Nucleotide Analysis on the Cyto-Scale

It is now possible to isolate and determine quantitatively the ribonucleic acid from individual nerve cells[1,2]. As ribonucleic acid can no longer be considered to be a tetranucleotide structure, methods for nucleotide analysis are of great interest. Thus, the composition of ribonucleic acid has been found to vary in different organs and even within the cell[3]. It takes a very active part in the different functional phases of the individual cell[4]. A knowledge of its purine–pyrimidine chemistry during these phases could therefore be expected to throw some light on the nature of its action. Existing methods for nucleotide analysis by paper chromatography[5] or ionophoresis[6] require amounts of ribonucleic acid in the range 100–1,000 μgm. For a more sensitive modification of these techniques, a few micrograms suffices[7]. Individual nerve cells contain 200–1,000 pgm. of ribonucleic acid (1 pgm. = 10^{-12} gm.)[2], or about one-millionth of that necessary by these methods. To permit analysis even of these small amounts the following method has been worked out.

A piece of copper silk is treated with alkali in order to produce swelling of its fibres. It is washed with water and immersed in a highly viscous citrate buffer of *p*H 3·6 containing glycerol and glucose. This buffer neither takes up nor gives off water at ordinary room humidity and temperatures, and is of high electrolytic strength. Its viscosity is 1,650 times greater than that of water. After at least 24 hours in this buffer the silk is taken out and the excess moisture removed by centrifugation. A fibre 1–2 cm. in length is stretched out on a quartz slide. Small dabs of paste containing the citrate buffer are smeared on both its ends. The quartz slide is then inverted over a groove in a thick glass slide with the fibre perpendicular to the groove. Hydrolysed ribonucleic acid (1 *N* hydrochloric acid for 1 hr. at 100° C.), from which the hydrochloric acid has been removed, is placed at some point along the fibre with a micropipette directed by a micromanipulator. It is applied slowly and is concentrated through the evaporation of the solvent. The work is controlled microscopically. When a suitable quantity of ribonucleic acid has been applied (100–1,000 pgm.), the chamber formed by the quartz slide and the groove is filled with liquid paraffin. Platinum micro-electrodes are then inserted into the dabs of buffer paste. A direct-current voltage (12 V./mm.) applied for two hours separates the four ultra-violet light-absorbing constituents of ribonucleic acid when the buffer has a viscosity of 1,650 centipoises. Less than 1 mm. of the length of the fibre is required, and several analyses can therefore be made simultaneously. The quartz slide is then removed from the oil chamber and provided with a quartz cover-glass. Liquid paraffin is used as the mounting medium. The fibre is photographed, together with a light-calibrating system, in monochromatic light of 257 and 275 mμ wave-lengths respectively. The plates are investigated photometrically and the absolute amount of each base and nucleotide can be computed.

An ionophoretic separation photographed at the two wave-lengths is shown in Fig. 1.

The method is a million times more sensitive than the conventional ones, and permits nucleotide analysis of individual cells.

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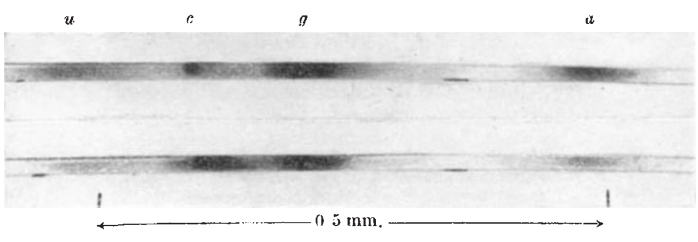

Fig. 1. A separation on a copper silk fibre of 15 μ diameter photographed at 257 mμ (upper) and 275 mμ (lower); *a*, *g*, *c* and *u* denote adenine, guanine, cytidylic and uridylic acid respectively

Identification of Amino-Acids Present in Purified Prothrombin by Circular Paper Chromatography

Seegers *et al.*[1] have very recently made an analysis on the amino-acid content, particularly tyrosine and tryptophan, of purified prothrombin. They have also shown by electrophoresis that their prothrombin preparation contains approximately 90 per cent of the protein in one component[1]. Several other workers[2–4] have investigated the nature of the protein in prothrombin. Astrup and Darling[4] have reported prothrombin to be a protein of globulin character. But the complete analysis of the prothrombin molecule with respect to different amino-acid contents is lacking.

In the present communication, the amino-acid composition of the purified prothrombin molecule has been determined by the circular paper chromatographic technique mainly developed by Giri and Rao[5].

Prothrombin was prepared from ox-blood by the method of Seegers *et al.*[6], and the activity of the prepared prothrombin found to be 1,800 units per mgm. protein nitrogen, as determined by the method of Ware *et al.*[7]. The nitrogen content of the preparation was found to be 13·36 per cent (micro-kjeldahl). The hydrolysis of the prothrombin molecule was carried out by autoclaving 100 mgm. of the dried prothrombin in 5 ml. of 6 *N* hydrochloric acid for a period of 6 hr. at 15 lb./sq. in. pressure. Excess hydrochloric acid was removed by vacuum distillation and lastly silver oxide was added to remove the last trace of acid. After filtration the filtrate was used for chromatography.

Chromatography was carried out on Whatman No. 1 filter paper of 18·5 cm. diameter in Petri dishes with solvent mixture containing butanol : acetic acid : water in the ratio of 40 : 10 : 50, the top layer being used. After the completion of the solvent run, the paper was air-dried and sprayed with a solution of 0·1 per cent ninhydrin in acetone and then heated at 60° C. for a period of 10–15 min. Amino-acids were