XLVI. THE EFFECT OF HALOGEN SALTS ON TRYPTIC DIGESTION.

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THE effect of halogen salts on salivary and peptic digestion has been described previously [Clifford, 1925; 1927; 1928], and in this paper an account is given of their action on tryptic digestion.

The earliest work on the subject appears to be that of Pfeiffer [1884] who found that, with the exception of sodium carbonate, all salts, especially sodium chloride, retard tryptic digestion. Later, Weiss [1904] found that sodium chloride in strong concentration retarded tryptic action whilst in weak concentration it accelerated it. With sodium bromide and iodide he obtained similar though less marked results, and the potassium salts acted in the same way as the sodium salts. Unfortunately, his experiments were carried out using solutions of equal percentages and not of equal molecular concentrations, and this makes the results difficult to compare.

Robertson [1906] found that all salts accelerated, but stated that chlorides, nitrates, and sulphates accelerated tryptic action least, as compared with cyanides and salts of fatty acids.

In the experiments to be described an attempt was made to investigate the action of equimolecular solutions of halogen salts on the tryptic digestion of protein.

EXPERIMENTAL METHODS.

The substrate used was dried ground fish protein prepared as follows.

Cod muscle, freed from skin and connective tissue, was passed through a fine mincer. It was then covered with three times its volume of 85 % alcohol, shaken well and allowed to stand for a week. During this period it was shaken at intervals. The protein was wrung as dry as possible through cotton winceyette, placed in a shallow dish covered with muslin and left in the air near a radiator for about 48 hours. It was then quite dry and could be ground into a fine powder which keeps indefinitely and gives a large surface for digestion. Unlike blood-fibrin it does not form lumps but gives a homogeneous product.

4 g. of this dried fish protein were weighed into a 500 cc. flask and were covered with 200 cc. of 0·4 % sodium carbonate solution previously warmed to 38°. The flask was placed in a water-bath at 38° for 30 minutes, when 10 cc. of 6 % pancreas substance, also at that temperature, were pipetted into the mixture.

25 cc. were removed at once, and about 100 cc. of boiling water were added to stop digestion. A Sørensen determination was carried out on the 25 cc. by adding 30 cc. of formalin (1 part formalin, 2 parts water), a few drops of phenolphthalein, and then titrating against 0·1 *N* H_2SO_4. Similar titrations

were carried out at intervals, using 0·1 *N* NaOH when the digestion mixture became acid to phenolphthalein.

For experiments where the effect of halogen salts was to be noted, the appropriate weight of salt was dissolved in the 200 cc. of 0·4 % Na_2CO_3 before adding it to the weighed protein.

A typical experimental result is as follows:

4 g. dried fish protein; 200 cc. 0·4 % Na_2CO_3 with 0·125 *M* NaI; 10 cc. 6 % pancreas substance; 25 cc. taken out at intervals, treated with formalin and titrated.

Minutes	cc. of 0·1 *N* acid or alkali	cc. of 0·1 *N* acid produced by tryptic hydrolysis
0	6·0 cc. 0·1 *N* H_2SO_4	0·0
30	4·6 ,,	1·4
60	3·5 ,,	2·5
90	1·3 ,,	4·7
180	3·7 cc. 0·1 *N* NaOH	9·7
210	5·6 ,,	11·6
270	8·2 ,,	14·2
300	10·9 ,,	16·9

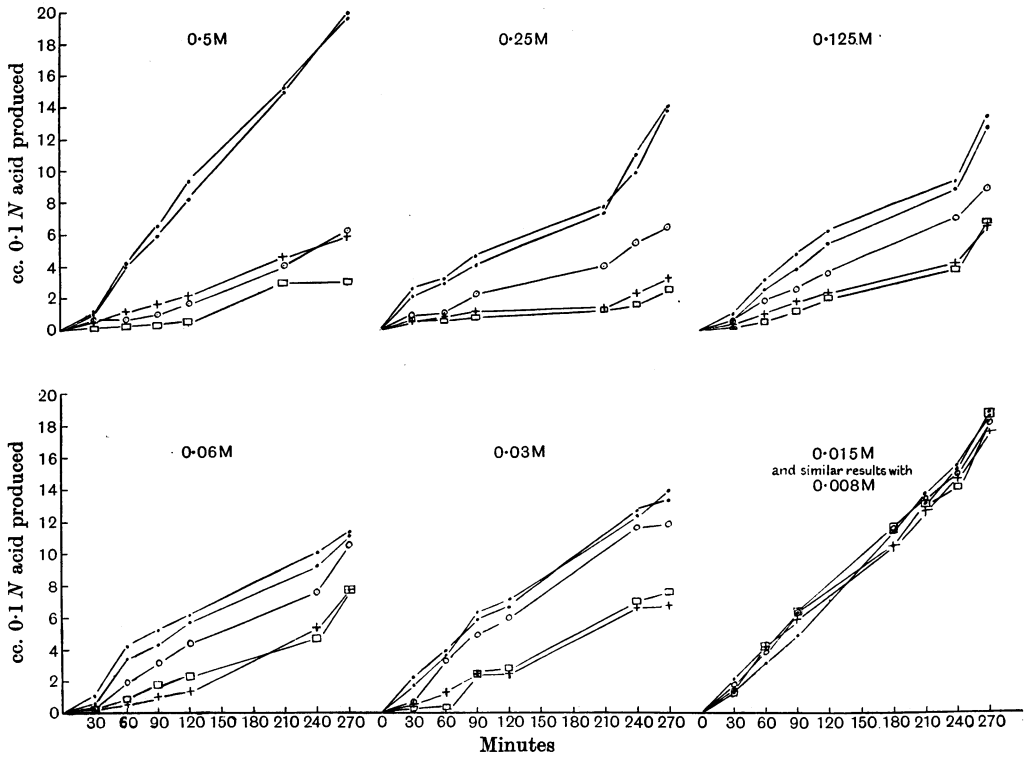

Fig. 1. Fluorides.

•—• Standard ×—× Lithium ○—○ Sodium +—+ Potassium □—□ Ammonium

In any set of experiments, two control flasks were used and one of each of the halogen mixtures of the concentration to be investigated.

Four separate batches of pancreas substance were used in the experiments

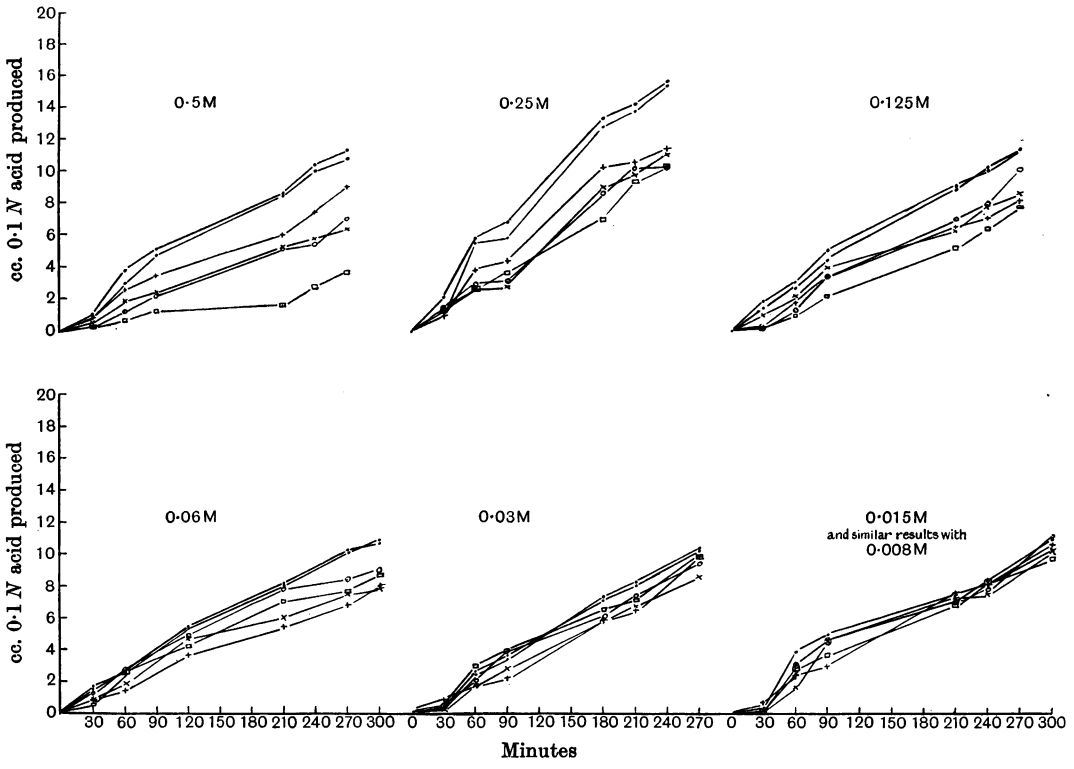

Fig. 2. Chlorides.

•—• Standard ×—× Lithium ○—○ Sodium +—+ Potassium □—□ Ammonium

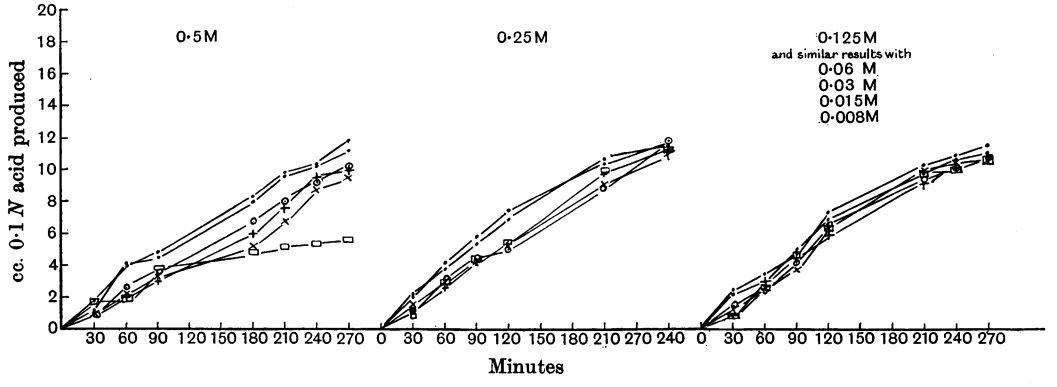

Fig. 3. Bromides.

•—• Standard ×—× Lithium ○—○ Sodium +—+ Potassium □—□ Ammonium

to be described, and at least three separate samples of each halogen salt. These various specimens all gave similar results. The concentrations of salt investigated varied from 0·5 *M* to 0·008 *M*.

Results.

Fluorides. Typical results with fluorides are shown in Fig. 1. From these curves it can be seen that sodium, potassium and ammonium fluorides in concentrations of 0·5–0·06 *M* are strongly inhibitory to tryptic digestion, but do

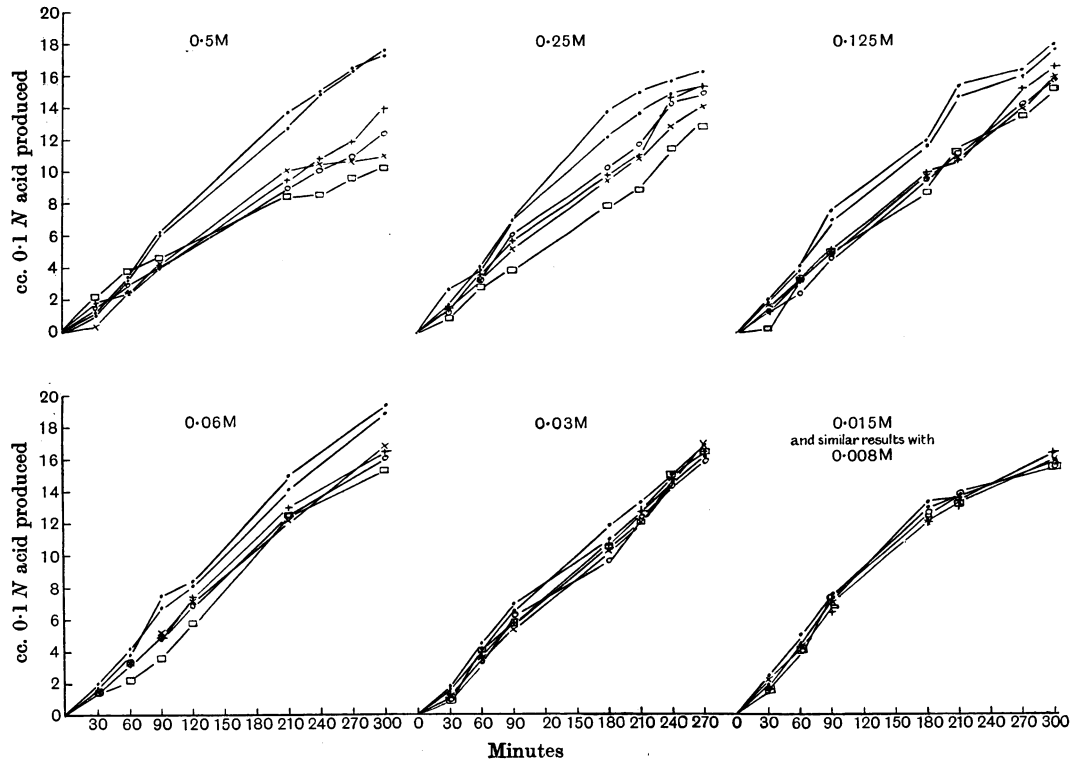

Fig. 4. Iodides.

•—• Standard ×—× Lithium ○—○ Sodium +—+ Potassium □—□ Ammonium

not actually stop it. This inhibition is greatest with ammonium and potassium fluorides, the sodium salt showing much less effect from 0·25 to 0·06 *M* and having hardly any action at 0·03 *M*. At concentrations of 0·015–0·008 *M* none of the three fluorides appeared to affect the rate of tryptic hydrolysis.

Chlorides. Graphs of experimental results are given in Fig. 2.

The chlorides, like the fluorides, have an inhibitory effect on tryptic digestion when used in the higher concentrations.

At 0·5 *M* this is most marked with the ammonium salt, but it is quite definitely seen with lithium, sodium and potassium chlorides.

At 0·25 *M* all four chlorides show the same depressant action, and this continues down to 0·06 *M*. With concentrations of 0·03–0·008 *M* there is little

if any effect on the rate of tryptic digestion. No accelerating action has ever been seen with any concentration of chloride.

Bromides. Representative graphs are shown in Fig. 3. The four bromides used showed the least effect of all the halogens. At a concentration of 0·5 *M* there was a slight depression with the lithium, sodium and potassium salts and a rather more marked one with ammonium bromide. At 0·25 *M* the inhibitory action was hardly noticeable, and in concentrations from 0·125 to 0·008 *M* there was no effect of any kind.

Iodides. Curves of typical results are shown in Fig. 4. From these it can be seen that there is a definite depressing action in concentrations of 0·5–0·25 *M* and a slight inhibition in concentrations of 0·125–0·06 *M*. With concentrations of 0·03–0·008 *M* there is no effect on the rate of tryptic hydrolysis.

Discussion.

From these results it appears that the halogen salts of lithium, sodium, potassium and ammonium in concentrations of 0·5 *M* depress the rate of the proteolytic action of trypsin, though in different degrees. Fluorides and chlorides exert the greatest depressant action, bromides the least, whilst iodides slow the rate a little more than do the bromides, but much less than the chlorides.

With low concentrations of halogen salts the rate of tryptic hydrolysis was unchanged, but in no case was it ever hastened. The concentration at which the salts ceased to affect trypsin varied, being 0·015 *M* for fluorides, 0·03 *M* for chlorides and iodides and 0·125 *M* for bromides.

In every case with the higher concentrations the ammonium salt exerted the greatest depressing effect. This is probably due to the acid reaction of the ammonium halide solutions. It is remarkable however that in many cases, *e.g.* with 0·25 *M* NH_4Cl, although the digestion mixture was acid to phenolphthalein even before the addition of formalin, tryptic digestion proceeded as well as in mixtures containing 0·25 *M* NaCl, KCl or LiCl, which were strongly alkaline. Again in the bromide experiments the ammonium solutions were always more acid than those of the sodium, potassium and lithium salts, yet except in the highest concentration (0·5 *M*) this increase in acidity caused no slowing of tryptic activity. The alterations in rate of hydrolysis cannot therefore be attributed solely to changes of p_H in the digestion mixture.

With fluorides the relatively slight action of sodium fluoride as compared with the lithium and potassium salts is remarkable between 0·25 and 0·03 *M*. Above this concentration it is equally inhibitory, and below, all the fluorides are without effect. This is not due to a less acid reaction as the p_H values of digestion mixtures with potassium and sodium fluorides were identical.

Summary.

1. The digestion of fish protein by trypsin is delayed by the addition of the halogen salts of Li, Na, K and NH_4.

2. This depression is greatest with fluorides and chlorides, less with iodides, and least with bromides.

3. The depression is greatest with high concentrations of salt (0·5 *M*) and disappears at low concentrations.

4. In no concentration investigated (0·5–0·008 *M*) was there any accelerating action.

5. In the more concentrated solutions the ammonium salts exerted the most powerful depressant action.

6. Sodium fluoride was less strongly inhibitory than potassium or ammonium fluoride.

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