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The frequency in Japanese of genetic variants of 22 proteins

I. Albumin, Ceruloplasmin, Haptoglobin, and Transferrin

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During 1972–5 aliquots of whole blood specimens collected in the course of the research program of the Atomic Bomb Casualty Commission (now the Radiation Effects Research Foundation) were processed for the occurrence of electrophoretic variants of 18 erythrocyte proteins and 4 serum proteins. This investigation was undertaken in connexion with a pilot study to determine the feasibility of carrying the appraisal of the potential genetic effects of the atomic bombs to the protein level. However, since the above-mentioned individuals were all born prior to the bombings, the possibility of genetic effects of the bombs does not enter into the findings of this investigation. The present series of papers will describe the results of the survey, as a contribution towards delineating the frequency and types of variation of these proteins in the Japanese population. This paper will present the findings with respect to albumin (ALB), ceruloplasmin (CRPL), haptoglobin (HP), and transferrin (TF). The following three papers will present results for the 18 erythrocyte proteins. Throughout the four data papers we shall routinely compare our findings with the results of other similar studies on Japanese. However, of the similar studies on other ethnic groups, only those on inhabitants of the British Isles and Western Europe will be routinely cited. In the final paper in the series the thesis will be developed that there are such similarities between the histories of the Japanese and British Islands as to make this comparison especially appropriate, and that such comparisons permit tentative inferences concerning similarities and differences in the mutation rates of the structural genes encoding for the proteins in question.

THE SAMPLE

The individuals upon whom this study is based are members of the Adult Health Study being carried out in Hiroshima and Nagasaki by the Radiation Effects Research Foundation. A description of this study, basically an ongoing medical surveillance, can be found in Hollingsworth *et al.* (1962) and Belsky, Tachikawa & Jablon (1973). The blood samples for these analyses were aliquots of samples drawn at the time of biennial physical examinations. Since the number of persons examined weekly in connexion with the Adult Health Study was in excess of the

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capacity of our laboratory to process samples, priority was given to those samples obtained from the parents of children included in the previous genetic studies of the Radiation Effects Research Foundation (Neel & Schull, 1956; Neel, Kato & Schull, 1974). Otherwise the selection of study samples from members of the Adult Health Study was essentially at random. The participants in the Adult Health Study are a group who received relatively large doses of radiation at the time of the atomic bombs and a matched control group who received little or no radiation exposure at that time. Although the two groups are largely composed of unrelated persons, the manner of selection of the groups did allow for some biologically related persons in the sample.

MATERIALS AND METHODS

The primary determinations were made on fresh plasma from whole blood, drawn in a mixed ammonium–potassium oxalate anticoagulant, between 8 August 1972 and 31 August 1975. Aliquots of each sample were stored at −70 °C and also in liquid nitrogen. When repeat determinations were necessary the fresh sample was first exhausted, then recourse was had to the sample stored at −70 °C, then to the sample in liquid nitrogen.

Ceruloplasmin, haptoglobin and transferrin were typed using vertical starch-gel electrophoresis on electrostarch (Electro-Starch Co., Madison, Wisconsin, lots 146 and 371) at 4 °C, as described by Smithies (1959) and modified by Weitkamp *et al.* (1972). Albumin was routinely typed using the pH 5·0 sodium acetate system of Weitkamp *et al.* (1969). Some comparison runs were made using the pH 6·9, tris-EDTA-borate system reported by the same authors and the pH 5·3 system of Arends *et al.* (1969). Albumin and transferrin were stained with Amido Black 10B, and haptoglobin was stained using 1% benzidine in 1·5 M sodium acetate, pH 4·47, followed by 3% hydrogen peroxide, or by the o-dianisidine procedure of Owen, Silberman & Got (1958). Ceruloplasmin was detected by the method of Shreffler *et al.* (1967). Critical comparisons of suspected variants of transferrin and ceruloplasmin were carried out using polyacrylamide gel electrophoresis (Beckman Microzone System, Beckman Instruments Co., Palo Alto, California), 7% acrylamide, 0·036 M tris-glycine, pH 8·4. Transferrin variants were also compared using the 0·096 M tris-glycine, pH 8·8, and the 0·005 M tris-borate, pH 6·5, polyacrylamide gel systems suggested by Sutton & Jamieson (1972), with samples of standard transferrin C–D_O and C–D_{Chi} provided by Dr H. E. Sutton (Department of Zoology, University of Texas, Austin, Texas). However, the rivanol precipitation step was omitted from our procedure.

As the number of reported rare variants in any system increases so does the problem of nomenclature. This is especially true with respect to the transferrin variants to be described in this study. For all systems, we have identified each variant which did not clearly correspond to a known type, in order of discovery and by the city where the variant was first observed, with abbreviations of HIR for Hiroshima and NGS for Nagasaki. Because the sample from Hiroshima was approximately twice the size of the sample from Nagasaki, electrophoretically indistinguishable variants which occur in both populations were generally encountered first in Hiroshima. When the electrophoretic identity of a variant encountered in Hiroshima or Nagasaki with a previously described variant was established to our satisfaction, the temporary terminology was abandoned and the previous terminology adopted. While we are aware that identical electrophoretic mobility for two variants does not mean chemical identity, we see no alternative to this procedure at the present time. Where for a system conventional nomenclature involves an electrophoretic classification, we have followed it.

In any study of this nature, there are always a few patterns which for a variety of reasons cannot be typed with confidence, even after repeated efforts. We omit these from the figures for 'numbers typed', recognizing that this group has a disproportionately high probability of containing poorly resolved variants.

FAMILY STUDIES

Throughout this work an effort was made to establish the genetic nature of each variant as it was encountered. However, some individuals with a variant protein had no relatives in the immediate vicinity of Hiroshima or Nagasaki, or did not desire their immediate relatives contacted. Furthermore, personnel shortages precluded extensive family studies. Therefore, the general philosophy was adopted of pursuing family studies only until one other family member with the variant was encountered, or until all the immediately available relatives had been found to be negative. However, because in some family studies samples were obtained from several relatives in the course of a single house call, we do have some instances of multiple affected family members in addition to the propositus. The family data will be presented in tabular fashion in the course of the description of the findings for each system.

RESULTS

Albumin

During the course of this investigation, among 4029 examinations, 10 individuals, 5 from Nagasaki and 5 from Hiroshima, were encountered who showed an albumin pattern with both the normal and a more cathodal migrating (slow) band. All 10 of these cases showed identical mobility patterns in the routine screening system and the two alternative methods already described. The variant was first observed in an individual from Nagasaki and therefore has been designated albumin Nagasaki 1 (NGS 1). We were able to compare this variant with two previously described albumin variants from Japanese populations, namely albumin Kyoto and albumin Otsu, for which some comparative data are available (Weitkamp, Yamamoto & Nishiyama, 1974). Fig. 1 shows the results of this comparison as well as a comparsion with three Amerindian variants, namely albumin Naskapi (Melartin, Blumberg & Martin, 1968), albumin Wapishana (Tanis *et al.* 1973) and albumin Yanomama 2 (Tanis *et al.* 1973). Albumin Nagasaki 1 is clearly different from the two available Japanese variants, as well as albumin Naskapi and albumin Wapishana. Although not clearly shown in Fig. 1, albumin NGS 1 tends to migrate more slowly than albumin Yanomama 2. (The degree of separation of Yanomama 2 from normal at a given pH varies with the lot number of the starch; resolution is poor in this figure but see Tanis *et al.* 1974.) Albumin NGS 1 is also clearly different from albumin Yanomama 2 and albumin Otsu when compared using the pH 6·1 gel system of Tanis *et al.* (1974). Limited family studies were carried out for 9 of the 10 cases, and the presence of the variant albumin pattern was observed in at least one first-degree relative in 7 of these cases (see Table 1). There seem to be no surveys on Japanese for the frequency of albumin variants comparable to this one.

Because an electrophoretically identical rare variant was observed in several individuals drawn from a random population, it was of interest to ascertain whether these individuals were related. For 7 of the 10 cases, it was possible to construct a genealogy including at least the three generations preceding that in which the variant was discovered; for no two cases could a common ancestor be demonstrated. The remaining three cases have not been investigated to date.

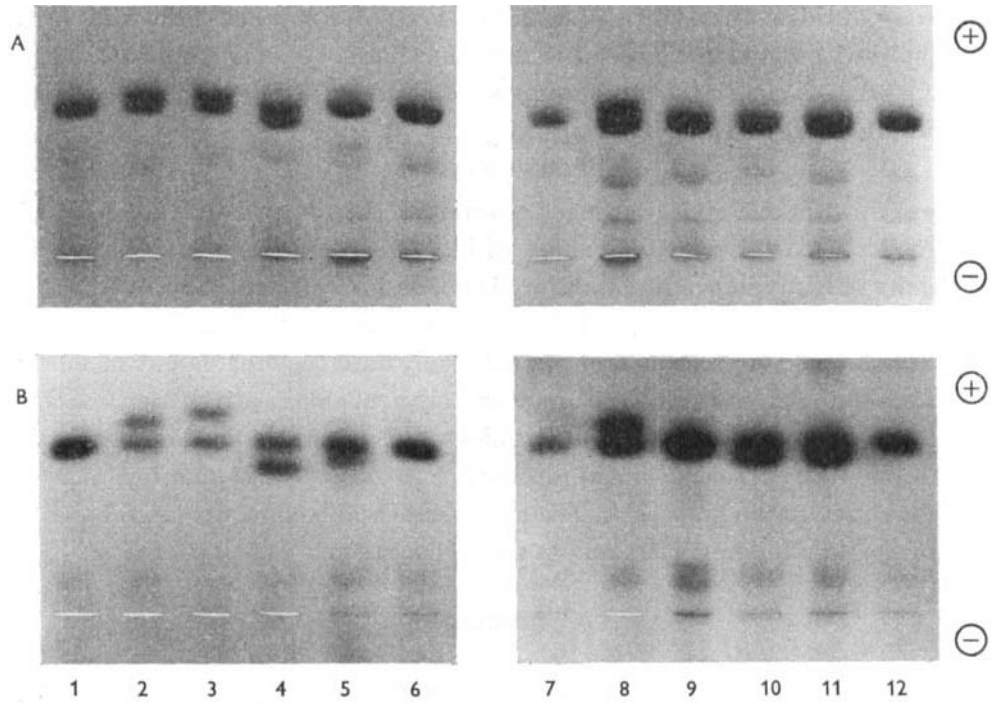

Fig. 1. Starch gel electrophoresis of albumin variants in two buffer systems: (A) Tris-EDTA-borate, pH 6·9; (B) sodium acetate system, pH 5·0. Wells 1, 6, 7, 9 and 12, normal albumin; well 2, N-Naskapi; well 3, N-Kyoto; well 4, N-Otsu; well 5, N-Yanomama 2; well 8, N-Wapishana; wells 10 and 11, N-NGS 1.

Ceruloplasmin

In this survey we have encountered two electrophoretic variants of plasma ceruloplasmin in 4026 individuals tested (2652 from Hiroshima and 1374 from Nagasaki). Three specimens could not be typed. Both of these variants occurred in individuals from the Nagasaki sample who also possessed the normal B phenotype, and have been designated ceruloplasmin $A_{\text{Nagasaki 1}}$ ($A_{\text{NGS 1}}$), for a sample showing a band migrating anodal to the normal B band, and ceruloplasmin $C_{\text{Nagasaki 1}}$ ($C_{\text{NGS 1}}$) for a sample showing a band migrating cathodal to the normal B band. We have not encountered any previous reports of ceruloplasmin variants in the Japanese.

Comparisons of the variants encountered in this study have been made with the A–B and B–C ceruloplasmin types encountered in American Negro populations by Shreffler *et al.* (1967). The results of this comparison are shown in Fig. 2A. As can be seen from this comparison, the two Japanese variants are clearly different from the ceruloplasmin variants from Negro populations. The $A_{\text{NGS 1}}$ variant appears to be electrophoretically similar to the A_{Cayapo} variant reported by Tanis *et al.* (1973) in South American Indian populations. The $C_{\text{NGS 1}}$ variant appears similar to the New Haven variant reported by Shokier & Shreffler (1970) (see also Giblett, 1969). No direct comparison with these variants was possible. Family studies could not be carried out on these variants.*

* Recently, $A_{\text{NGS 1}}$ was found to be present in the son of the propositus.

Table 1. *Summary of family studies for rare electrophoretic variants of albumin, haptoglobin and transferrin*

(The asterisk indicates the first family member to be contacted; solid symbol ♂ or ♀ indicates heterozygous presence of a variant.)

System	Variant	City	Propositus	Sex	Family studies: Mo	Fa	Sibs	Children
Albumin	NGS 1	N	000900	M	–	–	*♂♀	–
		N	034406	F	–	–	–	*♀
		N	091578	F	*♀	–	♂	♀♀
		H	203812	F	–	–	–	*♀♂
		H	203934	F	–	–	–	*♂
		H	296477	M	*♀	–	–	♂
		N	055736	M	–	–	–	*♀
		N	157425	F	♀	*♂	♀	–
		H	234199	F	–	–	–	*♂
Haptoglobin	$2_{HIR\ 1}$	H	202618	F	–	–	–	*♂
	$2_{NGS\ 1}$	N	013212	F	–	–	–	*♂♀
Transferrin	$D_{HIR\ 1}$	N	090457	F	–	–	–	*♂♀
	$D_{HIR\ 2}$	H	214801	F	–	–	–	*♂
	$D_{HIR\ 3}$	H	844205	M	–	–	–	*♀
		H	206921	M	–	–	–	*♀
		H	349608	F	–	–	*♀	–
	$D_{NGS\ 1}$	N	001842	M	–	–	–	*♂♂♀
	B_3	H	204257	M	–	–	–	*♂♂
		H	214501	F	–	–	–	*♂♂
		H	326266	F	–	–	*♀	–
		H	331469	F	–	–	–	*♀♂
		N	009831	F	–	–	–	*♂
	$B_{HIR\ 2}$	H	263809	F	–	–	–	*♂
		H	278094	M	–	–	–	*♂
		H	280300	M	–	–	–	*♂
		H	843778	F	–	–	–	*♂
		N	024386	F	–	–	*♀♂♂	♂
		H	363703	M	–	–	–	*♀
	$B_{HIR\ 4}$	H	204342	F	–	–	–	*♀

* First primary relative tested in the family study.

Haptoglobin

The well-known haptoglobin polymorphism due to the HP^1 and HP^2 alleles has been described in a large number of Japanese populations [summarized in Omoto (1975)]. The phenotypes and gene frequencies encountered in our screening of 3447 individuals are shown in Table 2. There is no significant departure from Hardy–Weinberg proportions in either city, and the gene frequencies for HP^1 do not differ between Hiroshima and Nagasaki. The gene frequencies for the HP^1 allele fall within the narrow range of 0·23–0·29 observed in other Japanese populations. The HP^1 frequency of 0·255 obtained for the Nagasaki sample is in agreement with the frequency reported in two previous studies in Nagasaki Prefecture (Ishimoto & Kuwata, 1973; Ohya, 1972). Although several samples failed to exhibit a haptoglobin pattern following starch gel electrophoresis, no effort was made to determine whether this was due to the presence of an HP^0 allele or to some non-genetic factor. Ogawa & Kawamura (1965) have reported the presence of a haptoglobin variant similar to haptoglobin Carlberg, first reported by Galatius-Jensen (1958),

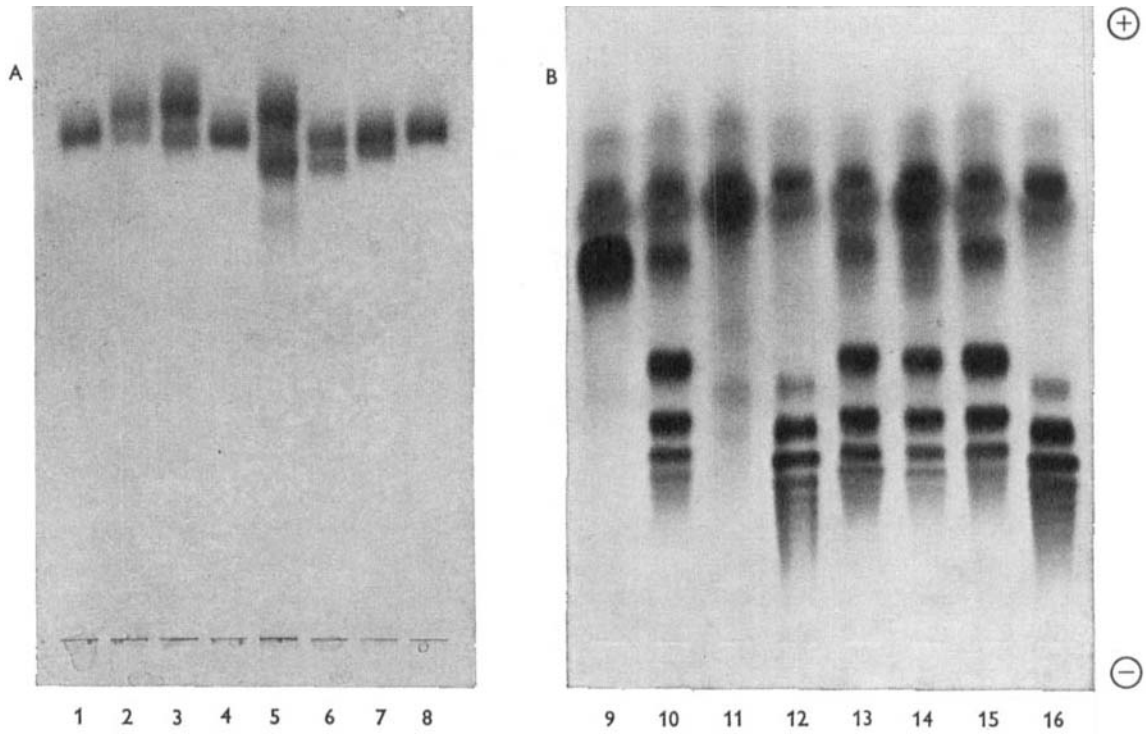

Fig. 2. (A) Polyacrylamide gel electrophoresis of Japanese ceruloplasmin variants, and comparison with previously reported variants. Wells 1, 4 and 8, normal ceruloplasmin B; well 2, type B–A from Negro populations; well 3, type B–$A_{NGS\ 1}$; well 5, type A–C from Negro populations; well 6, type B–C from Negro populations; well 7, type B–$C_{NGS\ 1}$. (B) Starch gel electrophoresis of haptoglobin variants from Hiroshima and Nagasaki. Well 9, HP 1; wells 10 and 15, HP 1–2; wells 12 and 16, HP 2; well 11, HP 1–$2_{HIR\ 1}$; well 13, HP 1–$2_{NGS\ 1}$, son of the propositus; well 14, HP 1–$2_{NGS\ 1}$, propositus (013212).

Table 2. *Phenotypes and gene frequencies for the haptoglobin polymorphism in Hiroshima and Nagasaki*

	Phenotype						Gene frequency†	
City	1	2–1	2	Variants	No type*	Total	HP^1	HP^2
Hiroshima	132	764	1165	1	11	2073	0·249	0·751
Nagasaki	98	500	767	1	8	1374	0·255	0·745
Total	230	1264	1932	2	19	3447	0·252	0·748

* These samples gave no haptoglobin pattern or a pattern too weak to be typed accurately.
† Calculation of gene frequencies excludes no-types and variants.

in 10% of the unrelated outpatients seen by Kyushu University Hospital. These results were obtained using polyacrylamide gel electrophoresis, and it is probable that this haptoglobin type would not have been detected by the starch gel method employed in the work reported here.

A single individual from the Hiroshima sample exhibited a variant haptoglobin 1 pattern, designated HP $1_{HIR\ 1}$. The variant pattern was characterized by a reduced staining intensity and an anodal shift of the bands characteristically present in an Hp 1–2 phenotype. This pattern is shown in Fig. 2B, well 11, and resembles closely the haptoglobin 2–H reported by Robson *et al.* (1964). In the figure, the anodal shift of the first Hp band is obscured by the presence of haemo-

Table 3. *Phenotype and gene frequencies for transferrin variants seen in Hiroshima and Nagasaki*

	Hiroshima	Nagasaki	Total
C	2580	1356	3936
$C\text{–}D_{Chi}$	36	11	47
$C\text{–}D_{HIR\ 1}$	1	1	2
$C\text{–}D_{HIR\ 2}$	2	0	2
$C\text{–}D_{HIR\ 3}$	3	0	3
$C\text{–}D_{NGS\ 1}$	0	1	1
$C\text{–}B_3$	6	3	9
$C\text{–}B_{HIR\ 2}$	15	4	19
$C\text{–}B_{HIR\ 4}$	1	0	1
No type*	9	0	9
Total	2653	1376	4029
TF^{C}†	0·9879	0·9927	
$TF^{D\ Chi}$	0·0068	0·0040	
$TF^{D\ HIR\ 1}$	0·0002	0·0004	
$TF^{D\ HIR\ 2}$	0·0004	0	
$TF^{D\ HIR\ 3}$	0·0006	0	
$TF^{D\ NGS\ 1}$	0	0·0004	
$TF^{B\ 3}$	0·0011	0·0011	
$TF^{B\ HIR\ 3}$	0·0028	0·0014	
$TF^{B\ HIR\ 4}$	0·0002	0	

* These samples gave a transferrin pattern which could not be typed accurately.
† Calculation of gene frequencies exclude 'no type'.

globin, but the existence of this band has been clearly demonstrated by gradient polyacrylamide gel electrophoresis employing a tris-boric acid-EDTA buffer at pH 8·4. Unfortunately only one family member was available for study and this individual did not show the variant phenotype.

Another single individual exhibited the unusual haptoglobin phenotype shown in Fig. 2B, characterized by an extra, weakly staining band, migrating cathodally to the normal HP^1 band, the remainder of the pattern being typical of a 1–2 type. This pattern does not conform to any of the previously reported haptoglobin variants. In order to rule out artifacts that might result from sample preparation or storage, we collected a second sample from the propositus, and also samples from two children of the propositus. The unusual pattern was confirmed in the propositus, and was also present in the son, the daughter showing a normal haptoglobin pattern. Although we have not done any detailed studies of this unusual haptoglobin, we have assumed that it represents a new genetic phenotype, designated HP $1\text{–}2_{NGS\ 1}$. The absence of any apparent doubling of the bands in the 1–2 region of the pattern is probably due to the low level of the variant material in the sample.

Ogawa & Kawamura (1965) encountered one individual showing the phenotype HP 1–2. Johnson in a survey of 1000 Japanese from Fukuoka Prefecture and Sugita & Tsunenari (1974) have reported a single case of the same type in 1100 individuals from Kumamoto Prefecture. This type was not seen in our study.

Transferrin

Eight different electrophoretic variants of transferrin, involving 84 persons, were encountered during the screening of 4020 persons; the frequency of each is shown in Table 3. Nine additional

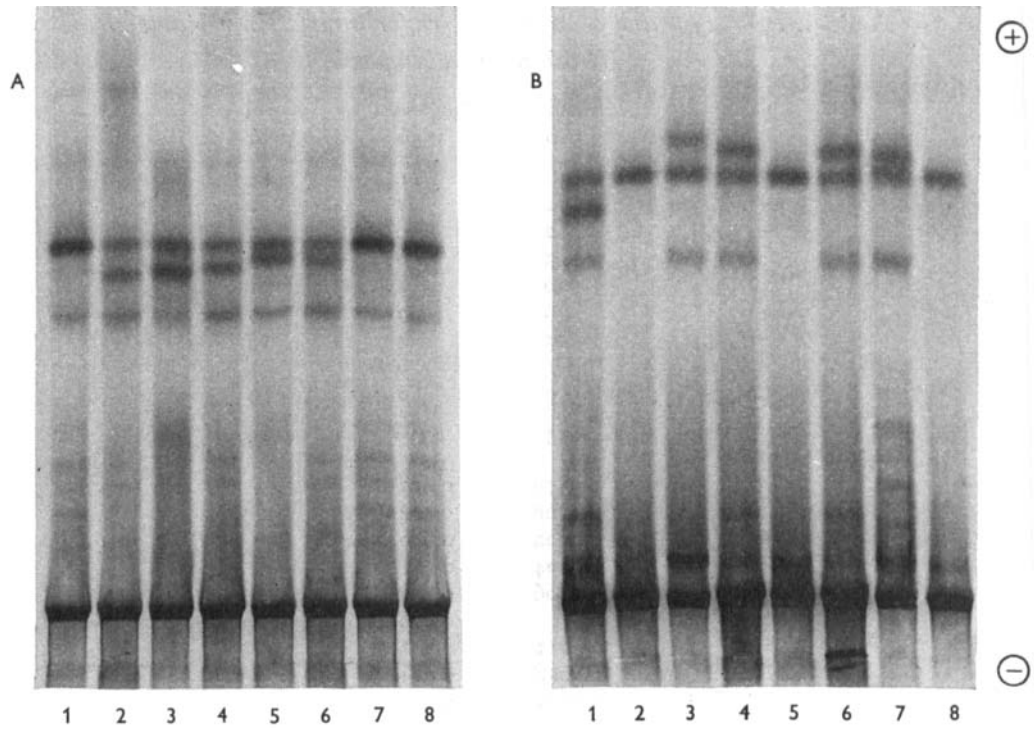

Fig. 3. Polyacrylamide gel electrophoresis in Tris-glycine buffer, pH 8·4 of transferrin variants encountered in this study. (A) Transferrins C–D: well 1, 7 and 8, normal controls, type C; well 2, C–$D_{HIR\ 3}$; well 3, C–D_{Chi}; well 4, C–$D_{HIR\ 2}$; well 5, C–$D_{HIR\ 1}$; well 6, C–$D_{NGS\ 1}$. (B) Transferrins C–B: wells 2, 5 and 8, normal controls, type C; well 7, C–B_3; wells 4 and 6, C–$B_{HIR\ 2}$; well 3, C–$B_{HIR\ 4}$; well 1, C–D_{Chi}.

samples were not typed because the transferrin region was obscured by haemoglobin due to haemolysis. Electrophoretic comparisons of these variants, using polyacrylamide gel electrophoresis, are shown in Fig. 3. Fifty-five of these, of five different mobilities, are variants showing a band cathodal to the normal C band, generically termed D variants. The most commonly encountered D variant in our study is identical in electrophoretic mobility to the variant D_{Chi}, first reported by Parker & Bearn (1961) in Chinese living in New York City. This comparison was made with a sample of transferrin C–D_{Chi} provided by Dr H. E. Sutton. The gene frequency for $TF^{D\ Chi}$ in our overall sample was 0·006. We have also observed two examples of a D transferrin with a mobility faster than D_{Chi}, and probably corresponding electrophoretically to the variant D_4 (see Sutton & Jamieson (1972) for the standard mobility table used). This variant has been designated $D_{HIR\ 2}$. As will be discussed later in this section, both of these variants have been encountered in previous Japanese studies.

We have also observed three D variants not previously reported in Japanese. Three individuals from the Hiroshima sample showed a variant, designated $D_{HIR\ 3}$ which migrates in a relative position equivalent to transferrin $D_{Ralford}$ in the polyacrylamide test system (Sutton & Jamieson, 1972). Two examples were encountered of a variant, designated $D_{HIR\ 1}$, which migrates near the normal C band on starch gel, but is well separated by polyacrylamide gel electrophoresis. This variant seems to correspond to the D_O variant reported by Giblett, Hickman & Smithies (1959). We have also found a single example of a variant, $D_{NGS\ 1}$, which is indistinguishable from

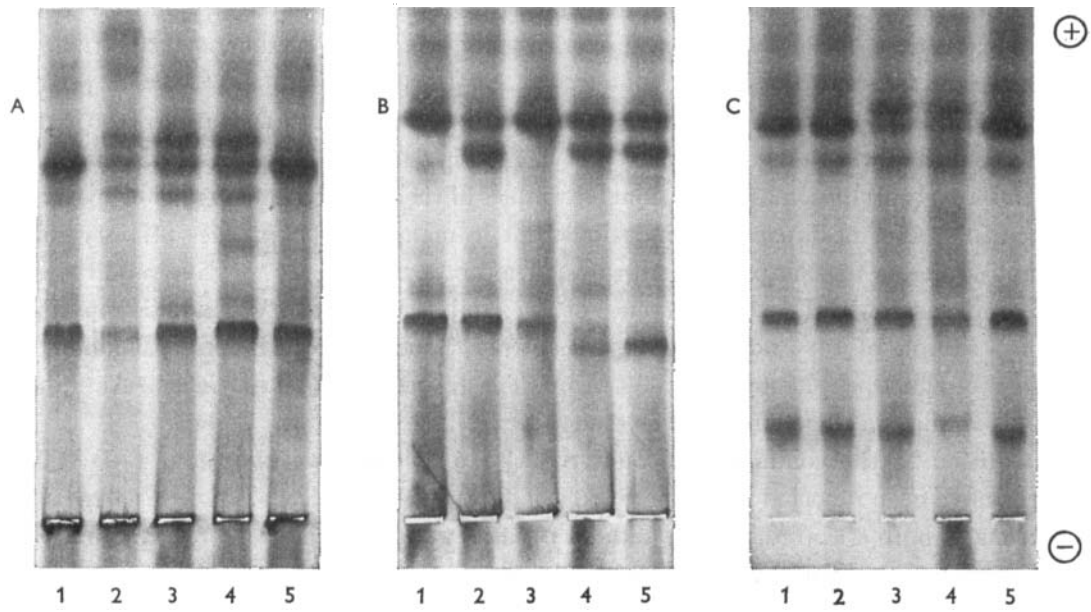

Fig. 4. Starch-gel electrophoresis of some transferrin variants encountered in this study. (A) Transferrin C–$B_{HIR\ 2}$: wells 1 and 5, normal controls, type C; well 2, C–$B_{HIR\ 2}$; well 3, propositus (263809); well 4, son of the propositus. (B) Transferrin C–D_{Chi}: well 1, normal control; well 2, propositus with C–D_{Chi} (267406); well 3, mother of propositus; wells 4 and 5, affected brothers of propositus. (C) Transferrin C–B_3: wells 1 and 5, normal controls; well 2, normal son of propositus; well 3, affected son of propositus; well 4, propositus (204257).

the normal C type in the routine starch gel screening system, and was discovered when run in the Tris-glycine, pH 8·4, polyacrylamide gel system as a putative normal (see Fig. 3). We have examined 100 randomly chosen samples presumed to be transferrin C by this polyacrylamide gel electrophoresis method without finding additional $D_{NGS\ 1}$ types. The frequency of this type in the total sample cannot be accurately estimated at this time, but it is probably quite low. Family studies have confirmed the genetic nature of 3 of the 5 D types. For $D_{HIR\ 2}$ the only family member available for testing was phenotypically normal; for $D_{HIR\ 1}$, two children were tested without finding the variant.

Parker & Bearn (1961) also reported the discovery of a single example of a rapidly migrating transferrin type, which they designated B_3, in a sample of 46 Japanese from Ube, Japan. In this study, we have seen 9 examples of a B type variant which has the same relative mobility as B_3 in the standard system of Sutton & Jamieson (1972). We have therefore designated this variant B_3, but no direct comparison has been made. We have also observed 19 examples of a B type variant which migrates anodal to the B_3 type, designated $B_{HIR\ 2}$. Its relative mobility in the standard system places it between the variants B_1 and B_3. However, it may be identical to the B variant designated B_1, seen by others in Japan (see below). In addition to these more common B types, we have observed a single variant, designated $B_{HIR\ 4}$, which has an electrophoretic migration anodal to $B_{HIR\ 2}$. Transferrin $B_{HIR\ 4}$ shows, at both pH 6·5 and pH 8·8, an electrophoretic mobility similar to that of $B_{Lambert}$ at pH 6·5 (Sutton & Jamieson, 1972). Family studies have confirmed the genetic nature of the three B types observed in this study. Table 1 shows the results of family studies for both the B and D types and Fig. 4 shows the results of family studies for three cases.

Following the discovery in 1961 of the transferrin variant designated D_{Chi} in New Yorkers of Chinese extraction, by Parker & Bearn, Japanese investigators soon demonstrated the presence of a D variant, presumed to be the same as D_{Chi}, in Japan. Matsumoto (1964) has reported 12 instances of the C–D type in 822 unrelated Japanese, and Omoto & Harada (1967) reported three examples of what they presumed to be C–D_{Chi} in 245 people from Amami Oshima Island. Since these early studies, many additional examples of the transferrin C–D phenotype have been reported in studies of Japanese populations, but in many of these studies primary standards were not available for comparison. However, Omoto & Harada (1972) have reported, using a direct comparison with D_{Chi}, the presence of 19 examples of a phenotype indistinguishable from D_{Chi} in a sample of 1085 non-Ainu Japanese from the district of Hidaka, Hokkaido, and 14 examples of the D_{Chi} phenotype in 466 Ainu. The frequency observed in non-Ainu Japanese (0·009) is similar to that found in this study.

In addition to the commonly encountered C–D_{Chi} phenotype, two other C–D transferrin patterns have been reported in earlier studies of Japanese populations. These involved a total of 29 persons. Omoto & Harada (1967) found a D variant with an electrophoretic mobility slightly faster than D_{Chi}, and Kudo & Ishimoto (1973) have observed a D variant with an electrophoretic mobility slower than D_{Chi} and possibly slower than D_1. Although no direct comparisons were possible, our variant $D_{HIR\ 2}$ exhibits an electrophoretic mobility similar to the fast variant seen by Omoto and Harada.

The transferrin variant B_3 has been shown to be widespread in Japanese populations. This variant has been reported in studies by Matsumoto (1964), Omoto & Harada (1972) and Kudo & Ishimoto (1973). Earlier studies in Japanese populations have also reported the presence of the transferrin variant B_1. Kudo & Ishimoto (1973) have observed this type more frequently than the B_3 variant in a sample of 630 individuals from Mie Prefecture. As discussed earlier, we also have encountered a variant of the B type more frequently than the B_3 variant, but our comparisons suggest that the variant encountered in this study is not electrophoretically the same as B_1. Because of the similarity in gene frequencies it seems likely that the variant designated B_1 by others and the variant designated $B_{HIR\ 2}$ represent the same variant. No direct comparisons with either a standard B_1 or the Japanese B_1 have as yet been carried out.

DISCUSSION

The most interesting finding with respect to these serum proteins has been the variety of transferrin variants present in Hiroshima and Nagasaki. We have encountered 84 individuals apparently heterozygous for one or the other of 8 distinct types of transferrin variants in a sample of 4020 individuals. The genetic nature of most of these variants has been verified by family studies, and their relative mobilities verified by electrophoresis in both starch gel and polyacrylamide gel under a variety of conditions. This work confirms the findings from other investigations of Japanese populations with respect to the occurrence of the variants D_{Chi}, B_3, a variant electrophoretically similar to B_1, and a D variant with an electrophoretic mobility slightly faster than D_{Chi}. Four of the variants encountered in this study have not been previously distinguished. As mentioned earlier, the comparison of observed electrophoretic variants, between and within populations, is complicated by the lack of adequate standard electrophoretic types in this and other studies. We have tried to standardize our results using polyacrylamide gel

electrophoresis at two pH values, one acidic and one basic, as suggested by Sutton & Jamieson (1972), but until this or some other standard system becomes more widespread, the comparison of findings from different studies remains somewhat arbitrary.

The variant D_{Chi} is widespread in Chinese and Japanese populations, but appears to be absent from Caucasian and African populations (Giblett, 1962). Arends & Gallango (1965, 1974) have reported a variant with the mobility of D_{Chi} in high frequency among the Yupa Indians of Venezuela, and Tanis *et al.* (1973) have observed a similar variant in the Piaroa of Venezuela. Others have reported an unspecified D type variant or a variant similar to D_1 in Indian tribes, but many tribes lack the D_{Chi} variant. If the D_{Chi} variant encountered in some Indian populations is indeed identical with the D_{Chi} of Chinese and Japanese, then this may be viewed as a Mongoloid trait of great antiquity which is lacking in Negro and Caucasian populations. The D_{Chi} variant also appears to be absent from Asiatic and Oceanic populations geographically close to the Japanese. We have confirmed the reports (Omoto, 1975) that the transferrin variant D_1, present in Negro, Asian and Melanesian populations, is not found in Japanese.

SUMMARY

This paper presents the results of an electrophoretic survey of approximately 4000 individuals from the cities of Hiroshima and Nagasaki, Japan, for four serum proteins: albumin, ceruloplasmin, haptoglobin and transferrin. The haptoglobin gene frequencies obtained for the HP^1–HP^2 polymorphism are in agreement with earlier reports. Rare electrophoretic variants of albumin, ceruloplasmin and haptoglobin occur with frequencies of 2·48, 0·50 and 0·58 per 1000 determinations, respectively. The noteworthy finding of 8 distinct transferrin variants in these populations, with a combined frequency of 20·90 per 1000 determinations, is also presented. Four of these variants (D_{Chi}, B_1, B_3 and $D_{HIR\,2}$ which corresponds electrophoretically to D_4) have been reported in other populations in Japan, but the other five have not previously been differentiated.

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