THE FREQUENCIES OF HAPTOGLOBIN TYPES IN FIVE POPULATIONS*

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The first report of the existence of the plasma proteins now known as 'haptoglobins' was made by Polonovski & Jayle (1938). In subsequent studies by Jayle and his co-workers it was demonstrated that haptoglobin, which combines specifically and stoichiometrically with haemoglobin, belongs to the α_2 fraction of blood and is a glycoprotein of molecular weight approximately 155,000 (Guinand, Tonnelat, Boussier & Jayle, 1956). An unusual form of haptoglobin of molecular weight 85,000 was detected in one nephrotic child (Guinand *et al.* 1956). It was postulated by the French workers that the usual form of haptoglobin is a dimer of the haptoglobin, formed in the nephrotic patient and that the dimer combines with two molecules of haemoglobin while the monomer combines with only one.

Smithies, using starch gel as the supporting medium for electrophoresis, discovered that under these conditions the proteins of human serum give a pattern quite different from that observed with 'free' electrophoresis or with agar or paper as the supporting medium (Smithies, 1955*a*). This difference consisted in a greatly increased resolution of proteins and, as shown later by him, a change in the relative positions of some of the proteins (Smithies, 1955*b*). He further observed that some of these proteins show qualitative variations among individuals and that these variable proteins form stable complexes with haemoglobin, thus identifying them with the haptoglobin of Polonovski & Jayle.

On the basis of the qualitative differences in the haptoglobins as revealed by the starch gel technique, Smithies was able to classify people into three types. Two of these types, however, actually are characterized by the presence of several different proteins each. With Walker, he demonstrated that these three haptoglobin types are determined by a pair of alleles (Smithies & Walker, 1955). Of particular interest in this system is the fact that the heterozygous individuals produce a series of haptoglobins different from those of either homozygous types. The genetic theory of Smithies & Walker has now been tested extensively, the largest series of families being that reported by Galatius-Jensen (1957), and no exceptions have been found if we ignore relatively minor variations or the rare individual in whom haptoglobin is difficult to demonstrate (see below). This system is of unusual interest, then, with respect to the genetic control of protein synthesis, for not only does the heterozygous individual produce proteins different from either homozygous type, but also in two of the types the haptoglobins consist of several fractions. The number of fractions was first set by Smithies at three in the heterozygote

* This work was supported in part by grant AT (11-1)-70 from the Atomic Energy Commission.

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(type 2-1) and in one homozygote (type 2-2), the remaining homozygote (type 1-1) possessing only one haptoglobin species. Allison & ap Rees (1957) reported that the presence of type 1-1 haptoglobin can also be demonstrated in heterozygous individuals when the more sensitive benzidine stain is used. Under conditions of optimum resolution we have been able to demonstrate the presence of five different haptoglobins in the type 2-2 individuals and six (including the 1-1) haptoglobins in the 2-1 heterozygotes (Sutton, unpublished). No other genetic system is known in which a single locus appears to control the specificity of more than one protein.

The existence of more than one allele raises questions concerning the distribution of the alleles in various populations and the relative selective advantages of the different genotypes. A previous publication from this laboratory reported the existence of both alleles in African Negroes as well as in Caucasians, although the frequencies differed in the two populations (Sutton, Neel, Binson & Zuelzer, 1956). The present report gives the results of haptoglobin determinations on additional individuals of these two populations with results also on American Negroes, American Indians (Apaches), and Asiatic Indians.

Technique

The blood samples from Caucasians were obtained primarily from laboratory personnel and from donors to the University Hospital Blood Bank. They were healthy so far as is known. The samples from American Negroes were obtained entirely from the Blood Bank. In some cases serum and in others citrated plasma were used. Samples from Africa were collected as part of a survey of abnormal haemoglobins and of serotypes in Liberia and the Ivory Coast. There was some haemolysis in all these samples, which were shipped oxalated, but only in exceptional cases, of a type to be discussed later, could this have prevented ascertainment of the haptoglobin type. The samples from Apaches were collected without anticoagulant at the Public Health Service Indian Hospital at Mescalero, New Mexico, and were shipped by air mail to Ann Arbor. Some of these subjects were healthy but most were under treatment for injury or infection. The Asiatic Indians were students at the University of Michigan. Forty-four of this group came from Bombay and vicinity, the remaining thirty coming from eleven different provinces.

The electrophoretic technique was essentially that of Smithies (1955*b*). The potato starch was obtained from Mallinckrodt Chemical Works and was hydrolyzed according to the procedure of Smithies. Insertion of the sample into the gel was usually by the starch method, which gives better results for most purposes than filter-paper insertion. Samples free of haemolysis were run both with and without addition of haemoglobin. The haemoglobin used was obtained by haemolysis of washed human erythrocytes and without further treatment. The gels were run at 6 V./cm. for 5 hr.

After the gel was sliced open, the protein bands were stained with amidoschwarz 10B dissolved in methanol-acetic acid-water (5:1:5) as outlined by Smithies. The excess dye was washed out with the same solvent. The haptoglobin-haemoglobin complex was also revealed by the classical reaction of haemoglobin with peroxide and benzidine. The latter reaction is extremely sensitive and is very useful in detecting small quantities of haptoglobin, particularly in the presence of large amounts of other proteins. Occasionally non-haptoglobin protein bands appear in the region occupied by the haptoglobins, and the benzidine test is very useful in clarifying such cases.

In blood samples with even moderate amounts of haemolysis, the positive identification of

Hp 1-1 is difficult because it is not sufficiently well separated from other proteins with the amidoschwarz stain and because the trailing edge of the free haemoglobin band may react sufficiently strongly with benzidine to obscure the *Hp* 1-1 position. In the absence of types 2-1 or 2-2 haptoglobin, such samples have been classified as 1-1. In both the African and American Indian samples it is possible that individuals who lack haptoglobin might be classified as 1-1. The importance of this will be dealt with in the discussion.

Results

In Table 1 are shown the frequencies of the three haptoglobin types for the five populations as well as the frequencies of the allele Hp^1. The nomenclature is that of Smithies & Walker (1956). In the case of the American Negroes, five additional individuals were tested in whom it was impossible to demonstrate haptoglobin. For reasons to be discussed later, these five were not included in the results of Table 1. In the samples from African Negroes, however, the presence of considerable haemolysis interfered with the detection of type 1-1 haptoglobin either by protein staining or by the benzidine reaction with haemoglobin. Accordingly, in the case of the specimens obtained from Africans, the classification of type 1-1 was usually based on a negative criterion, namely, the failure to demonstrate the type 2-1 or 2-2 pattern with benzidine staining. On the basis of the studies of Allison, Blumberg & ap Rees (1958), and our own results with American Negroes, it is probable that some of these 1-1 individuals actually do not have detectable amounts of haptoglobin. For this reason the frequencies of Hp^1 in Africans represent maximum values. All the populations differ significantly from each other by the χ^2-test except the African Negroes and Apaches. The relative frequencies of the three types are consistent with the two-allele hypothesis, assuming genetic equilibrium, in each population.

Table 1. *Number of individuals of each haptoglobin type and frequency of allele Hp^1 in five populations*

Population	*Hp* 1-1	*Hp* 2-1	*Hp* 2-2	Total	Hp^1
American whites	9	40	19	68	0·43
American Negroes	17	17	9	43*	0·59
African Negroes	327†	232	55	614	0·72
Apaches	34†	47	17	98	0·59
Asiatic Indians	3	21	50	74	0·18

* Does not include five individuals in whom haptoglobin was undetectable.

† The presence of haemolysis made it possible that ahaptoglobinemic individuals were erroneously classified as 1-1.

Of further interest are the possible variations in gene frequency among the tribes of Africa. In Fig. 1 and Table 2 are shown the frequencies of the Hp^1 alleles among those tribes represented by ten or more individuals. The terminology for the tribes is that of Schwab (1947) and Livingstone (1958). There is a considerable range—0·55 to 0·85—but the extreme values are from tribes represented by few individuals and tests for heterogeneity fail to show significance. On the other hand, if one plots the frequencies of Hp^1 against geographical location of the tribes, as is shown in Fig. 1, there is a suggestion of a lowered Hp^1 frequency in the south-eastern part of Liberia and the adjacent Ivory Coast, such as has also been demonstrated for the sickle cell gene (Livingstone, 1958; Binson, Zueler, Robinson & Neel, unpublished). This suggestion has been tested by plotting the Hp^1 gene frequency against sickle-cell trait frequency, as shown in

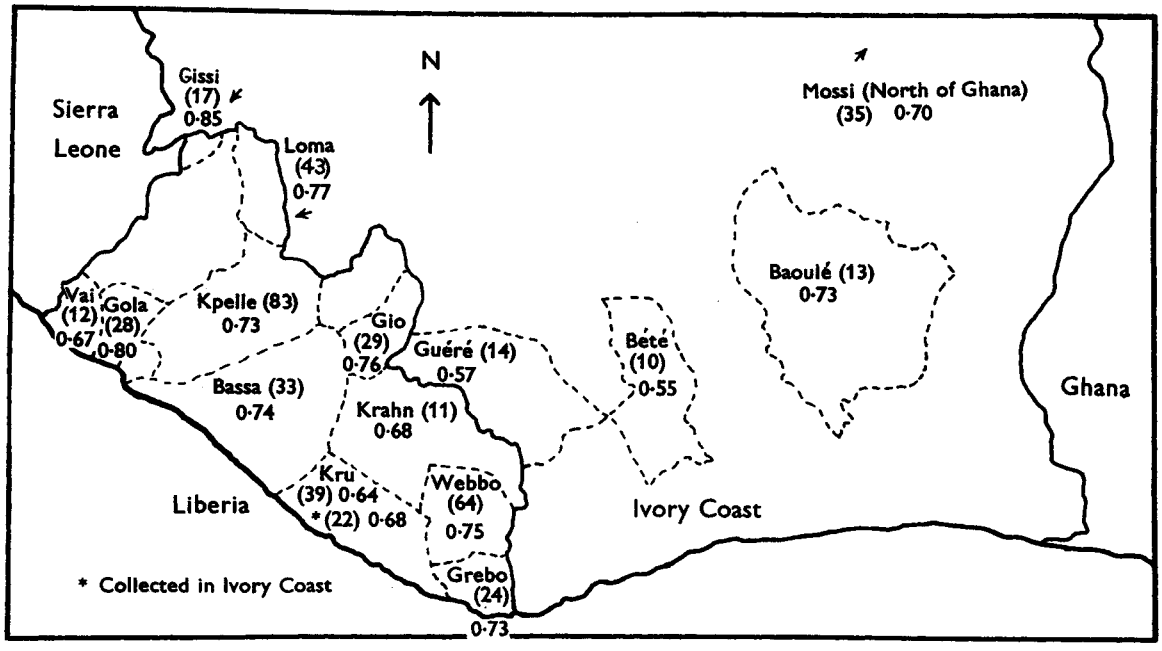

Fig. 1. The frequency of the Hp^1 gene in fifteen different West African tribes for which data are available concerning ten or more individuals. The number in parentheses indicates the number of persons studied; the fraction indicates the estimated gene frequency.

Table 2. *Number of individuals of each haptoglobin type and frequency in allele Hp^1 in various African tribes*

Tribe	*Hp* 1-1	*Hp* 2-1	*Hp* 2-2	Total	Hp^1
Liberia					
Bassa	18	13	2	33	0·74
Gio	17	10	2	29	0·76
Gissi	13	3	1	17	0·85
Gola	17	11	0	28	0·80
Grebo	15	5	4	24	0·73
Kpelle	44	34	5	83	0·73
Krahn	4	7	0	11	0·68
Kru	19	12	8	39	0·64
Loma	24	18	1	43	0·77
Vai	6	4	2	12	0·67
Webbo	36	24	4	64	0·75
Other*	17	9	3	29	—
Unknown	8	9	0	17	—
Ivory Coast					
Baoulé	7	5	1	13	0·73
Bété	4	3	3	10	0·55
Guéré	6	4	4	14	0·57
Krou	11	8	3	22	0·68
Mossi	17	15	3	35	0·70
Other*	44	38	9	91	—
Total	327	232	55	614	0·72

* Includes tribes represented by fewer than ten individuals.

Fig. 2. The weighted linear regression of Hp^1 frequency on sickle-cell trait frequency is significant at the 5% level ($\chi^2 = 4{\cdot}175$, 1 D.F., using the untransformed gene frequency; $\chi^2 = 4{\cdot}007$ using an arc sin transformation of the gene frequency). If the cline in the sickle cell gene is due to its recent advance through Liberia, as has been suggested by Livingstone (1958), then in this Hp^1 frequency cline we may be seeing other evidence for the postulated diffusion process.

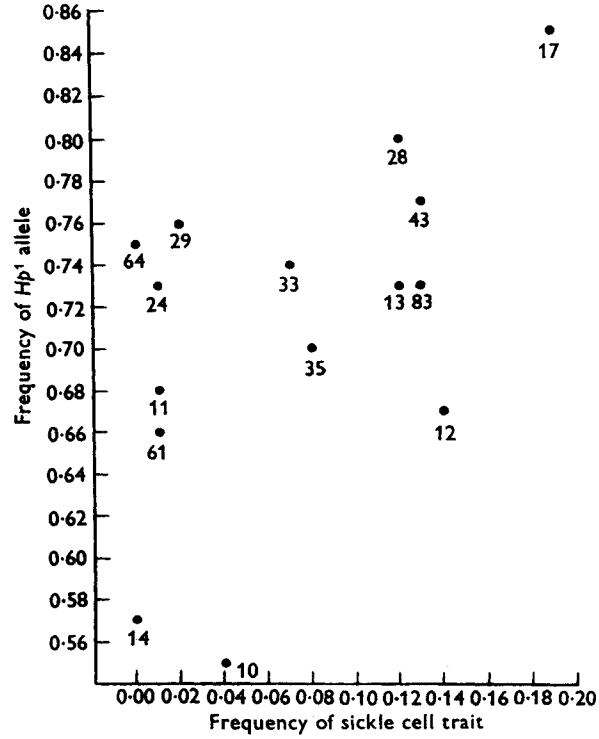

Fig. 2. Frequency of Hp^1 allele versus sickle cell trait in Liberia and the Ivory Coast. The number by each point indicates the number of individuals studied in that population. The Kr(o)us from the Ivory Coast and from Liberia have been combined. The values for sickle cell trait in Liberia have been taken from Livingstone (1958) and in the Ivory Coast from Neel, Binson, Robinson & Zuelzer (unpublished data).

An additional racial variation in haptoglobin levels was encountered with the Asiatic Indians. In this group the haptoglobin concentrations are so low that traces of haemolysis, which would be unnoticed with usual levels of haptoglobin, seriously interfere with the pattern of 'free' haptoglobin. The use of benzidine made recognition of the haptoglobin type a simple matter, but approximately half of the Indians showed concentrations low enough to lead to difficulties with protein stains only. While these students are living under essentially Western conditions now, the influence of early environmental effects cannot be excluded.

Other sources of difficulty in classifying haptoglobins are the appearance in aged blood samples of faint non-haptoglobin bands in the 2-1 and 2-2 region and the appearance of various faint haemoglobin bands under conditions of excess haemolysis. The latter may be due either to the existence of minor haemoglobin constituents, such as haemoglobin A_2, or to the binding of haemoglobin by proteins other than haptoglobins. Once these possibilities are recognized, they need not lead to error.

There are few reports of haptoglobin frequencies in populations with which to compare the present results. In his sample of forty-one individuals presumably of Caucasian ancestry, Smithies found a Hp^1 frequency of 0·45 (Smithies & Walker, 1955); Galatius-Jensen (1957) found a Hp^1 frequency of 0·403 among 1033 Danes. Allison *et al.* (1958) found distributions among British and Basque populations very similar to these studies and to the 0·43 reported in the present paper. Smithies has found a Hp^1 frequency of 0·59 among fifty New York Negroes (personal communication). In an earlier report from this laboratory, a frequency of 0·70 was reported for 142 Africans from Liberia and the Ivory Coast (Sutton *et al.* 1956). This value agrees well with the value of 0·72 reported in this paper.

Allison *et al.* (1958) found thirty-two ahaptoglobinemic individuals in their sample of ninety-nine Nigerians. Ahaptoglobinemic specimens certainly could and probably do occur in the present African series in the group we have designated as 1-1. The occurrence of large numbers of such specimens in our series would seriously distort any gene frequency estimation. In this connexion, however, it may be noted, that if one tests our African series for genetic equilibrium assuming the Smithies–Walker theory to be correct, the resulting χ^2 values are well below the level of significance. This suggests that the number of individuals incorrectly classified as 1-1 must be small. It should also be noted that the very much higher frequency of ahaptoglobinemic individuals found by Allison *et al.* (1958) in the Nigerians would not necessarily be expected in either of our Negro series, since the frequencies of 2-1 and 2-2 individuals are vastly different. For example, only three of ninety-nine Nigerians are reported to be type 2-2, while we found 8·9 % of our African sample to be 2-2. Likewise, eleven of the ninety-nine Nigerians were type 2-1 while we found 37·8 % of the subjects from Liberia and the Ivory Coast to be 2-1. The error in classifying type 2-1 and 2-2 individuals is always in the direction of failure to find the bands associated with these types; hence, our estimates of the frequencies of these two groups are minimum.

The finding that geographical variations in haptoglobin frequencies exist in Africa is interesting from the point of view of the degree of Caucasian admixture in American Negroes. If the American Negroes are assumed to be 30 % Caucasian (Neel, 1951; Glass, 1955), then on the basis of the gene frequencies reported here, the original African populations from which the American Negroes are derived can be computed to have a Hp^1 frequency of 0·66. With the small numbers of American Negroes reported and with the unknown importance of the ahaptoglobinemic individuals, such a calculation is at best a crude estimate. Nevertheless, it agrees as well as one might hope under the circumstances with the value of 0·72 reported here for West African tribes. It would not agree very well with the Nigerian data of Allison *et al.* (1958).

The existence of polymorphism in so many different populations suggests some selective advantage for the heterozygous individuals. What these advantages might be will probably remain unknown until more is known of the physiological function of haptoglobins. Both haptoglobins and the α_2-glycoproteins, which include haptoglobins, have been shown to increase in a variety of disease states involving increased tissue breakdown and synthesis (for example, see Jayle, Boussier & Badin, 1952; Sonnet, 1956; Winzler, 1955). Laurell & Nyman (1957) have demonstrated that free haemoglobin in the blood is bound by haptoglobin and does not appear in the urine until all the haptoglobin is bound. Nyman (1958) has shown that the haemoglobin combining power of the blood from the three types of individuals varies, the type 1-1 having the greatest combining power and type 2-2 the least. Whether this is due to increased concen-

trations of *Hp* 1-1 or to greater efficiency in complexing ability has not been determined. Allison & ap Rees (1957) found that the haemoglobin threshold is a function of the blood haptoglobin level and that the variations in expression of haemoglobinuria observed in certain diseases can be correlated with the haptoglobin level. Whether the complexing of free haemoglobin in the blood is the primary function of haptoglobin remains to be determined.

Discussion

In studying the distribution of haptoglobin types in two of the populations it was necessary to use blood samples which were not fresh. This raises questions regarding the reliability of results obtained with these populations since, under these circumstances, individuals of type 1-1 are so classified largely because they fail to show the bands characteristic of types 2-1 and 2-2. The type 1-1 band, either free or complexed with haemoglobin, migrates to a position which overlaps other plasma proteins on the gels. If appreciable haemolysis exists the free oxyhaemoglobin also confuses the picture, since it migrates to a position just ahead of the *Hp-Hb* complex and makes recognition of the complex, either with protein stains or with benzidine, very difficult.*

This problem is made more complex by the existence of individuals who have no demonstrable haptoglobin. Galatius-Jensen (1957) discussed these individuals in connexion with two he found among 593 Danish mothers and concluded that if this type is interpreted in terms of a third 'silent' allele, the upper limit to its frequency must be about 0·02. Allison *et al.* (1958), on the other hand, found six ahaptoglobinemic individuals among 218 British subjects and thirty-two among ninety-nine Nigerians. He designated these individuals as type 0-0 and considered them to belong to a group distinct from the three described by Smithies. As mentioned earlier, we found five individuals among our American Negroes in whom no haptoglobin could be detected in the single sample obtained from each. Smithies found only one ahaptoglobinemic individual among fifty New York Negroes (personal communication).

A classification of individuals which is based on the *failure* to detect an attribute must always be referred to the sensitivity of the examining procedure. The ahaptoglobinemic individuals unfortunately are so classified purely on negative findings. One may then ask whether or not these individuals show 'conventional' haptoglobin types at greatly reduced concentrations, or whether perhaps there are other factors, genetic or non-genetic, which prevent the production of haptoglobin in an individual with a conventional genotype. We have had occasion to examine one ahaptoglobinemic individual on four occasions extending over a period of a year. In three of the samples, it was impossible to demonstrate haptoglobin even with benzidine. In the fourth sample, however, the type 2-1 pattern was unmistakably present when the gel was stained with benzidine, suggesting that his genotype is actually Hp^2/Hp^1, the expression being altered by other factors. A further examination of his relatives has revealed the lack of detectable haptoglobin to be a characteristic of several individuals (Fig. 3). As yet these individuals have not been tested repeatedly.

The pedigree shown in Fig. 3 also provides evidence that the lack of haptoglobin is not due to an allele occurring at the Hp^1-Hp^2 locus. Individual III. 4, a male 9 years old, is the offspring of two individuals both of whom have type 2-1 haptoglobin. If we assume these individuals to

* Allison & ap Rees (1957) report the migration of free oxyhaemoglobin to be slower than that of β-globulin and of type 1-1 *Hp-Hb* complex. In our laboratory, free oxyhaemoglobin obtained from haemolysis of human erythrocytes has always migrated slightly faster than β-globulin, just as reported by Smithies (1955*b*).

be normal heterozygotes, then the possibility of a third allele being present in either is ruled out. For this reason we prefer to designate the ahaptoglobinemic individuals as *Hp* 0 rather than *Hp* 0-0 as Allison *et al.* (1958) have done, the latter suggesting a genotypic analogy to Hp^1 and Hp^2 at the recognized locus. At present, the data would suggest that ahaptoglobinemia may be of both genetic and environmental origin with, on the basis of this one pedigree, the simplest hypothesis concerning the basis of genetic ahaptoglobinemia being that the condition is due to homozygosity for a recessive gene. The observation that an individual with presumed genetic 'ahaptoglobinemia' may actually on occasion exhibit a clearly identifiable haptoglobin pattern indicates that the suppressor effect of the postulated recessive gene is not complete.

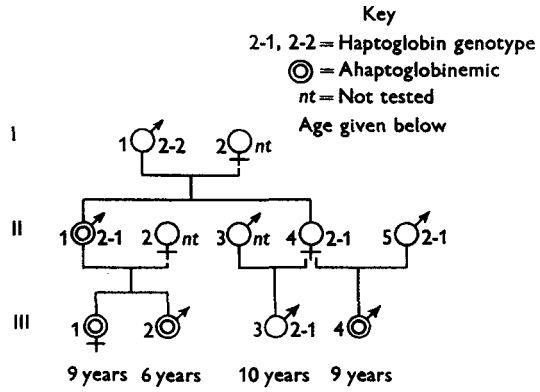

Fig. 3. Pedigree of a family containing several ahaptoglobinemic individuals. The numbers inside or to the right of the symbols represent the haptoglobin types, ◎ indicating no detectable haptoglobin, while a symbol with no entry means the individual could not be tested. Only subject II. 1 has been examined repeatedly, and on one occasion his blood contained traces of *Hp* 2-1. The remaining individuals had either normal haptoglobin levels or no detectable amounts.

Summary

Haptoglobin types have been determined by starch gel electrophoresis of blood from five populations. The gene frequencies obtained for allele Hp^1 were as follows: American whites, 0·43; American Negroes, 0·59; African Negroes, 0·72; Apaches, 0·59; and Asiatic Indians, 0·18. In tribes of the Ivory Coast and Liberia, there was a suggestion of a cline which parallels that for haemoglobin S.

Evidence is presented that the condition of ahaptoglobinemia is under genetic control but not by a gene allelic to the Hp^1-Hp^2 series. The importance of the ahaptoglobinemic individuals for genetic studies and the possibility of selection in the maintenance of the genetic polymorphism are discussed.

The authors wish to acknowledge the excellent assistance of Alojzia Sandor, who carried out the electrophoretic separations.

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