THE BLOOD GROUPS OF THE METIS OF ALBERTA :

With Special Reference To The

Rh Chromosome -D-.

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THE BLOOD GROUPS OF THE METIS OF ALBERTA:

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Introduction

In March, 1954, a Metisse patient of Rh genotype -D-/-D- was discovered in an Edmonton city hospital. Since her case represented the first of this nature to be found in Canada, an investigation of the patient's blood groups and those of her immediate family was made (Buchanan and McIntyre, 1954)[1].

The interest shown by geneticists and serologists in this Cree-Iroquois family and in their unusual Rh genotypes encouraged the writer to continue this work, and the results of an independant study of the blood groups of further related cases was presented at the Seventh Annual Meeting of the Canadian Society of Clinical Pathologists in June, 1955, at Toronto (Buchanan, 1956)[2].

With the generous assistance of a grant from the University of Alberta, for testing sera, it became possible to plan a more general investigation of the blood groups of the Metis of Alberta, upon which work this thesis is based.

The individuals whose blood groups and sera have been studied can be placed in two distinct categories: (1) One hundred and eighty-five who are not related by marriage or blood ties to the original family; and (2)

one hundred and forty-five who are related by blood or marriage to members of the original family possessing the Rh chromosome -D-.

For purposes of comparison, those in category (1) are referred to as the Unrelated group; and those in category (2) as the Related group.

Acknowledgments

The author wishes to thank several specialists in blood group research in Britain, Canada, and the United States for their valuable advice:-

Professor D.F. Cappell of the University of Glasgow outlined some errors in interpretation that I had made at an early stage in this research[3]. This caused me to evaluate the antigen-antibody reactions in more critical fashion before coming to a decision on the Rh genotypes of all of the unusual cases that were subsequently found.

Dr. Philip Levine of the Ortho Research Foundation, New Jersey, is thanked for his kindness in providing me with anti-K and anti-Fy^a testing sera during a visit to his laboratory in July, 1955, and for referring a rare case to me from California at a time when the patient and her newborn infant required a special type of donor blood from this district.

Dr. R.R. Race of the Blood Group Research Unit, the Lister Institute, London, and his colleague Dr. Ruth Sanger, kindly confirmed the results in many of the more

important specimens of Rh genotypes -D-/-D-, CDe/-D-, and cde/-D- that have since been obtained.

I am so indebted to the above authorities for their friendly criticism and advice that I fear that this appreciation is inadequate. One is often at some disadvantage, because distances are so great in Canada that the nearest major reference laboratories are many hundred miles away from this Alberta centre.

In conclusion, I should like to thank Professor J. Macgregor, Department of Pathology, University of Alberta, and Dr. W.S. Stanbury of the Canadian Red Cross Society for the early encouragement they gave me.

The Source of the Specimens Tested

Apart from two clotted blood specimens that were sent to Edmonton from the Sequoia Hospital, Redwood City, California in April, 1955, all the venous specimens tested were obtained from Canadian Indians living in northern Alberta.

As a general rule, five to ten ccs. of clotted venous blood were collected from each individual. The specimens were usually obtained in small numbers because the Indian or Metis donors live in small isolated family groups, but this had the advantage that a small number of samples could be tested while still fresh.

A special effort was made in testing each new specimen within 24 hours of collection. In this way,

there was less difficulty in assessing weak or negative agglutination reactions due to aging of the specimens, and to a possible weakening of the antigens.

In many instances, in dealing with the "related group", a second or third visit was made to the Indian donors concerned and in addition to fresh clotted blood samples, whole blood was collected into sterile disodium citrate-dextrose solution. Upon returning to the laboratory, such red cell plasma-citrate mixtures were then centrifuged, the plasma-citrate removed, and the packed red cells mixed in 30% glycerol-citrate and frozen at -20°C., by the method of Chaplin, Crawford, Cutbush, and Mollison[4].

Since the Indian donors live in numerous scattered villages thirty to over two hundred miles from the laboratory in Edmonton, this method of preserving red cells in the frozen state proved to be of great value, and any samples of special interest, and all control cells, came to be routinely treated in this fashion after detailed initial testing of the fresh specimens.

The Information Required

The work of collecting blood samples was slow and tedious at times, as information about racial origins, and family histories had to be recorded when the specimens were being taken from the Indian or Metis donors.

The experience gained in testing specimens from the

original Canadian Metisse patient and her immediate family proved to be useful in planning further research; as an example, it was apparent that consanguinity was an important factor in all of these cases[5].

Therefore, before proceeding with the testing of new specimens, a suitable type of mimeographed history form was prepared, and this was used in helping to clarify such blood relationships. Just prior to the taking of the labelled specimens, the following information was obtained on these history sheets:-

A. The individual's surname, and christian name or initials. The age, sex, and marital status.

B. The names of the person's children, if any. Their ages, and where applicable their marital status, and the addresses of these children if living elsewhere in the province.

C. The names of the grandchildren, if any.

D. The names and addresses of the donor's own parents, and grandparents, and their racial origin.

The immediate purpose in taking a detailed family history was to facilitate the finding of any "new" cases of a rare or unusual nature.

At the outset, in beginning the search, there was no way of predicting which cases would prove to have unusual features in regard to their blood groups. Naturally, the chief interest was to find further evidence

of Rh chromosomal "deletion" of the -D- specificity in any of the three hundred and thirty donors' blood specimens that were thus collected and tested.

It then became necessary to correlate this information with the official church records, and the writer is deeply indebted to the Superior of the Oblate Mission House of Saint Albert, Alberta, for his generous help in tracing the official daily records of Indian births, deaths, and marriages (written in French) that were so essential to this project.

The officials of the Department of Indian Affairs in Canada classify such persons as "Indians" if they occupy land that has been set aside by treaty for the native population, those living off these Reserves being referred to as "Metis". Since both groups are closely related in the general area within and surrounding each tract of reserved land, this arbitrary distinction has no particular anthropological value at the present time when more and more Indians are relinquishing their former status as "treaty Indians", and have left or are leaving the Reserves.

Those from whom a family history was obtained spoke the Cree language. The information obtained showed that they were all of mixed racial origin, being predominantly Cree, with some admixture of Blackfoot, Chipewyan, Iroquois, French-Canadian, European, and/or British

ancestry.

In addition to Cree, those in the oldest age groups spoke French with considerable fluency, while the majority, under approximately seventy years of age, were bilingual in the Cree language and in English.

The ages of those from whom blood specimens were procured ranged from a newborn infant to an old lady of ninety-five years.

Since the term "Metis" implies a mixed racial origin, it is employed extensively in this thesis. No purely Indian families could be traced through any official source of information.

Chapter 2

The Early History of the Indians of Western Canada

The classification of Canadian Indians into nations or tribes such as the Cree or Kristineaux of the Canadian prairies; the Chipewyan of the Northwest; the Blackfoot Confederacy of the southwestern plains; the Iroquois nations of eastern Canada, and many other tribes, is convenient, but misleading in an anthropological application of their blood groups.

Many, if not most, are of several racial blends, and the term Metis--métis de sang mêlé, or of mixed blood--is much more appropriate.

It has been suggested that the early inhabitants of the American continent were descended from Eastern Asians or Mongoloid peoples who had migrated across the narrow Bering (or Behring) Strait in small numbers sometime after the end of the last Glacial Era.

The existence of a land bridge between the Siberian and Alaska territories has frequently been postulated, but in the absence of any definite hypothesis, it is conjectured that the ancestors of these Indians drifted in small bands, generation after generation, into the northeastern corners of Siberia, whence the vision of new hunting grounds lured them across the narrow strait, at a time, perhaps fifteen thousand years ago or earlier, when the glaciers were in full retreat.

From Alaska, the most natural route would appear to be the ascent through the valley of the Yukon, whence small bands could have crossed to the upper reaches of the great Mackenzie river.

The vast river systems and their valleys form a chain that would lead these "early Americans" slowly southwards through the Northwest Territories into what are now the provinces of Alberta and Saskatchewan, progressing further south through present-day Montana, or eastwards to Manitoba and beyond.

Others may well have followed a westerly route across the Aleutians to Alaska, into the Yukon, and into what is now British Columbia.

The process of migration must have been an extremely slow one in traversing these vast regions. Extensive glacier systems still persist in the Columbia icefields at the source of the North Saskatchewan River in the Rocky Mountains adjoining western Alberta, and in the far northern territories.

It would seem inevitable that severe climatic conditions, and the nature of the land would permit the existence of only small bands of immigrants in any one locality. Their ancient means of livelihood by hunting necessitated a nomadic existence in following their prey, and in themselves these limiting factors would prevent the aggregation of major local communities in

the prairie regions of Canada.

Conceivably, the latest of these migratory people were the Eskimo, whose descendants now occupy the barren Canadian northlands, while the Chipewyan Indians, who live in small settlements in the most northern districts of Alberta and adjoining areas of the Northwest Territories, are also regarded as being descended from comparatively "recent" immigrants to the New World.

The Past Two Centuries in the History of Alberta

The year 1955 marked the fiftieth anniversary of the Province of Alberta. Before 1905, this young Canadian province, and the Province of Saskatchewan, formed part of the Northwest Territories.

The northern portion of Alberta was known as the Athabasca District, or "the forest of the Athabasca" to the early fur traders, this being then the ill-defined area north of the North Saskatchewan river and in the region of the Athabasca river further north.

It is only two centuries ago that a young fur trader, Anthony Henday, entered this province, having journeyed west in company with a band of Crees returning to their families after trading at the Hudson's Bay post of York Factory, on that great bay from which the company derived its name (Map, page 28).

Henday and his Indian guides are known to have wintered on the North Saskatchewan river between 1754 and

spring, 1755, at a site near the future Fort Edmonton. He had been instructed to keep an accurate daily journal[6], and thus the period 1754 to 1755 may be considered as the first brief interval of British contact with the Cree and Blackfoot tribes in Alberta.

The Crees, or Kristineaux

The Hudson's Bay Company's fort at York (York Factory) in northeastern Manitoba was in existence in the seventeenth century in territory occupied by bands of Crees. The proximity of such posts to the vast hunting grounds of the Crees in the region of James Bay and Hudson's Bay placed these Indians in a very favourable position in comparison to their enemies further west.

In exchange for furs, the Crees obtained European firearms, and their early acquisition of a superior weapon enabled them to venture west into Saskatchewan and Alberta, where they displaced all of the weakly armed tribes with whom they came into contact.

By the middle of the eighteenth century, they controlled all of northern Manitoba, northern Saskatchewan, and much of northern Alberta. According to Jenness[7], bands of Crees had even raided up the Peace river into the western mountains; others had voyaged down the Athabasca river to Lake Athabasca in the northeastern corner of Alberta, and on down the Slave and the

Mackenzie rivers to the Mackenzie delta almost within view of the Artic Sea, "preceeding in both directions the explorations of Sir Alexander MacKenzie".

The great westward expansion of the Crees became checked when the surrounding tribes--such as the Chipewyans to the north, and the Blackfoot Confederacy to the south of the North Saskatchewan river in Alberta--obtained firearms from the traders.

Finally, a series of epidemics of smallpox at varying periods in 1784, and again in 1838, and in subsequent years, devastated their ranks. From these disasters they never recovered, but remained scattered in small bands or family groups.

In Alberta, bands of Crees became more permanently associated with the areas north, west, and east of Fort Edmonton in the general district between the North Saskatchewan and the Athabasca rivers, and as far west as the Rocky Mountains.

Their women had a widespread reputation for beauty; in fact, so experienced a traveller as Sir Alexander MacKenzie considered "they were better proportioned, and possessed more regular features than any other Indians within the boundaries of Canada"[8].

As among most tribes in western Canada, the only real social units were the bands or family groups. Adolescents passed a period in seclusion "fasting for

visions", and men served their wives' parents for a term, although there was no formal marriage ceremony, until the Crees slowly adopted the white man's religions in the early nineteenth century.

From Hudson's Bay and from the Great Lakes westwards, Indians trading at the older forts had acquired the Europeans' common infectious diseases, to which they had no resistance. Epidemics so greatly weakened the Indians that they offered but little serious or prolonged threat to the few white traders who built new trading posts on the North Saskatchewan and other major rivers in Alberta, including the main fort at Edmonton.

Fort Edmonton

In 1794 to 1795 the first posts of Fort Augustus and Fort Edmonton were established "within a musket shot of each other" because of the abundance of fur-bearing animals between the North Saskatchewan and the Athabasca rivers[9].

The intense rivalry between the Northwest Trading Company's factors and those of the Hudson's Bay Company brought about many changes in the way of life of the local Cree bands. Voyageurs of French-Canadian descent, and the companies' Indian hunters were encouraged to live with the local family groups during the winters, returning with these Indians to the posts each spring with their harvest of furs.

Fort Chipewyan

Similarly, the earlier establishment of other posts such as Fort Chipewyan by Alexander MacKenzie in 1788 to 1789, on Lake Athabasca in the extreme northeastern portion of Alberta, created a comparatively sudden change in the lives of the Chipewyan Indians in the north.

There came to live around the posts, groups of mixed ancestry in whom flowed the blood of the Crees; that of the Chipewyan; that of French-Canadians from "Lower Canada"; and to a lesser extent, that of the Scots and English clerks and factors.

Year after year, the fur brigades voyaged by canoe from the posts in the Athabasca and North Saskatchewan river regions to distant Fort William and Lake Superior.

The voyages from Fort Chipewyan and the posts of the most westerly districts near the Rocky Mountains began in May as soon as the rivers were free of ice, and the brigades of canoes, manned principally by French-Canadian voyageurs and Indian hunters, eventually reached Lake Winnipeg in August, many voyaging on to distant Lake Superior.

At the Fort William rendezvous (Kamenistiquoia) the Athabasca brigades were met by the larger canoes of the factors from Montreal, with whom they exchanged their furs for the next winter's supply of trade goods.

Provisioning depots were rapidly built throughout

the western river systems and lakes so that the brigades could return home before winter set in--the Indians and voyageurs being unable to hunt for food, to any extent, while en route in either direction on a total journey exceeding two thousand miles.

The general effect of the fur trade was to bring about a mingling of races, and their descendants at the different forts came to have principally French surnames, and adopted the Roman Catholic faith of their French-Canadian forebears, while maintaining much of their Indian ancestors' outward appearance, customs and nomadic instincts.

The Blackfoot Confederacy, and Some Basic Blood Group Characteristics

The North Saskatchewan River, flowing in an easterly direction from the Rocky Mountains for over a thousand miles into the lakes of Manitoba, formed a natural boundary between the Blackfoot tribes in southern Alberta and their enemies the Crees, to the north of this river.

The two races differed in many respects. While the Crees were of medium height, the Blackfoot warriors were generally tall and approached six feet in stature.

Whereas the Crees excelled with the canoe, the Blackfoot tribes hunted the buffalo herds on horseback, and ranged from their northern river boundary south into the United States.

Before, and since the coming of the white man, the

Blackfoot Confederacy of tribes have occupied, until recent years, all the southern prairie regions of Canada, and much of northern Montana and the western plains in the United States.

The aggressive nature of the Blackfoot bands was, in all probability, a greater menace to the early fur traders than any danger from the Crees, and it is significant that the two trading companies built their principal forts on the northern banks of the North Saskatchewan river in Cree territory.

Raiding parties from both tribes entered each others' domain, and carried off female captives who were either killed, or occasionally adopted by their captors. The distinctive characteristics of these two major Indian races is reflected even in their major blood groups, the Blackfoot being predominantly group A, while the Crees are most frequently of group O[10]. (Also chapter 3).

It is interesting to speculate that the much lower frequency of group O in the Blackfoot bands on their Reserves in southern Alberta, and the comparatively low proportion of group A bloods in the Cree-Metis of central Alberta originated from, and may be attributed to their ancestor's preoccupation with making war upon each other and thus obtaining young captives who were adopted by either side before the coming of the British

and Europeans.

The earlier findings of Matson have been confirmed by Chown and Lewis[11]. In investigating the Blackfoot of Alberta, these authors have stressed that approximately eighty per cent of this tribe and the smaller related Blood tribe of southern Alberta are of group A, the A gene frequency being far too high to be accounted for by European admixture[12].

The history of the Crees and the Blackfoot, as well as their distinct blood group differences, offer many contrasts. Unlike the Blackfoot bands, the Cree-Metis of central Alberta and the north have declined in physique, and, unable to stifle their longing for the old free days of buffalo hunting and the excitement of the chase, now lead a precarious existence, particularly when they elect to leave their own reserved land.

Semi-starvation and diseases resulted in the pitying scorn of the white man, destroying their morale and robbing them of self-respect.

The Blackfoot, however, did not escape from the perils of smallpox, tuberculosis, epidemic measles, and other infections most probably introduced by Europeans. The dreadful nature of the epidemics of smallpox in the early nineteenth century is vividly conveyed in the narrative of Maximilian, Prince of Weid:

"The prairie all around is a vast field of death, covered with unburied corpses, and spreading, for miles, pestilence and infection..... The Assiniboines, nine thousand in number, roaming over a hunting territory to the north of the Missouri, as far as the trading posts of the Hudson's Bay Company, are, in the literal sense of the expression, nearly exterminated. They, as well as the Crows and the Blackfoot, endeavoured to flee in all directions, but the disease everywhere pursued them"[13].

Although greatly reduced in population by the end of the nineteenth century, many of the Blackfoot tribes have since made good use of the lands on their treaty reserves in southern Alberta, and many of their descendants in this province and northern Montana have become successful farmers. Their neighbours near the larger Canadian cities and towns have, as a general rule, been less able to adopt themselves to the ways of the white man.

The finding of a few unusual cases of Rh chromosomal "deletion" by the writer, in Metis of group A_1 who live in the Lesser Slave Lake area of Alberta, resulted in a detailed study of both their blood groups and their family history. It was found that an older generation had lived in Montana in Blackfoot territory where the population are chiefly of blood group A_1, their former home being some seven hundred miles south

of Lesser Slave Lake in Alberta.

These particular Metis speak only Cree and some English, and yet their racial origin includes forebears of Cree, French-Canadian, Iroquois, and Blackfoot descent. It is possible that more examples of the unusual Rh chromosome -D- will be found in northern Montana (U.S.A.) at some future date, a possibility that would justify further serological investigation.

The Iroquois

During the seventeenth century the French, through their missionaries, made numerous attempts to win the goodwill of the Iroquois within and to the south of the province of Quebec.

The Iroquois consisted of five major tribes or "nations", and the Jesuit missionaries succeeded in inducing a large number of these Indians, mainly Onondaga and Mohawks, to move to French Canada. There, they ultimately accepted Christianity, and joined their fortunes with the French and Algonkians, sometimes against the British.

The League of the five nations of the Iroquois then outlawed these proselytes[7a], but many of them and their families regrouped as bands living at Caughnawaga, near Montreal, at St. Regis, and at other settlements in the province of Quebec.

The remaining Mohawks and the other "nations" fre-

quently joined the British against the French, but one group of current scientific interest is the Caughnawaga band of Iroquois, whose forefathers manned the canoes of the fur traders after peace was established between the victorious British and the French. Several descendants of these Iroquois whose ancestors became guides and canoemen for the fur traders during the eighteenth and early nineteenth centuries, now occupy a small "Iroquois" or Metis reserve thirty miles from Edmonton, Alberta. Those still living on this reserve, and many living in the surrounding area have been the subject of an important portion of this study. ("The Related Group").

The knowledge of the early French settlers, and especially of Jesuit fathers about the Iroquois within the province of Quebec has enabled those interested in anthropology to garner more information than usual about their aboriginal condition.

Concerning the family customs, among the Iroquois it was the young man's mother who chose his bride, and arranged the match with the girl's mother without regard to the young couple's inclination or wishes. The two families then exchanged presents, and the youth was informed that henceforth he and the girl were man and wife; that they must live together in the hut of her family and provide for each other, he by hunting and

fishing, and she in the cornfields.

Fathers had no jurisdiction whatever in the match, and were usually not consulted, but the mothers might seek advice from elderly people in their own clans. Divorce was easy, but infrequent, because it brought discredit on both parties, and particularly on the man.

The name given to a child at birth received public confirmation at the feast of the green corn, and at the mid-winter festivals. However, at adolescence, the child discarded the given name at the same two yearly festivals, and assumed another name which the young person generally retained through life.

This changing of names caused great difficulty in tracing the Iroquois who voyaged west, but in one exceptional case it was possible to identify one young Iroquois whose descendants still live in the Edmonton region over two thousand miles from the Iroquoian village of Caughnawaga:-

Through the active participation of members of the Indian Health Services[14], and of the Jesuit fathers, the Church records at Caughnawaga, near Montreal, were examined for the period 1750 to 1800.

These old parchments, brown and crumbling with age, revealed that "on October 17, 1782, a male child was born to Thomas Anatoha (Kanakonme) son of Karakwentha or Kwarakwente, and Marie Anne Tekonwakwehnii. The child

was named Louis Karhiio, meaning "beautiful forest".

Since the band name of Callihoo or Kallihoo is that of the "Iroquois" Indian reserve at Edmonton, Alberta, it is considered that the name of Louis Karhioo became corrupted to Callihoo within the ensuing one and a half centuries, and for the following reasons:

The "r" sound in Iroquois, the Jesuit fathers stated, closely resembled that of the letter "l", while "iio" at the end of a word is pronounced rather like "ee-u". Hence it is reasonable to postulate that the surname Karhiio of the "beautiful forest" became changed to Callihoo in the west. Eighteen different spellings for this surname have been found in the old records of the mission houses at Lac Ste. Anne and St. Albert, near Edmonton, during the course of this investigation.

A notation in the Jesuit mission records after the name of Louis l'Iroquois, as he came to be known in this region, reads--"Alla au Nord, sy maria, y eut famille, n'en revint jamais". This, significantly, is the only notation of this type seen in the period covered.

His four brothers and a sister remained on their land near Montreal, three brothers reaching maturity--Jean Baptiste, Rene, and Thomas, who married locally at Caughnawaga.

One old Metis lady of 95 years, Victoria Callihoo, née Belcourt, suggests that a Bernard Belcourt joined the voyageurs, she having married Louis, a grandson of the original Iroquois of this history[15].

Sir Alexander MacKenzie's journal also reveals that "a small colony of Iroquois immigrated to the banks of the Saskatchewoine who had been brought up from their infancy by the Romish Missionaries, and had been instructed in their village nine miles from Montreal"[8a] (1799).

The rival companies' fur brigades were generally manned by a large number of Iroquois[16], and the colony referred to by MacKenzie may have become established a few years before that of Louis Karhiio, son of Karakwentha. Nevertheless, an Indian Reserve near Edmonton came to be named after Michel, the oldest son of Louis. (I.e., the Michel, or Callihoo Reserve)[17].

By virtue of their bilingual French or Iroquois background and their religion, voyageurs of French-Canadian and Indian descent such as Campion, Chalifoux, and L'Hirondelle became closely associated with the Karhiio family in marriage and their offspring became identified with the band (summary of descent, page 75a).

References to this band of Iroquois in the Journal of Father De Smet, indicate a strongly religious French-Canadian influence, at a time (1845) when the Iroquois

tongue had not yet been replaced by that of the Crees[18]:-

"4th October, 1845. I arrive at the Fort des Montagnes"--Rocky Mountain House--"there meeting the worthy commander of the fort M. Harriote. The Cree nation is considered very powerful, and numbers more than 600 wigwams".

"Lake Jasper, eight miles in length, is situated at the base of the first great mountain chain. The fort of the same name, and the second lake, are twenty miles higher, and in the heart of the mountains".

"On the banks of Lake Jasper, we met an old Iroquois called Kwaragkwante ("le soleil voyageur" or "the travelling sun") accompanied by his family, thirty-six in number".

"He has been forty years absent from his country, during which he has never seen a priest, having dwelt for the last thirty-four years in the forests of the Athabasca and on the Peace River, and subsisted by hunting and fishing. The good old man was overwhelmed with joy, and the children experienced similar feeling with their father. I will give you the old man's words in English, on learning that I was a priest...".

"How glad I am to have come here, for I have not seen a priest for many years. Today I behold one, as I did in my own country--my heart rejoices--wherever you go I shall follow you with my children--all will

hear the word of prayer, all will have the happiness to receive baptism. Therefore my heart rejoices and is happy".

"The little camp of Iroquois immediately set out to follow me to Fort Jasper. Most of them knew their prayers in Iroquois. Seven marriages were rehabilitated and blessed. The number baptised amounted to forty-four; among whom was the lady of M. Fraser, superintendent of the fort, and four of his children and two servants".

Joined by a few French-Canadians, this band later settled in the Lac Ste. Anne district near Fort Edmonton. Between 1860 and 1870, they came under the influence of the highly respected Oblate missionary, Father Albert Lacombe. As the great herds of bison began to disappear, within the same decade, the men of the Iroquois and Cree tribes became fishermen and hunters of smaller game for the forts.

By the end of the nineteenth century and in the early years of this century, all of these bands except a few in the mountainous regions gave up their vast hunting grounds by official treaty, and came to dwell on the reserves as individual bands comprised of large family groups related by close blood ties.

Metis of the Present Day: A Summary

In summary, it may be said that a slow integration of the Indian and White races has been occurring in

Alberta during the past one hundred and sixty years, but that the process is far from complete.

The history of the different Indian bands would indicate a relatively greater degree of blending between the Chipewyans and Crees of northern Alberta, and some "merging of blood" between these bands and the Blackfoot to the south.

Economically and socially, the invasion of their territories by Europeans created a most rapid change in their ancient way of life, and previously unknown infections decimated their ranks. The integration of these Indians with the French-Canadian voyageurs from "Lower Canada", or the province of Quebec was of significant degree, and far exceeded the racial influences of the relatively few Scots and British traders, and of the later settlers.

The arrival of colonists, and the rapid expansion of agriculture since approximately 1890 coincided with the withdrawal of the fur trade to more remote areas. Within a few years, moreover, the great majority of the existing Indian or Metis bands came to occupy their prescribed reserves.

Since the voyageurs themselves were of mixed French and Indian origin, many "Indians" on reserves in the general zone around Edmonton may well claim a mixed French and Iroquois lineage, in addition to a more local

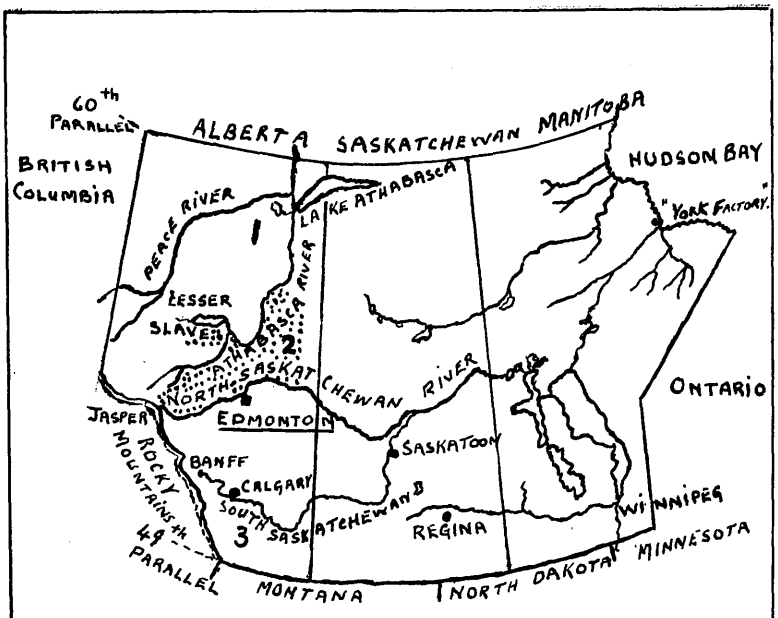
heritage.

They are truly métis de sang mêlé. Restricted to their own land, they have reverted to the ways of their Indian forebears, and they have mated or married within their own confined circles.

Within the past few decades there has been a growing tendency to disband, in a curiously nomadic and aimless fashion.

Culturally, they have already contributed nearly everything of value to our own civilization, except the anthropological knowledge that we may still glean from their specific characteristics, and from their history.

THE MAIN INDIAN TRIBAL DISTRICTS.



1, = CHIPEWYAN. 2, = CREE (ALBERTA)

3 = BLACKFOOT (ALBERTA).

= PRESENT DISTRIBUTION OF TRIBES IN ALBERTA.

⁘ = GENERAL AREA WHERE SPECIMENS WERE OBTAINED.

ALL CREE-MÉTIS SPECIMENS WERE OBTAINED IN AREA 2 NORTH, WEST, AND EAST OF EDMONTON.

Chapter 3

The Landsteiner, or ABO Groups of the Cree-Metis of Alberta

Methods

(a) Collection and Preservation of Specimens; and

(b) ABO Testing.

For convenience of presentation, the methods used for each blood group system are described in each successive chapter dealing, in turn, with the ABO, MN, Rh, Kell, Duffy, and Lewis systems.

(a) As noted on pages 3 and 4, the first sample collected from each Metis donor consisted of five to ten mls. fresh, clotted, venous blood. Further specimens were collected in the standard sterile acid-citrate-dextrose solution normally used in obtaining donor blood for transfusion purposes, or in tubes containing dry oxalate, or in modified Alsever's solution.

The modified Alsever's solution was found to be of great value as a collecting medium, prior to freezing specimens, or for sending blood samples from western Canada to Britain or to the eastern United States of America to reference laboratories, and the simple formulae used are as follows:

Modified Alsever's Solution:

A.	Dextrose	18.66 grams
	Sodium Chloride	4.18 grams
	Citric Acid	0.50 grams
	Sodium Citrate	8.0 grams
	Total	31.34 grams

Distilled water to 1,000 mls.

B.	Dextrose	20.5 grams
	Sodium Chloride	4.2 grams
	Citric Acid	0.55 grams
	Sodium Citrate	12.47 grams
	Total	37.72 grams

Distilled water to 1,000 mls.

A. The one litre of prepared solution was divided equally between two clean rubber-stoppered 500 mls. blood bottles, and autoclaved at 250°F. for 20 minutes.

A. In use, 5 mls. of the whole blood sample required were added immediately to 5 mls. of the above modified Alsever's solution in previously autoclaved rubber-stoppered test tubes, and then kept at 4°C. to 6°C.

Solution A, for modified Alsever's solution, was prepared after discussion with Professor Collier of the Department of Biochemistry, University of Alberta. Solution B represents a different modification recommended

to me by Dr. Amos Cahan[19a], and by Dr. Richard E. Rosenfield of New York.

In practice, solution A was used extensively as a collecting medium when the donor's blood group antigens were being retested, after initial work with the cells and sera obtained in clotted blood specimens.

It came to be preferred to acid-citrate-dextrose when the red cells were to be frozen, later, in glycerol-citrate. No difference in the effective length of red cell preservation was noted in comparing the two modifications of Alsever's solution, provided the donor's blood was added to the solution forthwith, before clotting had occurred.

The choice of a red cell preserving medium is probably a personal one, depending on local experience, and on the chemical reagents available. The addition of minute traces of antibiotics as recommended by Dr. Cahan[19b], was not found to be necessary, if due sterile precautions were taken in transferring the freshly taken donor's blood directly to the solution.

The Storage of Red Cells at -20°C.

A simple modification of one of the methods employed by Chaplin and his colleagues[4] was developed, since it was not possible, locally, to prepare the more complex buffered solutions that these authors have recommended for small specimens. Instead, one of the methods

(solution 4 of Chaplin et al) given for freezing larger volumes of blood was adapted to small red cell mixtures taken in modified Alsever's solution.

Most of the Metis donors live many miles from Edmonton, and by freezing the rare or less common of the specimens encountered, a useful panel of known test cells was created. This panel of known Metis' cells was extended by including those of members of the laboratory staff, and other White donors' erythrocytes--particularly those who were group O, and whose Rh genotypes, etc. were known in detail.

1. Preparation of "Freezing Solution"

150 mls. of group AB plasma-citrate were obtained from a bottle of normal donor blood collected in A.C.D. solution. Only group AB plasma-citrate that was free from cells was used.

To the 150 mls. of group AB plasma-citrate were added 90 mls. of pure glycerol, and 9 mls. of 30% trisodium citrate ("Reagent quality").

The above three components were carefully measured and mixed in a dry sterile 500 mls. blood bottle, and the mixture was stored in the frozen state, at -20°C. This mixture constituted the "freezing solution".

2.

The specimens of red cells that were selected for preservation were first "packed" in the centrifuge at

3,000 revolutions per minute for 30 minutes, or they were simply allowed to sediment down in the original test tubes in which they had been collected from the donors, after standing for 48 hours at 4°C. to 6°C. The supernatant mixture of donor's plasma plus modified Alsever's solution was then removed.

A small amount of the "freezing solution" was thawed out and brought to room temperature. This was then added slowly to the donor's "packed" or sedimented red cells until an equal quantity had been added to the red cell concentrate, with continuous mixing to obtain an even suspension. This mixture of red cells plus group AB plasma-glycerol-tri-sodium citrate was then frozen at -20°C.

3. Recovery of Red Cells Stored at -20°C.

It was found that accurate "washing solutions" were essential. Any deviation from detail resulted in haemolysis and total loss of the previously frozen cells.

The "washing solutions" consisted, in serial dilution, of 16%, 8%, 4%, 2%, of glycerol (w/v) each in 3% tri-sodium citrate, the final washing solution being 1% sterile saline.

In practice, the tube of frozen cells was thawed out by placing it in tepid water. It was then centrifuged at 3,000 r.p.m. for 5 minutes and the "freezing solution" removed.

16% glycerol-citrate was then added, and after complete mixing, the tube was again centrifuged at 3,000 r.p.m. for 5 minutes, and then the supernatant was removed.

Similarly, washing was carried out with each lower serial dilution of glycerol-citrate, and finally with three washings in 1% saline.

A high proportion of the frozen erythrocytes have been recovered even after several months at -20°C., and such specimens have been of great value as control cells, as each new group of specimens became available for testing.

(b) ABO Testing

"Reverse" blood grouping was performed in each case; i.e., in addition to testing each Metis donor's red cells with anti-A and anti-B sera, the first tests--using the donor's clotted blood specimens--included the use of mixed, known group A_1 plus A_2, and known group B cells in 3% saline suspensions against the donor's serum.

The anti-A and anti-B sera were obtained from stocks prepared in the Canadian Red Cross National Laboratory, Toronto, these sera being derived from group B and group A donors who had been previously stimulated with 0.15 ml. of substances A and B two weeks before their donation of serum. (A standard method used in Canadian Red Cross Laboratories for obtaining potent testing sera.)

Anti-A_1 serum was used in testing all group A and group AB Metis' red cells to distinguish the subgroups A_1,

A_2, A_1B, or A_2B. Group O serum (anti-A plus Anti-B) was not employed, although this is known to be of value in identifying weakly reacting cells such as A_4 or A^{o}[20].

The donor's serum, free from cells, was separated from the clot into a marked labelled tube. Fresh, 3% suspensions of the Metis' cells were set up in sterile, isotonic saline after preliminary washing in saline to remove traces of the donor's serum. To one volume of anti-B was added one volume of 3% unknown cells in a marked precipitin tube. To one volume of anti-A was added one volume of the unknown 3% cells in a second marked precipitin tube.

Simultaneously, each donor's serum was "controlled" by testing it against the 3% known group A_1 plus A_2, and B cell mixtures, in precipitin tubes, using equal volumes.

The marked identified tubes were left at 20°C. (room temperature) for 1½ hours, before being read, macroscopically, in numbered blocks.

Subtyping with anti-A_1 serum was also conducted at 20°C. A 3% red cell suspension of those classified as being of group A, or group AB was made in sterile, isotonic saline.

To one volume of anti-A_1 serum in a precipitin tube was added one volume of the fresh cell suspension. At the same time, the anti-A_1 serum was tested against 3% saline suspensions of known A_1B and A_2 red cells as controls of

the results obtained.

All results were read after 1½ hours at 20°C., and recorded forthwith against a list of the Metis donors' names. As with anti-A and anti-B testing, results were read macroscopically, but in addition, in anti-A_1 testing the degree of agglutination or its absence, was confirmed under the low power of the microscope.

The ABO Groups of (1) the "Unrelated" Metis, and (2) the "Related" Metis

In the introduction, and on pages 1, 2, 4 to 7, and 20, it has been explained how the two distinct categories were arrived at by taking a detailed family history, and by checking the birth records, etc., of the Mission Houses.

The ABO Groups of each category are listed below in Table 1.

Table 1

The ABO System: (1) 185 "Non-Related" Bloods
(2) 145 "Related" Bloods

"Non-Related"	O	A_1	A_2	B	A_1B	A_2B	Total
185 Specimens (1)	107	62	6	7	3	0	= 185
Percentages (1)	57.84	36.76		3.78	1.62	0	= 100%
Gene Frequencies (1)	O=0.760, A=0.214, B=0.026						= 1.000
"Related"	O	A_1	A_2	B	A_1B	A_2B	Total
145 Specimens (2)	78	52	4	9	2	0	= 145
Percentages (2)	53.79	38.62		6.21	1.38	0	= 100%
Gene Frequencies (2)	O=0.731, A=0.227, B=0.042						= 1.000

The gene frequencies for O, A, and B were calculated by the methods recommended by Dr. A.E. Mourant ("The Distribution of the Human Blood Groups", 1st edition, 1954)[21], the formulae used being:

Frequency of gene O = r = $\sqrt{\bar{O}}$, where $\bar{O}$ = proportion of O.

Frequency of gene A = p = $\sqrt{\bar{A} + \bar{O}} - \sqrt{\bar{O}}$, where $\bar{A}$ = proportion of A.

Frequency of gene B = q = $\sqrt{\bar{B} + \bar{O}} - \sqrt{\bar{O}}$, where $\bar{B}$ = proportion of B.

To obtain the corrected values, so that the total gene frequencies added up to unity, the difference, D, was obtained by adding up the frequencies obtained from the above equations, and subtracting from unity.

The corrected values p_c, q_c, and r_c were then obtained by the formulae:

$$p_c = \frac{p}{1 - D}, \quad q_c = \frac{q}{1 - D}, \text{ and } r_c = \frac{r}{1 - D}.$$

The corrected values are listed in Table 1, where it will be noted that in both series the métis of Alberta were found to be more predominantly of group O, the gene frequency of O, on a percentage basis, being 76 per cent, and 73.1 per cent respectively, while the gene frequencies for group A were 21.4 per cent and 22.7 per cent in comparing the two different population samples.

In neither the "non-related" nor the "related" groups were there any of group A_2B; and there were only 3 persons

of group A_1B in the "non-related" series, and only 2 of group A_1B in the "related" bloods. Group B bloods in both categories had a relatively low gene frequency of 2.6 per cent and 4.2 per cent, having the higher value in the "related" group.

Discussion

The ABO groups of these Cree-Metis of Alberta offer a remarkable contrast to the ABO blood groups of the Blackfoot Indians of southern Alberta and northern Montana. In the early work of Matson (1938)[10], the Blackfoot tribes in both Montana and southern Alberta were found to be predominantly of group A, and of six hundred and eighty-eight tested, 79.07 per cent were of group A, while only 20.20 per cent were of group O, the A gene frequency being reported as 54.3 per cent, while the group O gene frequency was 45 per cent.

None of those reported at that time by Matson were of group B, although five persons (0.73 per cent) were listed as being of group AB.

A comprehensive and recent study by Chown and Lewis (1953)[11], confirmed the high incidence of group A in the Blackfoot and the related Blood Indians of southern Alberta. Thus, of the two hundred and forty-one Blood Indians tested by these well-known Canadian authorities, only forty (16.60 per cent) were found to belong to group O, while one hundred and ninety-six

(81.33 per cent) were group A_1; none were of group A_2, while only two were group B, and three were of group A_1B.

The gene frequencies of those whose ABO groups were tested in the current series are contrasted with those found by Dr. Chown and Miss Lewis in 1953, in Table 2 below:

Table 2

ABO (Percentage) Gene Frequencies

Number	Origin	Gene Frequencies %			
		A	B	O	
185	Cree-Metis (1)	21.40	2.60	76.00	(Buchanan)
145	Cree-Metis (2)	22.70	4.20	73.10	(Buchanan)
241	Blood Indians	58.24*	1.04	40.72	(Chown and Lewis 1953)[11]

*All of subgroup A_1

It is generally agreed that the gene B does not occur in purebred Indians in North or South America. Thus, in the putatively pure Chippewa Indians whose blood groups were tested by Matson, Koch, and Levine (1954)[22] none of one hundred and sixty-one pure Chippewa were of group B, but B in a gene frequency of 3.7 per cent was noted in two hundred and six who were thought to be "less than $\frac{3}{4}$ pure".

The recent and extensive work of Mourant (1954)[21a], relating to the distribution of the human blood groups in many different races, would indicate that the finding of

group B or group AB in Canadian Indians and in North American Indians generally is an indication of European admixture within any particular tribe.

While Chown and Lewis[11] suggested that the dilution ratio of Indian by White blood was "roughly one-sixth" in their series, and while on the same basis the influx of White group B and AB bloods would seem much greater in the Cree-Metis of this series, marked consanguinity must be taken into account.

In Indian bands of this area, consanguinity and cousin marriages are so marked a feature that the introduction of only one or two "White" group B genes, for example, in early generations, would lead to a very high rate of recurrence of the same genes in their descendants.

This may account for the comparatively high frequency of gene B, and the relatively greater number of both group B and group AB bloods in the "related" group of metis.

Similarly, the presence of the subgroup A_2 is unusual. Since none were found in the Blood Indians[11], in spite of their high proportion of group A_1, the subgroup A_2 may well represent another European characteristic within both the "non-related" and the "related" Metis categories.

No "purebred" Crees are known to exist on any of the Indian Reserves in Alberta. It may be conjectured that the original Crees were predominantly a group O people,

and that the incidence of A_1, A_2, B, and A_1B reflects an admixture of Blackfoot or Blood Indian, and of European racial strains within the Cree-Metis of the present time.

The original blood groups of the Iroquois in Canada are unknown, there being no "pure" Iroquois remaining.

Conclusions

In considering the ABO blood groups of all the Cree-Metis whose specimens have been tested, due regard must be paid to the question of selection. The smaller number in category (2) was highly selected, and many, if not all, are believed to be blood relatives.

Thus, in comparing the blood groups of the Metis with those of other Indians it is better to exclude this "related group", and for this purpose the one hundred and eighty-five that were obtained at random are of value. These particular Metis differ markedly from the Blood Indians of southern Alberta[11] in that group O occurs much more frequently, while in the Bloods subgroup A_1 is common.

The higher incidence of group O in the Cree-Metis of this category (1) is in agreement with findings among other Indian tribes, with the exception of the Blackfoot and Bloods of Montana and southern Alberta. These Cree-Metis are comparable in this respect to the Chippewa Indians of Minnesota[22]. Of two hundred and six Chippewa who were regarded as "less than three-quarters" pure

Indian, 62.1% were of group O, the gene frequencies of the two different series being as follows:

	Gene Frequencies		
	A	B	O
185 Cree-Metis	0.214	0.026	0.760
206 Chippewa	0.193	0.037	0.770

Matson and his colleagues[22] suggest that the Chippewa are predominantly a group O people and that group A and group B represents the introduction of caucasoid blood, mainly French and English, into the Chippewa race.

In the case of the Cree-Metis of Alberta, however, it is most probable that in addition to French and British elements there is an admixture of Blood and Blackfoot within the Cree tribes, thus possibly increasing the proportion of group A.

Chapter 4

1. Testing with Anti-M and Anti-N

The sera used in the tests were purchased from a commercial source (Ortho Pharmaceutical Corporation) through a grant generously provided by the University of Alberta.

These sera had been prepared by injecting rabbits with appropriate washed, packed cells. The proper absorption of such sera is known to be difficult, and as an additional safeguard, the tests were controlled by comparing the results obtained with the same anti-M and anti-N sera against known group M, group MN, and group N red cells. No anti-S or anti-s sera were available.

Testing was performed by using precipitin tubes in numbered wooden blocks at room temperature, the same method being adopted for the known controls. The red cells to be tested were washed once in normal saline: 2% saline cell suspensions were then prepared. Two drops of the saline cell suspension were added to one drop of each of the anti-M and anti-N sera in the marked tubes. The results were read at room temperature after 30 minutes.

The results of the tests with two different anti-M and two different anti-N sera are shown in Table 3, as follows:

Table 3

The M - N System

(1) 185 "Non-related" Metis Specimens.

M	MN	N	Total
79	84	22	185 specimens
42.70%	45.41%	11.89%	= 100%
Gene M = 0.65405		Gene N = 0.34595	

The gene frequencies were calculated by the standard method used by Race and Sanger, "Blood Groups in Man", second edition, 1954, pages 56 and 57.

Thus, the gene frequencies for M and N were calculated as follows:

$$\text{Gene M} = 0.4270 + \frac{0.4541}{2} = 0.65405$$

$$\text{Gene N} = 0.1189 + \frac{0.4541}{2} = 0.34595$$

The "expected" and the observed numbers were then ascertained and compared, and in the one hundred and eighty-five "non-related" Metis the results were:

	Expected	Observed
MM =	79.1	79
MN =	83.7	84
NN =	22.1	22
	184.9	185

There was close agreement between the "expected" and the observed numbers. For example, the X^2 test for MN

was:

$$X^2 = 185 \frac{[84^2 - (4 \times 79 \times 22)]^2}{[(2 \times 79) + 84]^2 [84 + (2 \times 22)]^2}$$

$$X^2 = 0.002 \text{ for one degree of freedom.}$$

The smaller group of one hundred and forty-five related Metis, a highly selected group of persons related by blood and/or marriage, differed considerably from the "unrelated" series in their M and N gene frequencies.

Because of this high degree of selection, any comparison is of limited value, and of doubtful statistical significance in relation to other reported series. Since those whose case histories had been published under joint authorship[5] do not form a part of this group—all one hundred and forty-five being additional cases—their M and N groups were investigated with another object in view, it being thought that there might be some detectable variation in the M and N antigens.

In earlier unpublished work there had been some indication that three specimens gave very weak agglutination with anti-N, and that these might be examples of the rare N_2 type. Unfortunately the three individuals concerned could not be retraced within the nomadic band, and as in the case of those previously reported[5], they do not form a part of those presently being considered. If they can be found again, it is hoped that a more detailed study can be made.

Subject as they are to the above limitations, the M and N gene frequencies of the one hundred and forty-five in the related category (2) were as follows:

Table 4

The M - N System

(2) The 145 "Related" Metis Specimens.

M	MN	N	Total
46	79	20	145 specimens
31.73%	54.48%	13.79%	= 100%
Gene M = 0.5897			Gene N = 0.4103

Gene M = $0.3173 + \frac{0.5448}{2} = 0.5897$

Gene N = $0.1379 + \frac{0.5448}{2} = 0.4103$

(Expected MM = 0.5897^2 = 0.34775

(MM, absolute number expected = 50.4

(Expected MM = 0.5897 x 0.4103 x 2 = 0.48391

(MN, absolute number expected = 70.2

(Expected NN = 0.4103^2 = 0.16835

(NN, absolute number expected = 24.4

145 Related Metis

	Expected	Observed
MM =	50.4	46
MN =	70.2	79
NN =	24.4	20
	145.0	145

The considerable variation between the expected and the observed numbers caused considerable worry, since no immediate cause could be found. I.e., all the specimens had been tested with anti-M and anti-N within 24 hours of collection, and the controls with known red cells of M, MN, and N types were satisfactory.

It was thought that some variation was possible because of the small number of specimens tested; or that a few weakly reacting N_2 samples could have been missed, but this explanation did not seem to be satisfactory since there appeared to be an excess of MN.

X^2 tests, based on the standard formula $\frac{(\text{No. expected} - \text{No. observed})^2}{\text{No. expected}}$ gave the highest variation, as could be anticipated, in the MN group, the total X^2 for all three (MM, MN, and NN) being $X^2 = 2.28$.

On the whole, the results obtained were deemed to be unsatisfactory, and it is hoped that at least a proportion of those whose samples were tested can be traced again, and retested.

Discussion

Restricting comparisons to the one hundred and eighty-five specimens showing interwal consistency in regard to the M, MN, and N groups, a high frequency for the M gene is known to be a characteristic of the North American Indian tribes in general.

The only other tribe that has been tested in detail with anti-M and anti-N in Alberta is the Blood Indian group in the South. There, Chown and Lewis[11] found a very high proportion of M in 241 Blood Indians. Of this total, 181, or 75.1% were of group M, 59 or 24.48% were group MN, and only one, or 0.42% was of group N.

Comparison of the gene frequencies were as follows:

Blood Indians (total 241) M = 0.8734, N = 0.1266

Cree-Metis (total 185) M = 0.65405, N = 0.34595

If a high frequency of gene M is indeed characteristic of Indians of Alberta, this may be considered as an indication of dilution of Cree racial strains with much N derived from European and British elements, but Matson et al[22] noted gene frequencies of M = 0.718 and N = 0.282 in 161 putatively pure Chippewa in their Minnesota series, the N gene frequency rising to 0.449 in their group of 206 who were "less than three-quarters" Indian. These authors also suggest that the average M gene frequency for White races in the United States of America is approximately 55%.

It is very possible that more extensive testing with anti-M and anti-N sera would be of value, particularly in the Crees of northern Alberta.

On reading the published works of both these Canadian and American authorities, one is left with the impression that neither group is satisfied with all of their findings

in Indian tribes. Chown and Lewis[11], for example, indicate that some of their "population samples do not agree in their MN distribution", while Matson, Koch, and Levine[22], suggest the possibility of "chance variation" in 128 Chippewa who, although thought to be "more than three-quarters pure Indian", possess a higher N gene frequency of 0.492 than the more "diluted less than three-quarters" Indian of their series who have a frequency for the N gene of 0.449.

One difficulty that arises in most works relates to the relatively small number that can be tested in any one area. Similarly, it is doubtful if one can use the term "pure" Indian; certainly in Alberta, no known Crees exist who are truely Indian, all Indian Agency records suggesting most strongly that all are of mixed Indian and European or British descent.

Summary

The frequency of the M gene is high among all North American Indian races, the observed frequency in the one hundred and eighty-five Cree-Metis of this study being 65.4 per cent. Indeed, this proportion is lower than that reported for the M gene in other North American Indian tribes whose blood groups have been investigated, and an N gene frequency of 34.6 per cent in this series suggests that the population is of mixed racial origin, as their history indicates.

PART II

The Rh Factor

Notation

In Britain and elsewhere the outbreak of the Second World War delayed the complete appraisal of the now classical studies of Landsteiner and Wiener (1940) and the report of Levine and Stetson (1939)[23] at a critical period in world history.

At that time, Levine and Stetson had found an atypical agglutinin in the serum of a group O American patient whose infant had been stillborn, and who had received a single blood transfusion resulting in a severe reaction. Her serum agglutinated the red cells of some eighty per cent of the one hundred and four group O individuals then tested.

In the course of subsequent studies, this serum was found to be identical in reaction to the anti-rhesus serum Landsteiner and Wiener had produced in the sera of rabbits and guinea pigs by injections of blood of the monkey Macacus rhesus.

Having ascertained that about eighty-five per cent of the red cell samples of white people in New York were agglutinated by their anti-rhesus sera--these being called Rh positive, and the remaining fifteen per cent Rh negative--it is astonishing to find how quickly Karl

Landsteiner and Alexander Wiener, in conjunction with Albin Matson, applied their energies to other racial blood group studies, an outstanding paper[24] on the distribution of the Rh factor in American Indians appearing in 1942.

The clotted blood samples tested by these authorities came from North American Indians in the Tacoma Hospital and from Indian soldiers stationed at Fort Lewis, Washington. Altogether ten different tribes were represented.

Landsteiner and his associates prepared saline cell suspensions from the Indian donors' specimens, and after preliminary washing in saline, testing was done after adding either guinea pig immune Rh serum or one human anti-Rh serum and incubating at 37°C. The period of incubation was not stated, but equal volumes of the saline erythrocyte suspensions and sera were used.

Two categories were listed: (1) one hundred and twenty Indian samples from persons who were thought to be "pure" Indians on the basis of Indian Agency records, and (2) one hundred and fifty-five judged to be of mixed ancestry from these records.

Of the "full blooded" Indians, one hundred and nineteen were found to be Rh positive, and only one Rh negative. That is, 99.2 per cent were Rh positive, and

it was thought that the one Rh negative case in this category might be "attributable to some untraceable crossing with Whites, a possibility which may also apply to the single group B individual found". It was also noted that eighty-eight or 73.3 per cent were group O, and that thirty-one or 25.8 per cent were group A. None were of subgroup A_2.

In the second category of those known to be of mixed racial origin, one hundred and forty-eight or 95.5 per cent were Rh positive, and seven or 4.5 per cent were Rh negative. In this group of Indians the proportion of group O fell to 58.1 per cent; A_1 was 31.6 per cent; five were of subgroup A_2 (3.2 per cent); while seven group B cases were found (4.5 per cent).

It was concluded that the full blooded North American Indians in this series were predominantly, if not exclusively, Rh positive, and that the proportion of Rh negatives in those of mixed White and Indian ancestry reflected the extent of White racial admixture.

There was no suggestion by these authors that the results with the human anti-Rh serum used differed in any way from those obtained with the immune anti-Rh guinea pig serum. Limited use was made of a second human anti-Rh serum because it gave "about twenty-seven per cent negative

reactions instead of fifteen per cent" with White persons' erythrocytes.

Following this report, the interest in the anthropological application of such studies greatly increased. The absence or the very low incidence of Rh negative individuals appears to be peculiar to primitive races, as is clearly shown in the detailed review of the racial distribution of the human blood groups by Dr. A.E. Mourant (1954)[21].

In Britain, in the early years of the Second World War, much thought was being given to the Rh subtypes as new Rh sera of different specificity came to light. The culminating point was reached when Sir Ronald Fisher designated six possible specific Rh antisera by Greek letters, corresponding to the elementary antigens of his hypothesis, but the simplification proposed by Professor D.F. Cappell[25] of naming the various sera anti-C, anti-D (corresponding to the original anti-Rh serum of Landsteiner and Wiener), anti-E, etc., has now become standard usage in many countries of the world. In obstetrical practice the value of such knowledge was further emphasized by Professor Cappell in 1944[26]. The concept of three closely linked genes in different possible combinations, and of the allelomorphic pairs C-c, D-d, and E-e was reached well before the rarer sera such as anti-e were discovered.

This hypothesis of closely linked genes (while challenged bitterly by Dr. A.S. Wiener throughout the years[27]) and the CDE notation, offers a straightforward way of forming an easily understood picture of the Rh system.

D^u

The existence of at least one variant, D^u, forming an alternative third gene allelomorphic to D and d, has brought about no basic change in the accepted pattern of the CDE notation. Although it gives rise to such Rh chromosomes as CD^ue in lieu of CDe and Cde, this and other variants such as C^w at the C-c locus, nevertheless complicate the original hypothesis.

Dr. Richard Rosenfield[28] recently sent an unusual anti-D serum to this writer, stating that it came from an Rh (D) positive woman, whose possible Rh phenotype was ccDee. The serum was tested, at his request, against red cells of Rh genotype -D-/-D- and cde/-D- (these cases being discussed in a later chapter).

This serum was found to be of the incomplete type, and when tested locally against Rh positive cells in saline at 37°C. for ninety minutes, there was no agglutination. In 20 per cent bovine albumin the same Rh positive cells of genotypes CDe/cde, CDe/CDe and cDE/cde, etc., were agglutinated to a titre of 1:4. By the indirect Coombs method the titre was 1:8, weak 1:16. It was negative in saline, albumin, and by the indirect Coombs method against six Rh negative cells of genotype cde/cde, Cde/cde, and cdE/cde.

While giving negative reactions with group O, Rh

positive cells in two per cent saline suspension, and in half to one per cent saline, it agglutinated group O, -D-/-D- and group O, cde/-D- cells when one volume of the approximate one per cent saline cell suspension was added to two volumes of the patient's serum and incubated at 37°C., for ninety minutes. The extent of agglutination was one + with cells of Rh genotype -D-/-D-, and weak to (+) with the cde/-D- sample.

That is, this serum reacted in the same way as weak incomplete anti-D sera react in saline with Rh positive cells possessing the rare Rh chromosome -D-. Thus, this patient's cells may well possess a variant of the D antigen, and D^u may be further subdivided in the course of time.

Rh Investigations: Methods and Controls

The sera available for Rh testing consisted of complete and incomplete anti-D; complete anti-C plus anti-C^w; complete and incomplete anti-E; complete and incomplete anti-c; a small volume of incomplete anti-e; and lastly, one-half ml. anti-f--the last kindly given to the author by Dr. Richard Rosenfield of New York.

The anti-e and the anti-f sera had to be used sparingly, and tests with these sera were restricted to those persons' red cells who were thought to have a probable Rh "deletion"--that is, to those cases believed

to possess the Rh chromosome -D-, whether homozygous and of Rh genotype -D-/-D-, or heterozygous and of Rh genotype CDe/-D-, etc.

Since erythrocytes of this rare nature are agglutinated in saline cell suspensions by incomplete anti-D, as demonstrated in the original case of Race, Sanger, and Selwyn[29 and 30], care had to be taken in selecting all sera. Thus many anti-C and anti-E sera contain traces of incomplete anti-D, having been generally prepared from the sera of Rh negative maternity patients possessing incomplete anti-D as well as the desired specific antibody.

At least two and often three different saline anti-D were used; and at least two different lots of anti-C plus anti-C^w, anti-E, and anti-c sera, whether possessing saline or blocking forms of the antibody desired.

"Screening Test" with Incomplete Anti-D

A minimum of five incomplete anti-D sera were used in testing each red cell sample in two per cent saline suspension and in one per cent saline cell suspension for the agglutination reaction typical of cells bearing the Rh chromosome -D-.

All three hundred and thirty specimens were thus tested in as much detail as possible, and while the specimens were still less than twenty-four to thirty-six hours

old. Incomplete anti-D sera that agglutinated normal group O controls of Rh genotype CDe/CDe, CDe/cDE, CDe/cde and cDE/cDE in one per cent, and two per cent saline cell suspensions were discarded for the purposes of this study, it being necessary to avoid using incomplete anti-D sera having any residual saline anti-D activity against normal Rh positive cells.

The Selection of anti-Rh Sera, and Controls

The selection of suitable specific Rh testing sera occupied much time, in advance of the study. That this was necessary was made obvious to the writer because of the mistakes he made in trying to identify the proper Rh genotype of the original Metisse propositus, false impressions having been reached after tests with ordinary commercial anti-C, anti-E, etc., containing traces of incomplete anti-D.

The cells of this propositus[1] (subsequently identified as being of Rh genotype -D-/-D-) and those of certain of her close relatives of Rh genotypes -D-/-D- and CDe/-D- were used as control cells in this investigation, those selected being of group O.

As the investigation proceeded, it was found best to make use of both two per cent and one per cent cell suspensions in sterile 0.85 per cent saline, these two

per cent suspensions being used for tests with saline anti-D, anti-C plus anti-C^W, anti-E, and anti-c, while the one per cent saline cell suspensions were used in testing with incomplete anti-D. With the complete sera one volume of each two per cent cell suspension was added to one volume of the known serum in separate marked precipitin tubes. After one and a half hours incubation at 37°C., the results were read, and at the same time compared to the results obtained with known group O controls including those of the original family noted above.

The titres of these complete sera were not less than 1:64 (with serial saline dilutions of test cells homozygous for the corresponding D, C, E or c antigens). Before making up the unknown and the known cell suspensions, preliminary washing was done in sterile isotonic saline to remove traces of the donor's own serum: in the case of known control cells that had been kept frozen in glycerol-citrate, the final three washings were performed with one per cent sterile saline (pages 33 and 34).

Incomplete Anti-D Sera. Method and Technique.

In testing the donors' saline cell suspensions with the five or more selected incomplete anti-D, it was found that it was better to use the weaker one per cent saline cell suspension, the reason being that while homozygous -D-/-D- cells are intensely agglutinated in the two per cent saline medium, cells that are heterozygous CDe/-D-,

or cde/-D- may not be recognized as such on preliminary testing. This is particularly true of cells possessing the single D antigen in the form of the rare genotype cde/-D-.

Also, those reacting only with anti-c, saline active anti-D, and anti-e, and thus having the presumed phenotype ccDee may be thought to possess the possible Rh genotype cDe/cde, but by studying the exact family relationships and by testing very carefully with a larger number of twelve or more suitable incomplete anti-D in one per cent saline cell suspension, it has been found that the more probable Rh genotype in certain instances is cde/-D-.

The value of reconsidering the family pedigrees, and of questioning both the persons concerned and of studying the family Church records and blood relationships therein recorded cannot be overestimated. At the same time it is necessary to be certain of having normal and rarer control cells set up in the weaker one per cent suspension, in tests with incomplete anti-D, before coming to an opinion on the most probable Rh genotype, and after consideration of all the other "genotyping" tests.

The titres of those incomplete anti-D selected varied from 1:32 to 1:128 against normal Dd and DD erythrocytes. The exact titre was less important than selection on the basis of no discernible agglutination in one per cent

saline suspension of normal Rh positive controls of genotypes CDe/CDe, CDe/cDE, cDE/cDE, CDe/cde, and cDE/cde.

Typing with Other Rh Sera

The cells were prepared in two per cent saline suspension when being tested with complete anti-D, complete anti-C plus anti-C^w, complete anti-E, and complete anti-c sera--having first been washed with sterile isotonic saline to remove any traces of the individual donor's serum. One volume of the cell suspension was added to one volume of the known saline-active serum in numbered precipitin tubes, incubation being at 37°C., for ninety minutes. Results were read macroscopically, and simply confirmed with the low power of the microscope. Known control cells were first read, and then the test results.

In confirming the reactions under low power, care was taken to prevent "false negatives" due to destruction of the agglutinates that may occur with rough handling in spreading the droplet on a slide. (It is known that in many laboratories two hours incubation is preferred to the local practice of reading at the end of ninety minutes incubation, while in other laboratories results are sometimes read at the end of a shorter period at 37°C. to 39°C., following one minute at 500 - 1000 r.p.m. in the centrifuge). Local experience would indicate that a minimum of one and a half hours incubation at 37°C. is

satisfactory with the Rh sera available, and no final centrifuging is necessary or desirable.

Since the study was conducted with different Rh sera over a long period, it was considered well worth while to make periodic checks of the sera with various suitable group A_1, A_2, and B test cells, as anti-A and anti-B iso-antibodies may occasionally recur in anti-Rh testing sera, after storage[26a].

For grouping with incomplete anti-Rh sera such as anti-E, and anti-c, the cells to be tested were first washed free of the donor's serum, and then the packed cells were suspended in twenty per cent bovine albumin to make a two to three per cent albumin cell suspension. One volume of the testing serum plus one volume of the cell suspension were incubated at 37°C. for a minimum of one and a half hours. Reading was then done macroscopically, and as in the method for saline or complete anti-Rh sera.

Whenever possible, saline-active sera were used in preference to the blocking variety, except for tests with incomplete anti-D in saline cell suspensions.

Rarer Rh Sera

1. Anti-e

The anti-e serum first used was a commercially purchased product. Only when it was learned that it had

been prepared from the serum of a haemophiliac who had received multiple transfusions (in New York), was it realized that certain of the non-specific reactions it gave might be attributed to either a weak immune agglutinin of low activity at 37°C., or possibly to an auto-antibody in the patient's serum, of the type sometimes found in acquired haemolytic anaemia.

While the exact specificity of the additional antibody remained unknown, earlier tests with this anti-e serum created a great deal of confusion when it weakly agglutinated the cells of three related patients who were ultimately found to be of Rh genotype -D-/-D-, and not -De/-D- as had been thought. The writer is greatly in the debt of Dr. R.R. Race, and of Professor Cappell[3] for pointing out many of the genetical inconsistencies that arose in this author's first interpretation.

When a second "blocking" anti-e became available, this incomplete antibody was employed by testing fresh two per cent cell suspensions prepared in the patient's own, or in group compatible serum, or in group A_1B serum, as recommended by the pharmaceutical company who had prepared it (Ortho, New Jersey). Usually group A_1B serum, free from iso-antibodies, was used as the protein medium after inactivation at 56°C. for 30 minutes.

Again, as recommended, small precipitin tubes were

used, two drops of the light cell suspension being added to one drop of this typing serum. After mixing, the marked tube was incubated at 37°C. for one and a half hours before being read macroscopically, and under the low power of the microscope. In practice, macroscopic readings with or without a hand lens provided satisfactory clear-cut positive or negative results. When a small group of unknown cells were being tested with anti-e, group O cDE/cDE, and known group O -D-/-D- cells were used as negative controls while group O cde/cde or Cde/cde cells were used as positive controls.

2. Anti-f

The small amount of anti-f available was limited to testing those -D- cells considered to be of probable genotypes -D-/-D-, CDe/-D-, and cde/-D-. This incomplete antibody was employed using the same methods adopted for anti-e, but with an equal and a very minute volume of the serum and cells in inactivated group A_1B serum. Dr. Rosenfield, one of the co-discoverers of anti-f, now recommends the prior treatment of test cells with ficin, one of the enzymes used extensively in his laboratory[28a]. Cells of Rh genotype cde/-D- alone were agglutinated with this serum.

3. Anti-V

A generous supply of anti-V was sent to the writer

recently by Dr. Amos Cahan[19c], together with a specimen of control cells from a Negro who is V positive. To date, it has only been possible to re-test three Metis' specimens of Rh genotypes -D-/-D-, CDe/-D-, and cde/-D- respectively, using the Negro V positive cells as a control. These three Metis proved to be V negative.

The anti-V serum agglutinated the Negro control cells in two per cent cell suspension (20% bovine albumin medium) very strongly, but were negative in two per cent saline suspension, and in the bovine albumin, with the Metis' cells. Results were read after incubation at 37°C. for ninety minutes.

Chapter 5

The Rh System

1. One Hundred and Eighty-five Metis
The Unrelated Group

No other studies of this nature have been made in the Metis population of Alberta. Although the total number is rather small for accurate statistical analysis, this group was regarded as a control series, providing some indication of the commonly occurring Rh chromosomes in these people of mixed racial origin.

Their Rh phenotypes, the chromosome frequencies, and other details are shown in Table 5, page 68, the necessary calculations having been based on the simplified method of Race and Sanger (1950)[31]. It was regretted that a larger proportion could not be tested with anti-e serum, the estimations being thus based on the observed proportion of bloods reacting with the four sera anti-C, anti-c, anti-D, and anti-E in that order.

Of the total number, one hundred and seventy-one or 92.43 per cent proved to be Rh (D) positive, while the remaining fourteen or 7.57 per cent were Rh negative.

Those specimens that were D negative on testing with saline active anti-D sera showed no evidence of the D^u antigen when rechecked by the indirect antiglobulin method.

None of the one hundred and seventy-one Rh positive specimens were agglutinated by six different selected incomplete anti-D sera in saline cell suspension, nor did the results with the other regularly used anti-sera indicate any evidence of Rh chromosomal "deletion".

Because the Rh genotype CDe/cde appeared to be common in both this control group and in the related families discussed in Chapter 6, the expected frequencies of the component genotypes derived from the Rh phenotype CcDee were estimated, and X^2 tests were then applied.

This phenotype, R_1r, comprising of thirty-five specimens, and the Rh chromosome frequencies, provided the following analysis:

CDe/cde = 0.4411 x 0.2440 x 2 = 0.2153

CDe/cDe = 0.4411 x 0.0211 x 2 = 0.0186

cDe/Cde = 0.0211 x 0.0000 x 2 = 0.0000

Thus, the expectation within Rh phenotype R_1r = 0.2339, and the expected number 185 x 0.2339, or 43.27.

Total Tested	Expected	Observed	$X^2 = \frac{(Obs.-Exp.)^2}{(Exp.)}$
R_1r	185 x 0.2239 = 43.27	35	= 1.58
185			
the rest	185 x 0.7661 = 141.73	150	= 0.48
	185.00	185	X^2 = 2.06

Number of degrees of freedom (n) = 1. $p > 0.1$.

The Rh System

Table 5

(1) 185 Metis: The "Non-Related" Group

Rh * Phenotype	Most Frequent Genotype	Number Observed	Percentage Observed	Chromosome Frequencies
CCDee	CDe/CDe (R_1R_1)	36	19.46	
CcDee	CDe/cde (R_1r)	35	18.92	
ccDEe	cDE/cde (R_2r)	41	22.16	CDe=0.4411
CcDEe	CDe/cDE (R_1R_2)	56	30.27	cDE=0.2566
CCDEe x	CDe/CDE (R_1R_z)	1	0.54	CDE=0.0061
ccDee x	cDe/cde (R_or)	2	1.08	cDe=0.0211
Ccddee	Cde/cde (r'r)	0	----	Cde=0.0000
ccddEe x	cdE/cde (r"r)	3	1.62	cdE=0.0311
ccddee	cde/cde (rr)	11	5.95	cde=0.2440
Total		= 185	= 100%	=1.0000

*Due to supplies of anti-e being limited, only a small number of the above were tested with anti-e and thus reactions with this serum are not strictly represented. The probable Rh genotypes shown are the more common that maybe expected on the basis of tests with anti-C, anti-D, anti-E and anti-c. All samples were also tested with at least five incomplete anti-D in saline cell suspension.

x = Tested with anti-e serum.

Discussion

The three most frequently occurring Rh chromosomes in the group of one hundred and eighty-five Metis were CDe, cDE, and cde respectively, and in this they differ very considerably from the majority of the Indian tribes that have been studied in Canada and in the United States of America.

On a percentage basis, CDe = 44.1, cDE = 25.7, and cde = 24.4 in this group. Of two other tribes that have been investigated in recent years, they compare more closely to the two hundred and six "less than three-quarters" pure Chippewa bloods tested by Matson and his colleagues[22] in 1954; but the Metis also show a much reduced frequency of the cDE chromosome and a much greater frequency of cde than the two hundred and forty-one Blood Indians' samples investigated in southern Alberta by Chown and Lewis (1953)[11].

A comparison of these three different series is of value, since it points to the marked influence of European interbreeding with the Indians of this vicinity:

Rh Chromosomes	185 Metis (Northern Alberta) %	Blood Indians[11] (Southern Alberta) %	* Chippewa[22] (Minnesota) %
CDe	44.1	46.9	41.7
cDE	25.7	40.1	38.4
cDe	2.1	0.0	3.1
CDE	0.6	3.8	2.9
cde	24.4	6.5	13.9
cdE	3.1	2.7	0.0
Cde	0.0	0.0	0.0

*Matson, et al[22] "Less than three-quarters pure" specimens

The most significant single finding is the high incidence of the Rh chromosome cde. That eleven (or almost six per cent) were Rh negative and of probable genotype cde/cde suggests that they are rather less than fifty per cent purebred.

It is also interesting to note that the remaining three Rh negative samples were of probable Rh genotype cdE/cde; while the Cde chromosome and the genotype Cde/cde were not detected.

Summary

Of 185 Indians of mixed ancestry 171 or 92.4 per cent were found to be Rh positive, while 14 or 7.6 per cent were Rh negative. In such a group, it was surprising to find that 11 or nearly 6 per cent were of probable Rh genotype cde/cde, the remaining three Rh negatives being of probable Rh genotype cdE/cde.

No evidence of Rh chromosomal "deletion" was detected, although all those who were Rh positive were tested with selected incomplete anti-D sera in saline cell suspension, and each specimen was carefully screened with this possibility in mind. Those whose cells were negative with complete anti-D sera showed no indication of possessing the D^u antigen, on testing for D^u by the indirect Coombs method.

The three most frequent Rh chromosomes in this small series were CDe (44.1%), cDE (25.7%), and cde (24.4%). The group differs from other North American Indian tribes in that the Rh chromosome cDE is of comparatively low frequency, while the cde chromosome is greatly in excess of the proportion usually found in relatively pure Indian bloods.

Their history, and their Rh characteristics indicate a marked interbreeding with Europeans, and it is most probable that the Rh chromosome cde, in particular, was derived from their White ancestors.

Chapter 6

The Rh System

2. One Hundred and Forty-five Metis
The Related Group

As indicated in the introduction on page one, this unusual group does not include the original Metisse propositus and her family--many of whom possess the unusual Rh genotypes -D-/-D-, or CDe/-D-, (and possibly cDe/-D-)[1 and 5].

This additional series of related cases were traced, in small family groups, as it was believed that more specimens of this specific nature (-D-) might be found. It was particularly interesting to discover several examples of the rare Rh genotype cde/-D-, when the difficult problem of tracing more distant relatives had been successfully resolved.

Table 6, page 73, shows the phenotypes and the most probable Rh genotypes of the additional one hundred and forty-five specimens.

All the Rh (D) positive samples were tested with incomplete anti-D sera in saline cell suspension.

Table 6

Anti-sera					Rh Phenotypes	Probable Rh Genotypes	No. Obs.	% Obs.
C	c	D	E	e				
+	+	+	-	+	CcDee	CDe/cde	50	34.48
+	-	+	-	+	CCDee	CDe/CDe	35	24.14
+	+	+	+	+	CcDEe	CDe/cDE	22	15.17
-	+	+	+	+	ccDEe	cDE/cde	12	8.28
-	+	-	-	+	ccddee	cde/cde	12	8.28
-	+	+	-	+	c(-)De(-)	*cde/-D- (or cDe/-D-)	7	4.83
+	-	+	-	+	C(-)D(-)e	*CDe/-D-	3	2.07
-	-	+	-	-	(-)(-)D(-)(-)	*-D-/-D-	1	0.69
+	+	+	+	+	CcDEe	*CDe/cdE	1	0.69
-	+	-	+	+	ccddEe	*cdE/cde	1	0.69
+	+	+	+	-	CcDEE	CDE/cDE	1	0.69
							145	100%

*Deduced also from family specimens after repeated testing.

No samples of probable Rh genotype cDE/cDE found.

General Discussion of Table 6

One of the most striking features noted was the relative preponderance of the Rh chromosome cde. Twelve examples of probable Rh genotype cde/cde were found. These twelve, and the one sample of probable genotype cdE/cde showed no evidence of having D^u, on being tested by the indirect Coombs method.

The percentage of cde/cde specimens was higher (8.28 per cent) than that found in the non-related "control" group where 5.95 per cent were of this genotype (Table 5, page 68).

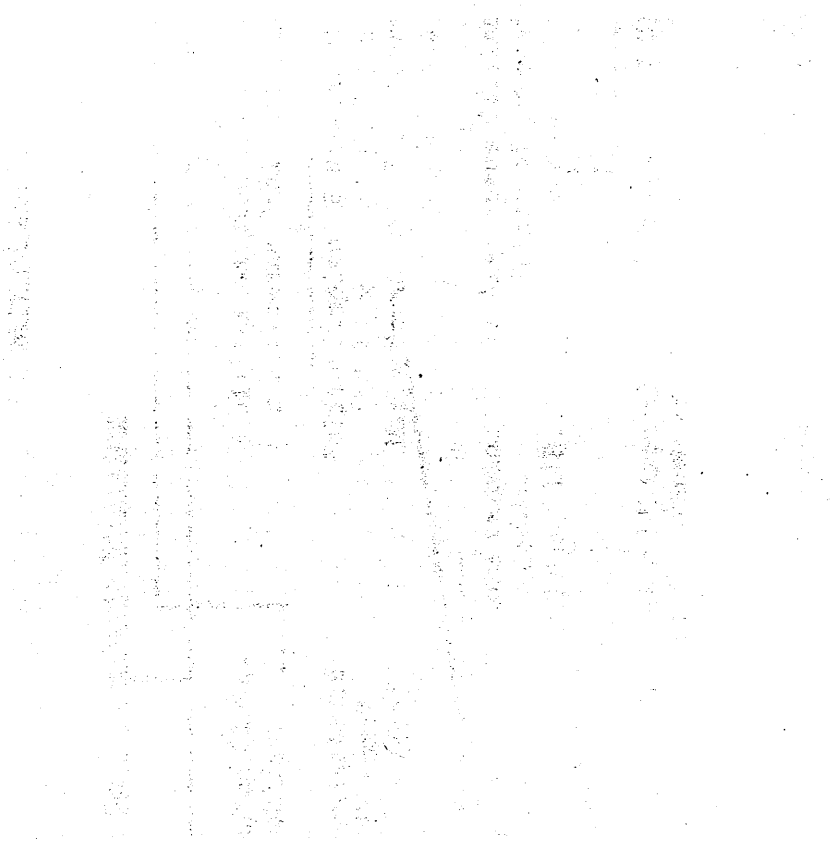
While the Rh phenotype CcDee, and the corresponding common genotype CDe/cde do not preclude the possible co-existence of the uncommon genotype CDe/cDe and the rare cDe/Cde, no evidence of these two much less frequent genotypes could be detected from the small individual family bloods that were studied.

Similarly, it was surprising that no example of the probable Rh genotype cDE/cDE occurred in this group, since cDE is a common chromosome in North American Indian tribes. As compared to the unrelated group, the genotype CDe/cDE could only be deduced in approximately 15 per cent of the related samples while the other non-related bloods had twice as many (i.e., 15.2 as compared to 30.3 per cent).

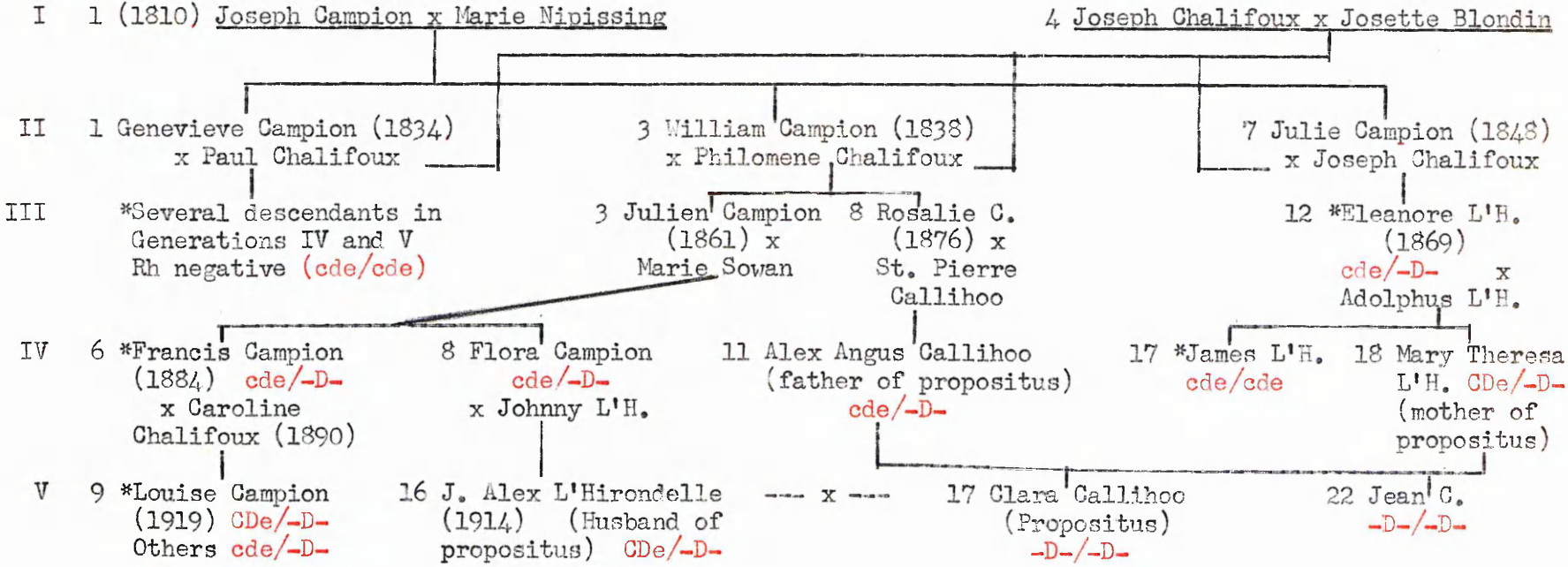
There was no reason to doubt the specific quality of the two anti-E and the one anti-e sera employed, as all the Rh sera used had given the expected results with appropriate known control cells of Rh genotypes CDe/cDE, CDe/cde, cde/cde, Cde/cde, cDE/cde, cDE/cDE and -D-/-D-.

The noteworthy features were therefore the comparatively high proportion of the rare chromosome -D-, the high CDe and cde, and the quite low proportion of the

chromosome cDE. This reduced proportion of cDE may well be of special significance. For example, not a single specimen of the Rh genotype cDE/-D- had been found in this consanguineous family group until June 1956, when a newborn infant of this most unusual genotype was born. (This will be discussed in Chapter 8).



Summary of Descent

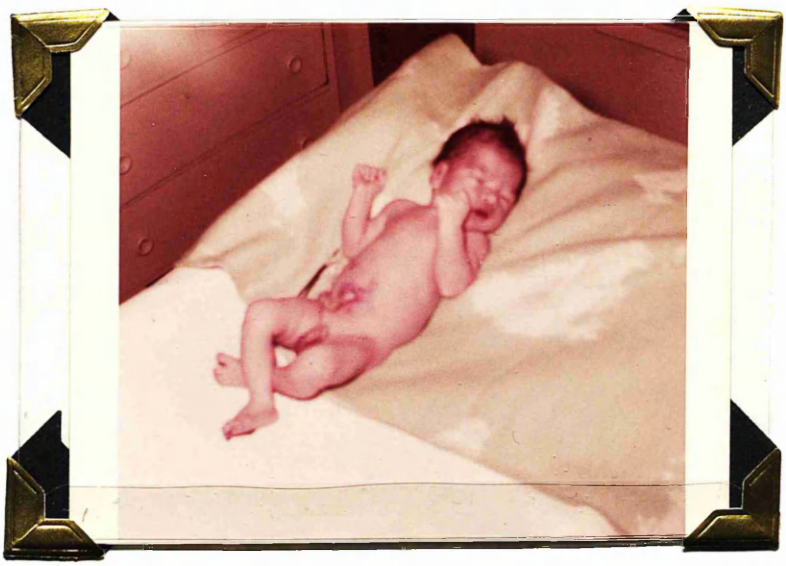


*Details of These Given in Case Reports

Francis Campion (IV-6), born 1884.
A_1O, MN, cde/-D-; and his wife
Caroline (IV-7), born 1890, OO, MN, CDe/cde

Emile Campion (V-10), born 1922.
OO, MN, cde/-D-.
Son of Francis and Caroline (above).

Jean Callihoo (V-22), O, MN, -D-/-D-.
Her daughter Barbara (VI-15), A_1, MN, CDe/-D-.



Bruce Callihoo (VI-18), born February 1955.
O, MN, -D-/-D-
Son of Jean C. (above).

Case Reports - 1

The Rh Genotypes cde/-D-, and CDe/-D-
The Family of Francis Campion (IV-6: 1884)

This elderly Metis, born in 1884, and his immediate family were traced in July 1955, to Lesser Slave Lake, Alberta, two hundred and thirty miles north of Edmonton. Until then, there had been no knowledge of his existence or whereabouts.

After extensive questioning and a search of the Church records, it was discovered that he is a brother of Flora Campion (1886), the mother of V-16, husband of the original Metisse propositus.

These new cases are of special value in that they illustrate many of the difficulties encountered in making a serological diagnosis, and for purposes of clarification, this family's blood relationships are indicated in Figure I, the major pedigree placed at the end of this volume.

(As required by the regulations governing this thesis, the family of the original Metisse propositus will not be discussed, and case reports will not include any that were investigated in 1954[1] and [5]. Figure I is attached to show the complicated inter-relationships of the different families involved, the extent of their consanguinity, and the most probable Rh genotype of each individual).

Table 7

The Family of Francis Campion

Name	Date of Birth	Relationship	Blood Groups							Incomplete Anti-D in Saline (Upon retesting)	Most Probable Rh Genotype
			ABO	MN	C	c	D	E	e		
IV-5	-	1st wife of IV-6									
IV-6	1884	Father	A_1O	MN	-	+	+	-	+	(+) to +	cde/-D-
IV-7	1890	Wife of IV-6	OO	MM	+	+	+	-	+	-	CDe/cde
V-3	-	Husband of V-4	OO	MM	+	+	+	-	+	-	CDe/cde
V-4	1903	Daughter	OO	NN	+	+	+	-	+	-	CDe/cde
V-5	1907	Son	OO	MN	-	+	+	-	+	(+) to +	cde/-D-
V-6	-	Son (Not traced)									
V-7	-	Son (Not traced)									
V-8	1906	Husband of V-9	OO	MM	+	-	+	-	+	-	CDe/CDe
V-9	1919	Daughter	A_1O	MM	+	-	+	-	+	++	CDe/-D-
V-10	1922	Son	OO	MN	-	+	+	-	+	(+) to +	cde/-D-
V-11	1929	Son	A_1O	MM	+	+	+	-	+	-	CDe/cde
V-12	1932	Daughter	A_1O	MM	+	+	+	-	+	-	CDe/cde
V-13	1928	Husband of V-12	B	MM	+	-	+	-	+	-	CDe/CDe
VI-1	1939	Daughter of V-4	OO	MN	+	-	+	-	+	-	CDe/CDe
VI-9	1954	Son of V-13	OO		+	+	+	-	+	-	CDe/cde

All fourteen red cell samples were Fy(a+) and K(-). All the eight group O's were Le(a-). The anti-Le^a serum contained an immune anti-A, and only group O cells were tested with this serum.

Serological Tests

The red cells of Francis Campion (IV-6) gave the following reactions with Rh testing sera: C-, c+, D+, E-, e+. On initial testing with incomplete anti-D sera in saline cell suspension, no agglutination was noted, and it was presumed that he was of possible Rh genotype cDe/cde. Anti-f also agglutinated these cells.

Had it not been found that one of his daughters was of probable Rh genotype CDe/-D- (V-9), the father's, and three other siblings' cells would have been considered as being of Rh genotype cDe/cde.

Two other reasons led to a further analysis of these cases: 1. The Rh chromosome cDe is uncommon in North American Indians, and 2. the possibility of the father being of Rh genotype cDe/cde could not be reconciled with the finding of a sibling of genotype CDe/-D-, her mother's cells being of most probable Rh genotype CDe/cde (IV-7).

The main findings in this family are shown in Table 7, page 77, and their blood relationships are indicated in Figure I.

Errors in Interpretation

The first (wrong) conclusion was that the chromosome cDe, descended from the father (IV-6), could only be represented in one child's genotype as -D- if his c and e

genes became "deleted":

(IV-6) A_1 MN $\underline{cDe/cde}$ x (IV-7) O MM $\underline{CDe/cde}$

Sibling (V-9) A_1 MM $\underline{CDe/-D-}$

It was assumed that the Rh genotype cDe/cDe was most unlikely, and there was no suggestion that the mother's cells possessed the rare chromosome -D- on testing with incomplete anti-D in saline. In any case, the mother's CDe chromosome appeared to have descended to this child. The possibility of suppression of c and e was considered, but these interpretations seemed too facile to be true. Similarly, while theoretically possible, it did not seem likely that the mother was of Rh genotype CDe/cDe, nor would this alternative account for the findings.

Two other alternatives were then considered, either IV-6 was of Rh genotype cDe/-D- or cde/-D-, and in either event the presence of the chromosome -D- had not been detected by the first six incomplete anti-D sera used in saline cell suspensions.

The sibling V-9's cells had given quite strong ++ agglutination by this method, employing a one per cent suspension of her cells and of normal CDe/CDe, CDe/cde, and cde/cde controls. All such normal controls were negative by this method.

It was then decided to revisit the family, when other

members were also found, and samples obtained. Because of the long distance from Edmonton, one day was spent on the outward journey from the laboratory, and the venous specimens were collected very early on the second morning so that they could be tested within twelve to twenty-four hours of being taken from each individual. They were kept in an insulated box containing ice, during the return journey.

Under these conditions, the red cells of IV-6, V-5, and V-10 showed (+) to + agglutination with seven incomplete anti-D sera in one per cent saline suspensions, while the probable CDe/-D- cells of V-9 were very strongly agglutinated by the same method (Table 7). Normal Rh positive controls were negative.

An Apparent "Dosage" Effect

Since the only test results favouring an exalted D antigen were those employing incomplete anti-D in saline cell suspension, it was thought that cells of possible Rh genotype cde/-D- might also give a reduced dosage effect with anti-c and anti-e sera.

Having very little anti-e serum available, and having almost exhausted the supply of complete anti-c, the following titrations were performed by the indirect Coombs method, using an incomplete anti-c as shown in Table 8, page 81.

Table 8

Titrations With an Incomplete Anti-c Serum
(Indirect Coombs Method)

Test Cells	Titre										
	1	2	4	8	16	32	64	128	256	512	Score*
O, cde/cde	+++	++	++	++	++	+	w	-	-	-	49
O, CDe/CDe	-	-	-	-	-						0
O, CDe/cde	+++	++	++	++	+	(+)	-	-	-	-	42
V-22 O, -D-/-D-	-	-	-	-	-						0
IV-6 A, cde/-D-	++	++	+	w	-	-	-	-	-	-	23
V-5 O, cde/-D-	++	++	+	w	-	-	-	-	-	-	23
V-9 A, CDe/-D-	-	-	-	-	-						0
V-10 O, cde/-D-	++	++	(+)	?	-	-	-	-	-	-	19

*The score in the titrations was obtained by assessing the intensity of agglutination in each agglutinin tube and adding the total obtained. +++ = 10, ++ = 8, + = 5, (+) = 3, w = 2, ? or - = 0. The first four test cells were used as controls, the fourth being a known related sample.

Table 8 shows that Francis Campion's cells and two of his children's erythrocytes gave a much reduced titre, assessed as 1:4, compared to normal cde/cde test cells, titre 1:32. The score obtained with these cells of apparent genotype

cde/-D- varied from 19 to 23, or approximately one half the score noted with the cde/cde control.

As expected, the cells of V-22, a known example of the Rh genotype -D-/-D-, were negative with the anti-c serum, as were the cells of Francis Campion's daughter V-9, CDe/-D-.

The surprising element was that control cells of Rh genotype CDe/cde proved to have a titre of 1:16 to 1:32, and a score of 42, a quite unexpected result. This sample was therefore retested, and CDe/cde was considered still to be the most probable Rh genotype, in agreement with family studies (parents cde/cde and CDe/cde respectively).

It was thought that this might be related in some unknown way to a "position effect".

Conclusions

The conclusions were reached that cells IV-6, V-5, and V-10 had a reduced amount of the c antigen, compatible with the Rh genotype cde/-D-, and that these erythrocytes contained less D antigen than cells of specificity CDe/-D-.

In the absence of anti-d testing serum, the Rh genotype cde/-D- cannot be differentiated from cDe/-D-. The cells of Francis Campion (IV-6) were agglutinated by anti-f, and his cells are very possibly of Rh genotype cdef/-D--. Again, however, cells having the chromosome

cde or cDe are known to be usually agglutinated with anti-f as shown by Rosenfield et al[32], so that anti-f serum is of no direct assistance in this particular connection.

Erythrocytes of Rh specificity CDe/-D- such as those of Louise Campion (V-9), presumably because they are homozygous for D, and because they possess the -D- chromosome are readily agglutinated by incomplete anti-D in saline at 37°C.

It may be that even specially selected incomplete anti-D sera that are negative with normal Rh positive cells in saline, contain sufficient complete anti-D to affect -D- cells. There is no doubt that many incomplete sera of this specificity agglutinate normal D containing cells in saline because they contain some complete agglutinin in addition to the blocking form of the antibody.

Nevertheless, selected incomplete anti-D sera provide a most useful screening test in cases such as these.

Francis Campion (IV-6) and his second wife Caroline Chalifoux (IV-7) are second cousins through his paternal grandmother II-4. The degree of consanguinity was so complex that it would have led to confusion if placed on the pedigree in Figure I. Of his first wife's (IV-5's)

children, one is of probable genotype CDe/cde and one of probable Rh genotype cde/-D-.

Of the four siblings V-9, -10, -11, and -12, by his second wife, their most probable Rh genotypes are CDe/-D-, cde/-D-, CDe/cde, and CDe/cde respectively.

No siblings of Rh genotype cde/cde were found in this particular family, so that there is no direct evidence that would distinguish the two chromosomes cde and cDe. The accumulated evidence in other related families is, however, in keeping with the probability of the chromosome cde being present in preference to the uncommon cDe chromosome.

In view of the rarity of some of these cases, specimens from Francis Campion and from his sister Flora Campion (IV-8) were sent to Dr. Ruth Sanger of the Blood Group Research Unit, the Lister Institute, London, in September 1955. She reported that both were of probable Rh genotype cDe/-D- or cde/-D-, and in her hands these cells were agglutinated by fourteen different selected incomplete anti-D sera in saline[33].

Case Reports - 2

The search for more related families was continued, and two journeys were made to Fort Assiniboine on the Athabasca river, one hundred miles north northwest of Edmonton, late in 1955.

In this carefully planned manner, specimens were obtained from IV-1, Veronique Courtepatte (1907), and from her brother IV-3, Hector Chalifoux (1916) and his wife IV-4.

Both Veronique Courtepatte and her brother Hector Chalifoux were found to be group O, MN, and of most probable Rh genotype cde/cde. They were found to be the descendants of II-1, Genevieve Campion (1834) and II-2 Paul Chalifoux, the sister and brother, respectively, of II-3, William Campion (1838) and II-4, Philomene Chalifoux, as indicated in Figure I: "the related families pedigree".

IV-2 has not yet been traced, but the two children of Hector Chalifoux and his wife IV-4 (1917), namely V-1 and V-2 were found to be of Rh genotype cde/cde and cDe/cde respectively.

By this stage in the development of the series it was not too surprising to find that IV-4 (cDE/cde, 1917) was a cousin of IV-10 (1893), and that the latter had also married a Chalifoux, IV-9.

Conclusions

Having disentangled the major consanguineous relationships, it became clear that the chromosome cde was very probably a frequently occurring chromosome in the closely intermarried families of generation II-1, -2, -3, -4, -5, -6, -7, and -8.

Since IV-1 and IV-3 are both of Rh genotype cde/cde it must be presumed that both of their parents III-1 (1871) and III-2 possessed the cde chromosome, and that it similarly occurs in the living descendants of II-3, and II-4.

If one did not know that these related groups were of mixed Indian and European descent, one might well surmise that they were of purely White origin, and indeed it seems possible that both the chromosome cde and the rare -D- chromosome originated from their European forebears.

It is possible that consanguineous matings resulted in the pairing of cde with cde, and of cde with -D-, giving rise to the Rh genotypes cde/cde in IV-1, and in IV-3; and to cde/-D- in such individuals as IV-6.

Case Reports - 3

Eleanore L'Hirondelle (1869), III-12

This elderly Metisse, now eighty-seven years old, is the oldest living descendant of II-7, and II-8, through whom she is the granddaughter of the original Campion (I-1) and of the original Chalifoux (I-4) of this anthropological history.

Her maiden name, Chalifoux, and her mother's surname before marriage, Campion, are repeated frequently

throughout the whole related series, and it was not too difficult to relate her in the pedigree to Francis Campion (IV-6) and to numerous others within this group.

By tracing two of her previously unknown children, and a previously unknown brother and sister, it became clear that her probable Rh genotype was cde/-D-, and not cDe/cde as had previously been thought.

The blood groups of III-12 and of four of her recently found family are listed in Table 9.

Table 9

Identity	Year of Birth	Relationship to III-12	Blood Groups, and Most Probable Rh Genotypes
III-12	1869		O, NN, cde/-D-*
III-13	1880	Brother	O, MN, CDe/cde
III-15	1888	Sister	O, NN, cde/-D-
IV-17	1895	Son	O, NN, cde/cde
IV-19	1898	Daughter	O, MN, CDe/cde

*III-12's cells were negative on testing with anti-V serum. (V-positive control satisfactory).

The erythrocytes of the two sisters, III-12 and III-15, gave identical reactions with the available test

sera, the plan of testing following the same methods used in investigating the family of Francis Campion (Case reports - 1). Using the same incomplete anti-c serum noted in Table 8, page 81, the two sisters' cells, when freshly obtained, gave a dosage effect with this anti-c. In the indirect Coombs test, titres of 1:4 were obtained as compared to 1:32 with normal cde/cde and cDe/cde controls. I.e., a single amount of the c antigen was thought to be present.

All five of the samples listed in Table 9 were Fy(a+), Kell (K) negative, and Le(a-). On testing with Rh sera, III-12's and her sister's, III-15's, cells were found to be C-, c+, D+, E-, e+, and f+; i.e., of probable genotype cdef/-D--.

Six out of seven selected incomplete anti-D sera caused definite (one plus) agglutination with one per cent saline suspensions of the cells of III-12 and III-15 upon incubation at 37°C. for ninety minutes, normal Rh positive controls being negative with all of the seven incomplete anti-D sera in one per cent saline suspension.

The cells of III-13 and of IV-19 were found to be C+, c+, D+, E-, and e+. These samples were not agglutinated by the seven blocking anti-D sera in saline, and the genotype CDe/cde appears to be highly probable.

IV-17's cells were also negative on testing for the

presence of the D^u variant, being C-, c+, D-, E-, and e+, and thus of most probable genotype cde/cde.

Discussion

Unless very extensive serological tests are made, the proving of the Rh genotype cde/-D- presents a difficult problem in blood group determinations.

The now almost classical method first described by Race, Sanger, and Selwyn[29], of testing a two per cent cell suspension in saline with incomplete anti-D sera may give ambiguous results unless these sera are specially selected. Such sera must give clearly negative results with normal Rh positive control cells, and be sufficiently avid to clearly agglutinate rare known controls of Rh genotypes -D-/-D-, CDe/-D-, and cde/-D-.

Local experience would indicate that a weaker cell suspension, one per cent in saline, is more satisfactory, two volumes of this cell suspension being added to two volumes of incomplete anti-D in agglutinin tubes, and read after incubation at 37°C. for ninety minutes. (Since the antigens c and e, and possibly f, may degenerate with aging or improper storage, fresh specimens are essential, for all such Rh testing procedures).

In the case of III-12 (1869), the evidence in favour of the Rh genotype cde/-D- may be summarized as follows:

1. Of her three living children, one sibling is of Rh genotype cde/cde, and thus one of his cde chromosomes must have been inherited from III-12. (This sibling, IV-17, married a second cousin, IV-15, and by chance coupled with intense consanguinity, one of his children is of probable Rh genotype cDE/cde (V-24') while the other is of possible Rh genotype cde/-D- (V-25'). It is presumed that the -D- chromosome of V-25' is inherited from the mother, who died shortly after this younger child was born).

2. Further evidence in favour of III-12's Rh genotype being cde/-D- is that one of her daughter's is of genotype CDe/-D-. (This sibling, IV-18, mother of the original propositus, had been previously identified as possessing the -D- chromosome, so that her case has not been included in Table 9, nor in the present study).

3. In the light of what is now known, however, it seems clear that the -D- chromosome of IV-18 was inherited from III-12 (Eleanore L'H.) and not from her deceased husband III-11, as I had previously and erroneously assumed.

4. Finally, the youngest living sibling, IV-19, is of probable genotype CDe/cde, while the -D- chromosome appears to co-exist in the cells of III-12's sister III-15. (It is hoped that the latter's immediate family can be

traced at some future date, since preliminary and recent serological tests suggest that a son of III-15, namely IV-21 (1914) is also of possible Rh genotype cde/-D-).

The foregoing rather complicated discussion can be reduced to a simplified list of the remaining living children of III-12, Eleanore L'H., and of her near relatives:

The three living children of Eleanore L'H., (1869): O, NN, cde/-D-

1. James L'H. (IV-17) O, NN, cde/cde.
2. Mary L'H. (IV-18) O, MN, CDe/-D-.
3. Margaret L'H. (IV-19) O, MN, CDe/cde.

The children of James L'H., (IV-17)

1. Irene L'H. (V-24') O, MN, CDe/cde.
2. Robert L'H. (V-25') O, MN, cde/-D-.

The brother and sister of III-12

1. Eugene Chalifoux (III-13) O, NN, CDe/cde.
2. Jeanne Chalifoux (III-15) O, NN, cde/-D-.

It is remarkable that Eleanore L'H., her brother Eugene, and her sister Jeanne are all three of the genotype OO, and that all three are NN, (or M-negative).

This would suggest that their parents II-7 and II-8 may have been blood relatives, and that each of these parents must have possessed genes O and N in common.

Since the genotype OO is of high frequency in the Cree-Metis, it may have been inherited from a Cree ancestor, or from a French forebear, Campion or Chalifoux. The N gene, however, suggests a more marked degree of European admixture, since North American Indians have a high frequency of gene M, N being relatively uncommon in purer Indian races.

One is left with the suspicion that their other inter-married relatives in generation II were perhaps cousins or second cousins. The intensity of consanguinity (or the related mechanism of genetic drift) is everywhere apparent throughout the whole group from II-1 to II-8, downwards, to the youngest children of generation VI.

Case Reports - 4

The Direct Descendants of the Iroquois (I-6)

A large proportion of the one hundred and forty-five related specimens came from the more direct descendants of I-6 (born 1782, died 1846). A number of these are represented to the right of Figure I, in the main pedigree, their family surname being Callihoo—see also pages 19 to 25, Chapter 2.

Of the children of II-11 and II-12, two sons, III-7 and III-9, mated with III-8, a Campion-Chalifoux descendant. On the early death of III-7 (born 1870), his younger brother III-9 (1871) mated with the same woman.

Nothing is known of the circumstances, and at the most it can only be assumed that the cDE chromosome of Veronique Callihoo, IV-10, 1893, and of the other sibling, IV-13, may have been inherited from their Iroquoian forefathers, while their cde chromosome may have been of Campion-Chalifoux origin--a possible explanation of their Rh genotype cDE/cde.

With the exception of the above cases, the more direct Iroquois descendants III-17, -19, -21 and -23 possess the probable Rh genotypes CDe/cde, CDe/cDE, CDe/cDE, and CDe/cDE, respectively. These four, bearing the surname Callihoo, are all over seventy years of age, and the males have been the official chiefs of the band, the title of "chief" or leader having been a source of great pride to the families concerned.

Perhaps with some justification, they claim to be of "pure" Iroquois lineage, and they despise their poorer Metis relatives who have "the strange White blood". All are Rh positive, the chromosomes CDe and cDE recurring in them and in their immediate descendants IV-23 to IV-28. The cde chromosome appears to be relatively uncommon, but its presence suggests a small degree of Caucasian or European influence.

In spite of thorough testing, there is no evidence of the existence of the -D- chromosome in III-17's to III-25's

red cells, nor in the erythrocytes of their immediate offspring in generations IV-23 to IV-28, and V-31 to V-33.

A Summary of Chapter 6

The serological investigations described in case reports 1, 2, and 3 indicate that the Rh genotype cde/-D- is likely to be confused with the "alternative" genotypes cDe/cde or cDe/-D-, unless detailed family studies are made.

In the consanguineous group under discussion, the commonly occurring Rh chromosomes are CDe, cde, and that otherwise rare chromosome -D-. The occasionally recurring genotype cde/cde favours the co-existence of cde with -D- in some relatives. The Rh chromosome cde is most probably of European origin, and this may also be true of the uncommon -D- chromosome.

Thus the Rh genotypes CDe/cde, cde/cde, CDe/-D-, and cde/-D- are surprisingly common in these related families, and none appear to be of Rh genotype cDE/cDE. Although the genotypes cDE/cde, and CDe/cDE do occur, the chromosome cDE is of low frequency for a native Canadian Indian tribe.

In the related group of Metis, only some fifteen per cent are of probable Rh genotype CDe/cDE (Table 6), whereas thirty per cent of the unrelated Metis (Table 5) are of this apparent genotype, but the former group were

highly selected.

Consanguinity is a marked feature, and just as the cde chromosome is probably of European origin, so does the high incidence of the gene N support the hypothesis that many of their blood group characteristics are inherited from a few European ancestors.

In case report 3, the Rh chromosome -D- has finally been traced back to generation III for the first time. The cells of the oldest living member, who is now eighty-seven years old, possessed this rare chromosome, and her probable genotype is cde/-D-.

Chapter 7

The Kell, Lewis, and Duffy Systems

The work of testing with anti-K, anti-Le^a, and anti-Fy^a sera is very incomplete. A shortage of suitable sera prevented thorough testing, and therefore no definite conclusions are inferred.

Under these conditions, only the specimens of the related group of one hundred and forty-five were tested with one anti-K, and with one anti-Fy^a serum.

The indirect Coombs method was employed, known K+, and Fy(a+) cells having been first tested with the appropriate serum. The anti-Le^a (Lewis) serum was obtained from a local donor who had become sensitized, following an ectopic gestation, with one transfusion of Le(a+) blood. She had also received a transfusion, in early childhood, of unknown type. As in the case of the anti-K and anti-Fy^a sera, the indirect Coombs method was used in testing with this proven immune anti-Le^a serum[34].

But there was one further problem--the locally obtained anti-Le^a serum also contained a potent immune anti-A agglutinin. With group O control cells that were Le(a+) the titre was 1:8 by the indirect Coombs method, but the immune anti-A agglutinin could not be absorbed out of the serum, which was most disappointing in that it was the only one available in relatively great volume.

1. It was found that all one hundred and forty-five related specimens were Kell (K) negative, and that all were Fy(a positive). Of this group of relatives, seventy-eight were of group O, and of these seventy-eight group O's, thirty were tested and found to be Le(a negative).

Group O controls, positive and negative for the corresponding antigen were satisfactory, but no conclusions are drawn other than that all the related group appear to be K-negative and Fy(a positive).

2. Only fifteen of the other unrelated series of one hundred and eighty-five specimens were tested with anti-K serum, it being found that no further supplies could be obtained. The fifteen were all Kell (K) negative.

Summary

A shortage of the valuable anti-K and anti-Fy^a sera prevented adequate testing. A much more readily available anti-Le^a serum was used in testing a limited number of group O relatives' samples, the co-existence of a potent immune anti-A preventing wider use of this serum.

No final conclusions are drawn. It appears that the closely consanguineous Metis' samples are all Kell (K) negative, and that they are Duffy, Fy(a positive).

Chapter 8

Studies of Families Possessing the Rh Chromosome -D-

Of the families recently studied, eleven possessed the Rh chromosome -D-, and of these eleven people one infant (VI-18, born February 1955) was homozygous, -D-/-D-[2]. Of the other ten, seven were heterozygous, cde/-D-, and three were heterozygous, CDe/-D-. (Table 6, page 73).

All are consanguineous, although each family lives from one hundred to over two hundred miles from each other, in northern Alberta.

In a vast area such as this it was difficult, at first, to relate them all on one pedigree (Figure I), but when this had been accomplished it was clear that all were of common descent, and all eleven proved to bear a blood relationship to the original Metisse propositus, and therefore to the fourteen people first described as possessing the Rh chromosome -D-[5].

A General Discussion

1. It seems incredible that so many individuals' red cells should bear a rare Rh chromosome. However, the families concerned are large, and all are blood relatives.

In a group of people having a mixed Canadian Indian and European ancestry, one would expect the Rh chromosome cDE to be more prevalent, since it has long been regarded

as a typical chromosome in North American Indian races.

In one of their original and detailed studies, Race and his two colleagues[29] referred to the Rh chromosome -D- as possibly representing a deletion of the corresponding allelomorphs C or c, and E or e. Although the cause of this apparent "deletion" within the Rh complex is unknown, it seemed to be possible that there was a direct bearing between the moderate incidence of the Rh chromosome cDE and the apparently "high" incidence of the -D- chromosome in these Metis families.

It would be unwise to postulate that the missing genes c and E account for the "deletion" in the Rh chromosome expressed as -D-. A more direct explanation is that (a) the Rh chromosome -D-, and the other commonly occurring CDe and cde chromosomes were inherited through six known generations in the line of descent, and (b) the -D- chromosome is not of recent origin.

Evidence that the Rh chromosomal entity D-- or -D- is not a very recent acquisition in these families' Rh blood groups is found in its presence in two members of generation III. The older of these two was born in 1869, and allowing for her age, she is in good general health.

It is only natural to ask if it is purely a coincidence that her red cells bear the chromosomes cde and -D-. (Proof that she is of Rh genotype cde/-D- is given in case

report 3, page 86).

Since her cells are also agglutinated by anti-f the possible Rh genotype is cdef/-D--, but until anti-F is found, and until such cells can be tested with this yet unknown serum, there must remain a doubt about the true nature of this extended genotype. The cells of this Metisse were negative with the anti-V serum kindly sent by Dr. Amos Cahan[19a], a Negro V-positive control and a random White blood being respectively V-positive and V-negative.

2. Leaving the presumed allelomorphs F-f, and "V-v" out of this account, the co-existence of cde and -D- in this and in six others of probable Rh genotype cde/-D- may indicate that the Rh genes of Indian origin have been overwhelmed and displaced by those of their few White forebears. Nevertheless, a more straightforward explanation is that both the cde and -D- chromosomes are of Caucasian (European) origin and that intense consanguinity and repeated cousin or second cousin matings in each generation of Metis have resulted in a most unusual but chance selection of Rh chromosomes of European and possibly French derivation--a form of "genetic drift" taking place in a short period of less than two centuries.

There is no evidence that the -D- chromosome is by any means common in North-American Indian races, nor has this rare chromosome been found in the small number of

other Metis' blood samples that have been tested in northern Alberta (Chapter 5, page 66).

In essence, it is suggested that one of the early French-Canadian voyageurs who journeyed to this region shortly before or after 1800 possessed the rare Rh chromosome -D-, and one or more of his companions bore the chromosome cde. Through subsequent consanguineous matings, their mixed Cree-Iroquois-Metis descendants came to possess only the Rh chromosomes -D-, cde, CDe, and cDE.

This explanation, in a highly inbred group of people, encompasses the view that the CDe and cDE chromosomes may be of either Indian or European origin. It permits the resulting genotypes CDe/cde, CDe/CDe, CDe/cDE, cDE/cde, cde/cde, cde/-D-, CDe/-D-, -D-/-D-, cDE/cDE, and cDE/-D-.

3. The curious absence of the Rh genotype cDE/cDE

As a result of serological tests, all of the foregoing Rh genotypes have been noted, excepting the genotype cDE/cDE. This is strangely absent, and one would have expected that it should be as common as the Rh genotype -D-/-D- in this particular group of persons.

Possible explanations are that (a) the genotype cDE/cDE might have been found if further studies had been made of each family's siblings, but no parents were found where the mating was CDe/cDE x cDE/cde etcetera, that is, where each parent's cells were at least heterozygous for

the cDE chromosome.

(b) In tracing the additional one hundred and forty-five people in the related group, however, it is unlikely that any persons of probable Rh genotype cDE/cDE were missed, because a special effort was made to find red cell samples of this particular nature--these being required in testing the sera of those -D-/-D- cases possessing immune Rh antibodies as a result of repeated pregnancies, or of earlier blood transfusions (Buchanan, 1956[2]). As it was, only two unrelated cDE/cDE samples could be used in such tests, these being obtained from White Canadians during extended Rh antenatal testing.

(c) The conclusion was reached that this genotype cDE/cDE did not exist in any of the known living relatives' cells.

4. The Rh genotype cDE/-D-

Of those possessing the -D- chromosome, the Rh genotypes that have been shown to occur beyond reasonable doubt are CDe/-D-, cde/-D-, and -D-/-D-. Although the genotype cDe/-D- appeared to exist[5], the evidence that has accumulated to date would indicate that the chromosome cDe has been confused with the more commonly occurring chromosome cde (Chapter 6, pages 78 to 84).

Until the present time, there was no direct evidence that the Rh genotype cDE/-D- existed in any of these

person's cells. By chance, another child was born recently to V-22, a young Metisse of Rh genotype -D-/-D- whose previous case history had been reported by this author in an earlier publication[2].

Briefly, this newborn infant's cord blood erythrocytes had the most probable Rh genotype cDE/-D-, being c+, D+, E+, but C- and e-. The cells were agglutinated with five out of eight random incomplete anti-D sera in saline. The direct Coombs test was positive, and the child showed other signs of erythroblastosis foetalis (June 1956).

This case has only recently come to light, and further study is necessary. When last tested in 1955, the mother's serum contained a weak incomplete anti-e^2, and both then and now all normal group O, Rh positive and Rh negative test cells are agglutinated by the maternal serum in saline and particularly in albumin, suggesting the presence of incomplete Rh antibodies.

On this occasion, at least, the father is of most probable Rh genotype CDe/cDE. Since this particular Metisse is not a reliable witness, further detailed investigation is necessary. However, in view of the clinical and other findings, the child recieved an exchange transfusion of group O, -D-/-D- donor blood from a relative, and it is presently making satisfactory progress.

Apart from the foregoing case, which should be of genetical value when all investigations are completed, the Rh genotype cDE/-D- is surprisingly rare. No known example of this particular Rh genotype has been recorded, to the best of this author's knowledge.

Chance alone may not account for its rarity. It is possible that in earlier generations, such as in generation II, or III (Figure I), that siblings of Rh genotype cDE/-D- did not survive. It may be assumed that to a mother of Rh genotype CDe/-D-, or cde/-D-, or -D-/-D- a mating with a partner heterozygous or homozygous for the Rh chromosome cDE was particularly lethal to the infant where the father's cDE chromosome descended to the child.

In such matings, the foetal antigens c and/or E might have sensitized the mother, with the resulting production of immune anti-c and/or anti-E. For example, where the female was of Rh genotype CDe/-D- and the male partner of Rh genotype CDe/cDE, children of Rh genotypes CDe/-D- and CDe/CDe would survive, but siblings of Rh genotypes cDE/-D- or CDe/cDE might succumb to the combined effects of haemolytic disease of the newborn associated with the immune antibodies anti-c plus anti-E.

In this hypothesis there would be a reasonable chance that both the parents were of group O, and that the combined effects of the antigens c and E were uninhibited.

In such instances, the two co-existing immune antibodies could be compared in virtual effect to immune anti-D in the case of White Rh negative females bearing a severely affected Rh positive infant.

Under these circumstances, the paternal and foetal c and E may have been at least as dangerously antigenic as that of D in the familiar case of the White mother of Rh genotype cde/cde who becomes sensitized to the D antigen of her Rh positive (DD or Dd) husband during repeated pregnancies.

In the cases under discussion, mothers of Rh genotypes CDe/-D- or -D-/-D- might become readily sensitized to the c and E antigens derived from the paternal and foetal cDE. Those females of Rh genotype cde/-D-, on the other hand, would not be exposed to more than the potential stimulus of the possible E antigen alone in the mating cde/-D- x cDE/cde, or cde/-D- x cDE/cDE.

The Rh genetical patterns in the existing families indicate that females of genotype cde/-D- have produced normal siblings of genotypes cde/cde, CDe/cde and cde/-D- where the father was most probably of Rh genotype CDe/cde. That is, the paternal C antigen, per se, appears to have had no discernible effect in stimulating anti-C where the mother is of genotype cde/-D-.

It is of interest, also, that a female of the rare Rh

genotype -D-/-D- may have several children of most probable Rh genotype CDe/-D-, before apparently becoming sensitized to the e and C antigens, but again it is possible that the combined antigens C and e are relatively less antigenic than the combined antigens c and E.

If the above interpretation is valid, a sibling of Rh genotype cDE/-D- has a better chance of survival if it happens to be born in one of the earlier of the CDe/-D- or -D-/-D- mother's pregnancies before sensitization to the c and E foetal and paternal antigens develops to a dangerous degree.

It is appreciated that chance alone is an important factor in studies such as this. Only a few members of generation III are still alive, in the group III-1 to III-15. That is, in those instances where consanguinity, and the above possible effects of Rh antigenic stimuli are likely to be encountered in their offspring of generation IV.

Group O Donors of Rh Genotype -D-/-D-

In several instances, group O donors of this rare genotype have been of value, principally in replacement transfusions of newborn children suffering from haemolytic disease caused by unusual combinations of immune Rh antibodies.

Locally, the author has obtained group O, -D-/-D-

donor blood on three occasions for related cases, during 1954, 1955, and 1956. In the first two instances, the Metisse propositus V-17 and her sister V-22 were transfused with such relatives' bloods following labour and associated postpartum haemorrhage. Very recently, the newborn child, VI-19, was given a successful replacement transfusion of this specificity, this case being still under study.

Donor bloods of this genotype have also been sent to both California and Toronto hospitals during 1955 and 1956, the group O, -D-/-D- donor's cells being first matched (without any difficulty) with the corresponding maternal serum. Crossmatching was performed in albumin, and also by the indirect Coombs method.

Since these last mentioned case histories may be published independently by the American and Canadian physicians concerned, it would be incorrect to report all of the known details, beyond indicating that the mother and father of the Californian infant are White people of Rh genotype $C^{W}De/-D-$ and cde/cde respectively. The exchange transfusions were successful in both this instance, and in the more recent one in Toronto where the mother was again of White (European) origin.

General Conclusions, and Summary

In an additional group of one hundred and forty-five Metis, consanguinity was apparent in that a large proportion possessed the Rh chromosome cde, and no less than eleven were of the rare Rh genotypes cde/-D-, CDe/-D-, or -D-/-D- (Table 6, page 73).

It is postulated that the Rh chromosome cde is almost certainly of European origin, and that very probably the Rh chromosome -D-, or D-- was also introduced by a White ancestor shortly before or soon after 1800 when the first few voyageurs reached this area.

Assuming that one White ancestor possessed the rare Rh chromosome -D-, and others cde, subsequent intensely consanguineous matings largely replaced the Rh chromosomes common in North American Indians--namely CDe and cDE.

There is no evidence that the chromosome -D- is of very recent origin in these families, since it has clearly descended, as such, through four living generations III, IV, V, and VI, the oldest living possessor having been born in 1869, and being of the unusual Rh genotype cde/-D-.

Several of their forebears of generation II were known to have been descended from common ancestors in generation I (I-1, and I-4) and all of these bore the surnames of French-Canadian voyageurs.

It is generally accepted that the chromosomes CDe and cDE are common in pure North American Indian tribes, and there was every indication that both are frequently occurring Rh constituents of other non-related Metis. For example, in a small separate group of one hundred and eighty-five of mixed European and Indian ancestry, CDe had a percentage frequency of 44.1, cDE 25.7, cde being almost as common with a frequency of 24.4 per cent.

As the unusual genotype -D-/-D- has been found, it is very strange that none of the related group are of Rh genotype cDE/cDE--surely an Rh genotype that might be expected in a population such as this.

Similarly, the Rh genotypes CDe/-D- and cde/-D- have been found, but only in one very recent case (a newborn infant suffering from haemolytic disease of the newborn) was the Rh genotype cDE/-D- probable.

The question thus arises, is it purely chance or intense consanguinity that has prevented the finding of at least one sample of Rh genotype cDE/cDE, and only one case of possible Rh genotype cDE/-D-?

Twenty-two of probable Rh genotype CDe/cDE and twelve persons of most probable Rh genotype cDE/cde were discovered, and yet another of the unusual Rh genotype CDE/cDE, (page 73). That is, twenty-four per cent of the normal heterozygotes possessed the cDE chromosome.

It is surprising that so many Metis of Rh genotypes CDe/-D-, cde/-D-, and -D-/-D- have survived.

No doubt many siblings, and especially those of Rh genotypes cDE/-D- did not survive as a result of erythroblastosis foetalis following maternal sensitization to the foetal and paternal c and E antigens.

For example, in the mating of a female of Rh genotype CDe/-D- with a male of genotype cDE/cde, the siblings might have been of the possible genotypes CDe/cDE, CDe/cde, cde/-D-, and cDE/-D-. In such a mating, there is the possibility of sensitization to the antigens c and E, in particular.

It would largely depend upon the number of pregnancies, the rapidity of sensitization, and on the order in which the children of different genetical combinations were born; but on an average, it is possible that more siblings of Rh genotype cde/-D- would survive than those of cDE/-D- and CDe/cDE. The latter two would be exposed to the combined danger of anti-c plus anti-E, rather than to anti-c alone, as in the instance of the infants of genotype cde/-D-, or CDe/cde.

Within the limits of four living generations, there is nothing to suggest that the C or c and E or e genes become suddenly "deleted", thus giving rise to the rare chromosome -D-, or D--. Similarly, there is no direct

evidence of gene suppression, or mutation.

As far as can be ascertained, the Rh chromosome D-- has descended, as such, through six generations in the course of over one and a half centuries.

Those possessing the Rh chromosome D-- (assuming that the order of the genes is DCE) are free from any other apparent congenital abnormality, and they are in reasonably good health--points favouring some other explanation than deletion of C or c and E or e as the genetical cause of this rarity.

The D gene and the corresponding D antigen are possibly the basic elements of the Rh system, and in the homozygous expression D--/D-- the D gene alone appears to be present.

It will be both interesting and valuable to follow up this large family group in future studies.

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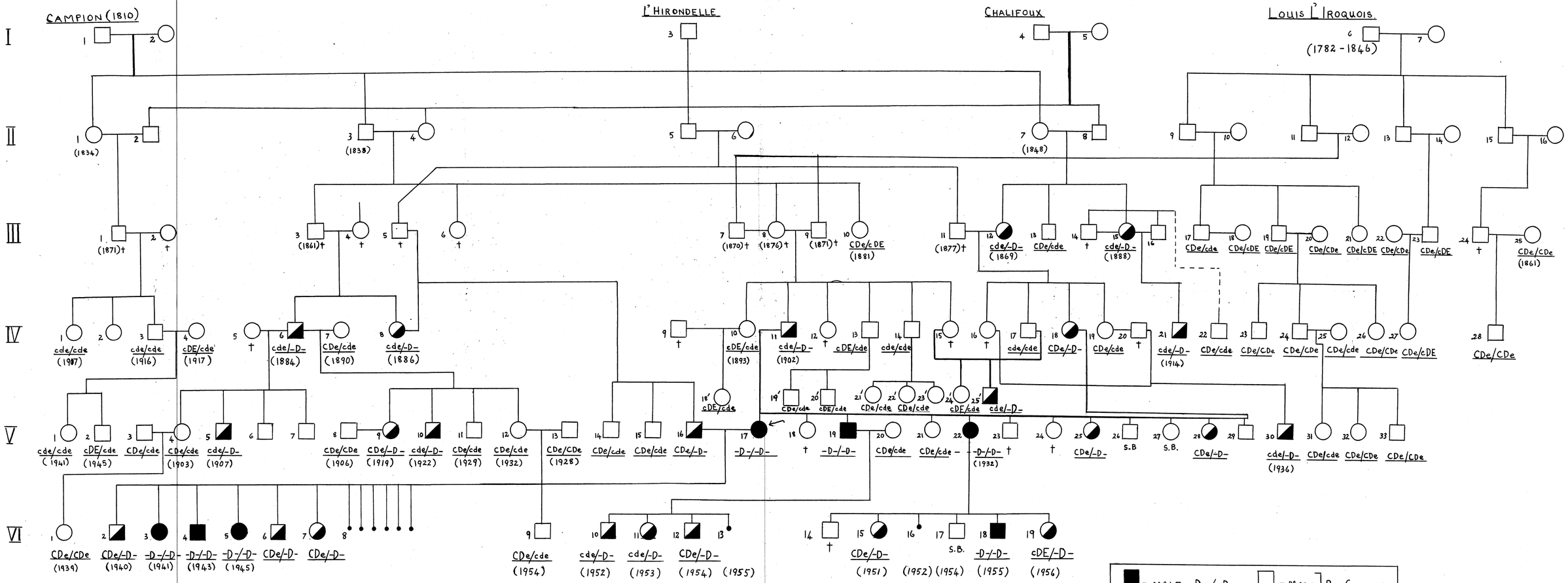
FIGURE I: RELATED FAMILIES' PEDIGREE.



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