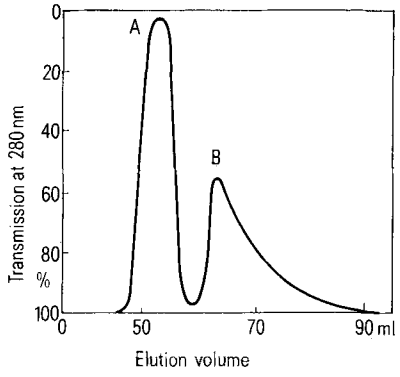
The figure shows the elution profile of the affinity chromatographic separation of the products. The eluted substances were analyzed by TLC, and DansAGlc was identified by a positive reaction with 50% H_2SO_4 and chloro-toluidine reagent. Peak A represents mainly 1-dimethylamino-naphthalene-5-sulfonic acid and unreacted D-glucosamine. Peak B consists only of Dans AGlc, which is retarded by its interaction with the matrix-fixed lectin. In the presence of the Con A-specific sugar, α-methyl-mannoside (1% w/v), all components of the reaction appear in peak A, indicating that the interaction of DansAGlc with Con A-sepharose is specific[6].



Affinity chromatographic purification of 2-deoxy-2-dansylamido-D-glucose. 0.2 ml of the solution as obtained from the reaction mixture (see text) were applied to a column (1.1 × 48 cm) of Con A-sepharose. Flow rate: 2.2 ml/h.

Con A-sepharose can be used for the purification of any sugar derivative possessing α-D-mannopyranoside end groups or internal 2-0-linked α-D-mannopyranosyl residues[7]. The experiment described here indicates that the aromatic substitution in position 2 of the sugar moiety strengthens the sugar-lectin interaction[8]. Thus, differential binding of 2-deoxy-2-amino-D-glucose and 2-deoxy-2-dansylamido-D-glucose can be used to isolate DansAGlc directly from the reaction mixture. To obtain optimal results, care should be taken not to overload the column. A slow flow rate (e.g. 2 ml $h^{-1}cm^{-2}$) will give good separations.

1 H. Lis and N. Sharon, A. Rev. Biochem. *42*, 541 (1973).
2 R. Axén, J. Porath and S. Ernback, Nature *214*, 1302 (1967).
3 J.P. Reeves, E. Shechter, R. Weil and H.R. Kaback, Proc. nat. Acad. Sci. USA *70*, 2722 (1973).
4 S. Schuldinger, G.K. Kerwar and H.R. Kaback, J. biol. Chem. *250*, 1361 (1975).
5 C. Gros and B. Labouesse, Eur. J. Biochem. *7*, 463 (1969).
6 Affinity chromatography, Principles and Methods: Pharmacia Fine Chemicals, Uppsala, Sweden 1974.
7 I.J. Goldstein, C.M. Reichert, A. Misaki and P.A.J. Goren, Biochem. biophys. Acta *317*, 500 (1973).
8 F.G. Loontiens, J.P. Van Wauwe, R. De Gussem and C.K. De Bruyne, Carbohydrate Res. *30*, 51 (1973).

Development of γ-glutamylcysteine synthetase and oxoprolinase in rat kidney[1]

E. Tsui and D. Yeung

Department of Biochemistry, University of Hong Kong, Hong Kong (Hong Kong), 27 December 1978

Summary. γ-Glutamylcysteine synthetase is present in barely detectable amounts in foetal kidney. Its activity starts to increase in postnatal life. In contrast, oxoproline is already found in significant quantities in the foetal tissue. Both enzymes show marked elevation in activities during the weaning period.

The ubiquitous occurrence of glutathione in both prokaryotic and eukaryotic cells suggests the importance of this compound in living system. Numerous biological roles have been proposed for the tripeptide, and more recently Meister et al.[2] formulated the γ-glutamyl cycle in the kidney. According to these authors, the cycle is catalyzed by 6 enzymes and serves as an amino acid transport system. Evidence has been presented pointing to the operation of the cycle in adult animal[3]. However, it is doubtful if the cycle is functional in the foetus. Tate and Meister[4] have shown that γ-glutamyl transpeptidase is almost undetectable in foetal rat kidney, but that the activity of the enzyme starts to appear in the immediate postnatal period. In terms of relative activities of the enzymes of the cycle, oxoprolinase and γ-glutamylcysteine synthetase may be considered rate limiting. It is therefore of interest to study the development of these enzymes in the kidney.

Materials and methods. Chemicals. Oxoproline, NAD, glutamic dehydrogenase, pyruvate kinase, phosphoenolpyruvate, dithiothreitol were obtained from Sigma Chemical, USA, C^{14}-glutamic acid was obtained from Amersham Radiochemical Centre, England.

Animals. Rats used were of the Wistar Albino strain. For the establishment of the developmental pattern of enzyme, 4-6 determinations were carried out to obtain the mean of each point. The foetal and neonatal animals were from both sexes, while adults were male.

Determination of enzyme activities. Oxoprolinase. Rat kidney was homogenized in 3 vol. of 50 mM triethanolamine pH 7.8, 2 mM oxoproline, 1% mercaptoethanol. The homogenate was assayed for enzyme activity by the method of Wendel and Flugge[5].

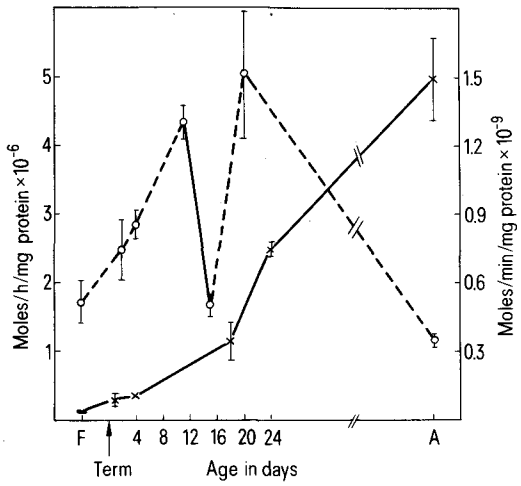
γ-Glutamylcysteine synthetase. Rat kidney was homogenized in 9 vol. of 150 mM KCl, 5 mM mercaptoethanol, 1 mM $MgCl_2$. The homogenate was centrifuged at 10,000 × g for 15 min. The supernatant was fractionated with ammonium sulfate and enzyme activity was assayed according to the method of Orlowski and Meister[6].

Results. The development of γ-glutamylcysteine synthetase and oxoprolinase is shown in the figure. Although γ-glutamylcysteine synthetase is barely detectable in late foetal kidney, its activity rises abruptly in the postnatal period. The increase appears to be biphasic, an early rise in the immediate postnatal life followed by a marked elevation during the weaning period. Oxoprolinase activity, on the other hand, is already present in substantial amounts in the foetus. Similar to the previous enzyme, there is a surge in activity after birth. However, there follows a significant drop in enzyme activity prior to weaning. Thereafter, the enzyme activity increases slowly to a peak at the 20th

postnatal day. Afterwhich it falls gradually to the adult value.

Discussion. Although the γ-glutamyl cycle has been demonstrated to occur in adult kidney[3], results obtained in this study as well as those of Tate and Meister's[4] suggests that it is unlikely to be operational in foetal tissue. The enzymes of other metabolic processes maintaining the homeostasis, like that of gluconeogenesis[7] and of the urea cycle[8], also appear only after birth.

In the present study, the estimation of γ-glutamylcysteine synthetase activity was performed in ammonium sulfate fractionated extract as interference of the assay occurs in crude extract. It might be argued that the absence of enzyme activity in foetal extract could be a result of loss of enzyme activity through fractionation with ammonium sulfate. Work from Orlowski and Meister[6] suggests this is unlikely, as more than 97% of enzyme activity might be recovered after the fractionation procedure. As for the assay of the enzyme, the method of Orlowski and Meister[6] was used as it is simpler than that of Minnich et al.[9]. The latter assay was only used to confirm the absence of enzyme activity in foetal samples, as it is more sensitive. Since both enzymes show marked increases in activities during weaning, it is likely that dietary factor is involved in the normal regulation of the 2 enzymes. In this respect, it is of interest to note that γ-glutamyl transpeptidase, another enzyme of the γ-glutamyl cycle, is reported to be affected by the nutritional state of the animal[10]. Thus if the γ-glutamyl cycle does participate in the manner proposed by Meister's group[2] it is logical to expect such a response to dietary intake of amino acids.



Development of oxoprolinase and γ-glutamylcysteine synthetase in rat kidney. Each point represents the mean of determination on 4–6 animals drawn from different litters. The vertical bars represent 1 SD of the mean. A, Adult rats, 3 months old. Activities of oxoproline (---○---○---) and γ-glutamylcysteine synthetase (——×——×——) are determined by procedures described in the text.

1 Acknowledgment. The work was supported by a research grant awarded by the Committee on Research and Conference Grants, University of Hong Kong.

2 M. Orlowski and A. Meister, Proc. nat. Acad. Sci. USA *67*, 1248 (1970).
3 A. Meister, in: Glutathione: Metabolism and Function, p. 35. Ed. I.M. Arias and W.B. Jacoby. Raven Press, New York 1976.
4 S.S. Tate and A.J. Meister, Biol. Chem. *250*, 4619 (1975).
5 A. Wendel and U.I. Flugge, Hoppe-Seyler's Z. Physiol. Chem. *356*, 881 (1975).
6 M. Orlowski and A. Meister, Biochemistry *10*, 372 (1971).
7 D. Yeung and I.T. Oliver, Biochem. J. *105*, 744 (1967).
8 N. Kretchmer, R. Hurwitz and N.C.R. Raiha, Biologia Neonat. *9*, 187 (1966).
9 V. Minnich, M.B. Smith, M.J. Brauner and P.W. Majerus, J. clin. Invest. *50*, 507 (1971).
10 T. Higushi, N. Tateishi, A. Naruse and Y. Sakamoto, Proc. 1st Congr. Fed. Asian Ocean. Biochem., p. 127 (1977).

Xanthumin and 8-epi-xanthatin as insect development inhibitors from *Xanthium canadense* Mill.[1]

K. Kawazu, S. Nakajima and M. Ariwa[2]

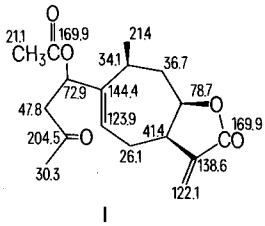
Department of Agricultural Chemistry, Okayama University, Tsushima, Okayama 700 (Japan), 19 December 1978

Summary. Two insect development inhibitors against *Drosophila melanogaster* have been isolated from the leaves of *X. canadense* and identified as xanthumin and 8-epi-xanthatin.

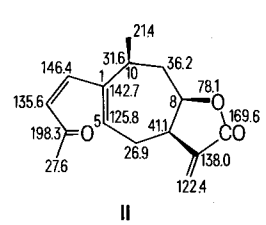
In recent years much interest has centered on the use of natural products for controlling insect pests[3–7]. For the purpose of exploring such natural products, suitable bioassay method, of which the criterion is development retardation or interruption as well as death, would be required. We devised a novel bioassay method using *Drosophila melanogaster*. The features of this method is to administer a plant extract orally, dermally and respiratorily to the insects throughout their development from egg to adult, and to observe precisely its effect on every stage of their development[8].

The bioassay was carried out as follows: 10 newly laid eggs of *D. melanogaster* were seeded on 2 g of the diet containing the test material in a glass tube and reared for 2 weeks with a photoperiod 16 h light/8 h dark at 25±2 °C and 93% relative humidity. The development state was observed, and the number of survivors at every stage of development was recorded every other day and compared with that of a control.

Of the methanol extracts of several hundred species of terrestrial and marine plants tested, the extract of the leaves of *Xanthium canadense* showed remarkable inhibitory ac-



I



II