IX. ON THE NATURE OF THE PROTEOLYTIC ENZYME OF YEAST.

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THE organism of the higher animals possesses a system of ferments hydrolysing the proteins to the stage of their final decomposition—to amino-acids. With regard to two components of this system—pepsin and erepsin [Cohnheim, 1901]—we have sufficient data to allow us to regard them as more simple ferments than animal trypsin. And indeed, pepsin converts proteins into peptones, erepsin acts on peptones, hydrolysing them into amino-acids, whilst trypsin unites in itself both these functions. The latter circumstance leads us to doubt the individual character of trypsin and allows us to regard it as a mixture of two ferments [Cohnheim, 1902; Salkowski, 1902]. However, trypsin cannot be regarded as a mixture of pepsin and erepsin, as, on the one hand, trypsin digests protein more slowly than pepsin, and on the other, it has been definitely shown that trypsin does not act on certain combinations of polypeptides, which are readily acted upon by erepsin.

In invertebrate animals, in which the differentiation of organs stands much lower, it is also much more difficult to observe the specific ferments. Here the hydrolysis of protein to amino-acids is attributed to tryptases [Oppenheimer, 1909, p. 222] which act most efficiently sometimes in an acid, and sometimes in an alkaline medium. Fredericq [1878], for instance, obtained from the tissue of a worm an enzyme which dissolved fibrin better in an acid medium (0.6–1.2 % HCl). We encounter phenomena of a similar kind in investigating the proteolytic ferments in plants. Owing to a weak physiological differentiation in plants it is difficult to solve the question, whether we are here dealing with one ferment hydrolysing the proteins to amino-acids, or whether there is also a system of ferments, which effects the hydrolysis of protein to the end by stages.

From the wide literature on this question I may mention the conclusion arrived at by Hahn [1903], who worked with the juice of yeast obtained by Buchner's method. Hahn thinks that the proteolytic ferment of yeast presents

a new type of digestive ferments, as, similarly to pepsin, the best medium for its action is acid, whilst in its final products of decomposition it corresponds to the tryptic ferment. Hahn named the proteolytic ferment of yeast endo-tryptase—since it acted inside the yeast cell, and although he was acquainted with the work of Cohnheim on ereptase, Hahn, and other investigators who preceded him, did not express themselves in favour of the complex character of the proteolytic ferments of plants. Butkewitsch [1904, see also Godlewski, 1911], who investigated autolysis of the seeds of *Lupinus angustifolius*, has shown that the process of decomposition of protein is inhibited in an acid medium (0.2 % HCl or 0.1–1 % HCN), and the correlation between the products of decomposition changes altogether, the greater part of them being precipitated by phosphotungstic acid. Evidently, the decomposition of proteins proceeds chiefly to peptones. The supposition that it is possible to alter the correlation between the products of decomposition of protein according to the conditions of the experiment, is corroborated by Fernbach and Hubert [1900]. These authors demonstrated that during the autodigestion of malt the products of decomposition were greatly influenced by the temperature. Thus, at 40° nearly the entire soluble nitrogen belonged to amino-acids, which are not precipitated by phosphotungstic acid. At 60° there were produced only 50–60 % of amino-acid nitrogen, and at 70° its amount did not exceed 40 %, the remaining quantity belonging to the peptones precipitated by phosphotungstic acid. Windisch and Schellhorn [1900] arrived at the same conclusion. The analogy involuntarily comes to mind that in the given case the phenomena taking place are similar to those in the fermentative metamorphosis of starch; and indeed, by heating diastase to 80° it is possible to destroy its function of sugar production, without influencing the dissolution of starch [Pottevin, 1899].

The quantitative alterations of the products of decomposition of protein, observed in different media and at different temperatures, indicate the possible presence in plant tryptase of at least two constituents. The numerous investigations of Vines ("The Proteases of Plants") on the proteolytic ferments of plants have provided experimental data on this question. Vines [1908] regards the protease of plants as an association of "peptase" and "ereptase." The first ferment dissolves the flakes of fibrin, and the second hydrolyses Witte peptone to products giving the tryptophan reaction. This author tried to obtain peptase and ereptase separately in extracts from leaves, bulbs and seeds. Thus, an extract from hemp seeds acidified with acetic acid produced a dense precipitate of protein, which on repeated dissolution in common salt

(NaCl) produced an opalescent solution, which was capable of digesting fibrin, but did not decompose Witte peptone to tryptophan; the filtrate from the protein precipitate contained an active peptonising enzyme (ereptase), but not "peptase." In the next paper [Vines, 1909] this author investigated papain in this respect. As regards the ferment of yeast, Vines [1902, 1904, 1905, 1906] discovered that a watery extract of dried yeast digested Witte peptone, but not fibrin, and an extract in 2 % NaCl digested both. The investigations of Vines are very illuminating, but his methods were far from perfect, and the author often sets forth unconvincing experiments for the confirmation of the correctness of his ideas. Thus, he founds all his views on the presence of ereptase in plants on the tryptophan reaction, whereas Butkewitsch [1904] had already shown that coloration by chlorine water does not present a criterion of the deep decomposition of protein, just as the reduction of an alkaline solution of copper oxide, due to the aldehyde group, does not correspond to the complete hydrolysis of the starch molecule to glucose, but appears in the dextrans together with the liberation of their aldehyde group. How careful one has to be in applying the tryptophan reaction for the establishment of the deep decomposition of protein is shown in the experiments of Levene and Rouiller [1907]. These authors decomposed the protein by barium oxide solution and observed coloration by bromine water even after slight hydrolysis; but in the latter case *the substance producing the tryptophan reaction was precipitated wholly by phosphotungstic acid*. As it was impossible to separate tryptophan from such a precipitate, the authors arrived at the conclusion that tryptophan was here in the form of a constituent of a complex compound, probably a polypeptide. Since the polypeptides manifest the tryptophan reaction, it cannot be used for the detection of ereptase at all. Of course, it was also impossible to identify the ereptase with Cohnheim's erepsin, as for this purpose it would have been necessary to show that ereptase is capable of hydrolysing combinations of amino-acids which are inaccessible to trypsin. In general, for the sake of uniformity, Vines' terms ought to be retained and a rational nomenclature of ferments adopted. The ferment splitting the peptones to amino-acids should be named, in agreement with Oppenheimer [1909] *peptase*, without deciding *a priori*, whether it is to be identified with Cohnheim's erepsin, or with one of the constituents of animal trypsin acting on peptones. The ferment which dissolves the protein and converts it into peptone should be named *protease*, using this term in a restricted sense, and not denoting by it the sum of proteolytic ferments. Thus, owing to Vines, we are able to distinguish in plant trypsin at least two constituents, the *protease* and the

peptase. As regards the protease, which is equivalent in its significance to pepsin, it became known after the fundamental investigations of Hooker [1874] and Darwin [1888] on insectivorous plants. These authors arrived at the conclusion that the secretions of insectivorous plants contain an enzyme similar to the *pepsin* of animals, *i.e.* digesting fibrin and other proteins in an acid medium. After the detailed investigations of Vines [1897, 1898, 1901] on the juice of *Nepenthes*, the experiments of Abderhalden and Terruchi [1906] have shown that this juice, just as in *Drosera*, does not hydrolyse the peptides, *i.e.* is identical with the pepsin of animals. Thus, the presence of pepsin in insectivorous animals points to a high degree of physiological differentiation, since pepsin appears only in the higher animals, whilst in the invertebrate animals it is either not observed at all, or its presence is disputable. The idea of the identity of pepsin in plants and animals (the "pepsin-idea" of Vines [1909]) was the starting-point in the investigation of the proteolytic ferments of plants, and only after Green [1890] had discovered in seeds a ferment hydrolysing proteins to amino-acids could be added to it the "trypsin-idea." At present Abderhalden's method [Abderhalden and Schittenhelm, 1906; Abderhalden and Dammhahn, 1908; Abderhalden and Rilliet, 1908; see also Euler, 1907], applied by himself and his pupils, has assumed great importance in the study of peptase in plants. It is founded on the change in the rotation of the solution of optically active polypeptides after the addition of the ferment. By this method it was possible to demonstrate [Abderhalden and Brahm, 1908] that, if the juice of yeast is diluted in a certain manner, it hydrolyses added polypeptides, similarly to intestinal juice, quite identically and at the same rate; the peptase of yeast could thus be identified with Cohnheim's erepsin.

In my experiments on the proteolytic ferments of yeast I adopted Stutzer's method of estimation of nitrogen in protein. This method of quantitative estimation of nitrogen in the precipitate of copper hydroxide has evoked wide criticism. The most essential arguments were raised by Schjerning [1900], who directed attention to the facts that peptones were not precipitated by copper, and that amides passed into the precipitate. However, in my experiments amides are not encountered in the process of autolysis of yeast and the peptones in the filtrate may be determined by other methods. Stutzer's method is inapplicable only in the presence of a large quantity of carbohydrates, for recently Kostytshev and Brilliant [1916, see also Maillard, 1913] have pointed to a remarkable fact—the union of amino-acids with sugar without any intervention of ferments. This product gave with copper hydroxide a compound insoluble in water. In other cases Stutzer's method gives good

results; it should only be remembered that *from the diminution of nitrogen in the precipitate of copper hydroxide the conversion of proteins can be guaranteed only to peptones*, as they are no longer precipitated by copper. For the estimation of the work of peptase, i.e. the conversion of peptones into amino-acids, other methods are required, and I estimated the nitrogen in amino-acids by means of the improved apparatus of van Slyke [Plimmer, 1908]. By combining both these methods it was possible to determine the action of both protease and peptase. My experiments were conducted either with hefanol (a Munich preparation of dried yeast), or with dried yeast (Lebedev). In 1912 I published the results of my experiments on the influence of phosphates upon the proteolytic decomposition during autolysis of yeast. Using different temperatures, adding K_2HPO_4 or KH_2PO_4 for the production of alkaline and acid media, I estimated in the filtrate—after treatment by copper, according to Stutzer—the amount of nitrogen in the precipitate produced either by lead acetate or phosphotungstic acid. It was found that the acid phosphate (KH_2PO_4) had the effect of considerably increasing the decomposition of protein, judging by the precipitate of copper oxide; however, additional precipitation of the filtrates by phosphotungstic acid showed that in proportion to the surplus amount of protein decomposed in the phosphate portion, the surplus of nitrogen in the phosphotungstic precipitate also rose. Thus after 67 hours' autolysis of hefanol at 52–58° the amount of nitrogen in the *normal portion* was:

in the copper precipitate	...	...	145.7 mg.
in the phosphotungstic acid precipitate			131.1 „
Total	...		276.8 „

and in autolysis with 1 % KH_2PO_4 :

in the copper precipitate	...	...	118.9 mg.
in the phosphotungstic acid precipitate			163.2 „
Total	...		282.1 „

The same results were obtained when lead acetate was employed for precipitation, instead of phosphotungstic acid. Assuming with Vines the presence of two constituents in the proteolytic ferment of yeast, I had already at that time arrived at the conclusion that KH_2PO_4 *increases the decomposition of protein only to the stage of the high-molecular products of decomposition of protein—peptones*—which, as is known, are precipitate dboth by phosphotungstic acid and lead acetate. Therefore, in the case quoted we can speak only of stimulation of protease¹.

¹ In my work of 1912 I used Vines' terms "peptase" and "ereptase," which must now no longer be employed.

In order to ascertain this point finally I have estimated the quantity of amino-groups by van Slyke's method in the normal and phosphate portions.

Exp. 1. Four portions of hefanol of 1.1 g. were set for autolysis with 25 cc. of water at 46°. To 2 and 4 were added 0.37 g. KH_2PO_4 in each and to all toluene. The experiment was conducted for 43 hours. The initial quantity of protein-nitrogen in the portions varied between 83.4 mg. and 84.2 mg.; the amounts of nitrogen obtained are, for the sake of convenience, re-calculated to portions containing 100 mg. of protein-nitrogen. In 1-2 the nitrogen was estimated according to Stutzer, in 3-4 according to van Slyke.

Nos. of portion	Composition of portion	Decomposed protein in mg. of protein-nitrogen	Newly formed amino-acid nitrogen
1 and 3	Water	37.8	14.6
2 and 4	1.5 % KH_2PO_4	51.8	14.9

Here, notwithstanding the greatly increased amount of protein decomposed in the presence of acid phosphate, the quantity of nitrogen according to van Slyke, i.e. the nitrogen of amino-acids, did not increase. It appears that *the given conditions have created surroundings favourable for protease, but these conditions (acid medium) have in no way accelerated the work of peptase.*

Saleski and Schatalov [1915], who have revised my previous experiments with phosphates, have arrived at the same results, but they could not explain the fact that in my experiments the difference between the normal and phosphate portions did not disappear with time, and at the end of autolysis remained the same as after 21 hours. Indeed, after 46 hours of autolysis at 54-58°, the decomposition of protein in my experiments was in the normal—58.5 %; phosphate—73.5 %, and after 72 hours—normal—56.9 %; phosphate—73.1 %. In all cases, when autolysis was conducted at a higher temperature, rising to 58°, this difference was maintained, but at a temperature of 34° the difference between the normal and phosphate portions disappeared after 7 days, and at 18° autolysis during 10 days gave:

normal—69.6 % of protein-nitrogen,
phosphate—68.4 % of protein-nitrogen.

This phenomenon is explained by the fact that *at high temperature the work of peptase is at first interrupted, and later altogether suppressed.* The high-molecular product of protein decomposition accumulated in these conditions is not hydrolysed further; that is why this difference is maintained.

Exp. 2. Portions of about 1 g. dried yeast (Lebedev) with 10 cc. water were set for autolysis with toluene. Amount of protein-nitrogen—100 mg. Temperatures—34° and 54°.

Nos.	Conditions of experiment	T	Amount of amino-acid nitrogen in mg.	
			in portion	newly formed
1	Autolysis 90 hours	34°	54.9 ¹	—
2	Same, added 0.2 cc. 10 % NaOH and 20 hours longer	34°	55.0	—
3	90 hours, added 0.25 g. peptone and 20 hours longer	34°	63.2	8.3
4	90 hours, added 0.25 g. peptone + 0.2 cc. 10 % NaOH, and 20 hours longer	34°	66.9	12.0
5	Same as 1, but higher temperature	54°	39.8 ¹	—
6	Same as 3, but higher temperature	54°	41.2	1.4
7	Same as 4, but higher temperature	54°	42.2	2.4

¹ To each of the control portions 1 and 5 was added 0.25 g. peptone previously to nitrogen estimation by van Slyke's method.

It follows clearly from the experiment that during 90 hours at 34° the peptase is not destroyed, as the peptone added is hydrolysed by it; this is seen from portions 3 and 4, in which an increase in the nitrogen, according to van Slyke, is observed, especially in the presence of a small amount of alkali. Autolysis at 54° has interrupted the work of peptase, and later has altogether inactivated it, as the peptone added was hardly decomposed, and there was no increase of amino-acid nitrogen, disregarding the 1.4 and 2.4 mg., which are due to a certain decomposition of peptone in water solution at the temperature of 54°, as control experiments have shown.

That acids and acid salts promote the work of the proteolytic ferment has been known since the time of the first investigations of Gorup-Besanez, but from the experiments with acid phosphate we observe that only the work of protease is increased, *i.e.* the conversion of proteins to the high-molecular products of decomposition. In animal physiology it is assumed that under the influence of hydrochloric acid the proteins are converted into acid albumin soluble in water and in this form become easily acted upon by pepsin. The data of Neumeister [1890] prove that before being digested by pepsin the coagulated protein is simply dissolved without any traces of denaturation. The addition of KH_2PO_4 in my previous experiments also evoked the dissolution of coagulated protein in the presence of the ferment. Thus, a watery extract of hefanol containing a large amount of heat-coagulable protein was submitted to autolysis with and without KH_2PO_4 , at temperatures of 32° and 54°. At 32° the experimental portions were quite transparent and the difference in the decomposition of protein was insignificant (54.3 % control and 64.5 % in phosphate). At 54° the control produced 38.5 % decomposition and contained a precipitate of coagulated protein, whilst the phosphate was transparent and the decomposition of protein in it was 65.1 %. The presence of

KH_2PO_4 , probably, evoked the formation of acid albumin and thus accelerated the work of protease; the importance of the acid phosphate was considerable at 54° , when coagulation of the protein took place. Besides this rôle, KH_2PO_4 could also serve to convert the pro-enzyme stage of protease into the active form, similarly to the manner in which the pro-pepsin (pepsinogen) of animals is transformed to pepsin by acids. My previous experiments have proved with full evidence that during autolysis at temperatures of 19° and 54° the final products of decomposition of protein will be different. At these lower temperatures hardly any intermediate high-molecular products of decomposition of protein¹ are observed; if the filtrate from the copper treatment is precipitated with lead acetate, the precipitates obtained in the portion autolysed at 19° only one-fifth the size of those obtained at 54° . That shows that at a high temperature the work of peptase is interrupted; this is corroborated by *Experiment 3*. After standing with toluene at 34° peptone does not increase the amino-acid nitrogen but at 54° this is observed to a small degree.

Exp. 3. Two portions of dried yeast (Lebedev) to the amount of 1 g. were set for autolysis with 25 cc. water. Toluene added; duration 94 hours. Temperatures 34° and 58° .

T.	Protein decomposed in mg.	Newly formed amino-acid nitrogen
34°	68.8	42.6
58°	50.4	20.9

Therefore, to each 100 mg. of protein decomposed at 34° correspond 61.8 mg. amino-acid nitrogen, and to the same amount decomposed at 58° there correspond only 41.5 mg. Since it is possible to estimate the action of peptase by the amount of amino-acid nitrogen, it is clear that high temperature inhibits its work.

In my preceding work I reduced the proteolytic ferment of yeast to an inactive state by heating to 80° in water. Hefanol heated in this manner did not produce decomposition of protein during further autolysis, but if acid phosphate (1.5 %) was added to the experimental portion, the decomposition of protein was again observed. In one experiment out of 106.4 mg. of protein-nitrogen, 5.9 mg. were decomposed; in another, in which the temperature was raised to 75° —even 21.3 mg. This decomposition may be ascribed either to the regeneration of the ferment caused by the acid salt, or, which I think more probable, to the conversion of the pro-enzyme under these conditions into the

¹ This is also mentioned by Hahn [1903], who found on autolysis traces of albumoses and a total absence of peptones.

active form. The pro-enzymes are in general more resistant to heating than the ferments themselves. In order to demonstrate that the decomposition of protein takes place here owing to the action of the ferment, and is not due to the influence of the acid phosphate, I carried out experiments with boiled portions and portions inhibited with formalin.

Exp. 4. Portions of hefanol of 1.1 g. set for autolysis at 46°, toluene added; 1-2 were treated with 25 cc. boiling water and boiled during 1 minute; to 3-4 were added 25 cc. 1.5 % formalin. Into each of the portions 2 (after cooling) and 4 was introduced 0.37 g. KH_2PO_4 . Autolysis during 42 hours.

Nos. of portions	Condition of experiment	Amount of protein-nitrogen previously to experiment	Amount of protein-nitrogen after experiment
1	Boiled	84.2	84.4
2	Boiled + KH_2PO_4	83.8	83.9
3	1.5 % formalin	84.9	84.1
4	1.5 % formalin + KH_2PO_4 ...	84.4	84.5

In the result no traces of decomposition of protein were observed even in the phosphate portions after the ferment was inactivated either by boiling or by formalin. What were the products of decomposition of protein obtained after the resumption of the activity of the proteolytic enzyme under the influence of KH_2PO_4 after heating at 75-80°? The question was, whether there took place a transition from the pro-enzyme state of protease and peptase, or only from the former. In my preceding work it was observed that in proportion as the decomposition of protein with phosphate was increased, the greater was the amount of nitrogen appearing in the precipitate with lead acetate, as compared with the control portion. I have arrived at the conclusion that in the given case one may only speak of the work of protease carrying out the decomposition of protein to the high-molecular products of digestion. That heating to 75° destroys peptase completely is seen from the following experiment.

Exp. 5. Portions of dried yeast (Lebedev) of 1 g. were heated 1.75 minutes to 75° on a water-bath with 25 cc. water. To each of 2 and 3 was added 0.25 g. peptone.

1.	Control, nitrogen estimated according to van Slyke	13.0 mg.
2.	Same, but previously to estimation 0.25 g. peptone added	15.7 ,,
3.	To portion heated to 75° added 0.25 g. peptone, and left for 20 hours at 56°.	16.0 ,,

Notwithstanding the 20 hours' digestion at 56°, the amount of amino-acid nitrogen did not increase, as compared with control portion 2. There-

fore, heating to 75° destroys peptase, and only the protease is regenerated in the given conditions under the influence of KH_2PO_4 .

This is seen from the following.

Exp. 6. Four portions of hefanol of 1.1 g. each with 25 cc. water were previously heated on the water-bath for 1.5 min. to 75°; then after cooling, to each of 2 and 4 were added 0.37 g. KH_2PO_4 and these allowed to remain 44 hours at 46°. In all toluene. Amount of protein nitrogen estimated to 100.

Nos. of portions	Nature of portion	Nitrogen in precipitate of $\text{Cu}(\text{OH})_2$	Protein decomposed, in mg. of N	Newly formed amino-acid nitrogen
1 and 3	Control	100	0	0
2 and 4	Phosphate	89.6	10.4	0.8

Only the work of protease is present; decomposition was conducted only to the high-molecular products not precipitated by $\text{Cu}(\text{OH})_2$, but precipitated by lead acetate.

Exp. 7. Same as preceding, but the portions were precipitated with lead acetate after autolysis.

Nature of portion	N in mg. before experiment	Nitrogen in lead acetate precipitate
Control	70.1	69.7
Phosphate	72.0	71.9

No difference was obtained between the control and phosphate portions, as the high-molecular products of decomposition, protein-albumoses and peptones, produced in the latter case, were precipitated by lead acetate.

In the experiments described above the same phenomenon was repeated as in the autolysis of yeast, in which the ferment was not inactivated by heating; in both cases in the phosphate portions was observed an accumulation of surplus high-molecular products of decomposition, but the quantity of amino-acids did not increase.

Involuntarily there come to mind the analogous results with the fermentative conversion of starch into glucose. I have already mentioned experiments in which by heating to 80° it was possible to inactivate the sugar-forming function of diastase without hindering the solution of starch and its conversion into dextrins. Evidently, in my experiments a complete inactivation of peptase also takes place. My data can be placed in parallel with the experiments on papain. Papain is regarded as a ferment of the tryptic type and Vines even discovered in it both constituents of the proteolytic enzyme. However, a series of authors [Delezenne, 1906; Jonescu, 1906; Sachs, 1907] had shown that papain digested egg-white best at 80–90°; *decomposition proceeds rapidly, but is carried on only to albumoses and peptones.*

While I dealt only with killed yeast—hefanol and zymin—I invariably obtained activation of the protease of yeast by acid phosphate, only sometimes in portions to which phosphate had not been added there was also observed a slight decomposition, probably due to incomplete inactivation of the ferment by heating. But when I carried out similar experiments with dried yeast (Lebedev), it occurred that *rapid* heating to 80° inactivated the ferment altogether, and after gradual heating during 2 min. to 75° decomposition of protein was observed both in the phosphate and control portions, but there was produced no increase of the amino-nitrogen in this decomposition.

Exp. 8. Portions of dried yeast (Lebedev) of about 1 g. with 25 cc. water, heated for 2 min. to 75°¹. Autolysis with toluene 20 hours at 45°. To each of 3 and 4 was added 0.37 g. KH_2PO_4 after cooling.

Nos. of portions	Nature of portion	Decomposition of protein in mg. of nitrogen	Newly formed amino-acid nitrogen
1 and 2	Watery	14.0	0
3 and 4	Phosphate	16.6	0

It is seen that the addition of phosphate hardly produced any effect; this is a general phenomenon in working with dried yeast (Lebedev), as even during autolysis of this yeast with the ferment *not inactivated by heating*, no increase of the decomposition of protein in the presence of KH_2PO_4 was observed. Probably, in the given case, the yeast possessed highly active enzymes, and, therefore, the addition of KH_2PO_4 , which in its essence serves as an activator, does not alter its work. It must also be noted that in Experiment 8 the product of decomposition of protein is nearly wholly precipitated by phosphotungstic acid; thus the amount of protein decomposed, expressed in mg. of protein-nitrogen, was 14.0, and in the phosphotungstic precipitate following copper, 11.9 mg. of nitrogen. This fact confirms the assumption that here are produced the primary products of decomposition of protein, the so-called albumoses and peptones. These primary products of decomposition are obtained in large quantities during autolysis of short duration; thus, during 3 hours' autolysis of dried yeast (Lebedev) at 45° in my experiment, 32.1 mg. of protein were decomposed, and only 2.1 mg. of amino-acid nitrogen were newly formed; or to 100 mg. correspond only 6.2 mg.

¹ It was proved by control experiments that during the 2 min. heating no visible decomposition of protein took place.

Besides high temperature, with the view of depressing the work of peptase I have conducted experiments on autolysis of dried yeast (Lebedev) in the presence of alcohol.

Exp. 9. Portions of dried yeast (Lebedev) of about 1 g. with 20 cc. water + 5 cc. 95 % alcohol. Autolysis during 20 hours at 45°.

Amount of protein decomposed in mg. of N 18.2.

Newly formed amino-acid N ... 0.

Thus, 19 % alcohol destroys the work of peptase similarly to heating.

In all my experiments which were carried out either in a natural slightly acid medium, or acidified, owing to the presence of KH_2PO_4 , I have not observed any stimulation of peptase; on the contrary, after a certain time, especially at high temperature, it was altogether inactivated. In the work of 1914 [Ivanov, 1914] I changed the acid medium of the experimental portion to alkaline during the autolysis of yeast, and allowed the decomposition of protein to proceed. I expected that in the alkaline medium, which is most favourable to animal trypsin and peptase [see experiments of Abderhalden and Terruchi, 1906], the decomposition of the intermediate compounds to amino-acids would proceed better; as a matter of fact I observed, on such a change of medium, accumulation of nitrogen determinable by Stutzer's method, and a corresponding diminution of nitrogen in the lead acetate precipitate, in which the presence of peptones could have been suspected. I at that time arrived at the conclusion that in the given conditions there was a possibility of fermentative synthesis from the products of primary decomposition of proteins. However, the question proved to be more complicated; if the medium became weakly alkaline from a slight addition of secondary phosphate (K_2HPO_4), then, as I have previously shown [Ivanov, 1916], in an alkaline medium at the cessation of the decomposition of protein increased formation of amino-nitrogen was observed, *i.e.* the work of peptase. Thus, in one experiment after complete cessation of the decomposition of protein, the amount of amino-acid nitrogen rose from 78.8 mg. to 102.2 mg. after an addition of alkali and renewed autolysis during 24 hours. At that time I concluded that toward the end of autolysis "there is accumulated a certain amount of polypeptides not precipitated by copper, and with difficulty acted upon by ferments; the addition of alkali and high temperature evoke a retardation in the decomposition of protein, but facilitate the decomposition of these polypeptides."

Thus, it was possible to separate the two constituents of the proteolytic ferment by function: *in this case, in the alkaline medium the protease was inactive, while the peptase acted*: and in the experiments described above the

peptase was inactivated by heating to 75° and only the protease manifested activity in the presence of acid phosphate.

In the above-mentioned work of 1916 I succeeded in establishing the presence of phenylalanine amongst the products of decomposition of protein in alkaline medium. E. Fischer and Abderhalden [1903] consider that during digestion of protein by animal trypsin, phenylalanine (and proline) never appear amongst the products of decomposition, on account of the polypeptides which contain them being resistant to the action of this ferment. And since the hydrolysis of these polypeptides is possible only by erepsin, the peptase of yeast may be regarded as identical with the erepsin of animals.

After the conclusion of the present investigation, I received the recently issued excellent paper by K. Dernby [1917]. The author applied exact methods for the determination of the concentration of the H-ions of the medium and demonstrated that there exist corresponding optima of the concentration of H-ions for pepsin, trypsin and ereptase of yeast. Thus, according to Dernby, the proteolytic ferment of yeast does not consist of two constituents—protease (“pepsin”) and ereptase, as might be supposed, on account of previous experiments, but of three; to them is added also tryptase, which is analogous in action to the trypsin of animals.

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