[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

THE LIPOLYTIC ACTIVITY OF THE CASTOR AND SOY BEAN.

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Since 1900 there have been many investigations of the behavior of animal and vegetable lipases. These investigations have had a number of objects in view, such as the determination of the effect upon the lipase enzyme of media containing various salts,[1] alkalies,[2] acids,[3,4,5,6] and preservatives,[7] and the relation of lipase to a variety of substrates, including natural fats,[8,9,10,11,12] ethyl butyrate,[13] ethyl acetate,[14] and amyl salicylate.[15] But there has been so much variation in the mode of attack used by different investigators that it is difficult to make comparisons between their results.

The present work was planned to determine a few of the properties of the lipases of the castor and soy bean. The effect of increasing concentrations of acidity from the neutral point on the activity of the lipases was determined. The amount of enzyme in the two beans was compared. And the activity of the lipase on different substrates, such as lard, olive oil and ethyl butyrate, was estimated.

Experimental Work.

Analytical Methods.

In following Falk's method[16,17] unsatisfactory results were obtained when such substrates as lard, olive oil, castor oil and soy oil were used. It was thought that the cause for this trouble was in the titration of organic acids in a watery medium, in which case the acids would not be in solution. Tests were made to determine this by the following method: Olive oil was saponified with potassium hydroxide and the resulting fatty acids purified. 0.1 cc. of these fatty acids was used in every case.

[1] Falk and Hamlin, THIS JOURNAL, 35, 210 (1913).
[2] Green, *Proc. Roy. Soc.*, 48, 370 (1890).
[3] Nicloux, *Mem. soc. c. Biol.*, 56, 701, 839, 868 (1904).
[4] Hoyer, *Z. physiol. Chem.*, 50, 414 (1904).
[5] Taylor, *J. Biol. Chem.*, 2, 87 (1906).
[6] Astrid and Euler, *Z. physiol. Chem.*, 51, 244 (1907).
[7] Kastle and Loevenhart, *Am. Chem. J.*, 24, 507 (1900).
[8] Connstein, *Ergebnisse Physiol. Biochem.*, 3, 194 (1904).
[9] Falk and Nelson, THIS JOURNAL, 34, 741 (1912).
[10] Pekelharing, *Z. physiol. Chem.*, 81, 355 (1912).
[11] Connstein, Hoyer, Wartenberg, *Ber.*, 35, 3988 (1902).
[12] Terroine, *Biochem. Z.*, 32, 429 (1910).
[13] Falk, THIS JOURNAL, 36, I, 1047 (1914).
[14] Hanriot, *Compt. rend. soc. biol.*, 124, 778 (1897).
[15] Kastle and Loevenhart, *Am. Chem. J.*, 24, 491 (1900).
[16] Falk, THIS JOURNAL, 37, 649 (1915).
[17] Falk and Nelson, *ibid.*, 34, 735 (1912).

TABLE I.

Titration of 0.1 cc. of Fatty Acids in Various Media.

25 cc. of water.	25 cc. of water and 30 cc. of alcohol.	25 cc. of water, 30 cc. of alcohol, 10 cc. of ether.	35 cc. of water, 30 cc. of alcohol, 10 cc. of ether.	25 cc. of absolute alcohol.
1.70	2.40	3.00	3.00	2.90
1.80	2.30	3.00	2.95	2.85
1.60	2.40	3.00	2.90	3.05
1.70	2.36	3.00	2.95	2.93

These results indicate that it is impossible to titrate all of the organic acid in a water medium.

To show that it is impossible to use the method employed by Falk, and Pennington and Hepburn,[1] two series of complete determinations were made. The first series, which is given in Table II, is the result of the titrations when made without the addition of alcohol and ether. The second series, Table III, is the result of the titrations after the addition of alcohol and ether. The differences given in the tables are the differences in titrations before and after incubation.

TABLE II.

Substrate.	% HCl. Acidity medium.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme, difference. Cc.	Lipase action. Cc.
Lard	0.1	0.1	0.2	1.5	1.2
Olive oil	0.1	0.1	0.2	1.2	0.9
Lard	0.2	0.2	0.2	0.9	0.5
Olive oil	0.2	0.2	0.2	1.6	1.2
				Av.,	0.95

TABLE III.

Substrate.	% HCl. Acidity medium	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme, difference. Cc.	Lipase action. Cc.
Lard	0.1	0.0	0.2	2.3	2.1
Olive oil	0.1	0.3	0.2	2.2	1.7
Lard	0.2	0.0	0.2	1.4	1.2
Olive oil	0.2	0.1	0.2	2.3	2.0
				Av.,	1.75

Considering these results, it is obvious that all of the acid is not measured when a direct titration is made. It was, therefore, decided to use the alcohol-ether method of titration.

There was the further possibility that error might arise by reason of undissolved fat, since fatty acids are more soluble in it than in any combination of water, alcohol and ether. A series of experiments was carried out to determine the amount of oil that would dissolve in a small

[1] Pennington and Hepburn, THIS JOURNAL, 34, 210 (1912).

quantity of water on the addition of a fixed amount of ether and alcohol. In 25 cc. of water, the amount used by some investigators,[1] it was found that 0.3 cc. of fat would dissolve on the addition of 50 cc. of alcohol and 20 cc. of ether.

It was found that the method of putting the materials together was important. The more intimate the contact of substrate and enzyme, the greater the activity of the lipase. Thorough breaking up and dissemination of the material through the liquid conduces to the best results. Even those titrated immediately show smaller values if not broken up.

In this work, then, the following method[2] was used: castor beans[3] freed from husks by hand were coarsely ground, washed with ether, ground finely, extracted with ether for a week or more in a Soxhlet apparatus, and finally passed through a No. 40 sieve.[4] Experiments with castor bean with and without the seed coat showed nearly twice the activity without the thick husk[5] as compared with the whole bean preparation. In these experiments the enzyme preparations used were castor bean husk-free, and soy bean with the husk, otherwise similarly prepared.

The method of procedure in the determination of lipase activity was as follows: 0.2 g. of the lipase preparation was placed in a 150 cc. Erlenmeyer flask. Upon this 0.3 cc. of substrate was poured and the two shaken together so that all of the bean powder was saturated with the substrate. 20 or 25 cc. of solution was added and any lumps broken up. All experiments were made in triplicate. Three flasks containing substrate and acid, 3 flasks containing enzyme and acid, and 3 containing enzyme, substrate and acid, were placed in a thermostat[6] and incubated[7] at 38° for 24 hours. A similar series of 9 was prepared at the same time and titrated immediately. In all cases, before titrating, 20 cc. of ether and 50 cc. of 95% neutral alcohol were added[8] and the flask vigorously shaken.[9] The end-point, using 1.0% phenolphthalein in 95% alcohol, was a deep pink[10] which lasted 30 seconds. Thymol was used as an antiseptic, 20 cc. of acid solution was used in all experiments except for 0.0 and 0.1 percentages, in which cases 25 cc. was used. To keep the volume of the standard alkali added less than 20 cc., 2 strengths were employed in some cases. 5.0 to 10.0 cc. of 0.5 *N* alkali was first added

[1] Falk and Nelson, THIS JOURNAL, **34**, 741 (1912).

[2] Taylor, *J. Biol. Chem.*, **2**, 87 (1906).

[3] The castor beans were obtained from the Baker Castor Oil Co., New York.

[4] Falk and Sugiura, THIS JOURNAL, **37**, 218 (1915).

[5] Nicloux, *Mem. soc. c. Biol.*, **56**, 701. 839, 868 (1904).

[6] Sigmund, *Sitzb. Akad. Wiss., Wien*, **407**, 328 (1891).

[7] Kastle and Loevenhart, *Am. Chem. J.*, **24**, 491 (1900).

[8] Allen, "Commercial Organic Analysis," II, part I, 3rd Ed., 104 (1899).

[9] Lewkowitsch, "Chemistry, Technology and Analysis of Oil, Fats, and Waxes, Etc.," 3rd Ed., 277 (1904).

[10] Falk and Nelson, THIS JOURNAL, **34**, 735 (1912).

and the titration completed with 0.1 *N* solution. All results are recorded in terms of 0.1 *N* standard.

The olive oil used was "Superfine" grade from Lautier Fils, Grasse. The butyric acid ester was Merck's "Concentrated Ethyl Butyrate." And the lard[1] was vacuum-kettle rendered, of fine texture and became liquid at 32°. The acid chosen was hydrochloric and the concentrations varied from 0.0 to 1.0% with intervals of 0.1%.

I. Castor Bean.

The determinations cover a series of tests of the lipolytic activity of the castor bean for the 3 substrates, lard, olive oil and ethyl butyrate, in an acid medium of various strengths. In the titrations made in triplicate the variations were very small, not exceeding 0.2 cc. The ethyl butyrate was the most subject to slight fluctuations.

The results obtained by the method outlined above are given in Tables IV to VII.

TABLE IV.

Action of Castor Bean Lipase on Lard.

Acidity medium. % HCl.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme, difference. Cc.
0.0	0.0	0.6	0.8
0.1	0.0	0.2	2.3
0.2	0.0	0.2	1.4
0.3	0.0	0.0	1.1
0.4	0.1	0.6	2.0
0.5	0.0	0.3	1.9
0.6	1.2	0.5	0.9
0.7	1.4	0.5	1.1
0.8	0.8	0.4	1.1
0.9	1.0	1.2	1.7
1.0	1.3	0.7	1.5

TABLE V.

Action of Castor Bean Lipase on Olive Oil.

Acidity, medium. % HCl.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme difference. Cc.
0.0	0.0	0.6	0.6
0.1	0.3	0.2	2.2
0.2	0.1	0.2	2.3
0.3	0.0	0.0	1.3
0.4	0.2	0.6	0.9
0.5	0.2	0.3	0.9
0.6	1.1	0.5	1.8
0.7	0.9	0.5	1.3
0.8	1.0	0.4	0.8
0.9	0.9	1.2	1.5
1.0	1.1	0.7	0.8

[1] The lard was furnished by Armour and Company, Chicago.

TABLE VI.

Action of Castor Bean Lipase on Ethyl Butyrate.

Acidity, medium. % HCl.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme difference. Cc.
0.0	0.0	0.6	0.6
0.1	3.3	0.2	2.0
0.2	5.8	0.2	2.6
0.3	7.2	0.0	5.8
0.4	8.9	0.6	8.7
0.5	8.7	0.3	10.2
0.6	10.4	0.5	11.7
0.7	12.5	0.5	10.6
0.8	13.7	0.4	11.6
0.9	14.6	1.2	11.6
1.0	14.9	0.7	11.4

TABLE VII.

Action of Castor Bean Lipase on Different Substrates.

Acidity medium. % HCl.	Lard. Cc.	Olive oil. Cc.	Ethyl butyrate. Cc.
0.0	+0.2	+0.0	—0.0
0.1	+2.0	+1.6	—1.5
0.2	+1.2	+2.0	—3.4
0.3	+1.1	+1.3	—1.4
0.4	+1.3	+0.1	—0.8
0.5	+1.6	+0.4	+1.2
0.6	—0.8	+0.2	+0.8
0.7	—0.8	—0.1	—2.4
0.8	—0.1	—0.6	—2.5
0.9	—0.5	—0.6	—4.2
1.0	—0.5	—1.0	—4.2

Discussion.

The values in Tables IV, V and VI are obtained by subtracting the titrations before incubation from those after incubation, and are expressed in cc. of 0.1 *N* reagent. They represent the mean of triplicate determinations. In order to conserve space these separate determinations and others, also, have been omitted.

In Table IV, Acid and Substrate column, successive differences are 0.0 cc. with one exception, up to 0.6%; from 0.6% to 1.0% they vary from 0.8 cc. to 1.4 cc. The differences are larger in the acid and enzyme column up to 0.6%. Except for 0.9%, there is no increase in this column for stronger acid solutions. Due to the combination of enzyme and substrate, and the varying influence of different percentages of acidity on each, and on the combination, the third column shows a less uniform variation. Here they differ from 0.8 cc. to 2.3 cc.

Table V contains differences for the substrate olive oil. Comparing the differences in the acid and substrate column with those in the acid

and enzyme column, the variation is larger in the latter column up to 0.6%. From 0.6% to 1.0% the effect of the acid on the olive oil is greater than on the enzyme except in the case of 0.9% acid. As previously noted, the combination of acid, substrate and enzyme produces the greatest differences, the largest being 2.3 cc.; the smallest, 0.6 cc.

Table VI gives the differences in the determination of the action of castor bean lipase on ethyl butyrate. They show a steady increase from 0.0 cc. to 14.9 cc. This indicates hydrolysis of the substrate with weak acid solution which was not shown when lard and olive oil were used in corresponding strengths of acid. It is, therefore, doubtful whether it is advisable to substitute ethyl butyrate for the more complex esters of the fatty acids such as olive oil, palm oil, lard, etc., in experiments with fat-splitting enzymes.

The results in Table VII are obtained by subtracting the sum of the "difference" Col. 1 and 2 from Col. 3, as given in Tables IV, V and VI, and show the comparative lipase action on the 3 substrates, lard, olive oil and ethyl butyrate. From this table it is apparent, first, that castor bean lipase is active toward the substrates lard, olive oil and ethyl butyrate in an acid medium up to 0.7%; second, that the optimum acidity is approximately 0.6% for the 3 substrates; third, that castor bean lipase is active toward ethyl butyrate in 0.5 and 0.6% acidity only; and fourth, that this lipase is more active toward lard and olive oil than toward ethyl butyrate.

These results are more or less in agreement with those obtained by other workers. The experiments of Green[1] led him to the conclusion that the lipase action was hindered by the presence of even small amounts of acid. This may have been due to his method of lipase preparation. Armstrong and Gosney[2] found that "Ricinus lipase has its maximum activity when the acidity does not exceed that of the oleic acid." It may be that such equilibrium is reached at approximately 0.5% acidity. H. E. Armstrong[3] found "Ricinus enzyme to have but little action on ethyl butyrate." Loevenhart[4] found entirely different results when he substituted amyl salicylate for ethyl butyrate with liver-lipase extract. Connstein had better results with olive oil, castor oil and palm oil than with ethyl butyrate and other simple esters when acted upon by castor bean lipase.

II. Soy Bean.

The seed-coat of the soy bean is comparatively thin so that its presence does not materially alter the lipase activity. Two soy bean prepara-

[1] Green, *Proc. Roy. Soc.*, **48**, 370 (1890).
[2] Armstrong and Gosney, *Proc. Roy. Soc.* (*B*), **88**, 176 (1914).
[3] Armstrong, *Proc. Royal Soc.* (*B*), **76**, 606 (1905).
[4] Loevenhart, *J. Biol. Chem.*, **51**, II, 393 (1906–7).

tions were tried for relative activity with and without the husk. The following table shows the results with olive oil and 0.2% hydrochloric acid. S. B. I is with seed-coat and S. B. II without.

COMPARATIVE TESTS OF SOY BEAN WITH AND WITHOUT SEED-COAT.

Acid and substrate difference.		Acid and enzyme difference.		Acid, substrate and enzyme difference.		Lipase action.	
S. B. I.	S. B. II.	S. B. I.	S. B. II.	S. B. I.	S. B. II.	S. B. I.	S. B. II.
0.1	0.1	0.1	0.2	0.5	0.7	0.3	0.4

In these experiments the soy bean preparation was made from the whole bean, otherwise prepared as was the castor-bean powder. The procedure was the same, *i. e.*, 0.2 g. portions were placed in 150 cc. Erlenmeyer flasks. Over this powder 0.3 cc. of substrate was poured, shaken together to insure through contact, and the mass broken up and completely scattered through the liquid. The ether-alcohol method of titration was used, all experiments being made in triplicate. The variation among these triplicates was small, being most marked in the case of ethyl butyrate.

Tables VIII to XI give the results in these determinations.

TABLE VIII.

Action of Soy Bean Lipase on Lard.

Acidity medium. % HCl.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme, difference. Cc.
0.0	0.0	0.4	0.8
0.1	0.0	0.4	0.5
0.2	0.0	0.1	0.6
0.3	0.0	0.4	0.9
0.4	0.1	0.5	1.2
0.5	0.0	0.4	0.8
0.6	1.2	1.9	2.1
0.7	1.4	1.8	2.5
0.8	0.8	1.0	1.7
0.9	1.0	1.5	2.3
1.0	1.3	1.5	2.4

TABLE IX.

Action of Soy Bean Lipase on Olive Oil.

Acidity medium. % HCl.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme, difference. Cc.
0.0	0.0	0.4	0.6
0.1	0.3	0.4	0.9
0.2	0.1	0.1	0.5
0.3	0.0	0.4	0.8
0.4	0.2	0.5	1.2
0.5	0.1	0.4	0.8
0.6	1.0	1.9	2.1
0.7	0.9	1.8	2.6
0.8	1.0	1.0	1.7
0.9	0.9	1.5	2.2
1.0	1.1	1.5	2.1

TABLE X.

Action of Soy Bean Lipase on Ethyl Butyrate.

Acidity medium. % HCl.	Acid and substrate, difference. Cc.	Acid and enzyme, difference. Cc.	Acid, substrate and enzyme, difference. Cc.
0.0	0.0	0.4	0.4
0.1	3.3	0.4	2.8
0.2	5.8	0.1	3.5
0.3	7.2	0.4	6.9
0.4	8.9	0.5	9.9
0.5	8.7	0.4	9.4
0.6	10.4	1.9	12.5
0.7	12.5	1.8	14.7
0.8	13.7	1.0	14.9
0.9	14.6	1.5	15.9
1.0	14.9	1.5	15.6

TABLE XI.

Action of Soy Bean Lipase on Different Substrates.

Acidity medium. % HCl.	Lard. Cc.	Olive oil. Cc.	Ethyl butyrate. Cc.
0.0	+0.4	+0.2	—0.0
0.1	+0.1	+0.2	—0.9
0.2	+0.5	+0.3	—2.4
0.3	+0.5	+0.4	—0.7
0.4	+0.6	+0.5	+0.5
0.5	+0.4	+0.3	+0.3
0.6	—1.0	—0.8	+0.2
0.7	—0.7	—0.1	+0.4
0.8	—0.1	—0.3	+0.2
0.9	—0.2	—0.2	—0.2
1.0	—0.4	—0.5	—0.8

Discussion.

Tables VIII, IX and X are made up of the differences of titrations before and after incubation. Mean values based upon triplicate determinations were used to obtain the results.

Table VIII is for the differences of titration with soy bean lipase on lard. It will be noticed that there is no incubation effect up to 0.6% acidity. The hydrolysis of the fat is shown by the increase in differences from 0.6% to 1.0% acid solution, the largest being 1.4 cc. in the acid and substrate column. In the acid and enzyme column the greatest difference is 1.9 cc. But in the third column the maximum difference is 2.5 cc. and the minimum is 0.5 cc.

In Table IX, where the differences for soy bean lipase on olive oil are given, the greatest difference in Col. 1 is 1.1 cc. for 1.0% acid. In Col. 2 the greatest difference is 1.9 cc., while in Col. 3, or in acid, substrate and enzyme combination, the largest value is 2.6 cc. in a 0.7% hydrochloric acid medium.

Table X gives the differences of soy bean lipase action on ethyl butyrate. It is noticeable that there is a very marked effect of the acid upon the substrate, the greatest value being 14.9 cc. for 1.0% acidity. The decrease in the effect is gradual from 1.0% to 0.0% acidity. In the third column, where the differences for the mixture of acid, substrate and enzyme are given, a gradual increase is observed from 0.4 cc. to 15.9 cc.

Table XI is a comparison of the lipase action of soy bean upon the 3 substrates, lard, olive oil and ethyl butyrate. The maximum acidity in which lipase action takes place is 0.8%. This is for ethyl butyrate. For lard and olive oil the maximum acidity is 0.5%. The maximum value is 0.6 cc. for lard, 0.5 cc. for olive oil, and 0.5 cc. for ethyl butyrate. The optimum acidity for the 3 substrates is 0.4%. The range of acidity in which there is positive action is 0.0 to 0.5%, inclusive, for lard and olive oil, and 0.4 to 0.8% for ethyl butyrate. It is interesting to note that the range of acidity of the medium in which ethyl butyrate is split is shorter than the range for lard and olive oil. Also that the range includes only a small part of the extreme ends of the ranges for lard and olive oil.

TABLE XII.

Lipase Action of Soy and Castor Beans on Lard.

Acidity medium. % HCl.	Soy bean. Cc.	Castor bean. Cc.
0.0	+0.4	+0.2
0.1	+0.1	+2.0
0.2	+0.5	+1.2
0.3	+0.5	+1.1
0.4	+0.6	+1.3
0.5	+0.4	+1.6
0.6	—1.0	—0.8
0.7	—0.7	—0.8
0.8	—0.1	—0.1
0.9	—0.2	—0.5
1.0	—0.4	—0.5

TABLE XIII.

Lipase Action of Soy and Castor Beans on Olive Oil.

Acidity medium. % HCl.	Soy bean. Cc.	Castor bean. Cc.
0.0	+0.2	+0.0
0.1	+0.2	+1.7
0.2	+0.3	+2.0
0.3	+0.4	+1.3
0.4	+0.5	+0.1
0.5	+0.3	+0.4
0.	—0.8	+0.2
0.7	—0.1	—0.1
0.8	—0.3	—0.6
0.9	—0.2	—0.6
1.0	—0.5	—1.0

TABLE XIV.

Lipase Action of Soy and Castor Beans on Ethyl Butyrate.

Acidity medium. % HCl.	Soy bean. Cc.	Castor bean. Cc.
0.0	—0.0	—0.0
0.1	—0.9	—1.5
0.2	—2.4	—3.4
0.3	—0.7	—1.4
0.4	+0.5	—0.8
0.5	+0.3	+1.2
0.6	+0.2	+0.8
0.7	+0.4	—2.4
0.8	+0.2	—2.5
0.9	—0.2	—4.2
1.0	—0.8	—4.2

Tables XII, XIII and XIV show the comparative lipase activity of castor and soy beans on the substrates, lard, olive oil and ethyl butyrate. In Table XII where the action of the enzyme is shown on lard, the range gives a positive action for 0.0 to 0.5%, inclusive, and negative for the remainder. The amount of lipase action of soy bean varies from 0.1 to 0.6 cc. The range of the acidity of the medium in which fat splitting takes place is from 0.1 to 0.5%. The amount of lipase action of castor bean ranges from 0.2 to 2.1 cc. The acidity of the medium in which action takes place amounts to 0.0 to 0.5%. The largest negative value for each enzyme is at 0.6%. For soy bean this value is 1.0 cc. and for castor bean 0.8 cc. Tests with acidities up to 2.0% gave still larger negative results.

Comparing the enzyme action of soy bean with that of castor bean on lard, some differences are evident. First, the maximum activity of castor bean is 2.0 cc., that of soy is 0.6 cc. Second, the maximum activity for castor bean occurs at 0.1% acidity, while for soy bean it is at 0.4%. And, third, for both enzymes the maximum acidity for lipase activity is 0.5%.

These results are, in general, comparable to those of other investigators. Connstein, Hoyer and Wartenberg[1] found that hydrolysis was not hindered by as much as 2.0% of acetic acid, and that 0.1 *N* sulfuric acid hastened fat-splitting. This is approximately 0.5% concentration. Further comparison with their results is impossible because of difference in material and method. Armstrong[2] obtained 4.1 g. of oleic acid from 5.0 cc. of olive oil with one g. of fat-free castor bean in 0.03 *N* (approximately 0.15%) sulfuric acid, after 18 hours of incubation at 38°.

In Table XIII is a comparison of the lipase action of both beans on olive

[1] Connstein, Hoyer and Wartenberg, *Ber.*, **35**, 3988 (1902).

[2] Armstrong, *Proc. Roy. Soc.* (*B*), **76**, 606 (1905).

oil. The positive action range for this substrate is from 0.0 to 0.5%, inclusive, for soy bean and from 0.0 to 0.6% for castor bean. For the remainder of the percentages of acidity the values are negative. The greatest soy bean activity, 0.5 cc., is manifest at 0.4%. For castor bean the maximum activity is 2.0 cc. at 0.2%. The maximum negative value is 0.8 cc. for soy bean at 0.6% and 1.0 cc. for castor bean at 1.0%. Higher percentages of acidity gave increased negative results. Therefore, using olive oil as a substrate, the comparative action of lipases from soy and castor beans is as follows: first, the castor bean is very much more active; and second, the range of acidity of the medium is practically the same in both cases. These results are very similar to those obtained with lard.

Table XIV gives the results of the action of soy and castor bean lipases upon ethyl butyrate. Soy bean shows a negative activity up to 0.4% acid, positive to 0.9%, and negative again for 0.9 and 1.0%. For castor bean lipase all values are negative except 0.5 and 0.6%. The highest positive value for soy bean lipase is 0.5 cc. at 0.4%. For castor bean, 1.2 cc. at 0.5% is the greatest positive value. Stronger acidity gave larger negative results. A comparison of the action of the enzymes of the 2 beans on ethyl butyrate shows: first, that soy bean lipase is active over a greater range of acidity, but activity is less than that of castor bean lipase for a given strength of acid; second, that the maximum activity for castor bean lipase is 1.2 cc. at 0.5%; for soy bean it is 0.5 cc. at 0.4% acidity; and third, that the activity of soy bean lipase ceases at 0.9% and that of castor bean lipase at 0.7%.

Comparing the various tables, especially Tables VII and XI, the following features are apparent: first, that castor and soy bean lipases are active toward the substrates, lard, olive oil and ethyl butyrate, in 0.4 and 0.5% medium; second, that the optimum acidity for castor and soy bean lipase is approximately 0.5% for lard and olive oil; third, that neither soy nor castor bean lipase is active toward ethyl butyrate in less than 0.4% acidity; fourth, that soy bean lipase does not show as great activity values as does castor bean lipase—the maximum ratio being 2.0 cc. to 0.6 cc.; fifth, with ethyl butyrate the castor bean lipase is active over a smaller range of acidity than is soy bean lipase; and sixth, that both soy and castor bean lipases are more active toward olive oil and lard than toward ethyl butyrate.

A graphic representation of the data would show that the castor bean has the more intense activity, that the forms of the curves for acidity of media and amount of activity do not have characteristic differences, and that the ranges of activity in increasing strengths of acid do not characteristically differ. These facts would go far in leading one to the conclusion that castor and soy beans contain the same lipase or lipases

but in different quantity. With this Falk and Sugiura,[1] in the main, agree; and Falk[2] concluded from his work with lipases from castor bean, soy bean, etc., that marked similarities in action are shown by lipases from different sources. He did not compare the relative amounts of activity for any range of acidity. Armstrong[3] found castor bean to have little action on ethyl butyrate. Taylor[4] recommended the use of triacetin as a substrate. Connstein[5] found that ethyl butyrate was acted upon to a much less satisfactory extent than was olive oil, castor oil and palm oil.

Summary and Conclusion.

The lipases of the castor and soy bean were studied with relation first to their ranges and amounts of activity in a watery medium with acid reaction and second to their actions in these media on 3 substrates, lard, olive oil and ethyl butyrate. The methods used in making these determinations were investigated.

The following conclusions were reached:

First, when lard or olive oil is used as a substrate, the liberated fatty acids cannot be titrated in a water mixture, with an aqueous standard alkali solution. Ether and alcohol must be added before titration in at least sufficient quantity to make a single and complete solution of all of the fat.

Second, of the preparations used, the castor bean lipase was more intense in its action than the soy bean lipase.

Third, the ranges of acidity of the media in which action took place were practically the same for castor and soy bean lipase, and were independent of the kind of substrate.

Fourth, lard and olive oil afforded practically equal degrees of activity in the various acidities and ranges of acidity in which activity took place.

Fifth, ethyl butyrate as a substrate differed characteristically from lard and olive oil in that the degree of activity was smaller and the range of acidity in which action took place was much higher and shorter.

The above conclusions in connection with the data presented, lend proof to the following hypotheses:

First, the castor bean lipase splits esters of the fatty acids to a greater extent than does soy bean lipase.

Second, soy and castor beans contain the same lipase or lipases.

Third, both soy and castor beans contain more than one lipase.

This work was outlined and begun under the direction of the late Dr.

[1] Falk and Sugiura, THIS JOURNAL, 37, 218 (1915).

[2] Falk, *ibid.*, 37, 649 (1915).

[3] Armstrong, *Proc. Roy. Soc.* (*B*), 76, 606 (1905).

[4] Taylor, *J. Biol. Chem.*, 2, 87 (1906).

[5] Connstein, *Ergebnisse Physiol. Biochem.*, 3, 194 (1904).

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CHICAGO, ILL.

[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, MINNESOTA AGRICULTURAL EXPERIMENT STATION.]

ON THE ORIGIN OF THE HUMIN FORMED BY THE ACID HYDROLYSIS OF PROTEINS. IV. HYDROLYSIS IN THE PRESENCE OF ALDEHYDES. III. COMPARATIVE HYDROLYSIS OF FIBRIN AND GELATIN IN THE PRESENCE OF VARIOUS ALDEHYDES.[1]

BY GEORGE E. HOLM AND ROSS AIKEN GORTNER.

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In the earlier papers of this series, by Gortner and Blish,[2] Gortner,[3] and Gortner and Holm,[4] we have studied the effect of certain aldehydes upon the acid hydrolysis of proteins with especial reference to the origin and mode of formation of the "humin" fraction. Detailed observations were made using various carbohydrates, which under the conditions of the experiment would yield furfural and formaldehyde, with a few observations, where benzaldehyde was present. It was noted that the hydrolysis of a protein in the presence of an aldehyde markedly altered the nitrogen distribution, so much so that when formaldehyde was present in excess the resulting nitrogen distribution bore no resemblance to the values obtained in the absence of the aldehyde. The most noteworthy changes in the nitrogen distribution were in the ammonia and humin fractions, but the fact that an excess of formaldehyde over that required to cause maximum humin formation apparently unites with the α-amino groups so that they no longer react with nitrous acid, causes the remaining nitrogen fractions to lose all resemblance to those of a normal hydrolysate.

We have furthermore shown that in all probability the black insoluble humin nitrogen is derived from the interaction of tryptophane and an aldehyde. However, tyrosine also reacts with aldehydes to form com-

[1] Presented before the Biological Division at the Philadelphia meeting of the American Chemical Society, Sept. 2–6, 1919. Published with the approval of the Director as Paper No. 190, Journal Series of the Minnesota Agricultural Experiment Station.

[2] R. A. Gortner and M. J. Blish, "On the Origin of the Humin Formed by the Acid Hydrolysis of Proteins," THIS JOURNAL, 37, 1630–36 (1915).

[3] R. A. Gortner, "The Origin of the Humin Formed by the Acid Hydrolysis of Proteins. II. Hydrolysis in the Presence of Carbohydrates and of Aldehydes," *J. Biol. Chem.*, 26, 177–204 (1916).

[4] R. A. Gortner and G. E. Holm, "On the Origin of the Humin Formed by the Acid Hydrolysis of Proteins. III. Hydrolysis in the Presence of Aldehydes. II. Hydrolysis in the Presence of Formaldehyde," THIS JOURNAL, 39, 2477–2501 (1917).