EFFECT OF Mg^{++} AND K^+ ON SMOOTH AND SKELETAL MUSCLE RESPONSES TO ADRENALINE, ACETYLCHOLINE AND 5-HT

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The effect of different concentrations of magnesium and potassium ions on the actions of adrenaline, acetylcholine and 5–hydroxytryptamine on the smooth and skeletal muscles has been studied. No significant change in the responses of smooth muscles to these hormones was observed with 0.05 mg/ml $MgCl_2$. Whereas quantitative reduction in the responses was noticed with 0.18 mg/ml and 0.35 mg/ml $MgCl_2$. A decrease in the action of acetylcholine on the skeletal muscle was observed with high concentrations (0.18 mg/ml and 0.35 mg/ml) of $MgCl_2$. In the presence of low concentrations of KCl in the Ringer, the effect of acetylcholine and 5–hydroxytryptamine on the smooth muscles was intensified and reduced with high concentration. Adrenaline produced less relaxation of the smooth muscles in the presence of low concentrations of KCl in the modified Ringer solution while this action of the hormone increased with high concentration of potassium ion. An increase in the response of the skeletal muscle to acetylcholine was observed in the presence of low concentrations of potassium chloride and a reduction was seen with high concentration.

Adrenaline, acetylcholine and 5–hydroxytryptamine have been termed as local harmones by Burn (1950). They have vital role to play in regulating tissue functions. Sharma (1958) observed the effect of ions on the action of adrenaline and acetylcholine on rabbit intestine. He showed that the relative state of equilibrium of ionic concentrations of the three bulk metals (Na, K, Ca) in the intracellular and extracellular fluids appears to play a very important role in modifying and probably determining the nature of effects which will be produced by a particular hormone. 5-hydroxytryptamine is a relatively new hormone. The effect of ions on the action of this hormone on smooth and skeletal muscle was undertaken for the present study.

MATERIALS AND METHODS

Perfusion fluid—To study the effect of Mg^{++} on smooth and skeletal muscle responses to adrenaline, acetylcholine and 5-hydroxytryptamine, the magnesium chloride was added in Ringer solutions in quantities of 50 mg (1.04 mEq) 180 mg (3.77mEq) and 350 mg (7.34 mEq) per litre.

Alterations in potassium ion concentrations were made either by reducing or by adding it in the Ringer solution. In the present study it was reduced to $\frac{1}{2}$ and $\frac{1}{4}$ and increased to double the quantity in three modified Ringer solutions respectively. In Frog Ringer's solution the concentration of KCl was 4.02 mEq/L while in modified Ringer it was 1.0, 2.01 and 8.05 mEq/L. In case of Ringer

Locke, the normal Ringer contains 5.63 mEq/L KCl and the modified ones contained 1.4, 2.83 and 11.2 mEq/L. In normal Kreb's Ringer solution the quantity of KCl was 6.4 mEq/L and in modified Ringer solutions it was 1.5, 3.02, and 12.08 mEq/L. Normally 2.7 mEq/L KCl is added in Tyrod's Ringer solution while in modified Ringer it was added in 0.6, 1.34 and 5.36 mEq/L.

When reduction in salt composition was made, appropriate quantity of sucrose was added to prevent alteration in osmolarity. In experiments where addition of salts was done, change in osmolarity was inevitable. The effect of this alteration of osmolarity was excluded by doing experiments with solutions of changed osmolarity without alteration in ion concentration. This too was achieved by sucrose.

The Scheme of dosages for the drugs (adrenaline, acetylcholine and 5-hydroxytryptamine) used in various experiments is given in table 1.

SMOOTH MUSCLE

I. Rat Ileum—The experiments were done on the isolated piece of ileum of albino rats.

The drugs (acetylcholine, adrenaline and 5-hydroxytryptamine) were added to the bath and allowed to act for 30 seconds in case of acetylcholine and adrenaline, and for 90 seconds with 5-hydroxytryptamine. The Ringer solution was then drained and replaced with fresh solution. The preparation was washed twice or thrice to restore the original activity of the muscle. Two consecutive contractions equal in amplitude served as controls. The normal Ringer solution was then replaced by modified Ringer solution. The modified Ringer solution was changed every 2 minutes for 10 minutes. After that, the drugs were added in the same doses to study their effect in the presence of modified Ringer solution on smooth muscle. The response was recorded for 30 seconds and then the preparation was washed several times at an interval of 2 minutes for 10 minutes with normal Ringer solution. The response was taken in normal Ringer solution again.

II. Dog's Tracheal Muscle Preparation—The method described by Sharma (1960) was employed. Responses were obtained by the drugs (Adrenaline, acetylcholine, 5-hydroxytryptamine). Each response was recorded for exactly 90 seconds. The drum was then stopped and the muscle band was thoroughly washed. An interval of five minutes was allowed between two responses.

Two such consecutive responses of equal amplitude acted as controls. Then the normal Ringer was replaced by modified Ringer solution. The response was observed again by adding the drug in the bath. After noting the responses in modified Ringer solution they were taken once again in normal Ringer solution.

III. Rat Fundus—The method described by Vane (1957) was employed.

Records were taken after adding the drug (5-hydroxytryptamine) for 90 seconds. The tissue was then washed. Usually 5-6 minutes were required to achieve the normal tone. Two controls of equal heights were recorded. Then the effect of the drug was seen on the tissue in the presence of modified Ringer solution after which the record was taken once again in the normal Ringer.

SKELETAL MUSCLE

Frog Rectus Abdominis Muscle Preparation: The preparation was set up according to the method described by Burn (1952). The contractions were recorded on slowly revolving kymograph by adding 5 μg of acetylcholine to the bath (giving a final dilution of 1:2,000,000) and leaving it in contact with the tissue for exactly 90 seconds. The strip was then washed and left to relax to the previous base line while the drum was stationary. An interval of at least five minutes was allowed between successive additions of acetylcholine. Two consecutive contractions to acetylcholine equal in amplitude served as controls. The normal Ringer solution was then replaced by modified one which was changed at an interval of a minute for 10 minutes. After that the record was taken for 90 seconds with 5 μg Ach. The strip was then thoroughly washed and left to relax to the previous base line. The control tracing in the presence of normal Ringer solution was taken again.

The data of the result are calculated as follows: First of all the effect of the hormone on the particular tissue was observed in the presence of normal Ringer solution. The response was measured in mm. Then the action of the hormone was seen in the presence of modified Ringer solution. The response thus obtained was also measured in mm. The difference of the two responses was found out. Finally the percentage increase or decrease in the respose was calculated in comparison with the control responses obtained in presence of normal Ringer solution.

RESULTS

Smooth Muscles

TABLE 1

S.No.	Experimental preparation	Drug	Dose	Final concentration in 10 ml. bath
1.	Rat ileum	Adrenaline	5.0μg	1: 2,000,000
		Acetylcholine	0.5μg	1: 20,000,000
		5-hydroxytryptamine	2.0μg	1: 5,000,000
2.	Dog Tracheal Muscle Preparation	Adrenaline	10.0μg	1: 1,000,000
		Acetylcholine	10.0μg	1: 1,000,000
		5-hydroxytryptamine	10.0μg	1: 1,000,000
3.	Rat Fundus	5-hydroxytryptamine	50.0ng	1:200,000,000
4.	Frog Rectus Muscle Preparation	Acetylcholine	5.0μg	1: 2,000,000

TABLE 2

Effect of Mg^{++} on the action of adrenaline, acetylcholine and 5-hydroxytryptamine on smooth and skeletal muscles.

Concentration of Mg^{++} in Ringer solution	Drug	No. of observations	Mean Per cent change in the response of hormone ± S. D. Increase (+), Decrease (−)			
			Rat Ileum	Tracheal Muscle	Rat Fundus	Frog rectus muscle
0.0005 M (0.05 mg/ml)	Adr.	6	0.0	0.0	—	—
	Ach.	6	0.0	0.0	—	0.0
	5-HT	6	0.0	0.0	0.0	—
0.002 M (0.18 mg/ml.)	Adr.	6	-7.9 ± 2.8 ($P<0.05$)	-4.6 ± 2.8 ($P<0.01$)	—	—
	Ach.	6	-47.8 ± 7.43 ($P<0.001$)	-28.1 ± 4.3 ($P<0.001$)	—	-26.6 ± 5.97 ($P<0.001$)
	5-HT	6	-50.6 ± 6.7 ($P<0.001$)	-19.7 ± 4.3 ($P<0.001$)	-25.4 ± 6.6 ($P<0.001$)	—
0.004 M (0.35 mg/ml).	Adr.	6	-23.12 ± 7.1 ($P<0.01$)	-7.0 ± 4.9 ($P<0.001$)	—	—
	Ach.	6	-56.5 ± 4.13 ($P<0.001$)	-37.02 ± 4.02 ($P<0.001$)	—	-32.97 ± 6.9 ($P<0.001$)
	5-HT	6	-61.6 ± 3.4 ($P<0.001$)	-41.14 ± 6.6 ($P<0.001$)	-44.98 ± 3.63 ($P<0.001$)	—

I. (A) Magnesium ion and Adrenaline : The antiacetylcholine activity of the hormone on rat ileum and tracheal muscle preparation was same in normal and modified Ringer solution when the latter contained 0.05 mg/ml. $MgCl_2$. There was a quantitative decrease in the response of these tissues to adrenaline in the presence of modified Ringer solutions, containing 0.18 mg/ml and 0.35 mg/ml $MgCl_2$ respectively. The findings are given in Table 2.

(B) Potassium ion and Adrencline : The response of rat ileum and tracheal muscle preparation to adrenaline was decreased when the quantity of potassium chloride was reduced in the Ringer solutions and increased when double the normal amount of potassium chloride was added in modified Ringer solution. The findings are given in Table 3.

II. (A) Magnesium ion and Acetylcholine : There was no change in the response of the smcoth muscles to acetylcholine when 0.05 mg/ml. $MgCl_2$ was added in the Ringer solution. The effect of acetylcholine decreased when the Ringer solutions contained 0.18 mg/ml and 0.35 mg/ml. $MgCl_2$ respectively. The findings are given in Table 2 and Figure 1.

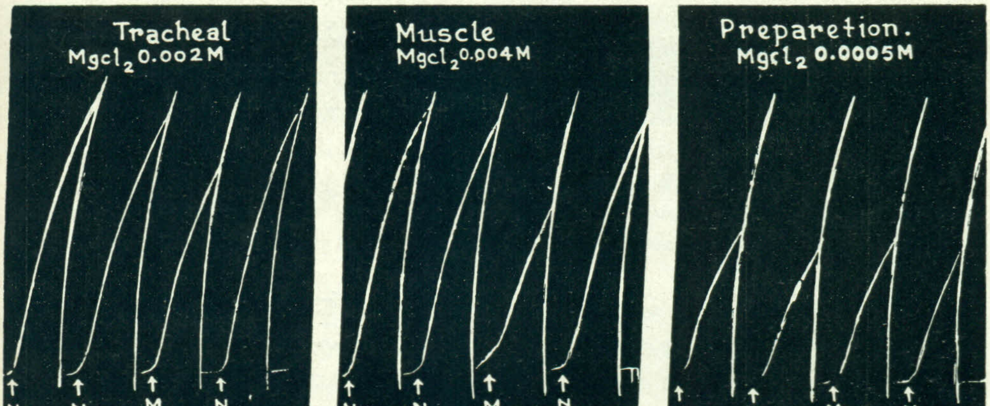

Fig. 1. Illustrates the modification in acetylcholine (1:1,000,000) responses of tracheal muscle of dog, produced by various quantities of $MgCl_2$ in the Ringer solution (N-Normal Ringer, M-Modified Ringer). The various concentrations of Mg^{++} in Ringer solution are indicated at the top of each series of contractions.

(B) Potassium ion and Acetylcholine : When the potassium chloride concentration was reduced to $\frac{1}{2}$ and $\frac{1}{4}$ of the normal, the response of the muscles to acetylcholine increased. They decreased when double the amount of KCl was added in the modified Ringer solution. The findings are given in Table 3.

TABLE 3

Effect of K^+ on the action of adrenaline, acetylcholine and 5-hydroxytryptamine on smooth and skeletal muscles.

Concentration of K^+ in Ringer solution	Drug	No. of observations	Mean per cent change in the response of hormone ± S. D. Increase (+), Decrease (–).			
			Rat Ileum	Tracheal muscle	Rat Fundus	Frog rectus muscle
Reduced to ¼ of the normal	Adr.	6	– 11.0±4.8 (P<0.001)	– 4.7±1.2 (P<0.001)	—	—
	Ach.	6	+ 25.13±7.5 (P<0.001)	+ 24.8±5.8 (P<0.001)	—	+ 12.2±1.7 (P<0.001)
	5-HT	6	+ 48.3±8.1 (P<0.001)	+ 24.7±4.3 (P<0.001)	+ 27.6±4.8 (P<0.001)	—
Reduced to ½ of the normal	Adr.	6	– 6.0±3.4 (P<0.05)	– 13.3±4.7 (P<0.001)	—	—
	Ach.	6	+ 14.5±3.5 (P<0.001)	+ 14.4±7.6 (P<0.001)	—	+ 10.9±2.5 (P<0.001)
	5-HT	6	+ 25.4±4.9 (P<0.001)	+ 13.0±0.95 (P<0.001)	+ 14.62±1.7 (P<0.001)	—
Double the normal amount	Adr.	6	+ 3.5±0.61 (P<0.05)	+ 8.9±1.5 (P<0.001)	—	—
	Ach.	6	– 23.9±2.7 (P<0.001)	– 41.8±13.3 (P<0.001)	—	– 32.5±3.9 (P<0.05)
	5-HT	6	– 42.3±13.0 (P<0.001)	– 19.6±5.7 (P<0.001)	– 25.7±5.3 (P<0.001)	—

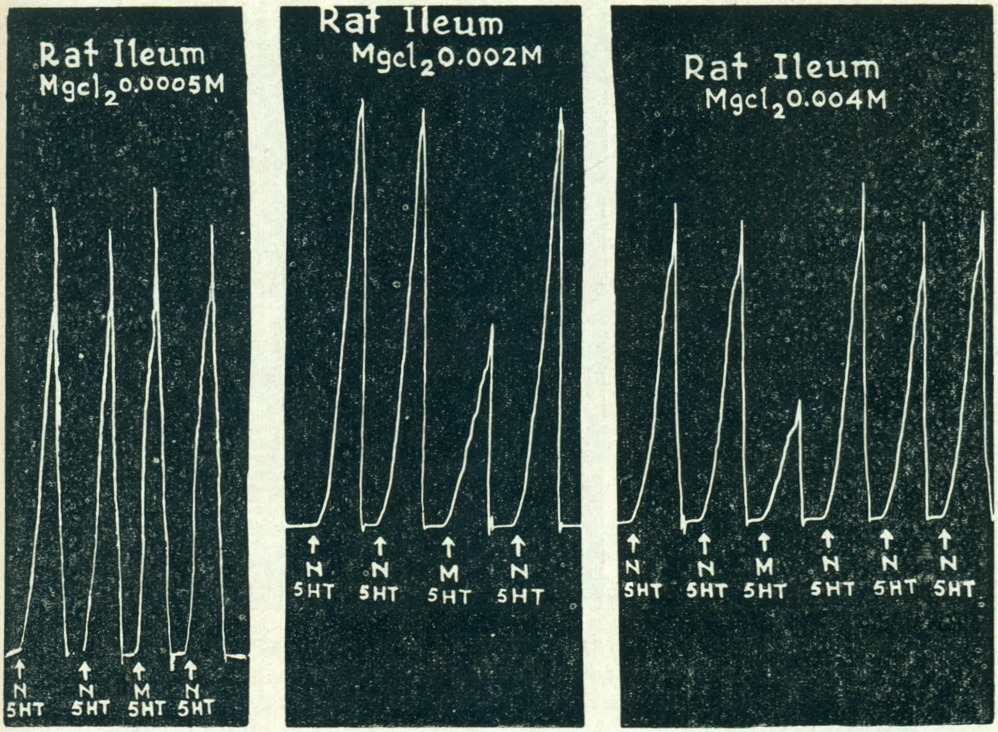

Fig 2. Illustrates the modification in 5-hydroxytryptamine (1:1,000,000) responses of the isolated rat ileum, produced by the addition of various quantities of $MgCl_2$ in the Ringer solution (N-Normal Ringer, M–Modified Ringer). The various concentrations of magnesium ion in the Ringer solution are mentioned at the top of each series of contractions.

III. (*A*) ***Magnesium ion and 5-hydroxytryptamine*** : The study was made on rat ileum, dog's tracheal muscle preparation and rat fundus. Similar modifications in the response of this hormone were observed on these muscles as seen with acetylcholine in the presence of modified Ringer solutions. The findings are shown in Table 2 and in Fig. 2.

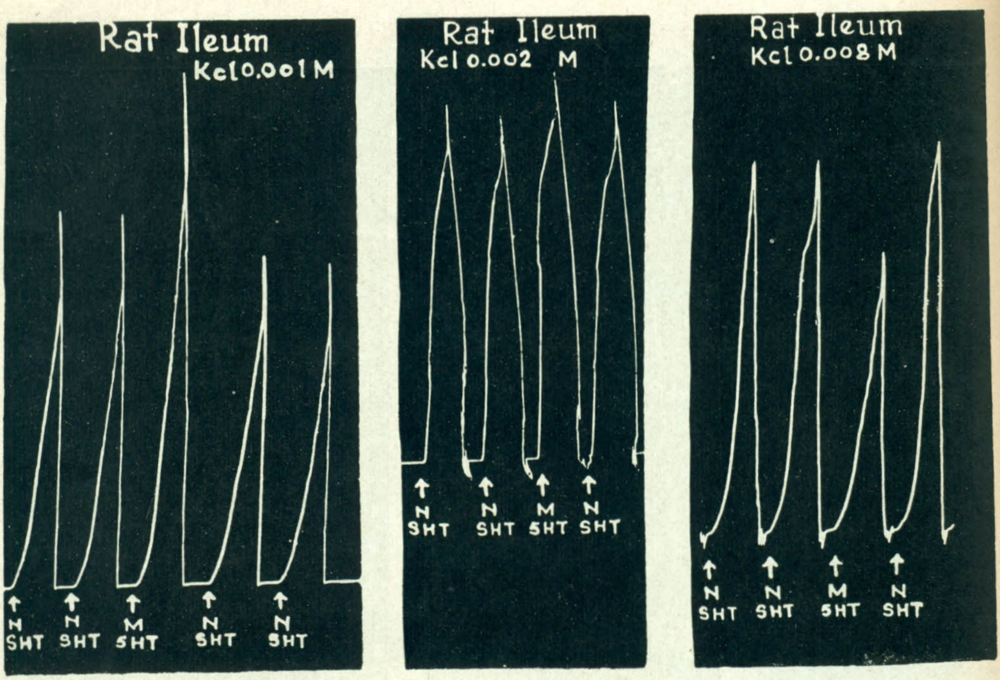

Fig. 3. Shows the modification in 5-hydroxytryptamine (1:5,000,000) responses of the isolated rat ileum, produced by the variations in the quantity of potassium in the Ringer solution (N-Normal Ringer, M-Modified Ringer). The potassium variations in the Ringer are indicated at the top of each series of contractions.

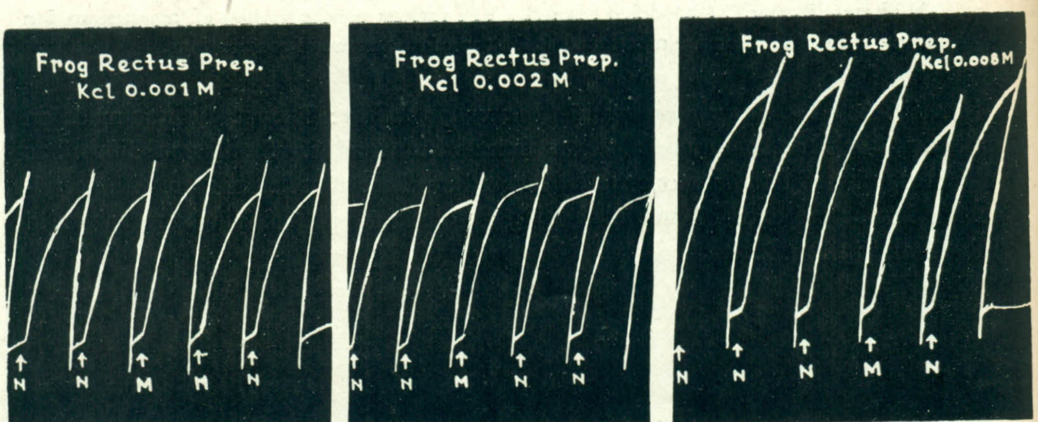

Fig. 4. Shows the modification in acetylcholine (1:2,000,000) responses of the isolated frog rectus muscle, caused by the variations in the quantity of K^{+} in the Ringer solution (N-Normal Ringer, M-Modified Ringer). The potassium variations are indicated at the top of each series of contractions.

(B) Potassium ion and 5-hydroxytryptamine: An increase in the response of 5-hydroxytryptamine on the smooth muscles was noted on reducing KCl concentration to ¼ and ½ of the normal while there was decrease in the action of the hormone in presence of modified Ringer solution which contained double the quantity of potassium chloride. The findings are given in Table 3 and figure 3.

Skeletal Muscle :

(A) Magnesium ion and Acetylcholine: There was no alteration in the effect of this hormone on frog rectus, in the presence of modified Ringer solution, containing 0.05 mg/ml $MgCl_2$. A quantitative reduction in the response was noted when 0.18 mg/ml and 0.35 mg/ml $MgCl_2$ was added respectively. The findings are given in Table 2.

(B) Potassium ion and Acetylcholine: A decrease in the response of acetylcholine was observed in the presence of modified Ringer solution containing double the normal amount of potassium chloride. There occurred an increase in the response of the muscle to the hormone when potassium chloride was reduced to ¼ to ½ of the normal. The findings are given in Table 3 and figure 4.

DISCUSSION

It has been extensively emphasized by various workers that Na, K and Ca ions in proper concentrations play an important role in maintaining the physiological state of the tissue. In the present study the effects of magnesium and potassium ions on adrenaline, acetylcholine and 5-hydroxytryptamine have been studied on smooth and skeletal muscles. In case of skeletal muscle the effect of ions on acetylcholine action alone was investigated.

Magnesium ion and local Hormones: The responses obtained on different smooth muscles were similar and hence are discussed together. The results show that $MgCl_2$ in concentrations of 0.35 mg/ml (7.34 mEq/L) and 0.19 mg/ml (3.77 mEq/L) reduced the effect of adrenaline, acetylcholine and 5-hydroxytryptamine on the smooth muscles. This is in conformity with the views of other workers. Stanbury (1948) showed that magnesium had direct inhibitory action on the nictitating membrane in addition to the blocking of the superior cervical ganglion. Haury (1938) showed a bronchodilator action of magnesium by perfusion of excised guineapig lungs. In isolated intestine, Zadine and Kriz (1948) observed that magnesium chloride reduced the height of contraction obtained with acetylcholine and decreased the sensitivity of the muscle. It has been generally reported that an increase in Mg^{++} concentration causes relaxation and a decrease in responsiveness of vascular smooth muscle, (Haddy et al., 1963; Hanenson, 1962; Scot et al. 1961). Sperelakis (1962) has observed that treatment with 20 mg arrests the mechanical activity of intestinal smooth muscle but permits continuation of action potentials.

In conc. of 0.18 mg./ml and 0.35 mg./ml. $Mgcl_2$ decreased the action of acetylcholine on the skeletal muscle.

A number of workers (Engbaek, 1949, Jenerick and Gerard, 1950) have found that magnesium depressed the excitability of amphibian muscle. Magnesium also has depressive action on rats diaphragm muscle fibres as reported by Paul (1960), Choa (1935), Ashkenaz (1938). Carleton et al., (1938), Jenerick and Gerard (1950) have suggested that the mechanism of depression of excitability of amphibian muscle by magnesium can be explained on the basis that it does so by raising the threshold to stimulation.

Potassium ion and local Hormones : The response of acetylcholine and 5-hydroxytryptamine on rat ileum and tracheal muscle preparation was reduced when the quantity of potassium chloride was doubled in the Ringer solution. This response was increased on reducing the amount of KCl to half and one-fourth of the normal. In case of 5-hydroxytryptamine similar findings were observed on rat fundus as seen on other smooth muscles.

By producing changes in the concentration of potassium in Ringer solution, a change in the action of adrenaline on the small intestine of rat and tracheal muscle of dog was observed. High potassium increased while low potassium decreased the relaxation caused by adrenaline.

An increase in the response of acetylcholine on the skeletal muscle was observed when the amount of potassium chloride was reduced to half and one-fourth of the normal in the modified Ringer solution. On the other hand it reduced when the quantity of potassium chloride was doubled in the modified Ringer solution.

The ionic basis of electrical activity of nerve and muscle is widely accepted. The resting action potential of muscle is due to high intracellular potassium and high extracellular sodium. Hodgkin and Horawickz (1959) have shown that the action potential is dependent on the concentration gradients of potassium and chloride ions across the membrane, in experimental animals the resting potential varies with these ingredients. Depolarization is accompanied by rapid entry of sodium into the cell and exit of potassium as studied by Hodgkin and Horawicz (1959). It is this transfer of ion which occurs in smooth as well as in skeletal muscle contraction.

According to Grob (1957) when extracellular potassium is high the movement of potassium from the muscle is reduced and hence there is decrease in depolarization activity of acetylcholine.

There was potentiation of adrenaline activity by increase in extracellular potassium and decrease in the adrenaline activity by decrease in potassium concentration. This can also be explained on the basis of Born and Bulbring

(1956) who have shown that the relaxation of the intestine and trachea is associated with uptake of potassium by the cell. Thus when the potassium ion is high in extracellular fluid, more of it can be taken off and hence there will be better relaxation and vice versa.

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