ON THE ANTAGONISM BETWEEN TWO DIFFERENT SALTS IN THE ENZYMATIC ACTIONS.

BY

TETSUTARO TADOKORO, *Nōgakushi.*

酵素作用に對する鹽類の頡頏現象に就て

田所哲太郎

To these days, some different experimental results for the influences of different neutral salts upon the enzymatic actions have been reported by many authors. Many contrary points, however, are found among those which are written in the OPPENHEIMER's "Fermente und ihre Wirkungen". These authors have reported always the influences of one salt alone and there are no experimental data upon the mutual and antagonistic actions between two different salts.

BANG[1] reported that when the human saliva was mixed with secondary phosphate, the diastase of saliva lost its action but if this fluid was mixed farther with common salt, the diastase recovered its action. Thereupon he explained these phenomena by introducing the fact that the compound of phosphoric-acid-ptyalin has no diastatic action while the compound of common-salt-ptyalin has.

Recently, MICHAELIS and PECHSTEIN[2] investigated the action of the diastase of saliva in the mixture of salts, and their reports are as follows. "The saliva-diastase acts better in N/500 NaCl-solution than in N/50 Na_2SO_4 + N/500 NaCl-solution, and it acts better in N/50 NaBr + N/5000 NaCl-solution than in N/5000 NaCl-solution alone. It acts better in N/50 NaCl-solution than in N/50 NaBr + N/50 NaCl-solution, and better also than in N/50 NaBr-solution, because here N/50 NaBr-solution, because here N/50 NaCl-solution acts better than N/50 NaBr-solution."

Thus until today, we can not find any report on the discussion of the antago-

1) BANG, E.—Biochem. Zeits., 32,417 (1911).
2) MICHAELIS, L. and PECHSTEIN, H.—Biochem., Zeits., 59, 77—99 (1914).

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nistic influence of two different salts upon any enzymatic action. And here I have the honor to report the following experiment on this field of investigation. As samples I took some fresh pressed juice of wheet-seedling and the 0.05 % solution of Takadiastase. At first I determined the injurious concentration of different salt solutions and got the following results.

1. The injurious concentration of different salt solutions upon the actions of two amylases.

20 ccm of 1.0 % solution of soluble starch were mixed with 2 ccm of fresh juice and farther with the following quantities of salts, and thus many series of these mixed solutions were placed in a thermostat at 40° C under the presence of 2 ccm toluol.

After 6 hours, the formed sugar in 10 ccm of each of the series of these mixed solutions were determined by the BERTRAND's method and the result are as follows :

The following numbers of reducing sugar are shown in mg.

Table 1.

The comparison of cathions

Concentration / salts	1/3 normal	1/5 normal	1/10 normal	1/20 normal	1/100 normal
$CaCl_2$	—	—	13,0	17,6	19,4
$MgCl_5$	—	17,8	19,2	19,6	24,6
KCl	14,4	16,2	18,0	17,8	17,6
NaCl	16,2	18,0	22,0	22,0	24,0

The comparison of anions

Concentration / salts	1/3 normal	1/10 normal	1/50 normal	1/1000 normal
KI	11,4	11,6	11,6	12,4
KCI	11,6	12,0	12,8	13,0
KNO_3	9,8	11,6	12,4	13,2
K_2SO_4	12,0	13,0	13,0	16,6
K_2HPO_4	14,4	14,0	14,6	14,0

40 ccm of 1,0 % solution of soluble starch were mixed with 1,0 ccm of 0,05 % Takadiastase solution and with the following quantities of different salts and placed in a thermostat at 40° C under the presence of 2 ccm toluol. After 6 hours 20 ccm of these mixed solution were treated in the same way as above mentioned and the results are as follows:

Table 2.

The comparison of cathions				
salts \ Concentration	1 normal	1/10 normal	1/50 normal	1/100 normal
$CaCl_2$	—	5,2	9,2	9,0
$MgCl_2$	—	8,8	10,8	11,0
KCl	8,8	9,2	9,6	10,0
NaCl	10,2	10,2	11,0	11,0
$BaCl_2$	—	10,0	11,0	11,4
$AlCl_3$	—	4,6	6,4	8,0
The comparison of anions				
salts \ Concentration	1 normal	1/10 normal	1/50 normal	1/100 normal
KCl	6,4	6,8	8,0	8,0
KNO_3	6,2	7,4	7,8	7,8
K_2SO_4	7,0	7,2	8,2	8,4
KI	0	5,8	7,8	8,0

From the above results of my experiment we know that the enzymatic action is evidently injured in the 1/10 normal concentration of Mg- or Ca- salts and in the normal solution of alkalisalts.

2 The antagonistic action between KCl and $CaCl_2$ for two amylases

50 ccm of 1,0 % soluble starch solution were mixed with 5 ccm of fresh juice of wheet-seedling or with 5 ccm of 0, 05 % Takadiastase solution, and the concentration of salts in the solution was kept as follows.

The farther treatment is the same as above mentioned, and the formed sugar was determined by the BERTRAND's method. (the following number shows sugar-quantity with mg. in 50 ccm of solution.)

Table 3.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
N-KCl + 2,5 cc H_2O	12,5	N/10 $CaCl_2$ + 2,5 cc H_2O	10,5
N-KCl + 2,5 cc N/10 CaCl	14,5	N/10 $CaCl_2$ + 2,5 cc N-KCl	12,2
N-KCl + 2,5 cc N/2 $CaCl_2$	13,2	N/10 $CaCl_2$ + 2,5 cc N/2 KCl	13,5
N-KCl + 2,5 cc H_2O	13,0	N/10 $CaCl_2$ + 2,5 cc H_2O	8,5
N-KCl + 2,5 cc N/10 $CaCl_2$	15,0	N/10 $CaCl_2$ + 2,5 cc KCl	9,4
N-KCl + 2,5 cc N/2 $CaCl_2$	14,2	N/10 $CaCl_2$ + 2,5 cc N/2 KCl	10,2

3 The antagonistic action between NaCl and $CaCl_2$ for two amylases

Table 4.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
N-NaCl + 2,5 cc H_2O	13,25		
N-NaCl + 2,5 cc N/10 $CaCl_2$	14,7		
N-NaCl + 2,5 cc N/2 $CaCl_2$	14,5		
N-NaCl + 2,5 cc N/10 $CaCl_2$	12,8	N/10 $CaCl_2$ + 2,5 cc H_2O	10,5
N-NaCl + 2,5 cc N/10 $CaCl_2$	14,5	N/10 $CaCl_2$ + 2,5 cc N-NaCl	11,25
N-NaCl + 2,5 cc N/2 $CaCl_2$	14,0	N/10 $CaCl_2$ + 2,5 cc N/2 NaCl	12,3

4 The antagonistic action between NaCl and KCl for two amylases

Table 5.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
		N-NaCl + 2,5 cc H_2O	14,25
		N-NaCl + 2,5 cc N-KCl	15,5
		N-NaCl + 2,5 cc N/2 KCl	15,0
N-KCl + 2,5 cc H_2O	10,5	N-NaCl + 2,5 cc H_2O	10,0
N-KCl + 2,5 cc N-NaCl	11,8	N-NaCl + 2,5 cc N-KCl	10,8
N-KCl + 2,5 cc N/2 NaCl	11,0	N-NaCl + 2,5 cc N/2 KCl	10,5

5 The antagonistic action between $CaCl_2$ and $MgCl_2$ for an amylase

Table 6.

Concentration	N/10 $CaCl_2$ + 2,5 cc H_2O	N/10 $CaCl_2$ + 2,5 cc N/2 $MgCl_2$	N/10 $CaCl_2$ + 2,5 cc N/5 $MgCl_2$
wheat-seedling	4,00	6,75	6,25

The results in the above tables show the antagonistic effects between different cathions for amylase of wheat-seedling and for Takadiastase and we can also observe that the antagonistic effect between monovalent cathion and bivalent one is greater than that between two monovalent ones.

Farther I exermined the antagonistic effects between different anions with same cathion for wheat-amylase and for Takadiastase.

6 The antagonistic action between KCl and K_2SO_4 for wheat-amylase

Table 7.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
N-KCl + 2,5 cc H_2O	12,5	N-K_2SO_4 + 2,5 cc H_2O	11,5
N-KCl + 2,5 cc N/2 K_2SO_4	13,25	N-K_2SO_4 + 2,5 cc N/2 KCl	12,25
N-KCl + 2,5 cc N-K_2SO_4	13,75	N-K_2SO_4 + 2,5 cc N-KCl	12,75

7 The antagonistic action between KNO_3 and K_2SO_4 for Takadiastase

Table 8.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
N-KNO_3 + 2,5 cc H_2O	8,0	N-K_2SO_4 + 2,5 cc H_2O	10,0
N-KNO_3 + 2,5 cc N/2 K_2SO_4	10,0	N-K_2SO_4 + 2,5 cc N/2 KNO_3	11,2
N-KNO_3 + 2,5 cc N-K_2SO_4	11,0	N-K_2SO_4 + 2,5 cc N-KNO_3	22,5

Here we can also observe the antagonistic effects between different anions for wheat-amylase and for Takadiastase, and then I exermined the antagonistic effects between different cathions and different anions for wheat-amylase.

8 The antagonistic action between K_2SO_4 and $MgCl_2$ for wheat-amylase

Table 9.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
$N-K_2SO_4+2,5$ cc H_2O	11,0	$N-MgCl_2+2,5$ cc H_2O	10,5
$N-K_2SO_4+2,5$ cc $N/2\,MgCl_2$	13,5	$N-MgCl_2+2,5$ cc $N/2\,K_2SO_4$	13,7
$N-K_2SO_4+2,5$ cc $N-MgCl_2$	15,25	$N-MgCl_2+2,5$ cc $N-K_2SO_4$	13,9

9 The antagonistic action between $CaCl_2$ and $MgSO_4$ for wheat-amylase

Table 10.

Concentration of salts	sugar mg.	Concentration of salts	sugar mg.
$N/10\,CaCl_2+2,5$ cc H_2O	10,0	$N/10\,MgSO_4+2,5$ cc H_2O	10,2
$N/10\,CaCl_2+2,5$ cc $N/2\,MgSO_4$	11,2	$N/10\,MgSO_4+2,5$ cc $N/2\,CaCl_2$	11,4
$N/10\,CaCl_2+2,5$ cc $N-MgSO_4$	11,5	$N/10\,MgSO_4+2,5$ cc $N-CaCl_2$	—

The results show the antagonistic effects between different cathions and anions for wheat-amylase. At last I exermined LOEB's socalled apparent antagonistic effects between the decomposition products of protein and potassium chloride for wheat-amylase and got the following results.

10 The antagonistic action between glycocoll and KCl for wheat-amylase

Table 11.

Concentration of salts	5 % glycocoll 2,5 cc H_2O	5 % glycocoll 2,5 cc N/2 KCl	5 % glycocoll 2,5 cc N-KCl
sugar mg.	11,0	11,5	12,7

In this case we can also observe the antagonistic effect between glycocoll and KCl for wheat-amylase.

CONCLUSTON

From the results we know the antagonistic effects between different salts — of different cathions and same anions as KCl and $CaCl_2$, NaCl and $CaCl_2$, NaCl and KCl or $CaCl_2$ and $MgCl_2$, and of different anions and same cathions as KCl and K_2SO_4, KNO_3 and K_2SO_4 and of different cathions and different anions as K_2SO_4 and $MgCl_2$ or $CaCl_2$ and $MgSO_4$ — for amylase of wheat-seedling and for Takadiastase. Farther more the apparent antagonistic effect is observed between glycocoll and KCl for the same enzym.

摘　　要

酵素作用に對する鹽類の影響に關しては從來幾多の研究結果を報告せしものありと雖も、此等は多く單獨の影響に關するものにして、未だ二種以上の鹽類の相互關係若くは同鹽類間に於ける頡頏現象に就きて試驗を試みしもの極めて稀なり是著者の本研究を企圖せし所以なり。

著者は先づ次の如き各種鹽類の陽**イオン**及び陰**イオン**が發芽小麥の**アミラーゼ**並に**タカヂアスターゼ**に對する阻遏作用を現すべき濃度を決定せり其結果は**カルシウム**及び**マグネシウム**の如き二價**イオン**の鹽類は十分の一規定液に達するに及んで有害なるを認めたり、反之**カリウム**及び**ナトリウム**の如き一價**イオン**の鹽類は一規定液の濃度に至りて初めて其阻遏作用を表はせり。

依て著者は酵素液を各種鹽類の有害作用を表はすべき濃度に保持せし後更に之に微量の他の鹽類を添加して此の場合に於ける相互作用の結果を試驗せり、其の結果同一陰**イオン**の鹽類にして陽**イオン**異なる場合即ち鹽化**カリウム**と鹽化**カルシウム**、鹽化**ナトリウム**と鹽化**カルシウム**、鹽化**ナトリウム**と鹽化**カリウム**若くは鹽化**カルシウム**と鹽化**マグネシウム**とを相共に作用せしめたる場合は勿論、同一陽**イオン**の鹽類にして相異なる陰**イオン**を有するもの例へば鹽化**カリウム**と硫酸**カリウム**、硝酸**カリウム**と硫酸**カリウム**とを添加せし場合更に陰陽兩**イオン**の全く異なる場合即ち硫酸**カリウム**と鹽化**マグネシウム**若くは鹽化**カルシウム**硫酸**マグネシウム**とを添加せし場合にありては單獨の鹽類を作用せしめたる場合に比較して常に其阻遏作用の輕減せらるゝことを認むるものなり、或は又蛋白質分解產物たる**グリココール**と鹽化**カリウム**とを作用せしめたる場合に於ても之と同樣なり。

玆に於てか吾人は各鹽類の相互作用は生物体に對して常に頡頏現象を表はすと同樣に酵素作用に對しても亦同樣に頡頏現象を表はすことを知るものなり更に生物体に對して所謂外觀的頡頏現象と稱せらるる現象も亦酵素作用に對して生物体と同樣の結果を與ふるものなることを知る。