sublimed substance consisted of the chlorine compounds of niobium, since it is quite possible that our tantalic oxid was not entirely free from an admixture of niobic oxid. This behavior suggests a method of purification of tantalic acid containing a little niobic acid; we will not lay much stress on this, however, untll we have done more work on the subject.

The readiness with which niobium oxychloride was formed in all our experiments contrasting with the comparatively small quantity of the pentachloride obtained illustrates once again the great inclination niobium has to enter into combination not as an individual element, but in the form of the radical *niobyl*. The action of sulphur and chlorine on NbO, investigated by Delafontaine, the numerous series of oxyfluo-salts prepared by Marignac, and the decomposition of niobyl chloride by magnesium are other facts of the same import. In this respect the analogy of niobium aud vanadium is very striking, but it is almost entirely lacking in niobium and tantalum.

THE CHEMICAL NATURE OF DIASTASE.[1]

SECOND PAPER.

By Thomas B. Osborne and George F. Campbell.

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In a former paper, by one of us,[2] the results of some attempts to isolate diastase have been detailed. This work has been continued, but as yet no preparations of diastase have been realized more active than those there described. The results given in the former paper, however, have been confirmed, and details of the process for obtaining highly active diastase have been determined more exactly.

Here follows a concise account of this later work so far as it is worth placing on record.

Fifteen kilograms of fine ground malt were treated with thirty liters of five per cent. sodium chloride brine, and after standing some time, with frequent stirring, the extract was pressed out and filtered, yielding sixteen liters of clear filtrate. The meal residue was again treated with fifteen liters of five per cent. brine

[1] From the Report of the Connecticut Agricultural Experiment Station for 1895.

[2] Eighteenth Annual Report of this Station, pp. 192-207; *J. Am. Chem. Soc.*, 17, 587-603.

and fifteen liters more of clear extract obtained. The united solutions were then saturated with ammonium sulphate and the precipitate filtered out, dissolved in brine and filtered perfectly clear. This liquid was saturated with ammonium sulphate, the precipitate was suspended in two liters of water and dialyzed two days. The ammonium sulphate which adhered to the precipitate, at first prevented solution of the substance, but after two days enough sulphate was removed by dialysis to allow the proteid to dissolve. The solution was filtered clear and dialyzed seven days longer. The globulin thus precipitated was filtered out and the solution, which measured 5800 cc., was dialyzed into an equal volume of alcohol of 0.86 sp. gr. for eighteen hours. The precipitate, VI,[1] was filtered out and the solution, which then measured 3500 cc., was again dialyzed into an equal volume of alcohol of 0.86 sp. gr. for eighteen hours yielding precipitate VII. The filtrate from VII measured 2700 cc. and was dialyzed into an equal volume of alcohol of 0.86 sp. gr. for eighteen hours, giving precipitate VIII, the filtrate from which measured 2000 cc. and was dialyzed into 2500 cc. of alcohol of 0.82 sp. gr. for eighteen hours. This gave precipitate IX, the filtrate from which measuring 1800 cc. was dialyzed into twice its volume of alcohol of 0.81 sp. gr., giving precipitate X. The solution filtered from X was then treated with absolute alcohol until nothing further separated, giving precipitate XI.

These six fractions were all, separately, treated with water; X and XI dissolved completely, the others partially. The aqueous solutions, filtered clear, containing the proteoses and albumin of the six fractions, were then separately dialyzed into water, to remove all freely diffusible substances and, as no globulin was precipitated from any of them, the dialyzers were transferred to alcohol, in order to concentrate their contents, and absolute alcohol was finaly added until the proteids were completely thrown down.

In this way six preparations were obtained, which, when dehydrated with absolute alcohol and dried over sulphuric acid, weighed respectively, 18, 1.37 gram; 19, 1.47 gram; 20, 4.05

[1] The precipitates and preparations described in this paper are numbered consecutively with those specified in the former article on Diastase: This Journal, 17. 587.

grams; 21, 4.82 grams; 22, 2.17 grams; and 23, 0.63 gram.

The disastatic power of these preparations was determined in the manner described in the former paper,[1] and found to be as follows: 18 — 0; 19 = 60; 20 = 300; 21 = 300; 22, trace, and 23 = 0.

It will be noticed that nearly all the enzyme was thrown down in fractions 8 and 9, which gave preparations 20 and 21. These were but half as active as preparation 15, described in the former paper.

In order to purify this diastase, 20 and 21 were united, dissolved in 100 cc. of water, the insoluble matter filtered out and washed with thirty-five cc. of water (these first washings being added to the filtrate), then with more water, and finally with absolute alcohol. Dried over sulphuric acid, this preparation, 24, weighed 0.23 gram. The filtrate and first washings from 24 were treated with 200 cc. of alcohol of 0.885 sp. gr., making a solution containing 36.5 per cent. of alcohol. A small precipitate resulted, 25, which when filtered out and dried over sulphuric acid, weighed 0.25 gram, and had a diastatic value of 15. The filtrate from this precipitate was mixed with 160 cc. of alcohol of 0.84 sp. gr., raising the per cent. of alcohol to 50.7, and the precipitate, 26, thus produced when dried as usual weighed 2.35 gram and had a diastatic value of 86. To the filtrate from 26, 100 cc. of alcohol of 0.84 sp. gr. and 100 cc. of absolute alcohol were added, raising the alcohol-content to 61.6 per cent. The precipitate, 27, which resulted, was filtered out, weighed 2.87 grams and had a diastatic value of 600, just twice that of 20 and 21, from which it had been derived, and just equal to that of the most active preparation, 15, of the former paper. To the filtrate 200 cc. of absolute alcohol were added, giving a precipitate, 28, which weighed 1.00 gram and had a diastatic value of 100.

The filtrate from 28 mixed with 200 cc. more of absolute alcohol, gave a precipitate, 29, which weighed 0.40 gram and showed only a trace of disastatic power. The filtrate from 29, mixed with 400 cc. of absolute alcohol, yielded 0.17 gram of substance, 30, that was totally inactive, and the filtrate from

[1] Report of Conn. Expt. Station for 1894, p. 194; *J. Am. Chem. Soc.*, 17, 587.

this when evaporated to dryness left a residue weighing 0.65 gram.

The results of this experiment showed that little diastase was precipitated by bringing the alcohol-content of the malt extract to fifty per cent. by weight, while nearly all the diastase was thrown down, under the conditions described, when the proportion of alcohol in the malt extract was made sixty per cent.

In order to still further concentrate or purify the diastase contained in precipitate 27, this was treated with 100 cc. of water and, without filtering from the substance which had been coagulated by precipitation and drying, 100 grams of absolute alcohol were added. The precipitate so produced was filtered out and extracted with water. The insoluble matter, after washing and drying, weighed 0.50 gram. The aqueous filtrate, from this insoluble matter, was then completely precipitated with absolute alcohol and 0.45 gram of substance, 31, obtained having a diastatic value of 200. The solution, filtered from the first precipitate, produced by adding an equal weight of alcohol to the solution of 27, as just described, was mixed with enough absolute alcohol to raise this ingredient to fifty per cent., and the substance thereby thrown down, 32, weighed, when dry, one gram, and had a diastatic value of 400. The filtrate from 32 was completely precipitated with absolute alcohol and yielded two-tenths gram of inactive proteid. It is thus seen that the diastase instead of increasing in power under this treatment declined to two-thirds of its original in activity.

Having thus learned more exactly the conditions under which diastase may be so far separated from the other malt proteids, an attempt was made to prepare a large quantity of material with which to carry the purification farther. Through the kindness of Mr. C. Von Eggloffstein, of the Maltine Manufacturing Company, at Yonkers, N. Y., a considerable supply of malt extract, rich in diastase, was placed at our disposal. For this favor and much information respecting malt extracts, we wish to express our especial thanks.

One gallon (3,785 cc.) of this malt extract, which had been concentrated at a low temperature *in vacuo* until it contained about fifty per cent. of solid matter, was dialyzed into water for

forty-eight hours, whereby a large part of the sugar was removed and a thin liquid remained. This was saturated with ammonium sulphate and the precipitated proteids were filtered out, suspended in water and dialyzed for five days. To the liquid contents of the dialyzer, filtered clear from insoluble matters, alcohol was added to make fifty per cent. of the resulting mixture. This threw down a precipitate which was filtered out, dehydrated with absolute alcohol, and dried over sulphuric acid.

This white, easily-powdered precipitate, XII, weighed ninety-five grams. One-half of it was insoluble in water and salt solution. By extraction with water and precipitation with alcohol, added first to fifty per cent. and afterward to sixty per cent., two preparations, 33 and 34, resulted, weighing respectively 4.85 grams and 7.21 grams, that had little diastatic power.

The solution from which the first precipitate, XII, had been separated was treated with enough alcohol to make seventy-five per cent., and the resulting precipitate, XIII, filtered out and found to weigh, when dried over sulphuric acid, seventy grams. This precipitate included the chief part of the diastase of this extract. It dried to a light, dusty powder of pale straw-yellow color, almost entirely soluble in water and had a diastatic value of 200.

XIII was dissolved in water and fractionally precipitated, but, for some unknown reason, the resulting fractions were almost entirely inactive.

In another attempt to make a large quantity of diastase, three gallons (11.4 liters) of the highly concentrated malt extract were mixed with half their weight of water and enough alcohol to make a mixture containing forty-six per cent. of alcohol. A very large precipitate, XIV, resulted, which was filtered out, and as it consisted almost entirely of insoluble matter (probably globulin), it was not further examined.

The filtrate from precipitate XIV was treated with alcohol, raising the strength to sixty per cent; the precipitate so produced was filtered out and, as it contained a large amount of sugar, it was dissolved in about five liters of water, the resulting solution was saturated with ammonium sulphate, the precipitate filtered out, suspended in one liter of water and dialyzed

for five days. The precipitate in the dialyzer was filtered out and the clear solution was treated with alcohol sufficient to make fifty per cent. of the resulting mixture, but as only a little substance separated, the amount of alcohol was increased to sixty per cent. This threw down a considerable precipitate, XV, which, when dehydrated with absolute alcohol and dried over sulphuric acid, weighed fifty-seven grams and had a diastatic value of 300.

Numerous attempts were made to obtain from portions of precipitate XV, by fractional precipitation with alcohol, diastase of greater power than 300, but without success.

Several hundred trials were made with the object to determine precisely the influence of certain conditions, such as the age of the diastase solution, and of certain substances, added in systematically varied quantities, especially sodium chloride, disodium orthophosphate, tripotassium orthophosphate, orthophosphoric acid, acetic acid, and citric acid (using the amount of copper reduction as the measure of effect), but, while the results were decisive in some cases, *e. g.*, citric acid, in the minutest quantities, always depressed or destroyed diastatic action, in the majority of instances, no such uniform results were attainable as would lead to safe conclusions in regard to the circumstances that insure a high degree of diastatic activity.

From our experience in testing these preparations it would seem that the purer the diastase is made, the more sensitive it is to external conditions, and that the method of testing the purity of the ferment by its maltose-producing power thus becomes of uncertain value and perhaps fails to furnish a safe criterion of the purity of the enzyme. That the proteid is not the only factor involved in the amylolytic action of diastase is indicated by the great influence on its activity that often accompanies the addition of various substances to its solution. In view of these facts it is not at all improbable that in thus attempting to purify diastase we remove some substance that favors, or is essential to its action, and that we may have in hand what may be properly termed the enzyme itself, which is feeble in its operation through the absence of deficiency of some accessory substance. Thus the addition of sodium chloride in

many cases increases the diastatic action several fold. That the albumin is an essential factor in diastatic action could not be positively proved, but the results of further experience have tended to strengthen this belief. Of all the preparations that we have made, none from which albumin was absent showed amylolytic power, and those containing the most albumin were the most active. It was always possible to roughly judge of the diastatic power of a preparation, by heating a portion of its solution to 65° C. and observing the amount of coagulum formed.

The fact that active diastase was obtained only from solutions whose alcohol content lies between fifty and sixty per cent., may, we think, be regarded as probable evidence that the enzyme is not something carried down mechanically with the proteid.

THE PROTEIDS OF MALT.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received May 15, 1896.

AS is well known, water extracts a considerable quantity of proteid matter from ground malt. This we find to consist of at least five distinct bodies, namely, a globulin, an albumin and three proteoses. Whether true peptones are present was not determined, for the malt extracts are so strongly colored that the biuret test entirely fails. Besides the proteids soluble in water another exists that may be taken up by dilute alcohol (of 0.9 sp. gr.). After extracting malt with saline solutions and alcohol, a further quantity of proteid matter remains, the nature of which we have not been able to determine.

Malt-Globulin.—Ten kilograms of air-dried malt, freshly prepared by ourselves in the laboratory, and ground to a fine meal were treated with twenty liters of water and, after standing three hours, were squeezed out in a press and the solution filtered clear. The residual meal was treated with eight liters more of water and the second solution was pressed out and filtered. The united solutions were saturated with ammonium sulphate, the precipitate was suspended in about four liters of water and dialyzed for three days, when it dissolved, with the exception of a

[1] From the Report of the Connecticut Agricultural Experiment Station for 1895. Communicated by the authors.