CHROM. 3299

Gel filtration of iodoamino acids

Iodoamino acids present in enzymatic digests of thyroglobulin and in blood plasma are commonly separated and identified by paper[1] or thin layer[2] chromatography. To prevent overloading of the chromatograms, a preliminary purification is usually carried out, either by ion exchange chromatography[3] or by gel filtration[4]. Such a purification is essential when the iodoamino acids are grossly contaminated as, for example, in digests of the unencapsulated thyroid gland of teleost fish. Moreover, the sparingly volatile nature of the eluants used in previously described preliminary purification procedures necessitates the extraction or sublimation of each column fraction in order to remove buffer solids prior to chromatography.

The modified gel filtration procedure, now described, uses readily volatile eluants and gives markedly improved separations with the same simplicity of operation.

Method

A 1.5 × 15 cm column was packed with Sephadex G-25, Fine (Pharmacia Corp.), previously equilibrated for a minimum of 2–3 h with a buffer of composition pyridine–acetic acid–water (45:11.5:1943.5, v/v), pH 5.6. The effluent was passed through the well of a crystal scintillation counter connected via a ratemeter to a chart recorder (3 cm/h), prior to collection in round bottom flasks. The sample under investigation was centrifuged, any precipitate washed with buffer and the combined supernatant and washings transferred on to the column. After the elution of the iodotyrosines (MIT and DIT) was complete (100 ml), the buffer was replaced by *tert.*-amylol saturated with 2 *N* ammonia which eluted the iodothyronines (T_3 and T_4). With a flow rate of 0.5 ml/min the separation was completed within 4 h.

Prior to purification by gel filtration, blood plasma samples were digested with papain[4] to release protein bound hormones. Samples of plaice thyroid gland were homogenised in saline–Tris buffer, pH 8.3 and hydrolysed with pancreatin according to the procedure of TONG AND CHAIKOFF[5].

Results

Fig. 1 shows the separation of a model mixture of millimicrogram quantities of ^{125}I labelled amino acids and thyroglobulin. This indicates the complete separation of MIT, DIT and I^- and of T_3 and T_4 from these three compounds. Although separa-

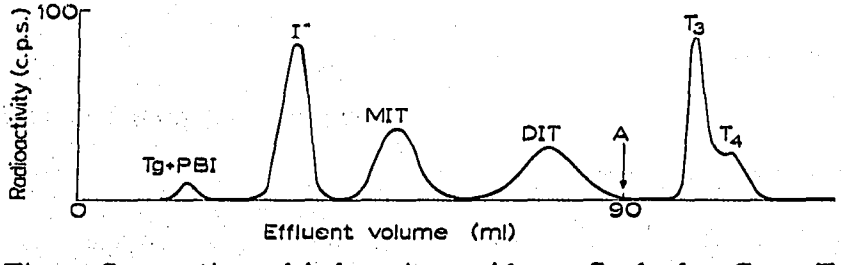

Fig. 1. Separation of iodoamino acids on Sephadex G-25. Tg = Thyroglobulin; PBI = protein-bound iodine; I = iodide; MIT = monoiodotyrosine; DIT = diiodotyrosine; T_3 = triiodothyronine; T_4 = thyroxine. A marks introduction of tertiary amylol saturated with 2 *N* ammonia.

tion of T_3 from T_4 was not sought in this study, it is expected that this may be achieved by elution with a slightly less polar solvent.

The purity of each of the fractions was established by thin layer chromatography; the eluates were prepared for chromatography merely by evaporation, *in vacuo* at 40°. Overall recoveries exceeded 95 % for each of the compounds examined.

Examinations of blood plasma iodoamino acids, performed on samples which had not been subjected to papain digestion, showed that the serum protein bound hormone (PBI) was eluted at the same time, relative to MIT and DIT, as thyroglobulin and that there were marked variations in the ratio PBI/(T_3 & T_4) in replicate analyses. This is in accord with a previous observation[6] that dissociation of PBI occurs during gel filtration, making the ratio dependent on such factors as column size and packing, rate of flow and pH.

The work described here forms a part of the programme of the Department of Agriculture and Fisheries for Scotland and was carried out during the tenure by one of us (R.H.O.) of a Natural Environment Research Council Studentship.

The Marine Laboratory,
Torry, Aberdeen (Great Britain)

R. H. OSBORN
T. H. SIMPSON

1 L. G. PLASKETT, in M. LEDERER (Editor), *Chromatographic Reviews*, Vol. 6, Elsevier, Amsterdam, 1964, p. 91.
2 T. SOFIANIDES, C. R. MELONI, E. ALGAR AND J. J. CANARI, *Proc. Soc. Exptl. Biol. Med.*, 123 (1966) 646.
3 R. J. BLOCK, R. H. MANDL, H. W. HOWARD, C. D. BAUER AND D. W. ANDERSON, *Arch. Biochem. Biophys.*, 93 (1961) 15.
4 S. LISSITZKY AND J. BISMUTH, *Clin. Chim. Acta*, 8 (1963) 269.
5 W. TONG AND I. L. CHAIKOFF, *J. Biol. Chem.*, 232 (1958) 939.
6 G. HOCMAN, *J. Chromatog.*, 21 (1966) 413.

Received November 6th, 1967