The Proteases of Plants (V).

BY

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Experiments with Seeds (*continued*).

In a previous paper[1] I gave an account of some experiments on seeds, both germinated and ungerminated, which led to the following conclusions: (1) that the ungerminated seeds contained (*a*) a protease that acted immediately on Witte-peptone, and (*b*) one or more proteases that acted more or less slowly upon the reserve proteids of the seeds; further (2) that the germinated seeds all contained a protease that digested fibrin, and that such a protease was, in certain cases, developed in the substance of the ungerminated seed during the experiment. The difference in point of time between the action on Witte-peptone and that on fibrin, led me to infer that probably the two actions were due to two distinct proteases. The seeds used were those of *Phaseolus multiflorus*, *Phaseolus vulgaris*, *Vicia Faba*, *Pisum sativum*, *Lupinus hirsutus*, and *Zea Mais*. All of them, except those of *Lupinus*, are starchy seeds; in *Lupinus*, which proved to be the most active proteolytically, the non-nitrogenous reserve-material consists mainly of the thickened cell-walls of the cotyledons (hemi-cellulose) and also of some fat in the cells.

Since then I have confined my attention to oily seeds, more especially those of the Hemp (*Cannabis sativa*). I may say at once that I have found oily seeds to be much more proteolytically active than starchy seeds; so much so, in fact, that I have experimented almost exclusively with ungerminated seeds. I should explain that I did not find it necessary to remove the oil from the seeds, as it did not appear to interfere with the progress of the experiments. I may add that various samples of Hemp-seed obtained from different sources were used, with but slightly varying results. The temperature of the incubator was 38° C.

[1] The Proteases of Plants (IV); Ann. Bot., vol. xx, 1906, p. 113.

Ungerminated seed of Cannabis sativa.

EXPERIMENT 1. In this case a mixture of crushed seed with water was used; all subsequent experiments were made with extracts of the seed.

15 grms. of crushed seed were placed in each of two bottles with 100 cc. distilled water; a sample of the liquid gave no tryptophane-reaction: to No. 1 nothing was added; to No. 2, 0·2 grm. of well-washed fibrin. After 20 hours in the incubator No. 1 gave distinct tryptophane-reaction; No. 2 gave a marked reaction, and the fibrin had entirely disappeared: 0·5 grm. fibrin was then added to No. 2. After 48 hours' digestion both gave strong tryptophane-reaction; the 0·5 grm. fibrin added to No. 2 disappeared in 48 hours. Subsequently 0·3 grm. fibrin was added to No. 1 and was digested within 24 hours.

These results prove that autolysis proceeded actively during the experiment, and that the mixture was able to digest fibrin: it may be inferred that the resting seed contains both peptonizing and peptolyzing enzymes. It should be explained that, in such experiments as these, it is difficult to demonstrate the actual formation of peptones, for the liquid gives the biuret-reaction to begin with, owing to the presence of albumoses extracted from the seed.

Some of these seeds were germinated with a view to comparative experiments with the ungerminated seeds and the seedlings; but they were not pursued when the seeds were found to be so active proteolytically. The only observation made was that a watery extract of the seedlings, a week old, gave a strong tryptophane-reaction.

EXPERIMENT 2. 10 grms. of seed were crushed with 100 cc. distilled water, and the same quantity with 100 cc. 2% NaCl-solution: the mixtures were left to filter in the cold all night. The filtered extracts were found to be acid, and to give a precipitate of proteid on boiling, rather more in the NaCl-extract; the NaCl-extract also gave a faint tryptophane-reaction.

About 30 cc. of each extract were put separately into each of two bottles; to one of the H_2O-extract bottles (1) and to one of the NaCl-bottles (3) nothing was added but some HCN (about 0·1%) as an antiseptic; to the other H_2O-bottle (2) and to the other NaCl-bottle (4) 0·2 grm. fibrin was added. After 24 hours' digestion the fibrin in the NaCl-bottle had disappeared; and after 48 hours that in the H_2O-bottle had disappeared. The precipitate on boiling the liquids was observed to diminish gradually. At the close of the experiment (96 hours) the precipitate was very slight in the two H_2O-bottles, rather more in the two NaCl-bottles; the tryptophane-reactions were distinct in all the bottles, except No. 4, in which it was strong.

The diminution of the precipitate on boiling affords evidence of digestive action on the coagulable proteids dissolved in the extract, that is of autolysis. The results confirm those of Experiment 1, and further show that the proteases of the seed can be dissolved out by both water and dilute NaCl-solution, apparently rather more by the latter than by the former.

In order to make sure that the more rapid action on fibrin of the NaCl-extract was not due to saline digestion, a control-experiment was made: 0·2 grm. fibrin was placed in a bottle with 70 cc. of 2% NaCl-solution; after remaining in the incubator for six weeks the fibrin was found to be quite unaltered.

Experiment 3. The question as to the greater solubility of the fibrin-digesting protease in NaCl-solution was further investigated by the method of preparing a relatively stronger H_2O-extract.

10 grms. of seed were extracted with 100 cc. 2% NaCl-solution, and 20 grms. with 100 cc. distilled water: 50 cc. of each extract were put into separate bottles with 0·2 grm. fibrin and a little HCN. After 24 hours the fibrin in the NaCl-bottle was broken up, that in the H_2O-bottle being unaltered; after 48 hours the fibrin had nearly disappeared in the former, and was breaking up in the latter; after 72 hours there was still some left in the former, and but little in the latter; after 96 hours the fibrin had completely disappeared in both. The tryptophane-reaction was strong in both, but distinctly stronger in the H_2O-bottle; neither liquid gave any appreciable precipitate on boiling. The interesting observation was made that the H_2O-liquid gave only a trace of biuret-reaction, whereas that of the NaCl-liquid was as strong as at the beginning of the experiment. Subsequent special experiments on autolysis confirmed this result (see Experiment 5, p. 107).

It appears from this experiment that the fibrin-digesting activity of the NaCl-extract was about the same as that of the H_2O-extract of twice the strength; the digestive action of the former, though more rapid at first, was eventually overtaken by that of the latter, so that the complete disappearance of the fibrin occurred at about the same time in both. It may be concluded that a 2% solution of NaCl dissolves about twice as much of the peptonizing protease from the seed as does an equal proportion of distilled water. But the possibility still remains that the greater digestive activity of the NaCl-extract may be due, at least in part, to the direct action of the salt upon the process of digestion. The following experiment was made for the purpose of determining this point:—

Experiment 4. 20 grms. of crushed seed were extracted with 125 cc. H_2O, and 10 grms. with half the quantity of 2% NaCl-solution.

Three bottles, each containing 40 cc., were prepared as follows:—No. 1, H_2O-extract; No. 2, H_2O-extract to which was added 0·8 grm. NaCl (= 2%); No. 3, 2% NaCl-extract: to each bottle were added 0·2 grm. fibrin and a few drops of HCN.

After 24 hours' digestion in the incubator the fibrin in No. 1 was apparently unaltered; in No. 2 it was breaking up; in No. 3 it had nearly all disappeared. 24 hours later the fibrin was breaking up in No. 1; it was about half gone in No. 2; and had entirely disappeared in No. 3. 24 hours later it was reduced in No. 1, and had nearly disappeared in No. 2. 48 hours later the fibrin had not quite gone in No. 1, but had entirely disappeared in No. 2. Hence the fibrin was digested in No. 3

within 48 hours; in No. 2 within 120 hours; in No. 1 digestion was not complete in 120 hours.

It is clear that the presence of the added NaCl in No. 2 promoted digestive activity, the salt apparently playing the part of an 'activator'. But since digestion was still more rapid in the NaCl-extract, it may be concluded that a considerable proportion of the greater activity of NaCl-extracts of seeds, as compared with H_2O-extracts, is to be attributed to the presence of a larger proportion of fibrin-digesting protease in the former than in the latter, enough to justify the inference that the fibrin-digesting protease is more soluble in NaCl-solutions than in distilled water.

The four experiments described suffice to prove that the ungerminated seed of the Hemp contains proteases which are capable (1) of digesting fibrin, and (2) of digesting the reserve-proteids of the seed (autolysis), as shown by the diminution of the precipitate on boiling, and by the development of the tryptophane-reaction. As might be expected, it was found in many experiments that Witte-peptone, when added to the liquids, was readily peptolyzed, as indicated by a more or less strong tryptophane-reaction. It should be mentioned that the extracts sometimes gave a tryptophane-reaction to begin with; a 10% H_2O-extract gave at most a faint reaction, whereas the reaction of 20% H_2O-extract or of a 10% NaCl-extract was distinct.

I did not devote much attention to the investigation of the effect of increasing the acid reaction by the addition of acid, or of diminishing the acidity, or of an alkaline reaction, by adding alkali, a matter that I have laid stress upon in previous papers. One such experiment is, perhaps, worth recording.

Experiment 5. 50 grms. of crushed seed were extracted with 350 cc. distilled water: about 250 cc. of filtrate were obtained; this liquid gave a dense precipitate on boiling, faint tryptophane-reaction, and distinct biuret-reaction. Six bottles, each containing 40 cc. were prepared as follows:—No. 1, extract alone; No. 2, added Na_2CO_3 to 1·25%; No. 3, added HCl to 0·05%; Nos. 4, 5, and 6 were exactly as Nos. 1, 2, and 3, except that 0·2 grm. of fibrin was added to each; HCN was added to all to 0·1%; Nos. 2 and 5 were distinctly alkaline. After 24 hours' digestion, the tryptophane-reactions were, Nos. 1 and 4, marked; Nos. 2 and 5, faint; Nos. 3 and 6, strong; the fibrin in No. 4 was visibly attacked, in No 5 it was swollen and gelatinous, in No. 6 it was quite broken up.

After 48 hours' digestion, the fibrin had almost disappeared in No. 6, and was broken up in Nos. 4 and 5. The reactions of Nos. 1, 2, and 3 was as follows:—

	Tryptophane.	*Boiling.*	*Biuret.*
No. 1,	marked	slight ppt.	scarcely perceptible
,, 2,	very faint	scarcely any	distinct
,, 3,	marked	,,	none.

After 72 hours the fibrin had almost entirely disappeared in Nos. 4, 5, 6; the reactions were:—

	Tryptophane.	*Boiling.*	*Biuret.*
No. 1,	strong	very slight ppt.	none
,, 2,	very faint	no ppt.	distinct
,, 3,	strong	turbidity	none
,, 4 (fibrin),	strong	slight ppt.	fair
,, 5 ,,	very faint	no ,,	distinct
,, 6 ,,	strong	no ,,	fair.

From this it appears that fibrin-digestion was not materially interfered with by the addition of either acid or alkali. The effect on autolysis could not be gauged by the presence or absence of a precipitate on boiling, inasmuch as the native proteids were converted into acid-albumin or alkali-albumin; but it is clear that peptolysis was prevented by alkalinity, since the liquids containing Na_2CO_3 gave but faint tryptophane-reaction, whilst retaining the biuret-reaction which disappeared in the two other liquids (Nos. 1 and 3). Thus the results, so far as they go, are differential as between peptonization and peptolysis in the presence of alkali.

This experiment, however, is not only of interest in this respect, but also in that it confirms the previous observation (Experiment 3, p. 105), that the biuret-reaction disappears during the autolysis of H_2O-extracts, and persists in NaCl-extracts; this result was further established by other experiments on autolysis, of which the following are examples:—

Experiment 6. 20 grms. crushed seed were extracted with 200 cc. 2% NaCl-solution; and 20 grms. with 100 cc. H_2O: 50 cc. of each filtered extract were placed separately in two bottles, to which some HCN was added: both extracts were acid, the H_2O-extract being darker in colour and less clear than the NaCl-extract; their reactions were:—

	Boiling.	*Tryptophane.*	*Biuret.*
NaCl-ext.	dense ppt.	faint	good
H_2O ,,	less ,,	distinct	,,

After 48 hours' digestion the reactions were—

NaCl-ext.	slight ppt.	distinct	good
H_2O ,,	rather more ppt.	marked	none

The experiment extended over six days; at the close the reactions were—

NaCl-ext.	very little ppt.	marked	good
H_2O ,,	slight ,,	,,	none.

In another experiment, Witte-peptone was added to the extracts with the object of ascertaining whether the H_2O-extract was capable of peptolyzing proteids in addition to those formed in autolysis.

Experiment 7. 20 grms. of crushed seed were extracted with 200 cc. H_2O, and 20 grms. with 200 cc. 2% NaCl; the filtered extracts both gave a considerable precipitate

on boiling, distinct tryptophane-reaction, and good biuret-reaction. About 50 cc. of the H_2O-extract were put into each of two bottles, to one of which 0·1 grm. of Witte-peptone was added, and two similar bottles of NaCl-extract were prepared; both the bottles to which Witte-peptone had been added gave strong biuret-reaction; some HCN was added to all the bottles. After 48 hours' digestion the reactions were:—

	Tryptophane.	*Boiling.*	*Biuret.*
H_2O-ext. without W.-peptone	marked	no ppt.	indistinct
NaCl „ „ „	less marked	slight ppt.	good
H_2O „ with „	strong	no „	distinct
NaCl „ „ „	„	slight „	good.
At close of experiment 4 days later—			
H_2O-ext. without W.-peptone	marked	no „	none
NaCl „ „ „	„	„ „	good
H_2O „ with „	strong	„ „	faint
NaCl „ „ „	„	„ „	good.

The marked reduction in the intensity of the biuret-reaction in the bottle to which Witte-peptone had been added proves that the H_2O-extract was able to peptolyze more proteid than it originally contained. Without this experimental addition of proteid the results of autolysis might have been attributed to the larger amount of proteid in solution in the NaCl-extracts as compared with the H_2O-extracts; but it is now made clear that the difference in the biuret-reactions of the two extracts is due to the difference in the relative amount or activity of the peptolyzing enzyme, and not in the relative amounts of proteid that they contain. Whilst NaCl-extracts, on the one hand, digest fibrin more actively on account of the larger amount of the protease that they contain, H_2O-extracts, on the other, are more active peptolytically. Consequently these results bear upon the question that I have raised in several papers, the question as to the number and nature of the proteases occurring in plants: to this I return in the latter portion of this paper.

Experiments with other oily seeds.

I have devoted so much time to the investigation of the Hemp that it has not been possible to make more than a few cursory observations on other oily seeds, those of the Mustard (*Sinapis alba*), the Hazel (*Corylus avellana*), the Castor-Oil plant (*Ricinus communis*), and the Flax (*Linum usitatissimum*).

Sinapis alba. Experiments on autolysis made with aqueous mixtures or extracts (10%) of ungerminated seeds gave negative results, nor did they digest fibrin; but a stronger extract, whether aqueous or of NaCl 2% solution, proved active.

Experiment 1. 15 grms. ground seed were extracted with 100 cc. H_2O, and the same weight with 100 cc. 2% NaCl-solution; the filtrates gave no tryptophane-

reaction; bottles, of 30 cc. each, were prepared as follows: No. 1, H_2O-extract alone; No. 2, 30 cc. H_2O-extract with 0·3 grm. Witte-peptone and 0·2 grm. fibrin; No. 3, NaCl-extract alone; No. 4, NaCl-extract with 0·3 grm. Witte-peptone and 0·2 grm. fibrin: some HCN was added to each bottle. After 24 hours' digestion the results were:—

	Tryptophane-reaction.	*Fibrin.*
No. 1,	faint	
„ 2,	distinct	attacked
„ 3,	„	
„ 4,	marked	gone.

0·3 grm. fibrin was put into No. 4: after 24 more hours' digestion—

No. 1,	marked	
„ 2,	strong	nearly gone
„ 3,	marked	
„ 4,	strong	gone.

These results afford evidence of autolysis, of fibrin-digestion, and of the peptolysis of Witte-peptone; fibrin-digestion was, as usual, more actively carried on by the NaCl-extract.

I found that seeds which had been kept moist for about two days at room-temperature gave more active extracts; and that an extract of seedlings three days old digested fibrin rapidly.

Corylus avellana. 20 grms. of ground kernels were put into each of two bottles with 80 cc. distilled water, and to one bottle 0·2 grm. fibrin was added. The fibrin gradually diminished, and at the end of 72 hours there was scarcely any left; both bottles then gave marked tryptophane-reaction.

Ricinus communis. 10 grms. ungerminated seed (without testa) were extracted with 100 cc. H_2O, and a similar weight with 100 cc. 2% NaCl-solution: both filtered extracts gave a precipitate on boiling; about 80 cc. of each were respectively put into bottles with 0·2 grm. fibrin and some HCN. After 72 hours' digestion the fibrin had disappeared in the NaCl-bottle, that in the H_2O-bottle was unaltered; the contents of the former gave distinct tryptophane-reaction, those of the latter none; the contents of both still gave a precipitate on boiling. Four days later, after a week's digestion, the fibrin in the H_2O-bottle was breaking up, and the liquid in the bottle gave a distinct tryptophane-reaction; the tryptophane-reaction of the contents of the NaCl-bottle was now marked; the H_2O-extract gave only turbidity on boiling, but the NaCl-extract still gave a precipitate.

I found that both H_2O and NaCl-extracts of germinated seeds (10 days) digested fibrin within a week, the NaCl-extract the more rapidly.

Linum usitatissimum. 20 grms. ground ungerminated seed were extracted with 200 cc. distilled water, and a similar weight with the same quantity of 2% NaCl-solution. 40 cc. of the filtered H_2O-extract were put into each of two bottles, to one of which 0·2 grm. of fibrin was added, and similarly with the NaCl-extract: HCN to 0·1% was added to each bottle; the extracts gave a trace of tryptophane-reaction

After 48 hours' digestion the fibrin in the NaCl-bottle had disappeared, and that in the H_2O-bottle was attacked; the contents of the NaCl-bottle gave a faint, those of the H_2O-bottle a distinct, tryptophane-reaction. Three days later the fibrin in the H_2O-bottle had disappeared. At the close of the experiment, after 10 days' digestion, the tryptophane-reactions were:—

H_2O-extract	with fibrin		strong
„	„	without fibrin	marked
NaCl	„	with fibrin	„
„	„	without fibrin	„

These experiments, incomplete as they are, show that all the oily seeds investigated either contained to begin with, or developed during the experiment, proteases that effected both peptonization and peptolysis, and proved themselves to be more proteolytically active than starchy seeds; but they were all much less active than Hemp-seed. There is, however, the serious difficulty in comparing the seeds in this way, that I had no means of knowing how old they were: accurate comparative results can only be expected when the seeds compared are known to be of the same harvest. It is well known that old seed does not germinate so well as new, and probably it is not so active proteolytically; in fact the capacity for germination may depend to some extent upon the presence of active proteases.

The Separation of the Proteases.

In the series of papers on the proteases of plants that I have published in previous volumes of this periodical (1903 to 1906), I have shown that a peptolyzing protease, an ereptase, is generally present, perhaps universally, in the tissues of plants. In some cases—for instance, ordinary foliage-leaves—it appeared that this was the only protease present, since the extract did not digest fibrin. In many cases, however, the extracts not only peptolyzed Witte-peptone, as indicated by the development of the tryptophane-reaction, but digested fibrin as well. The question remained—what is the nature of this fibrin-digesting protease? is it a tryptase or a peptase? In my last paper (April, 1906) I pointed out that though my experiments up to that time did not afford evidence to prove that there is no such thing as 'vegetable trypsin', yet they sufficed to prove that 'vegetable trypsin' is a mixture of proteases, and that ereptase is one of the constituents; and I expressed the opinion that it seemed more probable that the fibrin-digesting constituent was a peptase rather than a tryptase.

In the course of this year I have endeavoured to contribute something to the definite settlement of this vexed question. My idea was to obtain extracts which should contain only either the ereptase or the fibrin-digesting protease. It is, of course, quite easy to obtain an extract containing only ereptase; almost any leaf, bulb, or seed, extracted quickly with a relatively

large quantity of water, will yield an active solution. It is the fibrin-digesting extract that offers difficulties.

Whilst experimenting with Hemp-seed, it struck me that this would be suitable material for the attempt to isolate the proteases. It is unnecessary to give all the tentative efforts that were made. It will suffice to say that I was guided by the fact with which I had long been familiar, and which is especially brought out by the experiments described in the first part of the present paper, that the fibrin-digesting protease is more soluble in NaCl-solutions than in water. Moreover, I was aware that NaCl-solutions also extract a great deal of proteid from seeds; it therefore seemed to be probable that the precipitation of the proteid in such an extract would carry down with it the fibrin-digesting protease. This probability I have succeeded in realizing. I found it to be necessary to use a strong (10%) NaCl-solution, and to obtain a strong extract holding much proteid in solution. On acidifying such an extract with the least possible quantity of acetic acid, a dense precipitate of proteid was formed. Filtering this off, the acid filtrate was found to peptolyze actively, and to have no action on fibrin; the fibrin-digesting protease evidently remained in the precipitate on the filter. Washing the precipitate with 10% NaCl-solution, slightly acidulated with acetic acid, the washings were found at first to act upon Witte-peptone, but this action gradually diminished and eventually ceased. A portion of the washed precipitate was then extracted with distilled water and filtered; the somewhat opalescent filtrate was found to digest fibrin actively, and not to act upon Witte-peptone, as indicated by the absence of the tryptophane-reaction. Further details are given in the following account of typical experiments:—

50 grms. crushed Hemp-seed were extracted with 250 cc. 10% NaCl-solution, and left to filter all night at a low temperature. It may be remarked that, as filtration is slow, it is necessary that the temperature should be as low as possible during the process. The filtrate was a rather viscid liquid, giving dense precipitate on boiling, also a strong tryptophane-reaction, and digesting fibrin actively. To this filtrate acetic acid was now added to 0·2%, and a dense precipitate was produced; the liquid was then put to filter in the cold.

The *acid filtrate* gave turbidity on boiling, and marked tryptophane-reaction: its digestive properties were tested as follows:—40 cc. were placed in each of three bottles: to No. 1, no proteid was added; to No. 2, 0·1 grm. fibrin; to No. 3, 0·2 grm. Witte-peptone. After 24 hours' digestion in the incubator the tryptophane-reactions were:—No. 1, marked; No. 2, marked; No. 3, strong; the fibrin in No. 2 was unaltered. The experiment with No. 2 was continued for three days longer, at the end of which time the fibrin still remained unaltered. Thus the acid filtrate digested Witte-peptone but not fibrin.

The *precipitate produced by acetic acid* was washed on the filter with 100 cc. 10% NaCl-solution containing 0·2% acetic acid, and the digestive activity of the

filtered washings was tested: 40 cc. of the liquid, which gave no tryptophane-reaction, were put into a bottle with 0·2 grm. of Witte-peptone; after 24 hours' digestion the liquid gave distinct tryptophane-reaction. Consequently the precipitate on the filter was again washed with 100 cc. 10% NaCl-solution containing 0·2% acetic acid, and the washings were tested: 40 cc. were put into a bottle with 0·2 grm. Witte-peptone; no tryptophane-reaction was observable after 24 hours' or after 48 hours' digestion; it was therefore concluded that all the ereptase had been washed out of the precipitate.

It now remained to ascertain if the precipitate contained a fibrin-digesting protease. 2 grms. of the precipitate (which was kept in the cold all the time) were treated with about 70 cc. distilled water, and the mixture put to filter. Filtration soon became very slow; the filtrate was turbid, and seemed to continue precipitating; 40 cc. were put into a bottle with 0·5 grm. fibrin, and 30 cc. into another bottle with a solution of 0·2 grm. Witte-peptone in 10 cc. distilled water that had been boiled and filtered; after 24 hours' digestion the fibrin was seen to be much broken up, and in 72 hours it had entirely disappeared: at the end of this time the liquid to which Witte-peptone had been added gave no trace of tryptophane-reaction, nor did the other.

This last experiment was repeated with a more dilute solution: 1 grm. of the precipitate was treated with 50 cc. distilled water; 20 cc. of the filtrate were put into each of two bottles, to one of which 20 cc. distilled water and 0·5 grm. of fibrin were added, to the other 20 cc. of a boiled and filtered solution of 0·2 grm. of Witte-peptone; after 48 hours' digestion the fibrin was disintegrated, though it had not altogether disappeared, in the one bottle, and the contents of the other gave no tryptophane-reaction. Thus a very small quantity of the washed acetic acid precipitate sufficed to give a solution that readily digested fibrin.

In another experiment with the precipitate, 2 grms. were extracted with 80 cc. distilled water, and filtered through muslin instead of through filter-paper. The filtered liquid gave no precipitate on boiling, and no biuret-reaction: 35 cc. of the solution were put into each of two bottles, after boiling in one case; to each 0·2 grm. fibrin was added. After 24 hours in the incubator the fibrin had nearly disappeared in the unboiled liquid, which now gave a strong biuret-reaction; the fibrin in the boiled liquid was unaltered, and the liquid gave no biuret-reaction: 0·4 grm. of fibrin was now added to the unboiled liquid; in 24 hours the fibrin was quite broken up, and neither liquid gave tryptophane-reaction, nor did the boiled liquid give a biuret-reaction.

To return to the *acid filtrate*: I endeavoured to obtain from this a precipitate which, on being dissolved, would give a solution that would act on Witte-peptone. About 130 cc. of it were poured into twice the volume of strong alcohol; a precipitate was formed, and the liquid was filtered. 1 grm. of the precipitate was treated with 50 cc. distilled water and filtered; the filtrate gave no tryptophane-reaction: 20 cc. of it were put into each of two bottles, and to one of them 0·1 grm. Witte-peptone was added. After 24 hours' digestion the contents of the latter gave a faint tryptophane-reaction, which had become quite distinct after 48 hours; the contents of the other bottle gave no reaction.

In all these experiments HCN, 0·1%, was the antiseptic.

The results of these experiments can be very briefly summarized. I have succeeded in isolating from a vegetable tissue, I believe for the first time, a protease that is essentially peptic in its properties, digesting fibrin to albumose or peptone, but not acting on albumose or peptone whether produced by its own digestion of fibrin or added as Witte-peptone. The facts justify the conclusion that the Hemp-seed contains two proteases, the one a peptase, the other an ereptase. What now remains to be done is to apply this method of investigation, modified according to circumstances, to other cases, and so to arrive at a general conclusion as to the nature of 'vegetable trypsin'. With this work I am now occupied.