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The occurrence of haemoglobin-J (Tongariki) and of thalassaemia on Karkar Island and the Papua New Guinea mainland

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The known α-chain variant Hb-J (Tongariki) was found to occur at relatively high frequency in several villages on the island of Karkar, about 15 km from the New Guinea mainland. The same variant was also found in an adjacent coastal region of the mainland, but not in a large number of blood samples collected in the Central Highlands. This is the third Pacific location in which Hb-J (Tongariki) has been found, and this variant may be a useful genetic marker for the study of Melanesian populations.

Hb-J (Tongariki) is a 'high-level' α-chain variant; its mean proportion in heterozygotes is 41 %. Three homozygotes were found in which Hb-J (Tongariki) and Hb-$J_2(\alpha_2^J \delta_2)$ were the sole major and minor constituents respectively. These findings are of interest in relation to current studies on the possible duplication of the human α-chain gene locus.

Introduction

Blood samples collected from two selected communities in New Guinea during the joint Royal Society and Australian Academy of Science contribution to the Human Adaptability Section of the International Biological Programme were used to establish the presence of polymorphisms involving blood groups, serum proteins, red-cell enzymes and haemoglobins. The genetically determined abnormal haemoglobin Hb-J (Tongariki) was found to occur at relatively high frequency among the inhabitants of the Kaul Village complex of Karkar Island. This haemoglobin variant had been found previously in two other widely separated locations in the Pacific. Since it therefore appeared to be a useful genetic marker for Melanesian populations, the search for it was extended to many other villages on Karkar Island, the adjoining mainland and many locations of the Eastern Highlands of New Guinea. Evidence was also sought for the possible existence of thalassaemia in the communities studied.

Materials and methods

Blood was collected in venules containing an anticoagulant and flown to London on ice. The samples were collected with the least possible delay, centrifuged and the plasmas removed and put aside for other work. A part of each packed red-cell sample was washed three times with cold normal saline, haemolysed with one and a half volumes of water, and half a volume of toluene added, and after thorough shaking, kept at 4 °C overnight. The mixture was then centrifuged, and the clear haemolysate separated from the toluene supernatant and the intermediate layer of denatured stromal protein.

The haemolysates were analysed by electrophoresis in starch gel at pH 8.6 using the tris-EDTA-borate buffer system developed for the separation of haemoglobins (cf. Huehns & Shooter 1965). One slice of the gel was stained with Amidoblack in methanol/water/acetic acid (5:5:1 by vol.) and differentiated in the same solvent system. For quantitative work, a second slice was stained with the same dye dissolved in glycerol/water/acetic acid (5:5:1 by vol.) and differentiated in the same glycerol-based solvent mixture. The clear gel slice was transferred to glycerol and examined by transmission densitometry, as described by Gratzer & Beaven (1960) and Gordon (1969). The densitometer records, which were linear in absorbance, were evaluated by planimetry to obtain the proportions of the components of interest, namely Hb-J (Tongariki) when present, and the normal minor component Hb-A_2. Cord blood haemolysates were analysed by the same method, and also by electrophoresis in agar gel at pH 6.2 using a citrate buffer (Gratzer & Beaven 1961). Foetal haemoglobin (Hb-F) was estimated by spectrophotometric measurement of the rate of denaturation at pH 12.6 (Beaven, Ellis & White 1960).

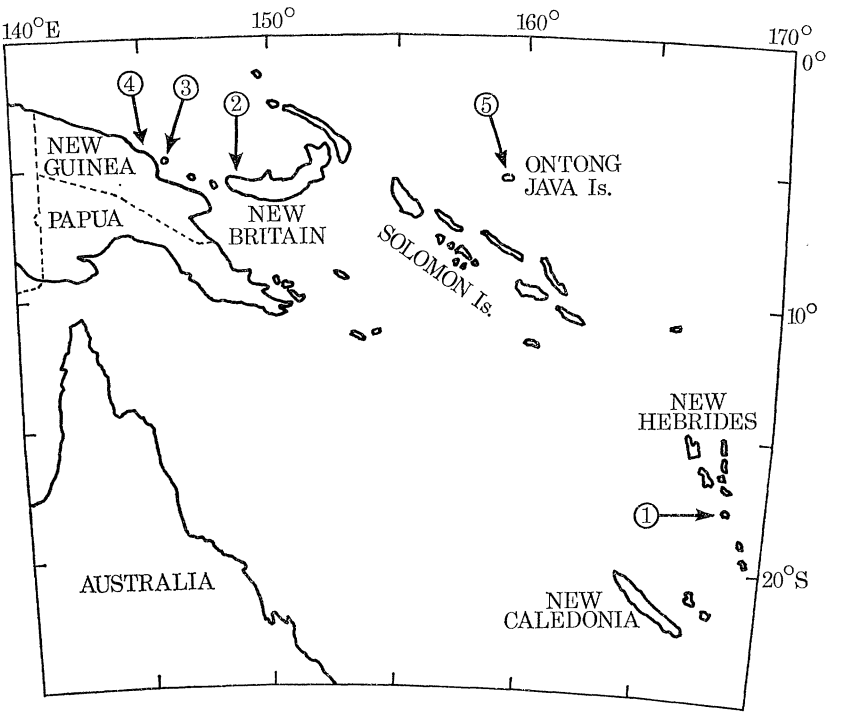

FIGURE 1. Map of Pacific Ocean area (long. 140–170° E, lat. 0–25° S) showing locations at which Hb-J (Tongariki) has been found. 1, Tongariki Island, Shepherd group, New Hebrides; 2, Kilenge, Sac Sac, New Britain; 3, Karkar Island; 4, Megiar, Papua New Guinea mainland; 5, Ontong Java Island.

The identification of the abnormal haemoglobin as Hb-J (Tongariki) was made by the standard methods used for the structural characterization of abnormal haemoglobins, as described by Beaven, Hornabrook, Fox & Huehns (1972), on the separated normal β and abnormal α chains obtained by the fractionation of Hb-J (Tongariki) globin.

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Results

Hb-J (Tongariki)

The locations on Karkar Island and the mainland from which the samples examined were taken are listed in table 1, together with the numbers of subjects from each location with Hb-J (Tongariki). With the exception of three homozygous inhabitants of the Kaul village complex, all the subjects with the abnormal haemoglobin were heterozygous for normal adult haemoglobin (Hb-A) and Hb-J (Tongariki). The distribution of the values found for the proportion of Hb-J (Tongariki) is shown in figure 2.

Table 1. Occurrence of Hb-J (Tongariki) in individual communities of Karkar Island and the New Guinea mainland

location	no. of subjects	no. with Hb-J	location	no. of subjects	no. with Hb-J
Karkar Island			New Guinea mainland		
Kaul village complex			Lufa (near Goroka)	424	0
initial survey	648	48†	Arora	30	0
selected families	186	22†	Okapa	224	0
Dorogodam	52	1	Kauna, Agomonofi, Hompazentu	50	0
Langlang	34	3	Wario Valley	137	0
Urara	36	4	Bib'be'ori	68	0
Mangar 2	39	6	Aronis and Megiar	76	2
Dangsai	47	0	Gogol	78	0
Katom	53	0	Asaro	38	0
Henganofi	90	0	Jimi Valley	400	0
Wadau	61	0	Bena Bena	33	0
Gamog	141	0	Kainantu	52	0
Boroman	39	3			
Bafor	19	0			
Did	17	0			
Kilden	25	1			
Kevasop	30	0			

† Including 1 homozygote.
‡ Including 2 homozygotes.

The starch-gel electrophoretic analysis of a haemolysate from one of the three subjects found to be homozygous for Hb-J (Tongariki) is shown in figure 3. No haemoglobin H(Hb-β_4), haemoglobin Barts (Hb-γ_4) or other abnormal haemoglobins were found.

Hb-A_2 and Hb-J_2 (Tongariki)

The electrophoretic analyses of all the haemolysates were inspected for any indication of elevated Hb-A_2 that might indicate the trait condition for β-thalassaemia. About 90 haemolysates were selected in this way for estimation of their Hb-A_2 levels together with a large number of apparently normal haemolysates as controls. Out of more than 3000 samples, 38 had Hb-A_2 levels at or above the upper normal limit of 3.5 %. For the normal haemolysates without Hb-J (Tongariki) the mean value was 2.2 % (range 0.8–2.4; n = 255, s.d. = 0.60). For the haemolysates with elevated Hb-A_2 the mean value was 4.5 % (range 3.5–8.1; n = 38, s.d. = 1.38). For the Hb-J (Tongariki) heterozygotes the mean value was 1.4 % (range 0.5–3.4; n = 7.9, s.d. = 0.74). The unusually low values of the lower normal limits undoubtedly arise from the poor condition of some of the blood samples, resulting in diffuse weak Hb-A_2

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zones on the analytical starch gels. The significantly lower mean Hb-A_2 levels in Hb-J (Tongariki) heterozygotes are due to the presence of Hb-J_2 (Tongariki). This was much more difficult to estimate with any precision, but for 11 haemolysates a mean value of 0.7 % was found (range 0.4–1.3). Four analyses on Hb-J homozygotes gave a mean value of 2.2 % Hb-J_2 (Tongariki), with no detectable Hb-A_2.

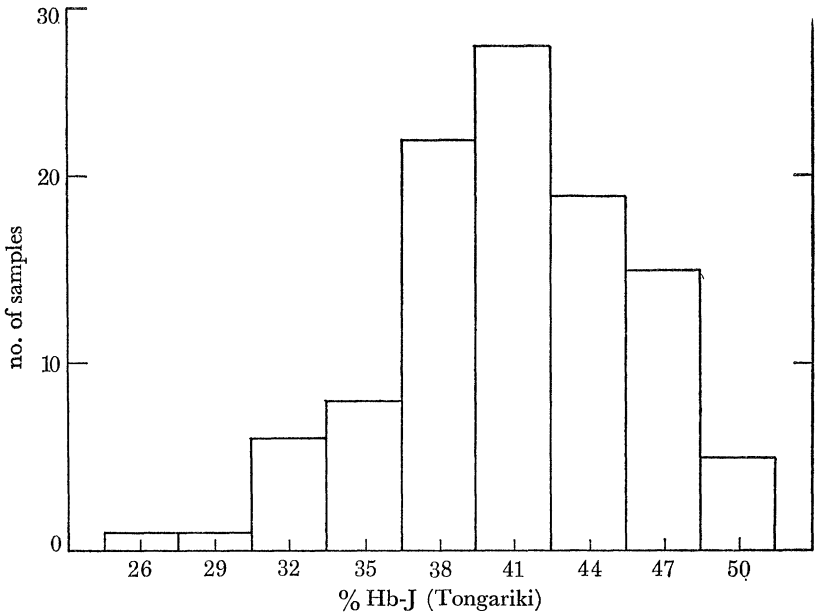

FIGURE 2. Distribution of proportions of Hb-J (Tongariki) in heterozygous subjects (n = 104; mean = 41.1 %, s.d. = 4.8 %).

TABLE 2. ANALYSIS OF CORD BLOODS FROM KARKAR ISLAND AND MADANG

origin of samples	
Karkar Island	73
Madang	9
Hb-F	
range	51–78 %
average	65 %
Hb-Bart	
not detected	56
possible trace	8
less than 1 %	7
at 1 % level	11†
more than 1 %	nil

† Range 0.7–1.3 %; average 1.0 %.

Cord bloods

The total number of cord bloods examined was 82; of these, nine were taken on the mainland at Madang and the remainder from Karkar Island. They were examined for the presence of Hb-Barts by electrophoresis in starch gel under the standard conditions, but using a benzidine stain to increase the sensitivity of detection of minor haemoglobin zones. When present, Hb-Barts was estimated as described above by densitometry of glycerol-cleared gel slices. The results obtained are shown in table 2. No definite evidence was obtained for the presence of Hb-F (Tongariki), i.e. $\alpha_2^J \gamma_2$, in any of the samples.

Identification of the variant haemoglobin

From the purified variant α-chain the chymotryptic peptide α(110–122), which is part of the tryptic peptide αTp XII(100–127) was isolated and shown to contain one residue of aspartic acid and a deficit, compared with normal α-chain, of one residue of alanine, as required for Hb-J (Tongariki) from the definitive work of Gajdusek *et al.* (1966, 1967). The sequence analysis of the α(110–122) peptide to confirm that the substitution is in fact at α-115 has not yet been completed, but until this is available it is assumed that the New Guinea variant is Hb-J (Tongariki).

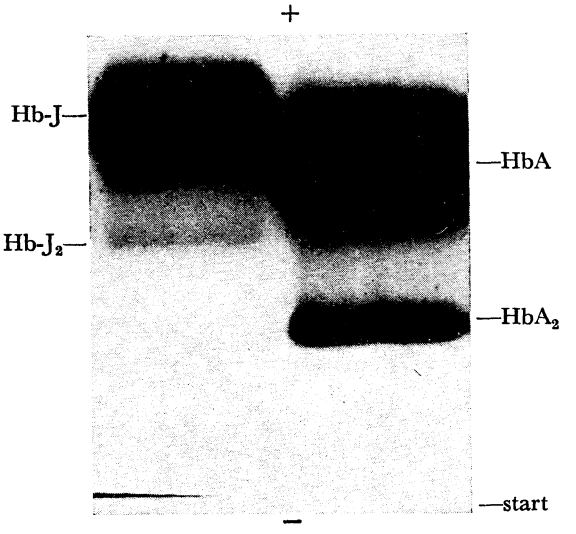

FIGURE 3. Starch-gel electrophoretic analysis of Hb-J (Tongariki) homozygote (left) and normal adult haemolysate (right). Tris-EDTA-borate buffer system, pH 8.6; benzidine stain.

DISCUSSION

The haemoglobin variant Hb-J (Tongariki) was discovered by Gajdusek *et al.* (1966, 1967) in an appreciable proportion (*ca.* 5%) of the population examined of Tongariki Island in the New Hebrides group. A pedigree study indicated no departures from simple Mendelian inheritance and also that the gene responsible for Hb-J (Tongariki) must have been present on the island at least four generations ago. The population on Tongariki is predominantly Melanesian, and pedigree analysis suggested that the occurrence of the haemoglobin variant was independent of the known small admixture of other populations.

The same haemoglobin variant was found in a Kilenji village near Sac Sac in New Britain by Abramson, Rucknagel, Schreffler & Saave (1970), as a result of an earlier study of blood groups and other genetic markers of several ethnic groups in New Britain by Booth, Vines & Saave (1969). The Kilenji are also a Melanesian population. Two apparently homozygous subjects with only Hb-J (Tongariki) and Hb-J$_2$ (Tongariki), i.e. $\alpha_2^J\delta_2$, were found in the New Britain location, which is nearly 2000 km distant from the New Hebrides. Very recently the variant has also been found at Ontong Java Island, north of the main group of Solomon Islands (personal communication, R. Carrell & A. Damon). The island of Karkar is about 260 km from New Britain and 15 km from the New Guinea mainland. As shown in table 1 the occurrence of Hb-J (Tongariki) on the mainland appears, at least on the basis of present evidence, to

be confined to the coast around Megiar, opposite Karkar Island. The known geographical distribution of Hb-J (Tongariki) is shown in figure 1. The distribution of the variant on Karkar Island itself is shown in figure 4. In general, it occurs in the northern half of the island inhabited by the Waskia, and is absent from the Takia communities of the south and east. There is some overlap between villages in the southwest of the island, with respect to the occurrence of Hb-J (Tongariki) but broadly the linguistic division running northeast to southwest across the island which separates the Waskia from the Takia also separates the communities with Hb-J (Tongariki) from those where it is absent.

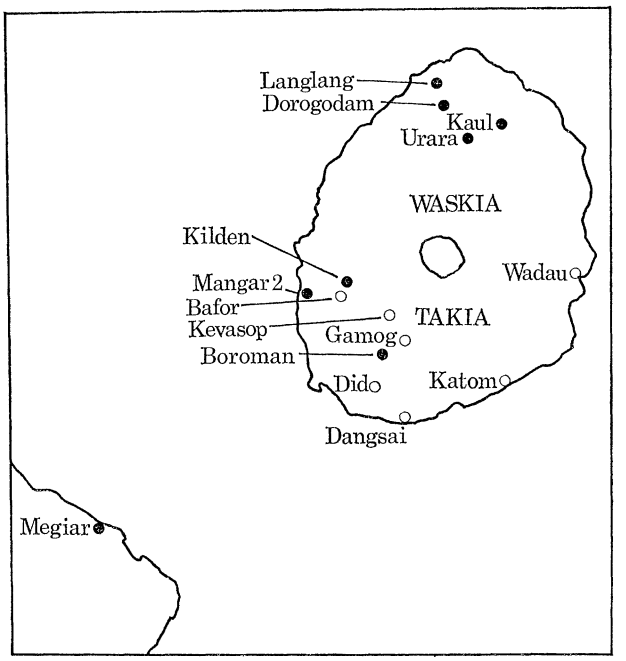

FIGURE 4. Locations of communities on Karkar Island examined for occurrence of Hb-J (Tongariki). ●, Hb-J found; ○, Hb-J not found. See also table 1.

As shown by Gajdusek *et al.* (1966, 1967), Hb-J (Tongariki) is an α-chain variant, the structural alteration being α-115, alanine → aspartic acid. In view of the large proportion of α-chain variants which occur in heterozygotes at the 20–30 % level (see De Jong 1969, for figures), it is of interest that Hb-J (Tongariki) is clearly a 'high-level' α-chain variant, like Hb-G (Honolulu) and Hb-G (Philadelphia). Abramson *et al.* (1970) reported that the heterozygotes studied in New Britain consistently showed 45–50 % of the variant, and the present findings (figure 2) amply confirm this point and define the range more precisely.

The fact that Hb-J (Tongariki) is a 'high-level' α-chain variant is of interest in relation to recent discussion concerning the possible duplication of the human α-chain gene. One argument (Lehmann & Carrell 1968) in support of this hypothesis is that, in the absence of β-thalassaemia as an agency for the depression of β-chain synthesis, low levels of α-chain variants in heterozygotes could then arise from a single mutant gene of the two pairs of genes normally coding for identical α-chains, comprising about 25 % of the total production of α-chains. The same authors also suggested that duplication of the α-chain locus could account for the notable variability and genetic complexity of α-thalassaemic states. In support of the hypothesis a

Hungarian family has been described by Hollán *et al.* (1970) in which there is unequivocal evidence for two major α-chain loci.

In the case of Hb-J (Tongariki), however, the high proportion of the variant in heterozygotes, and the total absence of components containing normal α-chains, namely Hb-A and Hb-A_2, in the homozygotes, together with the absence of any evidence for α-thalassaemia (see below) provide further support for the conclusion by Abramson *et al.* (1970) that in these particular Melanesian populations, at least, the α-chain locus is not duplicated. As these workers have emphasized, this does not preclude the possibility of duplication either in particular families, or in other populations in which heterozygotes for 'low-level' α-chain variants have only about 20 % of the variant. With respect to Hb-J (Tongariki), the present work adds a further three homozygotes to the two found by Abramson *et al.* (1970) and a further 68 heterozygotes to the 30 discovered by them in New Britain and the 28 found in Tongariki by Gajdusek *et al.* (1966, 1967). For the largest group of randomly selected samples studied in the present work (Kaul village complex) the observed frequency of heterozygosity for Hb-J (Tongariki) corresponds to an apparent gene frequency of *ca.* 0.04. For such small endogamous communities it is difficult to be certain that sampling is truly random, as Abramson *et al.* (1970) have noted, but it is clear that in all three areas in which Hb-J (Tongariki) has now been found the gene frequency is not low. A pedigree containing one of the homozygotes found in the Kaul village complex is shown in figure 5.

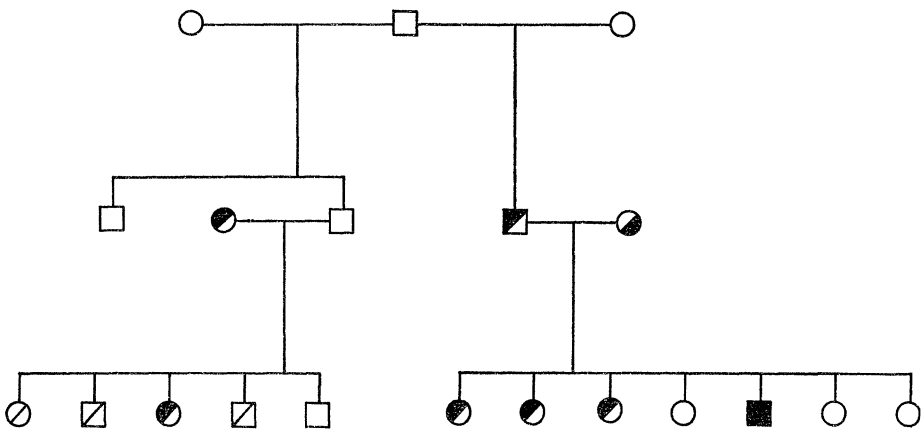

FIGURE 5. Family tree showing inheritance of the gene for Hb-J (Tongariki). □, male; ○, female; ◩, ◒, heterozygotes for Hb-J; ■, homozygote for Hb-J; ⊘, ⊠, normal haemoglobin; blank symbols denote subject not examined.

Thalassaemia

The 27 haemolysates with Hb-A_2 levels at or in excess of the upper normal limit of 3.5 % of the total haemoglobin suggest that the gene for β-thalassaemia may be present at low frequency in the region examined, both on Karkar Island (13 samples) and the mainland (14 samples). In an attempt to obtain confirmatory evidence for the existence of the β-thalassaemia trait a small number (23) of blood films were taken when a second group of blood samples was collected from selected families of the Kaul village complex which had previously been found to have Hb-J (Tongariki). The findings on this second group of samples are shown separately in table 1.

Definite red-cell abnormalities suggestive of β-thalassaemia trait (anisocytosis, +/++; poikilocytosis, +/++; hypochromasia −/++; polychromasia, −/+; anisochromasia, −/+;

macrocytosis, −/+; microcytosis, −; target cells, −/+: key, −absent, + slight, ++ moderate degree) were found in four of the films; these comprised two subjects with elevated Hb-A_2, one with electrophoretically normal haemoglobins, and one heterozygote for Hb-J (Tongariki). The small number of films examined, and the low incidence of relevant red cell abnormalities, notably microcytosis (absent from all the films) and target cells, provide very little additional firm evidence for the presence of the β-thalassaemia gene in this limited sample of the island population. In view of other evidence, discussed below, for the presence of β-thalassaemia in Papua New Guinea, further haematological studies will be required properly to assess the true incidence of β-thalassaemia on Karkar Island and the various mainland locations included in the present survey. Of more interest, in relation to α-thalassaemia, is the failure to find any evidence for thalassaemia in the films from six heterozygotes and a homozygote for Hb-J (Tongariki). This indicates that the high proportion of the variant haemoglobin in heterozygotes, and the total absence of Hb-A with normal α-chains in the homozygous state, are not likely to be due to any depression of α-chain synthesis associated with α-thalassaemic states. Further evidence for the absence of α-thalassaemia comes from the failure to find any occurrence of Hb-H in over 3000 adult blood samples, and the results of the cord blood studies (table 2), which are, however, not unequivocal. The fact that proportions of Hb-Barts in excess of 1 % were not present in any of the 82 cord bloods examined certainly suggests the absence of the gene for classical α_1-thalassaemia, using the criteria established by Na-Nakorn & Wasi (1970). As shown by these workers, the proportions of Hb-Barts in cord bloods can be used to detect the heterozygous states for both classical α_1-thalassaemia and the milder α_2-thalassaemia, as well as the doubly heterozygous state (α_1/α_2 thalassaemia) associated with Hb-H disease. Thus the occurrence of Hb-H in adults is indicative only of the latter genetic state, and very detailed haematological and family studies are needed to reveal the singly heterozygous α-thalassaemic states in adults.

For a northern Thailand population Na-Nakorn and Wasi found a striking trimodal distribution of Hb-Barts levels in cord bloods, and assigned the lowest peak with a mean value of 1.47 %, s.d. = 0.61 to the mild α_2-thalassaemic trait. Of the present samples the 11 samples with measurable proportions of Hb-Barts (range 0.7–1.3 %, mean 1.0 %) are all on the low side of this distribution. The nominal probability that the observed values are diagnostic for α-thalassaemia is appreciable, but subject to the poor precision with which such small proportions of Hb-Barts can be measured and, more importantly, to the reservation that such proportions of Hb-Barts may also be found in some cord bloods in the absence of α-thalassaemia (Weatherall & Clegg 1972). It appears that normal cord bloods may contain up to 0.5 % Hb-Barts, with higher levels in normal Negro infants, so that the detection of α_2-thalassaemia based solely on estimates of Hb-Barts in cord bloods is open to question. Here again more detailed haematological studies would be of value.

The presence of β-thalassaemia in the general area of Papua New Guinea has been firmly established since the first clinical reports by Ryan (1961 *a*, *b*, *c*). Subjects with elevated levels of Hb-A_2 (exceeding 4 %) were found by Curtain, Kidson, Gajdusek & Gorman (1962) in the Western Highlands, the Sepik River district and in New Britain. References to β-thalassaemia in other locations are given by Hornabrook, Fox & Beaven (1972).

As far as is known, the presence of Hb-J (Tongariki) is without clinical significance. The presence of thalassaemia would be of importance in relation to health. The present evidence for the existence of the β-thalassaemia gene at low frequency is based solely on elevated Hb-A_2

levels and requires confirmation by haematological studies. The possibility that the gene for the mild (α_2) variety of α-thalassaemia is present on Karkar Island, based on the findings of Hb-Barts at the 1 % level in an appreciable proportion of cord bloods, also requires confirmation, for the reasons discussed above. Nevertheless, the possibility of β-thalassaemia in particular as an explanation for anaemia, not attributable to iron-deficiency or other relevant factors, in the populations studied should certainly be kept in mind.

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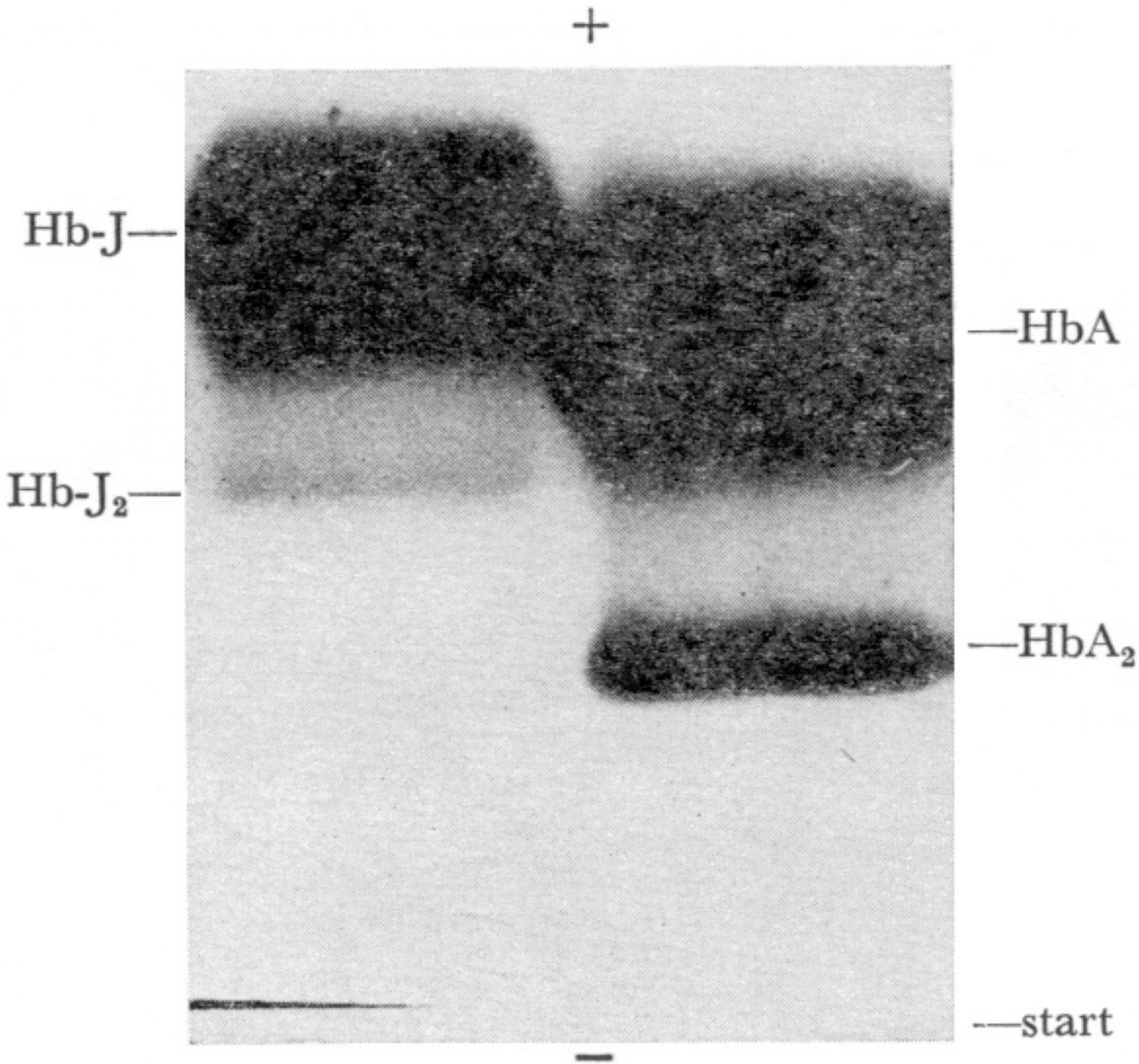

FIGURE 3. Starch-gel electrophoretic analysis of Hb-J (Tongariki) homozygote (left) and normal adult haemolysate (right). Tris-EDTA-borate buffer system, pH 8.6; benzidine stain.

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