Short Communications and Preliminary Notes

QUANTITATIVE DETERMINATION OF RIBONUCLEIC ACID FROM INDIVIDUAL NERVE CELLS

by

JAN EDSTRÖM

Department of Histology, Medical School, Gothenburg (Sweden)

Ultraviolet absorption measurements on histological sections have been used for quantitative determination of ribonucleic acid (RNA)[1,2,3]. As the reliability of these methods can be disputed[4,5,6], it might be of interest to be able to estimate RNA in cells by other means. A method which allows quantitative evaluation of the total amount and average concentration of RNA in individual free dissected cells will be presented here.

Carnoy fixed nervous tissue, embedded in paraffin, is cut into 70 μ thick sections which are attached to large coverglasses. A section is deparaffinized and transferred via graded alcohols to water. A drop of water is allowed to remain on the section while the rest of the glass is dried. A drop of ribonuclease solution and a drop of glycerol is placed on a dried part of the glass near the preparate. Behind it, on a free part, a small coverglass of quartz is fastened by means of tape. On this glass a narrow strip of cellophane has been stretched out and fixed with two small pieces of tape. The strip is 40 μ wide, 30 μ thick and 7–8 mm long. It is obtained by cutting a paraffin-embedded piece of cellophane with a microtome. After this the large coverglass is turned upside down and placed over a two mm deep, two cm broad groove in a thick object glass, thus forming the roof in a chamber which is filled with liquid paraffin. The section, the drops and the quartz glass with its strip are in that way immersed in paraffin oil and can be reached with instruments from in front. While under the control of a phase contrast microscope the nerve cells which lie wholly within the section are dissected free and parted from surrounding tissue by means of a needle, directed by a micromanipulator. They are placed in the glycerol drop, which has the same refractive index as the paraffin oil. The areas of the different optical sections of the cells are determined and their volumes can be calculated. They are then liberated from glycerol and placed on free parts of the glass where they are crushed and flattened with the needle. As a rule no free small fragments are formed. After this the cells are digested and extracted three times with 0.1–0.2 mμl volumes of buffered ribonuclease solution, which are taken with a micropipette from the drop. Each digestion is allowed to go on for 20 minutes at 20° C. A solution containing 0.2 mg enzyme per ml in a 0.2 N phosphate buffer of pH 7.6 is used. The extracts are sucked up with the pipette, and those from one and the same cell are placed on the same part of the cellophane strip. When all the extracts have been put on, the quartz glass is taken away, washed with petroleum ether and placed with the strip underneath on an object glass of quartz. Liquid paraffin is used as a mounting medium. The strip is photographed at 257 mμ in the regions where the extracts are placed, and the free parts of the strip are projected against a revolving sector, logarithmically cut out, for calibration of the blackening. The plates are investigated by photometry. The optical density is recorded photoelectrically along the whole length of the photograph of the strip with extract and calibration system. Knowing the breadth of the strip, the definite enlargement in the photometer curve and the extinction constant at 257 mμ for RNA, degraded through ribonuclease and at a neutral pH, the amount of RNA in each extract can be computed from the photometer curve. With the aid of the figures for the cell volumes, the average RNA concentration is calculated. A minor correction has to be made for contaminating protein.

A detailed account with a discussion of the errors will be given. The accuracy in the calculation of the RNA concentration in fixed nerve cells of size $(20–60)\cdot 10^3\mu^3$ is better than $\pm$ 4%.

Some preliminary results of analyses of motor anterior horn cells (ventrolateral nucleus) will be given here (Table I). The figure for the mean RNA concentration, 0.67% ($\pm$ 0.10%), can be compared with the value 1.7% for the cytoplasm of the corresponding cells from the same species of animal, obtained by ultraviolet microphotometry by HYDÉN AND HARTELIUS[7].

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.