The Immunoelectrophoretic Pattern of Properdin in Fresh and Aged Human Serum[1] (36786)

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The isolation and purification of properdin has provided the opportunity to study the role of properdin in renal diseases. By immunofluorescent microscopy, properdin was found to be deposited in the glomeruli of all patients with chronic membranoproliferative glomerulonephritis (CMPGN) and poststreptococcal glomerulonephritis (1, 2). A depression in the level of serum properdin, measured by the method of Pillemer *et al.* (3), and by immunodiffusion assay (4) has been reported in patients with poststreptococcal glomerulonephritis and CMPGN.

As originally described (5) properdin is a euglobulin of normal serum which *in vitro* activates C3 in the presence of at least two other factors: Factor A, a hydrazine sensitive factor, and Factor B, a heat-labile factor. Other euglobulin proteins have recently been shown to be necessary for the complexing of properin to zymosan (6). Polysaccharides and liposaccharides such as zymosan, inulin, endotoxin, and dextrans are required for *in vitro* activation of the properdin system, and the reaction is magnesium dependent. Gewurz, Shin and Mergenhagen (7) called attention to the possibility of an "alternate pathway" of complement (C) entering the cascade at C3. Recent investigations using cobra venom factor have demonstrated the existence of an "alternate pathway" activating C3. The role of properdin in this alternate pathway is an area of active current investigation (6, 8, 9).

We report here the first description of a technique which allows visualization of serum properdin by immunoelectrophoresis. Utilizing this technique, we have discovered changes in the electrophoretic migration of properdin on aging of normal serum.

Methods and Materials. Immunoelectrophoresis was performed in 2.0% Noble agar in a glycine saline EDTA buffer [glycine 7.5 g, sodium chloride 8.5 g, dissodium EDTA 0.744 g (0.002 *M*), sodium hydroxide (1 *N*) 2.5 ml, 10% sodium carbonate to pH 8.2, distilled water to 1000 ml]. Sodium azide 0.02% was present in the buffer and agar. Premade agar in glycine saline EDTA buffer was melted on the day of use and applied to microscopic slides in a 2–3 mm thickness. After hardening, 4 mm diameter wells were cut one third the distance from the cathodal end of the slide and 20–30 μl of the undiluted serum specimen were placed in the well. A control serum was run opposite each serum specimen on the same slide. Electrophoresis was performed for 3 to 4 hr at 4–5 V/cm (measured across bath) at room temperature in a humid environment.

Following electophoresis, a 1:12 dilution (in saline) of specific rabbit anti-human properdin antiserum was placed in the trough. Plates were put into a humid chamber at room temperature. Precipitin arcs were visible by 12–18 hr but full development took 72 hr. The plates were washed in saline for 48 hr with two changes of wash. For photographic purposes, goat anti-rabbit IgG could then be applied into the trough to react with rabbit IgG to augment the precipitin arcs (10). After further washing, staining with amidoschwarz was performed.

Pure properdin was isolated by the method of Pensky et al. (11). Rabbit antiserum was prepared as previously described and did not react with C1q, C5 or C3 (1). C3 proactivator (C3PA) was isolated according to the method of Gotze and Mueller-

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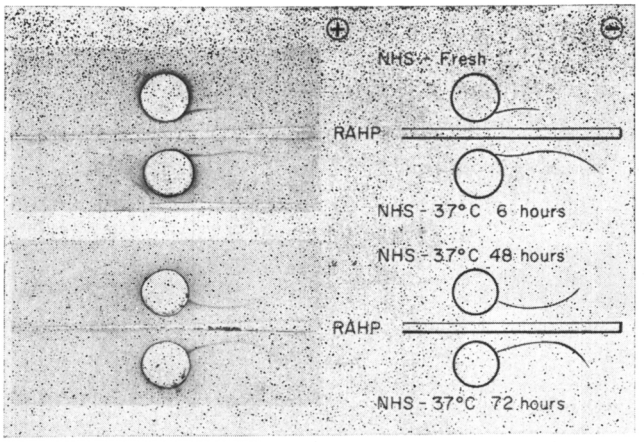

FIG. 1. Stained immunoelectrophoretic patterns for properdin of normal human serum (NHS) and the same serum after 6, 48, and 72 hr incubation at 37°. RAHP is rabbit anti-human properdin.

Eberhard (8) and β1C by column chromatography (12). Monospecific antiserum in goats was prepared against C3PA and β1C. No cross-reactivity was seen when antisera to C3PA, β1C, and properdin were reacted with human serum. In addition, antiserum to properdin did not react with isolated C3PA.

Properdin activity was measured by the hemolytic assay of Pillemer *et al.* (5). Modifications included formation of the properdin–zymosan complex at 17° and utilization of the assay system as recently described for measuring Factor B (9). Serum deficient in properdin (RP) was made by passing a normal serum over an immunoabsorbent column of sepharose 4B complexed to rabbit anti-human properdin (13). Properdin was undetectable by double diffusion assay and less than 14% fixation occurred in RP in the presence of zymosan.

Immunodiffusion assays for β1A were performed by the method of Mancini, Carbonara and Heremans (14). Goat antisera to β1C/β1A prepared by column chromatography was performed in duplicate in agarose and glycine–saline–EDTA. Serial dilution of a standard serum of known β1A concentration was run on each petri plate. Total hemolytic complement was measured by previously described methods (15).

Properdin assays were performed by a similar technique with a 1:900 dilution of antisera on sera stored at —70°. Following 72 hr incubation, the plates were washed for 48 hr and then stained. The method of staining was: 10 min with 0.1% tannic acid, 10 min with 0.2% 8-anilino-1-naphthalinesulfonic acid (Analytical Chemists, Inc.) in 5% acetic acid, 1 min with 5% acetic acid and 1 min rinse in saline.

Normal serum was incubated at 37°; aliquots were removed at varying intervals of time as indicated in the results, and frozen at —70° until assayed. Sera were not thawed more than twice.

Results. Immunoelectrophoresis of unincubated (time 0) normal serum, showed the following pattern: the arc originated near the front of the well, sloped away from the trough with the apex of the arc usually, but not always, being lost in the well; there was very little migration towards the cathode (Figs. 1 and 2).

Afer 6 hr of incubation at 37°, the immunoelectrophoretic pattern was clearly different: a longer precipitin arc was visible and the arc had shifted significantly towards the cathode [designated properdin cathode (Pc)]. The origin of the arc from the well in the 6 hr specimen was similar to the unheated serum but was shorter and completely fused with the more cathodally migrating arc described above (Fig. 1).

The only change which was seen on longer

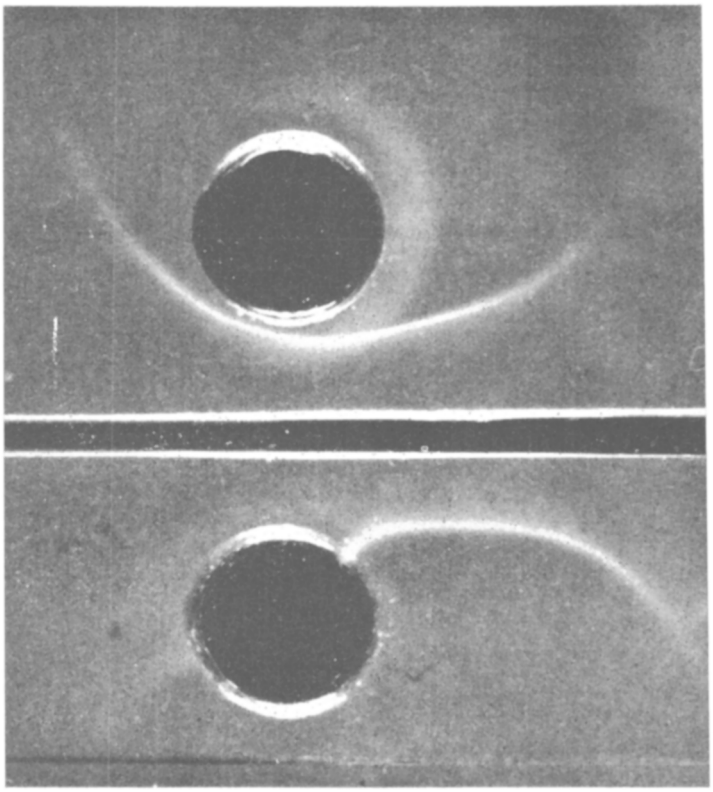

FIG. 2. Unstained immunoelectrophoretic pattern of normal human serum properdin comparing fresh serum (top well) and the same serum incubated at 37° for 7 days (bottom well). Cathode is on the right. The trough contained rabbit anti-human properdin antiserum. In this case the precipitin pattern of the fresh serum (top) does not end in the well. The aged serum (bottom) showed more cathodal migration.

incubation was the disappearance of that part of the arc near the well which sloped away from the trough. The precipitin arc after 12 hr or longer incubation was a symmetrically cathodally migrating arc which still touched the well, though more posteriorly than with unheated serum.

The functional (hemolytic) assay for properdin decreased by 12% at 6 hr and 20% by 12 hr (Table I). Thus, the immunoelectrophoretic changes were completed at a time when the functional assay for properdin still showed activity.

Changes in total hemolytic complement (CH50) titers paralleled those of functional properdin. Immunoelectrophoresis for β1C/β1A demonstrated conversion of β1C to a more anodal migrating protein by 12 hr consistent with a change in β1A. The concentration of β1C/β1A increased from 118 mg/100 ml at 0 time to 130 mg/100 ml at 7 days, consistent with conversion of β1C to β1A (Table I). The concentration of properdin showed no change during the 7 day period of incubation (Table I).

Discussion. The demonstration of properdin in fresh (unaged) normal serum as a cathodally migrating arc on immunoelectrophoresis has not previously been reported. This finding is important for several reasons. The role of the alternate pathway in the pathogenesis of human diseases, such as chronic membranoproliferative glomerulonephritis, is currently being investigated in several laboratories. In addition, the relationship of the properdin system to the C3 proactivator, the serum protein which complexes with the anticomplementary factor of cobra

TABLE I. The Effect of Incubation at 37° on Properdin (Hemolytic Assay), CH50, Properdin (Immunodiffusion), β1A and the Immunoelectrophoretic Patterns of Properdin in Normal Serum.

Duration of incubation at 37°	Hemolytic properdin[a] (% decrease)	CH50[b] (% decrease)	Properdin immunodiffusion assay[c] (% normal)	β1A immunodiffusion assay[d] (mg/100 ml)	Change in immunoelectrophoresis of properdin[e]
0	0	0	98	118	—
6 hr	12	36	93	112	±
12 hr	20	50	88	121	+
2 days	50	58	93	118	+
3 days	71	61	95	124	+
4 days	79	89	95	124	+
5 days	79	86	93	124	+
6 days	71	89	90	130	+
7 days	84	95	95	130	+

[a] Hemolytic assay for properdin using serum made deficient in properdin by immunoabsorbent column (RP).

[b] CH50 = total hemolytic complement.

[c] Normal properdin 106.7 ± 52 (2 SD) % of normal (reference) serum.

[d] Normal β1A 164 ± 82 (2 SD) mg/100 ml.

[e] — = no change from normal; + = cathodally migrating properdin (Pc) present.

venom, is presently unclear. In experiments reported here, incubation of normal serum for 12 hr at 37° produced a more cathodally migrating precipitin arc for properdin (Pc). The finding that the properdin migration changed on aging is similar to changes noted in other factors of the alternate pathway such as β1C (12) and glycine-rich beta glycoprotein II (16). A functional assay for properdin has been difficult to interpret because of the variables introduced by the many cofactors of the alternate pathway. The RP described in this communication, using an immunoabsorbent column for properdin, provided a serum deficient in properdin; utilizing this RP, the hemolytic assay of properdin decreased with aging yet continued to show functional activity at a time when immunoelectrophoretic changes were complete. This suggests that either the assay is dependent upon other factors besides properdin or that Pc retains some functional activity. A third possibility is that residual normal properdin is present in too small a concentration to be detectable by this technique. Thus, the question of the functional integrity of the cathodally migrating properdin in the hemolytic assay of properdin must await further investigation. We are unable to say at this time whether the molecular change is due to splitting of the properdin molecule or simply a change in charge due to other causes.

Summary. We have described for the first time a technique for demonstrating human properdin in whole serum by immunoelectrophoresis. Utilizing this method, we have noted a change in the immunoelectrophoretic migration of properdin by incubating serum at 37°. There was a concomitant decrease in the functional activity of properdin and CH50, as well as conversion of β1C to a β1A pattern on immunoelectrophoresis. It is probable that the changes in the electrophoretic mobility of properdin resulted from a fundamental change in the properdin molecule.

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