XV. CONTRIBUTIONS TO THE STUDY OF THE VEGETABLE PROTEASES:

I. INTRODUCTORY¹.

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ALTHOUGH the work that has hitherto been done on the proteoclastic enzymes and their actions is considerable, the great bulk of it has been carried out in connection with those enzymes which occur in the animal body. Comparatively little has been done on the vegetable proteases². This is possibly due to the fact that whereas the three types of animal proteases can be readily differentiated according to their origin, very different is the case with the

¹ This investigation was carried out at the South-Eastern Agricultural College, Wye, between 1912 and 1914. Publication was delayed owing to the author's absence on military service.

² Some confusion exists in the terminology of the proteoclastic enzymes. Euler [1912] calls them *carbamases* or *proteinases* and considers that animal pepsin is a *pepsinase* and trypsin a *tryptase*. Vines gives the name *peptase* to those enzymes which convert the higher more complex proteins into albumoses and peptones, and *ereptase* to those that split up albumoses and peptones into simple amino-acids.

The custom is rapidly becoming general of giving an enzyme a name derived from that of the substrate on which it acts by changing the final letter or syllable into *ase*. Thus lactase is the enzyme which hydrolyses lactose, urease that which decomposes urea into CO₂ and NH₃. According to this nomenclature the names used by Euler and by Vines are obviously incorrect for a tryptase would mean an enzyme which acts upon trypsin, an ereptase one that hydrolyses erepsin, and so on. It would be advisable in order to bring the terminology of the proteoclastic enzymes into line with that of other enzymes to apply the term *protease* to all those enzymes whether animal or vegetable in origin which will attack any of the proteins or their intermediate decomposition products. Those which attack the higher proteins only (*i.e.* those similar to pepsin) would be called *proteinases*, whereas those which split up albumoses, peptones, etc. but not the higher proteins (*i.e.* those similar to erepsin) would be *peptases*.

Animal trypsin is anomalous in some respects and has both proteinoclastic and peptoclastic properties. It cannot therefore be classed either as a proteinase or a peptase but only (generally) as a protease. It is not impossible though that trypsin may ultimately be found to be separable into a proteinase and a peptase [Vernon, 1904, 1; Vines, 1909]. Similar considerations apply to the vegetable ecto-proteases, *e.g.* of *Nepenthes*, which are considered to be mixtures of proteinases and peptases.

This terminology (which will be adhered to in the following paper) of course in no way conflicts with, or renders unnecessary, the classical individual names pepsin, trypsin and erepsin for the three particular enzymes so designated. Thus pepsin and erepsin are two particular enzymes belonging respectively to the classes proteinases and peptases.

vegetable proteases because a classification of these is not possible according to either the localisation of the enzymes or the nature of the media in which they act.

Three types of animal proteases are known:

1. *Pepsin*, which attacks so far as is known all true proteins. The products formed consist of albumoses and peptones, *i.e.* the hydrolysis is incomplete, lower polypeptides and amino-acids not as a rule appearing. Pepsin occurs in the gastric juice of all the vertebrates examined with the exception of certain fishes and it exerts its optimum activity in faintly acid solution, being quite inactive in an alkaline medium.

2. *Trypsin*, which is active in alkaline or neutral solution only, resolves proteins (as well as albumoses, peptones, etc.) into simple polypeptides and amino-acids. It is found in the pancreatic juice of all vertebrates, as well as in insects, protozoa, sponges, worms and molluscs.

3. *Erepsin* resembles trypsin in actively decomposing albumoses, peptones, protamines and polypeptides into simple amino-acids, but differs widely from it in its inability to decompose higher proteins such as fibrin and albumin. It occurs in the intestinal juice and intestinal mucous membrane and appears to be the most widely distributed of all the proteoclastic enzymes. According to Vernon [1904, 1, 2] the peptoclastic activity of trypsin is due to an ereptic enzyme (pancreato-erepsin) associated with it, so that it is conceivable that trypsin may be really separable into a pepsin and an erepsin (or rather a proteinoclastic and a peptoclastic part).

The vegetable proteases appear to be of two kinds which differ

- (a) in mode of occurrence; and
- (b) in the relations which exist between their digestive activity and the reaction, *i.e.* acidity or alkalinity, of the media in which they act.

One of these, and apparently the smaller class, consists of the extracellular or ecto-proteases which are found in the secretions of certain plants, *e.g.* in the antiseptic almost protein-free sap of the leaf pitchers of *Nepenthes*, in the leaf glands of *Dionaea* and *Drosera* and the leaf edge of *Pinguicula*, and also in bacteria especially those forms capable of liquefying gelatin. According to Vines [1897] the ecto-protease of *Nepenthes* is inactive in neutral and alkaline solutions, but is very active at the natural acidity of the pitcher liquid and also in the presence of free acid (up to 0.3 % HCl). This ecto-protease decomposes proteins not merely into albumoses and peptones but into amino-acids as well, and is considered by Vines to be a mixture of a proteinase and a peptase. On the other hand the ecto-protease of *Drosera* is active in alkaline and neutral as well as in acid media; it would appear therefore that the plant ecto-proteases are less rigid than the corresponding animal ones in respect to their activity in acid or alkaline media.

The second and larger class of vegetable proteases comprises the intracellular or endo-proteases most of our knowledge of which is due to Vines [1904, 1905, 1906, 1908, 1909, 1910]. They occur more especially in germi-

nating and ungerminated seeds and more abundantly in oil-bearing than in starch-containing seeds, and particularly in *Cannabis sativa*, *Sinapis*, *Ricinus* and *Linum*. They also occur in certain juicy fruits (*Ficus carica*) and leaves (*Agave*) and in many fungi such as yeasts. They belong to two groups (a) proteinases¹ and (b) peptases¹.

(a) The *proteinases* break down the true proteins such as fibrin and albumin, the decomposition however proceeding only as far as albumoses and peptones, behaving in this respect like animal pepsin. They are readily soluble in NaCl-solution but only slightly so in water or 50 % alcohol. Their extraction from seeds by means of water is therefore slow, as salts, etc., are extracted first, the enzymes dissolving in the solution so produced. Their digestive activity is greatest at the natural acidity of the plant extract. With pure aqueous extracts the activity is strong in neutral and even in slightly alkaline solution, but a slight addition of mineral acid (0.05 % HCl) or of rather more organic acid (0.30 % citric) arrests the action. Increase of alkalinity does the same. In this respect they differ from animal pepsin and the ecto-protease of *Nepenthes*, both of which show their optimum activity in the presence of free acids. In this connection it is worthy of note that Fernbach and Hubert [1900] regard the primary (acid) and secondary (basic) phosphates present in malt extracts as determining the course of protein-digestion, the former promoting, the latter retarding it.

The endo-proteinases very rarely occur alone in the organs of plants where proteins are decomposed but are usually (according to Vines always) accompanied by peptases which correspond with the erepsin of the animal body, differing from it only in their more extensive reaction range in the direction of acidity. As Vines points out, the fact that we never find proteinases apart from peptases must be correlated with the significance of the proteoclastic enzymes in the plant economy: proteinases alone would never be able to carry protein-hydrolysis far enough, as they hydrolyse only as far as albumoses and peptones.

(b) The *peptases* are readily soluble in water and in aqueous solutions of neutral salts. Their digestive activity is exclusively peptoclastic and is especially associated with an acid medium. The reaction range is not known as "pure" solutions of peptase have not been experimented with and the presence of other substances materially affects the action of acids and alkalies upon the enzyme.

Practically all the work done so far on the plant proteases has been confined to those that occur in seeds and in certain juicy fruits and insectivorous plants. Very little work has been done on the enzyme content of other parts of plants. The present investigation was undertaken with the object of determining whether proteoclastic enzymes exist in the ordinary foliage leaves and other parts of certain common farm crops and if so how far they

¹ Called by Vines peptases and ereptases respectively. Cf. note on p. 124.

resemble in their action the seed proteases. The crops experimented with, some fourteen in number, were grown on the college farm of the South-Eastern Agricultural College, Wye, in the spring and summer of 1912, some in the ordinary rotations and some specially for the purpose of this investigation. They belonged to the following species:

Cereals:

Barley	<i>Hordeum sativum</i> (Pers.)
Oats	<i>Avena sativa</i> (L.)
Maize	<i>Zea mais</i>
Rye	<i>Secale cereale</i>

Leguminous plants:

Red Clover	<i>Trifolium pratense</i> (L.)
White Clover	<i>Trifolium repens</i> (L.)
Lucerne	<i>Medicago sativa</i> (L.)
Sainfoin	<i>Onobrychis sativa</i> (L.)
Vetch	<i>Vicia sativa</i>
Field beans	<i>Vicia faba</i> (L.); <i>Vicia vulgaris</i> (Moench)
Peas (field var.)	<i>Pisum arvense</i> (L.)
„ (garden var.)	<i>Pisum sativum</i> (L.)

Other plants:

Buckwheat	<i>Polygonum fagopyrum</i> (L.)
White mustard	<i>Brassica alba</i> (Vis.); <i>Sinapis alba</i> (L.)

They were examined at two stages of growth: in full flower and at a comparatively young stage when the plants were about 8—10 ins. in height. The cereals, however, were examined in the grass stage only. They were gathered, dried and ground as finely as possible in a steel mill and only the part that passed through a sieve of 80 meshes per mm. was used for the work. Owing to the wet summer of 1912 it was found impossible to air-dry them sufficiently to admit of more than very coarse grinding; so recourse was had to oven-drying. The partially dried chopped material was placed in shallow trays in a large iron oven heated by means of Bunsen burners placed underneath to a temperature not exceeding 45°—50°. In these circumstances it is practically certain that the injurious effects of the increased temperature on the enzymes present would be a minimum. It is well known that diastase can withstand higher temperatures than this even in aqueous solution and in common with all enzymes can withstand much higher temperatures when *in situ* than when in solution; *e.g.* in plant material diastase can be heated to 80° without being destroyed although its hydrolysing power is somewhat impaired. Miss White [1909] observed that heating dry oats to a temperature of 100° for 4½ hours was without effect on the enzymes present although they were destroyed by heating to 130° for one hour. The present author has observed that heating dry linseed cake in a steam oven to a temperature of 99° for 4 hours caused only a small reduction (less than 10 %) in the activity of the cyanogenetic

enzymes present. It is true that proteoclastic enzymes are more sensitive to heat than is diastase particularly in solution, but both substrate and decomposition products exert considerable protecting action (Bayliss, Vernon). Moreover, the optimum temperature of animal protease is given as 40° . The presence of such large quantities of adsorbent material as are present in green plants combined with the comparative dryness would tend to make the enzymes still more resistant to heat; so that in all probability no more damage would be done than in the case of simple air-drying under glass. At the same time oven-drying at this temperature facilitates grinding, enabling a much finer powder to be obtained.

In the case of such crops as lucerne and sainfoin which lignify rapidly very early in the flowering period, the main woody stems were rejected and only the leaves, flowers and smaller and younger twigs retained.

Owing to the bulkiness and comparatively small proteoclastic activity of the plant material and the dark colour of the extracts considerable difficulty was experienced in devising a method for estimating any enzymic activity there might be.

The method followed by Vines was first tried. This consists in detecting the presence of tryptophan (a frequent product of tryptic hydrolysis) by means of the violet colour produced by bromine water in the presence of a little HCl. The method requires considerable care and experience in its use before it can be used successfully even with "pure" trypsin and substrate, *i.e.* in colourless or nearly colourless solutions. In the present case the *differences* in activity exhibited by the various plant materials used were insufficient to be detected—let alone estimated—by this method. It was, therefore, abandoned as a quantitative measure but was used as a qualitative test for proteoclastic action since in every instance the controls gave no tryptophan reaction.

The method finally adopted was Sørensen's [1908]. By this method the extent of protein-hydrolysis can be determined from the number of free carboxyl groups formed. By adding excess of formaldehyde solution the free amino-groups of the amino-acids produced were neutralised forming methylene compounds; the carboxyl groups can then be estimated by titrating with $N/5$ $\text{Ba}(\text{OH})_2$, using thymolphthalein as indicator. In this way the extent of protein-hydrolysis can be expressed by the number of cc. of $N/5$ $\text{Ba}(\text{OH})_2$ used in the titration. Also on the assumption that each carboxyl-group formed during the hydrolysis corresponds with one amino-group, the amount of hydrolysis can be stated as mg. of nitrogen, this being obtained by multiplying the number of cc. of $N/5$ $\text{Ba}(\text{OH})_2$ required by 2.8.

In using this method two solutions are required:

A. 0.50 g. of Grubler's thymolphthalein dissolved in 1000 cc. of 93 % alcohol;

B. 50 cc. of commercial formaldehyde (40 %) + 25 cc. of absolute alcohol + 10 cc. of thymolphthalein solution A. $N/5$ $\text{Ba}(\text{OH})_2$ is run in until a faint

green or blue colour is obtained. This solution must be prepared fresh for each series of experiments.

In practice 10 g. of the plant material + 250 cc. of distilled water which had been raised to 40° + a thin layer of toluene in a tightly corked flask served as a control.

10 g. of plant material + 5 g. of Witte's peptone + 250 cc. of water at 40° + a thin layer of toluene; and 10 g. of plant material + 3 g. of legumin + 250 cc. of water at 40° + a thin layer of toluene served as the experimental liquids. All three were contained in tightly corked flasks and were kept in an incubator at 37° for three days². At the end of that time they were filtered as rapidly as possible, and the residues were thoroughly washed with cold water until the volume of the filtrate and washings from each was 400 cc. Preliminary experiments had shown that all amino-acids were washed out when the filtrate and washings occupied this volume. A tenth, *i.e.* 40 cc., of each filtrate was then transferred to a flask, decolorised as far as possible by shaking with alumina cream³, filtered and the residue thoroughly washed. To the filtrate and washings from the control was added 15 cc. of solution *B* and $N/5$ $\text{Ba}(\text{OH})_2$ run in until a distinct blue or green colour⁴ was obtained. The other filtrates were then treated in the same way, $N/5$ $\text{Ba}(\text{OH})_2$ being added to them until their tint matched that of the control solution. To the actual readings of $\text{Ba}(\text{OH})_2$ used a correction had to be applied to allow for the amount of $N/5$ $\text{Ba}(\text{OH})_2$ absorbed by the undecomposed protein, *i.e.* either legumin or peptone, left in the solution.

The results obtained for the action of 10 g. of plant material on 5 g. Witte's peptone during 3 days' incubation at 37° are given in Table I, A.

A consideration of the results given in Table I, A, indicates that a protease capable of splitting up Witte's peptone into simple amino-acids is present in all the plants examined. The peptoclastic activity varies with the nature of the plant but is an individual rather than a generic character. Thus, contrary to expectation, the leguminous plants examined did not on the whole possess greater peptoclastic power than the non-leguminous ones. The activity of the foliage of young peas (field variety) was found to be some $2\frac{1}{2}$ times that of young oat plants; but the leguminous plants other than peas were in no way

¹ This temperature was chosen in order that the temperature of the plant-water mixtures might be in the neighbourhood of 37° at the beginning of each experiment.

² This period was shown by preliminary experiments to be a suitable time for digestion. It was sufficiently long to allow a measurable amount of hydrolysis to occur but not long enough for the reaction to have reached its limit.

³ The alumina cream was made by gradually adding excess of ammonia solution to saturated alum solution with constant shaking, allowing to stand for several hours, and then filtering off the precipitated alumina. It is very important that this should be washed until free from NH_3 and sulphate either of which would interfere with the titration. The alumina was then shaken up with water until a thin cream was obtained.

⁴ Blue if the original solution was colourless, green if it was yellowish or brownish. Most of the extracts dealt with were deep brown in colour and after shaking with alumina cream were slightly yellow or brownish. This did not interfere with the titration provided all three filtrates were of the same tint.

superior to the non-leguminous ones, while white mustard (a non-leguminous plant) especially in the flowering stage was distinctly more active than any of the other plants examined except peas.

Table I.

Name of plant	A. Witte's Peptone ¹ .		B. Legumin ² .	
	N lib. in mg. = c.c. N/5 Ba(OH) ₂ required for whole extract × 2·8	Percentage of peptone hydrolysed	N lib. in mg. = c.c. N/5 Ba(OH) ₂ required for whole extract × 2·8	Percentage of legumin hydrolysed
Cereals (grass stage only):				
Barley	128·80	18·68	30·66	5·68
Oats	78·40	11·37	18·93	3·51
Maize	103·60	15·02	37·80	7·01
Rye	109·20	15·84	16·80	3·11
Leguminous plants:				
Red clover (young)	135·80	19·70	24·92	4·62
„ (old)	166·60	24·16	35·28	6·54
White clover (young)	134·40	19·49	22·68	4·20
„ (old)	165·20	23·96	30·24	5·61
Lucerne (young)	85·60	12·41	21·28	3·94
„ (old)	158·50	22·99	25·56	4·74
Sainfoin (young)	98·00	14·21	8·93	1·65
„ (old)	152·00	22·04	10·22	1·89
Vetch (young)	120·40	17·46	19·15	3·55
„ (old)	144·20	20·91	24·27	4·50
Beans (young)	81·20	11·78	10·22	1·89
„ (old)	102·20	14·82	15·34	2·84
Peas (f. var.) (young)	196·00	28·43	38·07	7·05
„ „ (old)	236·60	34·31	50·10	9·36
Peas (g. var.) (young)	140·00	20·30	39·20	7·27
„ „ (old)	177·80	25·79	47·88	8·88
Other plants:				
Buckwheat (young)	89·60	12·99	7·675	1·42
„ (old)	112·56	16·32	8·50	1·57
White mustard (young)	125·20	18·16	17·92	3·32
„ (old)	199·40	28·92	25·56	4·74

¹ Percentage of nitrogen in Witte's peptone = 13·79.

² Percentage of nitrogen in legumin = 17·97.

The activity varies with the stage of development of the plant and is in every case greater at the flowering period than in the earlier ones.

In some cases the tryptophan reaction was applied and gave positive results in some few instances with the plant-peptone extracts; with most of the plant extracts, however, as with the controls, no colour change occurred. It does not follow of course that no tryptophan was formed in these plant extracts either from the splitting up of the plant proteins themselves or from the added protein. In any case very little would be formed and this would be distributed throughout 400 cc. of liquid of which 10—20 cc. were taken

for the test. The absence of any tryptophan reaction is therefore quite explicable even though some slight formation of tryptophan had occurred.

In Table I, B, are given the results obtained for the action of 10 g. of plant material on 3 g. of legumin during 3 days' incubation at 37°.

The results given in Table I, B, are interesting in view of Vines' statement that out of a very large number of leaves examined by him only those of the lettuce and of *Phytolacca decandra* would digest blood fibrin or milk caseinogen. All other leaves gave either a doubtful or negative result. From this he concluded that the hydrolysis of albumoses and peptones by green plants is related to the fact that those proteins which occur naturally in the plant are such as are readily digested; whereas the more resistant ones similar to fibrin and caseinogen (which possibly do not occur in plant tissues, but only in the seeds where proteinases do undoubtedly occur) are not attacked.

The two results are, however, not altogether incompatible. Vines used fibrin in the form of small shreds in his experiments and relied on the appearance or, rather, the disappearance, of the fibrin after incubation as a test of hydrolysis. The legumin used by the present author was in the form of a very fine powder; the surface exposed to any proteoclastic action there might be would be much larger than in the former case and digestion therefore more rapid. The method employed for detecting the hydrolysis was also a more delicate one and even under these favourable conditions the hydrolysis was slight as compared with that of the peptone. It is doubtful whether such a small hydrolysis would produce any *visible* effect on small shreds of fibrin suspended in the extracts.

That the activity indicated is enzymic in nature and not merely the result of imperfections in the conditions of experiment seems to be shown by the fact that, as with peptone, the activity is invariably greater at the flowering stage than at the earlier one, and the greater activity of peas is again evident when legumin is employed as the substrate.

Having established the presence of proteinoclastic and peptoclastic enzymes in all the green plants examined it was next decided to examine one or two plants in detail in order to find out how the different parts of the plants varied as regards enzyme content. For this purpose an examination was made of beans, peas (field variety) and buckwheat. In this portion of the work the different parts of the plant were gathered separately and dried—first by air-drying and then *in vacuo* over sulphuric acid.

To test the effect of germination the seeds were germinated in moist sand kept in a warm place, were dried as rapidly as possible in an air-oven at 40–45°, coarsely ground and finally dried *in vacuo* over sulphuric acid. The enzymic activity was determined by Sørensen's method as described above. The results are given in Table II, A and B.

An examination of the tables shows that all parts of the plant are active and at all stages of growth. The activity of the leaves and seeds presents features of particular interest. Both the proteinoclastic and peptoclastic

activity of the leaves increases with increasing maturity right up to the time of harvesting and does not fall off, as was at first anticipated, after the flowering stage when translocation of foodstuffs from the leaves to other parts of the plant is taking place and during the dying off of the leaves. In this connection it is interesting that increasing activity with increasing maturity of the foliage right up to the time at which the leaves begin to die off has been shown to hold also for the cyanogenetic enzymes present in flax foliage. Thus the foliage of flax in full flower has been found to be rich in both cyanogenetic enzyme and cyanophoric glucoside; whereas flax leaves which have turned brown at the edges are still as rich in enzyme some time after the corresponding glucoside has completely disappeared.

Table II.

Part of plant	A. <i>Witte's Peptone.</i>		B. <i>Legumin.</i>	
	N lib. in mg. = c.c. $N/5$ Ba(OH) ₂ required for whole extract × 2·8	Percentage of peptone hydrolysed	N lib. in mg. = c.c. $N/5$ Ba(OH) ₂ required for whole extract × 2·8	Percentage of legumin. hydrolysed
Beans:				
Leaves (plant 3-4 in. high)	81·20	11·77	10·22	1·73
„ (full flower) ...	102·20	14·82	15·34	2·84
„ (at harvest) ...	159·80	23·71	17·92	3·32
Flowers ...	153·40	22·25	19·18	3·56
Seeds (ungerminated) ...	154·65	22·43	14·06	2·61
„ (just germinated) ...	188·20	27·29	12·77	2·37
„ (germinated) ...	230·10	33·37	14·06	2·61
Pods ...	139·45	20·22	10·00	1·74
Stems ...	93·50	13·56	14·06	2·61
Peas (field var.):				
Leaves (plant 6-8 in. high)	196·00	28·42	38·08	7·06
„ (full flower) ...	236·60	34·32	50·96	9·45
„ (at harvest) ...	276·10	40·04	55·44	10·38
Flowers ¹ ...	171·40	24·85	25·56	4·74
Seeds (ungerminated) ...	120·40	17·46	24·08	4·47
„ (germinated) ...	244·10	35·41	47·04	8·80
Pods ...	145·00	21·03	12·60	2·34
Stems ...	83·44	12·10	13·44	2·49
Buckwheat:				
Leaves (v. young) ...	89·60	12·99	7·67	1·42
„ (full flower) ...	112·55	16·32	8·95	1·66
„ (at harvest) ...	222·40	32·25	10·22	1·90
Flowers ...	122·60	17·79	35·78	6·64
Seeds (ungerminated) (6 dys.)	103·40	15·00	5·18	0·96
„ (just germinated) ...	114·80	16·65	12·77	2·37
„ (germinated) ...	177·90	25·21	—	—

¹ This figure is only approximate as the pigment present interfered with the titration and made the end-point indeterminate.

The effect of germination on the enzyme content is also of considerable interest. It would appear from the results obtained that ungerminated seeds,

like all other parts of the plant, are active towards Witte's peptone and slightly so towards legumin. When germinated so that the young shoot is just showing itself a distinct increase in peptoclastic activity occurs; when the germination has proceeded until the shoots are one to two inches in length both the proteinoclastic and peptoclastic activity become much greater. This is shown best in the case of buckwheat and falls in line with Vines' observations that the activity is not at once evident but is developed during the germination process¹.

The above investigation also throws a little light on another interesting point. Vines [1903] first pointed out the possibility of the proteinoclastic and peptoclastic enzymes of green fodder crops being of use to the animal during the process of digestion. He pointed out that in the case of herbivorous animals and especially the ruminants the vegetable food that has been eaten is probably placed under conditions that are altogether favourable to the action of the proteases which it contains, so that there is reason to believe that digestion in these animals is, in no small degree, a process of autolysis, the food providing at once the nutriment and the means of digesting it. It is by no means impossible too that the enzymes present may even assist in the digestion of any additional protein that may be fed along with the greenstuff. The results obtained above indicate that the peptoclastic activity of green fodder plants is sufficiently great to be of assistance to the animal in the digestion of the simpler, more easily digested, proteins, but that the proteinoclastic activity is much too slight to be a serious factor in the digestion of the higher proteins that may be present in, or fed along with, the green food.

Whether the conditions operative in the animal's stomach are such as admit of auto-digestion of proteins taking place is another matter and cannot be determined until we know the quantitative relationship that exists between the peptoclastic activity of plant proteases and the reaction of the medium in which they act. Attempts were made to determine this but with little or no success. Various aqueous extracts of the plant material were obtained and the action of acids and alkalies upon their peptoclastic activity was investigated. In the case of the alkalies more or less bulky precipitates were formed in all cases during the incubation and no consistent results could be obtained. This is not very surprising when it is remembered that plant extracts are very slightly acid in reaction. On changing the reaction of the medium the internal equilibrium is disturbed and the colloidal substances present are partially precipitated carrying some enzyme down with them.

The whole extract is an extraordinarily complex colloidal system and even

¹ The sudden increase in peptoclastic activity that occurs in seeds during germination finds an interesting parallel in the case of the lipase of linseed. While a lipase is frequently found in many oil-bearing seeds—being especially abundant in species of *Ricinus*—its presence had not until recently been detected in linseed although many attempts have been made to find it. The present author, in conjunction with Dr J. V. Eyre, has succeeded in showing the presence of a lipase in *germinated* linseed and has measured its activity. It would appear to be developed during the germination process.

if no disturbing factors were introduced and it were possible to determine accurately the effects of acids and alkalies on the enzymic activity the results obtained would not necessarily correspond to those obtained when using "pure" enzyme preparations. All that can be deduced from these latter experiments is that the peptoclastic activity of plant extracts is not destroyed by the addition of small quantities (*e.g.* up to 0.05 %) of either acids or alkalies. But the direct action of acids or alkalies cannot be determined until the enzymes can be extracted in a less impure condition. This is being attempted, and it is hoped that a systematic study of the vegetable peptases may form the subject of a further communication.

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