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**Physiology.** - "*The Influence of Neutral Salts on the Action of Urease*". By J. TEMMINCK GROLL. (Communicated by Prof. G. VAN RIJNBERK).

(Communicated in the meeting of September 29, 1917).

Ferments being colloids it is not impossible that the influence that electrolytes exercise on the action of enzymes must be attributed to modifications of the dispersity of the ferment.

If this were really the case, then the character of one of the two ions would have a domineering influence over against that of the other, as was likewise found by HARDY for the flaking of colloids: it appeared namely that with colloids moving cathodically, which are consequently charged positively, the anion of an electrolyte has the greater influence, whilst with colloids moving anodically the influence of the kation was domineering.

A ferment will be either a positive colloid or a negative one, and a ferment that has its action especially in alkaline surroundings, will usually be negatively charged, whilst the ferments acting in acid surroundings will be positive. We can consequently expect a great influence either of the character of the anions or of that of the kations, according to the ferment being either positively or negatively charged. Moreover with colloids the phenomenon presents itself, that both the kations and the anions can be placed in a special series according to their being able to cause a modification of dispersity either in a greater or in a more restricted degree.

With regard to the kations this series is not always entirely the same, but in the main there is no difference with various colloid-phenomena.

Some metals occasionally change place, or as FREUNDLICH remarked: "Die Reihe der Kationen ist wieder etwas verschränkt".

Usually the series of the kations is:  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Ba}^{++}$ ,  $\text{Mg}^{++}$ .

The series of the anions is  $\text{CNS}'$ ,  $\text{J}'$ ,  $\text{Br}'$ ,  $\text{NO}'$ ,  $\text{Cl}'$ ,  $\text{SO}_4''$ .

In order to examine in how far the influence of salts on a ferment-action corresponds with that on colloid-phenomena, it is advisable to make use of an enzyme that decomposes a crystalline substrate to crystalline products of decomposition, for if one takes a colloidal

substrate, the salts might modify the substrate and make it consequently more or less susceptible to be influenced by the ferment.

The phenomenon to be studied would probably become more implicate.

The urease, the ureum decomposing ferment, which is found in soybeans is very fit for such like experiments.

The experiments were executed as follows: 3 cc of a solution of ureum ( $1\frac{1}{2}$  percent) a definite number of cc of the solution of salt and water to constantly the same volumes (100 cc.) were put in a number of flasks, as a rule 7. The flasks were placed in a flat-bottomed basin in water of the temperature of the room. After the flasks had been so long in the water till the temperature in each of them had become the same, 3 cc. of the urease-preparation, according to JANSEN, was added to them. One minute elapsed between the addition of the ferment to each flask. After 50 to 60 minutes the ammonia that had been formed, was titrated with methyl-orange as indicator, every time with an interval of one minute. Consequently the action was of equal duration in each flask. It appeared at a preliminary experiment that equal results were obtained indeed in flasks of the same composition.

### 1. *Kations.*

The kations used were  $K^+$ ,  $Na^+$ ,  $NH_4^+$ ,  $Mg^{++}$ ,  $Ba^{++}$ ,  $Sr^{++}$ ,  $Ca^{++}$ . Of all metals the chlorides were used.

The following results were obtained: (Vide Tab. on the next page).

If now we express the above figures graphically in such a way that the salt-concentration is indicated on the abscis, and the quantity of ammonia that has been formed on the ordinate, then irregularities strike us with two metals viz. calcium and magnesium.

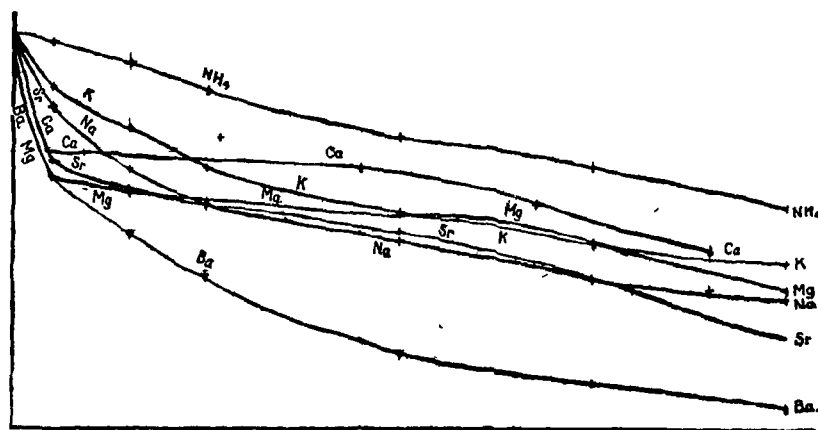


Fig. 1.

Concentration salt (grammol. per liter)	Number c.c. $\frac{1}{10}$ HCl.	Reduced	Concentration salt (grammol. per liter)	Number c.c. $\frac{1}{10}$ HCl.	Reduced
<b>K<sup>+</sup></b> 0	7.9	100	<b>Ba<sup>++</sup></b> 0	8.5	100
$\frac{1}{20}$	6.8	86.4	$\frac{1}{20}$	5.4	63.7
$\frac{3}{20}$	6.0	76.2	$\frac{3}{20}$	4.2	49.6
$\frac{5}{20}$	5.2	66.0	$\frac{5}{20}$	3.4	38.9
$\frac{10}{20}$	4.3	54.6	$\frac{10}{20}$	1.7	19.5
$\frac{15}{20}$	3.7 <sup>5</sup>	47.6	$\frac{15}{20}$	1.0	11.8
$\frac{20}{20}$	3.3	41.9	$\frac{20}{20}$	0.5	5.9
<b>Na<sup>+</sup></b> 0	7.6	100	<b>Sr<sup>++</sup></b> 0	8.1	100
$\frac{1}{20}$	6.2	81.6	$\frac{1}{20}$	5.5	67.7
$\frac{3}{20}$	5.0	65.8	$\frac{3}{20}$	4.9	60.3
$\frac{5}{20}$	4.4	57.9	$\frac{5}{20}$	4.6	56.6
$\frac{10}{20}$	3.6	47.4	$\frac{10}{20}$	4.1	50.4
$\frac{15}{20}$	2.9	38.2	$\frac{15}{20}$	3.1	38.1
$\frac{20}{20}$	2.5	32.9	$\frac{20}{20}$	1.9	23.4
<b>NH<sub>4</sub><sup>+</sup></b> 0	8.2	100	<b>Mg<sup>++</sup></b> 0	10.1	100
$\frac{1}{20}$	8.1	98.8	$\frac{1}{20}$	6.5	64
$\frac{3}{20}$	7.6	93.0	$\frac{3}{20}$	6.1	60
$\frac{5}{20}$	7.0	85.4	$\frac{5}{20}$	5.9	58
$\frac{10}{20}$	6.0	73.6	$\frac{10}{20}$	5.5	55
$\frac{15}{20}$	5.4	65.9	$\frac{15}{20}$	4.6	46
$\frac{20}{20}$	4.6	56.1	$\frac{20}{20}$	3.5	35
<b>Ca<sup>++</sup></b> 0	7.9	100			
0.9 twentieth	5.5	69.8			
1.8 "	5.5	69.8			
5.4 "	5.8	73.7			
9.0 "	5.2	66.0			
13.5 "	4.5	57.1			
18.0 "	3.5	44.5			

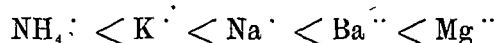
These curves indeed do not, like the others, descend regularly but show first, to about  $\frac{1}{20}$  grammolecule, a considerable descent. and afterwards suddenly descend much less, so that they cut a number of the other curves.

I constantly found this sudden modification of direction at  $\frac{1}{10}$  grammolecule in repeated experiments.

With regard to the other salts, they appear to retard the urease stronger in the following order :



Up to  $\frac{1}{20}$  grammolecule per Liter the curve for  $\text{Ca}^{++}$  corresponds about with that of  $\text{Sr}^{++}$ , and that of  $\text{Mg}^{++}$  with that of  $\text{Ba}^{++}$ . With the experiments with colloids we do not find as a rule the whole series mentioned, the series is most complete in the experiments of PAULI about the increase of the temperature of congelment of solutions of albumen viz. :



With the exception that here  $\text{Mg}^{++}$  comes after  $\text{Ba}^{++}$ , whilst these two metals on urease to  $\frac{1}{20}$  grammol. per Liter show an equal action, there is perfect agreement between the two series.

The order of succession  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{++}$  occurs a.o. in experiments about the coagulation of albumen by an earthalkali salt.

The coagulation is namely favoured by salts of these four metals in the indicated order.

The series  $\text{K}^+ < \text{Na}^+ < \text{Sr}^{++} < \text{Ba}^{++} < \text{Mg}^{++}$  is found at lowering the melting-point of a gelatine-gel by neutral salts.

One might draw the following normal series, from the different series that occur in literature for colloid-phenomena :

$\text{NH}_4^+ < \text{K}^+ < \text{Na}^+ < \text{Sr}^{++} < \text{Ba}^{++} < \text{Mg}^{++}$ , which corresponds consequently with that which is found for the influence of the action of urease. Occasionally  $\text{Ca}^{++}$  occurs in another place in one series than in the other, now between  $\text{K}^+$  and  $\text{Na}^+$ , now after  $\text{Ba}^{++}$ .

This depends presumably on the concentration that has been used; it occurs likewise with urease, as the calciumcurve cuts a number of other curves above the concentration  $\frac{1}{20}$  mol. per Liter.

## II. Anions.

When we studied the influence of anions on the action of urease we used potassium salts. Only those acids were used the potassium salts of which are neutral, the action of urease, like other ferment-processes being very sensitive to modification of the hydri-

concentration, so that a retardation or an acceleration might be caused both by a modification of the hydrions and by the anions.

The hydrion-concentration can indeed be kept constant by means of buffer-mixtures, but as these are likewise composed of salts, borates, phosphates, citrates, we should make the results more complicated by adding at the same time other anions.

The following potassium-salts have been made use of: KCl, KI, KBr, KNO<sub>3</sub>, KCNS, K<sub>2</sub>SO<sub>4</sub>.

The experiments were executed in entirely the same way as those with different kations. The obtained results were the following:

Concentration salt (grammolecule per liter)	Number of c.c. $\frac{1}{10}$ N · HCl.	Reduced	Concentration salt (grammolecule per liter)	Number of c.c. $\frac{1}{10}$ N · HCl.	Reduced
<b>Cl'</b> 0	7.9	100	<b>So<sub>4</sub>'</b> 0	9.1	100
$\frac{1}{20}$	6.8	86.4	0.7 twentieth	7.5	82.5
$\frac{3}{20}$	6.0	76.2	2.1 "	5.9	64.9
$\frac{5}{20}$	5.2	66.0	3.4 "	5.1	56.1
$\frac{10}{20}$	4.3	54.6	6.9 "	4.0	44.0
$\frac{15}{20}$	3.75	47.6	10.3 "	3.5	38.5
$\frac{20}{20}$	3.3	41.9	13.8 "	3.1	34.1
<b>CNS'</b> 0	9.9	100	<b>I'</b> 0	14.3	100
$\frac{1}{20}$	8.7	88.9	$\frac{1}{20}$	12.6	88.1
$\frac{3}{20}$	7.6	76.7	$\frac{3}{20}$	10.9	76.2
$\frac{5}{20}$	6.7	67.7	$\frac{5}{20}$	9.95	69.6
$\frac{10}{20}$	5.9	59.6	$\frac{10}{20}$	8.7	60.8
$\frac{15}{20}$	5.3	53.5	$\frac{15}{20}$	7.8	54.5
$\frac{20}{20}$	4.7	47.5	$\frac{21}{20}$	7.2	50.3
<b>NO<sub>3</sub>'</b> 0	8.9	100	<b>Br'</b> 0	9.1	100
$\frac{1}{20}$	7.8	87.4	$\frac{1}{20}$	7.9	86.9
$\frac{3}{20}$	6.5	72.8	$\frac{3}{20}$	6.7	73.7
$\frac{5}{20}$	5.9	66.1	$\frac{5}{20}$	5.9	64.9
$\frac{10}{20}$	4.8	53.8	$\frac{10}{20}$	4.9	53.9
$\frac{15}{20}$	4.2	47.0	$\frac{15}{20}$	4.3	47.3
$\frac{21}{20}$	3.8	42.6	$\frac{20}{20}$	3.8	41.8

If we represent these figures likewise graphically in the same way as with the kations, then we obtain a number of closely cramped curves from which appears, that with the same kation the nature of the anion has only an unimportant influence (fig. 2).

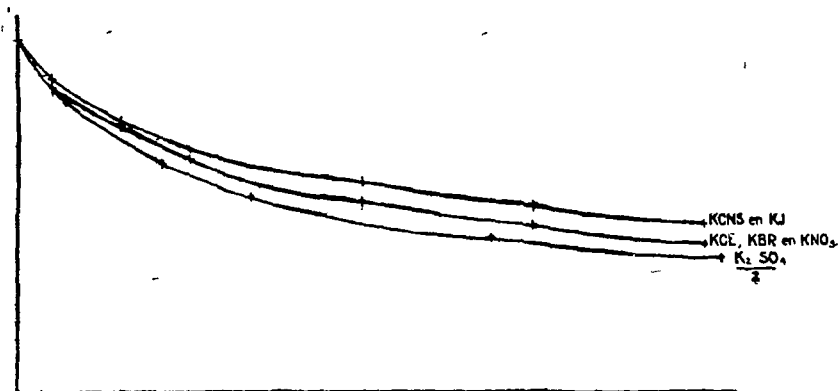


Fig. 2.

In so far as we can still speak of a regular succession it appears that  $\text{CNS}'$  and  $\text{I}'$  retard the action of urease least, and  $\text{SO}''_4$  most.  $\text{Cl}'$ ,  $\text{Br}'$ ,  $\text{NO}'_3$ , lying between these, are so close to each other, that the differences do not surpass the errors of observation. As has been remarked before, we find at different colloid-chemical phenomena the series  $\text{CNS}'$ ,  $\text{I}'$ ,  $\text{Br}'$ ,  $\text{NO}'_3$ ,  $\text{Cl}'$ ,  $\text{SO}''_4$ .

The faculty of precipitating colloids rises e. g. in this succession.

$\text{CNS}'$  and  $\text{SO}''_4$  are here likewise the two extremes of the series, whilst  $\text{I}'$ ,  $\text{Br}'$ ,  $\text{NO}'_3$  and  $\text{Cl}'$  lie between them. But as the differences between  $\text{CNS}'$  and  $\text{I}'$  and between  $\text{Br}'$ ,  $\text{NO}'_3$ ,  $\text{Cl}'$  are greater than at the urease-retardation, it is possible to place them in a definite succession.

The comparatively slight influence of the anions over against that of the kations is likewise the reason, why in fig. 1 the concentrations are indicated in grammolecules per Liter, whilst in fig. 2 at  $\text{K}_2\text{SO}_4$  they are indicated in half grammolecules per Liter, consequently as the normalities.

In the first case we have e. g. for  $\text{NaCl}$  and  $\text{BaCl}_2$ , it is true, the prejudice that the action of  $1 \text{Na}' + 1 \text{Cl}'$  is compared with that of  $1 \text{Ba}'' + 2 \text{Cl}'$ , but after the unimportant influence of the anions was proved by the 2<sup>nd</sup> series of experiments, this seems to me to be preferable to a comparison between  $1 \text{Na}' + 1 \text{Cl}'$  and  $\frac{1}{2} \text{Ba}'' + 1 \text{Cl}'$ , which would be obtained from the comparison of normal solutions instead of molar-solutions.

In Fig. 2 on the contrary e. g.  $\text{KCl}$  and  $\text{K}_2\text{SO}$  must be compared.

As now the influence of the kations is greater than that of the anions, we had better compare

than  $1 K + 1 Cl'$  and  $1 K + \frac{1}{2} SO_4''$   
 $1 K + 1 Cl'$  and  $2 K + 1 SO_4''$ .

It appears from a comparison of  $MgSO_4$  and  $MgCl_2$ , which exercise about the same influence at an equal concentration of magnesia, that really for the same kation the action of  $1 SO_4''$  corresponds with that of  $2 Cl'$ , consequently that of  $\frac{1}{2} SO_4''$  with that of  $1 Cl'$ .

Mg sulphate	Magnesium chloride	Concentration
100	100	0 grammol. p. L.
64	64	$\frac{1}{20}$ " " "
60	60	$\frac{3}{20}$ " " "
55	58	$\frac{5}{20}$ " " "
47	55?	$\frac{10}{20}$ " " "
45	46	$\frac{15}{20}$ " " "
35	35	1 " " "

We may deduce from the results that we have obtained, that for the action of neutral salts on the decomposition of ureum by urease the kations have a domineering influence, and that the succession in which those kations can be placed, in accordance with their faculty of retardation, is about the same as that which we find at the flaking and at other colloidal phenomena.

The nature of the anion has comparatively little influence; in so far as we can still observe at anions an increasing faculty of retardation, the series into which they can be placed corresponds likewise with the series occurring in colloidal chemistry.

Consequently it is possible that the influence that neutral salts exercise on a ferment-action consists in the fact that the dispersity of the ferment is modified by the ions in the same way as with other colloids.

*Physiol. Lab. of the Univ. of Amsterdam.*