Journal of Medical Genetics (1974). **11**, 341.

Haemoglobin D Punjab (D Los Angeles)

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Summary. A search for haemoglobin variants undertaken in Canada revealed 21 unrelated instances of Hb D Punjab amongst 207,300 specimens tested. Of these, eight came from East Indian immigrants and the rest from Canadians of United Kingdom origin. No instances of Hb D Punjab were found in 14,500 specimens from Canadian Indians that were tested. The geographical origins of 27 instances of Hb D Punjab characterized at the MRC Abnormal Haemoglobin Unit, Cambridge are presented. Of these five were natives of the British Isles. The results of surveys undertaken in the United Kingdom are summarized. The global distribution of Hb D Punjab is discussed.

Haemoglobin D (Hb D) was the third abnormal haemoglobin to be reported (Itano, 1951; Sturgeon, Itano, and Bergren, 1955). It was discovered in a family of mixed British and American-Indian origin from the Los Angeles area, of which some members had a haemolytic anaemia with a tendency for the erythrocytes to sickle slowly on reduction. The characteristics of Hb D were: (1) an electrophoretic mobility identical to that of Hb S at alkaline pH, (2) a normal solubility when in the reduced state, and (3) absence of sickling of erythrocytes which contain it in association with Hb A. The Hb D in this family has been characterized as $\alpha_2\beta_2^{121\ Glu\rightarrow Gln}$ and called D Los Angeles (Babin, Jones, and Schroeder, 1964).

Between the discovery of Hb D and the elucidation of its molecular structure, abnormal haemoglobins with the same properties were found to occur in many ethnic groups. Thus, they were reported in white persons of European extraction (Dacie, 1954; White and Beaven, 1954; Chernoff, 1958) as well as in English (Stewart and MacIver, 1956; Smith and Conley, 1959), Portuguese (Arends, Layrisse, and Rincon, 1959), Italian (Bowman and Ingram, 1961), German (Martin *et al*, 1960), Greek (Gouttas *et al*, 1960), Turkish (Aksoy and Lehmann, 1956; Gammack *et al*, 1961), Persian (Hynes and Lehmann, 1956), Indian (Bird and Lehmann, 1956; Jacob, Lehmann, and Raper, 1956), as well as American negro subjects (Chernoff, 1956; McCurdy, 1959; Marder and Conley, 1959). Five specimens of Hb D of widely differing ethnic origin—namely Hb D Chicago (Bowman and Ingram, 1961); Hb D North Carolina (Smith and Conley, 1959); Hbs D Punjab, D Portugal, and D Cyprus supplied by Dr H. Lehmann—were all shown to have the composition $\alpha_2\beta_2^{121\ Glu\rightarrow Gln}$ (Baglioni, 1962). These findings demonstrated that this variant has a wide distribution. It was soon demonstrated that the electrophoretic and solubility properties of Hb D were by no means specific for Hb D Punjab by the discovery of many instances of Hb D which have a different molecular structure from Hb D Punjab. For example, D Baltimore (Marder and Conley, 1959), D Washington (McCurdy *et al*, 1961), D St Louis (Minnich *et al*, 1962), G Azuakoli (Lehmann and Nwokolo, 1959), G Bristol (Raper *et al*, 1960), G Philadelphia (Atawater, Schwartz, and Tocantins, 1960) all of which have the structure $\alpha_2^{68\ Asn\rightarrow Lys}\beta_2$ (Baglioni and Ingram, 1961; Weatherall, Sigler, and Baglioni, 1962; Dance, Huehns, and Shooter, 1964) as do also G_{St-1} (Bowman *et al*, 1966) and G Knoxville (Chernoff and Pettit, 1965); while G Galveston is $\beta^{43\ Glu\rightarrow Ala}$ (Bowman *et al*, 1964); D Ibadan is $\beta^{87\ Thr\rightarrow Lys}$ (Watson-Williams *et al*, 1965); D Bushman is $\beta^{16\ Gly\rightarrow Arg}$ (Wade, Jenkins, and Huehns, 1967).

Some confusion arose from the different behaviour on starch gel electrophoresis and paper electrophoresis at pH 8·6 of some variants. Hb D Punjab moves in the same manner as Hb S on both techniques. Certain haemoglobins, however, move slightly faster than Hb S on paper electrophoresis

Received 14 February 1974.

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particularly if barbitone buffer is used and these were named Hb G, but on starch gel electrophoresis they behave like Hb D. Depending on which medium research workers used for electrophoresis they named the haemoglobin variant D or G.

This paper describes the occurrence of Hb D Punjab in Canada and in the United Kingdom and reviews its global distribution.

Hb D Punjab in Canada

Between July 1965 and April 1973, some 222,000 blood samples obtained from blood donors or from hospital patients in various parts of the four Canadian provinces of Ontario, Manitoba, Saskatchewan, and Alberta were screened during a deliberate search for haemoglobin variants that have a different net charge from that of normal haemoglobin. For this purpose, a filter-paper electrophoresis technique was used (TEB—barbiturate discontinuous buffer system, pH 8·9; Vella, 1967). Twenty-nine of these specimens were found to contain an abnormal haemoglobin fraction which had the following properties: (1) it accounted for 30–40% of the total pigment, (2) it had the electrophoretic mobility of Hb S at pH 8·9, (3) it did not separate from Hb A in citrate-agar gel electrophoresis at pH 6·1 (Robinson *et al*, 1957), (4) it was associated with a single haemoglobin A_2 band of normal concentration, and with normal amounts of alkali-resistant haemoglobin (0·5–1·5% by the one minute alkali denaturation method; Singer, Chernoff, and Singer, 1951), (5) it showed a normal solubility when reduced in 2·24 M phosphate buffer (Itano, 1953), and (6) it was not associated with sickling of erythrocytes when these were treated with metabisulphite. All these subjects were considered to be heterozygotes for an Hb D gene. In 21 of them, the abnormal haemoglobin was isolated, purified, and characterized in Saskatoon on the basis of fingerprint patterns of tryptic digests by methods that have already been described (Vella *et al*, 1967b). Confirmatory results were obtained on three of these subjects by fingerprinting and by amino-acid analysis of isolated abnormal peptides undertaken at Cambridge. In all cases, the fingerprints were indistinguishable from those of Hb D Punjab while the amino-acid analysis of aberrant peptides confirmed this characterization.

The incidence of Hb D Punjab in the four provinces is summarized in Table I. Eight of these subjects were of East Indian origin, while the remainder were white Canadians of British stock. The overall frequency appears to be 1/10,000 of the population tested. If only the instances in white subjects are considered, the overall frequency becomes 1/16,000 (ranging between 1/7,000 in Ontario and 1/27,000 in Alberta).

TABLE I

HAEMOGLOBIN D PUNJAB IN CANADA*

Province	No. of Samples	D Punjab	
		Total	No. in East Indians
Ontario	14,700	3	1
Manitoba	84,500	5	1
Saskatchewan	81,400	9	3
Alberta	26,700	4	3
Total	207,300	21	8

* No instance of this variant was encountered in 14,500 blood samples from persons of Canadian Indian origin.

Hb D Punjab in the United Kingdom

The first instances of Hb D in English persons were reported by Stewart and MacIver (1956) from Jamaica, and Smith and Conley (1959) from North Carolina. The haemoglobin of the family reported by the latter was characterized as β121 Glu→Gln by Baglioni (1962). Other instances of Hb D Punjab in English persons abroad have been reported from Ghana (Ringelhann *et al*, 1967) and Australia (Ungar *et al*, 1973).

Several extensive surveys have been undertaken in recent years to determine the incidence of abnormal haemoglobin variants in the United Kingdom. The frequency of Hb D Punjab revealed by these is summarized in Table II. The first instance to be detected was that in an East Anglian family (Huntsman *et al*, 1963; Konigsberg *et al*, 1965). Black (1969) found four instances amongst some 8,000 newborn English infants that he tested. However, other surveys have given only negative results and the overall frequency in British persons appears to be about 1 in 7,500. This is in good agreement with the frequency suggested by the Canadian results. The incidence is much higher amongst recent immigrants to Britain, especially amongst those of Indian origin. A recent survey amongst some 7000 immigrant school children in Birmigham showed Hb D to be present in 0·54% (Stuart *et al*, 1973).

During the last nine years, 27 instances of Hb D Punjab have been characterized at the MRC Abnormal Haemoglobin Unit in Cambridge. The geographical origin of these is shown in Table III.

TABLE II

INCIDENCE OF Hb D PUNJAB IN SURVEYS IN THE UNITED KINGDOM

Source	No.		References
	Examined	D Punjab	
British servicemen in Singapore and Malaysia	4,387	0	Vella (1961/1962)
Norfolk	1,000	1	Huntsman *et al* (1963) Konigsberg *et al* (1965)
Oxford and Peterborough	1,971	0	Liddell *et al* (1964)
Cambridge	3,000	0	Sick *et al* (1967)
London and other English cities*	10,000	6†	Black (1969)
London‡	10,000	0	F. E. Boulton and R. G. Huntsman (personal communication)
Scotland	3,968	0	Cook and Lehmann (1973)
Ireland	2,500	0	D. Tills (personal communication)
Total	36,826	7	

* Cord blood survey.
† Includes two instances among East Indian immigrants.
‡ Myoglobin survey. (This survey did detect some instances of Hb S and C.)

TABLE III

HAEMOGLOBIN D PUNJAB CHARACTERIZED AT CAMBRIDGE
(unpublished cases only)

Source of Specimens	D Punjab	
	Total	No. in East Indians
England	11	6
Canada	5	2
Australia	1	–
South Africa	2	1
Lebanon	2	–
Italy	1	–
Switzerland	2*	–
France	1	–
Portugal	1	–
Venezuela	1	–

* Both Italians.

Eleven of them had come from England. However, only five had come from English persons (two from Leeds and three from London) while the remainder came from Indian immigrants.

Global incidence and distribution

The distribution and incidence of Hb D Punjab is summarized in Table IV. While single instances of this variant have been reported in persons originating from various parts of Europe, the incidence increases appreciably from the Eastern Mediterranean to the western part of the Indian sub-continent, and especially in Iran, the Punjab, and the western half of India where between 1–2% may have the variant in their blood. It has been reported once from Thailand (Wasi *et al*, 1968) and has been found in a Batak Indonesian from Medan (L. E. Lie-Injo and J. Clegg, personal communication), but does not appear to have been encountered during extensive surveys in Taiwan (Blackwell and Liu, 1970; Blackwell *et al*, 1972) and Japan (Shibata, Iuchi, and Miyaji, 1966).

The spread of haemoglobin D Punjab to its present day geographical pattern may be explained on the basis of population migration occurring in two phases. The first phase would cover the period of the appearance of the mutation and its distribution to northern India, Iran, and Turkey and may reflect the pattern of migrations that occurred in that region during the times of Darius I of Persia or later during the Mogul invasions. The second would cover the spread of the gene to Western Europe and to the New World and may have resulted from intermarriage between Europeans and Indians over the last two or three centuries. Konigsberg *et al* (1965) have examined the evidence that this variant was introduced into Britain from the Punjab as a result of intermarriage of serving British troops with the Indian population during the 18th century. They concluded that the occasional examples of D Punjab found during surveys in Europeans (and especially in Britain, France, and Portugal) confirm the bonds that existed between these countries and India between the 17th and 20th centuries.

Though the earlier literature on haemoglobin variants contains many reports on the occurrence of Hb D in negro subjects, especially in America (Chernoff, 1956; Chernoff and Weichselbaum, 1958; Marder and Conley, 1959; Myerson, Harrison, and Lohmuller, 1959; McCormick, 1960; Cawein *et al*, 1966) only in a very few cases has this been identified as D Punjab (Rothman and Ranney, 1971; P. R. McCurdy and H. Lehmann, unpublished results). In fact the commonest variant with the properties of Hb D in American negroes is Hb G Philadelphia, and this appears to be widely distributed since it has also been found in the Congo (Dherte *et al*, 1959), in Nigeria (Lehmann and Nwokolo, 1959), and in the British West Indies

TABLE IV

INCIDENCE AND DISTRIBUTION OF HAEMOGLOBIN D PUNJAB

Country	No.		Reference	Reference to Isolated Instances
	Examined	D Punjab		
United Kingdom	36,826	7	Table II	Ringelhann *et al* (1967); Schneider *et al* (1968)
Portugal	3042	0	Trincao and Ferreira (1962)	Table III; Preto *et al* (1961); Baglioni (1962)
France	—	—	—	Table III; Labie and Rosa (1969); Wajcman *et al* (1969)
Holland	991	1	De Jong and Went (1968)	—
Denmark	5000	1	Sick *et al* (1967)	—
Norway	3000	1	Monn *et al* (1968)	—
Sweden	4171	0	Nilsson and Eriksson (1972)	—
Finland	4418	0	Nilsson and Eriksson (1972)	—
Russia	317	0	Nilsson and Eriksson (1972)	—
Germany	—	—	—	Martin *et al* (1960); Kohne *et al* (1972)
Switzerland	—	—	—	Table III
Italy	13,187 3000	0 0	Quattrin *et al* (1966) Licenziati (1969)	Table III; Bowman and Ingram (1961); Baglioni (1962); Ventruto *et al* (1963); Cortesi *et al* (1966); Ricco *et al* (1970); Rossi and Morelli (1970)
Bulgaria	—	—	—	Cholakov and Kanchev (1970)
Yugoslavia	2860	2	Sadikario *et al* (1969)	—
Greece	1000	0	Fessas (1959)	R. Rieder (personal communication)
Turkey	—	—	—	Baglioni (1962); Özsoylu (1970)
Lebanon	2938	0	Cabannes *et al* (1965)	Table III
Israel	397 190	0 0	Dreyfus and Pinkhas (1958) Beaven (1973)	Ramot *et al* (1969)
Iran	400 200 184	1 2 3	Rahbar *et al* (1967) Rahbar *et al* (1967) Lehmann *et al* (1973)	—
Indian sub-continent	1000 (Bengal) 129 (W. Pakistan)	1 1	Chatterjea (1959) Stern *et al* (1968)	Table I; Table II Table III; Baglioni (1962); Marengo-Rowe *et al* (1968)
Thailand	2790	0	Flatz *et al* (1965)	Wasi *et al* (1968)
Indonesia	5400	0	Lie-Injo (1959)	L. E. Lie-Injo and J. Clegg (personal communication)
Taiwan	100,000	0	Blackwell and Liu (1970)	—
Japan	130,000	0	Shibata *et al* (1966)	—
Australia	—	—	—	Ungar *et al* (1973)
South Africa	3125	0	Botha and van Zyl (1966)	Table III
Canada	207,300	21	Table I	Table III
Greenland	153	0	Nilsson and Eriksson (1972)	—
Iceland	1000	0	Nilsson and Eriksson (1972)	—
USA	—	—	—	Stout *et al* (1964; see Arends, 1966); Rothman and Ranney (1971); R. R. McCurdy and H. Lehmann (unpublished results); Imamura and Riggs (1972)
Jamaica	10,000 3449	0 0	Milner (1967) Ahern *et al* (1973)	— —
Venezuela	1666	0	Arends (1963)	Table III; Arends (1971)
Mexico	2336	0	Lisker *et al* (1966); Lisker (1971)	Schneider *et al* (1968); Bello *et al* (1969)

(Dance *et al*, 1964). Huisman (1969) has found 13 alpha-chain variants with the electrophoretic mobility of Hb D or G but only three beta-chain variants with a similar mobility amongst 8500 negroes from Georgia (USA) examined. Also frequent in negroes originating in West Africa is Hb Korle-Bu (β73 Asp→Asn) which has been reported from Ghana (Konotey-Ahulu *et al*, 1968) as well as Martinique (Bookchin, Nagel, and Ranney, 1970). This variant is identical with Hb G Accra (Lehmann, Beale, and Boi-Doku, 1964; Boi-Doku, Kinderlerer, and Lehmann, 1972) and is the commonest form of Hb D or G so far found in the British West Indies (Milner, 1967; Ahern, Swan, and Ahern, 1973). It has been suggested that the Hb D found amongst negro subjects in South America (Venezuela—Arends, 1961; Colombia—Restrepo and Londoño, 1965; Brazil—de Araujo and Jamra, 1966) may have originated from the Iberian peninsula (de Araujo and Jamra, 1966). However, the Hb D found in a Colombian negro family (Restrepo and Londoño, 1965) has been identified as Hb Korle-Bu (Restrepo, 1971). The Hb D reported from some parts of Portuguese Africa (Trinčao, 1967; David and Trinčao, 1963) has not been characterized.

Hb D Punjab has not been reported amongst Arab populations in North Africa. It has been suggested that the Hb D that is present at appreciable frequency in the Arabo-Berbers of Algeria (Cabannes, 1965) may in fact be an alpha-chain variant (de Traverse and Coquelet, 1961; Konigsberg *et al*, 1965). More recently, however, two new variants of Hb D have been reported in the Algerian population, one Hb D Ouled Rabah (β19 Asn→Lys) and the other D Iran (β22 Glu→Gln) (Elion *et al*, 1973) the latter having also been reported in an Iranian (Rahbar, 1973) and in a Pakistani family (Rohe, Sharma, and Ranney, 1973).

A variant with the electrophoretic properties of Hb D has been found amongst some groups of American Indians in the USA (Pollitzer *et al*, 1959; Githens *et al*, 1961) but not in South America (Colombia, Venezuela, British Guiana—Arends, 1963; de Pinango and Arends, 1965; Arends, 1971, Restrepo, 1971; Mexico—Lisker, Zarate, and Loria, 1966, Lisker, 1971; Brazil—Salzano and Tondo, 1968; de Araujo, 1971; San Salvador—Bloch and Rivera, 1969). Arends (1966) has summarized the results of haemoglobin studies of Indians of the American continent. The only variant with the properties of Hb D that was found in Canadian Indians in Canada is Hb G Coushatta which was reported in a large family in Saskatchewan (Vella, Isaacs, and Lehmann, 1967a; Vella and Guzak, 1968). It has since been found in 26 other Canadian Indians (12 each in Manitoba and Alberta and two in Ontario). This variant was originally discovered in the Alabama Coushatta Indians of Texas (Schneider *et al*, 1964; Bowman, Barnett, and Hite, 1967) and has more recently been reported in Korean and Chinese subjects (Blackwell *et al*, 1969).

Addendum

The following reported instances of Hb D Punjab were inadvertently omitted from Table IV: Greece (Deliyannis, Ballas, and Christakis, 1969) and Cuba (Uriarte, Perez Atencio, and Colombo, 1973).

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We are grateful to Drs F. E. Boulton, J. B. Clegg, R. G. Huntsman, L. E. Lie-Injo, R. Rieder, and D. Tills for permission to quote their unpublished data.

F. V. is grateful to A. Labossiere and Mrs M. Hurley for technical assistance and to the Medical Research Council of Canada for a grant.

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Oxford Symposium

A symposium on 'The eye in the inborn errors of metabolism' will take place at Oxford University, Oxford, England from 13 to 16 April 1975. Speakers will include: Norman Ashton, Elmer Ballintine, Elaine Berman, Anthony Bron, Donald Bergsma, Elliot Berson, Robert P. Burns, Ronald E. Carr, David G. Cogan, Patrick I. Condon, Edward Cotlier, Harold Cross, Glyn Dawson, Monte A. Del Monte, Albert T. Franceschetti, Jules Francois, Alec Garner, Morton Goldberg, Brian Harcourt, William F. Hughes, Barrie Jay, Kenneth Kenyon, Gordon Klintworth, Toichiro Kuwabara, Irene Hussels-Maumenee, Michael D. Sanders, Jack D. Singer, George Spaeth, C. Takki, Ramesh Tripathi, Ruth van Heyningen, J. M. Walshe, Warren Wilson, Jonathan Wirschafter, Vernon Wong, Wolfgang Zeman. A limited number of free papers will be accepted. Ophthalmologists, paediatricians, or basic scientists are invited to attend; register in advance. For information regarding registration or participation, contact the symposium organizers; Edward Cotlier, MD, University of Illinois Eye and Ear Infirmary, 1855 W. Taylor Street, Chicago, Illinois 60612, USA, or Anthony Bron, FRCS, University of Oxford Nuffield Laboratory of Ophthalmology, Walton Street, Oxford OX2 6AN, England.