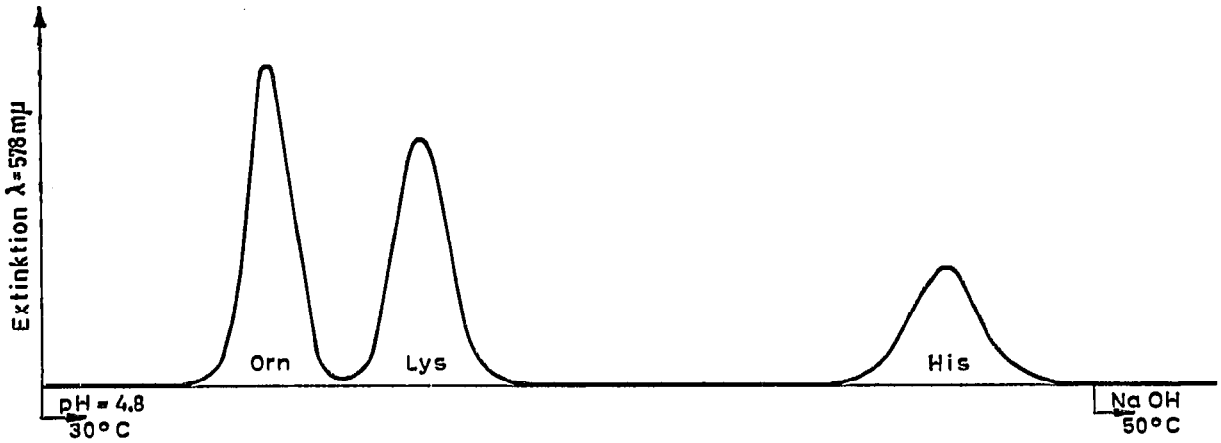

Fig. 1. Trennung von je 1 μMol Ornithin (Orn), Lysin (Lys) und Histidin (His) mit Na-Citrat-HCl-Puffer (0.12 *M*).

dadurch auch eine ausreichende Trennung von Ornithin und Lysin erreicht. Diese getrennte Arbeitsweise ermöglicht es, den Fehler bei der Lysin-Bestimmung, der in unserem Falle bis zu 20 % betragen konnte, auszuscheiden.

Institut für Pflanzenernährung, A. WÜNSCH
Technische Hochschule München-Weihenstephan (Deutschland)

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The separation of estrogens on Sephadex

Although Sephadex is mainly used as a molecular sieve, it has some adsorptive properties for many compounds, especially phenolic. In EECHAUTE AND DEMEESTER's[1] method, Sephadex G-25 medium was used for the purification of urinary estrogens. Further study of this method[2] indicated that the three estrogens, estrone (E_1), 17β-estradiol (E_2) and estriol (E_3), were adsorbed differently on the Sephadex matrix. In the present note the adsorption of these estrogens on Sephadex G-25 fine and on its 2-hydroxyethyl derivative LH-20 is compared.

For the chromatography on Sephadex G-25 fine a column (I.D. 1.27 cm), fitted with a glass filter and a glass stopcock, was packed with washed Sephadex to a height of 23.7 cm in order to obtain a bed volume of 30 ml. For the chromatography on Sephadex LH-20 a small glass column (I.D. 0.85 cm), fitted with a glass filter and the lower end not narrowed, in order to reduce the dead volume, was packed with 1.250 g of the dry Sephadex, which was allowed to swell overnight. The chromatographic separations were done at room temperature using water as eluant. Two ml of an aqueous solution of equal amounts of tritiated E_1, E_2 and E_3 were applied on to the

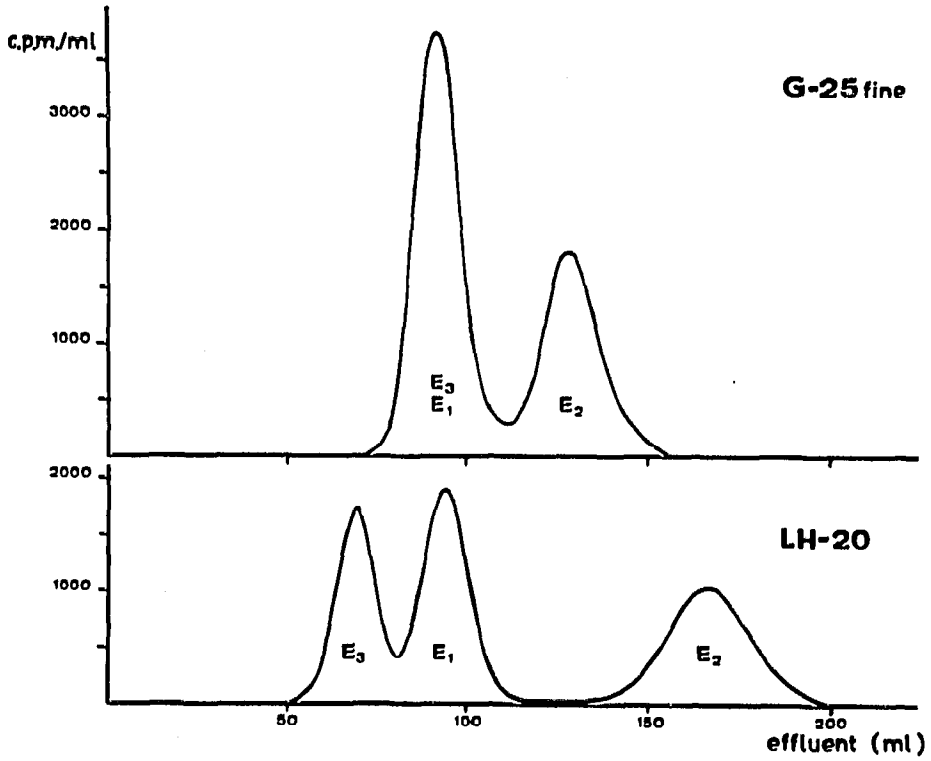

Fig. 1. Elution curves of estrone (E_1), 17β-estradiol (E_2) and estriol (E_3) on Sephadex G-25 fine and Sephadex LH-20 using water as eluant. For experimental details, see text.

top of the gel bed and after rinsing with 2 ml of distilled water the flow was regulated at 27.5 ml/h. Fractions of 1.5 ml were collected and the radioactivity was measured by liquid scintillation counting.

Fig. 1 gives the elution curves for the three estrogens studied on Sephadex G-25 fine and Sephadex LH-20. The sample and rinsing volume are included in the effluent. It appears that on Sephadex G-25 fine E_3 and E_1 are not separated from each other while E_2 forms a clearly distinct peak. On a much smaller column of Sephadex LH-20, the three estrogens are almost completely separated although large volumes of water are necessary for the elution. Hence, the introduction of 2-hydroxyethyl groups bound by an ether linkage to most of the free hydroxyls of Sephadex G-25 resulted in a higher adsorption and better separation. While at the present time large volumes of water are necessary for the separation of these estrogens on Sephadex LH-20, the use of solvents or mixtures of solvents[3] or the introduction of other radicals[4] may render this procedure more suitable for practical applications.

Rega Instituut, Laboratorium voor Experimentele Geneeskunde, Universiteitskliniecken St Rafaël, Leuven (Belgium)

H. Van Baelen
W. Heyns
P. De Moor

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