

fels, volcanic rock, sandstone, granite, and fragmentary belemnites and bryozoa.

All fossil collections were studied by G. D. Hanna and Leo Hertlein. Middle Cretaceous fossils reported as *Lytoceras* sp., *Phylloceras* sp., and *Prionotropis* sp. were collected from the sea cliffs at the mouth of the Douglas River. Sea-cliff exposures a short distance west of the southernmost mouth of the Douglas yielded *Phylloceras* sp. Though assigned to undifferentiated Cretaceous, the latter beds are believed to be stratigraphically close to the base of the Cretaceous of the Kamishak Bay area; better collections might establish the presence of Lower Cretaceous. Collections made high in the Kamishak Hills about 7 miles southeast of the mouth of the Kamishak River include the following Upper Cretaceous genera: *Parapachydiscus*, *Phylloceras*, *Turritites*, and *Inoceramus*.

Stratigraphic relations are uncertain between the Cretaceous at Kamishak Bay and the Lower (Albian) Cretaceous at Kaguyak, 30–35 miles south. At Kaguyak, Lower (Albian) Cretaceous fossils were collected a few hundred feet above the top of the Upper Jurassic Naknek formation.

## Lower Cretaceous Rocks at Cape Kaguyak North of Kukak Bay, Alaska

John C. Hazzard

Union Oil Company of California, Los Angeles

Beds of Lower Cretaceous (Albian) age occur at Cape Kaguyak on the western side of Shelikof Strait about 17 miles north of Kukak Bay. Cape Kaguyak is a flat-topped promontory separated from the main coast to the west by a half-mile wide swampy sand flat. About 400 feet of beds comprising fossiliferous, concretionary, black limy siltstone with thin beds of dark bluish-gray limestone are exposed in the seacliffs on the cape and in the surrounding reefs. The presumed base of the Cretaceous is a 30-foot greenish-gray, fine-grained sandstone cropping out at the mainland edge of the sandflat west of the cape. The nature of the intervening section is unknown. The basal sandstone rests with apparent conformity on the Upper Jurassic Naknek formation. North of Cape Kaguyak along the coast west of Swikshak Lagoon a thick section of bedded rocks is exposed. Regional relations indicate that this section includes the Naknek formation at its base, overlain by beds correlative with those at Cape Kaguyak, and the section may extend upward into the Tertiary. Atwood reported Cretaceous rocks in this vicinity (1, Pl. VI).

S. W. Muller reports the following Lower Cretaceous (Albian) forms from Cape Kaguyak: *Cleoniceras* sp., *Hamites* several species, *Beudanticeras* sp., *Phylloceras* sp.

Correlation between the Kaguyak Cretaceous and the Middle and Upper Cretaceous in the Kamishak Bay region 30–35 miles north is uncertain. Lower Cretaceous rocks in the Alaska Peninsula are known at Herendeen Bay and Port Moller (1, Pl. VIII).

## Reference

1. Atwood, W. W. U. S. Geol. Survey Bull. 467, 1911.

## Measurement of Ion Migration on Paper in an Electric Field. Transference Numbers of Nickel and Copper Sulfates

Hugh J. McDonald, Matthew C. Urbin, and Martin B. Williamson

Department of Biochemistry,  
Sritch School of Medicine, Loyola University, Chicago

While studying the separation of various organic and inorganic compounds of biochemical interest, a method was devised which yielded good results in some cases, and appeared to be of great promise in others. As far as the authors are aware, the method described here has not been reported previously.<sup>1</sup> Essentially, it is based on electrophoresis, in which a strip of filter paper serves as a path along which ions or charged particles migrate under the influence of a potential gradient.

The apparatus is illustrated in Fig. 1. A strip of filter paper,<sup>2</sup> *P*, 35–50 cm in length, was supported in a glass tube 2.5 cm in diam by means of two glass pins, *B*, piercing the paper strip and passing into small holes in the rubber stoppers at each end of the tube. The electrode vessels, *A*, fitted with platinum-wire electrodes, were filled with 0.1 *N* KCl and connected by means of an agar saltbridge with the large buffer vessels, *D*. The ends of the paper strip were then permitted to become completely wetted with the KCl solution by wick

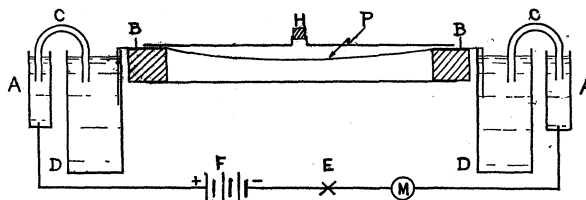


FIG. 1. Apparatus devised to study ion mobilities.

action. In certain experiments, particularly those with amino acids and proteins, solutions of electrolytes that acted as buffers were used to wet the paper strip. With the switch, *E*, closed, the circuit was completed. With a voltage of 135 v, provided by the batteries, *F*, a current of 1–3 ma was registered on the milliammeter, *M*.

Despite the superficial resemblance to paper chromatography, the method is basically electrophoretic in nature. Chromatographic processes depend on a distribution of some material between a mobile and a non-mobile phase. In the technique described, the separa-

<sup>1</sup> After this manuscript had been submitted for publication, an abstract by E. L. Durrum (1), which appears to embody some of the ideas of this paper, came to the attention of the authors.

<sup>2</sup> Eaton and Dikeman Paper No. 613 in rolled strip form, 8 mm wide.

TABLE 1  
ION MOBILITIES AND TRANSFERENCE NUMBERS FOR NICKEL AND COPPER SULFATE

| (+) Ion migration<br>(mm) | (-) Ion migration<br>(mm) | Transference number | Time<br>(sec)      | Voltage per cm | (+) Ion mobility<br>$\mu/sec/v/cm$ | (-) Ion mobility |
|---------------------------|---------------------------|---------------------|--------------------|----------------|------------------------------------|------------------|
| Nickel sulfate            |                           |                     |                    |                |                                    |                  |
| 139.0                     | 185.0                     | 0.429               | $2.16 \times 10^4$ | 2.31           | 2.79                               | 3.71             |
| 96.0                      | 128.5                     | 0.428               | $1.44 \times 10^4$ | 2.31           | 2.89                               | 3.87             |
| 88.5                      | 118.0                     | 0.428               | $1.44 \times 10^4$ | 2.31           | 2.66                               | 3.55             |
| Copper sulfate            |                           |                     |                    |                |                                    |                  |
| 62.5                      | 101.0                     | 0.382               | $1.44 \times 10^4$ | 2.31           | 1.88                               | 3.04             |
| 67.5                      | 113.0                     | 0.373               | $1.44 \times 10^4$ | 2.25           | 2.03                               | 3.40             |

tion results from the movement of oppositely charged ions or particles under the influence of a unidirectional potential gradient, rather than from distribution equilibria, adsorption-desorption effects, or countercurrent processes. The filter paper strip serves to hold the solvent or dispersion medium and to fix the migrating species in position when the current is turned off, thus preventing back diffusion. It is suggested that the term *ionography* be used to describe the technique.

The material to be studied was made up in a 0.038 M solution, and 0.045 ml of the solution was placed on the dampened paper strip midway between the electrodes by inserting a micropipette through opening, *H*. The initial substances whose ion mobilities were determined were nickel and copper sulfate. Under the influence of the potential, the positively and negatively charged ions migrated to opposite ends of the paper. In the case of such typical electrolytes, the circuit was opened after 3-6 hr and the paper dried.

The position of the ions on the paper was determined by the use of colorimetric reagents. Nickel ions were determined by spraying the paper with 0.1% dimethyl glyoxime in water-alcohol solution. The cupric ion was identified by the use of a 2% solution of potassium ferrocyanide. The position of the sulfate ion was determined by first spraying the paper with 0.1 M barium chloride followed by 0.1% potassium rhodizonate. The area covered by the sulfate ions remains uncolored, and the surrounding area turns red. The ion mobilities and transference numbers of nickel and copper sulfate are shown in Table 1.

The transference numbers of the ions of an electrolyte may vary with the concentration of the electrolyte. Except for the postulation of intermediate or complex ions, the mobility of a given ion depends not only on its nature and on the temperature, but also on the ionic strength of the solution, since the interionic forces have a considerable effect on the mobilities of the ions. These effects are, in general, different for the two kinds of ions of the electrolyte, and hence will give rise to transference numbers that vary with the concentration.

In the case of copper sulfate, the transference number of the cupric ion, as determined by the Hittorf method, increases from 0.327 at 0.5 *N* to 0.375 at 0.05 *N* (3). The values are in agreement with those determined previously. The transference numbers reported

here correspond to values obtained in very dilute solution by the Hittorf method, or to values computed from such data obtained at higher concentrations and extrapolated to lower concentrations.

The transference number of nickel in nickel sulfate has been reported to be 0.366 at 40° C in 0.1 *N* solution (2). The average value of 0.428 reported in this investigation is, therefore, not unreasonable as a limiting value, as the concentration of the salt approaches great dilution.

The endosmotic movement of the water itself will affect the movement of the ions to some extent. It would be expected that, in general, movement of the cations would be increased, while that of the anions would be retarded. That the effect is not a serious one is apparent from the relatively good agreement between transference numbers obtained by ionography and those obtained by the more time-consuming Hittorf method.

With inorganic ions, the leading edge of the ion bands was always quite sharp, but the trailing edge appeared to be rather diffuse. Preliminary experiments with inorganic ions seem to indicate that optimal separation can be secured when the paper strip is wetted with 0.1 *N* KCl. The purpose of the glass tube enclosing the paper strip is to maintain equilibrium between the moisture in the paper and in the environment. Usually a drop or two of the KCl solution was put into the glass tube to help establish this equilibrium.

Preliminary experiments indicate that the isoelectric point of proteins can be determined, and that amino acids and proteins can be separated by ionography. A number of complicating factors so affect the results, however, that further exploratory work is required to put these particular measurements on a sound basis. Even in the presence of a buffer in the solution, enough electrolysis takes place to cause a marked change in pH in the electrode vessels when the separate buffer vessels are omitted. This change in pH results in a change in charge on the amino acids or proteins with a consequent reversal in direction of movement part way through an experiment.

Indications are that amino acids and perhaps lower molecular weight proteins can be fractionated. A possible advantage over traditional electrophoresis techniques is that, in principle at least, it seems possible that complete separation of the constituent fractions of amino acid mixtures, or lower molecular weight pro-

teins can be achieved. This opens up the possibility of using the technique for preparative purposes, since that portion of the paper strip containing a particular fraction can be cut out and the fraction eluted by customary methods.

The fact that convection currents are of no great consequence in ionography will permit liquids other than water to be used. When electrophoretic measurements in water are made at any temperature other than in the neighborhood of 4° C, the point of maximum density, convection currents offer a serious difficulty. As very few nonaqueous liquids or liquid mixtures exhibit points of maximum density, the study of electrophoresis in the past has been restricted largely to water solutions or suspensions. In the technique described here, the elimination of convection currents means that electrophoretic studies can be extended to many organic liquids and to solutions of water with other liquids; this possibility has important implications in biochemical work, where many materials of great interest are soluble in water to only a very limited extent.

Experiments are contemplated—using sheets of filter paper instead of paper ribbon—in which the movement of charged particles or ions would be influenced not only by an electrical field but by a superimposed magnetic field as well. In effect, this system would be equivalent to a mass spectrograph applicable to charged particles in solution.

#### References

1. DURRUM, E. L. *Fed. Proc.*, 1950, 9, 166.
2. ZITEK, C. J. B., and McDONALD, H. J. *Trans. Electrochem. Soc.*, 1946, 89, 433.
3. *International critical tables*. New York: McGraw-Hill, 1929. Vol. VI, 309.

## Penetration of Benzpyrene into the Stomach Wall of Mouse

Kai Setälä and Per Ekwall

*Department of Roentgenology,  
Second Medical Clinic, University of Helsinki, and  
Institute of Physical Chemistry, Abo Akademi,  
The Swedish University of Abo, Finland*

Under what conditions and by which routes the carcinogenic hydrocarbons enter the living organism in general, and the individual cells expressly, are still almost unexplained questions.

Seeking an answer, we, among others, have painted the skin of newborn mice with carcinogen solutions and have found that the carcinogen will penetrate the epidermis directly, even though the pilosebaceous apparatus is still undeveloped and penetration through the follicular openings thus cannot take place (13). It was of interest to investigate whether other organs that come into contact with carcinogenic hydrocarbons will also absorb them. Particular attention was directed toward the alimentary canal.

In our experiments we have tried to use chemically well-defined solvents for the carcinogenic hydrocarbons,

especially those that are able to dissolve both water- and lipid-soluble substances. We have, for example, used water-soluble polyethylene glycols (Carbowaxes) as carriers for the carcinogenic hydrocarbons in our experiments (7-11, 13, 14). The hydrocarbons dissolved in these compounds penetrate easily into the skin and induce cutaneous tumors. Carbowaxes in aqueous solutions are also suitable carriers for carcinogenic hydrocarbons—for subcutaneous injections, for instance.

The so-called association colloids furnished another type of both water- and lipid-soluble solvents. These have the ability to bring carcinogenic hydrocarbons into clear and stable aqueous solutions (1, 2). Aqueous solutions of carcinogenic hydrocarbons will also induce cutaneous tumors (3, 4, 12), and tumors in the mouse forestomach (5), even when comparatively small quantities of the carcinogen are used.

The present communication deals with the penetration of benzpyrene, dissolved in Carbowax 1500, into the stomach wall of mice. The fluorescent microscope technique was used.

The animals used, about 75 in all, were adult mice of an anonymous, known strain employed for several years in our experiments on chemical carcinogenesis. 3:4-Benzpyrene dissolved in water-soluble Carbowax 1500 was introduced directly into the stomach of the animals by means of a stomach tube. The animals were killed immediately or 2-60 min or 24 hr after the application. The stomach (with or without preceding fixation in 10% neutral formalin solution) was cut on the freezing microtome at 10- $\mu$  thickness, and examined immediately with the fluorescent microscope (type Reichert Lux UV with a Philora-lamp HPW 125 w). Other specimens were embedded in paraffin in the usual manner and stained using the hematoxylin-van Gieson technique. Some unstained preparations were cut without prior application of the fluorescent substance. The concentration of 3:4-benzpyrene was 0.5%. (The investigative technique will be presented later in detail.)

The following results are reported:

**Forestomach:** Immediately after the application of benzpyrene in Carbowax 1500, the superficial keratinized layers showed a brilliant, almost dazzling, white fluorescence. All layers of the stratified squamous epithelium below these had taken up material with a strong blue fluorescence. The intensity of the fluorescence was much stronger than that seen in skin painted with the same solution. The fluorescent material was localized diffusely in the cytoplasm of all cells in all layers of the epithelium. Only the nuclei appeared optically empty. In other words, benzpyrene in this carrier immediately penetrates into the wall of the mouse forestomach (Fig. 1).

In addition, a strong blue fluorescence could be observed, almost without exception, in the region of both the circular and the longitudinal muscle layers of the forestomach. It was found that the fluorescent substance in this region of the stomach was gathered into a kind of fine network, which could be beautifully visualized with the fluorescent microscope (Fig. 1). We have not attempted to prove that this network of channels which