

about 1.0 per cent less  $\frac{4}{13}K$  than mineral potassium. Both living and necrotic parts of tumour were utilized in the preparation of these samples, but two other samples prepared from only living parts showed approximately the same percentage.

Similar slight deviations were obtained in potassium from mouse sarcoma 37 S and from some forms of human cancer tissue; so far no corresponding normal tissue has been taken for comparison, except human bone marrow, which gave results similar to rat bone marrow.

Detailed accounts of our investigations will appear elsewhere.

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#### Active Group of Papain

FROM their work on the active group of papain, Bersin<sup>1</sup> and Purr<sup>2</sup> concluded that the SH group was essential for the hydrolysis of gelatin by the enzyme, the activity disappearing with the oxidation of the SH to the SS form.

We have already shown that the papaya latex is rich in SH compounds (about 2 per cent) and that about a tenth of it is glutathione<sup>3</sup>. This observation led us to the examination of the activation of papain.

An aqueous extract of the fresh latex, previously extracted with ether, was treated with hydrogen peroxide to oxidize all the SH to the SS as shown by the nitroprusside test, and then precipitated with alcohol, washed with absolute alcohol and dried in vacuum. This preparation, while being completely inactive towards peptone, retained its capacity to hydrolyse gelatin. Its 'gelatinase' activity was comparable to that of the preparation activated by hydrogen cyanide or glutathione. Its optimum pH was in the neighbourhood of 3, far below the value (4.6-5.0) reported in the literature for papain. The reaction mixture before and after incubation did not answer the nitroprusside test. The results obtained are tabulated below.

(250 mgm. gelatin or peptone + 250 mgm. of SS prepn. in 20 c.c. of buffered solution, pH 3. Temp. 38° C. Time of incubation, 20 hr. The activity is measured by the increase in formal titration of 2 c.c. of the reaction mixture against 0.1 N caustic soda.)

	Gelatin	Peptone
1. SS preparation in citrate buffer	0.25	0.00
2. " in acetate buffer	0.27	0.01
3. " + hydrogen cyanide	0.38	0.30
4. " + glutathione	0.48	0.46
5. " + maleic acid	0.26	0.00
6. " + iodoacetic acid	0.00	0.00

Maleic acid, which has recently been shown to inhibit the activity of enzymes the activity of which depends on the presence of the SH group<sup>4</sup>, is without effect on the gelatinase activity of the preparation. Iodoacetic acid inhibits the activity irreversibly.

These results indicate that for the gelatinase activity of papain the SH group is not necessary; other groups which react irreversibly with iodoacetic acid appear to be essential. At the same time, it is evident that the SH group is essential for 'peptonase' activity.

We have also got evidence that the papain hydrolysis of the protein takes place in two definite stages, first to peptone and then to simpler amino acids; the activation mechanism being specific for the two stages.

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<sup>1</sup> Bersin, *Hoppe-Seyl. Z.*, **222**, 177 (1933).

<sup>2</sup> Purr, *Biochem. J.*, **29**, 5 (1935).

<sup>3</sup> Ganapathy and Sastri, *Curr. Sci.*, **6**, 330 (1938).

<sup>4</sup> Morgan and Friedmann, *Biochem. J.*, **32**, 862 (1938).

#### Excretion from Leguminous Root Nodules

IN previous communications<sup>1,2,3</sup>, I have reported my inability to detect appreciable excretion of nitrogenous substances from root nodules of leguminous plants, and thus to confirm the results obtained by Virtanen. During the past summer, further trials have been carried out with soya bean (4 varieties), pea (5 varieties), and broad bean. Three rooting media have been employed, namely, (a) a coarse quartz sand composed of particles of diameter mostly in the region of 0.5 mm.; (b) a fine quartz sand of particles diameter 0.1-0.3 mm.; and (c) a very fine quarry sand of particles 0.1 mm. and less. (b) appears to be very similar to the sand used by Virtanen. Open and closed containers have been used, while the tests for excretion consisted of analysis of rooting medium and of barley plants grown in association with the legumes.

Satisfactory growth and fixation were shown by the legumes, but in no instance was evidence of excretion obtained. The pea varieties included Torstai and Concordia, inoculated with bacillus strain HX, combinations which in Virtanen's experiments gave vigorous excretion. I have repeated Virtanen's arrangements and conditions so far as practicable, and yet have obtained very different results. It is clear that apart from the possible influence of factors such as identity of legume, bacterial strain and adsorptive capacity of rooting medium, some other factor has a controlling effect on excretion. Wilson and his collaborators<sup>4,5</sup>, recently reached a similar conclusion from their Wisconsin experiments, and advanced evidence that excretion depends on the maintenance of a certain relation between rate of photosynthesis and of fixation, and for this reason is liable to be affected by light intensity and temperature. These factors were not subject to close control in my experiments. The soya beans were grown in a lean-to greenhouse partly shaded by trees, with a day temperature of 65-75° F. The other legumes were placed outside on dry days in a position where they received a maximum of six hours sunshine daily. No difference in excretion has emerged in these experiments between soya bean and the so-called 'cool weather' legumes, negative results having been obtained in all cases.

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Glasgow. Aug. 25.

<sup>1</sup> Bond, *NATURE*, **139**, 675 (1937).

<sup>2</sup> Bond, *NATURE*, **140**, 683 (1937).

<sup>3</sup> Bond, *Ann. Bot. (N.S.)*, **2** (1938).

<sup>4</sup> Wilson and Burton, *J. Agric. Sci.*, **28**, 307 (1938).

<sup>5</sup> Wilson and Wyss, *Proc. Soil Sci. Soc.*, **289** (1937).