Rapid electrophoresis of RNA nucleotides in a dilute agar gel paper medium*

Much interest has been shown in the electrophoretic separation and subsequent quantitation of nucleotides in biological systems[1–4]. An attempt was made by GORDON AND REICHARD[5], to separate nucleotides in concentrated agar gel. However, this medium was found to be unsuitable[6] and most of the subsequent investigations used paper as the anticonvection medium. Several existing zone electrophoresis techniques appear to have been used successfully[7–9]. The EDSTRÖM procedure, which used alkali treated copper silk fibers as the anticonvection medium, required an application of 1800–6000V to the fiber[10]. Acid soluble nucleotides were separated by KLOUWEN using high voltage paper electrophoresis[11]. Others have used equally arduous techniques[12]. A need still exists for a simple system which can yield rapid well resolved separation of nucleotides and which possesses both quantitative and qualitative characteristics. In the present study agar gel overlaying vegetable parchment paper was the medium in which the electrophoresis took place[13,14]. Yeast ribonucleic acid nucleotides were used as a model for investigating the following:

1. The rapid electrophoretic separation of nucleotides in an open unequilibrated system.

2. The elution of the resolved and dried nucleotides for their subsequent qualitative elucidation by differential spectrophotometry.

3. The quantitative determination of separated nucleotides by ultraviolet scanning.

Materials and methods

Electrophoresis was carried out in agar gel overlaying vegetable parchment paper on a $3^1/_4$ in. × 4 in. lantern slide as previously described[13,14]. The gel was prepared by dissolving 300 mg of Ionagar No. 2 in acetate buffer of pH 3.1 and approximate ionic strength of 0.045. The buffer was made by diluting a stock buffer 1:4 where the stock was prepared by titrating 0.18 moles of sodium acetate to pH 3.1 with glacial acetic acid. 10 mg of each nucleotide were dissolved in 1 ml of distilled water, or shaken to form a saturated solution for those nucleotides which did not dissolve completely. Small strips of filter paper were impregnated with the nucleotides, blotted free of excess material by touching them to filter paper and then placed on the agar gel parallel to the 4 in. axis about 1 in. from the cathode. Application of 250 V for 20–30 min caused a rapid movement of the nucleotides toward the anode. The distance each moved was observed by holding a short wave U.V. light over the plate. In the case of mixtures, the electrophoresis was discontinued when clear separation of the components was observed, and this always occurred during the 20–30 min period. When separation was completed, the gel was quickly dried with an infrared lamp. The spots were penciled after ultraviolet visualization, cut out and eluted with 3 ml of 0.05 *N* NaOH. These solutions were then scanned across the ultraviolet spectrum with an automatic recording spectrophotometer.

Results and discussion

Fig. 1 shows an experiment which delineates the electrophoretic separation of RNA

* Supported in part by Grants-in-aid from the Receiving Hospital Research Corporation, and Research Grant G-16245 from the National Science Foundation.

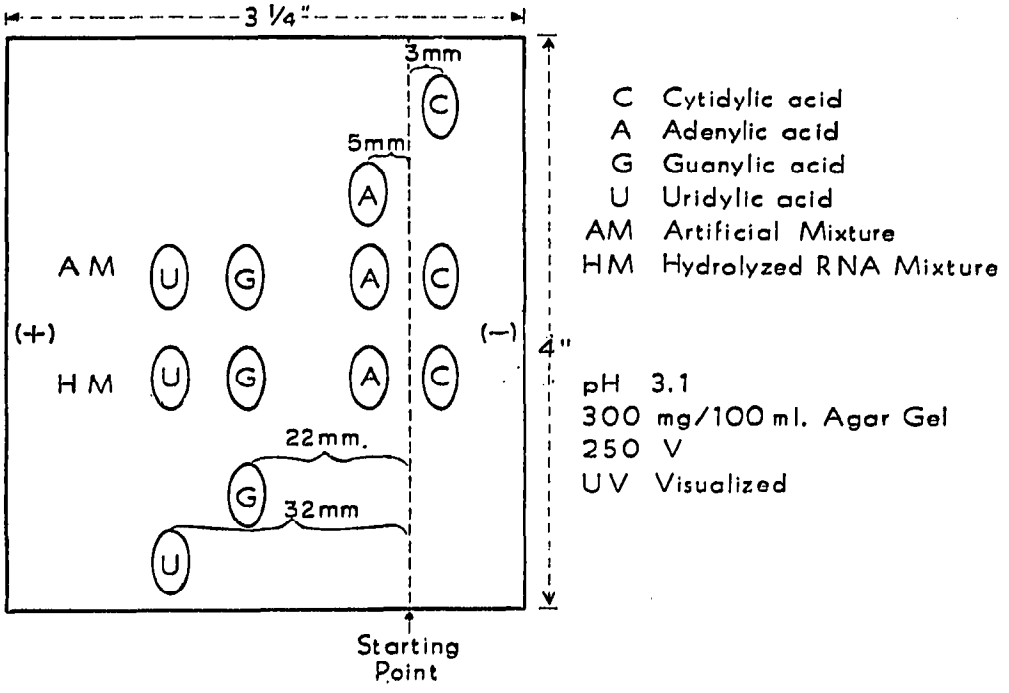

Fig. 1. Schematic representation of mobilities and separations of RNA nucleotides.

nucleotides in the pure state from artificially controlled mixtures and from natural mixtures obtained by mild hydrolysis of yeast ribonucleic acid. The upper and lower single components moved with exactly the same mobility as when they were present in the mixtures shown in the two center separations in the electropherograms. The ratios of the mobilities U:U, G:U, A:U and C:U were 1.00, 0.67, 0.15 and 0.08 respectively, as uridylic acid moved approximately 32 mm, guanylic acid 22 mm, adenylic acid 5 mm and cytidylic acid 3 mm. These ratios were the same for the

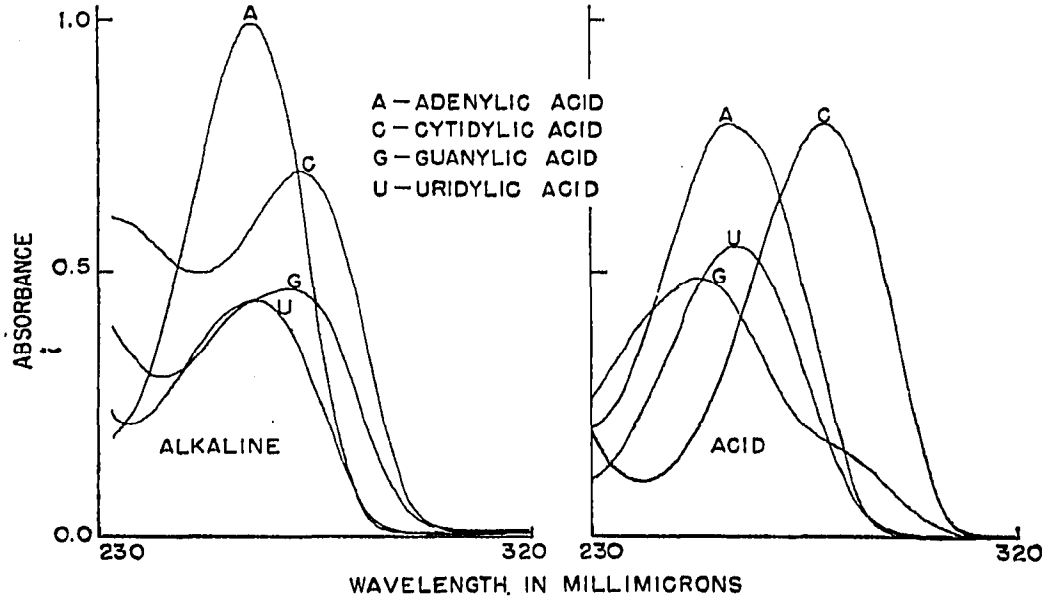

Fig. 2. Spectra of RNA nucleotides dissolved in alkali and acid.

artificially contrived mixtures, the hydrolysate as well as the individually moved components. This experiment showed a lack of influence on the mobility of any one nucleotide because of the presence of the other three nucleotides. When purified agarose was substituted for Ionagar No. 2, the endosmotic decrease caused the slowest nucleotide to remain at the starting line with equal diffusion to both sides of the line. This was not as clear cut a result as when the nucleotide moved behind the line and away from the filter paper site of application.

Fig. 2 shows the ultraviolet spectra obtained for the nucleotides when one of two

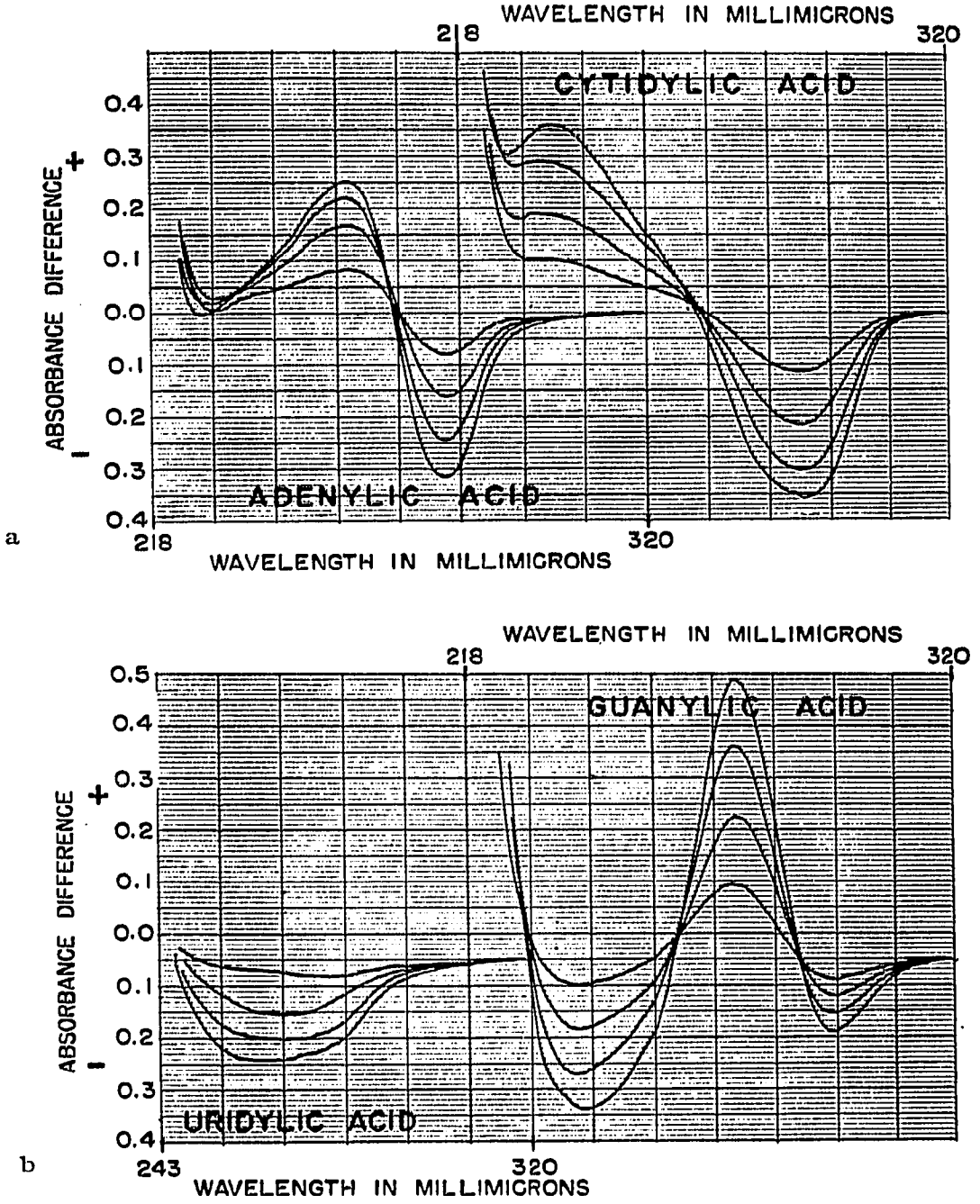

Fig. 3. (a) Ultraviolet differential spectra of cytidylic and adenylic acids. (b) Ultraviolet differential spectra of guanylic and uridylic acids.

aliquots of the same nucleotide was acidified with hydrochloric acid and the other alkalinized with sodium hydroxide. Samples prepared in this manner could be scanned simultaneously through the ultraviolet range by placement of one aliquot in the reference compartment and a second aliquot in the sample compartment of a double beam spectrophotometer. Fig. 3a and 3b show the distinct qualitative differences one can obtain with this automatic differential spectroscopy technique[15]. Ordinary ultraviolet readings at a fixed wavelength thus serve as a quantitative measure of the nucleotide being considered, while relative electrophoretic mobility and differential spectrophotometry can serve as a double checking qualitative system. The isosbestic points which are different for the several nucleotides can be seen as those wavelengths at which the differential spectra are isoabsorptive and cross the zero absorbance lines of Fig. 3a and 3b. These points may also be useful in the identification process. There is some acetate left in the parchment paper from which the elution of the resolved electropherogram of nucleotides was carried out. This did not appear to distort the spectra when compared to solution in the NaOH alone, although the peaks may shift several millimicrons. However, scanning of the entire spectra with an automatic recording instrument avoided any error due to bathochromic or hypsochromic shifting. Hyperchromic and hypochromic effects with small pH differences seem to be negligible.

Department of Pathology and Microbiology,
Wayne State University College of Medicine,
Detroit, Mich. (U.S.A.)

B. ZAK
L. M. WEINER

[1] A. M. CRESTFIELD AND F. W. ALLEN, *Anal. Chem.*, 27 (1955) 424.
[2] R. MARKHAM AND J. O. SMITH, *Nature*, 168 (1951) 406.
[3] J. N. DAVIDSON AND R. M. S. SMELLIE, *Biochem. J.*, 52 (1952) 594.
[4] T. WIELAND AND L. BAUER, *Angew. Chem.*, 63 (1951) 511.
[5] A. H. GORDON AND P. REICHARD, *Biochem. J.*, 48 (1951) 569.
[6] D. O. JORDAN, *The Chemistry of Nucleic Acids*, Butterworths, London, 1960, p. 83-84.
[7] R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 52 (1952) 552.
[8] M. LEDERER, *An Introduction to Paper Electrophoresis and Related Methods*, Elsevier, Amsterdam, 1957, p. 133.
[9] K. DIMROTH, L. JAENICKE AND I. VOLLBRECHSHAUSEN, *Z. Physiol. Chem.*, 289 (1952) 71.
[10] J. E. EDSTRÖM, *J. Biophys. Biochem. Cytol.*, 8 (1960) 39.
[11] H. M. KLOUWEN, *J. Chromatog.*, 7 (1962) 216.
[12] F. TURBA, H. PELZER AND H. SCHUSTER, *Z. Physiol. Chem.*, 296 (1954) 97.
[13] B. ZAK, F. VOLINI, J. BRISKI AND L. A. WILLIAMS, *Am. J. Clin. Pathol.*, 33 (1960) 75.
[14] L. M. WEINER AND B. ZAK, *Clin. Chim. Acta*, 8 (1963) 440.
[15] L. A. WILLIAMS AND B. ZAK, *Clin. Chim. Acta*, 4 (1959) 170.

Received May 1st, 1963