SERUM HAPTOGLOBINS IN AFRICA*

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The haptoglobins are serum proteins that combine with haemoglobins. They are part of the α_2-glycoprotein fraction and were first detected by Polonovski and Jayle in 1938.[1] Peroxidase activity is developed when haemoglobin forms a complex with haptoglobin,[2] and this property has been used to measure the concentration (or index) of haptoglobin in normal and abnormal sera.[3] The haptoglobin concentration has been found to be raised in a variety of diseases in which there is inflammation and tissue necrosis, including acute pyogenic infections, rheumatic fever, and some forms of cancer,[4] as well as in patients with rheumatoid arthritis.[5]

By means of starch-gel electrophoresis, Smithies[6] identified 4 different haemoglobin-binding proteins, which are the same as haptoglobins. Smithies and Walker[7] demonstrated that European populations have 3 major patterns, termed haptoglobin types 1 - 1, 2 - 1, and 2 - 2. These phenotypes are under the control of a single pair of allelomorphic genes designated Hp^1 and Hp^2.[8,9] Genotype Hp^1/Hp^1 produces haptoglobin type 1 - 1 (Hp1 - 1); Hp^2/Hp^1 produces Hp2 - 1; and Hp^2/Hp^2 produces Hp2 - 2.

Until quite recently, publications[10-21] concerning the haptoglobins emphasized that the Hp^2 gene is commoner than the Hp^1 in all European populations studied, and that in Africa Hp^1 is much more frequent than Hp^2. The rather simple story of a single pair of allelomorphic genes producing 3 genotypes in man, and the co-existence of these haptoglobins types in several populations providing a true state of polymorphism, became more intricate with the publication by Allison *et al.*[13] of an apparent fourth group, in which no haptoglobin at all is present, which they designated Hp0 - 0. Since then further rare modifications have been described. Furthermore, Sutton *et al.*[14] mention the presence of 5 different haptoglobins in the type 2 - 2 individuals and 6 haptoglobins in the 2 - 1 heterozygotes. The apparently clearcut racial distinction between Europeans and West African Negroes has also become more complex and less clear with publications on the incidence of the haptoglobins in Swedish Lapps;[15] in Japanese populations;[16] and in Malays, Chinese, and Indians in Malaya.[17]

TABLE I. DISTRIBUTION OF HAPTOGLOBIN TYPES IN VARIOUS POPULATIONS

	No.	Gene frequency		HpO	References
		Hp^1	Hp^2		
Europe					
Basques (Spain)	107	0·39	0·61	0·01	13
British	218	0·38	0·62	0·03	13
British	114	0·42	0·58	—	10
Swedish Lapps	329	0·28	0·72	0·02	15
Finns	891	0·36	0·64	0·002	35
Danes	1,033	0·40	0·60	—	9
Norwegians	1,000	0·36	0·64	—	36
Swedes	1,003	0·37	0·63	0·03	37
Italians	466	0·38	0·62	0·01	10
French	406	0·40	0·60	—	38
Americas					
Alaskan Eskimoes ..	418	0·29	0·71	—	12
Anaktuviak Eskimoes ..	57	0·50	0·50	0·04	12
Athabascan Indians ..	284	0·42	0·58	0·01	12
Apache Indians	98	0·59	0·41	—	14
US Whites	68	0·43	0·57	—	14
US Whites	54	0·38	0·62	—	14
Canadian Whites	49	0·44	0·56	—	5
US Negroes	48	0·59	0·41	0·10	14
US Negroes	760	0·54	0·46	0·04	39
Venezuelans (Caracas) ..	208	0·55	0·45	—	40
Central American Non-Maya	170	0·57	0·43	0·01	41
Central American Maya (Less Lacandon) ..	414	0·59	0·41	—	41
Central American Lacandon	31	0·93	0·07	0·10	41
Peruvian Indians	173	0·73	0·27	—	48
Africa					
Yoruba (Nigeria)	99	0·87	0·13	0·32	13
Yoruba (Nigeria)	30	0·72	0·18	0·23	10
Habe (Nigeria)	120	0·60	0·40	0·27	46
Fulani (Nigeria)	111	0·76	0·24	0·37	46

* Read in modified form at the Conference on Biochemical Anthropology held at Yellow Springs, Ohio, United States of America, in July 1960.

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Liberia and Ivory Coast Negroes	142	0·70	0·30	—	21
Liberia and Ivory Coast Negroes	614	0·72	0·28	—	14
Gambia	157	0·70	0·30	0·40	11
Ibo	70	0·49	0·51	0·48	11
Metropolitan Congo	186	0·60	0·40	0·05	42
Non-metropolitan Congo	468	0·57	0·43	0·21	42
Pygmy (Congo)	125	0·40	0·60	0·31	42
Zulus (South Africa)	113	0·53	0·47	0·03	18
Bushmen (South Africa)	113	0·29	0·71	0·02	18
Hottentots (South Africa)	59	0·51	0·49	—	18
Cape Coloured (South Africa)	88	0·47	0·53	—	18
Xhosa and Msutu (South Africa)	315	0·55	0·45	0·05	43
Asia and Oceania					
Malaya-Malays	236	0·24	0·76	0·01	17
Malaya-Chinese	167	0·28	0·72	0·01	17
Malaya-Indians	219	0·09	0·91	0·02	17
Asiatic Indians	74	0·18	0·82	—	14
Japanese (Sapporo)	349	0·24	0·76	0·01	16
Micronesians (Marshall Islands)	52	0·58	0·42	—	47
Borneo	22	0·50	0·50	—	11
Tongans	200	0·60	0·40	—	44
Australian Aborigines	133	0·17	0·83	0·01	45

Before discussing the current trends in the interpretation of and the difficulties in relation to the thus far very limited and discrepant African series, some information will be presented on the haptoglobin distribution in Bushmen, Hottentots, Cape Coloureds and Zulus, based on the field work of Dr. J. S. Weiner and myself, and published in collaboration with Drs. N. A. Barnicot and J. P. Garlick in *Nature* in December 1959.[18]

Blood samples were collected from (1) Naron Bushmen living their traditional hunting and food-collecting life and from Bushmen employed on farms near Khanzi in Bechuanaland; (2) Nama-speaking Hottentots or Khoi-Khoi in the Richtersveld region of Namaqualand; (3) Cape Coloureds in the Springbok and Steinkopf areas of Namaqualand, whose gene pool consists essentially of Caucasoid (Dutch, German and English) and Hottentot components with

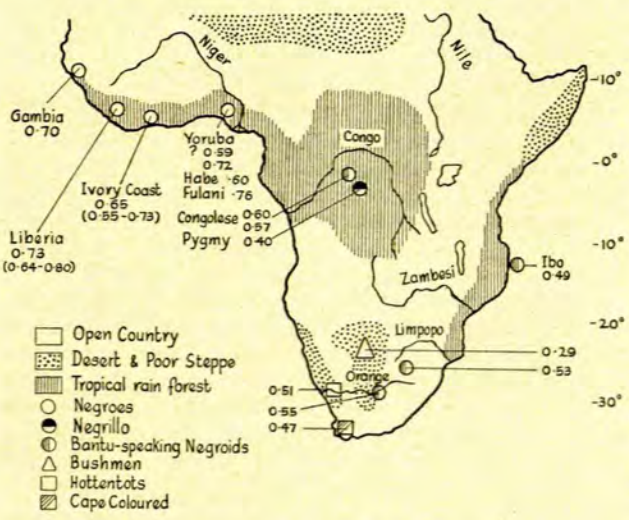

Fig. 1. Map of Africa: Frequency of Allele *Hp*.[1]

possibly slight Bushman and minimal, if any, Negroid admixture; and (4) Zulus working in Johannesburg.

The techniques used on the sera by Drs. Barnicot and Garlick were essentially those outlined by Smithies[6,19] and by Poulik.[20]

The most striking feature is the low frequency of the haptoglobin Hp^1 in the Bushmen as compared with the other 3 South African series, which are closely similar to one another in this respect. The Hp^1 frequency in the Zulus, Cape Coloured and Hottentots is lower than the lowest limit of the range reported for Negroes from Liberia and the Ivory Coast and the Nigerian Yoruba.[13,14,21] No haptoglobins were located in 4 Bushmen and 3 Zulus. These were excluded in the calculation of the gene frequencies since the genetical significance of this phenotype, which thus far is very common only among the Yoruba and some Liberians, is still not clear. Some typical examples of the modified 2 - 1 phenotype (2 - 1M) were found in the Zulu sera, but not in the other populations. However, in the Bushmen and Coloureds a few sera were noted in which the slower $\alpha\beta$ bands were relatively weak although the Hp^1 band was somewhat weaker than the first $\alpha\beta$ band.

TABLE II. THE INCIDENCE AND GENE FREQUENCY OF HAPTOGLOBIN PHENOTYPES IN SOME SOUTH AFRICAN PEOPLES

		Haptoglobin phenotypes					*Gene frequency*	
	No.	*1-1*	*2-1*	*2-1M*	*2-2*	*0-0*	Hp^1	Hp^2
Bushmen:								
(*a*) Tribal	71	7	29	0	34	1		
(*b*) Farms	42	5	11	0	25	1		
Total	113	12	40	0	59	2	0·29	0·71
Hottentots:								
(*a*) Random	34	10	16	0	8	0		
(*b*) Related	25	8	9	0	8	0		
Total	59	18	25	0	16	0	0·51	0·49*
Cape Coloured	88	17	49	0	22	0	0·47	0·53
Zulus	113	36	45	3	29	3	0·53	0·47

* Standard error=0·05

Information concerning the distribution of serum-protein variants in Africa is still very limited, and our material represents only a fraction of the widely dispersed Bantu, Bushman, and Hottentotoid peoples. Consequently, only tentative opinions rather than conclusive assessments are feasible.

Bushmen are distinct from other African populations both in morphology and blood-group frequencies,[22,23] and it appears that their haptoglobin frequencies may also be unusual. It is interesting that the general serum-protein pattern of the Okavango Bushman and Bantu tribes, as determined during the University of Cape Town 1952 Expedition, is quite distinct from the 'normal' pattern for urbanized Bantu groups in Cape Town.[24] Furthermore, the so-called 'abnormal' pattern of the Bushmen (which is probably normal for the Okavango and adjacent Kalahari regions), is related directly to the distance of their usual habitat from the Okavango River and its associated malaria and bilharzia. From this point of view it seems that there is an advantage in hunting and food-collecting in the desert away from the rivers.[25]

The Hottentots are generally thought to be closely related to the Bushmen on certain morphological and linguistic grounds, but substantial blood-group differences between them are known,[26,27] and it seems that the haptoglobins may be another point of distinction. It is doubtful whether any sizeable Nama-speaking Hottentot groups survive who are free from European ancestry (my estimate is about 2,000 individuals in the Richtersveld and adjacent regions). Intermixing with Dutch, German, and English has led to the formation of various 'hybrid' Coloured communities. From what is known of European haptoglobin frequencies, however, this would have made them more like the Bushmen in this respect, so that the earlier Hottentots may have been more distinct. The effect of intermixture with the Malays, brought to the Cape of Good Hope as skilled artisans in the 18th century, cannot at present be assessed. There is probably extremely little Malay admixture in those Cape Coloureds tested. It is interesting to note that in Malaya the Malays exhibit a gene frequency of 0·24 for Hp^1, which is slightly lower than that (0·28) of the Malayan Chinese, but significantly higher than the 0·09 of the Malayan Indians.[17] Asiatic Indian students studying in the USA have a frequency of 0·18.[14]

A slow transferrin variant was found to be quite common in the Bushmen, and one serum which showed this variant alone was identified (by Dr. O. Smithies) as βD_1 which was first described by Smithies[28] in Australian populations and American Negro sera, and is fairly common in some West African populations.[29] The variant is evidently less frequent in the Zulus and Cape Coloureds, and perhaps in the Hottentots, than in the Bushmen.

The distribution of the haptoglobins in a small region of West Africa shows a marked discrepancy for the frequency of haptoglobin deficiency or O-O, as indicated in the results of Allison *et al.*,[13] on the one hand, and Sutton *et al.*[21] on the other. In one-third of 99 Yoruba studied by Allison *et al.*,[13] haptoglobin was deficient. Most of the remainder were type 1. Sutton *et al.*[21] did not report any ahaptoglobinaemia in their series from Liberia and the Ivory Coast. In a subsequent study, Sutton *et al.*[14] found that the presence of considerable haemolysis interfered with the detection of 1 - 1, so that their diagnosis of 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. They state that probably some of these 1 - 1 individuals actually do not have detectable amounts of haptoglobin. For this reason their frequencies of Hp^1 (in their survey) represent maximum values. Allison *et al.*,[13] in comparing their data with the absence of ahaptoglobinaemia in the 1956 study[21] of Sutton *et al.*, attributed it to inclusion of group O-O in the 1 - 1 group, because the distinction between these two groups is difficult without specific staining of haemoglobin. However, if one takes Sutton *et al.*'s results[21] and assumes an approximate one-third of ahaptoglobinaemics, the frequency for Hp^1 would be reduced from 0·70 and 0·72 to 0·54 and 0·52, which brings the Ivory Coast and Liberia Negroes into line with Zulus and Venezuelans, and would bring their frequency lower than that of US Negroes, Apache Indians, and Micronesians. It would appear that the Hp^1 frequency, as seen in the Yoruba, has not been affected by the Caucasoid dilution in the US Negroes. The highest incidence of haptoglobin deficiency, other than in the Yoruba and Sutton's third series of Liberians, is only 4·2% in US Negroes, out of more than 33 world-wide surveys reported, in which 13 out of 33 displayed no ahaptoglobinaemia, and in the remainder the mean is less than 2% per population group.

It must be interesting to break down all the West African data to tribal distribution, and study their inter-relationships afresh. Certainly the significance of a 32% haptoglobin deficiency in the Yoruba is not at all obvious. Allison *et al.*,[13] in noting the difference between this figure and Sutton *et al.*'s data[14] for Liberia and Ivory Coast, stated that the discrepancy may represent a difference between Africans from these different regions, but they excluded this in preference to an explanation of difference in laboratory techniques. However, it should be noted that the haemoglobin *S* frequency in Liberians is relatively low compared with that in Nigeria, and that the haemoglobin *C* frequency is relatively low in Nigeria and in Liberia, and about the same cline. Whatever produces the discrepancy in O-O, it is not a constant in relation to abnormal haemoglobins. Haptoglobins are not present in most newborns,[9] but absence is unusual after the age of 4 months. Deficiency in the adult represents either a persistence of what could be called the foetal state, or a secondary loss from various causes. If haptoglobin deficiency is related to malaria somehow, one would expect a close correlation with high frequencies of sickle-cell homozygotes on the basis of the theory that the heterozygotes constitute an advantageous selection.

Ahaptoglobinaemia may occur for several reasons. Laurell and Nyman[30] have shown that, if the circulation is saturated with haemoglobin, as in acute haemolytic conditions, the plasma can be completely depleted of haptoglobins in 24 hours. It is possible that chronic haemolytic conditions, so common in Africa, may produce a similar picture. In the Japanese population of Sapporo, studied by Matsunaga and Murai,[16] 4 of the 5 haptoglobin-deficient cases had no history of anaemia or liver disease. The 5th had a history of hepatitis. They also found haptoglobin deficiency in 4 patients with liver cirrhosis, one case having liver metastases. Haptoglobins were also scarcely recognizable in 1 of 3 patients with hepatoma. Haptoglobin deficiency has also been observed in European and American Negro groups in whom there was neither evidence of a haemolytic condition nor a history of malaria. With respect to haptoglobin synthesis and the gene or genes controlling this, it is worthy of note that the liver in the African Negro has a rather unique metabolism (whether inherent or acquired, or both, is not certain), but African Negroes very commonly suffer liver cirrhosis and for some reason, probably related, have a high incidence of primary carcinoma of the liver, which is rare in other racial and geographic groups.

Giblett[31] pointed out that inheritance studies of ahaptoglobinaemia have so far been inconclusive. However, in some families, both parents of a subject with haptoglobin deficiency appear to carry a pair of normal haptoglobin genes. Thus it is clear that if a mechanism is involved, the responsible gene cannot be an allele at the *Hp* locus, nor can it be a Mendelian dominant. It has been suggested that the Hp O-O phenotype may be due to a modifying gene at a separate locus, or alternatively represents the homozygous condition for the Hp^{2M}. Thus it seems that in haptoglobin

deficiency one may encounter a primary, genetically-determined ahaptoglobinaemia and/or a secondary, acquired, disease-produced and temporary ahaptoglobinaemia. A solution to the significance of this phenomenon will only become possible when a laboratory-tested distinction between the two forms may be elicited. The inherited form does not appear to be controlled by alleles at the usual haptoglobin locus.[12,14] Some ahaptoglobinaemics possess small amounts of normal haptoglobin.[14]

It has been suggested[13] that the true polymorphism exhibited by the haptoglobins may have been maintained, like those of the blood groups and abnormal haemoglobins, by a balance of selective forces related to susceptibility to disease. They state that the absence of haptoglobins may be disadvantageous, particularly in regions where there is a deficiency in the diet. That so many Yoruba lack haptoglobins is presumably due to a compensatory advantage in persons heterozygous for the factor concerned. The low frequency of type 2 - 2 in the tropics was felt to confer protection against one or more conditions common in temperate, but not in tropical climates. However, the evidence available today does not obviously confirm these hypotheses, which are based on insignificant sampling of tropical areas, because other tropical areas also give high frequencies of type 2 - 2. It is clear that correlative studies with distributional incidence of schistosomiasis, yaws, onyalai, malaria, kalar-azar, helminthiases, etc. may provide some clue to selective advantage, but on the present evidence this is quite imperceptible.

Comparative anatomical studies have not yet helped in determining a positive direction towards the nature of the selective advantage of one or other form of haptoglobin in maintaining the balanced polymorphism. A number of investigations on non-human primates[33,34] indicate that all the animals studied had an electrophoretic band in the approximate position of haptoglobin 1 - 1. None had anything resembling the human 2 - 1 or 2 - 2. It would appear that type Hp1 - 1 is the primary or primitive type from which present types have mutated, and that a mutation occurred after *Homo* or his immediate ancestors had moved away from the Pongid line. The gene's favourable spread into various populations was limited by various factors, as yet unknown. It is at present not possible to assess whether such a mutation actually promoted hominid evolution in one or other direction. It appears reasonable to assume that the possession of the Hp^1 gene is an advantage among hominoids, at least.

TABLE III. RACIAL (ETHNIC) GROUPING ACCORDING TO FREQUENCY OF ALLELE Hp^1

Hp^1 frequency	
$<0{\cdot}25$	Malays, Asiatic Indians, Japanese
$0{\cdot}26-0{\cdot}40$	Swedish Lapps, Finns, Italians, Bushmen, Chinese, Alaskan Eskimoes, Danes, French, Swedes, US Caucasoids, Spanish Basques, British (Oxford), Norwegians, Pygmies (Congo)
$0{\cdot}41-0{\cdot}55$	Anaktuviak Eskimoes, Athabascan Indians, Canadian Caucasoids, Venezuelans (Caracas), British, North Italians, Cape Coloured, Zulus, Xhosa and Msutu
$0{\cdot}56-0{\cdot}70$	Apache Indians, U.S. Negroes, US Caucasoids, Congolese, Hottentots, Micronesians (Marshall Islands)
$>0{\cdot}70$	Liberia and Ivory Coast Negroes (mean), Yoruba (Nigeria)

If one takes the range of variation of Hp^1 within the Liberian and Ivory Coast tribes,[14] and uses this range arbitrarily for dividing up the racial and ethnic populations studied in respect of Hp^1, one finds a rather curious grouping, the significance of which is not apparent. But it should make us wary of the pitfalls inherent in over-hypothesizing and over-emphasizing inadequate data.

In conclusion, it seems that in the study of haemoglobin-binding proteins (haptoglobins), as well as the iron-binding serum proteins (transferrins), we have a rather useful genetic mechanism for marking out racial and ecologically-induced differences, and for indicating possible selective adaptations in man. The usefulness of such studies cannot yet be assessed in Africa because of the inadequacy of the available, limited data.

Grants from the Wenner-Gren Foundation for Anthropological Research, Inc., New York and the Dr. C. Herman Research Fund, University of Cape Town, made it possible for the field work to be carried out.

REFERENCES

1. Polonovski, M. and Jayle, M. (1938): C.R. Soc. Biol. (Paris), **129**, 457.
2. Jayle, M. F. (1939): Bull. Soc. Chim. biol. (Paris), **21**, 14.
3. *Idem* (1951): *Ibid.*, **33**, 876.
4. Jayle, M. F. and Boussier, G. (1935): Expos. ann. Biochim. méd., **17**, 157.
5. Allison, A. C. and Blumberg, B. S. (1958): Arthritis Rheum., **1** (3), 239.
6. Smithies, O. (1955): Biochem. J., **61**, 629.
7. Smithies, O. and Walker, N. F. (1956): Nature (Lond.), **178**, 694.
8. *Idem* (1955): *Ibid.*, **176**, 1265.
9. Galatius-Jensen, F. (1957): Acta genet. (Basel), **7**, 549.
10. Harris, H., Robson, E. B. and Siniscalco, M. (1959): *Ciba Foundation Symposium on Medical Biology and Etruscan Origins*. Boston: Little, Brown & Co.
11. *Idem* (1959): *Ciba Foundation Symposium on Biochemistry of Human Genetics*, p. 114. London: Churchill.
12. Blumberg, B. S., Allison, A. C. and Garry, B. (1959): Ann. Hum. Genet., **23**, 349.
13. Allison, A. C., Blumberg, B. S. and Rees, W. (1958): Nature (Lond.), **181**, 824.
14. Sutton, H. E., Neel, J. V., Livingstone, F. B., Binson, G., Kunstadter, P. and Trombley, L. E. (1959): Ann. Hum. Genet., **23** (2), 175.
15. Beckman, L. and Mellbin, T. (1959): Acta genet. (Basel), **9**, 306.
16. Matsunaga, E. and Murai, K. (1960): Nature (Lond.), **186**, 320.
17. Kirk, R. L., Lai, L. Y. C., Mahmood, R. and Singh, R. B. (1960): *Ibid.*, **185**, 185.
18. Barnicot, N. A., Garlick, J. P., Singer, R. and Weiner, J. S. (1959): *Ibid.*, **184**, 2042.
19. Smithies, O. (1959): Biochem. J., **71**, 585.
20. Poulik, M. D. (1957): Nature (Lond.), **180**, 1477.
21. Sutton, H. E., Neel, J. V., Binson, G. and Zuelzer, W. W. (1956): *Ibid.*, **178**, 1287.
22. Weiner, J. S. and Zoutendyk, A. (1959): *Ibid.*, **183**, 843.
23. Zoutendyk, A., Kopec, A. C. and Mourant, A. E. (1953): Amer. J. Phys. Anthrop., **11**, 361.
24. Brock, J. F. and Bronte-Stewart, B.: Personal communication.
25. Singer, R. (1960): Z. Morph. Anthrop., **51** (1), 1.
26. Zoutendyk, A., Kopec, A. C. and Mourant, A. E. (1955): Amer. J. Phys. Anthrop., **13** (4), 691.
27. Weiner, J. S. and Singer, R. In preparation.
28. Smithies, O. (1957): Nature (Lond.), **180**, 1482.
29. Giblett, E. R., Hickman, C. G. and Smithies, O. (1959): *Ibid.*, **183**, 1589.
30. Laurell, C. B. and Nyman, M. (1957): Blood, **12**, 493.
31. Giblett, E. R. (1959): Nature (Lond.), **183**, 192.
32. Galatius-Jensen, F. (1958): Acta genet. (Basel), **8**, 232.
33. Arends, T. and Gallango de Rodriguez, M. L. (1960): Nature (Lond.), **185**, 325.
34. Blumberg, B. S. (1960): Proc. Soc. Exp. Biol. (N.Y.), **104** (1), 25.
35. Mäkelä, O., Eriksson, A. W. and Lehtovaara, R. (1959): Acta genet. (Basel), **9**, 149.
36. Fleischer, E. and Lundevall, J. (1958): *Op cit.*[30]
37. Tarukoski, P. H. (1959): Nord méd., **62**, 1425.
38. Moullec, J. and Fine, J. M. (1959): Nature (Lond.), **184**, 196.
39. Giblett, E. R. (1960): *Op cit.*[31]
40. Gallango de Rodriguez, M. L. and Arends, T. (1959): Nature (Lond.), **183**, 1465.
41. Sutton, H. E., Matson, G. A., Robinson, A. R. and Koucky, R. W. (1960): Amer. J. Hum. Genet., **12** (3), 338.
42. Motulsky, A. G. and Giblett, E. R. (1960): *Op cit.*[41]
43. Giblett, E. R. and Zoutendyk, A. (1960): *Op cit.*[42]
44. Douglas, R. and Staveley, J. M. (1960): *Op cit.*[41]
45. Kirk, R. L. (1959): *Op cit.*[41]
46. Barnicot, N. A., Garlick, J. P. and Roberts, D.F. (1960): Ann. Hum. Genet., **24**, 171.
47. Blumberg, B. S. (1959): *Op cit.*[41]
48. Giblett, E. R. and Best, W. (1960): *Op cit.*[41]