The further evidence for the presence of lysolecithin in bile was provided using the combination of snake venom and autoradiography. A part of the labelled phospholipids was hydrolyzed with snake venom *(Crotalus adamanteus)* [10] and subjected to paper chromatography together with the original phospholipids. After hydrolysis with snake venom of the pure liver lecithin obtained by preparative paper chromatography, the lecithin spot was diminished greatly and transformed into a lysolecithin spot (Ib). The lecithin spot of biliary phospholipids (IIa) was diminished greatly after similar hydrolysis and transformed into a lysolecithin spot, thus confirming the presence of lysolecithin in bile (IIb).

This work is part of investigations supported by *The Swedish Medical Research Council.*

1. Thudichum, J. L. W. *A treatise on gallstones*, John Churchill and Sons, London 1863.
2. Johnston, C. G., Irvin, J. L. and Walton C. *J. Biol. Chem.* **131** (1939) 425.
3. Polonovski, M. and Bourillon, R. *Bull. soc. chim. biol.* **34** (1952) 720.
4. Nakayama, F. and Johnston, C. G. *Surg. Forum, Am. College Surgeons* **9** (1958) 518.
5. Blomstrand, R. *Acta Chem. Scand. In press.*
6. Folch, J., Ascoli, I., Lees, M., Meath, J. A. and LeBaron, F. N. *J. Biol. Chem.* **191** (1951) 833.
7. Borgström, B. *Acta Physiol. Scand.* **28** (1952) 101.
8. Marinetti, G. V., Albrecht, M., Ford, T. and Stotz, E. *Biochim. et Biophys. Acta* **36** (1959) 4.
9. Marinetti, G. V., Witter, R. F. and Stotz, E. *J. Biol. Chem.* **226** (1957) 475.
10. Dittmer, G. C. and Hanahan, D. J. *J. Biol. Chem.* **234** (1959) 1976.

Received May 31, 1960.

Separation of Nucleic Acids and Protein by Electrophoresis Combined with Counter Flow

R. ELIASSON, E. HAMMARSTEN and H. PALMSTIERNA

Department of Bacteriology Karolinska Institutet, Stockholm 60, Sweden

A good separation of proteins and nucleic acids from cell extracts could be achieved by electrophoresis in long columns stabilised with ethanolised cellulose [1]. The length of the path needed to affect such a separation was 300—320 cm. The diameters of the columns was 3.5 cm, and in a few cases 8.0 cm (unpublished). It became apparent that some material contained in the protein fractions was lost in the large amounts of cellulose (*e. g.* the neurotoxin from *Bordetella pertussis*).

The experiments to be described aim at a separation of the nucleic acids from the proteins in small columns as a preliminary to a further separation of these compound groups by means of chromatography. It was also desirable to let the proteins be in contact with as little cellulose as possible. Very small columns are also desirable for the separation of the components in biologically active nucleoproteins.

From these considerations we have tried to replace the length of the migration path by a steady flow of buffer against the direction of the electrophoretically migrating compounds. For this purpose we have used jacketed and cooled columns, with a length and diameter of 45 and 3.5 cm, respectively. We have also been working with 35 × 1 cm columns, about which will be reported later on. The packing, cooling, continuous buffer exchange and the buffer composition were exactly the same as described before [1]. The end of the side tube was, as usual, closed by a tightly fitting, thick dialysis membrane. Micropumps were constructed with capacities suitable for pumping the buffer in the direction opposite to the electrophoretic migration of the polyelectrolytes to be separated.

In the present paper we report experiments (*cf.* Table 1) where suction was simply applied to the buffer above the stabilised part of the column, with the purpose of finding the rate of flow that would permit the nucleic acid to migrate towards the anode at reduced speed, and drive the

Table 1.

Number of expt.	Sample mg Protein	DNA	Electrophoresis time, h	Counter flow cm/h	Migration velocities cm/h Protein	DNA
1	200	—	7	0	1,4 [e]	—
2	200 [c]	10 [a]	9	0	1,9	3,1
3	200 [c]	—	12	0	1.1 [e]	—
4	200 [c]	—	18	0	1.1 [e]	—
5	200 [c]	10 [a]	19.3	1.95	f	1.1
6	d	18 [b]	6	2.27	f	1.5
7	d	17.5 [b]	17.5	2.23	f	0.8

a) DNA prepared from calf thymus.
b) DNA prepared from *B. pertussis.*
c) Protein: Serum albumin containing praealbumun and some globulin.
d) Protein: Unknown amount of cell proteins from *B. pertussis.*
e) Not corrected for the path passed during the application of the sample before the electro phoresis was started.
f) The protein was driven out of the column by the counter flow. Measured by the photometer mentioned in the text.
g) The migration is measured from the front of the substances, when appearing after elution. The effective volume of the stabilised part of the column was measured by using phenol red.

proteins the opposite way or reduce their mobility to zero. In experiments to be published later suction has been applied with the view of isolating all protein. In Table 1 the mobilities are expressed as the position of the fronts of nucleic acids and proteins, as found after elution of the column following the electrophoresis either they have been performed with or without counter flow of the buffer solution. In cases were the proteins were driven against their electrophoretic mobility by the counter flow, the elution of the proteins was followed during the electrophoresis by a LKB Uvicord continuous photometer at 254 mμ (Svensson [2]). The nucleic acids recovered by elution after electrophoresis contained no protein as judged by the ninhydrin reaction (Moore and Stein [3]) after acid hydrolysis of solutions with 0.2 mg NA/100 ml and sublimation of the ammonium chloride *in vacuo*. Some dilution of the nucleic acid and protein fractions occurred, but the volumes could easily be reduced by a recently developed ultrafiltration method [4].

The authors are grateful to engineer E. Meyer for skilful technical assistance and the construction of the micropumps.

This work has been supported in part by the *Office, Chief of Research and Development, U.S. Department of Army,* through its European Office.

1. Hammarsten, E., Palmstierna, H. and Meyer, E. *J. Biochem. Microbiol. Technol. Eng.* **1** (1959) 273.
2. Svensson, H. *Science Tools* **6** (1960) 21.
3. Moore, S. and Stein, W. *J. Biol. Chem.* **211** (1954) 907.
4. Palmstierna, H. *Biochem. Biophys. Res. Comm.* **2** (1960) 53.

Received June 2, 1960.