Rapid micro-electrophoresis of five adenine compounds*

In a previous communication a method for the rapid separation and ultraviolet densitometry of a mixture of AMP, ADP and ATP was described[1]. Since mixtures of adenine compounds encountered may contain adenine and/or adenosine as well as the three phosphates, it was of interest to determine whether the same analytical system could be used to separate the five adenines regardless of how many of the compounds were present in solution at one time. The present communication also contains an attempt to isolate AMP, ADP and ATP from *Escherichia coli* W. and the MS tissue culture cell line. The electrophoretic procedure may be used to establish the purity of adenine compounds and is excellent for determining the stability of their solutions. For this purpose, the technique is more rapid and much simpler than other procedures[2,3].

Materials and methods

Reagents

Adenine, adenosine, adenylic acid, adenosine diphosphate and adenosine triphosphate were used as obtained from Mann Research Laboratories. Individual solutions and various mixtures were made up to 10 mg of each compound per ml of distilled water.

Procedure

Preparation of cultures. E. Coli W. was grown on tryptose agar in Kolle flasks. The cells were harvested with ice cold saline containing 5 % formaldehyde after an 18 h incubation at room temperature and washed several times with normal saline to remove medium constituents. The MS human epithelial tissue culture cell line was grown as monolayers in 32 oz. bottles containing minimum essential medium (Eagle), Hank's salts and 15 % calf serum. After 4 days' incubation at 37° the monolayers were removed with trypsin and the cells were washed several times in Hank's salt solution. Four ml of cold 10 % trichloroacetic acid (TCA) solution were added to either the bacterial or tissue culture cells and the mixture was centrifuged. The filtrate was extracted with ether to remove TCA according to the procedure of BARTLETT[4]. Three microliters of extract were pipetted into a sliver of Whatman No. 3 MM paper and dried with an air blower. This process was repeated several times to enable one to obtain strong absorbances of the subsequently eluted bands.

Electrophoresis. Agarose gel (0.5 %) was prepared as previously described[1] and was pipetted onto a lantern slide containing 25 mm × 60 mm coverglasses. The gel was connected to the buffer in the baffle boxes by Schleicher & Schuell No. 900 filter paper. The paper slivers impregnated with the mixtures of samples and controls were placed in the center of the gel and 250 V were applied to the buffer box-plate system for 8–10 min. The plate was immediately dried in an oven at 120° which minimized diffusion of the adenines and loss of resolution of the separated zones.

Quantitative analysis. The spots designated as adenine compounds were cut off with a diamond point and dropped into 1.0 ml of 0.001 N NaOH eluting fluid. This solution was scanned through the ultraviolet range against a similarly eluted blank and the absorbances were compared to those of adenine calibration standards[5].

* Supported in part by Public Health Service Grant CAO 8217-01 from the National Cancer Institute, the Michigan Heart Association and the Detroit General Hospital Research Corporation.

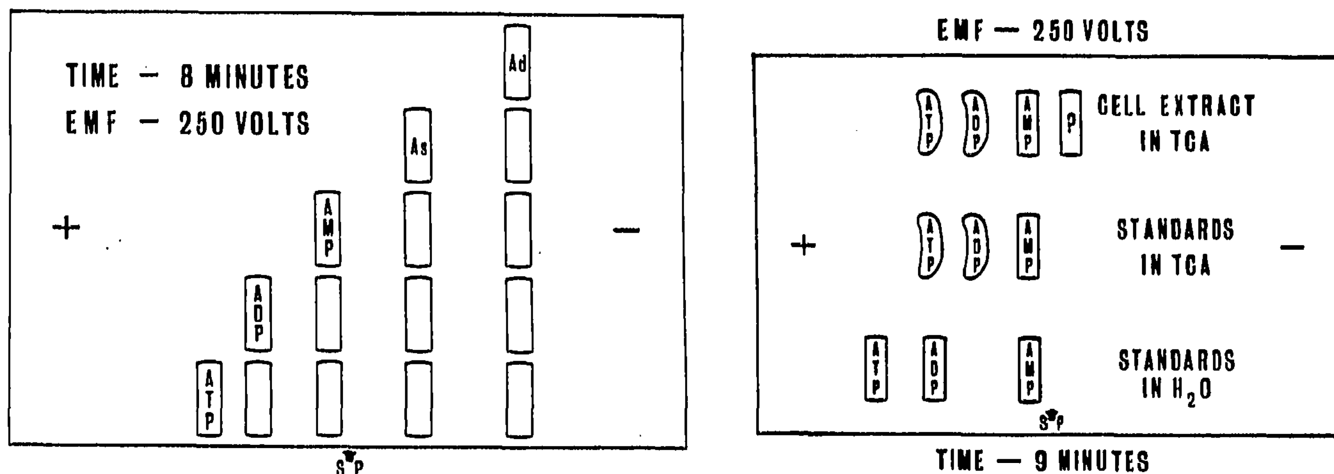

Fig. 1. Electropherogram indicating the mobility characteristics of the five adenine compounds moved as individuals and various mixtures.

Fig. 2. Mobility characteristics of adenine nucleotides in TCA from cell extracts and pure standards compared to mobilities for standards without TCA present.

Discussion and results

The results of the separation of the various adenine compounds are shown in Fig. 1. Each adenine compound moved with a definite mobility even when mixed with the other compounds. The time required to resolve the five compounds was 8–10 min with no tailing or overlap. In the interest of minimal handling of the adenines before electrophoresis, a study was made on the effect of the presence of TCA on the separation. The mobilities of the compounds appearing to be ADP and ATP in the TCA filtrate were slower than those of the known aqueous control compounds and this is shown in Fig. 2. This mobility difference between the controls and the TCA filtrate compounds was investigated. In one experiment the sample spots were eluted after electrophoresis and scanned spectrophotometrically in pH 2 and pH 11 solutions. They showed the spectral characteristics of pure adenine compounds similarly treated. In a second experiment the filtrate was fortified with varying concentrations of ADP and ATP and each time the increase in ultraviolet absorbing material appeared in the ADP and ATP zones of the electrophoretogram. In a third experiment, TCA was added to the controls and the mobilities decreased to equal those of the presumptive adenines of the culture filtrates. If the cell filtrates were extracted with ether to remove TCA the mobilities of the adenines increased and were similar to those of the standards. The mobilitiy differences with and without TCA present were attributed to the retarding influence of the TCA present in the samples at the starting point of electrophoresis. In any event, the spectral comparisons and the additions of known compounds with the determination of their mobilities in the sample separation under the described conditions yielded evidence that the zones were adenine compounds. Nonetheless there is the possibility of the presence of other TCA soluble nucleotides with relative mobilities similar to those of the adenines since other nucleotides are known to be present[3]. A fourth and still unidentified component appeared behind AMP in the cell extract pherogram, but did not interfere with the analysis of the presumptive adenine nucleotides. No other zones could be visualized under ultraviolet light or ascertained by ultraviolet spectrophotometry.

TABLE I

RECOVERY OF AMP, ADP AND ATP FROM SOLUTION AND FROM ELECTROPHEROGRAMS

Sample	*Present*	*Micrograms of adenine**	
		Found (solution)	*Found (electrophoresis)*
AMP	26.5	26.3	23.9
AMP	26.5	26.3	25.1
AMP	26.5	26.9	24.6
AMP	26.5	27.6	24.4
ADP	24.3	24.8	21.5
ADP	24.3	23.9	20.5
ADP	24.3	23.5	21.1
ADP	24.3	24.1	22.8
ATP	21.9	21.4	21.0
ATP	21.9	23.0	23.3
ATP	21.9	21.6	24.5

* All results represent duplicate values.

The last phase of the study was concerned with the quantitative characteristics of the procedure. The recoveries of AMP, ADP and ATP from 3 μl samples shown in Table I indicate a precise system. The results obtained on the same amounts of nucleotides when pipetted into solution and read in the spectrophotometer without prior electrophoresis are also shown in Table I. When the preparation of solutions of labile compound such as ATP were allowed to stand, they showed progressive breakdown. The electrophoretic process proved this since the concentration of the compound in its zone was lowered and the resulting compound reappeared as a second measurable zone in the same electrophoretogram.

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

L. M. WEINER
B. WELSH
B. ZAK

1 B. ZAK, L. M. WEINER AND E. BAGINSKI *J. Chromatog.*, 20 (1965) 157.
2 W. E. COHN AND C. E. CARTER, *J. Am. Chem. Soc.*, 72 (1950) 4273.
3 J. S. FRANZEN AND S. B. BINKLEY, *J. Biol. Chem.*, 236 (1961) 515.
4 G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 449.
5 B. ZAK AND L. M. WEINER, *J. Chromatog.*, 13 (1964) 255.

Received October 17th, 1966