grams of the isoamylurethane were treated, in alkaline solution, with 0.7 gram of chlorocarbonic ethyl ether. A heavy red oil separated which would not solidify until it was distilled *in vacuo* (185–200° at 15 mm. pressure). After several recrystallizations of the distillate the crystals became white and melted at 65–66°. They are insoluble in acids and alkalies, but quite soluble in alcohol, ether, chloroform and benzol. Mixed with the possible isomer, described above, the melting point was not depressed. Evidently in this case a molecular rearrangement occurs as expressed in the following equation:

$$KOC_6H_4NHCOOC_5H_{11} + ClCOOC_2H_5 \longrightarrow KCl + C_2H_5OOCOC_6H_4NHCOOC_5H_{11} \longrightarrow C_5H_{11}OOCOC_6H_4NHCOOC_2H_5.$$

The final product is carbamyl *o*-hydroxyphenylethylurethane.

Summary.—The work here outlined, together with that previously reported, shows that when two carboxyl radicals (COOR and $COOR_1$) are introduced into the molecule of *o*-aminophenol the lighter one becomes attached to nitrogen, the position not being influenced by the order in which the groups are introduced; and that to accomplish this, a molecular rearrangement occurs in one case. This is also true when both of the radicals are carbonyls (COR and COR_1). In case one radical is carbonyl and the other carboxyl the latter becomes attached to nitrogen without being influenced by the relative weights of the entering groups. The hope that the introduction of radicals of nearly the same weight (C_6H_5CO and $C_5H_{11}COO$) would result in the formation of isomeric substances was not realized, the velocity of the rearrangement being, apparently, almost instantaneous in every case. Consequently the mechanism of the rearrangement is still in doubt. It is possible that there is equilibrium of the two isomeric forms, one always being in too small amounts to be isolated, but there is little evidence to support this view. Work already begun with the orthoaminomercaptans may still throw light upon the problem.

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[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF COLUMBIA UNIVERSITY AND THE HARRIMAN RESEARCH LABORATORIES. NO. 227.]

A STUDY OF THE CHEMICAL CONSTITUTION OF INVERTASE.

[FIRST PAPER.]

BY J. M. NELSON AND SIDNEY BORN.

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Since enzymes play such important roles in most chemical reactions occurring in plants and animals, any knowledge as to their true chemical constitution is of obvious importance. In a study of this kind it is desirable to select an enzyme which can be had in comparatively large amounts and can be easily purified. Such an enzyme is yeast invertase. Pressed

yeast can be obtained from breweries and the invertase can be isolated by autolytic or extraction methods.

The constitution of invertase has already received considerable attention. In this connection may be mentioned the work of O'Sullivan and Tompson[1] who were probably the first to come to any important conclusions. Their results indicate invertase to be a water soluble substance made up of a carbohydrate and an "albuminoid" or protein. The latter seemed identical with the protein obtained from yeast itself. Another investigator, Salkowski[2] regarded the polysaccharide in invertase as an impurity which he called "yeast gum." He doubted the protein character of the nitrogenous part, because of its weak response to the Millon, xanthoproteic and biuret tests. Wroblewski[3] came to similar conclusions regarding the carbohydrate part, but differed with Salkowski in believing invertase to contain a very active protein, which he claimed he succeeded in isolating by salting out with ammonium sulfate. Osborne[4] prepared invertase which had a nitrogen content of about 6.1% and which gave Millon, xanthoproteic and biuret tests. In spite of these tests and on the basis of its empirical composition he likens it to chitin. Hafner,[5] showed that invertase contained phosphorus in organic combination, a fact neglected by most investigators. He could not obtain the biuret test, and thought invertase was a complicated substance containing besides phosphorus, a carbohydrate and a nitrogenous group which he was unable to isolate. Euler and his students[6] have succeeded in preparing the most active invertase known thus far ($=0^{\circ}$ = 10 to 20 minutes), and the nitrogen content of which varied from 1.85 to 4.59%. They suggest that invertase closely approaches yeast gum in its chemical constitution, but express no opinion as to the nature of the nitrogenous part. Mathews and Glenn[7] suggest that the active principle in invertase may be protein in character and conclude that the carbohydrate part serves as a colloidal ferment bearer for this active protein.

From the above it can be seen that, so far, very little has been definitely determined concerning the chemical constitution of invertase, except that all products prepared thus far contain a polysaccharide, a nitrogenous substance and phosphorus. The results obtained in this laboratory confirm in general the opinions of O'Sullivan and Tompson. More data was secured showing the nitrogenous part to be protein and the phosphorus to be chemically combined with the carbohydrate.

[1] *J. Chem. Soc.*, **57**, 834 (1890).
[2] *Z. physiol. Chem.*, **31**, 305 (1900).
[3] *J. prakt. Chem.*, **64**, 1 (1901); *Ber.*, **36**, 1134 (1898).
[4] *Z. physiol. Chem.*, **28**, 399 (1899).
[5] *Ibid.*, **42**, 1 (1904).
[6] *Ibid.*, **65**, 124 (1910); **69**, 152 (1910); **72**, 340 (1911); **73**, 335 (1911).
[7] *J. Biol. Chem.*, **9**, 29 (1911).

Preparation of Invertase.—Two hundred pounds of pressed yeast were placed in a barrel, some toluene added to prevent bacterial growth, and the yeast allowed to autolyze, at room temperature, from two to six weeks. At first, a strong fermentation, with the evolution of large amounts of carbon dioxide, took place and later liquefaction. Several types of filter presses and centrifuges were tried for filtering the large amount of liquid, but these were found to be unsuccessful and finally large folded papers with parchmentized tips were used. In this way five to six gallons of a clear yellow liquid were obtained to which was added an equal volume of 95% alcohol. A flocculent yellow precipitate settled out, the alcohol was decanted and the residue dissolved in water. There was left a considerable amount of insoluble matter, probably consisting chiefly of insoluble proteins. To this aqueous extract, alcohol was added. This time the precipitate was gummy instead of flocculent and, after decanting the alcohol, it dissolved almost completely in water, giving an opalescent solution which was filtered when necessary. A concentrated solution of lead acetate was then added with stirring, until practically no more precipitate formed, care being taken to avoid an excess of the reagent. After filtering off the lead precipitate, potassium oxalate was added to throw down the lead remaining in the solution, and after filtering, the filtrate was treated with kaolin and stirred constantly for an hour. The kaolin treatment was generally repeated two or three times. The resulting liquid was placed in collodion bags, containing some toluene, and dialyzed in running water from three to twenty days. After dialysis, the invertase, as O'Sullivan and Tompson pointed out, could no longer be precipitated by the addition of several volumes of alcohol alone, and it was necessary to add also some electrolyte to destroy the colloidal solution. For this purpose a small amount of sodium chloride, potassium acetate or acetic acid was found satisfactory. A mechanical stirrer was found useful in hastening the coagulation of the precipitate at this point. The gummy precipitate was ground in a mortar with absolute alcohol, whereupon the precipitate gradually changed into a white powder which was easily filtered on a suction filter and finally washed with absolute alcohol and ether. This product was then dried in a vacuum over sulfuric acid for several days. From two hundred pounds of yeast about two hundred grams of invertase were obtained.[1]

The above method of isolation and purification was adopted since it corresponded more closely with those used by such other investigators as O'Sullivan and Tompson, Euler, and Mathews and Glenn, both in procedure and composition of the preparations. It really amounts to a purification of the yeast gum or carbohydrate part, and experiments

[1] The authors wish to thank Messrs. C. W. Otto and L. Siegler for their valuable assistance in some of the experimental work.

are being conducted now, in this laboratory, to see whether it is advisable, or not, to change this form of procedure.

Properties of Invertase.—The invertase obtained by the above method was a white powder, soluble in water, and giving a slightly opalescent solution. It gave the biuret, Millon and xanthoproteic reactions for proteins, when sufficient material was used. Mathews and Glenn had great difficulty in obtaining the biuret test, and Hafner could not obtain it at all. These reactions are important in view of the fact that the preparations were as low in nitrogen content as that of any other investigator and the activity ($\pm 0^\circ$ = 37 minutes at 37°) was comparable with that of O'Sullivan and Tompson, and Mathews and Glenn.

The water solution gave no precipitate with phosphotungstic acid, mercuric acetate, nor lead acetate, and could not be salted out by ammonium, magnesium or zinc sulfates. It did not coagulate on heating. The average preparations had a nitrogen content of 1.3%; ash, 0.9%; and phosphorus, 0.3%.

Analysis of the Invertase Preparations.—The samples were dried at 70° to constant weight. The ash values were obtained with a special ashing burner at low red heat; the phosphorus was obtained by sodium carbonate and potassium nitrate fusion, dissolving in nitric acid, precipitating first with molybdate and finally with magnesia mixture and weighing as magnesium pyrophosphate; the nitrogen was determined by the ordinary Dumas method.

Activity of the Invertase Preparations.—This was determined according to the method of O'Sullivan and Tompson, subsequently used by Euler and his co-workers.[1] The principle underlying this method is the determination of the length of time required by a given amount of invertase to perform a definite amount of work, under standard conditions of temperature, concentrations of cane sugar, invertase and hydrogen ion. This amount of work is expressed as the time in minutes in which a definite amount of invertase reduces the rotation of 100 times its weight of cane sugar to $\pm 0^\circ$.

All determinations were carried out in a constant temperature water bath at 37°, and not varying more than $\pm 0.01^\circ$ and in an optimum hydrogen ion concentration. The rotation was read in a water-jacketed polariscope tube, kept accurately at 35°, and similar to the one used by Hudson.[2] The following determination is given in order to illustrate the method: Fifty milligrams of an invertase preparation were dissolved in 5 cc. of $N/2$ acid sodium phosphate solution and added to 20 cc. of a cane sugar solution, containing 4 grams of the sugar, both solutions being previously warmed to 37° C. At the end of half an hour the inver-

[1] *Z. physiol. Chem.*, **69**, 152 (1910).

[2] THIS JOURNAL, 30, 1564 (1908).

sion was stopped by the addition of 5 cc. of a strong sodium carbonate solution. Rotation of the solution without inversion (Blank) in a 220 mm. tube, temperature 35° and sodium light, was +18.97°, and after inversion +1.25°. Calculating, by means of $\alpha_D^{35°}$ (specific) rotation for cane sugar and invert sugar, the amount of inversion, and then applying the method given by O'Sullivan and Tompson, the activity was found to be $\pm 0° = 37\frac{5}{8}$ minutes at 37°.

The following analytical results were obtained for 9 samples:

No. of sample.	Period of dialysis. Days.	Ash. Per cent.	Nitrogen. Per cent.	Phosphorus. Per cent.	Activity at 37° = 0.[1] Minutes.
1	0	5.25	2.21	....	37
2	3	1.30	1.51	0.27	154
3	3	0.85	1.61	0.27	72
4	7	0.80	1.26	0.26	37
5	7	1.02	1.28	0.27	52
6	10	0.89	1.35	0.27	136
7	14	0.88	1.83	0.29	70
8	..	1.49	1.35	0.32	953
9	..	1.68	1.36	0.30	none

The Chemical Constitution of the Polysaccharide of Invertase.

Schützenberger[2] was the first to isolate yeast gum from yeast by extraction with water and precipitation with alcohol. Hessenland[3] showed that it gave mannose and glucose on hydrolysis. Salkowski[4] prepared it by precipitation from yeast extract with Fehling's solution. Meigen and Spreng[5] found Salkowski's method for isolating the gum the best, and by hydrolysis obtained twice as much mannose as glucose. Euler and Fodor[6] showed this gum was present in invertase preparations, a fact previously shown by Salkowski and Wroblewski,[7] and by hydrolysis found its composition to be between 4 mannose, 3 glucose and 4 mannose and 4 glucose. In the present investigation, the behavior of the gum towards acetolysis has been studied, also the phosphorus content of the acetylated products and the gum itself has been determined.

Preparation of the Yeast Gum.—The yeast gum was prepared from invertase by Salkowski's method.

Twenty grams of invertase were dissolved in 150 cc. of 3% potassium

[1] The degree of activity was not relied upon as a criterion of purity, as many investigators have done heretofore, since this property, besides being dependent upon the composition, is also influenced by the way it is precipitated with alcohol, and very likely many other conditions at the present little understood.

[2] *Bulletin*, [2] **21**, 204 (1874).

[3] *Z. Ver. Rübenzuckerind.*, **42**, 671 (1892).

[4] *Z. physiol. Chem.*, **31**, 305 (1900).

[5] *Ibid.*, **55**, 48 (1908).

[6] *Ibid.*, **72**, 340 (1911).

[7] *J. prakt. Chem.*, **64**, 1 (1901).

hydroxide solution, and warmed on the water bath for half an hour. About 200 cc. of Fehling's solution were then added, the copper gum precipitated and collected in a spongy mass. The excess of Fehling's solution was decanted and the gum washed with water to remove the excess alkali. It was then placed in a mortar, ground up with water and a few drops of concentrated hydrochloric acid, until all had gone into solution. The gum was reprecipitated from this solution by means of alcohol, redissolved in distilled water, and again precipitated by means of alcohol. This last treatment was repeated three times, the gum then put in absolute alcohol, and after it had crumbled to a fine, white powder, was washed with ether and dried in a vacuum over concentrated sulfuric acid. The gum left no ash on ignition, was free from copper and nitrogen, but contained phosphorus, a fact which seems to have been overlooked by most investigators. Phosphorus was determined in the same way as in invertase itself. One sample showed 0.32% phosphorus while another not quite so pure showed 0.23%.

To determine whether any of the phosphorus of the original invertase had been split off by the Fehling solution, the Fehling filtrate from the gum was acidified with hydrochloric acid, treated with hydrogen sulfide to remove copper, filtered, evaporated to dryness, and tested for phosphorus A very slight amount of molybdate precipitate was obtained, but it was so small that magnesia precipitation gave negative results. From these results it seems probable that all the phosphorus is chemically combined with the gum.

Action of Acetyl Bromide on Invertase.—Two grams of invertase, dried *in vacuo* at 55°, were treated with 10 cc. of acetyl bromide in a flask provided with a ground in reflux condenser surmounted by a calcium chloride tube. At first the reaction was rapid with evolution of hydrobromic acid but as it diminished in speed, it seemed best to heat gently on the water bath. Most of it went into solution rapidly, but a small amount required longer treatment. On account of the small amount of the latter, no attempt was made to isolate and to see whether it was different from the rest. After all had dissolved, the brown syrup was poured into ether (ethereal solution, see below), whereupon a yellowish white precipitate settled out. The precipitate was soluble in hot alcohol, and on cooling settled out in what seemed to be microscopic crystals but which, on further examination, were found to be amorphous.[1] This product was reprecipitated from alcohol four or five times. The substance did not reduce Fehling's solution, but gave a white precipitate. It was soluble in acetone, ethyl acetate, and hot alcohol, insoluble in ether. It began to soften at 178° and melted with decomposition at 198°. The substance

[1] In one or two instances we have noticed needles one or two mm. in length adhering to the stirring rod in the beaker.

contained phosphorus. The yield was about 1.5 grams. Upon analysis two samples gave, respectively, the following results:

C = 49.44, 49.03%; H = 6.27, 6.39%; P = 0.31, 0.29%; Acetyl value = 44.44%.

The acetyl values were obtained by dissolving a weighed amount in 50 cc. of methyl alcohol; 50 cc. of $N/10$ sodium hydroxide were then added, allowing the solution to stand over night and titrating with $N/10$ acid in the morning.

The ethereal solution, mentioned above, was washed with ice-cold sodium bisulfite solution, with ice water, with sodium carbonate solution, and again with ice water. It was then dried over calcium chloride and evaporated *in vacuo*. It left a very small amount of a brown syrup, insoluble in ligroin, and soluble in alcohol. So far, no crystals have been obtained from the syrup.

Action of Acetyl Chloride on Invertase.—Invertase was also treated with acetyl chloride, but even at boiling temperature there was very little action. This was to be expected from the behavior of acetyl halides on other carbohydrates.

Action of Acetic Anhydride and Sulfuric Acid on Invertase.—Five grams of invertase, dried *in vacuo* at 55°, were heated on a water bath with 50 cc. of acetic anhydride, to which 0.5 cc. of concentrated sulfuric acid had been previously added. In a short time, the mixture dissolved completely with slight darkening. The crude acetyl derivative was precipitated in light yellow flocks by pouring it into a mixture of one part gasoline and four parts of ether. These were redissolved in hot alcohol, boneblacked, filtered and allowed to cool, when the acetyl derivative usually separated as a snow white amorphous solid; but in one or two instances, we noticed needles 1 or 2 millimeters long adhering to the stirring rod in the beaker. It was very hydroscopic, soluble in acetone, ethyl acetate, hot alcohol. chloroform, slightly soluble in cold alcohol, insoluble in ligroin, gasoline, and ether. It melted with decomposition at 184–198°.

Analysis of two samples gave the following results, respectively:

Acetyl value = 42.5, 42.5%; P = 0.25, 0.28%; no N.[1]

To investigate the nature of the monosaccharides occurring in this acetyl derivative, two grams of the above acetyl derivative were hydrolyzed for 12 hours with 300 cc. of 5% sulfuric acid, neutralized with barium hydroxide, the excess of barium removed with CO_2, filtered, evaporated to small bulk, cooled, phenylhydrazine acetate added, allowed to stand 24 hours in cold, filtered on weighed Gooch, and the crystals washed with cold water. Two grams of acetyl invertase gave 0.8612 gram mannose hydrazone = 0.5741 gram mannose = 28.7% mannose. The filtrate,

[1] Of the many samples of this acetyl derivative examined, all values for phosphorus were between these two values except one which was 0.73%. As there seemed no error in the determination this result is given.

after heating on the water bath, gave a test for glucosazone. Time did not permit direct determination of glucose content, or testing for other monosaccharides.

The above series of experiments show that not only the polysaccharide in invertase contains phosphorus, but also the acetylated compounds derived therefrom.

This very interesting fact, that the phosphorus is combined with a polysaccharide, brings out another class of naturally occurring compounds, the phosphorized polysaccharides. Ford has stated[1] that it is very difficult to obtain starch free from phosphorus, and Kiliani[2] has noted the same fact about inulin. Perhaps these substances in their naturally occurring state are also partially phosphorized polysaccharides. While the acetylated polysaccharide contains almost the same per cent. of phosphorus as the original gum by allowing for the 44% acetyl, the phosphorus content of the polysaccharide itself has been almost doubled, showing that part of the polysaccharide has been split off by the acetolysis, and it is probably this part that goes into the ether solution.

The Chemical Constitution of the Nitrogenous Constituents.

O'Sullivan and Tompson fractionally separated their invertase into a series of seven substances which they called "invertans." The first member contained 8.35% nitrogen but was low in carbohydrates, while the last member contained only 1.05% nitrogen and had a high carbohydrate content. From the member high in nitrogen they isolated a nitrogenous substance, after removing the carbohydrates by precipitation with alkaline copper solution, and found it to be a protein (nitrogen 14%) identical with the protein in yeast itself. Furthermore, they concluded that the invertans consisted of various amounts of carbohydrates and this protein.

Wroblewski, as previously stated, claims to have salted out a very active protein from a Merck's preparation of invertase by means of ammonium sulfate. He gives no analysis, but seems to rely on biuret and Millon tests as an evidence for its being a protein. In this laboratory, it has been found impossible to salt out the protein in this manner. Nearly all the other investigators were unsuccessful in isolating any substance which, upon analysis, had a sufficiently high nitrogen value to be a protein. Moreover many were led to doubt the protein character of this constituent because of the weak biuret, Millon, and xanthoproteic tests.

Hafner[3] attempted, but failed, to isolate the nitrogenous constituent by hydrolysis of invertase with $2^1/_2\%$ sulfuric acid, and subsequent precipitation of the nitrogenous substance with phosphotungstic acid.

[1] *J. Soc. Chem. Ind.*, **23**, 414 (1904).

[2] *Ann.*, **205**, 147 (1888).

[3] *Z. physiol. Chem.*, **42**, 1 (1904).

It was found that, before hydrolysis, a 10% solution of invertase was not precipitated by any of the ordinary protein precipitants, while after hydrolysis with sulfuric acid and neutralization with barium carbonate, copious precipitates were obtained with phospho-molybdic acid, phosphotungstic acid, mercuric acetate, and mercuric chloride. This indicates that in the original invertase the nitrogenous or protein part is either combined with the carbohydrate or the latter acts as a protective colloid. In either event, the properties of the protein are effectually masked in the invertase.

The following series of experiments was made to determine the concentration of sulfuric acid to be used and the time necessary for this hydrolysis, in isolating the nitrogenous substance: Five grams of invertase were placed in each of four Erlenmeyer flasks with 50 cc. of 20, 10, 5 and $2^1/_2$% sulfuric acid, respectively, and immersed in a water bath kept at 100° C. At two-hour intervals, two samples of five cc. each were pipetted from each flask, and cooled to room temperature. To one, 5 cc. of a 10% solution of phosphotungstic acid were added, the other was neutralized with sodium hydroxide and then added to an equal volume of Fehling's solution.

H_2SO_4.	Precipitate with phosphotungstic.				Precipitate with Fehling's solution.			
Per cent.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
20	heavy.	heavy	heavy brown	very brown	none	none	none	none
10	heavy	heavy	heavy yellow	heavy brown	none	none	none	none
5	slight	moderate	heavy	heavy	heavy	moderate	none	none
$2^1/_2$	very slight	slight	moderate	moderate	heavy	moderate	moderate	moderate

The above results show that four hours' hydrolysis with a 10% sulfuric acid gives the best results.

Twenty grams of invertase were hydrolyzed four hours with 200 cc. of 10% sulfuric acid at 100°. The solution was then filtered from a slight amount of insoluble matter, cooled, and phosphotungstic acid added until no more precipitate collected, the precipitate was filtered, and washed with 10% sulfuric acid. The precipitate was then boiled for an hour or so with an excess of barium hydroxide solution, the precipitate of barium sulfate and barium phosphotungstate filtered off, the filtrate saturated with CO_2 to remove barium, solution filtered, evaporated to small bulk (5 cc.), filtered from a slight amount of barium phosphotungstate, and then added to three volumes of absolute alcohol; a flocculent precipitate settled out. This substance was quickly filtered on a platinum Gooch, washed with absolute alcohol and ether and weighed. Yield, about 0.25 gram. The yield was not always as satisfactory. This was due to the difficulties involved in the method, and also to the fact that a quantity of the protein itself was probably hydrolyzed in the preliminary treat-

ment. This protein was soluble in water, not coagulated either by heating or hydrochloric acid, was precipitated by phosphotungstic acid, and gave a very strong biuret reaction. It gave no test for phosphorous after digestion with nitric acid, etc., and no reduction or precipitate with Fehling's solution. It is extremely hydroscopic.

0.0534 gram gave 6.6 cc. N at 25° and 757 mm. = 13.7% N.

Another sample from a different invertase gave:

0.0063 gram gave 0.6 cc. N at 26° and 765 mm. = 10.7% N.

Due to the small amount of sample the latter value is not as trustworthy as the former.

In another experiment 20 grams of invertase were hydrolyzed for four hours with 10% sulfuric acid, the resulting solution neutralized with barium carbonate, filtered, evaporated to half bulk, mercuric acetate added instead of phosphotungstic acid, the flocculent precipitate filtered, washed, decomposed with hydrogen sulfide, solution filtered from the mercuric sulfide, evaporated to about 5 cc., and precipitated with absolute alcohol. A heavy flocculent precipitate was obtained which gave no reduction with Fehling's solution, and no test for phosphorus, but showed a strong biuret reaction. The yield was very poor.

Analysis:

0.0519 gram substance gave 4.8 cc. N at 26° and 757 mm. = 10.2% N.

This result is probably low because it was impossible to separate all the barium carbonate from the protein.

Assuming all the nitrogen to be protein, only about 10% of it could be isolated by either the phosphotungstic acid or mercuric acetate methods. This is probably due to experimental difficulties. Still, it is impossible to say that all of the nitrogen in invertase is protein in character. Although a great number of experiments were made, testing the presence of purines, pyrimidines, and volatile bases, no evidence was obtained that any of the nitrogen was other than protein in character.

Salting out Experiments after Partial Hydrolysis.—After the hydrolysis with 10% sulfuric acid, and neutralization with ammonium hydroxide, small amounts of a gelatinous precipitate were obtained with ammonium sulfate, magnesium sulfate and zinc sulfate, although before hydrolysis these salts gave no precipitate. These precipitates, when filtered, gave strong biuret reactions. The filtrates, however, also gave good biuret reactions, showing that all the protein is not salted out by these reagents.

Twenty grams of invertase were hydrolyzed with 10% sulfuric acid under the same conditions as above, and were then placed in collodion bags and dialyzed for thirty-six hours in running water. At the end of that time, the contents of the bags were tested for protein, but gave negative results, showing that all the protein had dialyzed.

The Action of Enzymes on Invertase.—Wroblewski[1] showed that invertase was not destroyed by the action of trypsin, and Mathews and Glenn found that the gum was not digested by diastase. Similar results were obtained in this laboratory with ptyalin and pancreatic amylase. They were allowed to stand in contact with the gum from invertase over 12 hours, and at the end of that time no reduction with Fehling's solution could be noticed. Castor bean lipase also gave negative results.

The authors wish to take this opportunity to express their thanks and appreciation to the Jacob Ruppert Brewery, New York City, for furnishing such ample amounts of pressed yeast.

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THE PICRATE COLORIMETRIC METHOD FOR THE ESTIMATION OF CARBOHYDRATES.

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The quantitative estimation of sugars and other carbohydrates involves the use of the polarimeter or the oxidizing power of copper solutions; as yet no accurate application of colorimetric methods[2] has been made to carbohydrates.

The method herein described depends upon the formation of a red-to-brown color when solutions of sugars are heated with *sodium carbonate solutions of picric acid.* These colors were first described by Braun;[3] other observers were Jaffé,[4] Johnson[5] and Chapman.[6] All these investi-

[1] *J. prakt. Chem.*, **64**, 1 (1901).

[2] Dubrunfaut devised a method for estimating glucose by comparing the color developed by treating sugar solutions with alkali. Heller (*Archiv.*, 1, 212; 4, 310; *Z. anal. Chem.*, **28**, 650; *Deut. med. Wochschr.*, **1888**, 451) made modifications of this method. Neitzel (*Chem. Ztg.*, **1894**, Rep. 93; *Z. Rübenzucker-ind.*, **1894**, 221) used a modification of Molisch reagent (*Monatsh.*, **7**, 198). Johnson (*Z. anal. Chem.*, **23**, 111; *Brit. Med. J.*, **1883**, 504; *Pharm. J. Trans.*, **54**, 24; *Deut. med. Wochschr.*, **1888**, 451, 479; *Pharm. Prax.*, 1880, 1, 103) used picric acid and potassium hydroxide. Autenreith and workers (*Münch. med. Wochsch.*, **57**, 1780; **58**, 899; **59**, 689) oxidized by Bang's solution (*Biochem. Z.*, **2**, 271; **32**, 443) and estimated colorimetrically the unchanged copper. Järvinsen (*Z. anal. Chem.*, **52**, 14) makes use of Almen-Nylander's reagent (*Ibid.*, **9**, 494; *Z. physiol. Chem.*, 50, 36; *Biochem. Z.*, **16**, 489). For other applications of colorimetry to carbohydrates see Neitzel, *Z. Spiritusind.*, **20**, 163; Ruini, *Gazz. chim. ital.*, **31**, 445; Lyons, *Pharm. Rev.*, **20**, 155; Dennstedt and Voigtländer, *Forschungsb. ü. Lebensmittel*, **2**, 173; Ambuhl, *Chem. Ztg.*, **19**, 1508.

[3] *Z. Anal. Chem.*, **4**, 185; *Chem. Zentr.*, **1866**, 219; **1874**, 825; *J. prakt. Chem.*, **96**, 412. Braun mentions that glucose, fructose and lactose give these colors.

[4] *Z. physiol. Chem.*, 10, 391.

[5] *Pharm. J. and Trans.*, **54**, 24.

[6] *Analyst*, **34**, 475.