itate on the addition of phosphotungstic acid. If it does precipitate it will be calculated as histidine.

6. Increases in the insoluble humin N due to prolonged hydrolysis we regard as due to carbonization.

ST. PAUL, MINN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF IOWA.]

SOME NITROGENOUS AUXOAMYLASES.

BY ELBERT W. ROCKWOOD.

Received August 18, 1917.

For some time it has been known that starch-splitting enzymes were made more active by some component of serum. Thus Chittenden and Ely[1] found that the hydrolysis of glycogen and starch by saliva was increased by peptones. Chittenden and Cummins[2] found that the action of malt diastase was stimulated by peptones and albumins.

Langley and Eves[3] ascribed this to the binding of sodium carbonate by peptone and found that other proteins acted similarly, myosin, alkali and acid metaproteins, egg albumin and blood serum. Wohlgemuth[4] extracted from serum a heat-stable activating principle and thought it might be a lipoid. Pozerski[5] obtained similar results with salivary and pancreatic amylases by the action of intestinal juice and press-juice from macerated glands and from serum. He believed that salts of serum were the activating agents, though possibly proteins or their decomposition products.

In the following experiment the effect of various nitrogen compounds have been tested as regards their ability to increase the hydrolytic activity of the amylases of the saliva on boiled starch. Those which do increase the activity of the starch-splitting enzyme are here called auxoamylases.

The substrate was 10 g. of cornstarch, moistened, boiled 15 minutes and diluted to a liter. The saliva was diluted with water and filtered before adding to the starch solution. The saliva percentages refer to the volume of saliva in the diluted solution which was added to the starch.

The digestions were carried out in flasks suspended in a water bath heated to 38° by an electric heater. Only those digested at the same time and with the same saliva and starch are compared. Ordinarily, 200 cc. were used and in all cases 5 cc. of toluene were added. 25 cc. samples were removed at intervals by a pipet and, after the addition of an excess of Fehling's solution, were heated for an hour on a steam bath or boiled

[1] Chittenden and Ely, *Am. Chem. J.*, 4, 107 (1882).

[2] Chittenden and Cummins, *Trans. Conn. Acad.*, 7 (1885).

[3] Langley and Eves, *J. Physiol.*, 4, 18 (1883).

[4] Wohlgemuth, *Biochem. Z.*, 33, 303 (1911).

[5] Pozerski, *Thesis*, Inst. Pasteur, Paris, 1902; *Compt. rend. soc. biol.*, 55, 429.

on a sand bath. The precipitated cuprous oxide was separated by an asbestos filter and after washing was dissolved on the filter with nitric acid (1 : 2). The nitrous acid was expelled by boiling, after adding a little powdered talc and a small crystal of urea. The solution was made slightly alkaline with ammonia, then acidified with acetic acid. An excess of KI crystals was added.

$$2Cu(NO_3)_2 + 4KI = Cu_2I_2 + 4KNO_3 + I_2,$$

and the iodine titrated with 0.1 *N* sodium thiosulfate.

In the following protocolls no assumption is made as to whether the decomposition product of the starch is a reducing dextrin, maltose, dextrose or other sugar, but the degree of hydrolysis is expressed by the reducing power of the mixture. This is here given in terms of cc. of 0.1 *N* thiosulfate used for 25 cc. of the digestive mixture; this corresponds to the precipitated cuprous oxide.' That is, the figures represent the relative amounts of starch hydrolyzed. Inasmuch as different salivas were used, one series is not strictly comparable with another. Since the reaction so markedly influences the action of the enzyme, care was taken to have the reaction of the solutions, after testing, the same as that of the control or standard. A neutral litmus solution was used as the indicator. The concentration of this indicator was the same in the solutions under comparison, and the same neutral color was maintained in all during digestion.

Typical protocolls follow, with some conclusions drawn from them.

Action of α-Amino Acids.—Of the aliphatic series glycocoll was tested; of the aromatic series, tyrosine. Some results are appended.

No. 30.—Glycocoll.

190 cc. of 1% starch solution with 10 cc. of 2% saliva.

Glycocoll used.	0.5 hour.	1.5 hours.	4 hours.	5.5 hours.
None	3.9	9.7	16.1	18.8
0.1 g	6.0	11.3	17.2	20.4
0.3 g	9.0	12.8	18.7	21.2
0.5 g	8.2	16.5	22.2	23.3

No. 35.—Tyrosine.

180 cc. of 1% starch solution, 10 cc. of 2% saliva, water to 200 cc.

Tyrosine used.	40 min.	1.75 hrs.	4 hrs.	6.25 hrs.	24 hrs.
None	1.3	5.4	9.7	11.2	18.6
0.05 g	2.9	6.2	11.3	13.7	22.3
0.1 g	3.4	6.6	12.1	13.7	24.0

The tyrosine was dissolved by boiling with the starch solution and cooled before adding the saliva.

The amino acids which contain the amino group in the α position with reference to the carboxyl, both of the aliphatic and the aromatic series,

are thus shown to act as auxoamylases with respect to ptyalin when present in the proportion of 1 : 2000–4000.

Action of the Amides.—Acetamide, propionamide and urea were tested.

No. 40.—ACETAMIDE.

180 cc. of 1% starch, 10 cc. of 3% saliva, water to 200 cc.

Amide used.	1 hr.	2 hrs.	4 hrs.	8 hrs.	24 hrs.
None	4.5	8.6	15.1	17.9	25.0
0.5 g	4.2	8.9	14.9	17.0	24.5

No. 26.—PROPIONAMIDE.

180 cc. of 1% starch solution, 20 cc. of 2.5% saliva.

Amide used.	0.3 hour.	1.3 hrs.	3.3 hrs.	5.3 hrs.
None	6.1	15.8	21.6	22.3
0.5 g	5.9	16.0	20.7	22.6

No. 38.—UREA.

180 cc. of 1% starch solution, 10 cc. of 1% saliva, 0.25 g. of urea, water to 200 cc.

	2 hrs.	4 hrs.	6 hrs.	24 hrs.
Standard	1.8	3.5	4.0	9.1
Urea	2.1	3.6	4.0	9.1

It is evident that when the amino group replaces the hydroxyl of the carboxyl group of acetic, propionic and carbonic acids the compounds formed do not act as auxoamylases, that is, the amino radical in this position is inactive.

That the effect is not due to the aliphatic anion is shown below:

No. 130.

100 cc. of 1% starch solution, 25 cc. of 1% saliva, 5 cc. water and acetate or propionate 0.01 *M*.

	1 hr.	2 hrs.	4 hrs.	6 hrs.
Standard	1.6	3.7	7.1	7.8
Sodium acetate	1.6	3.2	6.9	7.9
Sodium propionate	1.6	2.9	6.2	7.8

The stimulating effect is consequently due not to the acid radical but to the amino group. The influence of this group varies with its position.

Action of Compounds whose Amino Radical is not in or Adjacent to the Carboxyl.—Anthranilic acid (*o*-aminobenzoic acid) whose amino radical is in the benzene ring, not in the side chain, was used in different amounts.

No. 42.—ANTHRANILIC ACID.

(Neutralized by NaOH.)

180 cc. of 1% starch solution, 10 cc. of 3% saliva, water to 200 cc.

Anthranilic acid used.	1 hr.	3 hrs.	5 hrs.	25 hrs.
None	5.5	12.2	15.1	23.9
0.1 g	7.1	14.3	17.1	25.8
0.3 g	8.3	15.6	18.5	25.9
0.5 g	8.8	16.4	18.7	25.1

The amino radical is shown to increase the activity of the ptyalin even if it is not in the α position.

To determine whether the position of the amino radical in the benzene ring modifies the action of the auxoamylase the isomers of anthranilic acid were employed, the amino radical of these being in the meta and para position, respectively, with reference to the carboxyl group.

No. 45.—Isomers of Aminobenzoic Acid.
(Neutralized by NaOH.)
180 cc. of 1% starch solution, 10 cc. of 3% saliva, 0.3 g. of amino benzoic acid, water to 200 cc.

	1 hr.	3 hrs.	5 hrs.	7 hrs.	24 hrs.
Standard	3.6	6.0	10.8	11.5	20.9
Ortho	5.1	9.6	13.7	16.4	25.8
Meta	5.2	9.2	14.8	16.1	25.4
Para	4.5	9.2	13.5	16.2	26.4

All these isomers are auxoamylases and the position of the amino radical does not cause any variation in their activity.

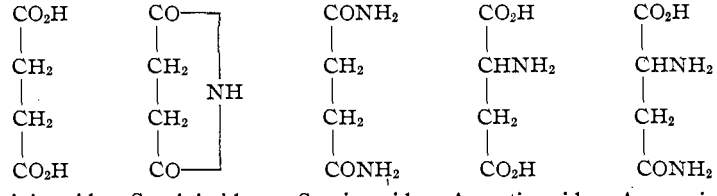
Effect of the Introduction of Other Radicals into Amino Compounds.—Sulfanilic acid, in which the carboxyl group of the *p*-aminobenzoic acid is replaced by the sulfonic acid radical, was tested.

No. 25.—Sulfanilic Acid.
(*p*-Anilinsulfonic acid neutralized by NaOH.)
180 cc. of 1% starch solution, 20 cc. of 2.5% saliva.

Acid used.	0.5 hr.	1.5 hrs.	4 hrs.	6 hrs.
None	7.4	14.3	18.9	20.8
1.0 g	7.5	14.6	19.8	21.0

The effect of the amino radical upon the ptyalin is inhibited by the sulfonic acid radical and the compound does not act as an auxoamylase.

Effect of Derivatives of Succinic Acid.—The nitrogen derivatives of succinic acid furnish a series for testing the effect of varying configuration upon the amylase, an α amino acid, an acid amide, a combination of the two and an imide.



Succinic acid. Succinimide. Succinamide. Aspartic acid. Asparagin.

No. 133.
100 cc. of 1% starch solution, 25 cc. of 1% saliva, asparagin and aspartic acid, respectively, 0.01 *M*.

	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
Standard	0.6	1.5	4.1	5.6	12.6
Asparagin	0.4	0.8	3.2	4.6	10.4
Aspartic acid	1.5	3.9	7.6	9.4	14.3

No. 124.

112 cc. of 1% starch solution, 6.25 cc., 3% saliva, water to 125 cc., succinimide 0.01 *M*.

	1 hr.	2 hrs.	4 hrs.	6 hrs.
Standard	1.6	3.6	6.5	9.7
Succinimide	1.7	3.9	6.5	9.5

No. 152.

100 cc. of 1% starch solution, 25 cc. of 1.5% saliva, succinic acid and succinamide, respectively, 0.01 *M*.

	1 hr.	2 hrs.	4 hrs.	6 hrs.
Standard	6.8	9.8	13.7	15.4
Succinic acid	7.5	11.3	15.1	16.1
Succinamide	6.2	9.6	14.4	15.3

Here, as with the monobasic acids, the α-amino derivative acts as an auxoamylase and the acid amide has no such effect. In asparagin, which is both an α-amino acid and an amide, the amide group appears to overcome the effect of the α-amino group. The imide is inactive. The nitrogen-free acid may have some effect, though it is not well marked.

Effect of Substitution Products of NH_2.—Hippuric acid (C_6H_5CO-OH HNHCH_2CO_2H) was taken as a type.

No. 24.

180 cc. of 1% starch solution, 1.2 g. of hippuric acid (neutralized), 10 cc. of 3% saliva, water to 200 cc.

Acid used.	1 hr.	1.5 hrs.	4.5 hrs.	5.5 hrs.	23 hrs.
None	8.4	14.5	14.9	17.4	21.7
1.2 g.	12.2	16.9	18.1	24.8	26.8

Therefore substituting the benzoyl group in place of hydrogen of the amino group does not destroy the stimulating effect of the glycocoll.

The Action of Proteins as Auxoamylases Compared with that of Their Proteolytic Products.—In the early experiments of Chittenden and others quoted above,[1] amylolysis was shown to be increased when proteins were present. If the active agent is the amino group the starch cleavage should be greater with increasing hydrolysis of the protein, that is, with the increase in the number of free amino groups. To test this theory two proteins, serum albumin and gelatine, were hydrolyzed with acid and the activity of equivalent amounts of the hydrolyzed and unhydrolyzed substances were compared.

Serum albumin was boiled with 10% sulfuric acid until there was no biuret reaction. The mixture was neutralized and an amount equivalent to 0.5 g. of the original dry albumin was used. Parallel with this was run a digestion containing 0.5 g. of albumin, also a standard digestion without protein.

Gelatine was boiled 3 hours with 10% sulfuric acid and tested in the same manner.

[1] *Loc. cit.*

No. 48.

100 cc. of 1% starch solution, 10 cc. of 3% saliva, 20 cc. protein solution (or water in standard).

	1 hr.	2.5 hrs.	4.5 hrs.	6.5 hrs.
Standard	1.8	4.6	7.3	8.6
0.5 g. albumin	16.1	21.8	24.7	25.0
0.5 g. hydrolyzed albumin	19.2	24.1	27.8	24.7

No. 47.

200 cc. 1% starch solution, 10 cc. 3% saliva, 0.5 g. gelatine or its cleavage products, water to 240 cc.

	1 hr.	2 hrs.	4 hrs.	6 hrs.
Standard	5.1	8.9	13.2	15.3
Gelatine	9.5	15.5	21.8	21.4
Hydrolyzed gelatine	11.7	16.0	18.1	22.2

Although there is a discrepancy in the fourth hour the hydrolyzed gelatine appears to have a greater power of activation than the gelatine itself.

Effect of Amines of the Methane Series.—These are evidently very active. That the activity is dependent upon the nitrogen and not related to the weight of the molecule seems proved by the substantial agreement in the amounts of starch digested in presence of the hydrochlorides of methylamine, CH_3NH_2, ethyl amine, $C_2H_5NH_2$, diethylamine, $(C_2H_5)_2NH$, and trimethylamine, $(CH_3)_3N$.

No. 143.

100 cc. of 1% starch solution, 25 cc. of 1.5% saliva, 6 cc. water, hydrochlorides of amines 0.01 *M* (in starch-saline mixture). The mixture was neutral to litmus.

	2 hrs.	4 hrs.	6 hrs.
Standard	8.5	13.0	15.0
Methylamine	21.6	24.4	24.4
Ethylamine	21.2	23.8	24.4
Diethylamine	21.6	23.9	24.9

No. 150.

Proportions taken as in No. 143.

	2 hrs.	4 hrs.	6 hrs.
Standard	8.4	12.0	13.4
Monomethylamine	17.2	19.0	19.5
Diethylamine	18.3	18.4	18.4
Trimethylamine	18.3	18.8	19.7

The Amylase of the Pancreas.—A few tests have been made with the pancreatic amylase.

No. 22.—Glycocoll.

180 cc. of 1% starch solution.

A. With 1 cc. extract of macerated dog's pancreas.

	30 min.	1 hr. 40 min.	2 hrs. 20 min.
No glycocoll	14.6	23.9	24.8
0.5 g. glycocoll	21.1	27.4	30.9

B. With 5 cc. pancreas extract.

	1 hr. 40 min.	2 hrs. 20 min.
No glycocoll	28.7	29.1
0.5 g. glycocoll	32.2	35.9

No. 118.—Aspartic Acid.

The pancreatic juice was from a dog, obtained by a fistula and preserved with a little glycerine. 2 cc. were used in 200 cc. of 1% starch solution and aspartic acid to 0.01 *M*.

	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
Standard	1.1	1.6	2.6	4.9	6.8
Aspartic acid	1.6	2.7	4.4	6.2	10.6

The action of the amino acids, then, as far as has been tried, is similar to that on ptyaline.

I am indebted to J. D. Boyd for part of the analytical work.

Conclusions.

1. α-Amino acids increase the power of the salivary ferment to hydrolyze boiled starch; that is, they act as auxoamylases.

2. This is true of both acyclic and cyclic compounds. Of the cyclic compounds both tyrosine, where the amino group is in the side chain, and the amino benzoic acids, where it is in the benzene ring, are active.

3. The salts of the acids from whence the amino acids are derived, such as acetic and propionic, are not auxoamylases; that is, it is the amino nitrogen which is the activating agent.

4. The position of the amino group in the benzene ring with reference to the carboxyl group does not appear to cause any difference in activity. Thus the three isomers of amino benzoic acid stimulate the salivary enzyme, and to the same degree.

5. The acid amides, acetamide, propionamide, urea, etc., do not increase the hydrolytic power of the amylase, that is, when the amino group is substituted in the carboxyl radical an auxoamylase is not formed. An amino group in the carboxyl radical also destroys the effect of an amino group elsewhere in the molecule, as shown in asparagin.

6. The sulfonic acid radical when introduced into an amino compound instead of the carboxyl group (even when neutralized) destroys the stimulating effect of the amino group, as illustrated by sulfanilic acid.

7. Replacement of one hydrogen of the amino group of glycocoll, as in hippuric acid, does not destroy its stimulating action. An imide, however (succinimide), is not an auxoamylase.

8. The proteins act as auxoamylases toward ptyalin because of their nitrogen content. As the number of free amino groups is increased by hydrolysis their activity also increases. Thus, the effect of the cleavage products of albumin and gelatin is greater than that of the proteins before hydrolysis.

9. The amino acids appear to act as auxoamylases toward the pancreatic enzyme also. Hence the amino acids produced in the intestine by digestive proteolysis will act as hormones in starch digestion and this factor should be taken into account in the study of normal digestion.

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[CONTRIBUTION FROM THE DIVISION OF LABORATORIES AND RESEARCH, NEW YORK STATE DEPT. OF HEALTH.]

PREPARATION OF A PRESERVATIVE FROM CRESOL.

BY MARY NEVIN AND BORIS MANN.

Received July 6, 1917.

Schering's "Trikresol" has been extensively used in this country as a preservative for biological products such as antitoxins, vaccines, etc. Since the beginning of the European war, however, no more "Trikresol" being available, our laboratory has been compelled to find a substitute of the same quality.

Commercial cresol is a mixture of all three isomeric cresols—ortho, meta and para. The boiling point of *o*-cresol is 188–190.8°, *m*-cresol 201–202.8°, and *p*-cresol 198–201.8°. Nördlinger[1] states that *o*-cresol is less poisonous than its isomers. Working on the supposition that the lower fractions of commercial cresol contained mostly *o*-cresol, we prepared a product with the boiling point 190–193° from Cresol U. S. P. The toxicity of this preservative, which until recently was used in our laboratory in the place of "Trikresol," was 0.00045 g. per body weight of a white mouse and the germicidal power was 1 : 150 in 5 minutes and 1 : 180 in 10 minutes.

In order to get a more desirable product and to confirm our supposition as to the toxicity of different fractions of cresol we decided to subject commercial cresol to a more thorough fractional distillation and to investigate the different fractions as to their toxicity and germicidal power.

Commercial cresol was distilled over a free flame and the distillate collected while the thermometer in the vapor recorded 188–205°.

First Fractional Distillation.—1000 cc. of this product were distilled, giving:

Fractional distillate.	Boiling point.	Quantity in cc.	Specific gravity.
1	188–192°	25	...
2	192–196°	250	1.039
3	196–199°	400	1.035
4	199–204°	250	1.030

Owing to the small amount of the first distillate no further work was done with it.

[1] Allen's "Commercial Organic Analysis," 3, 313.