is by no means proven that this is the case in animals treated with pyrithiamine and in fact it is not accepted by all investigators that all of the combined thiamine is identical with cocarboxylase. Consequently, the reply of DE CARO, RINDI, PERRI, and FERRARI to my previous objection is not convincing.

D. W. WOOLLEY

With this I declare: End of the discussion. The Redactor.

PRO EXPERIMENTIS

Detection of Dipeptides and Dipeptidase Activity on Paper

During the course of some work[1] on the purification of pig kidney cysteinyl-glycinase[2], the usefulness of working out a quick method to detect the dipeptidase activity became apparent.

The method is a modification of GIRI and NAGABHUSHANAN's test for the detection of amino acids on paper[3], and is based on the observation that, after spraying with naphtoquinonesulfonate and during the following alcohol-alkali treatment, some dipeptides change their colour at a lower rate or develop a different colour than the corresponding amino acids, thus enabling the use of this reaction for the detection of dipeptidase activity.

Procedure. Reagent *a:* 0·3 g of Na β-naphtoquinone-4-sulfonate (Eastman) is dissolved in 10 ml water, and distilled acetone is added to 100 ml. The reagent must be prepared immediately before use.

Reagent *b:* 2 ml of 4 *N* NaOH is diluted to 100 ml with 95% ethanol.

The paper is sprayed with reagent *a*, and heated in an oven at 100°C for 3–5 min. At this stage, amino acids and dipeptides develop colours which are often different enough to permit one to distinguish between them: cysteinyl-glycine, e.g., gives a pink-yellow colour; glycine and cysteine a violet one.

A better differentiation is obtained if the paper is then dipped in a bath of reagent *b*. The colours of most of the amino acids turn to grey-green within 10–15 min, whilst most of the dipeptides tested retain for a longer time the staining acquired during the heating, or develop a different colour. After some hours' bath, the contrast is much less clear.

Colours of some dipeptides and of the corresponding amino acids after 10 min treatment with alcohol-alkali.

Glycyl-glycine	–violet-brown	–Cysteine	– bluish-green
Glycyl-leucine	–violet-brown	–Glycine	– bluish-green
Glycyl-tyrosine	–violet-brown	–Leucine	– bluish-green
Glycyl-tryptophan	–violet-brown	–Alanine	– bluish-green
Leucyl-glycine	–green-yellow	–Tyrosine	– grey-brown
Alanyl-glycine	–green-yellow	–Tryptophan	– grey-brown
Cysteinyl-glycine (+ glutamic acid)	–pink-yellow		

Instead of cysteinyl-glycine, a mixture of cysteinyl-glycine and glutamic acid (partial acidic hydrolysate of glutathion[4]) was used. All substances were Hoffmann-La Roche products.

As seen from the above table, a differentiation is possible between several dipeptides and their respective component amino acids.

Test for the cysteinyl-glycinase activity. The following procedure was used either to detect the cysteinyl-glycinase on the electrophoresis paper, or as a spot test of a solution to be analyzed.

The filter paper carrying a drop of the solution, or the electrophoresis paper, was dipped in a uniform thin layer of the substrate-buffer-activator mixture: neutralized hydrolyzed glutathion[4] 3 to 4 mg per millilitre, corresponding to about 1·5 to 2 mg cysteinyl-glycine per millilitre, in 0·02 *M* THAM-HCl[5] buffer, pH 8·1 + 0·0005 *M* $MnCl_2$.

Paper electrophoresis of cysteinyl-glycinase. 0·04 *M* THAM-HCl buffer, pH 8·2, + 0,0005 *M* $MnCl_2$; 400 V during 3 h at room temperature on Whatman N 54. The cysteinyl-glycinase activity is shown by the green-grey spot (white in the figure) against a pink background (black in the figure).

Incubation was carried out at room temperature for 30–45 min, and stopped by heating the paper in the oven at 110°C for 5 min. The paper was then stained according to the procedure described above; the cysteinyl-glycinase activity was shown by the appearence of a violet (after the heating) or green-bluish (in the alcohol-alkali bath) spot, against a pink-yellow background.

Alternatively, the incubation was carried out by superposing the paper into a 1% agar gel, containing the substrate-buffer-activator mixture. The agar gel was then stained by spraying the reagent *a*: the colour developed slowly at room temperature, without any further treatment. The cysteinyl-glycinase activity was shown by the appearence of a brown spot, against a yellow background. The electrophoresis paper, in this alternative procedure, could of course be used for other analysis.

The sensitivity of these procedures is somewhat higher than the usual quantitative method in a tube[4].

To test other dipeptidase activities, an identical procedure was used. The reaction mixture contained about 3 mg of dipeptide per millilitre, the buffer and the suitable activator.

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Riassunto

Viene descritto un metodo rapido e sensibile per la rilevazione delle dipeptidasi su carta.

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