colorless and melts at 145–147° rather than 148°, the recorded melting point.

The experiments described above were repeated, the purified starting material being used. The yield of the free base was increased to 73% and of salophen to 80%.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 313.]

ACTION OF PANCREATIC ENZYMES UPON CASEIN.

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The fact that our purified pancreatic amylase preparations show proteolytic activity also[1] has led us to extend this phase of our enzyme investigation to a comparative study of the hydrolysis of casein by various preparations derived from the pancreas.

The data summarized in Table I permit quantitative comparison of the proteolytic activities (1) of high grade commercial pancreatins, (2) of the 3 principal fractions recovered from the pancreatin in the course of making from it the pancreatic amylase preparations previously described,[1] (3) of the most active trypsin which we have found commercially available.

In each case the proteolytic activity was determined by allowing from 0.25 mg. to 2.0 mg. of the enzyme preparation to act upon 1 g. of casein (weighed air dry and containing 135 to 140 mg. of nitrogen) in a slightly alkaline solution: hydrogen ion concentration $= 1 \times 10^{-8.3}$; or $P_H = 8.3$ (Sörensen). The methods used in measuring the extent of hydrolysis were those described in our previous paper,[2] of which the ones chiefly employed in this investigation are, (1) the determination of the total nitrogen of digestion products which have passed through the early proteose stage, (2) the nitrogen converted to the amino form as determined by the Van Slyke method.

Pancreatins 6 and 7 were high grade commercial preparations representing practically the whole gland freed from water and fat. These formed the starting point for the laboratory preparations.

Residue 78 (N 14) was the material remaining when pancreatin was extracted once with 9 times its weight of 50% alcohol (as the first step in the process of preparing pancreatic amylase) the residue being dried by washing with alcohol and ether.

"Sac precipitate" is the material which settles out of the amylase solution during the dialysis in 50% alcohol which precedes the final precipitation of the amylase preparation as previously described.[3]

[1] Sherman and Schlesinger, THIS JOURNAL, **34**, 1110 (1912); **37**, 1306 (1915); Sherman and Neun, *Proc. Soc. Expt. Biol. Med.*, **15**, 55 (1918).

[2] Sherman and Neun, THIS JOURNAL, 38, 2199 (1916).

[3] Sherman and Schlesinger, *Loc. cit.*

TABLE I.—PROTEOLYTIC ACTIVITIES OF VARIOUS PREPARATIONS FROM PANCREAS.

Nature of preparation	Weight. Mg.	Time of Action Hrs.	Total nitrogen digested. Mg.	% of total.	$k \times 10^4$.*	Nitrogen digested to amino form. Mg.	% of theory.	$k \times 10^4$.
Pancreatin 6	1	1	13.7	10.6	8	1.9	2.0	1.5
		2	26.7	20.7	8	3.0	3.2	1.2
		3	36.9	28.7	8	4.8	5.2	1.3
Pancreatin 6	2	1/2	14.1	10.4	16	..	..	..
Pancreatin 7	1	1	14.2	10.5	8	3.4	3.5	2.6
		2	26.1	19.3	8	5.0	5.2	1.9
Residue 78 (N. 14)	1	1	12.4	9.2	7	3.1	3.2	2.4
		2	25.1	18.6	7	3.7	3.8	1.4
Sac Precipitate 78 (N. 14)	0.25	1/2	31.6	23.4	39	3.9	4.0	5.9
		1	56.0	41.5	39	6.5	6.7	5.0
		2	94.5	70.0	44	12.0	12.4	4.8
Sac Precipitate 78 (N. 14)	0.5	1/2	61.0	45.2	87	9.4	9.7	14.8
		1	96.1	71.2	90	15.3	15.8	12.5
		2	121.3	89.9	83	20.3	20.9	8.5
Sac Precipitate 78 (N. 14)	1.0	1/2	94.0	69.6	173	11.0	11.4	17.4
		1	120.2	89.1	160	17.8	18.4	14.7
		2	131.9	97.7	137	24.2	25.0	10.4
Amylase Prep. 60	1	1/2	29.4	21.7	35	1.9	2.0	2.9
		1	56.0	41.3	39	4.3	4.5	3.3
		2	89.8	66.2	39	8.7	9.0	3.4
Amylase Prep. 62	1	1/2	42.9	31.4	55	3.1	3.2	4.6
		1	75.5	55.2	58	7.3	7.4	5.6
		2	106.2	77.7	54	11.9	12.2	4.7
Amylase Prep. 63	0.5	1/2	17.7	13.1	20	..	..	..
Amylase Prep. 63	1.0	1/2	32.9	24.4	40	..	..	..
Amylase Prep. 63	2.0	1/2	64.0	47.4	93	..	..	..
Amylase Prep. 64	0.5	1/2	16.7	12.4	19	..	..	..
Amylase Prep. 64	1	1/2	33.5	24.8	41	..	..	..
Amylase Prep. 64	2	1/2	59.4	44.0	84	..	..	..
Trypsin I	0.5	1/2	13.6	10.1	15	2.0	2.1	3.0
	1.0	1/2	28.3	21.0	34	3.3	3.4	5.0
	2.0	1/2	55.2	41.0	76	5.4	5.6	8.3

Amylase preparations 60, 62, 63, 64 were typical "purified pancreatic amylase preparations" as prepared in this laboratory at intervals during the past seven years.

The proteolytic activities of the various preparations may be approximately compared by finding the relative amounts required to digest

* From the formula $k = 1/t \log 1/1 - x$, in which t equals time in minutes and logarithms are to the base 10.

the same amount of nitrogen in the same time, or the relative times required to digest the same amount of nitrogen, as shown in our previous paper, provided the time is not too long and the amount of digested nitrogen selected as a basis of comparison is not too large a fraction of the substrate. The value of k in the formula, $k = 1/t \log 1/1 - x$, also serves as a means of comparing the proteolytic activities even though we are not dealing with a single unimolecular reaction.

By such comparisons it may be seen from the data (more particularly those for "total nitrogen digested") given in Table I that the pancreatic amylase preparations have proteolytic activity about 5 times that of the high grade pancreatins from which they were prepared, and equal to or somewhat higher than that of our best commercial trypsin. The residue remaining after extraction of pancreatin with 50% alcohol has a proteolytic power about equal to that of the pancreatin itself.

The sac precipitate shows 15 to 20 times the proteolytic activity of the pancreatin from which it was made, and 3 to 4 times that of the pancreatic amylase preparation or of the best commercial trypsin which has come to our notice.

In order that the comparisons of the proteolytic activities of the amylase preparations, the sac precipitate and the high grade commercial trypsin might be as conclusive as possible 3 different methods of measuring the extent of the hydrolysis have been employed and determinations made at fixed intervals for several hours in each case.

Table II shows the total nitrogen of digestion products for (1) sac precipitate, (2) amylase preparation, (3) trypsin I, (4) pepsin I. In the case of the sac precipitate data are given for the hydrolysis produced both by 1.0 mg. and by 0.25 mg. of this preparation upon 1 g. of casein in order to compare more closely its activity with that of the other preparations. In each of the other 3 preparations, 1.0 mg. was employed in each case.

It will be noted that in these experiments with more prolonged periods of digestion the sac precipitate and amylase preparation show the same relation to the trypsin in activity as in the experiments summarized in Table I, while as compared with high grade commercial pepsin their proteolytic powers are still more striking. Attention may also be called to the fact that with the sac precipitate, the amylase preparation and the trypsin the rate of hydrolysis (value of k) is approximately maintained up to a point at which 90 to 95% of the substrate has been digested whereas with pepsin the rate of hydrolysis of the casein diminishes steadily, k being reduced to one-half its initial value before one-half of the substrate is digested. In this as in other respects the proteolytic activities of the pancreatic amylase preparation and its by-product the sac precipitate are distinctly of the tryptic rather than the peptic type.

TABLE II.—COMPARISON OF PROTEOLYTIC ACTIVITIES AS MEASURED BY THE TOTAL NITROGEN OF DIGESTION PRODUCTS.

	Sac precipitate 78 (N. 14) 1.0 mg.			Sac precipitate 78 (N. 14) 0.25 mg.			Pancreatic amylase 62. 1.0 mg.			Trypsin I. 1.0 mg.			Pepsin I. 1.0 mg.		
Time. Hrs.	Nitrogen. Mg.	% of total.	$k\times10^4$.	Nitrogen. Mg.	% of total.	$k\times10^4$.	Nitrogen. Mg.	% of total.	$k\times10^4$.	Nitrogen. Mg.	% of total.	$k\times10^4$.	Nitrogen. Mg.	% of total.	$k\times10^4$.
1/2....	94.0	69.9	173	31.6	23.5	39	42.9	31.4	55	36.1	26.2	44	19.8	14.7	23
1......	120.2	89.4	160	56.0	41.6	39	75.5	55.2	58	61.4	44.5	43	31.2	23.1	19
2......	131.9	98.1	137	94.5	70.3	44	106.2	77.7	54	98.4	71.3	45	44.4	32.9	14
3......	132.7	98.7	98	...	..	..	119.1	87.1	49	119.1	86.3	48	55.6	41.2	13
4......	132.7	98.7	74	122.1	90.8	43	127.3	93.1	48	128.4	93.0	48	62.3	46.2	11
6......	134.5	100	..	128.8	95.8	38	133.2	97.4	44	133.4	96.6	40	73.9	54.8	10
8......	(133.8)	...	..	130.4	97.0	32	134.5	98.4	37	135.8	98.4	36	79.8	59.2	8

TABLE III.—COMPARISON OF PROTEOLYTIC ACTIVITIES BY MEASUREMENTS OF THE NITROGEN CONVERTED TO AMINO FORM.

	Sac precipitate 78 (N. 14). 1.0 mg.			Sac precipitate 78 (N. 14). 0.25 mg.			Pancreatic amylase 62. 1.0 mg.			Trypsin I. 1.0 mg.			Pepsin I. 1.0 mg.		
Time. Hrs.	Amino nitrogen. Mg.	Amino nitrogen × 100 ÷ Total N of substrate.	Amino nitrogen × 100 ÷ Total N of filtrate.	Amino nitrogen. Mg.	Amino nitrogen × 100 ÷ Total N of substrate.	Amino nitrogen × 100 ÷ Total N of filtrate.	Amino nitrogen. Mg.	Amino nitrogen × 100 ÷ Total N of substrate.	Amino nitrogen × 100 ÷ Total N of filtrate.	Amino nitrogen. Mg.	Amino nitrogen × 100 ÷ Total N of substrate.	Amino nitrogen × 100 ÷ Total N of filtrate.	Amino nitrogen. Mg.	Amino nitrogen × 100 ÷ Total N of substrate.	Amino nitrogen × 100 ÷ Total N of filtrate.
1/2...	11.0	8.1	11.7	3.9	2.9	12.2	3.1	2.3	7.2	3.0	2.2	8.4	0.7	0.5	3.6
1.....	17.8	13.2	14.8	6.5	4.8	11.6	7.3	5.4	9.6	6.5	4.8	10.6	1.6	1.2	5.0
2.....	24.2	17.9	18.3	12.0	8.9	12.7	11.9	8.8	11.2	10.9	8.0	11.0	3.2	2.3	7.1
3.....	26.2	19.4	19.7	..	..	..	13.9	10.3	11.6	14.1	10.4	11.8	3.8	2.8	6.9
4.....	28.2	20.9	21.3	23.2	17.2	19.0	16.7	12.4	13.1	17.3	12.8	13.4	4.5	3.4	7.3
6.....	31.8	23.5	23.6	26.0	19.2	20.0	19.5	14.5	14.7	19.6	14.5	14.7	5.2	3.9	7.1
8.....	36.1	26.7	27.0	26.3	19.5	20.1	22.4	16.6	16.6	22.0	16.3	16.2	6.1	4.5	7.8
48.....	..	..	..	..	..	..	..	..	..	..	..	..	10.6	7.9	9.8

TABLE IV.—COMPARISON OF PROTEOLYTIC ACTIVITIES AS MEASURED BY THE ACIDITY OF DIGESTION PRODUCTS.

Time. Hrs.	Sac precipitate 78 (N. 14). 1.0 mg.			Sac precipitate 78 (N. 14). 0.25 mg.			Pancreatic amylase 62. 1.0 mg.			Trypsin I. 1.0 mg.			Pepsin I. 1.0 mg.		
	Cc. 0.1 *M* NaOH.	% of maximum.	$k \times 10^4$.	Cc. 0.1 *M* NaOH.	% of maximum.	$k \times 10^4$.	Cc. 0.1 *M* NaOH.	% of maximum.	$k \times 10^4$.	Cc. 0.1 *M* NaOH.	% of maximum.	$k \times 10^4$.	Cc. 0.1 *M* NaOH.	% of maximum.	$k \times 10^4$.
1/2	13.3	48.7	97	3.4	13.3	21	5.7	23	38	4.4	18.2	28	1.8	11.1	17
1	18.1	66.3	79	6.9	27.1	23	10.6	43.1	41	8.2	33.5	28	2.8	18.0	14
2	21.4	78.1	55	13.2	51.8	26	15.5	62.9	36	13.6	55.7	30	4.5	28.6	12
3	24.8	90.5	57	17.0	66.5	26	20.0	81.2	40	18.0	73.8	30	5.8	37.0	11
4	26.9	98.3	74	18.8	73.6	24	22.0	90.0	42	19.6	80.2	27	6.8	42.9	10
6	27.35	100.0	..	19.6	76.7	18	23.9	97.1	32	22.8	93.5	28	8.9	54.5	9
8	(26.8)	...	..	22.4	87.9	19	24.5	100.0	..	24.5	98.3	27	9.3	59.2	8

TABLE V.—ACTION OF 1.0 MG. OF TRYPSIN I UPON 1.0 GRAM OF CASEIN IN PRESENCE OF THYMOL.

Time. Hrs.	Total nitrogen of filtrate.			Amino nitrogen.						Acidity.		
	Weight. Mg.	% of total 48 hrs.*	$k \times 10^4$.*	Weight. Mg.	% of total 48 hrs.*	$k \times 10^4$.*	% of theory.†	$k \times 10^4$ (theory).†	Amino N / Total N	Cc. 0.1 *M* NaOH.	% of total 48 hrs.*	$k \times 10^4$.*
1	59.1	46.1	45	5.5	19.4	16	6.0	4.4	9.3	8.0	30.1	26
2	86.6	67.6	41	10.9	38.6	18	11.8	4.6	12.6	13.5	50.9	26
3	97.7	76.2	35	14.2	50.3	17	15.4	4.0	14.5	16.1	60.7	23
4	104.2	81.2	30	16.3	57.9	16	17.8	3.5	15.7	18.0	68.1	21
5	107.0	83.4	26	18.1	64.2	15	19.7	3.2	16.9	19.0	71.8	18
6	112.5	87.8	25	19.7	69.7	14	21.4	2.9	17.5	20.4	77.2	18
7	113.4	88.5	22	20.4	72.4	13	22.2	2.6	18.0	20.4	77.0	15
8	115.7	90.2	21	21.5	76.3	13	23.4	2.4	18.6	21.3	80.4	15
9	117.6	91.7	20	21.5	76.2	12	23.4	2.1	18.3	22.1	83.3	14
10	119.0	92.8	19	22.3	79.2	11	24.3	2.0	18.8	22.8	86.0	14
12	118.1	92.1	15	22.3	79.2	9	24.3	1.7	18.9	23.9	90.2	14
15	119.0	92.8	13	24.1	85.3	9	26.2	1.5	20.2	24.5	92.6	13
18	120.8	94.2	11	25.5	90.4	9	27.8	1.3	21.1	25.0	94.4	12
21	121.3	94.6	10	26.6	94.2	10	28.9	1.2	21.9	25.4	95.9	11
24	122.6	95.6	9	25.2	89.3	8	27.4	1.0	20.6	25.6	96.8	10
48	128.2	100.0	..	28.2	100.0	..	30.7	0.6	22.0	26.5	100.0	..

TABLE VI.—ACTION OF 1.0 MG. OF PANCREATIC AMYLASE PREPARATION 60 UPON 1.0 GRAM CASEIN IN PRESENCE OF THYMOL.

Time. Hrs.	Total nitrogen of filtrate. Weight. Mg.	% of total 48 hrs.*	$k \times 10^4$.*	Amino nitrogen. Weight. Mg.	% of total 48 hrs.*	$k \times 10^4$.*	% of theory.†	$k \times 10^4$ theory.†	Amino N / Total N	Acidity. Cc. 0.1 *M* NaOH.	% of total 48 hrs.*	$k \times 10^4$.*
1.....	53.6	40.6	38	4.7	13.9	11.0	5.0	3.7	8.8	7.6	28.8	25
2.....	83.9	63.6	37	8.0	23.4	10.0	8.4	3.2	9.5	13.3	50.2	25
3.....	99.7	75.5	34	12.3	36.1	11.0	13.0	3.4	12.3	16.1	60.7	23
4.....	107.5	81.5	30	14.8	43.3	10.1	15.6	3.1	13.7	18.6	70.1	22
5.....	111.2	84.2	27	17.4	51.1	10.0	18.4	2.9	15.6	19.3	73.0	19
6.....	114.0	86.3	24	16.8	49.2	8.0	17.7	2.4	14.7	19.9	75.0	17
7.....	113.6	86.0	20	19.2	56.4	9.0	20.3	2.3	16.9	21.2	80.1	17
8.....	117.2	88.8	20	20.9	61.4	9.0	22.1	2.3	17.8	21.7	82.1	16
9.....	119.6	90.6	19	21.7	63.6	8.0	22.9	2.1	18.1	22.4	84.5	15
10.....	118.1	89.5	16	23.3	68.4	8	24.6	2.0	19.7	22.8	86.0	14
12.....	120.9	91.6	15	23.9	70.0	7	25.2	1.8	19.7	23.8	89.7	14
15.....	123.7	93.6	13	25.7	75.3	7	27.1	1.5	20.7	24.4	92.2	12
18.....	126.0	95.4	12	27.6	81.2	7	29.2	1.4	21.9	25.1	94.6	12
21.....	126.6	95.9	11	27.4	80.4	6	28.9	1.2	21.6	26.0	98.0	14
24.....	126.5	95.8	10	28.7	84.4	6	30.4	1.1	22.7	26.3	99.3	10
48.....	132.0	100.0	..	34.1	100.0	..	36.0	0.7	25.8	26.5	100.0	..

* These columns show the yields of total nitrogen of filtrate, of amino nitrogen and of acidity of digestion products, expressed as percentage, and the rate of digestion expressed as value of k, both calculated on the basis of the yield at the end of 48 hours as sufficiently approximating the completion of the process. Since, however, the transformation into amino nitrogen does not cease at this point it is calculated also on the basis explained in the next note.

† The yield of amino nitrogen and its rate of formation are here computed on the basis of a "theoretical" yield of 71.7% of the total nitrogen of casein in amino form as obtained on complete acid hydrolysis by Osborne and Guest (*J. Biol. Chem.*, **9**, 333 (1911)).

Table III compares the same 4 preparations as in Table II, but by measurements of the amount of nitrogen digested to the amino form instead of by total nitrogen of digestion products.

The data of Table III show that with the formation of amino nitrogen as the criterion of proteolytic activity, the amylase preparation again shows practically the same proteolytic power as the high grade commercial trypsin while the sac precipitate shows four to five times the activity of the trypsin.

Unlike the total nitrogen of digestion products, the production of amino nitrogen had not nearly reached its maximum at the end of 8 hours, except in the case in which the larger amount of sac precipitate was used. Since the formation of amino nitrogen continues after all the substrate has been converted into soluble digestion products, the ratio of amino to total nitrogen of the filtrate steadily rises.

At any given stage in the digestion, *i. e.*, when the same percentage of the nitrogen of the substrate has reached the amino form, the ratio of amino nitrogen to total nitrogen of the filtrate is about the same under the influence of the different proteases here compared.

In Table IV the same materials are compared as in Tables II and III but by the method of titrating the total acidity of digestion products, as described in our previous paper. The opalescence of the filtrate at an advanced stage of hydrolysis makes the titration very difficult, which accounts for the apparent irregularities in the experiment with 1.0 mg. of sac precipitate. In this case the region of unsatisfactory titrations was reached within an hour of the beginning of the hydrolysis.

The data of Table IV show the same general relationships among the activities of the four enzyme preparations as do those of Tables II and III.

For the sake of completeness we have also compared pancreatic amylase preparation with trypsin in respect to proteolytic activity as shown by increase of electrical conductivity, and by change of optical rotatory power, of the substrate solution. By each of these methods as well as by the 3 methods more fully described above, the pancreatic amylase preparation was found to show fully as high proteolytic activity as did our best commercial trypsin.

In none of the experiments summarized in Tables II, III and IV was any antiseptic used. Tables V and VI show the data of experiments in which trypsin I and pancreatic amylase preparation 60, respectively, were allowed to act for a longer time upon casein substrate in the presence of thymol as antiseptic.

Comparison of the data of these two tables will show that, with the exception of a few slight irregularities which are doubtless due to experimental error, the action of the two enzymes was practically identical throughout.

Summary.

The laboratory preparations here described were made from high grade commercial pancreatin.

Extraction of the pancreatin with 50% alcohol leaves a residue having about the same proteolytic activity as the original pancreatin.

The sac precipitate obtained during dialysis in 50% alcohol in the course of purification of pancreatic amylase had 15 times the proteolytic activity of the original high grade pancreatin and about 4 times that of the most active commercial trypsin which we have seen.

The final preparation of pancreatic amylase purified as described in previous papers from this laboratory has proteolytic activity fully equal to that of the high grade trypsin when tested by any of the 5 methods used for the measurement of proteolytic power.

Experiments designed to throw light upon the relation between the amylolytic and proteolytic activities of this product will be described in our next paper.

We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

NEW YORK CITY.

NEW BOOKS.

The Chemical Constitution of the Proteins. Part I. Analysis. By R. H. A. PLIMMER. Pp. xii + 174. Third Edition. Longmans, Green & Co., London, 1917. Price, $1.80.

One of the advantages alleged for the series of Monographs on Biochemistry edited by Plimmer and Hopkins, of which this volume forms a part, was the possibility of issuing new editions on each topic as rapidly as the progress in that field of the science might necessitate. In accordance with the demands of the workers in physiological chemistry it thus becomes possible to procure an up-to-date presentation of their special branches without the necessity of securing a re-issue of such chapters in the science as have not experienced equally rapid changes. The plan of publication has justified itself, if one may judge by the character of the successive editions of Dr. Plimmer's parts. The present one differs from Part I of the second edition, published in 1912, in being concerned with the *analysis* of proteins. The description of the amino acids, which is essentially cyclopedic in character and less subject to change than is the present conception of the detailed composition of the proteins and the methods applicable to its study, is now relegated to a separate part. The earlier editions have been reviewed in THIS JOURNAL. It is only necessary to add, therefore, that in the 1917 volume the important contributions of five years on the subject of the hydrolysis of proteins and the isolation and estimation of their constituent groups are included. Especially prominent is the technic of