The NMR-spectra were obtained at 40 Mc/s with a Varian Associates model V-4 300 B high resolution NMR-spectrometer and a flux-stabilized 12 in. electromagnet obtained from the same company. The magnet sweep was calibrated using the modulation side-band technique.

Acknowledgements. The author wishes to express his thanks to Professor Arne Fredga for his interest in this work and for all facilities put at his disposal. Thanks are also due to Mr. Bo Gestblom for recording the NMR-spectra. The elementary analyses were carried out by Dr. A. Bernhardt, Mülheim. A grant from the *Hierta-Retzius stipendiefond* is gratefully acknowledged.

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Received August 22, 1961.

Separation of Uronic Acids by Paper Electrophoresis

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The purpose of this work was to develop a rapid and convenient method for qualitative and quantitative analysis of mixtures of uronic acids. Paper chromatography of uronic acids in the solvents most commonly used for chromatography of sugars does not give satisfactory results. In basic solvents such as pyridine-ethyl acetate-water, the uronic acids do not move, and in acidic solvents such as acetic acid-ethyl acetate-water, the mobilities of the different uronic acids are nearly identical. The most satisfactory solvents are mixtures of both basic and acidic components, such as the solvent introduced by Fischer and Dörfel [1]: Pyridine-ethyl acetate-acetic acid-water, 5:5:1:3. However, the separation is not very satisfactory, it is not possible to distinguish between glucuronic and guluronic acid, it is time-consuming and for quantitative work it is necessary beforehand to transform all lactones to uronic acids. Paper electrophoresis of uronic acids has been used by Jayme and Kringstad [2] and Hoffman, Linker and Meyer [3] but no details of the mobilities of the different uronic acids were given. The possibility of using paperelectrophoresis for separation of uronic acids was therefore further investigated.

Materials and methods. The glucuronic and galacturonic acids used were commercial preparations. The guluronic and mannuronic acids were prepared from hydrolysates of alginic acid by chromatographic separation of the lactones [4] and transforming the lactones by addition of alkali to uronic acid salts. The determination of the pK values has been described earlier [5]. The electrophoresis was carried out in an LKB paper electrophoresis apparatus on Schleicher & Schüll 2043b paper strips. The

Table 1. pK_s values and mobilities in acidic medium.

	pK_s	M_m
Glucuronic acid	3.20	1.10
Mannuronic acid	3.38	1.00
Galacturonic acid	3.42	0.95
Guluronic acid	3.65	0.88

Table 2. Mobilities of uronic acids in borate buffer containing varying amounts of calcium.

Acid	M $CaCl_2$							
	0	0.0001	0.0003	0.0005	0.001	0.002	0.005	0.007
Guluronic	0.93	0.93	0.92	0.85	0.84	0.85	0.85	0.81
Mannuronic	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Galacturonic	0.97	0.97	1.01	0.98	1.00	1.09	1.18	1.16
Glucuronic	1.05	1.05	1.12	1.13	1.19	1.28	1.44	1.40
Glucose	0.71	0.76	0.80	0.88	1.00	1.01	1.14	1.17

current applied was 0.5 mA/cm. A 2.5 % solution of anilinetrichloroacetate in glacial acid was used as location reagent. After dipping, the strips were heated for 2—3 minutes at 100°C. It was found to be convenient to examine the strips in ultraviolet light.

Results. Acids having sufficiently different pK values may be separated by paper electrophoresis in acid buffer. The pK values of galacturonic, glucuronic, guluronic and mannuronic acids were determined and the results are given in Table 1. The same table gives the mobilities of the four acids relative to mannuronic acid (M_m) in phthalate buffer at pH 3.15. It may be seen from the table that it is possible to separate guluronic and glucuronic acid from each other and from galacturonic and mannuronic acid, while the two latter acids are not separated.

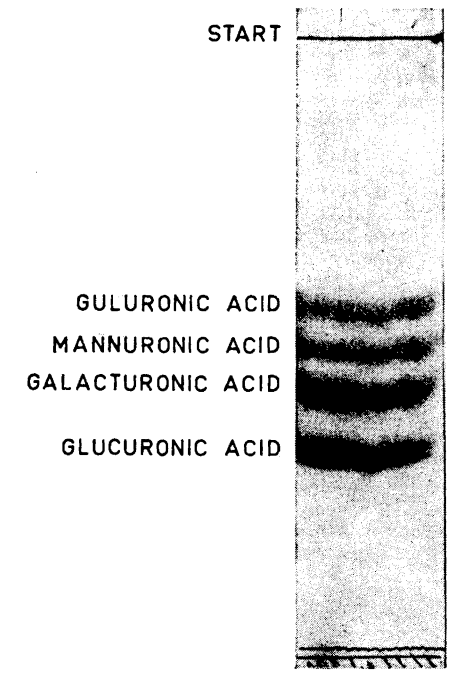

Fig. 1. Separation of uronic acids by electrophoresis in 0.01 M borax containing 0.005 M calcium chloride.

Borate buffers give good results for separation of neutral monosaccharides [6]. However, the separation of uronic acids in this buffer was not satisfactory. If the uronic acids show some tendency to complex formation with calcium at the pH of the borate buffer, the addition of small amounts of calcium to the buffer should reduce the mobility. If this tendency is different for the different uronic acids, the addition of calcium to the buffer might improve the separation. The mobilities of the four uronic acids in borate buffer containing varying amounts of calcium are given in Table 2. For comparison, the mobility of glucose is given in the same table.

As the table shows, the separation is very much improved by the addition of calcium. A 0.01 M borax solution (pH 9.2) containing 0.005 M calcium chloride gives in two hours a satisfactory separation of the four uronic acids investigated (Fig. 1). For quantitative determination the method has the advantage that the alkaline buffer automatically transforms all lactones to uronic acid salts.

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Received August 9, 1961.