THE BLOOD GROUPS OF THE NATAL INDIAN PEOPLE

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Frontispiece

Indians at the Bluff, Durban

By courtesy of the Natal Museum, Pietermaritzburg

PREFACE AND ACKNOWLEDGEMENTS

Hirsch (1958) and Ally (1967) recorded the ABO group frequencies, and Hirsch (1958) the Rhesus D+, D^u and D– frequencies, in the Indian people of Natal. In this thesis the work thus begun has been continued to the fullest possible extent that the reagents available to me would allow. It is well known that time is fast running out for studies to be made of the inherited factors in relatively unmixed populations, and I would have considered it a severe loss not to have given this detailed account now of the present blood group and gene frequencies in the Indian populations of Natal.

My thesis also contains full details of the rare blood groups in the ABO and MNSs systems found by myself among the Natal Indians. I considered it was important to record them, not only for the interest of other workers, but for posterity and in gratitude to the Indians and members of their families who so generously and willingly contributed samples of their blood and saliva for my studies. The findings in a number of subsequent investigations with their blood and saliva, which were either conducted by myself, by myself in co-operation with colleagues in Durban and other centres, or by other workers who requested samples from time to time for their own studies, are included, the former in full and the latter two in the form of summaries. The detailed investigation of a Natal Indian female dispermic chimaera has been described as well, for she was found as the result of a Rhesus blood group test and her blood contained two populations of red cells. All these represent the culmination of 20 most enjoyable and fruitful years spent in the field of blood group serology in Natal, for the abiding interest and the exciting opportunities unexpectedly offered during which I will always be extremely grateful.

The thesis was written and, except where indicated to the contrary in the text, all the studies presented in it were made by myself in my capacity as Serologist in Charge of the Red Cell Serology Reference Laboratory of the Natal Blood Transfusion Service in Durban. The help of Dr Joy Brain and Mrs Sabitha Jithoo, who read Chapter 1 and made profitable criticisms and suggestions, is thankfully acknowledged. For the excellent technical assistance of Mrs Elizabeth Smart in some of the blood grouping tests and the assistance of a number of others who, from time to time, helped with the red cell frequency studies, I am also extremely grateful. In addition, I would like to thank Mr D. Appadoo, Mr B. Lachman, Mr T. Marimuthu,

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Finally, I would like to dedicate my thesis in grateful appreciation to the Indian people of Natal and to the Natal Blood Transfusion Service which they loyally support with the blood donations.

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CHAPTER 1

HISTORY; CIRCUMSTANCES, CUSTOMS AND INHERITANCE; NAMES AND ETHNIC ORIGIN

1.1 INTRODUCTION

The colony of Natal was first settled by Europeans in about 1840 (Brookes and Webb, p 17--41, 1965). Some 15 years later, encouraged by farmers from Mauritius who assured them that the climate and soil near Durban was ideal for cultivating sugar cane, many Natal farmers began planting this crop on their land. However, to cultivate sugar cane on a large scale adequately, the farmers soon found that they needed a supply of reliable energetic labourers. In 1859, in response to persistent enquiries, the Government of Natal, with the consent of Lord John Russell, Secretary of State for the Colonies, and the British Colonial Office, successfully concluded an agreement with the Government of India for the importation of a large number of Indians as farm labourers under a system of indenture (Thompson, 1952). In 1911, when the Union of South Africa was formed and importation of the identured Indians ceased, the population of Natal comprised 133 420 Indians, 98 114 Whites, mainly from western Europe, 953 398 indigenous Blacks, chiefly of Zulu tribal origin (Southern African Negroes) and 9 111 persons of mixed racial descent known colloquially as "Coloureds" (Union Year Book, 1910–22). The small size of the "Coloured" population (10,85%) shows that little miscegenation had taken place in Natal (Brookes and Webb, p 248, 1965).

Since provision had been made in the 1859 agreement for land to be granted to some of the Indians when their period of indenture was over (Brookes and Webb, p 83, 1965), from the beginning a number at least were clearly expected to settle permanently in Natal, but there seemed to have been no conception that so many would have wanted to do so (Pachai, 1952). Together with the "passenger" Indians (merchants and traders who came later and paid their own fares), the Indians stayed on despite considerable White hostility in later years and despite the incentives and penalties imposed by the Natal Government in its efforts to induce them to leave (Kuper, 1960; Brookes and Webb, p 181–189, 1965). From 1910 onwards, all Indian immigration into South Africa was actively discouraged (Brookes and Webb, p 287, 1965); but gradually, from the Cape Town Agreement in 1927 in which the South African Government acknowledged that the Indians who remained part of the permanent population of

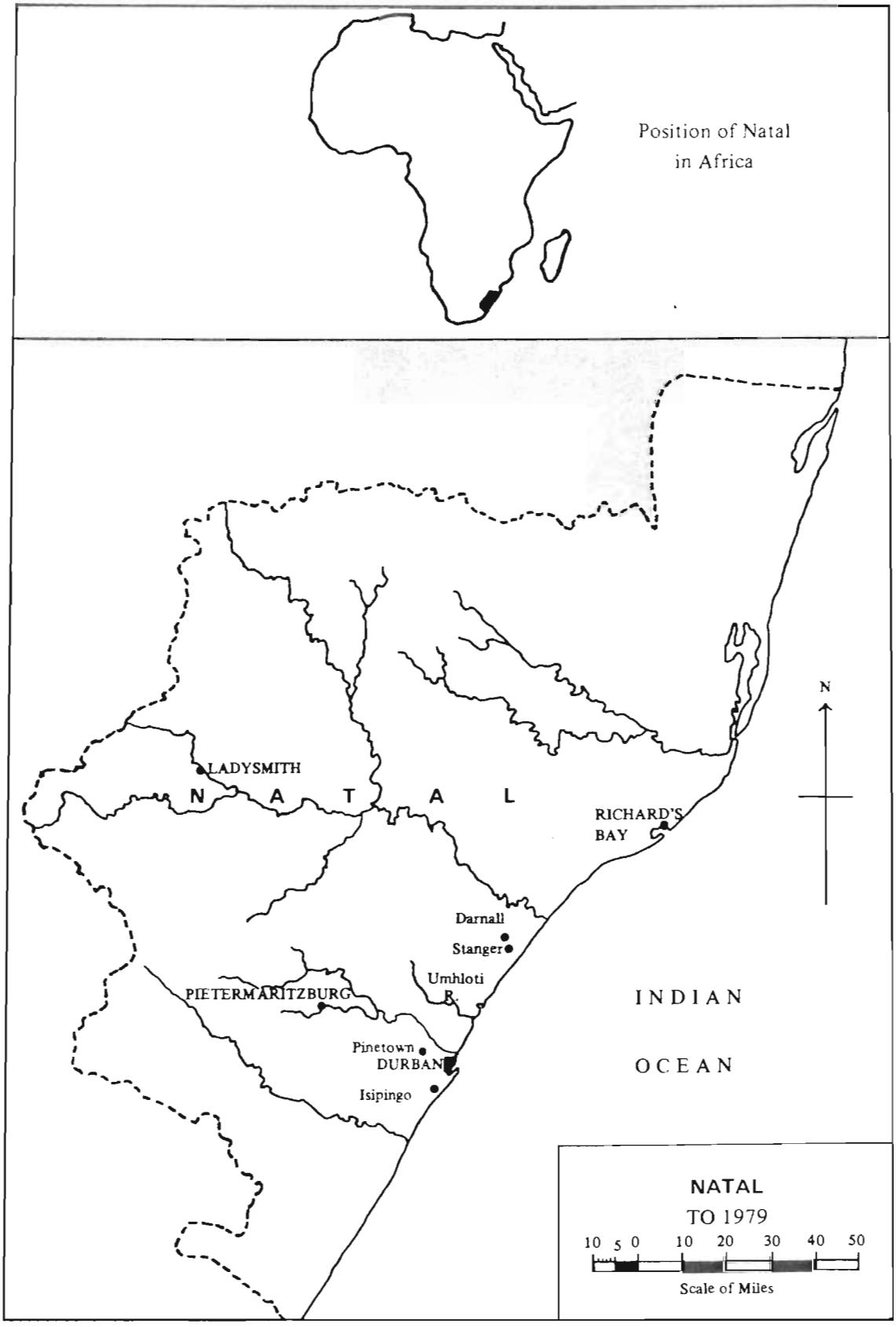
South Africa had a right to educational and other facilities, to the ministerial statement (Dept of Information, 1975) which confirmed their acceptance when South Africa became a Republic in 1961, all the Indians who had chosen to live in South Africa came to be regarded as permanent members of the South African population.

In 1960, just over 100 years after the arrival of the first Indians and immediately before the Republic of South Africa came into being, the population census (Statistical Year Book, 1964) showed that the number of people of Asian origin in South Africa had reached 477 125, of whom 1,26% or approximately 6 000 were non-Indians. The number of Asians in Natal was given as 394 854, or 82,76% of the Asians in South Africa, and 221 403, or 59,9% of them resided in Durban. Only 817, or 13,6% of the non-Indian Asians resided in Natal. The population census of 1970 (Dept of Statistics, 1971) unfortunately made no distinction between Indian and non-Indian Asians, but by then the number of Asians in Natal had increased to 514 810; a figure that comprised approximately 83% of the total Asian population of South Africa and some 3% of the South African population of all racial origins. During the ten years from 1960 to 1970, therefore, the Asian population in Natal rose by about 23%. In 1974, the total number of Indians in South Africa was conservatively estimated as being some 700 000 (Dept of Information, 1975) and, at the next census, taken in 1980, the number in Natal will almost certainly be over 600 000.

Whereas in India some 80% of the Indians reside in the rural areas, in Natal (Plate 1.1) more than 70% dwell in the urban districts of the two large cities, Durban and Pietermaritzburg (Burrows, 1943). The remainder of the Indians live mainly in the smaller towns and villages of Natal, especially in those situated in the sugar cane growing coastal regions. Very few Natal Indians now own large farms, the majority of their indentured forefathers, rather than acquiring land, apparently having found it more economical to become tradesmen, small-scale market gardeners, laundrymen, domestic servants, waiters and unskilled labourers when their years of indenture were over (Burrows, 1943; Kuper, 1955). Later, from among all these there appeared a middle class of doctors, lawyers, teachers and other professional men and women, many of whom achieved positions of eminence (Brookes and Webb, p 86, 1965).

The people of India are divided into two linguistic groups, situated geographically in the north and south of the sub-continent. The languages in the south, roughly from Cape Comorin as far north as the Narmada River and east of the Western Ghat mountains, are known as Dravidian, and those in the north as Aryan (Grierson, 1927).

Plate 1.1


Position of Natal
in Africa
LADYSMITH
N A T A L
RICHARD'S
BAY
N
Darnall
Stanger
Umhloti
R.
PIETERMARITZBURG
INDIAN
OCEAN
Pinetown
DURBAN
Isipingo
NATAL
TO 1979
10 5 0 10 20 30 40 50
Scale of Miles

Since there is no known instance of a Dravidian having superseded an Aryan language but numbers where the reverse has taken place, and there is no real evidence that similar languages exist elsewhere, the Dravidian languages are believed almost certainly to be the oldest, but opinions are divided as to whether or not the Dravidian language-speaking Indians are autochthonous in Southern India (Grierson, 1927; Encyclopaedia Britannica, 1973). The early ancestors of these Indians are nevertheless not thought to have been responsible for or associated with the remarkable Harappian Indus River Valley civilization which thrived in early north-west India (Encyclopaedia Britannica, 1973). History relates that, in about 2000 B.C., the Harappians were overrun by migrating fair-skinned blue-eyed invaders who are believed to have entered India through the north-west mountain passes (Grierson, 1927) from southern Russia or the Austria-Hungary region in eastern Europe. The invaders were Aryans, whose name meant "noble", and they settled and mixed with the Indus Valley people. The Aryans introduced a form of the Sanskrit language which differed from the dialect spoken by their countrymen who had settled earlier in Persia (Grierson, 1927). The ancient, sacred scriptures of the Hindus, known as the Vedas, which were transmitted orally for some 2 000 years, were written in Vedic-Aryan Sanskrit script prior to 600 B.C. (Encyclopaedia Britannica, 1973). In time, the Indo-Aryan people spread eastwards in India, incorporating into their already hybrid language as they did so, many words used by the local inhabitants. Their language eventually developed into Hindi, at present almost certainly the most widely spoken Aryan language in northern India.

In the 8th century A.D., India was again invaded, this time by people of the Islamic faith who came through the mountain passes in the north-west and overthrew the earlier Indo-Aryan rulers. The Moslems controlled northern India until the 14th century and, in their turn, were overthrown by the Mughals, who were Moslems of Mongol origin from central Asia. Under the Mughals, India enjoyed 300 years of prosperity, and many Hindus were converted to Islam during this period. In the west and north-west of the sub-continent, the Arabic and Persian languages of the Moslems in due course merged with Hindi and developed into Urdu, a language still spoken predominantly by Moslem Indians (Encyclopaedia Britannica, 1973).

The Dravidian and Aryan language speaking Indians are of many different physical types, but in Natal the typical 'Dravidian' Indian is short to medium in height and is lightly built. He is dolichocephalic, has a large nose which is often depressed at the root, and his eyebrows are fairly prominent. His skin colour is medium to dark brown, his eyes are brown and his hair, which is thick and black, is usually straight

but may be wavy or curly (Mistry, 1965). The typical 'Aryan' Indian in Natal is also lightly built, but he is taller. He is dolicho- or mesocephalic, has a nose of medium width, more often lacking than having a depression at the root, and his skin colour varies in different individuals from light to dark brown, sometimes even being white or black. His eyes are brown, and his hair, which is thick and black, is more often straight than wavy or curly (Mistry, 1965). A third group of Indians, the Indian Aborigines who inhabit the jungles and remoter regions of southern India, are short, sturdy food-gatherers whose thick, broad noses, thick lips and low facial angles give them a more negroid than Indian appearance (Risley, 1891). These Indians have very dark brown or black skins and black, curly hair. It is very unlikely that any aboriginal Indians ever came or were brought to Natal either in the early years of settlement or later.

1.2 BRIEF DETAILS OF NATAL INDIAN HISTORY

1.2.1 The indentured Indians

In 1849, about nine years after the arrival of the first White settlers in Natal, E.F. Rathbone, a farm overseer experienced in sugar cane cultivation, used the first four Indians to set foot in Natal, who had been brought over earlier from Mauritius, to help him plant five acres of sugar cane on some land near the Umhloti River, just north of Durban (Hattersley, 1950; Osborn, 1964). Soon, many Natal farmers, aware that sugar cane was potentially a very lucrative crop, were planting experimental patches, and the first cane crushing mill was started by Edward Morewood (Hattersley, 1940) who had to abandon it shortly afterwards because of insufficient funds (Brookes and Webb, p 80, 1965). The Natal farmers at that time relied for their labour on the Zulus, of whom there were vast numbers, but the Zulus, not yet familiar with the responsiblilities attached to earning money regularly and, looking upon cultivation of the soil as women's work, were unwilling and unreliable (Brookes and Webb, p 81, 1965). By 1855, the farmers had begun agitating for the importation of substitute labourers, and Colenbrander and van Prehn introduced a handful of Chinese and Malayans from Java who were accustomed to sugar cane cultivation. However, they were prevented from continuing with their scheme by the Colonial Secretary in Hong Kong who considered that the wages being offered were too low (Hattersley, 1940). Eventually, in 1859, the Government of Natal passed three laws, the most important of which, Law No. 14 of the Colony of Natal (Ordinances, Laws, 1845 –1874), contained details of the regulations and conditions under which a proposed

group of indentured Indians were to be cared for and employed. The laws were confirmed by the British Colonial Office and, after they had been approved by Lord Canning, first Viceroy of India, and by the Indian Government, in Act No. 33 of India of 1860 (Brookes and Webb, p 82–83, 1965), the importation of the indentured Indians to Natal was set in motion.

The system of indenture approved by Lord Canning bound the Indians to labour, as directed, at first for three but later for five years. They were then free to renew their indentures for five more years, offer their labour in the open market or even not work at all if they so wished. After ten years they were entitled to claim a free passage back to India or, subject to the approval of the Lieutenant Governor, might be granted a piece of Crown land in Natal equal in value to their passage money (Ordinances, Laws, 1845–1874). It is of interest that there was nothing either in the laws of Natal of 1859 or in the agreement concluded with India in 1860 which specifically compelled the Indians to return to India (Thompson, 1952).

After Act No. 33 of Natal had been proclaimed in 1860, the Post-master General of Natal, W.M. Collins, was despatched as a special agent to India to make arrangements for the Indian importations. His initial requisition was for 1 046 men and the prescribed proportion (35%) of women (Thompson, 1952; Brookes and Webb, p 86, 1965). The Government of India permitted Indians to be exported only from Madras, Calcutta and Bombay; and Collins, with the assistance of the agencies already established there for other parts of the British Empire, commenced his recruiting campaign in Madras and later moved to Calcutta (Plate 1.2). Collins successfully despatched five ships with the required number of Indians aboard to Durban in 1860 and 1861, and then returned himself, having made arrangements for fulfilling further requirements, should the need for them arise (Thompson, 1952).

The first ship to arrive at Durban, the "Truro", completed the 45-day voyage from Madras on 16th November, 1860, and put ashore 342 Indians, 203 of whom, or 59%, were adult men. The next, the "Belvedere", took 54 days to reach Durban from Calcutta and arrived on 26th November, 1860, bringing 351 Indians, 225 of whom, or 64%, were adult men (Brookes and Webb, p 10 and 85, 1965). These two ships were followed by the "Lord George Bentinck", the "Spirit of Trade" and the "Tyburnia" (Ship's lists), all of which increased the number of the indentured Indians in Natal in the next year to 1 029 (Thompson, 1952).

PLATE 1 2


KASHMIR
WEST PAKISTAN
PUNJAB
INDUS RIVER
DELHI
NEPAL
UTTAR PRADESH
RAJASTHAN
GONDA
BASTI
GOROKHPUR
LUCKNOW
FYZABAD
CHAMPARAN
MUZAFFAPUR
DHARBANGA
JAUNPUR
AZAMGHAR
SARAN
BALLIA
PATNA
GHAZIPUR
BANARAS
SHAHABAD
GAYA
MONGHYR
MIRZAPUR
BIHAR
S. PARGANAS
EAST PAKISTAN
PALAMAU
HAZARIBAGH
MADHYA PRADESH
KUTCH
GUJARAT
MANBHUM
BIRBHUM
WEST BENGAL
RANCHI
SINGHBHUM
CALCUTTA
KATHIAWAR
NARMADA RIVER
PORBANDER
SURAT
NAGPUR
ORISSA
MAHARASHTRA
BOMBAY
AHMADNAGAR
POONA
GANJAM
VISAGAPATAM
ANDHRA PRADESH
GODAVARI
GUNTER
MYSORE
NELLORE
MALABAR
BANGALORE
MADRAS
CHINGLEPUT
ARCOT
SALEM
MADRAS
TRICHINOPOLY
TANJORE
KERALA
MADURA
RAMNAD
TINNEVELLEY
CAPE COMORIN
SRI LANKA
(Ceylon)
N
INDIA
1969
200 0 200
Scale of miles

As soon as the advantages of the Indians as labourers became known in Natal, requests accumulated for more of them to be imported. As a result, between 14th August, 1863, and 21st May, 1866, 2 814 further Indian men were landed at Durban (Thompson, 1952). However, difficulty was experienced in recruiting the correct proportion of Indian women to accompany the men. Many Natal farmers also objected to the principle of accepting on their farms Indian women whom they regarded as merely a nuisance, and nothing was done to remedy this (Thompson, 1952). In all, approximately 6 000 Indians were imported from Madras and Calcutta between 1860 and 1866, and the importations then ceased for a time owing to a severe world trade depression (Burrows, 1943). In response to the complaints of the first Indians, who returned to India in 1871 after having completed their indenture period, about their treatment in Natal, the Indian Government also forbade all further recruitment for this region (Brookes and Webb, p 88–89, 1965). The complaints of the Indians were investigated by the Natal Government and, on the basis of the subsequent report, Law No. 12 of Natal of 1872 was passed which guaranteed the Indians better conditions and treatment. The Natal government also appointed a Protector of Indian Immigrants who had wide powers to look after the affairs of the Indians and act effectively on their behalf. The Government of India and the Viceroy approved the new conditions in 1873 (Stein, 1942), and the Indian importations then recommenced, to the obvious satisfaction of the farmers and without, at that stage, any apparent opposition from the White public of Natal (Brookes and Webb, p 88–89, 1965).

From the year 1880 onwards, strong anti-Indian feelings arose among the Natal Whites, their chief criticism being that many of the "passenger" and ex-indentured Indians had become trade competitors (Brookes and Webb, p 157 and 168, 1965). The resentment grew and, in 1897, a large party of Whites gathered on the quayside at Durban, intent on demonstrating vigorously against five or six hundred additional Indian passengers who had just arrived in two ships. The demonstration was quelled by the Attorney-General of Natal, Harry Escombe, who promised that the Government would stop subsidising indentured Indian transport immediately. However, Mahatma Gandhi, who happened to be returning to Natal in one of the ships after a brief visit to India, was attacked as he reached the quay and was rescued, just in time, by Mrs Alexander, wife of the Durban Superintendent of Police (Brookes and Webb, p 183–184, 1965). Gandhi, who came to Durban first in 1893, founded the Natal Indian Congress; and it was in Natal that he first used his principle of passive resistance to support the Indians in their campaigns against injustices and discrimination. The

Natal farmers, who by this time had been joined in their need for labour by the owners of the coal mines in northern Natal, took to paying the transport subsidy for the Indians themselves (Burrows, 1943); but in 1911, in response to requests by influential Indians in India, the Indian Government passed legislation prohibiting all further export of indentured Indians to Natal absolutely (Brookes and Webb, p 250, 1965). By the end of December, 1932, of the more than 152 000 Indians who had been brought to Natal since 1860, 32 031 remained, the rest having either returned to India or died in Natal (de Vos, 1947). Following the more vigorous recruiting measures introduced after 1874, the number of Indian women in Natal had increased and, by 1936, the earlier adverse numerical disparity between the sexes had virually disappeared. At that stage also, some 182 280 Indians had been born in Natal, of whom 91 287 were males and 90 993 females (de Vos, 1947).

1.2.2 The "Passenger" Indians

After the year 1870, poverty, the exorbitant rate of taxation in India and the promise of trade with their countrymen in Natal caused numbers of Moslem and Hindu merchants from the districts of Kathiawar, Surat and Porbander and from the northern Indian provinces to undertake the long sea voyage to Natal (Kuper, 1960). The merchants came as normal paying passengers mainly in ships from Bombay, and many brought their wives and children with them. As they were British subjects and could move about freely within the Empire, almost no records of the dates of their arrival exist but, by 1887, Natal appears to have contained about 4 000 of them (Brookes and Webb, p 158, 1965). A few traders and hawkers, most of whom were ex-indentured Indians, came also from Madras and Calcutta, less than 50 Moslems arrived from Mauritius and various parts of East Africa, and a few Tamil-speaking Hindus appeared from Mauritius and Ceylon (Brookes and Webb, p 86, 1965). Alluded-to, jokingly, as "Arabs" by the Whites, the "passenger" Indians in the early years are believed not to have constituted more than 10% of the Indian population in Natal (Statistical Report, 1953).

During the 1880's, the Indian merchants were subjected to strong anti-Indian feelings by the Natal Whites who, by then, had begun to regard them as trade competitors (Kuper, 1960; Brookes and Webb, p 181–189, 1965). This friction occasionally led to physical conflict, and it precipitated the curtailing of Indian immigration in 1897 (Act No. 1 of Natal of 1897). After 1913, when Indian immigration was restricted much more severely, further Indian merchants entered Natal illegally, but without

their families. In 1927, the Government of South Africa condoned their entry, but it did not permit their families to join them (Union Statutes, 1910--1947). However, just before 1956, when all further Indian immigration was forbidden, many Indian merchants took advantage of a temporary relaxation in the regulations and rushed their families from India to Natal (Kuper, 1960).

1.3 CIRCUMSTANCES, CUSTOMS AND BELIEFS RELATED TO GENETIC INHERITANCE

1.3.1 Early domestic difficulties

When Collins went to India in 1860 to recruit the first indentured Indians for Natal, his directions were to obtain young men fit enough for hard agricultural labour and, as far as possible, who were unencumbered by families (Kuper, 1955). A minimum proportion of 35% Indian women had been stipulated to accompany the men in the 1860 agreement with India, and Collins and Burton, Collins' successor as the Natal agent in Madras, both exceeded this (Thompson, 1952; Brookes and Webb, p 86, 1965). However, Hunt and Marriott, Collins' successors in Calcutta, experienced considerable difficulty in persuading Indian women to undertake the journey, and the true proportion in Natal in the early years was probably nearer 25% (Thompson, 1952). Many of the indentured Indian men also left India because of poverty and domestic problems and, although some brought their wives with them, a considerable number left them behind. In addition, among the women who came, both from Madras and Calcutta, there were many who were either young widows, deserted wives or prostitutes (Kuper, 1955).

From the beginning, caste among the indentured Hindus who were brought to Natal was seriously upset (Kuper, 1955). Many Hindus belonged to superior castes, and there were Moslems, Christians and others. In the ships in which they came, their cramped living conditions and absence of separate facilities frequently made it impossible for the Hindus to observe the proper caste "distances", and many "lost caste" as a result (Stein, 1942). On reaching Natal, large numbers found that they were expected to live together in barracks, in which there were no separate rooms for the unmarried women or for the married couples, and the acute shortage of Indian women which existed in the early years was also cruelly ignored by the Natal farmers, whose sole interest was to acquire labourers (Kuper, 1955). It is of interest that, during this difficult period, so little miscegenation appears to have taken place between Indian men and Zulu women. Perhaps it was because these two groups distrusted each other

(Thompson, 1952). The Natal Government made little attempt to register Indian marriages until 1874, and even then serious doubts arose as to what actually constituted them (Stein, 1942). The men frequently formed loose unions with the women, and some later abandoned them, together with their children. The Wragge Commission (Indian Immigrants Commission, 1887) which inquired into the affairs of the Indians, noted that to resort to registering only those Indian marriages which had taken place according to Hindu or Moslem rites would not be enough, for more than 115 mixed Moslem-Hindu couples were found, during the contracting of whose marriages no Moslem or Hindu religious ceremony would have been possible (Stein, 1942). Law No. 25 of Natal, passed in 1891, ensured that the marriages of the indentured Indians registered before a resident magistrate in Natal were accepted, both in Natal and in India, but the law was unsatisfactory as the "passenger" Indians were not accorded the same facility (Stein, 1942). These marriages were also regarded by the indentured Indians as being invalid unless a prior religious ceremony had taken place and, since the costs of any subsequent divorce proceedings were well beyond their ability to pay, they avoided them . Thus, the law increased rather than decreased the prevalence of profligacy and concubinage among the Indians (Stein, 1942). Their alternatives were, marriage across the barrier of caste, celibacy, homosexuality or returning to India (Kuper, 1955), and the latter expedient was sometimes accomplished by feigning illness or insanity. In view of all these adverse circumstances, it is little wonder that many of the Indians in Natal abandoned all their former caste associations completely. Later, when more Indian elders had arrived who were in a better position to exert their influence, order was restored again. After 1874, Lord Carnarvon, Secretary of State for the British Colonies, also raised the minimum proportion of women permitted in each consignment of indentured Indians to 50%. However, the ratio of Indian men to women continued to remain unbalanced in Natal until after the end of the 19th century (Meer, 1969). In modern Natal, the younger Indians are largely ignorant of caste. Many of them are adapting, in all aspects except religion, to the customs and ideals of a western-European society (Rambiritch and v.d. Bergh, 1961), and maximum fertility is the expected norm in most Natal Indian families (Burrows, 1943).

1.3.2 Aspects of Indian customs and beliefs in relation to marriage

After the arrival of the Aryans in north-west India, the Harappian people in the Indus river valley apparently adopted restrictive customs and practices involving their personal, home and working lives and in particular their choice of marriage partners.

The restrictions evolved into the laws of caste, and they persisted in India for nearly 4 000 years.

Caste is a form of class distinction and it is thought to have been encouraged in north-west India by the Aryans, who may have looked upon it as a means of reinforcing their own superior rank (Nowbath, 1964). The principles of caste would probably have been accepted by their subjects without too much concern, as it seems that, from antiquity, they had been accustomed to a socially stratified type of society. The stratifications were five in number, and the first four were known as the Varna. In descending order, the Varna were known as the Brahmin, the Kshatriya, the Vaishiya and the Sudra. The Brahmins were the priests and scholars, the Kshatriya were the noblemen and warriors, the Vaishiya were the merchants, traders, herdsmen, fishermen, etc., and the Sudra were the labourers. On the fifth level, the lowest, were the Pariahs and the untouchables who performed menial tasks in India such as sweeping the streets, clearing away rubbish and burying dead bodies (Kuper, 1955). In the early Indian communities, the restrictions imposed on the people by the Varna classifications and caste would probably have blended naturally with the Hindu philosophy that the social level of human existence was pre-ordained at birth. Escape to a higher or a lower social level, depending upon whether the conduct of the subject on earth had been good or bad, was believed to be achieved only when he was reincarnated after death. Under normal conditions, the Hindus are restricted by their caste laws (Kuper, 1955) to eating only certain foods, speaking to, touching, living near and working only with certain people, engaging only in certain pre-determined occupations, scrupulously observing certain religious and other rituals, and marrying only those individuals whose caste, religion, language and other cultural customs have been approved beforehand by their family elders. Perhaps the most important concern of the Hindu elders is that, at all costs, they must avoid the possibility that their children will knowlingly or unknowlingly contract an incestuous union (Tinker, 1974; Sanghvi, 1966).

Since the restricted choice of marriage partners may well have affected the distribution of inherited factors such as the blood groups, this aspect of the Indian caste system will be discussed in some detail. From ancient times, the Hindus in India have observed strict caste endogamy, which is the custom of marrying only within the caste or clan, tribe or group of birth (Sanghvi, 1966; Vyas *et al.*, 1958). More than 3 000 castes and sub-castes existed in India at the end of the 19th century (Vyas *et al.*, 1958) and they were adhered to tenaciously, particularly in the village communities (Kuper, 1955). Each caste contained very many small, mutually exclusive sections or enlarged families,

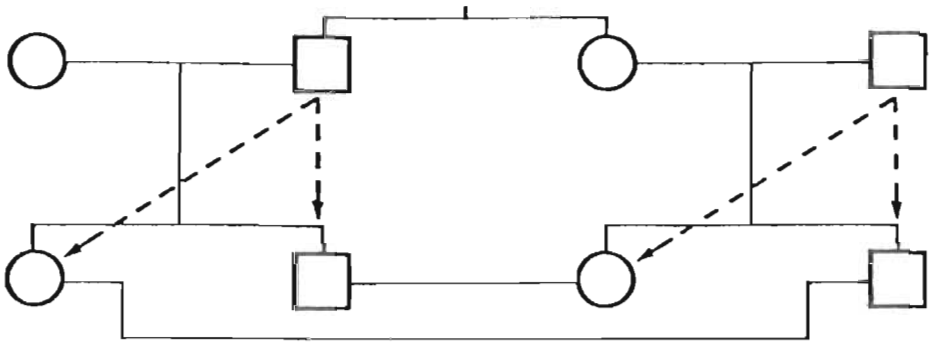
called Gotra, Nukh, Kul or Kutum, within which the members were forbidden to marry anyone who was considered a direct lineal relative, as far back as the seventh generation on the father's and the fifth generation on the mother's side (Sanghvi, 1966). The rule, which was regarded as a sure means of avoiding incestuous unions, was associated with the Hindu belief that the factors that govern inheritance were passed down to them only by their fathers. The Hindus regarded their women solely as repositories for the infants before birth and concerned only with their growth and development. This belief led the Hindus who spoke the Dravidian languages Tamil and Telegu to forbid marriages between the children of brothers but permit them between the children of sisters, between the children of brothers and sisters, and between uncles and their nieces (Figure 1.1). However, the Hindi-speaking and the Gujarati-speaking Hindus, whose Indo-Aryan background showed that they may have been influenced in this by other cultures, forbade all cross- and parallel-cousin marriages. The Moslem Indians accepted cross- and parallel-cousin marriages, the latter even between the children of brothers when a special reason for them existed in the family. Sanghvi (1966) said that a high consanguineous marriage rate caused a reduction in the incidence of persons heterozygous for genetic markers such as the blood groups, and that it led to a gradual increase in the frequency of the rarer markers. A marker that had originally had a frequency in India of 1 in 40 000, was said by him to have increased in frequency 13 times in this way. The Hindus believed that the surest means of avoiding incest was to marry the closest possible female relative who was not considered lineal, for inter-family connections that existed in the past in village communities might have become forgotton (Tinker, 1974). All non-family women were therefore suspect, unless evidence existed that marriages between other members in their respective sections (enlarged families) had been successful. The practice had the advantage that the family possessions were retained; but as all the members in a particular section usually remained within it, at least until the head of their section died, the men who were acquired as husbands from other sections were normally relegated to the status of family visitors (Tinker, 1974).

About 90% of the indentured Indians who were brought to Natal were Hindus, at least 12% were Moslems and less than 2% were Christians (Brain, 1980). Among the Hindus, there would almost certainly have been a large number of Sudra Indians, and it is said that there were also between 25 and 30% Vaishiyas, between 10 and 15% Kshatriyas and a few Brahmins (Kuper, 1960). A few Pariahs may also have accompanied them.

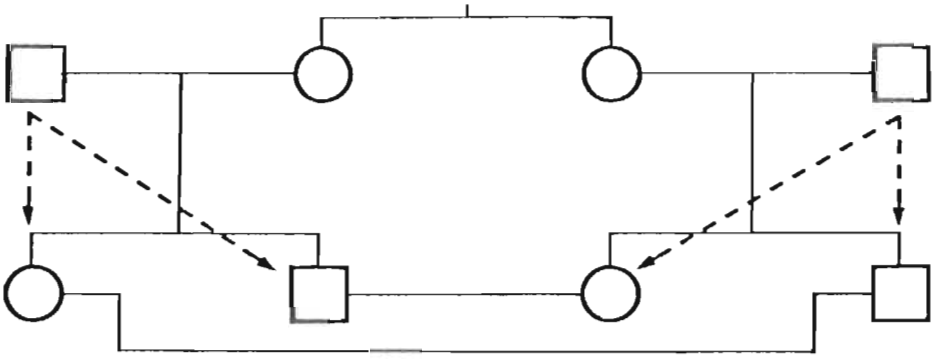
FIGURE 1.1

THE INDIAN CONCEPT OF GENETIC INHERITANCE

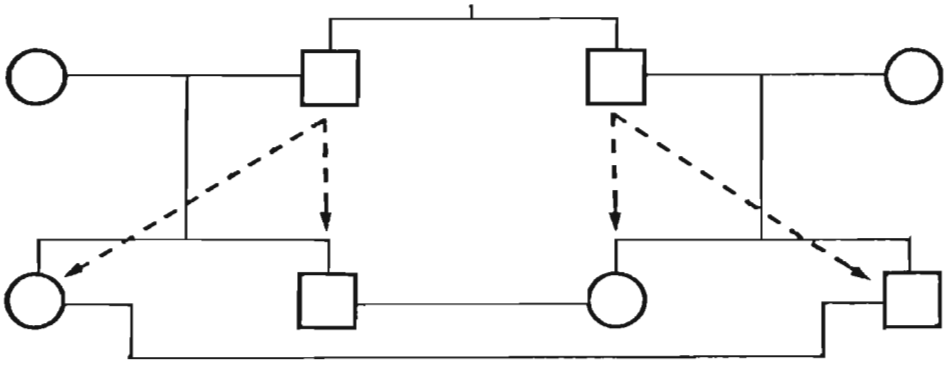
CROSS-COUSIN MARRIAGE



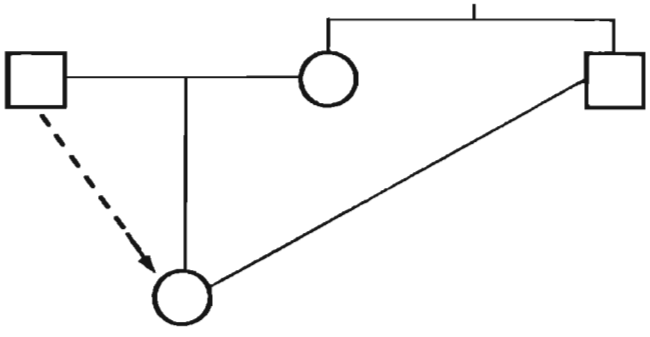
PARALLEL COUSIN MARRIAGE BETWEEN THE CHILDREN OF SISTERS



PARALLEL COUSIN MARRIAGE BETWEEN THE CHILDREN OF BROTHERS



UNCLE-NIECE MARRIAGE



KEY

(- - - - - = Lineal relatives, according to Indian custom.

The majority of the "passenger" Indians who came to Natal were Moslems who professed principally the Sunni tenets of Islam. Undoubtedly some of them were of Arab or Persian origin but, in the main, they were Hindus whose ancestors had been converted to this faith when India was conquered by the Moslems from central Asia in the 14th century A.D. A few further "passengers" from Bombay were Hindu merchants who became known as the "Gujarati-speaking Hindus", and their descendants have maintained their earlier social customs with the utmost strictness to the present (Kuper, 1955). A small number of ex-indentured Indians also returned of their own accord to Natal (Kuper, 1960). In 1960, the South African population census figures showed that 75,5% of the Indians in Natal were Hindus, 14,6% Moslems, 7,4% Christians and 0,1% Buddhists, Parsees (Zoroastrians), Jains and others. No Confucians were recorded, and 2,3% of the Indians indicated that they either had no religion, objected to being questioned or preferred to give no answer (Statistical Year Book, 1964).

1.4 INDIAN NAMES AND ETHNIC ORIGIN

1.4.1 Origin in relation to languages

The people of India and Pakistan are conveniently sub-divided by their languages into groups that correspond reasonably well with the known geographical centres of their ethnic origins (Grierson, 1927). As the majority of the descendants of the indentured Indians in Natal now have no knowledge of where their ancestors originally lived in India, in order to compare their blood group frequencies with the known frequencies of the Indians in India, it was decided to use the Indian languages spoken by the Natal Indians as a guide. The areas in India from which the Indians either came or were gathered for Natal were also known from historical records.

Although the main centres for indentured Indian recruitment were situated in the Indian cities of Madras and Calcutta, the Indians who were brought to Natal were collected from a wide area around them and spoke a number of different Indian languages. In 1860, the Madras Presidency extended from Cape Comorin in the south of India to the northern border of the district of Ganjam in the north-east, and it included most of the present-day State of Andhra Pradesh, the home of the Hindus who speak the Dravidian language Telegu. The Hindus in the city of Madras and in most of the Indian sub-continent from there to Cape Comorin, including the present-day State of Mysore, spoke the Dravidian language Tamil; and in Kerala, on the south-west coast of India, the people spoke the Tamil-related language Malayalam. In Calcutta and nearby

northern Indian provinces south of the Nepalese border, the principal language spoken by the people was Hindi, and in the west of India, in and around the city of Bombay, the languages were Gujarati, Urdu and Memon (Grierson, 1927).

Tamil and Telegu are ancient, vernacular languages of Dravidian origin in southern India and they are unlike any other languages known (Grierson, 1927). Although both are abundantly polysyllabic and agglutinative, Tamil, which is probably the older, has a richer, more extensive and well-cultivated vocabulary, while Telegu excels in the euphony of its words. In decreasing order of numbers, the Tamil-speaking indentured Indians were gathered for Natal in the south of the then Madras Presidency (Plate 1.2) mainly from the districts of Trichinopoly, Madura, Ramnad, Salem, Tanjore, Chingleput, North and South Arcot, Malabar and Tinnevelly, and those who spoke Telegu from the more northerly coastal districts of Vizagapatam, Ganjam, East and West Godavari, Gunter and Nellore (Tinker, 1974).

As already mentioned, the Indo-Aryan language Hindi, which is based on Sanskrit, arose in north-western India some time after the arrival and subsequent settlement there of the Aryan conquerors in about 2000 B.C. The word "satem", used in it for the number 100, shows that these Aryans spoke a different dialect from their countrymen in Europe who used the word "centum" (Grierson, 1927). The Hindi-speaking indentured Indians were gathered for Natal in the Province of Bihar, mainly from the hill districts of Santal Parganas, Hazaribagh, Ranchi, Manbhum, Birbhum, Singhbhum and Palaman, and from the Ganges Valley districts in this province of Shahabad, Patna, Gaya, Muzaffarpur, Champaran, Saran, Darbhanga and Monghyr. In addition, others were gathered in the then United Provinces districts of Ballia, Ghazipur, Azamghar, Fyzabad, Basti, Gonda, Gorakhpur, Banaras, Mirzapur and Jaunpur (Tinker, 1974).

The Indo-Aryan language Gujarati arose in western India in the areas known as Kathiawar and Kutch apparently some time between A.D. 400 and A.D. 600. The forerunner was Prakrit, a later form of the Vedic Sanskrit language. Gujarati evolved through Apabhramsa, the 12th century language of the grammarian Hemachandra, to its present form, the earliest known examples of which are found in the Mugdhavabodha-mauktika of 1394 (Grierson, 1927). The few indentured Indians who spoke Gujarati were gathered for Natal in the Bombay Presidency of their time from the district of Ahmadnagar (Tinker, 1974).

The "passenger" Indians who journeyed to Natal came mainly from Kathiawar, Surat and Porbander, at one time districts in the Bombay Presidency but now part of the Indian State of Gujarat (Kuper, 1955). A few came also from the more northerly states in India. They spoke Urdu, Gujarati and Memon. The Urdu language is Aryan in origin and was introduced into north-western India in the 18th century by the Moslem invaders who came from the east. It contains many Persian, Hindi and Arabic words and is still spoken mainly by Moslem Indians (Encyclopaedia Britannica, 1973). The "passengers" who spoke Urdu outnumbered those who spoke Gujarati and Memon by about two to one (Meer, 1969).

In the population census of 1960, 34,7% of the Asians in Natal spoke Tamil, 8,7% Telegu, 29,5% Hindi, 7,9% Urdu, 5,7% Gujarati and 0,2% other Indian languages. English was spoken by 13,2%, Afrikaans by less than 0,1%, Chinese by less than 0,1% and other languages by 0,1% (Statistical Year Book, 1964).

1.4.2 Languages in relation to names

Although the Indian languages provided an indirect means of identifying the ethnic origin of Natal Indian individuals, two further problems had to be overcome. They were, that for reasons of economy and time the blood donors could not be questioned and that an increasing number of Natal Indians now spoke only English. Consequently, the Indian language spoken either by the donor at home or that at one time had been spoken at home by his family had to be identified by means of his Indian name. Happily, a number of senior Indian staff members who, between them, spoke all the Indian languages and were conversant with the origin and derivation of Indian names, accomplished this for me successfully and without undue difficulty. However, I confirmed their findings by identifying from the early ships lists made by shipping clerks, the villages and districts, and through them the States and regions in India, from which the indentured Indians with certain names came or to which, when their period of indenture was over, they subsequently returned. The ships lists are preserved permanently in the Department of Indian Affairs in Durban and in the Natal Archives in Pietermaritzburg.

The task of relating the names of the Indians to a particular Indian language in this study was assisted by referring to Indian traditions. The Hindus, for example, customarily selected first names for their children from the early Vedic Sanskrit scriptures such as the Rig-veda of 1400 to 600 B.C. and the Ramayana and Mahabharata of 500 to

300 B.C. These books contained an abundance of stories about Hindu gods, mythology, Indian history and Hindu personalities of the past. To ensure that the names were propitious, the parents also consulted Hindu astrologers who advised them in accordance with the signs of the Zodiac and Tantric philosophy. Until quite recently the names selected were kept secret to avoid evils and a "calling name" was substituted. However, when schools required birth certificates to be produced, secrecy was no longer possible and the custom has largely fallen away. (Pillay *et al.*, 1973, 1974). Favourite Hindu first names for males were Brahma, Krishna, Shiva (Siva), Vishu and Rama (Ram), and for females Sakti, Lakshmi, Devi, Gangamai, Parvathi and Sarasvati. The first or family names of Pillay and Govender were Tamil caste terms, and Reddy and Naidoo (Naidu, Nydoo) were Telegu-, Naicker and Moodley (Mudali) were Tamil-, and Singh and Maharaj were Hindi titles. The Hindi title of Maharaj became a symbol of Brahmin (priest) status in Natal, and the popular Vaishiya title of Naidoo was adopted there by many Sudra Hindus (Rambiritch ad v.d. Bergh, 1961). On reaching Durban, the indentured Indians were frequently registered erroneously by their titles or the names used by them to denote their caste-orientated occupations. Some of the latter were Nao (barber), Chamaar (leather-worker), Lohar (blacksmith), Dhobi (laundryman), Soni (jeweller), Maistry (laundryman), Chakli (leather-worker), Chetty (businessman), Odde (leather-worker) and Sali (weaver). When their caste-orientated occupations became functionally meaningless in Natal, many Hindus dropped these names; and non-caste names were sometimes adopted by the Hindus to avoid caste laws (Kuper, 1955; Rambiritch and v.d. Bergh, 1961).

Family names appear not to have been customary among the Indians in northern India, but among the Telegu-speaking Hindus they were always important and were transmitted from one generation to the next in the male line, usually in the form of an initial letter only (Sjoberg, 1968). The Hindus who spoke Tamil regarded family names as important too, but used them very little, preferring instead to be identified by means of a patronymic, similarly reduced to an initial letter. Their children used the patronymic as a form of surname; but the patronymic used by the grandchildren differed as it consisted of the given or main element of the name of their own father. Consequently, the family "surname" changed with each generation (Sjoberg, 1968). To overcome the problems of identifying Indian families, the South African Government passed a law in 1963 which encouraged the Indians to nominate a permanent surname according to western practice (Law No. 81 of South Africa, 1963).

The names of the Moslem Indians were usually Arabic or associated with the many countries through which in the past Islam spread. Some names, such as Abdul and Mohammed or Muhammad, were from the Koran, while others, such as Israel, Lazarus, Adam and Yusuf, occurred in both the Koran and the Bible. The names of famous Moslem figures in history were also very popular, but those of important modern Indian personalities are no longer used as this sometimes causes difficulties in family legal matters (Mehtar, 1974). As many Hindus were converted to Islam during the prosperous Mughal period in India and it is customary for the converted to take Moslem names (Vanker, 1979), the Natal Moslems studied in this thesis had to be regarded as a collection of Indians of mixed Indian ethnic origin. Nevertheless, as the majority of their ancestors came in ships from the Indian port of Bombay, their descendants were considered more likely to be related to the Indo-Aryan than to the Dravidian Indian language groups. The Natal Indians who were converted to Christianity and who had taken European names also posed a problem but, as they were few in number, this was overcome by discarding their blood samples.

1.5 CONCLUDING COMMENTS

In this thesis, the use of the names of the Natal Indians to determine their ethnic origin was a departure from normal. However, while admittedly not as accurate as other methods, it seemed to be the only way in which the geographical centre of origin in India of the Natal Indians could reasonably be decided, now that so few of them knew where their ancestors originally lived there and many spoke only English. Despite the early severe shortage of Indian women, which also contributed to the early breakdown of the caste system in Natal, the Tamil-, Telegu-, and Hindi-speaking and the Moslem Indians appeared to have maintained their disinclination to marry outside their own cultures, except perhaps to a minor degree. In order to be able to compare the frequencies of the blood groups in the Natal Indians meaningfully with those determined for the people of India, it was therefore clearly important, at least at first, to sub-divide the Natal Indians as far as possible along ethnic lines.

With the exception of Sri Lanka, the largest group of Indians living permanently outside the continent of India is in South Africa. In practical terms, the Indians in Natal constitute a genetically isolated community within which, because the earlier circumstances of many were adverse, the incidence of consanguineous marriages was, for a time, almost certainly lower than usual in Indians. However, had the Natal Indians always been able to choose their marriage partners at random, there would still not

have been enough generations since 1860, when the first of them arrived in Natal, for the various Indian ethnic gene markers to have become uniformly distributed among them yet. Consequently, the blood group frequencies in the Natal Indians described in this thesis were expected to be fairly similar to the frequencies recorded by others in the Indians of India.

CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter contains details about the Indians whose blood samples were used in determining the blood group frequencies in this thesis. Some indication of the commoner Indian names by which the people are known in Natal and which were used to subdivide them by language and religion have also been included. The techniques by which the samples were tested are described here as well, together with other techniques and the methods used in recording and scoring the results. The reagents used and their origin are described in the 'Materials and Methods' sections in Chapters 3 to 17.

2.2 THE INDIANS

2.2.1 Their blood samples

The blood samples used for the frequency studies in this thesis were provided by Indian male and unmarried female blood donors of the Natal Blood Transfusion Service in Durban and, with their parents' informed consent, by a number of Moslem school children. All married female donors were excluded as their names were often those of their husbands and could not be used as a guide to their possible ethnic origins in India. The donors and children were all healthy, and the donors were adults of between 18 and 65 years of age. Care was taken, by testing the cells of the donors who were bled in the first 56 days following commencement of a series, and thereafter new donors only, that each donor was tested only once. Where known, the relatives of the donors and children were also excluded. The proportion of males to females tested was approximately 10 to 1, but this difference had occurred only because Indian women in Natal often failed the preliminary haemoglobin screen test and therefore could not be bled. The Indians whose blood groups were of special interest were either blood donors, antenatal patients from whom blood samples had been received for routine tests or hospital patients who had required blood transfusions. In addition, their families were also visited at home, and blood

and, where necessary, saliva was acquired from as many family members as possible with their informed consent.

2.2.2 Their names

The names of the Natal Indians were rather more sounds than words and, once familiar, on being voiced were easily recognised, even when the words had been spelled incorrectly. Among its nursing staff, the Natal Blood Transfusion Service employs Indian women who are responsible for all the activities connected with the giving of blood at Indian donation clinics. These nurses sometimes recorded the names of the donors phonetically in the donation registers, but this posed no problem, frequently the reverse. The names of the donors also had to be matched phonetically by myself with those in the lists of the earlier indentured Indians, as the shipping clerks of the time had used a different form of spelling. However, without the kind assistance of senior Indian staff members of the Natal Blood Transfusion Service, the subdivision of the Indian donors and others by name would certainly have been an immense problem.

Among the Tamil-speaking Indians, the sounds 'sammy' and 'money' and the names Arumugam, Chellan, Govender, Kisten, Log nathan, Manickam, Maistry, Mariemuthu, Moodley, Naicker, Nair, Padayachee, Pather, Perumal, Pillay, Shunmugam and Vadivelu were common. The names of the Telegu-speaking Indians often had long vowel sounds, like Appadoo, Naidoo, Rajoo and Appana, and other names used by them were Chembiah, Chetty and Reddy. The Hindi-speaking Indians' names had sounds such as 'ath', 'lall', 'ram', 'sadh', 'deo', and 'raj', and familiar names were Lutchman, Singh, Ramkisson, Rampersadh and Sukdeo. The Moslem Indians were often called Adam, Ashruf, Ahmed, Ali, Ebrahim, Hoosen, Ismail, Khan, Lockhat, Mahommed, Moosa, Omar, Sayed, Seedat or Vahed. It must be stressed here again that subdivision of the Indians by name was not expected to be 100% accurate, but their names provided a guide to their ethnic origins which it might not otherwise have been possible to identify.

2.2.3 The blood

The blood specimens used were 2–5 mls of clotted blood or unclotted blood drawn into ACD solution, and all were stored at 4°C and tested within five days of having

been collected. After centrifugation, the serum or plasma was recovered and the plasma was recalcified with 50% calcium chloride solution. The cells were washed three times with large volumes of 0,85% saline, care being taken to remove as much of the supernatant as possible after each wash. The cells were packed down, and cell suspensions were made by drawing a measured volume of the washed, packed cells into a known volume of saline, 6% bovine albumin, LISS (low ionic strength solution; Moore and Mollison, 1976) or the red cell preserving fluid of Burgess and Vos (1971). The concentration of the cell suspensions was 2–5% in all these media. When packed cells were required, for example to absorb a serum before preparing an eluate, the three-times washed cells were recentrifuged without saline being added to them, and the supernatant and top layer of cells were then discarded. The cell suspensions in saline, bovine albumin or LISS were all discarded after 24 hours, but those in red cell preserving fluid, which contained anti-bacterial and anti-fungal agents, were used for a maximum of three days.

2.3 THE TECHNIQUES

2.3.1 Saline Tube Test (Dunsford and Bowley, 1967, p 25)

Equal volumes (± 0,01 ml) of reagent and red cell suspension were mixed together in a 10 x 75 mm test tube and placed for one hour at the required temperature. The test was then read either by careful observation over a source of light, all doubtful and negative results being examined microscopically (x 100 magnification), or by microscopic examination only. When necessary, the tube was centrifuged lightly before reading in order to enhance red cell agglutination.

2.3.2 One-stage 0,5% Bromelin Tube Test (Pirofsky and Mangum, 1959)

Equal volumes (± 0,01 ml) of reagent, 0,5% bromelin and red cell suspensions were mixed together in a 10 x 75 mm test tube and placed for 15 minutes at the required temperature. The test was then centrifuged lightly and read macro- and microscopically.

2.3.3 One-stage Löw's Enzyme Test (B. Löw (1955)

The same as the 0,5% bromelin test but with Löw's enzyme added instead of bromelin.

2.3.4 Saline Tile Test (Simmons, Graydon, Jakobowicz and Bryce, 1944)

Equal volumes (± 0,01 ml) of reagent and cell suspension were placed in a 17x17 mm square outlined permanently by means of thin strips of plastic on a 28x12 cm glass tile. After gentle agitation and tilting to mix the cells with the reagent evenly, the tile was placed in a moist chamber for one hour. The tile was then carefully tilted to an angle of 45°, and the result was read after returning it to a horizontal position over a source of light. All negatives were checked with a x2 magnifying lens.

2.3.5 One-stage 0,25% Ficin Tile Test (after Simmons, Graydon, Jakobowicz and Bryce, 1944)

This test differed from the saline tile test only in that one volume (± 0,01 ml) of 0,25% ficin solution was added to the square on the glass tile immediately after the reagent and cell suspension.

2.3.6 Indirect Antiglobulin Test (Rosenfield, Vogel and Rosenthal, 1951)

Equal volumes (± 0,02 ml) of reagent and cell suspension were mixed together in a 12 x 75 mm test tube and incubated usually at 37°C for one hour. The cells were then washed four times with large volumes of saline and re-suspended in two drops of antiglobulin reagent. After 2–5 minutes, the test tube was centrifuged gently, and the result was read either by direct observation over a source of light, all doubtful and negative results being examined microscopically, or by microscopic examination only. The antiglobulin reagent used was of the 'broad spectrum' type and had been obtained from a commercial source.

2.3.7 Autoanalyser Test

The ABO and Rhesus D frequencies in Tables 3.3 and 13.12 respectively were obtained by consulting the records of the Natal Blood Transfusion Service which uses a Technicon model B.G. 15 automated blood grouping machine according to the methods recommended.

2.3.8 Microwell Test (modification of technique of Crawford, Gottman and Gottman. 1970)

Either one or two drops of reagent and cell suspension were added by means of a 1 ml tuberculin syringe, fitted with a 23 gauge needle, the tip of which had been ground flat, to a well in a conditioned microwell plastic plate. The reagents were mixed together by gentle agitation and the test completed by saline, one-stage 0,5% bromelin or indirect antiglobulin technique. The result was read microscopically by carefully transferring the contents of the well onto a glass microscope slide.

2.3.9 Titration (Dunsford and Bowley, p 285, 1967)

A row of ten 12 x 75 mm test tubes was prepared and one volume of the serum was placed in tubes 1 and 2. An equal volume of diluting medium (saline, 6% bovine albumin, LISS or red cell preserving fluid) was added to tubes 2 to 10 and the titration completed by the doubling dilution procedure. These dilutions constituted the 'master' titration. Pipetting from the weakest dilution back towards the neat serum, an equal volume of each 'master' dilution was placed in the appropriate test tube in a second row of tubes or in the appropriate square on a glass tile. The test was then completed in accordance with the method required.

2.3.10 Elution (Landsteiner and Miller, 1925)

The sensitized cells were washed six times with large volumes of saline, and part of the sixth saline wash was kept for testing later. To the washed, packed cells, an equal volume of saline, 6% bovine albumin or AB serum was added. The test tube was agitated gently at 56°C for 5 minutes and then centrifuged strongly for one minute in a warmed centrifuge bucket. The supernatant was recovered and recentrifuged to remove any deposit before use.

2.3.11 Elution (Vos and Kelsall, 1956)

The sensitized cells were washed six times with large volumes of saline, and part of the sixth saline wash was kept for testing later. To the washed, packed cells, an equal volume of 6% bovine albumin or AB serum was added, followed by a double volume (cells + albumin or serum) of ether. The test tube was stoppered firmly and the contents shaken hard for 5 minutes. The test tube was then centrifuged hard for 5

minutes. A Pasteur pipette, dipped through the layers of ether and of stroma, was then used to recover the eluate from the bottom of the tube. All residual ether was evaporated by passing a current of air over the eluate, and the eluate was recentrifuged to remove any deposit before use.

2.3.12 Enzyme-treated Cells

The cells were washed three times and packed down. For bromelin-treated cells, an equal volume of 0,5% bromelin solution was added and the suspension was incubated at 20° for 10 minutes. The cells were then re-washed three times and a cell suspension prepared from them for use. For ficin-treated cells, one volume of 0,25% ficin solution was added to four volumes of packed cells, and the suspension was incubated at 20°C for 10 minutes. The cells were then re-washed three times and a cell suspension prepared for use. Successful treatment was monitored by testing the D+ cells in saline with a D^u serum and the D- cells with an IgG anti-c.

2.3.13 Saliva Inhibition Test

The saliva was heated in a boiling waterbath for 20 minutes within one hour of having been obtained and was then centrifuged to remove the deposit. The clear supernatant fluid was recovered and stored at −28°C until required.

Two methods were used. In the first method, equal volumes of the reagent and saliva were mixed and incubated at 20°C for 30 minutes. The reagent was then tested by the optimum technique. In the second method, the reagent or the saliva was titrated, and an equal volume of the saliva or reagent, respectively, added subsequently to all the dilution tubes. The contents of each test tube were mixed together by agitation, and inhibition was allowed to take place for 30 minutes. An equal volume of cell suspension was now added to all the test tubes and the test reincubated for one hour. Finally, all the test tubes were centrifuged and the results were read macro- or microscopically.

2.3.14 2-mercaptoethanol and Dithiothrietol Techniques (Deutsch and Morton, 1957; Moores and Grobbelaar, 1969; Pirofsky and Rosner, 1974)

The serum was mixed with an equal volume either of 0,1 molar 2-mercaptoethanol

in saline or Dithiothrietol solution, the former for one hour and the latter for 15 minutes, at 37°C. The treated serum was then tested without having been dialysed beforehand to remove these substances.

2.3.15 Negative Control Tests

The cell suspensions were all controlled in strict parallel tests with each reagent using AB serum, anti-T (*Arachis hypogea*) and *Glycine soja* lectin.

2.4 METHOD OF RECORDING RESULTS AND OF SCORING TITRATIONS

The results were recorded by a numerical system in which 4+ indicated that all the cells were agglutinated in one large clump. A 3+ result was one of several largish clumps of cells, a 2+ result one of many smaller clumps and a 1+ result showed even granular agglutination, only just visible macroscopically. A ± result required microscopic confirmation, and thereafter was usually recorded as (3). In a (2) result, many small clumps were seen microscopically, often with unagglutinated cells in the background, and a (1) result was one of very small clumps of cells spread evenly. A (±) result indicated that doubtful agglutination had occurred, a – result that no agglutination had taken place.

The method of scoring, adapted from the method of Race and Sanger (2nd ed., p 275, 1954) was as follows: 4+ 10; 3+ 10; 2+ 8; 1+ 5; ± and (3) 2; (2) 1; (1) 0.

The saline used was always freshly made on the same day and the equipment was kept spotless and in good order by energetic teams of workers.

CHAPTER 3

THE ABO BLOOD GROUP SYSTEM
POPULATION FREQUENCIES

3.1 INTRODUCTION

The variations in frequency of the inherited characters in man are of abiding interest to serologists, and the diversity of the blood group frequencies in particular has been stimulating ever since the Hirszfelds first reported, in 1919, that the frequencies of the ABO groups differed between a number of populations. Except in one or two instances, the reasons for the variations are still largely unknown, but population migrations, hybidisation, genetic isolation, divers diseases and other forces in the environment connected with natural selection, operative both in the past and at present, are believed to have played and to continue to play important roles. The phenotype and gene frequencies in some blood group systems appear to have been more stable than in others in the long term, but nevertheless the differences have enabled workers to uncover population relationships, and they are thought to be reliable guides to them dating back for the last few thousand years (Mourant, Kopéc and Domaniewska-Sobczak, 1976).

In the people of India, the ABO blood group system is marked by a high group B phenotype frequency which exceeds 30% in many places. The *B* gene frequency is about 25% in the people of north and central India, and its prevalence there has been attributed by some to the invasions by Mongols, and by others to a selective advantage which it may enjoy in the local situation. As the Indians in northern India speak Hindi, Urdu, Gujarati and Memon, the Natal Indians who used these languages at home were expected to exhibit the same high *B* gene frequency. Similarly, the Natal Indians who spoke the Dravidian languages Tamil and Telegu were expected to exhibit the lower *B* and higher *O* gene frequencies characteristic of the people in southern and south-eastern India.

The sub-groups of the ABO system are also represented in India, and the A_2 gene is reported to have a frequency of 2,5% in the Indian population as a whole (Mourant *et al.*, 1976). Weak group A variant cells have been identified in 1 in 3 276 group A and AB Indians, or in 1 in 9 300 Indians of all ABO groups (Bhatia, 1976), and A_2

weak, A_3, A_x and A_m phenotypes have all been recorded. Weak group B variant cells have been identified as well, but they have a lower frequency and are present in only 1 in 9 362 group B and AB Indians or in 1 in 23 915 Indians of all ABO groups. However, the phenotypes B_x and B_m are both represented (Bhatia and Sathe, 1974; Bhatia, 1976). The majority of the Indians with weak group A variant or weak group B variant cells were discovered in the predominantly Aryan-speaking populations in northern India. The A_{int} phenotype was described in 2,05% group A Maharashtrians by Bird (1964), and the A_1 high H and B high H phenotypes were both recorded in 5% Maharashtrians and Gujaratis by Sathe and Bhatia (1974).

Almost without question, the most notable ABO group recorded from India was O_h, or the 'Bombay' phenotype, described from the city of Bombay in the State of Maharashtra by Bhende, Deshpande, Bhatia, Sanger, Race, Morgan and Watkins in 1952. Blood provided by donors of this rare type led to significant advances being made in blood group knowledge, particularly in the fields of biochemistry and of inheritance. Bhatia and Sathe (1974) recorded the frequency of the O_h phenotype in the population of Bombay as being 1 in 7 600 (*h* frequency 0,011), and 98 O_h Indians, the majority of whom were Maharashtrians, were identified by them in 23 years of study. The first three O_h Indians reported, and all those discovered subsequently in India, were classified by Bhatia (1976) as 'typical' O_h. Seven Indians whose cells were phenotype para-Bombay were recorded from India as well by Bhatia (1976).

In this chapter, the A, B and O, A_1 and A_2 and O_h phenotype and gene frequencies in the Tamil-, Telegu- and Hindi- speaking and Moslem Indians of Natal have been presented. Their frequencies were compared among themselves and with those recorded by other workers in Indian populations of similar composition in India and South Africa. The frequencies of the high H phenotypes, the subjects of a special investigation, have been recorded in this chapter also. However, my account of the identification of weak group A variant and weak group B variant phenotypes in the Natal Indians will be found in Chapters 4, 5 and 6, and my discovery of the first Natal O_h Indian is presented in Chapter 7. Chapters 8 to 11 contain the accounts of studies made with the cells and sera of other Natal O_h Indians, and Chapter 12 describes the para-Bombay Indians discovered in Natal. The A_{int} phenotype was not studied in this thesis, and all suspected examples were included as A_2 in the frequency tables.

3.2 MATERIALS AND METHODS

The Natal Indian 'first time' male and unmarried female donors of the Natal Blood Transfusion Service and others whose blood samples were used for the studies presented in this chapter, together with the preparation of their samples for the tests, are described in Chapter 2. The reagents used were pooled donations of human anti-A, anti-B and anti-A,B sera, some standardised for typing by 15 channel auto-analyser technique and others for manual testing by saline technique at 20°C. The anti-A_1 was a lectin extracted from Australian *Dolichos biflorus* seeds, and the anti-anti-H was a lectin extracted from English *Ulex europaeus* seeds, both in Durban. The anti-A_1 was used by one-stage 0,5% bromelin technique, and the anti-H, diluted serially in saline (doubling dilutions) for the titrations, by saline technique, also at 20°C. These methods are described as well in Chapter 2. All the group O blood samples were tested by saline technique at 20°C with anti-A+B+H from Natal O_h Indian donors. The sera from the samples were tested with A_2, B and O cells and, where necessary, with A_1 cells either by 15 channel autoanalyser or saline technique. The reagents were all checked for specificity both before use and daily with negative and positive control cells throughout the test period.

The p, q and r and the p_1, p_2, q and r frequencies in the Natal and other Indians recorded in this chapter were calculated by the first methods suggested by Mourant *et al.* (p 50–51, 1976). The χ^2 tests for internal consistency were calculated by the method suggested by Race and Sanger (p 15, 1968), and the population frequencies were compared by calculating χ^2 for 2 x 2 tables, without Yates' correction. The conventional standard of significance, $P \leq 0,05$, was employed.

3.3 RESULTS

Table 3.1 shows the A, B, O and AB phenotype and gene frequencies recorded by other workers in Indian populations of large size in India. These populations were chosen particularly for my studies as their places of origin in India, religions and home languages were the most likely among the populations recorded by Mourant *et al.* (1976) to be similar to those of the Natal Indians.

Table 3.2 contains the frequencies in the Natal Indians recorded by other workers, and Table 3.3 the frequencies presented by myself. The languages thought to have been spoken by the populations in Table 3.1 have been indicated in brackets under

TABLE 3.1

ABO phenotype and gene frequencies recorded in Indian populations of large size in India recorded by others

Category	Authors	Number tested		Phenotype frequencies				Gene frequencies			Calculations		
				A	B	O	ABO	p	q	r	Expected AB	χ^2	P
Madras donors (Tamil)	Hsuen *et al.*, 1963	1 000	No.	214	331	397	58	0,1509	0,2221	0,6270	68	0,91	<0.5
			%	21,40	33,10	39,70	5,80						
Madras donors (Tamil)	Povey & Horton, 1966	755	No.	162	260	282	51	0,1553	0,2354	0,6093	55	0,24	<0,7
			%	21,46	34,44	37,35	6,75						
Hindu donors in Andhra Pradesh (Telegu)	Dronomraju *et al.*, 1967	5 486	No.	1 088	1 727	2 402	269	0,1354	0,2052	0,6594	307	3,22	<0,1
			%	19,83	31,48	43,78	4,90						
Hindu controls in Uttar Pradesh (Hindi)	Jolly, 1965	19 255	No.	4 053	7 547	5 971	1 684	0,1642	0,2803	0,5555	1782	3,23	<0,1
			%	21,05	39,19	31,01	8,75						
Moslems in Uttar Pradesh	Jolly *et al.*, 1960	522	No.	141	182	152	47	0,2076	0,2578	0,5346	57	1,02	<0,5
			%	27,01	34,87	29,12	9,00						
Moslems in Uttar Pradesh	Jolly, 1965	5 191	No.	1 324	1 775	1 566	526	0,1971	0,2532	0,5497	517	0,09	<0,8
			%	25,51	34,19	30,17	10,13						

TABLE 3.2

ABO phenotype and gene frequencies in Natal Indians recorded by others

Category	Authors	Number tested		Phenotype frequencies A	B	O	AB	Gene frequencies p	q	r	Calculations Expected AB	χ^2	P
Antenatal	Hirsch, 1958	1 472	No.	321	473	574	104	0,1555	0,2192	0,6253	100	0,10	<0,8
			%	21,81	32,13	38,99	7,07						
Hindu	Ally, 1967	2 451	No.	546	821	842	242	0,1688	0,2365	0,5947	172	17,97	<0,001
			%	22,28	33,50	34,35	9,87						
Moslem	Ally, 1967	1 083	No.	242	361	400	80	0,1621	0,2304	0,6075	81	0,008	<0,95
			%	22,35	33,33	36,93	7,39						
Tamil	Ally, 1967	4 695	No.	996	1 576	1 809	314	0,1520	0,2281	0,6199	326	0,31	<0,7
			%	21,21	33,57	38,53	6,69						
Telegu	Ally, 1967	1 736	No.	344	576	723	93	0,1381	0,2189	0,6430	106	1,03	<0,5
			%	19,81	33,18	41,65	5,36						

TABLE 3.3

ABO phenotype and gene frequencies in 2 720 Natal Indian 'first time' male and unmarried female blood donors of the Natal Blood Transfusion Service

Category	Number tested		Phenotype frequencies A	B	O	AB	Gene frequencies p	q	r	Calculations Expected AB	χ^2	P
Tamil	1 211	No.	256	371	510	74	0,1465	0,2041	0,6494	72	0,03	$<0,9$
		%	21,14	30,64	42,11	6,11						
Telegu	493	No.	97	158	199	39	0,1408	0,2177	0,6414	30	1,98	$<0,2$
		%	19,67	32,05	40,36	7,91						
Hindi	644	No.	151	220	217	56	0,1756	0,2435	0,5809	55	0,01	$<0,95$
		%	23,45	34,16	33,69	8,69						
Moslem	372	No.	86	114	143	29	0,1653	0,2121	0,6226	26	0,25	$<0,7$
		%	23,12	30,64	38,44	7,80						

Significance of differences

All differences were not significant between the populations in Table 3.3 and the relevant populations in Tables 3.1, 3.2 and 3.3 at level $P < 0,05$ except:

Phenotype			Phenotype		χ^2	P
B	Natal Hindi	and	B	Hindu (Jolly, 1965)	6,6	0,01
O	Natal Tamil	and	O	Natal Hindi	12,5	$<0,001$
O	Natal Telegu	and	O	Natal Hindi	5,4	0,02
O	Natal Tamil	and	O	Madras donors (Povey *et al.*, 1966)	4,4	$<0,05>0,02$
O	Natal Tamil	and	O	Natal Tamil (Ally, 1967)	5,2	$<0,05>0,02$
O	Natal Moslem	and	O	Moslem (Jolly, *et al.*, 1960)	8,5	$<0,01>0,001$
O	Natal Moslem	and	O	Moslem (Jolly, 1965)	11,2	$<0,001$

the title 'Category', and the frequencies in all three tables were recalculated to ensure that they were comparable. The high B phenotype frequency, known to be more than 30% in India, was seen to pertain also in Natal. As expected, the highest B frequency in Table 3.3 was that of the Natal Indians who spoke Hindi; and the only significantly different B frequencies in the three tables were those of the Hindi-speaking Natal Indians and the Hindu controls who were likely to have spoken Hindi, from the north Indian State of Uttar Pradesh recorded by Jolly (Tables 3.1 and 3.3; P = 0,01).

A number of significant differences in O phenotype frequencies were detected in the populations shown in Tables 3.1, 3.2 and 3.3. As expected, the Natal Tamil- and Telegu-speaking Indians both had higher O frequencies than the Natal Hindi-speaking Indians (P = <0,001 and 0,02 respectively, Table 3.3), and the Natal Tamil-speaking Indians also seemed further removed from them than the Natal Telegu speaking Indians. This conformed with the higher O and lower B frequencies noticed in northern India by Mourant *et al.* (1976). Surprisingly, however, the O frequency in the Natal Tamil-speaking Indians differed statistically from the frequencies in the Madras donors recorded by Povey and Horton (< 0,05 > 0,02, Tables 3.1 and 3.3) and from those in the Natal Tamil-speaking Indians recorded by Ally (< 0,05 > 0,02, Tables 3.2 and 3.3). This was so despite the χ^2 tests for ABs having showed that all three populations were internally consistent. The lower O frequencies recorded in the Tamil-speaking populations were matched in them by higher B frequencies and, since the O frequencies in the Telegu-speaking Indians in all three tables were approximately equal, the O frequency in the Natal Tamil-speaking Indians was thought unlikely not to be correct.

In the Moslem Indian populations, the higher A frequency recognised in northern than in southern India, and apparent as well in Table 3.1, was not seen in the Natal Moslem pouplations either in Table 3.2 and 3.3. The higher O frequency seen in the Natal Moslem population in Table 3.3 differed statistically from the lower O frequency in the Moslem populations in India recorded by Jolly (p < 0,01 > 0,001, and 0,001) but not from that in the Natal Moslems recorded by Ally (Table 3.2).

No statistical differences were observed in the A phenotype frequencies between the four Natal Indian populations in Table 3.3 or between them and the Indian populations shown in Table 3.1 and 3.2.

The Natal antenatal Indian population of Hirsch was recorded in Table 3.2 solely for interest as it had not been subdivided by language and religion, and the Natal Hindu population of Ally was recorded there for interest as well. The latter was not discussed in this chapter as the number of ABs detected showed that it lacked internal consistency.

Table 3.4 contains the A_1, A_2, B, O, A_1B and A_2B phenotypes and gene frequencies recorded by other workers in Indian populations in India and South Africa subdivided by language and religion in the same manner as the Natal Indian populations, and Table 3.5 contains these frequencies recorded by myself in the Natal Indians. No statistically different A_1+A_2, B or O frequencies were observed between the Natal Indian populations in Table 3.5 or between them and their corresponding populations in Table 3.4. The frequencies in the Hindu populations in Table 3.4 were compared with an average frequency calculated for this purpose from the combined frequencies in the Tamil-, Telegu- and Hindi-speaking Natal Indians in Table 3.5. Since the number of Indians tested in the Moslem and Hindu populations in Johannesburg was less than 100, it was also appreciated that, in their case, the comparisons made might not reflect the true position.

The high B frequency characteristic in Indians, and the higher O frequency in those who spoke the Dravidian than the Aryan languages, was again apparent in Table 3.5. The number of ABs observed and expected in this table also confirmed that the frequencies were internally consistent. The A_2 gene frequency in the four Natal Indian populations was in accord with the 2,5% frequency noticed throughout India by Mourant *et al.* (1976), but a wider difference was noted between the A_1 and A_2 gene frequencies in the Hindi-speaking and Moslem than in the Tamil- and Telegu-speaking Natal Indians (Table 3.5). The significance of this difference was not calculated as the number of A Indians in the populations was considered to be too small.

The percentages of A_1 high H and B high H cell samples found in a special study of these phenotypes in the Natal Indian populations are shown by means of the histograms in Table 3.6. The strength of the cell H antigen was estimated in parallel titrations with *Ulex* anti-H lectin. The A_1 high H cells having the highest H antigen scores (< 41) were found among the Natal Tamil- and Telegu-speaking A_1 Indians (4,34% and 6,67% respectively), but when all the samples having scores above 17 were added together, B high H cells having the highest H antigen scores occurred in the Natal Moslem Indians (39,56%), those having the next highest in the Natal Tamil-

TABLE 3.4

A_1, A_2, B and O phenotype and gene frequencies in Indians of India and Johannesburg recorded by others

Category	Place	Authors	Reference no. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies A_1	A_2	B	O	A_1B	A_2B	Gene frequencies p_1	p_2	q	r
Hindu	Rourkela in Orissa	Seth, 1967	2 607 page 225	215	No.	45	7	70	82	9	2	0,1355	0,0247	0,2119	0,6279
					%	20,93	3,25	32,56	38,14	4,19	0,93				
Moslem	Chandernagore in W. Bengal	Bhattacharjee, 1956	297 page 224	354	No.	x		120	118			0,1671	0,0129	0,2441	0,5759
					%	(78,58)	(5,42)	33,90	33,33	(29,87)	(2,13)				
Moslem	Rourkela in Orissa	Seth, 1967	2 607 page 225	164	No.	38	6	59	43	14	4	0,1740	0,0379	0,2723	0,5158
					%	23,17	3,66	35,97	26,22	8,54	2,44				
Hindu	Johannesburg	Nurse and Jenkins, 1977		53	No.	6	3	24	17	2	1	0,0791	0,0422	0,2016	0,5771
					%	11,32	5,66	45,28	32,08	3,77	1,89				
Moslem	Johannesburg	Nurse and Jenkins, 1977		56	No.	14	2 *	17	16	3	4	0,1646	0,0665	0,2428	0,5261
					%	25,00	3,57	30,36	28,57	5,36	7,14				

Calculations		
Expected AB	χ^2	P
16	1,19	<0,3
	0,06	<0,9
20	0,08	<0,8
4	0,28	<0,7
6	0,15	<0,7

Key:
x 22A, 17AB not tested for A_1
* A_{int} included with A_2 sample

TABLE 3.5

A_1, A_2, B and O phenotype and gene frequencies in 666 Natal Indian males and unmarried females

Category	Number tested		Phenotype frequencies						Gene frequencies			
			A_1	A_2	B	O	A_1B	A_2B	p_1	p_2	q	r
Tamil	197	No.	34	9	71	73	7	3				
		%	17,26	4,57	36,04	37,05	3,55	1,52	0,1109	0,0351	0,2343	0.6197
Telegu	101	No.	14	4	32	44	5	2				
		%	13,86	3,96	31,68	43,57	4,95	1,98	0,0985	0,0334	0,2155	0,6526
Hindi	126	No.	27	2	45	45	6	1				
		%	21,43	1,59	35,71	35,71	4,76	0,79	0,1419	0,0141	0,2353	0,6087
Moslem	242	No.	48	7	80	82	19	6				
		%	19,83	2,89	33,06	33,88	7,85	2,48	0,1488	0,0320	0,2462	0,5730

Calculations		
Expected AB	χ^2	P
15	1,06	$< 0,5$
5	0,45	$< 0,7$
10	0,57	$< 0,5$
19	1,19	$< 0,3$

Significance of differences

All differences were not significant between the populations in Table 3.5 and the relevant populations in Tables 3.4 and 3.5 at level $P \leq 0,05$

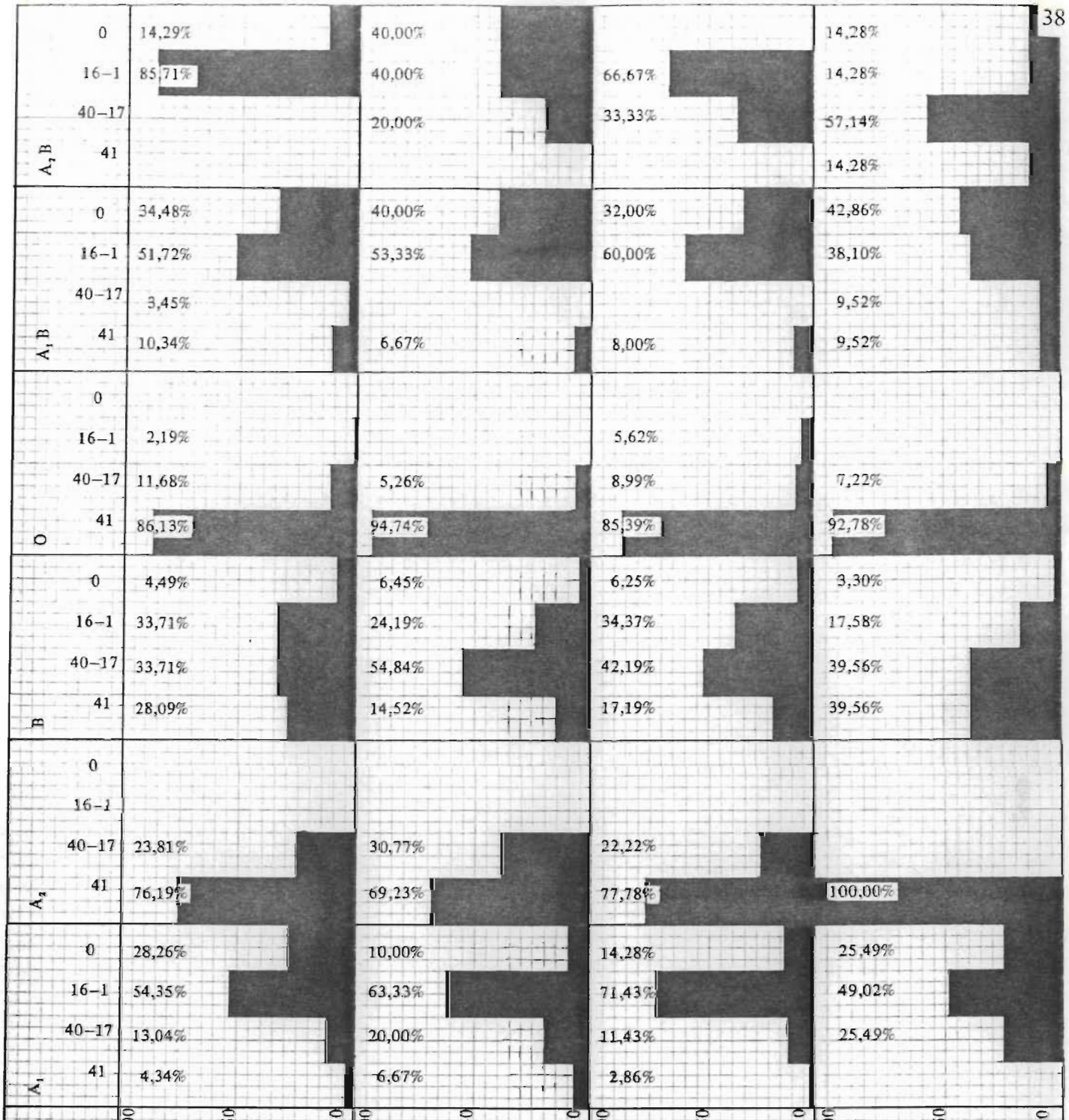


TABLE 3.6 RED CELL H ANTIGEN STRENGTH IN 1 033 NATAL INDIAN MALES AND UNMARRIED FEMALES TESTED WITH *ULEX* ANTI-H

speaking Indians (28,09%) and those having the lowest in the Natal Telegu- and Hindi-speaking Indians (14,52% and 17,19% respectively).

The frequency of the O_h phenotype in Natal 'first time' blood donors (0,0054%) was lower than the frequency recorded in Bombay (0,0131%) by Bhatia and Sathe (1974), but 24 O_h Indians in 11 apparently unrelated families had been identified in Natal through pregnancy and family studies. Nine were Tamil-speaking, seven Telegu-speaking and eight Hindi-speaking Indians (Table 3.7). No O_h Moslem Indians have been recorded yet in Natal. The number of Natal O_h Indians who spoke Dravidian therefore exceeded the number who spoke Aryan languages (16:8). The nine O_h Indians who spoke Tamil were provided by three families, the seven who spoke Telegu by four families and the eight who spoke Hindi by four families in Natal. Since the early indentured Indians in Natal had been gathered mainly near the city of Madras and in north-east India, the O_h Indians found in Natal were therefore unlikely to have had a Maharashtrian origin.

TABLE 3.7

The O_h phenotype in Indians of India recorded by Bhatia and Sathe (1974) and in the Natal Indians

Indian individuals in India		in India	in Natal	
Category	Number O_h	O_h not found	Category	Number O_h
S.W. Maharashtrians	33	Gujaratis	Tamil	9
Uttar Pradesh	2	Sindhis	Telegu	7
Madya Pradesh	1	Punjabis	Hindi	8
Andhra Pradesh	3	Rajput	Moslem	0
Mysore	2			
Kerala	3			
Tamil Nadu	1	O_h phenotype frequency in Indians		
Non-Marathas in Maharashtra	2	In Bombay 1 in 7 600 or 0,0131%		
Moslems	6			
Christians	2	In Natal 1 in 18 404 or 0,0054%		
Unknown	3	'first time' blood donors (1965–1979)		

3.4 DISCUSSION

The high O phenotype and gene frequency in the Natal Tamil- and Telegu-speaking Indians (gene frequency 62–65%) compared with the other Indian populations recorded in this chapter might be due to several reasons. Among them were that the Natal Indians might be more aware now of the greater value of O than of A, B or AB blood for transfusion purposes and already knew their ABO groups before donating blood for the first time. On reaching the minimum age for donation, perhaps the children of the active group O blood donors might also be more willing to donate their blood when the opportunity to do so was presented them. Alternatively, a factor in the environment, such as genetic drift or the diseases common in Natal, might be responsible. The reason for the higher A_1 in relation to A_2 phenotype frequencies noticed was not known, but the high B phenotype and gene frequency recorded (gene frequency 20–24%) was typically Indian.

The presence of A_1 high H and B high H phenotypes in the Natal Indians was interesting, and expected. However, more B than A_1 cells appeared to have high H. The O_h phenotype, potentially a grave Natal blood transfusion problem, had been largely overcome by keeping in contact with the propositi and their families and by making full family studies whenever new propositi were found.

3.5 SUMMARY

The Indian populations in Natal studied in this chapter all exhibited the high B phenotype frequencies typical in Indians and, as expected, the Tamil- and Telegu-speaking had higher O phenotype frequencies than the Hindi-speaking and Moslem Indians. However, all four populations had higher O phenotype frequencies than expected. A wider difference between the A_1 and A_2 frequencies in the Hindi-speaking and Moslem Indians than in those who spoke Tamil and Telegu was also observed. Both the phenotypes A_1 high H and B high H were detected, and it was suggested that the 24 O_h Indians found in Natal were probably not connected with the relatively high O_h-containing population in Maharashtra.

CHAPTER 4

WEAK GROUP A VARIANT PHENOTYPES IN THE NATAL INDIANS

4.1 INTRODUCTION

Three different backgrounds have been identified for weak group A variant red cells. They are, rare weak alleles of the *A* gene, expression of normal A antigen on the red cell membrane reduced by the inheritance of dominant or recessive inhibiting or modifying genes, and effects of the environment. The variants known as A_x (Fischer and Hahn, 1935), A_3 (Friedenreich, 1936) and A_m (described but not named as such by Gammelgaard in 1942), are all believed to be due to the inheritance of different weak *A* alleles. The phenotypes A_{end} (Weiner, Sanger and Race, 1959), A_{el} (Reed and Moore, 1964) and A_{bantu} (Brain, 1966) are further examples. However, the A_m-like cells now known as A_y (Weiner, Lewis, Moores, Sanger and Race, 1957) are thought to be due to suppression of a normal A_1 gene by the inheritance of *yy* recessive inhibitor or modifying genes, and those described by Rubinstein, Allen and Rosenfield (1975) to similar suppression by a dominant inhibitor gene. The environment is believed to underlie the mixture of A and weak A cells sometimes seen in advanced age and the weak A cells associated with leukaemia.

The reactions given by weak group A variant red cells vary from weak or negative with anti-A and 1+ positive with anti-A,B reagents to negative with both these reagents. A_{bantu} cells react perhaps the most strongly with anti-A, their mixed-field agglutination with this but not with anti-A,B reagents distinguishing them from A_3 and the weaker phenotype A_{end} cells which are agglutinated in mixed-field patterns both by anti-A and anti-A,B reagents. A_x cells, which are not agglutinated by anti-A, are agglutinated in normal rather than in mixed-field patterns by anti-A,B reagents, and A_m, A_{el}, A_y and the A_m-like cells reported by Rubinstein *et al.* (1975) are not agglutinated by either reagent. The four latter cells are distinguished from one another both by the ease with which anti-A may be eluted from them after they have been exposed to this antibody and by the amount of A substance detected in the saliva of the secretors. A_m cells elute anti-A readily, A_{el} and A_m-like cells elute it less readily, and A_y cells, from the first known examples of which no anti-A was eluted, may elute traces of anti-A (Cartron, Gerbal, Badet, Ropars and Salmon, 1975; Salmon, 1976). The saliva of the people who have A_m cells and who are

secretors contains plenty of A substance, the saliva of those who have A_y and A_m-like cells contains less A substance, and no A substance is detected in the saliva of those who have A_{el} cells.

Anti-A_1, but not anti-A, allo-antibodies are often detected in the serum of persons who have A_{bantu}, A_x and A_{el} cells, are detected occasionally in those who have A_{end} cells and are usually not detected in those who have A_m, A_m-like and A_y cells. The serum of those who have A_m cells also contains only 30–50% of the α-2-N-acetylgalactosaminyltransferase expected when the cell phenotype is A_1 or A_2 (Cartron *et al.*, 1975). This enzyme has not been detected in the serum of persons who have A_y cells.

The frequency of the A_x phenotype was assessed by Salmon (1960) as being 1 in 40 000 of French blood donors, and only a few families and individuals who have A_m, A_{end}, A_{el} or A_y cells have been recorded (Race and Sanger, 1975). A_m-like cells (the result of dominant suppression by an A_1 gene) appear to have been described only once, but A_{bantu} cells occur in approximately 4% of group A Natal Blacks (Brain, 1966). The relatively high frequency of the A_{bantu} phenotype in Natal has unfortunately led to weak group A variant cells from Blacks, Whites and Indians being seldom referred for further studies, and this is consequently the reason so few Indians with these cells have been included in my thesis. The frequency of weak group A variant phenotypes, taken as a whole but excluding A_2, in India was recorded by Bhatia and Sathe (1974) as being 1 in 6 000 of group A Indians (twice as many with A_x as with A_m variant cells were detected in their series), or 1 in 16 700 of the Indian population.

In this chapter, four Natal Indians with weak group A variant red cells will be described. The findings showed that the cells of two of the Indians were A_x and of two A_m-like. As the families of the two Indians who had A_m-like cells were not informative, the results obtained with their cells were compared with those of the individuals with A_m and A_m-like cells recorded elsewhere (Race and Sanger, 1975) and their cells were identified provisionally as A_y.

4.2 CASE HISTORIES

The two Natal Indians who had A_x cells in this study were Mrs K. Cas., a 24 year old Moslem who was three months pregnant, and Mrs Poon., a Tamil-speaker who was

also pregnant. Both women were in good health and were discovered when samples of their blood were received by the laboratory for routine antenatal tests. The two Natal Indians who had A_m-like cells (A_y) were Mrs S. Nai., who spoke Telegu, was pregnant for the second time and was discovered in the same way, and G. Pir., a healthy, 21 year old, Hindi-speaking blood donor.

4.3 MATERIALS AND METHODS

The samples used in this study were clotted blood, serum separated immediately the blood had been received, and saliva. The preparation of these samples for use has been described in Chapter 2. The ABO reagents used were standardised anti-A, anti-anti-B and anti-A,B, six high-titre human immune anti-A, 20 sera containing anti-A,B from White and four from Black antenatal patients who were selected at random, six sera containing low titre anti-A,B from unstimulated White male blood donors, two high-titre rabbit immune anti-A previously absorbed for unwanted agglutinins, anti-A_{HP} from a snail (kindly supplied by Dr M.C. Botha, Provincial Blood Grouping Laboratory, Cape Town) and *Lima* bean anti-A lectin. Anti-A+B+H from a Natal O_h Indian donor, two naturally-occuring anti-H from group A_1 donors. anti-I and anti-i from known donors, *Ulex* anti-H lectin and *Arachis hypogea* anti-T lectin were also used. The eluates were made either by the 56°C heat technique of Landsteiner and Miller (1925) or by the ether technique of Vos and Kelsall (1956) in saline from cells sensitized with human immune anti-A. These techniques are also described in Chapter 2. The tests were made by the saline, one-stage 0,5% bromelin and one-stage 0,25% ficin tile techniques at 22°C and at 4°C, and the eluates were tested by the indirect saline antiglobulin technique as well, using a broad-spectrum antiglobulin reagent obtained from a commercial source.

4.4 RESULTS

4.4.1 First two Natal Indians with weak A variant red cells

The cells of Mrs K. Cas. and Mrs Poon. were not agglutinated by standardised anti-A, anti-B or snail anti-A_{HP}, but they were agglutinated moderately strongly by commercial anti-A,B and microscopically by high titre human and rabbit immune anti-A sera (Table 4.1). Their cells were agglutinated normally in these tests, and not in the mixed-field pattern associated with A_3 and A_{end} cells tested with anti-A and anti-A,B reagents. Their cells were also not agglutinated in the mixed-field pattern

seen with A_{bantu} cells tested in parallel with anti-A. Neither the strength nor the number of the cells agglutinated was improved by incubating the tests at 4°C instead of at 20°C, and anti-B but no anti-A or anti-A_1 allo-antibodies were identified in their sera. It should perhaps be re-stated here that anti-A_1 is detected regularly in the serum of the Natal Blacks who have phenotype A_{bantu} cells.

TABLE 4.1

Results of tests with phenotype A_x cells and sera of two Natal Indians and with phenotype A_{bantu} cells and serum of a Natal Black

Cells and serum	Group	Anti-A	Anti-B	Anti-A,B	A_1 cells	A_2 cells	B cells	O cells
Mrs K. Cas.	A_x	–	–	1	–	–	4	–
Mrs Poon.	A_x	–	–	1	–	–	4	–
Natal Black	A_{bantu}	(2)$^{\cdot}$	–	1$^{\cdot}$	1	–	4	–
Control	Positive	4	4	4				
Control	Negative	–	–	–				

Key: (2)$^{\cdot}$, 1$^{\cdot}$ = mixed-field agglutination.

The I and i antigen strength of the cells of Mrs K. Cas. and Mrs Poon. appeared normal and their cells were not T-sensitized. Since it was evident that their cells were group A, no absorption-elution tests with anti-A were made. In their case, no saliva samples were received and, unfortunately, the families of the Indians were not available for family inheritance studies.

4.4.2 Second two Natal Indians with weak A variant red cells

The cells of Mrs S. Nai. and G. Pir. were not agglutinated by anti-A, anti-B or anti-A,B in normal tests or when nine volumes of these reagents were added to one volume of cell suspension (Table 4.2, part a). Their cells were also not agglutinated by 20 anti-A,B selected at random from White and four from Black antenatal patients, in some of whom this antibody was expected to be undergoing current stimulation by

TABLE 4.2

Results of tests with A_m-like cells and sera
of two Natal Indians

Part a

		anti-A			anti-B			anti-A,B			anti-A+B+H
		Saline	Bromelin	Ficin	Saline	Bromelin	Ficin	Saline	Bromelin	Ficin	Saline
Mrs S. Nai.	A_m-like	–	–	–	–	–	–	–	–	–	4
G. Pir.	A_m-like	–			–			–			4
Control	Positive	4	4	4	4	4	4	4	4	4	4
Control	Negative	–	–	–	–	–	–	–	–	–	–

Part b

		A_1 cells			A_2 cells			B cells			O cells		
		Saline	Bromelin	Ficin	Saline	Bromelin	Ficin	Saline	Bromelin	Ficin	Saline	Bromelin	Ficin
Mrs S. Nai.	(1)	1	2	–	–	–	4	4	4	–	–	–
G. Pir.	–			–			4			–			

an ABO incompatible fetus, by high-titre human and rabbit immune anti-A, by snail anti-A_{HP}, by *Lima* bean anti-A lectin or by low titre anti-A,B in the sera of six unstimulated male blood donors. The male donors' sera were included as they were more likely to contain a higher proportion of type IgM anti-A,B than the sera of stimulated donors. However, the cells of Mrs S. Nai. and G. Pir. were agglutinated by anti-A+B+H, by both examples of naturally-occurring anti-H from group A_1 donors and by *Ulex* anti-H lectin. In subsequent titrations with the *Ulex* anti-H, Table 4.3 shows that their cells were agglutinated to the same titre as the control O cells. The strength of the I and i antigens of their cells appeared normal and their cells were not T-sensitized.

TABLE 4.3

Ulex anti-H titration results with phenotype A_m-like cells of two Natal Indians

Cells	Group	*Ulex* anti-H Dilutions in saline						
		1	2	4	8	16	32	64
Mrs S. Nai.	A_m-like	4	4	4	4	3	1	–
G. Pir.	A_m-like	4	4	4	3	2	1	–
Control	A_1	1	(2)	–	–	–	–	–
Control	A_2	4	4	3	2	1	–	–
Control	B	2	1	–	–	–	–	–
Control	O	4	4	4	4	3	1	–

The eluates recovered from the cells of Mrs S. Nai., using the 56°C heat technique, and from the cells of G. Pir., using the ether technique, after their cells had been exposed to human immune anti-A, failed to agglutinate A_1 and A_2 cells in saline tests at 20°C. However, their eluates did agglutinate these cells, and not O cells, microscopically in one-stage 0,5% bromelin tests at this temperature. The weak reactions of their eluates was in marked contrast to the strong reactions obtained when similar eluates were recovered instead from A_{bantu} control cells and tested in the same way.

TABLE 4.4

Results of inhibition tests with saliva of Natal Indian with A_m-like cells

Saliva	Group	Anti-A and A_1 cells Dilutions in saline									
		1	2	4	8	16	32	64	128	256	512
Mrs S. Nai.	A_m-like	4	4	4	4	3	2	1	–	–	–
Secretor	O	4	4	4	4	4	3	2	1	(2)	–
Secretor	A	(1)	–	–	–	–	–	–	–	–	–
Non-secretor	A	4	4	4	4	3	3	3	1	(2)	–
Saline		4	4	4	4	3	3	2	1	(3)	–

Saliva	Group	Anti-B and B cells Dilutions in saline									
		1	2	4	8	16	32	64	128	256	512
Mrs S. Nai.	A_m-like	4	4	4	4	4	3	3	1	(2)	–
Secretor	O	4	4	4	4	4	3	2	1	(2)	–
Secretor	A	–	–	–	–	–	–	–	–	–	–
Non-secretor	A	4	4	4	4	4	3	2	1	(2)	–
Saline		4	4	4	4	4	3	2	1	(3)	–

Saliva	Group	Anti-H *Ulex* and O cells Dilutions in saline									
		1	2	4	8	16	32	64	128	256	512
Mrs S. Nai.	A_m-like	–	–	–	–	–	–	–	–	–	–
Secretor	O	–	–	–	–	–	–	–	–	–	–
Secretor	A	–	–	–	–	–	–	–	–	–	–
Non-secretor	A	4	4	2	1	(1)	–	–	–	–	–
Saline		4	4	3	2	1	–	–	–	–	–

Saliva inhibition tests confirmed that G. Pir., whose red cells typed as Le(a+b−), was a non-secretor of ABH substances. The saliva of Mrs S. Nai., who had Le(a−b+) red cells, inhibited anti-H strongly and anti-A weakly (Table 4.4). These results showed that Mrs S. Nai. was a secretor of H and of a small quantity of A substances.

In the serum of G. Pir., anti-B but no anti-A or anti-A_1 allo-antibodies were detected. However, the serum of Mrs S. Nai. contained anti-B and weak anti-A_1 (Table 4.2, part b).

The three-years old first child of Mrs S. Nai. was the only member of her family tested. Her cells were group O and her serum contained anti-A,B allo-antibodies of normal strength. Saliva inhibition tests showed that she was a non-secretor of ABH substances. The family of G. Pir. was unfortunately not available for study.

4.5 DISCUSSION

The cells of the first two Indians described in this study, Mrs K. Cas. and Mrs Poon., were identified confidently as phenotype A_x as their cells were not agglutinated by anti-A and were agglutinated in normal, rather than in mixed-field, patterns by anti-anti-A,B reagents. Although no anti-A_1 allo-antibodies were detected in their sera, this was not of serious concern as anti-A_1 is not detected invariably in A_x persons. Moreover, its absence in Mrs K. Cas. and Mrs Poon. leant support to the view that their cells were not phenotype A_{bantu}, the weak group A variant phenotype found in 4% Natal group A Blacks. A_{bantu} may also be distinguished from A_x cells by the former being agglutinated by anti-A in mixed-field patterns.

The cells of the second two Indians described in this study, Mrs S. Nai. and G. Pir., clearly had very little A antigen expression. As their cells were not agglutinated by anti-A or by anti-A,B reagents, the phenotype of their cells was either A_m, A_{el}, A_y or A_m-like. Since anti-A had been eluted with difficulty from the cells of both Indians and the saliva of Mrs S. Nai. contained a minimal amount of A substance, the most likely phenotype of their cells was A_y, but the serum of Mrs S. Nai. contained weak anti-A_1 allo-antibodies. The possibility that the Indians had inherited *yy* inhibitor genes which had suppressed the expression of normal A_1 genes in them had also not been confirmed through adequate family studies. As a result, the phenotype of their cells was identified as A_y with reserve (Table 4.5).

TABLE 4.5

Results reported for persons with weak group A variant cells elsewhere and those found with two Natal Indians whose cells were A_m-like

Phenotype	Reactions with anti-A	Reactions with anti-A,B	Strength of anti-A in eluate	A substance in saliva	anti-A_1 in serum
A_{bantu}	(2)$^{\cdot}$	+$^{\cdot}$	strong	no	yes
A_3	+$^{\cdot}$	+$^{\cdot}$	strong	yes	sometimes
A_{end}	(1)$^{\cdot}$	(2)$^{\cdot}$	strong	no	sometimes
A_x	–	+	strong	usually no	yes
A_m	–	–	strong	strong	no
A_y	–	–	moderate	weak	no
A_{el}	–	–	intermediate	no	yes
Dominant inhib. of A	–	–	weak	weak	no
G. Pir.	–	–	weak	non-secretor	no
Mrs S. Nai.	–	–	weak	weak	yes

Key: +$^{\cdot}$ = mixed-field agglutination.

No previous accounts of Indians from Natal with weak group A variant red cells were found.

4.6 SUMMARY

Four Natal Indians with weak group A variant red cells, the cells of two of whom were identified as phenotype A_x and the other two provisionally as phenotype A_y, established that weak A variant cells other than A_{bantu} were present in Natal.

CHAPTER 5

WEAK GROUP B VARIANT PHENOTYPES IN THE NATAL INDIANS

5.1 INTRODUCTION

Variants of blood group B first began appearing in the literature in 1955. In general their characteristics are: weak or no agglutination of the red cells by anti-B and Anti-A,B reagents, H antigen usually expressed strongly, anti-B usually easily eluted after their exposure to this reagent, no or at the most weak anti-B in the serum and B and H, but sometimes only H, substances present in the saliva of secretors. Some examples were considered to be due to the inheritance of an allele of the *B* gene (Sathe, Sharma, Bhatia and Sahiar, 1966), some to reduction of normal *B* gene expression on the membranes of the cells by an inhibiting or modifying gene which might be a linked (Bhatia, Undevia and Sanghvi, 1965) or an inherited (Gundolf and Anderson, 1970; Marsh, Ferrari, Nichols, Fernandez and Cooper, 1973) mutant regulator, some to a B-like antigen "acquired" by the cells as the result of action by certain bacteria (Cameron, Graham, Dunsford, Sickles, Macpherson, Cahan, Sanger and Race, 1959) and at least one to leukaemia (Undevia, Bhatia, Sharma and Parekh, 1966). The variant B_m is believed (Watkins, 1978) to be caused by an inhibitor gene which results in a genetic block at the level of B antigen expression on the red cell membrane as both B and H transferases are present in the serum, and the variant B_3 examples so far recorded appear to be heterogenous (Lopez, Bouguerra, Lemeud, Badet and Salmon, 1974).

Although the group B phenotype frequency is more than 30% in most Indian populations and it nearly always exceeds the Indian group A phenotype frequency (± 20%; Mourant, Kopeć and Domaniewska-Sobczak, 1976), the number of Indian individuals and families with weak group B variant red cells reported from India is almost three times lower than the number reported with weak group A variant red cells (Bhatia and Sathe, 1974). Bhatia and Sathe (1974) gave the weak group B variant phenotype frequency in India as being 1 in 9 300 group B Indians or 1 in 24 000 Indians of all ABO groups. Four of the 11 weak group B variant Indians in their series were Moslems, two were Christians, four were Hindus and the religion of one was not known. As these studies were made in the Indian States of Maharashtra and Gujarat, the language spoken by the non-Moslems is likely to have been Hindi. By contrast, 32 Indians with weak group A variant red cells were recorded in the same series.

In Natal, three Indian blood donors whose red cells were shown in my tests to be weak group B variant were found among 67 000 Indians who donated their first units of blood at Natal Blood Transfusion Service donor clinics in the years between 1965 and 1980. The likelihood that other donors with this phenotype were missed was low as it had always been customary to refer all donations giving unusual results for further tests. The frequency of this phenotype is therefore approximately 1 in 7 560 group B Natal Indian blood donors or 1 in 22 680 Natal Indian blood donors of all ABO groups, and it does not differ significantly ($P = < 0,98$) from the frequency reported for Indians in India. In addition, five further Natal Indians with weak group B variant red cells were investigated, supported wherever possible by family studies. The apparent scarcity of Indians with weak group B variant phenotypes is strange in view of the finding by Milner and Calitz (1968) that the strength of the B antigen on the red cells of normal group B Natal Indians varies considerably. In a quantitative study, the cells of some of the group B Natal Indians tested by these investigators were agglutinated more strongly by anti-B than the strongest-agglutinated group B Zulu cells tested (the cells of the Zulus often have extra-strong B antigen; Brain, 1966) while the cells of other group B Indians tested were agglutinated more weakly than the most weakly-agglutinated group B White cells tested.

The weak group B variant red cells which react weakly or not at all with anti-B and anti-A,B were sub-divided by Race and Sanger (1962) into three categories. In *Category 1* were placed those cells, called B_v, in the sera of the possessors of which anti-B was present and in the saliva of the secretors a kind of B substance in addition to H substance had been detected. In *Category 2*, the cells, usually called B_m, were from persons who had no or almost no anti-B in their sera and the secretors secreted both B and H substance in their saliva; and in *Category 3*, the cells, called B_3 or B_x, but not having quite the same characteristics as A_3 and A_x cells, belonged to persons who had no anti-B in their sera. The secretors in *Category 3* had H but no B substance in their saliva. Salmon (1976), however, who believes that the terminology should correspond as far as possible with the terminology used for weak group A variant red cells, recently suggested retaining the symbol B_3 for the cells which show 'mixed field' agglutination, consisting of small agglutinates among many unagglutinated cells in the presence of anti-B and anti-A,B, and using the symbol B_x for the cells which are not agglutinated by anti-B but are agglutinated normally although weakly by anti-A,B. According to Salmon (1976), B_3 secretors have B and H substances in their saliva and B_x secretors a B-like substance which inhibits the agglutination of their cells by the anti-B recovered, after adsorption, from their cells in eluates. The serum of B_x secretors also contains weak anti-B.

The first Indians in India with weak group B, *Category 3*, red cells were recorded by Vyas, Bhatia and Sanghvi in 1960; and the first Indians in India with weak group B, *Category 2* or phenotype B_m red cells by Sathe, Sharma, Bhatia and Sahiar in 1966. In 1974, Bhatia and Sathe reported that the serological reactions of the cells of all the weak group B variant members in some Indian families in India tested with anti-B and anti-A,B were similar, while in other Indian families there the cells of some of the weak group B variant members reacted with these reagents differently from others. In this chapter, the serological findings in the first two Indian families in Natal with *Category 2* or phenotype B_m red cells will be described. The families are believed to be only the second and third with this phenotype to be recorded in Indians, and the cells of all the 11 phenotype B_m members in the first family reacted similarly in the appropriate tests. Four other Natal Indian individuals whose cells and sera reacted in the same manner as those of the weak group B variant individuals in these two families and whose cells were called B_m-like as family studies were not made, and two Natal Indians whose cells were called *Category 3* – or phenotype B_3-like, will also be described.

5.2 CASE HISTORIES

An. Gov, the propositus in the first Natal Indian family with *Category 2* or phenotype B_m red cells, was 40 years of age, in good health and spoke Tamil. He was discovered when his first blood unit, donated at an N.B.T.S. clinic in 1968, was grouped in manual tests as O, but anti-A only was detected in his serum. An. Mur., the propositus in the second Natal *Category 2* Indian family, and M. Kri., a Natal Indian individual with *Category 3* – or phenotype B_3-like cells, both of whom were also healthy blood donors and who spoke Tamil, were detected in the same way in 1979 and 1978 respectively. The two Natal *Category 2* or phenotype B_m Indian women, Mrs Sob., aged 24 years and Mrs Sta., aged 16 years; and the second Natal Indian individual, with *Category 3* – or phenotype B_3-like cells, Mrs S. Pil., aged 19 years; all of whom spoke Tamil and who were pregnant, were identified when routine blood samples were submitted from them to the laboratory for antenatal tests. The two other Natal *Category 2* or phenotype B_m Indian individuals, Mrs Ch. and her young son G. Ch., who both spoke Telegu, were participants in a disputed paternity suit whose blood samples were received for confirmation of an apparently weak ABO group.

5.3 MATERIALS AND METHODS

The routine procedure consisted of testing the cells with from 6 to 10 different examples of standardised anti-A, anti-B and anti-A,B reagents, several special high titre immune examples of these antibodies, serial dilutions in saline of *Ulex* anti-H reagent, and AB serum. Tests with lectins specific for various types of red cell polyagglutination were included as well, when these became available. The anti-A, anti-B, anti-A,B and AB serum reagents were used by the saline, one-stage 0,5% bromelin and one-stage 0,25% ficin techniques, whenever possible at 22°C, 10°C and at 4°C, and the lectins were used in accordance with the techniques recommended for each. All these techniques are described in Chapter 2, and suitable positive and negative control cell samples were always included with the tests. The serum from the blood samples was tested by the techniques described in Chapter 2 as well, and the eluates were made in saline by the 56°C technique of Landsteiner and Miller (1925).

5.4 RESULTS

5.4.1 First Natal Indian family with *Category 2* or phenotype B_m red cells (Moores, 1970)

Table 5.1 shows that the red cells of An. Gov., II-4, (Figure 5.1), were not agglutinated in saline, bromelin and ficin tests either at 22°C or at 4°C by the anti-A and anti-B reagents used. However, his cells were agglutinated by the anti-A,B reagents used in saline tests at 4°C and in bromelin and ficin tests at 22°C. In the saline and bromelin tests the agglutination seen was microscopic only, and in the ficin tests weakly macroscopic. Anti-B but not anti-A was readily recovered from his cells in eluates, but the titre of the anti-B reagent absorbed by his cells in these tests was not seen to have been reduced when it was subsequently compared with the titre of the same reagent after absorbtion by group O cells (Table 5.2). The H antigen strength of An. Gov.'s cells was seen to be similar to that of the control group O cells (Table 5.3). A sample of his cells was washed three times, packed down and mixed with an excess of anti-A,B in a Petri dish by intermittent hand rotation at 22°C for one hour, but despite the opportunity provided for improved contact between his cells, they failed to produce macroscopic agglutinates by this technique. Anti-A but not anti-B was detected in An. Gov.'s serum, and his saliva, which inhibited anti-B and anti-H but not anti-A, showed by this that he was a secretor of B and H substances (Table 5.4). The amount of these substances secreted by An. Gov. was found to be similar to the

TABLE 5.1

Results of ABO grouping tests with the cells of An. Gov., phenotype B_m, and his family members

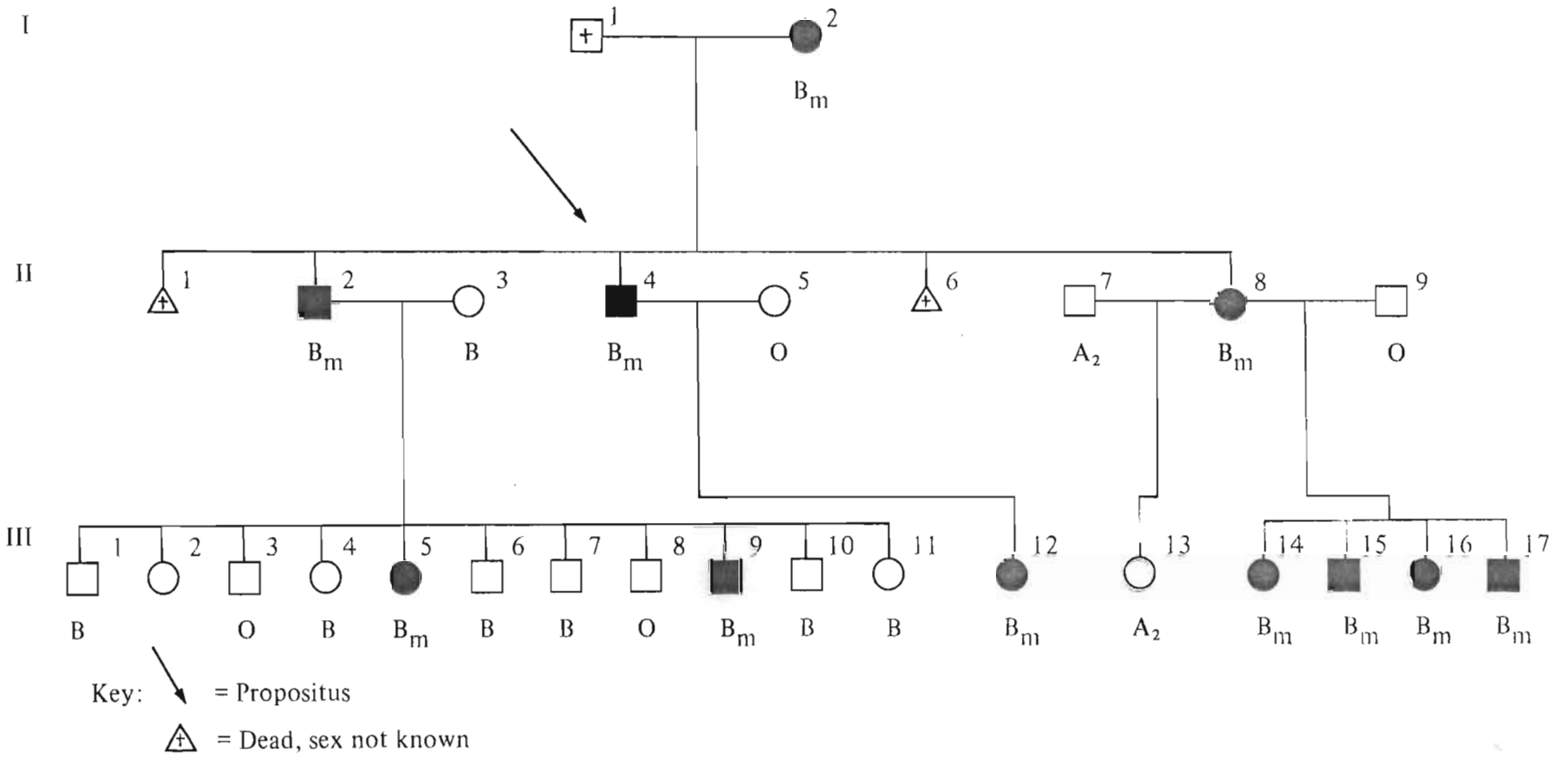
	SALINE						0,5% BROMELIN						0,25% FICIN					
	Anti-A		Anti-B		Anti-A,B		Anti-A		Anti-B		Anti-A,B		Anti-A		Anti-B		Anti-A,B	
	22°	4°	22°	4°	22°	4°	22°	4°	22°	4°	22°	4°	22°	4°	22°	4°	22°	4°
I-2	–	–	–	–	(1)		–	–	–	–	(2)		–	–	–	–	1	
II-2	–	–	–	–	–	(±)	–	–	–	–	(2)		–	–	–	–	1	
II-4 ↙	–	–	–	–	–	(3)	–	–	–	–	(3)		–	–	–	–	2	
II-8	–	–	–	–	–	(1)	–	–	–	–	(2)		–	–	–	–	±	
III-5	–	–	–	–	–	1	–	–	–	–	–		–	–	–	–		1
III-9	–	–	–	–	–		–	–	–	–	–		–	–	–	–		1
III-12	–	–	–	–	–	–	–	–	–	–	1		–	–	–	–	±	
III-14	–	–	–	–	–		–	–	–	–	(2)		–	–	–	–	1	
III-15	–	–	–	–	(±)		–	–	–	–	(3)		–	–	–	–	2	
III-16	–	–	–	–	–		–	–	–	–	(2)		–	–	–	–	1	
III-17	–	–	–	–	(±)		–	–	–	–	(2)		–	–	–	–	2	

Key: 22° = 22° Centigrade

↙ = propositus

FIGURE 5.1

Family of An. Gov.



amount secreted by his normal group B relatives (Table 5.4). These findings, confirmed when further samples were obtained from An. Gov. one year later and recorded as such by Race and Sanger in *Blood Groups in Man* (6th ed. p 20, 1975), established that An. Gov. had *Category 2* or phenotype B_m red cells.

TABLE 5.2

Results of titrating with B cells the anti-B and anti-A,B reagents absorbed with the cells of An. Gov.'s family members

Reagent	Absorbing cells	Dilutions of anti-B 1	2	4	8	16	32	64	128	256	512	1024	Score
Anti-B	II-4 B_m	4	4	4	4	4	4	3	2	2	(2)	–	87
	II-5 O	4	4	4	4	4	4	4	2	1	(1)	–	83
	Unabsorbed	4	4	4	4	4	4	4	4	2	(3)	(1)	90
Reagent	Absorbing cells	Dilutions of anti-A,B 1	2	4	8	16	32	64	128	256	512	1024	Score
Anti-A,B	II-4 Bm	4	4	4	4	4	4	4	2	1	(1)	–	83
	II-5 O	4	4	4	4	4	4	4	2	1	(2)	–	84
	Unabsorbed	4	4	4	4	4	4	4	3	2	(3)	–	90

5.4.1.1 Family study and discussion

Blood and saliva samples from members of An. Gov.'s family disclosed that, in addition to himself, 10 family members had *Category 2* or phenotype B_m red cells (Figure 5.1; Tables 5.1 and 5.5). The reactions of the cells of some of the family members were fractionally weaker with the anti-A,B reagents used than the cells of others (Table 5.1) but the small differences were not considered significant. In titrations with *Ulex* anti-H, the H antigen strength of their cells was seen to be similar to that of the group O family members' and the group O control cells (Table 5.3), and the serum of II-2, like that of II-4, contained anti-A but no anti-B allo-antibodies. The family members who had phenotype B_m cells and who were secretors secreted approximately the same amount of B and H substances as the group B secretor family members (Table 5.4). The group O phenotypes of III-3 and III-8, and the group A_2

TABLE 5.3

Results of *Ulex* anti-H titrations with the cells of An. Gov. and his family

Family number	ABO phenotype	Ulex anti-H titre				
		2	4	8	16	32
I-2	B_m	3	2	2	1	±
II-2	B_m	4	3	2	1	±
II-4	B_m	4	3	2	1	±
II-8	B_m	4	3	2	1	±
III-5	B_m	4	3	2	1	±
III-9	B_m	4	3	2	1	–
III-12	B_m	4	3	2	1	±
III-14	B_m	4	3	2	±	–
III-15	B_m	4	3	2	±	–
III-16	B_m	4	3	2	±	–
III-17	B_m	4	3	2	±	–
III-1	B	2	2	1	–	–
III-4	B	2	2	±	–	–
III-6	B	2	2	1	–	–
III-7	B	2	2	1	–	–
III-10	B	2	2	±	–	–
III-11	B	3	2	1	–	–
II-3	B	3	2	1	–	–
II-9	O	4	3	2	1	±
III-3	O	4	4	3	1	±
III-8	O	4	3	2	1	–
II-5	O	4	3	2	1	±
III-13	A_2	4	2	2	1	–
CONTROL	B	2	2	±	–	–
CONTROL	O	4	3	2	1	±
CONTROL	A_1	1	±	–	–	–

TABLE 5.4

Results of titrations in which saliva from An. Gov. and his family members were used to inhibit anti-B and *Ulex* anti-H reagents

Family No.	Group	Anti-B diluted 1 in 20 Group B cells									Ulex anti-H diluted 1 in 8 Group O cells								
		2	4	8	16	32	64	128	256	512	2	4	8	16	32	64	128	256	512
I-2	B_m		–	–	±	1	2	2	4	4	–	±	1	2	3	4	4	4	4
↘ II-2	B_m	–	–	–	–	–	–	±	1	2	–	–	–	±	1	2	3	4	4
II-4	B_m	–	–	–	–	–	±	1	1	2	–	–	±	2	4	4	4	4	4
II-8	B_m	–	–	–	–	–	–	±	1	1	–	–	±	1	2	3	4	4	4
III-5	B_m	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
III-9	B_m	–	–	–	–	–	–	±	1	1	–	–	±	1	2	3	4	4	4
III-12	B_m	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
III-14	B_m	–	–	–	–	–	±	1	1	2	–	–	±	2	2	3	4	4	4
III-15	B_m	–	–	–	–	–	±	1	1	2	–	–	±	1	1	3	4	4	4
III-16	B_m	–	–	–	–	–	±	1	1	2	–	–	±	1	2	4	4	4	4
III-17	B_m	–									–								
II-3	B	–	–	–	–	–	±	1	1	2	–	–	–	±	1	3	4	4	4
III-1	B	–	–	–	–	–	±	1	1	2	–	±	1	3	4	4	4	4	4
III-4	B	–	–	–	–	–	–	±	1	1	–	–	±	1	2	4	4	4	4
III-6	B	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
III-7	B	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
III-10	B	–	–	–	–	–	–	–	±	1	–	±	1	2	4	4	4	4	4
III-11	B	–									–								
II-5	O	4	4	4	4	4	4	4	4	4	–	–	–	–	±	1	1	2	2
II-9	O	not tested									not tested								
III-3	O	4	4	4	4	4	4	4	4	4	–	–	–	–	–	–	±	1	1
III-8	O	4	4	4	4	4	4	4	4	4	–	–	–	–	±	1	2	2	3
III-13	A_2	not tested									–	–	–	–	–	–	–	±	1
Control	B	–	–	–	–	–	–	–	–	±	–	–	–	–	–	±	1	1	2
Control	O	not tested									–	–	–	–	–	–	–	±	1
Control	Saline	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Key: ↘ = propositus

TABLE 5.5

Blood groups of An. Gov. and his family members

Family member	Age in years	ABO	Substances in saliva	MNSs	P_1	Rhesus	Lu^a	K	Lewis	Fy^a	I
I-2	60	B_m	B.H.	NS/Ns	–	CDe/CDe	–	–	Le(a–b+)	+	+
II-2	45	B_m	B.H.	Ms/NS	–	CDe/CDe	–	–	Le(a–b+)	+	+
II-3	adult	B	B.H.	Ms/Ns	+	cDE/cde	–	–	Le(a–b+)	+	+
II-4 ↙	40	B_m	B.H.	MNS	–	CDe/CDe	–	–	Le(a–b+)	+	+
II-5	adult	O	H	MNS	–	CDe/CDe	–	–	Le(a–b+)	+	+
II-8	38	B_m	B.H.	Ms/Ns	+	CDe/CDe	–	–	Le(a–b+)	+	+
II-9	adult	O	not tested	MS/NS	+	CDe/cde	–	–	Le(a–b+)	+	+
III-1	22	B	B.H.	Ms/NS	+	CDe/cDE	–	–	Le(a–b+)	+	+
III-3	20	O	H	Ms/NS	+	CDe/cDE	–	–	Le(a–b+)	+	+
III-4	16	B	B.H.	Ms/Ms	+	CDe/cde	–	–	Le(a–b+)	+	+
III-5	14	B_m	–	Ms/NS	+	CDe/cde	–	–	Le(a+b–)	+	+
III-6	13	B	–	NS/NS	+	CDe/cDE	–	–	Le(a+b–)	+	+
III-7	11	B	–	NS/Ns	+	CDe/cde	–	–	Le(a+b–)	+	+
III-8	8	O	H	Ms/Ns	–	CDe/cDE	–	–	Le(a–b+)	+	+
III-9	7	B_m	B.H.	NS/Ns	+	CDe/cde	–	–	Le(a–b+)	+	+
III-10	6	B	B.H.	Ms/Ns	+	CDe/cDE	–	–	Le(a–b+)	+	+
III-11	4	B	B.H.	Ms/NS	+	CDe/cde	–	–	Le(a–b+)	+	+
III-12	9	B_m	–	N.NS	–	CDe/CDe	–	–	Le(a+b–)	+	+
III-13	16	A_2	A.H.	Ms/Ms	+	CDe/cde	–	–	Le(a–b+)	+	+
III-14	13	B_m	B.H.	MNS	+	CDe/cde	–	–	Le(a–b+)	+	+
III-15	11	B_m	B.H.	NS/Ns	+	CDe/CDe	–	–	Le(a–b+)	+	+
III-16	9	B_m	B.H.	MNS	+	CDe/cde	–	–	Le(a–b+)	+	+
III-17	5	B_m	B.H.	MS/Ms	+	CDe/CDe	–	–	Le(a–b+)	+	+

Key: ↙ = propositus

phenotype of III-13 (Figure 5.1) showed by this that II-2 and II-8 were heterozygous B_m/O, and the family pedigree showed that the genes responsible for the B_m phenotype were being inherited in this family in an apparently straight-forward manner. As none of the children of II-4 and II-3 unexpectedly had normal group B cells, it seemed possible, in this family, either that the expression of a normal *B* gene was being affected by linked mutant regulator gene or that a true allele, B_m, of the *B* gene was being inherited.

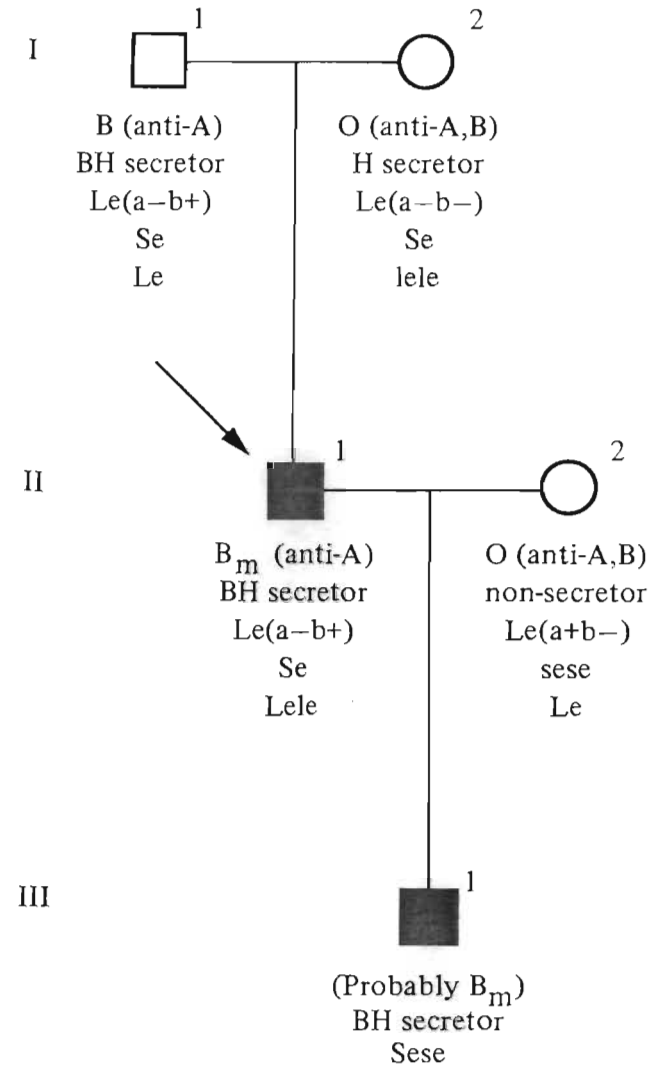
5.4.2 Second Natal Indian family with *Category 2* or phenotype B_m red cells

Unlike the red cells of An. Gov. and members of his family, the cells of An. Mun., II-1, (Figure 5.2) the propositus in the second family with the B_m phenotype, which were not agglutinated by anti-A and anti-B, were agglutinated by the anti-A,B reagents used only in one stage 0,25% ficin tile tests. However, the weaker results obtained with his cells were considered either to be due to a minor quantitative variation of B antigen strength in them or to fewer B antigen sites being available, rather than to a B_m sub-type. The H antigen strength of the cells of II-1 was found to be similar to that of the group O control cells (Tables 5.6 and 5.7) and anti-B but not anti-A was readily eluted from his cells after they had been exposed to these two reagents. Like the anti-B reagent absorbed by An. Gov.'s cells, the anti-B reagent absorbed by the cells of II-1 was shown subsequently not to have undergone a reduction in titre. Anti-A and a cold agglutinin of undetermined specificity, but not anti-B, were detected in the serum of II-1 (Table 5.6), and B and H substances were detected in his saliva (Table 5.8).

At birth, the red cells of III-1 (Figure 5.2) were found to group as O, but his saliva showed, by inhibiting anti-B and anti-H and not anti-A, that he was a secretor of B and H substances. As the ABO antigens are known to be weaker in the newborn than later in life, the normal B antigen is readily detected on cord blood cells and the ABH substances are well-developed in the saliva (Race and Sanger, p 38 and 314, 1975), the most likely phenotype of III-1 was B_m. The corresponding genes had almost certainly been inherited by III-1 from his father, and the non-agglutination of his cells by anti-A,B, unlike those of his father, was not considered significant at this early stage in his development.

Figure 5.2

Family of An. Mun.



Key: ↘ = Propositus

TABLE 5.6

Results of tests with the *Category 2* or phenotype B_m cells and sera of An. Mun. and B_m-like cells of four other Natal Indians

		CELLS									SERUM												Ulex	Anti-B
		Anti-A			Anti-B			Anti-A,B			A_1 cells			A_2 cells			B cells			O cells			anti-H	recovered
		22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	titre	in eluate
	Saline	–	–	–	–	–	–	–	–	–	4	4	4	4	4	4	–	–	–	–	–	–		
An. Mun.	Bromelin	–	–	–	–	–	–	–	–	–	4	4	4	4	4	4	–	(2)	1	–	(1)	(3)	32	yes
	Ficin	–	–	–	–	–	–	1	2	3	4	4	4	4	4	4	–	1	2	–	1	2		
	Saline	–			–			–			4			2			–			–				
Mrs Sob.	Bromelin	–			–			(2)																yes
	Ficin	–			–			–																
	Saline	–			–			–			4			4			–			–				
Mrs Sta.	Bromelin	–			–			–															32	yes
	Ficin	–			–			–			4						–			–				
	Saline	–			–			(±)			4			4			–			–				
Mrs Ch.	Bromelin	–			(±)			(2)			4			4			–			–			32	
	Ficin	–			–			–			4			4			–			–				
	Saline	–			–			–																
G. Ch.	Bromelin	–			–			1															32	
	Ficin	–			–			–																
Control B	Saline	–			4			4			4			4			–			–			4	yes
Control O	Saline	–			–			–			4			4			4			–			32	no

Key: 22° = 22° centigrade

Bromelin = one-stage 0,5% bromelin tube technique

Ficin = one-stage 0,25% ficin tile technique

5.4.2.1 Family study and discussion

I-1, the father of An. Mun., II-1, was group B with anti-A in his serum and I-2, his mother, group O with anti-A,B in her serum (Figure 5.2). As the other blood group systems studied revealed no evidence of illegitimacy in the family, the most likely genotype of I-1 was B/B_m. In titrations with *Ulex* anti-H, the H antigen of the cells of I-1 was found to be increased in strength in relation to that of the control group B cells (Table 5.7) and inhibition tests showed that his saliva contained less H substance than the saliva of the control group AB secretor. The apparent B_m phenotype of III-1 also provided support for the genotype proposed for I-1, for B_m appeared, in this family (and in the family of An. Gov.), to be due to the inheritance either of a true B_m allele or of a mutant regulator gene linked to the *B* gene. The increased H cell antigen of I-1 was therefore of interest in that it suggested that his ABO genotype was unusual. However, in addition to the wide variation of B red cell antigen strength in the Natal Indians reported by Milner and Calitz (1968), the H red cell antigen strength in them was shown in this thesis (Chapter 1) to vary widely. The quantity of the ABH substances secreted is also known to vary for reasons unconnected with the ABO groups, such as the subject's age. As a result, and in accordance with the well-known observation that group A and group B cell homo- and heterozygosity cannot be judged from the results of serological tests, the true genotype of I-1 was not considered to have been established by this means.

TABLE 5.7

Results of *Ulex* anti-H titrations with the cells of An. Mun. and members of his family which showed that the cells of his father, I-1, had increased H antigen

		Ulex anti-H							
Red cells	Phenotype	1	2	4	8	16	32	64	Score
I-1	B	4	3	1	(3)	(2)	(1)	–	28
I-2	O	4	4	4	4	4	2	–	58
II-1	B_m	4	4	4	3	1	(1)	–	45
II-2	O	4	4	4	3	2	1	–	53
Control	A_1	1	(2)	–	–	–	–	–	6
Control	A_2	4	4	3	2	1	–	–	43
Control	B	2	1	(2)	–	–	–	–	14
Control	O	4	4	4	3	1	(1)	–	45

TABLE 5.8

Results of titration-inhibition tests with anti-A, anti-B and *Ulex* anti-H reagents and the saliva of An. Mun. and his family members in which the saliva of his father, I-1, showed reduced inhibition of anti-H

Saliva sample	Blood group phenotype	A_2 cells Dilutions of anti-A						B cells Dilutions of anti-B						O cells Dilutions of Ulex anti-H					
		4	8	16	32	64	128	4	8	16	32	64	128	4	8	16	32	64	128
I-1	B	4	2	1	1	(2)	–	–	–	–	–	–	–	2	1	–	–	–	–
I-2	O	4	2	1	(3)	(2)	–	4	4	4	3	2	2	–	–	–	–	–	–
II-1	B_m	4	2	1	1	(2)	–	–	–	–	–	–	–	–	–	–	–	–	–
II-2	O	not tested						not tested						not tested					
Control secretor	AB	–	–	–	–	–	–	1	(2)	–	–	–	–	(1)	–	–	–	–	–
Control non-secretor		4	2	1	1	(3)	–	4	4	4	3	2	2	4	3	2	1	–	–
Control saline		4	3	3	2	1	–	4	4	4	3	2	2	4	4	2	1	–	–

5.4.3 Four Natal Indian invididuals with *Category 2-* or phenotype B_m-like red cells; phenotype study

Table 5.6 shows that the red cells of Mrs Sob., Mrs Sta., Mrs Ch. and G. Ch., like the red cells of An. Gov., the 10 other B_m members of his family and those of An. Mun., were not agglutinated by the anti-A and anti-B reagents used and that they were either not or were only very weakly agglutinated by the anti-A,B reagents used (Table 5.6). Similarly, the strength of the H antigen of the cells of Mrs Sta., Mrs Ch. and G. Ch. resembled that of the control group O cells (Table 5.6). Anti-B was eluted with equal readiness from the cells of Mrs Sob. and Mrs Sta. after they had been exposed to this reagent, and the sera of all except G. Ch., from whom no serum was received, contained anti-A but no evidence of anti-B allo-antibodies. No family studies were made. As the father of G. Ch. was not tested, he and his mother, Mrs Ch., were not regarded as a family for the purposes of this investigation. Table 5.6 also shows that the reactions of the cells of these four Indians were not completely identical in every instance.

5.4.4 Two Natal Indian individuals with *Category 3-* or phenotype B_3-like red cells; phenotype study

The *Category 3-* or B_3-like red cells of the first Natal Indian individual described in this section, M. Kri., were agglutinated both by anti-B and by anti-A,B, but not as strongly as normal group B cells, in saline tests at 22°C. The agglutination was 'mixed field' in type and many unagglutinated cells were present. By contrast, the cells of the second Natal Indian with this phenotype, Mrs S. Pil., were agglutinated in similar tests in 'mixed field' patterns only by the anti-A,B reagents used (Table 5,9).

In titrations, in which the anti-B reagent used was diluted serially in doubling dilutions with 6% bovine albumin in order to avoid the cells in the higher dilutions not being agglutinated because insufficient protein was present, the cells of M. Kri. were observed to agglutinate in 'mixed field' patterns to titre 32 and normal group B control cells to titre 256 (Table 5.10). The H antigen strength of the cells of M. Kri. and of Mrs S. Pil. was seen to be similar to that of the normal group O control cells (Table 5.9 and 5.11). Following their exposure to anti-A and anti-B in separate tests, anti-B but not anti-A was readily recovered from M. Kri.'s cells in eluates, but no attempt was made to separate the agglutinated from the unagglutinated cells first and ascertain whether or not anti-B could be eluted from them subsequently. The titre of the anti-B reagent,

TABLE 5.9

Results of tests with the *Category 3*- or phenotype B_3-like cells and sera of M. Kri. and Mrs S. Pil.

Name	Technique	CELLS									SERUM												Ulex anti-H titre	Anti-B recovered in eluate
		Anti-A			AntiB			Anti-A,B			A_1 cells			A_2 cells			B cells			O cell				
		22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°	22°	10°	4°		
M. Kri.	Saline	–			1•			2•			4	4	4	3	4	4	–	–	–	–	–	–		
	Bromelin	–			2•			3•			4	4		3	4		–	–		–	–		32	yes
	Ficin	–			3•			4•			4	4		4	3		–	–		–	–			
Mrs S. Pil.	Saline	–	–		–	–		1•	1•		4			3			–			–				
	Bromelin	–			–			1•															32	
	Ficin	–	–		–	–		±	±		4			4			–			–				
Control B	Saline	–			4			4			4			4			–			–			4	yes
Control O	Saline	–			–			–			4			4			4			–			32	no

Key: 1• = 1+ sized aggutinates amid many unagglutinated cells

22° = 22° centigrade

Bromelin = one-stage 0,5% bromelin tube technique

Ficin = one-stage 0,25% ficin tile technique

TABLE 5.10

Results of anti-B titration with the *Category 3-* or phenotype B_3-like cells of M. Kri.

Cells	Anti-B								
	1	2	4	8	16	32	64	128	256
M. Kri	2•	2•	2•	1•	(3)•	(1)	–	–	–
B	4	4	4	4	4	3	2	1	(3)
O	–	–	–	–	–	–	–	–	–

Key: 2• = 2+ sized agglutinates with some unagglutinated cells present

TABLE 5.11

Results of *Ulex* anti-H titration with the *Category 3-* or phenotype B_3-like cells of M. Kri. and Mrs S. Pil.

Red cells	Phenotype	Ulex anti-H 1	2	4	8	16	32	64	Score
M. Kri.		4	4	4	4	2	1	–	53
Mrs S. Pil.		4	4	4	3	2	±	–	49
Control	A_1	1	±	–	–	–	–	–	6
Control	A_2	4	4	4	3	1	±	–	46
Control	B	3	1	–	–	–	–	–	15
Control	O	4	4	4	4	2	1	–	53

after absorbtion with M. Kri.'s unseparated cells, was found to have been reduced from 1 024 to 64 (Table 5.12), and the serum of M. Kri. and Mrs S. Pil. contained anti-A but no evidence of anti-B (Table 5.9). The saliva of the two Indians could not be examined as no sample was received from Mrs S. Pil. and the Le(a+b−) phenotype of M. Kri.'s cells showed by this that he was almost certainly a non-secretor of ABH substances. Family studies were also not possible as the Indians were both uncooperative.

To exclude the 'mixed field' agglutination of M. Kri.'s cells seen with anti-B and anti-anti-A,B from being due to 'acquired B' antigen as the result of the effect of bacterial action *in vivo* (Race and Sanger, p 31, 1975), his cells were tested with BS I (*Bandeiraea simplicifolia*) lectin. Surprisingly, although normal group B control cells, as expected, were agglutinated strongly, M. Kri.'s cells were not agglutinated by this reagent. However, his serum did not contain the anti-B customarily found when 'acquired B' cells are present and, as 'acquired B' antigen is confined almost entirely to group A cells, the lectin's failure to agglutinate his cells strongly suggested that his B antigen was weaker than usual. Had M. Kri. been a B/O chimaera the proportion of the B cells in whose blood was sufficient to give the same sized agglutinates as those observed in the tests with anti-B, 'mixed fields' of agglutinated and unagglutinated cells would almost certainly have been observed with his cells when tested with this lectin. This was confirmed when the BS I lectin used was seen to agglutinate readily in 'mixed field' patterns artificially-prepared mixtures of from 10% to 50% group B in group O cell suspensions (in parallel tests, the agglutination values 1$^{\bullet}$ and 2$^{\bullet}$ (see Tables 5.9 and 5.10) were obtained in the 10% to 30% mixtures using anti-B). Polyagglutination was excluded by the negative reactions of M. Kri.'s cells with *Arachis hypogea, Dolichos biflorus, Salvia sclarea, Salvia horminum* and *Glycine soja* lectins.

5.4.5 *Category 3*- or phenotype B_3-like red cells; discussion

Like phenotype A_3 cells (Gammelgaard, 1942; Race and Sanger, p 14, 1975), the cells described as B_3 and grouped together in *Category 3* by Race and Sanger (see 5.1, introduction to this chapter) were characteristically agglutinated in 'mixed field' patterns by anti-B and anti-A,B reagents. The agglutinates were small in size and they were greatly outnumbered by unagglutinated cells. In absorbtion-elution tests, B_3 cells absorbed very little anti-B but gave it up readily in eluates and, with difficulty, two apparent cell populations were separated by means of differential agglutination

TABLE 5.12

Results of titrating with B cells the anti-B reagent absorbed with the *Category 3*- or phenotype B_3-like cells of M. Kri.

Reagent	Cells used for absorbtion	Dilutions of anti-B 1	2	4	8	16	32	64	128	256	512	1024	2048	Score
Anti-B	M. Kri.	4	4	3	2	1	(3)	(1)	–	–	–	–	–	45
	unabsorbed	4	4	4	4	4	4	3	2	2	(3)	(2)	–	86

(Sussman, Pretshold and Lacher, 1960) but not by differential centrifugation (Wiener and Cioffi, 1972).

Although Gammelgaard (1942) and Dunsford (1959) had shown that anti-A,B existed which would agglutinate all the cells in A_3 samples and Reed (1964) had successfully eluted anti-A from both the agglutinated and the unagglutinated cells after A_3 blood had been exposed to this antibody, no reports were found of similar attempts having been made to elute anti-B separately from the agglutinated and the unagglutinated cells in B_3 samples (perhaps because complete separation was too difficult to achieve). In all the papers studied, the authors confirmed that the unseparated cells would absorb and elute anti-B, and they then established by means of family studies that their propositi had probably inherited a weak *B* variant allele or mutant regulator gene. As a result, other causes of 'mixed field' agglutination, such as blood transfusion, a bone marrow transplant, a leukaemic change or a chimaera whose blood was a mixture of group B and group O cells were not excluded. From the group O cells (naturally not agglutinated by anti-B) in such a chimaera's blood, this antibody would not have been expected to have been recovered again in eluates. Provided the B_3 is the analogue of the A_3 phenotype, however, anti-B should be recovered from the cells not agglutinated by anti-B in B_3 blood samples.

Both M. Kri. and Mrs S. Pil. were healthy and no history of recent blood transfusions or bone marrow transplants was obtained. A fetal bleed was excluded in Mrs S. Pil.'s case as the proportion of group O cells which would have had to be supposed in her blood was too great to have been derived in this way. The possibility that she was group O with fetal group B cells circulating was excluded as well for the B cells would almost certainly have been haemolysed by the anti-A,B expected in a group O person, and no evidence of this antibody had been detected in her serum.

5.5 DISCUSSION

The findings in the healthy Indians with *Category 2* red cells in the two families described in this chapter established that the genes or alleles responsible for the weak group B variant phenotype B_m were present in Natal. Since the phenotypes of the four other Indians whose cells had reacted similarly and of the two Indians whose cells had reacted with anti-B in 'mixed field' patterns had not been shown by means of family studies to have been genetically inherited, their origin was uncertain and they were consequently called B_m-like and B_3-like respectively. The other possible causes of the characteristic

agglutination seen with the B_3-like cells, however, were discussed and shown to be unlikely in these two Indians. Moreover, direct evidence that the cells of one Indian had weak B antigen was obtained in tests with BS I lectin.

In my M.Sc. thesis (1976), weak group B variants had not been recorded in the Natal Negroes (Zulus) or in the Natal White controls, and it was therefore probable that the genes for the B_m (and possibly also for the B_3) phenotype had been brought to Natal from India by the Indian immigrants. The evident rarity of the weak group B variant phenotypes, both in Natal and in India, showed that they were unlikely to be due to local factors in the environment, and Lopez, Bouguerra, Lemeud, Badet and Salmon (1974) and Salmon (1976) are of the opinion, following the detailed quantitative studies of the former workers with weak group B variant cells and the same set of reagents, that the variants are genuinely-inherited genetic characters. Lopez *et al.* (1974) observed that the expression of the B antigen was invariably the same within a family but differed between families, and my findings in the Natal Indians described in this chapter support their view. Although my studies were made at different times, my standard anti-A, anti-B and anti-A,B reagents were obtained from a single commercial source which subjects them to rigorous quality control measures before issue, and my techniques have always been applied to every specimen investigated with the same stringent degree of personal care. It is of interest that the biochemists, who have studied the serum transferases in individuals with different weak group A variant, different weak group B variant and A, B and O H-deficient red cell phenotypes, also support the concept that they are genuinely inherited genetic characters (Watkins, 1980).

The Natal Indians described in this chapter spoke either Tamil or Telegu and not the Hindi Indian language expected to have been spoken by the non-Moslems with weak group B variant cells recorded from Maharashtra and Gujarat in India by Bhatia and Sathe (1974). This showed that the variants may occur in the Dravidian as well as in the Aryan language-speaking Indians.

5.6 SUMMARY

The weak group B variant phenotype B_m, or *Category 2* red cells, was described in 11 members of one and in two members of a second Natal Dravidian language-speaking Indian family. Within each family the B antigen expression of the cells was almost identical but between the families it was noted to vary minimally. Minor variations were also found in four further Natal Indians whose cells were described as *Category 2*- or B_m-like as family studies had not been made in their case. The reactions of the cells and

serum in two Natal Indians who had *Category 3*- or B_3-like cells, the B antigen in one being more weakly-expressed than in the other, were also described. The possibility that these Indians had group B and group O cell populations was discussed and in one Indian shown to be unlikely as his cells had weaker-than-normal B antigen.

CHAPTER 6

A_1B WEAK VARIANT RED CELLS IDENTIFIED IN A NATAL INDIAN BLOOD TRANSFUSION RECIPIENT

6.1 INTRODUCTION

Theoretically, as AB persons have no anti-A or anti-B allo-antibodies in their blood, their sera may be crossmatched for transfusion purposes with A, B, AB or O blood units. In Natal, the first units of blood selected for them are usually AB but, when they are in short supply, A or B units may be substituted. However, in the following weeks, further samples sent from the recipients will naturally be expected to contain a mixture of blood cells of AB and other groups.

Of course, blood transfusions are not the only reason mixtures of cells are detected in the blood in serological tests. True cell mixtures may be detected in the blood of blood group chimaeras, recipients of transplanted bone marrow and pregnant women whose fetuses have bled into their circulations. Apparent cell mixtures are also detected occasionally in blood samples from leukaemics, and they occur normally in persons who have some types of weak group A variant and weak group B variant red cells. In addition, cells with 'acquired B' antigen and those that are polyagglutinable are frequently agglutinated in mixed-field patterns in serological tests.

'Acquired B' antigen is considered by most workers to be an effect of the environment. The cells of a number of elderly patients who had carcinoma, intestinal infections or gangrene, and two who were apparently healthy, were noticed by Cameron, Graham, Dunsford, Sickles, Macpherson, Cahan, Sanger and Race (1959) to be weakly agglutinated by anti-B, but family studies disclosed that they could not have inherited a *B* variant allele. The source of their apparent B antigen was therefore surmised to be their disease. The list of disorders in which red cells with this antigen have now been identified includes appendicitis, peritonitis and urinary tract infections. In 1960, Springer and Ansell provided an explanation by showing that lipopolysaccharides from E. coli O_{86} and other organisms with B-like antigens would bind to red cells *in vitro*, and in 1970, Liberge, Lopez and Salmon suggested that 'acquired B' antigen, found so far only on group A cells, might be the result of

deacetylation of N-acetylgalactosamine (A antigen) to a B-like substance by bacterial activity. When a young Natal Indian girl who had recently received severe internal abdominal injuries, but who had apparently not been transfused, was grouped as A B weak, it was consequently easy to infer at first that her cells were A_1 with 'acquired B' antigen.

6.2 CASE HISTORY

In July 1977, Miss N.L. was the victim of a bus accident in Southern Natal. She was 16 years of age, unmarried and had previously been in good health. Her home language was Hindi. With many other young victims, she was taken to a local hospital and, on admittance, was found to have a fractured pelvis and other abdominal injuries. The hospital records do not mention that she was given a blood transfusion at this time. On the following day she was transferred by ambulance, first to the R.K. Khan Hospital at Chatsworth and then to the King Edward VIII Hospital in Durban. At the latter hospital, clotted and unclotted blood samples were drawn from her for cross-matching and haematological studies, and the staff member on duty at the blood bank who grouped her blood noticed that her ABO group was unusual. Her blood sample was therefore referred to me for further studies.

6.3 MATERIALS AND METHODS

The following samples were received from Miss N.L.

a. the initial blood sample drawn in Durban for cross-matching;

b. the initial blood sample drawn in Durban for haematological studies;

c. a blood sample drawn 12 days after the accident and seven days after she had been transfused with four units of group A blood;

d. a blood sample drawn in February 1978, seven months after her accident;

e. a saliva sample, obtained and processed correctly by an NBTS staff member.

Blood samples were received from Miss N.L.'s parents as well. The methods used to prepare the blood and saliva samples for use are described in Chapter 2. The reagents used were all either standardised commercial preparations, or were sera and lectins prepared and standardised according to the customary strict criteria by myself. The

techniques used in this study are also described in Chapter 2.

6.4 RESULTS

In tests made by the saline, one-stage 0,5% bromelin and one-stage 0,25% ficin techniques at 20°C and by the saline technique at 10°C and 4°C, the red cells of Miss N.L.'s first blood sample (Sample *a*) were agglutinated strongly and normally by anti-A, anti-A_1 and anti-A,B but weakly and in mixed-field patterns by anti-B reagents. The agglutinates with the anti-B reagent were just visible microscopically (1+), and large numbers of unagglutinated cells were present. Polyagglutination was excluded by the negative reactions of Miss N.L.'s cells with AB serum, *Arachis hypogea* anti-T lectin, *Salvia sclarea* lectin and a D^u ($\mathfrak{R}h_o$) reagent used in a saline test at 37°C (the Rhesus phenotype of her cells was CDe/cde or Rh_1 rh), and 'acquired B' antigen was suspected. However, surprisingly, her cells were seen to be agglutinated less strongly than the MN control cells by anti-M and anti-N, were agglutinated about as strongly as the weak P_1 control cells by the anti-P_1, and were agglutinated by the anti-Lu^a reagents. The direct antiglobulin test with her cells was negative.

Since Sample *a* from Miss N.L. was now exhausted, the tests were continued with Sample *b*. The findings, which confirmed those obtained with Sample *a*, established that Sample *a* was unlikely to have been contaminated by having accidentally had the blood of another person added to it before being received. Some cells from Sample *b* were mixed with an excess of anti-B reagent and left at 4°C overnight but, unlike 'acquired B' cells (Issitt and Issitt, p 84, 1975), this antibody was recovered readily from them the next day in an eluate made in saline by the 56°C heat technique of Landsteiner and Miller (1925). However, as reported in 'acquired B' cases (Issitt and Issitt, p 84–85, 1975), anti-B, albeit rather weak, was detected in saline tests at 10°C and 4°C in the serum separated from both these samples.

As Sample *b* was now also exhausted and some doubt had arisen as to the origin of Miss N.L.'s B antigen, Sample *c* was requested and was tested with BS I (*Bandeiraea simplicifolia*) lectin. This lectin agglutinated B cells strongly but cells having 'acquired B' antigen only weakly (Judd, Steiner, Friedman, Hayes and Goldstein, 1976). The results showed, although B and AB adult and cord cells were all agglutinated 4+ macroscopically immediately and A_1 and O adult cells were not agglutinated, that no agglutinates formed in Miss N.L.'s blood. Later, however, microscopic-sized agglutinates were detected in her blood and not in the negative control samples. A

mixture of cells in her blood was suspected, and enquiries showed that Sample *c* had indeed been drawn seven days after Miss N.L. had received four units of A blood. Artificial mixtures of different volumes of A_1 and A_1B cells from known donors were then prepared, and the mixture which was agglutinated by BS I lectin and by anti-B in the manner that most closely resembled the mixture in Miss N.L.'s blood was seen to contain 90% A_1 and 10% A_1B cells. The volume of A_1 in relation to A_1B cells in her blood thus suggested, was nevertheless considered too large, and the possibility that she had a B sub-type, was envisaged. To investigate this, the cells in Sample *c* were separated meticulously by differential agglutination, using anti-B, -C, c, -D, -M and -N, and five different kinds of cells were identified. Four were grouped as A and the fifth as AB. Moreover, the AB cells persisted in being agglutinated by anti-B in mixed-field patterns reminiscent of Sample *a*. Some saliva was requested from Miss N.L., and saliva inhibition tests confirmed that her phenotype was A_1B weak variant as she was seen to secrete abundant A and H and a small quantity of B substances (Table 6.1). The tests with Sample *c* were therefore discontinued, and Miss N.L. was discharged from hospital three weeks later in a statisfactory condition, no further blood transfusions having been required.

In February 1978, Sample *d* was drawn from Miss N.L. at home, seven months after her accident and when all the A donors' cells in her blood were expected to have disappeared. The cells in this sample were agglutinated as strongly (4+) and as normally as before by the anti-A and anti-A,B reagents, but they were also now agglutinated more strongly (2+ to 3+) by the anti-B reagents. However, the background,using the anti-B reagent, contained a minor proportion of unagglutinated cells. The *Ulex* anti-anti-H reagent also agglutinated her cells to approximately the same titre as the control group A_1B cells. The cells in Sample *d* and the cells of Miss N.L.'s parents were typed as fully as possible, and the results are given in Figure 6.1. Miss N.L.'s father, I-1, was seen to be A_1B, with no evidence of a weak B variant factor (see also Table 6.2), and her mother, I-2, to be A_1. Her father's serum also contained no evidence of anti-A, anti-B or anti-H antibodies and her mother's serum contained apparently normal anti-B. Weak anti-H but no anti-B was detected in Miss N.L.'s serum.

TABLE 6.1

Results of saliva inhibition tests showing that Miss N.L. secreted abundant A and H and a small quantity of B substances in her saliva

Saliva	Anti-A and A_2 cells, dilutions in saline									
	1	2	4	8	16	32	64	128	256	512
Miss N.L.	–	–	–	–	–	–	–	–	–	–
A secretor	–	–	–	–	–	–	–	–	–	–
B secretor	4	4	4	3	2	2	1	(±)	–	–
O secretor	4	4	4	3	3	2	1	(1)	–	–
AB secretor	–	–	–	–	–	–	–	–	–	–
AB secretor	–	–	–	–	–	–	–	–	–	–
AB secretor	–	–	–	–	–	–	–	–	–	–
Non-secretor	4	4	4	3	2	2	1	(1)	–	–
Saline	4	4	4	3	2	2	1	(1)	–	–

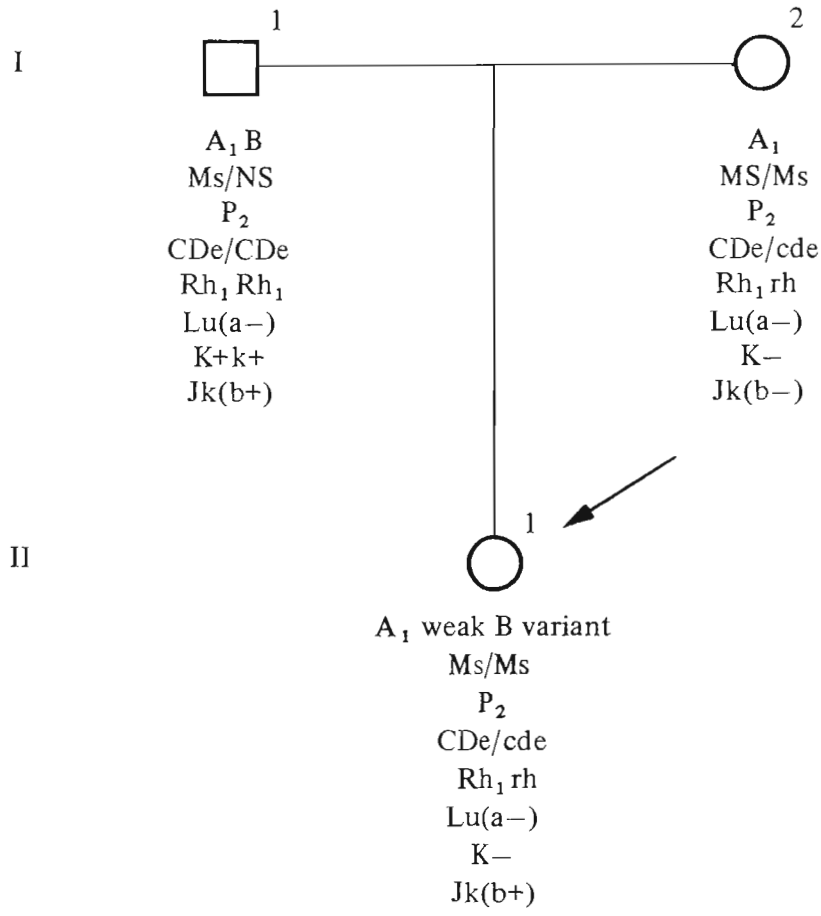
Saliva	Anti-B and B cells * dilutions in saline									
	1	2	4	8	16	32	64	128	256	512
Miss N.L.	4	4	4	3	1	(2)	–	–	–	–
A secretor	4	4	4	4	4	2	1	(2)	–	–
B secretor	–	–	–	–	–	–	–	–	–	–
O secretor	4	4	4	4	4	2	1	(1)	–	–
AB secretor	4	3	2	(±)	–	–	–	–	–	–
AB secretor	4	3	2	(±)	–	–	–	–	–	–
AB secretor	1	(2)	–	–	–	–	–	–	–	–
Non-secretor	4	4	4	4	4	2	1	(±)	–	–
Saliva	4	4	4	4	4	2	1	(2)	–	–

Saliva	Ulex anti-H and O cells, dilutions in saline									
	1	2	4	8	16	32	64	128	256	512
Miss N.L.	1	(±)	–	–	–	–	–	–	–	–
O secretor	–	–	–	–	–	–	–	–	–	–
Non-secretor	4	4	4	3	2	(3)	(±)	–	–	–
Saline	4	4	4	4	2	1	(1)	–	–	–

* A one-tube test, using the anti-B diluted 1 in 10, would have shown Miss N.L. as a weak secretor and the AB controls as secretors of B substances.

Figure 6.1

Family pedigree of Miss N.L. showing relevant blood groups



Key: ↙ = Proposita

TABLE 6.2

Results showing that the strength of the B antigen of I-1 was similar to that of the controls

Cells	Reagent	Titrations Dilutions 1	2	4	8	16	32	64	128	256	512
I-1		4	4	4	4	4	3	2	1	(2)	–
Control A_1B	anti-B	4	4	4	4	3	4	2	1	(3)	(1)
Control B		4	4	4	4	4	3	2	(3)	(1)	–
Control O		–									

The MN, P_1 and Lu^a groups of the cells in Sample *d* from Miss N.L. were seen, unexpectedly, to differ from those obtained with the cells in Sample *a*. The cells in Sample *d* were typed as MM instead of MN, P_2 instead of P_1 and Lu(a–) instead of Lu(a+). Moreover, the groups of her parents confirmed that the earlier results obtained with Sample *a* had not been a true reflection of her groups. The easiest explanation, despite the earlier information to the contrary, was that Miss N.L. had received a blood transfusion prior to the investigations which were made with Samples *a* and *b*.

Cytogenetic studies showed that Miss N.L. had an apparently normal female karyotype.

6.5 DISCUSSION

Although, at first, the red cells of Miss N.L. were thought to be A with 'acquired B' antigen, the subsequent studies designed to confirm this, failed to do so. They failed because anti-B, usually not eluted from 'acquired B' cells, was recovered readily from her cells and, since she secreted a small amount of B and normal quantities of A and H substances in her saliva, she clearly possessed a gene that coded for some form of B antigen. Nevertheless, her cells, shown not to be polyagglutinable, were not considered to be normal A_1B as, even though she was known to have received at least four units of A blood, they had reacted with BS I lectin remarkably weakly. After carefully separating the mixture of cells in her blood, the AB cells had also

persisted in being agglutinated in mixed-field patterns by anti-B. However, unexpectedly, the H antigen of her cells was not increased. The anti-B allo-antibodies detected in the sera of Samples *a* and *b*, which were in agreement with Miss N.L. having 'acquired B' antigen, could be disregarded, once these samples were shown, after all, to have contained a mixture of blood. The anti-B had probably been acquired by Miss N.L. from her donors' plasma.

Having established that Miss N.L.'s cells had an A_1B weak variant phenotype, it was disconcerting to find, in the family study, that her father had normal A_1B cells. The *B* variant gene which Miss N.L. appeared to have inherited was unlikely to have been passed on to her by her mother, for her mother had normal A_1 cells and her anti-B was of normal strength. Since the groups, other than ABO, in the family revealed no evidence of illegitimacy, chimaerism between genetically A_1 and A_1B tissues seemed excluded by the unequal amounts of A, B and H substances secreted in Miss N.L.'s saliva, she had not had a bone marrow transplant, was not pregnant and was not suffering from leukaemia, it was surmised that she might have inherited a double dose of a rare recessive inhibitor or modifying gene. The genes might be depressing, fractionally, the expression of a normal *B* gene on her cells, and suppressing markedly the secretion of B, but not of A or H, substance in her saliva. Recessive inhibitor genes with similar effects have been described previously by Gundolf and Anderson (1970). Their propositus, who secreted a small amount of B but a normal amount of H substance in his saliva, had parents who were both *BO*. However, his cells were said not to be agglutinated by anti-B. Alternatively, Miss N.L. might have inherited a *B* gene from her father which had mutated.

The possibility that organisms of gastro-intestinal origin had been present in Miss N.L.'s blood as the result of the internal injuries she had sustained, raised the question as to whether Miss N.L. might have depressed rather than 'acquired B' antigen on her red cells. No similar cases appeared to have been reported, but depressed A and B antigens were known to occur in leukaemia and in some refractory anaemias (see Race and Sanger, p 29–30, 1975, for references). In the earlier blood samples from Miss N.L., the strength of the A as well as of the B antigen of her cells might have become decreased, perhaps as the result of excessive erythropoesis. The A blood with which she was transfused might then have masked the depressed A but not the depressed B antigen in her blood.

The possibility that Miss N.L.'s cells had a *cis*-A_1B phenotype was excluded, for

these cells are almost always A_2B, are agglutinated strongly by anti-H reagents and are not agglutinated by anti-B reagents in mixed-field patterns (Pacuszka, Kośielak, Seyfried and Walewska (1975).

6.6 SUMMARY

The red cells of a young Natal Indian victim of a bus accident in which she sustained injuries to her abdomen were found to be agglutinated in mixed-field patterns by anti-B reagents. Although the results in the serological tests were complicated by an unknown prior and by subsequent blood transfusions, the phenotype of her cells was concluded to be A_1B weak variant. However, the antigens on her cells may have been depressed by increased erythropoesis. Since her parents were A_1 and A_1B, the origin of her weak B variant factor was attributed to the inheritance either of two recessive inhibitor genes or of a *B* gene that had mutated.

CHAPTER 7

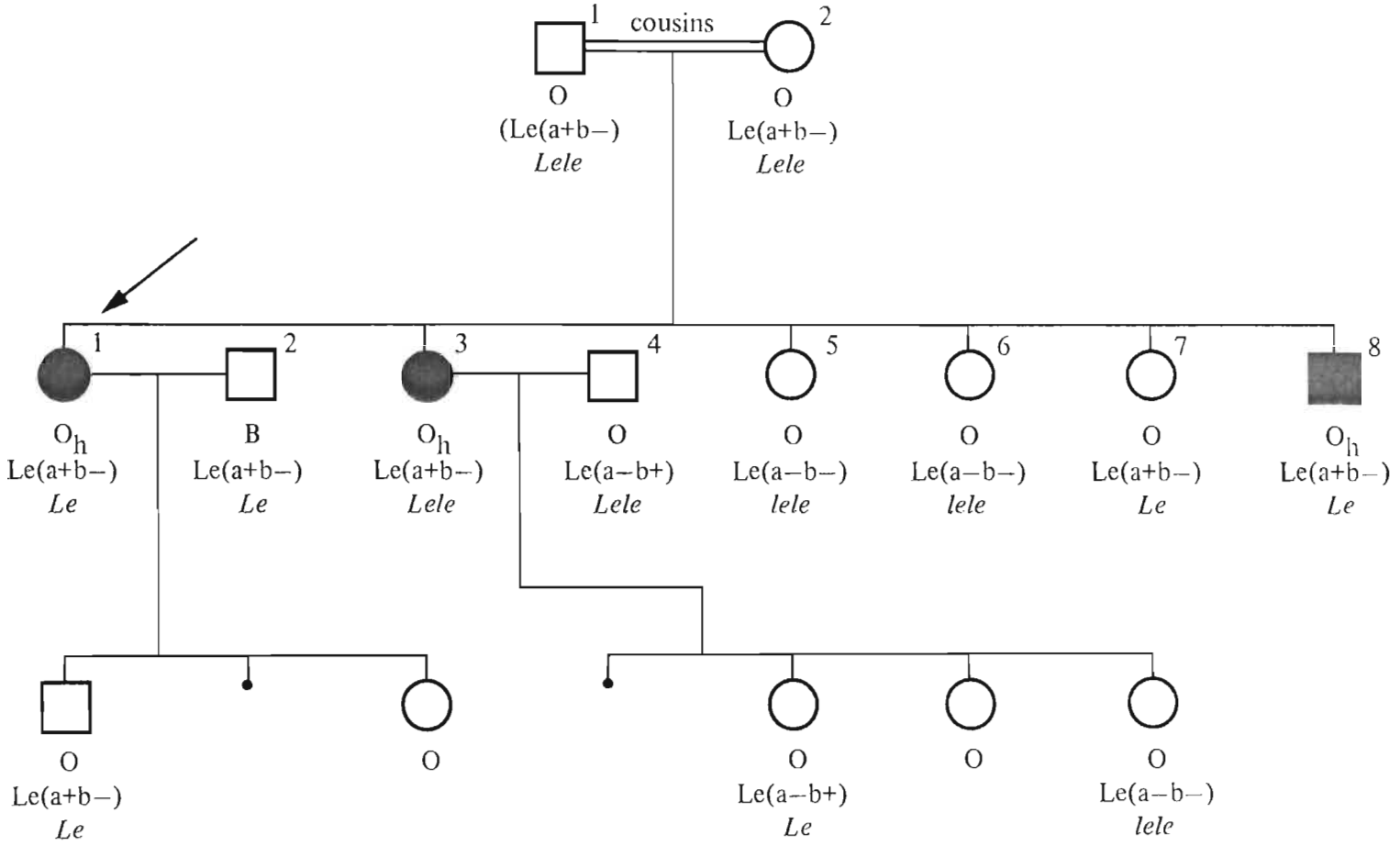
THE FIRST 'BOMBAY' O_h PERSON IN SOUTH AFRICA

7.1 INTRODUCTION

The O_h or 'Bombay' phenotype, discovered by Bhende, Deshpande, Bhatia, Sanger, Race, Morgan and Watkins in three Indians from Bombay in India in 1952, is characterised by no A, B or H antigens being present on the red cells and no A, B or H substances being secreted in the saliva. However, O_h people have normal but unexpressed *ABO* and secretor genes. This was demonstrated by Levine, Robinson, Celano, Briggs and Falkinberg in 1955 when they showed that the normally-expressed *B* and *Se* genes in the parents of an O_h American woman were not expressed in her but had been passed on by her to her children in whom they were being re-expressed normally. Aloysia, Gelb, Fudenberg, Hamper, Tippett and Race (1961) subsequently showed, almost certainly, that the A_1 gene in an American/French O_h woman, and certainly that the A_2 gene in two American/English O_h brothers, had also been transmitted unexpressed through them in this way. The *O* genes were described first as being unexpressed in two Natal O_h Indian sisters whose parents and children were all group O by Moores in 1972 (Moores, 1972b) (Figure 7.1). The absence of *A, B* and *H* gene expression in O_h people is now known to be due to the inheritance of rare *hh* instead of *HH* genes or a double dose of a rare recessive suppressor gene which inhibits *H* gene enzyme synthesis (Watkins, 1980).

The 'Bombay' O_h family reported by Levine *et al.* in 1955 showed that the expression of the *Le* gene was also affected in O_h people. In a figure in which the possible pathways of *ABO, Hh, Sese* and *Lele* gene interaction were displayed, Watkins (1965) explained that precursor substance was transformed by *Le*, but not by *lele* genes, into Le^a substance. The Le^a substance then underwent transformation by *H* and *Se*, but not by *H* and *sese* or by *hh* and either *Se* or *sese* genes, into H, Le^a and Le^b substances. An O_h person (who had *hh* genes) might therefore secrete Le^a substance or have untransformed precursor substance and, consequently, have either Le(a+b–) or Le(a–b–) cells respectively, but would not have Le(a–b+) cells. Further work has now shown that Le^b substance is a product of *H* and *Le* gene interaction and is not formed when *hh* genes have been inherited (Watkins, 1980).

Figure 7.1 Family pedigree of P. and B. Gov. in whom the O group is not expressed



Key: = Proposita

= Miscarriage

The first 'Bombay' O_h person described in South Africa, Mrs P.N., was identified by Moores on 30th December, 1964. She was a Natal Indian woman, and her discovery was reported by Moores in 1965 in a paper presented at the South African Blood Transfusion Congress held from 30th April to 1st May in Durban. This chapter contains an account of her discovery and of the serological studies which confirmed that she was O_h.

7.2 CASE HISTORY

The Indian woman, Mrs P.N., who spoke Telegu, was born at Isipingo, a coastal town near Durban, in about the year 1910. The place of origin of her family in India was not known either by her or her relatives and, as far as she was aware, her parents had not contracted a consanguineous marriage. She was found, suffering from cervical carcinoma in a hospital in Durban, when her doctor requested compatible blood to correct her anaemia before radiation therapy was commenced. Although apparently group O, her serum contained a strong saline- and enzyme- reacting antibody and had agglutinated the cells of 124 units of group O blood in the cross-matching tests. The auto-antibody control and direct antiglobulin tests had both been negative.

Since difficulty was being experienced in finding compatible blood for Mrs P.N., her blood samples were referred to me for further study. Although aware that O_h red cells grouped as O with anti-A, anti-B and anti-A,B reagents and were distinguished from normal O cells by a negative reaction with anti-H, I had not had the opportunity to identify such cells myself before. I confirmed that Mrs P.N.'s red cells were group O and, having found that her serum agglutinated the cells of 14 adult O donors (selected for their different groups in eight blood group systems) and two O cord cell samples, tested her cells and those of a known group O person (myself) with a known example of anti-H from a group A_1 patient (we had no *Ulex* anti-H lectin at that time). The results showed that Mrs P.N.'s cells were not agglutinated while the control cells reacted well with this serum. The negative reaction of Mrs P.N.'s cells with anti-H was confirmed subsequently using two commercial and five further examples of human anti-H, but the supply of her cells was too small for distribution to other centres for confirmation of her O_h status. However, later, the cells of other Natal Indians which were not agglutinated by her serum, were confirmed as being O_h in many centres in Britain, the United States, France and other parts of the world (see Chapter 9).

7.3 MATERIALS AND METHODS

Blood samples were received from Mrs P.N. on two occasions, the first in 1964 and the second early in 1965. She was not given a blood transfusion during this period as no compatible blood had been found for her and the red cells of all the relatives in her extensive family tested, who now lived in Durban and the north coast towns of Stanger and Darnall, had been agglutinated by her serum. A sample of her saliva was received and processed as described in Chapter 2. The preparation of the cell suspensions, the methods of absorbtion, elution and titration used and the saliva inhibition technique will also be found in this chapter.

7.4 RESULTS

Figure 7.2 shows that Mrs P.N., II-2, had seven children. Among them, III-3, III-7 and III-8 were A_1, III-5 was B and III-2 and III-6 were A_1B. Unfortunately, as her parents and husband were no longer living, neither the identity of her unexpressed *ABO* genes, which might have been A_1O, *BO* or A_1B, nor of her unexpressed secretor genes, could be determined. Her groups were identified as follows:–

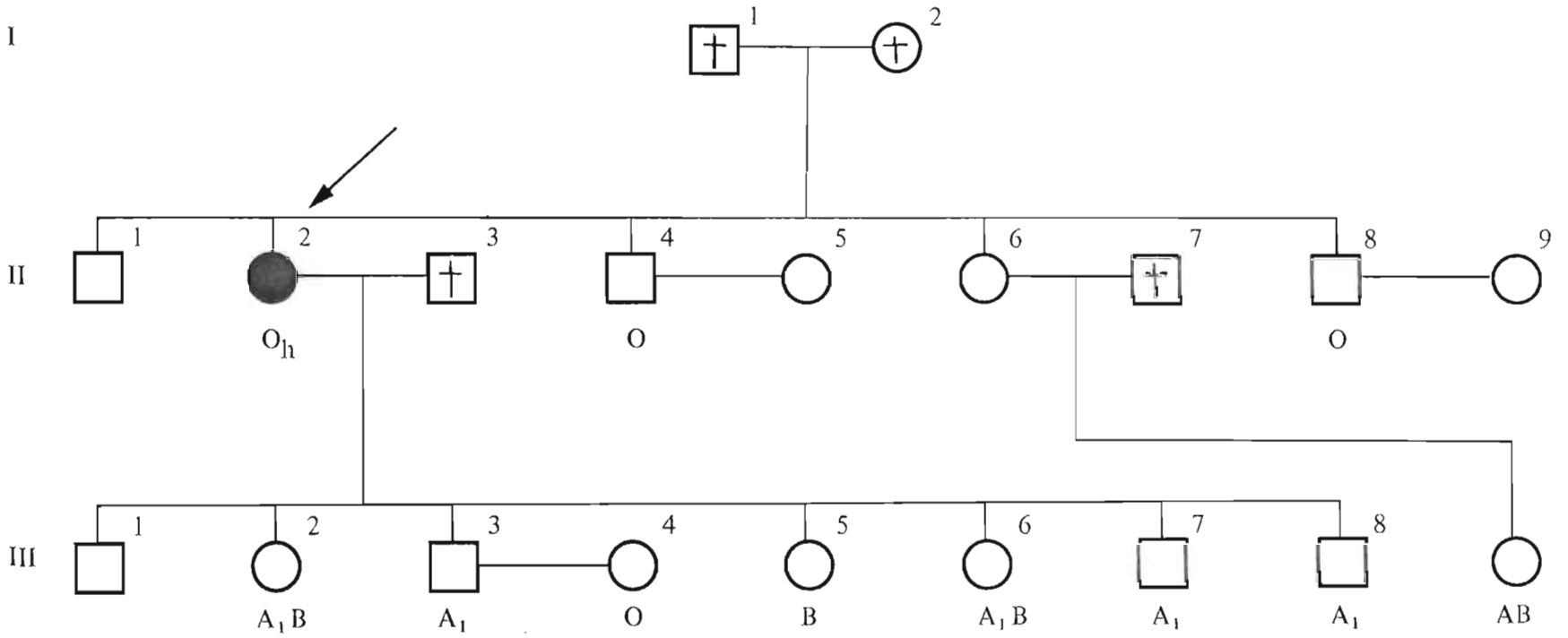
O_h, MNs, P_2, CDe/cde (Rh_1rh), K–, Le(a+b–), Fy(a+), I+, Yt(a+)

Saliva inhibition titrations showed that Mrs P.N. did not secrete A, B or H substances (Table 7.1, part a).

In comparative saline and one-stage 0,5% bromelin titrations at 20°C, Mrs P.N.'s serum agglutinated A_1, B and O cells to titre 32, in one-stage 0,25% ficin titrations to titre 256 and in indirect antiglobulin titrations to titre 512 (Table 7.1, part b). The identity of the antibodies in her serum was confirmed as anti-A+B+H when, following the addition of equal volumes of the saliva of A, B and H secretors to all the appropriate test tubes in further titrations, the agglutination was seen to have been inhibited. Two absorbtions with A_1 cells left anti-B, two absorbtions with B cells left anti-A and two absorbtions with O cells left anti-A and anti-B in her serum (Table 7.1, part c). The titres obtained when the absorbed sera were subsequently titrated were not found to have been reduced by this procedure. The antibodies recovered by the 56°C heat technique of Landsteiner and Miller (1925) in the eluate from the A_1 cells, agglutinated B and O cells weakly, and those recovered in the

Figure 7.2

Part of the family pedigree of Mrs P.N.



Key: † = dead

↙ = proposita

TABLE 7.1

Results of tests with the saliva and serum of Mrs P.N.

Part a

	Saliva inhibition titrations																	
	anti-A + A_2 cells						anti-B + B cells						anti-H + O cells					
	1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32
Mrs P.N.	4	4	3	3	2	1	4	4	4	3	2	1	4	3	(3)	(±)	–	–
AH secretor	–	–	–	–	–	–	4	4	4	3	2	1	–	–	–	–	–	–
BH secretor	4	4	3	3	3	2	–	–	–	–	–	–	–	–	–	–	–	–
Non-secretor	4	4	3	2	2	1	4	4	4	3	2	1	3	2	(3)	(1)	–	–
Control saline	4	4	4	3	2	1	4	4	3	2	1	1	3	2	1	(3)	(1)	–

Part b

Serum	Technique at 20°C	Cells	Serum titrations dilutions										
			1	2	4	8	16	32	64	128	256	512	1024
Mrs P.N. O_h	Saline	A_1	4	4	3	2	(3)	(1)	–	–	–	–	–
		B	4	4	3	2	1	(2)	–	–	–	–	–
		O	4	4	4	4	2	1	(2)	–	–	–	–
	One-stage 0,5% bromelin	A_1	4	4	3	2	1	(3)	(1)	–	–	–	–
		B	4	4	3	2	1	(2)	(±)	–	–	–	–
		O	4	4	4	3	2	(3)	(1)	–	–	–	–
	one-stage 0,25% ficin	A_1	4	4	4	4	4	4	4	2	1	–	–
		B	4	4	4	4	4	4	4	2	±	–	–
		O	4	4	4	4	4	4	4	2	1	–	–
	indirect antiglobulin at 37°C	A_1	4	4	4	4	4	4	3	2	1	(2)	–
		B	4	4	4	4	4	4	2	1	(3)	(1)	–
		O	4	4	4	4	4	3	3	1	1	(2)	–

Part c

Serum of Mrs P.N. absorbed with:–		Serum titrations dil utions						
		1	2	4	8	16	32	64	128
A_1 cells	A_1	(2)	–	–	–	–	–	–	–
	A_2	(3)	(1)	–	–	–	–	–	–
	B	4	4	3	1	(2)	(±)	–	–
	O	(3)	–	–	–	–	–	–	–
B cells	A_1	3	2	1	1	(3)	(2)	–	–
	A_2	3	2	1	(3)	(2)	–	–	–
	B	(1)	–	–	–	–	–	–	–
	O	(2)	–	–	–	–	–	–	–
O cells	A_1	3	3	2	1	1	(2)	(1)	–
	A_2	2	2	1	1	(2)	(2)	(±)	–
	B	4	4	2	1	(3)	(2)	(±)	–
	O	(2)	–	–	–	–	–	–	–

TABLE 7.2

Natal Indians identified as O_h between the years 1964 and 1980

Year	Name	Category	*ABO* genes unexpressed	Lewis phenotype
1964	Mrs P.N.	Telegu	A_1O, B or A_1B	Le(a+b–)
	Miss S. Hari.			Le(a+b–)
1965	Miss G. Hari.	Hindi	BO or OO	Le(a+b–)
	Miss P. Hari.			Le(a+b–)
1967	Mrs P.C.	Telegu	A_1O, BO or OO	Le(a+b–)
	M. Gov.			Le(a+b–)
1967	D. Gov.	Tamil	OO	Le(a+b–)
	Miss A. Gov.			Le(a+b–)
1969	Mrs L. Dew.	Hindi	A_1O, BO or OO	Le(a+b–)
	G. Nai.		* A_1O	Le(a+b–)
1969	S. Nai.	Tamil	* BO	Le(a+b–)
	Mrs P. Gov.			Le(a+b–)
1970	Mrs B. Gov.	Tamil	OO	Le(a+b–)
	T. Gov.			Le(a+b–)
	Miss N. Moon.			Le(a+b–)
1970	B. Moon.	Tamil	OO	Le(a–b–)
	V. Moon.			Le(a–b–)
	Miss R. Gan.			Le(a+b–)
1972	Mrs K. Ram.	Hindi	?	Le(a+b–)
	Mrs D. Oma.			Le(a+b–)
1974	Mrs D. Rug.	Hindi	?	Le(a+b–)
	A. Nar.		* BO	Le(a+b–)
1976	S. Red.	Telegu	* BO	Le(a+b–)
	R. Red.		* A_1O	Le(a+b–)

* Determined by Dr W.M. Watkins from transferases in their sera.

eluates from the B and O cells agglutinated O cells weakly. These antibodies were later identified as anti-B+H and anti-H respectively.

7.5 DISCUSSION

The realization that Mrs P.N. was an O_h Indian established that the *h* gene was present in South Africa. In due course, other O_h persons were expected to be found there, and the importance of being able to locate compatible blood donors for them in an emergency led me to make a full family study whenever one appeared. As a result, 24 O_h Indians have now been identified in Natal (Table 7.2). An Indian in the Transvaal and two persons of mixed race in the Cape Province have also been confirmed by me as O_h from blood samples sent by colleagues in other blood transfusion services. However, not all the O_h Indians in Natal were suitable as blood donors and some of them are still young children.

As anticipated in 1964, Mrs P.N. had Le(a+b−) red cells. However, three Natal O_h Indians and one of the two O_h Coloured people identified in the Cape Province have since been found to have Le(a−b−) cells. By giving approximately equal titres with A_1, B and O cells in titrations made by four serological techniques, the anti-A+B+H antibodies in Mrs P.N.'s serum showed that she belonged to the category of 'typical' O_h individuals. The absorbtions with A_1, B and O cells confirmed that her serum contained separable anti-A, anti-B and anti-H, but the eluates recovered subsequently from these cells revealed that her serum contained more than a simple mixture of these antibodies. This subject is returned to and discussed in more detail in Chapter 11.

7.6 SUMMARY

The red cells of the first Natal O_h Indian described in South Africa were not agglutinated by anti-A, anti-B or anti-H and she secreted no A, B or H substances in her saliva. Her serum contained anti-A+B+H antibodies which agglutinated A_1, B and O cells to almost identical titres. Absorbtion-elution studies suggested that her serum contained more than a simple mixture of anti-A, anti-B and anti-H antibodies.

Table 7.2 shows the Indians identified as O_h in Natal between the years 1964 and 1980.

CHAPTER 8

RED CELL AND SERUM STUDIES IN THE FIRST FAMILY TO HAVE BEEN RECORDED IN WHICH BOTH O_h Le(a+b−) AND O_h Le(a−b−) SIBLINGS WERE REPRESENTED

8.1 INTRODUCTION

Watkins (1959), Watkins and Morgan (1959) and Ceppellini (1959) described a theory of biosynthesis of the A, B, H and Lewis substances which made provision for a then unknown O_h Le(a−b−) red cell phenotype. The theory supposed that a precursor substance was transformed by the sequential activity of *Lele, Hh, Sese* and *ABO* genes into blood group antigens on the red cells and substances in the secretions. When an *Le* and *hh* gene had been inherited, the red cell phenotype was O_h Le(a+b−) and the individual secreted Le^a substance only, and when *lele* and *hh* genes had been inherited, the phenotype was O_h (Le(a−b−) and neither ABO nor Lewis substances was secreted. The investigations made by these and other workers which followed showed that the genes were more likely to act in the order *Sese, Hh, ABO* and *Lele* (Boettcher, 1978), but the possibility apparently also exists that independent recessive suppressor genes inherited in double dose may alter the α-2-L-fucosyltransferase expressed by normally-inhertied *H* genes in O_h individuals (Watkins, 1980).

The first known O_h Le(a−b−) individual was a Bengali Moslem from East Pakistan (Giles, Mourant and Atabuddin, 1963). As predicted, his red cells, which were not agglutinated by anti-A, anti-B, anti-A,B or by anti-H, were also not agglutinated by several examples of anti-Le^a, anti-Le^b, anti-Le^a+Le^b and one example of anti-Le^x reagents. Moreover, these reagents were also not inhibited on being mixed with his saliva. Unfortunately, the family study provided no information as to whether or not the *Hh* and *Lele* genes had been inherited independently in his family members.

Further examples of the O_h Le(a−b−) phenotype were recorded by Bond, Shirgaonkar, Randeria and Bhatia (1965), by Gandini, Sacchi, Reali, Veratti and Menini (1968) and by Pretty, Taliano, Fiset, Baribeau and Guévin (1969), but the Lewis types in the family described by Gandini *et al.* (1968) were questioned by Wiener (1969) who calculated that five Le(a−b−) among six children born to *Lele* parents

was likely to occur only once in 222 times. The family described by Pretty *et al.* (1969) strongly suggested, but failed to prove, that the *Hh* genes were not controlled from the *Le* locus (Race and Sanger, p 23, 1975), but the groups in the first family containing both O_h Le(a+b−) and O_h Le(a−b−) siblings, that of the Natal Indians, Mr and Mrs Moon. (Moores, 1972b) described here, confirmed that *h* segregated independently of the *Le* and *le* genes (Race and Sanger, p 23, 1975). In 1973 and 1976, additional Indian families containing both O_h Le(a+b−) and O_h Le(a−b−) members were reported by Sathe and Bhatia, and their 1976 family contained O_h Le(a−b−) members in two generations. The groups in these families provided further confirmation that the *Hh* and *Lele* genes segregated independently.

As the rare gene *h* (originally the *x* gene of Levine, Robinson, Celano, Briggs and Falkinberg, 1955), when homozygous, prevents expression of the *ABO* secretor genes, it was of interest in this study to determine whether or not the apparently heterozygous *Hh* parents of O_h persons had reduced H antigen expression on their red cells. The cells of members of the Moon. family were convenient for this purpose as the parents were both group O and different amounts of H, due to *ABO* genes of other types inherited, could be ignored. The apparently small amount of H antigen on enzyme-treated O_h cells, that had been detected with *Ulex europaeus* and *Cytisus sessifolius* anti-H lectins by Dodd and Lincoln (1978), was also investigated using *Ulex* anti-H, the anti-A+B+H antibodies of a number of Natal O_h Indians and the cells of the O_h members in the Moon. family and of controls.

8.2 CASE HISTORY

The O_h phenotype of the propositus in the Natal Indian family, Moon., V. Moon., was discovered in 1969 while he was in hospital suffering from an undisclosed illness. He was then three years old and no compatible blood for a blood transfusion had been found for him. A unit of blood was donated by a known Natal O_h Indian donor, and V. Moon.'s subsequent recovery was uneventful.

The blood groups in the Moon. family were studied in 1970 and again in 1973. On each occasion, blood samples were obtained from all the family members, and in 1973 saliva samples were obtained from them as well. In 1975, four family members each donated 100 mls of their blood for the preparation of ethanol soluble substances (ESS). The studies revealed that both V. Moon. and a sibling had phenotype O_h Le(a−b−) cells and that another sibling had phenotype O_h Le(a+b−) cells.

8.3 MATERIALS AND METHODS

The red cells and saliva for this study were prepared for use as indicated in Chapter 2. The reagents used consisted of several examples of standardised anti-A,anti-B and anti-A,B, *Ulex* anti-H lectin, anti-Le^a, anti-Le^{bL} (not inhibited by the saliva of a secretor of H but no Lewis substances), anti-Le^x, and antibodies of other specificities for determining the groups of the cells. The anti-A+B+H sera used were from several known Natal O_h Indians.

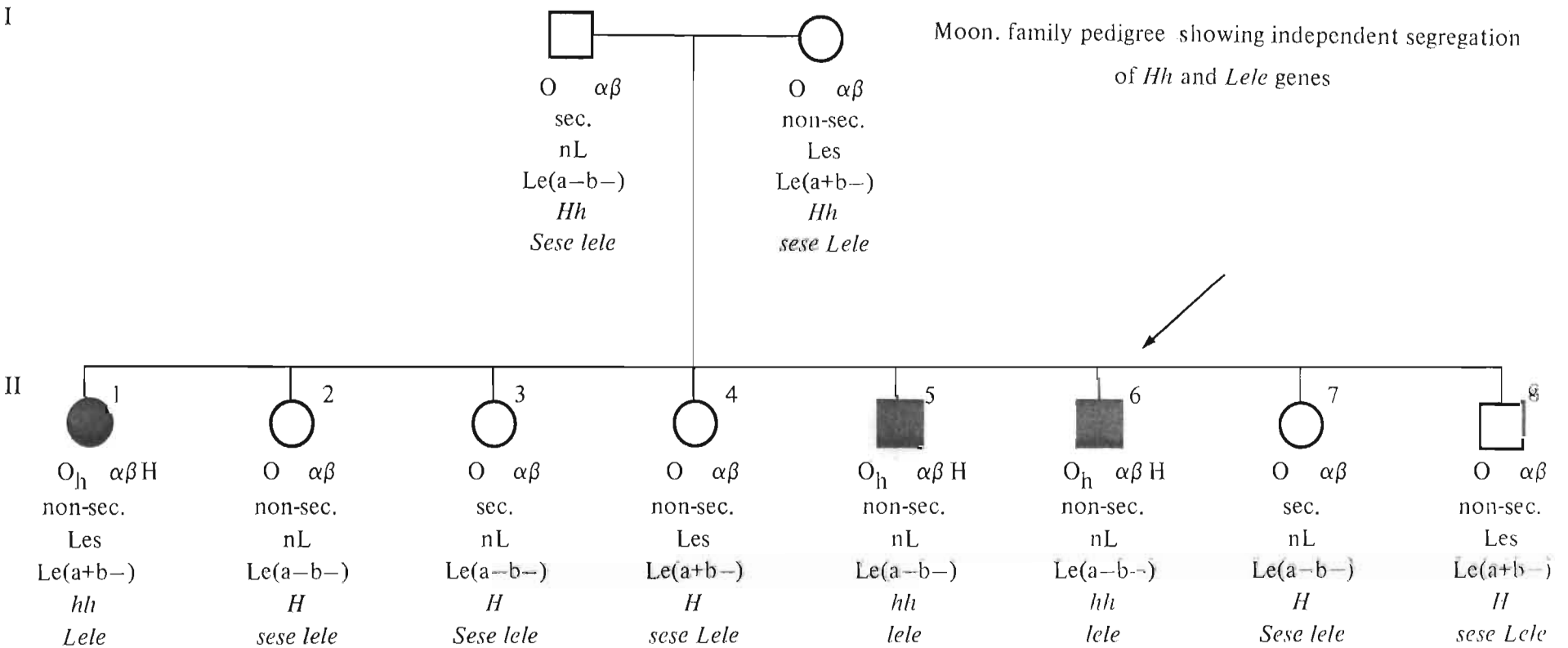
8.4 RESULTS

8.4.1 The family red cell and saliva groups

The red cells of the parents, I-1 and I-2, in the Moon. family (Figure 8.1), both of whom were group O, were agglutinated by *Ulex* anti-H lectin and by 'Bombay' anti-A+B+H sera, and their sera contained normal anti-A,B allo-antibodies. The cells of I-1 typed as Le(a−b−) and those of I-2 as Le(a+b−), and the saliva of I-1 inhibited *Ulex* anti-H only while the saliva of I-2 inhibited anti-Le^a only. I-1 was therefore a secretor of H substance and possessed *Se* and *lele* genes, while I-2 was a secretor of Le^a substance and possessed *sese* and *Le* genes. Among their eight children, the cells of II-1, II-5 and II-6 were not agglutinated by anti-A, anti-B, anti-A,B, anti-A+B+H or *Ulex* anti-H lectin, and their sera contained anti-A+B+H. The phenotype of their cells was therefore O_h. The cells of II-1 typed as Le(a+b−) and the cells of II-5 and II-6 as Le(a−b−). The saliva of II-1 inhibited anti-Le^a, while the saliva of II-5 and II-6 failed to inhibit anti-A, anti-B, anti-H, anti-Le^a or anti-Le^{bL} reagents. II-1 had therefore inherited an *le* gene from her father, I-1, and an *Le* gene from her mother, I-2, while II-5 and II-6 had inherited *le* genes from both I-1 and I-2. As II-2, II-3 and II-7 were non-secretors of Lewis substances and their cells were (Le(a−b−), I-2 was seen to be heterozygous *Lele*, and by not secreting H substance in their saliva, II-2, II-4 and II-8 confirmed that I-1 had heterozygous *Sese* genes. Provided that the O_h phenotype was due to the inheritance of *hh* genes (Watkins and Morgan, 1955), the inheritance by II-1 of *h* and *le* from her father, I-1, and of *h* and *Le* from her mother, I-2, and the inheritance by II-5 and II-6 of *h* and *le* from both I-1 and I-2, showed that the single *h* gene in I-2 had travelled independently of the *Lele* genes to these three children (Race, 1973).

Figure 8.1

Moon. family pedigree showing independent segregation of *Hh* and *Lele* genes



Key: ↙ = Propositus, V. Moon.
nL = No Lewis substances secreted
Les = Le^a substance secreted

8.4.2 The H antigen dosage studies

8.4.2.1 In titrations with various reagents

Since 'Bombay' O_h Indians are homozygous for the *h* gene (Watkins and Morgan, 1955) or homozygous for recessive suppressor genes inherited independently of the *ABO* genes (Watkins, 1980), it was not unreasonable to suppose that their parents and some of their siblings might be heterozygous for these genes. To determine whether the cells of heterozygous (*Hh*) individuals carried less H antigen than those of homozygous (*HH*) individuals, carefully matched cell suspensions from all the Moon. family members and a known control (PPM) were tested in strict parallel titrations with *Ulex* anti-H lectin and the anti-A+B+H antibodies of ten Natal O_h Indians. Five of the anti-A+B+H antibody donors were females, two of whom, as their infants had become jaundiced in the neonatal period, may have been stimulated antigenically, two were non-transfused males and the remaining three were the O_h children in the Moon. family. The anti-A+B+H antibodies of V. Moon., the three-years old propositus in this family, had the highest titres so far recorded in the laboratory. As far as it could be ascertained by testing the sera of the O_h donors with the cells of each other, their sera contained no other blood group antibodies.

Table 8.1 lists the results obtained with the *Ulex* anti-H lectin and two of the ten ant-A+B+H sera. The results with the remaining eight anti-A+B+H sera are not shown in this table as they were essentially the same. The *Ulex* and the anti-A+B+H sera detected no differences in H antigen strength between the cell samples tested or between them and the control 0 cells. The weaker H antigen expected on heterozygous *Hh* cells was therefore either not distinguished by the reagents used or both heterozygous *Hh* and homozygous *HH* cells carried the same amount of H antigen.

8.4.2.2 Using *H* gene-specified enzymes

In the second study, Dr W.M. Watkins kindly assayed the α-2-L-fucosyltransferase activities in the sera of the parents, one O and one O_h sibling of the Moon. family, and in four O controls who consisted of a White woman and three Natal Indian males. The assay was made by measuring the amount of radioactive [^{14}C] fucose incorporated into a low molecular weight acceptor. Although the results, shown in Table 8.2, suggested that a dosage effect existed between the parents (I-1 and I-2, Figure 8.1) and the controls, the intermediate level in the serum of II-2, who could

TABLE 8.1

H-antigen dosage titrations with red cells of Moon. family members

Cells	ABO Group	Suggested genes	cells in saline — Ulex anti-H dilutions									cells ficin-treated — Ulex anti-H dilutions									cells in saline — anti-A+B+H of Mrs P.N. 30 dilutions									cells ficin-treated — anti-A+B+H of Mrs P.N. 30 dilutions									cells in saline — anti-A+B+H of V. Moon. dilutions										cells ficin-treated — anti-A+B+H of V. Moon. dilutions									
			2	4	8	16	32	64	128	256	512	2	4	8	16	32	64	128	256	512	8	16	32	64	128	256	512	1024	2048	8	16	32	64	128	256	512	1024	2048	8	16	32	64	128	256	512	1024	2048	4096
I-1	O	Hh	4	1	±	–	4	3	2	1	–	4	3	1	(2)	–	4	3	1	–	4	3	3	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
I-2	O	Hh	4	2	±	–	4	3	2	1	–	4	2	1	(2)	–	4	2	1	–	4	3	3	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
II-1	O_h	hh	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
II-2	O	H	4	1	±	–	4	3	2	1	–	4	2	1	(2)	–	4	2	1	–	4	4	4	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
II-3	O	H	4	1	±	–	4	3	2	1	–	4	2	1	(2)	–	4	2	1	–	4	4	3	1	±	–	4	3	2	±	–	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
II-4	O	H	4	1	±	–	4	3	2	1	–	4	3	1	(2)	–	4	2	1	–	4	4	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
II-5	O_h	hh	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
II-6	O_h	hh	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
II-7	O	H	4	1	±	–	4	3	2	1	–	4	2	1	(2)	–	4	2	1	–	4	4	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
II-8	O	H	4	1	±	–	4	3	2	1	–	4	2	1	(1)	–	3	1	±	–	4	4	2	1	±	–	4	3	1	±	–	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–
Control	O	HH	4	1	±	–	4	3	2	1	–	4	2	1	(2)	–	4	2	1	–	4	4	2	1	±	–	4	2	2	1	–	–	4	3	2	1	±	–	4	3	2	1	±	–	4	3	2	1	±	–

TABLE 8.2

Assay of 2′-fucosyltransferase activity in the serum of Moon. family members

Details supplied by W.M. Watkins

Subject	Group	Genotype	2′-Fucosyltransferase activity (% incorp. [^{14}C] fucose
I-1	O	*Hh*	6,7
I-2	O	*Hh*	6,6
II-1	O_h	*hh*	nil
II-2	O	*HH* or *Hh*	8,1
Control S.N.	O	*HH*	10,5
Control A.K.	O	*HH*	10,7
Control S.B.	O	*HH*	10,2
Control P.P.M.	O	*HH*	10,6

have been either homozygous *HH* or heterozygous *Hh*, unfortunately rendered the findings inconclusive.

8.4.2.3 Using red cell substances

Vos, Moores, Downing and Mohideen (1976) described the isolation of ethanol-soluble substances (ESS) from human and animal red cells and reported that the ESS obtained from the cells of Gov. (G. Nai.), a Natal 'Bombay' O_h Indian, inhibited the agglutination of O cells by 'Bombay' anti-A+B+H antibodies weakly. The inhibition detected suggested that O_h cells had 'hidden' H antigen. In a later study (Vos and Moores, 1976), the ESS obtained from the cells of seven further Natal O_h Indians and controls were found to vary in activity from person to person. I-1, I-2, II-1 and II-2 in the Moon. family were included in these tests, but the ESS obtained from I-1, an obligatory *Hh, lele*, secretor of H substance, inhibited the anti-A+B+H antibodies of six other Natal 'Bombay' Indians to the same titre as the *HH, lele* and *HH, Le* controls who secreted H substance. Similarly, the ESS of I-2, an obligatory *Hh, Le* non-secretor of H substance, inhibited the anti-A+B+H antibodies to the same titre as the *HH, Le* non-secretor control. The findings suggested, either that the cells of the *Hh* members in this family had as much H antigen as the controls, or that the method used was not suitable for H antigen dosage studies. The ESS of II-2, who was an *HH* or *Hh, lele*, non-secretor of H substance, inhibited the anti-A+B+H antibodies to approximately the same titre as the ESS of her mother (I-2); and the ESS of II-1, who had *hh, Le* genes, inhibited these antibodies only minimally.

The following tests were made by myself as part of this study. The anti-A+B+H antibodies in a sample of serum from M. Gov. (167), and the anti-H lectin of *Ulex europaeus*, were standardised for inhibition tests by titrating them with ficin-treated O cells. The dilution selected for use was the highest which showed 4+ agglutination of these cells. Equal volumes of the reagents were mixed with the ESS of I-1, I-2, Mrs B. Gov. (a Natal O_h Indian control) and an O control (PPM), and inhibition was allowed to take place for 30 minutes at 20°C. One volume of ficin-treated O cells was then added, and the tests were read after a further hour at 20°C. In the second study, which was conducted in parallel, the *Ulex* anti-H lectin was titrated, and equal volumes of all the dilutions were tested for inhibition. In the third study, the ESS were titrated, and a volume of each dilution was mixed with the *Ulex* anti-H lectin which had been diluted 1 in 5 with saline. The inhibition test was then completed.

Table 8.3 shows the findings in this study. The ESS of I-1, I-2 and the control inhibited both the anti-A+B+H antibodies and the *Ulex* anti-H lectin, and the ESS of Mrs B. Gov. inhibited both partially (Table 8.3, part a). When the *Ulex* was titrated, the ESS of I-1 and I-2, both of whom were thought to have *Hh* genes, inhibited this reagent to the same degree as the ESS of the *HH* control; and when the ESS were tirated, the titres of these substances in the three individuals were all seen to be similar. By contrast, the ESS of Mrs B. Gov. inhibited the *Ulex* anti-H to a lesser extent, and its titre was also lower than those of the other three ESS (Table 8.3 part b). Although confirming that *HH* and *hh* cells were distinguishable from each other in these tests, the results therefore failed to show that the presumed *Hh* cells of I-1 and I-2 had less H antigen than the *HH* control cells.

8.4.3 Investigation of the H antigen apparently detected on the cells of a Natal O_h Indian

In 1974, using the cells of a Turkish O_h girl, Poschmann, Fischer, Seidl and Spielman suggested that H existed in the form of a cryptantigen on O_h red cells. Two years later, Vos and Moores (1976) suggested that molecules having H specificity were hidden within the red cell membranes of certain Natal O_h Indians, and in 1978, Dodd and Lincoln obtained very high haemagglutination titres with some preparations of *Ulex* anti-H lectin after the cells of 'W'. (A. Nar.), a Natal O_h Indian, and of other O_h persons had been treated with enzymes. Dodd and Lincoln (1978) suggested that the successful detection of H antigen on O_h cells required the use of 'incomplete' anti-H lectins, and these serologists also recorded that the anti-A+B+H antibodies of 'W'. and of another O_h individual had cross-reacted weakly with each others' cells in saline tests. In addition, slight auto-agglutination of their enzyme-treated cells had been recorded.

Prompted by these findings, a study was made of the reactions of bromelin-, ficin- and Löw 's-enzyme-treated O_h cells with anti-A+B+H sera from 19 Natal O_h Indians, including the sera of 'W'. (A. Nar. 202) and the three O_h members in the Moon. family. Four examples of *Ulex* anti-H lectin, examples of commercial and eel anti-H, human anti-H (group B) and anti-HI, and an extract of snail *Terebralia* were included as well. The object of the study was to discover whether reactions similar to those recorded by Dodd and Lincoln (1978) could be detected, and whether or not such agglutination could be inhibited with the saliva of a secretor of H but of no Lewis substances. The tests were made with bromelin- and ficin-treated cells, and also with

TABLE 8.3

Results of inhibition tests using ethanol-soluble substances (ESS) extracted from Hh, hh and HH cells of various Moon. family members and of control cells by Dr G.H. Vos

Part a

Subject	Group	Presumed genotype	Substance	Reagents: Ulex anti-H O cells	Reagents: anti-A+B+H O cells
I-1	O	*Hh*		–	–
I-2	O	*Hh*	ESS	–	–
Mrs B. Gov.	O_h	*hh*		2	1
Control (PPM)	O	*HH*		–	–
Saline				4	4

Part b

Subject	Group	Presumed genotype	Substance	Ulex anti-H dilutions 1	2	4	8	16	32	64	128	Ethanol-soluble substance dilutions 1	2	4	8	16	32	64
I-1	O	*Hh*		(3)	–	–	–	–	–	–	–	–	–	(3)	1	3	4	4
I-2	O	*Hh*	ESS	(3)	–	–	–	–	–	–	–	–	–	(±)	1	3	4	4
Mrs B. Gov.	O_h	*hh*		1	(3)	–	–	–	–	–	–	(3)	1	1	4	4	4	4
Control (PPM)	O	*HH*		(2)	–	–	–	–	–	–	–	–	–	–	(2)	2	4	4
Saline				4	4	4	3	(3)	(1)	–	–	4	4	4	4	4	4	4

Löw 's enzyme utilized in a one-stage test, as described in Chapter 2.

Table 8.4 is a summary of the findings obtained in these tests. The anti-A+B+H antibodies of Mrs L. Dew. (219), Mrs K. Ram. (254), Mrs R. Gan. and V. Moon. agglutinated the bromelin- and ficin-treated O_h cells weakly but, where the other results are shown as positive, the cells appeared merely to be aggregated. The H secretor saliva could not be said with certainty to have inhibited the reactions of any of the anti-A+B+H antibodies with the bromelin- or ficin-treated O_h cells, and no agglutination or aggregation of the O_h cells was detected in the presence of Löw 's enzyme. High titres were not obtained either with the bromelin- or the ficin-treated O_h cells, with these cells and Löw 's enzyme or with the four examples of *Ulex* anti-H lectin, but the anti-A+B+H antibodies which had reacted most strongly with the ficin-treated O_h cells were noticed to be those that had the highest titres against the untreated O cells.

8.5 DISCUSSION

In the first part of this chapter, it was shown that the Moon. family, which contained both O_h Le(a+b−) and O_h Le(a−b−) members, had made an important contribution towards our knowledge of the way in which the *Hh* and *Lele* genes are inherited by having kindly provided samples of their blood and saliva. In the second part, the H antigen dosage titrations with the cells of the presumed obligatory *Hh* parents in this family showed that their cells were indistinguishible from the cells of their five group O children, at least one of whom might have inherited *HH* genes, and from the known *HH* control cells. This finding was supported by the lack of significantly different amounts of α-2-L-fucosyltransferase activity detected in the family members' and control sera tested, and by the similar quantities of ethanol-soluble substances (ESS) extracted from the family members' and the control cells. While it was known that not all antibodies were capable of showing dosage, even in the MNSs system where many showed it regularly, these findings, which suggested that the parents might have *HH* genes, surprisingly lent some support to the view (see Watkins, 1980, p 58) that the absence of H antigen on O_h cells might be due to the inheritance of two recessive suppressor genes at a separate locus, rather than to the inheritance of *hh* genes.

In the third part of this chapter, the findings showed that anti-H specificity had not been confirmed as being the reason for the weak agglutination of two-stage enzyme-

TABLE 8.4

Tests with anti-A+B+H and other reagents using enzyme-treated cells from Natal O_h Indians

Reagent	antibody	Titre with untreated		Titre with ficin-treated O_h cells and		Titre with brom.-treated O_h cells and		O_h cells, Löw 's enzyme and	
		O cells	O_h cells	saliva	saline	saliva	saline	saliva	saline
Mrs P.N. 30	anti-A+B+H	64	0	±	1	1	1	0	0
Mrs P.C. 121	,,	16	0	0	±	0	0	0	0
G. Nai. 135	,,	32	0	0	0				
S. Nai. 212	,,	32	0	0	0				
Mrs D. Rug. 197	,,		0	1	2	0	1	0	0
Mrs D. Oma.	,,	128	0	0	1				
A. Nar. 202	,,		0	0	1				
S. Red. 215	,,		0	0	±				
R. Red. 216	,,		0	1	1				
M. Gov. 213	,,	16	0	0	0	0	0	0	0
Miss A. Gov. 214	,,		0	0	1				
Mrs L. Dew 219	,,	128	0	±	±	1	1	0	0
Mrs P. Gov. 230	,,	32	0	1	1				
Mrs B. Gov. 231	,,	64	0	±	±				
Mrs K. Ram. 254	,,	256	0	0	4	0	1	0	0
Mrs R. Gan.	,,	512	0	4	8				
N. Moon. II-1	,,	128	0	±	1				
B. Moon. II-5	,,	64	0	±	1				
V. Moon. II-6	,,	256	0	1	2	1	1	0	0
S. Mhlo.	anti-H	0	0	0	1				
M. Molet.	anti-HI	64	2	8	8				
S. Khan.	anti-HI	16	0	1	8				
Ulex 189	anti-H	32	0	0	2	0	0	0	4
Ulex 300	,,	32	0	0	0	0	0	0	0
Ulex 381	,,	32	0	0	0	0	0	0	0
Ulex R343	,,	32	0	0	0	0	0	0	0
Commercial	,,		0	1	8				
Eel	,,		2	4	>8				
Terebralia	unknown	>16	2	>8	>8				
Peanut	anti-T	0	0	0	0				
Control	D^u serum	0	0	>8	>8	>8	>8	>8	>8
Control	AB serum	0	0	0	0	0	0	0	0

treated O_h cells seen in the tests with anti-A+B+H antibodies. However, as the anti-A+B+H sera which had the highest titres had given the strongest reactions, the activity seemed to be a property of the sera, rather than of the cells. Perhaps the sera of O_h people contained a hitherto unsuspected antibody which had the capacity to react with an antigen exposed on the membranes of enzyme-treated O_h cells. Alternatively, enzyme-treated O_h might be more liable than O cells to aggregate non-specifically in the presence of some sera and lectins, especially the sera which contained highly concentrated antibody molecules, and the method of enzyme-treating these cells might therefore require some adjustment.

8.6 SUMMARY

Studies with the red cells and sera of members of the Moon. family showed that the gene *h* was inherited independently of the *Le* and *le* genes. The normal anti-H titres and normal amounts of ethanol-soluble substances (ESS) obtained from the cells, and the normal amount of H transferase activities detected in the sera, of the apparently *Hh* parents in this family showed that their cells and sera could not be distinguished by these methods from those of their group O children and the *HH* controls. The weak agglutination seen with the enzyme-treated cells of the O_h members in this family and in the families of other Natal O_h Indians using their own anti-A+B+H antibodies and those of others, which was not inhibited by H substance, was not thought to be due to serologically detectable H antigen on their cells.

CHAPTER 9

STUDIES WITH THE CELLS AND SERA OF SEVEN NATAL 'BOMBAY' O_h INDIANS IN THREE FAMILIES WHOSE BLOOD ASSISTED IN FURTHERING BLOOD GROUP KNOWLEDGE

9.1 INTRODUCTION

The important discovery by Levine, Robinson, Celano, Briggs and Falkenburg (1955) that an individual who had no expression of A, B, O or H antigens on her red cells or of ABH substances in her saliva had unexpressed *B* and *Se* genes and disturbed Lewis red cell antigen expression marked the beginning of a new era in blood group research. The genes A_1 and A_2 were shown to be equally unexpressed in other individuals by Aloysia, Gelb, Fudenberg, Hamper, Tippett and Race (1961), and in the same year Levine, Uhlir and White recorded the first para-'Bombay' phenotype characterised by weak A and no H antigen expression on the red cells.

Meanwhile, the significance of the sugars L-fucose in H antigen specificity and N-acetylgalactosamine in A antigen specificity, noticed by Morgan and Watkins in 1953, was confirmed by Kabat and Leskowitz and by Watkins and Morgan in 1955 (Watkins, 1980). The significance of D-galactose in B antigen specificity was also noticed by these workers in 1955, and the three sugars subsequently became very familiar in blood grouping circles. The sugars represent terminal non-reducing sequences of carbohydrate chains carried on glycoprotein molecules in the secretions, and on both glycoprotein and glycosphingolipid molecules on red cell membranes (Watkins, p 9, 1980). They are not the direct products of the ABO genes, however, but are serologically-active structures built up from precursor substrate material by enzymes controlled by these genes. The enzymes, which are glycosyltransferases, cause sugar units to be added in sequential order to carbohydrate chains in a precursor molecule, and as each unit is added, the structure formed becomes the substrate for the next enzyme (Watkins, p 32–33, 1980).

The unravelling by the biochemists of the steps involved in A, B and H antigen synthesis was assisted in no small measure by 'Bombay' O_h red cells and serum. As already mentioned, the O_h phenotype was first described by Bhende, Deshpande, Bhatia, Sanger, Race, Morgan and Watkins in Indians from Bombay, India, in 1952,

and the first South African Indian with phenotype O_h red cells was identified in Durban by myself on 30th December, 1964. The first Durban 'Bombay' O_h Indian was a woman of mature years who was in need of blood transfusions for the correction of anaemia, and her discovery was reported in a paper read at the South African Blood Transfusion Congress held in Durban in 1965. The nine studies by different authors, summaries of whose results have been given in this chapter, are presented as a gesture of appreciation to seven Natal O_h Indians and their families who contributed samples of their blood and saliva for them and for the work of many others. Except in Section 9.4.9, my part in these studies was only that I originally identified the O_h propositi, made full family studies wherever possible in my on-going search for more O_h family members and obtained or caused to be obtained the blood and saliva samples for local use or dispatch overseas. The propositi and their families will be described first, followed by the summaries and then my own investigations.

9.2 FAMILY HISTORIES

9.2.1 The family of P., B. and T. Gov.

As the Natal Blood Transfusion Service is the centre where blood samples from antenatal patients of all races are grouped and tested for blood group antibodies, the staff are in a particularly favourable position for patients with unusual blood groups to be found. Mrs P. Gov., also known as 'SA' and 'AFS', the proposita in the Gov. Indian family, was identified as 'Bombay' O_h in 1970 after her red cells were grouped as O but her serum was seen to contain a strong antibody that agglutinated, and also partially haemolysed, all the group O cells with which it was tested by all the serological techniques used. Her blood sample was submitted to me for further study and subsequent investigations showed that her cells were not agglutinated by *Ulex* anti-H lectin or by the anti-A+B+H antibodies in the serum of the first Natal 'Bombay' O_h Indian, Mrs P.N. (30). The serum of Mrs P. Gov. also failed to agglutinate the cells of two other Natal 'Bombay' O_h Indian donors, and the Rhesus negative phenotype of her cells detected in the earlier antenatal tests was confirmed.

Mrs P. Gov. was the first Rhesus negative Natal O_h Indian to be discovered, and the finding caused some consternation. She was a young primipara and, if she required a blood transfusion at delivery, the only compatible blood for her would have to be

provided by one of the five other adult O_h Indians found by then in Natal, all of whom were Rhesus positive. The two Rhesus negative O_h persons recorded by Aloysia *et al.* (1961) lived in Massachusetts, USA, but an investigation of Mrs P. Gov.'s family (Figure 9.1) disclosed that her younger sister, Mrs B. Gov., also known as G. Bal., had O_h Rhesus negative blood and was old enough to donate. Fortunately, Mrs P. Gov. did not require a blood transfusion and her group O infant was not affected by ABO haemolytic disease of the newborn.

The study of the family of Mrs P. Gov. and her sister showed that a brother, T. Gov., was also phenotype O_h, but he was Rhesus positive. The Lewis phenotype of the cells of all three O_h Indians was Le(a+b–). The parents, I-1 and I-2, who were cousins, were both group O, CDe/cde (Rh_1 rh), and the Le(a+b–) phenotypes of their cells made saliva studies to decide whether or not II-1, II-3 and II-8 had suppressed *Se* genes, impractical. However, the Le(a–b–) cells of II-5 and II-6 disclosed that I-1 and I-2 had heterozygous *Lele* genes.

9.2.2 The family of G. and S. Nai.

G. Nai., also known as 'Gov.' who was the propositus in this Indian family, was identified as phenotype O_h after he had donated a unit of his blood at an NBTS donor clinic in 1969. His serum was found to agglutinate all the cells with which it was tested, except O_h cells, and his cells were not agglutinated by *Ulex* anti-H lectin or by the serum of Mrs P.N. (30), the first known Natal O_h Indian. The family study (Figure 9.2) showed that an elder brother, S. Nai. (II-1), also known as 'Soo', had phenotype O_h cells as well, and the cells of both brothers (II-1 and II-2) were Le(a+b–). As their father, I-1, who was O, Le(a–b+), had married his niece, I-2, the marriage was consanguineous. The *sese* genes of the six children of II-1 and II-2 disclosed that II-1 had almost certainly inherited *sese* genes. I-1 must therefore be heterozygous *Sese*.

9.2.3 The family of A. Nar. and S. and R. Red.

The propositus, A. Nar., also known as 'W', in the Nar.–Red. Indian family, was discovered in 1975 after he had donated a unit of his blood at an NBTS donor clinic held in the Natal coastal town of Port Shepstone, south of Durban. Again, his red cells were not agglutinated by *Ulex* anti-H lectin or by the anti-A+B+H antibodies of other Natal O_h Indians, and his serum was compatible with known O_h red cells. With the help of Mr D. Naidoo, the technologist in charge of the NBTS blood

Figure 9.1

P. Gov. family pedigree

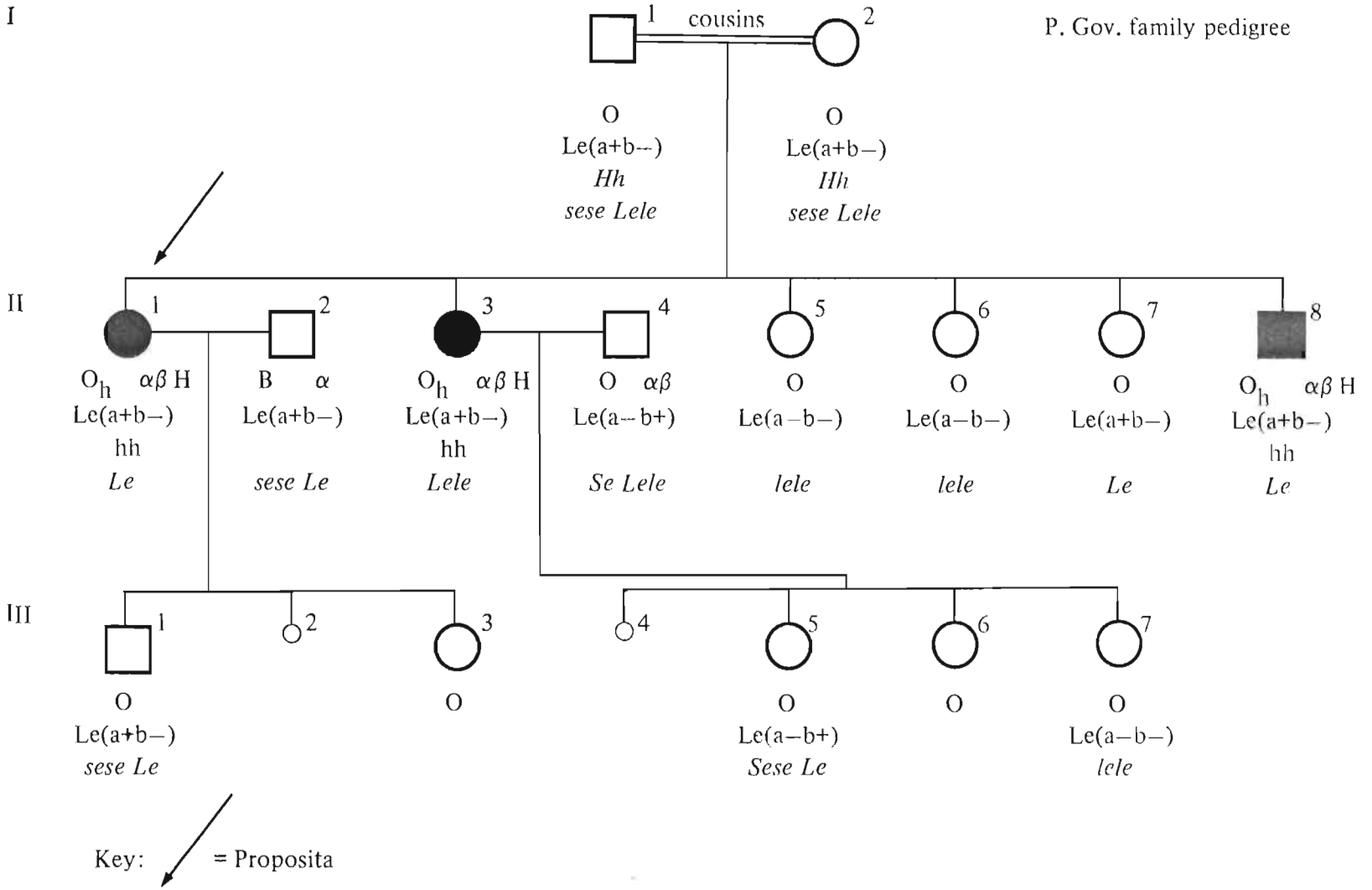
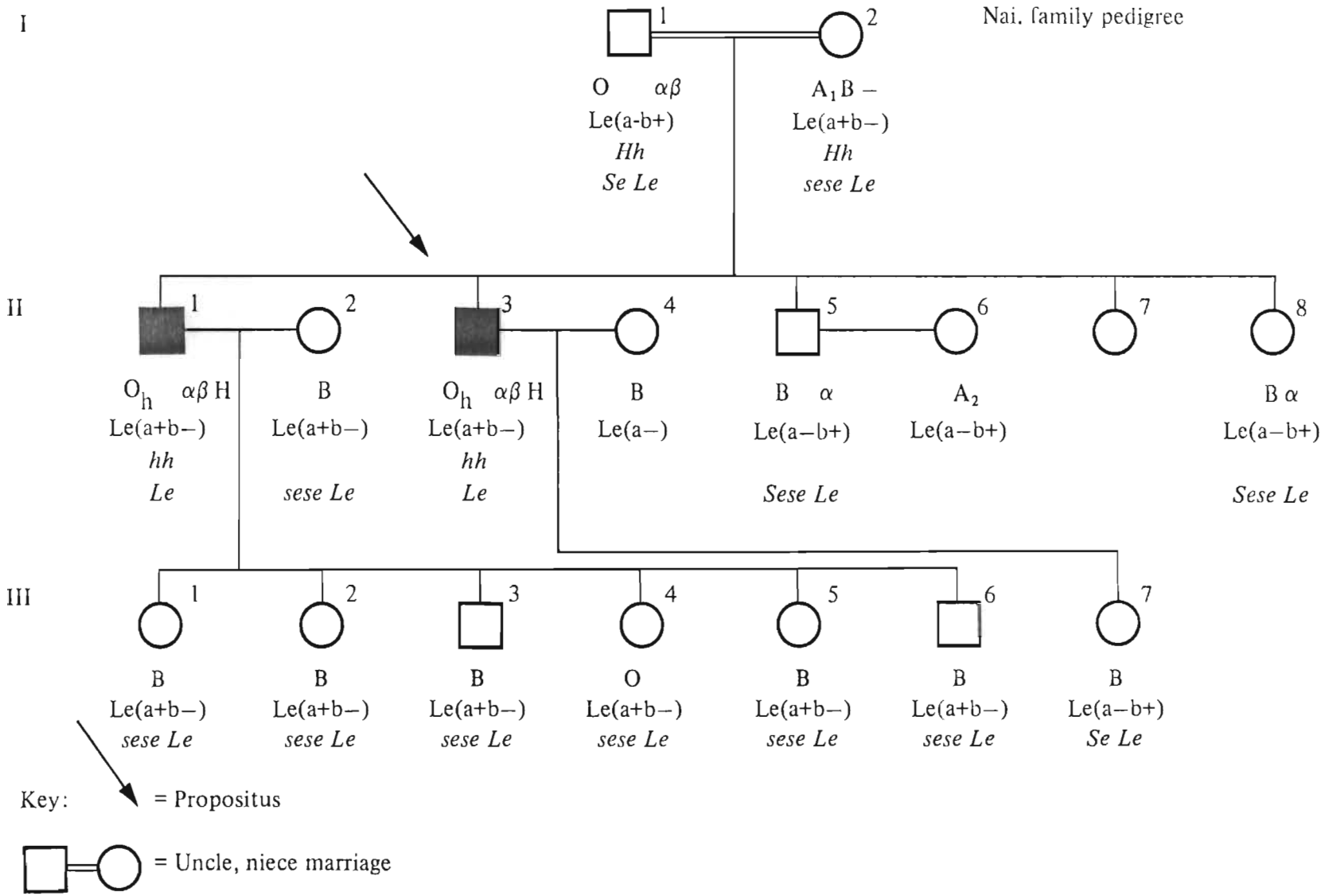


Figure 9.2

Nai. family pedigree



bank at Port Shepstone, a family study was made, and two brothers, S. Red. and R. Red., were also identified as phenotype O_h (Figure 9.3). The Lewis phenotypes of A. Nar. and R. Red. were Le(a+b–) and that of S. Red. Le(a–b–). Their family was therefore the second in Natal shown to contain both Le(a+b–) and Le(a–b–) Indians. Saliva studies were not made, and the Lewis phenotypes in few other family members were determined.

The Gov., the Nai. and the Nar.–Red. Indian families all spoke Tamil at home.

9.3 MATERIALS AND METHODS

The blood samples s for the studies described in Section 9.4.9 were prepared for testing, and the techniques used to study them, have been described in Chapter 2.

9.4 SUMMARIES OF THE WORK OF OTHERS

The blood samples requested for their studies by a number of scientists were donated by Mrs P. Gov. (SA., AFS), Mrs B. Gov. (G. Bal.), G. Nai. (Gov.), S. Nai. (Soo.), A. Nar. (W., Willie), R. Red. and S. Red. The findings, which are recorded in the literature, were as follows:

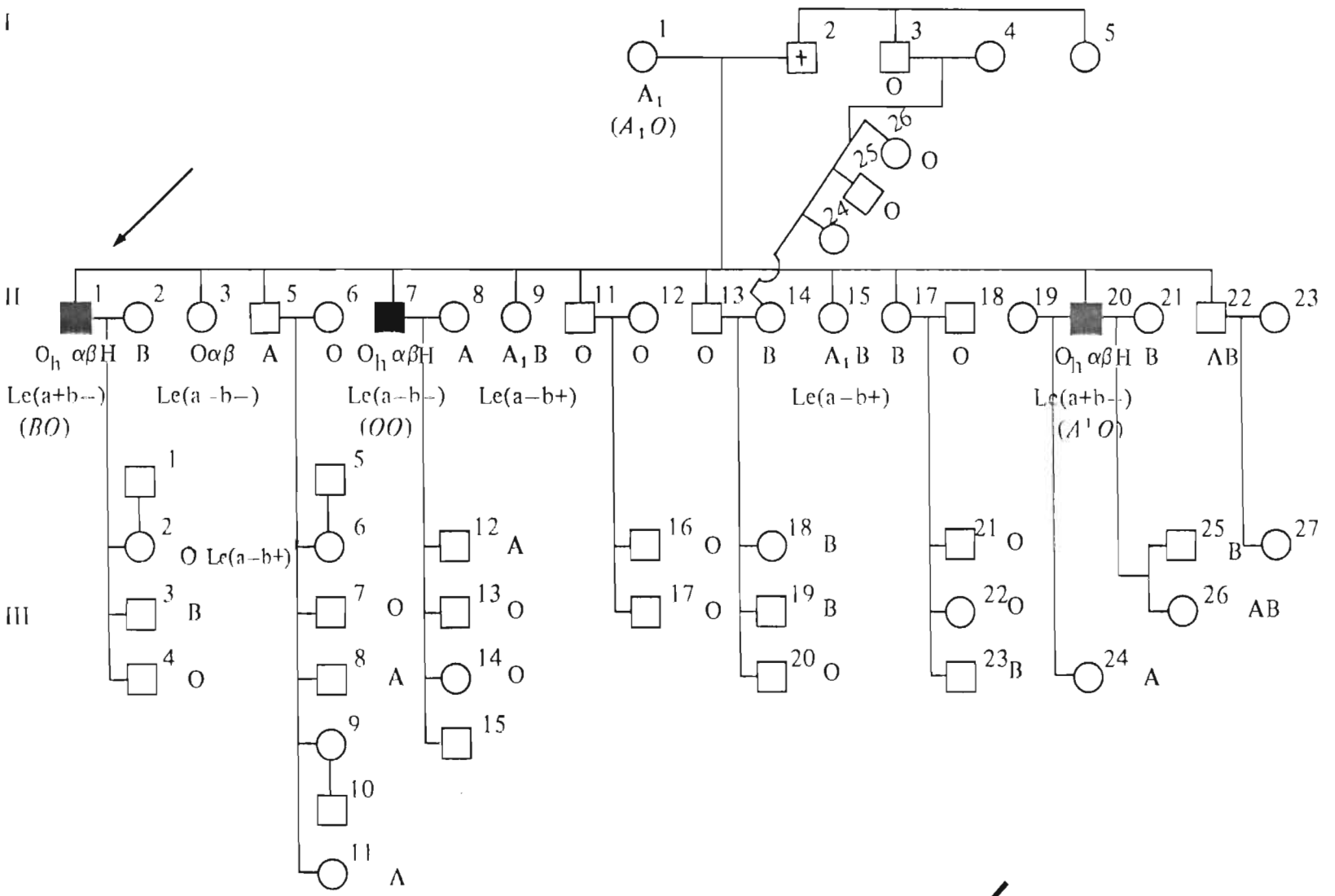
9.4.1 H. Schenkel-Brunner, M.A. Chester and W.M. Watkins, 1972

α-L-fucosyltransferases in human serum from donors of different ABO, secretor and Lewis blood group phenotypes

The discovery that α-fucosyltransferases were present in human milk, stomach mucosa and submaxillary glands, in Hela cells, in hog gastric mucosa, in rat intestinal mucosa and in pig liver, led Schenkel-Brunner *et al.* (1972) to study the distribution of these enzymes in human serum. For this purpose, the sera of individuals with different ABO, secretor and Lewis phenotypes were obtained, and a sample of serum from the Natal O_h Indian, Mrs P. Gov., was included. Since O_h individuals were believed to be genetically *hh*, her serum should not contain *H* gene-dependent α-2-L-fucosyltransferase. The findings, in which trisaccharides containing α-1,2-linked fucose were not detected when Mrs Gov.'s serum was used as the enzyme source, and N-acetyllactosamine or lacto-N-biose 1 as the sugar acceptor, showed that the premise was correct and strongly suggested that the α-2-L-fucosyltransferases present in other sera tested were concerned with the synthesis of H-active structures. The *Le* gene-dependant α-4-L-fucosyltransferase was not detected in the serum of any of the individuals, including Mrs P. Gov. who was known to possess an *Le* gene.

Figure 9.3

Nar. and Red. family pedigree



9.4.2 C. Race and W.M. Watkins, 1972 (a)

The action of the blood group B gene-specified α-galactosyltransferase from human serum and stomach mucosal extracts on group O and 'Bombay' O_h erythrocytes

In showing that group O red cells were converted into A-active cells with soluble enzymes extracted from human gastric mucosal microsomes and α-N-acetylgalactosaminyl transfer, and into B-active cells with these enzymes and α-galactosyl transfer, Schenkel-Brunner and Tuppy (1970) assumed that the H-active structures on the membranes of group O cells were acceptors for the transferred sugars. Race and Watkins (1972 a) supported this inference by establishing that the 'Bombay' O_h cells of Mrs P. Gov., known not to be agglutinated by anti-H reagents, were not converted in the presence of group B stomach extract into B-active cells. They also showed in this study that the sera of group B and AB persons constituted a more readily-available source than *post mortem* and post-operative gastric tissues of the α-galactosyltransferases necessary for converting group O into B-active cells. They suggested that it might be very rewarding to investigate the serum of persons known to have inherited rare *ABO* alleles.

9.4.3 C. Race and W.M. Watkins, 1972 (b)

The enzymic products of the human A and B blood group genes in the serum of 'Bombay' O_h donors

Although the red cells of 'Bombay' O_h individuals had been shown not to have H-active structures as the result of defective or absent serum *H*-gene-specified α-2-L-fucosyltransferase, it was not necessary to expect that their serum either lacked or had defective *A* gene-specified α-N-acetylgalactosaminyl- and/or *B* gene-specified α-D-galactosyltransferases. Race and Watkins (1972 b) tested nine 'Bombay' O_h individuals, including the Natal Indians Mrs P. Gov., G. Nai. and S. Nai., and established that the enzymes appropriate to the *ABO* genes surmised as being unexpressed in them were readily detectable in their sera (Table 9.1, part a). The serum of Mrs P. Gov., both of whose parents were group O (Figure 9.1), was not seen to convert normal group O into either A- or B-active cells in the presence of UDP-N-acetylgalactosamine or UDP-galactose respectively. This confirmed that her serum lacked both α-N-acetylgalactosyl- and α-D-galactosyltransferases and showed that her group was indeed O. The serum of G. Nai., one of whose parents was group O and the other group A_1B (Figure 9.2), converted the O cells into A-active cells, showing that it contained α-N-acetylgalactosyltransferase and thus that he had an

TABLE 9.1

Transferase activities in the sera of three Natal O_h Indians

Adapted from Race and Watkins, 1972 b.

Part a

Subject	Group	Genotype*	Radioactivity incorporated into 2′-fucosyllactose as α [^{14}C]Gal		α [^{14}C]GalNAc		Genotype predicted
			cpm	% of total	cpm	% of total	
Mrs P. Gov.	O_h	*OO*	0	0	0	0	*OO*
G. Nai.	O_h	A_1O or *BO*	0	0	17,229	8,6	A_1O
S. Nai.	O_h	A_1O or *BO*	16,247	8,1	0	0	*BO*
Control	A_1B	A_1B	18,510	7,7	15,954	7,3	
Control	O	*OO*	0	0	0		

Part b

Subject	Group	Genotype*	Haemagglutination end-point after treatment of O cells with serum and: UDP-Gal		UDP-GalNAc		Converted cell group
			anti-A	anti-B	anti-A	anti-B	
Mrs P. Gov.	O_h	*OO*	< 2	< 2	< 2	< 2	
G. Nai.	O_h	A_1O or *BO*	< 2	< 2	256	< 2	A
S. Nai.	O_h	A_1O or *BO*	< 2	128	< 2	< 2	B
Control	B	*BB* or *BO*	< 2	256	< 2	< 2	B
Control	A_1		< 2	< 2	128	< 2	A
Control	O	*OO*	< 2	< 2	< 2	< 2	

Key * determined from family studies

unexpressed *A* gene, and the serum of his brother, S. Nai., converted the O cells into B-active cells, showing that it contained α-D-galactosyltransferase and that he had an unexpressed *B* gene. The findings in this study are given in Table 9.1, part b.

The blood groups in the family of G. and S. Nai., while not proving that G. Nai. had an unexpressed A_1 gene and S. Nai. an unexpressed *B* gene, at least provided no evidence against this. Figure 9.2 shows that G. Nai., II-3, had one group B child by his group B wife and that S. Nai, II-1, whose wife was also group B, had a group O and six group B children. The most likely genotype of S. Nai. was therefore *BO* but no conclusion could be reached concerning the genotype of G. Nai.

9.4.4 W.M. Watkins, 1980

Biochemistry and genetics of the ABO, Lewis and P blood group systems

Further confirmation that the true genotype of 'Bombay' O_h persons could be discovered by determining the identity of transferases in their sera was provided by the members of the Nar.–Red. Natal O_h Indian family found in 1975. The transferases detected by Watkins and her staff in the serum of six members of this family and controls are given in Table 9.2. They showed that A. Nar., II-1 (Figure 9.3), whose serum contained α-D-galactosyltransferase, had an unexpressed *B* gene, his brother R. Red., II-7, did not have A or B genes and another brother S. Red., II-20, had an unexpressed *A* gene. *H* gene-specified α-2-L-fucosyltransferase was not detected in the sera of these three brothers.

TABLE 9.2

Transferase activities in the sera of members of the Nai.-Red. Natal O_h Indian Family.

adapted from Watkins, 1980

Family member	Group	Transferase activity % incorp. [^{14}C] A transferase pH 6	A transferase pH 8	B transferase	H transferase	Group predicted
I-1	A_1	18	13	0	4	
II-1	O_h	0	0	18	0	B
II-7	O_h	0	0	0	0	O
II-11	O	0	0	0	4	
II-13	O	0	0	0	6	
II-20	O_h	31	19	0	0	A_1
Control	A_1	20	13	0	7	
Control	B	0	0	21	5	
Control	O	0	0	0	5	

The blood groups in the Nar.–Red. family confirmed that II-20 had an unexpressed *A* gene as, unless III-26 was illegitimate, her AB group showed that she had inherited a *B* gene from her mother, II-21, and an *A* gene from her father. However, they provided no evidence against II-1 having an unexpressed *B* gene and II-1 having neither *A* nor *B* genes. The A, A_1B, B and O children in generation II and the A_1 group of their mother, I-1, showed that I-1 was genotype, A_1O and I-2 (deceased) genotype *BO*. Watkins, in her description of this family, said that, provided the 'Bombay' phenotype was due to the inheritance of *hh* genes, both I-1 and I-2 were genotype *Hh*. I-1 had therefore given her *O* and *h* genes to II-1 and II-7 and her A_1 genes and *h* to II-20 while I-2 had given his *O* and *h* genes to II-7 and II-20 and his B and h genes to II-1. The findings suggested that the *ABO* genes and the *Hh* genes in the family were segregating independently, and Dr Peter Cook of the MRC Human Biochemical Genetics Unit, London, who kindly calculated the log scores (log to base 10 of odds on linkage) for Dr Watkins, advised that the values obtained almost certainly eliminated very close linkage between the *ABO* and *Hh* gene loci.

9.4.5 M.A. Chester, A.D. Yates and W.M. Watkins, 1976
Phenyl β-D-galactopyranoside as an acceptor substrate for the blood-group H gene-associated guanosine diphosphate L-fucose: β-D-galactosyl α-2-L-fucosyltransferase

The assay of α-2-L-fucosyltransferase was complicated by the presence of other α-L-fucosyltransferases in human milk, serum, human stomach mucosa, submaxillary glands and bone marrow usually employed as the enzyme source. Instead of using low molecular weight oligosaccharides, N-acetyllactosamine, lacto-N-biose I and lactose or macromolecular substances, such as human blood group precursor glycoprotein, sialidase-treated α_1-acid glycoprotein, sialidase-treated 'Bombay' O_h red cells and porcine submaxillary mucin, Chester *et al.* (1976) utilized phenyl β-D-galactopyranoside as the acceptor substrate. The assay, which required only one chromatographic step, had the advantage of being relatively quick. The serum of Mrs P. Gov., one of four O_h donors whose sera were used in this study, as expected, failed to transfer L-[^{14}C]fucose to phenyl β-D-galactopyranoside, while all the other sera did so readily. Moreover, although 'Bombay' O_h serum contains α-3-L-fucosyltransferase activity, the absence of a radioactive product formed when it was employed as the enzyme source indicated that phenyl β-D-galactopyranoside was not an acceptor for GDP-fucose: N-acetyl-D-glucosaminyl α-3-fucosyltransferase.

9.4.6 L. Rodier, M. Lopez, G. Liberge, J. Badet, A. Gerbal and Ch. Salmon; 1974; and C. Salmon, 1976

Anti-H absorbed by, and eluted from O_h (Bombay) red blood cells

The apparent phenotype O_h cells of a woman (SCH) living in the east of France were studied with nine examples of potent anti-H reagents including the anti-A+B+H of Mrs P. Gov. Although SCH cells were not agglutinated either in saline tests or after they had been bromelin-treated, fixation-elution tests resulted in the recovery of anti-H from her cells. No α-2-L-fucosyl-, α-N-acetylgalactosaminyl- or α-D-galactosyltransferases were detected in the woman's serum. The authors concluded that some phenotype O_h cells had a small amount or a different quality of H antigen on their membranes.

Samples of red cells from Mrs P. Gov. (*OO*), G. Nai. (A_1O) and S. Nai. (*BO*) were studied in Paris with the same reagents and techniques that had been used to test the cells of SCH. However, their cells were not agglutinated by, and did not yield up after fixation-elution tests, anti-A, anti-B or anti-H antibodies.

9.4.7 G.H. Vos, P.P. Moores, H.J. Downing and A.F.C. Mohideen, 1976; and G.H. Vos and P.P. Moores, 1976

Hemagglutination inhibition studies for the evaluation of blood group antigens in ethanol soluble substances (ESS) obtained from human, baboon and Vervet monkey red blood cells

The results of tests in which ethanol-soluble substances obtained from the O_h red cells of some Natal Indians were found to inhibit the agglutination of O cells by their own antibodies are described in Chapter 8 (p 97). The inhibition was believed to show that H antigen was present below the O_h cell membrane surface. Among the 11 Natal O_h Indians who each contributed 500 mls of their blood for these studies were G. Nai., S. Nai. and Mrs B. Gov.

9.4.8 B.E. Dodd and P.J. Lincoln, 1978

Serological studies of the H activity of O_h red cells with various anti-H reagents

Papain- and neuraminidase-treated O_h red cells were found to be agglutinated to very high titres by some preparations of *Ulex europaeus* anti-H lectin. However, as judged by inhibition tests and in comparison with O cells, the amount of H antigen apparently detected on the O_h cells was said to be very small. A. Nar. (Willie) was one of the two O_h donors whose blood was used in this study.

9.4.9 P.P. Moores, P.D. Issitt, B.G. Pavone and B.G. McKeever, 1975.
Some observations on 'Bombay' bloods, with comments on evidence for the existence of two different O_h phenotypes

This study, which was conceived and written up by P.D. Issitt in Cincinnati, USA, will be described in more detail than those in paragraphs 9.4.1 to 9.4.8 as some of the serological tests were made by myself in Durban. The results of my subsequent tests, which supported the conclusions, will also be described.

Concerned by reports in the literature (see Moores *et al.*, 1975) that some serologists had eluted anti-A from O_h^A red cells and anti-B from O_h^B cells in absorbtion-elution tests while others had not been able to do so, and that successful elution was being used to indicate the presence on these cells of small amounts of A or B antigens, the authors decided to investigate whether or not weak anti-I, undetected in anti-A and anti-B reagents, could become attached to the known stronger I antigen on O_h cells. In the process, molecules of anti-A or anti-B might be adsorbed non-specifically into the I antigen-antibody complexes formed and later be eluted, in accordance with the phenomenon described by Ogata and Matuhasi (1962 and 1964) and supported by Allen, Issitt, Degnan, Jackson, Reihart, Knowlin and Adebahr (1969) and by McKeever (1974).

The absorbtion-elution studies were conducted in both Durban and Cincinnati using samples of O_h red cells supplied by Mrs P. Gov. (*OO*), G. Nai. (A_1O) and S. Nai. (*BO*), all of whose genotypes were known from the transferases detected in their sera by Race and Watkins (1972b). Their cells were divided into two portions, and one portion from each person was sent by air to Cincinnati, reaching there within three days. Samples from known A, B and O donors, drawn locally on the same date as the O_h cells, were used as control cells in both places. The O_h cells were identified only by the numbers 1, 2 and 3 at the request of the Cincinnati staff. In Durban the cells were prepared for use in saline and were ficin-treated as indicated in Chapter 2.

The anti-A and anti-B reagents used in Durban in this study were not known to contain anti-I but were high titre, immune antibodies obtained from women who had given birth to infants suffering from ABO haemolytic disease of the newborn. The immune nature of these antibodies was confirmed by showing that they were not inhibited in the combined 2-mercaptoethanol-secretor saliva inhibition test described by Moores, Grobbelaar and Ward (1970). In Cincinnati, the anti-A and anti-B reagents used contained strong anti-I antibodies, and aliquots from which the anti-I had been removed completely by absorbing them with ficin-treated I-positive

O cells but in which the anti-A or anti-B titre was not seen subsequently to have been reduced, were used in parallel tests. Each serum was absorbed with an equal volume of the appropriate cells at 4°C overnight in Durban, and eluates were made next day by the 56°C technique of Landsteiner and Miller (1925).

The strength of the reactions of nine local examples of allo-anti-I and two of auto-anti-I with the cells of the three Natal O_h Indians and controls was assessed in Durban. The titration scores obtained are presented in Table 9.3. They showed that the O_h cells all had stronger I antigen than the non-O_h control cells. In Cincinnati, however, parallel titrations with three American allo-anti-I, 10 auto-anti-I and two anti-I from I-positive patients suffering from cold haemagglutinin disease showed that all three allo- and two of the auto-anti-I failed to detect any titre or score differences between the O_h and the non-O_h control cells. The findings supported the suggestion made by Feizi, Kabat, Vacari, Anderson and Marsh, (1971) that anti-I reagents are heterogeneous, some recognising the type of I antigen expressed on normal and unusually strongly on O_h cells, while others detect a different type of I antigen expressed with the same strength on both normal and O_h cells.

Before describing the results of the absorbtion-elution tests with anti-A and anti-B, the attempt to detect increased Le^a, Sd^a and i antigen on the O_h cells used in this study will be presented. Two potent examples of anti-Le^x and one each of anti-Le^a, anti-Sd^a and anti-i were titrated by optimum techniques with the cells of the three Natal O_h Indians and suitable control cells. The results in Table 9.4 showed that none of these antigens was increased in strength on the O_h cells compared with the control cells. The anti-i reagent was especially selected for these tests as it agglutinated adult I (negative control cells) weakly.

The absorbtion-elution tests, in which three anti-A, six anti-B and two anti-A,B sera, one aliquot of each of which contained anti-I antibodies with titres ranging from 32 to 512 while from the other all the anti-I had been removed, showed that no anti-A or anti-B was absorbed by the O_h cells. This was revealed by the post-absorbtion titres being the same as the pre-absorbtion titres of all these reagents, and by the eluates from the O_h cells not agglutinating A_1 or B cells in saline tests at 4°C, in ficin and bromelin tests or in indirect antiglobulin tests. Anti-I was recovered in some of the eluates, however.

TABLE 9.3

Anti-I titration scores with the cells of Three Natal O_h Indians and many control cells

Reagent	Anti-I type	Titration scores O_h^O	O_h^A	O_h^B	Non-O_h
PPM 136	allo	86	77	86	67
PPM 56	allo	76	77	80	57
M370	allo	93	89	93	51
PPM 18	allo	35	35	47	26
PPM 117	allo	75	67	75	53
Mafuya	allo	79	79	79	57
PPM 73	allo	55	41	46	30
P. Zulu	allo	43	46	46	18
M 309	allo	35	35	38	18
M 261	auto	85	85	86	55
PPM 100	auto	82	67	77	44

TABLE 9.4

Scores obtained in titrations with various antibodies using the cells of Natal O_h Indians and control cells

Cells	Titration scores anti-Le^a	anti-Le^x	anti-Le^x	anti-i	anti-Sd^a
O_h^O	69	83	66	14	32
O_h^A	70	81	61	24	31
O_h^B	72	83	71	28	33
Control Pos	71	83	71	72	30
Control Neg	0	0	0	27	0

The *ABO* genotypes of the three Natal O_h Indians whose red cells were used in this study were guessed incorrectly from the titres obtained in Cincinnati with A_1, B and O cells and plasma recalcified from their coded blood samples. Moreover, after the samples had been uncoded, the titres were not seen to correlate with their genotypes in any way. Within the bounds of experimental error, the titres of the recalcified plasma from each Indian were identical with the A_1, the B and the O cells, in titrations both at room temperature and at 4°C.

Since anti-A and anti-B antibodies had not been eluted from the cells of the three Natal O_h Indians and titrations with the anti-A+B+H antibodies in their sera had not disclosed the true genotypes of the Indians, known from the transferases detected earlier in their sera and from family inheritance studies, the authors of the paper in which this investigation is described proposed that at least two categories of O_h individuals existed. Those whose cells, like the cells of the Natal O_h Indians, showed complete suppression of A, B and H antigens constituted one category, while those whose cells were not agglutinated by anti-A, anti-B or anti-H but which absorbed and eluted these antibodies constituted the other. The individuals in the first category were considered to have *hh* genes, while those in the second category had two alleles of *H* which resulted in weak H antigen expression, and therefore also of weak A and/or weak B antigen expression, on the red cell membrane. The alleles of *H* might constitute a series, each allele coding for the production of a small but different amount of H antigen, the *hh* genes might respond differently in different environments or the genes *H* and *h* might be subject to the activity of control genes situated at another genetic locus.

The finding in this study that the anti-A+B+H antibodies in the sera of the three O_h Indians tested reacted individually in titrations equally strongly with A_1, B and O cells supported the conclusion that their cells lacked all expression of A, B and H antigens. According to Bernstein's law and as confirmed by many workers, an antibody is not normally produced in the serum when the subject's tissues contain the corresponding antigen. It is therefore reasonable to expect, and the investigations of others have shown, when ABH antibodies are eluted successfully from cells which have an apparent O_h phenotype, that the corresponding antibody is weaker than the other ABH antibodies in the subject's serum. Following publication of the paper by Moores *et al.* (1975), I titrated the anti-A+B+H antibodies in the sera of 17 further Natal O_h Indians and included the three already recorded as controls. Table 9.5 shows, with differences amounting to not more than one doubling dilution

TABLE 9.5

Titres obtained with the anti-A+B+H antibodies of Natal O_h Indians using A_1, B and O cells

Subject		Saline tests			2-stage ficin tests		
		A_1	B	O	A_1	B	O
M. Gov. 69	Tamil	16	16	16	256	128	256
Miss A. Gov. 214	Tamil	128	128	64	256	256	128
G. Nai. 164	Telegu	16	16	16	64	64	128
S. Nai. 169	Telegu	256	256	128	512	512	256
Mrs P.N. 30	Telegu	16	32	64	256	256	256
Mrs L. Dew	Hindi	64	128	128	256	256	512
Mrs P. Gov. 165	Tamil	64	64	64	256	256	256
Mrs B. Gov.	Tamil	128	128	128	512	512	256
N. Moon.	Tamil	128	128	128	256	128	256
B. Moon.	Tamil	512	256	256	1024	1024	512
V. Moon.	Tamil	256	512	512	2048	2048	4096
Miss R. Gan.	Hindi	512	256	512	512	512	512
Mrs K. Ram.	Hindi	128	64	128	512	512	512
Miss S. Hari	Hindi	512	512	512			
Mrs P.C. 121	Telegu	32	32	32	128	128	128
A. Nar. 202	Telegu	64	128	128	256	512	512
R. Red. 216	Telegu	128	128	128	256	512	512
S. Red. 215	Telegu	64	64	64	256	128	256
Mrs D. Rug. 197	Hindi	32	16	32	256	128	128
Mrs D. Oma.	Hindi	256	128	64	512	256	256

either way attributed to small errors in recording the weaker results, that the individual titres of their antibodies with the A_1, B and O cells used were identical in each instance. Moreover, after the sera of some of the Indians had been treated with 2-mercaptoethanol to inhibit IgM antiA+B+H antibody activity, their titres with the A_1, B and O cells used were also all equal. It was therefore clear that the Natal O_h Indians found so far formed a homogeneous group that belonged in the first proposed category of phenotype O_h individuals, all the members in which had inherited *hh* genes. Bhatia (1976), who performed similar titration studies with the sera of 40 individuals in India, supported the view that two O_h categories existed and referred to them as 'typical' and 'atypical', respectively. His opinion that the Indians recorded by Moores *et al.* (1975) were serologically identical to his own 'typical' O_h Indians, including the three original examples (Bhende *et al.*, 1952), is compatible with my view that the genes responsible for the presence of the O_h phenotype in Natal (Moores, 1965 and 1972) were brought to Natal from India by the Indian immigrants some time during or after the year 1860.

9.5 SUMMARY

Blood samples provided by two female and five male Natal Indians whose phenotype was O_h and members of their families assisted several scientists to make major advances in their biochemical and serological research. This research culminated in the unravelling of the biochemistry of the A, B and H antigens and showed that the *A*, *B* and *H* genes coded for the production of separate glycosyltransferases each of which catalysed the addition of an immunodominant sugar to the appropriate precursor substrate. Since the cells of the Indians failed to absorb and elute ABH antibodies, and the titres of the anti-A+B+H antibodies in their sera were the same whether A_1, B or O cells were used as the indicator, the claim was made that two categories of phenotype O_h cells existed. Alteration of the membranes of the O_h cells with enzymes and extraction with ethanol after disrupting the cells mechanically also resulted in the discovery that a very small quantity of H antigen existed below the membrane surface. The presence of strong I antigen on the O_h cells was confirmed but it was shown not to be recognised by all examples of anti-I, and the strength of the Le^a, Sd^a and i antigens of these cells was shown not to be increased.

CHAPTER 10

Le GENE EXPRESSION IN THE PARENTS AND CHILDREN OF O_h PEOPLE

10.1 INTRODUCTION

The O_h phenotype, in which H, A and B antigens are absent from the red cells and the corresponding substances are absent from the saliva, has been explained by genetic failure to produce the *H* gene enzyme α-2-L-fucosyltransferase. This enzyme normally acts upon two different carbohydrate chain endings, known as Type 1 and Type 2, carried on macromolecular glycoproteins in the secretions and part of both glycosphingolipid and glycoprotein molecules on the membranes of the cells (Watkins, 1980). The structure of the chain endings is as follows:

Type 1: β-Gal(1−3)β-GlcNAc-- remainder of molecule
Type 2: β-Gal(1−4)β-GlcNAc-- remainder of molecule

(Gal = D-galactose; GlcNAc = N-acetyl-D-glucosamine)

When H antigen and substance are formed, the *H* gene enzyme adds L-fucose to both Type 1 and Type 2 carbohydrate chain endings in the β-Gal position (1,2 linkage), but it is only able to do so in the secretory tissues when the regulator or 'switch' gene *Se* has been inherited. In the presence of *H* and *Se* genes, the *Le* gene enzyme α-4-L-fucosyltransferase competes with the *H* gene enzyme for the Type 1 chain endings, and Le^b substance appears when the former enzyme has added L-fucose to them in the β-Gal position and the latter L-fucose to them in the β-GlcNAc position (1,4 linkage). Le^b substance is therefore a product of interaction between the genes *H* and *Le* and has no gene of its own. In the absence of *HH* genes, or when *sese* genes have been inherited, the *Le* gene enzyme converts all the available Type 1 chain endings to Le^a substance by adding L-fucose to them in the β-GlcNAc position (Watkins, 1980).

Genetic failure to produce the *H* gene enzyme is believed to be caused by the inheritance of the rare recessive alleles *hh* of the gene *H*. However, the possibility also exists that two equally rare recessive suppressor genes, situated at another chromosomal

locus, inhibit H enzyme production by normal *HH* genes (Watkins, 1980). Bhatia (1975) named the corresponding red cell phenotype 'typical' O_h, but a second phenotype, named 'atypical' O_h by him, may be due to the inheritance of a different allele of *H* or the suppressor gene. This allele is envisaged as allowing the production of a minimal amount of H antigen, which is then all converted into a minimal amount of A or B antigen or both. The A and B antigens thus formed are too weak for the cells of the individuals to be agglutinated by anti-A, anti-B and anti-A,B, but they will absorb and elute these antibodies (Moores, Issitt, Pavone and McKeever, 1975). Further alleles of *H* may also exist (Bhatia, 1977; Cartron, 1978), and para-Bombay cells, the weak A, weak B and weak AB antigens of which are often detected serologically, may be due to them. In addition, Mourant, Kopeć and Domaniewska-Sobczak (1976) suggested that the Le(a+b+) cells seen in some adults would be explained if less than the normal amount of H antigen was being produced. A weak but not fully-amorphic allele of *H* was thought to be present in the adults which allowed the production of more Le^a substance than usual. The Le(a+b−) cells of the Thais who secreted A or B but no H substance, reported by Chandanayingyong, Sasaki and Greenwalt (1967), were also considered by Mourant *et al.* (1976) possibly to be accommodated by this theory.

The belief that the O_h phenotype was due to the inheritance of two *h* or recessive inhibitor genes suggested that the parents and children of the propositi would be heterozygous for these genes. However, all attempts to detect less than the normal amount of H antigen on their cells have so far proved unsuccessful (Parkin, 1956; Haddad 1973; Vos and Moores, 1976). Vos and Moores (1976) concluded that a single *H* gene coded for the production of normal quantitites of H antigen on the cells, and there seems to be no way yet in which the *Hh* identity of a person may be established by serological methods.

This chapter describes the observations and reasoning which led me to conclude that the *Hh* genotypes of some parents and children of O_h people might be revealed by their Le(a+b−) red cell phenotypes.

10.2 MATERIALS AND METHODS

The O_h Indian family studies made in Natal were examined, and the literature was also searched for details of the families of as many other O_h individuals as possible, whose phenotypes were either 'typical' or 'atypical' according to the criteria favoured by Bhatia (1975) and supported by the studies of Moores *et al.* (1975). The Lewis groups of the parents were entered in my register only once when the parents had more than one O_h child but, where known, the Lewis groups of the spouse and children of all the O_h individuals were entered. Where the mother of the O_h individual was herself O_h, this was also noted. The spouses were included in Tables 10.1 and 10.2 as controls as it was appreciated that the O_h families were from a number of different population groups.

TABLE 10.1

Lewis groups of parents, spouses and children of Natal O_h Indians

Name	Mother	Father		Spouse	Children		
					Le(a+b−)	Le(a−b−)	Le(a−b+)
P.C.	Le(a+b−)	?	*Le*			1	1
Dew.	Le(a+b−)	?	*Le*				
Ram.	Le(a+b−)	Le(a−b+)	*Le*				
			Le				
Moon.	Le(a+b−)	Le(a−b−)	*lele*				
			lele				
P. Gov.	Le(a+b−)	Le(a+b−)	*Le*	Le(a+b−)	1		
			Le	Le(a−b+)		1	1
Nai.	Le(a+b−)	Le(a−b+)	*Le*	Le(a−b?)			1
			Le	Le(a+b−)	6		
M. Gov.	Le(a−b+)	Le(a+b−)	*Le*				
Hari.	?	Le(a+b−)	*Le*				

TABLE 10.2

Lewis groups of parents, spouses and children of O_h individuals reported by others

Authors	Parents		O_h subject	Spouse	Children			
	Mother	Father			Le(a+b–)	Le(a–b–)	Le(a–b?)	Le(a–b+)
Levine *et al.*, 1955	Le(a+b–)	Le(a–b+)	*Le*	Le(a+b–)	1		1	
Parkin, 1956	?	?	*Le*			1		
			Le	Le(a+b–)	2			
Hakim *et al.*, 1961	Le(a+b–)	Le(a+b–)	*Le*					
	?	?	*Le*	Le(a–b–)				3
			Le	Le(a+b–)	4			
	?	?	*Le*	Le(a+b–)	3			
	?	Le(a+b–)	*Le*					
Jakobowicz *et al.*, 1961	Le(a–b?)	Le(a–b?)	*Le*	Le(a–b?)	1			1
			Le					
Aloysia *et al.*, 1961	Le(a+b–)	Le(a–b+)	*Le*	Le(a+b–)	2			
	?	?	*Le*	Le(a–b+)	1			2
			Le	Le(a–b+)				1
Aust *et al.*, 1962			*Le*	Le(a–b?)			4	
	Le(a+b–)	Le(a–b?)	*Le*	Le(a+b–)	3			
			Le	Le(a–b?)	1		2	
Giles *et al.*, 1963	?	?	*lele*	Le(a–b+)	2			1
Gandini *et al.*, 1968	Le(a–b+)	Le(a+b–)	*lele*	Le(a–b+)				
Yunis *et al.*, 1969			*Lele*	Le(a+b–)	1	1		
	O_h Le(a+b–)	?	*Lele*	Le(a–b–)	1	1		
			Lele	Le(a–b+)	2			
	?	?	*Lele*	Le(a+b–)	3	1		
Pretty *et al.*, 1969	?	Le(a+b–)	*lele*	Le(a–b+)	1			2
Dzierzkowa-Borodej	Le(a–b+)	Le(a–b+)	*Le*					
et al., 1972			*Le*					
Sathe and Bhatia, 1973			*lele*					
	Le(a+b–)	Le(a–b–)	*lele*					
			Lele					
Haddad, 1974	Le(a–b+)	Le(a+b–)	*Le*	Le(a–b+)		2		
Poschmann *et al.*, 1974	Le(a+b–)	Le(a–b–)	*lele*					
Rodier *et al.*, 1974	Le(a+b–)	?	*Le*	Le(a–b+)				2
Sathe and Bhatia, 1976	Le(a+b–)	?	*lele*	Le(a–b+)				1
	O_h Le(a–b–)	Le(a–b+)	*lele*					
			Lele					
Sringarm *et al.*, 1977	Le(a–b+)	Le(a+b–)	*Le*	Le(a–b+)	2			2
			Le					1

10.3 RESULTS

Excluding two families in which a parent of an O_h individual was O_h as well, Tables 10.1 and 10.2 show, in 21 of the 25 O_h families recorded, that at least one parent had phenotype Le(a+b–) cells. One parent in three O_h families also, had phenotype Le(a–b–) cells. In the two remaining O_h families, the parents described by Jakobowicz, Simmons and Whittingham (1961) had been tested with anti-Le^a only and the parents reported by Dzierzkowa-Borodej, Meinhard, Nestorowicz and Pirǒg (1972) both had phenotype Le(a–b+) cells. All together, 23 of the 40 non-O_h parents of the O_h people had Le(a+b–), three had Le(a–b–) and 11 had Le(a–b+) cells; and three parents had Le(a–) cells which had not been tested with anti-Le^b. The Le(a+b–) phenotype frequency in the non-O_h parents was therefore 57,50%, and the Le(a–b–) phenotype frequency was 7,50%. When the number of parents with Le(a–b+) was added to the number with Le(a–b?) cells, the frequency of their pooled phenotypes became 35,00%.

The high Le(a+b–) phenotype frequency noted in the parents of the O_h individuals in Tables 10.1 and 10.2 was compared (Table 10.3, part a) with the non-secretor frequency in eight populations in India in which the population structure approached that of the Natal Indians (Mourant *et al.*, p 551–552, 1976). It was also compared with the Le(a+b–) phenotype frequency (Table 10.3, part b) in the single large sample of Indians recorded in India (Mourant, *et al.*, p 566, 1976), in pooled Natal Indian donors recorded in this thesis (Table 16.11) and in the 27 spouses (controls) of the O_h Indians recorded in Tables 10.1 and 10.2. The non-secretors were all arbitrarily assumed to have Le(a+b–) cells, although it was appreciated that a small minority would almost certainly have had Le(a–b–) cells. The secretor/non-secretor frequencies were selected for my purpose as more of these than of Lewis frequencies were known in random Indian populations, and the Indians were preferred to other population groups as at least 12 of the 25 O_h families in Tables 10.1 and 10.2 were of Indian origin. The average non-secretor, or Le(a+b–), phenotype frequency in Table 10.3 was 25,46% and the highest single non-secretor phenotype frequency 30,72%. The average non-secretor frequency was similar to the 23,05% Le(a+b–) frequency recorded in the Natal Indian donors, but it was lower than the 37,03% Le(a+b–) frequency recorded in the 27 spouses. However, in the latter group, no allowance had been made for consanguinous marriages. In the light of these frequencies, the 57,50% Le(a+b–) phenotype frequency in the parents was therefore indeed remarkable (χ^2 25,16; $P = < 0{,}001$).

TABLE 10.3

Secretor and Lewis phenotype frequencies in Indians of India recorded by others

Part a

Subjects	Authors	Number tested	Number detected		% detected	
			Secretors	non-secretors	Secretors	Non-secretors
South Indians	Rao, 1952	200	141	59	70,50	29,50
Controls	Anand, 1965	201	173	28	86,07	13,93
Hindu-Punjabi	Sharma, 1957	203	168	35	82,76	17,24
Controls	Sehgal & Dube, 1967	370	259	111	70,00	30,00
Bengali	Roy & Chatterjea, 1965	438	321	117	73,29	26,71
Donors	Tyagi *et al.* 1968	475	338	137	71,16	28,84
Moslems	Bhattacharjee, 1966	166	115	51	69,28	30,72
Moslems	Bhattacharjee, 1966	202	148	54	73,27	26,73
Average						25,46

Part b

Subjects	Authors	Number tested		Lewis phenotype frequencies		
				Le(a+b−)	Le(a−b+)	Le(a−b−)
Pathans	Alciati, 1968	132	No.	34	60	38
			%	25,76	45,45	28,79

Omitting the seven children who had not been tested with anti-Le^b and the seven who were Le(a–b–), the Lewis phenotypes of the children of the O_h people recorded in Tables 10.1 and 10.2 showed that the Le(a+b–), compared with the Le(a–b+), phenotype frequency in them was also increased (38 of 57 children, or 66,67%). After it was assumed that the Le(a–b–) children had all been grouped incorrectly as Le(b–) and they and the Le(a–b?) children had been included in the count with the Le(a–b+) children, the children's Le(a+b–) frequency became 53,52% (38 of 71 children). Nevertheless, this was still remarkably high (χ^2 20,08; P = < 0,001).

10.4 DISCUSSION

The high Le(a+b–) phenotype frequency in the parents and children of the O_h people shown in Tables 10.1 and 10.2 suggested, although the results in my serological tests with the cells of some and the results obtained in similar tests by other workers with the cells of others had indicated that they had *sese, Le* genes, that in fact they had *SeSe* or *Sese, Le* genes. For some reason, in them, these genes might not be able to convert their Type 1 substrate chain endings correctly into Le^b substance, and they might be secreting Le^a substance and have Le(a+b–) cells 'incorrectly'. Similarly, the parents and children who had Le(a–b–) cells might not, as supposed, have *Se, lele* or *sese, lele* genes but, in fact might have *SeSe, Le* or *Sese, Le* genes. In them, possibly for a different reason, perhaps even Le^a substance could not be formed or the production of this substance was inhibited to the extent that it could not be detected in serological tests. They might therefore be secreting Lewis substances and have Le(a–b–) cells also 'incorrectly'. Since there was no evidence that Type 1 and Type 2 chain endings in the precursor substrate were faulty in O_h persons (Schenkel-Brunner, Prohaska and Tuppy, 1975; Watkins, 1980), perhaps the explanation was, either than the *H* gene enzyme, which in non-O_h persons who have *Se* genes competes with the *Le* gene enzyme for Type 1 chain endings, or the *Le* gene enzyme was malfunctioning in some way. However, as O_h people have Le(a+b–) cells, *Le* gene enzyme synthesis is known probably not to be affected when *hh* genes have been inherited. A malfunctioning *H*, rather than a malfunctioning *Le*, gene enzyme was therefore preferred as the possible solution. My theory, in connection with the Le(a–b–) parents and children of O_h people, consequently fell away and, instead, the *Hh* status of the parents and children was envisaged as being revealed, often but not always, by secretion of Le^a substance in the saliva and Le(a+b–) cells. A precedent had been set for this theory by Mourant *et al.* who recorded, in 1976, that the Thais who had Le(a+b–) cells and who were secretors had a weak but not

fully amorphic allele of *H* which allowed production of more Le^a substance than usual in them.

10.5 SUMMARY

The parents and children of 'typical' and 'atypical' O_h people were noticed to have Le(a+b−), and perhaps also Le(a−b−), cells more often than random non-O_h controls. The reason was thought to be that the *H* gene enzyme was malfunctioning in them in some way. As a result, more Le^a substance than usual was being produced, and their correct Lewis phenotypes were being masked. In some but not all the parents and children of O_h people, *Hh* genes might therefore be revealed by the secretion of Le^a substance and their Le(a+b−) red cell phenotype.

CHAPTER 11

SEROLOGICAL STUDIES OF ANTI-A+B+H ALLO-ANTIBODIES IN THE SERA OF THREE NATAL 'BOMBAY' O_h INDIANS

11.1 INTRODUCTION

Few technical details are available in the literature of studies with anti-A+B+H allo-antibodies in the sera of O_h individuals. However, Parkin (1956), who examined the allo-antibodies in the sera of two Irish O_h siblings and commented subsequently that her findings were remarkably constant, and Dzierzkowa- Borodej, Meinhard, Nestorowicz and Pirog (1972), who investigated them in the sera of two Polish O_h siblings, included tables in their reports in which the results suggested strongly that the siblings did not have simple mixtures of anti-A, anti-B and anti-H antibodies in their sera. Parkin (1956) commented as well that the Lewis phenotypes of the cells used by her to absorb the Irish siblings' sera had not been significant in this context.

In this chapter, the studies made with the allo-antibodies of three Natal O_h Indians, whose immunological backgrounds varied, will be described. The studies included saliva inhibition tests, and absorbtion-elution tests which went further than the single absorbtion and elution studies of Parkin (1956) and of Dzierzkowa-Borodej *et al.* (1972). The results showed that anti-H, rather than anti-A or anti-B, was almost certainly the most serologically significant antibody specificity in the Indians' sera. Moreover, surprisingly, in each instance a stronger anti-H antibody was recovered in the eluate from the A_1 than from the B cells and from the B than from the O cells. This finding contrasted strangely with the known agglutination by anti-H in A_1 individuals, and by *Ulex* anti-H lectin, of A_1, B and O cells, and therefore also the expected absorbtion of these reagents by A_1, B and O cells, from strong to weak in the order $O \rightarrow A_2 \rightarrow B \rightarrow A_1$ (Race and Sanger, p 50, 1975).

11.2 MATERIALS AND METHODS

11.2.1 The Subjects

Samples of serum from a Natal O_h Indian man and two Natal O_h Indian women, all of whom were known not to have received transfusions of blood, were used in this study.

The Indian man, M. Gov. (69), was a healthy, Tamil-speaking adult of about 25 years of age. The O_h phenotype of his cells was recognised following the donation of his first unit of blood at a Natal Blood Transfusion donation clinic in 1967. The ABO group unexpressed on his cells was O and the Lewis phenotype of his cells was Le(a+b–). The anti-A+B+H allo-antibodies in his serum confirmed, by agglutinating A_1, B and O cells to approximately equal titres in parallel titrations, that he belonged to the category of 'typical' O_h individuals. The titres of his antibodies were the lowest recorded in the three Indians.

The first Indian woman, Mrs P.N. (30), spoke Telegu and is described in further detail in Chapter 7. Approximately 55 years of age, she had a carcinoma, was anaemic, and her pregnancies had numbered at least seven. The ABO group unexpressed on her cells was either A_1, B or A_1B and the Lewis phenotype of her cells was Le(a+b–). The approximately equal titres of her anti-A+B+H allo-antibodies with A_1, B and O cells showed that she, too, was a 'typical' O_h individual. In her case, however, the antibody titres were higher than those of M. Gov.

The second Indian woman, Mrs L. Dew., who spoke Hindi, was 17 years of age, healthy and pregnant for the first time. The O_h phenotype of her cells was discovered in 1969 when a sample of her blood was received for routine antenatal tests. A family study failed to reveal the ABO group unexpressed on her cells and the Lewis phenotype of her cells was Le(a+b–). The approximately equal titres with A_1, B and O cells of her anti-A+B+H allo-antibodies confirmed that her cells were also 'typical' O_h. In due course, Mrs L. Dew. was delivered of a group B infant who suffered mildly from haemolytic disease of the newborn. The eluate made from the infant's cord cells contained an antibody at first identified as anti-B but which was later re-identified as anti-H. Anti-H was also identified in the cord serum. The titres of Mrs L. Dew.'s anti-A+B+H allo-antibodies were the highest recorded in the three Indians.

11.2.2 The cells and sera

The A_1, A_2, B and O cells used in this study were from 475 ml donations of blood in ACD from White donors, and titrations confirmed that their cells were agglutinated by *Ulex* anti-H lectin to the titres expected in relation to their ABO groups. The Lewis phenotype of the group O donor's cells was Le(a+b–). The donations were stored at 4°C and the method of preparing the packed cells and saline and

0,5% bromelin-treated cells from them are described in Chapter 2. The O_h cells, donated by another Natal O_h Indian, were stored at −28°C in glycerol freezing solution (Mollison, p 726, 1979) and were dialysed in saline (Weiner, 1961) for use as required. The samples of serum used from the three Natal O_h Indians were not of the same age but had all been stored continuously at −28°C and appeared in excellent condition. The sera were not inactivated before use as complement was no longer demonstrable in them.

11.2.3 The tests

The saliva inhibition titration technique has been described in Chapter 2. The salivas were from secretors of A and H, B and H, and H substances, and an equal volume of the appropriate saliva was added to all the dilutions of serum in each titration instead of to the neat serum only.

The absorbtion-elution tests were conducted as follows. Three volumes of serum were prepared for use from each O_h Indian. The first volume was absorbed with an equal volume of packed A_1 cells four times, twice at 4°C, once at 37°C and once at 22°C, for periods of one hour each. The second volume was absorbed in the same way with packed B cells and the third volume with packed O cells. The absorbed sera were recovered and stored at −28°C until required, and the packed cells from the second, third and fourth absorbtions were discarded.

The packed cells from the first absorbtions were washed six times with saline at 4°C and in each instance part of the sixth saline wash was retained. The washed cells were eluted into saline by the 56°C heat technique of Landsteiner and Miller (1925), this elution method having been chosen specifically in order that the antibodies in the eluates might be directly compared with those recovered, using the same method, by Parkin (1956) and Dzierzkowa-Borodej *et al.* (1972). The elution technique of Landsteiner and Miller (1925) is still used extensively, and it is quoted as a standard method in the following textbooks: Applied Blood Group Serology (P.D. Issitt and C.H. Issitt, 1975, p 28), Technical Manual of the American Association of Blood Banks (Publ. J.B. Lippincott Co. Philadelphia, USA, p 186) and Blood Transfusion in Clinical Medicine (P.L. Mollison, 1979, p 478). The elution procedure provided nine eluates, or the eluate 1 samples, each of which was now subdivided into four portions.

The first portions of the eluate I samples were all absorbed once with an equal volume of packed A_1 cells, the second portions absorbed once with packed B cells

and the third portions once with packed O cells, all at 4°C for one hour. The cells were washed six times with saline at 4°C and, again, part of the sixth saline wash was retained. The cells were eluted into saline by the 56°C technique of Landsteiner and Miller (1925), providing 27 further eluates, or the eluate 2 samples.

The nine unabsorbed fourth portions of eluates 1.1 to 1.9, the 27 eluate 2 samples and the 36 saline wash samples were tested with saline suspensions of A_1, B and O cells by the indirect antiglobulin technique, using a broad-spectrum antiglobulin reagent as described in Chapter 2. The results were read both macro- and microscopically, and none of the 36 sixth saline wash samples were found to have agglutinated these cells.

Eluates 1.3, 1.6 and 1.9 were tested as well with 0,5% bromelin-treated A_1, B and O cells, and the nine absorbed serum samples were tested by the indirect antiglobulin technique with saline suspensions of these cells.

11.3 RESULTS

11.3.1 The saliva inhibition titrations

Table 11.1 shows the findings in this study. The reciprocal of the highest dilutions in the titrations to which saline instead of saliva had been added and in which agglutinated cells could still be detected represented the titres of the unabsorbed anti-A+B+H antibodies in the sera of the three Indians. The titres were one dilution tube lower than those which would have been expected had saline not been added to these titrations. The titres of the antibodies of M. Gov. were seen to be 64 with A_1 cells, 32 with B cells and 64 with O cells; those of Mrs P.N. to be 128 with A_1 cells, 256 with B cells and 256 with O cells, and those of Mrs L. Dew. to be 256 with A_1 cells, 512 with B cells and 512 with O cells.

The titres detected after A_1 cells and saliva containing B and H substances, and after B cells and saliva containing A and H substances, had been added to the titrations of the O_h Indians' sera represented the titres of their specific anti-A and anti-B antibodies. This was due to their anti-H antibodies having been inhibited in these titrations by H substance. The titre of M. Gov.'s anti-A was seen to be 32 and of his anti-B 16, the titres of Mrs P.N.'s anti-A and anti-B were both seen to be 64, and the titre of Mrs L. Dew.'s anti-A was seen to be 128 and of her anti-B 32. As their anti-A and anti-B titres were all lower than their anti-H titres (64, 256 and 512 respectively, observed in the control titrations with O cells), their anti-A and anti-B were consequently not seen to be their most serologically significant antibodies. Moreover, the higher titres obtained in the control titrations with the B and O, rather than with the

TABLE 11.1

Saliva inhibition tests with anti-A+B+H antibodies
in the sera of three Natal O_h Indians

Sera	Cells	Substrate	Anti-A+B+H antibodies Dilutions 1	2	4	8	16	32	64	128	256	512	Score	Apparent antibody specificity
	A_1	A+H	–	–	–	–	–	–	–	–	–	–	0	
	A_1	B+H	4	4	4	2	1	(2)	–	–	–	–	44	Anti-A
	A_1	Saline	4	4	4	3	2	1	(2)	–	–	–	54	Anti-A+H
	B	B+H	–	–	–	–	–	–	–	–	–	–	0	
M. Gov. (69)	B	A+H	4	3	2	1	(2)	–	–	–	–	–	34	Anti-B
	B	Saline	4	4	3	3	1	(2)	–	–	–	–	46	Anti-B+H
	O	A+H	(3)	–	–	–	–	–	–	–	–	–	2	
	O	B+H	–	–	–	–	–	–	–	–	–	–	0	
	O	H	(2)	–	–	–	–	–	–	–	–	–	1	
	O	Saline	4	4	4	3	2	1	(2)	–	–	–	54	Anti-H
	A_1	A+H	–	–	–	–	–	–	–	–	–	–	0	
	A_1	B+H	4	4	4	3	1	(3)	(1)	–	–	–	47	Anti-A
	A_1	Saline	4	4	4	3	3	2	1	(2)	–	–	64	Anti-A+H
	B	B+H	–	–	–	–	–	–	–	–	–	–	0	
Mrs P.N. (30)	B	A+H	4	4	3	3	2	1	(1)	–	–	–	53	Anti-B
	B	Saline	4	4	4	4	4	4	3	2	(3)	–	80	Anti-B+H
	O	A+H	(2)	–	–	–	–	–	–	–	–	–	1	
	O	B+H	–	–	–	–	–	–	–	–	–	–	0	
	O	H	(2)	–	–	–	–	–	–	–	–	–	1	
	O	Saline	4	4	4	4	4	4	3	2	(3)	–	80	Anti-H
	A_1	A+H	(2)	(1)	–	–	–	–	–	–	–	–	1	
	A_1	B+H	4	4	4	4	3	2	1	(1)	–	–	63	Anti-A
	A_1	Saline	4	4	4	4	4	3	2	1	(2)	–	74	Anti-A+H
	B	B+H	–	–	–	–	–	–	–	–	–	–	0	
Mrs L. Dew.	B	A+H	4	4	3	2	(3)	(1)	–	–	–	–	40	Anti-B
	B	Saline	4	4	4	4	4	4	3	2	(3)	(1)	80	Anti-B+H
	O	A+H	3	2	1	(1)	–	–	–	–	–	–	23	
	O	B+H	–	–	–	–	–	–	–	–	–	–	0	
	O	H	1	(3)	(2)	–	–	–	–	–	–	–	8	
	O	Saline	4	4	4	4	4	4	3	2	(3)	(1)	80	Anti-H

A_1 cells, using the serum of Mrs P.N. and Mrs L. Dew., supported the view, in these two Indians at least, that the identity of their serologically most significant antibody was anti-H. The anti-H in Mrs L. Dew.'s serum, which was not inhibited as completely as the anti-H in the sera of the other two Indians by the salivas containing A+H and H substances, showed by this as well that it was almost certainly her most serologically potent antibody. The complete inhibition of anti-H in the sera of all three Indians, noted with the saliva containing B+H substances, was attributed to the donor being a natural secretor of a greater quantity of saliva substances than the other two donors.

11.3.2 The absorbtion-elution studies

Table 11.2 shows that the antibodies in eluates 1.1 and 1.7 agglutinated A_1, B and O cells almost all equally strongly and that the antibodies in all the other eluate 1 samples agglutinated them as if they had anti-B+H or anti-H specificity. The anti-H specificity of these antibodies was identified by their agglutination of A_1, B and O cells from strong to weak in the order of O → B → A_1 and their non-agglutination of the O_h control cells.

After absorbtion with A_1 cells, eluates 1.1.d, 1.4.d, and 1.7.d and, after absorbtion with O cells eluates 1.1.f, 1.4.f, 1.5.f and 1.7.f, all appeared to contain anti-B. The curious elution of anti-B from A_1 cells may have been due to the Matuhasi-Ogata phenomenon (Ogata and Matuhasi, 1962 and 1964) but Bird (1953 and 1954) noticed that anti-A,B antibodies which agglutinated B more strongly than A cells were eluted readily from A cells, while those which agglutinated A more strongly than B cells were not eluted readily from B cells. Since Bird's observations with anti-A,B were remarkably similar to mine with the anti-A+B+H antibodies of the O_h Indians, in my tests the only evidence that anti-A had been weakly eluted from B cells had been seen in eluates 1.2.e and 1.2.f, and anti-A and anti-B had not been eluted equally readily from B and A cells respectively, the likelihood that the Matuhasi-Ogata phenomenon was the correct solution was discarded.

Eluates 1.1.e, 1.2.e and 1.4.e absorbed with B cells, and eluates 1.1.f, 1.2.f and 1.4.f absorbed with O cells, were all seen to contain anti-A. However, the weak agglutination of A_1 cells by eluates 1.4.d, 1.7.d, 1.8.d and 1.9.d absorbed with A_1 cells and by eluates 1.8.f and 1.9.f absorbed with O cells was considered rather to be a manifestation of anti-H, as they had agglutinated O more strongly than B and B more

TABLE 11.2

Results of tests with eluate 1 samples obtained from A_1, B and O cells used to absorb the sera of three Natal O_h Indians, with the same eluates after they had been absorbed with A_1, B and O cells and with the eluate 2 samples obtained subsequently from these cells

Sera	Cells used to absorb sera	Eluate 1 samples	Results Eluate 1 samples A_1	B	O	O_h	Cells used to absorb eluate 1 samples	Absorbed eluate 1 samples	Results Absorbed eluate 1 samples A_1	B	O	Eluate 2 samples	Results Eluate 2 samples A_1	B	O
							A_1	d	–	1	–	1	–	–	–
	A_1	1	4	3	4	–	B	e	2	–	–	2	–	–	–
							O	f	2	1	–	3	–	–	–
							A_1	d	–	–	–	4	–	–	<1
M. Gov. (69)	B	2	1	1	2	–	B	e	<1	–	–	5	–	1	1
							O	f	<1	–	–	6	–	–	<1
							A_1	d	–	–	<1	7	–	–	–
	O	3	1	1	2	–	B	e	–	–	–	8	–	–	1
							O	f	–	–	–	9	–	–	–
							A_1	d	<1	3	2	10	<1	2	4
	A_1	4	2	4	4	–	B	e	<1	–	<1	11	–	1	3
							O	f	<1	4	–	12	–	<1	1
							A_1	d	–	2	2	13	–	1	3
Mrs P.N. (30)	B	5	1	4	4	–	B	e	–	<1	<1	14	–	1	2
							O	f	–	1	<1	15	–	<1	1
							A_1	d	–	1	2	16	–	1	1
	O	6	<1	4	4	–	B	e	–	–	<1	17	–	<1	1
							O	f	–	–	<1	18	–	–	–
							A_1	d	<1	4	2	19	<1	3	4
	A_1	7	4	4	4	–	B	e	–	1	1	20	–	1	3
							O	f	–	2	–	21	–	1	1
							A_1	d	1	4	4	22	<1	4	3
Mrs L. Dew.	B	8	1	4	4	–	B	e	–	1	1	23	<1	2	3
							O	f	<1	1	1	24	<1	2	3
							A_1	d	1	4	4	25	1	3	3
	O	9	1	4	4	–	B	e	–	2	2	26	<1	2	3
							O	f	<1	2	2	27	<1	2	3

strongly than A_1 cells. A stronger anti-H was apparent in the absorbed eluates 1.4.d, 1.5.d, 1.6.d, 1.7.d, 1.8.d and 1.9.d than in the absorbed eluates 1.4.e, 1.5.e, 1.5.f, 1.6.e, 1.6.f, 1.7.e, 1.8.e, 1.8.f, 1.9.e and 1.9.f, and this was believed to show that the A_1 cells used to absorb them had removed less anti-H than the B cells and the B cells less anti-H than the O cells.

All the eluate 2 samples agglutinated O cells more strongly than B and B more strongly than A_1 cells. Consequently they were all believed to contain anti-H. However, except in eluates 2.1 to 2.9, a stronger anti-H had been eluted from the A_1 cells (eluates 2.10, 2.13, 2.16, 2.19, 2.22, 2.25) than from the B cells (eluates 2.11, 2.14, 2.17, 2.20, 2.23, 2.26) and a stronger anti-H from the B than from the O cells (eluates 2.12, 2.15, 2.21). Eluates 2.23, 2.24, 2.26 and 2.27 all appeared to contain anti-H antibodies of almost equal strength; and the agglutination of A_1, B and O cells by eluates 2.1 to 2.9, while not exhibiting exactly the same pattern as anti-H, at least showed that the pattern exhibited had a certain degree of regularity.

Since Mrs L. Dew.'s eluates 2.22 to 2.27 were found to contain stronger anti-H antibodies than her eluates 2.19 to 2.21, while Mrs P.N.'s eluates 2.10 to 2.12 were found to contain stronger anti-H antibodies than her eluates 2.13 to 2.15 and her eluates 2.13 to 2.15 stronger anti-H antibodies than her eluates 2.16 to 2.18, more potent anti-H antibodies appeared to have been recovered in the eluate 1 samples from the B and O than from the A_1 cells used to absorb Mrs L. Dew.'s serum. As a result, in her case, these findings were at variance with my view that anti-H had been recovered from the A_1, B and O cells from strong to weak in the order $A_1 \rightarrow B \rightarrow O$. Although Mrs L. Dew.'s eluate 2 samples were not subjected to further absorbtion-elution tests, the anti-H agglutination pattern seen with her absorbed eluate 1 samples suggested that a similar pattern might have been observed after her eluate 2 samples had been absorbed and tested with A_1, B and O cells. If so, then the pattern, when her eluate 3 samples were tested, was expected to have been the same as the pattern observed when the eluate 2 samples of Mrs P.N. were tested with A_1, B and O cells. This prediction was supported by the evidence that Mrs L. Dew. had a more potent anti-H antibody than Mrs P.N. (see Table 11.1) and by the knowledge that at birth her infant had been affected by haemolytic disease of the newborn due to anti-H.

Table 11.3 shows, by bromelin-treated A_1, A_2, B and O cells having been agglutinated in the order $O \rightarrow A_2 \rightarrow B \rightarrow A_1$, that the antibodies in eluates 1.3 of M. Gov., 1.6 of Mrs P.N. and 1.9 of Mrs L. Dew., almost certainly all contained anti-H.

TABLE 11.3

Results of tests with eluates 1.3, 1.6 and 1.9 recovered from O cells used to absorb the sera of three Natal O_h Indians

Sera	Eluate 1 samples	Bromelin-treated cells			
		A_1	A_2	B	O
Mr M. Gov. (69)	3	–	1	< 1	2
Mrs P.N. (30)	6	–	2	< 1	2
Mrs L. Dew.	9	–	2	1	2

The results obtained when the Indians' sera, absorbed four times with A_1, B or O cells, were tested with A_1, B and O cells are presented in Table 11.4. Separate anti-A and anti-B antibody specificities were evident in all three absorbed sera; but in the absorbed sera from Mrs L. Dew. the absorbtions were seen not to have been complete. In separate tests, the weak antibodies in her absorbed sera were all shown to have anti-H specificity. The finding was confirmed by the stronger agglutination of O than of B and of B than of A_1 cells by her absorbed sera shown in Table 11.4.

11.4 DISCUSSION

As many serologists have described in studies of their own with the anti-A+B+H antibodies in the sera of other O_h people, the results in this chapter showed that the sera of the three Natal O_h Indians all contained separable anti-A, anti-B and anti-H antibodies. In the eluates recovered from the A_1, the B and the O cells used to absorb their sera, the expected anti-A, anti-B and anti-H antibodies were also detected, but in most instances their specificities were not demonstrated clearly until after the eluates had been partially absorbed. However, the antibodies in the second set of eluates made from the cells used to absorb the first set showed, by agglutinating A_1, B and O cells from strong to weak in the order O → B → A_1, that they all had only anti-H specificity.

TABLE 11.4

Results of tests with the sera of three Natal O_h Indians after absorbtion four times with A_1, B or O cells

Sera	Cells used to absorb sera	Results cells A_1	B	O
M. Gov. (69)	A_1	–	4	–
	B	4	–	–
	O	4	4	–
Mrs P.N. (30)	A_1	–	4	–
	B	4	–	–
	O	4	4	–
Mrs L. Dew.	A_1	(1)	4	2*
	B	4	(2)	(3)
	O	4	4	(2)

Key: * Further tests confirmed that the antibody causing less than 4+ agglutination was anti-H

The recovery of anti-H in all the eluate 2 samples in itself was not extraordinary, as A_1, B and O cells were all known to have some H antigen, O more than B cells and B more than A_1 cells (Race and Sanger, p 35, 1975). The most interesting finding in this study, and one which has apparently not been recorded before, was that stronger anti-H antibodies had been recovered in the eluate 2 samples of Mrs L. Dew. and Mrs P.N. from the A_1 than from the B and from the B than from the O cells used to absorb their eluate 1 samples. The anti-H agglutination pattern, which was not as obvious with the antibodies in Mrs L. Dew.'s eluate 2 samples as it had been with those in the eluate 2 samples of Mrs P.N., was attributed to Mrs L. Dew. having a more potent anti-H antibody. The potency of Mrs L. Dew.'s anti-H antibody was observed in the saliva inhibition titrations and tests with her absorbed sera, and her infant had also suffered from haemolytic disease of the newborn due to anti-H.

Possibly her infant, still unborn when her sample of serum was acquired, had been the source of antibody stimulation in her case. The reason anti-H was present in all the eluate 2 samples was thought to be, not that the A_1 had absorbed more anti-H than the B and the B more than the O cells but on being eluted, that the A_1 had yielded up more anti-H than the B and the B more anti-H than the O cells. This may have been due either to the anti-H antibodies having bound more firmly to the O than to the B, and more firmly to the B than to the A_1 cells, or to the smaller proportion of H antigen sites known to exist on the A_1 having been more easily disrupted by the 56°C heat elution procedure than on the B and on the B than on the O cells. The finding seemed to echo Bird's observations (1953 and 1954), and it was also similar to the finding (Voak and Lodge, 1968) that A_2 cord cells absorbed more *Ulex* anti-H lectin and eluted less than A_1 cord cells. In addition, Voak and Lodge (1968) described their results as not being as marked as those of Fischer and Hahn (1935) who had used anti-A and A cells. Finally, it was reminiscent of the familiar elution from cells with some weakly expressed A and B antigens of stronger anti-A and anti-B antibodies, respectively, than from cells with normally expressed A and B antigens (Celano, Levine and Lange, 1957).

The stronger anti-H antibodies eluted from the B than from either the A_1 or the O cells in the eluate 2 samples used to absorb the eluate 1 samples of M. Gov. (Table 11.2) suggested that, in his case, the A_1 cells might not have been able to retain sufficient antibody molecules for enough to be eluted and cause even the O cells to be agglutinated in the tests made later. However, the B cells had apparently been able to do so, and the O cells may have retained them so firmly that they were either not eluted or were eluted from them much less readily.

In the eluate 1 samples of M. Gov., Mrs P.N. and Mrs L. Dew., and in the single set of eluates recovered by Dzierzkowa-Borodej *et al.* (1972) from A_1, B and O cells used to absorb the sera of her two Polish O_h siblings, the anti-H specificity of the antibodies was thought to have been masked by their own strength and by the anti-anti-A and anti-B antibodies also present in them. However, in the single set of eluates recovered by Parkin (1956), from the A_1, B and O cells used by her to absorb the sera of her two Irish O_h siblings, the anti-H specificity of the antibodies was demonstrable, in spite of the presence of anti-A, eluted as well from the A_1 cells. Parkin (1956) must either have failed to observe the anti-H specificity of the antibodies in her eluates or have deemed the antibodies to be too weak for comment.

The results in this study revealed that there were marked differences between the anti-A+B+H antibodies in the sera of M. Gov., Mrs P.N. and Mrs L. Dew. In M. Gov.'s serum, perhaps because he was a male, the antibody titres recorded were the lowest, and his anti-H antibodies appeared the least concentrated. In Mrs P.N.'s serum, the antibody titres recorded were higher, and her anti-H antibodies appeared relatively more concentrated, as befitted an elderly woman who had been pregnant with incompatible fetuses several times in the past. In Mrs L. Dew.'s serum, the antibody titres recorded were the highest, and her anti-H antibodies appeared exceptionally concentrated, as befitted a currently pregnant woman whose infant later suffered from haemolytic disease of the newborn due to anti-H. The observation that A_1 cells absorbed less but yielded up more anti-H than B cells and B cells absorbed less but yielded up more anti-H than O cells also suggested that group O infants might be at an equal, if not at a greater, risk than their group A, B and AB siblings from haemolytic disease of the newborn due to their mother's anti-A+B+H antibodies.

Perhaps it should be mentioned here that this entire study was repeated in 1980 for confirmation and that the same results were obtained. The double (and perhaps even treble) elution procedure described was also seen unexpectedly to have provided a method of obtaining anti-H in pure form from anti-A+B+H antibodies.

It might be interesting, on some future occasion, to study in a similar manner the antibodies obtained in the eluates recovered by a different elution method, such as the ether technique of Vos and Kelsall (1956).

11.5 SUMMARY

The most serologically significant of the anti-A+B+H antibodies in the sera of three Natal O_h Indians was found to be their anti-H antibody. This was revealed when anti-H was detected in almost all the eluates recovered from the A_1, B and O cells used to absorb the initial set of eluates recovered from A_1, B and O cells used to absorb their sera. The anti-H antibodies in these eluates, which agglutinated A_1, B and O cells from strong to weak in the order $O \rightarrow B \rightarrow A_1$, were surprisingly found to be eluted from strong to weak in the order $A_1 \rightarrow B \rightarrow O$. A possible explanation for this so far apparently unrecorded finding was advanced.

CHAPTER 12

PARA-BOMBAY PHENOTYPES IN THE NATAL INDIANS

12.1 INTRODUCTION

Para-Bombay red cells are not agglutinated by anti-H and they are either weakly or are not agglutinated by anti-A, anti-B and anti-A,B reagents. Their A, B or AB antigens are also sometimes only detected by means of absorption-elution and other studies. The secretors secrete normal amounts of A, B and H substances in their saliva, and their serum often contains an anti-'O'-like antibody, not inhibited by secretor saliva, which may be anti-HI. The non-secretors usually have anti-H in their serum (Race and Sanger, p 26, 1975; Watkins, 1980).

Not everyone uses the same terminology for para-Bombay cells. The term A_h was chosen for the first example by Levine, Uhlíř and White, who discovered it in 1961; and Beranová, Prodanov, Hrubiško and Smálik reported the first cells with a B_h phenotype in 1969. Solomon, Waggoner and Leyshon (1965), however, and Kitahama, Yamaguchi, Okubo and Hazama (1967), used the terms A_m^h, B_m^h and O_m^h for their apparently weaker examples. In 1970, Hrubiško, Laluha, Mergancová and Zakovicová suggested using the terms A_{Hm} and B_{Hm} for the para-Bombay cells which were agglutinated by anti-A, anti-B and anti-A,B reagents, and using the terms O_{Hm}^A, O_{Hm}^B, O_{Hm}^{AB} and O_{Hm} when the cell antigens had to be identified indirectly. Later, Race and Sanger (p 26, 1975) suggested retaining A_h and B_h for the cells of the non-secretors and using A_m^h and O_{Hm}^A etc. for the secretors. The suggestions made by Race and Sanger have been followed in this thesis.

Since the secretors clearly have *H* genes, in these individuals, the para-Bombay red cell phenotype is regarded as being due to the production of less than the normal amount of H antigen on the membranes of their cells. For some reason, production of their *H* gene-specified α-2-L-fucosyltransferase is partially inhibited and, as the *A* and *B* gene-specified glycosyltransferases are present in normal amounts in their sera and haemopoetic tissues, all of the H antigen-specific glycoprotein and glycosphingolipid of their cells is apparently converted to A, B or AB antigens (Watkins, 1980). The cause, in the para-Bombay secretors, has been attributed to the inheritance of a gene (or genes) which may be a mutant regulator. Solomon, Waggoner and Leyshon

(1965) suggested that this gene and its allele should be called *Z* and *z* respectively, and para-Bombay people may therefore have *zz* genes. Mulet, Cartron, Lopez and Salmon (1978) suggested as well that a more appropriate notation for para-Bombay secretors would be O^{A}_{HZ}, O^{B}_{HZ} etc.

In this chapter, the results of my studies with three Natal Indians who had para-Bombay cells are presented. Their phenotypes were identified as O^{A}_{Hm}, O^{B}_{Hm} and O_{Hm} (or perhaps O^{A}_{HZ}, O^{B}_{HZ} and O_{HZ}) respectively.

12.2 CASE HISTORIES

12.2.1 Mrs G.N. (O^{A}_{Hm}) was discovered in 1977 when she donated a unit of her blood at an NBTS blood donation clinic in Durban. An antibody which agglutinated all of a collection of eight panel cells of known groups and two group O cord cells, but not her own cells, was detected in her serum. The antibody was identified as anti-H, but her cells appeared to be group O. Her blood samples were therefore referred to me for further studies. Mrs G.N. was in excellent health, and her home language was Tamil.

12.2.2 Miss S.M. (O^{B}_{Hm}), was the 18 months-old infant daughter of Moslem parents. She was found in 1977 as well, while in hospital for corrective hip surgery. Although she was apparently group O, no units of O blood crossmatched for her had so far been found compatible with the saline- and enzyme-reacting antibody in her serum.

12.2.3 Mrs G.G. (O_{Hm}) was encountered first in 1967 when she was 26 weeks pregnant. She was 36 years of age and was in hospital suffering from nephritis and severe anaemia. Her cells were apparently group O but 54 units of O blood has been cross-matched for her and all had been found incompatible. Further blood samples were received from her in 1968, four weeks after her infant had been delivered, and also when she was again pregnant, in 1969. However, her phenotype was not identified correctly until 1977.

As far as it was known, Mrs G.N., Miss S.M. and Mrs G.G. were not related.

12.3 MATERIALS AND METHODS

The blood samples from Mrs G.N., from Miss S.M. and members of her family and

from Mrs G.G. and her family were of clotted blood and were prepared for testing as indicated in Chapter 2. The saliva samples obtained from Miss S.M. and her family members and from Mrs G.G. were processed correctly by the technologist in charge at the blood bank in their respective hospitals before being despatched to my laboratory. The reagents used, which were all of commercial origin or had been obtained and standardised by myself according to customary strict criteria, were also used by the techniques described in Chapter 2. The anti-Le^b reagents used had been tested with the saliva of a secretor of H substance only and had been found to be type anti-anti-Le^{bL}.

12.4 RESULTS

12.4.1 *Mrs G.N.* The red cells of Mrs G.N. were not agglutinated by standard anti-A or anti-B reagents in saline or one-stage enzyme tests at 20°C, but were agglutinated weakly by anti-A,B reagents and by the anti-A+B+H antibodies of several 'Bombay' O_h donors in saline and one-stage enzyme tests at this temperature (Table 12.1, parts a and b). Her cells were also agglutinated weakly by some but not all the anti-A reagents and not by the anti-B reagents used, in saline tests at 10°C, and they were agglutinated by all the anti-A and by none of the anti-B reagents used, in saline tests at 4°C. A known high-titre immune anti-A, but not a known high-titre immune anti-anti-B, reagent agglutinated her cells weakly in one-stage enzyme tests at 20°C (Table 12.1, part a), and her cells were not agglutinated by *Ulex* anti-H lectin (Table 12.1, part c).

After exposing Mrs G.N.'s red cells to anti-A and anti-B reagents at 20°C for two hours, anti-A, and not anti-B, was readily recovered from them in eluates made in 6% bovine albumin by the 56°C technique of Landsteiner and Miller (1925). The I-antigen strength of her cells was not seen to be increased in titrations with three different anti-I reagents, and her serum was found to contain anti-B and IgM cold antibody that agglutinated O and A_2 adult cells moderately well (2+), A_1 adult and O cord cells weakly and failed to agglutinate O_h cells in saline and one-stage enzyme tests (Table 12.1, part d). The auto-antibody control test with her serum was negative. The specificity of the cold antibody in her serum was surprisingly confirmed as anti-H, when it was seen to be inhibited by an equal volume of commercial ABH blood group specific substances and not by an equal volume of saline in parallel tests.

TABLE 12.1

Reactions of red cells and serum of Mrs G.N. with various reagents

Part a

Reagent	Technique	°C	Mrs G.N.	Cells Positive	Negative Control
Anti-A			–	4	–
Anti-B		20	–	4	–
Anti-A,B			(3)	4	–
AB serum			–		
Anti-A	Saline		–	4	–
Anti-A		10	(2)	4	–
Anti-B			–	4	–
Anti-A			(3)	4	–
Anti-A		4	(3)	4	–
Anti-B			–	4	–
Anti-A	one stage 0,5% bromelin		–	4	–
Anti-B		20	–	4	–
Anti-A,B			1	4	–
AB serum			–		–
Anti-A	one stage 0,25% ficin		–	4	–
Anti-B		20	–	4	–
Anti-A,B			1	4	–
AB serum			–		
Immune anti-A	0,5%	20	2	4	–
Immune anti-B	Bromelin		–	4	–

Part b

'Bombay' anti-A+B+H sera	°C	Mrs G.N. Saline	Mrs G.N. 0,5% brom.	O cells Saline	O cells 0,5% brom.	O_h cells Saline	O_h cells 0,5% brom.
Mrs P. Gov. 165		(3)	2	4	4	–	–
S. Red. 215		(1)	1	4	4	–	–
R. Red. 223	20	(3)	1	4	4	–	–
Mr S. Nai. 212		–	(3)	3	4	–	–
Mrs P.C. 121		–	(3)	4	4	–	–

Part c

	Ulex anti-H dilutions 1	2	4	6	16	32	64
Mrs G.N.	–	–	–	–	–	–	–
A_1	1	(3)	(3)	(1)	–	–	–
A_2	4	4	4	3	3	1	(2)
B	2	2	1	1	(1)	–	–
O	4	4	4	3	1	(2)	(2)
A_1B	(3)	(±)	–	–	–	–	–
O_h	–	–	–	–	–	–	–

Part d

Cells	Mrs G.N. Serum Saline 20°C	0,5% brom. 20°C	0,25% ficin 20°C
A_1	(2)	(2)	1
A_2	2	2	3
B	4	4	4
O	2	2	4
O_h	–	–	–
Cord O	1	1	2

Other groups on Mrs G.N.'s cells were identified as follows:

MNSs, P_1, CDe/cde (Rh_1rh), hr^S+, hr^B+, C^W-, Lu(a–), K–, Kp(a–), Le(a–b+), Fy(a+), Jk(b+), I+, Sd(a+).

A saliva sample was not received from Mrs G.N. and, unfortunately, her family was not available for a family study. However, since the Lewis phenotype of her cells was Le(a–b+), she was presumed to be a secretor of ABH substances.

12.4.2 *Miss S.M.* Like the cells of Mrs G.N., the cells of Miss S.M. were not agglutinated by standard anti-A or anti-B reagents in saline or one-stage enzyme tests at 20°C, but they were agglutinated weakly by anti-A,B reagents (Table 12.2, part a). These tests were not repeated at lower temperatures in her case. Her cells were also not agglutinated by *Ulex* anti-H lectin in saline or in one-stage 0,5% bromelin tests at 20°C (Table 12.3, part a), but her cells were agglutinated strongly (4+) in saline and in one-stage 0,5% bromelin tests by some of the examples of anti-A+B+H, were not agglutinated in saline but were agglutinated weakly in one-stage 0,5% bromelin tests by some of the examples, and were not agglutinated by either technique by other examples of this antibody (Table 12.3, part b). However, the anti-A+B+H antibodies of R. Red. (216), which had reacted 4+ with her cells in saline tests, agglutinated them only to titre 4, while those of Mrs P. Gov. (165), which had not reacted with her cells, agglutinated the B and O control cells to titre 128 (Table 12.3, part a).

After the cells of Miss S.M. had been exposed to anti-A and anti-B for two hours at 20°C, anti-B but not anti-A was recovered readily from them in eluates made in saline by the 56°C heat technique of Landsteiner and Miller (1925). The I antigen strength of her cells was not found to be increased. Her serum was seen to contain a strong anti-A allo-antibody and a weak IgM cold antibody that agglutinated all the group O cells with which it was tested 1+ in saline tests and 3+ both in one-stage 0,5% bromelin and in one-stage 0,25% ficin tests at 20°C. Group B adult and group O cord cells were agglutinated by this antibody weakly, and the serum auto-antibody control test was negative (Table 12.2, part b). The identity of her cold antibody, the titre of which was 8 with O cells in one-stage 0,25% ficin tests, was thought to be anti-H; but no further studies were possible as the small quantity of her serum available had to be retained for crossmatching tests. Saliva inhibition tests showed that Miss S.M. was a secretor of B and H but not of A substances.

TABLE 12.2

Reactions of red cells and serum of Miss S.M. with various reagents

Part a

Reagent	Technique	°C	Miss S.M.	Cells Positive control	Cells Negative control
Anti-A			–	4	–
Anti-B	saline	20	–	4	–
Anti-A,B			(1)	4	–
Anti-A	one-stage		–	4	–
Anti-B	0,5%	20	–	4	–
Anti-A,B	bromelin		1	4	–
Anti-A	one-stage		–	4	–
Anti-B	0,25%	20	–	4	–
Anti-A,B	ficin		1	4	–

Part b

Cells	Miss S.M. serum Saline 20°C	0.5% brom. 20°C
A_1	4	4
A_2	3	4
B	(2)	1
O	1	3
O_h	–	–

TABLE 12.3

Reactions of anti-A+B+H with cells of Miss S.M., group O and group B adult cells and group O cord cells

Part b

'Bombay' anti-A+B+H	Miss S.M. cells		Adult O cells		Cord O cells		O_h cells	
	Saline	0,5% brom.	Saline	0,5% brom.	Saline	0,5% brom.	Saline	0,5% brom.
Mrs P. Gov. 165	–	–	4	4	4	4	–	–
,, 230	–	1	4	4	4	4	–	–
Mrs B. Gov. 166	1	2	4	4	4	4	–	–
,, 188	1	2	4	4	4	4	–	–
,, 231	2	4	4	4	4	4	–	–
S. Red. 203	–	3	4	4	4	4	–	–
,, 215	–	4	4	4	4	4	–	–
,, 227	(1)	4	4	4	4	4	–	–
R. Red. 204	2	4	4	4	4	4	–	–
,, 216	4	4	4	4	4	4	–	–
,, 223	4	4	4	4	4	4	–	–
Mrs L. Dew. 195	3	4	4	4	4	4	–	–
,, 219	3	4	4	4	4	4	–	–
Miss A. Gov. 214	–	(2)	4	4	4	4	–	–
M. Gov. 153	–	1	4	4	4	4	–	–
M. Gov. 213	(1)	1	4	4	4	4	–	–
S. Nai. 169	–	–	4	4	4	4	–	–
S. Nai. 212	–	–	4	4	4	4	–	–
S. Nai. 168	–	–	4	4	4	4	–	–
S. Nai. 211	–	(1)	4	4	4	4	–	–
A. Nar. 202	3	3	4	4	4	4	–	–
Mrs P.C. 121	1	3	4	4	4	4	–	–

Part a

Reagent	Technique	Cells	Dilutions 1	2	4	8	16	32	64	128	256	512
		Miss S.M.	–	–	–	–	–	–	–	–	–	–
	Saline	A_1	1	(3)	(2)	(1)	–	–	–	–	–	–
Ulex	20°C	A_2	4	4	4	4	3	2	1	(2)	–	–
anti-H		B	1	1	(3)	(2)	–	–	–	–	–	–
		O	4	4	4	4	3	2	1	(2)	–	–
Ulex	one-stage	Miss S.M.	–	–	–	–	–	–	–	–	–	–
anti-H	0,5%	O	4	4	4	4	4	4	4	4	2	1
	brom. 20°C	O_h	–	–	–	–	–	–	–	–	–	–
R. Red		Miss S.M.	4	2	1	–	–	–	–	–	–	–
216	Saline	B	4	4	4	3	3	2	1	(2)	–	–
anti-A+B+H	20°C	O	4	4	4	4	3	2	1	(2)	–	–
		O_h	–	–	–	–	–	–	–	–	–	–
Mrs P. Gov.		Miss S.M.	–	–	–	–	–	–	–	–	–	–
165	Saline	B	4	4	4	4	4	2	1	1	(2)	–
anti-A+B+H	20°C	O	4	4	4	4	2	1	1	(2)	–	–
		O_h	–	–	–	–	–	–	–	–	–	–

Other groups on Miss S.M.'s cells were identified as follows:

MN, U+, P_2, CDe/CDe (Rh_1Rh_1), hr^S+, hr^B+, Lu(a−), Le(a−b+).

The samples of blood and saliva from members of Miss S.M.'s family showed, in the appropriate tests, that her father, I-1, was O and secreted H but not A or B substances, and that her mother, I-2, and two elder sisters, II-1 and II-2, were all B and secreted B and H but not A substances (Figure 12.1). As expected, the cells of I-1 were agglutinated strongly, and the cells of I-2 and II-1 weakly, by *Ulex* anti-H lectin, but the cells of II-2 were agglutinated unexpectedly almost as strongly as the control A_2 cells by this reagent (Table 12.4). The Lewis phenotypes of the B as well as the O members in this family were determined with confidence using the anti-Le^a, anti-Le^{bL} and anti-Le^x reagents.

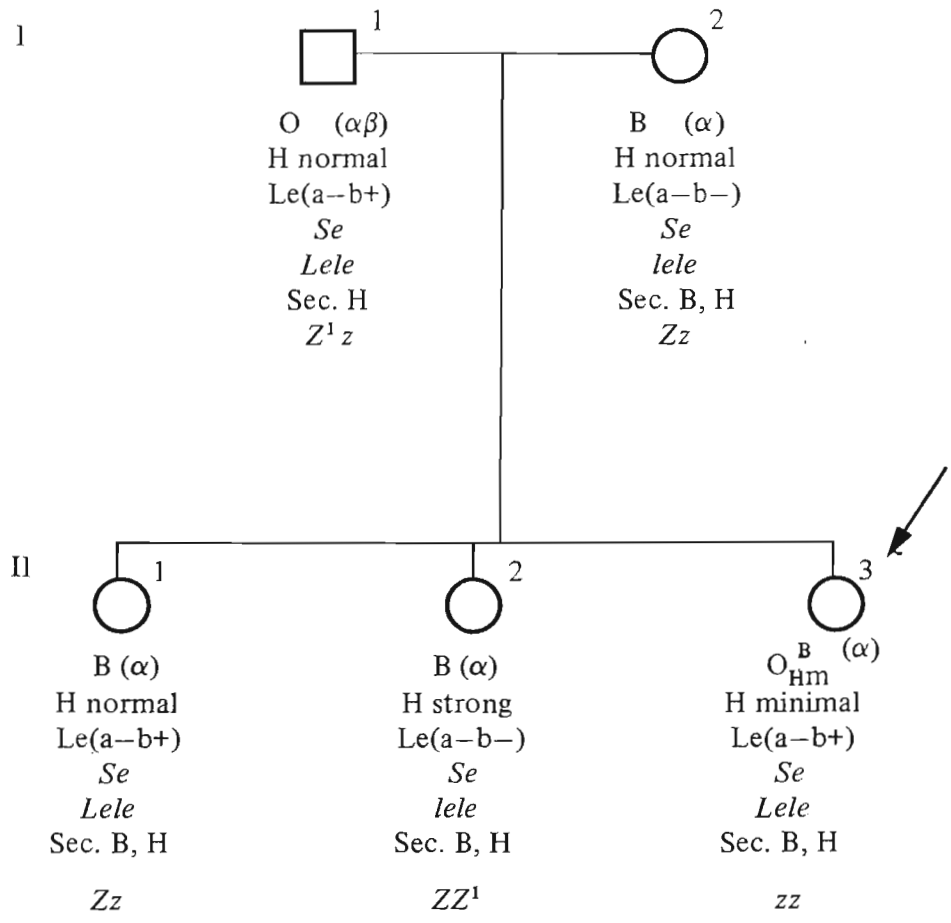
TABLE 12.4

Reactions of cells of members of Miss S.M.'s family with *Ulex* anti-H lectin

Cells	Ulex anti-H dilutions						
	1	2	4	8	16	32	64
I-1	4	4	4	4	4	2	1
I-2	1	(2)	–	–	–	–	–
II-1	1	1	(2)	–	–	–	–
II-2	4	4	3	3	1	(3)	–
A_1	(2)	–	–	–	–	–	–
A_2	4	4	4	4	2	1	–
B	1	(2)	–	–	–	–	–
O	4	4	4	4	3	2	1
A_1B	(1)	–	–	–	–	–	–

Figure 12.1

Family of Miss S.M., phenotype O^{B}_{Hm}



Key: ↙ = Proposita

Z, Z^1, z = Suggested regulator genes

12.4.3 *Mrs G.G.* The cells of Mrs G.G. were not agglutinated by standard anti-A or anti-B or by high titre immune anti-A and anti-B reagents. However, unlike the cells of Mrs G.N. and Miss S.M., her cells were also not agglutinated by anti-A,B reagents. *Ulex* anti-H lectin failed to agglutinate her cells, and her cells were agglutinated weakly by three examples of anti-A+B+H in saline tests at 20°C (Table 12.5, part a). No tests were made at temperatures lower than 20°C, and her cells were not subjected to absorption-elution studies. The I antigen strength of her cells was not found to be increased in titrations with several different anti-I reagents.

Mrs G.G.'s saliva did not inhibit a single dilution (1 in 8 in saline) of anti-A or anti-B, but it did inhibit a single dilution of *Ulex* anti-H lectin (diluted 1 in 4 in saline). In the subsequent titration, in which her saliva was diluted serially in saline and the anti-H reagent was used undiluted, she was seen to secrete as much H substance as the control O secretor (Table 12.5, part b). An anti-Le^{bL}, but not an anti-Le^{a}, reagent was inhibited by her saliva, but only the dilutions from 1 to 32 inhibited this antibody, whereas all the dilutions from 1 to 512 of the saliva of the control O secretor did so readily. The anti-Le^{bL} reagent (PPM 39) was known not to be inhibited by the saliva of a secretor of H but of no Lewis substances.

The titres of Mrs G.G.'s allo-antibodies were found to be 128 with A_2 cells and 64 with B cells, in saline titrations at 20°C. The titres therefore confirmed that her cells did not have A or B antigens. Her serum agglutinated adult O cells to titre 4 in a saline titration, and to titre 8 in a one-stage 0,5% bromelin titration, at 20°C. Cord O cells were agglutinated weakly, and the auto-antibody control test was negative. The reaction of her serum with O cells was not inhibited by the addition of an equal volume of the saliva of different O secretors, and one absorption of her serum with O cells removed all her antibody completely. As a result, the identity of her antibody was seen almost certainly to be anti-'O'-like, and it was similar to the antibodies often found in para-Bombay secretors, now thought to be anti-HI (Race and Sanger, p 26, 1975). The antibody was identified again in Mrs G.G.'s serum in 1968, and also in 1969.

In 1967, Mrs G.G.'s cells were typed with other reagents as follows:

MNs, P_1, CDe/CDe (Rh_1Rh_1), Lu(a−), K−, Le(a−b−), Fy(a+), I+

TABLE 12.5

Reactions of red cells, serum and saliva of Mrs G.G. with various reagents

Part a

Reagent	Technique	°C	Cells Mrs G.G.	Pos. control	Neg. control
anti-A			–	4	–
anti-B			–	4	–
anti-A,B	saline	20	–	4	–
anti-A+B+H			(3)	4	–
Ulex anti-H			–	4	–
immune anti-A			–	4	–
immune-anti-B			–	4	–

Part b

Subject	Reagent	Saliva dilutions 2	4	8	16	32	64	128	256	512
Mrs G.G.	Ulex	–	–	–	–	–	1	2	3	4
O secretor	anti-H	–	–	–	–	–	2	4	4	4
O non-secretor	undiluted	4	4	4	4	4	4	4	4	4
Mrs G.G.		–	–	–	–	–	1	2	3	3
O secretor	anti-Le^b	–	–	–	–	–	–	–	–	–
O non-secretor	undiluted	1	2	3	3	3	3	3	3	3

Part c

Cells	Technique	°C	Serum dilutions 1	2	4	8	16	32	64	128	256
A_1			4	4	4	3	3	2	(3)	(2)	–	–
B	Saline	10	4	4	4	4	3	1	(1)	–	–	–
O			3	2	(2)	–	–	–	–	–	–	–
A_1			4	4	4	4	3	2	2	1	(1)	–
B	Bromelin	20	4	4	4	4	3	2	(2)	–	–	–
O			4	3	1	(1)	–	–	–	–	–	–

The Le(a−b−) phenotype of her cells was thought to be false, both because she secreted Le^b substance and because Le^b antigen is commonly absent from the cells during pregnancy (Brendemoen, 1952). However, as her cells still typed as Le(a−b−) when she was not pregnant in 1968, this finding, and the normal secretion of H but weak secretion of Le^b substance noted in her saliva in 1967, suggested, in her, that production of Le^b substance was partially inhibited. Mrs G.C.'s siblings were all group O (Figure 12.2) and their cells were all agglutinated by *Ulex* anti-H lectin to the same titre as the control O cells. Their sera also contained anti-A,B allo-antibodies of normal strength, and no other antibodies were identified in their sera.

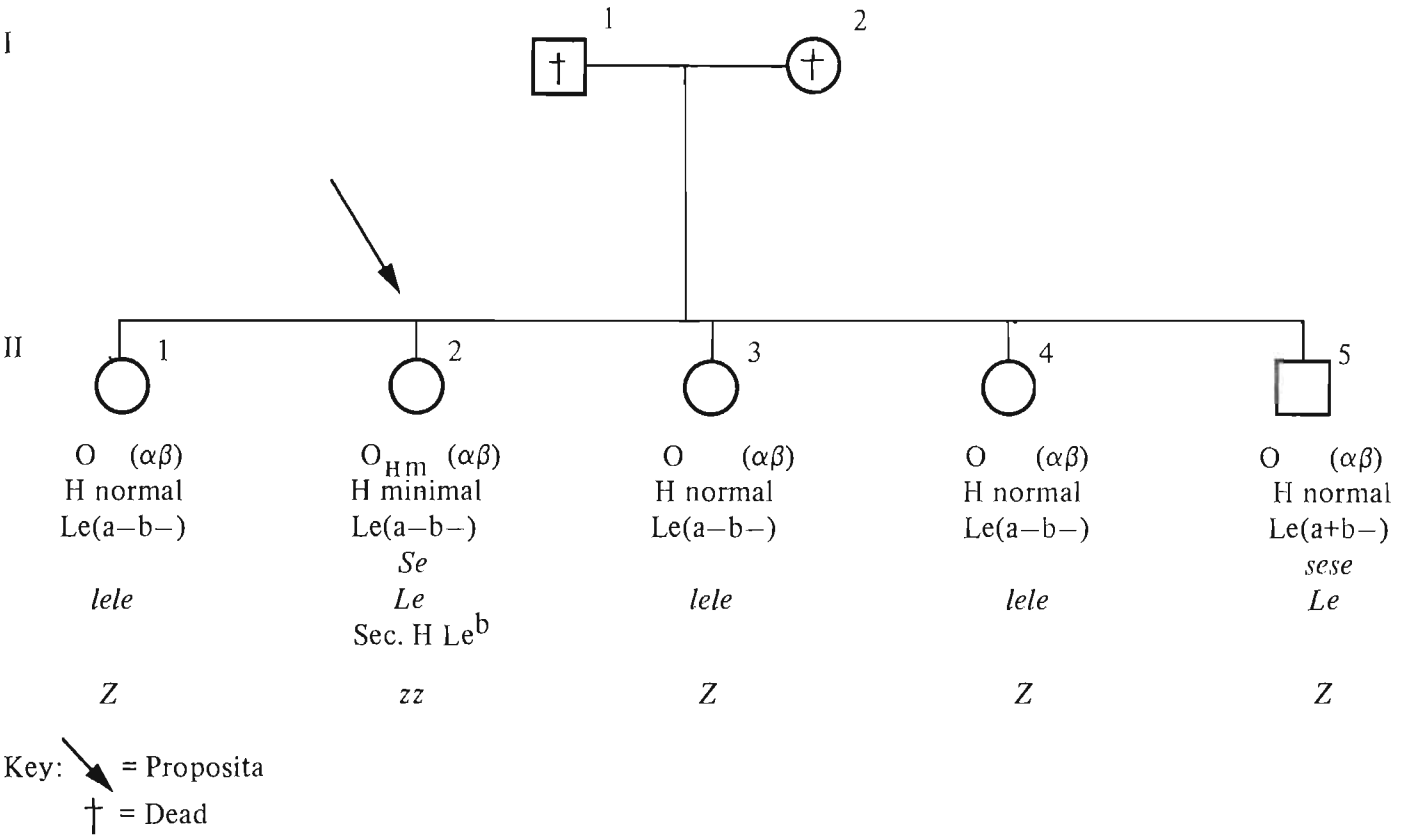
12.5 DISCUSSION

The cells of Mrs G.N., Miss S.M. and Mrs G.G. were identified as para-Bombay, phenotype O^A_{Hm}, O^B_{Hm} and O_{Hm} respectively as, although the groups of their cells appreared in tests with anti-A, anti-B and anti-A,B to be weak group A variant, weak group B variant and O respectively, their cells were not agglutinated by *Ulex* anti-H lectin and were only weakly agglutinated by anti-A+B+H. In addition, the two women and the child were all secretors of ABH substances, and their sera, which contained anti-B, anti-A and anti-A.B respectively, had been found to contain weak IgM cold antibodies that agglutinated all the O cells with which they were tested. The cold antibody in the serum of Mrs G.N., which was surprisingly seen to be inhibited by H substance, appeared to be anti-H, the cold antibody in the serum of Miss S.M. might have been either anti-H or anti-HI, and the cold antibody in the serum of Mrs G.G., was confirmed as being anti-HI. Perhaps the identification of this antibody as anti-H or anti-HI in the para-Bombay's who are secretors depends upon the amount of H antigen present on the owner's cells.

Sringarm, Chupungart and Giles (1972) suggested that O_{Hm} cells were agglutinated weakly by anti-A+B+H because their weakly-expressed H antigen was not recognised by *Ulex* anti-H lectin. In the present study, it was difficult to be certain whether the cells of Mrs G.N. and Miss S.M. had been agglutinated weakly by the anti-A+B+H used, because their cells had weak A or weak B as well as weak H antigen. The cells of Mrs G.N. had been agglutinated by the anti-A+B+H of S. Red. (215), R. Red. (223) and Mrs P.C. (121) less strongly than the cells of Miss S.M., but her cells had also been agglutinated more strongly than those of Miss S.M. by the anti-A+B+H of Mrs P. Gov. (165) (Tables 12.1 and 12.3). If this agglutination was solely due to

Figure 12.2

Family of Mrs G.G., phenotype O_{Hm}



anti-H, the four examples of anti-A+B+H used would have been expected to have reacted equally strongly with the cells of both Mrs G.N. and Miss S.M. The problem might have been resolved if the anti-A+B+H antibodies had been inhibited beforehand with saliva containing H but no A or B substances.

The strong H antigen expressed on the cells of II-2 in the family of Miss S.M. suggested, in addition to a normal Z regulator gene, that II-2 might have inherited a mutant regulator gene, arbitrarily called Z^1 by myself, which coded for excess H antigen on the membranes of her cells. The Z^1 gene would have been inherited by her from her father, I-1 (Figure 12.1), in whom its influence was likely to have been masked by the strong H antigen expected in a group O environment. The gene was not present in her mother, I-2, or in her sister, II-1, as their cells possessed the normally weak H antigen expected in individuals who were group B. Moreover, as the para-Bombay phenotype (and therefore the *zz* genes) of II-3 showed that I-1 was heterozygous Z^1z, the Z^1 gene was clearly capable of expressing itself in single dose. Some support for the existence of a Z^1 gene in the Indians of Natal was provided earlier in this thesis by the 14,52 to 39,56% Indian group B donors who had increased (or 'high') H antigen (Table 3.6, p 57). It was tempting to suggest as well that the A_1 'high' H phenotype recorded in Indians elsewhere (Sathe and Bhatia, 1974) might also be due to the inheritance of a Z^1 gene.

The reduced secretion of Le^b substance and the phenotype Le(a−b−) instead of Le(a−b+) cells in Mrs G.G. suggested that the expected weak *H* and normal strength *Le* gene-specified enzymes in her case might be unable to convert as much precursor substrate as usual into Le^b substance. This situation might have been due either to the limited amount of her H enzyme, or to her *H* gene or her H enzyme malfunctioning in some way. Her limited amount of H enzyme, or her malfunctioning *H* gene or H enzyme, in conjunction with her normal *Le* gene enzyme, might not be able to convert or promote the conversion of precursor glycoprotein substrate into Le^b substance as efficiently as in other people. The situation seemed to be an echo of the Lewis phenotype findings in the parents and children of O_h people, described in Chapter 10, and in the Indian dispermic chimaera shose unusual Lewis phenotype is described in Chapter 14.

No Indians with para-Bombay phenotypes appeared to have been reported before from Natal.

12.6 SUMMARY

Three Natal Indians whose red cells were para-Bombay, phenotype O_{Hm}^{A}, O_{Hm}^{B} and O_{Hm} respectively, have been described. The Indians were all secretors of ABH substances and, as well as their normal ABO allo-antibodies, their sera contained cold antibodies which were identified, in the first Indian as anti-H, in the second Indian as either anti-H or anti-HI and in the third Indian as anti-HI. The family of the Indian whose cells were phenotype O_{Hm}^{B} contained evidence that a mutant regulator gene, Z^{1}, may have caused increased H red cell antigen expression in her family.

CHAPTER 13

THE MNSs AND RHESUS BLOOD GROUP SYSTEMS POPULATION FREQUENCIES

13.1 INTRODUCTION

13.1.1 The MNSs system

In India, the MNSs system is characterised by a high *M* gene frequency of between 65 and 70%. The high frequency pertains throughout the continent, except in Pakistan and Gujarat, where it is lower; and in the north-east it exceeds 70%. The gene *S* has a frequency of approximately 30% in the Aryan language-speaking Indians in northern India, but in the central and southern regions, where the Dravidian languages are spoken, its frequency is less than this. A relatively high *MS* chromosome frequency everywhere is considered appropriate to the Caucasoid background of the Indians (Mourant, Kopéc and Domaniewska-Sobczak, 1976).

The S-s-U- blood group phenotype, the allele or alleles responsible for which are known by the symbol *u* or S^u (Race and Sanger, p 101, 1975) is thought to be a Negroid character and has so far not been recorded in Indians from India. However, Moores recorded an Indian family with these cells in Natal in 1972. The family study and the subsequent S and s antign dosage titrations with the members' cells are described in Chapter 15.

The phenotype and gene frequencies in the MNSs system in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal are presented in this chapter. Their MN, MNS and MNSs frequencies were studied separately, and were compared both between themselves and with those in Indian populations of similar composition recorded in India and South Africa by other workers. The cells of the Natal Indians were also tested for evidence of the low frequency phenotypes M_1, Henshaw, Mo(a+), s^D, Sk(a+), Ny(a+), V^W+ and U–; and their sera were tested with rare M^g, Mo(a+), Ny(a+), Ri(a+) and Sk(a+) cells in an attempt to find examples of the corresponding antibodies.

13.1.2 The Rhesus system

The Rhesus chromosome and gene frequencies vary considerably in Indians, and in the north-west of India are similar to those of the people in the Mediterranean area. In the north-east, however, and in the Dravidian language-speaking people in southern India, the frequency of the chromosome *CDe* is higher and of *cde* lower. The *cDE* chromosome occurs in a little less than 10% of the Indians throughout the continent, except in the north, east and extreme south, and the chromosomes *CDE* and *Cde* are present in about 2% of Indians. Surprisingly, the *cdE* chromosome has been seldom recorded, even among the Aryan language-speaking Indians in the north-west of India (Mourant *et al.*, 1976).

The studies of Rhesus phenotype, chromosome and gene frequencies in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal are presented in this chapter. Their D^u and C^w frequencies were also determined. As in the MNSs system, the frequencies obtained were compared with each other and with those recorded in similar Indian populations elsewhere by other workers. The cells of the Natal Indians were tested as well for evidence of the low frequency phenotypes C^wde, Cde^S or VS, D+G−, hr^S- and hr^B-, and the findings have been recorded here for interest.

13.2 MATERIALS AND METHODS

13.2.1 The MNSs system

The blood samples used in this study were from Natal Indian males and unmarried females, as described in Chapter 2. The reagents used were standardised examples of human anti-M, anti-M_1, anti-S and anti-s, *Vicia graminea* anti-N lectin, and rabbit immune anti-Henshaw absorbed previously for unwanted agglutinins. The anti-Henshaw, prepared by myself, had been supplied to other laboratories in South Africa and Australia for their work. The rare anti-Mo^a and anti-Ny^a antibodies were kindly sent by Dr L. Kornstad of Norway, the anti-Sk^a by Dr C. Giles of London and the anti-V^w by D.F. Skov of Copenhagen. The sample of the first anti-s^D to have been described was kindly sent by Dr M. Shapiro and Miss M. Le Roux of the South African Blood Transfusion Service in Johannesburg. The discovery and identification of this antibody and its corresponding antigen, s^D, were reported in a poster exhibited by Shapiro and Le Roux at the South African Blood Transfusion Congress held at Port Elizabeth in 1979. The anti-U was an eluate prepared by myself in

Durban in 6% bovine albumin by the ether technique of Vos and Kelsall (1956) from serum donated by the proposita in the Natal S-s-U- Indian family described in Chapter 15. The elution, from CDe/CDe cells, was necessary as the proposita had anti-c as well as anti-U in her serum. The rare Mo(a+), Ny(a+), Sk(a+), M^g, Ri(a+) and V^W+ cells were gifts from colleagues through the SCARF exchange system, and the rare s^D+ cells were kindly sent by Dr M. Shapiro and Miss M. Le Roux.

The techniques used in these studies are described in Chapter 2. The anti-M, anti-M_1, anti-N, anti-Henshaw, anti-Mo^a, anti-Ny^a, anti-Sk^a and anti-V^W were used by saline technique at the optimum temperature for each reagent. Where necessary, the cells were also suspended in LISS. The anti-s^D reagent was used either by one-stage 0,5% bromelin or saline antiglobulin technique. The anti-S, anti-s and anti-U reagents were used either by saline or albumin indirect antiglobulin technique. Some of the anti-S reagents were incubated at 20°C instead of at 37°C, and the anti-s reagent was conserved as far as possible by using it only for the S+ cell samples. The S- cell samples were tested for s with anti-U. The sera of the Natal Indians were tested with the rare phenotype cells by saline technique at 20°C. The specificity of each reagent was checked before use and also daily with negative, heterozygous positive and, wherever possible, homozygous positive control cells throughout the test period.

The gene frequencies in the MNSs system were calculated by the method suggested by Mourant *et al.* (p 52–53, 1976), and the frequency comparisons were made by the 2 X 2 table and χ^2 method without Yates' correction.

13.2.2 The Rhesus system

Again, the blood samples used were from Natal Indian males and unmarried females, their samples being prepared for these tests as described in Chapter 2. The reagents used were standardised examples of pooled anti-D sera; anti-C, anti-E, anti-c, anti-C^W and anti-K from local donors, and an anti-e+K reagent kindly supplied by Dr M. Contreras and Dr Cleghorn of London. The anti-e+K and anti-K reagents were used in parallel throughout. All e+, K+ samples were subsequently tested with a pure anti-e reagent. The anti-G, anti-hr^S and anti-hr^B reagents were from local donors, and the rare anti-VS(–e^S) reagent had been kindly supplied by P. Issitt of Cincinnati.

The techniques used in these tests were also those described in Chapter 2. Some of the anti-D reagents were used by saline technique at 37°C with cells suspended in

saline, and others by one-stage 0,5% bromelin technique or in the 15 channel autoanalyser. The D^u reagents were tested by saline indirect antiglobulin technique. All the cell samples identified as negative with saline, bromelin and autoanalyser anti-D reagents were subsequently tested with D^u serum. The anti-C, anti-E, anti-c, anti-e+K and anti-hr^S reagents were used by one-stage 0,5% bromelin technique, and the anti-C^w and anti-hr^B reagents were used by one-stage 0,25% ficin technique. The anti-K, anti-G and anti-VS (–e^S) reagents were employed by saline indirect antiglobulin technique. All the reagents were tested before use to ensure that they were specific, and they were also tested with negative and heterozygous positive control cells daily in parallel with the tests.

The Rhesus antigen, chromosome and gene frequencies were calculated by the methods suggested by Mourant (p 53–54, 1976), and the χ^2 and P values were calculated as before by the 2 X 2 table method without Yates' correction.

13.3 RESULTS

13.3.1 The MNSs system

Table 13.1 shows the MN phenotype and gene frequencies in two Indian populations from a predominantly Hindi-speaking part of India recorded by other workers, and Table 13.2 the frequencies in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal. . Significantly different M and N frequencies between the Natal Hindi-speaking Indians and the Bengali population of Roy *et al.* and between the Natal Hindu population (Natal Tamil-, Telegu- and Hindi-speaking populations pooled) and this population were observed. However, they were disregarded as the χ^2 for internal consistency in the population of Roy *et al.* was 3,62. No other significant differences were found.

The frequencies estimated with anti-M, anti-N and anti-S only in an Indian population from a predominantly Hindi-speaking part of India by Boyd and Boyd are shown in Table 13.3, and those in the Natal Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Table 13.4. A significantly different NNS frequency between the Natal Hindi-speaking and Natal Moslem Indian populations was found, but no other comparisons made were significant. The known high M frequency in Indians was observed in Table 13.4, and the typically high Caucasoid MS chromosome frequency

TABLE 13.1

MN phenotype and gene frequencies in Indians of India recorded by others using anti-M and anti-N

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies MM	MN	NN	Gene frequencies *M*	*N*	χ^2
Bengali (Hindi)	Calcutta in W. Bengal	Macfarlane, 1939	1 793 page 263	130	No.	46	66	18	0,6077	0,3923	0.55
					%	35,38	50,77	13,85			
Bengali (Hindi)	Calcutta in W. Bengal	Roy *et al.*, 1964	2 436 page 263	1 123	No.	348	582	193	0,5690	0,4310	3.62
					%	30,99	51,82	17,19			

TABLE 13.2

MN phenotype and gene frequencies in 514 Natal Indian males and unmarried females tested with anti-M and anti-N

Category	Number tested		Phenotype frequencies M	MN	N	Gene frequencies *M*	*N*	χ^2 (1 d.f.)
Tamil	113	No.	48	53	12	0,6593	0,3407	0,2
		%	42,48	46,90	10,62			
Telegu	109	No.	37	58	14	0,6056	0.3944	1,5
		%	33,95	53,21	12,84			
Hindi	104	No.	43	52	9	0,6635	0,3365	1,5
		%	41,35	50,00	8,65			
Moslem	188	No.	65	97	26	0,6037	0,3963	2,7
		%	34,57	51,60	13,83			

Significance of differences

All differences were not significant between the populations in Table 13.1 and between the relevant populations in Tables 13.1 and 13.2 at level $P \leq 0,05$ except:–

	χ^2	P
M in Natal Hindi and Bengali (Roy *et al.*, 1964)	4,7	$< 0,05 > 0,02$
M in pooled Natal Hindu and Bengali (Roy *et al.*, 1964)	7,8	$< 0,01 > 0,001$
N in Natal Hindi and Bengali (Roy *et al.*, 1964)	5,0	$< 0,05 > 0,02$

was apparent there as well.

Table 13.5 contains the MNSs chromosome frequencies recorded by other workers using anti-M, anti-N, anti-S and anti-s in Hindu and Moslem Indian populations in India and Johannesburg, and Table 13.6 the frequencies in the four Natal Indian populations. Significantly different MMSS, MMSs, MMss and MNSs frequencies

TABLE 13.3

MNSs phenotype and chromosome frequencies in Indians of India recorded by others using anti-M, anti-N and anti-S only

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies MMS	MsMs	MNS	MsNs	NNS	NsNs	χ^2 (2 d.f.)
	Born in	Boyd and	428		No.	44	35	62	47	21	21	
Bengali (Hindi)	Bengal	Boyd, 1954	page 287	230								0,52
					%	19,13	15,22	26,96	20,43	9,13	9,13	

Chromosome frequencies			
MS	*Ms*	*NS*	*Ns*
0,2019	0,3785	0,1289	0,2907

between the Natal Telegu-speaking Indians and the presumed predominantly Telegu-speaking Hindu population of Seth (1967) were found. However, the χ^2 test for internal consistency in the latter population was 10,77 (Mourant, *et al.*, 1976). The MNSs frequency shown in Table 13.6 in the Natal Moslem Indian population differed significantly from that in the Moslem population of Nurse and Jenkins shown in Table 13.5, and the MMss, MNSs and NNss frequencies in the combined Hindu population in Natal differed significantly from the corresponding frequencies in the Hindu population of Nurse and Jenkins. The characteristically high M frequency in Indians and MS frequency in Caucasoids was observed again in the populations in Table 13.6.

An interesting diversion was provided by estimating the M_1 phenotype and gene frequencies in the four Natal Indian populations, and the results are shown in Table 13.7. The anti-M_1 reagent had recently been identified in a local Zulu donor. Low M_1+ phenotype frequencies, varying from 0,47% to 4,81%, were found in all four populations, and were significantly different between the Natal Tamil and Natal Moslem Indian populations. The M_1 antigen was known to occur more frequently in Blacks than in Whites, but no estimates appeared to have been made before in Indians (Race and Sanger, p 107–108, 1975).

None of the M_1-positive samples were tested with anti-S and anti-s as the latter reagent was in very short supply.

TABLE 13.4

MNSs Phenotype and chromosome frequencies in 496 Natal Indian males and unmarried females tested with anti-M, anti-N and anti-S only

Category	Number tested		Phenotype frequencies						Chromosome frequencies				
			MMS	MsMs	MNS	MsNs	NNS	NsNs	*MS*	*Ms*	*NS*	*Ns*	χ^2
Tamil	105	Number	26	17	32	20	4	6	0,2772	0,3799	0,0829	0,2600	2,4
		%	24,76	16,19	30,48	19,05	3,81	5,71					
Telegu	101	Number	28	15	21	28	5	4	0,2312	0,4372	0,0867	0,2449	4,0
		%	27,73	14,85	20,79	27,72	4,95	3,96					
Hindi	100	Number	24	12	26	24	9	5	0,2247	0,3853	0,1350	0,2550	2,0
		%	24,00	12,00	26,00	24,00	9,00	5,00					
Moslem	190	Number	48	23	56	41	6	16	0,2845	0,3445	0,0666	0,3044	1,9
		%	25,26	12,11	29,47	21,58	3,16	8,42					

Significance of differences

All differences were not significant between the populations in Table 13.4 and between the relevant populations in Tables 13.3 and 13.4 at level $P \lesssim 0,05$

TABLE 13.5

MNSs phenotype and chromosome frequencies in Indians of India and Johannesburg recorded by others using anti-M, anti-N, anti-S and anti-s

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies									
						MMSS	MMSs	MMss	MNSS	MNSs	MNss	NNSS	NNSs	NNss	χ^2 (2 d.f.)
Hindu (Telegu)	Rourkela in Orissa	Seth, 1967	2607 page 302	215	No.	2	19	71	4	20	64	2	5	28	
					%	0,93	8,84	33,02	1,86	9,30	29,77	0,93	2,33	13,02	10.8
Hindu	Johannesburg	Nurse and Jenkins, 1977		53	No.	1	14	10	6	6	10	0	0	6	
					%	1,89	26,41	18,87	11,32	11,32	18,87	0,00	0,00	11,32	*
Moslem	Johannesburg	Nurse and Jenkins, 1977		56	No.	2	9	15	2	4	13	0	0	11	
					%	3,57	16,07	26,79	3,57	7,14	23,22	0,00	0,00	19,64	*

Chromosome frequencies

MS	*Ms*	*NS*	*Ns*
0,0874	0,5452	0,0522	0,3152
0,3207	0,3585	0,0000	0,3208
0,1874	0,4465	0,0000	0,3661

* χ^2 not calculated due to small size of sample

TABLE 13.6

MNSs phenotype and chromosome frequencies in 618 Natal Indian males and unmarried females tested with anti-M, anti-N, anti-S and anti-s

Category	Number tested		Phenotype frequencies MMSS	MMSs	MMss	MNSS	MNSs	MNss	NNSS	NNSs	NNss	χ^2 (2 d.f.)
Tamil	199	Number	11	33	29	8	40	48	3	12	15	1,5
		%	5,53	16,58	14,57	4,02	20,10	24,12	1,51	6.03	7,54	
Telegu	102	Number	10	19	12	5	19	23	1	4	9	13,5
		%	9,80	18,63	11,77	4,90	18,63	22,55	0,98	3,92	8,82	
Hindi	113	Number	8	19	20	3	19	29	1	7	7	2,8
		%	7,08	16,81	17,70	2,66	16,81	25,66	0,89	6,19	6,20	
Moslem	204	Number	13	35	27	12	47	41	3	7	19	3,4
		%	6,37	17,16	13,24	5,88	23,04	20,10	1,47	3,43	9,31	

Category	Number tested	Chromosome frequencies *MS*	*Ms*	*NS*	*Ns*
Tamil	199	0,2142	0,3938	0,1100	0,2820
Telegu	102	0,2118	0,4206	0,1509	0,2167
Hindi	113	0,2105	0,4310	0,0948	0,2637
Moslem	204	0,2635	0,3493	0,0918	0,2954

Significance of differences

All differences were not significant between the populations in Table 13.6 and between the relevant populations in Tables 13.5 and 13.6 at level $P < 0,05$ except:–

	MMSS,	MMSs,	MMss,	MNSs	NNss	
χ^2	15,0	6,3	16,2	5,6		Telegu and Hindi (Seth)
χ^2			6,3	4,1	10,1	Natal Hindu and Hindu (N. & J.)
χ^2				4,1		Natal Moslem and Moslem (N. & J.)

TABLE 13.7

M_1 phenotype and gene frequencies in 579 Natal Indian males and unmarried females tested with anti-M_1

Category	Number tested		Phenotype frequencies		Gene frequencies	
			M_1+	M_1-	M_1	non-M_1
Tamil	215	No.	1	214	0,0024	0,9976
		%	0,47	99,53		
Telegu	105	No.	2	103	0,0095	0,9905
		%	1,90	98,10		
Hindi	155	No.	2	153	0,0065	0,9935
		%	1,29	98,71		
Moslem	104	No.	5	99	0,0243	0,9757
		%	4,81	95,19		

All M_1 samples were phenotype MM except one Moslem who was phenotype MN

Significance of differences

All differences were not significant between the populations in Table 13.7 at level $P < 0,05$ except:

	χ^2	P
Natal Tamil and Natal Moslem	7,2	$< 0,01 > 0,001$

A search was conducted in the four Natal Indian populations with anti-Henshaw, anti-Mo^a, anti-s^D, anti-Sk^a, anti-Ny^a, anti-V^W and anti-U, and with Mo(a+), Ny(a+), Sk(a+), M^g and Ri(a+) cells, for evidence of the corresponding low frequency antigens and antibodies respectively. The results are shown in Tables 13.8 and 13.9. Two examples of anti-M^g and 12 of anti-Sk^a were identified, but these antibodies were known to occur frequently in human sera. The findings were included here for interest.

TABLE 13.8

Natal Indians tested for phenotypes of low frequency in the MNSs system

Reagent	Number of Natal Indians tested					
	No positives found					
	Tamil	Telegu	Hindi	Moslem	Random	Total
Anti-Henshaw	265	137	161	258		821
Anti-Mo^{a}					384	384
Anti-s^{D}	455	161	259	107		982
Anti-Sk^{a}	205	118	130	214	115	782
Anti-Ny^{a}	158	110	110	143	121	642
Anti-V^{w}	115	103	103	180		501
	No negatives found					
Anti-U					1 248	1 248

TABLE 13.9

Natal Indians tested for antibodies with cells of rare low frequency phenotypes

Category	Cells	Number tested	Antibodies detected	Antibodies not detected	%
Random	Mo(a+)	82	0	82	0
Random	Ny(a+)	381	0	381	0
Random	Sk(a+)	365	12	353	3,29
Random	M^{g}+	487	2	485	0,41
Random	Ri(a+)	667	O	667	0

TABLE 13.10

Rhesus D phenotype and gene frequencies in Indians of India recorded by others in tests with anti-D

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies D+	Phenotype frequencies D−	Gene frequencies *D*+	Gene frequencies *D*−
Hindu	Madras	Ranganathan *et al.*, 1948	2344 page 365	222	No.	206	16	0,7315	0,2685
					%	92,79	7,21		
South Indians (Tamil)	Madras	Rao, 1952	2349 page 365	132	No.	127	5	0,8053	0,1947
					%	96,21	3,79		
Bengali (Hindi)	Calcutta	Roy *et al.*, 1959	2435 page 364	1 435	No.	1 359	76	0,7698	0,2302
					%	94,70	5,30		
Hindu (Hindi)	Aligarh in Uttar Pradesh	Tyagi and Hameed, 1968	2952 page 364	1 511	No.	1 463	48	0,8217	0,1783
					%	96,82	3,18		
Bengali (Hindi)	Calcutta	Sen *et al.* 1959	2593 page 365	2 200	No.	2 134	66	0,8268	0,1732
					%	97,00	3,00		
Moslems	Aligarh in Uttar Pradesh	Tyagi and Hameed, 1968	2952 page 364	1 343	No.	1 293	50	0,8071	0,1929
					%	96,28	3,72		

13.3.2 The Rhesus system

The D+ and D– phenotype and gene frequencies recorded by other workers in Indian populations in India of comparable composition to the Natal Indian populations in this thesis, and estimated using anti-D but not D^u serum, are shown in Table 13.10. Table 13.11 contains the frequencies determined by Hirsch in random Indians in Natal with both anti-D and D^u sera, included here for interest, and Table 13.12 the frequencies in 'first time' Tamil-, Telegu- and Hindi-speaking and Moslem Indian male and unmarried female blood donors in Natal. The frequencies in the latter were determined using anti-D by one-stage 0,5% bromelin technique and using anti-CD, anti-E and D^u serum to test all the D– cell samples. The languages considered likely to have been spoken by the populations in Table 13.10 are shown in brackets under 'Category', and the gene frequencies in this table were recalculated to ensure that they were comparable. An exceptionally high D+ frequency was noted in all three tables, and the *cde* frequencies between the Natal Hindi- and the Natal Telegu-speaking Indians, and between the Natal Hindu Indians and the Madras Hindu population of Ranganathan *et al.*, differed significantly. However, no significant difference in *cde* frequencies between the Natal Tamil-speaking Indians and the latter population was found.

TABLE 13.11

Rhesus D phenotype and gene frequencies in random Natal Indians recorded by others in tests with anti-D and D^u serum

Category	Author	Number tested		Phenotype frequencies D+	D^u	D–	Gene frequencies D^+	D^u	D–
Ante-			No.	1 366	2	104			
natal	Hirsch, 1958	1 472					0,7317	0,0026	0,2657
			%	92,80	0,14	7,06			

Table 13.13 shows the Rhesus phenotype and chromosome frequencies recorded by other workers using anti-C, anti-D, anti-E anti-c and anti-e in Hindu and Moslem populations in India and Johannesburg, and Tables 13.14 and 13.15 the frequencies in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal. The high

TABLE 13.12

Rhesus D and other phenotype frequencies in 2 442 Natal Indian male and unmarried female 'first time' blood donors of the Natal Blood Transfusion Service tested with anti-CD, anti-D, D^u serum and anti-E only

Category	Number tested		Phenotype frequencies D+	Low grade CD^ue	Cde	cdE	cde	Gene frequencies *D*+	*D*−
Tamil	987	No.	939	2	1	0	45	0,7865	0,2135
		%	95,14	0,20	0,10	0,00	4,56		
Telegu	398	No.	370	4	3	0	21	0,7702	0,2298
		%	92,96	1,00	0,75	0,00	5,28		
Hindi	570	No.	553	0	2	0	15	0,8378	0,1628
		%	97,02	0,00	0,35	0,00	2,63		
Moslem	487	No.	465	0	1	1	20	0,7973	0,2027
		%	95,48	0,00	0,20	0,20	4,11		

Significance of differences

All differences were not significant between the populations in Table 13.12 and between the relevant populations in Tables 13.10, 13.11 and 13.12 at level $P \leq 0,05$ except:

	χ^2	P
cde in Natal Hindi and Natal Telegu	4,6	$<0,05>0,02$
cde in Natal Hindu and Hindu (Ranganathan et al., 1948)	4,4	$<0,05>0,02$

CDe chromosome frequencies in the Natal Tamil-, Telegu- and Hindi-speaking and the lower *CDe* chromosome frequency in the Natal Moslem Indians agreed with the *CDe* frequencies noted by Mourant *et al.* (1976) in similar populations in India, and the χ^2 test for CDe/CDe frequencies between the Tamil-speaking and Moslem Indians in Natal was statistically significant ($\chi^2 = 9,3$; $P < 011 > 0,001$). Significant χ^2 tests were also observed for CDe/CDe between the Hindi-speaking and both the Moslem and Telegu-speaking Indians in Natal ($P < 0,001$ and $< 0,02 > 0,01$ respectively). However, the high *CDe* frequency in the Natal Hindi-speaking Indians (69,66%) was exceeded by the frequency in the Hindu of Johannesburg (72,65%; Table 13.3), and

TABLE 13.13

Rhesus phenotype and chromosome frequencies in Indians of India and Johannesburg recorded by others using anti-C, anti-D, anti-E, anti-c and anti-e

Category	Hindu (Telegu)		Moslem		Hindu		Moslem	
Place	Rourkela in Orissa		Punjab and W. Pakistan		Johannesburg		Johannesburg	
Authors	Seth, 1967		Chaudhri *et al.*, 1952		Nurse and Jenkins, 1977		Nurse and Jenkins, 1977	
Reference No. in Mourant *et al.*, 1976	2607 page 444		593 page 484					
Number tested	200		101		53		56	
	Phenotype frequencies							
	No.	%	No.	%	No.	%	No.	%
cde/cde	15	7,50	5	4,95	3	5,66	6	10,71
cDe/ce	5	2,50	0	0,00	0	0,00	2	3,57
CDe/ce	65	32,50	33	32,67	14	26,42	22	39,29
CDe/CDe	71	35,50	46	45,55	29	54,72	15	26,79
CDe/cDE	32	16,00	10	9,90	5	9,43	7	12,50
cDE/cde	6	3,00	5	4,95	2	3,77	4	7,14
cDE/cDE	1	0,50	0	0,00	0	0,00	0	0,00
Cde/cde	3	1,50	1	0,99	0	0,00	0	0,00
cdE/cde	2	1,00	0	0,00	0	0,00	0	0,00
CDE/cDE	0	0,00	1	0,99	0	0,00	0	0,00
	Chromosome frequencies							
CDe R^1	0,5772		0,6489		0,7265		0,5268	
cDE R^2	0,0769		0,0768		0,0660		0,0983	
Cde r'	0,0278		0,0221		0,0000		0,0000	
cde r	0,2533		0,2449		0,2075		0,3247	
cdE r''	0,0256		0,0000		0,0000		0,0000	
cDe R^O	0,0392		0,0000		0,0000		0,0502	
CDE + CdE $R^Z + R^y$	0,0000		0,0073		0,0000		0,0000	
χ^2 (3 d.f.)	5,27		13,35		*		*	

* χ^2 not calculated due to small size of sample.

TABLE 13.14

Rhesus phenotype frequencies in 1 168 Natal Indian males and unmarried females tested with anti-C, anti-D, anti-E, anti-c, anti-e and D^u serum, D^u included with D+ Indians

Category	Number tested		Phenotype frequencies										χ^2 (3 d.f.)
			CDe/CDe Rh_1Rh_1	CDe/cde Rh_1rh	CDe/cDE Rh_1Rh_2	cDE/cde Rh_2rh	cDE/cDE Rh_2Rh_2	Cde/cde rh′rh	cde/cde rh	cdE/cde rh″rh	cDe Rh_o	CDE/CDe Rh_zRh_1	
Tamil	423	No.	201	138	44	13	4	3	15	0	4	1	1,6
		%	47,52	32,62	10,40	3,07	0,94	0,71	3,55	0,00	0,95	0,24	
Telegu	171	No.	70	66	14	8	4	0	5	0	3	1	4,9
		%	40,94	38,60	8,19	4,68	2,34	0,00	2,92	0,00	1,75	0,58	
Hindi	260	No.	138	78	14	8	1	2	14	0	4	1	3,3
		%	53,08	30,00	5,38	3,08	0,38	0,77	5,38	0,00	1,54	0,38	
Moslem	314	No.	114	107	39	21	7	2	15	1	7	1	1.9
		%	36,30	34,07	12,42	6,69	2,23	0,64	4,78	0,32	2,23	0,32	

Significance of differences

All differences were not significant between the populations in Table 13.14 and between the relevant populations in Tables 13.13 and 13.14 at level $P < 0,05$ except

	χ^2	P
CDe/CDe in Natal Tamil and Natal Moslems	9,3	$<0,01>0,001$
CDe/CDe in Natal Hindi and Natal Moslems	16,2	$<0,001$
CDe/CDe in Natal Hindi and Natal Telegu	6,1	$<0,02>0,01$
cDE/cde in Natal Tamil and Natal Moslems	5,3	$<0,05>0,02$
cDE/cde in Natal Hindi and Natal Moslems	3,9	$<0,05>0,02$
CDe/cDE in Natal Hindi and Natal Tamil	5,2	$<0,05>0,02$
CDe/cDE in Natal Hindi and Natal Moslem	8,4	$<0,01>0,001$
CDe/cDE in Natal Telegu and Hindu	5,2	$<0,05>0,02$

(Seth, 1967)

TABLE 13.15

Rhesus chromosome frequencies in 1 168 Natal Indian males and unmarried females, D^u included with D+ Indians

Category	Number tested	Chromosome frequencies						
		CDe R_1	*cDE* R_2	*Cde* r'	*cde* r	*cdE* r''	*cDe* R^O	*CDE and CdE* R^Y R^Z
Tamil	423	0,6755	0,0762	0,0190	0,2021	0,0000	0,0254	0,0018
Telegu	171	0,6446	0,0861	0,0000	0,2093	0,0000	0,0554	0,0046
Hindi	260	0,6966	0,0454	0,0162	0,2109	0,0000	0,0283	0,0026
Moslem	314	0,5847	0,1126	0,0146	0,2310	0,0058	0,0487	0,0026

the latter finding suggested that the Johannesburg Hindu population might have contained a large number of Indians who spoke Hindi. By contrast, the Hindu in Orissa, who may have been Telegu-speakers, and the Moslem Indians were shown, in Tables 13.13, 13.14 and 13.15, all to have similar *CDe* and CDe/CDe frequencies. Significantly different cDE/cde frequencies were obtained between the Natal Moslem and the Natal Tamil- and Hindi-speaking Indians and significantly different CDe/cDE frequencies between the Natal Hindi- and the Natal Tamil-speaking and Natal Moslem Indians. The CDe/cDE frequencies in the Natal Telegu-speaking Indians and the presumed predominantly Telegu-speaking Hindu population of Seth also differed significantly. As in India, the cde/cde frequencies in all four Indian populations in Natal were low (2,9–5,38%), and no significant χ^2 differences were found. The highest *Cde* frequency (1,62%) was identified in the Hindi-speaking Natal Indians, and only the Natal Moslem Indians had *cdE* chromosomes. The 2,5 to 5,5% *cDe* frequency in the Natal Indians was similar to the 2–3%frequency noted in India.

The results of a search for strong D antigen in the Natal Indian populations are given in Table 13.16. The reagent for this test was a D^u serum, and it was used by saline technique at 37°C with cDE/cDE as the negative control cells. Although strong D+ cells were known to occur regularly in the Natal Blacks, only two Natal Indians with similar cells were found. The results supported the view that little Black admixture had taken place in the Natal Indians.

TABLE 13.16

High D phenotype frequencies in 1 624 Natal Indian males and unmarried females tested with D^u serum by saline technique

Category	Number tested		Saline D^u test (more than 1+) Positive	Negative
Tamil	618	No.	2	616
		%	0,32	99,68
Telegu	272	No.	0	272
		%	0,00	100,00
Hindi	381	No.	0	381
		%	0,00	100,00
Moslem	353	No.	0	353
		%	0,00	100,00

Table 13.17 shows the results obtained in tests with anti-C^w in the Natal Indians. Only type *C^wDe* chromosomes were detected, and they were found most often in the Moslem Indians (0,94%). A low frequency was expected, as Bird *et al.* (1956) had detected 1,47% *C^wDe* chromosomes in Sikh Indians from northern India.

The findings, in a search for the low frequency phenotypes C^wde and Cde^s (VS+) in the Natal Indians who had phenotype Cde cells, for phenotype D+G– in the Natal Indians who had D+ cells and for phenotype hr^s– and hr^B – in all except the Natal Indians who had cDE/cDE cells, are presented in Table 13.18. None were found, and the figures are presented here for interest only.

TABLE 13.17

C^W antigen frequencies in 1 556 Natal Indian males and unmarried females

Category	Number tested (total population)		Phenotype frequencies C^W+ CDe/CDe Rh_1Rh_1	C^W+ CDe/cde Rh_1rh	C^W+ CDe/cDE Rh_1Rh_2	C^W+ Other Rh	C^W- All Rh
Tamil	627	No.	0	0	0	0	627
		%	0,00	0,00	0,00	0,00	100,00
Telegu	264	No.	0	0	0	0	264
		%	0,00	0,00	0,00	0,00	100,00
Hindi	344	No.	2	0	0	0	342
		%	0,58	0,00	0,00	0,00	99,42
Moslem	321	No.	3	2	1	0	315
		%	0,93	0,62	0,31	0,00	98,13

TABLE 13.18

Natal Indians tested for phenotypes of low frequency in the Rhesus system with the corresponding reagents

Reagents	Cell type of Indians tested	Number Natal Indians tested No positives found: Tamil	Telegu	Hindi	Moslem	Total
Anti-C^W	Cde	37	6	15	5	63
Anti-VS ($-e^S$)	Cde	12	2	5	1	20
Anti-G	D+	281	141	171	173	766
		No negatives found				
Anti-hr^S	all except	577	224	389	256	1 446
Anti-hr^B	cDE/cDE	215	100	134	241	690

(The full Rh types of the D+ Indians tested with anti-G was not recorded).

13.4 DISCUSSION

13.4.1 The MNSs system

Except in NNS between the Hindi-speaking and Moslem Indians, no significantly different frequencies in the MNSs system between the Indian populations in Natal were found. However, those detected between the Natal Hindu and the Hindu population in Johannesburg, and between the Natal Moslem population and the Moslem population in Johannesburg, recorded by Nurse and Jenkins (1977), were thought probably to be the result of the small numbers tested in the two latter. The low M_1 frequency in all four Natal Indian populations was in accord with the frequency known in Whites in Europe (3%). The frequencies in this system therefore failed to distinguish between the four Indian populations in Natal.

13.4.2 The Rhesus system

The typically high CDe frequency, especially in the north-east and extreme south of India, was apparent also in the four Indian populations tested in Natal. The Natal Hindi-speaking Indians clearly had the highest and the Natal Moslem Indians the lowest CDe frequencies, and the CDe/CDe, cDE/cde and CDe/cDE frequencies in these two populations suggested that they formed independent groups. However, the corresponding frequencies showed that the Natal Tamil- and Natal Telegu-speaking Indians were fairly similar to one another. The low cde/cde frequencies detected in all four populations, and the low $C^{W}De$, Cde and cdE frequencies found in the Natal Hindi-speaking and Natal Moslem Indians, were in accord with the frequencies known in Caucasoids. The low cDe frequencies detected, and the few Indians found whose cells had strong D antigen, again supported the view that the Natal Indian populations had little Black admixture.

13.5 SUMMARY

In the MNSs sytem, the only significantly different frequencies found between the populations tested were those for NNS in the Natal Hindi-speaking and Natal Moslem Indians. However, the χ^2 tests for other chromosome frequencies in this system showed that the populations in Natal differed significantly from the corresponding populations in Johannesburg. A low M_1 frequency was detected in all

four Natal Indian populations.

The frequencies detected in the Rhesus system were similar to those known in Indians and distinguished successfully between the Natal Moslem and Natal Hindi-speaking Indian populations. They also distinguished between these two populations and the Natal populations who spoke Tamil and Telegu, but not between the two latter. The cde/cde frequencies detected were low in all four Indian populations, and low $C^{w}De$, Cde, cdE and strong-D phenotype frequencies were found.

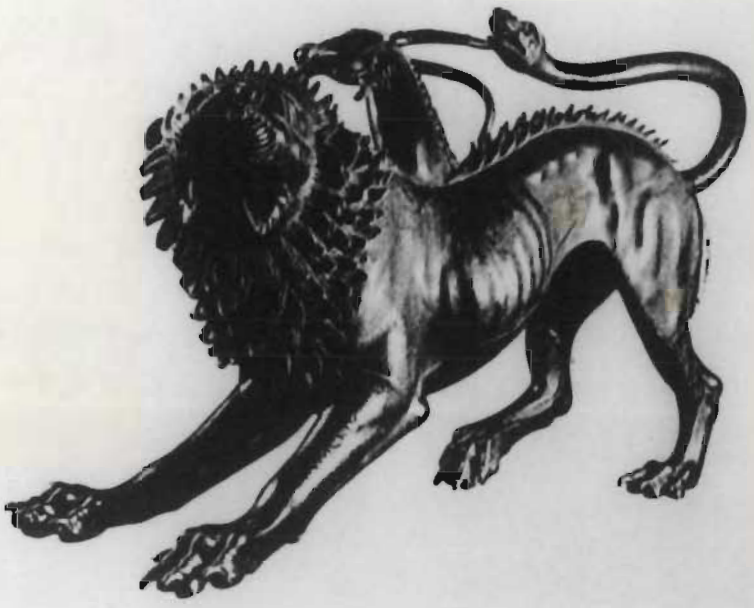


PLATE 14.1

Mythical Greek Chimaera; a monster, part lion, part goat, part serpent.

CHAPTER 14

NATAL INDIAN DISPERMIC CHIMAERA

14.1 INTRODUCTION

Far from being monsters, like the mythical part lion, part goat, part serpent Chimaera of ancient Greece (Plate 14.1), human chimaeras look like ordinary people. However, some or all of their tissues are composed of cells which originated from more than one fertilized human germ cell nucleus. Two types of human chimaeras are recognised. The first type are the twin chimaeras whose circulations contain not only their own blood cells but also the blood cells of their twin. This is caused by the migration of precursor blood-forming tissue cells from one twin to the other through a rare blood vessel anastomosis in their placentas. The second type are the dispermic chimaeras who begin life as separate twins *in utero* but, for some reason, either the fertilized cells or the developing cell morulas became fused together and only one individual was produced (Race and Sanger, p 519–539, 1975).

The first human dispermic chimaera was described by Gartler, Waxman and Giblett in 1962. Outwardly female, except for an enlarged clitoris, this two-year old child had one hazel and one brown eye, an ovary and an ovo-testis, genetically XX and XY leucocytes, and MS^{u}/MS, CDe/cDE ($Rh_1 Rh_2$) and MS^{u}/Ns, CDe/cde (Rh_1 rh) red cells in approximately equal proportions in her blood. The authors deduced that she was the result of double fertilization, two identical maternal germ cell nuclei having presumably united with two sperms, one of which had carried an X and the other a Y chromosome. The second example, reported by Beattie, Zuelzer, McGuire and Cohen and by Zuelzer, Beattie and Reisman in 1964, had gynacomastia and excreted more than the normal amount of oestrogen, but otherwise he appeared to be a normal male. Skin pigment mosaicism was present in the form of dark-coloured wedges and bands on his body, and these extended laterally in both directions from the midline on his body in front and behind. Chromosomally XX and XY tissue cells were identified in biopsies of his darker skin, and his blood was found to contain 90% A_1, Jk(a–) red cells which were positive for sickling and 10% B, Jk(a+) normal red cells. Double fertilization was again diagnosed, one of his father's sperms having presumably carried an X and the other a Y chromosome.

In this chapter, details will be given of a Natal Indian woman who was the first dispermic chimaera to be recognised who had only XX tissue cells. Unlike the other six dispermic chimaeras then known, she was fertile, having had four normal, healthy children. Her skin showed patchy pigmentation and she had two populations of red cells and of leucocytes in her blood (Moores, 1966; Moores, 1973).

14.2 MATERIALS AND METHODS

Blood samples were received from Mrs T.R. on many occasions between 1965 and 1980, and her family members were also most co-operative. Saliva samples were provided by them as well, and Mrs T.R. kindly agreed to be examined and photographed in my presence by Professor G.H. Findlay of the Department of Dermatology, University of Pretoria, Pretoria, and by Professor P. Brain of the Natal Blood Transfusion Service, Durban.

The serological methods used to study the blood samples were the saline, one-stage 0,5% bromelin, one-stage 0,25% ficin and indirect antiglobulin techniques at various temperatures, and they and the saliva inhibition tests are described in Chapter 2. The red cell isozymes were kindly determined for me by Professor Trefor Jenkins and D. Dunn of the Department of Human Sero-genetics, South African Institute for Medical Research, Johannesburg, and the comments on the skin pigment distribution were from a report on skin pigmentation in dispermic chimaeras by Findlay and Moores (1980). For the HL-A typing, I am indebted to Dr M. Hammond, for the karyotyping to Dr H.J. Grace, and for the Ashby red cell counting to Miss M. Jamieson, all of whom are of the Natal Blood Transfusion Service, Durban.

14.3 CASE HISTORY

Mrs T.R., who spoke Tamil, was discovered in 1965 when a sample of her blood was submitted for routine antenatal tests. She was then six months pregnant with her fourth child. Her blood was typed as AB, Rhesus D^u, but in the subsequent studies made by myself, the weak positive agglutination originally detected with the D^u reagent was seen to be caused by the presence of agglutinated (D-positive) and unagglutinated (D-negative) red cells in her blood. Further studies then showed that Mrs T.R.'s blood contained a mixture of B, D-positive and A_1B D-negative red cells.

Enquiries disclosed that Mrs T.R. was healthy, she had not had either a recent blood transfusion or a blood transfusion before birth, and she had not received a transplant of bone marrow. Her mother was not aware that a twin or other unexpected tissue had been present when her daughter was born; but a history of twins in the family of Mrs T.R.'s maternal grandfather was noted. After Mrs T.R. had given birth to her fourth infant, fetomaternal haemorrhage was eliminated as her infant had group O blood, and the persistence of two populations of red cells in her blood was confirmed on many occasions during the following years when she was not pregnant. A diagnosis of dispermic rather than twin chimaerism was made when, in addition to the absence of a twin, Mrs T.R. was seen to have mosaic skin pigmentation.

14.4 CASE REPORT

14.4.1 Red cell studies

Using a number of different anti-D and D^u reagents, the mixture of Rhesus D-positive and D-negative red cells in Mrs T.R.'s blood was confirmed. Mixed fields of agglutinated and unagglutinated red cells were also detected when her blood was tested with anti-A, anti-C, anti-S and anti-Fy^b reagents.

In order to separate the red cells which were not agglutinated by anti-A in Mrs T.R.'s blood from those that had been agglutinated by this reagent, a sample of her cells was washed three times with saline, packed down and mixed manually in a Petri dish at 22°C with an excess of anti-A reagent, as described by Booth, Plaut, Jones, Ikin, Moores, Sanger and Race (1957). The cells in the dish were then centrifuged and resuspended several times to concentrate the agglutinates, and the agglutinates were allowed to settle out of the mixture by gravity. The unagglutinated cells were then recovered, rewashed three times and grouped. Their groups were found to be B, MNs, CDe/cde (Rh_1 rh), Fy (a+b−).

To separate the red cells not agglutinated by D^u serum in Mrs T.R.'s blood from those agglutinated by this reagent, a similar procedure was adopted using D^u serum in place of the anti-A reagent and incubating the contents of the dish at 37°C for one hour. The cells were then washed four times with saline, and an excess of anti-globulin reagent was added to them. The unagglutinated cells were separated as already described, rewashed three times and grouped. Their groups were found to be A_1B, MNSs, cde/cde (rh), Fy(a+b+).

Both the B and the A_1B red cells in Mrs T.R.'s blood were found to be Le(a+b−), see Figure 14.1, and no mixed fields of agglutinated and unagglutinated cells were detected when her unseparated cells were tested with the following reagents: anti-A,B, −M, −N, −s, $-P_1$, $-PP_1P^k$, −c, −e, $-C^w$, $-hr^S$, $-hr^B$, −f (ce or hr), $-VS(e^S)$, $-Lu^a$, $-Lu^b$, −K, −k, $-Kp^a$, $-Kp^b$, $-Js^a$, −I, $-Co^a$, $-Co^b$, −Vel, $-Xg^a$, $-Do^a$, $-V^w$, *Arachis hypogea* lectin, *Glycine soja* lectin and AB serum.

The ratio of the B D-positive to the A_1B D-negative red cells in Mrs T.R.'s blood was assessed visually in 1965 in tests with anti-A and D^u (plus antiglobulin) reagents as being approximately 50:50. However, in 1973, a more accurate estimate of the proportion of the unagglutinated cells in these tests was possible using a Coulter electronic counter. Several counts (Mollison, p 729, 1979), in which saline was substituted for the anti-A and the antiglobulin reagents in the control samples, showed that Mrs T.R.'s blood contained 45,25% B D-positive and 54,75% A_1B D-negative red cells. These figures agreed reasonably well with the original estimates, but it will be appreciated, as the red cells of the blood are undergoing continual removal and regeneration, that the proportion of these cells in Mrs T.R.'s blood might not always have been exactly the same.

Plate 14.2 shows (a) the agglutination when one volume of washed, packed, unseparated cells from Mrs T.R. was mixed with two volumes of anti-A in a Petri dish and gently agitated by hand for 5 minutes (Booth *et al.*, 1957). The agglutination may be compared with (b) the control which consisted of a mixture of equal quantities of similarly-treated B D-positive and A_1B D-negative cells from blood donors. Plate 14.2 also shows (c) the agglutination detected when one volume of Mrs T.R.'s unseparated cells was treated instead with 0,5% bromelin and mixed similarly with two volumes of bromelin anti-D reagent in a Petri dish, compared with (d) the similarly-treated control cell mixture.

14.4.2 Lewis groups and secretion

Saliva inhibition tests with anti-A, anti-B, anti-H, anti-Le^a and anti-Le^{bL} reagents showed that Mrs T.R. secreted B, H, Le^a and Le^b substances (Table 14.1). This was a surprise, for persons who have Le(a+b−) cells usually secrete Le^a substance only (Race and Sanger, 1975). The problem was resolved when the results were compared with those reported for the Detroit chimaera (Beattie *et al.*, 1964). This chimaera secreted A, H, Le^a and Le^b substances, but his blood contained a mixture

a

b

PLATE 14.2

Mrs T.R.'s unseparated red cells.

a. Mixed with anti-A.

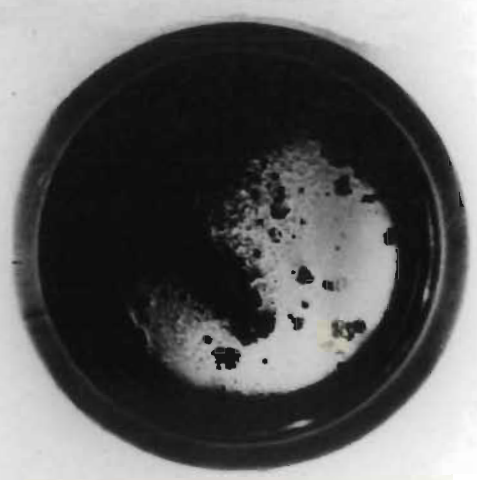
b. Mixed with anti-D.

Control cells (50% B and 50% A_1 B)

c. Mixed with anti-A.

d. Mixed with anti-D.

c



d

Figure 14.1

Pedigree of Mrs T.R.

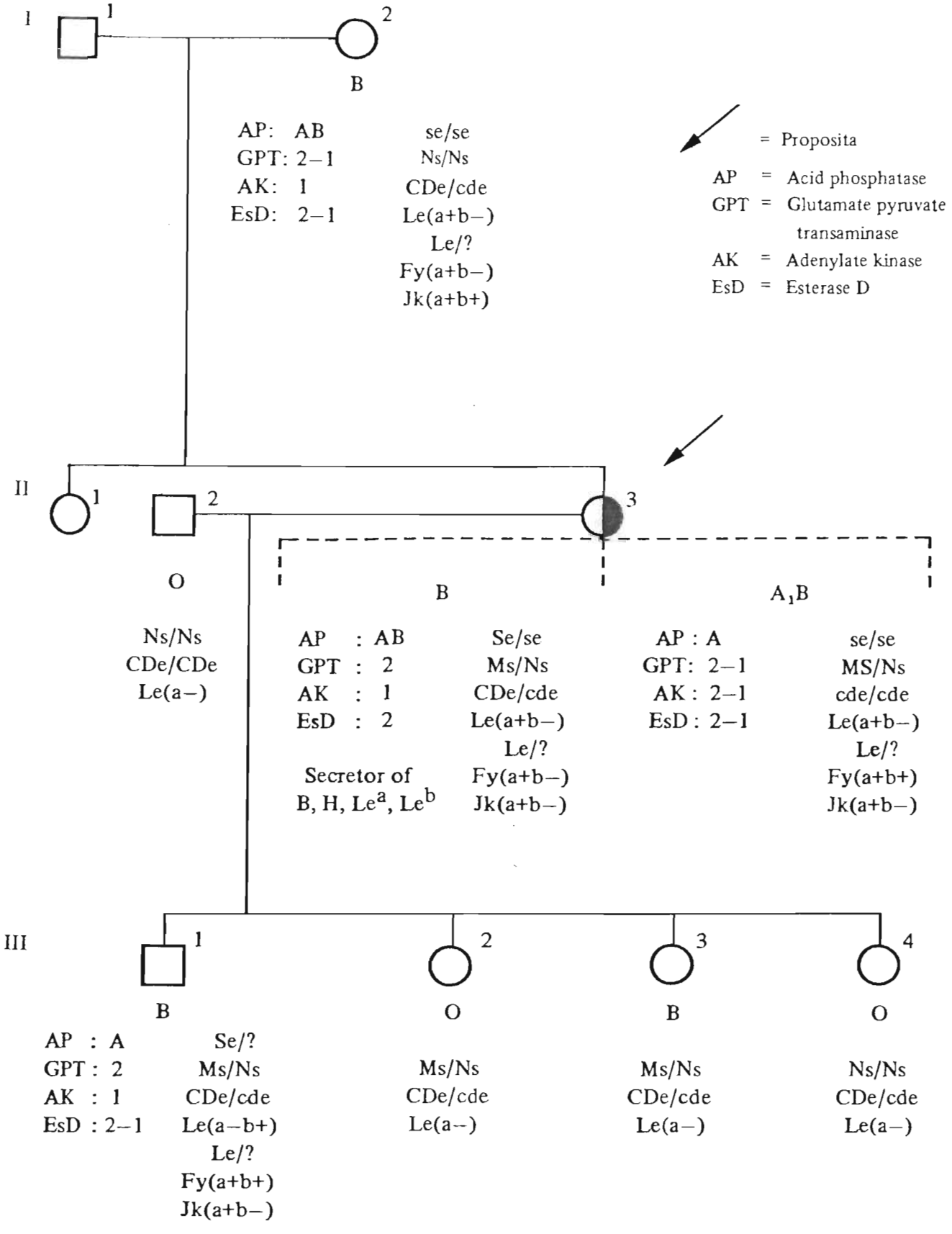


TABLE 14.1

Results of inhibition tests with the saliva of Mrs T.R.

	Anti-A dilutions in saline							
Saliva	1	2	4	8	16	32	64	
Mrs T.R.	4	3	2	1	(2)	–	–	A_2 cells
Control A secretor	–	–	–	–	–	–	–	
Control non-secretor	4	3	2	1	(2)	–	–	

	Anti-B dilutions in saline							
Saliva	1	2	4	8	16	32	64	
Mrs T.R.	–	–	–	–	–	–	–	B cells
Control B secretor	–	–	–	–	–	–	–	
Control non-secretor	4	4	3	2	2	1	(2)	

	Ulex anti-H dilutions in saline							
Saliva	1	2	4	8	16	32	64	
Mrs T.R.	–	–	–	–	–	–	–	O cells
Control H secretor	–	–	–	–	–	–	–	
Control non-secretor	4	4	3	1	(1)	–	–	

Saliva	anti-Le^a Le(a+b–) cells	anti-Le^b Le(a–b+) cells
Mrs T.R.	–	–
Control Le^a and Le^b secretor	–	–
Control Le^a secretor	–	2
Control non-secretor	2	2
Saline	3	3

of 90% A_1 Le(a+b−) and 10% B Le(a+b−) cells. The authors concluded that his genetically *BB* or *BO*, *sese*, *Lele* tissues were secreting enough Le^a substance into his plasma to be adsorbed not only onto his B cells but also onto the A_1 cells derived from his genetically *AO*, *Sese*, *Lele* tissues. Similarly, the genetically A_1B tissues in Mrs T.R. were judged to be *sese* and either *LeLe* or *Lele* and to be secreting sufficient Le^a substance into her plasma to be adsorbed not only onto her A_1B red cells but also onto the B red cells derived from her genetically *BB* or *BO*, *Sese* and either *LeLe* or *Lele* tissues.

14.4.3 Red cell enzyme studies

The two populations of red cells in samples of Mrs T.R's blood were separated by means of differential agglutination using anti-A and D^u serum (plus antiglobulin reagent), her B cells and her A_1B Rhesus-negative cells respectively being not agglutinated. The separated cells were washed with saline, haemolysed and subjected to analysis by starch gel electrophoresis for 14 polymorphic red cell enzyme systems (Harris and Hopkinson, 1976). No variation was found in the phosphoglucomutase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, peptidase A, peptidase B, glyoxalase I, carbonic anhydrase I, carbonic anhydrase II, adenosine deaminase and haemoglobin systems, but variation was detected in the acid phosphatase (AP), glutamate pyruvate transaminase (GPT), adenylate kinase (AK) and esterase D (EsD) systems (Table 14.2 and Figure 14.1).

Mrs T.R.'s B cells were AP-phenotype AB, GPT-phenotype 2, AK-phenotype 1 and EsD-phenotype 2; and her group A_1B cells were AP-phenotype A, GPT-phenotype 2−1, AK-phenotype 2−1 and EsD-phenotype 2−1. The isozymes detected in her mother's cells (I-2, Figure 14.1) were consistent with two identical maternal germ cell nuclei having been present at the time of Mrs T.R.'s conception the chromosomes in which had carried AP-type A, GPT-type 2, AK-type 1 and EsD-type 2 genes (Table 14.2). These genes were also seen to be present in Mrs T.R.'s son (III-1), but the phenotypes detected in his cells unfortunately did not disclose from which of his mother's two cell lines he had inherited them.

14.4.4 Tissue-typing studies

HL-A typing tests showed that an excess of antigens was present in Mrs T.R.'s lymphocytes. At the first or LA locus, the antigens detected were HL-A1, HL-A10

TABLE 14.2

Red cell enzyme factors in Mrs T.R.
and her family members

Enzyme system	I-2 (mother of Mrs T.R.)	II-3 (Mrs T.R.)		III-1 (Son of Mrs T.R.)
		B cells	A_1 B cells	
Informative				
Acid phosphatase	AB	AB	A	A
Glutamate pyruvate transaminase	2–1	2	2–1	2
Adenylate kinase	1	1	2–1	1
Esterase D	2–1	2	2–1	2–1
Non-informative				
Phosphoglucomutase	1^+1^+	1^+2^+	1^+2^+	1^+2^+
Glucose 6-phosphate dehydrogenase	B	B	B	B
6 phosphogluconate dehydrogenase	A	A	A	A
Peptidase A	1	1	1	1
Peptidase B	1	1	1	1
Glyoxalase I	2–1	2–1	2–1	2
Carbonic anhydrase I	1	1	1	1
Carbonic anhydrase II	1	1	1	1
Adenosine deaminase	1	2–1	2–1	2–1
Haemoglobin	$A + A_2$	$A + A_2$	$A + A_2$	$A + A_2$

and W28 and, at the second or Four locus, the antigens detected were HL-A8, HL-A13 (±) and W5. In all of the tests which were considered positive, approximately half the number of leucocytes reacted with the reagent used. Together with the excess number of antigens detected, this was consistent with the presence of two populations of lymphocytes in Mrs T.R.'s blood.

14.4.5 Skin pigmentation

A more detailed investigation of the distribution of the light-and dark-brown skin pigmentation in Mrs T.R. than had been possible in the first study (Moores, 1973) was reported by Findlay and Moores in 1980. Fundamentally, Mrs T.R.'s skin appeared light-brown in colour, over which, in places, a much darker-brown pigmentation had intruded. In the dark-brown coloured areas, her skin was not raised or rough, but this colour, which varied in depth from place to place, was darkest on her right forearm, lower abdomen and sites exposed to friction and sunburn. Also, many more dark-brown coloured areas were evident on the right than on the left half of her body.

The pattern of the dark-brown pigmentation in Mrs T.R. was divisible into four types. The first type was finely granular, speckled or mottled, and this coarsened gradully into the second which was denser and had a more reticulated appearance. The third type was one of fine streaks or striae which broadened into the fourth, a system of bands and block-like masses. The striae were sited at or near the midline of her body, and stretched down the length of her limbs or encircled her trunk. They were arranged like the epidermal naevi which sometimes occur along the so-called naevus lines of Blaschko. Plate 14.3.a shows the parallel streaks at the back of Mrs T.R.'s neck and the increased depth of colour on the right side of her neck in the position normally occupied by a necklace. The striae and bands in her right armpit and which extended along the underside of her right arm are shown in Plate 14.3.b,, and the bands, blocks and mottling which were more extensive on the front aspect of her right than on her left leg may be seen in Plate 14.3.d. Plate 14.3.c shows the bands and striae of dark-brown pigment which followed the line of the sartorius muscle on Mrs T.R.'s right leg, and this photograph also shows the mottling which was associated with the areas of friction on the right side of her body. The quadrants and wedges of dark and light skin pigmentation reported by Corey, Miller, MacLean and Chown (1967) and by Zuelzer, Beattie and Reisman (1964) respectively in their dispermic chimaeras were noticably absent in Mrs T.R. No biopsies of Mrs T.R.'s

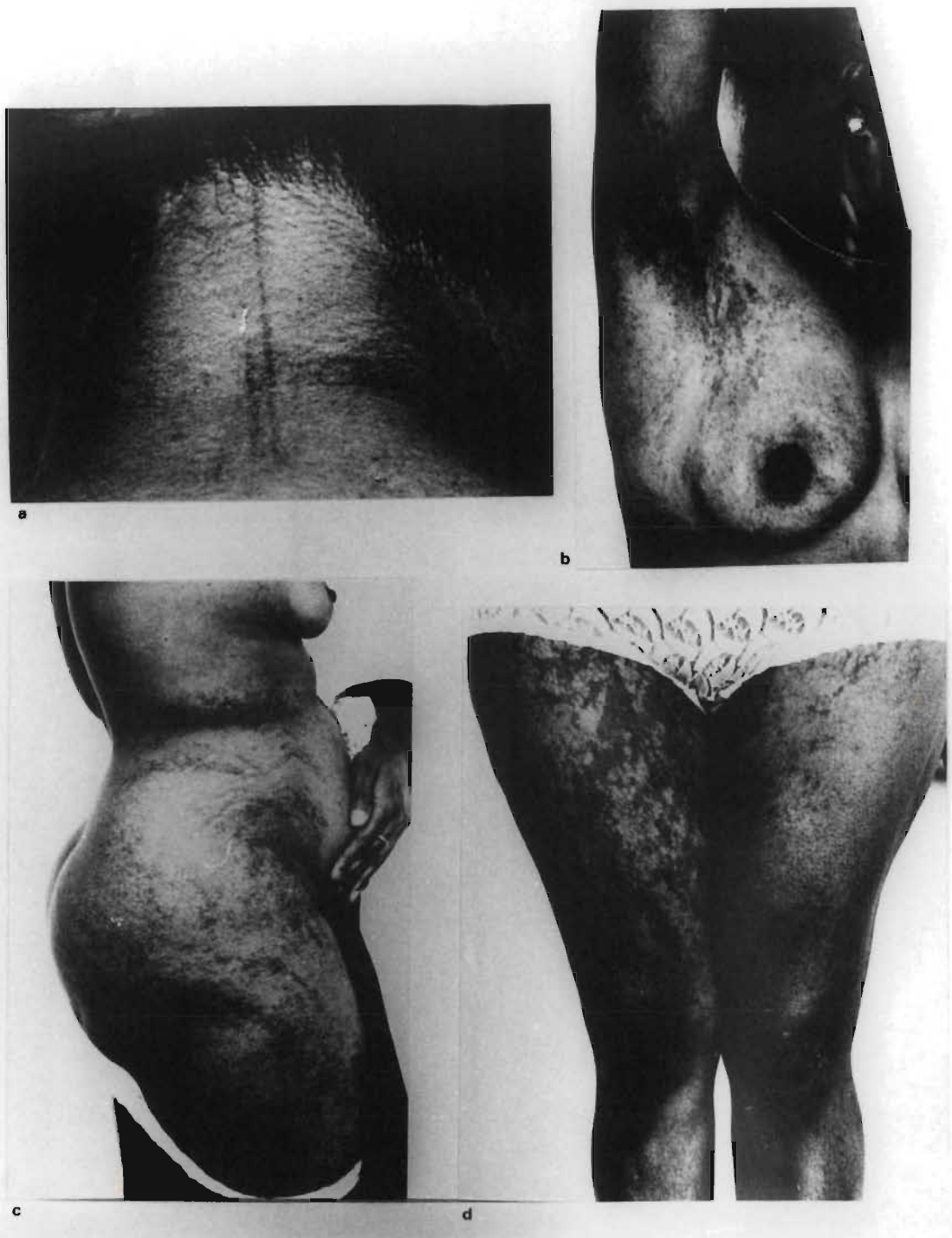


PLATE 14.3

a. Streaks of dark-brown pigmentation on the back of Mrs T.R.'s neck, and the darker pigmentation on the right than on the left side of her neck in the position of a necklace.

b. Mrs T.R.'s right armpit showing centrally-placed bands and striae of dark-brown pigmentation.

c. Bands and striae of dark-brown pigmentation following the line of the sartorius muscle, and mottled areas where friction was expected, on the right side of Mrs T.R.'s body.

d. Bands, blocks and mottled areas of dark-brown pigmentation more evident on the front aspect of the right than of the left leg of Mrs T.R.

skin tissues were made.

14.4.6 Cytogenetic studies

In 16 karyotypes of lymphocytes cultured from Mrs T.R.'s peripheral blood, only the 46,XX pattern was identified (Plate 14.4). These results were consistent with the diagnosis of XX/XX dispermic chimerism in her.

14.4.7 Family pedigree

The informative blood group factors, chromosomes and isozymes in Mrs T.R. and members of her family are shown in Figure 14.1. A fresh blood sample from Mrs T.R.'s mother, I-2, was found to be Le(a+b−), showing that the Le(a−) result recorded earlier (Moores, 1973) had been incorrect. However, fortunately, this made no difference to the interpretation of the inheritance in the family. Mrs T.R.'s groups showed that she had inherited two identical sets of chromosomes from her mother the genes on which were specific for the following: B, se, Ns, cde, Fy^a, Jk^a, AP-type A, GPT-type 2, AK-type 1, EsD-type 2. Mrs T.R.'s father, I-1, had contributed two non-identical sperms, on the chromosomes in which the genes were specific for the following:

(i) B (or O), Se, Ms, CDe, Fy^a, Jk^a, AP-type B, GPT-type 2, AK-type 1, EsD-type 2.

(ii) A_1, se, MS, cde, Fy^b, Jk^a, AP-type A, GPT-type 1, AK-type 2, EsD-type 1.

The B and O groups of Mrs T.R.'s four children by her group O husband showed that her ova were from the same cell line as her B red cells. Group A_1 and group B children (and perhaps even *cis* A_1B and group O children) would have been expected if her ova had been from the same cell line as her A_1B cells. III-1 had also inherited *Se* and *Ms* from the same cell line in his mother which was responsible for producing her B but not her A_1B cells.

Table 14.3 shows the non-informative blood factors in I-2, II-3 (Mrs T.R.) and III-1.

Plate 14.4

Karyotype of Mrs T.R. showing XX chromosomes

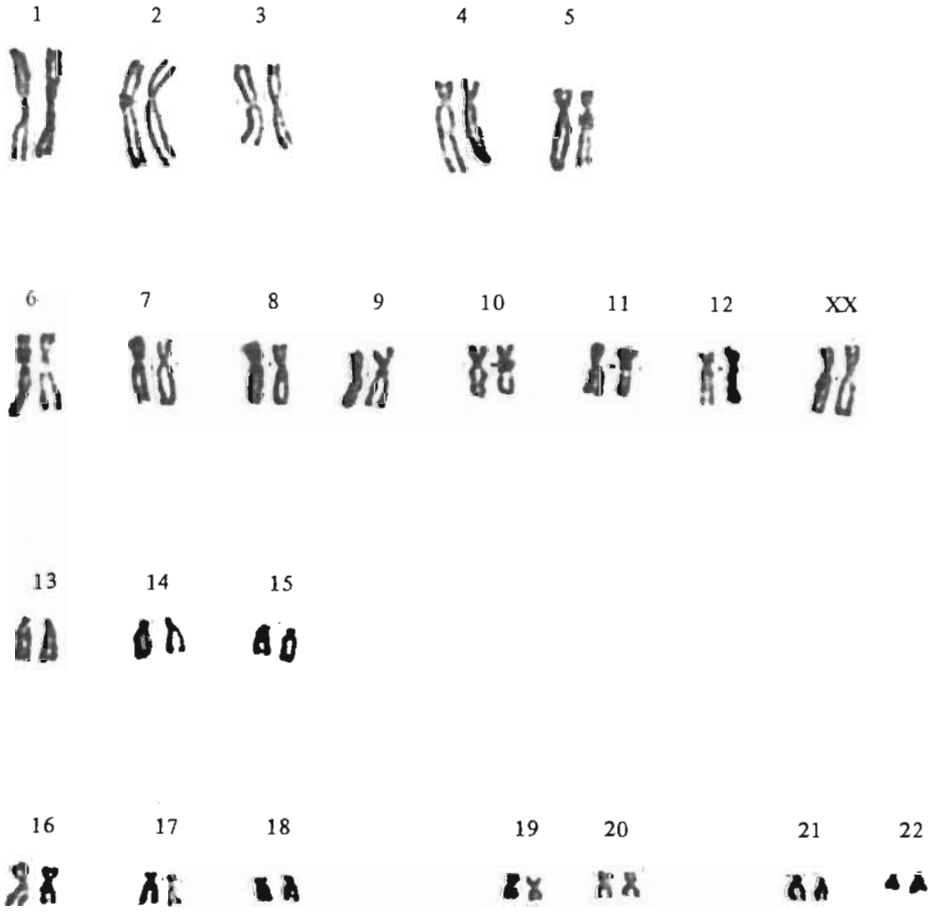


TABLE 14.3

Non-informative blood groups of Mrs T.R. and her family members

System or antigen	I-2 (Mother of Mrs T.R.)	II-3 (Mrs T.R.) B cells	II-3 (Mrs T.R.) A_1 B cells	III-1 (Son of Mrs T.R.)
MNSs	Henshaw–			Henshaw–
P_1–P_2	P_2	P_2	P_2	P_1
	PP_1P^k +	PP_1P^k +	PP_1P^k +	
Rhesus	C^w–	C^w–	C^w–	C^w–
	f+	f+	f+	f+
	hr^S +	hr^S +	hr^S +	hr^S +
	hr^B +	hr^B +	hr^B +	hr^B +
		VS –	VS –	
Lutheran	Lu(a–b+)	Lu(a–b+)	Lu(a–b+)	Lu(a–b+)
Kell	K–k +	K–k+	K–k +	K–k+
	Kp(a–b+)	Kp(a–b+)	Kp(a–b+)	Kp(a–b+)
	Js(a–b+)	Js(a–b+)	Js(a–b+)	Js(a–b+)
Ii	I +	I +	I +	I +
Xg	Xg(a+)	Xg(a+)	Xg(a+)	Xg(a+)
Colton	Co(a+b–)	Co(a+b–)	Co(a+b–)	Co(a+b–)
Sid	Sd(a+)	Sd(a+)	Sd(a+)	Sd(a+)
Vel	Vel +	Vel +	Vel +	Vel +
Dombrock		Do(a+)	Do(a+)	
Verweyst		V^w–	V^w–	
H		Ulex titre 16	Ulex titre 4	

14.5 DISCUSSION

The 21 dispermic chimaeras recorded in the 6th edition of *Blood Groups in Man* (Race and Sanger, p 531–535, 1975) were all found though having something amiss with their sex, unusual skin pigmentation or two populations of blood cells. Seventeen were hermaphrodites, apparently formed when genetically XX and XY zygotes became fused together *in utero*, two were genetically XY/XY males and two were genetically XX/XX females. Mrs T.R. was the first genetically XX/XX female dispermic chimaera to have been recorded. The second example was a Natal Zulu baby girl (Moores, recorded by Race and Sanger, p 533, 1975), the third a Natal Zulu woman (Moores, Findlay, Dunn, Watkins, Greenwell and Bird, in the press) and the fourth a woman from Austria (Mayr, Pausch and Schnedl, 1979).

The identity of Mrs T.R. as an XX/XX female dispermic chimaera rested upon her normal appearance, fertility, mosaic skin pigmentation and two populations of red cells and lymphocytes in her blood. The groups of her red cells showed that her mother had almost certainly provided two germ cell nuclei at her conception, each of which had contained an identical set of chromosomes. Gartler *et al.* (1962) suggested, regarding their chimaera. that the two germ cell nuclei might be the mitotic products of one meiotic nucleus; and Zuelzer *et al.* (1964), whose chimaera contained a widely disproportionate number of red cells derived from each zygote, suggested that the nucleus of an ovum and its relatively cytoplasm-deficient second polar body might have been fertilized. Since Mrs T.R.'s two cell lines had apparently each provided approximately 50% of her red cells and a large proportion of her skin pigment, it seemed likely that her mother had contributed two identical ova. Like the chimaera of Gartler *et al.* (1962), these ova may have been formed as the result of a single ovum having duplicated itself mitotically, some time after having achieved maturity and before fertilization. Mrs T.R.'s father had clearly provided two sperms the nuclei in which had contained X but otherwise non-identical chromosomes.

The question arose as to why Mrs T.R., who appeared to be the product of fusion of two zygotes of equal size, secreted B and H but not A, B and H substances and why she had B and O, but not A and B, or perhaps even O and *cis* AB, children by her group O husband? Race and Sanger (1975) concluded from the dispermic chimaera of Beattie *et al.* (1964), whose tissues were genetically *A, Sese* and *B, sese*, that, in order to secrete ABH substances, an *Se* gene must be present in the same cell as the ABO gene. The findings with Mrs T.R. confirmed this view, but the reason her germ

cells were apparently being provided by only one of her cell lines was not understood. Perhaps *Se* genes also coded for secretion of substances that inhibited or interfered with the maturation of ova provided by genetically *sese* gonads. Alternatively, perhaps it was only by chance that her genetically A (or *cis* AB) ova had not so far been fertilized. No records of other XX/XX female dispermic chimaeras, formed apparently by the fusion of two zygotes of equal size, were found.

Mrs T.R.'s red cell populations were both Le(a+b−), yet she secreted B, H, Le^a and Le^b substances in her saliva. The question thus also arose as to why her red cells were not Le(a+b+). The dispermic chimaera of Beattie *et al.* (1964), whose two red cell populations were Le(a+b−) as well, had inherited an *Le* gene from his mother in both of his cell lines. The minor (10%) B, *sese, Lele* tissue in him was said by the authors in his case to be producing enough Le^a substance for all of his red cells to be converted into Le(a+b−) cells. However, his major (90%) A_1, *Sese, Lele* tissue should have been able to produce abundant Le^b substance. While it was known that A_1 cells were difficult to type with anti-Le^b reagents, the studies of Watkins (1965, 1980), who, with others, identified the biochemical structure of A, B, H, Le^a and Le^b substances, had not at that time been published. Watkins (1980) showed that Le^b substance was formed by the addition of α-fucose to both the β-Gal and GlcNAc sub-units of the terminal portion of Type 1 carbohydrate chain endings on the precursor glycoprotein substrate. Le^a substance was formed by the addition of α-fucose to the GlcNAc sub-units on these chains only, and H substance was formed by the addition of α-fucose to the β-Gal sub-units only (Figure 14.2). When both *Se* and *sese* genes were inherited in different *Le* gene-containing tissue cells in a dispermic chimaera, perhaps, because their tissues were competing for conversion of Type 1 carbohydrate chain endings, only the more simple H and Le^a and not the more complex Le^b substances could be formed. Mrs T.R., who had B, *Sese, Le* and A_1B, *sese, Le* tissues, was therefore surmised not to have Le(a+b+) red cells for this reason. The chimaera of Beattie *et al.* (1964) had 90% *AO, Sese* and 10% *BO, sese* cells, yet he secreted only A, and not A and B substances. Similarly, Mrs T.R. had approximately 50% B, *Sese* and 50% A_1B, *sese* cells, yet she secreted only B, and not A and B substances. The secretion of A, B and H substances therefore seemed to be a property of intra- rather than inter-cellular action between the *ABH* and *Se* genes.

FIGURE 14.2

Structure of terminal portion of Type 1 carbohydrate chains

from Watkins (1980)

Substance	Biochemical structure
H	β-Gal (1–3) GlcNAc ——R ↑ α-Fuc
Le^a	β-Gal (1–3) GlcNAc ——R ↑ α-Fuc
Le^b	β-Gal (1–3) GlcNAc ——R ↑ ↑ α-Fuc α-Fuc

Key: R = Remainder of molecule

The mosaic skin pigmentation in Mrs T.R. and other dispermic chimaeras was described by Findlay and Moores (1980) as probably not being due to the production of different shades of skin pigment in their two cell lines. The more darkly-pigmented areas on Mrs T.R.'s skin were reminiscent of some types of skin naevi, and it was suggested that they might be composed of irregular skin tissue. This tissue might be the result of competition between underlying connective tissue cells of different genetic origin endeavouring to stimulate skin pigment synthesis in the melanocytes sited above them. The arrangement of the darker skin patches was also believed to confirm that the melanocytes had migrated during embryonic life away on both sides from the neural crest along pre-determined paths around the body, ending at the ventral midline. However, the reason for the greater amount of dark-brown skin pigmentation on the right than on the left half of Mrs T.R.'s body was not understood.

14.6 SUMMARY

An Indian woman with two populations of blood cells and mosaic skin pigmentation was shown, by the normal 46,XX karyotype of her lymphocytes and her fertility, to be an XX/XX dispermic chimaera. The family study disclosed that the woman's mother had almost certainly provided two identical ova when her daughter was conceived. Despite the Le(a+b−) phenotype of both of the chimaera's cell lines, she was a secretor of B, H, Le^a and Le^b substances. This phenomenon, her group B and O children, the absence of Le(a+b+) red cells from her blood and the origin of her skin pigmentation, were discussed.

CHAPTER 15

S-s-U- RED CELLS IN A NATAL INDIAN FAMILY

15.1 INTRODUCTION

The U- phenotype was discovered in 1953 by Wiener, Unger, Gordon and Cohen in an American Black woman. The woman's serum contained a hitherto unknown antibody and had agglutinated the red cells of 1 100 Whites and all except 12 of 989 Blacks in New York. A family study disclosed that the woman's cell phenotype was due to the inheritance of two rare recessive genes, and the gene was given the symbol *u*. The corresponding very common allele of the *u* gene was called *U*. The *u* gene frequency was estimated as being 0,011 in American Blacks.

Soon afterwards, U- cells were noticed to occur more frequently in West African than in American Blacks (Mourant *et al.*, 1976), and Frazer, Giblett and Motulsky (1966) detected them in over 34% Efe Pygmies in the north-east region of the Congo river basin. Vos, Moores and Lowe (1971) and Lowe and Moores (1972) detected them in 15 Blacks in Central Africa; Hoekstra, Albert, Newell and Moores (1975) in six Xhosa Blacks in the Eastern Cape region of South Africa, and Moores, Marks and Botha (1980) in two Coloured people in Cape Town. So far, U- cells have not been recorded in Whites, in the Khoisan or in the Transvaal Blacks, and in Natal, the cells of only three Zulu Blacks have been identified with this phenotype in 14 years of continuous studies with anti-U. Until 1972, U- cells had not been recorded in Indians.

A year after the discovery of the *Uu* genes, Greenwalt, Sasaki, Sanger, Sneath and Race (1954) noticed that U- cells were not agglutinated by anti-S or anti-s. For a time, anti-U was considered a cross-reacting anti-Ss antibody, but when S-s-U+ cells were identified and found difficult to type with anti-U, the theory was discarded. The possible circumstances of the U- phenotype put forward were that it was due to an allele S^u of the *Ss* genes, a linked inhibitor gene suppressing *S* or *s* gene expression in *cis*, an unlinked inhibitor gene inherited at a locus different from the *Ss* genes or the result of missing genetic material (Race and Sanger, p 101, 1975). To assist in determining the correct solution, interested workers were urged to establish, in antigen dosage titrations, whether or not the cells of the parents and children of U-

people had single or double doses of S or s antigens.

In 1966, the cells of a young Natal Indian woman, whose serum contained strong IgG antibodies, were identified as phenotype U-. One of her antibodies was also identified as anti-U. The family study and S and s antigen dosage titrations made with the cells of her parents and siblings (Moores, 1972a) are presented in this chapter.

15.2 CASE HISTORY

The Indian woman, Mrs S. Mah., who spoke Hindi, was three months pregnant with her third infant. Three years earlier, at her first confinement, she had been given two units of blood to correct her anaemia, but otherwise she was in good health. Her first two infants had both been normal at birth. A blood sample was received from her for routine antenatal tests on 22nd December 1965, and her group was determined as A_1, Rhesus positive. However, her serum reacted strongly in both one-stage 0,5% bromelin and indirect antiglobulin tests with the antibody screening but not with her own cells. In subsequent tests, all the cell samples in a panel of donors selected for their groups and the cells of 1 075 Whites, 217 Blacks, 298 Indians and 23 persons of mixed race were agglutinated by her serum. The members of her family were contacted and tested, and the cells of two of her siblings were found to be compatible with her serum.

Mrs S. Mah.'s infant, a male, was born at full term on 22nd May 1966 by normal delivery. He weighed 3 kilograms. The direct antiglobulin test on his cord cells was strongly positive, packed cell volume 45% and total serum bilirubin 1,9 mgs per 100 mls. At 12 hours his serum bilirubin had increased to 5 mgs per 100 mls and at 36 hours to 8,5 mgs per 100 mls. As no further rise occurred, an exchange transfusion was not given and, in due time, the mother and infant were discharged from hospital.

15.3 ANTIBODY IDENTIFICATION

Samples of blood from both mother and infant were sent to the Philip Levine laboratories in New York for identification of the antibody in Mrs S. Mah.'s serum. Her cells were tested by Miss Romola Lucia with many reagents and antibodies specific for high frequency antigens and were identified as U-. However, the antibody in her serum was not identified until after her infant's cord serum had been

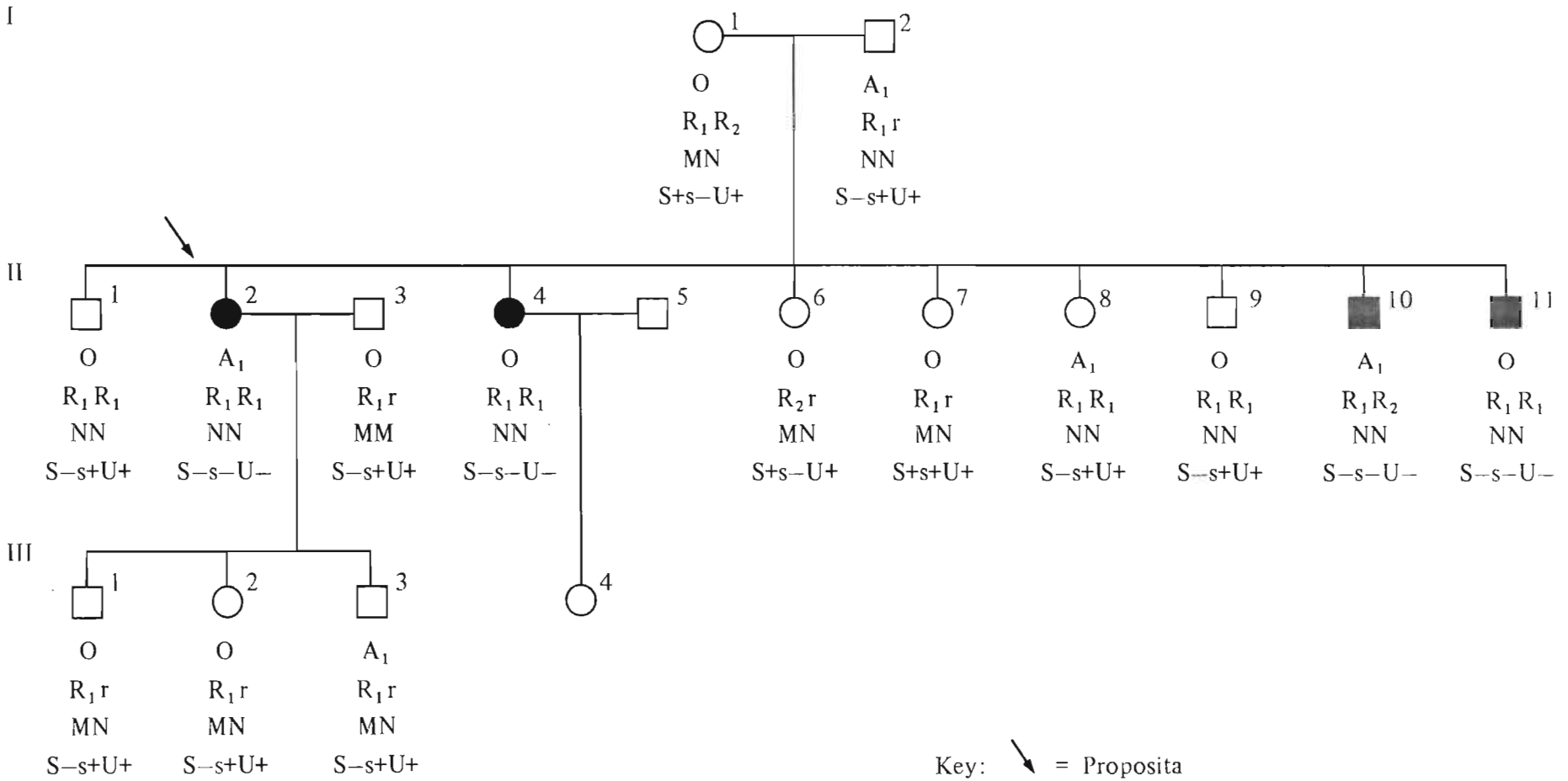
tested and found to contain anti-c. Both anti-c and anti-U were suspected in Mrs S. Mah.'s serum, and both were proved to be present when her serum failed to agglutinate a sample of Cde/Cde U- cells. Since the cord serum had contained anti-c only, but both anti-c and anti-U had been recovered in an eluate prepared by the 56°C heat technique of Landsteiner and Miller (1925) from the infant's cord cells, Mrs S. Mah.'s most serologically potent antibody was seen to be her anti-U. The titre of her anti-c antibody was determined in Durban to be 16 and of her anti-U 256. As neither of her two antibodies was inhibited by prior treatment with 2-mercapto-ethanol in a one-stage technique, both were determined to be IgG antibodies. Her antibodies were also easily removed from her serum by one absorbtion with c-U+ or c+U- cells. Mrs S. Mah.'s cells were typed as A_1 CDe/CDe and those of her infant as A CDe/cde.

15.4 FAMILY STUDY

Figure 15.1 shows that Mrs S. Mah., II-2, had three U- or S^uS^u, siblings, II-4, II-10 and II-11, and that their cells and her own were phenotype NN, U- (NS^uNS^u). Her mother, I-1, and sister, II-6, appeared to have homozygous SS cells, and her father, I-2, brother, II-1, sisters, II-7 and II-8, and three children, III-1, III-2 and III-3, all to have homozygous ss cells. The phenotypes of their cells were determined using four anti-S, six anti-s and two anti-U reagents of known strengths and reliable specificities. The ABO, MN and Rhesus groups, shown also in Figure 15.1, and the P_1, Lu^a, K, Lewis and Duffy phenotypes, revealed no evidence of illegitimacy in the family. Mrs S. Mah.'s parents had no knowledge of a consanguineous marriage relationship.

Since U- cells had not been identified before in persons other than Blacks and Pygmies, it was clearly important to establish whether or not Mrs S. Mah. and her family members had any of the genes or phenotypes characteristic in these races. The cells of all the family members except III-1, III-2 and III-3 were tested for strong H, M_1, cDe, weak C or Cde^S, V or ce, hr^S-, hr^B-, Js(a+), Le(a−b−) and Fy(a−b−), but they were not found. In addition, the HL-A factors of Mrs S. Mah.'s lymphocytes were kindly identified by Dr M. Hammond as being A2, A 11, Bw35, Bw40 and Cw4. While A2, Bw35 and Cw4 all failed to distinguish between Indians and Blacks, A 11 occurred in 25 to 38,8% Indians and 0,3% Blacks and Bw40 occurred in 29,4% Indians and 0,8% Blacks. The family lightly-built physique, straight black hair, brown skin colour and caucasoid faces also belied all suggestions that they had Black

Figure 15.1 Family of Mrs S. Mah.



admixture. The brown instead of black hair and short, stubby fingers of II-6 were also not considered in any way to denote a Black origin.

15.5 S AND s ANTIGEN DOSAGE STUDIES

Fresh blood samples, all drawn into ACD solution on the same day, were obtained from I-1, II-1, II-6, II-7, II-9 and from known SS, ss and Ss controls and were prepared for these tests as described in Chapter 2. Four anti-S and two anti-s reagents, known to distinguish between SS and Ss and between ss and Ss cells respectively, were titrated with extreme care, using matched cell suspensions and measured volumes of the cell suspensions and sera. The tests were then completed in accordance with the optimum technique for each reagent. The results (which were the same with all the reagents used) with one anti-S and one anti-s reagent only are shown in Table 15.1. They suggested that the cells of all the family members tested had single doses of either S or s antigen. The heterozygous sS^u and SS^u cells of the siblings also confirmed that I-2 had sS^u cells.

15.6 DISCUSSION

The U- cells of Mrs S. Mah. and the anti-U and anti-c antibodies identified in her serum confirmed that she was a U- person. The absence of evidence of Black admixture in the family also confirmed that she was the first U- person to be recorded outside the Black and Pygmy races. The three U- family siblings showed that the phenotype of Mrs S. Mah.'s cells was almost certainly not due to chance but to the inheritance of *uu* (or S^uS^u) genes; and antigen dosage titrations confirmed that the cells of I-1 and I-2 were heterozygous SS^u and sS^u respectively. The latter finding supported the view that *u*, or S^u, was a third allele at the *Ss* locus rather than the result of inhibition by a gene inherited at another chromosomal locus.

The serological coincidence of the anti-c and anti-U in Mrs S. Mah.'s serum was remarkable. Since anti-c was usually a potent antibody and, when present, often caused haemolytic disease of the newborn, it was interesting that her infant's cells appeared to have absorbed more anti-U than anti-c. However, neither antibody had caused severe haemolytic disease of the newborn in her infant. On many occasions subsequently, samples of Mrs S. Mah.'s serum were absorbed with A_1 CDe/CDe U+ cells and eluates containing potent anti-U recovered from them by ether technique of Vos and Kelsall (1956). As the cells of one of her three U- siblings were A_1

TABLE 15.1

S and s antigen dosage titrations with the cells of members of the S. Mah. U- Indian family

Family	Group	Dilutions of anti-S						
		2	4	8	16	32	64	Score
I-1	SS^u	2	1	(2)	–	–	–	14
II-6	SS^u	2	1	(2)	–	–	–	14
II-7	Ss	2	1	(2)	–	–	–	14
Control	SS	2	2	1	1	(2)	–	27
Control	Ss	2	1	(2)	–	–	–	14
Control	ss	–	–	–	–	–	–	0

Family Generations	Group	Dilutions of anti-$\bar{s}$						
		4	8	16	32	64	128	Score
II-1	sS^u	2	1	1	(2)	–	–	19
II-7	Ss	2	1	1	–	–	–	18
II-9	sS^u	2	1	(1)	–	–	–	13
Control	ss	3	3	2	1	1	–	38
Control	Ss	2	1	1	–	–	–	18
Control	SS	–	–	–	–	–	–	0

CDe/cDE, only two siblings were suitable for Mrs S. Mah. as blood donors, and it was not known whether she had borne further children.

15.7 SUMMARY

Four Natal Indians, the proposita and three siblings, all of whom had S-s-U- cells, represent the first U- persons to be recorded outside the Black race. The proposita had anti-c and anti-U in her serum and her infant was mildly affected by haemolytic disease of the newborn. Titrations with anti-S and anti-s showed that the cells of her parents were heterozygous SS^u and sS^u respectively.

CHAPTER 16

THE P, LUTHERAN, KELL AND LEWIS BLOOD GROUP SYSTEMS POPULATION FREQUENCIES

16.1 INTRODUCTION

16.1.1 The P system

The P_1, P_2 phenotype frequencies in the Indian populations in India are similar to those recorded in the literature in the populations of Europe (Mourant, Kopéc and Domaniewska-Sobczak, 1976). However, the frequencies were known to be difficult to compare with one another, as the P_1 antigen strength varied widely on the red cells. In this study, the P_1, P_2 frequencies in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal were estimated using two anti-P_1 reagents of good quality in parallel, and care was taken throughout the study to ensure that all the reagents used were capable of agglutinating a sample of cells that had exceptionally weak P_1 antigen. The cells of the Natal Indians were also tested for evidence of the p phenotype; and the percentage of samples positive with anti-P_1H, a 'new', antibody, discovered but not yet reported by myself which agglutinated only cells that had their P_1 and H antigens both expressed strongly, was recorded for the first time.

16.1.1.1 The P_1H antibody

16.1.1.1.1 Introduction

In 1967, an antibody of unknown specificity which reacted weakly in saline tests at 20°C with a few cell samples from group O Negroes (Zulus) was detected in the serum of a group B Indian blood donor. Microscopically, the agglutination was similar in type to that observed when (among other antibodies) anti-P_1, human anti-H, and anti-I reagents agglutinate red cells. The reaction might even be described as 'mixed field' in type, for some of the cells were agglutinated more strongly than others while a minority appeared unagglutinated. A further example of this antibody was discovered in the serum of a White donor, and another in the serum of a second Indian donor, in 1968, and the opinion of Dr Giles in London was sought. Dr Giles suggested that the antibody was a type of anti-P_1 but made no other comments.

16.1.1.1.2 Titrations

Since all the cell samples positive so far with the anti-P_1-like antibody had been from Zulus and Zulu cells were known to have strong H antigen (Brain, 1968), in 1978 the H and P_1 antigen strengths of some newly-detected anti-P_1-like positive cell samples and negative control cells of the same ABO group were estimated in careful parallel titrations using measured volumes of *Ulex* anti-H and several anti-P_1 reagents. The cell samples for the titrations were selected for being of the same age, and 5% suspensions of them were prepared in 1 ml volumes of the red cell suspending fluid of Burgess and Vos (1971) using measured volumes of washed, packed cells (matched cell suspensions). The anti-P_1 reagents had been standardised for use by different techniques. The results showed that the 15 anti-P_1-like positive group O samples tested all gave unusually high H and high P_1 antigen titre results and scores with these reagents, while among the 10 anti-P_1-like negative control group O samples, some gave similar high H but lower P_1, others lower H but similar high P_1 and still others lower H and lower P_1 titres and scores with them. A similar pattern of reactions was observed when two anti-P_1-like positive and one negative group B samples were tested in the same way (Table 16.1.1). Group A and group AB cells could not be tested as the three donors of the anti-P_1-like antibodies were all group B.

16.1.1.1.3 Family studies

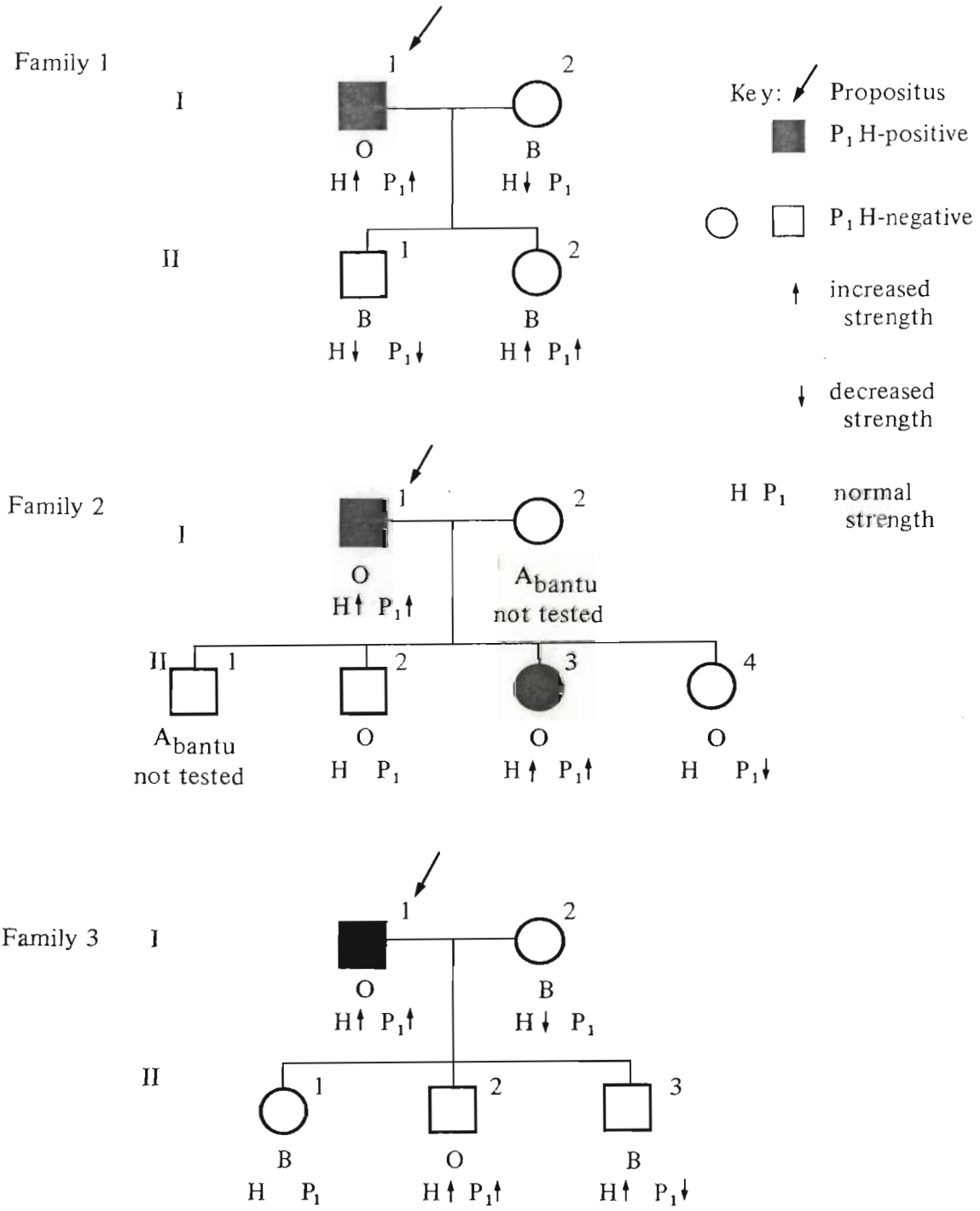
Three family studies (Figure 16.1) were made in 1978 with the much valued help of the Eastern Province Blood Transfusion Service in Port Elizabeth. The propositi (adult Xhosa Negro men who were all blood donors) were all found and members of their immediate families tested by this Service using a sample of the anti-P_1-like antibody sent to Port Elizabeth by myself for this purpose. On being received in Durban subsequently, the cells of the family samples were tested for their H and P_1 antigen strength by the technique described, and the concurrent exceptionally high titres and scores obtained with the cells of the three propositi using both the anti-H and the anti-P_1 reagents were noted. The cells of a daughter (group O) of one propositus, which were also agglutinated by the anti-P_1-like antibody, as expected, were agglutinated both by the anti-H and the anti-P_1 reagents almost as strongly as those of her father. However, the cells of all the anti-P_1-like negative family members tested were seen to react less strongly with both the anti-H and anti-P_1 reagents, or

TABLE 16.1.1

Titrations showing that anti-P_1 – like positive cells have strongly-expressed H and P_1 antigens

Sample No.	ABO group	P_1-like type	Anti-H dilutions 1	2	4	8	16	32	64	128	256	512	Score	P_1 type	Anti-P_1 dilutions 1	2	4	8	16	32	64	128	Score
1	O	+	4	4	4	4	4	3	2	(3)	–	–	70	+	4	3	2	1	1	(2)	–	–	39
2	O	+	4	4	4	4	4	3	2	(2)	–	–	69	+	4	3	3	2	1	(2)	(±)	–	44
3	O	+	4	4	4	4	3	2	1	(1)	–	–	63	+	3	3	2	1	(2)	(±)	–	–	34
4	O	+	4	4	4	4	4	3	2	1	(±)	–	73	+	4	4	3	3	1	(1)	–	–	45
5	O	+	4	4	4	4	4	4	3	1	(±)	–	75	+	4	4	3	2	1	(2)	–	–	44
6	O	+	4	4	4	4	4	4	4	2	(3)	–	80	+	3	3	2	1	1	(3)	–	–	40
7	O	+	4	4	4	4	4	3	1	(2)	–	–	66	+	4	4	3	3	2	1	–	–	53
8	O	+	4	4	4	4	4	3	2	(1)	–	–	68	+	4	4	3	2	1	(2)	–	–	44
9	O	–	4	4	4	2	1	(1)	–	–	–	–	43	+	4	3	3	1	(2)	–	–	–	36
10	O	–	4	4	4	3	2	1	(1)	–	–	–	53	+	2	1	(2)	(1)	–	–	–	–	14
11	O	–	4	4	4	2	(3)	–	–	–	–	–	40	+	1	(3)	(2)	(1)	–	–	–	–	8
12	O	–	4	4	4	4	4	2	(3)	–	–	–	60	+	3	3	2	1	(1)	–	–	–	33
13	O	–	4	4	4	3	3	1	(1)	–	–	–	55	+	3	2	1	1	(1)	–	–	–	28
14	O	–	4	4	4	3	3	1	(1)	–	–	–	55	+	4	3	2	1	(3)	(2)	–	–	36
15	B	+	3	2	1	(3)	(2)	(1)	–	–	–	–	26	+	4	3	2	1	(3)	(1)	–	–	35
16	B	+	3	3	2	1	1	(2)	–	–	–	–	39	+	3	2	1	(3)	(1)	–	–	–	25
17	B	–	4	3	2	2	1	(3)	–	–	–	–	43	+	1	1	(3)	(1)	–	–	–	–	12
Control	O	–	4	4	3	3	2	1	–	–	–	–	53	+	4	4	3	2	1	–	–	–	43
Control	B	–	3	2	1	(2)	–	–	–	–	–	–	24	+	2	1	(3)	(2)	–	–	–	–	16
Control	Cord O	–	2	1	1	(±)	–	–	–	–	–	–	18	+	–	–	–	–	–	–	–	–	0

Figure 16.1
Inheritance of P_1-like (P_1H) factor, strong H and strong P_1 antigens in three Xhosa Negro families



Note: In Family 3, II-2 had lower H and P_1 red cell antigen strength than I-1

In Family 1, II-2 had relatively high H and P_1 red cell antigen strength but not enough for his cells to be P_1 H-positive

strongly with one of these reagents, but in no instance as strongly with both of these reagents concurrently as the cells of the propositus in their families.

16.1.1.1.4 Inhibition tests

Inhibition tests using cyst fluid containing P_1 substances, saliva containing (1) H and Lewis substances from a group O secretor, (2) Le^a substance from a group O non-secretor, (3) H substance only from a group of Le(a—b—) secretor, and Guinea-pig urine containing Sd^a substance showed that the anti-P_1-like antibody was inhibited only by the cyst fluid. Further tests in which the cyst fluid was used unbuffered and buffered to pH 7,0 also confirmed that this substance did not inhibit anti-P_1 antibodies non-specifically through being over-alkaline (Marsh and Øyen, 1978).

16.1.1.1.5 The name

In selecting a suitable name for the anti-P_1-like antibody, the term anti-P_1H was chosen to indicate that it appeared to be recognising a product of strong P_1 and strong H antigen produced when these antigens were present on the same cell simultaneously but not when either strong P_1 or strong H antigen was present on them separately. As with the anti-IH of Rosenfield, Schroeder, Ballard, van der Hart, Moes and van Loghem (1964), the inclusion of the letter H was not meant to suggest that the antibody was inhibited by H substance. Moreover, no evidence suggesting that the anti-P_1H antibody was reacting in the same manner as the anti-IP_1 of Issitt, Tegoli, Jackson, Sanders and Allen (1968) was found and no studies with examples of pure anti-I (as distinct from anti-IH) which might have suggested that the cells of some Natal Negroes had stronger than usual I antigen were known to me.

16.1.1.1.6 Discussion

The anti-P_1H antibody was thought possibly to be reacting with an antigenic structure or structures formed by steric interaction between strong P_1 and strong H determinants on the red cell membrane. This was not unexpected as the biochemists have shown that the antigens of the ABO and P_1 systems are closely associated at the molecular level. It appears that a precursor, lactosyl ceramide, may be converted through ceramide trihexoside (P^k) to globoside (P) or through the sequential action of N-acetylglucosamine and galactose to paragloboside (lacto-N-neotetraosyl ceramide). The paragloboside is then converted either to a type 2 H structure (and so to

A and B structures), to a P_1 determinant by the addition of an α-galactosyl residue, or to sialoparagloboside by the addition of sialic acid (Watkins, 1980, p 109). The biochemists' views on the specificity of the anti-P_1 H antibody will be most welcome. The family studies suggested that one but not both of the two O genes in the propositi was coding for increased H antigen expression on the family members' cells but, since no family member was P_2, it was not possible to assess the inheritance of the P_1 gene in the families. Further work therefore seems called-for.

16.1.2 The Lutheran system

The Lu^a gene is reported to be either absent or to occur only rarely in Indians (Mourant *et al.*, 1976). A search was made with a potent anti-Lu^a reagent for Lu(a+) cells in the four Indian populations in Natal, and the findings have been included in this chapter.

16.1.3 The Kell system

The *K* gene, which has its highest frequency in the Caucasoid race, has been identified in 4–8% of the Indians in populations of large size in India. The frequency of the Kp^a gene in India, however, has not so far been recorded (Mourant *et al.*, 1976). In this study in the Indians of Natal, their K and Kp(a+) phenotype, and their *K* and $k+K^o$ and Kp^a and Kp^b+K^o gene frequencies were estimated. A search was also made in them with anti-Js^b, anti-Ku(K5) and anti-K13 for cells with the corresponding low frequency phenotypes, and a sample of Wk(a+) cells was used to investigate their sera for possible examples of anti-Wk^a.

16.1.4 The Lewis system

The most reliable method of determining the frequency of the *Le* and *le* genes is unquestionably by means of saliva inhibition tests. However, no saliva samples were obtained from the Natal Indian populations whose blood group frequencies were being studied. Their *Le/le* gene frequencies were therefore estimated by means of their Lewis red cell phenotypes, and care was taken to ensure that at least two examples of anti-Le^a, anti-Le^{bL} and anti-Le^x were always used in parallel for this purpose. The *Le/le* frequencies in the people of India appeared in the tables provided by Mourant *et al.* (1976) to be fairly similar to those of the people in Europe.

16.2 MATERIAL AND METHODS

The blood samples and their method of preparation for these tests have been described in Chapter 2. Only Natal Indian males and unmarried females were tested. The reagents consisted of several examples of potent anti-P_1 employed in pairs, their titres ranging between 32 and 512 by the technique used, and an anti-PP_1P^k serum kindly supplied by Mrs A. Hoppe of Washington. The anti-P_1H sera used were from a local Indian and a local White blood donor, and the anti-Lu^a reagent, from a local Black donor, was a strong example which had been distributed to other laboratories as well for their work. The anti-K reagents were standardised sera from local White donors, and the anti-Kp^a was a reagent prepared by absorbing a local serum containing both anti-CD and Kp^a with CD+Kp(a–) cells. The anti-Js^b reagents comprised sera from local Black donors, a serum kindly supplied by Dr R. Lowe of Salisbury and a placental eluate prepared by myself by the method kindly sent by J. Moulds of Houston. J. Moulds also kindly sent the anti-Ku, or anti-K5, reagent, and the anti-K13 (Sgro) was received with grateful thanks from L. Marsh of New York. The anti-anti-Le^a, anti-Le^{bL} and anti-Le^x reagents were all from local Black and Indian donors, their titres being 16 to 32 by the technique employed. The anti-Le^{bL} reagents were tested and found not to be inhibited by the saliva of a secretor of H but no Lewis substances. The specificity of all the reagents was checked before use and daily with both negative and heterozygous or weak positive control cells throughout the test period. To avoid weakening them, the anti-P_1 and Lewis reagents were not absorbed for ABO allo-antibodies, but the former were used instead with ABO compatible and the latter only with O cells. The Wk(a+), p, $P_1{}^k$ and K^o control cells were the gifts of colleagues through the SCARF exchange system, and the P_1H-positive, Lu(a+), K+, Kp(a+) and Js(b–) control cells were from local donors.

The techniques used in these studies have been described in Chapter 2. The anti-P_1, anti-PP_1P^k, anti-P_1H, anti-Lu^a, anti-Le^a, anti-Le^{bL} and anti-Le^x reagents were used by saline, one-stage 0,5% bromelin or one-stage 0,25% ficin techniques at 20°C, 10°C or 4°C. The anti-K, anti-Kp^a, anti-Ku(K5), anti-K13 and anti-Js^b reagents were used either by saline or LISS indirect antiglobulin techique, and the sera of the Natal Indians were tested with Wk(a+) cells by saline indirect antiglobulin technique. The gene frequencies in this chapter were calculated by the methods suggested by Mourant *et al.* (p 48–56, 1976), and the tests for significance were made by the 2 X 2 table and χ^2 method without Yates' correction.

16.3 RESULTS

16.3.1 The P system

The P_1, P_2 frequencies in the Natal Tamil-, Telegu- and Hindi-speaking and Moslem Indians are presented in Table 16.1.2. The frequencies were similar to those reported by other workers in populations in both India and Europe, and three of the χ^2 tests for P_1 frequencies between the populations in Table 16.1.2 were found to be significant.

In a small study made to detect p cells in the Natal Indians, none were found. Also, no examples of anti-P^k were detected in the sera of 667 random Natal Indians. These results were recorded in Table 16.2 for interest.

The frequencies of the curious 'new' P_1 H-positive phenotype in random group O Indians, Zulu Blacks and Whites in Natal are presented in Table 16.3. The highest P_1 H-positive frequency was noted to occur in the Zulus, possibly reflecting their known high mean red cell H antigen strength (Brain, 1968). The χ^2 tests for significance in the frequencies between the three races were as follows:

Populations	χ^2	*P*
Natal Indians versus Natal Whites	2,8	not significant
Natal Indians versus Natal Zulus	72,0	$<0,001$
Natal Whites versus Natal Zulus	93,8	$<0,001$

16.3.2 The Lutheran system

No Lu(a+) cell samples were found in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal. However, the number of Indians tested with anti-Lu^a has been recorded in this chapter for interest. (Table 16.4)

16.3.3 The Kell system

Table 16.5 shows the K+ (K:1) and K– (K:–1) phenotype and the *K* and $k+K^o$ gene frequencies recorded by other workers in Hindu and Moslem Indian populations in India and Johannesburg, and Table 16.6 the frequencies in the four Indian populations studied in Natal. Significantly different frequencies were detected between the

TABLE 16.1.2

P_1, P_2 frequencies in 716 Natal Indian males and unmarried females estimated using anti-P_1 and anti-PP_1P^k

Category	Number tested		Phenotype frequencies P_1	P_2	p	Gene frequencies P_1	P_2
Tamil	211	No.	153	58	0	0,4757	0,5243
		%	72,51	27,49	0,00		
Telegu	115	No.	80	35	0	0,4484	0,5516
		%	69,57	30,43	0,00		
Hindi	142	No.	117	25	0	0,5804	0,4196
		%	82,39	17,61	0,00		
Moslem	248	No.	173	75	0	0,4501	0,5499
		%	69,76	30,24	0,00		

Significance of differences

All differences between the populations in Table 16.1.2 were not significant at level $P < 0,05$ except:

	χ^2	P
Natal Hindi and Natal Tamil	4,6	$<0,05>0,02$
Natal Hindi and Natal Moslem	7,6	$<0,01>0,001$
Natal Hindi and Natal Telegu	5,8	$<0,02>0,01$

TABLE 16.2

Natal Indians tested with anti-PP_1P^k only and with $P_1{}^k$ cells

Number Natal Indians tested
No negatives found

Reagent	Tamil	Telegu	Hindi	Moslem	Random	Total
Anti-PP_1P^k	157	105	118	100		480
			No positives found			
$P_1{}^k$ cells					667	667

TABLE 16.3

P_1 H phenotype frequencies in group O Natal Indians, Blacks and Whites estimated using anti-P_1 H

Category	Number tested		Phenotype frequencies P_1 H +	P_1 H –	Gene frequencies *P_1H*	*non-P_1H*
		No.	10	1 094		
Indians	1 104				0,0046	0,9954
		%	0,91	99,09		
		No.	51	501		
Blacks	552				0,0473	0,9527
(Zulus)		%	9,24	90,76		
		No.	4	1 141		
Whites	1 145				0,0018	0,9982
		%	0,35	99,65		

Significance of differences

All differences between the populations in Table 16.3 were not significant at level $P < 0,05$ except:

Blacks and Whites χ^2	93,8	$< 0,001$
Blacks and Indians	72,0	$< 0,001$

TABLE 16.4

Lutheran phenotype frequencies in 725 Natal Indian males and unmarried females estimated using anti-Lu^a only

Category	Number Tested		Phenotype frequencies Lu(a+)	Lu(a−)
Tamil	255	No.	0	255
		%	0,00	100,00
Telegu	108	No.	0	108
		%	0,00	100,00
Hindi	143	No.	0	143
		%	0,00	100,00
Moslem	219	No.	0	219
		%	0,00	100,00

TABLE 16.5

Phenotype and gene frequencies in the Kell system in Indians of India and Johannesburg recorded by others

Category	Place	Authors	Reference No. in Mourant *et al.* 1976	Number tested		Phenotype frequencies K+ K:1	Phenotype frequencies K− K:−1	Gene frequencies K	Gene frequencies k + K^o
Hindu	Rourkela in Orissa (Telegu)	Seth, 1967	2 607 page 522	201	No.	30	171	0,0777	0,9223
					%	14,93	85,07		
Hindu	Johannesburg	Nurse and Jenkins, 1977		53	No.	14	39	0,0858	0,9142
					%	26,42	73,58		
Moslem	Johannesburg	Nurse and Jenkins, 1977		56	No.	9	47	0,0916	0,9084
					%	16,07	83.93		

TABLE 16.6

Phenotype and gene frequencies in the Kell system in 1 165 Natal Indian males and unmarried females estimated using anti-K (K1) only

Category	Number tested		Phenotype frequencies K+ K:1	K− K:−1	Gene frequencies K	$K + K^O$
Tamil	399	No.	8	391	0,0101	0,9899
		%	2,01	97,99		
Telegu	181	No.	2	179	0,0055	0,9945
		%	1,10	98,90		
Hindi	289	No.	2	287	0,0035	0,9965
		%	0,69	99,31		
Moslem	296	No.	16	280	0,0274	0,9726
		%	5,41	94,59		

Significance of differences

All differences between the populations in Table 16.6 and between the relevant populations in Tables 16.5 and 16.6 were not significant at level $P \preccurlyeq 0,05$ except:

	χ^2	P
Natal Moslem and Natal Tamil	5,9	$< 0,02 > 0,01$
Natal Moslem and Natal Telegu	5,7	$< 0,02 > 0,01$
Natal Moslem and Natal Hindi	10,9	$< 0,001$
Natal Hindu and Hindu (Seth, 1967)	79,4	$< 0,001$
Natal Hindu and Hindu (N. & J., 1977)	14,2	$< 0,001$
Natal Moslem and Moslem (N.& J., 1977)	8,1	$< 0,01 > 0,001$

Natal Moslem and each of the three Natal Hindu populations. The differences were also significant between the Natal Hindu and the two Hindu populations in Table 16.5 and between the Natal Moslem Indians and the Moslem population in this table. As normal 4,3% and normal 1,1% *K* frequencies had been recorded in my M.Sc thesis in 1976 for the Natal Whites and Natal Blacks respectively, and the anti-K reagents, which were standardised, had been checked for specificity and also daily with known K+ and K− control cells during these tests, the 2,74 to 1,0% *K* frequency found in the Natal Indian populations was believed to be correct.

The Kp(a+) or K:3 phenotype and the Kp^a and Kp^b+K^o gene frequencies in the Natal Tamil-, Telegu- and Hindi-speaking and Moslem Indians are presented in Table 16.7. No Kp(a+) cell samples were found among the Tamil- and Telegu-speaking or in the Moslem Indians tested but three (1,18%) were detected in the Hindi-speaking Indians.

In Table 16.8, the results of a search for the low frequency phenotypes Js(b−), K:−13 and K:−5 (Ku−), and for anti-Wk^a, in the Natal Indians have been shown. None were found, and the number of Indians tested was included here for interest.

16.3.4 The Lewis system

In the first series of tests for the Lewis phenotype and *Le/le* gene frequencies in four Natal Indian populations studied, anti-Le^a only was used, and in the second series, anti-Le^a, anti-Le^{bL} and anti-Le^x were all used in parallel. The results, presented in Tables 16.10 and 16.11 respectively, were compared with one another; and the Le(a+) frequency in the Natal Hindi-speaking Indians in Table 16.10 was compared with the frequency in the probably predominantly Hindi-speaking Indian population in India of Bird, shown in Table 16.9. None of the χ^2 tests for Le(a+b−) or Le(a−b−) phenotype frequencies in these tables was found to be significant. However, the χ^2 tests for Le(a−b+) phenotype frequencies differed significantly between the Natal Tamil- and Natal Telegu-speaking, Natal Hindi-speaking and Natal Moslem Indian populations and between the Natal Hindi-speaking and Natal Moslem Indian populations (see Table 16.11)

TABLE 16.7

Phenotype and gene frequencies in the Kell system in 1 041 Natal Indian males and unmarried females estimated using anti-Kp^a (K3) only

Category	Number tested		Phenotype frequencies Kp(a+) K:3	Kp(a−) K:−3	Gene frequencies Kp^a	$Kp^b + K^o$
Tamil	358	No.	0	358	0,0000	1,0000
		%	0,00	100,00		
Telegu	148	No.	0	148	0,0000	1,0000
		%	0,00	100,00		
Hindi	254	No.	3	251	0,0059	0,9941
		%	1,18	98,82		
Moslem	281	No.	0	281	0,0000	1,0000
		%	0,00	100,00		

TABLE 16.8

Natal Indians tested for phenotypes and antibodies of low frequency in the Kell system

Reagents	Number of Indians tested No negatives found					
	Tamil	Telegu	Hindi	Moslem	Random	Total
Anti-Js^b	103	101	107	100		411
Anti-K13	102	103	102	100		407
Anti-K5 (Ku)	108	104	103	101		416
	No positives found					
Wk(a+) cells					667	667

TABLE 16.9

Le^a phenotype frequencies in Indians of India recorded by others

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies Le(a+)	Le(a−)
Group B and O Indians (Hindi)	India	Bird, 1953a page 558	324	200	No.	57	143
					%	28,50	71,50

TABLE 16.10

Le^a phentoype frequencies in 427 Natal Indian males and unmarried females estimated using anti-Le^a only

Category	Number tested		Phenotype frequencies Le(a+)	Le(a−)
Tamil	125	No.	33	92
		%	26,40	73,60
Telegu	102	No.	25	77
		%	24,51	75,49
Hindi	100	No.	22	78
		%	22,00	78,00
Moslem	100	No.	23	77
		%	23,00	77,00

TABLE 16.11

Lewis phenotype and *Le:le* gene frequencies in 1 175 Natal Indian males and unmarried females estimated using anti-Le^a, anti-Le^{bL} and anti-Le^x

Category	Number tested		Phenotype frequencies Le(a+b−)	Le(a−b+)	Le(a−b−)	Le(a+b+)	Gene frequencies *Le*	*le*
Tamil	426	No.	125	228	69	4	0,5975	0,4025
		%	29,34	53,52	16,20	0,94		
Telegu	198	No.	43	125	28	2	0,6240	0,3760
		%	21,72	63,13	14,14	1,01		
Hindi	269	No.	63	167	39	0	0,6192	0,3808
		%	23,42	62,08	14,50	0,00		
Moslem	282	No.	50	199	33	0	0,6579	0,3421
		%	17,73	70,57	11,70	0,00		

Significance of differences

All differences between the populations in Table 16.11 and between the relevant populations in Tables 16.11 and 16.9 were not significant at level $P \leq 0,05$

16.4 DISCUSSION

The P_1 frequencies in the Tamil- and Telegu-speaking and Moslem Indians in Natal were not significantly different from the 50% frequency known in Europe and India but they differed significantly from the P_1 frequency in the Natal Hindi-speaking Indians. This indicated that the latter populations probably formed a separate group in Natal. The frequencies in all four populations were surprisingly low and differed significantly from the more normal K frequencies recorded in the Indian populations in Johannesburg by Nurse and Jenkins (1977). It was thought that perhaps the environment in Natal was selecting against Indians with K+ cells or that the differences were due to genetic drift. The low Kp(a+) frequency detected in the Hindi-speaking Indians was interesting and was in accord with the frequency in Europe. The Le(a+b−) frequencies in the four Indian populations agreed with those in Europe but the Le(a−b−) frequencies in them were all higher than expected. Their Le(a−b+) frequencies, which differed the most in this system, suggested that the Natal Tamil-speaking Indians, and again that the Natal Moslem Indians, formed separate groups. As expected, no Indians with Lu(a+) cells were found, and the low P_1 H-positive frequencies, which failed to distinguish between the Natal Indians and Whites but distinguished both markedly from the Natal Blacks, agreed with the view that the Natal Indians had little Black admixture.

16.5 SUMMARY

The P_1 phenotype frequencies in the Natal Hindi-speaking Indians were found to be significantly higher than those in the Natal Tamil- and Telegu-speaking and Natal Moslem Indians, and the Le(a−b+) phenotype frequencies in the Natal Tamil-speaking and Natal Moslem Indians were both seen to be lower than in the Natal Telegu- and Hindi-speaking Indians. The Le(a+b−) and Le(a−b−) phenotype frequencies in the four Indian populations were similar, and no Indians with Lu(a+) cells were found. The K+ frequencies were all lower than expected, and Kp(a+) cells were detected in the Hindi-speaking Indians. The P_1 H-positive frequency in the Natal Indians was similar to that in the Natal Whites, but both differed significantly from the frequency in the Natal Blacks. The phenotypes p, Js(b−), K:−13 and K:−5 (Ku−) were not detected in the four Natal Indian populations.

CHAPTER 17

THE DUFFY AND KIDD BLOOD GROUP SYSTEMS AND OTHER BLOOD GROUP ANTIGENS POPULATION FREQUENCIES

17.1 INTRODUCTION

17.1.1 The Duffy system

The frequency of the Fy^a gene is 40–60% in the people of India and is therefore higher in them than in the people of Europe (Mourant, Kopéc and Domaniewska-Sobczak, 1976). No records were found of the frequencies in the Duffy system determined in India using anti-Fy^a, anti-Fy^b and anti-Fy3. Since, in Durban, anti-Fy^a and small quantities of both anti-Fy^b and anti-Fy3 were available, all three reagents were used to determine the frequencies in the Natal Tamil-, Telegu- and Hindi-speaking and Moslem Indians recorded in this chapter.

17.1.2 The Kidd system

The frequencies in the Kidd system in the world's populations appear in most instances to have been estimated using anti-Jk^a. In Durban, as only anti-Jk^b was available, the Jk(a+) and $Jk^a + Jk$ frequencies in the Natal Indian populations had to be assessed indirectly with this antibody. The Jk^a frequency in India is lower than in Europe, and the average frequency there was said by Mourant *et al.* (1976) to be 51,4%. Both the Jk^a and Jk^b frequencies estimated in the Natal Tamil-, Telegu- and Hindi-speaking and Moslem Indians are included in this chapter.

17.1.3 The Xg^a blood group antigen

Bhatia (1963), who studied the Xg^a phenotype and Xg^a gene frequencies in Indian males and females in Bombay, India, recorded that their Xg^a gene frequency was 65,20%. This figure was within the known range for the people of Europe (50,31% in Finland and Norway to 76,12% in Italy; Mourant *et al.*, 1976) and close to the 62,97% average frequency in Europe for 13 populations. No records of other Xg^a

frequency studies in Indians were found. As only a limited volume of anti-Xg^a (group A) was available in Durban and more Indian males than females customarily presented themselves there as blood donors, the corresponding phenotype and gene frequencies presented in this chapter were estimated in group A and group O Natal Indian males only.

17.1.4 The Cad and Sd^a blood group antigens

Sringarm, Chupungart and Giles (1972) and Sringarm, Chiewsilp and Tubrod (1974) recorded Cad, or Sd(a+++), red cells in 0,24% group O Thai and 0,25% grop B Thai blood donors. Since Thailand was relatively near India and, consequently, Indians might also have these cells, a search was made for Cad+ cells in the group O and B Natal Indians. Thr reagent used for this purpose was *Dolichos biflorus* anti-A_1. In addition, the Sd(a++), Sd(a+) and Sd(a–) phenotype frequencies in the Natal Indians were estimated using anti-Sd^a, and the incidence of anti-Sd^a in the Indians' sera was studied.

17.1.5 The Colton blood group antigens

The Co^a gene frequency is 96,3% in Caucasoids, but it has not yet been recorded specifically in Indians (Mourant *et al.*, 1976). A small quantity of both anti-Co^a and anti-Co^b was available in Durban, and the latter reagent was employed to study the Co^b phenotype and Co^b gene frequencies in the Natal Indians. All the Co(b+) cell samples were subsequently tested with anti-Co^a. The frequencies found, and the results of a search with Co(b+) cells for anti-Co^b in the sera of the Natal Indians, are recorded here. The Co^b gene frequency in Europe varied between 4,2 and 7,7% (Mourant *et al.*, 1976).

17.1.6 The Diego blood group antigens

Although almost certainly a Mongoloid marker gene, Di^a has been found in low frequencies in Caucasoids (Simmons, Albrey, Morgan, Smith, 1968), including 2–3% Indians in northern India. An insufficient amount of anti-Di^a was available in Durban for a full frequency study to be made, but the reagent was used to test the cells of a small number of Natal Indians in an attempt to establish whether the gene was present in Natal. The study was successful and the results are presented in this chapter.

17.1.7 The In blood group antigens

The first example of anti-In^a was reported from Bombay in India by Badakere, Joshi, Bhatia, Desai, Giles and Goldsmith in 1973, and the first example of anti-In^b by Giles in London in 1975. In 1974, Badakere, Parab and Bhatia described the In(a+) phenotype frequency as being 2,3% in Maharashtrians and 4,5% in Gujaratis in Bombay, and the In^a gene frequency was estimated by them in random Bombay hospital patients and blood donors as being 3,17%. A small quantity of anti-In^a was available in Durban to study the In(a+) phenotype and In^a gene frequencies in the Natal Indians, and all the cell samples agglutinated by this reagent were kindly tested with anti-In^b in London by Dr Giles. The frequencies estimated, and the results of a search for examples of anti-In^a in Natal Indian hospital patients, are recorded here.

17.1.8 Other blood group antigens

As limited quantities of antibodies specific for the low frequency blood group antigens Mit, Wb and Wr^a, and for the high frequency antigens Gy^a, I, Jr^a, Lan, Vel and Yt^a were available in Durban, they were all used to test the cells of the Natal Indians for the corresponding low frequency phenotypes. No records of previous studies with these antibodies in the Indians of India were found. The sera of the Natal Indians were also tested with Wr(a+) and Rd+ cells in an attempt to identify examples of anti-Wr^a and anti-Rd in them. The findings are included in this chapter for interest.

17.2 MATERIALS AND METHODS

As before, the blood samples used for these studies were from Natal Indian males and unmarried females. The method used to prepare their samples for testing will be found in Chapter 2. The anti-Fy^a, anti-Jk^b, anti-Sd^a, anti-Co^a, anti-Wr^a and anti-I were standardised reagents prepared from the sera of local blood donors. The anti-Fy^b, anti-Xg^a and anti-Di^a reagents were kindly supplied by Dr G.H. Vos of Durban, the anti-Fy3, anti-Co^b, anti-Wb and Wb+ controls cells by J. Albrey of Brisbane, the anti-In^a by Dr C. Giles of London and Dr H.M. Bhatia of Bombay, the anti-Mit and Mit+ control cells by Dr Stout of Edmonton, the anti-Gy^a and anti-Jr^a by J. Moulds of Houston, the anti-Lan by Dr D. Smith of Winchester, the anti-Yt^a by Dr T. Greenwalt of New York and the anti-Vel by Dr A.P. Albert of Port Elizabeth. The

anti-A_1 lectin was prepared in Durban from *Dolichos biflorus* seeds obtained in Australia. The Cad+, Co(a–), Di(a+), In(a+), Rd+, Gy(a–), Jr(a–), Lan-, Vel- and Yt(a–) control cells were gifts from colleagues through the SCARF exchange system, and the Fy(a–b–), Co(b+) and Wr(a+) control cells were from local donors.

The techniques used in these studies are described in Chapter 2. The anti-Fy^a, anti-Fy^b, anti-Fy3, anti-Xg^a, anti-Sd^a, anti-Co^a, anti-Di^a, anti-Gy^a, anti-Jr^a, anti-Lan, anti-Mit, anti-Vel and anti-Yt^a were used by saline or LISS indirect antiglobulin technique, whichever of these was optimal for each reagent. The anti-Jk^b was used by LISS indirect antiglobulin technique as well but with complement added. The anti-A_1, anti-Wb and anti-In^a were used by saline technique, and the anti-I, anti-Wr^a and anti-Co^b by one-stage 0,5% bromelin technique at the optimum temperature for each reagent. The sera of the Natal Indians were tested for anti-Wr^a by saline, for anti-Co^b by one-stage 0,5% bromelin, and for anti-In^a, anti-Rd and anti-Sd^a by saline indirect antiglobulin, techniques. The reagents were all checked for specificity before being used, and also daily with negative, heterozygous positive and, where possible, homozygous positive control cells throughout the test period.

The gene frequencies in this chapter were calculated by the methods suggested by Mourant *et al.* (p 48–56, 1976). The frequencies in each table were compared with one another and, where possible, with those determined by other workers in Indian populations whose languages or religions were similar to those of the Natal Indians. The 2 X 2 table and χ^2 method was used for this, without Yates' correction.

17.3 RESULTS

17.3.1 The Duffy system

Table 17.1 contains the Fy(a+) phenotype and the Fy^a and $Fy^b + Fy$ gene frequencies estimated with anti-Fy^a only by other workers in Hindu and Moslem Indian populations in India and Johannesburg, and Table 17.2 the frequencies recorded in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal. As expected, the Fy(a+) phenotype frequencies in Table 17.2 were high and the average Fy^a frequency in the non-Moslem populations in this table (66%) exceeded the 40–60% frequency known from the lists provided by Mourant *et al.* (1976) to be characteristic in Indians. The Fy(a+) frequency in the Natal Moslem Indians, however, differed

TABLE 17.1

Phenotype and gene frequencies in the Duffy system in Indians of India and Johannesburg recorded by others

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies Fy(a+)	Fy(a−−)	Gene frequencies Fy^a	$Fy^b + Fy$
Hindu (Telegu)	Rourkela in Orissa	Seth, 1967	2 607 page 581	209	No.	140	69	0,4255	0,5745
					%	66,99	33,01		
Hindu	Johannesburg	Nurse and Jenkins, 1977		53	No.	45	8	0,6115	0,3885
					%	84,91	15,09		
Moslem	Johannesburg	Nurse and Jenkins, 1977		56	No.	47	9	0,5991	0,4009
					%	83,93	16,07		

TABLE 17.2

Phenotype and gene frequencies in the Duffy system in 813 Natal Indian males and unmarried females tested with anti-Fy[a]

Category	Number tested		Phenotype Fy(a+)	Phenotype Fy(a−)	Gene frequencies Fy^a	Gene frequencies $Fy^b + Fy$
Tamil	144	No.	130	14	0,6882	0,3118
		%	90,28	9,72		
Telegu	118	No.	102	16	0,6318	0,3682
		%	86,44	13,56		
Hindi	147	No.	130	17	0,6600	0,3400
		%	88,44	11,56		
Moslem	100	No.	72	28	0,4709	0,5291
		%	72,00	28,00		
Random	304	No.	264	40	0,6372	0,3628
		%	86,84	13,16		

Significance of differences

All differences between the populations in Table 17.2 and between the relevant populations in Tables 17.2 and 17.1 were not significant at level $P \leq 0,05$ except:

	χ^2	P
Fy(a+) in Natal Tamil and Natal Moslem	13,8	$< 0,001$
Fy(a+) in Natal Telegu and Natal Moslem	7,0	$< 0,01 > 0,001$
Fy(a+) in Natal Hindi and Natal Moslem	10,8	$< 0,001$
Fy(a+) in Natal Telegu and Hindu (Seth, 1967)	14,8	$< 0,001$

significantly from the frequencies in the Tamil-, Telegu- and Hindi-speaking Indians (Table 17.2), the probability being less than 1 in 100 between the Moslems and the Telegu-, and less than 1 in 1 000 between the Moslems and the Tamil- and Hindi-speaking Indians, that these differences were due to chance. The reason was that the Natal Moslem Indians had the lowest Fy(a+) frequency of the four populations. In the Natal Telegu-speaking Indians, the Fy(a+) frequency also differed significantly from that in the Hindu population of Seth shown in Table 17.1, despite the latter population being from a predominantly Telegu-speaking part of India. The reason for this finding was not known.

The high Fy(a+) and Fy^a frequencies observed in the four Natal Indian populations studied were seen again when a further series of Indian donors was tested with anti-Fy^a, anti-Fy^b and anti-Fy3. The findings in this investigation are presented in Table 17.3. None of the differences in Fy(a+b–) phenotype frequency between the populations shown in this table was significant, but significant differences were observed in the Fy(a–b+) frequency between the Natal Moslem and Natal Hindi Indians and in the Fy(a+b+) frequency between the Natal Telegu and Natal Hindi Indians. The χ^2 values calculated for the various Duffy frequencies between the populations who spoke the same language or had the same religion shown in Tables 17.2 and 17.3 were not significant. Only one Indian, a Natal Moslem, was found whose cells were phenotype Fy(a–b–). As his Rhesus phenotype was CDe/cDE, his cells were tested with anti-f (–ce) to determine whether his genotype contained the *CDE* chromosome sometimes found in Indians. A negative result indicated that this was not so.

17.3.2 The Kidd system

The frequencies in the Kidd system in the four Indian populations studied in Natal, detected using anti-Jk^b only, are shown in Table 17.4. No statistically significant differences in Jk(b+) frequency were found between any of these populations. The Jk^a frequency in the Natal Moslem Indians, however, was higher than the 51,4% average for European populations given by Mourant *et al.* (1976). The high average frequency (67,19%) in the non-Moslem populations in Table 17.4 was also interesting, but as no frequency studies had been made before with anti-Jk^b in Natal, it was unfortunately not possible to judge the efficiency of the reagents used by referring to frequencies in other populations there.

TABLE 17.3

Phenotype and gene frequencies in the Duffy system in 389 Natal Indian males and unmarried females tested with anti-Fy^a, anti-Fy^b and anti-Fy3

Category	Number tested		Phenotype frequencies Fy(a+b−)	Fy(a−b+)	Fy(a−b−)	Fy(a+b+)	Gene frequencies Fy^a	Fy^b	Fy
Tamil	114	No.	47	15	0	52	0,6404	0,3596	
		%	41,23	13,16	0,00	45,61			
Telegu	74	No.	42	9	0	25	0,7095	0,2905	
		%	54,06	12,16	0,00	33,78			
Hindi	97	No.	40	9	0	48	0,6598	0,3402	
		%	41,24	9,28	0,00	49,48			
Moslem	104	No.	41	22	1	40	0,5373	0,3703	0,0924
		%	39,42	21,16	0,96	38,46			

Significance of differences

All differences between the populations in Table 17.3 were not significant at level $P \lesssim 0,05$ except:

	χ^2	P
Fy(a−b+) in Natal Moslem and Natal Hindi	5,4	0,02
Fy(a+b+) in Natal Telegu and Natal Hindi	4,2	$< 0,05 > 0,02$

TABLE 17.4

Kidd phenotype and gene frequencies in 535 Natal Indian males and unmarried females tested with anti-Jk^b

Category	Number tested		Phenotype frequencies		Gene frequencies	
			Jk(b−)	Jk(b+)	$Jk^a + Jk$	Jk^b
Tamil	124	No.	55	69	0,6659	0,3341
		%	44,35	55,65		
Telegu	108	No.	50	58	0,6804	0,3196
		%	46,30	53,70		
Hindi	125	No.	56	69	0,6693	0,3307
		%	44,80	52,20		
Moslem	178	No.	73	105	0,6404	0,3596
		%	41,01	58,99		

Significance of differences

All differences were not significant between the populations in Table 17.4 at level $P \leq 0,05$

17.3.3 The Xg^a blood group antigens

The 64,00% to 77,14% Xg^a antigen frequencies in Natal Indian group A and O males presented in Table 17.6 differed little from the 65,20% frequency in Indians of Bombay in India recorded by Bhatia (1963) and shown in Table 17.5. The frequencies in the Natal Tamil-, Telegu- and Hindi-speaking Indians were all higher than in the Natal Moslem Indians, and the difference between the frequencies in the Tamil-speaking and Moslem Indians was statistically significant ($P < 0,05 > 0,02$). Using the same reagent, a 46,5% Xg^a frequency had been detected earlier in Natal Black males (Moores; M.Sc. thesis, 1976) but, as the frequency in Natal White males had not been studied, the efficiency of the reagent could not be assessed by comparing the latter

TABLE 17.5

Xg^a frequencies in Indians of India recorded by others

Category	Place	Authors	Reference No. in Mourant *et al.*, 1976	Number tested		Phenotype frequencies Males Xg(a+)	Males Xg(a−)	Females Xg(a+)	Females Xg(a−)	Gene frequencies Xg^a	Gene frequencies Xg
			295		No.	34	16	43	7		
Indians (Gujarati)	Bombay	Bhatia, 1963	page 630	100						0,6520	0,3480
					%	34,00	16,00	43,00	7,00		

with the frequencies in Europe. The Xg(a+) frequency differences between the Natal Moslems and the Bombay population of Bhatia, and between the Natal Tamil-, Telegu- and Hindi-speaking populations pooled together and this population, were not statistically significant ($P < 0,05$).

TABLE 17.6

Xg^a phenotype and gene frequencies in 412 Natal Indian males tested with anti-Xg^a

Category	Number tested		Phenotype frequencies Xg(a+)	Xg(a−)	Gene frequencies Xg^a	Xg
Tamil	105	No.	81	24	0,7714	0,2286
		%	77,14	22,86		
Telegu	106	No.	77	29	0,7264	0,2736
		%	72,64	27,36		
Hindi	101	No.	72	29	0,7129	0,2871
		%	71,29	28,71		
Moslem	100	No.	64	36	0,6400	0,3600
		%	64,00	36,00		

Significance of differences

All differences were not significant between the populations in Table 17.6 and between the relevant populations in Tables 17.6 and 17.5 at level $P \lesssim 0,05$ except:

	χ^2	P
Xg(a+) in Natal Tamil and Natal Moslems	4,3	$< 0,05 > 0,02$

17.3.4 The Sd^a blood group antigen

The numbers and frequency of Sd(a++) red cell samples detected with *Dolichos biflorus* anti-A_1, and of Sd(a++), Sd(a+) and Sd(a–) cell samples detected with anti-Sd^a, in the four Indian populations studied in Natal, are presented in Tables 17.7 and 17.8 respectively. A low Sd(a++) frequency was detected in all four Indian populations with both reagents (total Sd(a++) detected = 1,0–2,9%). The Sd(a–) frequencies in the four populations varied from 11,44 to 17,78%, and the differences in Sd(a+) frequencies between them were not statistically significant. The Sd(a–) frequency was higher than the 4,6% in the Natal Whites and lower than the 15,3% in the Natal Blacks recorded in my 1976 thesis. Five examples of anti-Sd^a were identified in the sera of 2 255 random Natal Indians (0,22%) tested using Sd(a+) cells having strongly-expressed Sd^a antigen.

TABLE 17.7

Sd(a++) phenotype frequencies in 571 Natal Indian group O and B males and unmarried females tested with *Dolichos biflorus* anti-A_1

Category	Number tested		Phenotype frequencies Dolichos positive	Dolichos negative
Tamil	185	No.	4	181
		%	2,16	97,84
Telegu	100	No.	3	97
		%	3,00	97,00
Hindi	122	No.	3	119
		%	2,46	97,54
Moslem	164	No.	1	163
		%	0,61	99,39

Significance of differences

All differences between the populations in Table 17.7 were not significant at level $P \leq 0,05$

TABLE 17.8

Sd^a phenotype frequencies in 577 Natal Indian males and unmarried females tested with anti-Sd^a

Category	Number tested		Phenotype frequencies Sd(a++)	Sd(a+)	Sd(a−)	Total Sd(a++) in Tables 17.7 and 17.8	
Tamil	201	No.	1	177	23	No.	5
		%	0,50	88,06	11,44	%	1,29
Telegu	107	No.	3	89	15	No.	6
		%	2,80	83,18	14,02	%	2,90
Hindi	135	No.	2	109	24	No.	5
		%	1,48	80,74	17,78	%	1,94
Moslem	134	No.	2	116	16	No.	3
		%	1,49	86,57	11,94	%	1,01

Significance of differences

All differences between the populations in Table 17.8 were not significant at level $P < 0,05$

17.3.5 The Colton blood group antigens

Table 17.9 shows the Co(b+) phenotype and the $Co^a + Co$ and Co^b gene frequencies detected in the Natal Tamil-, Telegu- and Hindi-speaking and Moslem Indians using anti-Co^b only, all the Co(b+) samples having been tested with anti-Co^a. A low Co(b+) frequency was detected in all four populations, the highest (1,82%) being in the Tamil-speaking Indians. The differences in the various frequencies between the populations, however, were not significant. No Co(a+b−) cell samples were found, and no examples of anti-Co^b in the sera of 667 random Natal Indians were identified using Co(a+b+) cells.

TABLE 17.9

Colton phenotype and gene frequencies in 1 016 Natal Indian males and unmarried females tested with anti-Co^b

All Co(b+) tested with anti-Co^a

Category	Number tested		Phenotype frequencies Co(a+b+)	Co(a−)	Gene frequencies $Co^a + Co$	Co^b
Tamil	275	No.	5	270	0,0091	0,9909
		%	1,82	98,18		
Telegu	144	No.	1	143	0,0035	0,9965
		%	0,69	99,31		
Hindi	154	No.	1	153	0,0033	0,9967
		%	0,65	99,35		
Moslem	216	No.	2	214	0,0047	0,9953
		%	0,93	99,07		
Random	227	No.	0	227	0,0000	1,0000
		%	0,00	100,00		

Significance of differences
All differences between the populations in Table 17.9 were not significant at level $P < 0,05$

17.3.6 The Diego blood group antigens

The numbers of Indians in the four populations studied in Natal whose cells were tested with anti-Di^a are shown in Table 17.10. Two Indians, a Tamil- and a Telegu-speaker, were found to have Di(a+) cells. The frequencies were not estimated or compared with one another as the number of Indians tested was too small for this. However, it was noted that Di^a had been detected in the Dravidian , as well as by others in the Aryan language-speaking Indians.

TABLE 17.10

Diego phenotype frequencies in 129 Natal Indian males and unmarried females tested with anti-Di^a

Category	Number tested	Phenotype frequencies Di(a+)	Di(a−)
Tamil	58	1	57
Telegu	28	0	28
Hindi	30	1	29
Moslem	13	0	13

17.3.7 The In blood group antigens

The In(a+) phenotype and In^a gene frequencies in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal are presented in Table 17.11. A total of 13 Indians (2,2%) had In(a+) cells. Their cells were all subsequently identified in London as being phenotype In(a+b+). The In(a+) phenotype frequencies in the Natal Indian populations, which varied from 1,64% in the Natal Moslem to 2,91% in the Natal Telegu-speaking Indians, were not significantly different from one another.

One example of anti-In^a was identified in 872 (0,11%) random Natal Indian hospital patients whose sera were tested with In(a+) cells.

17.3.8 Other blood group antigens

The results of my studies with a number of rare antibodies specific for blood group antigens or phenotypes of low frequency, and with cells having rare low frequency antigens, in the four Natal Indian populations and in random Natal Indians are given in Table 17.12. None were found, but the number of Indians tested has been included in this chapter for reference purposes.

TABLE 17.11

In^a phenotype and gene frequencies in 583 Natal Indian males and unmarried females tested with anti-In^a

Category	Number tested		Phenotype frequencies In(a+b+)	Phenotype frequencies In(a−)	Gene frequencies In^a	Gene frequencies In
Tamil	228	No.	4	224	0,0088	0,9912
		%	1,75	98,25		
Telegu	103	No.	3	100	0,0147	0,9853
		%	2,91	97,09		
Hindi	191	No.	5	186	0,0132	0,9868
		%	2,62	97,38		
Moslem	61	No.	1	60	0,0082	0,9918
		%	1,64	98,36		

Significance of differences

All differences between the populations in Table 17.11 were not significant at level $p \leq 0,05$

17.4 DISCUSSION

The high Fy(a+) and Jk(a+) frequencies recorded in this chapter in the Tamil-, Telegu- and Hindi-speaking and Moslem Indians in Natal were unexpected, and the Sd(a−) frequency in them (14,6% average), which fell between the frequencies in the Natal Black and Natal White races, also exceeded the 8,6–9% frequency recorded in England (Mourant *et al.*, 1976). As the reagents, which had all been standardised for both specificity and titre, were unlikely to have contained other antibodies, the higher frequencies were thought to represent real differences between the Natal Indians and their kinsmen in India. However, since only 120 years had passed since the first indentured Indians arrived in Natal, the frequencies detected were thought to be the result either of genetic drift or of inbreeding.

TABLE 17.12

Natal Indians tested for low frequency blood group antigens and antibodies

Reagent	Number of Natal Indians tested					
	No positives found					
	Tamil	Telegu	Hindi	Moslem	Random	Total
Anti-Mit	223	103	114	101	228	769
Anti-Wb	358	130	194	152		834
Anti-Wr^a	105	102	105	100		412
Wr(a+) and Rd+ cells					667	667
	No negatives found					
Anti-Gy^a	375	157	243	238	633	1 646
Anti-I	313	141	239	271	278	1 242
Anti-Jr^a	101	102	103	100		406
Anti-Lan	101	100	100	102		403
Anti-Vel	516	200	308	206	909	2 139
Anti-Yt^a	146	103	104	100		453

The low Di(a+) and In(a+) frequencies detected in the Natal Indian populations were expected, but the low Sd(a++) and Co(b+) frequencies in them represented new observations of interest in Indians. The Sd(a++) or 'Super Sid' phenotype had strong Sd^a antigen (Race and Sanger, p 395, 1975) but these cells were known to have less than Sd(a+++), or Cad+ cells. The finding suggested that in due course Cad+ cells might be identified in Indians as well as in Thais. The 0,3–1,8% Co(b+) frequency in the Natal Indians was lower than the approximately 10% frequency known in Australian and New Zealand blood donors (Case, 1971) and less than the 4,2 and 7,7% known in Europe.

The single Natal Indian with Fy(a–b–) cells found among 389 tested (0,25%) was again evidence that the Natal Indians had little Black admixture.

17.5 SUMMARY

In the four Natal Indian populations studied, the Fy(a+) and Sd(a–) phenotype frequencies were higher, and the Jk(b+) phenotype frequency lower, than expected. One Indian with Fy(a–b–) cells was found. The phenotypes Di(a+), In(a+), Sd(a++) and Co(b+) were also identified in these populations in low frequencies, the latter two both in Indians for the first time.

CHAPTER 18

DISCUSSION

The frequencies of the various blood group antigens, chromosomes and genes in the Natal Indians recorded in this thesis showed that the early decision to subdivide the Indians into four populations, one religious and three according to home language, had been taken correctly. In the Natal Moslem Indian population, some frequencies were found to differ significantly from those in the non-Moslem group and, among the non-Moslems, some in the Natal Hindi-speakers differed significantly from those in the Natal Tamil- and Natal Telegu-speaking Indians. Between the two latter, although showing more similarity, the frequencies also suggested that the populations were not identical. The frequencies estimated corresponded reasonably satisfactorily with those known for populations of similar type in India, verifying as well that the names of the Natal Indians, which had been used as a guide to subdivide them, had been allocated to the four populations appropriately. The Natal Moslem Indians who, according to their assumed background as a collection of Indians of differing Indian ethnic origins, were expected to have intermediate blood group frequencies, instead were seen to constitute a separate population in which the frequencies were more like those of the people in the west than in the north, east and south of India.

Although inbreeding, which in India is almost certainly the result of the Hindu caste system and the Indian concept of genetic inheritance, was believed to underlie the increased blood group homozygosity observed in Indians (Sanghvi, 1966), in Natal it was thought possible that this might have decreased in the early years as the caste problems in the ships and the marked shortage of Indian women, which persisted for many years, had led numbers of Natal Indians to abandon their laws of caste completely. Consequently, the different blood group frequencies in the populations in Natal might not now be as characteristic of the people as they were in India. However, the frequencies found showed that this was not so. The higher O, Fy^a and Jk^a frequencies in Natal even suggested that blood group homozygosity in the Indians might have increased. Alternatively, the high frequencies might be the result of genetic drift. They also showed that the apparent recent tendency among the younger Natal Indians to marry at random among themselves had not resulted in any marked reduction of differences between the blood group frequencies yet. The low M_1, *cDe*, increased D, P_1H and Fy(a–b–) frequencies observed

suggested as well that little miscegenation had occurred in the past between the Indian and Black (Zulu) races in Natal.

Among the ABO variant phenotypes identified in Natal, indeed the most interesting, and almost certainly the most useful, was O_h. The Indians whose cells had this phenotype and who had contributed their blood for research studies, had helped substantially to further blood group knowledge, and the surprisingly high evidence of Le(a+b−) phenotypes in their parents and the suggestion that their most serologically important antibody was anti-H, showed that the studies might not yet be complete. The Natal O_h Indians, like the O_h Indians in India, were shown, by anti-A and anti-B not being eluted from their cells, to belong to the category of 'typical' O_h Indians whose phenotype almost certainly represented the ultimate as far as serologically detectable H antigen on the cells was concerned.

The Natal Indians, whose cells were phenotype para-Bombay (O^{A}_{Hm} , O^{B}_{Hm}, O_{Hm}) were also of great interest, and the increased H antigen on the cells of a sibling of one of them led to the suggestion that a variant Z^1 allele might be present in her family. It was tempting to suggest that Z^1, active when heterozygous, might even be responsible for the B high H, and even the A_1 high H, phenotypes of some Indians. However, high H may be only one manifestation, and O_h another, of irregular H red cell antigen synthesis in Indians. Most of the other weak ABO variant phenotypes known in India were found in Natal as well, Indians with A_x, A_m-like (possibly A_y), B_m, B_3-like and A_1B weak variant cells having all been recorded.

Apart from the O_h phenotype, the discovery of the Indian XX/XX dispermic chimaera, and the S-s-U- Indian family, were of great importance to serologists and geneticists and had aroused world interest, even before details about them had been published. As a result, fertility was now known to be a possibility in chimaeras and U- cells were known to exist outside the Black race.

It is a tribute to Landsteiner who, in 1900, was the first person to notice that human red cells were agglutinated by some but not all human sera, that so many people, including myself, have had the opportunity of enjoying such a rewarding career as blood group serology.

GENERAL SUMMARY

The physical features, numbers, religions and languages of the Natal Indians were described and an outline of their history given. As the blood group frequencies and languages corresponded with the geographical distribution of the people in India and their blood group frequencies were to be compared with those found in the Natal Indians, the backgrounds of the Indian languages were also sketched briefly. The languages provided a basis for the subdivision of the Natal Indians into Tamil-, Telegu- and Hindi-speaking and Moslem Indian populations, for of necessity, now that the Natal Indians were mostly unaware of where their ancestors had originally lived in India, the subdivision had to be made by using their Indian names as a guide. Some details of the Indian caste system and Indian beliefs regarding genetic inheritance, both of which may lead to inbreeding, were given as well, since these customs might have affected the frequencies of the various blood groups in Natal.

The blood group frequencies of the Tamil-, Telegu- and Hindi-speaking Indians in Natal were found, for the most part, to resemble those of the corresponding populations in India fairly closely, and the Natal Moslem Indians to form a unit within which the frequencies were similar to those known in western India. However, the O, and Fy^a phenotype frequencies in all four populations were higher, and their K+ and Jk(b−) phenotype frequencies lower than in India. The high *B*, *M* and *CDe* and low M_1, *cDe*, Lu(a+), Di(a+) and In(a+) frequencies found were characteristic in Indians, but the Co(a+b+), Kp(a+), Sd(a++) and P_1 H+ phenotypes identified had not been recorded in Indians before.

Among the known blood group variant phenotypes in the ABO system, two Indians with A_x, two with A_m-like (possibly A_y), 12 with B_m (and an infant whose cells were almost certainly also of this phenotype), four with B_m-like, two with B_3-like, one with A_1B weak and numbers with A_1 high H and B high H cells were recorded in Natal. The O_h and the para-Bombay phenotypes were represented as well, 24 examples of the former in 11 families and three of the latter (O^A_{Hm}, O^B_{Hm} and O_{Hm}) in three families having been identified. The blood samples of a number of O_h Indians were used in studies outside South Africa and the findings were summarised. The first study made in Natal resulted in the discovery that anti-H was eluted more readily from A_1 than from B, and from B than from O, cells in complete reverse of the known order of absorption of this antibody by these cells. The most serologically significant of the anti-A+B+H antibodies in O_h Indians was seen to be their

anti-H, and the elution procedure used unexpectedly yielded anti-H in pure form.

The second study in Natal confirmed that the strength of the I antigen on O_h cells was increased, and absorption-elution tests made by colleagues in the United States showed that anti-A and anti-B were not eluted from Natal O_h Indian cells. My colleagues went on to postulate that at least two alleles of *H* existed, one coding for no H, as in the Natal O_h Indians, and the other for a small amount of H antigen on O_h cells. In the third study in Natal, the parents and children of O_h people were shown to have an increased frequency of the Le(a+b−), and possibly also the Le(a−b−), phenotype, and possible reasons for this were suggested. The fourth study found no evidence that H antigen dosage could be demonstrated in serological tests on the cells of the parents and children in a Natal O_h Indian family.

The study with the cells of a para-Bombay Indian and her family members suggested that another allele of *H*, coding for more strongly-expressed H antigen than usual, might have been inherited by one sibling; and this allele was given the symbol Z^1. The first known XX/XX dispermic chimaera was described, and the curious finding that this Natal Indian woman secreted B and H substances, yet had Le(a+b−) cells, and the reason her cells were not Le(a+b+), were discussed. The first instance of U-cells having been identified in a race other than the Black, in four members of a Natal Indian family, and the titrations which confirmed that the parents and siblings had half doses of S or s antigens, was described as well.

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