TABLE I

R =	Monoalkylamino alcohols $(CH_3C(NHR)(CH_3)CH_2OH)$ M. p., °C.	B. p., °C.	Mol. formula	Nitrogen, % Calcd.	Found	*p*-Nitrobenzoates of monoalkylamino alcohols M. p., °C.	Mol. formula	Nitrogen, % Calcd.	Found
Ethyl	75.5–76.5	162–163	$C_6H_{15}ON$	11.95	11.91	206.5–207.0	$C_{13}H_{18}O_4N_2$	10.52	[a]
n-Propyl	59.5–60.5	183–185	$C_7H_{17}ON$	10.67	10.55	185.0–185.5	$C_{14}H_{20}O_4N_2$	9.99	9.75
i-Propyl	43.0–45.0	165–166	$C_7H_{17}ON$	10.67	10.64	140.0–141.0	$C_{14}H_{20}O_4N_2$	9.99	[a]
n-Butyl	69.5–70.0	195–196	$C_8H_{19}ON$	9.64	9.48	163.5–164.0	$C_{15}H_{22}O_4N_2$	9.52	9.22
i-Butyl	51.0–52.5	185–186	$C_8H_{19}ON$	9.64	9.70	165.0–166.0	$C_{15}H_{22}O_4N_2$	9.52	9.27
n-Amyl	60.0–60.5	216–217	$C_9H_{20}ON$	8.80	8.78	151.0–151.5	$C_{16}H_{24}O_4N_2$	9.09	9.03
i-Amyl	76.5–77.0	205–207	$C_9H_{20}ON$	8.80	8.76	168.0–168.5	$C_{16}H_{24}O_4N_2$	9.09	9.10

[a] Discordant results probably due to persistent impurities.

non-reproducible results. The nitro esters were very pale yellow solids.

The *p*-aminobenzoates can be obtained by reducing a paste of the nitro ester in concentrated hydrochloric acid with powdered tin. The temperature must not exceed 40–45°. The *p*-aminobenzoic acid ester hydrochlorides are then prepared by treating an ether solution of the free base with dry gaseous hydrogen chloride. The hydrochlorides are white, extremely hygroscopic solids.

The physiological effect of various derivatives of the anesthetic bases will be reported elsewhere.

Experimental

The 2-amino-2-methyl-propanol-1 was purchased from the Commercial Solvents Corporation, and the alkyl bromides from the Eastman Kodak Company. All of the materials were purified by redistilling.

Preparation of Monoalkylamino Alcohols.—Molar quantities of the alkyl bromide and 2-amino-2-methyl-propanol-1 were refluxed in ethanol solution from fifteen to forty-eight hours. The ethanol was then distilled off and the residue treated with 30% sodium hydroxide solution. The warm solution separated into two layers. The aqueous layer was drawn off and the oily layer transferred to a beaker, where it rapidly solidified. The monoalkylamino alcohols can be purified by recrystallization from petroleum ether. Melting points and analyses of the alcohols are given in Table I.

Preparation of the *p*-Nitrobenzoic Acid Esters.—To 14.5 g. (0.1 mole) of the monobutylamino alcohol dissolved in 50 ml. of freshly distilled pyridine, was added 18.6 g. (0.1 mole) of *p*-nitrobenzoyl chloride. The addition was made in small portions and with constant stirring. Care is necessary in maintaining the temperature between 30 and 40°. After addition of the *p*-nitrobenzoyl chloride was completed, the mixture was allowed to stand for twenty-four hours and then diluted with 400 ml. of water. A voluminous curdy yellow precipitate formed and was filtered off, washed with 50 ml. of 2% sodium carbonate solution and allowed to dry. The nitro ester thus obtained was purified by recrystallization from ethanol. Melting points and analyses of the nitro esters are given in Table I.

Summary

1. A series of new monoalkylamino alcohols is reported.

2. The *p*-nitrobenzoates of these amino alcohols have been prepared.

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[CONTRIBUTION FROM THE ELECTROPHORESIS LABORATORY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

The Effect of Urea on the Electrophoretic Patterns of Serum Proteins

BY DAN H. MOORE

The effect of urea on the electrophoretic patterns of serum proteins has been examined in the electrophoresis apparatus of Tiselius.[1] One of the important characteristics of the Tiselius method is its ability to reduce convection in the cell (U-tube) caused by heat generated by passage of current. This is done by submerging the apparatus in a bath which should be maintained at the temperature where the change of density with temperature is least, *i. e.*, about 4° for dilute aqueous solutions. The patterns depending upon refraction gradients at the protein boundaries were obtained by means of the scanning method of Longsworth.[2]

Normal human serum was diluted 1:4 with a 0.02 *M* phosphate buffer containing physiological saline and 2.8 *M* urea, and dialyzed against the same buffer containing the urea and saline. The electrophoretic pattern is illustrated in Fig. 1a. The albumin is *apparently* broken up into three

(1) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(2) L. G. Longsworth, THIS JOURNAL, **61**, 529 (1939).

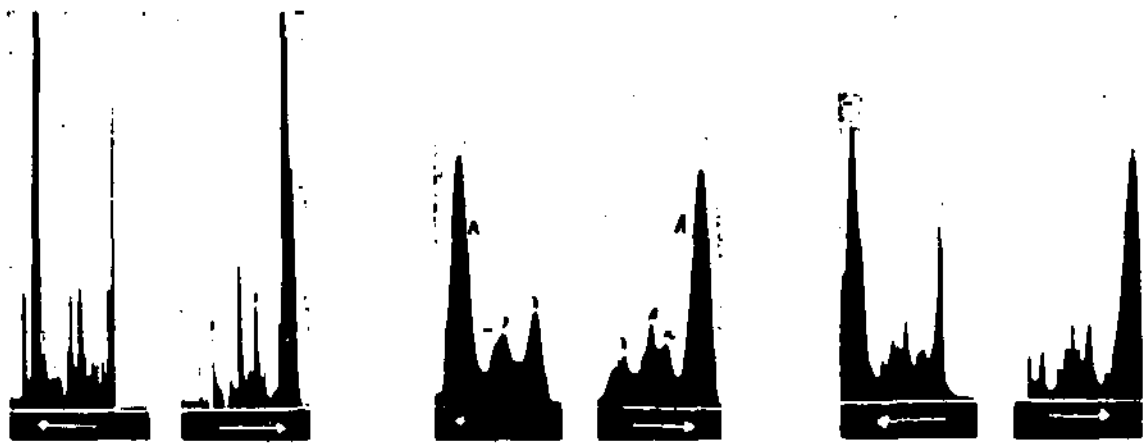

Fig. 1.—Electrophoresis patterns of human serum in the presence of 2.8 *M* urea in a temperature bath at 1.5°; left ascending pattern, right descending pattern: (a) after 4.8 volts/cm. had been applied for four hours; (b) after standing twelve additional hours without voltage; (c) after voltage was applied an additional thirty minutes, then switched off for one hour.

components and instead of the usual three globulins, alpha, beta and gamma, there are apparently a great number with extremely sharp boundaries. This photograph was taken after 4.8 volts/cm. had been applied for four hours. The voltage was then turned off and the serum allowed to stand in the electrophoresis cell for twelve hours, whereupon it gave an approximately normal serum pattern as is illustrated in Fig. 1b. The original voltage was again applied and within thirty minutes the pattern assumed the same shape as in Fig. 1a. The pattern of Fig. 1c was taken after the voltage had again been turned off for one hour. It was found that by applying and removing the voltage one could secure patterns intermediate between that found in Fig. 1a and that of a normal serum in a phosphate buffer without urea. Upon dialyzing out the urea from the same serum sample, a normal pattern was obtained.

These experiments were conducted at a bath temperature of 1.5°, which has been shown[3] to be about the correct temperature for dilute aqueous buffer solutions. In order to determine whether the pattern illustrated in Fig. 1a is the result of convection, the change of density with temperature in the region of 0° for the urea solution was studied. An improvised dilatometer of high sensitivity was made by taking a Pyrex bulb of about 25 ml. capacity with neck which fitted over a discarded thermometer stem, the capillary of the stem being open at both ends. The thermometer stem was ground into the neck of the bulb to give a good ground glass fit. The bulb and neck were filled with the solution whose change in density with temperature was to be measured and cooled to a low temperature before the thermometer capillary was inserted. A drop of dye was added to the open end of the capillary and the bulb was further cooled to draw the dye down in the stem and form an indicator the top of which could be read on the thermometer scale. By taking into account the thermal expansion of the Pyrex bulb, high precision of volume changes could be obtained. In the region of 0° a 2.8 *M* urea solution gave a change in scale reading of 125 units per degree change in temperature.

In Fig. 2 the slopes of the various curves indicate the relative changes in density with tem-

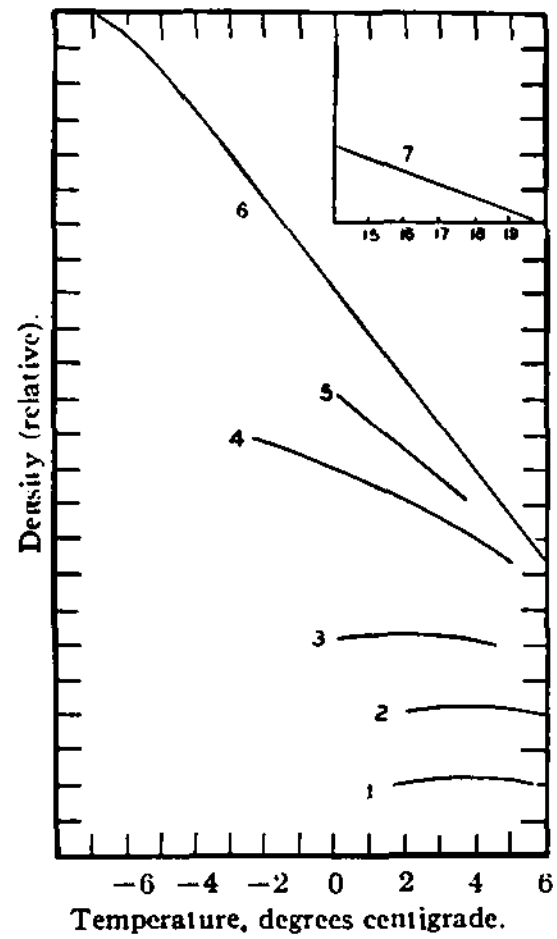

Fig. 2.—Density[a]-temperature curves for some common aqueous solutions: (1) water, (2) 0.05 *M* lithium barbiturate + 0.025 *M* LiCl, (3) 0.15 *M* NaCl + 0.02 *M* sodium phosphate, (4) 1.0 *M* urea, (5) 1.8 *M* sucrose, (6) 2.8 *M* urea + 0.15 *M* NaCl + 0.02 *M* sodium phosphate, (7) water.

[a] Densities are relative, consequently the slopes of the curves and not their positions are significant.

(3) L. G. Longsworth and D. A. MacInnes, *Chem. Rev.*, **24**, 271 (1939).

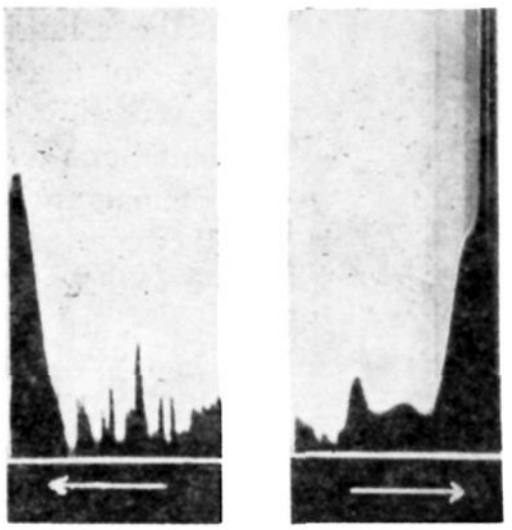

Fig. 3.—Electrophoresis patterns of normal human serum in 0.02 *M* sodium phosphate buffer containing 0.15 *M* NaCl at *p*H 7.4 in a temperature bath at 17°. Left ascending pattern, right descending pattern.

perature. Solutions of ordinary dilute buffers give almost a horizontal line between 1 and 6°. The 0.02 *M* phosphate buffer with 0.15 *M* sodium chloride (curve 3) has a maximum density of about 1.3° but the curve is still comparatively horizontal at 4°. The curves for the more concentrated urea and sucrose solutions have steep slopes at temperatures as low as −4°, which would result in marked convection if the heat dissipation in the electrophoresis cell were not kept at a low value. It is observed that curve 6 begins to flatten out at about −5°. An electrophoresis experiment was carried out in a bath at −7° whereupon the serum with 2.8 *M* urea gave only slight indications of the anomalies found in Fig. 1a. If the voltage were dropped to 2.4 volts/cm. (half the original value), no effect of urea was observed. These experiments lend evidence to the belief that the jagged pattern is a result of convection. Another indication that the pattern of Fig. 1a was caused by convection is the pattern for normal serum illustrated in Fig. 3 which was obtained in phosphate buffer without urea but in a bath at 17° instead of the usual 1.5°. This pattern is similar to that of Fig. 1a but is not identical. Even at this high temperature the slope of the density–temperature curve (curve 7, Fig. 2) is not as great as that of the 2.8 *M* urea solution (curve 6).

These experiments illustrate the importance of carrying out electrophoresis analyses at a temperature where the change in density with temperature is small.[4] For dilute aqueous solutions which are represented by curves 2 and 3 of Fig. 2, this temperature is between 0 and 4°. Protein concentrations of 2 or 3% do not shift the temperature of maximum density appreciably so that a bath temperature of 0 to 2° is ideal for general electrophoretic analysis.

I wish to express my appreciation to Mrs. Vernon Strub and the Misses Helen Sikorski and Evelyn Kulp for their contributions of sera and technical assistance, and my indebtedness to Dr. H. A. Abramson of Columbia University and to Dr. L. G. Longsworth of the Rockefeller Institute for their valuable suggestions and interest.

Summary

1. Electrophoretic patterns giving a large number of components for human serum in the presence of 2.8 *M* urea have been shown to be a result of convection currents in the electrophoresis cell. The change of density with temperature for a urea solution of this concentration is comparatively large in the region of 0 to 4°.

2. Density–temperature curves for a few common solutions are given.

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(4) Data on temperature of maximum density for a number of solutions may be found in the "International Critical Tables," Vol. III, McGraw-Hill Book Company, Inc., New York, N. Y., 1929, p. 108.