COMPARATIVE STUDIES ON THE PHYSICAL CHARACTERISTICS OF THE HERITABLE HAPTOGLOBIN GROUPS OF HUMAN SERUM*

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In a series of publications Jayle has described the existence and properties of certain serum proteins which have the unique capacity to combine irreversibly with hemoglobin (1–3). These proteins, which have an electrophoretic mobility of an α_2-globulin, have been called haptoglobins and their complex with hemoglobin called the haptoglobin hemoglobin complex (3, 2). Increased interest in these serum proteins followed the demonstration by Smithies (4) that it was possible, by means of starch gel electrophoresis, to distinguish three types of hemoglobin binding proteins in normal serum and, further, that these corresponded to the haptoglobins previously reported by Jayle (5). Genetic studies have indicated that the three major serum haptoglobin groups are heritable and are probably determined by two autosomal genes with incomplete dominance (5, 6). On the basis of these studies one homozygote has been called Group 1-1, the other homozygote 2-2. The heterozygote is designated Group 2-1. Recently minor subgroups also have been recognized (7–9).

Ultracentrifugal studies of the chemically isolated haptoglobins by Jayle and coworkers have disclosed two haptoglobins with different properties. One haptoglobin is a monomeric protein and has an observed s rate of 4.1 S and a molecular weight of approximately 85,000, the other is the dimeric form with a molecular weight of approximately 169,000. The first combines with hemoglobin in equimolecular quantities while the second combines with two molecules of hemoglobin (2, 3). Previous studies from this laboratory in which the types of haptoglobin hemoglobin complexes were isolated by electrophoretic techniques have also shown differences in the ultracentrifugal properties (9).

Ultracentrifugal studies of the serum α_2-globulins by a number of observers have revealed at least four major ultracentrifugal components whose s rates are approximately 4, 7, 10 to 12, and 19 S (10, 11). Most of these peaks appear broad and inhomogeneous and comprise a number of well characterized proteins, some of which have been isolated in a high state of purity. These include the haptoglobins, ceruloplasmin (12), the α_2-glycoprotein of Schmid (3 S) (13), thyroid binding protein (3.3 S) (14),

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and a number of enzymes present only in trace amounts. The 19 S peak consists primarily of the α_2-glycoprotein of Brown (15). Comparison of the ultracentrifugal composition of the α_2-globulins isolated from different sera have shown more variation than is usually encountered with other electrophoretically defined serum proteins. Since the haptoglobins in different individuals have been shown to fall into one of three groups which differ in their electrophoretic and ultracentrifugal (4, 9) properties, it seemed possible that some of the observed variations in the α_2-globulins from different sera might be due to the presence of different haptoglobins.

Ultracentrifugal studies were carried out on electrophoretically isolated α_2-globulin fractions from sera of each of the three haptoglobin groups to determine whether sufficient differences existed to have contributed to some of the discrepancies noted in the past and whether the appearance of the ultracentrifugal patterns of each of the three groups might be sufficiently characteristic to provide an additional independent method to define accurately individual serum groups.

Materials and Methods

Fasting serum was drawn into siliconized syringes, transferred to siliconized tubes, and immediately centrifuged. Following centrifugation the supernatant plasma was allowed to clot at room temperature, and the tubes were recentrifuged to separate the fibrin clot from the clear supernatant serum. Using this method serum free from recognizable traces of hemolysis could be obtained readily.

Starch Block Electrophoresis.—The electrophoretic separation of serum was carried out by the method of starch zone electrophoresis (16) using barbital buffer pH 8.6 ionic strength 0.1. Two equal aliquots (usually 7.0 ml.) of the same serum were separated simultaneously on a starch block. One of these was free from hemolysis while to the other hemoglobin, obtained from normal hemolyzed red cells (17), was added in amounts sufficient to insure saturation of the hemoglobin-binding capacity of the haptoglobin (18). After separation, the starch block was cut into half-inch segments and the protein from each segment was eluted by displacement filtration with 8.0 ml. of barbital buffer. The protein concentration was determined by a modification of the Folin-Ciocalteu procedure. Particular care was taken to exclude any contaminating protein corresponding to the β- and α_1-globulin regions. Under the conditions employed the α_2-peak usually separated completely from the β- and the α_1-proteins. The eluates corresponding to the α_2-region of each specimen were pooled separately, and the two samples, one free of hemoglobin and one containing hemoglobin bound to haptoglobin, were concentrated to about 1.0 to 1.5 ml. by ultrafiltration. Approximately 50 per cent of the α_2-protein was recovered. Some of the loss of protein was due to the need for completely eliminating the adjoining β- and α_1-globulins. After concentration, the material was centrifuged for 10 minutes to remove small particulate material. The protein concentration of the clear supernatant was determined and the material examined in the ultracentrifuge.

Isolated haptoglobin hemoglobin complexes of the serum from the three groups were prepared in the following manner. Fifteen to twenty ml. of serum free from hemolysis were separated on a broad starch block using barbital buffer pH 8.6 ionic strength 0.1. After electrophoresis the α_2-globulin peak was eluted and concentrated and hemoglobin C added in amounts sufficient to ensure that all the haptoglobin became bound to hemoglobin. The mixture of α_2-globulin and hemoglobin C was separated on a second starch block using barbital buffer pH 8.6 μ 0.05. The presence of the hemoglobin C resulted in a slowing of the haptoglobin

hemoglobin complex so that the complex migrated as a separate peak slower than the remainder of the α_2-proteins. A similar purification could not be achieved with normal hemoglobin since the faster mobility of hemoglobin A resulted in a haptoglobin hemoglobin complex which migrated with the rest of the α_2-proteins. By this technique, the haptoglobin hemoglobin complex was considerably purified in a single electrophoretic separation.

Starch Gel Electrophoresis.—Starch gel electrophoresis of whole serum was performed according to the method described by Smithies (4). The pattern obtained enabled the serum to be placed into one of the three main inherited haptoglobin groups; Groups 1-1, 2-2, and 2-1. It became apparent during the course of this work that many additional minor hemoglobin binding proteins could be demonstrated in serum from individuals of Group 2-2, and, to a lesser extent, in individuals of Group 2-1. Because these proteins were present in only small quantities they were more difficult to analyze with precision and have been disregarded in the experiments reported in this paper.

Ultracentrifugation.—Ultracentrifugal examination of the isolated protein fractions was carried out in a Spinco model E analytical ultracentrifuge at a speed of 52.640 R.P.M. and a centrifugal force of 2×10^5 g, using a double sector center piece. Sedimentation coefficients and protein concentrations were calculated with the aid of a comparator as described by Trautman (19). The observed s rates in barbital buffer were multiplied by 1.10 to obtain the $s_{20,w}$. In a number of instances dilution studies were carried out, and an s rate at infinite dilution was calculated by plotting the observed s rates against the concentrations.

Immunologic Studies.—Antisera to the isolated haptoglobin hemoglobin complexes of each of the three groups were prepared in rabbits by the subcutaneous injection of the electrophoretically isolated protein mixed with Freund's adjuvant. Quantitative precipitin studies were carried out as described previously using 0.1 to 0.15 ml. antiserum. Protein was estimated by the modified Folin-Ciocalteu procedure. Double diffusion studies in agar were performed by the method of Ouchterlony (20).

RESULTS

Fig. 1 illustrates the ultracentrifugal patterns of the α_2-globulins obtained from an individual of Group 1-1. The two slowly sedimenting peaks with an observed $s_{20,w}$ of approximately 2.8 and 5.1 S were present in a ratio of approximately 5:1. From a large number of experiments with whole α_2-fractions at different protein concentrations it could be calculated that the sedimentation coefficients at infinite dilutions of these two peaks would be approximately 4 S and 6 S respectively. In the presence of hemoglobin (Fig. 1 *b*) there was a marked decrease in the amount of the slower component with a concomitant rise in the 6–7 S peak which was now present at a concentration slightly greater than the 4 S peak. A deeply pigmented band was associated with this 6 S peak due to the binding of hemoglobin to the haptoglobin. Free hemoglobin was not present in preparations obtained from Group 1-1 since its mobility under the conditions used for isolation was sufficiently different to allow complete separation. The sharp 19 S peak and the intermediate 10–12 S peak which were clearly visible in the initial preparations of all three groups were partially obscured by the deep pigmentation of the solution containing the haptoglobin hemoglobin complex. However their relative concentrations appeared to have been unaffected by the addition of hemoglobin. No additional protein peaks were seen in serum from Group 1-1 after the addition

of hemoglobin. Qualitatively similar results were obtained with a second serum of Group 1-1. It would thus appear that in Group 1-1, approximately 40 to 50 per cent of the 3–4 S peak consisted of haptoglobin, and that following its

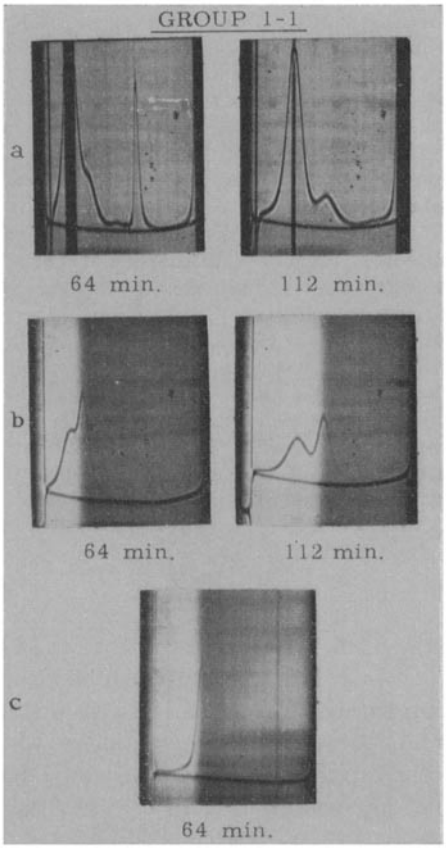

FIG. 1. Selected ultracentrifugal patterns of the serum α_2-proteins of Group 1-1 (E. F.). In this and the two subsequent figures, the upper patterns (*a*) represent the isolated α_2-protein free of hemoglobin, the middle patterns (*b*) represent the α_2-protein isolated after the addition to serum of hemoglobin, and the lower patterns (*c*) illustrate the isolated haptoglobin hemoglobin complex. Sedimentation proceeds from left to right and the time is indicated under each pattern.

combination with hemoglobin the haptoglobin hemoglobin complex had an approximate sedimentation coefficient of 6 S.

In an effort to confirm this observation the haptoglobin-hemoglobin complex was separated from the rest of the α_2-globulins and examined in the analytical ultracentrifuge. Fig. 1 *c* shows the complex isolated in this manner. It consisted

of a deeply pigmented fairly sharp homogeneous peak with an observed $s_{20,w}$ of approximately 6 S, which corresponded quite closely to the pigmented peak in the whole α_2-fraction.

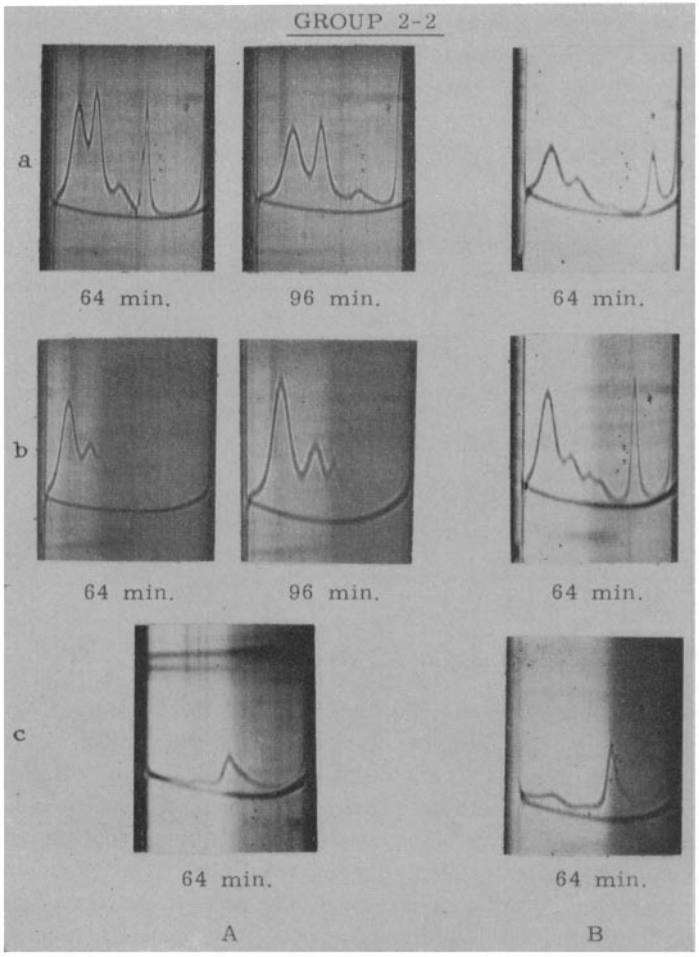

FIG. 2. Selected ultracentrifugal patterns of the serum α_2-proteins of Group 2-2. A (H. H.); B (H. D.).

The ultracentrifugal patterns of the α_2-globulin fraction from an individual of Group 2-2 are shown in Fig. 2A *a*. Prior to the addition of hemoglobin there were two major rather broad peaks with observed $s_{20,w}$ of 3.0 and 5.8 S which at infinite dilution correspond to the 4 S and 6–7 S components. Following the addition of hemoglobin there was a decrease in the relative concen-

tration of the 6–7 S peak accompanied by the appearance of a pigmented peak with an observed $s_{20,w}$ of 8.4 S (Fig. 2A *b*). It would appear that the haptoglobin made up almost one-third of the 6–7 S peak and that combined with hemoglobin it had an approximate sedimentation coefficient of 8 S. Although the observed *s* rates in this experiment were lower owing to their dependence on concentration, the isolated haptoglobin hemoglobin complex shown in Fig. 2A *c* consisted of one major rather asymmetric pigmented peak with an observed $s_{20,w}$ of 10.1 S. The asymmetry indicates that several components were present in this peak.

TABLE I

Comparison of the Ultracentrifugal Components Characteristic of the α_2-Proteins of Groups 1-1 and 2-2 with and without added Hemoglobin

Group		α_2			α_2+ Hb		
		Per cent 3-4 S	Per cent 6-7 S	Per cent 8-9 S	Per cent 3-4 S	Per cent 6-7 S	Per cent 8-9 S
1-1	E. F.*	82.2	17.8	0	48.4	51.6	0
	M. P.	90.0	10.0	0	47.5	52.5	0
2-2	H. H.‡	50.5	49.5	0	54.8	29.8	15.4
	H. D.§	61.9	38.1	0	61.3	26.4	12.3
	S. O.	47.1	52.9	0	58.4	18.7	22.9

* Fig. 1.
‡ Fig. 2A.
§ Fig. 2B.

Qualitatively similar results obtained with another serum of Group 2-2 are illustrated in Fig. 2B. Although in this instance the relative concentration of the 6–7 S peak was low, the addition of hemoglobin resulted in a further fall in concentration with the appearance of the more rapidly sedimenting pigmented peak, thus clearly differentiating this serum from Group 1-1. The isolated haptoglobin hemoglobin complex from the same serum, shown in Fig. 2B *c*, had an observed $s_{20,w}$ of 10.6 S. The relative concentrations of the 3–4 S, 6–7 S and the more rapidly sedimenting haptoglobin hemoglobin complex in the α_2-globulins prepared from two individuals of Group 1-1, and three from Group 2-2 are shown in Table I. The differences noted in the α_2-globulins are further accentuated by the addition of hemoglobin which resulted in a significant increase only in the 6–7 S peak in Group 1-1 whereas in Group 2-2 it resulted in the appearance of an additional more rapidly sedimenting peak.

The results obtained with serum from the heterozygote Group 2-1 were considerably more difficult to interpret. The isolated α_2-fraction resembled that found in Group 2-2 (Fig. 3A *a*.) It consisted of one slowly sedimenting peak

with an observed $s_{20,w}$ of 3.4 S and another with an approximate $s_{20,w}$ of 5.5 S which corresponded to the major 4 S and 6–7 S peak observed in Group 2-2. It differed from the α_2-protein from Group 2-2 however in that the more rapidly

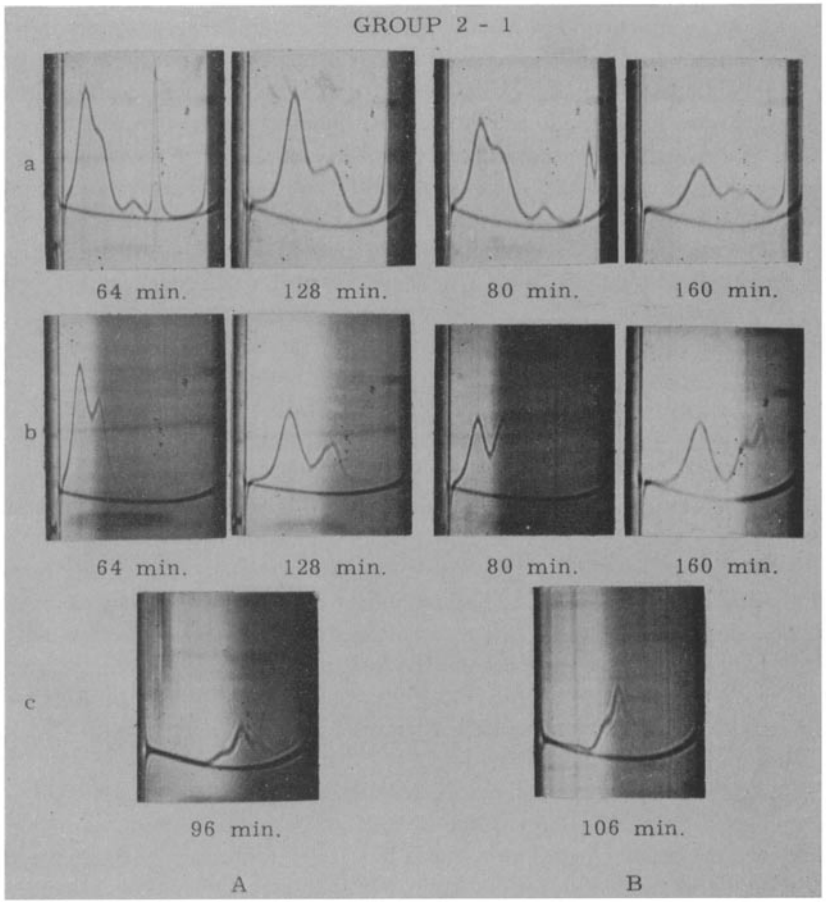

FIG. 3. Selected ultracentrifugal patterns of the serum α_2-proteins from two heterozygotes of Group 2-1. A (A. B.); B (R. K.). A comparison of these patterns with those illustrated in Fig. 2 demonstrates a greater degree of heterogeneity of the 6–7 S peak.

sedimenting of these peaks was more inhomogeneous and was composed of at least two major peaks with an observed $s_{20,w}$ of approximately 5.0 and 5.7 S respectively.

Following the addition of hemoglobin the relative concentration of the 6–7 S peak compared to the 3 S peak increased slightly. As sedimentation progressed it became apparent that most of the observed increase had occurred in the

faster of these two complexes which had become deeply pigmented. The isolated haptoglobin hemoglobin complex consisted of three major components with an observed $s_{20,w}$ for each peak of 6.6, 7.7, and 8.9 S (Fig. 3A *c*). The asymmetry of the fastest peak suggests, as it did in serum from Group 2-2 that further purification may resolve this peak into several distinct components. Study of a number of similar preparations at different concentrations indicated that the sedimentation coefficients of the three major components were approximately 7, 9, and 11 S at infinite dilutions. Although the color was usually most marked in the intermediate peak the presence of hemoglobin in association with the more slowly sedimenting component could not be ruled out. In the whole α_2-fraction the peak corresponding to the 11 S component was obscured by the dense pigmentation. Fig. 3B shows similar results obtained with the α_2-fractions from another individual of Group 2-1. The 6–7 S peak was again broad and had two major components. Following the addition of hemoglobin there was again a slight increase in the faster peak. The isolated complex also had three major peaks with observed $s_{20,w}$ of approximately 6.2, 7.8, and 8.9 S.

In an effort to resolve which of the two components of the 6–7 S peak of the α_2-globulins was characteristic of the heterozygote and which was common to all three groups, the α_2-fraction from an individual of Group 2-2 was mixed with one from Group 2-1. This resulted in a small increase in the more rapidly sedimenting of the two peaks. Similar results were obtained with the addition of the 6–7 S peak of Group 1-1. These observations suggest that the slower sedimenting peak contained a haptoglobin which was undetectable in the serum of either homozygote. The direct demonstration of the identity of two closely related peaks in such a complex mixture of proteins by direct *s* rate determinations proved difficult since it was not possible to isolate them in a state of sufficient purity to permit accurate calculations of infinite dilution sedimentation coefficients.

Electrophoretic Properties of the Haptoglobins.—An attempt was made to determine whether the correct assignment of an individual serum haptoglobin group could be made by ordinary starch block electrophoresis without the use of the gel procedure. The results indicated that although in the absence of hemoglobin the three haptoglobin groups were indistinguishable, the addition of hemoglobin in amounts sufficient to saturate the hemoglobin-binding capacity of the serum resulted in a characteristic electrophoretic pattern which allowed a correct classification to be made (9). While this work was in progress, the ability to distinguish the haptoglobin groups by use of paper electrophoresis was reported (21). Individuals of Group 1-1 showed two clear cut red bands in the starch block, with an intervening clear area. The more rapidly migrating band corresponded to the fast α_2-region and represented the haptoglobin hemoglobin complex, and the slow colored band, migrating in the β-globulin region, was due to free hemoglobin. In Group 2-2 the two bands were less distinct,

the slower band again had the mobility of a β-globulin but the faster band representing the haptoglobin hemoglobin complex migrated in the slow α_2-region. In Group 2-1 the band of the haptoglobin hemoglobin complex was intermediate in position between Groups 1-1 and 2-2, while the position of the free hemoglobin was again in the β-globulin region.

Although this method of grouping human haptoglobins is somewhat less reliable than starch gel electrophoresis it has the advantage that many sera can be grouped simultaneously. Using a broad starch slab (32 by 45 by 0.7 cm.) as many as 20 sera (1.0 ml. samples) can be run at the same time. The proper application of the serum required considerable care since imprecision in the alignment of the serum samples at the point of origin could result in difficulty in the interpretation of the results. However with some experience this difficulty was largely overcome. It was found that it was considerably easier to group sera with confidence if samples of standard sera from the three haptoglobin groups were separated at the same time for comparative purposes.

Immunologic Studies.—To determine the antigenic relationships of the haptoglobins derived from the three serum groups, antisera were produced in rabbits to the electrophoretically isolated haptoglobin hemoglobin complex of each group. The antisera were freed as completely as possible of antibodies to contaminating proteins by repeated absorptions with normal human umbilical cord serum which has been shown to contain inappreciable amounts of haptoglobin (22). Following absorption, these antisera failed to react with umbilical cord serum and free hemoglobin but reacted with normal serum and isolated haptoglobin hemoglobin complexes. Nevertheless they were not completely specific for haptoglobin since more than one line was usually visible by agar diffusion studies when tested with the haptoglobin hemoglobin complexes.

The isolated haptoglobin hemoglobin complexes of each group were tested with a homologous antiserum as well as with antisera to each of the two other groups and their reaction compared by the agar diffusion method of Ouchterlony and by quantitative precipitin studies. In the agar diffusion system more than one precipitin line was usually present, probably owing to antibodies to minor impurities which were not completely removed by absorption, or possibly owing to the presence of several antibodies to each type of haptoglobin. However the main line formed by the interaction of the antiserum with the haptoglobin hemoglobin complex, which could be identified by its reddish color, showed a reaction of identity when the isolated complexes of each of the three types were tested simultaneously against their homologous as well as against the two heterologous antisera.

Quantitative precipitin curves confirmed the identity. Fig. 4 shows that in the region of antibody excess and up to equivalence, the amount of precipitate produced by each of the three haptoglobin hemoglobin complexes with an antiserum to Group 2-2 was similar. However in the region of antigen excess,

some differences became apparent. These were probably due to the presence of varying amounts of impurities in different preparations. Qualitatively similar results were obtained with antisera to the other two groups, suggesting that the three haptoglobins were indistinguishable antigenically by the methods employed.

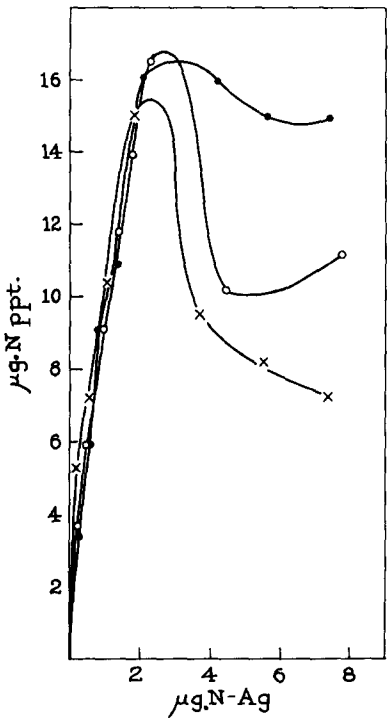

FIG. 4. Quantitative precipitin curves of the three haptoglobin hemoglobin complexes against an absorbed antiserum to the haptoglobin hemoglobin complex of Group 2-2. ●-●-●, Group 2-2, O-O-O, Group 2-1; X-X-X Group 1-1.

DISCUSSION

There is increasing evidence that many serum proteins in man and other animals possess certain distinct heritable differences (5, 23–25). Minor but consistent differences in the free electrophoretic patterns of serum obtained from various individuals have been described by Bernfeld and coworkers (26) and by Berry and Chanutin (27). More recently the development of starch gel electrophoresis has enabled the α_2-proteins of normal human serum to be subdivided readily into at least three major distinct heritable groups (4).

The precise reasons for the increased resolution of the α_2-proteins in a "starch

gel," composed of partially hydrolyzed starch, compared to that obtained using a "starch block" are incompletely understood. It has been suggested that the passage of proteins through the gel is tantamount to passing them through a sieve of fixed pore size and that, consequently, the proteins are separated not only by their differences in electrical charge but also by their molecular dimensions. Although the results of this study lend some support to this hypothesis, the importance of the nature of the buffer and its ionic strength should not be discounted.

The haptoglobin from Group 1-1 when combined with hemoglobin has an *s* rate of approximately 6 S whereas the haptoglobin hemoglobin complex of Group 2-2 has a sedimentation coefficient of approximately 11 S at infinite dilution. The electrophoretic mobility of the smaller molecule was somewhat faster in the granular starch medium. However the greatest difference in mobility was observed in the starch gel where the relative restriction of the larger Group 2-2 haptoglobins may be assumed to be playing a greater role. The difference in the electrophoretic mobility between the haptoglobins of 2-1 and 2-2 and their respective complexes is slight and not significantly greater in the starch gel medium than in the conventional starch zone electrophoresis and, similarly, more difficulty was encountered in differentiating Groups 2-1 and 2-2 by ultracentrifugation. A similar retardation in the starch gel has also been noted in the case of the 19 S α_2-globulin component which cannot be separated from the remainder of the α_2-globulins by starch or paper electrophoresis.

Before the genetic significance of the haptoglobin system had become apparent, Jayle and coworkers had made extensive studies (1–3) of the physicochemical properties of chemically isolated haptoglobins and their capacity to bind hemoglobin. Two haptoglobins were described in detail, one with a molecular weight of 85,000 and an *s* rate of 4.1 S and the other with a molecular weight of 169,000. The haptoglobin hemoglobin complex of the former had an *s* rate of approximately 6.3 S and the latter of 9.9 S. These *s* rates correspond closely with those obtained in this study from individuals of Groups 1-1 and 2-2.

The α_2-globulins from Group 1-1 can be readily identified by their characteristic ultracentrifugal patterns. The addition of hemoglobin accentuates the singularity of this group. However the distinction between Groups 2-2 and 2-1 is not easy in the absence of hemoglobin although there appears to be a greater inhomogeneity of the 6–7 S peak in the α_2-globulin prepared from Group 2-1. Following the addition of hemoglobin to the α_2-fraction however the peaks due to the complexes are sufficiently distinctive to permit differentiation of the two groups. The difference between them is even more pronounced when the isolated haptoglobin hemoglobin complex was examined.

While significant differences in the physical properties of the different

haptoglobins are readily detectable by electrophoretic, ultracentrifugal, and a number of other physical techniques (1, 2), it has not been possible to detect any immunologic differences using highly specific antisera to each of the three haptoglobin hemoglobin complexes. A similar situation also exists for a number of abnormal hemoglobins which differ in electrophoretic mobility but which fail to show significant immunologic differences (28). This result is in accord with the apparent immunological indifference of man to plasma transfusions.

Although genetic studies are usually compatible with the hypothesis that the synthesis of the haptoglobins is controlled by two autosomal genes with incomplete dominance, the undoubted complexity of the problem is rapidly becoming apparent (6–8). There are several minor proteins in serum from individuals of Groups 2-1 and 2-2 which, using special techniques, can be shown to have the capacity to bind hemoglobin but their biochemical properties and their genetic characteristics are at present hard to evaluate since they are present only in trace quantities. Moreover, single symmetrical peaks in the ultracentrifuge are not necessarily indicative of single proteins. The asymmetry of the haptoglobin hemoglobin peak of Group 2-2 suggests that further purification may resolve this peak into its constituent components. The presence of multiple bands in the starch gel also indicates the heterogeneity of the Group 2-2 haptoglobin hemoglobin complex. In contrast the narrow symmetrical peak obtained in Group 1-1 suggests that this may represent a relatively homogeneous protein complex. This peak corresponds to the single band found by starch gel electrophoresis. Because of its slower electrophoretic mobility the techniques employed permit isolation of the Group 2-2 complex in a greater state of purity than that of Group 1-1. Thus the greater asymmetry of the complex from Group 2-2 would suggest that other methods will resolve it into several discrete peaks. The ultracentrifugal finding of a haptoglobin hemoglobin complex in individuals of Group 2-1 which was not present in either homozygote suggests that the inheritance of the haptoglobins may be more complicated than is usually considered. The appearance of the new component of intermediate s rate in the heterozygote bears some similarity to the observations of Irwin (29) and Cohen (30) in which genetic interaction has been shown to influence the specific gene product, and particularly to the observations of Morgan and Watkins (31) in which a specific gene product in individuals belonging to the human blood group AB has been described.

SUMMARY

Ultracentrifugal studies of the α_2-proteins of normal human serum before and after the addition of hemoglobin have revealed three separate and clearly distinguishable patterns based on the three major serum haptoglobin groups. Isolation of the three haptoglobin hemoglobin complexes disclosed characteristic patterns for each group. The heterozygote was found to possess electrophoretic and ultracentrifugal components not seen in either homozygote.

Separation of normal human serum, to which hemoglobin had been added, by conventional starch zone electrophoresis resulted in three characteristic patterns which also permitted the ready identification of the serum haptoglobin group. The haptoglobin hemoglobin complex from Group 1-1 had the fastest mobility and that of Group 2-2 the slowest. The heterozygote Group 2-1 had an intermediate mobility. Immunologic differences between the haptoglobin hemoglobin complexes of the three groups could not be detected.

Some of the variations in the reported electrophoretic and ultracentrifugal patterns of normal human serum could be reasonably ascribed to the group variation of the haptoglobins in man. The physical studies imply that the proteins characteristic of the heterozygote differ in size and charge from those present in either of the two homozygotes and indicate that the genetic control of the synthesis of the serum haptoglobins is probably exceedingly complex.

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BIBLIOGRAPHY

1. Jayle, M.-F., and Boussier, G., Les séromucoides du sang; leurs relations avec les mucoprotéines de la substance fondamentale du tissu conjonctif, *Exposés Ann. Biochem. et Méd.*, 1955, **17,** 157.
2. Guinand, S., Tonnelat, J., Boussier, G., and Jayle, M-F., Propriétés physiques de l'haptoglobine, seromucoide α_2 et de sa combinaison hémoglobinique; (ler memoire), *Bull. Soc. chim. biol.*, 1956, **38,** 320.
3. Jayle, M.-F., Bouisser, G., and Tonnelat, J., Isolement à partir du sérum humain de la combinaison hémaglobine-haptoglobine à l'état homogène; (2e mémoire), *Bull. Soc. chim. biol.*, 1956, **38,** 343.
4. Smithies, O., Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults, *Biochem. J.*, 1955, **61,** 629.
5. Smithies, O., and Walker, N. F., Notation for serum-protein groups and the genes controlling their inheritance, *Nature*, 1956, **178,** 694.
6. Sutton, H. E., Neel, J. V., Binson, G., and Zuelzer, W. W., Serum protein differences between Africans and Caucasians, *Nature*, 1956, **178,** 1287.
7. Allison, A. C., Blumberg, B. S., and Rees, A. P., Haptoglobin types in British, Spanish Basque, and Nigerian African populations, *Nature*, 1958, **181,** 824.
8. Boussier, G., Obtention a l'état d'une mucoprotéine sérique: l'haptoglobine, Compt. rend. Acad., 1958, **246,** 1769.
9. Bearn, A. G., and Franklin, E. C., Some genetical implications of physical studies of human haptoglobins, *Science*, 1958, **128,** 596.
10. Brattsen, I., Continuous zone electrophoresis by crossed velocity fields in a supporting medium. Analysis of protein fractions obtained from normal human serum, *Ark. Kemi*, 1955, **8,** 347.
11. Wallenius, G., Trautman, R., Kunkel, H. G., and Franklin, E. C., Ultracentrifugal studies of major non-lipide electrophoretic components of normal human serum, *J. Biol. Chem.*, 1957, **225,** 253.
12. Holmberg, C. G., and Laurell, C-B., Investigations in serum copper. II. Isolation

of the copper containing protein, and a description of some of its properties, *Acta Chem. Scand.*, 1948, **2**, 550.
13. Schmid, K., Isolation of a group of α_2-glycoproteins from human plasma, *J. Am. Chem. Soc.*, 1955, **77**, 742.
14. Robbins, J., Petermann, M. L., and Rall, J. E., Electrophoresis of the thyroxine-binding protein of serum at pH 4.5, *J. Biol. Chem.*, 1955, **212**, 403.
15. Brown, R. K., Baker, W. H., Peterkofky, A., and Kauffman, D. L., Crystallization and properties of a glycoprotein isolated from human plasma, *J. Am. Chem. Soc.*, 1954, **76**, 4244.
16. Kunkel, H. G., Zone electrophoresis, *Methods Biochem. Anal.*, 1954, **1**, 141.
17. Kunkel, H. G., Ceppellini, R., Müller-Eberhard, U., and Wolf, J., Observations on the minor basic hemoglobin component in the blood of normal individuals and patients with thalassemia, *J. Clin. Inv.*, 1957, **36**, 1615.
18. Laurell, C-B., and Nyman, M., Studies on the serum haptoglobin level in hemoglobinemia and its influence on renal excretion of hemoglobin. *Blood*, 1957, **12**, 493.
19. Trautman, R., Operating and comparating procedures facilitating schlieren pattern analysis in analytical ultracentrifugation, *J. Physic. Chem.*, 1956, **60**, 1211.
20. Franklin, E. C., and Kunkel, H. G., Immunologic differences between the 19 S and 7 S components of normal human γ-globulin, *J. Immunol.*, 1957, **78**, 11.
21. Poulik, M. D., and Smithies, O., Comparison and combination of the starch-gel and filter-paper electrophoretic methods applied to human sera: Two-dimensional electrophoresis, *Biochem. J.*, 1958, **68**, 636.
22. Tuttle, A. H., Demonstration of hemoglobin-reactive substance in human serum, *Science*, 1955, **121**, 701.
23. Ashton, G. C., Serum protein differences in cattle by starch gel electrophoresis, *Nature*, 1957, **180**, 917.
24. Ashton, G. C., A genetic mechanism for thread protein polymorphism in cattle, *Nature*, 1958, **182**, 65.
25. Horsfall, W. R. and Smithies, O., Genetic control of some human serum β-globulins, *Science*, 1958, **128**, 35.
26. Bernfeld, P., Donahue, V. M., and Homberger, F., Characteristic individual electrophoretic patterns in humans, *Proc. Soc. Exp. Biol. and Med.*, 1953, **83**, 2129.
27. Berry, E. R., and Chanutin, A., Detailed electrophoretic analyses of sera of healthy young men, *J. Clin. Inv.*, 1955, **34**, 1513.
28. Itano, H. A., The human hemoglobins: their properties and genetic control, *Advances Protein Chem.*, 1957, **12**, 215.
29. Irwin, M. L., Immunogenetics, *Advances Genetics*, 1947, **1**, 133.
30. Cohen, C., Influences upon the agglutinability of rabbit erythrocytes in the presence of iso-antibody: Genetic Factors, *J. Immunol.*, 1958, **80**, 73.
31. Morgan, W. T. J., and Watkins, W. M., The product of the human blood group A and B genes in individuals belonging to Group AB, *Nature*, 1956, **177**, 521.