CXXXVIII. THE EFFECT OF FLUORIDES AND IODIDES ON THE CLOTTING OF MILK BY PEPSIN.

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SOME effects of halogen salts on the clotting of milk by pepsin have been described in a previous paper [Clifford, 1927]. With the fluorides and iodides of Na, K, and NH_4 inhibitory actions were found which varied with the concentration of salt used. It was therefore thought advisable to continue the research with a view to locating more exactly the molar concentration at which the inhibitory influence reached its maximum.

In the experiments published earlier, results were obtained of which the following with NaF is typical:

Molar concentration 0.035. Coagulation time 1 min. 43 secs.

„ 0.0175. „ no clot formed in 6 hrs.

Similar changes occurred with K and NH_4 fluorides and with Li, Na, K and NH_4 iodides. These results are in agreement with those of McMeekin [1928], who, using egg-white and pepsin, finds Na and K iodides to exert an inhibitory effect when added in a concentration greater than $N/256$. But, as previously shown [Clifford, 1927], the writer cannot agree that a similar inhibitory effect is shown by the bromides and chlorides of these metals, which actually hasten coagulation up to a concentration of 0.14–0.28 *M*. The differences may be due to the fact that, in one case the reaction is a hydrolysis, and in the other an aggregation of a colloidal system.

In this paper, experiments are described using halogen salt solutions of strengths intermediate between that causing very little, if any, alteration in the rate of coagulation of milk by pepsin, and that which caused complete inhibition of clotting for a period of $2\frac{1}{2}$ – $6\frac{1}{2}$ hours.

EXPERIMENTAL METHOD.

The method of determining the end-point of the reaction (*i.e.* the first formation of a clot) has been described in a previous paper [Clifford, 1927]. As before, a solution of commercial pepsin scales was used which gave a coagulation time of $1\frac{1}{2}$ –3 minutes when 2 cc. of it were added to a mixture of 10 cc. milk and 2 cc. distilled water, all three solutions having first been brought to a temperature of 37° .

A series of 21 test-tubes was then taken, and into each tube 10 cc. milk were pipetted. Then

	to tube	(1)	2 cc.	dist. water	and 0.0 cc. halide
		„	(2)	1.9 cc.	„ 0.1 cc. „
		„	(3)	1.8 cc.	„ 0.2 cc. „
and		„	(21)	0.0 cc.	„ 2.0 cc. „

were added.

The concentration of the salt solution used was obtained from the results of the experiments published earlier [Clifford, 1927]. It was the lowest concentration which was first found completely to inhibit coagulation when 2 cc. of it were added to 10 cc. milk and 2 cc. of pepsin. In every case the experiments were repeated with two separate batches of commercial pepsin scales, and at least two samples of each halide.

RESULTS.

Sodium fluoride. Concentration of solution added: 0.5 *M*.

Tube no.	cc. of 0.5 <i>M</i> NaF in 14 cc. mixture	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	2	35	0.0000
(2)	0.1	1	59	0.0036
(3)	0.2	1	51	0.0072
(4)	0.3	2	52	0.0108
(5)	0.4	5	0	0.0144
(6)	0.5	—		0.0180
(7)	0.6	No clot in 6½ hours		
(21)	2.0	No clot in 6½ hours		

Potassium fluoride. Concentration of solution added: 0.25 *M*.

Tube no.	cc. of 0.25 <i>M</i> KF	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	1	16	0.0000
(2)	0.1	1	11	0.0018
(3)	0.2	1	11	0.0036
(4)	0.3	1	12	0.0054
(5)	0.4	1	21	0.0072
(6)	0.5	1	30	0.0090
(7)	0.6	1	46	0.0108
(8)	0.7	2	12	0.0126
(9)	0.8	2	6	0.0144
(10)	0.9	2	15	0.0162
(11)	1.0	2	15	0.0180
(12)	1.1	2	28	0.0198
(13)	1.2	No clot in 3½ hours		0.0216
(21)	2.0	No clot in 3½ hours		

Ammonium fluoride. Concentration of solution added: 0.25 *M*.

Tube no.	cc. of 0.25 <i>M</i> NH ₄ F	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	0	53	0.0000
(2)	0.1	0	55	0.0018
(3)	0.2	0	54	0.0036
(4)	0.3	1	12	0.0054
(5)	0.4	1	15	0.0072
(6)	0.5	2	4	0.0090
(7)	0.6	2	34	0.0108
(8)	0.7	2	51	0.0126
(9)	0.8	4	57	0.0144
(10)	0.9	11	31	0.0162
(11)	1.0	13	13	0.0180
(12)	1.1	No clot in 4½ hours		0.0198
(21)	2.0	No clot in 4½ hours		

Lithium iodide. Concentration of solution added: 0.25 *M*.

Tube no.	cc. 0.25 <i>M</i> LiI	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	2	30	0.0000
(2)	0.1	2	27	0.0018
(3)	0.2	2	13	0.0036
(4)	0.3	2	26	0.0054
(5)	0.4	2	48	0.0072
(6)	0.5	3	4	0.0090
(7)	0.6	4	0	0.0108
(8)	0.7	4	7	0.0126
(9)	0.8	4	53	0.0144
(10)	0.9	5	4	0.0162
(11)	1.0	5	0	0.0180
(12)	1.1	8	7	0.0198
(13)	1.2	No clot in $4\frac{1}{2}$ hours		0.0216
(21)	2.0	No clot in $4\frac{1}{2}$ hours		

Sodium iodide. Concentration of solution added: 0.5 *M*.

Tube no.	cc. 0.5 <i>M</i> NaI	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	1	42	0.0000
(2)	0.1	1	37	0.0036
(3)	0.2	1	41	0.0072
(4)	0.3	1	43	0.0108
(5)	0.4	2	0	0.0144
(6)	0.5	2	13	0.0180
(7)	0.6	2	21	0.0216
(8)	0.7	2	33	0.0252
(9)	0.8	2	43	0.0288
(10)	0.9	3	0	0.0322
(11)	1.0	No clot in $3\frac{1}{2}$ hours		0.0360
(21)	2.0	No clot in $3\frac{1}{2}$ hours		

Potassium iodide. Concentration of solution added: 0.5 *M*.

Tube no.	cc. 0.5 <i>M</i> KI	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	1	25	0.0000
(2)	0.1	1	28	0.0036
(3)	0.2	1	27	0.0072
(4)	0.3	1	38	0.0108
(5)	0.4	1	47	0.0144
(6)	0.5	1	52	0.0180
(7)	0.6	2	2	0.0216
(8)	0.7	2	15	0.0242
(9)	0.8	2	21	0.0288
(10)	0.9	2	25	0.0324
(11)	1.0	3	8	0.0360
(12)	1.1	No clot in $3\frac{1}{2}$ hours		0.0396
(21)	2.0	No clot in $3\frac{1}{2}$ hours		

Ammonium iodide. Concentration of solution added: 2 *M*.

Tube no.	cc. 2 <i>M</i> NH ₄ I	Coagulation time		Molar concentration
		m.	s.	
(1)	0.0	2	5	0.0000
(2)	0.1	1	27	0.0143
(3)	0.2	1	19	0.0286
(4)	0.3	1	23	0.0429
(5)	0.4	1	45	0.0562
(6)	0.5	2	0	0.0715
(7)	0.6	2	30	0.0848
(8)	0.7	3	7	0.1001
(9)	0.8	3	25	0.1144
(10)	0.9	4	12	0.1287
(11)	1.0	4	37	0.1430
(12)	1.1	4	45	0.1573
(13)	1.2	6	17	0.1716
(14)	1.3	10	22	0.1859
(15)	1.4	13	47	0.2002
(16)	1.5	No clot in $4\frac{1}{2}$ hours		0.2145
(21)	2.0	No clot in $4\frac{1}{2}$ hours		

The outstanding feature, as can be readily seen from Fig. 1, is the sudden change from a coagulation time of, at the utmost, 14 minutes, and generally 3–5 minutes, to a completely inhibitory effect, with a very small alteration in concentration of salt in the mixture, amounting to an increase of 0.0036 *M* only.

The order of magnitude of the change in concentration and also the actual low molarity of salt in the experimental mixture (*e.g.* 0.018 *M* NaF) seems to preclude the idea that the results are a consequence of a change in hydrogen ion concentration, especially in a well buffered system such as milk.

Fig. 1.

There is a marked difference between the behaviour of NH_4I as opposed to the iodides of Li, Na and K since the molar concentration for inhibition has to be about ten times greater, but the shape of the curve obtained is the same as for the other iodides. Taken together with the fact that NH_4F showed a rather less sudden change from coagulation to inhibition of reaction than did either NaF or KF, it appears that the NH_4 radicle favours clotting, though the halide radicle is prepotent.

Curves from three experiments with NH_4I using two different samples of the salt are given. Several others have been obtained, and all fall within the limits of these curves.

SUMMARY.

- (1) The clotting of milk by pepsin is inhibited by the addition of the fluorides of Na, K and NH_4 and the iodides of Li, Na, K and NH_4 .
- (2) This inhibitory action depends on the concentration of the halide.
- (3) The inhibition of coagulation occurs suddenly at a definite molarity of the halide added, a difference of 0.0036 M , changing the rate from 5–13 minutes to absence of change for 4–6 hours.
- (4) The inhibitory action of fluorides is greater than that of iodides.
- (5) The concentration of NH_4I has to be six times that of NaI or KI , and ten times that of LiI to produce a similar inhibitory effect.

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REFERENCES.

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