## Germination of Leguminous Seeds and Urease Activity.

THE enhanced urease activity accompanying the germination of urease-containing seeds[1,2] may be due to (1) an increase in the absolute quantity of the enzyme, (2) an elaboration of activators or elimination of inhibitors and/or (3) a greater extractability of the enzyme. A series of experiments designed to test these possibilities was carried out in which the activities of the aqueous extracts of defatted powders were compared with those of the powders themselves.

0·1 g. of the powder or a quantity of the extract corresponding to 0·1 gm. of powder, was incubated for 30 minutes with 10 c.c. of a 1·0 per cent. solution of urea (in phosphate buffer of pH 6·9) at 30°·0 C. and the ammonia liberated was estimated by the aeration method. The results obtained are tabulated below.

TABLE I.

(*Urease Activity expressed in mgms. of Ammonia Nitrogen.*)

| Seed Material | Extract | | Powder–100 mesh | |
|---|---|---|---|---|
| | U.G.* | G.§ | U.G.* | G.§ |
| *Dolichos biflorus*, Linn. | 2·4 | 8·7 | 8·5 | 9·0 |
| *Glycine hispida* (Mœnch) | 5·4 | 8·2 | 9·7 | 9·6 |
| *Cajanus indicus*, Spreng. | 5·7 | 8·2 | 8·1 | 8·5 |
| *Canavalia ensiformis*, Dc. | 19·1 | 27·3 | 26·1 | 28·1 |

*U.G. = Ungerminated.
§G. = Germinated.

The results show (1) that the activities of the germinated seed extracts are invariably greater than those of the ungerminated seed extracts, and (2) that in the case of powders, the differences between the activities of germinated and ungerminated seed materials are not marked. The activities of the powders represent the total urease content of the seed, as the enzyme is capable of acting on its substrate in the absorbed state.[3] Calculated on the basis of the activities of powders, the percentage of extractable urease is above 95 in the case of germinated seeds, and less than 70, in the case of the ungerminated seeds. Activators and paralysers, if present, will influence the activities of both extracts and powders equally.

These considerations lead to the conclusion that part of the urease exists in the seeds in an adsorbed and unextractable condition—*desmo*-urease, and that during the process of germination, it is converted into an extractable, *lyo*-form. Further work is necessary to characterise the two forms of urease.

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[2] Wei Sun Tao and Shigeru Komatsu, *Mem. College of Sciences, Kyoto Imp. Univ.*, Ser. A, **1931**, **14**, 293.
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## Ascorbic Acid Oxidase from Drumstick, *Moringa pterygosperma*.

IT has been shown[1] that extracts from different plants exhibit great variations in the rate of oxidation of ascorbic acid. As resistance to oxidation was usually shown by extracts which contained reducing substances other than ascorbic acid, it was inferred that such substances exerted a protective action on the acid. An example of a material in which ascorbic acid was extremely stable to oxidation and which contained a large proportion of substances titrating against iodine but not against Tillmans' reagent, was the press juice of the Indian gooseberry, *Phyllanthus emblica*. In order to examine if this juice contained protective substances, it was decided to try the effect of its addition to the press juice of drumstick, *Moringa pterygosperma*, which on account of the identity of iodine and Tillmans' titres, was known to contain ascorbic acid as the only reducing substance. The surprising result was, however, obtained that, in contradistinction to trichloroacetic acid extracts of drumstick, its *press juice* did not reduce 2 : 6-dibromophenol-indophenol: further, that the juice rapidly oxidised the reducing factor in gooseberry or in orange juice, as also solutions of ascorbic acid (B.D.H.). It was also found that if the intact drumsticks were immersed in boiling water for a short time, press juice containing the usual amount of ascorbic acid could be obtained. Thus, in drumstick juice, the presence of an enzyme system capable of

oxidising ascorbic acid and which was inactivated either by boiling, or by treatment with trichloroacetic acid, was clearly indicated. Such a system, termed "hexoxidase" was shown to be present in cabbage juice by Szent-Györgyi in 1931.[2] Recently,[3] the isolation of an ascorbic acid oxidase from *Cucurbita maxima* was reported.

A preparation of the enzyme from drumstick was obtained as follows:—The press juice was centrifuged, the supernatant liquid treated with 5 per cent. ammonium sulphate (solid) and left in the ice-chest overnight. Next morning, the heavy precipitate that had formed was filtered off and the active filtrate further treated with ammonium sulphate to 35 per cent. final concentration (about 75 per cent. saturation). The resulting fine precipitate was separated by centrifuging, dispersed in a small volume of water (about a sixth of the original volume of press juice) and filtered. Thereby a clear yellow filtrate possessing very powerful oxidising action was obtained.

The action of the enzyme was studied with ascorbic acid as substrate at the optimum pH of 5·3 (9·75 ml. primary + 0·25 ml. secondary phosphates according to Sorensen) at 37° C. The amount of ascorbic acid present at any time in the reaction mixture was determined by titrating the latter against standard solution of 2 : 6-dibromophenol-indophenol. In a typical experiment, 0·1 ml. of the enzyme solution, obtained as above, was found to completely oxidise 0·4 mg. of ascorbic acid to its reversible oxidation product in 3 minutes in a total volume of 5 ml. From studies so far made of the kinetics, it appears that the reaction is monomolecular and the rate of oxidation is directly proportional to the quantity of enzyme and independent of substrate concentration.

At low concentrations of cyanide and $H_2S$ ($10^{-3}$ M) the enzyme is greatly inhibited, which thus differs from the enzymes obtained by Szent-Györgyi (*loc. cit.*) or Tauber and co-workers (*loc. cit.*).

Guaiacum, catechol, pyrogallol and *para*-phenylenediamine were not oxidised by the enzyme preparation except in the presence of $H_2O_2$. This peroxidase action exhibited by the preparation is, however, totally unconnected with the oxidative mechanism which does not require $H_2O_2$ for its action. That the ascorbic acid oxidase acts independently of the co-existing peroxidase was also shown by the complete disappearance of peroxidase activity in presence of $10^{-4}$ M cyanide, when the oxidising action was still very definite. Further, alcohol and acetone were found to destroy almost completely the ascorbic acid oxidising constituent, but left the peroxidase unaffected.

These results confirm the findings of Tauber *et al.*[3] that there exists in plants, apart from the complex mechanism postulated by Szent-Györgyi (*loc. cit.*) and by Szent-Györgyi and co-worker[4], a specific enzyme capable of oxidising ascorbic acid.

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## A New Apparatus for Carbonic Acid Estimations in Soils.

GASOMETRIC estimations of carbonic acid in soils are generally carried out with the apparatus designed by Collins. Although convenient for many purposes, Collins's Calcimeter suffers from certain inherent defects. The intervention of a rubber tubing between the reaction flask and the measuring burette may occasionally cause considerable errors. As one end of the tube adjacent to the measuring burette is open to the air, the instrument is very sensitive to atmospheric pressure fluctuations. To circumvent these difficulties, an apparatus for carbonic acid determinations has been developed in this Laboratory. This apparatus is independent of atmospheric pressure variations, and the use of a modified Thunberg[1] tube for the reaction makes it possible to mix the reactants very effectively. The apparatus can also be employed for the study of physiological functions provided the experiments are to be carried out at the room temperature.

The principle of the apparatus (Fig. 1) is the same as employed by the authors for another purpose.[2] The amount of $CO_2$ evolved will be proportional to the increase of pressure in the reaction bulb. If $h$ is the increase of pressure in mm. of Brodie solution and $x$ is the amount of $CO_2$ evolved in c.mm. at N.T.P. (dry), we have