# The Proteases of Plants (II).

BY

S. H. VINES,

*Sherardian Professor of Botany in the University of Oxford.*

IN the interval that has elapsed since the publication (April, 1904) of my last paper on this subject (1) in this periodical, various facts have come to light that contribute not a little to the interest and to the intelligent apprehension of it: although progress in so large a field has not been rapid, for the number of investigators exploring it is still relatively small.

## Papaïn.

This material has been more fully investigated than any other in the vegetable kingdom, and yet it cannot be said to be by any means fully understood. The occasion of my reverting to the subject was the reading of a paper on it by Emmerling (2), who found that papaïn digested fibrin most actively in a slightly alkaline liquid; and that, although the amount of amido-acids, &c., produced was relatively small, its action was 'specifically tryptic.'

As his results do not altogether agree with those published by me (3), I have repeated some of my former experiments and have made some under the conditions adopted by Emmerling. His method was somewhat as follows:—1,000 grms. of dry fibrin were covered with feebly alkaline water, but the degree of alkalinity is not stated: 20 grms. of Merck's papaïn were added, as also some toluol. After fourteen days in the incubator at 37°C., 10 grms. of papaïn were added; and after fourteen days more, further 10 grms. of papaïn. At the close of this prolonged digestion, the products were found to be much albumose and peptone, and small quantities of arginin, tyrosin, leucin, asparaginic acid, glycocoll, glutaminic acid, alanin, and phenyl-alanin. No account is given of any experiments made with acid or neutral liquid.

Apart from the alkaline medium, the conditions of Emmerling's experiments differed from mine in that he used Merck's preparation of papaïn, whereas I used Christy's preparation; and further, in that he used toluol, and I chiefly HCN, as the antiseptic. Moreover, the relative weight of the fibrin to that of the papaïn was very much larger in his than in mine.

[Annals of Botany, Vol. XIX. No. LXXIII. January, 1905.]

I felt sure that the points of divergence between his results and mine—such as the slowness of the digestive action on fibrin that he observed, and the relatively small production of amido-acids and hexon-bases—could be accounted for by some or all of these differences of method; and experiment has largely realized this anticipation. The method I adopted was to institute comparative experiments with papaïns derived from several sources, with acid and alkaline liquids, and with toluol and HCN as antiseptics. The quantity of fibrin used was both relatively and absolutely small. The following description of an experiment will make the method clear.

*Fibrin-Digestion.* Three samples of papaïn were used, obtained respectively from Messrs. Christy, Finkler, and Merck.

4 grms. of each sample of papaïn were mixed with 160 c.c. of distilled water, and after standing for some time were strained through muslin: the liquids obtained were turbid, and gave no tryptophane-reaction; the Christy and Merck liquids were slightly acid, the Finkler liquid neutral.

The 160 c.c. of each papaïn-liquid were put into four bottles, 40 c.c. in each, and were further treated as follows:—

No. 1, added toluol to 1%, and citric acid to 0·5 %
„ 2, „ „ „ $Na_2CO_3$ „ 0·75 %
„ 3, „ HCN to 0·2%, „ citric acid „ 0·5 %
„ 4, „ „ „ $Na_2CO_3$ „ 0·75 %

The contents of Nos. 2 and 4 were distinctly alkaline, and remained so throughout the experiment. To each bottle ½ grm. moist fibrin was added. There were thus twelve bottles in all put to digest in the incubator at about 40° C.

After 18 hours' digestion, the effect upon the fibrin was as stated below:—

| | (1) *Toluol acid.* | (2) *Tol. alk.* | (3) *HCN acid.* | (4) *HCN alk.* |
|---|---|---|---|---|
| Christy | not gone | not gone | gone | gone |
| Finkler | not gone | not gone | not gone | nearly gone |
| Merck | not gone | not gone | partly gone | gone |

Four hours later the fibrin had completely disappeared in the Finkler bottle No. 4.

After 24 hours' digestion the state of the fibrin was:—

| | *No.* 1. | *No.* 2. | *No.* 3. |
|---|---|---|---|
| Christy | not gone | not gone | — |
| Finkler | not gone | not gone | nearly gone |
| Merck | not gone | not gone | nearly gone |

after 44 hours' digestion the results were:—

| | *No.* 1. | *No.* 2. | *No.* 3. |
|---|---|---|---|
| Christy | nearly gone | nearly gone | — |
| Finkler | going | unaltered | gone |
| Merck | going | unaltered | gone |

after 68 hours' digestion :—

| | *No.* 1. | *No.* 2. |
|---|---|---|
| Christy | gone | gone |
| Finkler | nearly gone | unaltered |
| Merck | gone | unaltered |

after 92 hours' digestion the final results were :—

| | *No.* 1. | *No.* 2. |
|---|---|---|
| Finkler | gone | unaltered |
| Merck | — | swollen, but not digested. |

From these observations it may be concluded that :—

(1) the action on fibrin of these different samples of papaïn was not uniform, Christy's proving to be the most active ;

(2) the action was much slower in the presence of toluol than in the presence of HCN ;

(3) in the presence of HCN, the action was on the whole more rapid in the alkaline than in the acid liquid ; whilst in the presence of toluol it was more rapid in the acid than in the alkaline.

These conclusions afford the explanation why Emmerling found the process of digestion to be so slow in his experiments. It was slow (1) because he used Merck's papaïn and in relatively small quantity ; (2) because he used toluol as the antiseptic ; and (3) because the reaction of the digesting liquid was alkaline. It is clear, from the observations given above, that this was a singularly unfortunate combination of conditions, inasmuch as Merck's papaïn, though active enough under other circumstances, proved to be altogether inert in an alkaline liquid containing toluol, although in my experiment the amount of fibrin to be digested was relatively small.

The other point noted by Emmerling—the smallness of the amount of amido-acids, &c., formed in his digestion-experiments—remains to be considered. With this in mind, I made some observations by means of the tryptophane-method from time to time during the progress of the fibrin-digestion, with the following results :—

*Tryptophane-reactions.*

After 20 hours' digestion :—

| | *No.* 1. | *No.* 2. | *No.* 3. | *No.* 4. |
|---|---|---|---|---|
| Christy | — | — | marked | distinct |
| Finkler | — | — | — | distinct |
| Merck | — | — | — | faint |

after 44 hours' digestion :—

| | | | | |
|---|---|---|---|---|
| Christy | — | — | marked | distinct |
| Finkler | — | — | distinct | strong |
| Merck | — | — | distinct | distinct |

after 68 hours' :—

| | *No.* 1. | *No.* 2. | *No.* 3. | *No.* 4. |
|---|---|---|---|---|
| Christy | faint | distinct | — | — |
| Finkler | distinct | marked | — | — |
| Merck | none | distinct | — | — |

The tryptophane-test was applied when the fibrin had disappeared in each bottle, except in the case of those No. 2 bottles (Finkler, Merck) where it did not disappear at all.

Seeing that the liquids gave no tryptophane-reaction to begin with, it follows (1) that all the samples of papaïn tested proved capable of effecting complete proteolysis, or at any rate peptolysis, in various degree ; (2) that on the whole their action was more vigorous in the presence of HCN than in the presence of toluol ; and (3) that the action was on the whole stronger in the alkaline than in the acid liquids, though the difference was not great. The explanation of Emmerling's result is that probably his sample of Merck's papaïn, like mine, did not actively peptolyse in alkaline liquid containing toluol ; and, more certainly, that the quantity of papaïn used by him was too small in proportion to the fibrin.

But the evidence of the tryptophane-reactions given above is not conclusive, and is even to some extent paradoxical. For instance, the Finkler bottle No. 2 (toluol-alk.) gave almost as good a reaction as the Finkler No. 4 bottle (HCN-alk.), though in the latter case the fibrin had been digested and in the former it had not. It is obvious, therefore, that these tryptophane-reactions do not necessarily indicate the complete proteolysis of the fibrin supplied for digestion in the experiment; on the contrary, they indicate, in some cases at any rate, the proteolysis of some proteid other than fibrin, and one that is more readily proteolysable. The probable explanation is that all specimens of papaïn contain, in addition to protease, more or less proteid matter. In order to obtain some idea of the extent of this self-digestion, I made a series of experiments without any added proteid ; and, to connect them with my earlier observations (3), I included a series of bottles with sodium fluoride (NaF) as an antiseptic. The experiments may be summarized as follows :—

### *Autolysis-Experiments.*

The method adopted was to put 0·5 grm. of papaïn in each bottle with 40 c.c. distilled water (=1·25 %), containing the antiseptic (toluol 1 %, or HCN 0·2 %, or NaF 1 %): then the contents of the bottles were made either acid (citric acid 0·5 %), or alkaline ($Na_2CO_3$ 0·5 %), or were left at their natural reaction, which was slightly acid in the case of Christy's and Merck's samples, neutral in Finkler's. The mixtures thus prepared of Christy's and Finkler's gave no tryptophane-reaction: but the fresh sample of Merck's used in these experiments (but not in the one previously described)

gave a distinct reaction before digestion. There were thus nine bottles in each experiment.

The application of the tryptophane-test in comparative experiments requires some care in order to ensure that the maximal reaction is really obtained. The reaction of the liquid must be acid; and then the chlorine-water should be added gradually until a vol. equal to that of the liquid to be tested (I usually took 5 c.c.) has been used: after standing some minutes a second vol. of chlorine-water should be added, and if necessary a third vol., until further addition does not intensify the reaction, but only weakens it. I found that the maximum was generally obtained with about 2 vols. of chlorine-water: but sometimes with less, sometimes with more.

The duration of the digestion was 48 hours: the $Na_2CO_3$ bottles remained alkaline throughout the experiment. The terms used in describing the intensity of the tryptophane-reaction are, as in previous papers, the following: faint, distinct, marked, strong, very strong.

It must also be explained that the sample of Merck's papaïn used in this and subsequent experiments was not the same as that used in the previous experiments (see p. 150), but a fresh and apparently much more active sample.

The resulting tryptophane-reactions were as follows:—

*A.* Christy's Papaïn.

| | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|
| Toluol 24 hours | none | faint | none |
| „ 48 „ | none | faint | none |
| HCN 24 „ | distinct | none | distinct |
| „ 48 „ | marked | none | distinct |
| NaF 24 „ | faint | none | none |
| „ 48 „ | faint | none | faint |

*B.* Finkler's Papaïn.

| | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|
| Toluol 24 hours | faint | strong | distinct |
| „ 48 „ | distinct | strong | distinct |
| HCN 24 „ | marked | faint | distinct |
| „ 48 „ | marked | faint | distinct |
| NaF 24 „ | faint | distinct | faint |
| „ 48 „ | faint | marked | distinct |

*C.* Merck's Papaïn.

| | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|
| Toluol 24 hours | distinct | distinct | faint |
| „ 48 „ | marked | distinct | distinct |
| HCN 24 „ | marked | faint | distinct |
| „ 48 „ | marked | faint | distinct |
| NaF 24 „ | marked | distinct | distinct |
| „ 48 „ | marked | distinct | distinct |

The autolysis-results bring out once more the specific differences in activity between the three samples of papaïn. The most striking tryptophane-reaction was given by Finkler's papaïn, on account, probably, of its containing the largest amount of readily proteolysable proteid. With respect to the reaction of the liquid, Christy's and Merck's samples gave better results in acid than in alkaline liquids, whilst Finkler's was more active in alkaline than in acid. As to the antiseptics employed, Christy's papaïn was active in the presence of HCN, but its action was almost entirely inhibited by both toluol and NaF: Finkler's and Merck's showed about the same activity with all three antiseptics, Finkler's being especially active in the presence of toluol (alkaline). It must be remembered that the Merck solution gave a tryptophane-reaction to begin with.

With the object of obtaining further information as to the relative peptolytic activity of the three samples under various experimental conditions, an experiment was prepared in which such a quantity of readily peptolysable proteid was added—in the shape of Witte-peptone—as to render negligible, on the whole, whatever amount of proteid may have been originally present in the papaïn.

I desired, further, that the experiment should be such as to permit of some comparison between the fibrin-digesting and the peptolysing activity of the papaïn in each case. In previous observations (3) I had found that these two processes do not necessarily run parallel, and that the one may take place without the other. I therefore now added fibrin, as well as Witte-peptone, to the bottles in the following experiments.

### *Fibrin-digestion and Peptolysis.*

The general method adopted was to extract 8 grms. of papaïn for 2–3 hours with 400 c.c. distilled water, and filter. To the filtered liquid 6 grms. of Witte-peptone were added, causing a considerable precipitate in every case. To 120 c.c. of this liquid toluol was added to 1 %: to 120 c.c., HCN to 0·2 %; and to 120 c.c., NaF to 1 %. 40 c.c. of each of these liquids were put into each of three bottles, to which were added respectively either citric acid to 0·5 %, or $Na_2CO_3$ to 0·5 %, or nothing: in the last case the reaction was neutral or slightly acid. Finally 0·3 grm. fibrin was added to each bottle. The effect of digestion on the fibrin, and the tryptophane-reactions were:—

#### *A.* Christy's Papaïn.

| | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|
| Toluol 24 hours fibrin | unaltered | gone | unaltered |
| „ „ „ tryptophane | distinct | distinct | faint |
| „ 48 „ fibrin | not gone | gone | gone |
| „ „ „ tryptophane | distinct | marked | faint |

| | | | | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|---|---|---|
| HCN | 24 | hours | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | very strong | marked | distinct |
| „ | 48 | „ | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | very strong | marked | strong |
| NaF | 24 | „ | fibrin | gone | gone | nearly gone |
| „ | „ | „ | tryptophane | faint | distinct | faint |
| „ | 48 | „ | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | faint | marked | distinct. |

### *B.* Finkler's Papaïn.

| | | | | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|---|---|---|
| Toluol | 24 | hours | fibrin | unaltered | nearly gone | unaltered |
| „ | „ | „ | tryptophane | none | none | distinct |
| „ | 48 | „ | fibrin | unaltered | nearly gone | unaltered |
| „ | „ | „ | tryptophane | faint | distinct | faint |
| HCN | 24 | „ | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | distinct | faint | faint |
| „ | 48 | „ | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | distinct | faint | faint |
| NaF | 24 | „ | fibrin | unaltered | partly gone | unaltered |
| „ | „ | „ | tryptophane | none | none | none |
| „ | 48 | „ | fibrin | partly gone | gone | unaltered |
| „ | „ | „ | tryptophane | faint | faint | faint |

### *C.* Merck's Papaïn.

| | | | | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|---|---|---|
| Toluol | 24 | hours | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | very strong | distinct | marked |
| „ | 48 | „ | tryptophane | very strong | marked | marked |
| HCN | 24 | „ | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | strong | distinct | distinct |
| „ | 48 | „ | tryptophane | strong | marked | strong |
| NaF | 24 | „ | fibrin | gone | gone | gone |
| „ | „ | „ | tryptophane | very strong | distinct | marked |
| „ | 48 | „ | tryptophane | very strong | strong | marked |

The maximum reactions, in this case, were not obtained until 3–4 vols. of chlorine-water had been added.

This sample of Merck's papaïn proved itself to be so exceptionally active that there were but slight differences in the results. With the object of obtaining clearer differentiation, I repeated the experiment, using an extract of half-strength (4 grms. papaïn to 400 c.c. distilled water).

*D.* Merck's Papaïn (half-strength).

| | | | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|---|---|
| Toluol | 24 hours | fibrin | nearly gone | gone | nearly gone |
| „ | „ | „ tryptophane | strong | strong | strong |
| HCN | 24 „ | fibrin | gone | gone | gone |
| „ | „ | „ tryptophane | marked | distinct | distinct |
| NaF | 24 „ | fibrin | gone | gone | gone |
| „ | „ | „ tryptophane | very strong | strong | strong |
| Toluol | 48 „ | fibrin | gone | gone | gone |
| „ | „ | „ tryptophane | strong | very strong | very strong |
| HCN | „ | „ „ | strong | distinct | marked |
| NaF | „ | „ „ | very strong | strong | strong |

The results were still insufficiently differentiated, although some differentiation was indicated in the HCN bottles. Following this up, I made a further experiment with still weaker extract (1 grm. to 200 c.c. water) in the presence of HCN, with striking results; the other details were as before.

*E.* Merck's Papaïn (quarter-strength).

| | | | *Acid.* | *Alkaline.* | *Natural.* |
|---|---|---|---|---|---|
| HCN | 24 hours | fibrin | gone | gone | gone |
| „ | „ | „ tryptophane | marked | none | faint |
| „ | 48 „ | „ | marked | faint | distinct |

It will be observed that the results of the experiments *C*, *D*, *E*, with Merck's papaïn, differ in degree only: in all the HCN bottles the alkaline one was that which gave the least marked, and the acid that gave the most marked tryptophane-reaction.

No detailed analysis of the foregoing results is necessary to show that the various samples of papaïn experimented upon differed widely in their general proteolytic activity, and in their relation as well to acid and alkali as to the various antiseptics employed. Moreover, they account for the conflicting and sometimes contradictory observations not only of Emmerling and myself, but also of earlier observers such as Mendel (4), Martin (5), and Wurtz (6). One thing, at any rate, is made clear, that the last word as to the properties of papaïn will not have been pronounced until a series of careful observations shall have been made with perfectly fresh material, so as to avoid all those modifications that must necessarily accompany the preparation of the varieties of dried papaïn which have hitherto been used in experiments. Here is a subject for research that might well engage the attention of one of the botanical laboratories in the tropics.

This somewhat negative conclusion is not, however, the only or the most important one to be drawn. The results show that, as I have already

pointed out, fibrin-digestion and the peptolysis of Witte-peptone are not always similarly affected by the various experimental conditions provided. For instance, Christy's papaïn readily digested fibrin in the presence of NaF, whilst there was at the same time little or no peptolysis; and the same was the case with Finkler's papaïn and with weak Merck's papaïn (*E*), in the presence of HCN. The converse does not come out quite so clearly: but there are indications, as for instance in the toluol-acid experiment with Christy's papaïn (p. 154), of some degree of peptolysis with little or no digestion of fibrin. These facts are susceptible of two interpretations:— (1) that a single protease is present, capable of both fibrin-digestion and peptolysis, and that one or other of these activities may be more or less inhibited by the antiseptics: or (2) that two proteases are present in papaïn, which may respond differently and independently to the action of antiseptics. Of the two alternatives, the former is the one that seems to offer the greater difficulties. It is not easy to imagine how one part of the work of the protease could be arrested without the other: it is more natural to conclude that if the protease were affected at all, the whole of its functional activity would be interfered with. If, then, the second alternative be accepted, the interesting conclusion is arrived at that papaïn contains a fibrin-digesting, but not peptolytic, protease of the nature of a pepsin; as well as a peptolytic, but not fibrin-digesting, protease of the nature of an erepsin. If this be so, it will be the first clear demonstration of the existence of a pepsin in the Vegetable Kingdom.

I do not claim that my experiments suffice to establish this conclusion beyond the possibility of doubt. But they at least indicate a method by which a physiological analysis of possible mixtures of enzymes may be effected. It is, I believe, by the application of this method to the fresh juices of the Papaw that the question will be settled for that plant. In the meantime I am applying it to the investigation of other plants, and have already obtained some confirmatory results in the case of the Hyacinth-bulb, of the Pine-apple, and of Yeast, of which I hope to give an account in a subsequent paper. An investigation of animal trypsin along these lines would, I believe, yield results of considerable interest.

## Digestion by Leaves.

In a previous paper (7) I have given an account of some experiments upon the proteolytic action of the foliage-leaves of various plants, viz.: *Spinacia oleracea*, *Dahlia*, *Mirabilis Jalapa*, *Tropaeolum majus*, *Prunus Laurocerasus*, *Ricinus communis*, *Helianthus tuberosus*, *Pelargonium zonale*, *Brassica oleracea*, *Holcus mollis*, *Phalaris canariensis*, *Apium graveolens*, *Scolopendrium vulgare*, *Lactuca sativa*. The conclusion to be drawn from those experiments was that the leaves could peptolyse but not peptonise; in other words, that they contain an erepsin but no fibrin-digesting protease.

Since then I have made further investigations in this direction, and I avail myself of this opportunity to place them on record. I have confirmed my previous results in many of the cases mentioned above, and have extended them to the Lime (*Tilia vulgaris*), the Rhubarb (*Rheum officinale* and *undulatum*), and *Phytolacca decandra*. The observations on *Rheum* and *Phytolacca* present features of sufficient interest to justify special mention.

**Rheum officinale and undulatum.**

These leaves were selected with the object of testing the digestive activity of tissues known to be strongly acid.

In preparing the leaf for the experiment, the petiole and the lamina were kept separate: on grinding in the mincing-machine, a quantity of clear acid liquid was obtained from the petioles; a watery extract was made of the lamina, and strained through muslin: the liquids were strongly acid. The earlier experiments gave a purely negative result: e. g.—

40 c.c. of petiole-liquid were put into each of two bottles: to the one 0·2 grm. of fibrin was added, to the other 0·5 grm. of Witte-peptone; the antiseptic was toluol 1 %; two similar bottles of lamina-extract were prepared. After digestion for about 70 hours the fibrin was unaffected, and no tryptophane-reaction could be detected in any bottle.

It then occurred to me that possibly digestive action had been inhibited by the acidity of the liquids. I therefore made an experiment in which excess of $CaCO_3$ had been added to the liquids, but the results were still negative.

I repeated the experiment with the modification that the tissue was extracted with water for several hours after mincing: the effect was that the bottles containing Witte-peptone gave more or less distinct tryptophane-reaction. The inference to be drawn seemed to be that the protease is not readily to be extracted from the tissues of the Rhubarb. Acting on this, I made experiments in which the minced tissue itself was used, with complete success, e. g.—

250 grms. of lamina were minced, a little distilled water was added, and the mixture left to stand for 20 hours; the liquid was then strained through muslin; about 200 c.c. were obtained, giving no tryptophane-reaction. Four bottles, of 100 c.c. each, were then prepared as follows:—

No. 1. 100 c.c. strained liquid + 1 grm. Witte-peptone;
„ 2. „ „ „ „ + 5 grms. $CaCO_3$;
„ 3. 10 grms. lamina, 100 c.c. distilled water + 1 grm. Witte-peptone;
„ 4. „ „ „ „ „ + 5 grms. $CaCO_3$.

Toluol was added to 1 %.

After 22 hours in the incubator, the tryptophane-reactions were:—

No. 1, no reaction (liquid acid); No. 2, no reaction (liquid neutral);
„ 3, strong „ „ ; „ 4, marked „ „ .

Hence it appears that peptolysis is effected readily by the tissue itself, whether the reaction be acid or neutral, whilst extracts may be inert. It should be added that I failed to detect any digestion of fibrin, whether extracts or tissue were used; so that this leaf, like so many others, seems to contain only erepsin.

### Phytolacca decandra.

The interest attaching to the observations on the leaves of this plant is that they afford the first instance of fibrin-digestion by ordinary foliage-leaves, as distinguished from the leaves of carnivorous plants (*Nepenthes*, &c.), on the one hand, and the leaves of laticiferous plants (e. g. the Fig) on the other

70 grms. of fresh leaves were minced with the machine and were extracted with 150 c.c. distilled water containing 1 % toluol for 22 hours: the liquid was then strained off through muslin: 40 c.c. liquid were placed in each of four bottles, to which was added—No. 1, nothing; No. 2, ½ grm. Witte-peptone; No. 3, ½ grm. casein; No. 4, 0·2 grm. fibrin.

After 21 hours' digestion the fibrin had disappeared in No. 4. The tryptophane-reactions were—in No. 1, distinct; No. 2, strong; No. 3, marked; No. 4, distinct.

In another similar experiment, 50 c.c. of leaf-extract digested ½ grm. fibrin within 48 hours, the liquid then giving strong tryptophane-reaction.

### Ficus Carica.

It is well known that the latex of the Fig digests proteids (see my paper 8): but I thought that the investigation of the leaves from this point of view might yield interesting results, especially if experiments were made at different times of the year.

The first experiment was made on June 4, when the leaves were young, and did not seem to contain any milky latex.

Experiment 1. 60 grms. fresh leaves were minced with the machine, the material being then extracted for a short time with 200 c.c. distilled water, containing 1 % toluol. 50 c.c. of the strained liquid were put into each of two bottles: to the one was added ½ grm. of fibrin, to the other ½ grm. of casein. At the end of 48 hours in the incubator the fibrin remained unaltered, and neither bottle gave any tryptophane-reaction.

The second experiment was made on August 11, when the leaves contained latex abundantly; digestion was then rapid.

Experiment 2. 90 grms. of leaves were extracted with about 200 c.c. distilled water with toluol 1 %. 50 c.c. of the strained liquid were put into each of two bottles; to the one was added ½ grm. fibrin, to the other ½ grm. Witte-peptone.

After 23 hours' digestion the fibrin had disappeared in the one bottle, and the contents gave strong tryptophane-reaction; the contents of the other bottle also gave a strong reaction.

It would appear from these results that the protease is contained in the latex and not in the tissues. In connexion with Experiment 2 I incidentally made an observation of some interest that has a bearing upon the foregoing conclusion. On testing the reaction of the leaf-extract, I was surprised to find that it was not acid, as is generally the case with vegetable extracts. It seemed to be somewhat alkaline, and further inquiry proved it to be amphoteric. The latex dropping from an injured leaf was strongly acid. However, at the close of the digestion-experiments the reaction of the liquid had become distinctly acid.

### Asparagus officinalis.

Though the material for these observations consisted not of leaves but of shoots, they may be conveniently introduced here. Their interest lies in the fact that they afford yet another instance of fibrin-digestion by plants.

(June). On mincing a number of shoots with the machine, enough juice was obtained for the purpose of experiment. It was an acid, turbid liquid; when further acidified with acetic acid, boiled and filtered, it gave a marked tryptophane-reaction on the addition of 2–3 times its volume of chlorine-water. Since the liquid gives the tryptophane-reaction to begin with, it cannot be used for experiments on peptolysis.

50 c.c. of the expressed juice, with toluol added to 1 %, were put into each of three bottles, with ½ grm. fibrin; to No. 1, nothing further was added; to No. 2, $Na_2CO_3$ to 1 %; to No. 3, HCl to 0·16 %.

After 22 hours in the incubator, the fibrin was found to be mostly digested in Nos. 1 and 3, and apparently unaltered in No. 2; 24 hours later it had disappeared in Nos. 1 and 3, and was partly digested in No. 2.

### Cucurbita Pepo var. ovifera.

I may also include some observations on the digestion of fibrin by the Vegetable Marrow. I have not always succeeded with this material: but the following are the details of a successful experiment.

Part of a green, not quite ripe, Marrow, with the rind, was minced, and from the tissue 300 c.c. of expressed juice were obtained: 50 c.c. of it were put into each of five bottles with 0·2 grm. fibrin, and there was further added—to No. 1, nothing; to Nos. 2, 3, 4, 5, HCl to 0·1, 0·2, 0·5, 0·5 % respectively, and to No. 5 some toluol. After 22 hours in the incubator, the fibrin had disappeared in all the bottles; and their contents all gave marked tryptophane-reaction.

It may be useful to those interested in the investigation of proteolysis in plants if I append a chronological summary of all the known cases, which have been adequately examined from the chemical point of view, of the digestion of fibrin or albumin—cases, that is, which indicate the presence of a protease other than erepsin. The dates given are those of the publication of papers.

Germinating seeds: von Gorup-Besanez ('74), Green ('86, '90), Neumeister ('94), Vines (Wheat-Germ; '03), Weis ('03).
*Nepenthes*: von Gorup-Besanez ('76), Vines ('77, '97, '98, '01), Clautriau ('00).
*Carica Papaya* (papaïn): Wurtz ('79), Martin ('84, '85), Vines ('01, '03, '05), Mendel and Underhill ('01), Emmerling ('02).
*Ficus Carica* (Fig): Hansen ('86, '87), Mussi ('90), Vines ('02, '05).
Myxomycetes: Krukenberg ('79), Greenwood ('85, '87).
Bacteria: Bitter ('87), Lauder-Brunton and McFadyen ('89), Fermi ('90, '91), Emmerling and Reiser ('02).
Moulds (*Aspergillus*, &c.): Bourquelot ('93), Malfitano ('00), Butkewitsch ('02).
Yeast (*Saccharomyces*): Beyerinck ('97), Hahn and Geret ('98, '00), Vines ('02, '04).
Basidiomycetous Fungi (Mushrooms, &c.): Hjort ('97), Vines ('03, '04).
Fruits: *Ananas sativus* (Pine-apple): Chittenden ('91, '94), Vines ('02, '03).
*Cucumis Melo* var. *utilissimus*: Green ('92).
*Cucumis Melo* (Melon): Vines ('03).
*Cucumis sativus* (Cucumber): Vines ('03).
*Cucurbita Pepo* var. *ovifera* (Vegetable Marrow): Vines ('05).
*Asparagus officinalis* (shoots): Vines ('05).
*Phytolacca decandra* (foliage-leaves): Vines ('05).
Bulbs: *Hyacinthus orientalis* and *Tulipa* sp.: Vines ('03, '04).

For the sake of completeness I may add that I have found evidence of the presence of erepsin in a great variety of plants: so many indeed that it may be assumed that this protease is present in some part, or most parts, of every plant at one stage or other of its development. In two cases (Yeast and Mushroom) I have satisfied myself of the simultaneous presence of erepsin and a fibrin-digesting protease (1).

In conclusion, I would draw attention to the striking fact that the apparently universal distribution of erepsin in the tissues of plants that I have demonstrated, is paralleled by a similar distribution in the tissues of animals. This important discovery has recently been made by my friend and colleague, Dr. Vernon. His paper on the subject will shortly appear in the Journal of Physiology; in the meantime I have his permission to make use of the notes with which he has kindly supplied me. He has found that glycerin-extracts of the different organs of various animals, both vertebrate and invertebrate (e. g. frog's liver, pancreas, and ovary; cat's ovary, liver, lung, spleen and kidney; rabbit's kidney and liver; pigeon's kidney and liver; eel's kidney and liver; sheep's liver; lobster's muscle, liver, and kidney; Anodon's kidney, &c.), have no action on fibrin, but peptolyse Witte-peptone with various degrees of activity, tryptophane being always formed in the process. The protease is clearly erepsin. It acts more vigorously in dilute alkaline (0·1 % $Na_2CO_3$) than in dilute acid (0·1 % acetic) liquids, differing in this respect from the erepsin of plants, which acts most vigorously, as I have shown, in liquids of the natural degree

of acidity. But Dr. Vernon's results indicate a remarkable and interesting convergence between the tissue-erepsins of animals and of plants in this respect, inasmuch as he finds the protease of the lower animals to be relatively more active in acid liquids than that of the higher. For instance he determined the ratio of the activity of glycerin-extract of cat's kidney in alkaline to that in acid liquids to be 76 : 1 ; cat's liver 12 : 1 ; rabbit's kidney 42 : 1 ; pigeon's kidney 5 : 1 ; frog's liver 1·8 : 1 ; eel's kidney 1·4 : 1 ; lobster's liver 8 : 1 ; Anodon's kidney 2 : 1. It is not inconceivable that, with a more extended range of observation, animals will be found whose erepsin, like that of plants, is more active in acid than in alkaline liquids.

In this connexion I may quote an interesting passage from von Fürth's recent work on the chemical physiology of the lower animals (9). Comparing the proteolysing secretions of the Invertebrates with the gastric juice of the Vertebrates, the author states that 'so far the presence of free acid in the digestive secretions has not been demonstrated in the case of any Invertebrate. In certain cases, where the matter was especially investigated, it was clearly ascertained that the acid reaction was due to the presence of acid salts, and that accordingly the proteid-digestion was rather tryptic than peptic.' This agrees in a remarkable manner with the view, first expressed by Fernbach and Hubert with regard to malt, that the natural acidity which is so favourable to the activity of the vegetable proteases is due to the presence of acid salts such as monobasic phosphate of potash (see my paper, No. 1, p. 297).

---

## List of Papers referred to.

1. Vines : The Proteases of Plants ; Annals of Botany, vol. xviii, 1904, p. 289 (April).
2. Emmerling : Ueber die Eiweissspaltung durch Papayotin ; Ber. deutsch. chem. Ges., vol. 35, p. 695, 1902.
3. Vines : Proteolytic Enzymes in Plants (II) ; Annals of Botany, vol. xvii, 1903, p. 606 (June).
4. Mendel and Underhill : Observations on the Digestion of Proteids with Papaïn ; Trans. Connecticut Acad. of Arts and Sciences, xi, 1901.
5. Martin : Papaïn Digestion ; Journ. of Physiol., v, 1884, p. 213 : also, Nature of Papaïn, and its Action on Vegetable Proteids ; Journ. of Physiol., vi, 1885, p. 336.
6. Wurtz : Recherches cliniques et chimiques sur la Papaïne ; Paris Médical, 1879.
7. Vines : Proteolytic Enzymes in Plants ; Annals of Botany, vol. xvii, p. 249, 1903 (January).
8. Vines : Tryptophane in Proteolysis ; Annals of Botany, vol. xvi, p. 7, 1902 (March).
9. von Fürth : Vergleichende chemische Physiologie der niederen Thiere ; 1903, p. 254.