On the Occurrence of Vegetable Trypsin in the fruit of Cucumis utilissimus, Roxb.

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FEW lines of research into the processes of vegetable physiology have led in recent years to more important results than that which has been concerned with the distribution, character, and action of the several so-called unorganised

NOTE.—In 1885–6, Brigade-Surgeon E. Bonavia, M.D., sent to Kew seeds of several Cucurbits. Of these he gave the following particulars :—

'Etawah, N.W.P., 20 Oct., 1885.

'Next mail I shall send you seeds of three varieties of wild *Cucumis*, which I firmly believe are the wild parents of the famous Lucknow melons called "Chitla Kharbooja" (Spotted Melon) and others. These wild melons are called "Kachree" by natives. In common with the *Carica Papaya* (Papīta) they soften muscular fibre, and natives cook them with tough meat to make it tender. The milk of the common fig (*Ficus Carica*) has similar properties. It is not improbable that all contain Papaine.'

Two of the varieties were grown at Kew and proved to be the *Cucumis utilissimus* of Roxburgh, who gives Kakri as the vernacular name. *C. utilissimus* in the Flora of British India is reduced to a variety of *C. Melo*, Linn. It is a variable plant; but the fruit is always elongate or cylindrical.

'Etawah, N.W.P., 9 Feb., 1886.

'This hot weather I shall try and secure for you some seed of the Lucknow spotted melon, which I think owes its origin to the Kachree wild cucurbit I sent you seeds of. Please note that the papaine-quality of the latter was told me by a very intelligent native gentleman, and he was very sure that this quality was possessed to his knowledge by only three plants :—Papau, Kachree, Anjir. Anjir is the vernacular for the common Fig. He said he often used the Kachree fruit cut

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ferments or enzymes. The action of diastase in converting starch into sugar, established by Baranetzki [1], has been shown to be intimately concerned alike with the ordinary processes of nutrition of the adult plant, and with the phenomena of the germination of seeds and tubers, and further to be the means in some cases of starting the growth of the pollen-tube [2]. Side by side with diastase other similar bodies have been identified, by whose agency cellulose [3] and inulin [4] undergo similar transformation. The nitrogenous reserve materials deposited in temporary reservoirs have also been shown to be called into active consumption by other members of the same group, while glucosides and oils have also special enzymes to attack them.

The ferments which have the property of digesting nitrogenous substances like fibrin and albumin have been less conspicuous than diastase, but their existence has been clearly established by Reess and Will [5] in the leaves of *Drosera*; by Gorup-Besanez [6] and by Vines [7] in the pitchers of various

up with tough meat and stewed together. He is clear about the fact of its making tough meat tender. I have never tried it, but have tried the milk of the fig, and it does soften tough meat.'

Dr. Bonavia also sent to Kew seeds of a very pretty gourd. He remarked :— 'They are new to me, although I have been looking out for such things for the last twenty-eight years. They were sent to me by Major Buller, police officer, Gouda Oudh. He says they are largely grown in the Nepal Terai.'

They turned out to be a form of the pumpkin (*Cucurbita maxima*, Duch.).

The Kachree fruited rather sparingly at Kew, and it was some little time before fruits were available for examination. However, in 1891, all the available material was placed in the hands of Professor Green. By an unfortunate mistake Dr. Bonavia's pumpkin was sent him in the first instance instead of the Kachree. It is interesting to observe that the results of its examination were purely negative. In the Kachree, as will be seen, he had no difficulty in detecting a tryptic ferment. It is, however, clear that this is not characteristic of the Cucurbitaceae generally.

W. T. T. D.

[1] Die stärkeumbildenden Fermente in der Pflanze, 1878.

[2] Green, On the occurrence of diastase in pollen. Brit. Assoc. Reports, Cardiff, 1891.

[3] Brown and Morris : Journal of the Chem. Soc., lvii. June, 1890.

[4] Green : this Journal, vol. i. 1888.

[5] Bot. Zeit., Oct. 29, 1875.

[6] Ber. d. deutsch. Chem. Gesellsch., May, 1876.

[7] Journal Linn. Soc. Botany, vol. xv.

species of *Nepenthes*; by Wurtz[1] and later by Martin[2] in the fruit of the Papau (*Carica Papaya*); by Wittmack and by Hansen in the latex of the Fig. (*Ficus Carica*)[3]; and by the writer in the germinating seeds of the Lupin[4] and the Castor Oil plant[5]. To these another Indian plant may now be added in *Cucumis utilissimus*, Roxb., the Kachree gourd.

During the present autumn I have had, through the kindness of the Director of the Royal Gardens, Kew, the opportunity of examining the fruit of this plant, which has in India the reputation of possessing the same properties as those of the Fig and the Papau. The fruit is in appearance much like a small vegetable marrow, about six inches in length. It is yellow in colour, and when cut has an aroma similar to that of the water-melon. Its pulp is extremely succulent and the expressed juice is faintly acid in reaction.

The first series of experiments made were directed only to ascertain whether the juice has, as suggested, any action upon a proteid body. It was pressed from the central pulp, and filtered till quite clear. Two equal volumes were taken, and one of them boiled for a few minutes to destroy any enzyme that might be present. The two volumes were then put into labelled beakers with a little thymol, and to each a measured quantity of egg-albumin was added. The albumin was prepared by boiling white of egg for about five minutes and then forcing it through very small-meshed wire gauze, which reduced it to a fine state of sub-division, so that it could be accurately measured. The two beakers with their contents were then set in an incubator at a temperature of 34° C.

The action began slowly in the unboiled portion, and proceeded continuously and gradually. In two days about half the albumin had been dissolved. In the control-beaker, with the boiled extract, no change could be observed, either in the quantity or the appearance of the albumin. Microscopic

[1] Wurtz: Comptes Rendus, June, 1880.
[2] Martin: Journal of Physiology, vol. v. 1884, p. 213.
[3] Hansen: Arb. d. bot. Inst. in Würzburg, iii. 1885.
[4] Green: Phil. Trans., vol. 178 B, 1887, p. 39
[5] Green: Proc. Roy. Soc., vol. 48, 1890, p. 370.

examination of both liquids showed them to be free from micro-organisms.

The juice therefore possesses the property of dissolving coagulated egg-albumin and loses this power on being boiled.

While this experiment was proceeding, part of the pulp immediately under the rind of the fruit, was extracted with water, in which ·2 % of potassic cyanide had been dissolved as an antiseptic. The extract was filtered from the pulp after standing over it for twenty-four hours, and was alkaline in reaction, owing to the presence of the cyanide. The pulp itself, like the expressed juice, was faintly acid. With this extract a similar experiment to the first one was carried out, and similar results were obtained, showing that the power of dissolving coagulated proteid, presumably a ferment-action, extends to the pulp as well as to the juice of the fruit.

On boiling the extracts in both these experiments the liquid became opalescent. This was found to result from the presence of a proteid body coagulating by heat. Both the liquid and the granular deposit which formed gave a good xanthoproteic reaction. So many of the enzymes known to physiologists having been found to be associated with such proteids, and particularly with members of the globulin class, it seemed not unlikely that such an association might be found here. Globulins are characterised especially by their insolubility in pure water, and their ready solubility in solutions of common salt of about 3–10 % strength. A series of experiments was therefore arranged with a view to ascertaining whether the enzyme is more easily extracted by salt solution than by water or faintly alkaline fluids.

Two portions of the pulp, of equal bulk, were taken and mashed up separately in a mortar, one with 70 *cc.* of a solution containing 3 % Na. Cl. and ·2 % KCN; the other with 70 *cc.* of ·2 % KCN solution only. After twenty-four hours the two extracts, labelled for convenience of reference C and D, were filtered and carefully neutralised with very weak acid. The antiseptic action of the potassic cyanide was always found to be sufficient to prevent any contamination with micro-organisms, but as neutralisation involved the decomposition of the cyanide, it was thought advisable to add another preservative agent. Attention has recently been directed by several observers to oil of mustard [1] as possessing strong power in this direction, and it was therefore chosen. About 30 *cc.* of each extract was put into a bottle furnished with a graduated scale, and a measured quantity of egg-albumin, prepared as before, was added. Each was then shaken up with 1 *cc.* of oil of mustard, and placed in the incubator. The oil of mustard is very slightly soluble, and a good deal of it floated on the top of the liquid. Being somewhat volatile, the air in the upper part of the bottles contained some of its vapour. As the action of the extracts proceeded the antiseptic was found to work admirably, no putrefactive changes taking place all the time it was continued—a period of several days.

The mode of observation was to shake the graduated bottles every morning, and on the albumin settling to the bottom, as it did in about 2–3 minutes, to measure the quantity remaining by the scale.

The following table will enable a comparison of the activity in the two cases to be made :—

Time of digestion.	Diminution of albumin in C.	Diminution of albumin in D.
36 hours	16%	8%
60 ,,	25%	18%
108 ,,	29%	20%

The extract C had thus evidently greater ferment-power than D, and it is fair therefore to infer that the enzyme is more easily extracted by a weak salt solution—a fact which points either to its being a globulin in nature, or more probably associated with a globulin constituent in the cells of the plant. The fact that the first watery extract made possessed ferment-power would be explained by the fact that in the plant are many inorganic salts, by whose assistance it would be dissolved on the addition of water.

[1] Among others, Cadeac et Mennier, Recherches expérimentales sur l'action antiseptique des essences, Annales de l'institut Pasteur, iii. 1889 ; also Roux.

The association with a globulin would also be suggested by the observation already mentioned, that on boiling, the extract became turbid from the presence of coagulated proteid.

The most powerful proteo-hydrolytic ferment that has hitherto been found in plants is the vegetable trypsin occurring in the Papau, which has been carefully worked out by Martin[1]. The gourd under examination appearing to resemble this fruit in many respects, experiments were next undertaken to see if the two ferments also are alike. The points investigated were (1) the medium in which the *Cucumis*-ferment is most active, (2) the products of the decomposition which it initiates.

An extract prepared by salt solution from the pericarp, and one obtained by similar treatment of the central pulp in which the seeds were embedded, were carefully neutralised. Three tubes of each were prepared--one contained the extract diluted with an equal volume of ·4 % H. Cl., one the same with an equal volume of water, and the third the same with an equal volume of 3 % $Na._2$ $Co._3$. Boiled controls of all were exposed side by side with them. To each was added a measured quantity of the egg-albumin, and the two sets with their controls were put side by side in an incubator at 34° C. They were labelled E_1 E_2 E_3 and F_1 F_2 F_3, and their controls E_{1b} F_{1b}, &c. The digestion was in this case continued for three days. During the experiment and at its conclusion the greatest activity was found to be shown by the alkaline tubes of both sets, while the acid had least power. Like papaïn, therefore, the ferment acts most advantageously in a faintly alkaline medium.

The products of the decomposition were examined in a digestion carried out in dialysing tubes, controls being used, in which the extract was boiled before adding the albumin. These may be referred to as G and H. After twenty-four hours peptone was found to be present in the dialysate of G in

[1] Op. cit.

sufficient quantity to give a good biuret reaction. After 2 days the experiment was stopped, and the dialysates compared. That of the control H gave no evidence of peptone by the biuret test. Both were then evaporated to dryness over a water bath. The residue from the dialysate of G was much the more copious of the two.

These residues were dissolved in a small quantity of distilled water, and precipitated by neutral acetate of lead, a reagent which throws down peptones, and forms an insoluble compound with leucin, an amide body which occurs in the profound decomposition of proteids brought about by tryptic ferments. The appearance of leucin in the dialysate would afford evidence that the ferment under examination is a trypsin and not a vegetable pepsin.

Comparing the two after addition of the acetate of lead it was found that there was a precipitate in both, but much the greater quantity in G. After well washing, these precipitates were suspended in water, and a stream of H_2S passed through, till the liquid was saturated with gas and all the lead thrown down as Pb. S. Most of such peptone as was present in G was by this treatment rendered insoluble and was removed by filtration with the lead sulphide. The filtrate, containing now any leucin that might have been present, and any diffusible bodies that, being present in the original extract, behaved with regard to lead acetate in the same way as leucin, was concentrated to a very small bulk, and treated with boiling alcohol, in which leucin is soluble. After filtering, this alcoholic extract was in turn concentrated, and allowed to deposit the crystalline matter which it contained. Both dialysates, treated thus, threw down crystals, G depositing the greater amount. The crystals from G were found to be of two kinds: one, not very plentiful, separating in rosettes, which were doubly refractive; the others, in much greater quantity, had not a very definite crystalline form, and were only very feebly, if at all, doubly refractive. H contained only the rosettes. The crystals were then purified by repeated crystallisation from water, till they were nearly free from admixture, and were finally allowed to form very slowly in a watch-glass. Those from G were found, when examined under the microscope, to be of two kinds, the rosettes already spoken of, and others having the characteristic appearance of leucin crystals aggregated into rough rounded clumps. Those from H, as before, only showed the doubly refractive rosettes.

G then contained crystals of two kinds, H only one, and those absent from H were found to resemble leucin in appearance. The doubly refractive rosettes were present in the two extracts in about equal amounts. These were probably present in the fruit before extraction, while the leucin was formed during the digestion.

From the crystallised residue of G so obtained, several of the rounded clumps were separated and carefully dried. They were then put into a small hard glass tube and heated strongly in a flame. They sublimed without melting, and were deposited again in crystalline form on the upper cool portion of the tube. This is strong confirmation of their being leucin, as this is the only body derived from proteo-hydrolytic decomposition of albumin that will sublime unchanged.

I had unfortunately at this point come to the end of my material, and was therefore unable to investigate the ferment further in the direction of isolation.

To summarise my results, I find that:—

(1) The fruit of *Cucumis utilissimus*, Roxb., contains in its juice and in its pericarp a proteo-hydrolytic ferment, capable of dissolving coagulated egg-albumin.

(2) This ferment is either globulin in nature, or associated with a globulin in the cells of the plant.

(3) Like papaïn, it works best in a slightly alkaline medium; less readily in a neutral one, and least of all in the presence of acid.

(4) Like papaïn, again, it effects a very complete decomposition of the albumin, giving rise to peptone, and later to leucin. It is a ferment, therefore, allied to the trypsin, rather than to the pepsin, of the animal organism.