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THE CHEMICAL NATURE OF DIASTASE.[1]

BY THOMAS B. OSBORNE.

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FEW substances are of more importance or of more interest than the enzymes or unorganized ferments, yet our knowledge relating to these bodies is almost wholly confined to the products of their activity and the conditions under which this is manifested. Although the existence of these ferments was recognized early in the present century, our information in respect to their true nature is exceedingly limited and unsatisfactory. It was for a long time supposed that the active substance causing a fermentative change is a soluble proteid, and the power of inducing such change seems by many to have been ascribed to soluble proteid matter in general. Later, this power was thought to be restricted to special forms of proteid, but no sufficient evidence was brought forward. Of late years investigators have undertaken to isolate ferments and prepare them in a state of purity. The results of these attempts have led to very conflicting conclusions respecting the character of these bodies. Some of the so-called pure preparations of ferments have had the properties of the proteids, and have more or less agreed with them in composition, while others have differed widely from the proteids in both respects.

It still seems to be the opinion of many that the enzymes are

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in fact true proteids and that the ferments thus far supposed to be obtained in a state of purity were simply somewhat contaminated with other substances. This opinion is based on the fact that all those changes which are ascribed to the action of enzymes occur only in solutions which contain proteid matter, and that the activity of the ferment is greatly influenced by conditions known to have a pronounced action on proteids, such as heat, the presence of acids and alkalies, salts of the heavy metals, etc.

The first discovered and one of the most carefully studied of these ferments is diastase. The practical application of the action of diastase in the manufacture of alcohol and of malt liquors has given rise to careful and extended studies of the conditions affecting the activity of this ferment, and the result of these studies has led some to the opinion that the active substance is the albumin present in the malt extracts. The conversion of starch into maltose and dextrin by diastase increases in rate and extent as the temperature of the solution is raised, until the heat reaches the point at which the albumin begins to coagulate. The ferment then begins to lose power, and, when the heat is sufficient to completely coagulate the albumin, its amylolytic action ceases entirely. In 1883, C. Lintner, Jr., showed that the diastatic power of fifteen different samples of malt was very nearly proportional to the amount of coagulable albumin which they contained. In 1886, however, C. J. Lintner prepared diastase in, as he supposed, a state of purity, and came to the conclusion that the results of his analysis of diastase indicate that, in the ferments, we have a special class of proteid substances. The composition of Lintner's purest diastase differed much from that of the proteids, since it contained only two-thirds as much nitrogen and also less carbon. His diastase furthermore, failed to give the reaction with cupric sulphate and potassium hydroxide which is characteristic of proteid matter. These results of Lintner's threw much doubt on the hypothesis that the vegetable albumin is identical with diastase.

In my investigations of the proteids of wheat, rye, and barley, I found in all these grains the same albumin and was impressed with the close relation between the temperature at which this albumin coagulates and the temperature at which diastase begins

to lose its activity. The aqueous extracts of these seeds, as is well known, possess considerable diastatic power, and it seemed to be more than probable that this was due to the albumin. I accordingly undertook an investigation of this subject, and I now offer the results thus far obtained, which are preliminary to a more extended study.

The usual method of preparing vegetable enzymes is to treat the aqueous or glycerol extract containing them with alcohol as long as a precipitate, having fermentative power appears, to purify this by repeated precipitation from its solution in water, by means of alcohol, and finally to subject the aqueous solution to dialysis to remove salts. This method is wholly unsuited to yield pure preparations, because the precipitate produced by alcohol contains not only a large amount of carbohydrates and salts, but also nearly all of the various forms of proteid matter present in the extract. Lintner employed this method, and there can be no doubt that he obtained a mixture of proteids with other substances which defied all attempts at further separation.

The most rational method (hitherto very little used) is first to separate the proteids from the carbohydrates and other soluble substances by saturating the extract with ammonium sulphate, thereby precipitating the ferment and proteids together, next to remove the proteid existing as globulin, by dialysis, and then, if possible, to separate the albumin and proteoses by fractional precipitation with alcohol. In following this method, a measured quantity of malt extract was saturated with ammonium sulphate, the precipitated proteid matter was filtered out, dissolved in water, and the clear filtered solution made up to the volume of the original extract. This solution was found to have the same diastatic power as before precipitation, thus showing that ammonium sulphate had not injured the diastase. Throughout my work diastatic power has been measured by Lintner's method, which gives a very ready means of accurately comparing different preparations. This method consists in adding to each of a series of carefully measured volumes of the solution containing definite amounts of the diastatic preparation, ten cc. of a two per cent. solution of soluble potato starch, and allowing the ferment

to act upon the starch for one hour at the ordinary temperature of the room. At the end of this time five cc. of Fehling's solution are added to each portion and the mixtures are heated for ten minutes in a boiling water-bath. After the precipitated cuprous oxide has settled, where too little sugar has been formed to precipitate all the copper, the liquids will be blue; if sugar is in excess they will be yellow. The one colorless liquid that should result gives the measure of diastatic power. Lintner represented the value of his most active preparation by 100, and that of the other preparations by figures stating the amount of each necessary to give a complete reaction with Fehling's solution under the above conditions, in comparison with his most active preparation, of which, under the conditions of the test just described, twelve one-hundredths of a milligram completely reduced the five cc. of Fehling's solution.

For the sake of comparison I have measured the activity of my preparations by the same standard, so that a preparation whose activity is given as 200 means that six one-hundredths of a milligram sufficed to give a complete reduction.

As Lintner recommended extracting the malt with water containing twenty per cent. of alcohol instead of pure water, since thereby less foreign matter was removed with the proteid, this procedure was first tried. Fifteen hundred grams of ground air-dried malt, prepared in the laboratory, were treated with three liters of twenty per cent. alcohol, the extract squeezed out in a press, and the residue again treated with another liter of the same dilute alcohol. Three liters of extract were obtained which, after being filtered clear, were saturated with ammonium sulphate. Owing to the presence of the alcohol much less ammonium sulphate was dissolved than by a water extract, and the proteids were consequently incompletely precipitated. The precipitate obtained was treated with water and a considerable quantity of insoluble matter, consisting mostly of globulin, rendered insoluble by contact with the reagents, was filtered out. The solution was saturated with ammonium sulphate, and the precipitate dissolved in water. This clear solution was then dialyzed in water for some days, and after filtering from a slight deposit was dialyzed in alcohol of 0.845 sp. gr. for forty-eight hours.

As the water passed out of the dialyzer faster than the alcohol entered, the solution became concentrated and a considerable precipitate formed. This was filtered out and washed, first with dilute alcohol and afterwards with absolute alcohol, and dried over sulphuric acid. This preparation, 1, when thus dried, dissolved in water with the exception of a not inconsiderable residue. When filtered clear, the solution, on heating, gave an abundant coagulum, and after boiling and filtering out the coagulum, the filtrate gave a strong pink color with cupric sulphate and potassium hydroxide, showing the presence of proteose. The diastatic power of this preparation, in comparison with Lintner's best was eighty-six, but, as it was afterward found to contain a comparatively small amount of ash, the test was repeated with the addition of a few milligrams of sodium chloride and then found to equal 150.

The composition of preparation 1 was as follows:

PREPARATION 1.

	Ash-free.
Carbon	52.55
Hydrogen	6.48
Nitrogen	16.41
Sulphur, Oxygen	24.56
	100.00
Ash	2.29

These figures indicate that this preparation consisted almost wholly of proteid matter, and the reactions proved the presence of at least three forms; namely, coagulated proteid, albumin, and proteose. This mixture was one and a half times more active than Lintner's most energetic preparation, and contained about six per cent. more nitrogen and one-half as much ash. The composition of the preparation is very similar to that of the coagulated albumin-like body obtained from wheat, rye, and barley, and for which I have adopted the name leucosin. As this albumin has been found to have the same composition, whether coagulated by heat or by alcohol, and as most, if not all of the proteids have identical composition (so far as analysis can show), in the soluble and the coagulated states, it seems probable that

preparation 1 consisted mostly of coagulated and soluble leucosin together with a little proteose.

The filtrate from this preparation on addition of absolute alcohol, yielded a small precipitate, 2, which dissolved wholly in water and gave only a very slight coagulum on heating, but a strong pink biuret reaction, showing it to be mostly proteose. Its diastatic power was only nineteen.

As above stated, owing to the presence of alcohol, saturation of the original extract of malt with ammonium sulphate, precipitated only a part of the proteids. Accordingly the filtrate from this first precipitation was dialyzed for twenty-four hours, so as to remove most of the alcohol, and was again saturated with ammonium sulphate. The resulting precipitate was dissolved in water, filtered from a slight residue, and the clear solution dialyzed until nearly all the ammonium sulphate was removed. The dialyzer was then transferred to alcohol and left for forty-eight hours. The resulting precipitate was then filtered out and treated in the manner before described. After drying, this substance, preparation 3, like preparation 1, consisted of insoluble proteid, soluble leucosin, and proteose. Its diastatic power was 133, and it had the following composition:

PREPARATION 3.

	Ash-free.		
	I.	II.	Average.
Carbon	52.34	....	52.34
Hydrogen	6.73	....	6.73
Nitrogen	15.90	15.92	15.91
Sulphur } Oxygen }	....	....	25.02
			100.00
Ash			0.82

The filtrate from this preparation was next treated with a large quantity of absolute alcohol, and the contained proteid completely thrown down. This substance, preparation 4, dissolved entirely in water; its solution yielded but a trace of coagulum on heating, and when boiled and filtered gave a strong proteose reaction. It contained, ash-free, only 12.02 per cent. of nitrogen, and had a diastatic activity of 11.

These results prove that extraction of the malt with twenty per cent. alcohol is not suited for a subsequent precipitation of the proteids with ammonium sulphate; that otherwise the method is capable of yielding preparations of diastase of high fermentative power, which to a certain extent can be separated into fractions containing the different forms of proteid matter; that the fractions including the greatest amount of soluble albumin have the greatest diastatic power; and that a part at least of the proteose is almost, if not entirely, free from this power.

Another extraction was made on a much larger scale, so that the fractional precipitations might be more numerous, and the fractions examined more closely.

Ten kgms. of malt were exhausted with water and the extract was saturated with pure and neutral ammonium sulphate. The very bulky precipitate was suspended in four liters of water and dialyzed until much of the sulphate had been removed and the precipitated proteid largely dissolved. The solution was then filtered from an insoluble residue consisting mostly of globulin, and the clear filtrate was saturated with ammonium sulphate. The precipitate thus obtained was suspended in 1500 cc. of water and was dialyzed until nearly all the sulphate had been removed and the precipitate mostly dissolved. The globulin contained in the extract was thus largely separated and, after it had been filtered out, the clear solution was dialyzed into an equal volume of alcohol of 0.84 sp. gr. After forty-eight hours the precipitate, number 1, which had separated, was filtered out and set aside for further examination. The filtrate was again dialyzed into an equal volume of alcohol of 0.84 sp. gr., and after forty-eight hours another precipitate, II, obtained. The filtrate was further dialyzed into a rather large quantity of somewhat stronger alcohol, and precipitate III separated, and by similarly treating the filtrate from this, precipitate IV was obtained, the filtrate from which, on adding a large quantity of absolute alcohol, yielded precipitate V. All the proteid in the extract was thus separated. Precipitate I was much contaminated with coloring-matter, II less so, and III was nearly colorless, as were also IV and V.

The approximate weights of each of these precipitates was as

follows: I, thirteen grams; II, eight; III, six; IV, five; and V, three, a total of thirty-five grams.

Precipitate I was treated with water and found to be very largely insoluble. The insoluble matter was filtered out and washed with water, and the clear solution was dialyzed for several days to remove all the salts. No proteid was thus precipitated, and the dialysis was continued in strong alcohol, thereby throwing down all but a trace of proteid. The precipitate, preparation 5, weighed 2.11 grams. After drying, it dissolved in water with the exception of a small residue, and its solution when slowly heated became turbid at 65° and deposited flocks at 70°. After boiling and filtering out the slight coagulum, the solution gave a strong pink reaction with the biuret test. These tests show the preparation to consist largely of proteose. Its composition was as follows:

PREPARATION 5.

	Ash-free.
Carbon	53.16
Hydrogen	7.03
Nitrogen	16.50
Sulphur	1.50
Oxygen	21.81
	100.00
Ash	0.49

With Lintner's test this preparation showed a diastatic power of thirty.

The insoluble residue, remaining after treating precipitate I with water, was thoroughly extracted with ten per cent. sodium chloride solution; what remained insoluble in this menstruum was filtered out and the clear solution dialyzed until free from chlorides. The precipitate thus formed, preparation 6, weighed one and two-tenths grams, and after drying was not soluble in water, but dissolved readily and nearly completely in salt solution, having, as was to be expected, the properties of a globulin. This substance had a very slight diastatic power, and its sodium chloride solution when heated slowly became turbid at 60°, a few flocks appearing at 65°, due to a trace of albumin. Its composition was as follows:

PREPARATION 6.

	Ash-free.
Carbon	53.11
Hydrogen	6.45
Nitrogen	15.78
Sulphur } Oxygen }	24.66
	100.00
Ash	0.75

The filtrate from preparation 6 still contained proteid matter which was separated by dialysis in alcohol. Preparation 7 was so obtained, weighing 1.54 grams, having the same properties as 6, and the following similar composition :

PREPARATION 7.

	Ash-free
Carbon	53.58
Hydrogen	6.70
Nitrogen	15.87
Sulphur } Oxygen }	23.85
	100.00
Ash	1.43

After extracting precipitate I with water and salt solution a very considerable part still remained undissolved. This was treated with water to remove all the salt, and then with alcohol, and was dried over sulphuric acid. This preparation, 8, weighed eight grams and was quite dark in color. It had the properties of an insoluble form of globulin, being dissolved in one-half per cent. sodium carbonate solution and precipitated therefrom by neutralization. Its composition was nearly the same as that of the two last globulin-like preparations and is probably a so-called "albuminate" derived from that substance. The composition of preparation 8 was :

PRRPARATION 8.

	Ash-free.
Carbon	53.55
Hydrogen	7.01
Nitrogen	15.72
Sulphur	1.23
Oxygen	22.49
	100.00
Ash	1.09

Precipitate II was treated with water and the solution thus

formed was dialyzed in water for several days and then in alcohol for twenty-four hours. A quantity of absolute alcohol was finally added to the contents of the dialyzer, thus completely precipitating the proteid. This preparation, after drying, was almost wholly soluble in water, and when heated slowly its solution became turbid at 60° and deposited flocks at 66°. The amount of proteid thus coagulated was somewhat greater than was given by preparation 5, and its diastatic power was likewise greater, being seventy-five. Analysis showed its composition to be as follows:

PREPARATION 9.

	Ash-free.
Carbon	53.19
Hydrogen	6.71
Nitrogen	16.74
Sulphur	1.38
Oxygen	21.98
	100.00
Ash	0.78

This preparation contained a slight amount of insoluble matter, some albumin and much proteose.

The residue of precipitate II, which was not dissolved by water, was treated with sodium chloride solution and the clear extract dialyzed till free from chlorides, but as no precipitate was produced, the dialyzer was transferred to alcohol when preparation 10 separated, weighing 0.49 gram, and containing, ash-free, 15.18 per cent. of nitrogen. It is probable that this is the same globulin obtained in larger quantity from precipitate I, but less pure. That part of precipitate II which remained undissolved after extracting with water and salt solution, was then washed thoroughly with water and with alcohol, yielding preparation 11, which weighed 5 grams and had the following composition:

PREPARATION 11.

	Ash-free
Carbon	53.51
Hydrogen	6.75
Nitrogen	15.76
Sulphur	1.12
Oxygen	22.86
	100.00
Ash	0.66

These figures show that precipitate II contained less globulin and proportionately more leucosin and proteose than precipitate I and it was accordingly found to be more powerfully diastatic.

Precipitate III was in turn treated with water, the resulting extract filtered clear, dialyzed for several days in water, and then in alcohol, absolute alcohol being finally added in quantity to the contents of the dialyzer. The resulting precipitate, preparation 12, weighed three grams. It was almost completely soluble in water, and its solution when slowly heated became turbid at 55° and flocculent at 60°. The amount of this coagulum was much greater than that yielded by preparation 9. The filtrate from the coagulum gave a strong proteose reaction. The diastatic power was 222, indicating the presence of much more diastase than any of the preceding preparations. Its composition was as follows:

PREPARATION 12.

	Ash-free.
Carbon	52.80
Hydrogen	6.96
Nitrogen	16.09
Sulphur	1.45
Oxygen	22.70
	100.00
Ash	0.59

The residue of precipitate III was digested with salt solution, the filtered extract was dialyzed in water till free from chlorides, and then, as no proteids separated, the dialysis was continued in alcohol. Only 0.28 gram of proteid resulted, which, without correction for ash, contained 12.53 per cent. of nitrogen. This was marked preparation 13, and considered to be impure globulin.

The part of precipitate III still undissolved was washed with water and with alcohol, yielding preparation 14, which weighed 2.87 grams. This had the following composition:

PREPARATION 14.

	Ash-free.
Carbon	53.25
Hydrogen	7.65
Nitrogen	16.12
Sulphur	1.38
Oxygen	21.60
	100.00
Ash	0.55

This preparation has a somewhat higher nitrogen and lower carbon content than preparations 8 and 11, which is probably due to its being a mixture of the insoluble form of the globulin with some insoluble albumin coagulated by the long contact with alcohol to which it had been subjected. This is to be expected, as precipitate III contained relatively more albumin than precipitates I and II.

Precipitate IV was next treated with water, the solution filtered clear, dialyzed for some days in water, and afterwards transferred to alcohol, and the dialysis continued. Absolute alcohol was then added to the contents of the dialyzer, giving preparation 15, weighing four grams. This substance dissolved in water to a nearly clear solution, which, when filtered perfectly clear and heated carefully, became turbid at 50° and gave a large coagulum at 56°. After heating the solution and filtering off the coagulum, a good reaction for proteose was obtained with the biuret test. This preparation had a diastatic power of 600. As this was a much more powerful ferment than any yet produced, its properties were carefully studied and will be described at length later. When analyzed this substance was found to have the following composition:

PREPARATION 15.

	Ash-free.
Carbon	52.50
Hydrogen	6.72
Nitrogen	16.10
Sulphur	1.90
Oxygen	22.78
	100.00
Ash	0.66

It will be noticed that the sulphur in this preparation is a little higher than in the preceding preparations, which is probably due to its containing some sulphate.

The part of precipitate IV which did not dissolve in water was treated with salt solution, but no globulin was extracted. The residue was then washed with water, giving preparation 16, which weighed 0.9 gram and had the following composition:

PREPARATION 16.

	Ash-free.
Carbon	53.42
Hydrogen	7.15
Nitrogen	16.65
Sulphur } Oxygen }	22.78
	100.00
Ash	0.24

The composition of this insoluble product shows it to be probably coagulated leucosin.

A portion of precipitate V, when treated with water, was found to dissolve completely. It was therefore washed with absolute alcohol, yielding preparation 27, which weighed 2.87 grams. The clear solution of this substance when heated became turbid at 50°, and yielded a small coagulum at 58°. Boiled and filtered, a strong pink coloration was given with the biuret test, thus showing it to consist mostly of proteose. The diastatic power of this substance was 60, only one-tenth that of preparation 15. Its composition was:

PREPARATION 17.

	Ash-free.
Carbon	51.21
Hydrogen	6.52
Nitrogen	15.40
Sulphur } Oxygen }	26.87
	100.00
Ash	2.37

The lower nitrogen content of this preparation indicates that the strong alcohol had thrown down, together with the proteids, some non-nitrogenous substances.

Much is to be learned by studying these results which will be of service in future attempts to isolate pure diastase.

In the first place, it is plain that we have in our malt extract a globulin, an albumin, and at least one, more probably two, forms of proteose. I believe the substance soluble in salt solution to be a true globulin, since it so readily assumes an insoluble form, and also because a much larger quantity of the same

body was obtained by extracting with ten per cent. salt solution, the malt residue remaining after the extraction with water. I also think that at least two forms of proteose are present, for the water-soluble portion of precipitate I consisted chiefly of proteose, as did also precipitate V. The amount of proteose diminished from precipitate I to precipitate IV, which contained the least, while precipitate V, which, it will be remembered, was thrown down by adding to the solution a very large amount of absolute alcohol was mainly proteose. A part of the proteose was precipitated by alcohol more readily than the albumin, while another part was less readily precipitated. Beside the albumin, globulin, and proteose, we have also to take account of the "albuminate" or insoluble forms of the albumin and globulin. The results of this extraction show that the globulin is rendered insoluble more rapidly than the albumin, so that precipitation with alcohol and solution in water, repeated a few times, may be depended upon to remove the former. Whether repeated fractional precipitation can be employed to completely separate the albumin from the proteoses is not so certain. The albumin is thrown down from the malt extract by saturation with magnesium sulphate, and it is not unlikely that a complete separation can be accomplished by this reagent. It is, however, not to be forgotten that the diastase may be a substance which, when heated to from 50°–60°, splits apart into an albumin and a proteose, and that the proteose found in the solutions which have been heated is a decomposition product of the diastase. Kühne's attempts to produce pure trypsin led him to suspect that this ferment is a body which, when heated, yields a coagulable fraction and a proteose-like substance. Hammarsten's more recent work on a neucleoproteid obtained from the pancreas also points strongly in this direction.

Now that we have some precise knowledge of the associated substances, it seems probable that we may succeed in obtaining diastase nearly, if not quite, pure, and arrive at a clearer and more positive knowledge of this ferment, and also have a guide in further study of other enzymes, which will lead to a more satisfactory understanding of this whole subject. It is probable that the ferments contained in seeds are much easier to prepare than

those of animal origin, since the substances with which they are associated are largely non-proteid and comparatively easy to separate. It is also certain that the amylolytic ferments present an easier problem than the proteolytic, for the products of the activity of the latter are so similar, in their nature, to that which the ferment is supposed to possess, as to make it always a matter of great uncertainty whether the separated enzyme is free from those bodies or not.

As already stated, preparation 15 was a very energetic ferment, and on this account its properties were more fully studied, with the following results:

Dissolved in water this substance gave all the usual proteid reactions, and when heated slowly became turbid at 50° and gave a flocculent coagulum at 56°. This is exactly the temperature of coagulation of the albuminum (leucosin) which I have prepared from wheat, rye, barley, and malt, with identical composition and properties. The aqueous extracts of these grains have, moreover, a strong diastatic action on starch. The amount of coagulable albumin in preparation 15 was determined and found to be 53.2 per cent. of the dry substance.

These facts point strongly to the albumin as being the diastatic substance, yet there are several facts hard to explain, if this be true, which cannot be overlooked. Although in general the diastatic power of my preparations was greater the larger the amount of coagulable albumin they contained, I have never yet been able to establish any numerical relations between the two. In no case have I found any diastatic action with solutions free from albumin. Furthermore, the activity of my preparation 15 is such as to require a much greater diastatic power for malt than this shows if its coagulable albumin is the enzyme.

A malt extract corresponding to a solution of the diastase in five milligrams of malt had the same diastatic power as 0.02 milligram of preparation 15. As the preparation contained but a little over fifty per cent. of coagulable albumin, this would correspond to only 0.01 milligram of albumin in the five milligrams of malt, or two-tenths per cent. The amount of albumin in malt is much greater than this, as it is also in wheat, rye, and barley, whose diastatic power is greatly inferior to that of malt. It is

not probable that the *separated* diastase is more active than that *in the seed*, especially in view of the experiments which follow, comparing the action of malt extract and preparation 15. The only explanation of this that occurs to me, is that the active diastase is a combination of albumin with some other body, presumably the proteose, which breaks up on heating, yielding coagulated albumin, and that, besides this combined albumin, free albumin is also present, which has no diastatic power, but which is coagulated at the same time. There is no direct evidence, however, that this hypothesis is correct.

Compared with other so-called pure ferments, preparation 15 is very active. At 20° it was in a condition to produce, from soluble starch, over 2000 times its weight of maltose and a further undetermined quantity of dextrin, within one hour. After having been dried over sulphuric acid and kept for six months, its activity was reduced to one-half, but in this condition it produced in seventeen hours, at 20°, 10,000 times its weight of maltose besides an unknown quantity of dextrin. At 45° the same quantity of maltose was produced in one hour as at 20°. At 50° much less and at 55° very little maltose was formed. These tests were made by using an amount of diastase solution just sufficient to produce enough maltose at 20° to exactly reduce five cc. of Fehling's solution.

Compared with malt extract of the same diastatic strength, as measured by the amount of maltose produced in one hour at 20°, the distilled water solutions of preparation 15 have a less powerful action in liquefying starch paste. Five cc. of malt extract added to ten cc. of a starch paste containing two per cent. of starch, dissolved the starch completely in eight minutes, while the solution of preparation 15 required thirty-seven minutes.

The malt extract is also more energetic in converting starch completely into bodies giving no color with iodine. Five cc. of the same malt extract added to ten cc. of soluble starch solution caused the blue reaction with iodine to disappear in thirteen minutes, while it required thirty-eight minutes to reach the same result with the solution of the separated diastase. When, however, the diastase was dissolved in malt extract, whose enzymes

had been previously killed by heating, the difference between the separated diastase and that in the malt extract nearly disappeared.

Two test-tubes were each charged with ten cc. of starch paste. To one tube were added five cc. of fresh malt extract, and to the other the same amount of boiled and cooled malt extract in which had been dissolved a quantity of preparation 15, sufficient to make a solution of the same sugar-producing power as the fresh malt extract itself.

The fresh malt extract liquefied the starch in seven minutes, the mixture of preparation 15 and boiled malt-extract in fourteen minutes, while thirty-seven minutes were required to produce the same result with a distilled water solution of preparation 15. In completely converting starch into bodies giving no color with iodine, the solution of preparation 15 in boiled malt extract gave exactly the same result as the fresh malt extract, showing that the difference first noticed was due to the conditions and not to the ferment.

In view of these results, it seems highly probable that diastase is a true proteid, for if we consider the extremely minute quantity of preparation 15 required to produce large amounts of maltose, it is hard to believe that this action is due to some substance adhering to the proteid to the extent of only three or four per cent. at the most. If such were the case it is also remarkable that the enzyme should adhere in so much greater quantity to the particular precipitate represented by preparation 15 than to any of the other numerous fractions. If diastase, then, is to be considered as a true proteid, it is evidently either an albumin, a combination of an albumin with a proteose, or a proteose. We have seen that those fractional precipitates which consist largely or wholly of proteose have little or no diastatic action, amylolytic power being manifested most strongly in the fractions containing the most albumin, and least in those containing but little, though not in strict proportion to the amount of the albumin. It is to be concluded that the diastatic enzyme is most closely related to the albumin, named leucosin, and it is not improbable that further careful study will show more clearly what this relation is.