 6.6, phosphate.

chemical stimulation may be affected oppositely. Thus in frog's stomach muscle, small concentrations of atropine may potentiate the response to electric current and nervous stimulation, but completely suppress the response to acetylcholine (Singh and Singh, 1947). In frog's stomach muscle, small concentrations of eserine sulphate have a peculiar effect, that the response to acetylcholine may be completely suppressed, but that to electric

current and nervous stimulation potentiated. Adrenaline also has similar action.

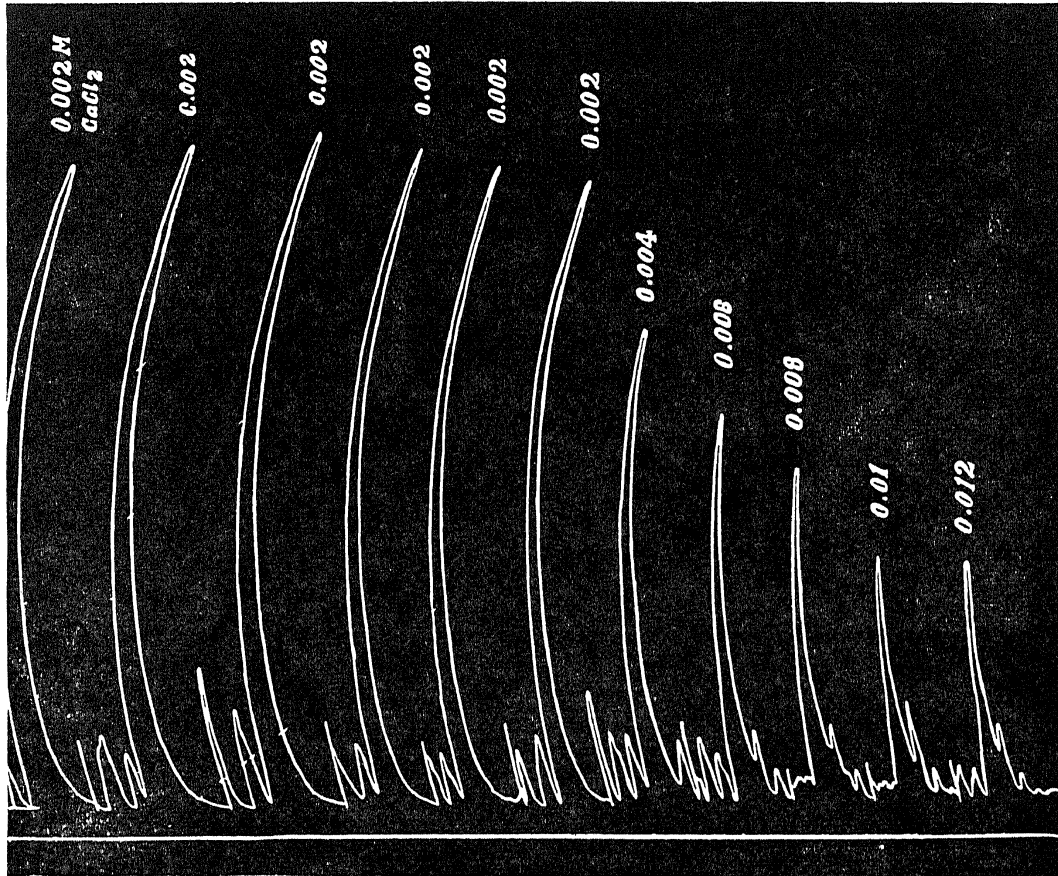


FIG. 13. Dog's stomach muscle. Effect of excess calcium on the response to alternating current (12 volts/10 sec.). Response every 15 min. pH 8, borate.

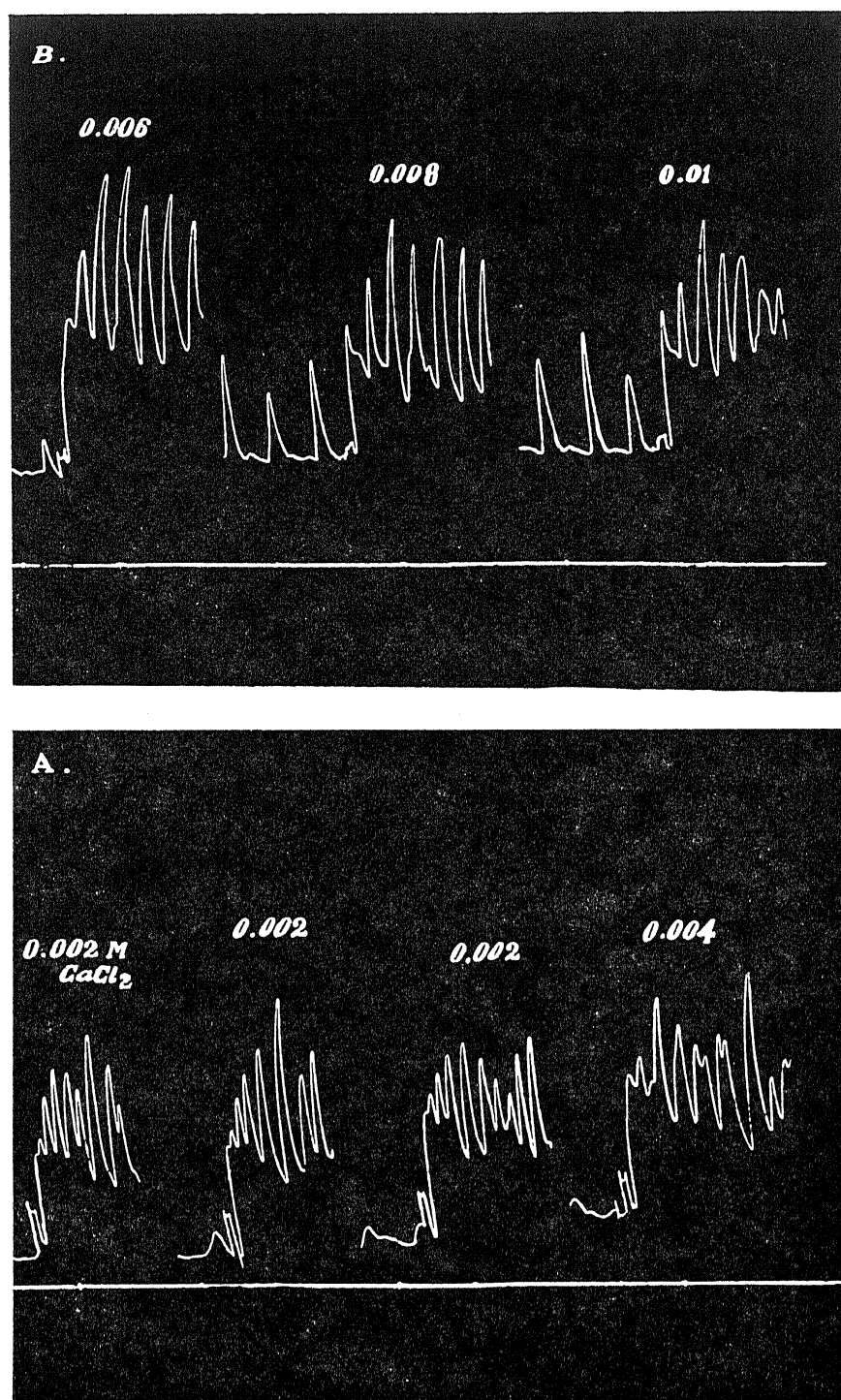


FIG. 14. Dog's stomach muscle. Effect of excess calcium on the response to acetylcholine (1 in 10⁶). Response every 15 min. pH 8, borate.

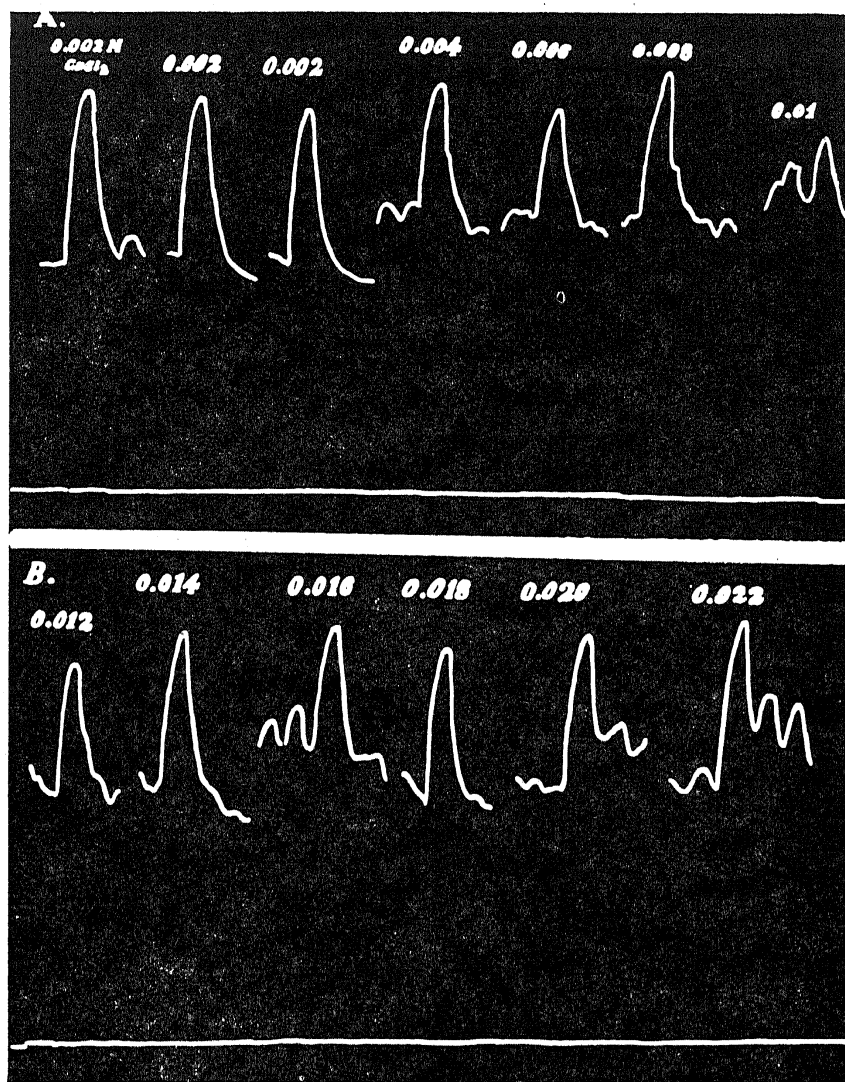


FIG. 15. Dog's stomach muscle. Effect of excess calcium on the response to nervous stimulation for 15 sec. every 15 min. pH 8, borate.

SUMMARY AND CONCLUSIONS

1. Unstriated muscle shows two kinds of responses; one kind is produced by electric current and the other by chemicals such as potassium or acetylcholine.
2. Certain reagents affect these two kind of responses differentially.
3. In dog's stomach muscle, the responses produced by electric current are inhibited by tone-producing substances such as bromide, nitrate, iodide, thiocyanate. The responses to chemical stimulation are potentiated. The

response to nervous stimulation are inhibited. It is concluded, therefore, the response produced by nervous stimulation is produced by electric current, and that can only be the action potential.

4. Excess of calcium potentiates the response to nervous stimulation, acetylcholine and potassium, but inhibits that to electric current. So it is concluded that the response due to nervous stimulation is also due to liberation of a chemical substance, presumably acetylcholine.

5. Neuromuscular transmission in unstriated muscle is therefore electro-chemical, produced both by the action potential of nerve and liberation of a chemical substance. The former produces the initial response, and the latter sustains it, and imparts to it, tonic properties.

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