LXXVII. THE EFFECT OF HALOGEN SALTS ON PEPTIC DIGESTION.

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IN a previous paper [Clifford, 1925] the effects of the halogen salts of sodium, potassium, ammonium, and calcium on salivary digestion were described. These salts were found to vary in their action on ptyalin, some delaying digestion, others being inert, and some increasing the speed of reaching the achromic point.

It was thought advisable to continue the investigation with other enzymes, and the experiments to be described give results obtained as to the effects of halogen salts on the action of pepsin. Very little work appears to have been done on the influence of salts on peptic digestion. Hamburger and Halpern [1916] found that sodium chloride in "high concentration" only (0.25 %) inhibited peptic activity, whilst in "low concentration" (0.170 %) it accelerated it. They obtained similar results with sodium phosphate.

Rona and Kleinmann [1924] investigated the effects of the chlorates, nitrates and sulphates of various metals, and using a nephelometric method found that all the salts hastened peptic digestion if the p_H of the solution were high and the concentration of the salts low, whilst the reverse was the case if the p_H were low or the salts more concentrated. Cole [1926, p. 248] says "neutral salts inhibit the action of pepsin, this being in marked contrast to the influence of sodium chloride on ptyalin." Bayliss [1924] states, as a generalisation on ferments, that "neutral salts also have an influence, as a rule, of a favourable kind."

EXPERIMENTAL METHOD.

The end-point, or any specific intermediate point of ordinary peptic digestion is difficult to ascertain, since there is no ready method of estimating the amount of peptone and polypeptides produced. Precipitation methods, such as Fuld's described by Cole [1926, p. 252], could not be used in experiments involving the use of halogen salts, for such salts would precipitate part of the edestin and obscure the end-point of the experiment. Finally it was decided to use the clotting of milk, recommended by Cole [1926, p. 249], as a measure of peptic activity, since it is a definite and easily seen change.

The actual experimental method was as follows.

10 cc. milk were pipetted into each of several test-tubes, standard solutions of the halogen salts in other tubes, and for control experiments distilled water in another tube. These solutions were brought to 37° in a water-bath. Then to one of the milk tubes 2 cc. of the distilled water or salt solution were added, and 2 cc. of the pepsin solution. The tube was returned to the bath, and a stop-watch started. At the moment when clotting began the watch was stopped. In this way a definite end-point could be seen and the effect of the salts on the rate of coagulation noted. The strength of the pepsin solution was adjusted till 10 cc. milk, 2 cc. water, and 2 cc. pepsin clotted in 1½ to 3 minutes.

To determine the moment of clotting, the test-tube was removed from the water-bath, tilted gently and replaced as rapidly as possible. This produced a thin film of milk which trickled down the side of the tube. The tilting was repeated every 30 seconds. When clotting was incipient the film became flocculent instead of homogeneous, and the whole tube of milk became solid about 2-5 seconds after the formation of the clotted film.

After the first tube had been roughly tested in this manner, the next tube was left in the bath till 15 seconds before the anticipated time of coagulation. It was then tilted as described, returned to the bath, and generally the film had not time to fall before becoming flocculent. If this were not the case, the tube was again tilted, and the start of coagulation looked for in the new film. In this manner cooling with consequent retardation of enzyme action was minimised.

The figures given in the following tables for each strength of each salt represent the average of results obtained from six tubes. The greatest difference in any one set of six was 8 seconds.

Actual figures for one of the standard experiments with 2 cc. water are 112, 108, 108, 110, 111, 112 seconds.

Two separate batches of commercial pepsin scales were used in these experiments, one requiring a 0.25 % solution, and the other a 0.4 %. At least two separate samples of each halogen salt were also tested.

The concentration of halogen salts varied from 2 cc. of 2*M* solution to 2 cc. of 0.062 *M* solution, each successive dilution being 50 % of the previous one.

Since these 2 cc. were in a bulk of 14 cc. liquid, the actual experimental concentration was $\frac{1}{7}$ of the weighed halogen solution.

EXPERIMENTAL RESULTS.

		<i>Fluorides.</i>					
Conc. <i>M</i>	NaF		KF		NH ₄ F		
0.28	2 hrs.	30 mins.	} No clot	2 hrs.	15 mins.	} No clot	3 hrs. 35 mins.
0.14	2 "	30 "		2 "	15 "		3 " 35 "
0.07	2 "	30 "		2 "	15 "		3 " 35 "
0.35	1 min.	43 secs.		2 "	15 "		3 " 35 "
0.0175	1 "	23 "		16 mins.	25 secs.		1 min. 10 secs.
0.00875	1 "	30 "		0 "	31 "		—
0.0 (control)	1 "	46 "		1 "	46 "		1 min. 46 secs.
Standard with water							

From these results it is seen that these soluble fluorides completely inhibit the clotting of milk by pepsin in concentrations greater than 0.035 *M* for the Na salt and 0.0175 *M* for the K and NH_4 salts. In the lower concentrations the action is reversed and the rate of coagulation hastened by the fluorides.

Chlorides.

Conc. <i>M</i>	LiCl	NaCl	KCl	NH_4Cl
0.28	1 min. 7 secs.	1 min. 22 secs.	1 min. 50 secs.	0 min. 40 secs.
0.14	1 " 0 "	0 " 57 "	1 " 27 "	0 " 34 "
0.07	0 " 53 "	0 " 49 "	1 " 0 "	0 " 30 "
0.035	0 " 56 "	0 " 50 "	1 " 2 "	0 " 32 "
0.0175	1 " 3 "	1 " 0 "	1 " 2 "	0 " 32 "
0.00875	1 " 12 "	—	1 " 53 "	1 " 30 "
0.0 (control)	1 " 45 "	1 min. 45 secs.	2 " 6 "	2 " 6 "

These figures show that the chlorides hasten the rate of clotting, the ammonium salt being the most active.

There is also an optimum concentration for this reaction ranging between 0.07 molar and 0.018 molar, above and below which the acceleration is less marked.

As far as this phase of digestion is concerned, there is certainly no inhibitory effect exerted by NaCl on pepsin in a concentration of approximately 1.5 %, though Hamburger and Halpern [1916] state that a concentration of NaCl above 0.25 % inhibits peptic activity.

Conc. <i>M</i>	MgCl_2	CaCl_2	BaCl_2
0.28	0 min. 41 secs.	Instantaneous	Instantaneous
0.14	0 " 4 "	"	"
0.07	Instantaneous	"	"
0.035	"	"	"
0.0175	"	"	"
0.00875	"	"	"
0.0 (control)	2 mins. 40 secs.	1 min. 47 secs.	3 mins. 0 secs.

The salts of this group, as expected from the well-known action of CaCl_2 on clotting reactions, produced a great increase in the rate of coagulation. This increase was much less marked with MgCl_2 in the higher concentrations, though in smaller concentration it equalled that given by the Ca and Ba salts.

Bromides.

The results obtained with the bromides are given in the curves in Fig. 1. They are similar to those obtained with chlorides.

The existence of an optimum concentration, however, is more strongly marked (Fig. 1) where it can be seen that with NaBr and KBr a concentration of 0.28 *M* causes definite retarding action. With KBr the hastening is only feebly shown with a concentration as low as 0.0175 *M*. Again the NH_4 salt is the most active.

Conc. <i>M</i>	MgBr_2	CaBr_2	BaBr_2
0.28	1 min. 17 secs.	Instantaneous	Instantaneous
0.14	0 " 9 "	"	"
0.07	0 " 4 "	"	"
0.035	0 " 3 "	"	"
0.0175	0 " 3 "	"	"
0.00875	—	"	"
0.0 (control)	1 min. 54 secs.	1 min. 54 secs.	1 min. 54 secs.

These results are similar to those with the corresponding chlorides, viz. a marked hastening which is more marked with Ca and Ba than with Mg. Again the higher concentrations of the Mg salt are less powerful than the lower.

Fig. 1.

Iodides.

Conc. <i>M</i>	LiI	NaI	KI	NH ₄ I
0.28	3 hrs. 0 mins. } No	2 hrs. 20 mins. } No	1 hr. 54 mins. } No	5 mins. 7 secs.
0.14	3 " 0 " } clot	2 " 20 " } clot	1 " 54 " } clot	3 " 8 "
0.07	3 " 0 " }	2 " 20 " }	1 " 54 " }	2 " 34 "
0.035	41 mins.*	3 mins. 36 secs.	3 mins. 18 secs.	1 " 26 "
0.0175	2 mins. 15 secs.	1 " 54 "	1 " 42 "	1 " 29 "
0.00875	1 " 43 "	—	—	—
0.0	1 " 43 "	1 min. 54 secs.	1 min. 54 secs.	2 mins. 55 secs.
(control)				

* This clot was very fine in each of the six tubes. The milk never became solid, but showed a distinctly granular appearance.

This series shows marked differences. The iodides of Li, Na and K completely stop the coagulative action of pepsin on milk up to a concentration of 0.07 *M*, whilst the NH₄ salt causes a comparatively slight retardation up to a concentration of 0.14 *M*, and then accelerates the rate.

The Li, Na, and K salts in low concentrations appear to have no effect on the rate of coagulation of milk by pepsin.

Conc. <i>M</i>	MgI ₂	CaI ₂	BaI ₂
0.28	0 mins. 51 secs.	Instantaneous	Instantaneous
0.14	0 " 14 "	"	"
0.07	0 " 9 "	"	"
0.035	0 " 10 "	"	"
0.0175	0 " 11 "	"	"
0.00875	—	"	"
0.0 (control)	3 mins. 0 secs.	3 mins. 0 secs.	3 mins. 0 secs.

This series is similar to the other two alkaline earth series. All show a marked hastening influence on coagulation which is much less pronounced with the Mg salt than with the salts of Ca and Ba.

From the results with the Na, K and NH₄ series, it seems that the halide radicle is the primary factor in altering the coagulation rate. The ammonium radicle, however, influences the reaction in a favourable manner.

With the Mg, Ca, Ba series the quickening influence is a function of the metallic radicle, Mg being by far the least potent.

These results are dependent on concentration, for in any series a high concentration of salt was accompanied by an increase in the time for coagulation to occur, except with salts of Ca and Ba where the influence of the metallic radicle is overpowering in any of the strengths used.

SUMMARY.

(1) The clotting of milk by pepsin is greatly affected by the addition of halogen salts.

(2) The reaction is hastened by the chlorides and bromides of Na, K and NH₄, the NH₄ salts having the strongest effect.

(3) There is an optimum concentration of Na, K and NH₄ chlorides and bromides which lies between 0.07 and 0.018 *M*.

(4) The iodides and fluorides of Na, K and NH₄ on the whole retard or inhibit the action of pepsin in coagulating milk.

(5) The inhibitory action of NH₄I is very weak compared with that of NaI and KI.

(6) This retarding action is also a function of concentration, since at low concentrations the iodides do not affect the rate, whilst the fluorides actually hasten it.

(7) The NH₄ radicle favours the coagulation of milk by pepsin.

(8) In the presence of Mg, Ca and Ba halides there is a great increase in the rate of coagulation of milk by pepsin. This action is weakest with the Mg salts.

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