LIX. THE EFFECT OF HALOGEN SALTS ON STEAPTIC DIGESTION.

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FOLLOWING on work as to the effect of halogen salts on salivary, peptic and tryptic digestion [Clifford, 1925; 1927; 1928; 1933], it was decided to carry out experiments on their action on lipase.

The effect of halogen salts on lipolytic activity has been investigated many times with varying results. Loevenhart and Pierce [1907], using pancreatic and hepatic lipases, found sodium fluoride strongly inhibitory, as also, though to a far less degree, were sodium chloride and iodide, potassium chloride and iodide and potassium bromide. Sodium bromide, however, was found by these observers to exert a slight accelerating action. Amberg and Loevenhart [1908] confirmed the inhibitory action of sodium fluoride on hydrolysis by hepatic lipase.

Mellanby and Woolley [1914] found no effect on lipolytic activity with the chlorides of sodium, calcium and magnesium, but Falk [1918] stated that sodium chloride accelerated the action over a period of 30 minutes. This acceleration disappeared if the fat were previously emulsified and was replaced by a retardation if the concentration of chloride were increased.

Leake *et al.* [1929] found that sodium fluoride in a concentration of 1 : 50,000 inhibited hepatic lipase *in vitro* and extended the work to observations *in vivo*, obtaining similar results. In the same year Corran [1929] found the chlorides of sodium and potassium to be slightly augmentative, and calcium chloride without effect. McGillivray [1930] confirmed these observations for sodium chloride but stated that calcium chloride also hastened lipase action.

These results, except for agreement as to the inhibitory action of sodium fluoride, vary considerably, and it was decided to investigate the action of various concentrations of halogen salts on the rate of lipolytic activity.

EXPERIMENTAL METHODS.

Since in the digestion of fats emulsification is obviously of great importance, the substrate used was the relatively stable emulsion found naturally in cow's milk.

A solution of commercial pancreas substance was used as the source of lipase, since in a low concentration it showed marked lipolytic activity together with a negligible tryptic action. A solution of the pancreas substance in the concentration used in these experiments showed no proteolytic action (biuret reaction unchanged in each case and this negative result confirmed by Sørensen titrations with fish-protein) in 5 hours, when incubated together with sodium carbonate at 37° with caseinogen, fish-protein or hard-boiled egg-white.

In any set of experiments 200 ml. of milk were placed in a flask together with the requisite weighed amount of halogen salt. The flasks were allowed to reach a

Time of digestion in minutes		ml. 0.1 N acid produced.													
		15	30	45	60	75	90	105	15	30	45	60	75	90	105
		0.5 M							0.25 M						
Control	A	0.3	2.4	4.6	6.2	7.2	8.3	8.9	0.9	2.1	4.0	5.0	6.0	6.6	7.1
"	B	0.8	3.0	5.0	6.4	7.4	8.9	9.1	0.5	2.4	3.8	4.8	5.6	7.0	7.1
Na		0	0	0.7	1.5	1.9	2.1	2.3	0	0	0.1	0.3	0.5	0.5	0.6
K		0	0	0	0.4	1.0	1.5	2.1	0.1	0.4	1.0	1.2	1.7	1.7	2.1
NH ₄		0	0	0	0.1	0.5	1.6	2.1	0	0	0.1	0.1	0.1	0.2	0.7
		0.125 M							0.06 M						
Control	A	2.1	4.6	6.4	7.9	9.1	10.7	11.0	2.1	4.8	6.4	8.2	8.9	9.7	10.6
"	B	2.0	4.4	6.3	7.8	8.7	10.5	10.9	2.5	4.7	6.3	8.0	8.7	10.2	10.4
Na		1.0	2.6	3.6	4.2	4.4	4.9	5.7	1.3	2.5	3.5	4.8	5.7	6.3	7.6
K		0.8	2.5	3.6	3.8	4.3	4.5	5.5	0.9	2.7	3.2	3.8	4.8	5.6	6.0
NH ₄		0.6	0.8	0.9	1.4	1.4	1.6	2.4	0.2	0.4	1.3	2.5	3.7	4.5	4.6
		0.03 M							0.015 M						
Control	A	2.9	4.8	7.6	9.0	9.9	10.3	11.2	2.2	5.3	6.9	8.6	9.2	10.2	11.3
"	B	3.0	5.3	7.2	8.8	9.9	10.8	11.0	2.5	5.2	7.1	8.5	9.5	9.9	11.2
Na		2.1	4.0	5.0	6.4	7.1	7.4	8.7	1.7	4.5	5.4	6.2	7.3	8.9	9.0
K		2.2	3.9	5.7	7.7	7.9	8.6	8.9	1.7	4.4	5.7	7.0	7.5	7.8	9.0
NH ₄		2.3	4.2	5.8	6.8	7.6	8.6	8.9	1.1	4.2	5.8	6.9	7.6	8.1	9.2
		0.008 M													
Control	A	0.3	2.4	4.3	4.5	5.9	6.9	8.9							
"	B	2.4	3.0	4.1	4.4	5.6	6.8	8.3							
Na		1.1	1.8	2.6	3.2	3.6	5.0	6.5							
K		0.6	1.2	2.4	2.6	3.5	4.4	5.4							
NH ₄		1.4	1.8	3.2	3.8	4.2	4.7	5.9							

Table II. *Chlorides.*

Time of digestion in minutes	ml. 0.1 <i>N</i> acid produced.													
	15	30	45	60	75	90	105	15	30	45	60	75	90	105
				0.5 <i>M</i>							0.25 <i>M</i>			
Control A	1.2	3.3	6.3	8.4	8.7	9.6	10.0	2.3	4.5	6.2	7.1	9.1	9.7	10.6
Li " B	1.4	4.5	5.9	8.3	8.9	9.5	10.1	2.0	4.4	5.9	7.0	9.2	9.9	10.4
Li	1.2	4.8	6.0	7.8	9.0	9.9	10.3	1.6	4.4	6.0	7.9	9.1	9.4	9.9
Na	0.9	4.5	6.6	8.0	8.3	8.9	9.5	1.4	4.6	5.8	7.1	9.3	9.5	10.0
K	0.8	4.7	5.8	8.2	8.8	9.2	9.7	1.7	3.8	5.7	7.9	9.0	9.9	10.0
NH ₄	0.8	3.6	6.3	7.5	8.6	9.0	9.6	1.8	4.5	5.2	7.3	9.7	10.0	10.4
				0.125 <i>M</i>							0.06 <i>M</i>			
Control A	2.1	4.9	7.1	8.8	10.6	11.1	12.0	1.7	3.4	5.6	7.7	8.5	9.7	10.4
Li " B	2.3	4.4	6.2	7.8	10.8	10.8	11.4	1.5	3.0	5.3	7.4	8.3	9.1	10.7
Li	2.1	4.5	6.7	7.3	9.6	10.2	12.1	1.8	2.9	5.5	7.5	8.0	8.9	10.2
Na	1.9	5.0	7.6	8.6	10.3	11.4	11.5	1.5	3.1	5.4	7.6	8.4	9.3	10.5
K	1.4	4.7	6.4	7.9	10.0	10.7	12.0	1.6	3.5	5.2	7.7	8.2	9.5	10.6
NH ₄	1.8	4.2	6.1	7.3	9.4	11.3	11.3	1.8	3.3	5.5	7.6	8.4	9.4	10.3
				0.03 <i>M</i>							0.015 <i>M</i>			
Control A	2.5	4.7	6.3	8.0	8.7	10.2	10.4	3.3	6.6	8.4	9.5	11.9	12.2	12.3
Li " B	2.0	4.5	6.1	8.2	8.9	10.3	10.6	3.0	6.4	8.5	9.8	11.7	12.0	12.5
Li	1.9	4.0	5.9	8.0	8.7	10.4	10.7	3.1	6.3	8.4	9.6	11.6	11.9	12.2
Na	2.3	4.4	6.0	8.4	8.8	10.1	10.5	3.3	6.5	8.5	9.7	11.5	12.1	12.4
K	2.4	4.3	6.2	7.9	8.5	10.4	10.6	3.1	6.2	8.6	9.4	11.3	12.2	12.4
NH ₄	2.2	4.4	6.4	8.0	8.6	9.9	10.3	3.2	6.6	8.5	9.5	11.5	12.3	12.5
				0.008 <i>M</i>										
Control A	1.9	3.4	6.5	7.7	9.5	10.7	11.6							
Li " B	2.0	3.5	6.7	7.9	9.8	10.8	11.9							
Li	1.9	3.3	6.4	7.8	9.7	10.7	11.5							
Na	2.1	3.5	6.6	7.5	9.6	10.6	11.7							
K	2.3	3.6	6.6	7.7	9.8	10.8	11.5							
NH ₄	2.0	3.4	6.5	7.6	9.7	10.7	11.8							

Table III. *Bromides.*

Time of digestion in minutes	ml. 0.1 <i>N</i> acid produced.													
	15	30	45	60	75	90	105	15	30	45	60	75	90	105
				0.5 <i>M</i>							0.25 <i>M</i>			
Control A	1.0	2.5	4.3	5.8	6.6	7.5	7.5	1.3	5.0	7.0	9.2	9.7	10.1	12.3
Li " B	1.0	2.6	4.3	6.3	6.6	7.2	7.6	1.1	4.9	6.8	9.0	9.5	9.9	11.8
Li	0.3	1.9	3.9	4.3	5.1	5.7	6.1	0.7	4.5	7.2	8.5	9.2	9.4	11.2
Na	0.2	1.5	3.3	4.5	5.1	6.7	6.7	0.9	3.4	6.7	7.7	8.6	9.6	11.5
K	0.5	1.9	3.5	4.6	5.3	5.8	6.6	0.5	3.4	6.6	8.6	9.0	9.8	10.8
NH ₄	0.3	1.1	3.7	5.0	5.2	5.6	6.5	3.1	4.3	7.7	8.4	8.6	9.4	11.7
				0.125 <i>M</i>							0.06 <i>M</i>			
Control A	2.2	5.3	6.9	8.6	9.2	10.2	11.3	1.4	3.4	6.5	8.6	8.9	9.9	10.2
Li " B	2.3	5.4	7.0	8.3	9.3	10.0	11.1	1.5	3.7	6.4	8.5	9.1	9.8	10.3
Li	2.0	5.5	7.1	8.5	9.1	10.3	11.0	1.5	3.6	6.6	8.4	9.0	10.0	10.4
Na	2.1	5.4	7.0	8.4	9.4	10.2	11.2	1.3	3.5	6.4	8.5	8.9	10.1	10.3
K	2.3	5.6	7.2	8.5	9.2	10.1	11.3	1.2	3.4	6.3	8.6	9.2	9.9	10.3
NH ₄	2.2	5.1	7.2	8.6	9.5	10.4	11.4	1.5	3.8	6.6	8.7	9.0	10.1	10.4
				0.03 <i>M</i>							0.015 <i>M</i>			
Control A	2.0	4.8	7.1	8.6	10.4	11.0	11.9	2.3	4.3	7.2	8.9	9.7	10.5	11.0
Li " B	2.1	4.0	6.0	7.6	10.7	11.0	11.5	2.0	4.7	7.0	8.9	9.9	10.4	10.9
Li	2.0	4.3	6.5	7.1	10.3	10.9	11.8	2.1	4.3	7.5	9.1	9.8	10.6	10.8
Na	1.9	4.7	7.2	8.4	10.5	11.2	11.7	2.1	4.5	6.9	9.3	9.8	10.5	10.7
K	1.6	4.8	6.5	8.3	10.4	11.1	11.7	2.2	4.7	7.4	9.2	10.0	10.6	11.1
NH ₄	2.1	4.6	6.5	7.7	10.6	11.5	11.6	1.9	4.4	7.1	8.9	9.7	10.5	10.9
				0.008 <i>M</i>										
Control A	1.7	2.9	5.0	7.5	9.4	10.0	10.5							
Li " B	1.3	2.8	5.0	7.4	9.2	9.8	10.1							
Li	1.5	2.7	4.9	7.3	9.0	9.9	10.4							
Na	1.8	3.4	5.3	7.5	8.9	9.7	10.5							
K	1.4	2.9	5.1	7.6	9.0	9.8	10.3							
NH ₄	1.5	2.8	5.3	7.5	9.4	9.9	10.4							

Table IV. *Iodides.*

Time of digestion in minutes	ml. 0.1 <i>N</i> acid produced.													
	15	30	45	60	75	90	105	15	30	45	60	75	90	105
0.5 <i>M</i>														
Control A	3.1	5.7	7.9	8.5	9.7	10.4	10.7	1.3	2.4	4.6	6.2	7.2	8.3	8.9
Li " B	3.2	5.3	7.8	8.4	9.2	9.9	10.0	1.8	3.0	5.0	6.4	7.4	8.9	9.4
Na	2.7	5.2	7.3	7.6	9.0	9.4	10.4	0.9	2.5	4.7	6.0	7.1	8.5	9.3
K	3.6	6.5	8.7	8.9	9.7	10.4	10.6	1.1	2.7	4.8	6.3	7.3	8.5	9.2
NH ₄	3.5	5.3	8.1	8.6	9.2	10.7	10.9	1.3	2.3	4.5	6.3	7.4	8.6	9.2
	3.1	5.5	8.3	8.5	9.7	10.7	11.2	1.2	2.6	4.9	6.4	7.5	8.9	9.4
0.125 <i>M</i>														
Control A	0.8	2.0	3.1	4.9	5.2	6.1	6.9	2.1	4.4	6.2	7.7	9.0	10.6	10.9
Li " B	0.7	2.1	3.4	4.8	5.9	6.5	7.1	2.0	4.2	6.1	7.9	8.9	10.5	11.0
Na	1.5	2.8	4.2	5.5	5.7	6.7	7.0	1.9	4.0	6.3	8.0	9.1	10.7	11.2
K	0.8	2.4	3.5	4.9	5.4	6.2	7.4	2.0	4.4	6.4	7.8	9.0	10.4	10.8
NH ₄	0.5	2.2	3.1	4.9	5.8	6.6	7.2	2.1	4.3	6.4	7.6	9.1	10.6	11.0
	0.6	2.5	3.2	4.7	5.6	6.5	7.1	1.9	4.2	6.5	7.8	8.9	10.6	11.1
0.03 <i>M</i>														
Control A	2.5	4.4	7.5	9.0	9.7	10.5	11.2	2.9	5.2	7.5	8.0	9.2	10.1	10.7
Li " B	2.3	4.7	7.3	8.9	9.5	10.2	10.7	3.0	5.3	7.8	8.2	9.0	9.9	10.4
Na	2.3	4.5	7.9	9.1	9.9	10.4	11.0	2.8	5.2	7.4	8.3	9.3	10.2	10.6
K	2.1	4.3	7.4	9.0	9.8	10.5	10.8	2.9	5.5	7.9	8.1	9.2	10.4	10.6
NH ₄	2.2	4.7	7.8	9.2	10.0	10.7	11.1	3.1	5.6	8.0	8.6	9.2	10.1	10.8
	2.0	4.4	7.8	9.5	9.9	10.5	10.9	2.9	5.5	7.8	8.5	9.1	10.4	10.9
0.008 <i>M</i>														
Control A	2.0	5.0	7.1	8.5	10.4	10.9	11.5							
Li " B	2.3	4.6	6.9	8.8	10.5	11.0	11.4							
Na	2.1	4.7	6.7	8.3	10.5	10.8	11.6							
K	2.0	4.9	7.1	8.6	10.3	11.1	11.7							
NH ₄	2.2	4.7	6.8	8.4	10.4	10.9	11.6							
	1.9	4.5	6.6	8.4	10.6	11.3	11.7							

inhibitory in all concentrations from 0.5 to 0.06 *M* and definitely so even at a concentration of 0.008 *M*.

In Fig. 1 a series of experiments with sodium fluoride in varying concentrations shows how the inhibitory action is a function of concentration. Ammonium fluoride is more inhibitory than either sodium or potassium fluoride up to a concentration of 0.03 *M*. Below this strength all three salts have an equal effect.

Chlorides (Table II). In concentrations of 0.5–0.008 *M* the chlorides of lithium, sodium, potassium and ammonium have no effect on the rate of lipolytic action.

Bromides (Table III). With a concentration of 0.5 *M* there is a slight depression of rate, but from 0.25 to 0.008 *M* no effect can be demonstrated.

Iodides (Table IV). As with chlorides, there appears to be no acceleration or retardation of lipolytic action when iodides of lithium, sodium, potassium or ammonium are added in concentrations varying from 0.5 to 0.008 *M*.

DISCUSSION.

From these results it appears that the halogen salts of lithium, sodium, potassium and ammonium are without effect on the rate of lipase action in concentrations

Fig. 1. Sodium fluoride 0–0.5 *M*.

of 0.5–0.008 *M*, except in the case of the fluorides. Fluorides exert a strongly depressant action which varies directly with concentration of salt. Ammonium fluoride at 0.25–0.06 *M* is more inhibitory than either the sodium or potassium salt, but at 0.5 *M* and from 0.3 to 0.008 *M* all three fluorides show an equal effect.

During the course of these experiments an unexpected effect of halogen salts on the clotting of milk by very weak trypsin was observed. Though the proteolytic enzyme present with the lipase was too weak to show any digestive action on fish-protein, caseinogen or hard-boiled egg-white in five hours, yet in all the concentrations of ammonium fluoride, a junket was formed 15 minutes after the addition of 20 ml. of pancreas substance to 200 ml. milk.

Similar thick soft clots were formed with all four salts of chlorine, bromine and iodine and with potassium and sodium fluorides, except that at the lowest concentration (0.008 *M*) a fine flocculation rather than complete coagulation took place. From these results it seems that the coagulating effect of added calcium salts when rennin, pepsin or trypsin is added to milk may be due to the effect of the acid radical as well as to the metallic ion.

Further experiments on this coagulating action of the halogen salts are in progress.

SUMMARY.

1. The lipolytic activity of pancreas substance is strongly inhibited by sodium, potassium and ammonium fluorides.
2. This inhibition varies directly with the concentration of fluoride.
3. Sodium, potassium and ammonium chlorides and iodides show no effect on the rate of lipase action in concentrations of 0.5–0.008 *M*.
4. Sodium, potassium and ammonium bromides depress lipolytic activity to a slight extent at a concentration of 0.5 *M* but are without effect at 0.25–0.008 *M*.
5. None of the halogen salts, in the concentrations used (0.5–0.008 *M*), accelerated lipolytic action.

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