CXVI. THE ENZYMIC DEAMIDATION OF ASPARAGINE IN THE HIGHER PLANTS.

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THE occurrence of asparagine in the higher plant and its rôle in protein metabolism have formed the subject of numerous investigations.

It has been found that the amount normally present in the green plant is very small and it is only under artificial conditions, associated with a carbohydrate deficiency, that an accumulation of this substance is ever obtained. E. Schulze [1906], in a series of papers, showed that in etiolated seedlings most of the protein reserve is converted into asparagine. In the mature plant, in which the leaf is the seat of metabolic activity, it has been found since Schulze's work that, in general, starvation results in a conversion of protein to asparagine.

Chibnall [1924], in the course of investigations on the nitrogenous metabolism of the higher plants, concludes that there is a constant production of asparagine from protein in the normal mature leaf, and that in plant metabolism asparagine has the rôle of a translocatory substance and is the chief medium whereby nitrogen, in a form suitable for subsequent resynthesis, can be conveyed from one part of the plant to another. According to Prianischnikov [1916] asparagine is formed as a temporary nitrogen reserve whenever there occurs, through some derangement of the normal course of protein metabolism, an excess of ammonia which is toxic to the plant.

Important as is the part played by asparagine in protein metabolism, the reactions whereby protein and asparagine are interconverted are still far from being clearly understood. On the one side the degradation of protein through proteoses and polypeptides to amino-acids is well known, and a similar reverse process is probable; but there is little or no evidence regarding the steps intervening between these amino-acids and asparagine. It has been assumed that the intermediate forerunner of asparagine in the plant is aspartic acid, and that an enzyme, for which the name asparaginase has been used, controls the reaction

The presence of aspartic acid in the plant had never been demonstrated until Kiesel [1924] claimed to have isolated it in the course of a separation of the nitrogen compounds of ripening rye ears. As no asparagine was found even in the later stages of ripening, Kiesel considered that aspartic acid cannot be the forerunner of asparagine. It is probable, however, that the reverse reaction is here occurring—that there is a breakdown of asparagine to aspartic acid; but instead of the deamidation only taking place at a stage still earlier than those investigated, as Kiesel suggests, it is more likely that there is a continuous diffusion of asparagine into the grain with almost simultaneous deamidation. Schulze and Winterstein [1910] came to the conclusion that asparagine migrates into the ripening seed where it is rapidly utilised for the production of protein.

In the earlier experiments on the autolytic splitting of ammonia from plant material, the whole process was referred to as one of “desamidation,” and the inability to distinguish between the processes of deamination and deamidation, *i.e.* the removal of ammonia from the amino- and amide-groupings respectively, has given rise to considerable confusion and controversy. The enzymes responsible for the reactions leading to the liberation of ammonia were indiscriminately termed amidases or de-amidases, and these expressions are still often employed.

Many attempts have been made to effect a definite enzymic deamidation of asparagine by means of plant material. The only instance, however, in which this is claimed to have been achieved is that recorded by Kiesel [1909]. By the autolysis of etiolated seedlings or of the expressed juice, the whole of the asparagine originally present was broken down. The time period of four months, however, was somewhat excessive, for asparagine solutions, especially in the presence of plant material, are extremely susceptible to bacterial infection. It was not stated whether any sterility tests were carried out at the end of autolysis. The products formed by the breakdown of the asparagine were not identified and the attempt to obtain a preparation active on asparagine by precipitation of the expressed juice with alcohol was unsuccessful.

Schulze and Winterstein [1910] tried to detect in unripe pea and bean seeds an enzyme which would liberate ammonia from asparagine, but without success; in both cases practically the same amount of asparagine was recovered in the active and control experiments. Kato [1911] found that the press-juice of bamboo shoots contained an enzyme which would liberate ammonia from asparagine. The amount formed, however, was almost inappreciable, being of the order of 0.3 mg. from 0.2 g. of asparagine after 50 hours' incubation.

The presence of “amidases” in moulds was first demonstrated by Shibata [1904] who found in the mycelium of *Aspergillus niger* an enzyme which acted on certain amides including asparagine. The ammonia set free in the case of asparagine was only about 0.5 %. Pringsheim [1908], using an acetone-dried preparation of yeast, was unable to obtain any “desamidation” of amino-

acids or asparagine and considered that the ability of moulds to liberate ammonia from nitrogenous substances was essentially associated with a living condition of the mould.

The "de-amidases" of yeast were briefly dealt with by Dernby [1917] in connection with a study of the yeast proteolytic enzymes. Yeast press-juice was found to be inactive on amino-acids but asparagine was readily broken down. As the ammonia formed was always less than 50 % of the total nitrogen, it was concluded that it had been set free by the yeast ereptase from the —CO.NH_2 group which was regarded as a peptide linkage. This point will be discussed later in connection with some results obtained in the present research.

Effront [1917], in the course of a discussion on amidases, describes the preparation of a "butyro-amidase" which acted on acetamide and asparagine with liberation of ammonia. When a solution of asparagine only was used, the action was limited to the amide group; if, however, oxidisable substances were also present—*e.g.* products of proteolysis—then the amino-group of the asparagine was also attacked. This is one of the very few doubtful instances in which the amino-group has been considered to have been displaced by enzyme activity apart from the living cell. In the rather vague account of his experiments on butyro-amidase, Effront makes no reference, unfortunately, to the original publication of the results which are quoted.

In the sphere of animal physiology, the earliest investigations on the existence of amidases in the tissues of the animal organism were attended by the same lack of differentiation between deamination and deamidation; resulting from this, divergences of opinion and conflicting results are again observed. S. Lang [1904] found that minced organs were able to effect the removal of ammonia not only from asparagine but also from certain amino-acids. Abderhalden and Schittenhelm [1906], using the press-juice from liver, obtained no action on amino-acids. Fürth and Friedmann [1910], experimenting with various organs of the horse and pig, found that, in general, they were all capable of bringing about *in vitro* the hydrolysis of the amide group of asparagine. Further, the amounts of ammonia formed were usually no greater than could be accounted for by the amide nitrogen. It was suggested that the ability to break down asparagine was related to the general autolytic processes taking part in the physiological degradation of proteins.

More recently, Clementi [1922] carried out a systematic study of the enzymic deamidation of asparagine by the animal organism. Contrary to the findings of Fürth and Friedmann, Clementi asserts that the enzyme, for which he uses the name "asparaginase," is present only in certain animals and in certain tissues. It is interesting to note that whereas Fürth and Friedmann obtained the maximum activity with intestinal mucous membrane of the horse, Clementi, using the same material, found only a slight deamidation. The intestine of the pig was found to be quite inactive, but this result was not confirmed in the present research. With regard to the physiological significance

of the appearance of asparaginase in the animal organism, Clementi elaborated the theory that this enzyme is related, not to the processes of metabolic degradation of the protein molecule, but rather to the nature of the alimentation of the species, and that the phenomenon is one of biochemical adaptation of the organism to the presence or absence of asparagine in the food. The experimental data obtained by Clementi appear to conform very well to this attractive hypothesis.

From the above introductory review it is apparent that the only clear-cut evidence for the occurrence of the enzymic deamidation of asparagine apart from the living cell is that obtained by experiments with animal material. Even these investigations, however, have had as their object merely the proof of the existence of an enzyme capable of liberating ammonia from asparagine. No attempt has been made to furnish experimental proof that the product of the deamidation of asparagine is aspartic acid and, except in the researches of Clementi, no active preparation of the enzyme by alcohol or acetone precipitation has been obtained. Further, the factors which condition the activity of the enzyme have received little attention. Clementi found that a slightly alkaline medium was favourable but did not determine the optimum p_H for the enzyme or the range over which it was active.

In the higher plant even the existence of this enzyme has not yet been satisfactorily demonstrated, although the deamidation of asparagine is a phenomenon of the greatest importance in the metabolic processes of the plant. The primary object of this research, therefore, was to prove conclusively the presence of this enzyme in the higher plant; a further consideration was the need of obtaining some information as to the nature of the enzyme.

EXPERIMENTAL.

General methods.

In the following experiments, the young rootlets (culms) of germinated barley were used as the material from which to obtain the enzyme and investigate its nature. The barley was obtained from the malthouse (Messrs Watney, Combe and Reid, Mortlake) in the fresh or "green" condition, *i.e.* before the kilning process. It had been allowed to germinate 8–9 days and the rootlets were 1–1.5 cm. long. For the experiments on asparagine, a $N/10$ solution containing 15 g. per litre was used throughout; the solution contained 0.5 % cresylic acid (triclesol) as antiseptic except where otherwise stated. Cresylic acid was used in preference to toluene partly because the trouble of separating the toluene before estimation was avoided and, more especially, because it had been found by other investigators to be more efficient. In general, the experiments were carried out at 37° and the ammonia formed was estimated by distillation with magnesia and absorption of the ammonia in $N/10$ HCl.

The amount of asparagine broken down in the course of the distillation is very small; for 10 cc. *N*/10 asparagine the blank titration is approximately only 0.3 cc. This was always accounted for in the parallel control experiments which were carried out under the same conditions except that the enzyme had been destroyed by previously heating the material.

Activity of barley culms on asparagine.

For a preliminary test of activity, 4 g. of fresh roots were picked off from 100 g. of the barley and ground to a thin cream with water to 25 cc. The activity of the fresh roots is shown in Table I, 5 cc. of the preparation being equivalent to 0.8 g. of roots. As the separation of the fresh culms by hand picking was an extremely tedious and lengthy process, the remainder of the germinated barley was spread out in an incubator at 37° and allowed to dry for 3 days. The dried culms were then fairly easily separated by vigorously shaking the barley and afterwards sifting through a coarse mesh. From 9.5 kg. of the dried barley, 200 g. of culms were obtained and ground to a fine powder. By comparison with the yield of fresh culms, it is seen that the culms had lost at least half their weight by drying. If the enzyme had been unaffected by the drying process, therefore, the dried culms should have had about double the activity of the same weight of fresh culms. Actually the activity of the dried culm powder was certainly no greater than that of the same weight of fresh material. 10 g. of the root powder were ground to a thick paste with water to 80 cc. and 5 cc. (= 0.6 g. of roots) used for the activity tests given in Table I.

Table I. *Activity of fresh and dried barley roots on asparagine.*

(Maximum ammonia formation = 10 cc. *N*/10.)

Fresh roots:	Time (hours)	cc. <i>N</i> /10 NH ₃
5 cc. preparation + 10 cc. <i>N</i> /10 asparagine + 5 cc. buffer <i>p</i> _H 7.5	88	8.75
5 cc. preparation + 10 cc. 0.5 % tricoresol + 5 cc. buffer <i>p</i> _H 7.5	88	0.7
5 cc. (boiled) preparation + 10 cc. <i>N</i> /10 asparagine + 5 cc. buffer <i>p</i> _H 7.5 	88	0.6
Dried roots:		
5 cc. preparation + 10 cc. <i>N</i> /10 asparagine + 5 cc. buffer <i>p</i> _H 7.5	88	7.5
5 cc. preparation + 10 cc. <i>N</i> /10 asparagine + 5 cc. buffer <i>p</i> _H 7.5	161	8.55
5 cc. preparation + 10 cc. 0.5 % tricoresol + 5 cc. buffer <i>p</i> _H 7.5	161	1.45
5 cc. (boiled) preparation + 10 cc. <i>N</i> /10 asparagine + 5 cc. buffer <i>p</i> _H 7.5 	161	1.25

Enzyme preparation by alcohol precipitation. 200 g. of dried barley culms were ground with 1½–2 litres of water for about 2 hours, sand being added to facilitate the grinding. The mass was wrapped in a thick filter-cloth and pressed out in a Buchner press. The residue was returned to the mill, ground with a further 1½ litres of water for 1 hour and again pressed out. The combined extracts measuring 2½ litres were centrifuged, and the clear liquid was precipitated with 4½ litres of ice-cold 90 % alcohol. The precipitate was centrifuged off

and washed with absolute alcohol and twice with ether, being centrifuged after each washing. The precipitate was air-dried as far as possible by breaking up in a small mortar, and the last traces of ether removed in a vacuum desiccator. Those steps in which the enzyme was in the presence of alcohol were carried out as rapidly as possible in order to minimise inactivation. The pale creamy-brown powder thus obtained weighed 8.85 g. or 4.4 % of the original dried root material; it was only partially soluble and when ground with water gave a thin suspension which slowly deposited. This alcohol precipitate is referred to below as the enzyme preparation.

To determine the activity of the preparation, 0.5 g. were ground with water to 25 cc. and 5 cc. of this suspension (= 0.1 g. of preparation) used for each of the experiments represented in Table II. Since 0.5 g. of the dried roots used for this preparation produced a break-down of 5.8 cc. $N/10$ (corrected) in 144 hours under the same conditions, it can be roughly calculated that the activity of the enzyme preparation was about 4–5 times that of the whole roots.

Table II. *Activity of the enzyme preparation.*

(Maximum ammonia formation = 10 cc. $N/10$.)

No.		Time (hours)	cc. $N/10$ NH_3
1.	5 cc. suspension. 10 cc. asparagine. 5 cc. buffer p_H 7.5	50	3.0
2.	5 cc. suspension. 10 cc. asparagine. 5 cc. buffer p_H 7.5	118	5.3
3.	5 cc. suspension. 10 cc. asparagine. 5 cc. buffer p_H 7.5	170	6.7
4.	5 cc. suspension. 10 cc. asparagine. 5 cc. buffer p_H 7.5	218	7.8
5.	5 cc. (boiled) suspension. 10 cc. asparagine. 5 cc. buffer p_H 7.5	218	0.8

The activity of the enzyme preparation under sterile conditions was confirmed, both in the presence of toluene and of cresylic acid. The extent of hydrolysis was practically the same as that obtained under the ordinary experimental conditions. The sterility tests were kindly carried out by Dr Wright and Mrs Channon of the Bacteriological Department, University College Hospital Medical School.

Isolation of aspartic acid as the end-product of the deamidation of asparagine. 2 g. of the enzyme preparation ground with water were added to 150 cc. $M/5$ asparagine solution (containing 0.5 % tricresol) together with 1.5 cc. of 2 N Na_2CO_3 . The volume was made up to 200 cc. in a stoppered measuring cylinder and incubated at 37°. 5 cc. portions were drawn off and estimated at intervals until the maximum theoretical amount of ammonia was nearly obtained.

The results of these determinations are given in Table III; the action is slower than usual and this may be due to the higher concentration of tricresol in the mixture. The values for the velocity constant (monomolecular), calculated after allowing for an arbitrary blank of 0.6 cc., are also given. The slight fall in the values has been noted in the case of other enzymes, e.g. trypsin and lactase, and is due in part to the effect of the products of the reaction.

Table III.

Time (hours)	cc. $N/10$ NH_3 (max. = 7.5 cc.)	k
46	1.6	229×10^{-5}
90	2.3	207
164	3.3	192
260	4.5	189
380	5.7	188
520	6.65	179
640	7.35	176

At the end of the 27 days, the enzyme material was precipitated from the remainder of the mixture by addition of alcohol. The solution was evaporated to about 50 cc. and the aspartic acid precipitated with the requisite amount of HCl. After standing in the ice-chest overnight, the aspartic acid was filtered off, washed with 50 % alcohol, absolute alcohol and ether successively, and finally dried in a desiccator. 1.96 g. were thus obtained. By concentrating the mother-liquor and adding an equal volume of alcohol, a further 0.46 g. was obtained. The total yield was therefore 2.42 g. or 76 % of the theoretical 3.2 g. The loss is due in part to an incomplete separation of the aspartic acid and possibly, also, to anhydride formation. The crude aspartic acid on recrystallisation gave 2.16 g.

Analysis.

1. *Amino-nitrogen* (Van Slyke). Found 10.38 %, calculated 10.53 %.
2. *Total nitrogen* (Kjeldahl). Found 10.57 %, calculated 10.53 %.
3. *Formaldehyde titration* (method of L. G. Harris). The solution of aspartic acid used contained 0.4018 g. per 100 cc.

1. COOH estimated	cc. $N/10$ NaOH titrating to phenolphthalein
(a) 5 cc. of aspartic acid solution + 50 cc. alcohol + 10 cc. neutralised formaldehyde	2.92 (corr.)
...	Theory 3.02
2. NH_2 estimated	cc. $N/10$ HCl titrating to methyl red
(b) Back titration of solution (a)	1.4 (corr.)
...	Theory 1.51

Relation between quantity of enzyme and extent of hydrolysis. Fig. 1 shows the curve obtained by plotting the corrected values of the percentage hydrolysis against enzyme concentration. The extent of hydrolysis is fairly directly proportional to the quantity of enzyme preparation, but the curve falls off slightly at the higher concentrations.

Effect of temperature on activity. For this experiment a new enzyme preparation was used, 0.1 g. being taken for each estimation; both active and control experiments were carried out in duplicate at each temperature.

The curve obtained by plotting the corrected mean values of the percentage hydrolysis is shown in Fig. 2. The preparation used was unfortunately of a low activity but the curve shows that the optimum temperature for the enzyme is about 38°. The time period for this curve was 120 hours.

Effect of p_H on activity. As this series was started at the same time as the previous one, the same preparation was used, but the time of incubation was increased to 12 days. 0.1 g. of enzyme ground with water to a thin suspension

was added to 10 cc. of the asparagine solution and 5 cc. of the buffer indicated in each case. The actual p_H of each mixture was determined electrometrically after equilibrium had been reached in the course of some hours. The preparation had a very high buffering power, this being the more in evidence the further the p_H from neutrality. The optimum p_H was found to be approximately 7; the phosphate buffer was more favourable for the enzyme than the phthalate or borate buffers at the same p_H . Fig. 2 shows the curve obtained by plotting the actual p_H values against the corrected mean figures for percentage hydrolysis.

Fig. 1.

Fig. 2.

Specific action of the enzyme preparation on l-asparagine. The asparagine used in the previous experiments was the naturally occurring laevo-form with which the enzyme normally has to deal. It was interesting to find out whether the enzyme was specific for this form or was also capable of effecting the hydrolysis of the dextro-modification. It has been shown by Bergell and Wülfing [1910] that the laevo-component of *dl*-leucinamide is hydrolysed by pancreatin very much more readily than the dextro-form.

A preparation of asparagine containing an excess of the dextro-component was prepared according to the method of Pringsheim [1910]. The rotation in 10 % HCl was $[\alpha]_{\text{H}_2\text{O}}^{18} = -11.31^\circ$ as compared with $+38.8^\circ$ for the natural laevo-form. Table IV shows the hydrolysis of a solution containing 0.6202 g. per 50 cc. by a water extract of the whole barley roots. Only about 50 % of the maximum ammonia formation was obtained, and it would appear

Table IV. *Activity of enzyme preparation on a mixture of d- and dl-asparagine.*

(Maximum ammonia formation = 4.2 cc. N/10.)
cc. N/10 NH_3

Time (hours)	Active	Control	Corrected
44	4.4	2.6	1.8
68	4.6	2.6	2.0
92	4.7	2.6	2.1
212	4.8	2.6	2.2

that the enzyme has little or no activity on the dextro-form of asparagine. Ravenna and Bosinelli [1919] showed that when moulds are grown on a mixture of *d*- and *l*-asparagine, the laevo-form is readily attacked but the dextro-form is unaffected.

The specificity of the enzyme preparation and the nature of deamidation. The simple amides, acetamide and propionamide, were entirely resistant to the action of the enzyme, but *d*-glutamine, which is closely associated with asparagine both in structural form and in its natural occurrence, was hydrolysed fairly rapidly. In these experiments the ammonia estimations were carried out by aeration with potassium carbonate in the cold. It is interesting to note that urea also was broken down but at a very much slower rate than with soya-bean urease.

With the object of obtaining some information on the nature of the enzyme and of the process of deamidation, the experiments described below were carried out. In order to ascertain whether deamidation is related to the processes underlying protein degradation, the action of the proteolytic enzymes pepsin, trypsin and, more particularly, erepsin was investigated.

Pepsin and trypsin were both found to be inactive on asparagine; the inactivity of trypsin had been previously demonstrated by Schwarzschild [1904].

A preparation of pig erepsin, however, proved to be readily active on asparagine, although Clementi's results with this material were negative. Bergell and Wülfing [1912] in their experiments on the splitting of leucineamide by pancreatin concluded that there was present a specific enzyme responsible for the amide cleavage. Levene, Simms and Pfaltz [1926], using intestinal erepsin, were able to obtain a slight hydrolysis of glycineamide. In a recent study of the peptidases, Waldschmidt-Leitz and his collaborators [1927] found that pig erepsin had a slight activity on glycineamide but hydrolysed leucineamide fairly readily.

The results obtained by acting on asparagine with an aqueous-glycerol extract of the mucous membrane of pig intestine are given in Table V. The p_H values in each case were measured electrometrically. It is seen that below p_H 6 the enzyme has only a very slight activity and this is the minimum p_H for the action of erepsin on dipeptides. The control figures were obtained by using the same amount of boiled extract.

Table V. *Action of pig erepsin on asparagine.*

(Maximum ammonia formation = 10 cc. $N/10$.)

p_H	Time (hours)	cc. $N/10$ NH_3		
		Active	Control	Corrected
3.0	68	2.15	2.2	0
4.0	68	2.35	2.2	0.15
5.1	68	4.0	2.2	1.8
6.0	68	7.4	2.2	5.2
7.1	50	7.4	2.25	5.15
8.0	50	8.7	2.25	6.45

From a consideration of these results, the question arises whether the enzyme in the barley preparation responsible for the splitting of the amide group of asparagine is merely a plant erepsin or—to use the preferable nomenclature—a peptidase. The action of the barley enzyme on a dipeptide (glycylglycine) was therefore investigated.

Activity of the barley enzyme towards glycylglycine.

For each estimation 10 cc. of barley root extract (containing 0.2 % tri-cresol) were added to 2 cc. of *M*/10 glycylglycine together with 0.25 cc. of *N*/10 NaOH to raise the p_H to 7.6. At the end of the indicated time at 37°, 40 cc. of alcohol were added and the mixture filtered. The filtrate was titrated with *N*/10 NaOH forward to phenolphthalein and with *N*/10 HCl back to methyl red. Table VI shows the increase in the titration values over the initial values showing that the hydrolysis has proceeded up to 70 % of the theoretical maximum.

Table VI. *Action of barley enzyme on glycylglycine.*

(Maximum value for both titrations = 2 cc. *N*/10.)

Time (hours)	Titrations, cc. <i>N</i> /10	
	Forward	Back
0	0.0	0.0
20	0.6	0.55
24	0.75	0.65
48	0.95	1.85
72	1.30	1.20
96	1.50	1.30

DISCUSSION.

The results of the foregoing experiments prove beyond question the existence in the higher plant of an enzyme capable of effecting the deamidation of asparagine. The fact that its action had not previously been satisfactorily demonstrated is probably due to the comparatively low activity of the enzyme or to the difficulty of obtaining it in a sufficiently concentrated form.

With regard to its identity, the experiments on the hydrolysis of glycylglycine and on the behaviour of the proteolytic enzymes towards asparagine would seem to indicate that we are here dealing with an enzyme in the nature of a plant erepsin. This is in accordance with the suggestion made by Dernby from his results on the hydrolysis of asparagine by autolysed yeast. As was pointed out in the introduction, Dernby considered that the amide group of asparagine was to be regarded as similar to a peptide linkage and that it was the yeast ereptase which was responsible for its cleavage.

The enzyme which breaks down asparagine has hitherto been included in the group of amidases under the name of asparaginase. The nomenclature of this group is discussed by Oppenheimer [1926] who separates the amidases into two sections. Those which are responsible for the liberation of ammonia from amino-acids—and which have a very doubtful existence apart from the living cell—he terms *amino-acidases*.

The name *amidases* he retains for the enzymes which, in general, are capable of hydrolysing the —CO.NH_2 grouping only. There is, however, still no sharp line of demarcation between this group and the peptidases to which the cleavage of the peptide linkage —CO.NH— is due.

Levene and Waldschmidt-Leitz have both probably assumed the peptide nature of the amide group in studying the action of peptidases on such amides as glycineamide as well as on true dipeptides. By adopting the view that the amide group of asparagine is essentially a peptide linkage and that both asparagine and glycylglycine are hydrolysed by a barley peptidase, our conception of the protein metabolism of the plant is considerably simplified. Asparagine need then no longer be regarded as a substance apart and distinct from the usual products of protein metabolism but simply as a dipeptide.

If the deamidation of asparagine is indeed due to a peptidase, as is here considered to be the case, then the conclusions of Clementi on the specific distribution of "asparaginase" in the animal kingdom would have very little significance. Since erepsin is an essential factor in the protein metabolism of all animals, the ability to break down asparagine should be universal.

SUMMARY.

A review of the literature shows that in spite of the great importance of asparagine in plant metabolism the enzyme responsible for its deamidation had not been satisfactorily demonstrated.

This enzyme has been shown to be present in the rootlets of germinating barley, and an active preparation has been obtained by alcoholic precipitation of the aqueous extract. It appears to be specific for the naturally occurring *l*-asparagine. The optimum p_{H} is about 7 and the optimum temperature about 37° .

A preparation of pig intestinal erepsin was found to be active on asparagine. As the barley preparation is able to effect the hydrolysis of glycylglycine a peptidase (barley erepsin) must be present.

It is concluded that the asparagine is also hydrolysed by this enzyme, and that it is unnecessary to assume the presence of any specific amidase (asparaginase).

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