phreys, S. R., Ashenbrucker, H., Worth, W., and Kramer, R., *J. Clin. Invest.*, 1947, v26, 103.

8. McCarthy, M. D., *Ann. Surg.*, 1952, v136, 546.

9. Fruhman, G. J., and Gordon, A. S., *Anat. Rec.*, 1953, v117, 603.

10. Endicott, K. M., and Ott, M., *ibid.*, 1945, v92, 62.

11. McCarthy, M. D., Amrein, M., Cobb, M., Neerhout, R., and Blackburn, V., *Am. J. Physiol.*,

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## Effect of Temperature on Ionographic Mobilities in Paper Stabilized Media.* (22989)

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In a recent article on paper electrophoresis (1), Forbes and Taylor reported that although the rate of electromigration of beta lipoproteins was considerably less at 5°C than at room temperature or at 35°C, no effect of temperature was evident in lipoproteins migrating with the albumin, alpha$_1$ globulin. They also reported that rate of movement of the main serum components was apparently the same at different temperatures. The non-uniformity of the effect of temperature on mobilities of the substances studied by them was in such marked contrast to those observed in this laboratory(2,3) that it was decided to reinvestigate the effect of temperature on ionographic mobilities in paper stabilized media for a range of substances varying from low molecular weight materials, such as a simple dye, to more complex substances such as plasma proteins and lipoproteins.

*Methods and material.* The Precision Ionograph,§ which utilizes the horizontal paper strip in a water-saturated atmosphere(4), was used with Whatman No. 1 filter paper strips (0.5 inch in width and 40-45 cm in length) and a veronal buffer of pH 8.6 and ionic strength of 0.03 and 0.01. The apparatus permits use of 7 paper strips simultaneously. In nearly all cases, the runs on each migrant were made at 4 different temperatures, namely 25°C, 15°C, 9°C and 4°C. During each run the temperature was maintained at $\pm$ 0.2°C by circulating liquid of constant temperature through the double-walled shell of the ionographic apparatus. The substances whose mobilities were determined included bromphenol blue (Hartman-Leddon Co., Philadelphia), crystalline bovine plasma albumin (Armour Laboratories, Chicago), and the following serum fractions, obtained from fresh pooled human serum, namely, albumin, alpha lipoprotein, alpha globulin, beta lipoprotein and gamma globulin. The veronal buffer having an ionic strength of 0.01 was employed for the bromphenol blue and bovine plasma albumin, while an ionic strength of 0.03 was used for all determinations on human serum fractions. The average time of a run was 3-4 hours, and a potential gradient of 4-5 volts/cm was used, maintained constant during the run. For the runs at 25°C helium was introduced into the water-saturated atmosphere while at lower temperatures, the water-saturated atmosphere alone was employed. Equilibration time, *i.e.*, the time the paper strips were permitted to stand, after wetting and with the current on, to reach a constant value for the ratio of "weight of buffer solution" to "weight of paper" was determined for the particular paper and buffer used, before beginning the runs.

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§ Precision Scientific Co., Chicago, Ill.

TABLE I. Ionographic Mobilities of 7 Different Substances as a Function of Temperature.

| Migrant | Temp., °C | Mobility, $\mu$/sec./v/cm | Stand. dev. | No. of runs |
|---|---|---|---|---|
| Bromphenol blue | 25 | 1.21 | .03 | 4 |
| | 15 | .917 | .03 | 1* |
| | 9 | .773 | .01 | 2 |
| | 4 | .685 | .06 | 2 |
| Bovine plasma albumin | 25 | .884 | .14 | 5 |
| | 9 | .688 | .03 | 2 |
| | 4 | .553 | .02 | 2 |
| Human serum albumin | 25 | .725 | .04 | 4 |
| | 15 | .599 | .04 | 1* |
| | 9 | .545 | .04 | 5 |
| | 4 | .491 | .03 | 5 |
| $\alpha_2$ globulin | 25 | .448 | .03 | 4 |
| | 15 | .374 | .03 | 1* |
| | 9 | .339 | .03 | 5 |
| | 4 | .307 | .03 | 4 |
| $\gamma$ globulins | 25 | .245 | .04 | 4 |
| | 15 | .206 | .03 | 1* |
| | 9 | .170 | .03 | 5 |
| | 4 | .174 | .02 | 4 |
| $\alpha$ lipoproteins | 25 | .733 | .05 | 4 |
| | 15 | .564 | .06 | 1* |
| | 9 | .509 | .07 | 5 |
| $\beta$ lipoproteins† | 25 | .225 | | 1 |
| | 15 | .173 | | 1 |
| | 4 | .132 | | 1 |

* Since only one run was made at 15°C, the stand. dev. was calculated by taking avg of stand. dev. at other temperatures.

† Figures for mobility represent avg of group of 7 ionograms run simultaneously.

The ratio was constant at a value of 1.58, after the strips were equilibrated for 30 minutes. It was also determined that movement of the migrant was a linear function of time and of the potential gradient, respectively, under otherwise constant experimental conditions. These are fundamental criteria which must be met if mobility measurements are to be meaningful(4,5). Movement of a particular component, for determining its mobility, was taken as the distance between forward or leading edge of a given band and the forward edge of initial narrow streak of the material which was applied initially to the paper strip at point of origin. The protein fractions were stained with bromphenol blue, while Sudan black B was used to develop lipoprotein ionograms.

*Results.* As pointed out in an earlier publication(3), the electromigration of a charged particle through the buffer solution saturating the paper strip, is fundamentally a simple rate process and it would therefore be expected to increase with increasing temperature. It would moreover be expected to exhibit the usual temperature dependency of such processes(6), namely, that when the natural logarithm of mobility is plotted as a function of the reciprocal of the absolute temperature, a straight line would result. Mobilities of various substances studied are listed in Table I. When these were plotted in the manner described above, the results were as shown in Fig. 1 and 2.

Each experiment consisted of 7 simultaneous runs. Figures in the mobility column of Table I represent averages, *e.g.*, for human serum albumin; 0.725 represents the average from 28 individual ionograms, run in 4 groups of 7. The standard deviation is based on the number of "groups of 7 strips," rather than on the number of individual ionograms. It must be borne in mind that the value for standard deviation is accentuated by this technic, and would be much smaller, numerically, if based on the number of individual ionograms. The reason for using the above

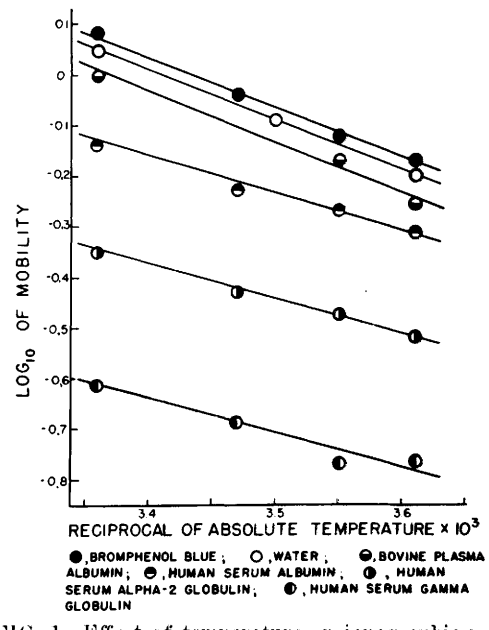


FIG. 1. Effect of temperature on ionographic mobility of bromphenol blue, on plasma protein fractions, and on coefficient of viscosity of water. The ordinate scale, for water, represents logarithm of reciprocal of coefficient of viscosity.

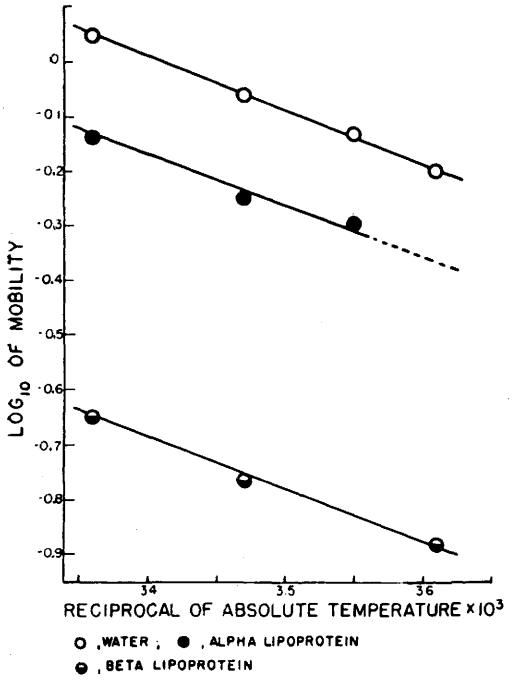


FIG. 2. Effect of temperature on ionographic mobility of human plasma lipoproteins. The ordinate scale, for water, represents logarithm of reciprocal of coefficient of viscosity.

procedure is that any human error, such as a wrong voltage reading, would cause a given group of 7 ionograms all to be in error by the same amount.

Since the normal increase in mobility of a migrant with increase in temperature would parallel the increase in fluidity of water with the same temperature increase, it would be expected that if the "logarithm of the reciprocal of the coefficient of viscosity of water" is plotted as a function of the "reciprocal of the absolute temperature," a straight line should be obtained. This linearity is shown in Fig. 1 and 2.

In the case of the beta lipoproteins, erratic mobility results were obtained in the earlier experiments. In general, as a serum sample aged, under refrigeration at 4°C, the mobility values dropped. This confirms the observations made earlier by Gottfried *et al.*(7). When, however, mobilities at 25°C, 9°C, and 4°C, were determined by running the experiments simultaneously, but using 3 separate ionographic apparatuses, the results were consistent with the behavior observed for all other migrants.

Since the effect of temperature on mobilities of all substances studied was nearly the same, it is possible to represent temperature dependency of a wide variety of migrants, under otherwise constant experimental conditions, by the following simple equation.

$$\ln u = \frac{m}{T} + A$$

where u = mobility of migrant in some definite system of units, ln = natural logarithm, T = temperature in degrees Kelvin, m = slope of line, for water itself, and is the value to be used for all other substances where the lines parallel that of water, and A is a constant which is characteristic for each migrant. Converting to logarithms to base 10 the equation becomes:

$$\log u = \frac{2.303 \times m}{T} + 2.303\ A$$

or

$$\log u = \frac{2.303 \times m}{T} + B$$

For a given migrant, then, the mobilities at all temperatures in the range 4°C to 25°C can be computed from a value determined at any one temperature within this range. As an example, consider the case of bromphenol blue. At 25°C, the mobility was 1.21 $\mu$/sec. per volt/cm. Insertion of the proper values in the equation then yields:

$$\log 1.21 = \frac{-980}{298} + B$$

Where -980 represents the slope of the lines shown in Fig. 1, which parallel that for water itself, and is therefore applicable to the case of bromphenol blue. From the equation the constant B is determined to be 3.37. The mobility at 4°C, then, can be computed as follows:

$$\log u_{4°C} = \frac{-980}{277} + 3.37$$

whence u is 0.681 $\mu$/sec. per volt/cm. The experimental value at that temperature is listed in Table I as 0.685.

Since it is now generally conceded that no single electroosmotic indicator is satisfactory for migrants of different molecular volumes, a real doubt arises about the advisability of applying so-called correction factors unless absolutely necessary in order to interpret the results. In the experiments described here, the *change* in the mobility was explored as a function of the temperature. Since the effect of temperature on electroosmotic movement appears to parallel that on electromigration mobility, the application of an electroosmotic correction would simply shift the lines, in Fig. 1 and 2, bodily, by a small amount but would not alter the slope or the nature of the equation expressing the effect of temperature on mobilities.

*Summary.* The effect of temperature on velocity of electromigration through paper stabilized electrolytes, of a variety of substances, including bromphenol blue, bovine plasma albumin, human serum albumin and globulin fractions and human serum lipoproteins, was investigated. The ionographic apparatus employed utilized horizontal paper strips in a water saturated atmosphere. The runs were made at temperatures from 4°C to 25°C and under constant conditions of buffer, ionic strength, pH and potential gradient. For all substances studied, the relationship between the mobility and temperature can be represented by the equation:

$$\log \text{ (mobility)} = m \cdot 1/T + B$$

where T is °Kelvin, B is a constant and m is the slope of the line obtained when the logarithm of the mobility is plotted against the reciprocal of the absolute temperature.

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1. Forbes, J. C., and Taylor, P. C., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 411.

2. McDonald, H. J., *J. Chem. Educ.*, 1952, v29, 428.

3. McDonald, H. J., and Spitzer, R. H., *Circulation Res.*, 1953, v1, 396.

4. McDonald, H. J., Lappe, R. J., Marbach, E. P., Spitzer, R. H., and Urbin, M. C., Ionography; Electrophoresis in Stabilized Media, Year Book Publishers, Chicago, Ill., 1955.

5. Paper Electrophoresis, A Ciba Foundation Symposium; Edited by Wolstenholme, G. E. W., and Millar, E. C. P., J. and A. Churchill, London, W. 1, England, 1956, p. 117.

6. Johnson, F. H., Eyring, H., and Polissar, M. J., The Kinetic Basis of Molecular Biology, John Wiley and Sons, Inc., New York, N. Y., 1954, chap. 8.

7. Gottfried, S. P., Pope, R. H., Friedman, N. H., Akerson, I. B., and DiMauro, S., *Clin. Chem.*, 1955, v1, 253.

Received November 19, 1956. P.S.E.B.M., 1957, v94.