Weintraub, A. H., Abstr. 7th Meet. Am. Soc. Hemat., Seattle, 1964, p30.

11. Krebs, Sir Hans, in Regulation of Cell Metabolism, G. E. W. Wolstenholme, C. M. O'Connor, Eds., Little, Brown & Co., Boston, 1958, p3.

12. Lowry, P. H., Borsook, H., in Erythropoiesis, L. O. Jacobson, M. Doyle, Eds., Grune & Stratton, 1962, p33.

Received May 11, 1965. P.S.E.B.M., 1965, v119.

Demonstration of the Inherited Serum Group Specific Protein by Acrylamide Electrophoresis.* (30401)

F. DAVID KITCHIN (Introduced by Alexander G. Bearn)
The Rockefeller University, New York City

The inherited group specific protein (Gc) was first recognized by the technique of immunoelectrophoresis(1) and also demonstrated by the method of vertical starch gel electrophoresis(2) as a system of protein staining bands in the immediate post-albumin region(3). In a later modification the use of a discontinuous buffer system incorporating lithium hydroxide(4), improved the resolution and revealed more clearly the heterogeneous nature of the Gc system. This method suffers from the limitations imposed by variations in the behavior of different batches of hydrolyzed starch, a moderately long running time and the proximity of the leading Gc protein bands to the trailing border of the albumin. For routine typing of the common Gc phenotypes these difficulties are of no great importance, but it has been almost impossible to separate albumin from the genetic variants of Gc which possess a component with an electrophoretic mobility greater than that of the normal fast Gc 1-1 phenotype.

In these circumstances the method of acrylamide gel electrophoresis developed by Raymond(5) and applied by Peacock(6) offers some advantages. Surface effects, which are apparent in the starch gel system, are abolished and the geometry of the apparatus ensures a linear field within the cell. With the additional advantage of a stable uncharged gel matrix of uniform pore size, it seemed likely that with minor changes in the electrophoretic conditions, it would be possible to resolve the post-albumin region more clearly. This paper is concerned with the resolution of the Gc proteins in the post-albumin region of serum obtained by the method of vertical slab acrylamide gel electrophoresis.

Materials and methods. Apparatus: A vertical slab of acrylamide is prepared in a 3 mm thick mold as described by Raymond (7). The apparatus is constructed so that the gel mold, cooling plates electrodes and buffer reservoirs are in a single plexiglass unit. The apparatus employed was obtained from the E. C. Apparatus Corp., Philadelphia, Pa. The cooling bath circulator was obtained from Forma Scientific Inc. and the circulating buffer pump from Gorman-Rupp Industries, Inc. Variations of the standard Trishydroxymethylaminomethane (TRIS), disodium ethylenediaminetetraacetic acid (EDTA) and boric acid were used. (a) 10.09 g Tris, 1.3 g EDTA, 0.81 g boric acid in 1 liter pH 8.91, (b) 10.75 g Tris, 0.93 g EDTA, 5.04 boric acid in 1 liter pH 8.28. *Cooling:* In the initial experiments cooling was obtained by circulating tap water at approximately 12°C, but in the later experiments by circulating water at a temperature of 4°C. *Gel:* A 5% acrylamide gel was used throughout and made by dissolving 7.5 g of Cyanogum-41 (electrophoresis grade) in 150 ml of buffer. Gel formation was catalyzed by adding 0.3 ml of N,N,N′,N′,-tetramethylethylenediamine (TMED) and 300 mg of ammonium persulphate

* This research was aided by a grant from The National Foundation and supported in part by USPHS Research Grant FR-00102, from the General Clinical Research Center Branch, Division of Facilities and Resources.

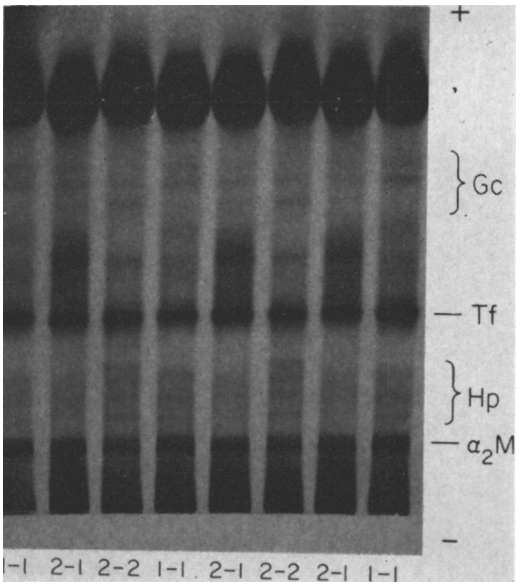

FIG. 1. Gc phenotypes 1-1, 2-1 and 2-2 showing the fast and slow bands in the 2 homozygotes and 3 bands in the heterozygotes. Conditions: 5% acrylamide gel. Electrophoresis in borate tris EDTA buffer pH 8.28 for 5 hr at 10°C.

The transferrin band is labelled, Tf; Haptoglobin, Hp; and the α_2 macroglobulin, α_2M.

immediately before the gel was poured. *Electrophoresis:* Equilibrium between the gel and the buffer reservoirs was obtained by a prerun of 20 minutes before the samples were applied. Twenty μl of fresh serum was pipetted into each of the 8 slots and electrophoresis allowed to proceed until the albumin had migrated 10 cm from the origin. *Staining:* The gel slabs after suitable trimming were stained for ½ hour in a 0.6% solution of amido-black. The stain was prepared by adding 135 ml methyl alcohol, 30 ml glacial acetic acid and 135 ml of distilled water to 2.0 g of amido Schwartz. Destaining was performed with either an electrical destaining apparatus (EC Corp.), or by washing in 5% acetic acid solution overnight.

Results and discussion. The post-albumin region, and in particular the Gc protein bands, were well resolved with each of the buffer concentrations employed. With the pH 8.28 buffer, tap water cooling and the current restricted to 60 ma, the albumin migrated 10 cm from the origin in 5 hours. A higher current produced overheating with swelling and distortion of the gel. A reduction of the running time to 4¼ hours could be achieved by using the buffer of pH 8.91 and increasing the current to 75 ma under similar cooling conditions. With an increase in pH to 8.91 the fast Gc bands were well separated from the albumin, but the separation between the individual components was decreased and the lines were not as sharp. The buffer of pH 8.21 resolved the Gc protein bands clearly and the separation between the individual components appeared optimal. A photograph demonstrating the separation obtained with the common Gc phenotypes is shown in Fig. 1. Experiments designed to shorten the running time have been performed. By cooling the gel to 4°C the current can be increased to 140 ma at 300 volts without overheating. Separation can be completed in 2½-3 hours and, although the stained pattern is readable, some blurring of the Gc bands is apparent. The slight loss of resolution, however, is not pronounced and for routine typing the pattern is satisfactory.

Identification of the Gc protein bands was established by running a gel under standard conditions and transferring an unstained 6 cm strip extending from the center of the albumin to the transferrin band to a 1% agarose plate containing rabbit antiserum containing antibodies of Gc albumin and α_1-antitrypsin. Electrophoresis in a second dimension was carried out for one hour at 40 volts/cm. During this time the proteins migrated from the acrylamide gel into the agarose and reacted immunologically with their corresponding antibodies. This method, based on the techniques of Ressler(8) and Laurell(9), identified the components of the Gc system as a series of normal curves and revealed small quantities of antitrypsin and albumin in the Gc region. The fast and slow bands of the homozygote Gc 1-1, could be seen as a 2-peaked curve; the heterozygote Gc 2-1 as a 3-peaked curve and the Gc 2-2 as a skewed curve with the fast band of the Gc 2-2 in a concentration of approximately half that of the slow band. Although the sharpest definition of the bands was obtained without the benefit of electrical destaining, in

the interests of speed, a short period (20 minutes) of electrical destaining followed by washing of the gel in 5% acetic acid overnight gave satisfactory results.

Summary. Acrylamide gel electrophoresis in a vertical slab has been adapted to the routine analysis of the Gc protein. The band patterns do not differ in principle from those obtained by starch gel electrophoresis using a discontinuous buffer system; however, the Gc bands are very much sharper and the fast Gc components are well separated from the trailing edge of the albumin. The method has good reproducibility and may be capable of still further refinement. At present it provides a simple and convenient way of confirming the results obtained from immunoelectrophoresis and is particularly advantageous in investigation of rapidly migrating Gc variants which are only with considerable difficulty distinguished by starch gel electrophoresis.

We wish to thank Dr. Samuel Raymond for his kindness in demonstrating his method of acrylamide gel electrophoresis and Dr. Andrew Peacock for his guidance in interpreting normal serum protein patterns in acrylamide gels.

1. Hirschfeld, J., Acta Path. et Microbiol. Scand., 1959, v46, 229.
2. Smithies, O., Biochem. J., 1955, v61, 629.
3. Parker, W. C., Cleve, H., Bearn, A. G., Am. J. Human Genet., 1963, v15, 353.
4. Bearn, A. G., Kitchin, F. D., Bowman, B. H., J. Exp. Med., 1964, v120, 83.
5. Raymond, S., Weintraub, L., Science, 1959, v130, 711.
6. Peacock, A. C., Bunting, S. L., Queen, K. G., ibid., 1965, v147, 1451.
7. Raymond, S., Ann. N. Y. Acad. Sci., 1964, v121, 350.
8. Ressler, N., Clin. Chim. Acta, 1960, v5, 795.
9. Laurell, C-B., Anal. Biochem., 1965, v10, 359.

Received May 12, 1965. P.S.E.B.M., 1965, v119.

Plasma Trapping in Hematocrit Determination. Differences Among Animal Species.* (30402)

SHU CHIEN, ROBERT J. DELLENBACK,† SHUNICHI USAMI AND MAGNUS I. GREGERSEN
Department of Physiology, Columbia University College of Physicians and Surgeons, New York City

When a blood sample is centrifuged for the determination of hematocrit, there is some plasma trapped in the packed cell column, resulting in a higher hematocrit reading than the actual cell percentage. The plasma trapping correction factor (cell percentage/hematocrit reading) for a given blood sample depends upon the force and duration of centrifugation. When normal human blood or dog blood is centrifuged for 30 minutes at 1500 $\times$ *g*, the plasma trapping correction factor averages 0.96(1,2,3). In the course of investigating the influence of hematocrit on the viscosity of blood in several animal species (4), it was discovered that the plasma trapping correction factor shows a considerable species difference.

Methods. Blood samples were drawn from elephant (ear vein), man (antecubital vein), dog (femoral artery or vein), sheep (external jugular vein) and goat (external jugular vein) into heparinized containers. The plasma trapping correction factor (F_t) was determined on heparinized whole blood, and, for all species except the elephant, also on suspensions of cells in Ringer's solution. To prepare the cell suspensions, plasma was removed from heparinized blood after centrifugation, and the cells were washed 3 times with a modified Ringer's solution (NaCl 9 g/l, KCl 0.42 g/l, $CaCl_2$ 0.24 g/l, $NaHCO_3$ 0.2 g/l and heparin 10,000 units/l).

* This investigation was supported by USPHS Research Grant HE-06139 from Nat. Heart Inst., and by U. S. Army Medical Research and Development Command Contract DA-49-193-MD-2272.

† Supported, in part, by a general research support grant from Nat. Inst. of Health.