

genase, β -hydroxybutyric dehydrogenase, lactic acid dehydrogenase, 3-phosphoglyceraldehyde dehydrogenase, and α -glycerophosphate dehydrogenase.

Triphenyltetrazolium chloride was also reduced by isocitric dehydrogenase, a pyridine nucleotide dehydrogenase requiring TPN, but the reduction was very slow as compared to the DPN enzyme systems.¹

When sodium glutamate was employed as the substrate, no color developed, although glutamic dehydrogenase has been shown to be present in seeds (8). Since the redox potential of this dehydrogenase system has been reported as -0.03 v, it is possible that the enzyme, even though present, would not reduce the tetrazolium salt.

The addition of sodium succinate to the enzyme mixture prepared from corn embryos did not result in the reduction of the tetrazolium salt at pH 6.6. At this pH the redox potential of the succinate/fumarate system probably is above the redox potential of the tetrazolium salt, and a reaction should not be expected. However, the addition of coenzyme and sodium succinate to the enzyme preparation resulted in a reduction of the salt. Possibly this indicates that succinic dehydrogenase was present in the enzyme mixture and

¹ The dimethyl ester of isocitric lactone was kindly supplied by H. B. Vickery, of the Connecticut Agricultural Experiment Station.

that the reduction of the tetrazolium salt only took place through the action of pyridine nucleotide systems which were one or more steps removed from the succinate reaction.

It is probable that the reduction of triphenyltetrazolium chloride by viable corn embryos can take place if one or more dehydrogenase systems with favorable redox potentials are present. The presence of active enzyme systems does not necessarily indicate seed viability, but the absence of active dehydrogenases probably indicates loss of germinating ability.

Because of the simplicity of the equipment and procedure, the elimination of the use of flavoprotein and cytochrome preparations, and the stability of the formazan, the use of triphenyltetrazolium chloride provides a convenient method for determining the presence of certain dehydrogenases.

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Comments and Communications

Paper Ionophoresis

IN A recent communication by McDonald, Urbin, and Williamson (*Science*, **112**, 227 [1950]), and experimental technique was described for the separation of various ions on filter paper strips saturated with electrolyte solutions across which an electric potential had been impressed. They also used this same method to determine mobilities of certain ions. It is certainly true that this method is, in many cases, capable of giving good ionic separations, as these authors have concluded. However, it is open to question whether the experimental conditions reported are satisfactory for determination of ion mobilities.

In a recent publication (*J. Am. Chem. Soc.*, **72**, 2943 [1950]) we pointed out that the progression of certain ions along filter paper under conditions essentially similar to those described by McDonald *et al.* was not a linear function of time. Although it is true that our experimental conditions were not exactly the same as those reported by McDonald *et al.*, all the essential elements upon which the following discussion bears were present in both studies. That is to say, electrolyte was fed continuously to the filter paper by capillary forces from both its ends, which were immersed in electrolyte supply vessels during the time a current flowed through the paper; the paper hung

free in a surrounding enclosed vapor space; and the mixture to be separated was applied to the paper at an intermediate point.

It is manifest that, for any method to be suitable for ion mobility studies, the following minimum conditions be met:

1. Adsorption must not be a factor, or must be corrected for if present.
2. Electroendosmosis must be taken into account in cases where appreciable zeta potentials are to be anticipated.
3. The path through which the ion migration is observed must be uniform with respect to the applied electrical field, with respect to its ionic composition, and with respect to temperature.

None of these factors apparently has been given consideration in the paper under discussion.

With respect to point 1, it is conceded that some ions behave on paper saturated with electrolyte solutions in a manner similar to their behavior in "free solution." However, this factor is strictly dependent upon the system under consideration and, in certain instances in our own experience (e.g., with the dye acridine orange in certain electrolytes), strong adsorption may altogether preclude the application of this technique.

With respect to point 2, the authors make the statement that electroendosmotic flow did not appear to be

a serious effect. However, they base this conclusion upon the fact that their experimental values happen to agree (within 17% in the case of nickel in nickel sulfate) with values obtained by the conventional Hittorf method. In our own experience at pH's far removed from pH 2, electroendosmosis may require a correction factor of as much as 20% (see above-mentioned reference, paragraphs dealing with apparent cathodic migration of gamma globulin at pH 8.6). However, even in a system in which adsorption between the ion to be measured and the filter paper does not occur, and in which correction is made for the electroendosmotic flow, it is probable that insurmountable difficulties would exist under the experimental conditions reported.

When currents of the order reported (1–3 ma) are caused to flow through narrow strips of filter paper that have been saturated with most electrolytes, appreciable heat is developed in the strip. As a result, water distills from the paper. (We have checked this point using the conditions reported—i.e., with E. & D. No. 613 paper strips 1 and 2 cm wide saturated with 0.1 N KCl. When currents even as low as 1 ma were caused to flow through the paper, gross distillation from the paper, with condensation on the enclosing vessel, was observed.)

Since the paper is dried by this procedure and its state of capillary saturation is decreased, capillary forces continuously "siphon" fresh electrolyte from the buffer vessels into the paper. The fact that the electrolyte solute used is not volatile adds further complications. As a result of the above distillation, the KCl concentration is being continuously increased on the paper during the course of the experiment. This is not, of course, a linear function along the path of ion migration.

Each succeeding increment of filter paper beyond the buffer vessel receives less volume of electrolyte by the amount that has been evaporated in the preceding increment. Since the actual cross section of the paper is constant, a *flow rate gradient* results in the fluid flow along the length of the filter paper strip from each buffer vessel, decreasing as the center of the paper is approached. (Electroendosmosis augments this flow rate on the anode side of the center and diminishes this flow rate on the cathode side.) Then, in analogy, the ions applied at the center of the paper must "swim" upstream against a current that is constantly increasing in swiftness as the electrode vessels are approached. In fact, this effect is so definite that we have been able to design experiments (in press) in which ions falling within certain ranges of mobility actually reach stationary positions of mobility equilibrium where their tendency to flow upstream in consequence of their charge in the electric field is exactly counterbalanced by the countercurrent flow of electrolyte toward the center of the paper. Furthermore, these final equilibrium positions are quite *independent of the point of application* of the particular ion to the paper.

The complications resulting from the progressive increases in concentration of solute in cases of non-volatile solutes could be avoided theoretically by utilizing as electrolyte a volatile solute which forms, with water, an azeotrope of constant composition, and by utilizing this particular mixture as electrolyte so that, when distillational equilibrium was achieved, a constant ionic strength would be maintained on the paper. However, this would not eliminate the flow gradients that we have observed.

In our own experience, temperature control of the strip is very difficult to achieve under the conditions reported by McDonald *et al.*, except in cases where very low currents are permitted to flow, since cooling, limited to the processes of convection and radiation, does not seem to be adequate under these conditions to dissipate the heat produced.

We have found that, if the vapor space surrounding the strip is eliminated, temperature control is simplified by permitting the process of conduction to be used for cooling. At the same time, the distillational equilibria and accompanying fluid flow gradient can be eliminated. This can be carried out in a number of ways, perhaps most conveniently by clamping the paper strip between plate glass strips (preferably treated with an antiwetting agent—for example, one of the silicone preparations). "Edge effects" can be reduced by limiting observations to migration near the center axis of relatively wide strips or, in the case of narrow strips, by utilizing gaskets which are placed at the edges of the paper strip, and which are conveniently made of the filter paper under investigation, saturated with a silicone grease to define an enclosed channel completely filled by the filter paper strip. Another satisfactory method is to place the paper in carefully fitted shallow channels machined in lucite blocks that are provided with carefully fitted covers.

Where currents are high and heating must be eliminated, we have found that it is satisfactory to place the strips on aluminum blocks partially immersed in equilibrium ice baths, a thin layer of electrical insulation being placed between the paper strip and the metal. (G. W. Smith and K. A. Kraus have independently used a similar method for this purpose [personal communication].)

An ingenious method of correcting these difficulties has been suggested by A. Tiselius (personal communication), wherein the paper strips saturated with electrolyte solution are immersed in water-immiscible, nonpolar liquids to provide a seal about the paper and a medium for removing by conduction any heat developed. The latter method would presumably be suitable for systems in which the components to be separated do not possess large nonpolar constituent groups that might interact with the nonpolar sealing and cooling medium at the polar-nonpolar interface. (In certain polar-nonpolar systems we have experimental evidence, however, that this interaction at the interface may lead to "chromatographic" separation in the absence of any electrical potential.)

In view of the above considerations, it seems probable that it is only fortuitous that the results reported by McDonald *et al.* approach the Hittorf values as closely as they appear to.

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On a Recent Proposal to Correct an Error Committed by the International Commission on Zoological Nomenclature at the Paris 1948 Meeting

ON October 28, 1950, Francis Hemming, Secretary to the International Commission on Zoological Nomenclature, undertook to circularize by mail the taxonomists working on the insect order Lepidoptera to determine their views on a proposition to correct a serious error made by the commission in its haste at Paris in 1948. Several important principles are involved in this proposal, with implications far beyond the name of the Monarch Butterfly, which was directly involved.

In brief, the proposal is a further extension of Mr. Hemming's campaign to replace the Law of Priority with *Nomina Conservanda*. Like so many previous cases, his proposal is accompanied by almost no justification beyond the statement that "greater confusion than uniformity" would result. His direct statements that "the name universally applied to" the Monarch is *plexippus* and that the Indo-Oriental species involved "is now universally known as *Danaus genutia*" are not only in error but are not even close to the truth.

Furthermore, Mr. Hemming proposes to take no account whatever of a careful lectotype designation already published; he proposes to designate a published illustration as a type of a species; and he proposes to designate a new type locality for that species which is not even the locality of the figured specimen. In choosing this figure, in a group of animals where color is of great importance in taxonomy, he chooses a black-and-white figure, stating that no colored figure is available. At least 4 of the best-known works on American butterflies, from 1904 to 1948 in at least 18 editions, have colored figures that could have been used.

In circulating his proposal Hemming neglected to send a copy to several prominent interested lepidopterists, including the author of the only modern revision of the subfamily involved. (We note with interest that this man had previously taken a stand opposite to that now taken by Hemming.)

These principles and the method of action proposed by Hemming are so objectionable to us that we feel it necessary to present our criticisms and suggestions. We believe that many taxonomists working in other groups will be equally interested in the case.

At the 13th meeting of the International Commission at Paris in 1948, it was the intention of the commission to fix the trivial name *plexippus* Linnaeus, 1758, to the North American species known as the

Monarch Butterfly. Linnaeus had proposed *plexippus* for specimens collected by Peter Kalm in North America and for his own specimens from China, and the name had since been applied to the 2 separate and distinct species found in these two regions. But lepidopterists were not agreed as to whether there was a type for *plexippus*, and the application of the name was thus a matter of opinion. The commission attempted to carry out their intention by designating an official figure (Holland. *Butterfly Book*, pl. 7, Fig. 1, [1931]) to replace the type. They erred in carelessly assuming that this represents the North American Monarch, for it actually is a figure of a distinct subspecies of that butterfly occurring in northern South America and parts of Central America. Thus the name *Danaus plexippus plexippus* (L.), 1758, was fixed to the South American subspecies, and the name of the North American subspecies became *Danaus plexippus menippe* (Hübner), 1816. Hence, the Commission's hurried action resulted in quite a different fixation than that intended. This error has already been discussed by one of the present writers (Field. *Proc. Entomol. Soc. Wash.*, 52, 234 [Oct. 1950]).

Hemming's proposal would correct this error by deleting from the Opinion to be rendered the reference to Holland's figure and substituting a reference to another figure in its place (Clark. *Proc. U. S. Nat. Museum*, 90, pl. 71, Fig. 1 [1941]) and further by designating a type locality, "Pennsylvania."

We are strongly against this proposal for three reasons.

First, because we believe that a strict application of the Law of Priority will serve the best interests of all concerned. Linnaeus' own material came from China and represented the Indo-Oriental species (sometimes referred to as *genutia* Cramer). We know this because he definitely states (in *Mus. Lud. Utr.*, 262 [1764]) "*meus e China*." It is clear that Linnaeus confused several species under the name *plexippus*, for although 2 of the 4 literature references listed by Linnaeus in his original description (*Systema Naturae*, Ed. X, 471 [1758]) refer to the North American Monarch, the other 2 refer to still another distinct New World species. The first part of the Linnaean description would fit either of these American species or the Indo-Oriental species, but he added at the end of his description a sentence ("*alæ primores fascia alba, ut in sequente*" [*chrysippus*] "*cui similis*") that could apply only to the Indo-Oriental species. The fact of the matter is that Linnaeus intended the name for the Indo-Oriental species (his Chinese specimens) and at the same time misidentified these American species with this Indo-Oriental species.

Corbet (*Proc. Roy. Entomol. Soc. Lond.*, 18, pts. 9-10, 188 [Oct. 1949]) has already presented a perfect case for applying the name to the Indo-Oriental species and has designated a Chinese specimen in the Linnaean Collection in London as the lectotype, for he says, "I have no hesitation in taking the male specimen bearing the Linnaean name label" (in Linnaeus'