CCLXXXIX. THE EFFECT OF HALOGEN SALTS ON SALIVARY AND PANCREATIC AMYLASE.

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In a previous paper [Clifford, 1925] an account was given of the effect of some halogen salts on the rate of salivary digestion. At that time the author had not realized the important part played by salt concentration in enzyme action, and therefore the results referred to one strength of halide only. The present paper is a continuation of this work using varying concentrations of salts and both salivary and pancreatic amylases.

The importance of amount of salt in amylase action has been shown by Omari [1931] and by Ambard & Trautmann [1933] who state that amylase is fixed to starch proportionately to the amount of NaCl present. McClure [1933] again quotes a statement that the activity of the amylase of potato is increased by solutions of NaF up to 1/1·75 *M*.

Experiments were therefore undertaken to determine the effects of various halogen salts on amylolytic action.

Experimental.

The method used was based on the time taken by a digestion mixture of starch, salt and enzyme to reach the achromic point with iodine.

10 ml. of 0·5 % soluble starch solution and 2 ml. of distilled water or halogen salt of requisite strength were warmed in a test-tube to 37°. 1 ml. of amylase solution was then added, the tube inverted to mix and a stopwatch started. At intervals 5 drops of the mixture were placed in 2 ml. of iodine solution (2 ml. *N*/20 I made to 300 ml. with distilled water) until no colour change resulted.

The average time taken to reach this achromic point by a series of six tubes was taken as that for any given concentration of salt. In no series was the variation greater than 10 sec. unless the time taken was above 15 min. when differences of 30 sec. were sometimes met.

The amount of halogen salt varied so that the final concentration in the tube ranged from 0·2 *M* to 0·000008 *M*, each dilution being half the previous one.

The source of pancreatic amylase was a 0·3 % solution of commercial pancreas substance, and for ptyalin saliva was collected directly into distilled water as described by Cole [1933].

Parallel experiments were made with three separate batches of pancreas substance and three different samples of saliva.

The figures given in this paper represent the results obtained with one batch of pancreas substance; the results with the other samples were similar in all respects, as were those with ptyalin except that with the latter enzyme both accelerations and relative inhibitions were slightly less marked.

Results.

Fluorides (Fig. 1).

From Fig. 1 it can be seen that K and NH_4 fluorides exercise an inhibitory action on amylase activity, this being specially marked with the NH_4 salt. This action continues to a definite concentration below which no effect whatever is seen. The Na salt is quite inert at concentrations varying from 0·5 to 0·000008 *M*.

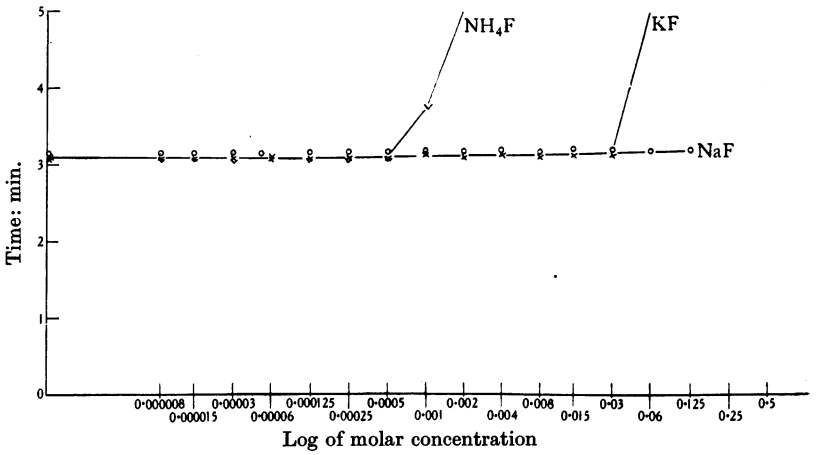

Fig. 1. Fluorides. Higher concentrations KF 10–40 min. Higher concentrations NH_4F no digestion in 4 hr.

o—o—o NaF. ×—×—× KF. v—v—v NH_4F.

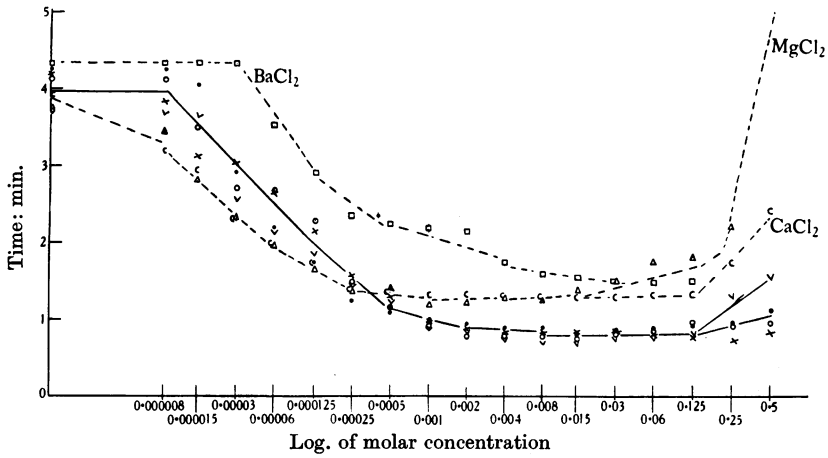

Fig. 2. Chlorides. $MgCl_2$ 0·5 *M* 9 min. 38 sec. for digestion.

•—•—• Li. o—o—o Na. ×—×—× K. v—v—v NH_4.
△—△—△ Mg. c--c--c Ca. □—□—□ Ba.

Chlorides (Fig. 2).

All the chlorides investigated accelerated diastatic action, this being more marked with the Li, Na, K and NH_4 salts than with those of the alkaline earths.

In the case of $MgCl_2$ a concentration of 0·5 *M* actually caused inhibition, and with the Li, NH_4 and Ca salts solutions of this strength were less powerful accelerators than weaker ones. The activating power could be detected in concentrations as low as 0·00003 *M* except with $BaCl_2$ which, besides being less potent at all strengths, showed no effect below 0·0006 *M*. There was no apparent difference of acceleration with concentrations between 0·25 and 0·002 *M*, but below these the action was progressively less marked.

This is in agreement with Cole [1903] who states that solutions of NaCl between concentrations of 0·3 and 0·003 *M* are equal as accelerators of amylase activity.

Bromides (Fig. 3).

The results with these salts were similar to those with chlorides, but less marked.

Again the highest concentration of the Mg salt retarded the action of the enzyme, and the Ba salt was less potent than any other at all strengths.

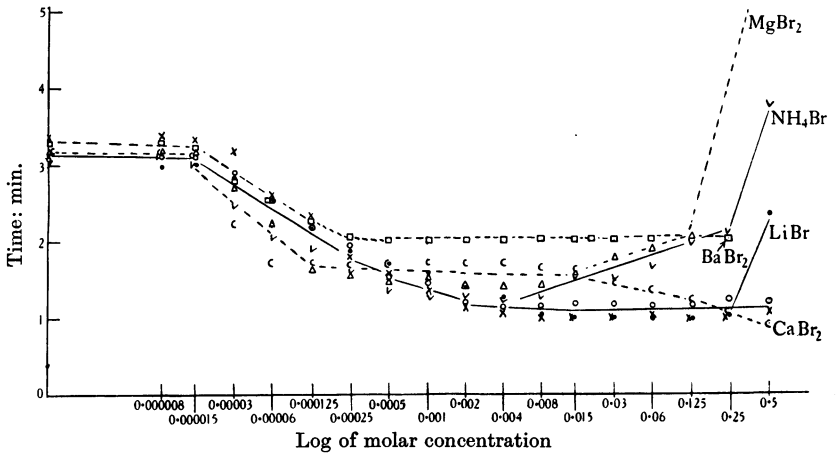

Fig. 3. Bromides. $MgBr_2$ 0·25 *M* 5 min. 23 sec. for digestion.

•—•—• Li. o—o—o Na. × — × — × K. v—v—v NH_4.
△—△—△ Mg. c--c--c Ca. □—□—□ Ba.

The optimum range of concentration was between 0·125 and 0·008 *M* for the first four salts; above and below these limits acceleration was less marked. With the alkaline earths, the Mg salt gave optimum quickening between 0·03 and 0·005 *M*, the Ca salt at 0·5 *M* with slightly less activity from 0·25 to 0·006 *M*, whilst the Ba salt gave least acceleration of all the salts of this series but acted at concentrations of 0·25–0·0006 *M*.

Iodides (Fig. 4).

The results with iodides were even more varied than with fluorides.

Li, NH_4, Mg and Ca iodides at 0·5 *M* showed inhibitory actions extending over a period of hours. This inhibition could be seen at 0·06 *M* with CaI_2, at 0·03 *M* with LiI and MgI_2 and at 0·25 *M* with NH_4I.

On the other hand the iodides of Na, K and Ba hastened diastatic activity at 0·5 *M*.

At lower concentrations all the iodides hastened the digestion of starch, Li, K, Mg and Ca showed effects at 0·0005 *M* whilst the Na, NH_4 and Ba salts were less potent. Again the Ba salt showed the least action.

From these results it can be seen that all halogen salts (except fluorides) quicken the rate of starch hydrolysis by salivary and pancreatic amylases, but this acceleration is a function of salt concentration. The effect is greatest and shows over the widest range of concentrations with chlorides, the next most powerful activators being the bromides.

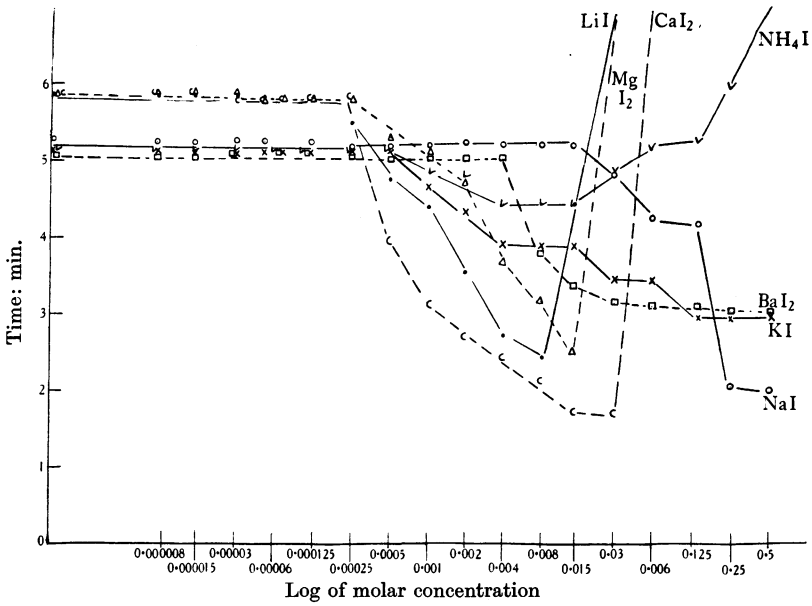

Fig. 4. Iodides. MgI_2 0·06–0·5 *M* 13 min.–2 hr. 30 min. CaI_2 0·06–0·5 *M* 12 min. 13 sec.–2 hr. 15 min. LiI, 0·3 *M* 17 min. 10 sec.; above 0·3 *M* no digestion 2 hr. 40 min. NH_4I, 0·4 *M* partial digestion 4 hrs. 30 min.

•—•—• Li. o—o—o Na. ×—×—× K. v—v—v NH_4.
△—△—△ Mg. c--c--c Ca. □—□—□ Ba.

With iodides there is still less acceleration of amylase action, and at the higher concentrations used there may be complete or relative inhibition.

Fluorides have never given any acceleration. NaF is completely inert, whilst the K and NH_4 salts inhibit at higher concentrations and are inert at lower ones.

The relative effects of halides in hastening amylase action are therefore in the order chlorides > bromides > iodides > fluorides.

The cation is not without effect since all the alkaline earth salts are less potent than those of Li, Na, K and NH_4 and the Ba salt is consistently the least potent in the series, whilst the Mg salt in the higher concentrations is more inhibitory than either the Ca or Ba salt.

Summary.

1. Chlorides, bromides and iodides, of Li, Na, K, NH_4, Mg, Ca and Ba hasten the hydrolysis of starch by pancreatic and salivary amylases.

2. The relative potencies are in the order chloride > bromide > iodide > fluoride.

3. Na, K and NH_4 fluorides do not hasten amylolytic action and at higher concentrations the two latter salts inhibit.

4. Li, NH_4, Mg and Ca iodides inhibit amylase activity at higher concentrations, but accelerate at lower ones.

5. The Ba halides are less potent in their action on amylolytic activity than any other halide investigated.

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