# QUANTITATIVE STUDIES IN ION ANTAGONISM

## I. Experiments on the Striated Muscle of the Frog

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Although the study of ion antagonism goes back to the year 1882 when Ringer published his first paper on ion antagonism in the heart, quantitative study of the subject has just begun. Loeb (1915*b*) suggested the interesting theory that the quantitative relationship in ion antagonism depends upon the function concerned. If ions influence irritability, a linear relation is supposed to exist between the antagonistic ions, while a parabolic relation expresses the antagonism if permeability is concerned. Recently Rubinstein (1928) performed experiments on a polychæte worm (*Fabricia sabella*) and found a parabolic relationship $[K] = a\sqrt{[Na]}$, although there was no proof that this antagonistic action was based upon changes in permeability. It is the writer's opinion that a sharp distinction between the two groups as suggested by Loeb is not possible at the present time. Changes in the behavior of the surface of cells may often initiate changes in permeability (Gellhorn, 1930). In spite of this, the ingenious idea of Loeb, that the quantitative study of ion antagonism may reveal different kinds of antagonism and may finally lead to a better understanding of that problem, merits further investigation. In contrast to the work of Loeb (1915*a*) on *Balanus* larvæ and that of Rubinstein (1926) on *Fabricia*, in which several tissues were involved, the writer has considered it preferable to examine ion antagonism as it affects one single structure. This is possible in muscle, for Kato (1929) has shown in histological studies that the antagonistic ions act upon the same anatomical structure.

It is apparent from the investigations of Overton (1904) that the irritability of striated muscle depends chiefly upon NaCl, KCl, and $CaCl_2$. Therefore a quantitative study of ion antagonism was carried out between these ions. The effect of the trace of $NaHCO_3$ added to the Ringer's solution to preserve neutrality was not taken into consideration.

## Method

The experiments were chiefly performed on the sartorius muscle of *Rana esculenta* between January and April, 1930. Frogs of medium size, which did not vary more than 10 per cent in weight, were chosen. The sartorii were prepared carefully in the usual manner and suspended between platinum electrodes. The muscles were kept in the salt solution, which was aerated with purified oxygen. At intervals of about thirty minutes the threshold of the muscle was determined

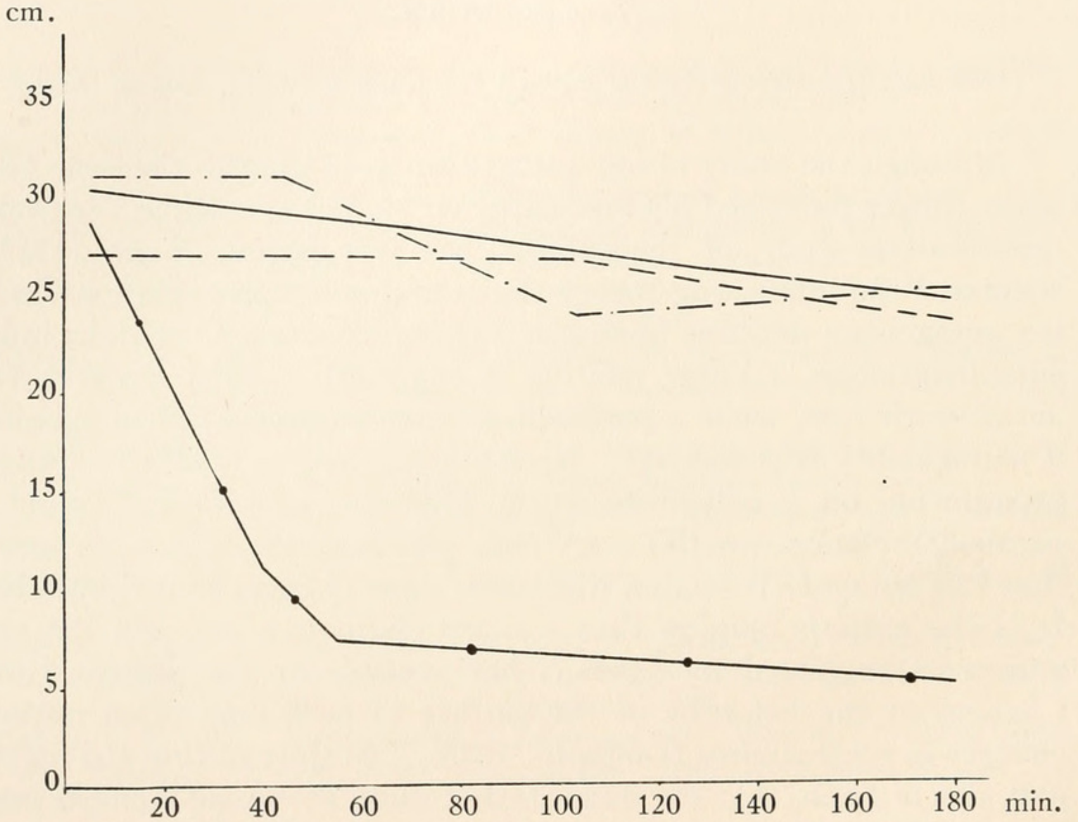


Fig. 1. The influence of the variation of KCl—NaCl and $CaCl_2$ being constant—upon the irritability of the sartorius.

Ordinate: threshold in cm. distance between primary and secondary coil.

Abscissa: time in minutes.

| | | | |
|---|---|---|---|
| 0.042 M NaCl | + 8.1 M × $10^{-4}$ $CaCl_2$ | + 11.3 M × $10^{-4}$ KCl | ———————— |
| " | " | + 17 M × $10^{-4}$ KCl | — — — — — — |
| " | " | + 20 M × $10^{-4}$ KCl | —·—·—·—· |
| " | " | + 23 M × $10^{-4}$ KCl | —●—●—●— |

after having removed the muscle from the liquid. The interrupted current of an induction coil, the primary circuit of which was supplied with one dry cell (1.5 volts), was used. In a series of preliminary experiments it was found that if at constant NaCl and $CaCl_2$ content the influence of increasing KCl concentration was investigated during the observation time of three hours, a sharp distinction between two groups of KCl effects could be made. In the first group, containing the lower KCl concentration, the threshold increased very gradually within three hours and the final value was indicated by a distance of at least 20 cm. between primary and secondary coils. In the greater concentrations, however, the irritability decreased very rapidly and fell far below 20 cm. The initial value, which varied between 25 and 35 cm. distance, had no influence on this typical behavior. Thus it was possible to determine exactly the threshold of the KCl effect which just leads to a loss of irritability below 20 cm. distance. In about 200 experiments, there were very few cases where the classification was doubtful. In such cases several repetitions of the experiments gave decisive results. A typical example illustrating the accuracy of the method is reproduced in Fig. 1. Further on it is shown in Fig. 2 that the antagonistic action of $CaCl_2$ can be determined exactly in the same manner.

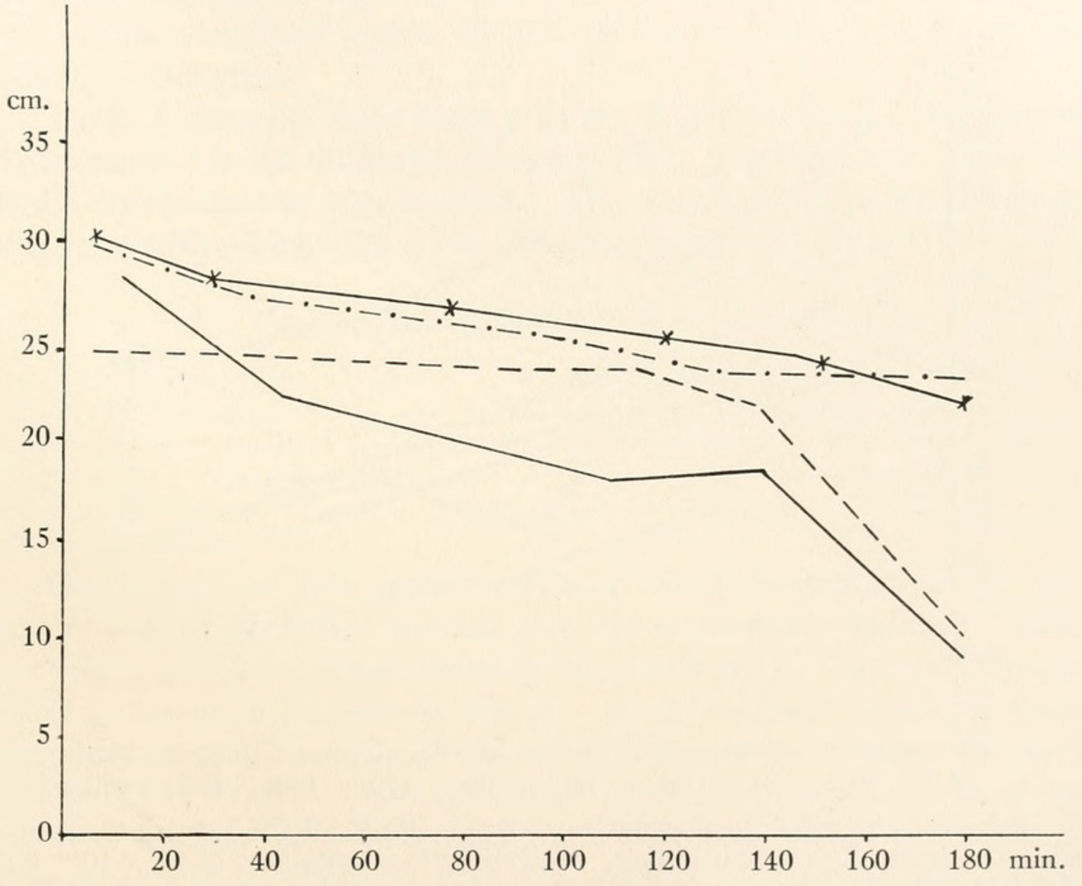


FIG. 2. The influence of variation of $CaCl_2$—NaCl and KCl being constant—upon the irritability of the sartorius.

Ordinate and abscissa as in Fig. 1.

0.084 M NaCl + 84 M × $10^{-4}$ KCl + 46.57 M × $10^{-4}$ $CaCl_2$ ————

" " 48.6 " — — — —

" " 50.62 " —·—·—·—

" " 52.65 " —x—x—x—

The solutions used had a freezing point of $\Delta = -0.39$. They were 0.113 M NaCl, 0.113 M KCl and 0.081 M $CaCl_2$. Greater concentrations of NaCl were obtained with different amounts of 1 per cent NaCl. The amount of liquid used was always 20 cc.

## I. *The Antagonism between Na and K*

In a series of experiments the dependence of the KCl effect upon the NaCl concentration was studied. The $CaCl_2$ concentration, $8.1\ M \times 10^{-4}$, was constant in all experiments. There were two ways of varying the NaCl concentration: first, by replacing a part of the NaCl solution by isotonic glucose solution, thus avoiding changes in the osmotic pressure for all solutions containing less NaCl than corresponds to the isotonic concentration; second, without the

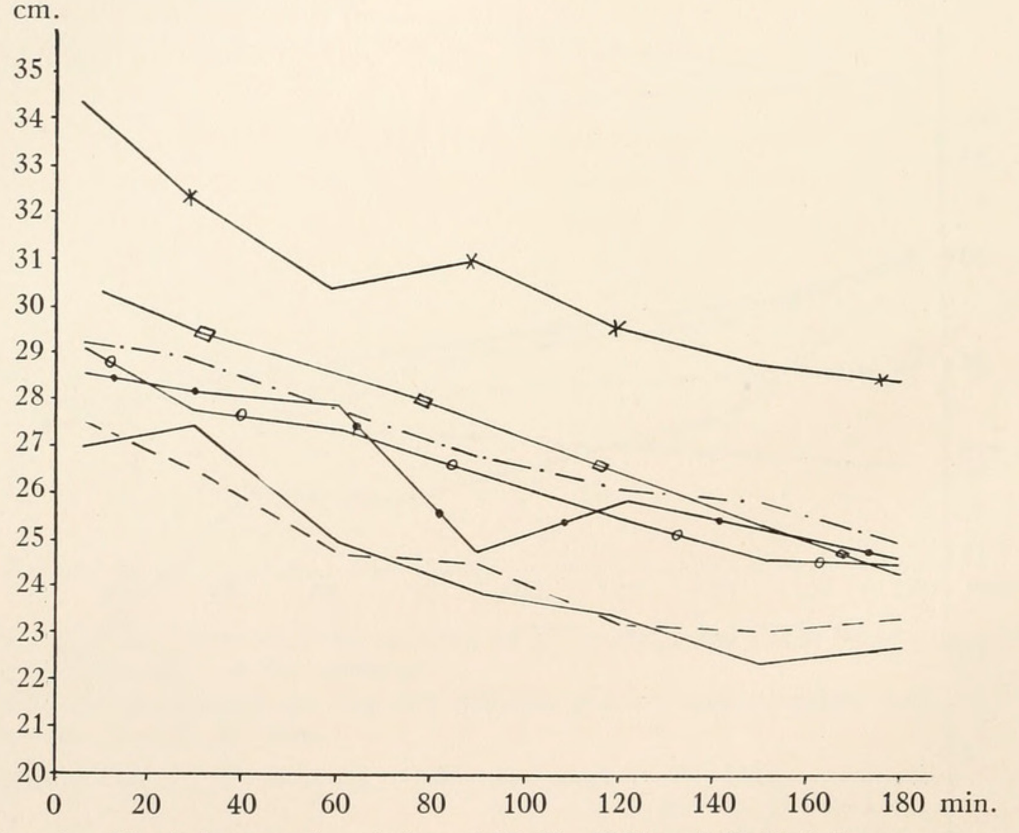


FIG. 3. The influence of variation of NaCl—KCl and $CaCl_2$ being constant—upon the irritability of the muscle. Each solution contains $5.65\ M \times 10^{-4}$ KCl $+ 8.1\ M \times 10^{-4}$ $CaCl_2$.

Ordinate and abscissa as in Fig. 1.

0.042 M NaCl —□—□—
0.056 M " — — — —
0.071 M " ————
0.08 M " —x——x—
0.09 M " —o——o—
0.113 M " —·—·—·—
0.127 M " —●——●—

addition of any non-electrolytes. The experiments were performed in both ways and it was found that glucose even in small amounts in the presence of NaCl is not indifferent to the muscle. The effective minimal concentration of KCl was greater with the addition of glucose than without it. This shows that glucose protects the muscle to a certain extent from the harmful effect of KCl. Similar observations were made in a study of the KCl contracture in muscle (Gellhorn, 1926). Because the osmotic pressure can be maintained constant for various concentrations of NaCl only by the addition of varying amounts of non-electrolytes, the effective minimal concentration of KCl would also be dependent upon the sugar concentration. In order to avoid these complications, salt solutions without non-electrolytes were used in spite of differences in the osmotic pressure. It is apparent from Fig. 3 that it was possible to study the effect of NaCl concentration between the values 0.042 M and 0.127 M because the threshold of the muscle remained above 20 cm. for three hours in spite of the osmotic differences.

Table I contains data from a series of experiments in which the dependence of the minimal effective KCl concentration upon the NaCl concentration was studied. The experiments show the great accuracy with which this value can be determined. It is:

| Concentration KCl ($M \times 10^{-4}$) | for M NaCl |
|---|---|
| 23 | 0.042 |
| 43 | 0.056 |
| 51 | 0.071 |
| 68 | 0.092 |
| 85 | 0.113 |

One recognizes that with increasing NaCl concentration the KCl concentration must also increase in order to become effective. There is therefore an antagonism between NaCl and KCl. The quantitative relationship is to be seen in Fig. 4. It shows that there is a linear dependence of $[K^+]$ upon $[Na^+]$ within the limits of 0.049 M NaCl and 0.113 M NaCl. Only in the extreme NaCl concentrations are small deviations from the straight line observed. They are probably due to a greater sensitivity of the muscle to KCl because of the extremely abnormal osmotic pressure. Therefore, further experiments were restricted to the range in which the linear dependence is exactly correct. According to these experiments the ratio of Na to K is constant as may be seen from Table II.

## Table I

*The Dependence of the KCl Effect upon the NaCl Concentration*

Group I. 0.042 M NaCl + 0.00081 M $CaCl_2$

| | | | | | | |
|---|---|---|---|---|---|---|
| $M \times 10^{-4}$ KCl concentration | 5.7 | 11.3 | 17 | 20 | 23 | 26 |
| Threshold in cm. after 5′ | 28.0* | 30.4 | 27.1 | 31.1 | 29.0 | 26.4 |
| " " " " 180′ | 21.8 | 24.6 | 23.5 | 24.7 | 7.3† | 12.0 |

Group II. 0.056 M NaCl + 0.00081 M $CaCl_2$

| | | | | |
|---|---|---|---|---|
| $M \times 10^{-4}$ KCl concentration | 34.0 | 40 | 43 | 45 |
| Threshold in cm. after 5′ | 29.4 | 31.3 | 31.0 | 31.4 |
| " " " " 180′ | 26.9 | 23.0 | 6.3 | 7.9 |

Group III. 0.071 M NaCl + 0.00081 M $CaCl_2$

| | | | | | | |
|---|---|---|---|---|---|---|
| $M \times 10^{-4}$ KCl concentration | 48 | 50 | 51 | 54 | 57 | 68 |
| Threshold in cm. after 5′ | 31.5 | 35.5 | 34.5 | 32.0 | 32.1 | 36.5 |
| " " " " 180′ | 22.2 | 21.8 | 14.5 | 4.5 | 7.5 | 5.7 |

Group IV. 0.092 M NaCl + 0.00081 M $CaCl_2$

| | | | | |
|---|---|---|---|---|
| $M \times 10^{-4}$ KCl concentration | 62 | 65 | 68 | 71 |
| Threshold in cm. after 5′ | 27.4 | 27.3 | 27.0 | 26.8 |
| " " " " 180′ | 26.0 | 20.5 | 9.0 | 7.5 |

Group V. 0.113 M NaCl + 0.00081 M $CaCl_2$

| | | | |
|---|---|---|---|
| $M \times 10^{-4}$ KCl concentration | 79 | 82 | 85 |
| Threshold in cm. after 5′ | 36.5 | 27.2 | 27.5 |
| " " " " 180′ | 23.2 | 20.5 | 9.0 |

* The numbers represent the threshold of the muscle measured by the distance between primary and secondary coil.

† The underlining indicates that the threshold has fallen below 20 cm. distance.

## Table II

*Ratio of Na to K for Different Na Concentrations*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M NaCl | 0.042 | 0.049 | 0.056 | 0.071 | 0.085 | 0.092 | 0.099 | 0.113 | 0.127 |
| $\frac{\text{M NaCl}}{\text{M KCl}}$ | 1.87 | 1.33 | 1.39 | 1.36 | 1.36 | 1.35 | 1.34 | 1.34 | 1.41 |

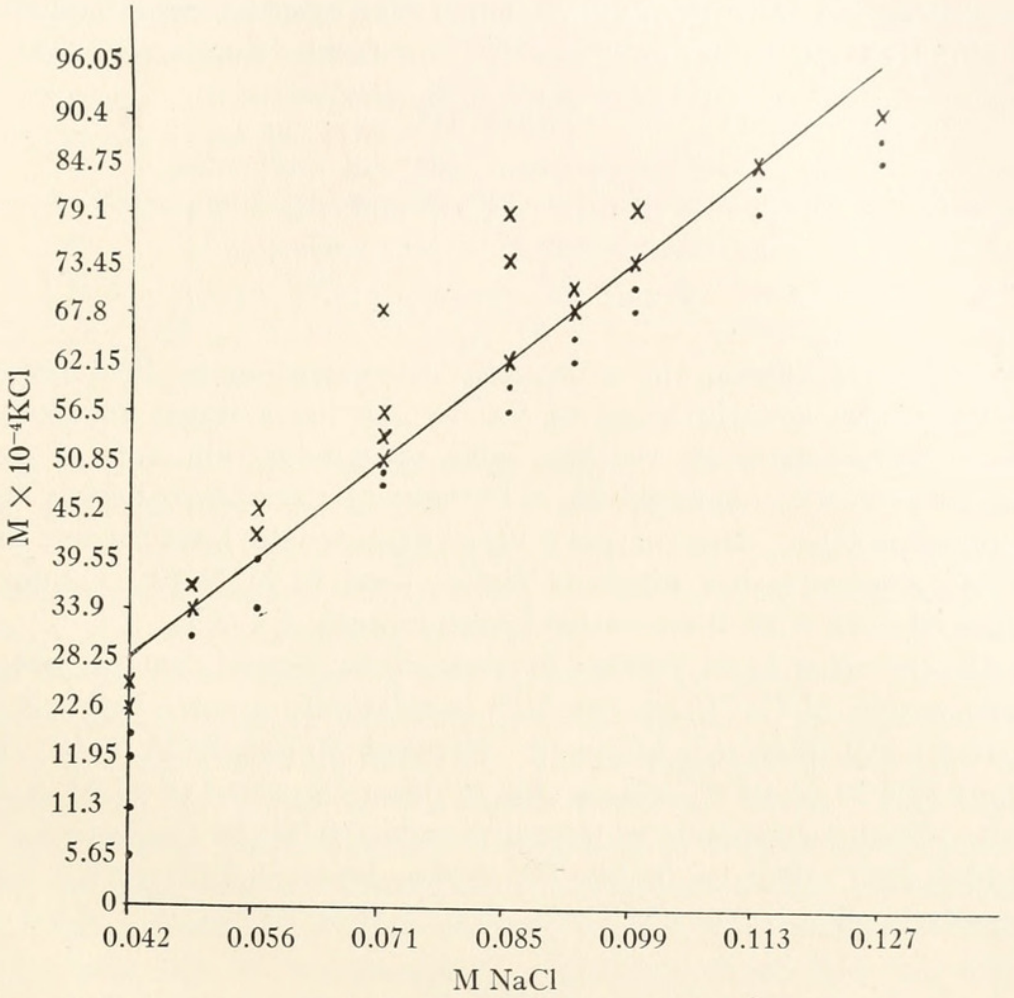


FIG. 4. The influence of the Na concentration upon the K effect.
Abscissa: NaCl concentration.
Ordinate: KCl concentration.

x = Rapid loss of irritability.
· = Preservation of irritability.

## II. *The Antagonism between K and Ca*

The solutions, the effects of which on irritability of the sartorius muscle were detailed in Part I, contained 8.1 M $\times 10^{-4}$ $CaCl_2$. Because it is known that Ca is the antagonist of K in muscle, the K effect in our experiments was balanced by NaCl + $CaCl_2$. In order to learn the quantitative relationship between K and Ca, the KCl concentration was varied and that $CaCl_2$ concentration which just sufficed to maintain the irritability above the 20 cm. threshold was determined. In these experiments the NaCl concentration was constant (0.071 mol.). As shown in Fig. 4, 8.1 M $\times 10^{-4}$ $CaCl_2$ was able to equilibrate the effect of 50 M $\times 10^{-4}$ KCl, but at the same $CaCl_2$ concentration 51 M $\times 10^{-4}$ KCl led to a rapid decrease in irritability. In a series of experiments the corresponding $CaCl_2$

concentrations were determined when the solution contained 68 M $\times$ $10^{-4}$ KCl and 85 M $\times$ $10^{-4}$ KCl. The results were as follows:

TABLE III

*The Ca → K Antagonisms in* 0.071 *mol. NaCl Solution*

| | | | |
|---|---|---|---|
| M $\times$ $10^{-4}$ KCl.......... | 50 | 68 | 85 |
| M $\times$ $10^{-4}$ $CaCl_2$......... | 8.1 | 40.5 | 72.9 |

As Table III shows, the increasing concentration of KCl requires an increasing concentration of $CaCl_2$. Hence a linear relationship seems to exist between the two salts, since equal amounts of $CaCl_2$ above the basic value of 8.1 M $\times$ $10^{-4}$ antagonize approximately equal increases of KCl. But there is a very characteristic limit for this ratio. A KCl concentration which is higher than 91 M $\times$ $10^{-4}$ cannot be balanced even with the greatest concentration of $CaCl_2$.

On the other hand, further investigations showed that the antagonistic action of $CaCl_2$ against KCl is relatively greater below 8.1 M $\times$ $10^{-4}$ $CaCl_2$ than it is above it. Between 50 and 80 M $\times$ $10^{-4}$ KCl about 32.4 M $\times$ $10^{-4}$ $CaCl_2$ is able to offset the effect of 17 M $\times$ $10^{-4}$ KCl. That corresponds to the relationship, 0.001 M $CaCl_2$ to 5.25 M $\times$ $10^{-4}$ KCl. But as Table IV shows, below 8.1 M $\times$ $10^{-4}$ $CaCl_2$ the relationship is 0.001 M $CaCl_2$ to 27.7 M $\times$ $10^{-4}$ KCl.

TABLE IV

*The Ca → K Antagonism in* 0.07 *mol. NaCl Solution*

| | | | | |
|---|---|---|---|---|
| M $\times$ $10^{-4}$ KCl conc......... | 34 | 39 | 44 | 50 |
| M $\times$ $10^{-5}$ $CaCl_2$ conc....... | 20.25 | 40.5 | 60.75 | 81 |

These data lead to the conclusion that the Ca → K antagonism is represented by two straight lines forming an angle with each other. However, as Tables III and IV indicate, there is no constancy of the ratio of K to Ca when the $CaCl_2$ concentration is either below or above 8.1 M $\times$ $10^{-4}$. The difference in the quantitative behavior of the Na → K antagonism and that between Ca → K can be expressed mathematically. The equation of the straight line $y = ax + b$ holds for both antagonisms, but since in the former case $b$ equals 0, $a = y/x =$ const., while in the Ca → K relationship $b$ has a negative value. The constants of the experiments of Table III are $a = 1.9$, $b = -87.9$; those of Table IV, $a = 0.36$, $b = -9.7$.

Summing up these results and those of Part I, it may be said that Na and Ca ions are antagonistic to K. Leaving out of consideration the case in which the $CaCl_2$ concentration is below 8.1 M $\times$ $10^{-4}$, the data show that 11.3 M $\times$ $10^{-4}$ KCl can be equilibrated by 20.25 M $\times$ $10^{-4}$ $CaCl_2$. Furthermore, it is apparent from Fig. 4 that 0.014 M NaCl balances 11.3 M $\times$ $10^{-4}$ KCl.

### III. *The Theoretical Calculation of the Sodium, Potassium, and Calcium Antagonism and its Experimental Proof*

It was pointed out that the results showed the relationship 0.014 M NaCl $\rightarrow$ 11.3 M $\times$ $10^{-4}$ KCl and 20.25 M $\times$ $10^{-4}$ $CaCl_2$ $\rightarrow$ 11.3 M $\times$ $10^{-4}$ KCl in which the arrow indicates that the first salt offsets the effect of the second one. On the other hand, the experiments reproduced in Fig. 4 show the minimal effective KCl concentration for different concentrations of NaCl in the presence of 8.1 M $\times$ $10^{-4}$ $CaCl_2$. Therefore, if the suppositions are correct, it should be possible to calculate the minimal effective KCl concentration if either the NaCl or the $CaCl_2$ concentration or both vary.

A series of experiments was performed in order to check the theoretical calculations and experimental facts. The experimental resu'ts are reproduced in Table V. The composition of the solutions is calculated according to the data described above. The $CaCl_2$ concentration of these solutions is supposed to be just insufficient to offset the KCl effects. Therefore it was expected that the addition of a small amount of $CaCl_2$ would warrant the maintenance of irritability above the 20 cm. threshold. Solutions which had this effect are marked with +, solutions causing rapid loss of irritability below 20 cm. threshold are marked with −. The calculated and the experimentally determined values check very well and show again that a linear dependence of KCl upon NaCl and $CaCl_2$ is characteristic of the ion antagonism in striped muscle.

In order to check these experiments still another series of experiments was performed on the gastrocnemius muscle of *Rana esculenta.* The muscle weight varied between .45 gram and .50 gram. Because these muscles are larger and the specific surface smaller, it was to be expected that they would be less sensitive to KCl than the sartorius muscles with their large specific surface. Correspondingly, it was found necessary to extend the time of observation to four hours in order to find differences in the irritability. Under these conditions the concentration of KCl was determined which just led to a marked decrease in irritability if NaCl and $CaCl_2$ were constant. This is a typical example:

| | | | | |
|---|---|---|---|---|
| M NaCl | 0.084 | 0.084 | 0.084 | 0.084 |
| M $\times$ $10^{-4}$ KCl | 68 | 71 | 74 | 80 |
| M $\times$ $10^{-4}$ $CaCl_2$ | 18.23 | 18.23 | 18.23 | 18.23 |
| Threshold after 4 hours, in cm. | 31.6 | 32.6 | 23.8 | 11.0 |

## Table V

*The Antagonism between Na, K and Ca*

+ = maintenance of irritability above the 20 cm. threshold.
− = rapid decrease of irritability; threshold below 20 cm.

| | | | | | $M \times 10^{-4}$ $CaCl_2$ | |
|---|---|---|---|---|---|---|
| | | | | | Observed | Calculated |
| Group I. 0.056 M NaCl + 0.0051 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 28.35 | 30.38 | 32.4 | | 28.35 | 28.35 |
| Irritability | − | + | + | | | |
| Group II. 0.056 M NaCl + 0.0068 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 58.73 | 60.75 | 62.78 | | 60.75 | 58.73 |
| Irritability | − | − | + | | | |
| Group III. 0.084 M NaCl + 0.0068 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 16.2 | 18.23 | 20.25 | | 16.2 | 18.23 |
| Irritability | − | + | + | | | |
| Group IV. 0.084 M NaCl + 0.0085 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 46.57 | 48.6 | 50.62 | 52.65 | 48.6 | 48.6 |
| Irritability | − | − | + | + | | |
| Group V. 0.092 M NaCl + 0.0085 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 36.45 | 38.48 | 40.5 | | 36.45 | 38.48 |
| Irritability | − | + | + | | | |
| Group VI. 0.098 M NaCl + 0.0085 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 28.35 | 30.38 | 32.4 | | 28.35 | 28.35 |
| Irritability | − | + | + | | | |
| Group VII. 0.098 M NaCl + 0.0102 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 52.65 | 56.70 | 60.75 | 64.80 | 52.65 | 58.70 |
| Irritability | − | + | + | + | | |
| Group VIII. 0.098 M NaCl + 0.0097 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 40.5 | 44.55 | 48.6 | 52.65 | 40.5 | 48.6 |
| Irritability | − | + | + | + | | |
| Group IX. 0.113 M NaCl + 0.0097 M KCl | | | | | | |
| $M \times 10^{-4}$ $CaCl_2$ | 24.3 | 28.35 | 32.4 | | 24.3 | 28.35 |
| Irritability | − | + | + | | | |

In this series 74 $M \times 10^{-4}$ KCl was the smallest concentration which was effective. In the same way, the KCl concentrations in the presence of different amounts of NaCl and $CaCl_2$ which led to the threshold below 25 cm. distance between primary and secondary coils were determined. The results have been reproduced in Table VI.

They indicate that the calculated values are generally a little smaller than the observed ones, but that this is almost a constant error, showing that the same relations hold as well for the gastrocnemius as for the sartorius.

TABLE VI

*The Antagonism between Na, K, and Ca (Experiments on Gastrocnemius)*

| No. | M NaCl | $M \times 10^{-4}$ $CaCl_2$ | $M \times 10^{-4}$ KCl | |
|---|---|---|---|---|
| | | | Observed | Calculated |
| 1 | 0.056 | 28 | 54 | 51 |
| 2 | 0.056 | 59 | 71 | 68 |
| 3 | 0.071 | 18 | 57 | 57 |
| 4 | 0.071 | 49 | 79 | 76 |
| 5 | 0.084 | 18 | 74 | 68 |
| 6 | 0.084 | 49 | 91 | 85 |
| 7 | 0.092 | 38 | 91 | 85 |
| 8 | 0.098 | 28 | 88 | 85 |
| 9 | 0.098 | 49 | 99 | 97 |
| 10 | ·0.113 | 28 | 102 | 97 |

IV. *The Limits of the Antagonistic Efficiency of* $CaCl_2$

In the last paragraph the possibility of calculating the minimal effective KCl concentration for different amounts of NaCl and $CaCl_2$ was indicated and a fairly good agreement was shown between experimental and calculated values. In these experiments the difference between the effect of NaCl and $CaCl_2$ in antagonizing KCl was of quantitative nature. But further experiments showed that this holds only within certain limits because the maximal concentration of KCl which can be balanced by $CaCl_2$ is determined by the concentration of NaCl. In Table VII it is shown that the maximal concentration of KCl which could be balanced by $CaCl_2$ varied from 71 to 108 $M \times 10^{-4}$ if the NaCl concentration varied.

TABLE VII

*Limits of the Antagonistic Action of Ca toward K in Dependence upon the NaCl Concentration*

| M NaCl concentration | Maximal tolerated K concentration ($M \times 10^{-4}$) |
|---|---|
| 0.056 | 71 |
| 0.071 | 91 |
| 0.084 | 102 |
| 0.098 | 108 |

The experiments make it probable that NaCl, although comparatively weaker than $CaCl_2$ in antagonizing KCl, exerts in part a specific influence. In this respect it cannot be replaced by $CaCl_2$. It might be thought that variation in the osmotic pressure might influence these results. But as shown in Fig. 4, the linear relationship between Na and K, in spite of a great variation of osmotic pressure, makes this view rather improbable. The direct proof is hardly possible because, as already shown, non-electrolytes such as dextrose are not indifferent to the muscle but reduce its susceptibility to KCl.

Towards the upper limit determined by the NaCl concentration the antagonisms followed the quantitative rules described above. Much greater concentration of $CaCl_2$ than that required by our equations had almost no influence. From Table VIII it is apparent that in 0.056 M NaCl the maximal tolerated concentration of KCl increased from 71 M $\times$ $10^{-4}$ to 74 M $\times$ $10^{-4}$ if greater amounts of $CaCl_2$ than calculated were used. For the other concentration of NaCl the limiting concentration of KCl was identical no matter whether $CaCl_2$ was added in calculated amounts or in larger ones. Only in 0.098 M NaCl the limiting KCl concentration was sometimes increased from 108 M $\times$ $10^{-4}$ to 113 M $\times$ $10^{-4}$ if greater amounts of $CaCl_2$ were added.

TABLE VIII

*Determination of the Maximal Concentration of KCl which can be Balanced by $CaCl_2$*

| | | If added in calculated amounts | | | If added in far greater amounts | | |
|---|---|---|---|---|---|---|---|
| M NaCl 0.056 | M $\times$ $10^{-4}$ KCl | 71 | 74 | 74 | 74 | 80 | |
| | M $\times$ $10^{-4}$ $CaCl_2$ | 69 | 75 | 79 | 162 | 180 | |
| | Irritability | +* | – | – | + | – | |
| M NaCl 0.071 | M $\times$ $10^{-4}$ KCl | 91 | 97 | | 97 | 102 | 113 |
| | M $\times$ $10^{-4}$ $CaCl_2$ | 83 | 93 | | 162 | 162 | 202 |
| | Irritability | + | – | | – | – | – |
| M NaCl 0.084 | M $\times$ $10^{-4}$ KCl | 102 | 108 | | 108 | | |
| | M $\times$ $10^{-4}$ $CaCl_2$ | 83 | 95 | | 126 | | |
| | Irritability | + | – | | – | | |
| M NaCl 0.098 | M $\times$ $10^{-4}$ KCl | 108 | 113 | | 113 | | |
| | M $\times$ $10^{-4}$ $CaCl_2$ | 73 | 83 | | 122 | | |
| | Irritability | + | – | | + or – | | |

* + = preservation of irritability above the 20 cm. threshold.
– = loss of irritability below the 20 cm. threshold.

## V. *The Antagonism Na → Ca*

In order to analyse completely the relationship between Na, K, and Ca, experiments were carried out to decide whether still other antagonisms exist than those described above. It was also found that $CaCl_2$ can reduce the irritability of the sartorius muscle if added in a sufficient amount. But, of course, the quantitative difference between the K and the Ca effect is very marked because one needs far larger amounts of $CaCl_2$ than of KCl in order to reduce the irritability of the muscle. On the other hand, the effective concentration of $CaCl_2$ depends upon the NaCl concentration in both cases and increases with increasing NaCl concentration (Table IX). While in a 0.042 M NaCl solution only 81 M $\times$ $10^{-4}$ $CaCl_2$ is required in order to reduce the irritability of the muscle below the 20 cm. threshold, three times as much is necessary in order to exert the same effect in a 0.056 M NaCl solution. The corresponding concentration in a 0.07 M NaCl is far greater, but the latter was not determined because of the interference with an increase of the osmotic pressure.

TABLE IX

*The Na → Ca Antagonism.* The effect of different $CaCl_2$ concentrations on irritability.

| | M $\times$ $10^{-4}$ $CaCl_2$ | | | | |
|---|---|---|---|---|---|
| 0.042 M NaCl<br>5.7 M $\times$ $10^{-4}$ KCl<br>Irritability | 8.1<br>+ | 40.5<br>+ | 60.75<br>+ | 81<br>– | 101.25<br>– |
| 0.056 M NaCl<br>5.7 M $\times$ $10^{-4}$ KCl<br>Irritability | 162.0<br>+ | 182.25<br>+ | 202.5<br>+ | 222.75<br>+ | 243.0<br>– |
| 0.071 M NaCl<br>5.7 M $\times$ $10^{-4}$ KCl<br>Irritability | 299.7<br>+ | | | | |

The experiments prove that Na is an antagonist of K and Ca because the effective concentrations of K and Ca increase with increasing concentrations of Na. Experiments of Part II showed that Ca is the antagonist of K, since the effective concentration of K varied in the same direction with varying Ca concentration. It still remains a question whether K also is the antagonist of Ca. If this be the case, it was to be expected that the addition of K could reduce the harmful effect of $CaCl_2$ or that it would increase the effective concentration of the latter. Neither was the case.

## SUMMARY

The irritability of the sartorius muscle of *Rana esculenta* has been studied in different mixtures of NaCl, KCl, and $CaCl_2$. The method

permits the detection of very small differences in the KCl effect. Usually the minimal effective KCl and $CaCl_2$ concentration is determined which leads to a rapid loss in irritability. The dependence of the KCl effect upon the NaCl and the $CaCl_2$ concentration is represented by a straight line. Concerning the antagonism between Na and K the result is that the ratio Na : K is constant. That does not hold for the antagonism between K and Ca although this also corresponds to a straight line. But in the latter case the relation is represented by the equation $y = ax + b$ and $b$ has a definite value, while in the first case $b = 0$ and therefore $a = y/x$. The quantitative results show that within certain limits 0.014 M NaCl has the same antagonistic value against KCl as 0.00081 M $CaCl_2$. Therefore, it is possible to predict the behavior of the muscle in any other solution if the antagonism has been investigated for one single mixture of these three salts. The results agree rather exactly with this assumption. There is, however, a very characteristic limit of the validity of these equations. It is found that the NaCl concentration determines the highest KCl concentration, the effect of which can still be offset by the addition of $CaCl_2$. It is higher the greater the NaCl concentration. Therefore the antagonistic action of Na against K is not only quantitatively but also qualitatively different from the antagonistic effect of Ca against K.

An antagonism between Na and Ca was also found. The complete analysis leads to the qualitative formula: $\underset{\searrow \mathrm{Ca} \nearrow}{\mathrm{Na} \rightarrow \mathrm{K}}$ in which the arrow indicates the cation which is able to balance the toxic effect of another cation.

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