strict equality of this concentration in both solutions, but the further fact, shown in this table, that in every case except the first, the action on ethyl butyrate is less in the absence of glycine, makes this strict equivalence unnecessary. It proves that whatever the hydrogen ion concentrations in the two solutions are, the variation that causes an increase of hydrolysis of methyl acetate causes a decrease in that of ethyl butyrate. This further confirms the results given in Table VI of the seventh paper of this series already referred to. Here the ratio of hydrogen ion concentrations in two solutions is $10^{-2.607}/10^{-3.341} = 5.42$, while the ratios of hydrolysis under like conditions of three esters in these solutions are, for methyl acetate, $17.41/2.14 = 8.13$; for ethyl acetate $13.34/1.45 = 9.40$, and for ethyl butyrate, $1.18/0.35 = 3.36$. Clearly, in the presence of glycine the velocity of hydrolysis of the methyl acetate and ethyl butyrate is not proportional to the hydrogen ion concentration.

III. Summary.

1. The selective lipolytic actions of glycine, alanine, phenylalanine and castor bean lipase on methyl, ethyl, glyceryl tri- and phenyl acetates, ethyl butyrate, and ethyl and phenyl benzoates have been compared, and that of castor bean lipase on castor oil and olive oil has been measured.

2. It has been shown that the selective lipolytic action of glycine on methyl acetate and ethyl butyrate is more marked in the presence of certain concentrations of hydrochloric acid, and this selective action has been measured.

3. It has been shown that in the glycine-hydrochloric acid solution used the hydrolysis of methyl acetate and ethyl butyrate is not proportional to the hydrogen ion concentrations of the solutions, and that the disproportionality, which has been measured, is comparatively large.

[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK.]

STUDIES ON ENZYME ACTION. IX. EXTRACTION EXPERIMENTS WITH THE CASTOR BEAN LIPASE.

BY K. GEORGE FALK.

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The experimental study of the lipolytic activity of the castor bean preparation, the results of which were presented in previous papers,[1] dealt with the mixed soluble and insoluble substances present in the preparation. This method of working was essential in the first part of an extended investigation, for it is evident that any treatment of the lipase material, which, in part, showed the properties or characteristics of a colloid, will produce some change in the material and undoubtedly modify the behavior

[1] THIS JOURNAL, **34**, 375 (1912); **35**, 210, 602, 616 (1913).

of the enzyme. In papers V and VI of this series,[1] the changes in lipolytic action, which resulted on the addition to the mixture of neutral salts and organic substances through wide ranges of concentration, were given in detail, and an explanation of some of the specific selective actions of lipases outlined.

With these results as a basis for further work, it then seemed desirable to attempt the separation of the preparation into soluble and insoluble material in the absence and presence of neutral substances, in order to study further the behavior of the active lipase under different conditions, as well as to find means of separating, as far as possible, the enzyme from the inactive substances present in the preparation. This work will be described in the present paper.

The lipase preparation was obtained in the manner described in the previous papers and consisted of husk- and oil-free, finely ground castor beans.[2]

In each experiment 0.2 gram lipase preparation and 25 cc. of water or solution were used. The usual lipase preparation—water and ester-water-blanks were run in every series of experiments and the lipase preparation-ester-water results corrected for these blanks. The values obtained in the blanks will not be given in detail for each set of experiments, but will only be mentioned if of special interest. The values which are given for the actions are corrected for the blanks and the results indicate, therefore, the acid produced by the lipase acting on the ester. These results are given in terms of cc. of 0.1 normal sodium hydroxide solution required to neutralize the acid formed from the ester. Five to 10 drops of a 1% solution of phenolphthalein in 95% ethyl alcohol were used as indicator in the titrations.

In order to effect the separation of the soluble and insoluble materials of the lipase preparation, the latter was treated with the requisit quantity of water, or solution, for various lengths of time and then filtered with suction through a Gooch crucible containing a thin mat of long-fibered asbestos. The asbestos was washed thoroughly with acid and water and contained no soluble matter. In most of the experiments, the activity was tested with two esters, ethyl butyrate and triacetin (glyceryl triacetate). Ethyl butyrate was chosen as an example of a simple ester and because comparison with other work in which it has been used is facilitated, and triacetin as a type of ester more closely related to the naturally occurring fats but more soluble in the solutions used than these, and showing less inhibiting action on the lipase than ethyl butyrate. In each experiment with ethyl butyrate 1 cc. of ester was used, while with triacetin 0.5 cc. was used.

[1] THIS JOURNAL, 35, 602, 616 (1913).

[2] Furnished by the Baker Castor Oil Company, New York.

Four different periods of time for the extractions were used—one minute, six hours, 24 hours, and six days. For the one-minute extractions, the lipase preparation and water (or solution) in a stoppered 150 cc. Erlenmeyer flask were shaken vigorously for one minute and then poured on the filter and filtered as rapidly as possible. For the six-hour extractions, the lipase preparation and solvent were vigorously shaken two or three times, for a few seconds at a time, during the six hours, allowed to stand at room temperature the remainder of the time, and then filtered. For the longer time periods, the procedure was similar except that a larger number of shakings was used, corresponding to the longer times. Clear filtrates were obtained by this method of working, and with the quantities of material used (0.2 gram lipase preparation and 25 cc. solvent) the time required for complete filtration seldom exceeded ten minutes and was generally considerably less. With a greater proportion of lipase preparation, or with larger quantities of the materials, the time of filtration was too great to give comparable results of practical value. In carrying out the extractions as described, the filtrate in each experiment was poured from the suction flask into an Erlenmeyer flask in which the lipolytic activity was to be determined. One to two cc. of solution remained in the suction flask, adhering to its walls, and was lost since it was not considered desirable to wash it out with fresh solvent. The insoluble residue on the filter, together with the asbestos mat, was removed with a spatula and the activity tested with water or solution and ester. One to two cc. toluene were present in every solution and were also added to the residues as soon as the filtrations were complete. The lipolytic actions were determined at 38–40° for the periods of time of 20, 24, or 48 hours, as indicated in the headings of the tables. The same procedure was followed in the blank experiments in which no ester was added, as when ester was added to test the activity. Each result in the following tables gives the corrected lipolytic action in cc. of 0.1 normal acid formed from the ester by the lipase, and is the mean of from four to ten experiments comprising all the determinations.

The results will be given in tabular form and will be elaborated further as far as may be necessary to explain their significance.

The results will be taken up in the order in which they appear in the table. The first result shows the direct action of the lipase preparation on the ethyl butyrate in water without any treatment whatsoever. As filtering the lipase preparation-water mixture may result in altering the activity of the enzyme, a series of experiments were carried out in which the filtrate and residue (with the asbestos mat) were combined immediately after filtering a one-minute extraction mixture, and the activity tested. The second result in the table shows that the activity was about 12% less than if the mixture was not filtered. This may be partly, if not

altogether, accounted for by mechanical losses incident to the manipulation, such as the liquid adhering to the suction flask, etc. That there was no deep-seated change in the activity due to filtration, but only a very slight decrease, if any, may be concluded from these results.

TABLE I.—ACTION OF AQUEOUS EXTRACTIONS OF CASTOR BEAN LIPASE PREPARATION ON ETHYL BUTYRATE (48-HOUR TESTS).

Direct tests, not filtered	2.50
One minute extraction, filtrate and residue combined	2.20
24 hours extraction, filtrate and residue combined	2.00

	Filtrate.	Residue (+ H_2O)[1].	Total.
One minute extraction	0.78	1.36	2.14
6 hrs. extraction	1.05	0.89	1.94
24 hrs. extraction	1.30	0.65	1.95
6 days extraction	1.39	0.53	1.92

Three successive extractions, one minute each.

Filtrate I.	Filtrate II.	Filtrate III.	Residue (+ H_2O).[1]	Total.
0.73	0.28	0.11	0.60	1.72

Two successive extractions, 24 hours each.

Filtrate I.	Filtrate II.	Residue (+ H_2O).[1]	Total.
1.34	0.10	0.48	1.92

The next set of results shows the separate and total activities of the filtrates and of the residues in water from extractions of various lengths of time. In the one-minute extraction, 36% of the activity was found in the filtrate and 64% in the residue. On longer extraction, more of the active substance appeared in the filtrate and less in the residue, reaching a value of 72% in the filtrate after six days extraction. There was comparatively little difference in the amount of active substance extracted during one day and 6 days. This is brought out again in the later experiments. The total actions, as given in the last columns, show that the total activity of the preparation decreased somewhat on standing in water[2] and that for the one-minute or 24-hour extractions, the total action in testing the filtrate and residue separately was practically the same as when they were combined and tested. It is true that the conditions differed slightly; in the former the total amounts of water and of ethyl butyrate were double the amounts in the latter, but it was thought advisable, especially in view of the experiments to be given later, to carry out the comparisons as described. The amount of ethyl butyrate added does not increase the amount of action, as shown in the VI paper,[3] after a certain concentration of ester, corresponding to about 1 cc. in 25 cc. solution, has been reached.

Since apparently the amount of active enzyme extracted reached or

[1] Residue treated with 25 cc. water and the activity tested.

[2] Cf. Paper III of this series, THIS JOURNAL, **35**, 210 (1913).

[3] *Loc. cit.*, p. 620.

approached a limit with the increase in the time of treatment, experiments were carried out as shown in the next series in Table I. The residue after a one-minute extraction, the filtrate of which is designated Filtrate I, was treated with 25 cc. water, agitated thoroughly for one minute, filtered through fresh asbestos, the filtrate designated as Filtrate II, the process repeated with fresh water yielding Filtrate III and the activity of the residue tested with water. The results show that the first filtrate contained about 42.5% of the active lipase, the second filtrate about 16%, the third filtrate 6.5%, and the residue 35%. The total activity was less than that of a single extraction, due doubtless to the losses incident to the manipulation. The interesting fact is brought out that about two-thirds of the active material was removed in the first extraction, much less in the second, and a very much smaller amount in the third, and presumably still less in a fourth or fifth extraction, although the residue still showed an activity corresponding to one-third of the total active material. The final results in Table I, showing similar series of experiments with two successive extractions each of 24 hours' duration, showed the same relations still more clearly. Filtrate I contained 70% of the active material, while Filtrate II contained only 5%, showing that almost all of the soluble active material was extracted in the first treatment and that the residue contained active material not soluble in water.

The results given in Tables II and III will be discussed together. They show the results of extraction experiments similar to those given in Table I except that the lipolytic action was tested with triacetin, the times of testing for the results given in Table II being 20 hours, and in Table III, 48 hours.

The first point of interest in connection with these results is that the action after extraction, when filtrate and residue were combined, was 15% less than unfiltered material (due mostly to manipulative losses) but 30% less when filtrate and residue (+ H_2O) were tested separately. This would indicate that extraction removed some substance which accelerated the action of the lipase on triacetin, analogous to the co-enzyme described by Rosenheim and Shaw-Mackenzie.[1] The results of the extraction experiments show that a one-minute extraction did not remove more than 6% of the active lipase, and that repeated one-minute extractions removed only slightly more. More active substance was taken out by the 24-hour extractions, since the two extractions removed 44%, but it is evident from the separate extractions that this also reached a limit, and that at this limit at least 50% of the active material remained in the residue.

On comparing the results of Table I with the results of Table II and III, it will be seen that 70–75% of the material showing activity toward

[1] *J. Physiol.* (*London*), 40, VIII–XVI (1910).

ethyl butyrate can be extracted by water, while 50% of the material showing activity toward triacetin can be extracted. The simplest explanation of this fact is to assume the existence of two enzymes showing lipolytic action in the lipase preparation, one of which is soluble in water, the other much less soluble under the conditions of the experiments. Both lipases showed action toward ethyl butyrate and triacetin, but the lipase soluble in water exerted a greater action on ethyl butyrate than the insoluble, while the insoluble lipase exerted a greater action on triacetin than the soluble. That surface actions, in which the ester solutions play a part, are involved in the action of the insoluble lipase cannot be doubted. The selective actions of the two lipases toward the two esters have been duplicated with simple soluble amino acids.[1]

TABLE II.—ACTION OF AQUEOUS EXTRACTION OF CASTOR BEAN LIPASE PREPARATION ON TRIACETIN (20 HOURS TESTS).

Direct tests, not filtered	2.00
One minute extraction, filtrate and residue combined	1.70

	Filtrate.	Residue (+ H_2O).[2]	Total.
One minute extraction	0.09	1.37	1.46
Six hours extraction	0.23	1.08	1.31
24 hours extraction	0.35	0.95	1.30

Three successive extractions, one minute each.

Filtrate I.	Filtrate II.	Filtrate III.	Residue (+ H_2O).[2]	Total.
0.07	0.03	0.05	0.98	1.13

Two successive extractions, 24 hours each.

Filtrate I.	Filtrate II.	Residue (+ H_2O).[2]	Total.
0.38	0.17	0.70	1.25

TABLE III.—ACTION OF AQUEOUS EXTRACTIONS OF CASTOR BEAN LIPASE PREPARATION ON TRIACETIN (48 HOURS TESTS).

Direct tests, not filtered	3.00

	Filtrate.	Residue (+ H_2O).[2]	Total.
One minute extraction	0.08	2.02	2.10

Three successive extractions, one minute each.

Filtrate I.	Filtrate II.	Filtrate III.	Residue (+ H_2O).[2]	Total.
0.19	0.03	0.02	1.90	2.14

Two successive extractions, 24 hours each.

Filtrate I.	Filtrate II.	Residue (+ H_2O).[2]	Total.
0.58	0.20	1.35	2.13

Since ethyl butyrate and triacetin represent two different types of ester and showed such marked differences in behavior with the castor bean lipase preparation, it seemed of interest to test the action on another ester.

[1] Cf. Papers II, VII and VIII of this series, THIS JOURNAL, **34**, 828 (1912); **35**, 624 (1913); and the preceding paper in this number.

[2] Residue treated with 25 cc. water and the activity tested.

Phenyl acetate was shown by Hamlin[1] to be very readily hydrolyzed by the lipase preparation and was, therefore, tested. Table IV gives the results obtained on extraction (with 25 cc. H_2O as before) and testing the lipolytic action with 1 cc. phenyl acetate for 24 hours at 38–40°.

TABLE IV.—ACTION OF AQUEOUS EXTRACTIONS OF CASTOR BEAN LIPASE PREPARATION ON PHENYL ACETATE.

Direct tests not filtered			3.05
One minute extraction, residue and filtrate combined			2.96
	Filtrate.	Residue (+ H_2O).[2]	Total.
One minute extraction	0.44	2.40	2.84

The results show that for a one-minute extraction, 15% of the material active toward phenyl acetate appeared in the filtrate, and 85% in the residue. These values are intermediate between those found for ethyl butyrate and for triacetin under similar conditions. A complete series of determinations was not carried out with phenyl acetate as with the other two esters and it is, therefore, not possible to tell with certainty the behavior of the two lipases (soluble and insoluble) toward it, but the results appear to show that the insoluble lipase exerted greater action on it than the soluble lipase. In all of this work it is of course evident that nothing is known as to the actual amounts of the two lipases present and that all of the actions referred to are comparative actions of the active material in the same quantity of preparation. The preparation used showed fairly constant properties, except for some of the results with salt solutions to be referred to later.

The results given in Paper V showed that the salts of the uni-univalent type inhibited the action of the lipase preparation on ethyl butyrate to a greater or less extent, depending upon the salt used and its concentration in the solution. The greatest inhibiting action was shown by the fluorides, for which at a concentration of 0.1 molar only very slight lipolytic activity was apparent. Similar results were given for methyl and ethyl alcohols and acetone in Paper VI, except that the concentrations at which the action became negligible were greater. The explanation was advanced that the inhibiting action of the lipase was due to a coagulating or precipitating action of the added substance on the active enzyme which resulted in removing it from the sphere of action. The results so far given in this paper relate to aqueous extractions. It seemed best to take up next the extreme case in which lipolytic action due to added substance had become zero or approached zero and to consider what light the extraction of the lipase preparation by solutions of these substances will throw upon the behavior of the enzyme. The solutions chosen for this study were 0.1 molar sodium fluoride and molar methyl alcohol and

[1] Preceding paper.

[2] Residue treated with 25 cc. water and the activity tested.

series of extraction experiments similar to those already given with water were carried out, the lipolytic actions again being tested with ethyl butyrate and with triacetin. The results for these solutions are given in Tables V–VIII and will be discussed together.

TABLE V.—ACTION OF 0.1 MOLAR SODIUM FLUORIDE SOLUTION EXTRACTIONS OF C. B. LIPASE PREPARATION ON ETHYL BUTYRATE (48 HOURS TESTS).

Direct tests, not filtered 0.20

	Filtrate.	Residue (+ H_2O).[1]	Total.
One minute extraction	0.01	0.49	0.50
Six hours extraction	0.09	0.37	0.46
24 hours extraction	0.07	0.32	0.39

TABLE VI.—ACTION OF 0.1 MOLAR SODIUM FLUORIDE SOLUTION EXTRACTIONS OF C. B. LIPASE PREPARATION ON TRIACETIN (20 HOURS TESTS).

Direct tests, not filtered 0.38

	Filtrate.	Residue (+ H_2O).[1]	Total.
One minute extraction	0	0.89	0.89
Six hours extraction	0.02	0.81	0.83
24 hours extraction	0.05	0.73	0.78

TABLE VII.—ACTION OF MOLAR METHYL ALCOHOL SOLUTION EXTRACTIONS OF C. B. LIPASE PREPARATION ON ETHYL BUTYRATE (48 HOURS TESTS).

Direct tests, not filtered 0.34

	Filtrate.	Residue (+ H_2O).[1]	Total.	Residue (+ CH_3OH).[2]	Total.
One minute extraction	0.06	1.29	1.35	0.16	0.22
Six hours extraction	0.13	0.85	0.98	....	....
24 hours extraction	0.12	0.67	0.79	0.20	0.32

TABLE VIII.—ACTION OF MOLAR METHYL ALCOHOL SOLUTION EXTRACTIONS OF C. B. LIPASE PREPARATION ON TRIACETIN (20 HOURS TESTS).

Direct tests, not filtered 1.33

	Filtrate.	Residue (+ H_2O).[1]	Total.	Residue (+ CH_3OH).[2]	Total.
One minute extraction	0.02	1.38	1.40	1.01	1.03
Six hours extraction	0.19	1.20	1.39	....	....
24 hours extraction	0.18	1.03	1.21	....	....

The filtrates from the sodium fluoride extractions showed practically no lipolytic action. This might be taken to be due to the presence of the fluoride in the filtrate and the fact that the lipase is present also, the fluoride inhibiting the action in some unknown manner. As fluorides exert perhaps the most marked coagulating action on colloids of any of the simple salts, it seems much more reasonable to attribute the lack of activity in the filtrates to this coagulating property, the lipase, or material with which the lipase is associated, becoming insoluble in the solution. The coagulation or precipitation is reversible in part, as is shown by the activity of the residues when tested in water. These lipolytic actions were markedly

[1] Residue treated with 25 cc. water and the activity tested.

[2] Residue treated with 25 cc. molar methyl alcohol solution and the activity tested.

greater than the activities of the unfiltered material in the fluoride solutions. The residues from the longer extractions showed less activity. This may be due either to more adsorbed fluoride which inhibited the action even when the residues were taken up with water, or to the coagulation or precipitation which was reversible at first becoming irreversible on longer treatment. The fact that the inhibiting action of the flouride on animal lipase was reversible and that the activity returned when the fluoride was removed was shown clearly by Loevenhart and Peirce.[1]

Essentially the same relations are shown by the methyl alcohol extractions, the results of which are given in Tables VII and VIII. The lipolytic actions of the filtrates were very slight. The activity of the residues, when treated with water, toward ethyl butyrate for the one-minute extractions was comparatively large (about half of that of untreated material when tested in aqueous solution) but decreased with the longer extractions, until for the 24 hours treatment the activity was only one-fourth that of untreated preparation. Since practically no methyl alcohol adhered to the residue after filtration, it is evident that the protracted treatment intensified the change produced by the slight treatment, or in other words, the coagulating, precipitating, or inactivating action brought about by the treatment with the alcohol became less and less reversible, bearing out the indications from the fluoride extractions. It is possible to go somewhat farther with these results. On comparing the actions of the residues in water given in Tables VII and VIII, where methyl alcohol was used as extracting solution, with those of Tables I and II with aqueous extractions, it will be seen that almost the same values were obtained. This may mean either, that in both cases the soluble lipase was extracted but did not show its activity in the presence of the methyl alcohol, or that the soluble lipase was rendered inactive by the treatment with methyl alcohol but remained in the residue as reversibly coagulated or precipitated material. The insoluble lipase showed the same action toward ethyl butyrate and triacetin, when extracted with methyl alcohol solution, as when extracted with water, but if the tests of the residues were carried out in methyl alcohol solution, practically the same total actions (filtrate + residue in methyl alcohol) were obtained as with unfiltered material in methyl alcohol solution directly.

TABLE IX.—ACTION OF MOLAR SODIUM CHLORIDE SOLUTION EXTRACTIONS OF C. B. LIPASE PREPARATION ON ETHYL BUTYRATE (48 HOURS TESTS).

Direct tests, not filtered			0.57
	Filtrate.	Residue (+ H_2O).[2]	Total.
One minute extraction	0.19	0.43	0.62
Six hours extraction	0.23	0.28	0.51
24 hours extraction	0.34	0.24	0.58

[1] *J. Biol. Chem.*, 2, 406 (1906).

[2] Residue treated with 25 cc. water and the activity tested.

TABLE X.—ACTION OF MOLAR SODIUM CHLORIDE SOLUTION EXTRACTIONS OF C. B. LIPASE PREPARATION ON TRIACETIN (20 HOURS TESTS).

Direct tests not filtered			1.55
	Filtrate.	Residue (+ H_2O).[1]	Total.
One minute extraction	0.44	0.99	1.43
Six hours extraction	0.65	0.57	1.22
24 hours extraction	0.72	0.53	1.25

It appeared to be of interest to determin the extracting action of a molar sodium chloride solution on the lipase preparation because its inhibiting action in the direct tests was less than that of the sodium fluoride solution. The results are shown in Tables IX and X. The action of the filtrates toward ethyl butyrate was less than the action of the aqueous filtrates and greater than the action of the fluoride filtrates, as would be expected, but the residues with water showed very nearly the same (comparatively small) action as the fluoride extraction residues and water. Evidently the amount of the adsorbed salt was large, or the coagulating (inactivating) action was very marked. The total action (filtrate + residue and water) was the same as that of unfiltered material in the sodium chloride solution, while for the fluoride, the former was markedly greater.

The filtrates from the sodium chloride extractions showed a greater action toward triacetin than any found previously, while the residues with water showed actions considerably less than those shown by the residues from aqueous extractions. This apparently indicates that the water-insoluble lipase is somewhat soluble in the sodium chloride solution. The inhibiting action of the sodium chloride toward triacetin, while still evident when the lipase preparation was tested without separation into soluble and insoluble material, was not apparent when the total lipolytic action was considered, although the active material was divided in a different ratio between the soluble and the insoluble portions.

A longer set of experiments in which a large number of separate portions of material was used may be summarized as follows:

24 HOURS EXTRACTION.—FILTER 1.

Filtrate I.—Stand 70 hrs. Tests, 24 hrs.: EtBu 0.63; Triac. 0.23.

Filtrate I.—CH_3OH added to make solutions 2 M. White ppt. Stand 70 hrs. Tests, 24 hrs.: EtBu 0.07; Triac. 0.26.

Residue I.—21 hrs. extraction with 1 M. NaCl solution. Filter 11.

Filtrate II.—Dialize 14 hrs. White ppt. Tests, 23 hrs.: EtBu 0.06; Triac. 0.46.

Residue II.—Tests, H_2O, 24 hrs.: EtBu 0.18; 20 hrs.: Triac. 0.71. Tests, 1 M. NaCl, 24 hrs.: EtBu 0.13; 20 hrs.: Triac. 0.41.

This set of experiments confirms the results already given. Water extracted a lipase active toward ethyl butyrate, much less active toward triacetin. Methyl alcohol inactivated (precipitated) the former and did not affect the latter. Sodium chloride solution extracted partly the water-

[1] Residue treated with 25 cc. water and the activity tested.

insoluble enzyme more active toward triacetin than toward ethyl butyrate, while, finally, the sodium chloride solution in addition showed marked inactivating action on the lipase active toward the triacetin.

The results which were obtained on extraction with aqueous, fluoride, and methyl alcohol solutions can be interpreted satisfactorily, as extreme conditions were present. The results with sodium chloride solutions are not as simple to interpret, as the conditions here are more involved, all of the possibilities occurring instead of only one set as with the other solutions. The inhibiting actions of the esters themselves on the active material must be taken into account in a thorough and complete analysis of the results (which was not attempted here).

Taking the facts as presented into consideration, it seems justifiable to adhere to the explanation advanced in the sixth paper for the inhibiting actions as due to a coagulation or precipitation of active material or of substances with which the active material is associated, and to consider that the experimental evidence so far presented in this paper confirms this explanation. In addition the existence of two lipases, one soluble in water and the other insoluble follows from the results.

The results given so far included extraction by water and by solutions showing inhibiting action toward the lipase. The solutions of salts which show activating action toward the lipase compared to water remain to be considered. Extended series of extraction experiments with solutions of 0.05 molar magnesium sulfate, and 0.002 and 0.005 molar manganous sulfate were, therefore, made, the manipulations being similar to those of the experiments already outlined.

The results of these experiments will not be given in detail. In the first place the accelerations observed in the direct tests without filtration were not as great with ethyl butyrate as those given in Paper V, although still marked. In the second place the results obtained in the filtration experiments in testing the filtrates and residues did not give constant results so that though the general trend of the actions could be followed, it was not feasible to attempt to obtain average values for the results. Only the qualitative results will, therefore, be given.

The accelerating action of the magnesium and manganous sulfates in the direct tests with triacetin were very much greater than with ethyl butyrate. This was foretold in the VI Paper in discussing the selective actions of lipases, where the smaller inhibiting actions of the uni-univalent salts on the triacetin tests were also predicted. The results given above have borne out the latter prediction.

In considering the magnesium sulfate extractions, it was found that the filtrates showed small activities toward both esters, about the same as those found with the sodium chloride extraction filtrates. The residues with water gave actions corresponding to the aqueous extraction resi-

dues and water, but with the magnesium sulfate solutions showed marked accelerations, especially toward triacetin. In no case, however, was the sum of the actions of the filtrates plus residues and water or magnesium sulfate equal to the action of unfiltered lipase preparation and magnesium sulfate solution.

The same results were found with the two manganous sulfate solutions. It appears probable, therefore, that the mechanical act of filtration produced a change in the lipase preparation which made it more difficult for the salts to produce acceleration. It was found, however, that the acceleration produced by the action of the salts, whatever may be the cause, was due to their action on the residues and not on the filtrates. This last observation appears certain from the experiments, but the results obtained do not throw further light on the changes involved.

Conclusions.

The result of extraction experiments in which the lipolytic activities of the filtrates and residues of a castor bean preparation after treatment with water, 0.1 molar sodium fluoride, molar sodium chloride, and molar methyl alcohol solutions, were tested toward ethyl butyrate and triacetin, confirmed the conclusion given in the fifth and sixth papers that the inhibiting actions of these solutions on the lipase were due to precipitating or coagulating actions.

Two lipases were shown to be present in the preparation. One of these was soluble in water, the other insoluble. The former exerted a comparatively greater action toward ethyl butyrate than toward triacetin, the latter a greater toward triacetin than toward ethyl butyrate.

Extraction experiments with solutions of magnesium and manganous sulfates which showed acceleration with unfiltered preparations indicated that the accelerations were due mainly to the action of the salts on the residues. Filtration also appeared to decrease the accelerating actions of these salts.

NEW BOOKS.

"Einführung in die Thermodynamik." By R. BLONDLOT. Translated into German by Carl Schorr and Friedrich Platschek. Leipzig: Verlag von Theodor Steinkopff, 1913. 102 pages, paper. Price, 4.00 Marks.

The chief merit of this little book, first published in 1888 and now appearing in its second edition translated into German, lies in a really clear and logical presentation of the first and second laws of thermodynamics and the immediate consequences to be drawn therefrom. The earlier experimental determinations of the mechanical equivalent of heat by Joule and Hirn, are interestingly described, and the ideas of Carnot and others which have led to the second law of thermodynamics are well presented. As the book was designed merely as an introduction to the