STUDIES ON ENZYME ACTION. V. THE ACTION OF NEUTRAL SALTS ON THE ACTIVITY OF CASTOR BEAN LIPASE.

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Introduction.

In the course of experiments with castor bean lipase, it was found that filtrates from extractions of the preparation by aqueous solutions of various salts, when dialyzed in collodion bags with running water, formed precipitates, soluble in minute quantities of acid or alkali. These suspensions showed lipolytic activities, which were greater or less than those obtained with aqueous extractions, depending upon the salt used and its concentration in the extracting solution. In order to carry out a complete study of this and the accompanying phenomena, it was necessary to determin the action of neutral substances upon the lipolytic activity of the castor bean preparation in aqueous solution or suspension. The results obtained with salts are presented in this paper. In the next paper the results obtained with a few neutral organic substances will be presented, together with an explanation of the selective actions of lipases upon various esters.

A complete review of the literature of the action of neutral salts on the lipolytic activity of animal or vegetable substances will not be attempted. In a number of cases the results obtained by different observers have been apparently contradictory, but if the details of the methods used are examined, it will generally be found that the experimental methods differed sufficiently to account for these differences, especially if the considerable changes which may be caused by small amounts of neutral substances are kept in mind.

Pekelharing[1] studied the action of a number of the halides of the alkali and alkaline earth metals on the activity of (pig) pancreatic lipase in hydrolyzing olive oil. He considered the increase in action caused by these salts due to the formation of insoluble soaps from the metallic element added and the oleic acid formed, whereby the latter is removed from the sphere of action. Sodium fluoride caused only a slight acceleration. Terroine[2] found that the amount of action of pancreatic lipase on olive oil was increased by sodium chloride, bromide, iodide and fluoride in dilute solution, but decreased in more concentrated solution. The explanation advanced by Pekelharing will evidently account for the former action, especially as Terroine described experiments with methyl acetate, triacetin, and ethyl butyrate in place of olive oil, in which no increase in action but only a retardation was shown. The retardation due to the different sodium salts increased in magnitude in the order chloride, bromide, iodide, fluoride, and it is pointed out that the retarding action found

[1] *Z. physiol. Chem.*, 81, 355 (1912).

[2] *Biochem. Z.*, 23, 429 (1910).

by Loevenhart and Pierce[1] for sodium fluoride on animal lipase and studied exhaustively by them, is not limited solely to the fluoride, but is shown as well by the other halides, the only difference consisting in the concentrations which show the actions for the different salts. These results with animal lipase are of interest in connection with the results of the present investigation with a vegetable lipase.

The result of Hamsik,[2] who found only a retarding action of sodium chloride and calcium chloride with pancreatic lipase and olive oil, may be ascribed to the different conditions of his experiments. He used a glycerol extract, a comparatively small amount of solution, and a large amount of oil.

Further work with animal lipases will not be taken up in detail, but it may be mentioned that the use of olive oil in testing the activities is liable to introduce complications, due to the formation of insoluble soaps when salts are added. Armstrong and Ormerod[3] considered the proper solution or emulsification of a fat of prime importance in comparing animal and vegetable lipases. Bradley[4] found parallel actions for added substances on ethyl butyrate and olive oil by (human) pancreatic lipase. It seems therefore best, in studying the action of salts on the activity of lipase, to use a lower ester instead of olive oil in order to avoid the complications mentioned.

No extended investigation of the action of neutral salts on the activity of a vegetable lipase appears to have been published. Hoyer[5] found that manganese salts produce a greater increase in the action of castor bean lipase than did any other salt studied by him. Aside from the harmful effects of sodium fluoride and mercuric chloride, the results of Connstein, Hoyer and Wartenberg[6] led to no definit conclusion as to the action of neutral salts on the castor bean lipase. Tanaka[7] found an increased activity caused by some neutral salts, and decreased activity by others, in the action of a lipase preparation obtained from castor beans after extracting the soluble constituents by dilute acid. He used one gram of the preparation, 25 grams soya bean oil and 6 cc. of solution in each experiment.

Experimental.

A. Uni-Univalent Salts.

In this section, the general method of carrying out the experiments will be described and the results which were obtained with the chlorides,

[1] *J. Biol. Chem.*, **2**, 397 (1907).
[2] *Z. physiol. Chem.*, **71**, 246 (1911).
[3] *Proc. Roy. Soc. London*, **78**, 376 (1906).
[4] *J. Biol. Chem.*, **6**, 133 (1909).
[5] *Z. physiol. Chem.*, **50**, 414 (1907).
[6] *Ber.*, **45**, 3988 (1902).
[7] *Orig. Com. 8th Intern. Congr. Appl. Chem.*, **11**, 37 (1912).

iodides, fluorides, and nitrates of sodium and potassium, potassium bromide, lithium chloride, lithium nitrate, and sodium acetate, presented.

General Method.—The lipase preparation used in this study was prepared from cold pressed castor beans.[1] The husks were removed by hand, the kernels ground, extracted with ether in a Soxhlet apparatus for a week or more in order to remove the oil completely, allowed to stand at the ordinary temperature until only a faint odor of ether remained, and then ground to an impalpable powder. The preparation obtained in this way was white in color and the lipolytic activity found with portions prepared at different times showed remarkably constant results. 7.5% of ash, white in color, was found on ignition. An analysis of this ash, carried out by Dr. M. L. Hamlin,[2] showed 0.5% of the ash as SiO_2, 3.8% as CaO, 26.0% as MgO, 48.2% as P_2O_5, a distinct trace of manganese, and a trace of chloride.

The hydrolysis of ethyl butyrate by the lipase preparation was used throughout these experiments as a measure of the activity. As stated in the introduction, the use of olive oil is liable to introduce complications, and since the primary object of this study was to obtain comparable results with different salts, a lower ester was used. The same amounts of the ester, of water or solution, and of lipase preparation were used in each set of experiments. The amount and the nature of the added salt were the only variable factors, so that all of the experiments are strictly comparable.

The salt solutions were made up volume molar. Most of the salts dissolved in water to form neutral solutions. Attention will be called to the solutions in which this was not the case in the discussion of the experiments.

In each experiment 0.2 gram of lipase preparation, 25 cc. of solution, 1 cc. of ethyl butyrate, and $^1/_2$ to 1 cc. of toluene were used. Three sets of solutions were made up; one set with lipase preparation, ester, and solution; and two blank sets, one with lipase preparation and solution, and one with ester and solution. These were allowed to stand in the incubator for from 23 to 25 or from 43 to 50 hours at 38–40° and then titrated with standard (approximately 0.1 normal) sodium hydroxide solution, with 5 to 10 drops of a 1% alcoholic phenolphthalein solution as indicator. The results were calculated to the common standard of 0.1 normal sodium hydroxide solution and 24 or 48 hours action. Proportionality of amount of action to time was assumed. While this is not strictly true, the error introduced in the results by this assumption is negligible. From two to ten

[1] Supplied by the Baker Castor Oil Company, New York.

[2] For details of the analysis, cf. *Biochem. Bull.*, 2, March, 1913.

solutions were tested for each concentration of each salt, and the final results taken as the means of these. In presenting the results, the separate experiments will be not given. Instead, the number of determinations with the given solution, the mean value of the action found for 24 or 48 hours in cc. of 0.1 normal sodium hydroxide solution, and the average deviation of the mean (A. D.)[1] will be given. The number of the determinations and the average deviation of the mean will show the agreement obtained in the separate measurements for each mean value given.

The activities were tested for either 24 hours or 48 hours; the former if an acceleration or small retardation was observed, the latter if the retardation was at all marked. More constant results were obtained by stopping the tests before a large amount of acid had been produced in the mixtures.

Uni-Univalent Salts.—Since only retardations were observed with the uni-univalent salts, the duration of the tests in this section was 48 hours in every case. The concentrations of the salts were varied from 2.0 molar to 0.0005 molar (for sodium and potassium fluorides).

Lipase Preparation-Solution Blanks.—The lipase preparation on standing at 38–40° in aqueous solution or suspension increased in acidity in 48 hours, as pointed out in the first paper.[2] The presence of sodium chloride, potassium chloride, sodium iodide, potassium bromide, lithium chloride, sodium nitrate, potassium nitrate, lithium nitrate, and sodium acetate, even in the most concentrated solutions, exerted no influence on this increase in acidity. The average of all the results gave a value of 0.52 cc. 0.1 normal sodium hydroxide solution as the equivalent of the acid present after 48 hours (the value at the beginning being 0.20 cc.). For potassium iodide and the fluorides of sodium and potassium, lower values were found for the more concentrated solutions. The following were obtained for these by plotting the mean experimental values and the concentrations and drawing smoothed curves:

Molar conc. =	2.0	1.0	0.5	0.2	0.1	0.05	0.02	0.01	0.005	0.002	0.001	0.0005
KI	0.30	0.36	0.42	0.48	0.50	0.52	..	..	...	...	...	...
NaF	..	0	0.06	0.18	0.26	0.33	0.40	0.44	0.47	0.50	0.51	0.52
KF	..	0	0.06	0.18	0.26	0.32	0.39	0.42	0.46	0.50	0.51	0.52

The sodium fluoride solution was slightly alkaline and the potassium fluoride solution slightly acid in reaction. The action of these two fluorides in decreasing the acidity of the preparation until, with molar solutions, no acidity is shown is noteworthy, as well as the marked agreement between the results at different concentrations, in view of the acidity of the one solution and the alkalinity of the other.

[1] Cf. Second Paper of this series, THIS JOURNAL, 34, 832 (1912).

[2] THIS JOURNAL, 34, 736 (1912).

Ester-Solution Blanks.—One drop of sodium hydroxide solution was required to produce a pink color in testing the solutions of ester and sodium chloride, potassium chloride, sodium iodide, potassium iodide, potassium bromide, sodium nitrate, potassium nitrate, or lithium nitrate. The ester blanks with these substances were therefore not considered in the final values. For the remaining substances, the following values were obtained by plotting the curves of the mean experimental results[1] and the concentrations:

Molar conc. =	2.0	1.0	0.5	0.1	0.2	0.05	0.02	0.01
NaF	..	0.13	0.06	0.02	0	..	..	..
KF	..	0.37	0.19	0.08	0.04	0.02	0	..
LiCl	0.15	0.12	0.09	0.05	0.03	0	..	..
$NaC_2H_3O_2$	..	0.43	0.33	0.23	0.15	0.09	0.03	0

Experimental Results.—The results are given in every case in terms of the amount of acid formed from the solutions in question, in 48 hours at 38–40°, expressed as cc. of 0.1 normal sodium hydroxide solution. The blanks have been subtracted in each case. With water alone, an action of 2.50 ± 0.02 was found as the mean value of eleven determinations carried out at different times. This was taken as the value for zero concentration for all of the salts. In Table I the results for the uni-univalent salts are given. The first column shows the molar concentration of the solutions; the second, the salt used; the third, the number of measurements; the fourth, the average deviation of the mean for the actions found, and the fifth, the mean values of the actions found. The third and fourth columns indicate the agreement of the various results obtained for each concentration. The values of the actions found, as given in column 5, were plotted against the logarithms of the concentrations and a smoothed curve drawn. The results in column 6 were taken from these curves and will be used in discussing the results. Columns 7–11, and 12–16, repeat columns 2–6 for different salts.

Discussion of Results.—The first fact which appears from the experimental results, is the continuous change in the activity which is produced in increasing the concentration of the salt. There is in no case a sudden change in activity. The same fact appears with salts of the uni-bi- and bi-bivalent types and with organic substances. Since the changes in activity are continuous, a more detailed discussion of the results of Table I will be given for the two concentrations 0.2 molar and 0.02 molar.

[1] Less 0.04, in order to use the results directly in the calculations. 0.04 cc. corresponds to one drop of the sodium hydroxide solution and was subtracted since the end point was attained twice in the blank experiments.

TABLE I.—ACTION OF CASTOR BEAN LIPASE ON ETHYL BUTYRATE IN THE PRESENCE OF UNI-UNIVALENT SALTS.

Molar conc.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Ave. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.
2.0	*NaCl*	6	±0.03	0.51	0.52	*KCl*	4	±0.03	0.50	0.52	*NaI*	2	±0.01	0.13	0.19
1.0	"	6	2	0.58	0.57	"	4	3	0.59	0.55	"	4	4	0.25	0.26
0.5	"	4	2	0.69	0.64	"	6	3	0.62	0.61	"	4	4	0.41	0.35
0.2	"	8	2	0.76	0.76	"	4	4	0.74	0.74	"	6	3	0.50	0.53
0.1	"	8	3	0.93	0.93	"	4	3	0.88	0.88	"	4	1	0.70	0.70
0.05	"	4	8	1.23	1.22	"	4	6	1.10	1.10	"	4	1	0.92	0.90
0.02	"	4	6	1.79	1.76	"	6	6	1.61	1.61	"	2	4	1.52	1.29
0.01	"	4	12	1.97	2.04	"	6	10	1.99	1.96	"	4	2	1.63	1.63
0.005	"	4	5	2.23	2.22	"	6	6	2.13	2.13	"	2	3	1.98	1.98
2.0	*KI*	4	±0.03	0.08	0.09	*KBr*	.	....	..	..	*NaF*	.	....	..	..
1.0	"	4	2	0.17	0.16	"	2	±0.08	0.26	0.34	"	2	±0.02	0.52	..
0.5	"	4	1	0.27	0.26	"	4	3	0.51	0.46	"	4	6	0.48	..
0.2	"	4	2	0.42	0.43	"	6	3	0.63	0.67	"	4	2	0.24	(0.13)
0.1	"	4	1	0.61	0.61	"	6	4	0.85	0.85	"	4	4	0.20	0.20
0.05	"	4	2	0.82	0.82	"	4	3	1.12	1.05	"	4	4	0.30	0.30
0.02	"	4	2	1.14	1.17	"	6	5	1.41	1.41	"	4	8	0.47	0.45
0.01	"	6	2	1.57	1.51	"	7	7	1.74	1.77	"	6	5	0.62	0.62
0.005	"	6	3	1.76	1.84	"	7	5	2.08	2.07	"	8	4	0.80	0.82
0.002	"	6	4	2.10	2.12	"	.	.	..	..	"	2	4	1.18	1.18
0.001	"	.	.	..	..	"	.	.	..	..	"	2	16	1.70	1.52
0.0005	"	.	.	..	..	"	.	.	..	..	"	2	6	1.88	1.88

2.0	*KF*	.	..	..	..	*LiCl*	2	±0.01	0.11	0.39	$NaNO_3$	2	±0.01	0.30	0.22
1.0	"	4	±0.06	0.32	..	"	4	2	0.41	0.44	"	4	3	0.35	0.32
0.5	"	2	4	0.43	..	"	4	5	0.50	0.50	"	4	1	0.37	0.44
0.2	"	2	1	0.32	0.31	"	4	4	0.61	0.60	"	4	5	0.61	0.64
0.1	"	4	3	0.39	0.34	"	4	7	0.69	0.69	"	4	3	0.80	0.85
0.05	"	2	6	0.41	0.39	"	2	1	0.82	0.82	"	3	7	1.24	1.15
0.02	"	2	7	0.38	0.50	"	2	4	1.10	1.10	"	4	8	1.54	1.61
0.01	"	4	3	0.63	0.63	"	2	4	1.46	1.46	"	3	9	1.93	1.93
0.005	"	6	2	0.80	0.80	"	2	3	1.84	1.84	"	4	15	2.17	2.15
0.002	"	4	4	1.10	1.10	"	.	.	..	..	"	.	.	..	..
0.001	"	4	8	1.31	1.37	"	.	.	..	..	"	.	.	..	..
0.0005	"	2	7	1.67	1.67	"	.	.	..	..	"	.	.	..	..
1.0	KNO_3	2	±0.06	0.32	0.50	$LiNO_3$	2	±0.03	0.37	0.30	$NaC_2H_3O_2$	4	±0.13	1.87	..
0.5	"	2	3	0.58	0.58	"	4	5	0.30	0.45	"	6	8	1.20	..
0.2	"	4	4	0.66	0.73	"	4	2	0.71	0.68	"	6	4	1.14	1.10
0.1	"	2	8	0.88	0.88	"	4	8	0.74	0.89	"	4	2	1.18	1.25
0.05	"	2	2	1.10	1.09	"	6	8	1.07	1.14	"	6	2	1.48	1.48
0.02	"	4	4	1.59	1.57	"	4	14	1.47	1.48	"	6	3	1.84	1.84
0.01	"	4	3	1.96	1.94	"	4	23	1.84	1.72	"	6	8	2.17	2.06
0.005	"	4	3	2.32	2.17	"	4	15	1.89	1.92	"	6	3	2.23	2.23
0.002	"	.	.	..	..	"	.	.	..	..	"	2	8	2.38	2.38

TABLE II.—SUMMARY OF RESULTS FOR UNI-UNIVALENT SALTS FOR 0.2 AND 0.02 MOLAR CONCENTRATIONS.

0.2 M.	Cl.	Br.	I.	F.	NO_3.	$C_2H_3O_2$.
Na	0.76	..	0.53	0.13	0.64	1.10
K	0.74	0.67	0.43	0.31	0.73	..
Li	0.60	..	..	..	0.68	..
0.02 M.						
Na	1.76	..	1.29	0.45	1.61	1.84
K	1.61	1.41	1.17	0.50	1.57	..
Li	1.10	..	..	..	1.48	..

For the sodium and potassium halides, the action decreases in the order chloride, bromide, iodide, fluoride. This is the same order which Terroine found for the sodium halides and pancreatic lipase, and indicates that the lipase from the different sources, while not necessarily identical, belongs to the same class of substances as far as their behavior toward neutral salts is concerned. Whether this similarity can be extended to the chemical properties cannot of course be foretold, but it is fair to assume that any light thrown upon the chemical properties of a vegetable lipase. will also be of value in studying animal lipases. The four halides cause similar effects, as pointed out by Terroine in his studies, only the amount of retardation for the same concentration differing for the different salts. Little difference is shown between the actions of the corresponding sodium and potassium salts, potassium decreasing the activity more with the chloride, iodide, and more concentrated fluoride solutions, sodium more for the more dilute fluoride and nitrate solutions. Whatever difference exists appears to show that potassium salts inhibit the actions more than do sodium salts.

Lithium as the chloride retarded the activity more than sodium chloride or potassium chloride; as the nitrate, the same was shown in the more concentrated solutions.

The activity shown by the fluoride solutions at concentrations greater than 0.2 molar appears to be due to some secondary cause and will be studied further. The actions of these salts were studied down to a concentration 0.0005 molar but no sign of an acceleration in action was observed. These concentrations were, however, not as small as those at which Loevenhart and Pierce found accelerated action with animal lipase.

In the more dilute solutions sodium acetate decreased the activity to an extent comparable to the action of sodium chloride; as the concentration of the sodium acetate became greater another effect is shown, in that the retarding action is counterbalanced by an accelerating action so that a minimum activity is shown at 0.2 molar.

It may be concluded from these results that both the metallic and non-metallic parts of the salts take part in affecting the activity of the lipase. For uni-univalent salts, the action is shown mainly, if not entirely as a

TABLE III.—ACTION OF CASTOR BEAN LIPASE ON ETHYL BUTYRATE IN THE PRESENCE OF THE CHLORIDES AND NITRATES OF BARIUM, CALCIUM, AND MAGNESIUM.

Molar conc.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.
1.0	$BaCl_2$	4	±0.03	0.13	0.13	$Ba(NO_3)_2$	.	..	..	..	$CaCl_2$	2	±0.01	0.14	0.15
0.5	"	5	2	0.46	0.46	"	.	..	..	..	"	5	6	0.49	0.46
0.2	"	4	4	1.03	0.86	"	4	±0.05	0.18	0.18	"	4	7	0.81	0.83
0.1	"	4	4	1.04	1.03	"	4	3	0.57	0.57	"	5	7	1.07	1.07
0.05	"	6	2	1.15	1.15	"	6	7	0.95	0.95	"	4	5	1.33	1.32
0.02	"	3	5	1.38	1.38	"	4	18	1.40	1.46	"	4	9	1.73	1.73
0.01	"	6	13	2.01	2.00	"	4	5	2.28	2.38	"	4	10	2.21	2.21
0.005	"	8	15	3.06	3.06	"	5	6	2.96	2.96	"	4	12	2.72	2.62
0.002	"	6	9	3.47	3.47	"	6	10	3.09	3.14	"	4	5	2.84	2.84
0.001	"	4	8	3.24	3.24	"	4	11	3.20	3.16	"	.	.	..	..
1.0	$Ca(NO_3)_2$	2	±0.02	—0.04	..	$MgCl_2$	4	±0.12	0.75	0.84	$Mg(NO_3)_2$	4	±0.02	0.19	0.19
0.5	"	4	0	0	0	"	3	11	1.03	1.01	"	4	4	0.54	0.58
0.2	"	3	7	0.52	0.54	"	4	8	1.23	1.19	"	4	10	0.93	0.95
0.1	"	3	3	1.01	0.97	"	4	6	1.24	1.22	"	4	2	1.13	1.10
0.05	"	4	5	1.30	1.40	"	4	6	1.28	1.31	"	4	7	1.16	1.22
0.02	"	4	1	1.98	1.95	"	4	11	1.54	1.55	"	4	6	1.35	1.41
0.01	"	4	12	2.28	2.41	"	4	5	1.93	1.90	"	4	6	1.64	1.64
0.005	"	3	9	2.87	2.84	"	4	11	2.20	2.20	"	4	2	1.93	1.93
0.002	"	.	.	..	..	"	2	3	2.45	2.41	"	.	.	..	..

retardation. In view of the similar actions of these salts, the results showing that the differences are only of degrees of retardation, the further study of this subject can be carried out with a few typical representatives of this type.

B. Uni-Bi and Bi-Bivalent Salts.

In this section the results obtained for the chlorides and nitrates of barium, calcium, and magnesium will first be presented, then those for sodium sulfate, potassium sulfate, and sodium oxalate, and finally those for manganous chloride, manganous sulfate and magnesium sulfate.

Since marked retardation in the lipolytic activity was produced in moderately concentrated solutions of the chlorides and nitrates of barium, calcium, and magnesium, the action of the lipase with solutions of these salts was tested for 48 hours. The results are presented in Table III. The method of experimenting was outlined in Section A. The significance of the columns is the same as in Table I. The action at zero concentration of the salts was again taken to be 2.50 ± 0.02, and the lipase blank under the same conditions as 0.52.

Lipase and Ester Blanks.—For some of the calcium chloride and magnesium chloride solutions, a value for the lipase blank about 0.1 cc. higher than with the purely aqueous solutions was indicated, but as these results were not perfectly definit, the smaller value was used. The calcium nitrate solutions required a different correction for the lipase blanks. The ester blanks were zero only for the solutions of barium chloride. For the rest, the corrections as given were taken from the smoothed curves obtained by plotting the observed values against the concentrations.

Molar conc. =	1.0	0.5	0.2	0.1	0.05	0.02	0.01
Lipase Blank.							
$Ca(NO_3)_2$	0.30	0.40	0.47	0.50	0.52	..	..
Ester Blanks.							
$CaCl_2MgCl_2$, $Mg(NO_3)_2$	0.06	0.03	0.02	0	..	..	..
$Ba(NO_3)_2$	..	..	0.15	0.08	0.04	0.02	0
$Ca(NO_3)_2$	0.14	0.07	0.03	0.02	0	..	..

For concentrations less than 0.01 molar, the actions for the barium and calcium salts increased very rapidly, reaching values greater than when no salt at all was added. For greater concentrations than 0.01 molar for these salts and throughout the range of concentrations studied with the magnesium salts, the retardations observed were of the magnitude of those observed with the uni-univalent salts (excepting the fluorides). A few regularities, connected with the specific nature of the substances may be mentioned. The chlorides showed less retardations than the nitrates for the barium salts for concentrations greater than 0.02 molar, for the calcium salts for concentration greater than 0.05 molar, and for the magnesium salts throughout. For the chlorides, the values for the

TABLE IV.—ACTION OF CASTOR BEAN LIPASE ON ETHYL BUTYRATE IN DILUTE SOLUTIONS OF THE CHLORIDES AND NITRATES OF BARIUM, CALCIUM, AND MAGNESIUM.

Molar conc.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.
0.01	$BaCl_2$	4	±0.02	1.24	1.24	$Ba(NO_3)_2$	4	+0.08	1.24	1.24	$CaCl_2$	4	±0.01	1.41	1.41
0.005	"	3	3	1.63	1.62	"	4	5	1.54	1.54	"	4	9	1.84	1.78
0.002	"	4	2	1.96	1.93	"	4	4	1.81	1.74	"	4	4	1.89	1.89
0.001	"	4	4	2.02	2.02	"	4	6	1.72	1.80	"	4	6	1.86	1.88
0.01	$Ca(NO_3)_2$	4	±0.09	1.17	1.15	$MgCl_2$	2	±0.02	1.21	1.31	$Mg(NO_3)_2$	2	±0.01	1.25	1.26
0.005	"	4	7	1.43	1.50	"	2	9	1.48	1.43	"	2	0	1.33	1.38
0.002	"	4	4	1.74	1.72	"	2	4	1.64	1.53	"	2	1	1.53	1.51
0.001	"	4	6	1.68	1.72	"	2	1	1.53	1.58	"	2	0	1.62	1.58

barium and calcium salts are practically identical for concentrations of 0.1 molar and greater, while for the nitrates the values for barium are less than those for calcium at concentrations of 0.01 molar and greater. The values of the magnesium salts (both nitrate and chloride) are greater than those for the corresponding barium and calcium salts at the higher concentrations and less at the lower concentrations. The change in relative values takes place at about 0.05 molar with the chlorides, and 0.2 molar with the nitrates.

The rapid increase in action for the dilute solutions of these salts made it desirable to study their actions on the ester for 24 hours in place of 48 hours. The results are presented in Table IV. The mean value from ten experiments of the action shown in 24 hours by the lipase preparations under the same conditions with no salt present was 1.66 ± 0.02. The lipase blanks gave a mean value of 0.43. The different columns in the table have the same significance as before.

The marked increase of action observed with the barium and calcium salts at these small dilutions compared with the action when no salt was added is apparent. The chlorides showed a greater increase than the nitrates and the barium salts a greater than the corresponding calcium salts. Since the time of testing the activities was shorter and therefore less probability of disturbances of a secondary nature existed, the results in Table IV may be looked upon as more reliable than the results in Table III for the same concentrations. The magnesium salts gave no indication of an increase in action above that observed when no salt was added.

Barium has been known to act as an oxygen carrier,[1] and it may be suggested that the increased action is due to an effect similar to that proposed to account for the accelerating action of manganous sulfate in the third paper[2] of this series. By implication, the calcium salts, to judge from the results, exert a similar action but to a much smaller extent.

The results for the sulfates of sodium and potassium and for sodium oxalate are presented in Table V. As before the action for zero concentration was taken as 1.66 ± 0.02, the lipase blanks were found to be 0.43, and the ester blanks zero.

These results show that, for the sulfates, an antagonistic action is involved. Sodium and potassium as found in Section A tend to retard the action, while the SO_4 appears to show a tendency to accelerate it. With potassium sulfate these actions were practically equal, so that no difference was observed whether the salt was added or not. With sodium sulfate,

[1] Engler and Weissberg, "Kritische Studien über die Vorgänge der Autoxydation" (1904). Cf. also Abel, *Monatsh.*, 34, 171 (1913).

[2] Falk and Hamlin, THIS JOURNAL, 35, 210 (1913).

TABLE V.—ACTION OF CASTOR BEAN LIPASE ON ETHYL BUTYRATE IN THE PRESENCE OF SODIUM SULFATE, POTASSIUM SULFATE, AND SODIUM OXALATE.

Molar conc.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.
0.5	Na_2SO_4	.	..	..	2.17	K_2SO_4	.	..	..	..	$Na_2C_2O_4$	.	..	..	..
0.4	"	8	±0.10	2.02	2.08	"	.	..	..	..	"	.	..	..	..
0.2	"	12	11	1.93	1.86	"	5	±0.03	1.58	1.66	"	.	..	..	..
0.1	"	8	9	1.87	1.70	"	5	5	1.66	1.66	"	4	±0.01	1.10	1.10
0.05	"	10	5	1.61	1.60	"	4	5	1.71	1.66	"	4	4	1.07	1.25
0.02	"	8	6	1.53	1 55	"	7	5	1.66	1.66	"	4	9	1.47	1.42
0.01	"	10	5	1.57	1.55	"	4	4	1.65	1.66	"	4	3	1.48	1.51
0.005	"	6	6	1.65	1.57	"	4	4	1.65	1.66	"	4	4	1.61	1.57
0.002	"	6	4	1.62	1.60	"	7	2	1.67	1.66	"	2	3	1.60	1.62

the action of the sodium predominated in the dilute solutions and that of the SO_4 in the more concentrated. This resulted in a minimum action at a concentration of 0.01 to 0.02 molar, the action rising for the concentrated solutions to values considerably greater than when no salt was added. With sodium oxalate, the retarding action of the sodium over-balanced any accelerating action of the C_2O_4 if such existed, only a marked retardation being observed in the solutions studied.

In Table VI the results obtained with magnesium sulfate for a series of concentrations and for dilute solutions of the sulfate and chloride of manganese are presented. The color of the precipitated manganese hydroxide interfered with the end points in more concentrated solutions of manganous salts. The lipase blanks for the solutions containing the salts were found to be as follows:

Molar conc. =	1.0	0.5	0.2	0.1	0.05	0.02	0.01	0.005	0.002	0.001	0.0005
$MgSO_4$.....	0.67	0.64	0.58	0.53	0.49	0.46	0.45	0.44	0.43	0.43	0.43
$MnCl_2$, $MnSO_4$....	..	..	..	..	..	..	..	0.74	0.64	0.57	0.48

The ester blanks were found to be zero possibly for the most concentrated magnesium sulfate solution and for the solutions of the manganous salts. In these cases the values were not more than 0.06 cc. at most, and since even these increases were doubtful, they will not be considered.

Since accelerations are produced by the solutions of these salts, the times of testing refer to 24 hours.

The magnesium sulfate solutions gave a marked increase in the action as the solutions became more concentrated until a maximum value was reached at 0.1 molar when a small decrease below this maximum action was shown. It would appear that the accelerating action of the SO_4 predominated over any retarding action of the magnesium throughout the whole range of concentrations studied, since the values found are all greater than when no salt was present. In the more concentrated solutions the effect of the magnesium is relatively greater than in the dilute solutions, to judge from the fact of a maximum value for the action. Manganous sulfate exerted such a marked accelerating action, incomparably greater than that observed for any other salt studied (except for the 0.001 molar solution of barium chloride), that it appears as if the reason must be sought in specific chemical influences. The acceleration of the oxidation of the zymogen by manganous sulfate to form the active enzyme was suggested in the third paper as the probable explanation. Manganous chloride showed only a small acceleration compared to the sulfate. Evidently this is due to the retarding action on the active enzyme of the chlorine in the one case, and the accelerating action of the SO_4 in the other as found in the study of the other salts.

TABLE VI.—ACTION OF CASTOR BEAN LIPASE ON ETHYL BUTYRATE IN THE PRESENCE OF MAGNESIUM SULFATE, MANGANOUS SULFATE, AND MANGANOUS CHLORIDE.

Molar conc.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Molar conc.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.	Salt used.	No. of determ.	Aver. dev. of mean.	Action found.	From curve.
1.0	$MgSO_4$	6	±0.12	2.17	2.15	0.0062	$MnSO_4$	6	±0.13	4.02	..	$MnCl_2$	.	..	..	..
0.5	"	4	10	2.18	2.20	0.005	"	.	..	..	3.58	"	4	±0.05	1.87	1.85
0.2	"	6	7	2.30	2.31	0.0025	"	5	6	2.59	..	"	.	..	..	..
0.1	"	10	10	2.46	2.43	0.002	"	.	..	..	2.44	"	4	6	1.74	1.83
0.05	"	10	12	2.56	2.39	0.0013	"	4	11	1.99	..	..	.	..	..	..
0.02	"	9	7	2.18	2.20	0.001	"	.	..	..	2.00	"	2	2	1.82	1.80
0.01	"	8	5	1.99	2.03	0.0006	"	6	9	1.79	..	"	.	..	..	..
0.005	"	8	5	1.89	1.90	0.0005	"	.	..	..	1.76	"	2	1	1.79	1.76
0.002	"	4	4	1.65	1.78	....	"	.	..	..	..	"	.	..	..	..
0.001	"	4	3	1.62	1.72	....	"	.	..	..	..	"	.	..	..	..

Summary.

The actions of neutral salts of the uni-uni-, uni-bi-, and bi-bivalent types were tested on the activity of a castor bean lipase preparation toward ethyl butyrate under comparable conditions.

In every case the change in activity, whether increase or decrease, was found to be a continuous function of the concentration of the salt added.

Decreased activities, as compared with aqueous solutions, were shown by all the uni-univalent salts, by the chlorides and nitrates of barium and calcium (except for the most dilute solutions) and magnesium, by sodium oxalate, and by dilute solutions of sodium sulfate.

Increased activities were shown by dilute solutions of the chlorides of barium and calcium, by more concentrated solutions of sodium sulfate, by magnesium sulfate, and by the chloride and sulfate of manganese. Potassium sulfate solutions gave the same results as purely aqueous solutions.

The observed regularities for the actions of the individual substances of each type are discussed in the body of the article following the tables in which the results are presented.

If an explanation of the retarding actions of the various salts be looked for, it may perhaps be found in the coagulation of the enzyme (either alone or together with other substances) by the addition of the salts, the ions of which produce their individual specific effects in each case. The unionized molecules may also take part in these reactions. The accelerations cannot be explained in as simple a manner except, perhaps, for the cases where increased formation of active lipase (as by manganous salts)[1] may be assumed.

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STUDIES ON ENZYME ACTION. VI. THE SPECIFICITY OF LIPASE ACTION.

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In the preceding paper, the action of neutral salts on the activity of a castor bean lipase preparation was described in detail. In this paper, the action of some organic substances on the activity of the lipase preparation will first be described and then the possibility of explaining the selective action of lipases on different esters as a logical deduction from these results will be discussed.

The following substances were studied: methyl alcohol, ethyl alcohol, acetone, glycerol, and glucose. On account of the presence of a urease

[1] Cf. Falk and Hamlin, Third Paper, *loc. cit.*