trast Katz and Stamler(11) induced marked cholesterolemia in the growing chick by addition of the same level of cholesterol (2%) and 5% cottonseed oil to a diet containing 18% protein. This level of protein, in the authors' experience, is inadequate for proper metabolism of the absorbed cholesterol.

Weight gains and feed consumption in our study (Table I) indicate uniformity at each protein level. This is of particular interest since the caloric density of the diets varies considerably between the 3 and 9% levels of dietary fats. The uniformity of feed consumption might well be a factor in uniformity of the plasma cholesterol values.

Summary. The effects on plasma cholesterol level of 4 fats each supplied at 3 levels in a high and a low protein diet were studied in the cholesterol-fed chicken. On the low protein diet a marked hypercholesterolemia was observed which was not affected by type of fat studied or level of supplementation. On the high protein diet, plasma cholesterol levels were essentially normal regardless of source and level of dietary fat. Since the fats used represented a wide range in the product EFA x TSFA, a lack of relationship between this product and plasma cholesterol level in the growing chicken is indicated.

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Received May 19, 1958. P.S.E.B.M., 1958, v98.

Use of Ionophoretic Mobility Equilibria in Fractionation of Mixtures Containing Indoles.* (24131)

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Chromatographic separations of indoles in urine samples are often complicated by interfering substances which mark the spot, distort the Rf value and spot size, or cause streaking (1,2). It is also quite difficult to separate certain alkaloids from pigments and other "interfering substances" in urine by the conventional methods of paper electrophoresis; however, good separations are practicable by the mobility equilibrium method (unpublished data, 1952). The phenomenon of mobility equilibrium (M.E.) was first described in open-hanging-strip electrophoresis by Durrum (3). Separations by this method are time-consuming and require high current flow with volatile electrolyte systems. Some of the theoretical considerations presented in this paper have also been discussed by Macheboeuf, *et al.*(9). The purpose of this report is to describe a less time-consuming method in which low current flow may be utilized; the use of horizontal or vertical types of open paper electrophoresis units is practicable; and volatile or non-volatile electrolytes may be employed. It is thus possible to separate neutral molecules and charged ions (amphoteric and non-

* This investigation was supported in part by research grant from Central Ohio Heart Assn. Most indole derivatives were kindly supplied by Dr. M. E. Speeter of Upjohn Co.

amphoteric) on a single strip of paper.

Methods. Two procedures have been used to determine M.E. values for compounds under specific conditions. *Strip test.* Strips of Whatman 3 MM filter paper (1½" x any convenient length) are moistened with the electrolyte. The samples are applied as indicated in Fig. 1A. The electric current is then applied for 1 to 2 hours in an open type paper electrophoresis unit. The spots are detected by their fluorescence in ultraviolet light, subsequent to drying the paper in an oven containing an atmosphere of formaldehyde. This treatment, however, precludes good color development with p-dimethyl-amino-benzaldehyde and with 1-mitroso-2-naphthol. Alternatively, the spots are detected by immersing or spraying the dried paper with the latter reagents to produce colored spots on a faint yellow background(4,5). The spots are then encircled, their total movement measured (Figs. 1B & 1C), and plotted as in Fig. 1D. The intercept of the locus of movement and the locus of origin delineates the M.E. Its numerical value is obtained by measuring the distance from this point to the center line. A negative or positive sign is then affixed. *Sheet test.* Samples are applied to a sheet of Whatman 3 MM filter paper as depicted in Fig. 2, and processed as were the strips. The terminal points of migration are connected at their centers. The intercept of this line and the locus of origin represents the M.E.

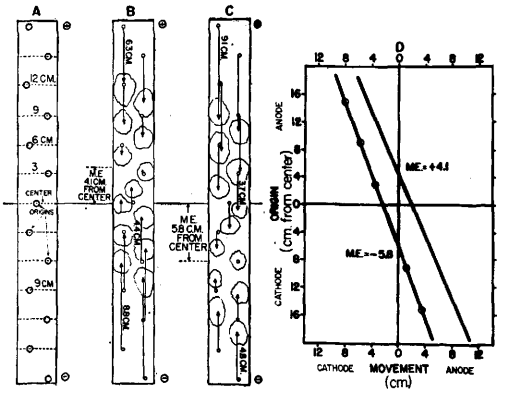

FIG. 1. Determination of M. E. values by the Strip Test, for 5-hydroxyindoleacetic acid (−4.1) and 5-hydroxytryptamine (+5.8), with a vertical type electrophoresis unit (apex height, 24 cm), in 0.05 M phosphate buffer, pH 7.0.

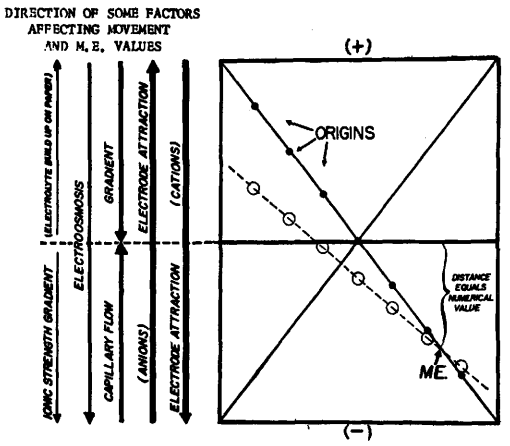

FIG. 2. Schematic representation of direction and relative magnitude of some factors on movement and M. E. of a cation in the Sheet Test. Compare with Fig. 3.

Results. The strip test is convenient and more economical for a single compound, while the sheet test is most practical for resolving mixtures. Fig. 3 illustrates an application of the method in fractionation of a mixture of urea and 3 indoles (5-hydroxy-tryptamine, 5-hydroxy-indoleacetic acid and 5-hydroxytryptophan). In this experiment the sheet test reveals: (1) the M.E. position of a substance in the mixture (#3, zone I and #9 zone III), (2) the position on the paper at which 2 or more substances may be separated from one another in the shortest interval of time, without resolving the other components in the mixtures (1A from 1B zone I 5A from 5D zone II), and (3) the zone in which best separations, with minimal spot spreading occurs (#4, zone II). Use of the sheet-test provides the maximal amount of information on the paper electrophoretic behavior of the components in a mixture in a minimal amount of time, and makes the use of the usual trial and error methods unnecessary. The determined M.E. value or a specific zone on the paper may then be selected for routine fractionations in accordance with experimental needs. Fig. 2 depicts the direction and relative magnitude of the major factors affecting movement of these compounds and producing the mobility equilibrium phenomenon. The ions are attracted by electrodes of opposite charge. Their mobilities are inversely proportional to

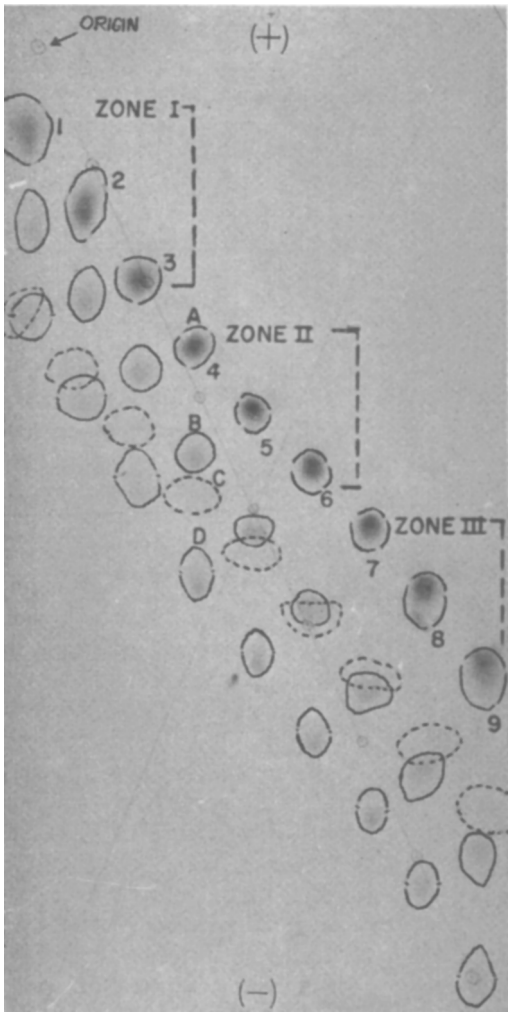

FIG. 3. Results of the Sheet Test performed on mixture of 5-hydroxyindoleacetic acid (A), 5-hydroxytryptophan (B), urea (C), and 5-hydroxytryptamine (D), using 0.05 M barbital buffer, pH 8.6, and a horizontal electrophoresis unit.

the ionic strength of the electrolyte, approaching the Y-axis asymptotically(6). In these studies a discreet stepwise decrease in numerical M.E. values was demonstrated by progressively increasing the starting concentration of the electrolyte. Evaporation as a sequel of the heat generated by the electric current passing through the filter paper, causes capillary forces to constantly draw fluid from the electrolyte compartments, with a flow rate gradient being set up toward the drier center. As evaporation of the solvent ensues, especially for non-volatile electrolyte systems, the amount of solute on the paper increases in such a manner that highest concentration is found midway between the electrodes. Filter paper or cellulose is structurally an aggregate of submicroscopic colloidal particles in the form of a reticulum. A certain number of carboxyl groups are usually found in filter paper, which may ionize, depending upon the pH. When alkaline electrolytes are used the ionized groups give the network of cellulose a negative charge; however, since the components of the reticulum are fixed and cannot migrate toward the anode in the imposed electrical field, the fluid in the interstices of the network moves toward the cathode. This movement of fluid accounts for the cathodal displacement of non-charged components in a mixture (*e.g.* urea, #5C zone II, is more cathodally displaced in this experiment than 5-hydroxytryptophan, #5B zone II, which possesses a weak negative charge at pH 8.6). Electroosmosis is the major factor responsible for the difference in absolute values of mobility equilibria for cations and anions of

TABLE I. Some Factors* Affecting the Movement and Mobility Equilibria of Indoles in Open-Type Paper Electrophoresis.

Factor	Movement			
	Cations		Anions	
	Anodal	Cathodal	Anodal	Cathodal
1. Electrode attraction	+	+	+	+
2. Capillary flow gradient	+	—	—	+
3. Electroosmosis	+	+	—	—
4. Ionic strength gradient (due to electrolyte build-up on paper)	—	+	+	—
5. pH	Significant effect on amphoteric compounds			
6. Adsorption and diffusion	Minimal			

* The factors are listed in order of decreasing magnitude.
+ = enhancement, — = inhibition.

otherwise molecularly similar species. In moderate concentrations of buffers, the compounds used in these experiments migrated as round spots (#3 zone I), indicating minimal adsorption(7). Spot spreading usually was most salient near the electrolyte compartments (compare #1A and #9A with #5A, Fig. 3). Lateral diffusion at M.E. occurs in experiments of long duration. Changes in the pH of the buffer in the electrolyte vessels are minimal even when volatile electrolytes are used(3). Positive ions exhibit maximal and negative ions show minimal mobilities on the anodal side of center. Conversely, cation mobility is lowest, and anion mobility highest on the cathodal side. Advantage may be taken of these regions of increased and decreased mobility by using longer strips of paper in a standard open-type electrophoresis unit. The extra length may be folded into the electrolyte vessels or counterpoised rollers may be inserted into these compartments. If the unresolved components of a mixture migrate into a region where differences in mobility are very slight (*e.g.* #5B & C zone II or #1C & D zone I) the paper may then be moved toward that electrode where mobility differences are greater (*i.e.* anode for #5 and cathode for #1) and electrophoresis resumed. This principle has been used to separate certain alkaloids from urinary pigments (unpublished data 1952) and more recently by Wolfson(8) and by Lederer *et al.*(8) to separate normal and abnormal adult hemoglobins. These investigators made successive analyses on the cathodal side of center. The zone of mobility equilibrium for a given ion: (1) represents the resultant of several forces, (2) occurs on the side of least movement (cathodal for cations, anodal for anions), and (3) is independent of the point of application of the sample. Its numerical value is equivalent to the distance from the center of this zone to the center line of the paper, with appropriate sign affixed.

Summary. (1) A method for rapidly determining the mobility equilibrium of a compound is described. The procedure provides an analytical rather than the conventional trial and error method for determining paper electrophoretic characteristics of the components in a mixture. (2) Some factors affecting ion movement and mobility equilibria in open-type paper electrophoresis units are discussed. (3) An application of the method in separating urea and several indole derivatives is presented.

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Received May 20, 1958. P.S.E.B.M., 1958, v98.

Blood Plasma Proteins in Fetal Goats and Sheep.* (24132)

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The electrophoretic patterns of fetal plasma proteins have been reported to differ from their maternal counterparts mainly by the presence of a special protein, fetuin(1), and in some species by the lack of γ-globulins

* Aided by grants from N.I.H., Josiah, Macy, Jr. Fn., and James Hudson Brown Memorial Fund of the Yale School of Medicine.