TABLE I

TOTAL CONTENT AND AVERAGE CONCENTRATION OF RIBONUCLEIC ACID IN MOTOR ANTERIOR HORN CELLS FROM A RABBIT

Cell No.	*Volume* (10^3 μ^3)	*Content RNA* (10^{-12} g)	*% RNA*
1	25	320	1.29
2	36	370	1.03
3	50	470	0.94
4	28	210	0.76
5	25	180	0.72
6	18.5	110	0.61
7	53	310	0.59
8	29	170	0.59
9	37	210	0.57
10	36	190	0.52
11	30	74	0.25
12	36	66	0.18
			mean 0.67

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THE USE OF RUBBER COLUMNS IN THE ISOLATION OF OESTRIOL FROM PREGNANCY URINE

by

L. BOSCH

The Netherlands Cancer Institute, Department of Endocrinology, Amsterdam (Netherlands)*

BOLDINGH'S[1] chromatographic separation of fatty acids by means of rubber columns was adapted by NYC *et al.*[2] to the separation of pure oestrone, oestradiol and oestriol in amounts of 400 μg of each oestrogen.

Little is known as yet, in this respect, of the behaviour of urine extracts containing oestrogens. GARST AND FRIEDGOOD[3,4] chromatographed the oestrogenic fraction of hydrolyzed urine and claimed to have isolated a hitherto unknown urinary substance with oestrogenic characteristics.

It is the object of this report to indicate that the use of rubber columns in the isolation of oestriol from pregnancy urine greatly facilitates the procedure. In a single chromatographic run, oestriol is separated from oestrone and oestradiol; during this operation the oestriol fraction is also cleared to a considerable extent of urinary pigments.

* Head Dr. O. MÜHLBOCK.

PROCEDURE

The urine is extracted with *n*-butanol, whereupon the butanol extract is concentrated in vacuo and hydrolyzed as indicated by STIMMEL[5].

The hydrolysate is extracted with ether; the ether extract thus obtained is shaken with 10% KOH-solution. The pH of the alkaline solution is brought to 9.0 ± 0.5[6] and extraction with ether is repeated. The extract of 12 l of urine, treated in this way, is evaporated to dryness and the residue is dissolved in 0.3 ml of a mixture of alcohol and benzene (1:1 v/v).

The rubber* is prepared as described by NYC *et al.*[2] and suspended in 60% methanol saturated with benzene. In this 60% methanol solution the rubber particles settle much better than in a 20% methanol solution. The top of the column (150 mm × 15 mm) is covered with a Monel gauze and the column is drained with 20% methanol.

When the urine extract (0.3 ml) is transferred to the top of the column, the Monel gauze must be dry, but not the rubber. If this is not the case, the extract is not quantitatively transferred to the column and fatty residues may adhere to the glass wall of the tube. Hereupon the vessel, which has contained the urine extract, is rinsed twice with 0.1 ml alcohol/benzene and these solutions are also transferred to the column.

Swelling occurs when the extract is brought into the column. As a result, the upper part of the column may become obstructed, but this is avoided if the columns are not packed too tightly.

Elution with 20% methanol (saturated with benzene) is then carried out. The dark urinary pigments remain in the upper part of the column; a light yellow eluate is obtained in which, after some time, small oestriol crystals may be observed. Starting with 12 l of urine, 230 ml of the eluant suffice to elute all oestriol from the column.

When larger quantities of urine are worked up, the dimensions of the column and the quantity of eluant used should be adjusted in accordance. In order to determine whether all oestriol has been eluted, use is made of paper chromatography according to HEFTMANN[7], MITCHELL[8], HEUSGHEM[9], BUSH[10]. The chromatographic procedure is carried out at a room temperature of 22–23° C (constant).

The eluate is evaporated in vacuo to dryness and pure crystalline oestriol is obtained by sublimation of the dry residue in a high vacuum (10^{-4} mm Hg).

In this way, 95 mg oestriol (m.p. 280–282° C uncorrected) has been isolated from 100 l pregnancy urine (9th month).

The oestriol obtained showed the correct ultraviolet absorption spectrum and specific absorption characteristics following reaction with sodium-*p*-phenol sulphonate in phosphoric acid (BACHMAN[11]).

The method described offers the advantage that the frequent tedious shaking in separating funnels is eliminated by a single chromatographic procedure.

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* Vulcanized rubber powder is called mealorub. The author is indebted to the "Rubberstichting", Delft, Holland, which provided him with the mealorub.