found to be free of anti-complementary components. Hamsters apparently make a potent anti-C′ when immunized with fresh human serum. Rivanol fractionation does not affect the anti-complementary character. The antibody obtained at 14 days was not 2-mercaptoethanol sensitive.

Serum and ascites from Syrian hamsters were found to contain at least 8 protein fractions similar to human serum proteins when developed with high titer horse, rabbit and goat antihuman sera. The two most important were found with albumin and γG globulin fraction. These possibly blocked production of antibody to albumin and γG globulin in the hamsters.

More "normal" antibody production was found in Korean hamsters immunized with the same antigens. The Korean hamster *Cricetulus triton* is quite unlike the Syrian hamster *Mesocricetus auratus* and more closely resembles the cotton rat or a large field mouse; however, it is classified as a hamster, and was included here for purposes of comparison. The Korean hamster produced antibodies to albumin, γG globulin and to several α and β globulins. It did not possess many of the cross-reacting antigens found in Syrian hamster sera and ascites.

In general antibody titers of the ascitic fluids were comparable to those of the sera; however, the ascitic fluid yield was 20-30 times that of serum.

Summary. Hamsters offer several likely aspects in their use as producers of antibodies. (1) They produce large volumes of ascitic fluid containing high titer antibody. (2) They provide a source of antibody free of anti γG globulin and, therefore, rich in non γ antibody specificity. (3) The hamster provides suitable system for preparation of homologous antibody to viruses which either require the hamster as a host or for which the hamster is used to isolate and/or maintian the virus. An interesting facet of this study was the finding of extensive cross-reactions between human and hamster plasma proteins.

1. Wiebenga, N. H., Am. J. Trop. Med. & Hyg., 1965, v14, 802.
2. Takemoto, K. K., Habel, K., Proc. Soc. Exp. Biol. & Med., 1965, v120, 124.
3. Munoz, J., ibid., 1957, v95, 757.
4. Lieberman, R., Mantel, N., Humphrey, W., Jr., ibid., 1961, v107, 163.
5. Hermann, E. C., Engle, C., ibid., 1958, v98, 257.
6. Sartorelli, A. C., Fischer, D. S., Downs, W. G., J. Immunol., 1966, v96, 676.
7. Gornal, A. G., Bardawill, C. J., David, M. M., J. Biol. Chem., 1949, v177, 751.
8. Williams, C. A., Grabar, P. J., J. Immunol., 1955, v74, 158.
9. Usui, M., Rinsho-Kensa (Clinical Diagnosis) (Jap.), 1964, v8, 5.
10. Ouchterlony, Ö., Arkiv Kemi Mineral. Geol., 1948, v26, B1.
11. Crowle, A. J., Immunodiffusion, Academic Press Inc., New York, 1961.
12. Feeley, J. C., Sword, C. F., Manclark, C. R., Pickett, M. J., Am. J. Clin. Path., 1958, v30, 77.
13. Singer, J. M., Plotz, C. M., Goldberg, R., Arthr. & Rheum., 1965, v8, 194.
14. Sever, J. L., J. Immunol., 1962, v88, 32.
15. Mettler, N. E., Casals, J., Shope, R. E., Am. J. Trop. Med. & Hyg., 1963, v12, 647.
16. Muller-Eberhard, M. J., Nilsson, C., Arronson, T., J. Exp. Med., 1960, v111, 201, 217.

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Differentiation of Pathological Conditions by Visual Evaluation of Serum Protein Electrophoretic Patterns. (32242)

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A clear electrophoretic pattern of serum proteins which can be inspected visually is a useful aid in the diagnosis of disease. Among the various electrophoretic methods used for fractionation of serum proteins, agar gel electrophoresis is distinguished for the clear separation of the protein fractions and speed of electrophoresis.

A modification of the agar gel electrophoretic method of Wieme(1), which was employed for the separation of lactate dehydrogenase isoenzymes(2), has been applied to the fractionation of serum proteins. Separation of proteins on a microscope slide, followed by fixation and staining, produced clear patterns which allowed comparison and differentiation of samples from various pathological conditions by visual inspection. Examples are presented which illustrate a correlation of protein patterns with pathological conditions.

Procedure. A plexiglass electrophoresis tank (1) was used which is now available commercially.* The agar blocks in the tank were prepared with an agar solution 0.9% in barbital buffer pH 8.4 and ionic strength 0.045. The same buffer was used in the electrophoresis vessels and the same agar solution for covering the microscope slides as previously described(2). Five microliters of serum previously diluted with saline to contain about 100 μg of total protein was placed in the slot and electrophoresis was accomplished at 140 V, with the run lasting 15 minutes at room temperature. Two samples were analyzed in each run. The slides were then immersed for 15 minutes in a fixing solution of 5% acetic acid in methanol; after fixation they were air dried with the aid of a filter paper placed on top of the gel. The dried slides were rinsed with water to remove any filter paper particles attached to the agar film and air dried again. The protein zones were detected by immersing the slides for 20 minutes in a staining solution of 1% aniline blue black in 2% acetic acid and destained by 2 changes in 2% acetic acid.

Results. A typical protein electrophoretic pattern of a normal human serum is shown in Fig. 1.† Six fractions are demonstrated. clearly delineated on the microscope slide and well removed from the slot of origin.

Albumin appears as a homogeneous zone. Alpha$_1$-globulin, close to the albumin, is a weak but visible zone. Alpha$_2$-globulin ap-

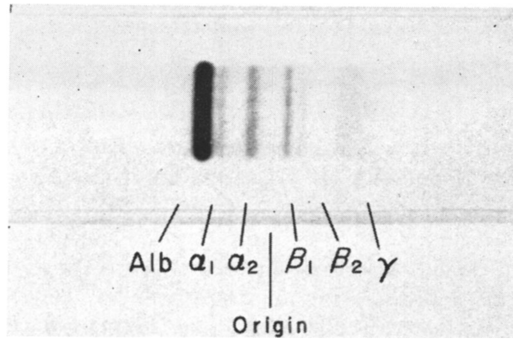

FIG. 1. A normal serum protein pattern by agar gel electrophoresis.

pears sometimes as a narrow zone and at other times as a wide band indicating the variation of α_2-globulins in normal individuals. The β-globulins appear as two distinct zones in fresh serum but the β_2 disappears and β_1 increases in sera stored for one week or longer. The γ-globulin is a diffuse broad band separated from the β-globulins.

Serum protein changes in pathological conditions are demonstrated by quantitative and qualitative differences from the normal pattern, which can be distinguished visually. Examples of 4 types of quantitative changes from normal patterns in pathological conditions are shown in Fig. 2. 1) Decreased density of the γ-globulin band as in hypogammaglobulinemia. 2) Increased density of the γ-globulin as in the diffused type of hypergammaglobulinemia. 3) Increased α-globulins. 4) Increased α- and γ-globulins. (A normal pattern is included in the same figure for comparison.) These pattens, obtained by the agar gel electrophoretic technique, have been associated with the following pathological conditions: 1) Various types of hypogammaglobulinemia, verified by immunoelectrophoresis; 2) elevation of γ-globulin with normal α-globulins found in chronic infections due to bacterial, viral, or protozoal agents and in sarcoidosis; 3) increase of α-globulins found in conditions of tissue injury such as recent myocardial infarction; 4) elevation of both α- and γ-globulins was associated with various types of carcinomas, Hodgkin's disease and active infectious conditions.

Patterns with both quantitative and qualitative differences from the normal were obtained in two types of hepatic disease and in

* Phipps and Bird, Inc., Richmond, Va.

† The patterns of the original slide are more distinct than their reproduction in the figures.

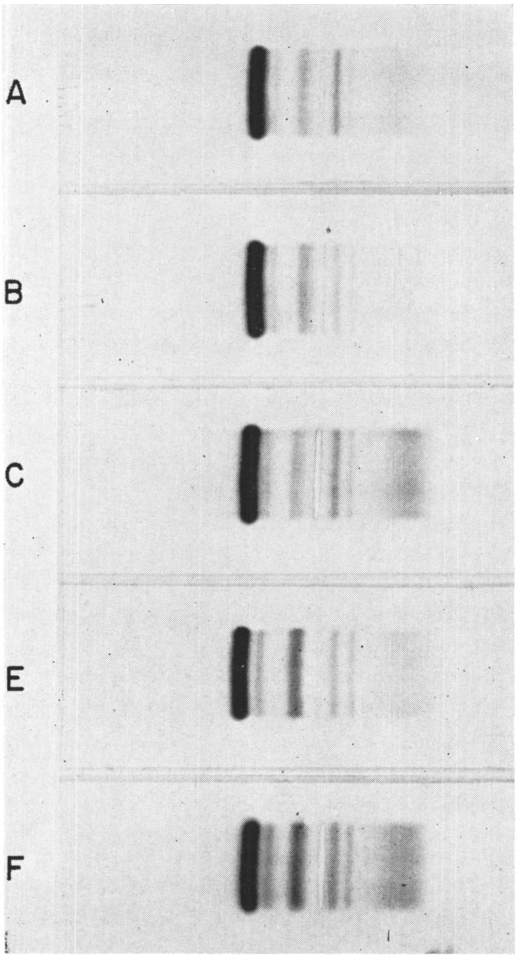

FIG. 2. Protein patterns by agar gel electrophoresis in: A, Normal serum; B-E Pathological sera depicting: B, hypogammaglobulinemia; C, Diffused hypergammaglobulinemia; D, Increased α-globulins; E, Increased α- and γ-globulins.

paraproteinemias. In Fig. 3 the patterns of serum samples from cirrhosis and hepatitis cases are shown. The increased γ-globulin in cirrhosis is characterized by a sharp leading edge and a trailing edge which extends into the β region and forms the so-called β-γ bridge. The increased γ-globulin band in hepatitis is distinguished by a sharp leading edge and a diffuse trailing edge clearly separated from the β-globulins.

The sharp homogeneous zones of paraproteinemias are illustrated in Fig. 4 by the patterns of serum samples from β- and γ-myelomas and macroglobulinemia. In the same figure a pattern is shown of a serum sample from a patient with lung carcinoma. A sharp homogeneous zone was found in the middle of the diffuse γ-globulin band.

Discussion. Studies of proteins in health and disease by a variety of electrophoretic techniques have been extensive; see, for example, Petermann(3), Sunderman(4), and Wieme(1). The information gained from these studies has been utilized here by the application of a simple and rapid system for correlation of serum protein patterns with clinical conditions. While numerical data as well as diagrammatic and graphic representation of the serum protein fractions were reported in previous studies, direct photographic reproduction of the slides is presented here. Analysis of samples can be completed in about 2 hours. Variation of protein patterns can be distinguished by visual inspection of the slides.

For reproducible results and good comparison of different samples, a constant amount of about 100 μg total protein was analyzed, and a control was run with each group of unknowns. In addition to the visual inspection of the serum protein patterns, quantitative determination of the intensity of the zones can also be performed by densitometry. The same basic system of agar gel electrophoresis described can serve for immunoelectrophoresis for further identification of proteins. It has also been adapted for the separation of lactate dehydrogenase (LDH) isoenzymes(2). Thus, both the protein and isoenzyme patterns of a serum sample can be obtained by running two slides simultaneously.

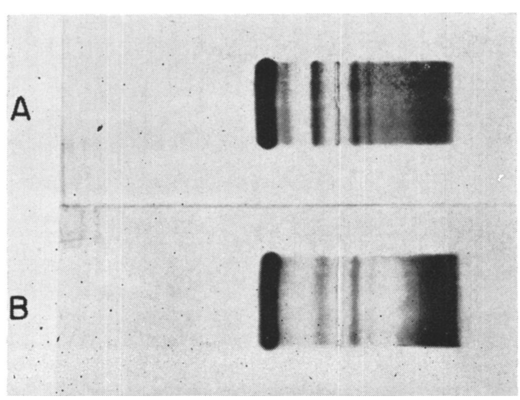

FIG. 3. Serum protein patterns by agar gel electrophoresis in: A, Cirrhosis; B, Hepatitis.

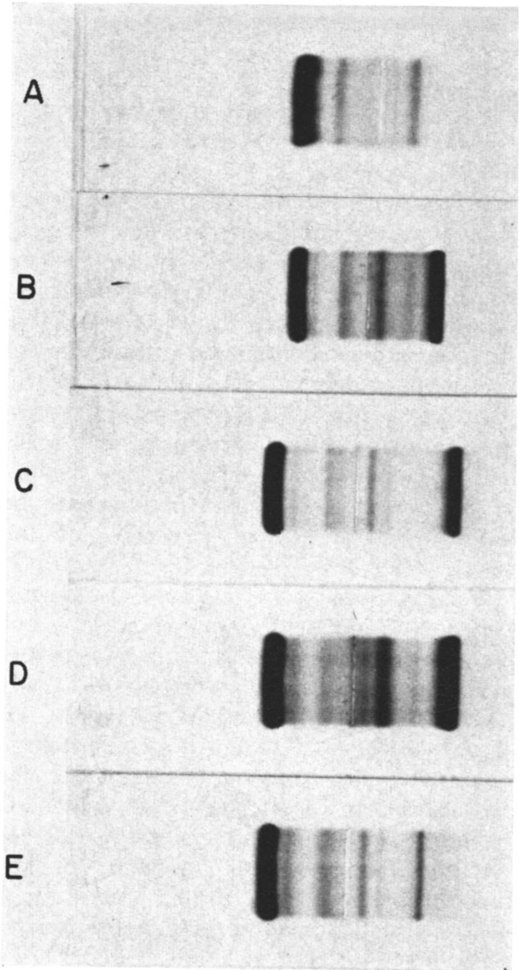

FIG. 4. Serum protein patterns by agar gel electrophoresis in: A, β-myeloma; B, Macroglobulinemia; C, γ-myeloma; D, β-γ-myeloma; E, Lung carcinoma.

The low cost of the apparatus and reagents, the relative simplicity of the procedure, the high resolution of the protein fractions, and the speed of electrophoresis, make this an excellent system for screening and monitoring serum protein patterns as a diagnostic aid and for following the progress of disease. Since this system does not require the use of a densitometer or other expensive apparatus, it can be utilized in a small laboratory or doctor's office. Valuable information can be obtained by visual evaluation of serum protein patterns.

Summary. Human serum proteins have been fractionated by agar gel electrophoresis. Clear resolution was achieved which allowed direct comparison of patterns from various samples by visual inspection. Quantitative and qualitative changes from normal patterns were correlated with clinical conditions. The procedure is rapid and useful for screening and monitoring serum protein patterns and for differentiation of pathological conditions by visual evaluation of their serum protein patterns.

1. Wieme, R. J., Agar Gel Eletrophoresis, Elsevier Publishing Co., New York, 1965.

2. Papadopoulos, N. M., Kintzios, J., Am. J. Clin. Path., 1967, v47, 96.

3. Petermann, M. L., in The Plasma Proteins, Putnam, F. W., ed., Academic Press, New York, 1960, v2, p310.

4. Sunderman, F. W., Sunderman, F. W., Jr., Serum Proteins and Dysproteinemias, Lippincott Co., Philadelphia, 1964.

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