for the glycogen reserve as influenced by fasting. Pflüger's method[1] for the quantitative determination of glycogen was employed. *No glycogen was present.* A portion of muscle subjected to the same method also yielded negative results.

To our mind, the unusual length of the fast is associated in a very important manner with the finding of a "glycogen-free" liver. May it not be possible that the nutritional régime of a dog hardy enough to withstand a fast of 100 days will become so altered during the closing days of such a test as to render the organs and tissues glycogen-free? The dog was also a very active animal and delighted to jump in and out of his cage,[2] and, therefore, inasmuch as muscular work will increase the glycogen consumption it is possible that this factor had some slight influence upon the final result. It should also be borne in mind that Oscar was a "repeated" faster. It is possible that the liver of a "repeated faster" subjected to fasts of a very prolonged nature may be rendered glycogen-free, whereas glycogen will remain present in the liver of an "initial faster" through a single protracted fast.

STUDIES ON ENZYME ACTION. II. THE HYDROLYTIC ACTION OF SOME AMINO ACIDS AND POLYPEPTIDES ON CERTAIN ESTERS.

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1. Introduction.

Very little is known at the present time of the chemical nature of lipase. Differences in the substances which occur in lipase preparations obtained from animal or vegetable sources greatly complicate the investigation into the identity of their active constituents. It is therefore preferable to speak with Loevenhart[3] of the hydrolytic action of enzymes as a definit property without assuming that this property is limited to a single substance or group of substances, until more chemical evidence of the nature of these substances is obtained. Loevenhart, in the paper referred to, discussed in detail the question "*Are the animal enzymes concerned in the hydrolysis of various esters identical?*" and concluded tentatively that while the action of the liver and the pancreas is probably to be attributed to a different single enzyme in each of these tissues, the variations in the actions on different esters by each enzyme are due to variations in the

[1] Pflüger, *Loc. cit.*

[2] Howe, Mattill and Hawk, *Loc. cit.*

[3] *J. Biol. Chem.*, **2**, 427 (1907).

admixture of substances in the extracts. A. E. Taylor[1] considers that "the question of the identity of the animal and vegetable ferments is a vain discussion. Both animal and vegetable lipases are able to accelerate the cleavage of all the simple esters of the monatomic alcohols and of glycerin, as well as of many synthetic esters."

A number of conclusions of other workers in these fields might be quoted, but as these are to be found summarized in suitable text-books, only some of the chemical evidence of the nature of lipase will be mentioned. The activity of the castor bean lipase is destroyed by pepsin or trypsin. While the activity is not destroyed by dry heating to 100°, it is lost in aqueous solution or suspension even at 65°.[2] The loss of activity by heating in aqueous solution is characteristic of both animal and vegetable lipases. The asymmetric hydrolysis of esters by lipase preparations has been described a number of times.[3] The acceleration of lipolytic action by various substances, more or less specific for a lipase from a definit source, may for the present at any rate, be considered to be of secondary importance even though the acceleration due to these substances, the coferments, is in some cases of unusual and unexpected magnitude.

From the chemical evidence which has been quoted and from a study of the literature of lipase action, it seems reasonable to suggest as a tentative hypothesis that the hydrolytic action of lipase is due to an optically-active substance of protein character, readily hydrolyzed in aqueous solution to form lipolytically inactive substances. The characteristic groups of protein substances are the amino and carboxyl groups, free or combined in some manner. The possible hydrolytic actions of a few of the simpler amino acids and polypeptides were therefore studied.

2. Experimental Method.

The quantity of acid produced when methyl acetate, ethyl butyrate, and olive oil were dissolved or suspended in aqueous solutions containing glycine, alanine, phenylalanine, leucine, glycylglycine, leucylglycine, glycylleucine, diglycylglycine, aspartic acid and glutamic acid at 38°, was determined. The esters were neutral or very slightly acid in reaction initially (less than 0.1 cc. of 0.1 *N* alkali was required to neutralize 1 cc. ester in the extreme cases), and corrections were introduced for the amounts of acid formed from these alone in aqueous solution. Some of the amino acids were obtained from Kahlbaum; the rest of these, as

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

[2] Taylor, *Loc. cit.*

[3] Dakin, *J. Physiol.*, 30, 84 (1903); 32, 199 (1905). Mayer, *Biochem. Z.*, 1, 39 (1906). Warburg, *Z. physiol. Chem.*, 48, 205 (1906). Neuberg and Rosenberg, *Biochem. Z.*, 7, 191 (1907).

well as the polypeptides, from the stock prepared at different times in the Harriman Research Laboratory.[1]

Three sets of measurements were necessary for each experiment: with solutions containing amino acid or polypeptide alone and ester alone, and with the mixture containing both. The formol method for which the details and necessary precautions were given by Sörensen[2] was used for the estimation of the amino acids and polypeptides; namely, the addition of neutralized formaldehyde solution to mask the action of the amino group and subsequent titration with alkali. A few modifications were introduced for the present work. Sörensen recommended 0.2 *N* alkali for the titrations, but because of the small increase in acidity due to any hydrolysis of ester taking place, the strength of the solutions of sodium hydroxide used was in the neighborhood of 0.1 *N*. Sörensen also recommends titrating to a definit red color with phenolphthalein as indicator. Partly on account of the presence of ester in the solutions, most of the measurements to be described were made using the first distinct pink color as end point. As it was desired to know the amount of alkali used by the amino acid or polypeptide in the solutions alone or in the presence of alkali by titrating both sets in the same way, comparable results were obtained without considering necessarily the question of the quantitative equivalence of carboxyl groups present and sodium hydroxide used. In some cases the addition of neutralized ethyl alcohol to the solutions before titration gave a more satisfactory end point. In some experiments, the titrations were carried to the definit red color, as recommended by Sörensen. Attention will be called to the experiments in which these different methods were used in connection with the tabulated results.

The method of carrying out an experiment may be outlined briefly with glycine as example. Amounts of glycine, weighing in the neighborhood of 0.1 gram each, were placed in eight flasks, 25 cc. water added to each, and 1 cc. methyl acetate each to two of them, 1 cc. ethyl butyrate to two, and 1 cc. olive oil to two. Six blanks were made up each containing 1 cc. ester (two of each ester) and 25 cc. water. The fourteen flasks were placed in the thermostat at 38° for 24 hours and then taken out and the amount of acid present determined by the formol method as follows: Five cc. of 40% formaldehyde solution, neutralized with a sodium hydroxide solution approximately 0.1 *N* with 5 drops of a 1% phenolphthalein solution in 95% ethyl alcohol as indicator, were added to each solution containing glycine, and the acid present titrated with the sodium hydroxide, using the first distinct pink color as the end point.

[1] Thanks are due to Mr. P. A. Kober and the late Dr. A. H. Koelker for having prepared and purified these substances.

[2] *Biochem. Z.*, **7**, 45 (1907).

From the amounts of alkali used for the glycine solutions alone, and for the ester solutions alone, the amounts used by the glycine and ester in the mixtures were calculated and the differences between these and the quantities actually used in the experiments (increased by 0.04 as explained later) show the amounts of acid formed from the esters due to the presence of glycine in aqueous solution.

In all of the experiments given in the following tables (except for two sets with aspartic and glutamic acids, in which 40 cc. of solution were used), the solutions were made up to 25 cc. with water initially, 5–15 cc. of 40% formaldehyde solution (neutralized) were used in carrying out the formol method, the quantity depending upon the weight of amino acid or polypeptide used. Phenolphthalein was used as indicator throughout.

3. Experimental Results.

The experimental results are shown in Tables I–VI. In every set of experiments with each amino acid or polypeptide, simultaneous experiments were made with amino acid or polypeptide in water, amino acid or polypeptide and ester in water, and ester in water, and the amounts of ester hydrolyzed calculated in each series. In presenting the results here however, the data have been collected so that all the experiments for each amino acid and polypeptide alone in aqueous solution are given together, and from the mean of these results the quantity of alkali required for the amino acid or polypeptide in the experiments with mixtures calculated. The separate results for the ester-water and the amino acid (polypeptide) ester-water experiments are however retained.

In Table I, the results found in titrating the amino acids and polypeptides by the formol method are given. The first column indicates the substance used, the second column the weight of substance in milligrams in each experiment, and the third column the number of cubic centimeters of sodium hydroxide solution required to give a faint but distinct pink color after the addition of the neutralized formaldehyde solution. In those experiments in which the titration was carried to a red color as described by Sörensen, the letter R is placed in the first column. Where 25 cc. of neutralized ethyl alcohol were added with the formaldehyde solution before titration (Alc.) was written in the first column. The letters preceding the amounts of alkali added refer to the normality of the sodium hydroxide solutions used in titrating: a = 0.0909, b = 0.1040, c = 0.1100, d = 0.1125, e = 0.1139, f = 0.1272. In the fourth column the quantities of 0.1 N sodium hydroxide required for 1 mg. substance as calculated from the experimental data in the second and third columns are given. These are then averaged for each amino acid and polypeptide assigning to each result a weight proportional to the weight of amino acid taken initially, and the result given as "W't'd

mean." From the weighted mean, the average deviations of the separate values (a. d.) of each set of results was calculated, and dividing this a. d. by the square root of the number of values, the average deviation of the mean[1] (A. D.) was obtained. This shows the accuracy of the results obtained in the different titrations.

TABLE I.—DETERMINATION OF AMINO ACIDS AND POLYPEPTIDES WITH ALKALI BY THE FORMOL METHOD.

Substance.	Wt. subst. in mg.	Cc. NaOH required.	Cc. 0.1 *N* NaOH for 1 mg. subst.
Glycine	78.1	*d* 9.15	0.1318
	83.8	*d* 9.82	0.1318
	91.2	*d* 10.72	0.1322
R.	99.5	*e* 11.47	0.1313
	99.7	*b* 12.66	0.1320
R.	106.2	*e* 12.25	0.1314
	113.8	*d* 13.27	0.1312
	115.1	*b* 14.60	0.1319
	158.0	*b* 19.96	0.1314
	161.4	*d* 18.88	0.1316
R.	169.1	*e* 19.60	0.1320
	220.7	*b* 27.96	0.1318
R.	239.3	*e* 27.76	0.1321
		W't'd mean.	0.1317
		a. d.	0.00027
		A. D.	0.00007
Alanine	60.0	*d* 5.60	0.1050
	73.8	*d* 6.88	0.1049
	83.7	*d* 7.68	0.1032
	92.1	*d* 8.60	0.1050
	98.0	*d* 8.88	0.1019
	125.3	*d* 11.65	0.1046
	155.2	*d* 13.96	0.1012
		W't'd mean.	0.1034
		a. d.	0.00140
		A. D.	0.00053
(Alc.)	46.7	*d* 4.65	0.1120
(Alc.)	66.5	*d* 6.50	0.1100
(Alc.)	91.7	*d* 9.38	0.1151
(Alc.)	118.4	*b* 12.98	0.1140
(Alc.)	119.8	*b* 12.52	0.1087
		W't'd mean.	0.1121
		a. d.	0.0027
		A. D.	0.0012
R.	58.4	*e* 5.50	0.1073
R.	62.0	*e* 5.92	0.1088
		W't'd mean.	0.1081
Phenylalanine (Alc.)	41.2	*b* 2.46	0.0606
	56.0	*d* 3.08	0.0619
(Alc.)	57.4	*b* 3.42	0.0620
	64.9	*a* 4.33	0.0606

[1] Equal to 1.18 times the "probable error."

TABLE I (*continued*).

Substance.	Wt. subst. in mg.	Cc. NaOH required.	Cc. 0.1 *N* NaOH for 1 mg. subst.
R.....	77.2	*e* 4.05	0.0598
	89.5	*d* 4.83	0.0607
	96.9	*d* 5.22	0.0606
	107.8	*b* 6.26	0.0604
	117.2	*a* 7.96	0.0617
	127.8	*d* 6.90	0.0607
	134.8	*d* 7.26	0.0606
	158.5	*d* 8.45	0.0600
	191.1	*a* 12.77	0.0607
		W't'd mean...	0.0607
		a. d.....	0.00045
		A. D.....	0.00012
Leucine.....	51.0	*d* 3.43	0.0757
	66.9	*d* 4.55	0.0765
	78.9	*d* 5.30	0.0756
	90.7	*b* 6.76	0.0775
	94.7	*d* 6.40	0.0760
	97.8	*d* 6.59	0.0758
	99.0	*d* 6.54	0.0743
	102.9	*b* 7.52	0.0760
	130.0	*d* 8.58	0.0743
	131.4	*b* 9.54	0.0755
	158.0	*c* 10.76	0.0749
		W't'd mean...	0.0755
		a. d.....	0.00069
		A. D.....	0.00021
Glycylglycine.....	50.4	*d* 3.34	0.0746
	54.5	*d* 3.59	0.0741
	61.3	*b* 4.30	0.0730
	89.2	*d* 5.94	0.0749
	99.9	*d* 6.65	0.0749
	131.3	*d* 8.68	0.0744
		W't'd mean...	0.0744
		a. d.....	0.00048
		A. D.....	0.00020
Leucylglycine.....	104.3	*c* 5.10	0.0538
	119.8	*f* 5.02	0.0533
	152.7	*f* 6.38	0.0531
	156.7	*c* 7.62	0.0535
		W't'd mean...	0.0534
		a. d.....	0.00023
		A. D.....	0.00011
(Alc. R).....	109.0	*e* 4.83	0.0505
(Alc. R).....	130.4	*e* 5.92	0.0517
(Alc. R).....	215.3	*e* 9.77	0.0517
		W't'd mean...	0.0514
		a. d.....	0.00050
		A. D.....	0.00029

TABLE I (*continued*).

Substance.	Wt. subst. in mg.	Cc. NaOH required.	Cc. 0.1 *N* NaOH for 1 mg. subst.
R.....	114.7	*e* 5.32	0.0528
R.....	120.7	*e* 5.54	0.0523
R.....	138.5	*e* 6.40	0.0526
		W't'd mean...	0.0526
		a. d.....	0.00017
		A. D.....	0.00010
Glycylleucine (Alc. R).....	51.8	*e* 2.40	0.0528
(Alc. R).....	79.8	*e* 3.62	0.0517
	87.8	*c* 4.22	0.0529
(Alc.).....	89.3	*c* 4.25	0.0524
	112.7	*c* 5.38	0.0525
(Alc. R).....	120.2	*e* 5.65	0.0535
		W't'd mean...	0.0527
		a. d.....	0.00043
		A. D.....	0.00018
Diglycylglycine R.....	94.5	*e* 4.22	0.0509
R.....	97.0	*e* 4.34	0.0510
R.....	117.3	*e* 5.36	0.0520
	124.5	*c* 5.73	0.0506
R.....	130.9	*e* 5.98	0.0520
R.....	132.8	*e* 6.00	0.0515
	141.5	*f* 5.71	0.0513
	154.0	*c* 7.22	0.0516
	193.2	*c* 9.04	0.0515
	194.9	*f* 7.92	0.0517
		W't'd mean...	0.0515
		a. d.....	0.00035
		A. D.....	0.00014
Aspartic acid.....	70.6	*c* 8.92	0.1390
	133.5	*c* 16.82	0.1386
	195.1	*c* 24.33	0.1372
		W't'd mean...	0.1380
R.....	125.3	*e* 15.89	0.1444
R.....	144.9	*e* 18.39	0.1446
		W't'd mean...	0.1445
Glutamic acid.....	175.2	*c* 20.10	0.1262
	212.7	*c* 24.30	0.1257
	249.4	*c* 28.48	0.1256
		W't'd mean...	0.1258
R.....	127.4	*e* 14.85	0.1328
R.....	178.3	*e* 21.04	0.1344
		W't'd mean...	0.1337

The results shown in detail in Table I are summarized in Table II. The first column shows the substance used; the second column the number of cc. 0.1 *N* sodium hydroxide solution required for 1 mg. substance as given by the weighted means of Table II and the average deviations of these means (A. D.). The third column shows the number of cc. of 0.1 *N*

sodium hydroxide solution which 1 mg. of the substance would require theoretically; the fourth column the amount of alkali used as percentage of the theoretical amount. In the fifth column the results found by Sörensen are given, those marked R referring to a strong red color as end point, the others to a distinct red color. The results of Table I and column 2 of Table II show that no difference is obtained in titrating to different end points and correcting these in the usual way, with glycine (agreeing with Sörensen's results), glycylleucine, and diglycylglycine. With the other substances where different end points were used or where alcohol was added, different results were obtained, constant for any one method. In using these values in the subsequent tables, therefore, it is necessary in order to calculate the amount of alkali required for the amino acid taken, to use the standard corresponding to the method used in the solution titrated. As the conditions for the experiments are the same in the blanks (in Tables I and II) and when ester was present (in Tables III–VI) no error is introduced in this procedure.

TABLE II.—SUMMARY OF RESULTS OF THE DETERMINATION OF AMINO ACIDS AND POLYPEPTIDES WITH ALKALI BY THE FORMOL METHOD.

Substance.	Cc. 0.1 *N* NaOH for 1 mg subst. Found.	Cc. 0.1 *N* NaOH for 1 mg. subst. Calc.	$\frac{\text{NaOH f'd.}}{\text{NaOH calc.}} \times 100$.	Sörensen.
Glycine	0.1317±0.00007	0.1332	98.9	98.5
Alanine	0.1034±0.00053	0.1124	92.0	93.5
(Alc.)	0.1121±0.0012	"	99.7	..
R	0.1081	"	96.2	98.0 R
Phenylalanine	0.0607±0.00012	0.0606	100.2	97.5
	.....	"	...	99.5 R
Leucine	0.0755±0.00021	0.0763	99.0	95.0
	.....	"	...	97.0 R
Glycylglycine	0.0744±0.00020	0.0757	98.3	97–99
Leucylglycine	0.0534±0.00011	0.0532	100.4	...
R	0.0526±0.00010	"	99.0	...
(Alc. R)	0.0514±0.00029	"	96.6	...
Glycylleucine	0.0527±0.00018	0.0532	99.1	...
Diglycylglycine	0.0515±0.00014	0.0529	97.4	...
Aspartic acid	0.1380	0.1503	91.8	96.5
R	0.1445	"	96.1	97.5 R
Glutamic acid	0.1258	0.1360	92.5	99.0
R	0.1337	"	98.3	99.5 R

Although this work was not done with the intention of studying the formol method, the results in column 4 may be of interest in this connection. These results are obtained using the first faint but distinct pink or strong red (marked R) colors as end points. With glycine, glycylleucine, and diglycylglycine, for which the end point used exerted no apparent influence, the amount of alkali used varied from 97.4–98.9% of the calculated quantity. For the substances which showed differences

depending upon the end point, results more nearly approaching the theoretical were obtained by using a deeper red color as pointed out by Sörensen. The results for leucylglycine, glycylleucine, and diglycylglycine, which were not included in Sörensen's article, approach closely to the theoretical. For the rest, there is on the whole a satisfactory agreement between the results obtained here and those given by Sörensen.

The experimental results obtained from amino acid and polypeptide and ester in aqueous solution with the production of acid are given in Tables III, IV, and V. In these tables column 1 indicates the amino acid or polypeptide used; column 2, its weight in milligrams in the experiment; column 3, the time in hours for which the experiment was allowed to run at 38°; column 4, the normality of the sodium hydroxide solution used in titrating as indicated by the letter (see above), and the number of cubic centimeters of this solution required in titrating by the formol method by the substance-ester solution at the end of the experiment; column 5, the amount of alkali required by the ester-water mixtures under the same conditions (practically the same results were obtained here whether neutralized formaldehyde solution was added or not); column 6, the number of cubic centimeters of alkali required for the amino acid or polypeptide calculated from the standards from Table II. Column 7 contains the amount of acid produced in each experiment in terms of the normality of the sodium hydroxide solution used in titrating from the amino acid or polypeptide and ester and is equal to the result in column 4 minus the sum of the results in columns 5 and 6, plus 0.04. The end point was produced twice in the blank experiments (columns 5 and 6) and once in the mixture (column 4), and since it was found that one drop of the alkalin solution produced the necessary color in titrating to the end point used, corresponding to 0.04 cc., this quantity must be added as indicated. In order to furnish a satisfactory basis of comparison for each series of experiments and to obtain a mean value, the results given in column 8 are calculated. These show the number of cubic centimeters of 0.1 *N* acid produced by the action of 0.1 gram of the substance on the ester in question for 24 hours. These standards of comparison were chosen as they correspond to the average values used in the separate experiments, and no great error was introduced by assuming proportionality of action under these conditions. The weighted means of these results are also given for each series, assigning to each result the weight proportional to the amount of amino acid or polypeptide used in that experiment. The average deviation of the separate values from the mean (a. d.) and the average deviation of the mean (A. D.) was calculated for each series and given in column 8.

Twenty-five cc. of water were used in each experiment and 1 cc. of ester unless stated to the contrary.

Table III contains the results obtained with methyl acetate, Table IV those obtained with ethyl butyrate, Table V those obtained with olive oil.

TABLE III.—RESULTS OBTAINED WITH METHYL ACETATE AND AMINO ACIDS AND POLYPEPTIDES.

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
Glycine	53.7	17	*d* 6.42	0.15	6.29	0.02	0.05
	60.7	24	*b* 7.82	0.09	7.68	0.08	0.13
	101.0[1]	44	*a* 15.40	0.14	15.14	0.16	0.08
	119.0	18	*b* 15.12	0.12	15.08	—0.04	—0.05
R	121.9	23	*e* 14.23	0.26	14.10	—0.09	—0.09
	129.4	18	*b* 16.56	0.12	16.40	0.08	0.08
	134.5	17	*d* 15.95	0.15	15.75	0.09	0.10
R	154.6	23	*e* 18.04	0.26	17.89	—0.07	—0.05
					W't'd mean...		0.02
					a. d.		0.074
					A. D.		0.026
Alanine	52.1	19	*d* 5.00	0.22	4.79	0.03	0.08
R	55.2	19	*e* 5.37	0.11	5.24	0.06	0.16
R	58.9	19	*e* 5.73	0.11	5.59	0.07	0.17
	60.8	17	*d* 5.70	0.15	5.59	0	0
	92.0	17	*d* 8.60	0.15	8.46	0.03	0.05
					W't'd mean...		0.09
					a. d.		0.057
					A. D.		0.026
Phenylalanine	48.4	17	*d* 3.08	0.14	2.61	0.37	1.21
R	61.6	25	*e* 3.53	0.26	3.28	0.03	0.05
	62.3	23	*d* 3.78	0.20	3.36	0.26	0.49
	82.7	17	*d* 5.02	0.14	4.46	0.46	0.88
	99.2	23	*d* 5.80	0.20	5.35	0.29	0.34
	101.3	18	*d* 6.15	0.16	5.47	0.56	0.83
	116.0	43	*a* 11.35	0.82	7.75	2.82	1.23
	146.3	18	*d* 8.76	0.16	7.89	0.75	0.77
	234.8	43	*a* 18.05	0.82	15.68	1.59	0.34
					W't'd mean...		0.65
					a. d.		0.328
					A. D.		0.109
Leucine	43.2	18	*d* 3.08	0.14	2.90	0.08	0.28
	48.0	18	*d* 3.56	0.14	3.22	0.24	0.75
	59.5	20	*d* 4.08	0.20	3.99	—0.07	—0.16
	63.9	20	*d* 4.40	0.14	4.29	0.01	0.02
	64.9	20	*d* 4.35	0.20	4.36	—0.17	—0.35
R	82.1	24	*e* 5.45	0.26	5.44	—0.21	—0.29
	84.3	20	*d* 5.94	0.14	5.66	0.18	0.29
	116.4	22	*c* 8.05	0.22	7.99	—0.12	—0.12
R	127.1	24	*e* 8.36	0.26	8.43	—0.29	—0.26
	187.2	22	*c* 12.80	0.22	12.85	—0.23	—0.15

[1] One-half cc. of methyl acetate used.

TABLE III *continued*).

Substance.	Wt. subst. in mg.	Time in hrs.		Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
						W't'd mean...		—0.07
						a. d.....		0.254
						A. D.....		0.080
Glycylglycine.....	46.2	18	*d*	3.14	0.10	3.06	0.02	0.07
	72.7	20	*d*	4.98	0.16	4.81	0.05	0.09
	80.0	18	*d*	5.56	0.10	5.29	0.21	0.39
	111.6	20	*d*	7.56	0.16	7.38	0.06	0.07
						W't'd mean...		0.16
						a. d.....		0.120
						A. D.....		0.060
Leucylglycine.....	100.6	25	*c*	5.00	0.22	4.88	—0.06	—0.06
R.....	115.8	19	*e*	5.45	0.11	5.35	0.03	0.04
(Alc. R).....	122.7	26	*e*	5.80	0.26	5.54	0.04	0.03
(Alc. R).....	125.5	26	*e*	5.95	0.26	5.66	0.07	0.06
R.....	138.8	19	*e*	6.42	0.11	6.41	—0.06	—0.06
R.....	171.5	19	*e*	8.07	0.11	7.92	0.08	0.07
	209.9	25	*c*	10.20	0.22	10.19	—0.19	—0.10
						W't'd mean...		—0.01
						a. d.....		0.060
						A. D.....		0.023
Glycylleucine R.....	80.9	20	*e*	3.73	0.24	3.74	—0.21	—0.36
R.....	90.9	20	*e*	4.20	0.24	4.21	—0.21	—0.32
	107.5	24	*c*	5.28	0.22	5.15	—0.05	—0.05
	118.2	24	*c*	5.79	0.22	5.66	—0.05	—0.05
						W't'd mean...		—0.17
						a. d.....		0.143
						A. D.....		0.072
Diglycylglycine R.....	119.2	20	*e*	5.33	0.11	5.39	—0.13	—0.15
	128.5	23	*c*	6.16	0.38	6.02	—0.20	—0.18
R.....	129.1	20	*e*	5.83	0.11	5.84	—0.08	—0.09
R.....	131.0	20	*e*	6.06	0.26	5.92	—0.08	—0.08
R.....	151.4	20	*e*	6.83	0.11	6.85	—0.09	—0.08
	193.2	23	*c*	9.18	0.38	9.05	—0.21	—0.13
R.....	211.9	20	*e*	9.78	0.26	9.58	—0.02	—0.01
						W't'd mean..		—0.12
						a. d.....		0.044
						A. D.....		0.017
Aspartic acid.....	83.8	19	*c*	14.62	0.12	10.51	4.03	6.68
	158.1	19	*c*	25.20	0.12	19.83	5.29	4.65
	20 cc.[1]	22	*f*	10.14	0.24	8.09	1.85	3.44
	40 cc.[1]	21	*f*	19.00	0.12	16.19	2.73	2.66
						W't'd mean...		4.18
						a. d.....		1.23
						A. D.....		0.62

[1] The amount of amino acid present in these solutions was determined by titrating a number of portions; the mean values are used in column 6.

TABLE III (*continued*).

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
Glutamic acid R.....	151.5	22	*e* 19.84	0.18	17.78	1.92	1.58
	157.5	21	*c* 20.85	0.12	18.02	2.75	2.15
	308.3	21	*c* 38.40	0.12	35.27	3.05	1.25
	15 cc.[1,2]	44	*a* 18.30	0.14	14.36	3.84	1.60
	20 cc.[2]	22	*a* 20.30	0.15	19.15	1.04	0.65
					W't'd mean...		1.40
					a. d.....		0.40
					A. D.....		0.18

TABLE IV.—RESULTS OBTAINED WITH ETHYL BUTYRATE AND AMINO ACIDS AND POLYPEPTIDES.

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
Glycine.....	59.5	24	*b* 7.78	0.12	7.54	0.16	0.28
	75.1	17	*d* 9.06	0.04	8.79	0.27	0.57
	78.8	19	*b* 10.30	0.19	9.98	0.17	0.28
	101.0[3]	44	*a* 15.28	0.09	15.14	0.09	0.04
	106.0	17	*d* 12.61	0.04	12.41	0.20	0.30
R.....	113.0	23	*e* 13.24	0.08	13.07	0.13	0.14
	146.1	19	*b* 19.06	0.19	18.51	0.40	0.36
	146.7	24	*b* 19.04	0.12	18.59	0.37	0.26
R.....	212.4	23	*e* 24.91	0.08	24.57	0.30	0.17
					W't'd mean...		0.25
					a. d.....		0.107
					A. D.....		0.036
Alanine R.....	68.5	19	*e* 6.82	0.04	6.54	0.28	0.59
	71.1	17	*d* 6.56	0.04	6.53	0.03	0.07
R.....	82.6	19	*e* 8.49	0.04	7.84	0.65	1.13
	118.5	17	*d* 10.92	0.04	10.89	0.03	0.04
					W't'd mean...		0.42
					a. d.....		0.403
					A. D.....		0.202
Phenylalanine.....	46.2	23	*d* 2.48	0.10	2.49	—0.07	—0.18
	46.2	17	*d* 2.76	0.16	2.49	0.15	0.52
R.....	54.1	25	*e* 2.95	0.08	2.88	0.03	0.06
	77.6	17	*d* 4.48	0.16	4.19	0.17	0.35
	89.5	43	*a* 6.25	0.35	5.98	—0.04	—0.02
	90.4	18	*d* 5.03	0.04	4.88	0.15	0.25
	90.7	18	*d* 5.08	0.04	4.89	0.19	0.31
	113.2	23	*d* 6.10	0.10	6.11	—0.07	—0.07
	125.0	43	*a* 8.76	0.35	8.35	0.10	0.04

[1] One-half cc. of methyl acetate used.

[2] The amount of amino acid present in these solutions was determined by titrating a number of portions; the mean values are used in column 6.

[3] One-half cc. of ethyl butyrate used.

TABLE IV (*continued*).

Substance.	Wt. subst. in mg.	Time in hrs.		Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
						W't'd mean...		0.13
						a. d.....		0.192
						A. D.....		0.064
Leucine.....	38.5	18	*d*	2.78	0.20	2.58	0.04	0.16
	44.7	18	*d*	3.08	0.20	3.00	—0.16	—0.54
	48.7	20	*d*	3.24	0.10	3.27	—0.09	—0.25
R.....	50.1	24	*e*	3.99	0.08	3.32	0.63	1.43
	61.6	20	*d*	4.36	0.16	4.13	0.11	0.24
	65.0	22	*d*	4.16	0.09	4.36	—0.25	—0.47
	65.7	20	*d*	4.44	0.10	4.41	—0.03	—0.06
	83.0	20	*d*	5.90	0.16	5.57	0.21	0.34
	95.0	22	*d*	6.12	0.09	6.38	—0.31	—0.40
R.....	95.6	24	*e*	6.60	0.08	6.34	0.22	0.29
	115.9	22	*c*	7.94	0.12	7.96	—0.10	—0.10
	117.3	22	*c*	7.96	0.12	8.05	—0.17	—0.17
						W't'd mean...		0.01
						a. d.....		0.373
						A. D.....		0.108
Glycylglycine.....	45.7	18	*d*	3.18	0.14	3.02	0.06	0.20
	61.5	18	*d*	4.20	0.14	4.07	0.03	0.07
	87.6	20	*d*	5.96	0.04	5.79	0.17	0.26
	108.2	20	*d*	7.25	0.04	7.15	0.10	0.16
						W't'd mean...		0.13
						a. d.....		0.069
						A. D.....		0.034
Leucylglycine (Alc. R)....	109.3	26	*e*	5.08	0.08	4.93	0.11	0.11
R.....	109.7	19	*e*	5.24	0.04	5.07	0.17	0.22
	117.5	25	*c*	5.78	0.12	5.70	0	0
	144.6	25	*c*	7.12	0.12	7.02	0.02	0.01
R.....	161.5	19	*e*	7.70	0.04	7.46	0.24	0.21
R.....	194.2	19	*e*	9.22	0.04	8.97	0.25	0.19
(Alc. R).....	285.3	26	*e*	13.02	0.08	12.87	0.11	0.04
						W't'd mean...		0.11
						a. d.....		0.081
						A. D.....		0.031
Glycylleucine.....	88.3	24	*c*	4.43	0.12	4.23	0.12	0.15
R.....	116.6	20	*e*	5.69	0.09	5.40	0.24	0.28
R.....	121.9	20	*e*	5.79	0.09	5.64	0.10	0.11
	222.8	24	*c*	10.92	0.12	10.67	0.17	0.08
						W't'd mean...		0.14
						a. d.....		0.058
						A. D.....		0.029
Diglycylglycine R.....	96.9	20	*e*	4.68	0.04	4.38	0.30	0.42
R.....	110.5	20	*e*	5.12	0.04	5.00	0.12	0.15
R.....	116.1	20	*e*	5.51	0.04	5.25	0.26	0.31
R.....	117.8	20	*e*	5.65	0.08	5.33	0.28	0.33

TABLE IV (*continued*).

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
R.....	125.5	20	*e* 6.23	0.08	5.67	0.52	0.57
	159.4	23	*c* 7.54	0.14	7.46	—0.02	—0.01
	196.5	23	*c* 9.23	0.14	9.20	—0.07	—0.04
					W't'd mean...		0.21
					a. d.....		0.190
					A. D.....		0.072
Aspartic acid.....	91.0	19	*c* 12.77	0.10	11.44	1.27	1.94
	97.1	19	*c* 13.31	0.10	12.18	1.07	1.53
R.....	138.9	22	*e* 18.41	0.08	17.62	0.75	0.67
R.....	146.4	22	*e* 19.34	0.08	18.57	0.73	0.62
	20 cc.[1]	22	*f* 8.66	0.08	8.09	0.53	0.99
	40 cc.[1]	21	*f* 16.79	0.09	16.19	0.55	0.54
					W't'd mean...		0.95
					a. d.....		0.438
					A. D.....		0.179
Glutamic acid R.....	128.4	22	*e* 15.66	0.08	15.07	0.55	0.53
R.....	178.0	22	*e* 21.22	0.08	20.89	0.29	0.20
	185.9	21	*c* 22.60	0.10	21.26	1.28	0.87
	247.9	21	*c* 29.90	0.10	28.35	1.49	0.76
	15 cc.[1,2]	44	*a* 15.11	0.09	14.36	0.70	0.29
	20 cc.[1]	22	*a* 19.40	0.08	19.15	0.21	0.13
					W't'd mean...		0.50
					a. d.....		0.255
					A. D.....		0.104

TABLE V.—RESULTS OBTAINED WITH OLIVE OIL AND AMINO ACIDS AND POLYPEPTIDES.

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
Glycine.....	33.3	24	*b* 4.36	0.04	4.22	0.14	0.44
	65.3	19	*b* 8.36	0.04	8.27	0.09	0.18
	69.3	17	*d* 8.28	0.07	8.12	0.13	0.30
	73.0	17	*d* 8.68	0.07	8.55	0.10	0.22
	87.1	18	*d* 10.36	0.04	10.20	0.16	0.28
	101.0[3]	44	*a* 15.42	0.06	15.14	0.26	0.13
	125.5	24	*b* 16.04	0.04	15.90	0.14	0.12
	128.2	19	*b* 16.52	0.04	16.24	0.28	0.29
R.....	131.8	21	*e* 15.24	0.08	15.25	—0.05	—0.05
R.....	138.5	21	*e* 16.38	0.08	16.02	0.32	0.30
	141.1	18	*d* 16.60	0.04	16.52	0.08	0.09
R.....	176.2	23	*e* 20.69	0.07	20.39	0.27	0.18

[1] The amount of amino acid present in these solutions was determined by titrating a number of portions; the mean values are used in column 6.

[2] One-half cc. of ethyl butyrate used.

[3] One-half cc. olive oil as emulsion used.

TABLE V (*continued*).

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.		Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
						W't'd mean...		0.18
						a. d.....		0.098
						A. D.....		0.028
Alanine R.....	35.8[1]	19	*e*	3.39	0.08	3.40	—0.05	—0.20
R.....	58.7[1]	19	*e*	5.65	0.08	5.57	0.04	0.10
	84.3	17	*d*	7.90	0.07	7.75	0.12	0.23
	120.5	17	*d*	11.20	0.07	11.08	0.09	0.12
						W't'd mean...		0.11
						a. d.....		0.112
						A. D.....		0.056
Phenylalanine.....	50.5[1]	21	*f*	2.28	0.08	2.41	—0.17	—0.49
	60.9	17	*d*	3.48	0.04	3.29	0.19	0.50
	77.0	23	*d*	4.15	0.06	4.15	—0.02	—0.03
	82.8	18	*d*	4.64	0.15	4.47	0.06	0.11
	95.8	43	*a*	6.86	0.24	6.40	0.26	0.14
	97.4	18	*d*	5.44	0.15	5.26	0.07	0.11
R.....	105.6[1]	25	*e*	5.65	0.07	5.63	—0.01	—0.01
	165.4	43	*a*	11.18	0.24	11.05	—0.05	—0.02
						W't'd mean...		0.04
						a. d.....		0.174
						A.D		0.061
Leucine.....	31.8	20	*d*	2.23	0.06	2.13	0.08	0.34
	55.8	20	*d*	3.71	0.06	3.74	—0.05	—0.12
	67.5	22	*d*	4.45	0.16	4.53	—0.20	—0.36
	70.8	22	*d*	4.90	0.16	4.75	0.03	0.05
	74.3	20	*d*	4.98	0.04	4.99	—0.01	—0.02
R.....	90.8[1]	24	*e*	6.05	0.07	6.02	0	0
	98.5	22	*c*	6.95	0.10	6.76	0.13	0.16
	99.8	22	*c*	7.03	0.10	6.85	0.12	0.14
						W't'd mean...		0.02
						a. d.....		0.155
						A. D.....		0.055
Glycylglycine.....	32.7	18	*d*	2.24	0.04	2.16	0.08	0.37
	51.3	18	*d*	3.43	0.15	3.39	—0.07	—0.20
	60.4	18	*d*	4.06	0.04	3.99	0.07	0.17
	108.4	18	*d*	7.26	0.15	7.17	—0.02	—0.03
						W't'd mean...		0.04
						a. d.....		0.195
						A. D.....		0.097
Leucylglycine R.....	125.8[1]	19	*e*	5.80	0.08	5.81	—0.05	—0.06
R.....	127.0	19	*e*	5.87	0.08	5.87	—0.04	—0.05
	137.3	25	*c*	6.82	0.15	6.67	0.04	0.03
(Alc. R).....	149.8[1]	26	*e*	7.03	0.07	6.76	0.24	0.17
R.....	180.1[1]	19	*e*	8.39	0.08	8.32	0.03	0.02
	187.0[1]	21	*f*	8.04	0.08	7.85	0.15	0.12

[1] One-half cc. olive oil as emulsion used.

TABLE V (*continued*).

Substance.	Wt. subst. in mg.	Time in hrs.	Cc. NaOH required. Subst. ester water.	Ester water.	Calc. for subst.	Subst. ester.	Cc. 0.1 *N* NaOH for 0.1 gm. subst. for 24 hrs.
	192.0[1]	21	*f* 8.25	0.08	8.06	0.15	0.11
	209.6	25	*c* 10.32	0.15	10.18	0.03	0.02
(Alc. R).....	275.8[1]	26	*e* 12.70	0.07	12.45	0.22	0.08
					W't'd mean...		0.06
					a. d.....		0.064
					A. D.....		0.021
Glycylleucine.....	103.3	24	*c* 5.12	0.10	4.95	0.11	0.12
	104.7[1]	20	*e* 4.79	0.08	4.84	—0.09	—0.12
	119.9	24	*c* 5.96	0.10	5.74	0.16	0.15
	216.0[1]	20	*e* 10.19	0.08	9.99	0.16	0.10
					W't'd mean...		0.07
					a. d.....		0.084
					A. D.....		0.042
Diglycylglycine R.....	55.0[1]	20	*e* 2.50	0.08	2.49	—0.03	—0.08
R.....	77.4[1]	20	*e* 3.42	0.08	3.50	—0.12	—0.21
R.....	79.8[1]	20	*e* 3.65	0.08	3.61	0	0
R.....	105.6[1]	20	*e* 4.95	0.07	4.77	0.15	0.19
R.....	133.3[1]	20	*e* 6.22	0.07	6.03	0.16	0.16
	154.3	23	*c* 7.41	0.08	7.22	0.15	0.11
	158.8[1]	20	*f* 6.45	0.08	6.43	—0.02	—0.02
	173.9[1]	20	*f* 7.07	0.08	7.04	—0.01	—0.01
	183.6	23	*c* 8.73	0.08	8.60	0.09	0.06
					W't'd mean...		0.04
					a. d.....		0.100
					A. D.....		0.033
Aspartic acid.....	91.2	19	*c* 11.73	0.04	11.44	0.29	0.44
	132.0	19	*c* 16.60	0.04	16.56	0.04	0.04
	20 cc.[2]	22	*f* 8.59	0.06	8.09	0.48	0.89
	40 cc.[2]	21	*f* 16.63	0.08	16.19	0.41	0.40
					W't'd mean...		0.39
					a. d.....		0.230
					A. D.....		0.115
Glutamic acid R.....	159.6[1]	22	*e* 18.54	0.08	18.73	—0.23	—0.18
R.....	179.7[1]	22	*e* 21.02	0.08	21.09	—0.11	—0.08
	217.0	21	*c* 25.40	0.04	24.82	0.58	0.34
	243.1	21	*c* 28.30	0.04	27.80	0.50	0.26
	15 cc.[1,2]	44	*a* 14.65	0.06	14.36	0.27	0.11
	20 cc.[2]	22	*a* 19.30	0.05	19.15	0.14	0.09
					W't'd mean...		0.11
					a. d.....		0.146
					A. D.....		0.060

[1] One-half cc. olive oil as emulsion used.

[2] The amount of amino acid present in these solutions was determined by titrating a number of portions; the mean values are used in column 6.

TABLE VI.—SUMMARY OF RESULTS OBTAINED WITH ESTERS AND AMINO ACIDS AND POLYPEPTIDES.

(Amounts of acid produced in equivalents $\times 10^{-4}$ in 24 hours at 38°.)

	Methyl acetate.	Ethyl butyrate.	Olive oil.
Glycine	0.02±0.026	0.25±0.036	0.18±0.028
Alanine	0.09±0.026	0.42±0.202	0.11±0.056
Phenylalanine	0.65±0.109	0.13±0.064	0.04±0.061
Leucine	—0.07±0.080	0.01±0.108	0.02±0.055
Glycylglycine	0.16±0.060	0.13±0.034	0.04±0.097
Leucylglycine	—0.01±0.023	0.11±0.031	0.06±0.021
Glycylleucine	—0.17±0.072	0.14±0.029	0.07±0.042
Diglycylglycine	—0.12±0.017	0.21±0.072	0.04±0.033
Aspartic acid	4.18±0.62	0.95±0.18	0.39±0.12
Glutamic acid	1.40±0.18	0.50±0.10	0.11±0.06

4. Discussion of Results.

The results given in detail in Tables III–V are summarized in Table VI in which the weighted means and average deviations of these means for each set of results are given for the equivalents $\times 10^{-4}$ of acid produced from the ester heading columns 2, 3, and 4 by 0.1 gram of the amino acid or polypeptide in the first column in 24 hours at 38°. The actions caused by 0.1 gram of substance instead of by equivalents are given since the experimental results corresponded more closely to the former.

Glycine and alanine show the greatest amount of action with ethyl butyrate and least with methyl acetate. Phenylalanine on the other hand shows a markedly greater action with methyl acetate, less with ethyl butyrate, and least with olive oil. Leucine gave practically no action with any of the three esters. Glycylglycine gave the same slight action with methyl acetate and ethyl butyrate but none with olive oil. With leucylglycine, glycylleucine, and diglycylglycine, maximum, though small, actions were obtained with ethyl butyrate, very slight but distinct with olive oil, while with glycylleucine and diglycylglycine and methyl acetate, negative values were obtained. Not enough measurements were made with the former to make this last result certain. A possible explanation for these two results may be the comparatively large correction of the ester-water blank experiments which may not be applicable directly to the polypeptide-ester-water mixture. With aspartic and glutamic acids, the order of magnitude of action is methyl acetate, ethyl butyrate, olive oil. Considerably greater action was caused by the aspartic acid than by the glutamic acid as would be expected from the greater ionization constant of the former. A comparison of these actions with the action of the mixture of glycine and acetic acid containing the same amount of carboxyl groups is of interest. From some results which will be communicated later, it was found that the hydrolytic action of this mixture corresponded very closely to that of the glutamic acid solution.

5. Conclusions.

The hydrolyses of the esters by the amino acids and polypeptides which are described in this paper are in themselves not unexpected. The interesting feature of these actions is however their selective charactor. With amino acids which differ from each other to such a small extent as glycine and phenylalanine, and with the similar esters methyl acetate and ethyl butyrate, glycine has the greater action on ethyl butyrate, and phenylalanine on methyl acetate. The greater action of the dipeptides on ethyl butyrate and of the dibasic amino acids on methyl acetate is also of interest. This selective action with different esters is strongly suggestive of the selective action of lipases from different sources with different esters. It seems probable that many of these selective actions of the lipases may be reproduced with amino acids and polypeptides of varying structure or in the presence of other substances.

On the other hand, there is no evidence that the hydrolytic action of lipase is to be attributed to amino acids or polypeptides. The specific groupings present in the amino acids or polypeptides which show this activity may be present in more complex substances such as the proteins, and from this point of view the study of the hydrolytic actions of the decomposition products, such as the amino acids from preparations possessing lipolytic activity, and of the more complex polypeptides or other substances synthesized from them, may throw light upon the substances capable of causing such lipolytic action. The study of the influence of various added substances upon these hydrolytic actions is a necessary accompaniment of an investigation of this nature.[1]

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NEW BOOKS.

The Elements of Qualitative Chemical Analysis, with Special Consideration of the Application of the Laws of Equilibrium and of the Modern Theories of Solution. By JULIUS STIEGLITZ, Professor of Chemistry in the University of Chicago. Two Volumes. Vol. I, Parts I and II. Fundamental Principles and their Application. pp. x + 312. Vol. II, Parts III and IV. Laboratory Manual. pp. viii + 151. New York: The Century Co. 1911. Price, I, $1.40; II, $1.20.

The first volume of this text on qualitative analysis represents the greatest advance in the presentation of the principles of analytical chemistry which has been made since the publication in 1894 of the first edition of Ostwald's "Wissenschaftliche Grundlagen der Analytischen Chemie."

[1] A large number of experiments were carried out in which glycine, alanine, phenylalanine, and tyrosine, in the presence and absence of acids and alkalis in solutions of known H^+ ion concentration, and also in the presence of phosphates, were dissolved with cane sugar in water and the optical rotations determined at stated intervals. No evidence was obtained in any of these experiments to show that the amino acid affected the course of the hydrolysis of the cane sugar in any way.