4600

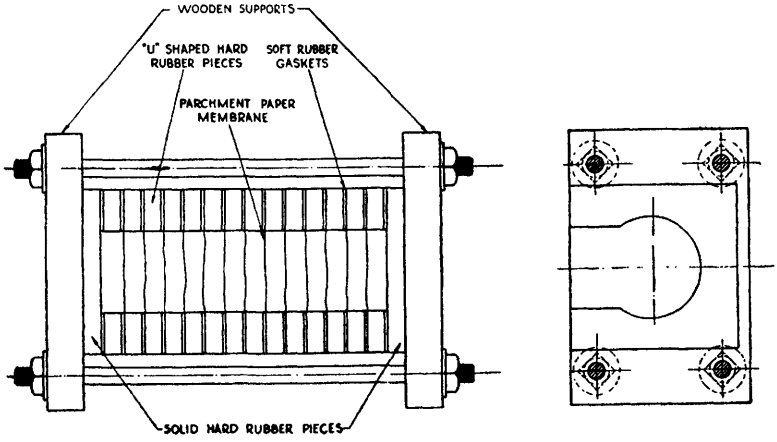
Electrodialysis as a Means of Characterizing Ampholytes.

ROBERT R. WILLIAMS AND ROBERT E. WATERMAN.

From the Laboratory of Physiological Chemistry, Teachers College, Columbia University.

In the course of our studies of water soluble vitamins and bioses we have had frequent occasion during the past 5 years to enquire whether the physiological activity of an extract of a foodstuff was due to a single substance or a plurality of substances. Information was also often needed as to whether the substances involved were colloidal or crystalloidal, and if the latter, whether acidic, basic, amphoteric or non-electrolytic. This paper describes a method which often suffices to secure the answer to these questions by a single simple experiment, thus laying a good foundation for any attempt at isolation. The method also indicates the approximate isoelectric point of the substance in case it proves to be an ampholyte, a fact which is often of great service in suggesting the proper choice of precipitants. Kerr[1] has cited an instance in which the method was of use, namely in detecting β bios.

The method consists of electro-dialysing the solution in question in a multiple compartment cell. A convenient form of such a cell is illustrated in Fig. 1. After electrolysis the contents of the compartments are separately removed, their H^+ ion concentrations are determined and the solutions are assayed for the constituents of interest by chemical means if available, or by physiological test such as an



[1] Kerr, Ralph W., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 344.

animal feeding experiment. If the substance sought is an ampholyte it may be concluded that the pH of that portion of the solution which contains the substance in maximum concentration approximates the isoelectric point of the substance. For at any pH greater or less than that of the isoelectric point, the substance will be ionized more strongly as a base than as an acid or vice versa and will therefore migrate toward the region at which ionization as acid and as base are equal, namely the region of its isoelectric point.

In order to test the validity of this reasoning we have electrolysed in a 12 compartment cell a solution composed as follows:

Quinine Sulphate (saturated sol.)	.346 gm.
Anthranilic Acid	.685 gm.
Valine	.585 gm.
Water to make 250 cc.	

These substances were chosen as representing a considerable range from acid to base and as being subject to ready analysis. The solution was apportioned equally among the compartments except in the case of the two terminal compartments which were of small volume, sufficient only to accommodate the electrodes. Electrolysis was carried out with platinum gauze electrodes with 110 volts, using a suitable resistance and milliammeter in series to avoid excessive current density (*i. e.*, over 2 milliamperes per sq. cm.) and consequent heating. As the resistance of the cell rose the line resistance was reduced and finally removed altogether. After 60 hours the current had dropped from 40 milliamperes to about 13 milliamperes, corresponding to a current density of 0.65 milliamperes per square cm. of cross section of liquid path. The potential drop from the cathode to the next adjoining compartment (No. 2) was 60 volts, and that from No. 2 to No. 3 was about 25 volts. Through the rest of the system the potential drop from cell to cell varied from 1 to 5 volts.

The solutions were removed and the H^+ concentration was determined colorimetrically in each. Sulphuric acid was determined by titration with barium hydroxide. Quinine was determined by extracting each solution with ether after rendering alkaline with excess barium hydroxide. After quantitative removal of barium as sulphate, the solutions were evaporated to dryness and the residues were extracted with ether, which dissolves free anthranilic acid but not free valine. Finally the valine was roughly determined as the water soluble portion of the ether insoluble residues.

The results are shown in Table I. It is to be noted that the SO_4 ion does not extend beyond the fourth cell from the anode and the quinine is confined to the cathode compartment in which it precipi-

TABLE I.
Composition and Characteristics of the Contents of 12 Compartments After Electrodialysis.

Compartment No.	Volume of liquid cc.	pH	Quinine + 3 H_2O mg.	SO_4 mg.	Valine mg.	Anthranilic acid mg.
1(Cathode)	5	8.6	147	0	39	10
2	24	5.4	Trace	0	196	2
3	24	4.2	0	0	140	25
4	23	3.8	0	0	82	52
5	23	3.7	0	0	55	75
6	22	3.5	0	0	26	75
7	20	3.5	0	0	22	85
8	19	3.4	0	0	12	66
9	14	3.3	0	20	*17	37
10	12	3.0	0	93	*25	*27
11	13	<2.8	0	160	*25	*27
12	5	<2.8	0	30	* 8	* 4

*Material was gummy, probably representing in part products of electrochemical decomposition reactions. The regions of maximum concentration were conspicuously the regions of most complete and perfect crystallization and freedom from color. The residues at the anode end were highly colored.

tated copiously. The maximum concentrations of valine and anthranilic acid respectively lie near the points where the H^+ concentrations approach those of pure solutions of each of the 2 substances, as determined by us in an independent experiment, *viz.*, pH 7.2 and pH 3.8 respectively. Electrokinetic redistribution of water took place during electrolysis, as is indicated by volume of solutions in Table I, but this does not account for the conspicuous concentration of each constituent.

This method is useful for the purification of ampholytes. The valine used in this experiment was obtained from Eastman. It was found by electrolysis to contain a considerable amount of a component of greater acidity than valine. This was removed by electrolysis in the same apparatus and the residual valine recovered by evaporation and recrystallization for use as above described. The anthranilic acid was conveniently purified by sublimation.

The above method applied to yeast extract has served to confirm certain suspicions which we have long entertained about the multipartite nature of what has been called vitamin B. Unfortunately the 2 factors which can be recognized most definitely by our present feeding technique migrate toward the alkaline region where they undergo fairly rapid decomposition, so that high concentrations of them cannot be obtained by this means. These factors are the antineuritic vitamin and the one which we have heretofore designated only as "the third factor".[2]

[2] Williams, R. R., and Waterman, R. E., *J. Biol. Chem.*, 1928, lxxviii, 311.

Grateful acknowledgment is made to the Carnegie Institution of Washington for financial support of this work.

4601

Further Consideration of Transmissibility of Human Upper Respiratory Infections (Common Cold) to the Ape.

G. S. SHIBLEY, K. C. MILLS AND A. R. DOCHEZ.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

In a previous communication[1] we have reported the suitability of the anthropoid ape as an experimental animal for the study of the upper respiratory tract infections usually grouped under the term "common cold".

We showed (1) that the upper respiratory flora of these animals during periods of normal health very closely resembles that of humans, and (2) that these animals are extremely susceptible to "colds" when exposed to humans suffering from such infections and that the clinical manifestations of these infections in the ape are more or less identical with those observed in human beings similarly affected.

Further, in an effort to ascertain the possibility of communicating to anthropoids, by means of a filterable agent, upper respiratory infections comparable to the human cold, it was shown that filtered nasal washings obtained from humans suffering with typical colds when injected intranasally into apes produced typical colds in about half of the instances attempted. In all positive experiments Gram-negative anaerobes of the type described by Olitsky and Gates were cultivated. However, no etiological significance was assigned to these organisms.

The importance of control experiments was recognized and early in the above investigations, plain broth and heated filtrate intranasal inoculations were carried out but were soon given up as inadequate. It was felt that it would be of more value to use for controls filtered nasal washings obtained from humans who were not suffering from colds. However, in view of the difficulty of excluding, with any degree of certainty, carriers of the active agent

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 562.