Results. The concentration of PMNs in the washings increased for all the patients during the intermenstrual period. For the volunteer who had been followed over the longest period (12 cycles), the average concentration of PMNs at menstruation was of 46,900 and reached the peak of 118,000 during the intermenstrual period. The mean increase (M in figure 1) for the 5 patients amounted to 56,000 PMNs/ml.

The number of epithelial cells/ml of washing also increased significantly during the intermenstrual period ($M = 4900$ cells/ml; $Li > 0$).

Parallel to the increase in the number of cells, all the free and total activities of cathepsin D, elastase and β-glucuronidase showed a significant rise during the intermenstrual period. As an example, figure 2 shows the significant increase of the free activity of cathepsin D.

As for the intracellular specific activity (total - free activity/N° of cells) this parameter was found, for the 3 enzymes, to be maximum at menstruation and to decrease during the intermenstrual period. This is illustrated for cathepsin D in figure 3.

Discussion. The cellular and enzymatic analysis presented here show that the free and total activities of cathepsin D, elastase and β-glucuronidase, having a well-known intracellular distribution[16], followed the same pattern of cyclic variations as the number of PMNs, with a low activity during menstruation and an increase during the intermenstrual periods.

A bacterial origin of the 3 enzymes cannot be entirely excluded; however it should be emphasized that the hygiene of the volunteers was excellent (very little bacterial plaque). Moreover, recent investigations from our laboratory have shown the absence of bacterial isoenzymes of β-glucuronidase in gingival washings collected from healthy gingiva[17].

Gingiva can be considered as a target tissue for the female sex hormones. In ovariectomized rats, it has been observed that gingiva can quickly accumulate oestradiol-17β, a derivative of oestrone, having a marked hyperemic action[18]. Investigations in vitro have shown that gingiva, particularly when inflamed, possesses an enzymatic system capable of converting oestrone into oestradiol-17β[19]. Female sex hormones are known to increase circulating PMNs[20] and to induce a labilization of lysosomal membranes[8,9]: the increase in the free activities of the 3 lysosomal enzymes found in this study could be the result of both the increase in the number of PMNs and the increased fragility of the lysosomal membranes within these cells. As shown by the results concerning the intracellular concentration of enzymes, the PMNs indeed tended to lose more enzyme during the intermenstrual phase, when the amount of circulating (and probably gingival) sex hormones is at its maximum.

A high concentration of extracellular lysosomal enzymes could sensitize the gingival tissue and contribute to the aggravation of periodontal inflammation when bacterial plaque is present.

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Purification of 2-deoxy-2-dansylamido-D-glucose by affinity chromatography on a lectin-loaded agarose column

W. Bessler and P. Schindler

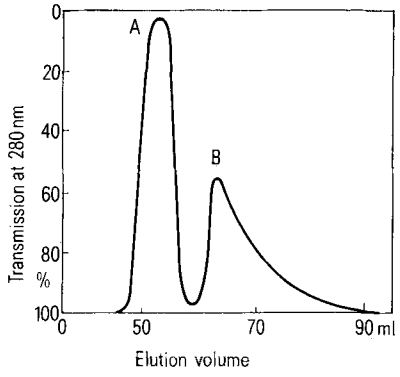
Institut für Mikrobiologie der Universität Tübingen, Auf der Morgenstelle 28, D-74 Tübingen (Federal Republic of Germany), 22 December 1978

Summary. A method is demonstrated to purify 2-substituted derivatives of glucose and sterically related sugars by affinity chromatography on Con A-sepharose. The method seems to be of rather general applicability.

Lectins are proteids mainly occurring in the seeds of plants, especially legumes, which have the property of binding specifically to mono- and oligosaccharides[1]. Lectins can be coupled to carrier materials, e.g. agarose; these matrix-bound lectins are powerful tools for separating sugar derivatives by affinity chromatography[2]. Agarose-bound lectins specific for mannose and glucose residues (Glycosylex, Miles; Con A-sepharose, Pharmacia), agarose and fucose (Fucosylex, Miles), or N-acetyl-D-galactosamine (agarose-soy bean agglutinin, Miles) are commercially available.

Fluorogenic or fluorescent sugar derivatives are very useful for the characterization of sugar transport systems and sugar carrier proteins in *Escherichia coli*[3,4]. Being interested in the transport mechanism of N-acyl-D-glucosamines, we synthesized the non-metabolizable fluorescent dansylated analogue of D-glucosamine (DansAGlc) by the reaction of dansyl chloride with glucosamine[5]. Looking for a method to purify the dansylated sugar from the reaction mixture, we found affinity chromatography on Con A-sepharose to be a new, powerful tool.

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.

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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