RHESUS BLOOD GROUPS IN FAMILIAL HODGKIN'S DISEASE

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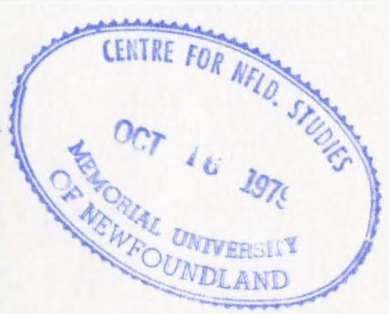
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RHESUS BLOOD GROUPS IN FAMILIAL HODGKIN'S DISEASE

by

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To my son

IAN HEATH NEWTON

ABSTRACT

The aetiology of Hodgkin's disease is unknown, some studies suggest an environmental and others a genetical basis, as the underlying cause: both are almost certainly involved. As an isolated population which included a large kindred containing seven cases of Hodgkin's disease and fourteen cases of "related conditions" was available, the opportunity was taken to study the Rhesus blood group antigens in patients, relatives and others. Previous studies of random Hodgkin's disease patients had demonstrated an association with Rh (D) negative status, but there was no information concerning Rh haplotypes.

The objectives were to study as many members of the population as possible, and using pedigree data to assign actual Rh geneotypes where possible; to use this data to compare haplotype frequencies of this population with that of the United Kingdom; and to compare frequencies for the patients and relatives of three disease groups, (Hodgkin's disease, immunodeficiencies and "embryonic tumours"), with two control groups in this population in order to seek associations of Rh haplotypes with disease.

All disease groups differed significantly from controls, the Hodgkin's disease and immunodeficiency groups were marked by a complete absence of R_o and r", replaced by r. The embryonic deficiency group had an excess of r" and a corresponding deficiency of R_2.

The whole population deviated significantly from the expected (U.K.) frequencies for all haplotypes but r". R_1 and R_2 were reduced, r', r^y and R_z were absent, while R_o and r were elevated. These differences have been ascribed to founder effects.

In conclusion, the association of Rh (D) negative status with Hodgkin's disease was strongly supported by this study, as there was an excess of Rh negatives in the population, this excess being confined to the kindred. Both R_o and r" in this family appeared to be protective against Hodgkin's disease and immunodeficiency, but r" seemed to be involved in susceptibility to "embryonic tumours".

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INTRODUCTION

In 1973 a patient suffering from Common Variable Immunodeficiency, (C.V.I.D.; Primary acquired immunodeficiency) was admitted to the General Hospital, St. John's, Newfoundland, for investigation. During this admission the information that there had been many cases of cancer in his extended family was revealed. The types and numbers of cases being; Hodgkin's disease 7, Lymphosarcoma 4, Leukaemia 3 and 1 case of each of the following, Retinoblastoma, Rhabdomyosarcoma and Neuroblastoma, grouped together as "Embryonic tumours". In addition to these cases of neoplastic disease and counting the above patient, there were 3 cases of C.V.I.D. and 1 case of benign thymoma, (Buehler et. al, 1974).

These findings led to a large multidisciplinary investigation of the isolated population of which this family forms a major fraction. The present study is part of this investigation.

A cluster of cases of a specific disease or group of related diseases occuring in a limited geographical area suggests that an environmental agent plays a major aetiological role. Environmental agents active in such situations are infective organisms, physical agents such a radiation and chemical agents for example mercury or asbestos. In the case of pathogenic organisms, limitation to a defined area suggests two possible explanations, first that the organism is transmitted by a vector or possibly a human carrier, or second that a septic focus exists, for example a water supply contaminated by sewage.

Although a circumscribed occurance most immediately suggests an environmental basis the alternative explanation of a genetical predisposition of the population should not be overlooked. This is

even more important where there is reason to suspect that the population is relatively isolated, whether by geography or social custom and there is therefore a higher than usual co-efficient of inbreeding present, as is the case for the studied population.

Although diseases are often referred to as genetical or environmental there is basically no sharp distinction between the two. In the vast majority of conditions both will play a part, often in varying degrees making it difficult to decide which has the greater influence. Bubonic plague of the Middle ages was undoubtedly a plague because of the poor sanitation and personal hygiene. However the decline of this disease may be due not only to improved environmental conditions but also to a change in population structure, due to selection of more resistant individuals as the breeding population.

A relatively recent and compelling example of this is the effect of myxomatosis on the rabbit population of Europe. This disease was spread deliberately in a susceptible population by man, the disease gave an extremely high death rate in the population and the number of rabbits was markedly reduced. Since then the population has recovered, the disease has a much lower rate of infection and the death rate in infected animals is low; these changes are presumably due to selection of resistant individuals as the breeding stock. An interesting behavioural change in the population has also occurred, in that prior to the disease rabbits tended to live underground in burrows whereas the present population spends much more time above ground and tend to have less contact with their fellows.

In occupational diseases environmental factors are of paramount importance, for example, mesoepithelioma caused by inhalation

of asbetos fibres of a very limited size range. However even here genetical factors almost certainly play a role, not all workers exposed to the correctly sized particles develop the condition. Although the incidence is somewhat dose dependant the correlation is not absolute and rare cases occur in which no exposure to asbestos can be demonstrated.

In Medelian diseases, for example, Tay-Sachs where the genetical aspects are of overwhelming importance, it is still not possible to rule out some environmental influence. It is possible that a suitable diet may lead to a relatively normal life and life expectancy as is the case for those individuals having phenylketonuria, and in these the diet needs to be maintained for a relatively short period of time.

Most conditions fall somewhere between these two extremes as is almost certainly the case for the population studied herein. It may be possible to assign a relative risk for each factor and studies are being pursued towards this end.

The study of genetical factors may be more easily accomplished since there are so many markers available and even in an inbred population these may be informative. The Rhesus blood group system, having eight haplotypes, has a high power of discrimination and would be an excellent marker should it be linked to a susceptibility gene for Hodgkin's disease or other malignancies.

The Rhesus system has been implicated in Hodgkin's disease by earlier studies on randomly collected patients. In five separate studies, performed in several different countries (Table 1) there has been a slight but consistent excess of Rhesus (D) negative

persons compared to the control groups. When these studies are analyzed together there is a significant excess of D negatives.

In our studies an early observation, prior to examination of the pedigrees, was of an increase in the numbers of D negatives compared with the expected for a Caucasian population, approximately two fold. It seemed probable that by examination of the haplotypes derived from the pedigrees, we could analyse this association of Hodgkin's disease with D negative at a higher level. Not all patients were alive at the time of the study and we have therefore relied upon studies of their first, second and third degree relatives in the greater part.

Table IA. Comparisons of random Hodgkin's disease patients with Normal controls for D antigen status in five studies.

Study	D+	D-	D+/D-	X_1^2	
Májský, 1964; Kout, 1959.	70	15	0.8732	0.2101	P
Czechoslovakia.	962	180			C
Does et. al., 1972	39	7	0.5865	0.6402	P
Netherlands	38	4			C
Cichecka, 1965	125	35	0.7843	1.6000	P
Poland.	17695	3886			C
Kay & Shorter, 1956,	53	20	0.5513	5.0983	P
Discombe & Meyer, 1952.	8278	1722			C
United Kingdom.					
Walther et. al., 1956	8	3	0.5363	0.8403	P
Kopeć, 1970.	1626	327			C
United Kingdom.					

X_4^2 for homogeneity of areas = 1.9268

X_1^2 for comparisons = 6.4621

Reproduced by permission of Dr. A.E. Mourant, Blood Groups & Diseases, 1978 (In press).

P = Patients C = Controls.

$$D+/D- = \text{Relative incidence} = \frac{D+/D- \ (P)}{D+/D- \ (C)}$$

Combined relative incidence = 0.7890

Table 1B. Comparisons of random Hodgkin's disease patients with Normal controls for the ABO A & O groups in thirteen studies.

Study	O	A	A/O	X_1^2	
Májský, 1964; Kout 1959. Czechoslovakia.	29	39	0.9356	0.0682	P
	359	516			C
Adamo, 1961; Boero & Coraddu, 1965. Italy	3	6	3.5346	3.1797	P
	2043	1156			C
Ninni & Bedarida, 1959. Italy.	14	16	1.1225	0.0947	P
	276	281			C
Visconti et. al., 1961. Italy	44	54	1.1798	0.06548	P
	3974	4143			C
Benda & Menghini, 1957. Italy.	51	31	0.6148	4.5061	P
	3163	3127			C
Does et. al., 1972 Netherlands.	25	19	1.0133	0.0008	P
	20	15			C
Cichecka, 1965. Poland.	54	58	0.8677	0.5606	P
	12457	15419			C
Krolikowska & Zupanska, 1964; Charzewski et. al., 1956. Poland.	88	86	0.8988	0.4865	P
	4719	5131			C
Kay & Shorter, 1956; Discombe, 1954. United Kingdom.	35	39	1.2122	0.6774	P
	4556	4188			C
Walther et. al., 1956. United Kingdom.	3	8	2.7892	2.2861	P
	1070	1023			C
Harris et. al., 1972. United Kingdom.	25	17	0.7695	0.4627	P
	43	38			C
Bubnov & Kirdan, 1972. Union of Soviet Soc. Republics.	90	73	0.7758	2.1662	P
	395	413			C
Levitan et. al., 1959. United States of America.	224	174	0.8488	2.5393	P
	5531	5062			C

X_{12}^2 for homogeneity of areas = 14.3602

X_1^2 for comparisons = 3.3228

Modified from Blood Groups & Diseases, 1978, Mourant et. al. (In press).

P = Patients C = Controls

$$O/A = \text{Relative incidence} = \frac{O/A\ (P)}{O/A\ (C)}$$

Combined relative incidence = 0.8625

OBJECTIVES OF THIS WORK

The objectives were, first to type individuals in the study population for the antigens D, C, c, E & e controlled by the Rhesus blood group locus, second to use this data in conjunction with pedigrees to obtain the actual genotype of each tested person where possible. Third, to use this data to derive frequencies for phenotypes, haplotypes and individual antigens in this population and compare these with frequencies for other populations and finally to examine the Rhesus data for the Hodgkin's disease patients and their families, the immunodeficiency patients and their families and the embryonic tumour patients and their families in order to seek associations between Rhesus haplotypes and disease.

The WORKING HYPOTHESIS derived from observations on page 3 and in Table 1 states that "The Rhesus locus or a linked locus may control in part the susceptibility of an individual to development of Hodgkin's disease or may contribute in other ways to the epidemiology of the disease".

LITERATURE REVIEW

Hodgkin's disease.

Associations between genetic markers and Hodgkin's disease.

Common variable immunodeficiency.

Blood group systems and associations with disease.

HODGKIN'S DISEASE

The recognition and classification of neoplasms of lymphoid tissues commenced in 1832 when Thomas Hodgkin first described the condition which was later to bear his name. The process may not yet be complete, since as recently as 1958 Dennis Burkitt described a "new lymphoma" which now bears his name. Leukaemia was described independantly in 1845 by Craigie & Bennett and by Virchow, the latter obviously having a clearer concept of the disease, also differentiating two forms of the disease, leukaemic and aleukaemic.

The next advance came much later when Dreschfeld (1892) and Kundrat (1893) distinguished from aleukaemic leukaemia another group of cases. In the latter the malignant cells remained confined to the lymphatic system for relatively long periods of time. These were termed lymphosarcoma. The follicular (nodular) lymphomas were the next to be defined by Brill, Bach & Rosenthal (1925); two cases were described and the exquisite sensitivity of the tumour cells to radiation therapy was noted. Oberling (1928) separated reticuloendothelial sarcoma from myeloma and Roulet (1930) separated a further group, the reticulum cell sarcomas. The most recent entrant to the lists is Burkitt's lymphoma, (Burkitt, 1958).

Hodgkin described mainly the gross anatomical findings of the disease whilst Langhans (1872) and Greenfield (1878) were among the first to give a histopathological description. They both recognised the presence of large multinucleated cells and Greenfield made drawings of these as seen by low power microscopy. Sternberg (1898) and Reed (1902) are however credited with making the first definite descriptions of the pathology, the latter publishing excellent

sketches of the large multinucleate cells now termed Sternberg-Reed cells.

Wilks (1865) recognised the anaemia and cachexia that accompany Hodgkin's disease and in at least one case noted an intermittant fever, but it was Pel (1887) who described the peculiar cyclical nature of the pyrexia which is occasionally seen in this disease.

Reed noted that in five cases tuberculin was administered without eliciting a response, even though one case had concurrent active tuberculosis.

Pusey (1902) was the first to use X-ray therapy in this condition and in the early 1940's the nitrogen mustard derivatives became available for chemotherapy to be followed in 1948 by the antimetabolites which were introduced by Farber and colleagues.

Aeliology & Epidemiology of Hodgkin's Disease

Due to the lack of an acceptable animal model for Hodgkin's disease experimental work on the aetiology has been severely handicapped. Although a morphologically similar or identical tumour occurs in dogs the incidence is so low and sporadic as to render them almost valueless (Moulton & Bostick, 1958; Squires, 1969).

The age specific incidence curves for Hodgkin's disease are in most populations, bimodal. The two known exceptions being Japan and the southern United States of America (MacMahon, 1966). MacMahon argues that Hodgkin's disease may not be a single entity but probably two or more having distinct aetiologies.

The disease is generally more prevalent in males, but the sex ratio is not the same for all age groups. In the 15 to 34 year old bracket the male to female ratio is low, being 0.91 - 1.20, but

increases sharply in the 35 - 49 year old bracket and reaches its highest on the 50+ age group with values of 1.44 - 2.35. In children under 10 years of age 85% of the cases are male, this proportion falling in the 11 - 19 year age group to about 60%. After age 11 the incidence rate increases and the increase is greater for females, thereby decreasing the male/female ratio. Miller (1966), concludes that the change in incidence and sex ratios occurs at about age 9 and he suggests a possible association between the disease and the prepubertal involution of lymphatic tissue.

Cases of more than one case of Hodgkin's disease occuring in a family have been reported (DeVore & Doan, 1957: Razis, Diamond & Craver, 1959). Most of these reports involve siblings with the times of onset for the disease being often relatively close together. The mean difference in onset for siblings being 2.6 years while the mean age difference between affected siblings was 7.9 years. In seven of twelve cases in which a parent and child were affected the onset was relatively close in time. There have been three reports of the occurance of the disease in husband and wife, in two of these cases the onset for each member of the pair was close in time (Mazar & Strauss, 1951; Brennan, 1956 and DeVore and Doan, 1957). Priesel & Winkelbauer (1926) reported an instance of apparently congenital Hodgkin's disease in a girl of four months old in whom lymphadenopathy was present from birth, her mother had been diagnosed in the last month of pregnancy by cervical node biopsy. These data suggests the involvement of an environmental agent in the aetiology of the disease.

There appears to be an association between prior infectious mononucleosis and subsequent development of Hodgkin's disease. Lukes et.

al. (1969) report that cells indistinguishable from Sternberg-Reed cells appear in the lymph nodes of individuals suffering from infectious mononucleosis, and have suggested that "infectious mononucleosis on rare occasions may not be a self limiting lymphoid proliferative condition, but the initial infectious episode preceeding neoplastic transformation".

Immunological Defects

Mention has been made of the lack of delayed hypersensitivity to tuberculin, but anergy is not confined to this material. Trichophyton gypseum, Candida albicans and Mumps skin test antigens have also failed to produce delayed hypersensitivity reactions in the majority of patients. Similar results have been obtained when using chemical allergens, such as 2,4-dinitrochlorobenzene, as the test reagents. Rejection of homografts is likewise impaired in most patients, Kelly et. al. (1958) applied full thickness skin grafts to fifteen patients, only in three cases was the time of rejection normal while in two cases it appeared that the graft had taken permanently.

Except in the terminal stages of the disease the antibody response appears to be normal, at least for antigens previously experienced. However, the question of whether or not primary antibody responses are normal is controversial. The investigations of Aisenberg (1964 & 1966) in which untreated patients were tested, suggest that here too the responses are normal except in the far advanced cases.

Immunodeficiencies, whether hypogammaglobulinaemias, cell mediated immune deficiencies or both, and whether natural or the result of immunosuppresive therapy appear to considerably increase the risk of development of neoplasms, mainly of the lymphoreticular system.

Kersey et. al. (1973) reported that data from the Immunodeficiency-Cancer registry demonstrated the occurence of 151 tumours in 145 immunodeficient patients. The risk of a tumour developing in any given patient being 2 to 10% depending upon the type of immunodeficiency. Of the tumours reported 75% were either lymphoreticular neoplasms or leukaemias. This is consistent with the hypothesis that individuals with a primary immunodeficiency have intrinsic abnormalities of the lymphoid tissue which predisposes them to an increased frequency of transformed cells and/or an inability to eliminate transformed cells. In patients suffering from C.V.I.D. the incidence of neoplastic disease is approximately 8% with a peak in the age group of 51 to 60 years, although the tumours occur at all ages from 0 to 70+. Of 41 cases involving C.V.I.D. patients 26 had either lyphoreticular tumours or leukaemias, approximately 63%. Penn (1974) confirmed this on a smaller series of patients and extended the findings to include those individuals undergoing immunpsuppressive therapy for a variety of reasons, mainly for organ transplants, here too the incidence of neoplastic disease was markedly elevated compared to the population as a whole, again with lyphoreticular tumours and leukaemias forming a large proportion of the tumours present.

Cook & Shepherd (1977) interviewed 25 Rhesus non-responder and 21 Rhesus responder male blood donors concerning their medical and family histories. In 267 relatives of the non-responders there were 17 cases of malignant disease (13 confirmed from case notes, death certificates or both), while in 297 relatives of the responders there were only 8 cases of neoplasia (6 confirmed). Rhesus non-responders are Rhesus (D) negative individuals who appear unable to respond to the D

antigen by producing anti-D, approximately 30% of D negative persons are non-responders (Mollison, 1972), this inability to respond to what is a powerful immunogen in the responder group may be regarded as a subtle immunodeficiency.

ASSOCIATION OF GENETIC MARKERS WITH HODGKIN'S DISEASE

Amiel (1967) reported that HLA antigen frequencies in Hodgkin's disease patients were abnormal, he demonstrated an increase in the frequency of the 4c antigen in this group, the 4c antigen has since been subdivided into the cross-reacting HLA antigens B5, B18 & B35. These findings have been confirmed and extended by other workers (Zervas et. al., 1970; Thorsby et. al., 1971 and Forbes & Morris, 1972). A different deviation was independantly reported by Kissmeyer-Nielsen et. al. (1971) and Falk & Osoba (1971) when they demonstrated an increase in the frequency of the HLA antigens A1 & B8 in Hodgkin's disease patients. Svejgaard et. al. (1975) have published an analysis of the data available for 1500 patients and found a significant deviation for each of these antigens in the Hodgkin's disease group, B18 shows the highest association and B8 the lowest, in this group of antigens.

Falk & Osoba and Kissmeyer-Nielsen have pointed out that the duration of survival following onset of the disease may play a role in the observed antigen frequencies of the Hodgkin's disease group. To fully evaluate the part played by HLA antigens in relation to susceptibility to Hodgkin's disease the patients should be studied as soon after onset as possible. By, then following each patient it is possible to determine the relationship between the various antigens and long term survival. The HLA antigens A19 & B5 are associated with a poor prognosis, but B8 seems to be associated with a high survival rate at the five year point. In patients who had recent onset of the disease there was a decreased frequency of the antigens HLA A3 & A11 which suggests that these are associated in some manner with an increased

an environmental basis the alternative explanation

predisposition of the population should not be overlooked. This is

resistance to this condition (Falk & Osoba, 1974).

The data on association of both the Rhesus antigens and the ABO antigens with Hodgkin's disease has been reviewed by Mourant (1978), the association of the Rhesus antigen d with Hodgkin's disease is stronger than the corresponding association of the ABO antigen O and both are weaker than any of the associations between Hodgkin's disease and the above HLA antigens. In the Hodgkin's disease patients both O and d demonstrate an increased frequency when compared with control groups.

COMMON VARIABLE IMMUNODEFICIENCY (C.V.I.D.)

This group of immunodeficiencies is presumably heterogenous, with a fairly wide range of age of onset, differing considerably in the component or components of the immune system which are affected and in the degree to which these are involved.

The aetiology is obscure, low immunoglobulin levels are common, with one or more classes of immunoglobulin involved. The condition usually occurs in adults in the absence of any obvious cause. In approximately 10% of cases there is an association with thymoma and some pedigrees suggest a genetic basis. Lymphopenia may be present while relatives may show auto-immune manifestations such as lupus erythromatosus, haemolytic anaemia, thrombocytopenic purpura, pernicious anaemia and positive serological tests for rheumatoid arthritis amongst others (Rosen, 1971).

THE RHESUS BLOOD GROUP SYSTEM

Levine & Stetson (1939) described a haemolytic blood transfusion reaction in a post-partum woman recently delivered of a still-born child. The blood transfused had been donated by the ABO compatible husband. The patient's serum contained a blood group antibody reacting with her husband's red cells and those of 80 of 104 ABO compatible random donors. The antigen detected was shown to be genetically independant of the then known blood group antigens, A, B, O, M, N & P.

In a brilliant synthesis the authors suggested that the woman's antibody reulted from immunisation by the paternal antigen which had been carried by the stillborn child and that this antibody caused the haemolytic transfusion reaction in the maternal circulation. They didnot propose a name for the antigen. Later it was postulated that this antibody by crossing the placenta and destroying the infant's red cells had caused the infant's death. This postulated aetiology of Haemolytic Disease of the Newborn (H.D.N.) has been confirmed on innumerable occassions for many blood group systems.

A year later Landsteiner & Wiener (1940) immunised rabbits and guinea pigs with the red cells of the monkey, Macacus rhesus, to find that antibodies produced reacted not only with the monkey but with human red cells also. The erythrocytes of approximately 85% of random Caucasian New York donors were agglutinated by these sera. These individuals were termed Rhesus or Rh positive while the 15% whose cells were not agglutinated were termed Rhesus negative. This remains a basic and very important distinction in this system.

It was then shown that the specificity of these sera was apparently the same as that present in the case of Levine & Stetson. With the passage of time discrepancies between the reactions given by the animal and human sera came to light. All newborn infants irrespective of their Rh group as defined by human antisera gave positive reactions with the animal antisera, though those who were negative with the human material gave weaker reactions with the animal reagents than did those who were positive (Fisk & Foord, 1942).

Murray & Clark (1952) then made the surprising observation that injection of heat extracts of human Rh negative cells into guinea-pigs led to production of an apparent anti-Rh in a large proportion of these animals. This finding was confirmed and extended by Levine et. al. (1961) who reported that this antibody could not be blocked by prior exposure of the erythrocytes to the human IgG antibody although human IgM anti-Rh could be blocked in this way.

Human IgM antibodies are frequently termed complete and the IgG antibodies termed incomplete. This refers to the ability of these antibodies to give agglutination with positive cells suspended in a saline medium. Under these conditions both will bind to the antigen, but only IgM is capable of the second stage of the reaction, agglutination.

Race & Sanger (1958) investigated the blood of two unrelated individuals, both were Rh or D positive as the antigen had been termed. Both had apparent anti-D in their serum which failed to react with their own red cells and with the red cells of the other, but did react with all other D positive samples tested. In retrospect it is obvious that these were the first examples of human anti-LW. LW was the name given, in honour of Landsteiner & Wiener, to the antigen detected by the animal

antisera when it became obvious that antigens detected by the animal and human "anti-D" were not the same.

In man both the Rh and LW systems are found, in the rhesus monkey only LW. It was however impossible to change the name of the human system at that time so the name LW was given to the system common to man and monkey.

In man the two systems are genetically independant, but they interact at the phenotypic level which suggests that there may be a common metabolic pathway, at least in part, leading to the formation of antigens of the two systems. Extremely rare individuals exist whose cells lack all representation of the Rh antigens, these are termed Rh_{null} and they also lack the common LW antigen. The converse does not apply, individuals exist who are LW negative but have the normal complement of Rh antigens. The present view of the interaction is that in man the Rh genes are mandatory in order to supply a precursor substance on which the LW gene can act, hence all Rh_{null} individuals must be LW negative. Those persons who have normal Rh antigens but who are LW negative are regarded as homozygous for the rare recessive allele lw.

In the vast majority of cases the Rh_{null} individual is LW/LW or rarely LW/lw. The situation must be more complex than the above suggests since the strength of the LW antigen is influenced by the D status of the individual, LW positive red cells from D negative persons frequently fail to agglutinate with anti-LW antisera though they do absorb the antibody. This may be due to a difference in the efficiency with which D positive and D negative red cells produce the required precursor substance. An alternative possible explanation is the

effect of one antigen on another, as is relatively common in the Rhesus system. C when in transposition to D frequently weakens the D antigen so that it types as a D^u antigen, hence the presence of d in double dose may weaken the LW antigen.

Since the LW negative individual, lw/lw is exceedingly rare and the heterozygote LW/lw cannot be distinguished from the homozygote LW/LW the study reported in this thesis has been confined to the Rhesus blood group system.

By 1941 it was obvious that the Rh system was not as simple as positive or negative, by 1943 the American workers had three and the British four different antisera all of which had strong associations with one another but giving different patterns of reactions. Fisher (1943) studied the results of testing red cells of given individuals with all four British antisera and noted that two were giving antithetical results. That is if one was negative with a given cell sample then the other was invariably positive. These two he named C & c, the other two did not give antithetical results to one another so he named these D & E and postulated that the antithetical antibodies would be found, that is anti-d and anti-e. Anti-e was later detected but to date no example of anti-d has been found; it is therefore generally accepted that the d gene is an amorph. If this is the case it provides yet a further explanation of the weaker reactions given by D negative LW positive cells with anti-LW.

Anti-D was the specificity of the antibody discovered by Levine & Stetson. The distinction between D positive and D negative individuals remains one of paramount importance in this system.

There is considerable controversy over the exact genetics of

the Rhesus system, one group of workers insist that there are three extremely tightly linked loci each of which has two major alleles, these being C & c, D & d and E & e (Race, 1944). The linkage must be extremely tight as there is only one commonly accepted example of a probable cross-over, in a Hutterite family the father's probable genotype is CDe/cde while the mother's genotype is cde/cde, there are seven children with the two possible non-recombinant genotypes, that is CDe/cde or cde/cde but there is one with the genotype Cde/cde, all other genetic markers studied fit with the parents and in view of the religous beliefs of the parents illegitimacy is unlikely (Steinberg 1965). The second group of workers are insistent that there is only one locus for the system with eight major alleles each of which produces many blood group factors (antigenic determinants) (Wiener, 1944). The difference is largely academic, the practical difference between the two hypotheses is minute. The three locus hypothesis has been used throughout this thesis since it is felt to be a little more understandable and the terminology more managable.

The two allels of the three loci occur in all possible combinations to give the eight possible haplotypes, a haplotype being a set of linked genes which tend to be passed down the generations unchanged. Fisher (1946) suggested that the rarer haplotypes had resulted from cross-overs involving the three most common haplotypes and that the extremely rare haplotypes had resulted from cross-overs involving one of the rarer haplotypes. In the Rhesus system not all haplotypes occur in all populations, cde, r, for example is practically absent in Chinese. Even where all do occur the frequencies may differ markedly from one population to another, as they do for Caucasians and

Negroes. The possible haplotypes are listed below, together with a shorthand notation and the approximate frequencies for Caucasian populations.

Table 2: Rhesus haplotypes, shorthand notation and frequencies.

Haplotype	Shorthand	Frequency
CDe	R_1	0.42
cde	r	0.39
cDE	R_2	0.14
cDe	R_o	0.03
cdE	r''	0.01
Cde	r'	0.01
CDE	R_z	0.00
CdE	r^y	0.00
		1.00

In the shorthand notation R denotes that D is present in the haplotype while r denotes it's absence. In a population these haplotypes occur in pairs, one inherited from each parent, there are therefore 36 possible combinations (genotypes) not all of which can be distinguished from one another. On testing with the five commonly available antisera, anti-D, anti-C, Anti-c, anti-E and anti-e, only 18 different reaction patterns are found, these are termed phenotypes. Each phenotype will contain from 1 to 6 possible genotypes. The number of recognisable phenotypes can be increased by the use of rarer antisera, for example anti-ce (anti-f), this antibody recognises a specificity produced by red cells in which the c and e genes are in cis position, that is on the same chromosome or part of the same haplotype. The phenotype D+, C+, c+, E+ and e+ contains the possible genotypes; CDe/cDE, CDe/cdE,

Cde/cDE, CDE/cde, CDE/cDe and CdE/cDe, use of anti-ce will differentiate the first three from the last three, the former being negative and the latter positive.

The other method of distinguishing between the different genotypes which form a phenotype is by family studies, but these too may not be informative. The greater the number of individuals tested and the more generations these fall in, the greater the chance of being able to deduce an actual genotype for any given individual.

In most laboratory situations where the five antisera have been used for Rhesus typing the most probable genotype is used to express the results. The most probable genotype is the most frequent genotype for the population to which the individual belongs which fits the phenotypic result. In Caucasians the phenotype D+, C+, c+, E-, e+ most often represents the genopyte CDe/cde, but CDe/cDe and CDe/cDe are both possible. In Negroes the genotypes CDe/cde and CDe/cDe have almost the same frequency so no most probable genotype can be given, in both races the genotype Cde/cDe is much rarer.

Table 3: Rhesus phenotypes, most probable and alternative genotypes.

Phenotype	Genotypes	
D C c E e	Most Probable	Alternative
+ + + - +	CDe/cde	CDe/cDe; Cde/cDe.
+ + - - +	CDe/CDe	CDe/Cde.
- - + - +	cde/cde	
+ + + + +	CDe/cDE	CDe/cdE; Cde/cDE; CDE/cde; CDE/cDe; CdE/cDe.
+ - + + +	cDe/cde	cDE/cDe; cdE/cDe.
+ - + - +	cDe/cde	cDe/cDe.
+ - + + -	cDE/cDE	cDE/cdE.

- - + + +	cdE/cde.	
- + + - +	Cde/cde.	
- + + + +	Cde/cdE	CdE/cde.
- - + + -	cdE/cdE.	
- + - - +	Cde/Cde.	
+ + - + +	CDe/CDE	CDE/Cde; CDe/CdE.
+ + + + -	cDE/CDE	CDE/cDE; cDE/CdE.
- + + + -	cdE/CdE.	
- + - + +	Cde/CdE	
+ + - + +	CDE/CDE	CDE/CdE
- + - + -	CdE/CdE.	

As can be seen, eight of the phenotypes represent only one genotype, in these cases the phenotype and actual genotype are synonymous.

This is the level of complexity at which the Rhesus system is commonly used, but the system is in fact much more complex and may indeed be the most complex genetical system yet found in man, the HLA system is a strong contender and the Kell blood group system reveals more complexities each year.

For each of the three loci of the Rhesus system there are other rarer alleles, such as C^W & C^X, E^u & e^s and D^u. For the C and E loci the additional alleles appear to be just that, for the D-d locus however the situation is not as straightforward. The D^u antigen is not recognized by a specific antibody, but reacts with some though not all anti-D sera. The antigen appears to exist in two forms termed high or low grade. The former reacts with many IgM anti-D sera and practically all IgG anti-D sera, while the latter reacts with a few IgM anti-D and most but not all IgG anti-D sera. Frequently potent IgG anti-D

serum and the anti-human globulin test (A.H.G.) must be used to detect the low-grade D^u.

The high-grade D^u is almost certainly not due to an alternative allele, in most cases family studies show it to be inherited as a normal D antigen in the majority of family members. In those persons in which it is expressed as a D^u antigen it appears to have been weakened by the effects of Rhesus genes on the partner chromosome, that is by Rhesus genes in the trans position. The simplest case is that of the phenotype D^u+, C+, c+, E-, e+ and family studies show that the actual genotype is cDe/Cde, the D of one haplotype being weakened by the C of the other. When this apparent cD^ue is paired with cde as may occur in parent or child of the carrier it is revealed as a normal cDe haplotype, with the D antigen fully expressed. Both C & E are capable of weakening D when in the trans position, but in the majority of cases do not reduce the strength of the D to D^u.

The low-grade D^u acts as a D^u antigen in all testable situations no matter what the partner chromosome bears, in terms of Rh haplotypes, and seems to be an alternative allele for the locus. Neither type of D^u antigen is detectable in the presence of a normal D antigen.

The next complexity is the existance of compound antigens, these are detected by specific antisera in each case and all share the property of being coded for only when the two required alleles are in cis position, that is in the same haplotype. The first reported was anti-ce, at first thought to be anti-d, later when it became obvious that it was not, the name was changed to anti-f and it may still be so referred to in some publications. Other examples of compound antigens exist in this system, including anti-Ce ans anti-cE.

The G antigen is usually included amongst the compound antigens in discussions, but is strictly not a compound antigen, it is produced by any Rhesus haplotype which has either C and/or D, but not by those with c and d, except for the rare Rhesus haplotype r^G. This r^G haplotype appears to be cde, but the c seems to be a varient producing the G antigen and some sort of C antigen. The term compound antigen may well be a misnomer, the rare cells CD-/CD- which lack all representation of any alleles at the E/e locus do in fact react with anti-Ce sera, although more weakly than do cells with more pedestrian and appropriate haplotypes. cD-/cD- cells also exist and these react with anti-ce in an analogous manner. It seems possible that the absence of E in the cis position alters C & c in some manner and that the altered C or c antigen can stimulate the production of an antibody specific for the compound antigen.

The type of cell mentioned above, CD-/CD- & cD-/cD- are termed deletion cells, in addition the cells C^wD-/C^wD- & -D-/-D- also occur. These cells lack all representation of antigens coded for by alleles at the locus or loci represented by -. The ultimate deletion cell also exists, ---/--- or Rh_{null}. The reason that all the above cells are homozygous is due to the fact that deletion haplotypes are so very rare. The probability of two non-related carriers mating is practically non-existent. All cases so far reported have been the results of consanguinous matings, usually first cousins. In first cousin matings any rare gene or haplotype present in the grandparents has a 1/4 chance of going to both cousins, so that the chance of a homozygous child being born to them is 1/16.

The Rh_{null} cases form a heterogenous group, in some cases the

--- haplotype is passed on in a simple Mendelian manner, but in others the Rh_{null} individual can be shown to possess normal Rhesus haplotypes since these are passed onto the offspring.. The explanation for the first type is that there is either an actual deletion of the Rhesus loci or possibly that there is a defective operator locus, while for the second group the Rh loci and the operator gene, if any, are normal but a very common gene needed to supply a precursor substance in the pathway leading to the Rh antigens is inoperative, possibly a mutant gene is present or an operator gene for this common gene is defective. Whichever is the case this very common gene is not linked to the Rhesus loci and it appears that one functional copy of this gene can supply sufficient precursor to allow the Rhesus genes to function. (Race & Sanger 1968). (Figures 1 & 2)

Figure 1.

Inheritance of Type I Rh_{null}

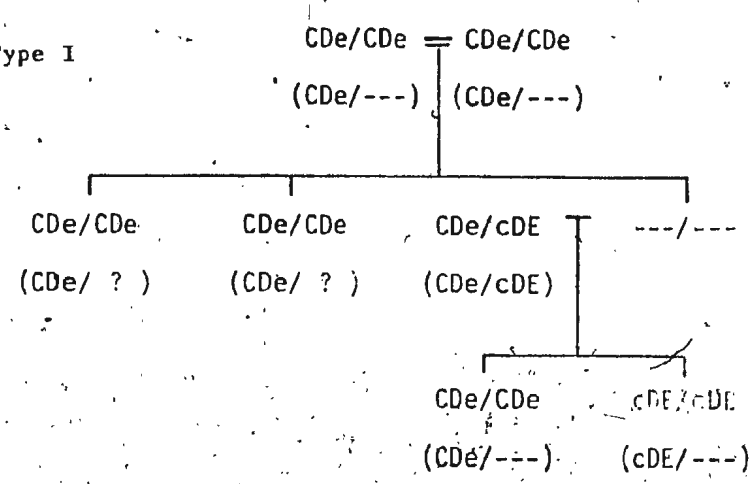
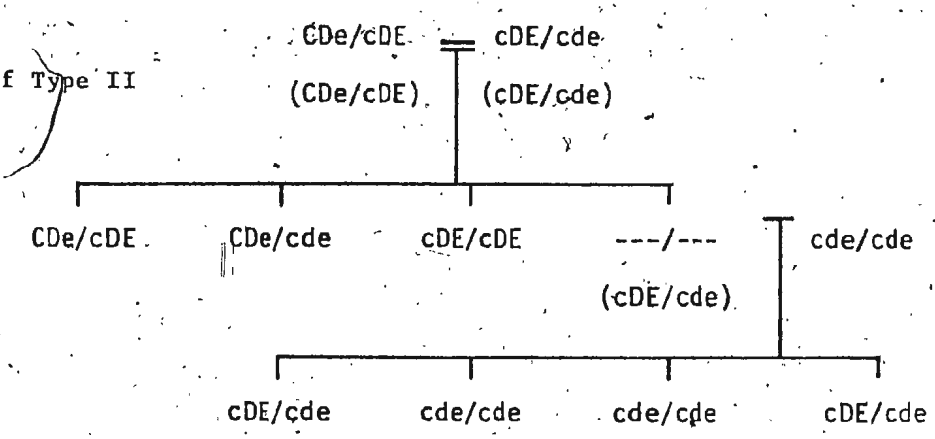


Figure 2.

Inheritance of Type II Rh_{null}



BLOOD GROUP SYSTEMS & ASSOCIATIONS WITH DISEASES

The associations of the blood group systems with various diseases may be classified as three types, 1) Genetic linkage, 2) Associations with unknown mechanisms and 3) Associations in which the blood group antigens are intimately involved in the disease mechanism.

Genetic Linkage

In this type of association the two factors travel together in individual families because of the linkage of the loci. In the population as a whole however there may be no association apparent due to the fact that in different families the disease gene will tend to travel with a different blood group allele due to occassional recombination between the two loci. All the diseases which show this type of association are Mendelian diseases due to a single locus in which the disease gene is an allele. This class includes the following associations; Rhesus and elliptocytosis (Morton, 1956; 1957), ABO and nail-patella syndrome (Renwick & Lawler, 1955), ABO and xeroderma pigmentosum (El-Hafnaw et. al., 1965) and Duffy and a congenital cataract (Renwick & Lawler, 1963). One interesting association is between Lutheran, Secretor and dystrophica myotonia (Dm); originally the linkage was believed to be between Lewis and Dm but this is a pseudolinkage due to the interaction of the Lewis, Secretor, Hh and ABO genes in a metabolic pathway (Renwick et. al. 1971, Harper et. al. 1972).

The number of the chromosome on which Lutheran, Secretor and Dm are located is not known at this time. The relative probabilities for the three possible orders of loci on the chromosome are: Lu - Se - Dm, 1.7; Dm - Lu - Se, 1.4 and Se - Dm - Lu, 1.0.

Associations With Unknown Mechanisms

These are not due to linkage and the associations are apparent when testing random sufferers of the disease. The first to be described was the association of group O individuals with duodenal ulcer, it was later shown that the association with this type of ulcer was with group O non-secretors (Clarke et. al., 1959), still later it was shown that these individuals have a greater tendency to haemorrhage and this affects ascertainment (Langman & Doll, 1965). This report was followed by others of similar associations. Clarke (1961) reported that the number of persons of group A was increased over expected in patients with stomach carcinoma and in those with pernicious anaemia. Cameron (1958) and Osborne & De George (1962) found an association between group A and salivary gland tumours; the latter workers also reported further associations of this blood group with primary ovarian and secondary ovarian tumours, the latter being more pronounced than the former. Glynn et. al. (1956) reported that there was an excess of non-secretors in patients with rheumatic heart disease. In none of these examples is the genetical or biochemical basis of the association known.

Associations In Which The Blood Group Antigens Are Intimately Involved In The Mechanism Of The Disease

This type is at present represented by only one example, which is the role of the Duffy blood group system in the aetiology of Plasmodium vivax (tertian) malaria. In Negro populations where P. vivax infection is endemic the Duffy phenotype Fy(a-b-) reaches high levels, but is very rare in other populations. It appears that both the Fy^a and Fy^b antigens permit or facilitate the entry of the parasite into the erythrocytes, this tentative conclusion being reached by

epidemiological studies.

In addition P. knowlesi can be used in an in vitro test with erythrocytes of known phenotypes in order to determine the proportion of cells which are parasitized under standard conditions, and this test correlates well with the behaviour of P. vivax in vivo (Miller et. al., 1975a; 1975b and 1976).

The hypothesis that both the Fy^a and the Fy^b antigen are directly involved in the susceptibility to parasitized erythrocytes is supported by the P. knowlesi test using the erythrocytes from three non-Negro Fy(a-b-) individuals, two North American Cree Indians and one Inuit, (Mason et. al., 1977), all of whom were resistant to infection. It seems very improbable that in three populations so widely divergent genetically that a P. vivax resistance gene would accompany the Fy(a-b-) phenotype unless it were either extremely closely linked to or an integral part of the Duffy locus.

POPULATION, MATERIALS & METHODS

Population.

Materials.

Methods.

POPULATION, MATERIALS & METHODS

POPULATION

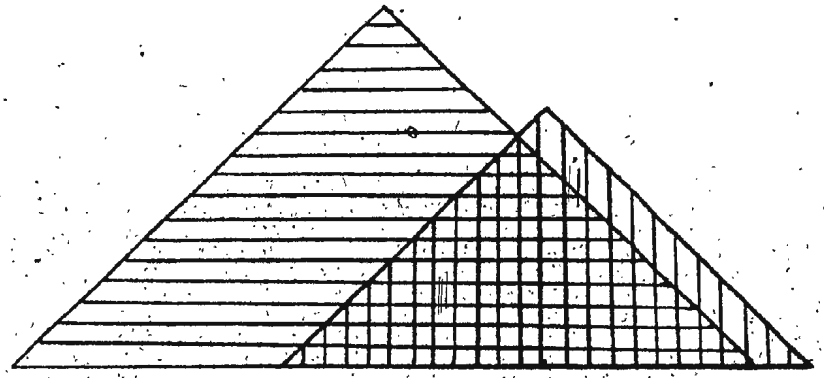
The area was settled originally in the early 1800's by a single couple, several of their children moved a little up and down the coast to start two new communities. All three communities are within a fifteen mile stretch of coastline, have similar geography and environment and there has been considerable traffic between them.

Until the late 1950's all travel was by sea, a gravel road then reached the area and has been in the process of being paved during this decade and will be completed by 1980.

The ancestry of approximately 85% of the population can be traced back to the original pair on one or both sides (1,277 individuals of a population of 1,518). These people form a very large extended family in which all the above reported cases occur. Due to the relatively small number of immigrants since the original couple arrived there is a high degree of inbreeding. The population structure can be conveniently diagramed as in Figure 3.

Figure 3.

Population structure



Descended on both sides from founding pair.

Descended on one side from founding pair.

Not descended from the founding pair.

The area of each segment is proportional to the number of individuals in the population having the appropriate lines of descent with respect to the original couple.

MATERIALS

Venous blood samples were collected from as many members of the population as possible, 1,133 being available for blood typing tests. The blood was collected into either acid-citrate dextrose (A.C.D.) or heparin and those collected into the latter anticoagulant were converted to A.C.D. by the addition of the appropriate quantity of blood to A.C.D. tubes within 24 hours of collection.

The blood samples were transported to St. John's where typing for the ABO & Rhesus antigens was performed within three weeks of collection. Aliquots were stored frozen in glycerol at -20^{o}C. for tests for antigens of other blood group systems, for example, K, k, Kp^{a} & Kp^{b}.

Antisera were obtained either from commercial sources or from the Canadian Red Cross Society, Blood Transfusion Service, whom we wish to thank for their gift of antisera.

The anti-human globulin serum (A.H.G.) was purchased from Burroughs-Wellcome, U.K.

METHODS

Red Cell Freezing

The freezing solution and it's use in cell freezing are as follows, tripotassium citrate, monohydric 9.70g; sodium dihydrogen phosphate, anhydrous 1.19g. and disodium hydrogen phosphate, anhydrous 1.40g. were dissolved in 300ml. of distilled water, 200ml. of glycerol added and the solution well mixed. Cells to be frozen were collected into A.C.D. or converted to A.C.D. within 24 hours of collection, the blood-anticoagulant mixture was centrifuged at 3,000 rpm. for 15 minutes to pack the red cells and the plasma-anticoagulant mixture was removed and discarded. A volume of freezing solution equal to the

volume of red cells was slowly added and this was gently mixed. This mixture was then frozen in 0.5ml. aliquots at -20°C.

Recovery of Frozen Red Cells

The recovery solutions are 5% trisodium citrate containing 16, 12, 8 and 4% of glycerol respectively. The cells were thawed at room temperature (18-22°C.), and washed once in each solution in descending order of glycerol concentration, this was followed by three washes in isotonic saline (0.85% sodium chloride solution). The washed cells were then suspended in saline at the required cell concentration.

Low's Papain Solution

2.0g of papain powder (B.D.H.) was wieghed out and titurated with a small volume of phosphate buffered saline (P.B.S.) pH 5.4 with a pestle and mortar, the volume was then brought to 100ml. with additional P.B.S. pH 5.4. This was stored overnight at 4°C., then centrifuged at 2,000 rpm. for 20 minutes and the supernatant fluid transferred to another container.

10ml. of freshly prepared L-cystein hydrochloride 0.5M, was added, the volume was brought upto 200ml. with additional P.B.S. pH 5.4, dispensed in 0.5ml. aliquots and frozen at -.20°C until required.

Once an aliquot was thawed it was used for the remainder of the day, being stored at 4°C. when not in actual use and then discarded.

Chown's Capillary Method

One volume of antiserum was allowed to enter the tube by capillary flow, the end of the tube wiped and an equal volume of a 10%

suspension of test red cell allowed to enter the tube taking care to have no air bubbles at the interface. The tube was then placed in a special rack which held the tubes at an angle of approximately 30^{o} from vertical with the lower end of the tube sealed by being pushed into a layer of plasticine. The tubes were left for the period of time recommended for each individual antiserum then read macroscopically. Appropriate controls were included with each batch of tests for each antiserum.

Chown's Capillary Papain Method

As for the above method except that the reaction mixture contains two volumes of Low's 1% papain solution, the antiserum was added to the tube first, followed by the two volumes of papain and then the one volume of test cells. The appropriate controls being performed for each batch of tests for each antiserum.

Anti-Human Globulin Test

This test was used for the detection of the D^{u} antigen using a selected anti-D serum. In an 8mm. I.D. test-tube (Luckham's LP3) two volumes of anti-D were placed and one volume of a 5% suspension of the test cells added, this mixture was then incubated at $37^{o}C$ for 60 to 90 minutes. A large volume of saline was then added to each tube, the tube centrifuged at 2,000 rpm. for 2 minutes and the saline poured off. The tubes were then given a sharp flick downward to remove most of the remaining saline, this process was then repeated twice more. To each tube 1 volume of appropriately diluted A.H.G. was added the contents of the tube mixed by gently tapping with the forefinger and the tubes recentrifuged at 1,000 rpm. for 30 seconds. The red cells in each tube were then examined macro- and micro-scopically for agglutination. Appropriate controls were set up with each batch of tests and carried

through with the tests. Since the antibody-antigen reaction is reversible the whole washing procedure was performed as rapidly as possible to minimise dissociation which could lead to false negatives.

Controls

These were set up in parrallel with the tests, carried through and read with them. The positive control cells used were always from a heterozygous donor while the negative control cells were from a homozygous donor, for example in C-typing with anti-C, the positive cell was CDe/cde and the negative cell was cDE/cde.

In the A.H.G. test an additional control was included to ensure that the washing procedure was adequate, since any free IgG would neutralise the A.H.G. giving false negative results. This consisted of group O, D+ red cells treated as follows. To 10 volumes of washed packed cells was added 1 volume of an incomplete (IgG) anti-D serum having a titre of 1/256, this mixture was incubated at 37°C. for 60 minutes, then washed thrice and the cells resuspended to a 10% suspension in saline. After completion of the A.H.G. test on each sample 1 drop of these sensitized cells were added to each and the tube recentrifuged at 1,000 rpm. for 30 seconds and read macroscopically for agglutination. Any tube in which the washing process had been insufficient would fail to give a positive reaction with the D sensitized cells. In no case was there a failure of this control to be positive. The reference for this section from cell freezing on was Issitt & Issitt (1975).

Antisera

All the antisera used were gifts from the Canadian

Red Cross Blood Transfusion Service, the anti-E supplied was reactive with saline suspended red cells and was used by Chown's capillary method, the anti-D, anti-C, anti-c and anti-e were all used by Chown's capillary papain method while the anti-D serum provided for the detection of the D^u antigen was used by the A.H.G. method.

ASSIGNMENT OF GENOTYPES

All the common antigens of the Rhesus system can be detected in the heterozygote with the exception of d, which is believed to be an amorph and no antiserum reacting with this antigen is known. At the basic level there are 8 haplotypes in the system, which are:-

Table 4 Rhesus haplotypes

R_1	CDe	r'	Cde
R_2	cDE	r"	cdE
R_o	cDe	r	cde
R_z	CDE	r^y	CdE

These can be combined 2 at a time to give the following 36 genotypes:- Table 5 Rhesus genotypes

R_1R_1	R_2R_2	R_oR_o	R_zR_z	r'r'	r"r"	r r	r^yr^y
R_1R_2	R_2R_o	R_oR_z	R_zr'	r'r"	r"r	r r^y	
R_1R_o	R_2R_z	R_or'	$R_zr"$	r'r	r"r^y		
R_1R_z	R_2r'	$R_or"$	R_zr	r'r^y			
R_1r'	$R_2r"$	R_or	R_zr^y				
$R_1r"$	R_2r	R_or^y					
R_1r	R_2r^y						
R_1r^y							

Even when tested with the five most commonly available antisera, anti-D, anti-C, anti-c, anti-E and anti-e, not all of these

genotypes can be distinguished from one another. The most common genotype which matches the phenotype is usually quoted as the "most probable genotype". Even this much is not always possible with some of the very rare phenotypes, for example, the phenotype D-, C+, c+, E+, e+, has the two possible genotypes Cde/cdE and CdE/cde, and it is not possible to give the most probable genotype on statistical grounds, since both are of about the same rarity.

This problem is compounded by the different frequencies of the haplotypes in different populations, in Caucasian populations the phenotype D+, C+, c+, E-, e+, will, in the vast majority of cases, represent the genotype CDe/cde rather than CDe/cDe or Cde/cDe, the latter being the rarest. In Negro populations the haplotypes cde & cDe have almost the same frequency, Cde/cDe remains the rarest possibility but it is practically impossible to call either CDe/cde or CDe/cDe the most probable genotype.

Together with the phenotypes, the most probable and alternative genotypes are given for Caucasian populations in general. In those cases where it is possible the percentage error occassioned by using the most probable genotype for the phenotype is also given.

Table 6 Rhesus phenotypes and genotypes

Phenotype					Genotypes		%
D	C	c	E	e	Most Probable	Alternatives	Error
+	+	+	+	+	CDe/cDE	CDe/cdE; cDE/CDe; cDe/CdE; CDE/cde; CDE/cDe.	13
+	+	+	-	+	CDe/cde	CDe/cDe; cDe/Cde.	7
+	-	+	+	+	cDE/cde	cDE/cDe; cDe/cdE.	6
+	+	-	-	+	CDe/CDe	CDe/Cde.	5
+	-	+	+	-	cDE/cDE	cDE/cdE.	16

+ - + - +	cDe/cde	cDe/cDe.	4
- - + - +	cde/cde.		
- + - - +	Cde/Cde.		
- + + - +	Cde/cde.		
- - + + -	cdE/cdE.		
- - + + +	cdE/cde.		
- + + + +	Cde/cdE	CdE/cde.	
+ + - + -	CDE/CDE	CDE/CdE.	
- + - + -	CdE/CdE.		
+ + + + -	cDE/CDE	cDE/CdE; cdE/CDE.	
+ + - + +	CDe/CDE	CDe/CdE; Cde/CDE.	
- + - + +	Cde/CdE.		
- + + + -	cdE/CdE.		

Assignment of the correct genotype to an individual where one or more fit the phenotype is possible only by family studies. It may be possible to assign actual genotypes to the majority of family members on two generation trees, but often data from three or even four generations is needed, even here it may not be possible to assign actual genotypes to all family members with any degree of confidence. These points are illustrated by the fictitious families of Figures 4, 5a and 5b, Table 6 is included for ready reference here.

Figure 4 demonstrates a family in which assignment of actual genotypes is relatively straightforward. Individual II 4 has the phenotype (actual genotype) of r"r, only I 2 could have passed on the haplotype r", I 1 must therefore have donated the r haplotype. I 1 must have the genotype R_1r and I 2 the genotype $R_or"$. II 1 & 2 are phenotypically R_1r but must be genetically R_1R_o, while II 3 & 5 must be

R_1r'' not R_1R_2.

The grandparent I 4 is r r which is the actual genotype and the spouse, I 3, is phenotypically R_1R_2, if this is the actual genotype then all children should be either R_1r or R_2r, as they are, I 3 must be R_1R_2, II 6 & 7 R_1r and II 8 R_2r.

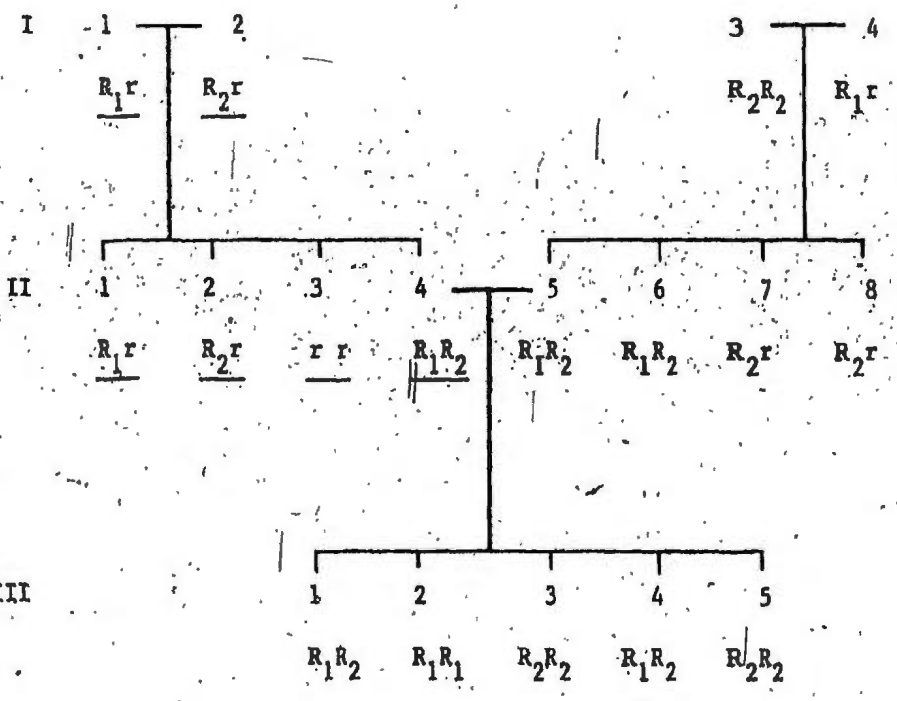
In generation III the phenotypes and genotypes are identical except for III 4 where the phenotype is R_1R_2 but the actual genotype can only be R_1r''.

Figure 5a shows a more common stituation where the genotypes of some family members can be assigned relatively readily but cannot be assigned to the majority of the family members without further data being available.

I 1 & 2 have produced four children, II 3 being r r which is the only possible genotype for that phenotype, the parents therefore must both have a r haplotype and their genotypes must match their phenotypes, this being so the genotypes of all their offspring must also match their phenotypes. In no other case can the actual genotype be deduced.

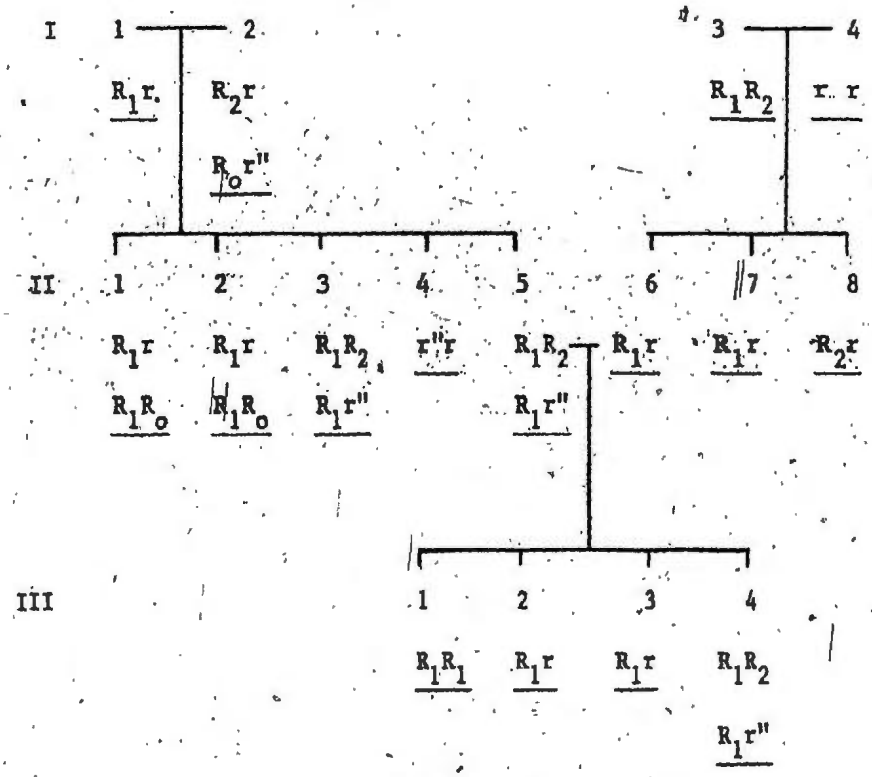
In figure 5b the family has been extended to the fourth generation, where the r'' reveals itself in IV 3 whose phenotype and genotype is r''r. It follows that III 5 must carry r'' in addition to R_2 and that this individual must have received this haplotype from II 5 since II 4 can only be R_1R_2, hence I 3 must have the genotype R_2r''. Both II 4 and 5 must have the R_1 haplotype since III 3 is R_1R_1, hence I 4 must have the genotype of R_1r. II 7 and 8 are therefore actually R_2r. In generation III, 3 and 5 must both have the genotype R_2r'', but III 1 and 4 may be either R_1R_2 or R_1r'', the probabilities for each being equal in both cases.

Figure 5a. Fictitious pedigree illustrating the assignment of actual genotypes to family members.



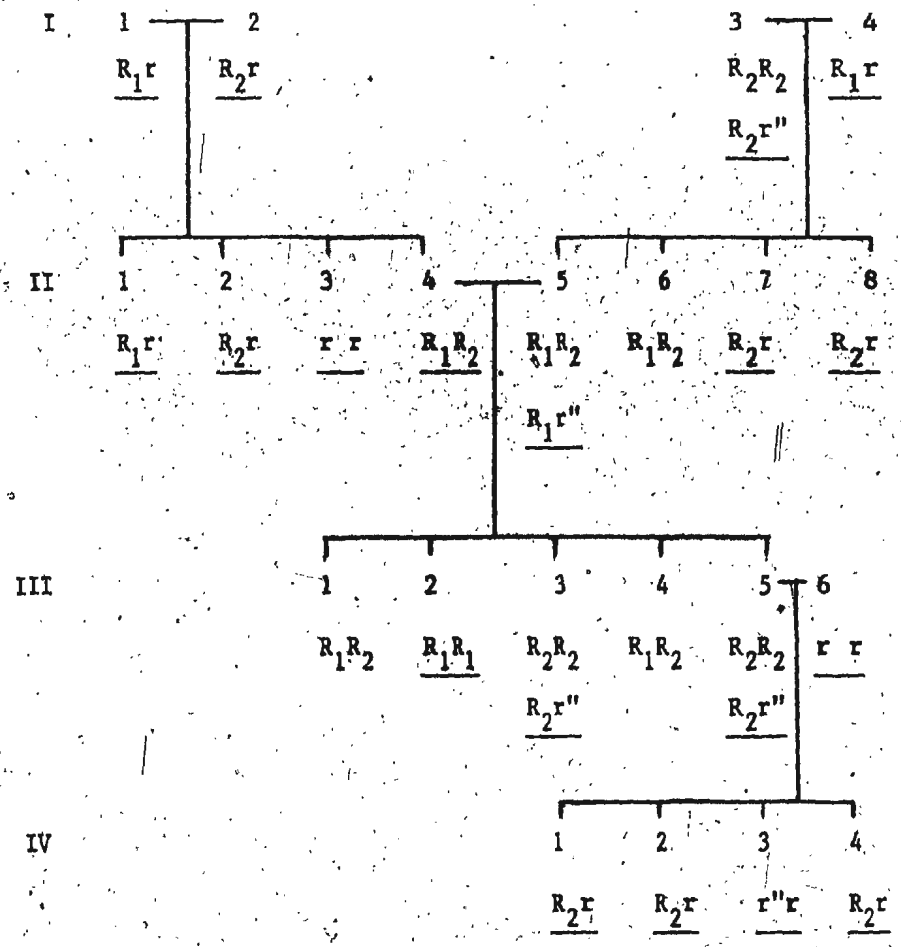
Actual genotypes are underlined.

Figure 4. Fictitious family illustrating the assignment of actual genotypes to family members.



The actual genotype is underlined.

Figure 5b. Fictitious pedigree illustrating the assignment of actual genotypes to family members.



Actual genotypes are underlined.

Since III/6 has the genotype r r then all members of generations IV have the most probable genotype which matches the phenotype.

In this family it is possible to assign genotypes to all but three members, all three have the phenotype R_1R_2 and all have an equal probability of being either R_1R_2 or R_1r''.

In these fictitious families all offspring have been considered legitimate, this is standard procedure unless there is evidence to the contrary.

This type of analysis has been applied to all the family trees of the study population. Pedigrees are available for all members of the population and in the appropriate cases go back to the original founding couple. All phenotypes have been entered on the appropriate trees which in the vast majority of cases are overlapping, so that each individual may enter thrice, once as a child, once as a parent and again as a grandparent where appropriate. There will be in most cases additional entrances as aunts or uncles. Each family tree has then been examined in order to define where possible the actual genotype of each individual. A list of each individual who has been typed for the Rhesus system has been prepared and for each the phenotype and where possible the actual genotype has been entered.

Estimating Haplotype Frequencies Of A Population

Two methods of obtaining the gene (haplotype) frequencies are available, the first is by direct counting of the genes, the second utilises the Hardy-Weinberg equation.

The first method is normally limited to those situations in which all the alleles of a locus can be distinguished no matter which

allele occupies the corresponding locus on the partner chromosome. That is all homozygotes and heterozygotes can be distinguished. The method consists of merely counting the number of each type of gene and expressing this value as a proportion of 1.0 which represents the total genes in the sample and this is always twice the number of individuals in the sample.

A good example of such a system is the Lutheran blood group system, this actually contains three alleles, Lu^a, Lu^b & Lu, the last of which is exceedingly rare, if we ignore Lu we can consider it to be a two allele co-dominant system in which the gene products can be recognised by the appropriate antiserum, anti-Lu^a or anti-Lu^b. Ignoring the very rare allele, Lu, unless the extremely rare phenotype Lu(a-b-) occurs in the sample, will lead to very minute errors in the estimates of the gene frequency of the other two alleles.

In a fictitious sample of 950 individuals the following phenotype numbers were detected.

Lu (a+b-)	4
Lu (a+b+)	97
Lu (a-b+)	849

Then counting the genes gives,

Lu^a 105, a frequency of 105/1900 = 0.05526

Lu^b 1795, a frequency of 1795/1900 = 0.94474

Substituting these values in the Hardy-Weinberg equation permits calculation of the expected numbers of each phenotype with which the observed numbers can be compared and tested for goodness to fit by the X^2 test.

Lu (a+b−) = 0.05526^2 X 950 = 2.90

Lu (a+b+) = 0.05526 X 0.94474 X 2 X 950 = 99.19

Lu (a−b+) = 0.94474^2 X 950 = 847.91

950.00

In this example there is no need to perform the X^2 test as the fit is obviously very close. This method usually gives excellent estimates of gene frequencies.

In several important respects the Kell & Rhesus blood group systems are very similar, the antigens appear to be coded for by three extremely tightly linked loci each of which has two relatively common alleles. So that K & k are analogous to D & d, Kp^a & Kp^b to C & c and Js^a & Js^b to E & e. The two vital aspects in which they differ from Rhesus are, first that all the antigens of the Kell blood group system can be tested for as there is no amorphic allele representing d of the Rhesus system, the second is that the Kell system has only 4 haplotypes instead of the 8 of the Rhesus system. The four known haplotypes of the Kell system are:

k, Kp^b, Js^b.

K. Kp^b, Js^b.

k, Kp^a, Js^b.

K, Kp^b, Js^a.

The other four which are theoretically possible have not been found, despite extensive searches for them, they do however remain possibilities with very remote probabilities. They are;

K, Kp^a, Js^b.

K, Kp^b, Js^a.

k, Kp^a, Js^a.

K, Kp^a, Js^a.

By far the most common haplotype is k, Kp^b, Js^b, if this is regarded as the original wild type then each of the other observed haplotypes represents a mutation at a different locus. The first three so far unobserved haplotypes then represent either mutations at two of the three loci or a crossover between two of the three mutated haplotypes above. The final possible haplotype then represents mutations at each of the three loci, or a crossover between a haplotype carrying at least one mutation with a second carrying mutations of the other two loci.

These two differences, only four haplotypes and all antigens detectable enables the direct counting method to be used in estimating the haplotype frequencies of Kell but not of Rhesus.

The second method of estimating gene frequencies is by the Hardy-Weinberg equations. These are used in any case where the genes or haplotypes cannot be directly counted, such as systems containing one or more recessive alleles or where phenotypes contain two or more possible genotypes. An example of the first is the P blood group system where P_1 is dominant to P_2 and this in turn is dominant to the rare allele p. The homozygote P_1P_1 cannot be distinguished from the heterozygote P_1P_2 or P_1p and the homozygote P_2P_2 is indistinguishable from the heterozygote P_2p. A good example of the second type is the MNSs blood group system, M & N and S & s are alleles at two very tightly linked loci, all the genotypes can be differentiated from one another with the sole exception of the double heterozygote M+, N+, S+, s+, which has two genotypes MS/Ns & Ms/NS, the former being the most common.

The Hardy-Weinberg equations are applicable only in populations where mating is random and there is no large scale immigration nor

emmigration. For a two allele system, where p = the gene frequency of one gene and q = the gene frequency of the other and p + q = 1, then the genotype frequencies are given by $(p + q) \times (p + q) = (p + q)^2 = p^2 + 2pq + q^2 = 1$. Homozygotes for the first allele are represented by p^2, for the second by q^2 and the heterozygotes are represented by 2pq. As an example we may use a fictitious sample typed for the P blood groups and ignore the very rare p allele. Of a sample of 750 individuals 600 were P_1+ and 150 were P_1-. Of the 600 we cannot tell which are P_1P_1 and which are P_1P_2, but all the 150 may be regarded as being P_2P_2. If we let p = frequency of P_1 and q = frequency of P_2, then; $q^2 = 150/750 = 0.20000$, therefore $q = \sqrt{0.2000} = 0.44721$. Since p + q = 1. then 1 - q = p, therefore; p = 1 - 0.44721 = 0.55279.

We cannot use the gene frequencies derived above to test the data for goodness of fit as this is tautology. We can however use the gene frequencies to test for goodness of fit with a second sample drawn from the same population. For example suppose another 575 individuals were tested with the following results, P_1+ 440, P_1-135. Then substituting the above frequencies in the Hardy-Weinberg equation we obtain the following expected numbers;

$P_1P_1 = 0.55279^2 \times 575 = 175.71$

$P_1P_2 = 0.55275 \times 0.44721 \times 2 \times 575 = 284.30$

$P_2P_2 = 0.44721^2 \times 575 = 114.99$

Or,

P_1+, 460.01 & P_1-, 114.99 these can be used in the Chi-square test for goodness of fit.

	Obs.	Exp.	O-E	$(O-E)^2/E$
P_1+	440	460.1	-20.1	0.8781
P_1-	135	114.9	+20.1	3.5162

$X_1^2 = 4.3943$

$p = 0.05$

The Chi square test can be performed without calculating the gene frequencies first for comparison of two samples or populations as follows:-

	Sample 1	Sample 2	Sample 1	Sample 2			
	Obs.		Exp.		O-E	$(O-E)^2/E$	
P_1+	600	440	588.7	451.3	11.3	0.2169	0.2829
P_1-	150	135	161.3	123.7	11.7	0.7916	1.0323

$X_1^2 = 2.3237$

$p = 0.10$

When it is intened only to compare two samples or populations the latter method is most commonly used. However there are situations in which gene frequencies are required, these permit a check on the accuracy of the typing results or may be used to give an indication that the Hardy-Weinberg equilibrium is being circumvented, for example by assortive rather than random mating or because of recent immigration or emmigration from the population. In such cases The Hardy-Weinberg equation does not accurately describe the population.

For a three allele system such as the ABO system, the three alleles are represented by p, q & r; $p + q + r = 1$ and the genotype

frequencies are given by; $(p + q + r)^2 = p^2 + 2pq + q^2 + 2pr + r^2 + 2qr$, where p = frequency of A, q = frequency of B and r = frequency of O, then the terms in the expansion represent, AA, AB, BB, AO, OO & BO respectively.

Similar calculations can be used for the Rhesus blood group system with 8 haplotypes, but the formulae are complex and time consuming where manual calculations are used. Computer programs for the calculation of gene frequencies are now available.

Normally for calculation of the Rhesus haplotype frequencies the Hardy-Weinberg equations must be used for the reasons given above. In the case of the population studied in this publication it has been possible to use the direct count method for the following two reasons.

First that in a large proportion of cases the actual genotypes are known, 697/1133 or 61.5%, second that in this population three of the Rhesus haplotypes appear to be completely absent, these are R_z (CDE); r^y (CdE) and r' (Cde), which considerably reduces the number of possible phenotypes.

Summarising the steps taken in the present work we have;

1. Derived the haplotypes for each individual where possible by pedigree analysis (61.5%).
2. For the remaining individuals the most probable genotype was assigned.
3. The haplotype frequencies were derived by direct counts.
4. Then, assuming that the Hardy-Weinberg equilibrium exists in this population, the expected phenotype numbers were calculated from the haplotype frequencies.
5. The expected and assigned phenotypes (at the haplotype level) were then compared for goodness of fit by the Chi-square test.
6. Since the only place where the goodness of fit was poor involved a

reduction in the number of assigned phenotypes of $R_o r$ and an excess of rr and since R_o will be incorrectly described as r in a proportion of cases an adjustment was considered to be in order. This adjustment was made by calculating the frequency of r from r r and using this value as the "correct" value. The difference was then added to the R_o frequency to give a "correct" value here.

As revealed in the result section this maneuver gave an excellent fit when the phenotype frequencies were recalculated from the "corrected" haplotype frequencies and used as expected values in a second Chi-square goodness of fit test. (Table 7 to 17)

RESULTS

Typing and Haplotyping.

Measure Of Significance.

Population As A Whole.

Analysis In Relation To Disease.

RESULTS

The Rhesus phenotype and where possible the actual genotype of each individual tested in the study population are listed in Appendix I. From these the following data has been extracted and used in the calculation of frequency of the different haplotypes for this population.

Table 7 Phenotypes of the studied population

Phenotype	Number	Frequency
R_1R_1	95	0.08383
R_1r	402	0.35481
R_1R_2	76	0.06708
R_2R_2	17	0.01500
R_2r	147	0.12974
R_or	72	0.006355
$r''r$	12	0.01054
$r'r$	296	0.26125
R_1R_o	3	0.00265
R_1r''	4	0.00353
R_2r''	3	0.00265
R_or''	3	0.00265
	1133	1.00000

From this we obtained by direct counting the following haplotype frequencies;

Table 8 Haplotypes of the studied population

Haplotype	Number	Frequency
R_1	675	0.29788
R_2	263	0.11606

R_o	81	0.03575
R_z	0	
r'	0	
r''	22	0.00971
r	1225	0.54060
r^y	0	
	2266	1.00000

The following expected numbers were obtained by substituting the above haplotype frequencies in the Hardy-Weinberg equation and the Chi square test for goodness of fit applied.

Table 9 Chi square test for goodness of fit.

Phenotype	Exp.	Obs.	O - E	$(O - E)^2/E$
R_1R1	100.5	95	5.5	0.3010
R_1r	389.0	405	16.5	0.6581
R_1R_2	84.9	80	4.9	0.2828
R_2R_2	17.8	20	2.2	0.2719
R_2r	152.4	153	0.6	0.0024
R_or	45.4	72	26.6	15.5850
r''r	11.9	12	0.1	0.0008
r r	331.1	296	35.1	3.7210

$X_7^2 = 20,8230$ $p = 0,0005$

Only two phenotypes demonstrate a poor fit and are responsible for practically the whole of the Chi square value, these are R_or which is far too low and rr which is elevated. In combination with any other haplotype bearing D, R_o will be termed r in the most probable genotype for Caucasians giving a low estimate for the frequency of R_o with a correspondingly high estimate for the frequency of r.

In order to obtain a more accurate estimate for the frequency of r the Hardy-Weinberg equation has been resorted to in this case. Since rr represents the fully recessive homozygote then the frequency of r is given by, $r^2 = 296/1133 = 0.26125$, and $r = \sqrt{0.26125} = 0.51113$. The overestimate of frequency of r - 0.54060 - 0.51113 = 0.02947. The underestimate of the frequency of R_o is by the corresponding amount and the "corrected" value is 0.03575 + 0.02947 = 0.06522. The ammended haplotype frequencies now become;

Table 10 Haplotype "corrected frequencies".

Haplotype	Frequency
R_1	0.29788
R_2	0.11606
R_o	0.06522
r"	0.00971
r	0.51113

Again substituting in the Hardy-Weinberg equation to obtain the expected number for each phenotype and performing the Chi square test for goodness of fit, we obtain;

Table 11 Chi square test for goodness of fit.

Phenotype	Exp.	Obs.	O - E	$(O - E)^2/E$
R_1R_1	100.5	95	5.5	0.3010
R_1r	389.0	405	16.0	0.6581
R_1R_2	84.9	80	4.9	0.2828
R_2R_2	17.8	20	2.2	0.2719
R_2r	152.9	153	0.1	0.0001
R_or	80.4	72	8.4	0.8776
r"r	11.4	12	0.6	0.0316

r r * 296.0 296

X^2_6 = 2.3915 p = 0.9

*Since the haplotype frequency of r was derived from the frequency of the rr phenotype, this entry cannot be used in the goodness of fit test.

The study population has been subdivided into three groups, the first contains those individuals descended from the original couple on both sides, the second contains those descended from the original couple on one side and the third group are those who are not descended from the founding pair.

These groups have been treated in the same manner as the whole population for calculation of haplotype frequencies and goodness of fit test. In each case the calculation of a "correct" value for r has been performed and an ammended value for frequency of R_o obtained.(Table 16)

Table 12 Phenotype & Haplotype Numbers for the Three Groups by Descent from the Founding Couple.

Phenotype	Descendants		Non-Descendants
	Both Sides	One Side	
R_1R_1	50	25	20
R_1r	221	138	43
R_1R_2	26	30	20
R_2R_2	7	6	4
R_2r	71	48	28
R_or	44	23	5
r"r	10	1	1
r r	179	95	22
R_1R_o		2	1

R_1r''	1	3	
R_2R_o	1	2	
R_2r''	3		
R_or''	3		
	616	373	144

Table 13

Haplotype			
R_1	348	223	104
R_2	115	92	56
R_o	48	27	6
r''	17	4	1
r	704	400	121
	1232	746	288

Table 14 Haplotype Frequencies of the Three Groups by Direct Counting.

Haplotype	Descendants		Non-Descendants.
	Both Sides	One Side	
R_1	0.28247	0.29893	0.36111
R_2	0.09334	0.12332	0.19444
R_o	0.03896	0.03619	0.02083
r''	0.01380	0.00536	0.00347
r	0.57143	0.53619	0.42014

Table 15 Chi Square Goodness of Fit Tests for the Three Groups

Phenotype	Observed	Expected	O - E	$(O - E)^2/E$
Descended on Both Sides				
R_1R_1	50	49.2	+ 0.8	0.0130
R_1r	221	212.4	+ 8.6	0.3482
R_1R_2	27	37.3	-10.3	2.8442
R_2R_2	10	7.0	+ 3.0	1.2857
R_2r	75	70.9	+ 4.1	0.2371
R_or	44	28.4	+15.6	8.5690
$r''r$	10	9.7	+ 0.3	0.0093
$r\,r$	179	201.1	-22.1	2.4287
$X_7^2 = 15.7352$		$p = 0.025$		
Descended on One Side				
R_1R_1	25	33.3	- 8.3	2.0688
R_1r	140	127.7	+12.3	1.1847
R_1R_2	33	28.7	+ 4.3	0.6443
R_2R_2	6	6.2	- 0.2	0.0065
R_2r	50	52.8	- 2.8	0.1485
R_or	23	15.0	+ 8.0	4.2667
$r''r$	1	2.1	- 1.1	0.5762
$r\,r$	95	107.2	-12.2	1.3884
$X_7^2 = 10.2841$		$p = 0.1$		

Table 15 continued

Phenotype	Observed	Expected	O - E	$(O - E)^2/E$
Non-Descended				
R_1R_1	20	18.8	+ 1.2	0.0766
R_1r	44	45.9	- 1.9	0.0786
R_1R_2	20	20.6	- 0.6	0.0175
R_2R_2	4	5.6	- 1.6	0.4571
R_2r	28	24.7	+ 3.3	0.4409
R_or	5	2.6	+ 2.4	2.2154
r"r	I	0.4	+ 0.6	0.9000
r r	22	25.4	- 3.4	0.4551

$X_7^2 = 4.6412$ $p = 0.7$

The "correct" frequencies of R_o & r were obtained for each group as they were for the total sample, that is by recalculating the frequency of r from the Hardy-Weinberg equation, $r = \sqrt{rr}$. The difference in the two values for the frequency of r being added to the original frequency of R_o.

Descended on Both Sides

$r = \sqrt{179/616} = 0.53906$

$R_o = 0.03896 + 0.57143 - 0.53906 = 0.07133$

Descended on One Side

$= \sqrt{95/373} = 0.50467$

$R_o = 0.03619 + 0.53619 - 0.50467 = 0.06771$

Non-Descended

$r = \sqrt{22/144} = 0.39087$

$R_o = 0.02083 + 0.42014 - 0.39087 = 0.05010$

Table 16 Ammended Haplotype Frequencies for the Three Groups

Haplotype	Descendants		Non-Descendants
	Both Sides	One Side	
R_1	0.28247	0.29893	0.36111
R_2	0.09334	0.12332	0.19444
R_o	0.07133	0.06771	0.05010
r"	0.01380	0.00536	0.00347
r	0.53906	0.50467	0.39087

Table 17 Ammended Chi Square Tests for Goodness of Fit for the Three Groups

Phenotype	Observed	Expected	O - E	$(O - E)^2/E$
Descended on Both Sides				
R_1R_1	50	49.2	+ 0.8	0.0130
R_1r	221	212.4	+ 8.6	0.3482
R_1R_2	27	37.3	-10.3	2.8442
R_2R_2	10	7.0	+ 3.0	1.2857
R_2r	75	71.4	+ 3.6	0.1815
R_or	44	50.5	- 6.5	0.8366
r"r	10	9.2	+ 0.8	0.0696
r r	179	179.0		

$X_6^2 = 5.5788$ $p = 0.4$

Table 17 continued

Phenotype	Observed	Expected	O - E	$(O - E)^2/E$
Descended on One Side				
R_1R_1	25	33.3	- 8.3	2.0688
R_1r	140	127.6	+12.4	1.2050
R_1R_2	33	28.7	+ 4.3	0.6443
R_2R_2	6	6.2	- 0.2	0.0065
R_2r	50	52.9	- 2.9	0.1590
R_or	23	27.2	- 4.2	0.6485
r"r	1	2.0	- 1.0	0.5000
r r	95	95.0		
X_6^2 = 5.2321		p = 0.5		
Non-Descended				
R_1R_1	20	18.8	+ 1.2	0.0766
R_1r	44	45.9	- 1.9	0.0786
R_1R_2	20	20.6	- 0.6	0.0175
R_2R_2	4	5.6	- 1.6	0.4571
R_2r	28	24.8	+ 3.2	0.4129
R_or	5	6.0	- 1.0	0.1666
r"r	1	0.4	+ 0.6	0.9000
r r	22	22.0		
X_6^2 = 2.1093		p = 0.9		

TYPING & HAPLOTYPING

1133 samples from this population were typed for the Rhesus antigens D, C, c, E & e. Of these 616 were from individuals descended on both side, 373 from individuals descended on one side and 144 from individuals who were not descended from the original settleing couple. From the data haplotypes were derived by pedigree studies for 61% of the individuals, the remainder were assigned haplotypes on the basis of the most probable genotype which matched their phenotype. The phenotypes and where known the genotypes for these individuals are listed in Appendix I. It will be noted that some 4% of the results do not fit with the pedigrees as they are presently assembled on the basis of either ABO or Rhesus typing. Of these 47 cases of parental exclusion 4 were concordant for both ABO & Rhesus. The causes for these errors has not been established.

Parental exclusions are always disturbing but to a much greater extent when it is a maternal exclusion, 16 of the exclusions are maternal, and of these four demonstrate exclusion in more than one blood group system. There are only three explanations of maternal exclusions, first the information regarding the relationship is erroneous, second that a clerical error of some description has occurred or third that the actual tests were incorrect.

The finding that in 25% of the maternal exclusions more than one blood group system demonstrated exclusion is evidence against errors of the third type, in addition some of the exclusions were on the basis of ABO groups in which technical errors in reasonably practiced hands are almost unknown and this applies also to the Rhesus system when the antisera are potent as was the case in this study.

furthermore all D negatives were retested with a second anti-D serum by the A.H.G. method in order to detect any D^u samples present, none were found. It is felt that the actual tests themselves were not in error for the above reasons and because each batch of tests was accompanied by a positive and negative control which in all cases gave the expected results. Unfortunately by the time the three generation pedigree sheets were available the red cells were no longer suitable for testing and to date it has not been possible to obtain further samples from these individuals and their "parents".

Although great care was taken in the collection and labelling of the samples it is not possible to be sure that none were inadvertently mis-labelled.

The HLA system demonstrated 12 exclusions, 4 of which were maternal, a rate very close to those given by the blood groups and the overall rate was very close also since only approximately 300 samples were typed.

The pedigree data may be incorrect in some cases. In Newfoundland it is quite common for the maternal grandparents of an illigitimate child to "adopt" the child as theirs and to rear the child as a sibling of the biological mother. In such cases the true relationship may have been deliberatly or inadvertently concealed. We have very little hard evidence of such generation shifts in the studied population, but it remains a distinct probability since it undoubtedly occurs in most other similar Newfoundland populations.

MEASURE OF SIGNIFICANCE

The Chi square test has been used throughout this study for the analysis of the different sub-groups of the population and the

significance level of 0.01 was set prior to commencing the study.

POPULATION AS A WHOLE

Grouping of the population

One "super-family" represents 85% of the population and are the descendants of the original pair. This "super-family" contains all the cases of neoplastic disease and immunodeficiency. For some purposes the division into descendants of the original couple has been used and the subgroups, descended on one side and descended on both sides used in other cases.

Haplotype frequencies

Haplotype frequencies for the population as a whole have been given, (Table 10) these being the corrected haplotype frequencies, derived by an adjustment described under "Estimating Haplotype Frequencies of a Population" (Page 55). When these frequencies are compared with those for the population of the United Kingdom, where the majority of the founders originated, it is found that there are significant differences (Table 18).

Table 18 Haplotype frequencies of the study population (Obs.) compared with those of the United Kingdom (Exp.).

Haplotype	Obs.	Exp.	O - E	$(O - E)^2/E$
R_1	675	961	286	85.1155
R_2	263	322	59	10.8106
R_o	81	24	57	135.3750
R_z	0	2		
r'	0	7		
r''	22	10	2	14.4000
r	1225	940	285	86.4096
Pooled	(0)	(9)	9	9.0000

$X_5^2 = 341.1107$ $p << 0.0005$

Table 19.

Rhesus Haplotype & Genotype Frequencies for the United Kingdom.

Haplotype	Frequency	Genotype	Frequency	n
R_1	0.41932	R_1R_1	0.1819	206
R_2	0.13374	R_1r	0.3577	405
R_o	0.02563	R_1R_2	0.1256	142
R_z	0.00228	R_2R_2	0.0209	24
r	0.40042	R_2r	0.1145	130
r'	0.00728	R_or	0.0212	24
r''	0.01133	r'r	0.0058	7
r^y	0.00000	r'r'	0.0001	0
	1.00000	r'r''	0.0002	0
		r''r	0.0091	10
		r''r''	0.0001	0
		rr	0.1603	182
		R_1R_z	0.0019	2
		R_2R_z	0.0006	1
			0.9999	1,133

The most stricking differences are that in this population R_1 is much reduced while R_o and r are elevated, p much less than 0.0005.

When the population is divided into three groups on the basis of descent from the original couple, a). those descended on both sides, b). those descended on one side and c). those not descended

from this particular pair, it is obvious that the major differences are due to the frequencies within the "super-family" in the main (Table 20), and that in all cases that observed numbers for the descendants on both sides deviate more than do those for the descendants on one side and these in turn deviate more than those for the individuals who are not descendants of the founders. As an example the relative frequency for r in the three groups where the expected number based on the U.K. data is 1.0 are as follows:

a). Descended on both sides, 1.43

b). Descended on one side, 1.34

c). Non-descendants, 1.05

As a whole the differences between the two groups of descendants do not react significance statistically (Table 21). It is interesting to note that the non-descendants have haplotype frequencies much closer to those of the U.K.

It appears that there is a founder effect operating in this genetic isolate which is most marked in the "super-family", more especially in those with the greatest degree of inbreeding.

ANALYSIS IN RELATION TO DISEASE

Disease groups

As outlined in the introduction there were 21 patients with either immunodeficiency or tumours of the lymphoreticular system (Page 3). In order to determine if certain Rhesus haplotypes were associated with some of the disease families, the Rhesus data on the relatives of each disease group were collected together. The three disease groups selected were Hodgkin's disease, Immunodeficiency and Embryonic tumours, in each of which the relatives selected were

first, second and third degree. Since all 20 cases occurred in the superfamily of 1,277 of a population of 1,518, $X_1^2 = 3.8317$, $p = 0.05$, which is suggestive but by no means conclusive of a genetic basis.

The findings of other workers of an excess of dd individuals in random Hodgkin's disease patients is also suggestive of a genetic background to the disease, at least in part. In the present study it has been possible to assign actual genotypes to 61% of the population, here although an excess of dd may be expected among the relatives of Hodgkin's disease there may also be a deficiency of one or more Rhesus haplotypes too.

The data herein are felt to confirm, complement and extend the previous findings, even though these were on random patients as opposed to a family situation. The two types of study may not be as divergent as first appears. Since there is an excess of dd persons in the randon patients it seems reasonable that if their relatives were to be typed there would be an excess of the r haplotype present, at least in the first degree relatives, since all the parents of the dd patients must have had at least one r haplotype present, and again the patients and their siblings must have passed on such an excess to their offspring.

This study includes an attempt to define which Rhesus haplotypes are involved in resistance or susceptibility to the three studied conditions and towards this end the data on the first, second and third degree relatives of each disease group has been utilised.

Although 85% of the population must have some degree of relationship to all the patients, those where the relationship to all the patients is more remote than third degree, the "Rest of the

Descendants" have been pooled with the unrelated members of the population to form the "Rest of the Population" for some comparisons. Only those comparisons in which the level of significance reaches or is less than 0.01 against the "Rest of the descendants" have been accepted as valid. This has been done in view of the differences mentioned above between the three groups defined by descent from the founding couple.

This tends to bias the tests towards non-significance, but it was felt that a false non-significant result was preferable to a false significant result.

Hodgkin's disease

From actual tests where these have been performed in conjunction with analysis of the relevant family trees, an attempt has been made to define the possible phenotypes and hopefully the actual genotypes of the Hodgkin's disease patients. In all seven cases this was possible to some extent (Table 22). The only Rhesus haplotype which could have been common to all seven was r, but only four could have been homozygous rr. The frequency of this haplotype in the patients was between 0.50 and 0.75. The next most common haplotype was R_1 which could have been present in six of the seven with a frequency between 0.25 and 0.5. The only other haplotype which may have been present in this group was R_2 with a frequency of 0.00 to 0.08. These ranges compare well with the frequencies of these haplotypes in the first, second and third degree relatives, as would be expected from the method of derivation (Table 23).

When each group of relatives was compared seperately with

the "Rest of the population", significance was attained only with the third degree relatives, $p = 0.01$. When the analogous comparison was made with the pooled data for all three degrees of relatives the p value reached high significance at less than 0.0005, the same comparison of the pooled data against the "Rest of the descendants" was also highly significant with p less than 0.0005. (Tables 25, 26 & 27).

Examination of the numbers of each haplotype reveals that for each degree of relationship the percentage for each haplotype in each degree of relationship is consistent, that is there are no differences between the first second and third degree relatives in terms of haplotype frequencies, (Tables 23 & 24), the gradient of significance when each group of relatives is compared with the "Rest of the population" and the "Rest of the descendants" is an artifact due to the relatively small sample size for each degree of relationship. More specifically the small numbers of first and second degree relatives.

In all cases the major differences were due to the total lack of r" and the almost complete lack of R_o in the relations of Hodgkin's disease patients. r tended to be the replacing haplotype.

Similar analyses have been performed for each of the Rhesus genes D, C & E and there are no significant differences in any comparison (Tables 28 & 29).

The lack of r" in the relatives of the Hodgkin's disease patients may well be due to chance, as indeed may be the lack of R_o also, however the lack of R_o seems to be much more significant as the "Rest of the descedants" have a high frequency of this haplotype.

Immunodeficiency

This group of patients, and hence their 1st., 2nd. and 3rd. degree relatives, are very closely inter-related to the Hodgkin's disease group, so much so that this group can be regarded as providing supportive evidence of the findings in the Hodgkin's disease group.

Here too only one haplotype could have been present in all three patients, though with a low probability in the third. The frequency would have been in the range 0.6667 and 0.8333, the only other haplotype which could have occured is R_1 with a frequency between 0.1667 and 0.3333. Two of the three were homozygous for r, but the third was almost certainly homozygous for R_1R_1, $p = 0.94$, the probability for R_1r being 0.04 (Table 22).

There is a hint of a gradient in the haplotype frequencies in the relatives which is a distinction from that of the Hodgkin's disease group (Table 30 & 31). Only the comparison of the 1st. with 3rd. degree relatives reaches significance at $p = 0.01$. The frequency of R_1 decreases in the direction from 1st. to 2nd. to 3rd. degree relatives while the frequencies of both R_2 and r increase in this direction.

As with the analysis of the Hodgkin's disease data, there were no significant differences when each degree of relationship was compared with either the "Rest of the population" or the "Rest of the descendants". However when the data for the three degrees of relationship were pooled and the same comparisons made both were highly significant, $p = 0.001$ and 0.005 respectively (Tables 32 & 33 & 34).

Again the two haplotypes involved are r" and R_o both are completely absent and again r is acting in the main as the replacement. Analysis of the data according to D, C or E status demonstrates no

significant deviation in any comparison (Table 35 and 26).

Embryonic tumours

The only haplotype which could have occured in all three patients was r and all three may have been homozygous, the frequency range for this haplotype was in the range of 0.50 to 1.00, the other possible haplotype which may have occured in this group were R_1 with a frequency of 0.00 to 0.333 and r" with a frequency of 0.00 to 0.1667. (Table 22).

The data on the relatives of patients with these conditions demonstrated a gradient with r" decreasing from 1st. to 2nd. to 3rd. degree relatives, while R_2 had an equal but opposite gradient. Only the comparison of the 1st. with the 3rd. degree relatives showed significance, p less than 0.0005. (Tables 37 & 38).

None of the comparisons of the three classes of relatives with the "Rest of the population" reach significance, (Table 39). This was due mainly to the small groups for each haplotype which had to be pooled and some differences then cancelled out.

With the three degrees of relatives pooled data both the comparisons with the "Rest of the descendants" and the "Rest of the population" were significant at p less than 0.0005 (Table 40 & 41).

In the case of the three Rhesus genes D, C & E there are no significant comparisons, (Table 42) which was rather surprising since the most obvious difference in this disease group was the excess of r", cdE, however this excess was counterbalanced by the reduction of R_2, cDE, and D demonstrated no deviation because the numbers of R_2 replaced by r" were too small.

Other comparisons

For each disease group the haplotype numbers for 1st., 2nd.,

and 3rd. degree relatives has been pooled. Each of these has then been compared with the other two disease groups (Table 43). As expected there is excellent agreement between the Hodgkin's disease and the immunodeficiency groups, $p = 0.40$ and both differ significantly from the embryonic tumour group, p less than 0.0005, in both cases.

Pedigree correlations

The data and analyses of the Hodgkin's disease and immunodeficiency patients and their 1st., 2nd. and 3rd. degree relatives may be regarded as identical. There is a complete lack of r" and an essentially complete lack of R_o, and in both cases r tends to be the replacing haplotype. There are no or only slight gradients from one degree of relationship to the next.

The embryonic tumour patients with their 1st., 2nd. and 3rd. degree relatives form a second group which is distinct from the Hodgkin's disease-immunodeficiency group. Here gradients of haplotype frequency do occur from one degree of relationship to the next, the gradient for r" runs in one direction and the gradient for R_2 in the other, so that these two balance.

We have therefore two distinct disease groups which differ from one another and more importantly from the "Rest of the descendants" also.

The minimal pedigrees of the 13 patients demonstrated that all but 2 of the Hodgkin's disease-immunodeficiency group could be traced back to a single common ancestor on both sides, the other two could also be traced back to this common ancestor, but on one side only, even here one of the two had two lines of descent from this individual both of which entered on the same side.

The common ancestor was a child of the original couple and both patients whose ancestry could be traced to this individual on one side only suffered from Hodgkin's disease.

The 3 patients with embryonic tumours can also be traced back to the same common ancestor, but in all cases on one side only, on the other side they all share a second common ancestor, also a child of the original pair.

Individuals having a common ancestor, for example cousins usually in fact have two common ancestors, in the case of cousins a pair of grandparents. This is not the case for the patients studied herein, the one ancestor common to them all married twice, some of the patients are descended from one union, others from the second and others from both.

The minimal pedigrees tracing the lines of descent of each of these patients are presented in Appendix III.

This tracing of the pedigrees correlates well with the Rhesus data in the separation of the two main groups of patients, Hodgkin's disease-immunodeficiency from the embryonic tumour patients. It is a little less satisfactory in dividing these from the rest of the descendants of the original pair, but does so to some extent. Only two of the children of the original couple are common ancestors to more then one patient, while the original couple had nine children, if each of these has bequeathed an approximately equal genetic donation to succeeding generations then the patients have received about one-third of the diversity of the rest of the descendants, or may be regarded overall as being perhaps inbred as much as three times the rate of inbreeding for the remainder of the "super-family".

Table 20 Comparison on the Observed Haplotype Numbers in the Three Subgroups of the Population with the Expected Haplotype Numbers for the Population of the United Kingdom.

Haplotype	Observed	Expected	O - E	$(O - E)^2/E$
Descended on Both Sides.				
R_1	348	516.6	168.6	56.1113
R_2	115	164.8	49.8	15.0488
R_o	48	31.6	16.4	8.5114
r''	17	14.0	3.0	0.6429
r	704	493.3	210.7	89.9949
Other	0	11.7	11.7	11.7000
	1232	1232.0	$X_5^2 = 182.0093$	$p << 0.005$
Descended on One Side.				
R_1	223	312.8	89.8	25.7802
R_2	92	99.8	7.8	0.6096
R_o	27	19.1	7.9	3.2675
r''	4	8.5	4.5	2.3824
r	400	298.7	101.3	34.3545
Other	0	7.1	7.1	7.1000
	746	746.0	$X_5^2 = 73.4942$	$p << 0.005$
Un-Descended.				
R_1	104	120.8	16.8	2.3364
R_2	56	38.5	17.5	7.9545
R_o	6	7.4	1.4	0.2649
r''	1	3.3	2.3	1.6030
r	121	115.3	5.7	0.2818
Other	0	2.7	2.7	2.7000
	288	288.0	$X_5^2 = 15.1406$	$p = 0.01$

Table 21 Comparisons of Haplotype Numbers Between the Three Groups

Haplotype	Observed		Expected		O - E	$(O - E)^2/E$	
Descended on Both Sides with Descended on One Side							
	B.S.	O.S.	B.S.	O.S.			
R_1	348	223	355.7	215.3	7.7	0.1646	0.2718
R_2	115	92	128.9	78.1	13.1	1.5050	2.4855
R_o	48	27	46.7	28.3	1.3	0.0356	0.0588
r"	17	4	13.1	7.9	3.9	1.1748	1.9402
r	704	400	687.6	416.4	16.4	0.3897	0.6436
$X_4^2 = 8.6696$		$p = 0.1$					
Descended on Both Sides with Non-Descended							
	B.S.	U.D.	B.S.	U.D.			
R_1	348	104	366.4	85.6	18.4	0.9201	3.9361
R_2	115	56	138.6	32.4	23.6	4.0185	17.1901
R_o	48	6	43.8	10.2	4.2	0.4088	1.7491
r"	17	1	14.6	3.4	2.4	0.3981	1.7033
r	704	121	668.7	156.3	35.3	1.8656	7.9804
$X_4^2 = 40.1701$		$p < 0.0005$					
Descended on One Side with Non-Descended							
	O.S.	U.D.	O.S.	U.D.			
R_1	223	104	235.9	91.1	12.9	0.7076	1.8327
R_2	92	56	106.8	41.2	14.8	2.0458	5.2996
R_o	27	6	23.8	9.2	3.2	0.4274	1.1073
r"	4	1	3.6	1.4	0.4	0.0421	0.1094
r	400	121	375.9	145.1	24.1	1.5464	4.0059
$X_4^2 = 19.1238$		$p = 0.0005$					

Table 22 Possible Phenotypes of Hodgkin's disease, Immunodeficiency & Embryonic tumour Patients.

Hodgkin's disease	
1005 R_1r.	Tested.
6019 R_1r, R_2r or r r.	
6086 R_1r or r r.	
6731 R_1r.	On pedigree r r is possible, but grouped as D+.
6765 R_1R_1 or R_1r.	
6790 r r.	
6800	Mother rx, may have been rr.
Immunodeficiency	
1141 r r.	Tested.
1148 r r.	Tested.
6500 R_1R_1 or R_1x.	Probability of R_1R_1 = 0.94, one parent is R_1R_1 as are all five siblings tested.
Embryonic tumours	
6189 R_1r, r r, R_1r'' or r''r,	
6589 R_1r.	
7238 r r.	

Table 23 Hodgkin's Disease. Number & Percentage of First, Second & Third Degree Relatives for each Phenotype & Haplotype.

Phenotype	First		Second		Third		Other	
	n	%	n	%	n	%	n	%
R_1R_1	3	10.7	4	7.5	11	7.1	77	8.6
R_1r	11	39.3	24	45.2	61	39.1	309	34.5
R_1R_2			3	5.6	11	7.0	66	7.4
R_2R_2			1	1.9	1	0.6	18	2.0
R_2r	5	17.9	2	3.8	22[1]	14.1	124	13.8
R_or					2	1.3	70	7.8
$r''r$							12	1.3
rr	9	32.1	19	35.8	48	30.8	220	24.6
	28	100.0	53	100.0	156	100.0	896	100.0
Haplotype								
R_1	17	30.4	35	33.0	94	30.1	529	29.5
R_2	5	8.9	7	6.6	35	11.2	216	12.1
R_o					3	1.0	78	4.4
r''							22	1.2
r	34	60.7	64	60.4	180	57.7	947	52.8
	56	100.0	106	100.0	312	100.0	1792	100.0

1. Includes one R_2R_o.

Table 24 Hodgkin's Disease. Comparisons Between Relatives of Different Degree For Rhesus Haplotype Numbers.

Haplotype	Degree of Relationship Observed		Expected		O - E	$(O-E)^2/E$	
	1st.	2nd.	1st.	2nd.			
R_1	17	35	18.0	34.0	1.0	0.0556	0.0294
R_2	5	7	4.1	7.9	0.9	0.1976	0.1025
R_o							
r''							
r	34	64	33.9	64.1	0.1	0.0003	0.0002
	56	106	$X_2^2 = 0.3856$		$p = 0.85$		
	2nd.	3rd.	3rd.				
R_1	35	94	32.7	96.3	2.3	0.1618	0.0549
R_2	7	35	10.7	31.3	3.7	1.2794	0.4374
R_o		3	0.8	2.2	0.8	0.8000	0.2909
r''							
r	64	180	61.9	182.1	2.1	0.0712	0.0242
	106	312	$X_2^2 = 3.1198$		$p = 0.30$		
	1st.	3rd.	1st.	3rd.			
R_1	17	94	16.9	94.1	0.1	0.0006	0.0001
R_2	5	35	6.1	33.9	1.1	0.1984	0.0357
R_o		3	0.5	2.5	0.5	0.5000	0.1000
r''							
r	34	180	32.6	181.4	1.4	0.0601	0.0108
	56	312	$X_2^2 = 0.9066$		$p = 0.80$		

Table 25 Hodgkin's Disease. Comparisons Between Relatives of Different Degree & The "Rest of the Population" (Other).

Haplotype	Degree of Relationship Observed		Expected		O - E.	$(O-E)^2/E$	
	1st.	Other	1st.	Other			
R_1	17	529	16.5	529.5	0.5	0.0152	0.0005
R_2	5	216	6.7	214.3	1.7	0.4313	0.0135
R_o	0	78	2.4	75.6	2.4	2.4000	0.0762
r"	0	22	0.7	21.3	0.7	0.7000	0.0230
r	34	947	29.7	951.3	4.3	0.6226	0.0194
	56	1792	$X_4^2 = 4.3017$		$p = 0.40$		
	2nd.	Other	2nd.	Other			
R_1	35	529	31.5	532.5	3.5	0.3889	0.0230
R_2	7	216	12.5	210.5	5.5	2.4200	0.1437
R_o	0	78	4.4	73.6	4.4	4.4000	0.2630
r"	0	22	1.2	20.8	1.2	1.2000	0.0692
r	64	947	56.5	954.5	7.5	0.9956	0.0589
	106	1792	$X_4^2 = 9.6993$		$p = 0.05$		
	3rd.	Other	3rd.	Other			
R_1	94	529	92.4	530.6	1.6	0.0277	0.0048
R_2	35	216	37.2	213.8	2.2	0.1301	0.0226
R_o	3	78	12.0	69.0	9.0	6.7500	1.1739
r"	0	22	3.3	18.7	3.3	3.3000	0.5824
r	180	947	167.1	959.0	12.9	0.9959	0.1734
	312	1792	$X_4^2 = 13.1808$		$p = 0.01$		

Table 26 Hodgkin's Disease. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Population" (Other).

Haplotype	Degree of Relationship: Observed		Expected		O - E	$(O - E)^2/E$	
	Rels.	Other	Rels.	Other			
R_1	146	529	141.2	533.8	4.8	0.1632	0.0432
R_2	47	216	55.0	208.0	8.0	1.1636	0.3077
R_o	3	78	16.9	64.1	13.9	11.4325	3.0142
r''	0	22	4.6	17.4	4.6	4.6000	1.2161
r	278	947	256.2	968.8	21.8	1.8550	0.4905
	474	1792	$\chi^2_4 = 24.2860$		$p < 0.0005$		

Table 27 Hodgkin's Disease. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients with the Rest of the Descendants (Desc) of the Original Couple.

Haplotype	Degree of Relationship: Observed		Expected		O - E	$(O - E)^2/E$	
	Rels.	Desc.	Rels.	Desc.			
R_1	146	425	136.8	434.2	9.2	0.6187	0.1949
R_2	47	160	49.6	157.4	2.6	0.1363	0.0429
R_o	3	72	18.0	57.0	15.0	12.5000	3.9484
r''	0	21	5.0	16.0	5.0	5.0000	1.5625
r	278	826	264.6	839.4	13.4	0.6786	0.2139
	474	1792	$\chi^2_4 = 24.8952$		$p < 0.0005$		

Table 28 Hodgkin's Disease. Comparisons Between 1st., 2nd. & 3rd. Degree Relatives (Rels) and the "Rest of the Population" (Other) for the Three Rhesus Genes, D, C & E.

	Degree of Relationship						
D	Observed		Expected		O - E	$(O - E)^2/E$	
Status	Rels.	Other	Rels.	Other			
D +	161	664	172.6	652.4	11.6	0.7796	0.2063
D -	76	232	64.4	243.6	11.6	2.0894	0.5524
	237	896	$\chi_1^2 = 3.6277$		$p = 0.05$		
C							
Status							
C +	128	452	121.3	458.7	6.7	0.3701	0.0979
C -	109	444	115.7	437.3	6.7	0.3880	0.1027
	237	896	$\chi_1^2 = 0.9587$		$p = 0.30$		
E							
Status							
E +	45	220	55.4	209.6	10.4	1.9523	0.5160
E -	192	676	181.6	686.4	10.4	0.5956	0.1576
	237	896	$\chi_1^2 = 3.2215$		$p = 0.10$		

Table 29 Hodgkin's Disease. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients, and the "Rest of the Descendants of the Original Couple" (Desc.) for the Three Rhesus Genes, D, C & E.

	Degree of Relationship						
D	Observed		Expected		O − E	$(O-E)^2/E$	
Status	Rels.	Desc.	Rels.	Desc.			
D +	161	543	168.7	535.3	7.7	0.3515	0.1108
D −	76	209	68.3	216.7	7.7	0.8681	0.2736
	237	752	$\chi_1^2 = 1.6040$		$p = 0.20$		
C							
Status							
C +	128	368	118.9	377.1	9.1	0.6965	0.2196
C −	109	384	118.1	374.9	9.1	0.7012	0.2209
	237	752	$\chi_1^2 = 1.8382$		$p = 0.20$		
E							
Status							
E +	45	167	50.8	161.2	5.8	0.6622	0.2087
E −	192	585	186.2	590.8	5.8	0.1807	0.0569
	237	752	$\chi_1^2 = 1.1085$		$p = 0.30$		

Table 30 Immunodeficiency. Number and Percentage of Firest, Second and Third Degree Relatives for each Phenotype and Haplotype.

Phenotype	First		Second		Third		Other	
	n	%	n	%	n	%	n	%
R_1R_1	5	27.8	4	19.0	4	6.7	82	7.9
R_1r	8	44.4	8	38.1	21	35.0	368	35.6
R_1R_2			1	4.8	2	3.3	77	7.4
R_2R_2							20	1.9
R_2r					10	16.7	143	13.8
R_0r							72	7.0
$r''r$							12	1.2
rr	5	27.8	8	38.1	23	38.3	260	25.2
	18	100.0	21	100.0	60	100.0	1034	100.0
Haplotype								
R_1	18	50.0	17	40.5	31	25.8	609	29.4
R_2			1	2.4	12	10.0	250	12.1
R_0							81	3.9
r''							22	1.1
r	18	50.0	24	57.1	77	64.2	1106	53.5
	36	100.0	42	100.0	120	100.0	2068	100.0

Table 31. Immunodeficiency. Comparisons Between Relatives of Different Degree For Rhesus Haplotype Numbers.

Haplotype	Degree of Relationship						
	Observed		Expected		O - E	$(O - E)^2/E$	
	1st.	2nd.	1st.	2nd.			
R_1	18	17	16.2	18.8	1.8	0.2000	0.1723
R_2	0	1	0.5	0.5	0.5	0.5000	0.500
R_o							
r''							
r	18	24	19.4	22.6	1.4	0.1010	0.0867
	36	42	$X_2^2 = 1.5600$		$p = 0.50$		
	2nd.	3rd.	2nd.	3rd.			
R_1	17	31	12.4	35.6	4.6	1.7065	0.5944
R_2	1	12	3.4	9.6	2.4	1.6941	0.6000
R_o							
r''							
r	24	77	26.2	74.8	2.4	0.1847	0.0647
	42	120	$X_2^2 = 4.8444$		$p = 0.10$		
	1st.	3rd.	1st.	3rd.			
R_1	18	31	11.3	37.7	6.7	3.9726	1.1907
R_2	0	12	2.8	9.2	2.8	2.8000	0.8522
R_o							
r''							
r	18	77	21.9	73.1	3.9	06945	0.2081
	36	120	$X_2^2 = 9.7181$		$p = 0.01$		

Table 32 Immunodeficiency. Comparison Between Relatives of Different Degree and the "Rest of the Population" (Other).

Haplotype	Degree of Relationship Observed		Expected		O - E	$(O - E)^2/E$	
	1st.	Other	1st.	Other			
R_1	18	609	10.7	616.3	7.3	4.9804	0.0865
R_2	0	250	4.3	245.7	4.3	4.3000	0.0753
R_o	0	81	1.4	79.6	1.4	1.4000	0.0246
r''	0	22	0.4	21.6	0.4	0.4000	0.0074
r	18	1106	19.2	1104.8	1.2	0.0750	0.0013
	36	2068	$X_4^2 = 11.3505$		$p = 0.025$		
	2nd.	Other	1st.	Other			
R_1	17	609	12.5	613.5	4.5	1.6200	0.0330
R_2	1	250	5.0	246.0	4.0	3.2000	0.0650
R_o	0	81	1.6	79.4	1.6	1.6000	0.0322
r''	0	22	0.4	21.6	0.4	0.4000	0.0074
r	24	1106	22.5	1107.5	1.5	0.1000	0.0020
	42	2068	$X_4^2 = 7.0596$		$p = 0.15$		
	3rd.	Other	3rd.	Other			
R_1	31	609	35.1	604.9	4.1	0.4789	0.0278
R_2	12	250	14.4	247.6	2.4	0.4000	0.0233
R_o	0	81	4.4	76.6	4.4	4.4000	0.2527
r''	0	22	1.2	20.8	1.2	1.2000	0.0692
r	77	1106	64.9	1118.1	12.1	2.2559	0.1309
	120	2068	$X_4^2 = 9.2387$		$p = 0.05$		

Table 33 Immunodeficiency. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Population" (Other).

Haplotype	Degree of Relationship Observed		Expected		O - E	$(O - E)^2/E$	
	Rels.	Other	Rels.	Other			
R_1	66	609	59.0	616.0	7.0	0.8305	0.0795
R_2	13	250	23.0	240.0	10.0	4.3478	0.4167
R_o	0	81	7.1	73.9	7.1	7.1000	0.6821
r"	0	22	1.9	20.1	1.9	1.9000	0.1796
r	119	1106	107.0	1118.0	12.0	1.3458	0.1288
	198	2068	$X^2_4 = 17.0108$		$p = 0.001$		

Table 34 Immunodeficiency. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Descendants" (Desc).

Haplotype	Degree of Relationship Observed		Expected		O - E	$(O - E)^2/E$	
	Rels.	Desc.	Rels.	Desc.			
R_1	66	505	57.2	515.8	8.8	1.3538	0.1507
R_2	13	194	20.7	186.3	7.7	2.8643	0.3183
R_o	0	75	7.5	67.6	7.5	7.5000	0.8333
r"	0	21	2.1	18.9	2.1	2.1000	0.2333
r	119	985	110.5	993.5	8.5	0.6538	0.0727
	198	1780	$X^2_4 = 16.0802$		$p = 0.005$		

Table 35 Immunodeficiency. Comparisons Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Population" (Other) for the Three Rhesus Genes, D, C & E.

	Degree of Relationship						
D	Observed		Expected		O - E	$(O - E)^2/E$	
Status	Rels.	Other	Rels.	Other			
D +	63	762	72.1	752.9	9.1	1.1485	0.1100
D -	36	272	26.9	281.1	9.1	3.0784	0.2946
	99	1034	$X_1^2 = 4.6315$		$p = 0.05$		
C							
Status							
C +	53	580	55.3	577.7	2.3	0.0957	0.0092
C -	46	454	43.7	456.3	2.3	0.1211	0.0116
	99	1034	$X_1^2 = 0.2376$		$p = 0.60$		
E							
Status							
E +	13	252	23.2	241.8	10.2	4.4845	0.4303
E -	86	782	75.8	792.2	10.2	1.3728	0.1313
	99	1034	$X_1^2 = 6.4187$		$p = 0.01$		

Table 36. Immunodeficiency; Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Descendants" (Desc) of the Original Couple.

D Status	Degree of Relationship Observed Rels.	Desc.	Expected Rels.	Desc.	O - E	$(O - E)^2/E$	
D +	63	641	70.5	633.5	7.5	0.7979	0.0888
D -	36	249	28.5	256.5	7.5	1.9737	0.2193
	99	890	$X_1^2 = 3.0797$		$p = 0.20$		
C Status							
C +	53	443	49.7	446.3	3.3	0.2191	0.0244
C -	46	447	49.3	443.7	3.3	0.2209	0.0245
	99	890	$X_1^2 = 0.4889$		$p = 0.50$		
E Status							
E +	13	199	21.2	190.8	8.2	3.1717	0.3524
E -	86	691	77.8	699.2	8.2	0.8643	0.0962
	99	890	$X_1^2 = 4.4846$		$p = 0.05$		

Table 37. Embryonic tumours. Number and Percentage of First, Second and Third Degree Relatives for each Phenotype and Haplotype.

Phenotype	First		Second		Third		Other	
	n	%	n	%	n	%	n	%
R_1R_1			1	4.4	2	3.0	92	9.0
R_1r	4	23.5	10[2]	43.4	26	39.4	365	35.5
R_1R_2			1[3]	4.3	3	4.6	76	7.4
R_2R_2			1	4.4	2	3.0	17	1.7
R_2r	2[1]	11.8	1	4.3	6[4]	9.1	144	14.0
R_or	1	5.9	1	4.4	6	9.1	64	6.2
$r''r$	3	17.7	3	13.0			6	0.6
rr	7	41.1	5	21.7	21	31.8	263	25.6
	17	100.0	23	100.0	66	100.0	1027	100.0
Haplotype								
R_1	4	11.8	13	28.2	33	25.0	625	30.4
R_2			3	6.5	12	9.1	248	12.1
R_o	3	8.8	2	4.4	7	5.3	69	3.4
r''	5	14.7	4	8.7	1	0.8	12	0.6
r	22	64.7	24	52.2	79	59.8	1100	53.5
	34	100.0	46	100.0	132	100.0	2054	100.0

[1] Includes two R_or''.

[2] Includes one R_1R_o.

[3] Includes one R_1r''.

[4] Includes one R_or''.

Table 38 Embryonic Tumours. Comparisons Between Relatives of Different Degree for Rhesus Haplotype Numbers

Haplotype	Degree of Relationship Observed		Expected		O - E	$(O - E)^2/E$	
	1st.	2nd.	1st.	2nd.			
R_1	4	13	7.2	9.8	3.2	1.4222	1.0449
R_2	0	3	1.3	1.7	1.3	1.3000	0.9941
R_o	3	2	2.1	2.9	0.9	0.3857	0.2793
r''	5	4	3.8	5.2	1.2	0.3789	0.2769
r	22	24	19.6	26.4	2.4	0.2939	0.2182
	34	46	$X_4^2 = 6.5941$		p = 0.20		
	2nd.	3rd.	2nd.	3rd.			
R_1	13	33	11.9	34.1	1.1	0.1017	0.0355
R_2	3	12	3.9	11.1	0.9	0.2077	0.0730
R_o	2	7	2.3	6.7	0.3	0.0391	0.0134
r''	4	1	1.3	3.7	2.7	5.6077	1.9703
r	24	79	26.6	76.4	2.6	0.2541	0.0885
	46	132	$X_4^2 = 8.3910$		p = 0.10		
	1st.	3rd.	1st.	3rd.			
R_1	4	33	7.6	29.4	3.6	1.7053	0.4408
R_2	0	12	2.5	9.5	2.5	2.5000	0.6579
R_o	3	7	2.0	8.0	1.0	0.5000	0.1250
r''	5	1	1.2	4.8	3.8	12.0333	3.0083
r	22	79	20.7	80.3	1.3	0.0816	0.0210
	34	132	$X_4^2 = 21.0732$		p = 0.0005		

Table 39 Embryonic Tumours. Comparisons Between Relatives of Different Degree (Rels) and the "Rest of the Population" (Other).

Haplotype	Degree of Relationship						
	Observed		Expected		O - E	$(O - E)^2/E$	
	1st.	Other	1st.	Other			
R_1	4	625	10.2	618.8	6.2	3.7686	0.0621
r	22	1100	18.2	1103.7	3.7	0.7522	0.0124
Other	8	329	5.5	331.5	2.5	1.1364	0.0189
	34	2054	$X_2^2 = 5.7506$		$p = 0.05$		
	2nd.	Other	2nd.	Other			
R_1	13	625	14.0	624.0	1.0	0.0714	0.0016
R_2	3	248	5.5	245.5	2.5	1.1364	0.0255
r	24	1100	24.6	1099.4	0.6	0.0146	0.0003
Other	6	81	1.9	85.1	4.1	8.8474	0.1075
	46	2054	$X_3^2 = 10.2947$		$p = 0.025$		
	3rd.	Other	3rd.	Other			
R_1	33	625	39.7	618.3	6.7	1.1307	0.0726
R_2	12	248	15.7	244.3	3.7	0.8545	0.0549
r	79	1100	71.2	1107.8	7.8	0.8545	0.0549
Other	8	81	5.4	83.6	2.6	1.2519	0.0809
	132	2054	$X_3^2 = 4.3735$		$p = 0.3$		

Table 40 Embroyonic Tumours. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Population" (Other).

Halpotype	Degree of Relationship Observed		Expected		O - E	$(O - E)^2/E$	
	Rels.	Other	Rels.	Other			
R_1	50	625	63.2	611.8	13.2	2.7570	0.2850
R_2	15	248	24.6	238.4	9.6	3.7463	0.3866
R_o	12	69	7.6	73.4	4.4	2.5474	0.2638
r''	10	12	2.1	19.9	7.9	29.7190	3.1362
r	125	1100	114.6	1110.4	10.4	0.9438	0.0974
	212	2054	$X_4^2 = 43.8825$		$p < 0.0005$		

Table 41 Embryonic Tumours. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Descendants (Desc).

Haplotype	Degree of Relationship Observed		Expected		O - E	$(O - E)^2/E$	
	Rels.	Desc.	Rels.	Desc.			
R_1	50	521	61.2	509.8	11.2	2.0497	0.2461
R_2	15	192	22.2	184.8	7.2	2.2251	0.2805
R_o	12	63	8.0	67.0	4.0	2.0000	0.2388
r''	10	11	2.3	18.7	7.7	25.7783	3.1706
r	125	979	118.3	985.7	6.7	0.3795	0.0455
	212	1766	$X_4^2 = 36.5241$		$p < 0.0005$		

Table 42. Embryonic Tumours. Comparison Between 1st., 2nd. & 3rd. Degree Relatives (Rels) of the Patients and the "Rest of the Population" (Other) for the Three Rhesus Genes, D, C & E.

	Degree of Relationship						
D	Observed		Expected		O - E	$(O - E)^2/E$	
Status	Rels.	Other	Rels.	Other			
D+	67	758	77.2	747.8	10.2	1.3477	0.1391
D-	39	269	28.8	279.2	10.2	3.6125	0.3726
	106	1027	$X_1^2 = 5.4719$		$p = 0.025$		
C							
Status							
C+	47	533	54.3	525.7	7.3	0.9814	0.1012
C-	59	494	51.7	501.3	7.3	1.0308	0.1063
	106	1027	$X_1^2 = 2.2197$		$p = 0.20$		
E							
Status							
E+	22	243	24.8	240.2	2.8	0.3161	0.0326
E-	84	784	81.2	786.8	2.8	0.0966	0.0100
	106	1027	$X_1^2 = 0.4553$		$p = 0.50$		

Table 43 Embryonic Tumours. Comparison Between 1st., 2nd. & 3rd. Degree Relatives and the "Rest of the Descendants" of the Original Couple (Desc.) for the Three Rhesus Genes, D, C & E.

	Degree of Relationship						
D	Observed		Expected		O − E	$(O-E)^2/E$	
Status	Rels.	Desc.	Rels.	Desc.			
D+	67	637	75.5	628.5	7.5	0.7450	0.0895
D−	39	246	30.5	254.4	7.5	1.8443	0.2210
	106	883	$X_1^2 = 2.8999$		$p = 0.10$		
C							
Status							
C+	47	449	53.2	442.8	6.2	0.7226	0.0868
C−	59	434	52.8	440.2	6.2	0.7280	0.0873
	106	883	$X_1^2 = 1.6247$		$p = 0.20$		
E							
Status							
E+	22	190	22.7	189.3	0.7	0.0216	0.0026
E−	84	693	83.3	693.7	0.7	0.0059	0.0007
	106	883	$X_1^2 = 0.0308$		$p = 0.80$		

Table 44 Comparisons Between the 1st., 2nd. & 3rd. Degree Relatives of the Hodgkin's Disease, Immunodeficiency & Embryonic Tumour Patients.

Hodgkin's Disease & Immunodeficiency.

Haplotype	Observed		Expected		O - E	$(O-E)^2/E$	
	H.D.	I.D.	H.D.	I.D.			
R_1	146	66	149.5	62.5	3.5	0.0819	0.1960
R_2	47	13	42.3	17.7	4.7	0.5222	1.2480
R_o	3	0	2.1	0.9	0.9	0.3857	0.9000
r"	0	0					
r	278	119	280.0	117.0	2.0	0.0143	0.0342
	474	198	$X_3^2 = 3.3823$		p = 0.40		

Hodgkin's Disease & Embryonic Tumours

	H.D.	E.T.	H.D.	E.T.	O - E	$(O-E)^2/E$	
R_1	146	50	135.4	60.6	10.6	0.8298	1.8541
R_2	47	15	42.8	19.2	4.2	0.4121	0.9188
r	278	125	278.5	124.5	0.5	0.0009	0.0020
Other	3	22	17.3	7.7	14.3	11.8202	26.5571
	474	212	$X_3^2 = 42.3950$		$p < 0.0005$		

Immunodeficiency & Embryonic Tumours

	I.D.	E.T.	I.D.	E.T.	O - E	$(O-E)^2/E$	
R_1	66	50	56.0	60.0	10.0	1.7857	1.6667
R_2	13	15	13.5	14.5	0.5	0.0185	0.0172
r	119	125	117.8	126.2	1.2	0.0122	0.0114
Other	0	22	10.6	11.4	10.6	10.6000	9.8561
	198	212	$X^2 = 23.9678$		p = 0.0005		

DISCUSSION

Genetic Linkage.

Association Via A Metabolic Pathway.

Association Via An Immune-Response (Ir) Or Immune-Associated (Ia) Locus.

The Rhesus Locus As a Susceptibility Locus.

Further Studies.

DISCUSSION

There seems little doubt from other studies (Mourant, 1978) and the data herein, that there is an association between the Rhesus blood group system and Hodgkin's disease and possibly with other malignancies. The essential differences between the prior studies and those reported here has been discussed earlier (Page 72). Possible mechanisms for such an association are;

1. Genetic linkage between the Rhesus locus and a susceptibility locus for Hodgkin's disease.
2. A metabolic pathway involving the Rhesus blood group system and a second locus which is involved with susceptibility to Hodgkin's disease.
3. The Rhesus blood group system is involved in some functional manner with either an Immune-response (Ir) or Immune-associated (Ia) locus which is involved in conferring either susceptibility or resistance to Hodgkin's disease.
4. The Rhesus locus is itself a resistance/susceptibility locus, involved in the aetiology of certain tumours.

These hypotheses are discussed in order with the Hodgkin's disease/immunodeficiency group of this study used as an example, similar reasoning will apply equally to the embryonic tumour group.

GENETIC LINKAGE

If we postulate that a susceptibility locus is on chromosome 1 within measurable distance of the Rhesus (Rh) locus, then the linkage would need to be very close in order to account for the association of dd status in random Hodgkin's disease patients. If we assume only two alleles at the susceptibility locus, Su & su, with Su conferring resistance and su susceptibility, then there are three possibilities; 1. The susceptibility allele, su, is recessive.

2. the susceptibility allele, su, is dominant and 3. The alleles are co-dominant. These possiblities can be considered seperately.

1. The Susceptibility Allele (su) is Recessive

This hypothesis is contradicted by neither this study nor the work performed on random patients. In the present study a "super-family" is involved, linkage of the su allele to a specific Rh haplotype for example r, would be expected to result in all patients being homozygous for that Rh haplotype. This is not so. It is possible that due to a rare cross-over event that in this family the su gene is travelling with two Rh haplotypes, for example r & R_1, some r & R_1 haplotypes carrying su and the others carrying Su, the proportions of the r and R_1 haplotypes carrying su would be expected to differ.

In such a case three genotypes could confer susceptibility in a proportion of cases by all being su homozygous but Rh homo- or heterozygous, the three genotypes being rr, R_1r & R_1R_1. There is a hint from the data presented herin that any susceptibility gene involved is recessive. In every case of Hodgkin's disease, immuno-deficiency and embryonic tumour, all matings in the direct ancestral line have been consanguinous over the last three generations, a pattern strongly suggestive of a recessive mode of inheritance. In addition there is the remarkable absence od two Rh haplotypes from the Hodgkin's disease and immunodeficiency patients and their first, second & third degree relatives, R_o & r". This suggests that in some manner both are able to confer resistance to these conditions to their bearers in this family, though this may be due to chance. Since both are rare haplotypes and would in the vast proportion of cases occur in the heterozygous state this suggests that su is recessive and that

the Su gene in single dose could be sufficient to confer a high degree of protection.

2. The Susceptibility Allele (su) is Dominant.

In the family studies this mode of inheritance is not shown by the data as there are no affected parent-child combinations, but it is possible that the su allele demonstrates imcomplete penetrance. Since we are studying a family all the affected individuals would be expected to share at lease one Rh haplotype in common, and this is possible for the nine for whom we can suggest possible haplotypes. The common allele being r, which fits well with the data on the random patients with Hodgkin's disease. Only a proportion of the r haplotypes of the family would be expected to carry the su allele, the majority would be associated with the Su allele.

3. The Two Alleles (Su & su) are Co-dominant.

If we argue, for the sake of simplicity, that all the su alleles are linked to r, but that not all r haplotypes carry su, then those individuals having rr would have the highest risk, those with a Rr genotype would have a median risk and those with a RR haplotype would have the lowest risk. This hypothesis is not contradicted by the data presented here nor by that on random patients.

In all three cases the strength of the association will depend upon the frequency of the su allele and the closeness of linkage, the linkage would have to be very close in order to give the association with the random patients.

The actual mode of inheritance would also affect the strength of the association. If for all three hypotheses the frequency of su and the degree of linkage were identical, then a recessive mode of

inheritance would give the strongest association and the dominant the weakest.

All three hypotheses have their weaknesses, in the first in order to explain the present findings a cross-over event, either in the family or prior to the original couple settling in Newfoundland, must be postulated. The cross-over may have occured in recent generations, or may have entered the family twice, either through two seperate paths or from a remote common ancestor, in either case such an occurance must have a very low probability.

In the second hypothesis incomplete penetrance of the gene for susceptibility has to be postulated, otherwise the family data does not fit a dominant mode of inheritance, furthermore the penetrance would have to be extremely partial. This criticism applies equally to the third hypothesis.

ASSOCIATION VIA A METABOLIC PATHWAY

The postulate here is that some Rh haplotypes, or all to a varying degree produce a precursor substance which is essential for the su gene to exert it's effect, the su gene is not linked to the Rh locus.

In such a case an association between the Rhesus blood group system and susceptibility to Hodgkin's disease would be expected in both family studies and studies of random Hodgkin's disease patients. In order to develope Hodgkin's disease the individual would have to inherit both the appropriate Rh haplotype(s) and the su gene. The su gene may be recessive, dominant or co-dominant. If we again use the r haplotype as an example, this haplotype being the only one producing the required precursor and being able to do so in both the homo- and hetero-

zygous state, then only those individuals having a r haplotype would be at risk.

In the case where su is recessive, two su alleles and one r haplotype would be required for the condition to develope, but where su is either dominant or co-dominant only one su allele and one r haplotype would be needed. Where su is co-dominant individuals having different relative risks would be expected to occur depending upon the Rh and Su/su genotype. Those with the greatest risk would be r, su/r, su or R, su/r, su, those with median risk would include r, Su/r, su; R, Su/r, su and R, su/r, Su, while the lowest risk would occur with the genotypes R, Su/R, Su, R, Su/r, Su or r, Su/r, Su.

If all Rh haplotypes produce the needed precursor, but in different amounts, then all individuals will be at risk depending upon the combined efficiency of the Rh haplotypes forming the geneotype, here the individual homozygous for the Rh haplotype having the greatest efficiency would also have the greatest risk.

Although there is no data to support this hypothesis, there is none to refute it. At this time it appears that it is a reasonable hypothesis but until the metabolic pathways involving the Rh system have been elucidated the only test must involve fully genotypeing Hodgkin's disease patients for Rh. If these can be ranked by Rh phenotype for relative risk of developing Hodgkin's disease then this would strongly support this hypothesis.

ASSOCIATION VIA AN IMMUNE-RESPONSE (Ir) OR IMMUNE-ASSOCIATED (Ia) LOCUS.

That such an interaction may be the basis of the observed association of the Rh system with Hodgkin's disease is suggested by the data of Cook & Shepherd (1977), regarding the differences in the incidence

of malignant disease in the relatives of Rh "non-responder" compared with those of "responders", (see page 14).

All studies so far performed on defining the genetical basis (if any) of the difference between Rh "responders" and "non-responder" have been concerned with demonstrating an association with HLA. This is based on the assumption that this difference is inherited and is due to either an Ir or Ia locus. In those species studied, including man, the Ir and Ia loci appear to be situated within the boundaries of the major histocompatibility locus of the species, in man within the HLA region of chromosome 6.

If there were an interaction between the Rh locus and either an Ir or Ia locus situated within the HLA boundaries, that is between HLA-A and HLA-D inclusive, then the HLA system would be expected to demonstrate associations with the Rh locus due to the linkage disequilibrium which affects the whole of the HLA region.

A working hypothesis can be developed as follows.

A common Ir or Ia gene exists in man which does not permit the carrier to respond to the Rh D antigen by producing anti-D, this gene is situated within the HLA region of Chromsome 6 and is therefore subject to linkage disequilibrium with the HLA system. This Ir or Ia gene itself predisposes to the development of certain maligancies, for example Hodgkin's disease, which therefore also demonstrates an association with the HLA system. A feedback mechanism exists between Rh and the Ir (Ia) gene by which the different Rh genotypes exert a modifying effect on the Ir (Ia) gene in such a manner as to increase or decrease the predisposition to development of the malignancy.

All searches for association between Rh and HLA have been

negative, Petranyi et. al. (1972) could not demonstrate any associations between HLA and Rh "responder" or "non-responder" status, however the numbers available were small, the authors themselves suggest that in view of the limited numbers tested failure to demonstrate such an association is not conclusive.

This hypothesis has the advantage of unifying to an extent the findings in Hodgkin's disease, that is it links the association of both HLA and Rh to Hodgkin's disease. The negative findings with respect to association between Rh and HLA are disappointing, but the crucial association of this hypothesis is that which should be detectable between HLA and Rh "responder" and "non-responder" status. If all Rh haplotypes modify to different extents the action of the Rh "responder gene" (Ir or Ia gene), then a direct association between Rh and HLA may be extremely difficult to demonstrate. This hypothesis would still be tenable in a modified form if the Rh "responder gene" were shown to be located outside the HLA region, but would lose a great deal of it's attraction.

THE RHESUS LOCUS AS A SUSCEPTIBILITY LOCUS

It is a basic tenet of genetics that in all loci where alleles occur the alleles are subject to selective pressures. It therefore follows that in any genetical locus exhibiting a balanced polymorphism, all alleles must be selected for and against by different environmental agents. Though some geneticists claim that neutral genes exist.

There are two apparent exceptions to this, the first is where the locus has one very common allele and one or more rare alleles, where the frequency of the rare alleles can be maintained by mutation, not by selection; as fast as the rare allele is lost by selection it is replaced

by a fresh mutation. The second case is where the rarer allele has obvious disadvantages but these develope after the reproductive life is over, for example Huntington's chorea, but here too the situation must be that the gene is maintained by mutation and that there is some subtle selective pressure acting on the heterozygote in early life in addition to the severe pressure commencing in the third or fourth decade.

In the Rh system which is highly polymorphic, one selective pressure is known, this is haemolytic disease of the newborn (H.D.N.). Well over 90% of cases involve the D+/D- distinction and with each foetus or infant lost the population loses both a D+ and a D- haplotype, in such a situation the selective pressure is directed against the D- haplotypes. Over the geological period of time during which Rh must have existed in man it would be expected that all D- haplotypes, that is r, cde; r', Cde and r'', cdE, would be lost. In most populations the whole system seems to be balanced and though r' and r'' are rare they are not at the level at which they could be replaced by mutation. In Caucasian populations Fisher's cross-over theory fits their frequency so they may be maintained in a population in this manner, however the figures did not fit nearly as well for other populations, such as Negroes.

As they are in balance they must have an advantage which offsets the known selective pressure against them. It may be assumed that all Rh haplotypes are subject to different selective advantages and disadvantages.

It appears reasonable to suppose that amongst the conflicting advantages and disadvantages different susceptibilities to different neoplastic diseases occur.

The data of Petranyi et. al. (1972) would lend some support to

this hypothesis since there were marked differences in the immunological responses of the D+ and D- groups, as measured by their responses to bacterial antigens by antibody titre, numbers of PHA reaponsive lyphocytes and PHA dose needed for maximal response by ^{3}H-thymidine uptake in tissue culture. These authors suggested that in some manner Rh was associated with a gene involved in regulation of immune responses.

If the Rh haplotypes of themselves predisposed to development of malignant conditions it would be expected that many more associations than are presently known would have been reported, in addition the relative weakness of the association between D negative state and Hodgkin's disease in random patients tends to suggest the link is not as direct as this.

FURTHER STUDIES

Studies of other families containing multiple cases of Hodgkin's disease (or other malignancies) are of primary importance and should include the full Rh and HLA typing wherever possible, followed by assignment of actual genotypes as far as possible. Should the results support those herein the intimate involvement of the Rh system in development of malignancy would be strongly supported.

If it were possible in the present study to include work on the other chromosome 1 loci which are known or suspected to be within measurable distance of the Rh locus the value would be much increased. The other loci which are known to be close to Rh, or in that general area of the chromosome, are;

Enolase-1

6-phophgluconate dehydrogenase

Adenylate kinase 2

Phosphoglucomutase-1

Uridine monophosphate kinase

Scianna blood group

One or more of these may show a stronger association with Hodgkin's disease than does Rh, which would suggest that there is in fact a susceptibility locus on chromosome 1, and might even give some clue to location. In the absence of such associations the work may still be of value in differentiating between homologous Rh haplotypes, for example the Scianna blood group locus is in the general area of the Rh locus and could possibly be used in such a manner, but the actual distance between the two is not yet known.

The Scianna system at present consists of two alleles, Sm & Bu^a, with the latter occurring in about 1/130 Caucasians. If in a family both were present and Bu^a were travelling with some of the r haplotypes this would permit differentiation of two types, r-Bu^a & r-Sm. Any individual of genotype rr could then be placed in one of three groups, r-Sm/r-Sm, r-Sm/r-Bu^a or r-Bu^a/r-Bu^a, the first may or may not be homozygous for homologous r haplotypes, the second are not likely to be homologous with respect to r while the third group probably would be depending upon how frequently cross-overs occur which is directly related to the distance between the two loci.

However assuming a relatively close linkage in a family which demonstrated a degree of inbreeding any homozygous r-Bu^a individual would almost certainly have recieved both twice from a single ancestor. However since Bu^a is a rare antigen the families in which it occurs and would be informative are also extremely rare.

Since the association between Hodgkin's disease and Rh is demonstrable at the population level, studies of the actual Rhesus genotypes occuring in Hodgkin's disease patients together with the haplotype frequencies may well be of value. This can be done relatively easily by typing the parents or spouse and children of each patient to enable assignment of an actual genotype to the patient.

If Rh is itself a susceptibility locus the above studies would be invaluable, in this case any associations between other chromosome 1 markers and Hodgkin's disease would be weaker than the association with Rh itself, but the associations would be expected and the strength of association should reflect the distance between Rh and the marker locus. If the Rh, Hodgkin's disease association is due to a susceptibility locus on chromosome 1, then associations with other chromosome 1 markers would be expected to be either stronger or weaker depending upon the position of the susceptibility locus among other factors.

In the other two hypotheses, Rhesus involved in a metabolic pathway, and Rh involved via an Ir (Ia) locus these chromosome 1 markers should show no association whatsoever with Hodgkin's disease.

Another important study is that of the basis for the Rh "responder" "non-responder" difference, as this has been tentatively implicated in susceptibility to malignancy. Although a genetic basis has been assumed it has not been investigated by means of family studies. Family studies are required to confirm or refute the hypothesis that this distinction is an inherited characteristic. Suitable family studies are difficult to perform since kindreds with a large number of Rh negative members are needed of which only the males and post-menopausal females can be tested, as the testing involves repeated injections of

small amounts (circa 1 ml.) D+ red cells untill anti-D is formed or the individual has been injected at least five times. These difficulties are almost certainly the reason such studies have not been performed.

It may be possible to carry out such a study on a prospective basis. In some countries D negative males volunteers are being immunized in order to produce anti-D for blood grouping. Blood donation is frequently a family affair with both parents attending and bringing their children along as these reach the age of 18 years and wish to donate themselves. Such a study would be expected to be very informative as to whether or not Rh is itself a susceptibility locus.

The present data support the reported association of Rh with Hodgkin's disease as there is a gradient of rr frequency in this population. The group which is not descended from the founding couple shows no increase in frequency, the group descended on one side shows an increase of 34%, while the group descended from the original pair on both sides demonstrates a 43% excess of the genotype when compared with the expected figures for the U.K.

In this family both R_o & r" appear to be exerting some kind of protective effect in both Hodgkin's disease and immunodeficiency, as both are practically completely absent from the relatives of these disease groups, though this may be due to chance.

The r" frequency does not differ significantly in any group from that expected for the U.K., but R_o does, in the group not descended from the founder couple the frequency is the same as that of the U.K., but the descendants of this couple show an increase of 48%.

In the embryonic tumour group r" appears to be involved since the frequency is increased and there is a frequency gradient from first to second to third degree relatives of the patients.

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APPENDIX I

APPENDIX I

RHESUS PHENOTYPES & GENOTYPES OF ALL MEMBERS OF THE STUDY POPULATION FROM WHOM BLOOD WAS AVAILABLE FOR TESTING.

The number, following the four digit sample number, codes for descent from the original couple.

0 = not descended.

1 = descended on one side.

2 = descended on both sides.

An * following the sample number indicates that on pedigree analysis the ABO &/or Rhesus type of that individual did not fit with the ABO &/or Rhesus types of the parents.

A list of this together with the blood group systems demonstrating exclusion and the parent excluded, follow after the phenotype and genotype list.

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
1001	2	R_1r	R_1r	1003	2	R_1r	
1004	2	R_2r		1005	2	R_1r	
1006	2	R_1r		1008	2	R_1r	
1009	1	R_1R_2		1011	1	R_1r	
1012	1	rr	rr	1013	1	R_1r	R_1r
1014	2	R_1r		1015	2	R_2r	R_2r
1016	1	R_1R_1		1018	1	rr	rr
1020	2	R_or	R_or	1021	2	R_2R_2	R_2r''
1022	2	R_2r	R_2r	1023	2	rr	rr
1024	2	rr	rr	1027	2	R_1R_1	R_1R_1
1028	2	rr	rr	1029	2	R_1r	R_1r
1030	2	R_1r	R_1r	1032	2	R_2r	
1033	2	R_or	R_or	1034	2	R_or	R_or
1035	2	R_or	R_or	1036	2	R_2r	R_2r
1037	1	R_1r	R_1r	1038	1	R_1r	R_1r
1039	2	rr	rr	1040	1	rr	rr
1041	2	R_2r		1042	2	rr	rr
1043	2	R_2r	R_2r	1044	2	R_2r	R_2r
1045	2	R_2r	R_2r	1046	2	rr	rr
1047	1	R_2R_2		1049	2	R_1R_2	
1051	2	R_1r		1052	2	R_1r	R_1r
1053	2	R_2r	R_2r	1054	1	R_2r	
1055	1	rr	rr	1056	2	R_2r	R_2r
1057	1	R_2r		1058	2	R_2R_2	R_2r''
1059	2	R_2R_2	R_2r''	1060	2	R_2r	
1061	1	R_2r	R_2r	1062	0	R_1r	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
1063	1	R_2r	R_2r	1064	2	R_1r	R_1r
1065	2	R_1r	R_1r	1066	2	rr	rr
1067	2	rr	rr	1068	2	R_1r	R_1r
1069*	2	R_1r		1070	1	R_1R_2	
1071	2	rr	rr	1072	2	rr	rr
1077	2	rr	rr	1078	2	R_1R_1	
1079	2	R_1r	R_1r	1080	1	rr	rr
1081	2	R_2r	R_2r	1082	1	R_2r	R_2r
1083	1	R_1r		1084	2	R_1r	R_1r
1085	0	R_1R_1		1086	2	rr	rr
1087	2	R_2r	R_2r	1090	2	R_1r	R_1r
1091	2	rr	rr	1093	2	rr	rr
1094	1	rr	rr	1095	2	R_1r	
1096	1	R_1R_2		1097	1	R_1R_2	
1098	1	R_2R_2		1099	1	rr	rr
1100	2	rr	rr	1101	2	rr	rr
1102	1	R_1r	R_1r	1103	0	R_1r	R_1r
1104	1	R_1r		1105	2	R_1r	
1112	2	rr	rr	1113	2	rr	rr
1115	2	R_2r	R_2r	1116	2	R_2r	R_2r
1117	2	rr	rr	1118	2	R_1r	R_1r
1119	2	R_1R_1		1120	2	R_1R_2	
1123	1	R_2r	R_2r	1124	1	R_1r	R_1r
1125	1	R_1r	R_1r	1126	1	R_2r	R_2r
1127	1	R_2r	R_2r	1128	2	rr	rr
1129	0	R_1R_2	R_1R_2	1130	2	R_2r	R_2r

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
1131	0	R_1r	R_1r	1132	2	R_1R_2	R_1R_2
1133	2	R_1r	R_1r	1134	2	R_2r	R_2r
1135	1	R_2r	R_2r	1136	1	R_2r	R_2r
1137	2	rr	rr	1138	1	rr	rr
1139	2	rr	rr	1141	2	rr	rr
1142	2	rr	rr	1143	2	rr	rr
1144	2	rr	rr	1145	2	R_1R_1	R_1R_1
1146	1	rr	rr	1147	1	R_1R_1	R_1R_1
1148	2	rr	rr	1149	2	R_1r	R_1r
1150	2	R_2r	R_2r	1151	2	R_1R_1	
1152	2	R_1R_1	R_1R_1	1153	2	R_1r	R_1r
1154	1	R_1R_1		1155	0	R_2R_2	
1157	1	R_1r	R_1r	1158	2	R_1r	R_1r
1159	2	R_2R_2		1160	2	R_1r	R_1r
1161	2	R_1r	R_1r	1162	2	R_1r	R_1r
1163	2	R_1r	R_1r	1164	2	rr	rr
1165	2	R_1R_1		1166	2	R_1R_1	
1168	2	R_1R_1		1169	2	rr	rr
1170	2	rr	rr	1171	2	rr	rr
1172	2	rr	rr	1173	2	rr	rr
1174	2	rr	rr	1175		R_1r	R_1r
1177	2	R_1r	R_1r	1178	2	R_2r	R_or''
1179	1	R_2r		1180	2	R_1r	
1182	1	R_1r		1183	1	R_or	R_or
1184	1	R_1r		1185	2	rr	rr
1186	2	R_1r	R_1r	1191	2	R_1r	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
1196	1	R_2r		1197	1	R_1r	
1198	1	R_2r		1199	1	rr	rr
1200	1	rr	rr	1201	1	R_2r	
1202	2	R_2r	R_2r	1203	2	R_1r	
1204	2	R_1r		1205	1	rr	rr
1206	1	rr	rr	1207	2	R_1r	R_1r
1208	1	rr	rr	1209	2	R_1r	
1210	2	R_1r	R_1r	1211	2	R_1r	R_1r
1212	2	R_1r	R_1r	1213	2	R_1R_1	R_1R_1
1214	2	R_2r	R_2r	1217	2	R_1r	R_1r
1218	2	rr	rr	1220	1	rr	rr
1221	2	R_1r	R_1r	1222	2	rr	rr
1223	2	R_1r	R_1r	1225	2	R_1r	R_1r
1226	2	r"r	r"r	1229	2	R_1r	R_1r
1230	2	rr	rr	1231	2	rr	rr
1232	2	r"r	r"r	1233	0	R_1r	
1234	0	R_1R_1		1235	2	R_2r	$R_or"$
1236	2	R_1r	R_1R_o	1237	2	rr	rr
1238	2	rr	rr	1239	2	rr	rr
1240	2	rr	rr	1241	2	R_1r	R_1r
1242	2	R_1r		1243	1	rr	rr
1245	2	R_1r		1247	2	R_1r	
1248	2	rr	rr	1250	1	rr	rr
1251	1	R_1r		1252	2	R_2r	
1253	2	R_1r		1254	1	rr	rr
1255	2	R_1r	R_1r	1256	2	R_or	R_or

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
1257	1	r"r	r"r	1258	2	r"r	r"r
1259	2	R_2r	R_2r	1260	1	R_1R_1	
1289	1	rr	rr	1343		rr	rr
1344	1	rr	rr	1345	2	R_1R_2	R_1r"
1346	2	R_1r	R_1r	1347	2	rr	rr
1348	2	r"r	r"r	1349	2	r"r	r"r
1350	2	rr	rr	1351	1	rr	rr
1503	1	R_or	R_or	2001*	2	R_2r	
2002*	2	R_2r		2003	2	R_2r	R_2r
2004	2	R_1r	R_1r	2005	2	R_1r	R_1r
2006	2	R_1r	R_1r	2007	2	R_1r	R_1r
2008	2	R_1R_1	R_1R_1	2009*	2	R_1R_1	
2010	2	R_1r	R_1r	2011	2	rr	rr
2012	2	rr	rr	2013	2	rr	rr
2014	2	R_1r		2015	2	R_1r	
2016	2	R_1r		2017	2	R_1r	
2018	2	R_or	R_or	2019	1	R_1	R_1r
2020	1	R_1r	R_1r	2021	1	rr	rr
2022	2	R_2r	R_2r	2025	2	rr	rr
2026	2	R_1R_1	R_1R_1	2028	2	R_2r	R_2r
2029	2	rr	rr	2030	1	R_1r	
2031	2	R_1r		2032	2	rr	rr
2033	2	rr	rr	2034	2	R_1r	R_1r
2035	1	R_1r		2036	2	rr	rr
2037	2	R_1r	R_1r	2039	2	R_1r	R_1r
2040	1	R_1r		2041	1	R_1R_2	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
2044	2	R_1r	R_1r	2045	2	R_2r	R_2r
2046	2	rr	rr	2047	2	R_2r	R_2r
2048	2	R_2R_2	R_2R_2	2049	2	R_2r	R_2r
2052	1	R_1r	R_1r	2053	1	R_1r	R_1r
2054	1	R_1r	R_1r	2055	1	R_1r	R_1r
2058	1	R_2r	R_2r	2060	1	R_2r	R_2r
2061	0	R_1R_1		2063	2	rr	rr
2064	2	R_1r		2065	2	rr	rr
2066	2	R_1r		2067	1	R_1r	R_1r
2068	2	R_1r	R_1r	2069	2	R_1r	
2070	1	R_1r		2071	2	rr	rr
2072	1	R_1r		2073	2	R_1r	R_1r
2074	2	rr	rr	2075	2	R_1r	R_1r
2076	2	rr	rr	2077	2	R_1r	R_1r
2078	2	rr	rr	2080	1	rr	rr
2081	2	R_2r	R_2r	2082	1	R_2r	
2083	1	R_1r		2084	2	R_2r	R_or''
2085	2	R_or	R_or	2086	2	R_or	R_or
2088	2	rr	rr	2089	1	R_1r	R_1r
2090	2	R_1r	R_1r	2091	2	R_1r	R_1r
2092	1	R_2r		2093	1	rr	rr
2094	2	R_1r	R_1r	2096	2	rr	rr
2097	2	R_1r	R_1r	2100*	2	R_2R_2	
2101	2	R_1R_2	R_1R_2	2102	2	rr	rr
2103	2	rr	rr	2104	2	R_1r	R_1r
2105	2	R_2R_2	R_2R_2	2106	2	R_or	R_or

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
2107	2	R_1r	R_1r	2108	2	R_1r	
2109	2	rr	rr	2111	2	R_2r	
2112	2	R_1R_2		2114	2	R_1r	R_1r
2115	2	rr	rr	2116	1	R_1r	R_1r
2118	1	R_1r	R_1r	2195	2	R_1r	R_1r
2196	1	R_1r	R_1r	2197	2	rr	rr
2198*	2	rr	rr	2199	2	R_1r	
3001	2	rr	rr	3002	1	R_or	R_or
3003	1	R_1r	R_1r	3004	1	rr	rr
3006	1	rr	rr	3007	1	rr	rr
3009	0	R_1r	R_1r	3010	1	R_or	R_or
3011	2	R_1r		3012	2	R_2r	
3013*	2	rr	rr	3014	1	R_1r	
3015	1	rr	rr	3016	2	R_1R_2	
3017	2	R_or	R_or	3018	1	R_1r	
3019	1	R_or	R_or	3020	1	R_1r	R_1r
3021	2	R_1r		3023	2	r"r	r"r
3025	2	R_1r		3026	2	R_or	R_or
3027	2	R_1r		3029	2	R_or	R_or
3031	2	R_or	R_or	3032	1	R_1r	
3034	0	R_2r		3035	1	R_1r	
3037	2	R_1r	R_1r	3038	2	R_1r	R_1r
3039	0	rr	rr	3040	0	R_1R_2	
3042	0	R_1r		3043	1	R_or	R_or
3045	2	rr	rr	3046	0	R_1R_1	
3048	2	R_1r		3049	2	R_1r	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3050	2	$R_o r$	$R_o r$	3054	2	$R_1 r$	$R_1 r$
3055	2	$R_1 r$	$R_1 r$	3056	2	$R_1 R_2$	
3057	0	rr	rr	3058	0	rr	rr
3039	1	$R_1 r$	$R_1 r$	3060	2	$R_1 r$	$R_1 r$
3063	2	$R_2 r$		3064*	2	$R_1 R_1$	
3065	1	$R_2 r$		3066	1	$R_o r$	$R_o r$
3067	1	$R_1 r$		3068	2	$R_o r$	$R_o r$
3069	2	rr	rr	3072	2	$R_o r$	$R_o r$
3073	2	$R_o r$	$R_o r$	3074	2	$R_2 r$	$R_2 r$
3075	2	$R_1 R_1$		3076	1	$R_1 r$	$R_1 r$
3077	2	$R_1 r$		3080	2	rr	rr
3081	2	$R_1 r$	$R_1 r$	3082	2	$R_1 r$	$R_1 r$
3083	2	rr	rr	3084	2	$R_1 r$	$R_1 r$
3085	2	$R_1 r$	$R_1 r$	3086	1	$R_1 r$	
3088	1	$R_o r$	$R_o r$	3090	2	rr	rr
3091*	2	$R_1 R_2$		3092	2	$R_1 r$	$R_1 R_o$
3093*	2	$R_2 r$		3095	1	$R_1 R_1$	$R_1 R_1$
3096	3	$R_1 r$	$R_1 r$	3097	0	$R_1 R_1$	
3098	2	$R_1 r$	$R_1 r$	3099	2	rr	rr
3102	1	$R_1 R_1$		3103	2	$R_1 r$	$R_1 r$
3104	0	$R_2 r$	$R_2 r$	3105	1	$R_o r$	$R_o r$
3106	2	rr	rr	3110	2	$R_1 r$	
3114	0	$R_2 r$	$R_2 r$	3116	1	rr	rr
3117	1	$R_o r$	$R_o r$	3119	2	$R_1 R_2$	
3120	1	rr	rr	3122	1	rr	rr
3123*	2	r"r	r"r	3124	2	$R_2 R_2$	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3125	0	R_2r	R_2r	3126	2	R_1r	R_1r
3127	1	R_2r	R_2r	3129	2	R_1r	R_1r
3130	1	R_1r	R_1R_o	3133	2	R_1r	R_1r
3135	2	R_1r	R_1r	3141	2	R_1R_2	
3143	0	R_2r	R_2r	3144	0	rr	rr
3146	0	R_1r		3147	2	rr	rr
3148*	2	R_1R_1		3149	2	R_or	R_or
3150	1	R_1r		3151	2	rr	rr
3152*	2	R_1R_1		3153	1	rr	rr
3155	2	rr	rr	3156	1	R_1r	R_1r
3157	0	R_or	R_or	3158	2	R_1r	
3159	2	R_1r		3164	2	rr	rr
3166	0	R_1r		3171	0	R_1r	t
3172	2	R_1R_1		3173	0	rr	rr
3174	0	R_1R_1		3175	2	R_or	R_or
3176	0	rr	rr	3177	0	rr	rr
3178	2	R_or	R_or	3179*	2	R_1R_1	
3180*	2	R_1R_1		3181	2	R_1R_1	
3183	2	R_1r	R_1r	3186	2	rr	rr
3188*	2	R_1r		3189	2	R_or	R_or
3190	1	R_or	R_or	3191	1	R_or	R_or
3194	1	rr	rr	3195	1	R_1r	
3197	1	R_1r		3199	1	rr	rr
3202	1	R_1r		3203	0	R_1r	R_1R_o
3204	1	R_1r		3205	0	R_or	R_or
3206	0	R_1R_1		3208	1	rr	rr

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3209	2	R_2r	R_2r	3210	1	r_2r	R_2r
3212	0	rr	rr	3212	0	R_or	R_or
3214	0	R_1R_1		3215	0	rr	rr
3217	0	R_1r		3219	1	R_2r	R_2r
3220	0	R_1r		3221	1	R_1r	
3222	1	R_2r	R_2r	3223	1	R_1R_2	R_1R_2
3224	1	rr	rr	3226	1	rr	rr
3227	1	R_1R_2	R_1R_2	3228	1	R_1R_2	R_1R_2
3229	2	R_2r	R_2r	3230	0	R_1r	R_1r
3231	1	rr	rr	3232	1	rr	rr
3233	2	R_1r		3234	2	rr	rr
3235	0	rr	rr	3236	0	R_1r	R_1r
3237	0	R_2r	R_2r	3238	1	rr	rr
3242	2	R_1R_2		3243	2	R_1R_2	
3244	1	R_1r		3246	2	R_1r	
3247	1	R_1R_2		3248	2	R_1R_1	
3249	1	R_2r	R_2r	3250*	2	R_1R_2	
3251	2	R_1r		3252*	2	R_1r	
3255	1	R_1r		3256	1	R_2R_2	
3257	2	R_1R_2		3258	1	R_2r	R_2R_o
3259	2	R_2r	R_2r	3260	2	R_1R_2	
3261	1	rr	rr	3262	1	R_2r	
3263	2	rr	rr	3265	1	rr	rr
3266	2	rr	rr	3267	2	rr	rr
3269	1	rr	rr	3270	0	R_2r	
3271	0	R_2r	R_2r	3272	1	R_2r	R_2r

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3273	2	R_1r		3274	2	rr	rr
3275	2	R_2r	R_2r	3276	1	rr	rr
3278*	2	rr	rr	3279*	2	R_1R_1	
3281	2	R_1r	R_1r	3286	2	R_1R_1	
3287*	1	R_1r		3288*	2	R_1R_1	
3289	1	rr	rr	3294	2	R_1R_1	
3296*	2	R_1R_1		3297	0	R_1R_2	
3298*	1	R_1R_1		3300	0	rr	rr
3301	2	R_1R_1	R_1R_1	3302	2	R_1r	R_1r
3304*	1	R_1R_1		3305	0	R_2r	
3306	0	R_1R_1		3307	0	R_1r	
3308	0	R_1R_1		3309	0	R_1r	
3310	1	R_1r	R_1R_o	3311	1	R_1R_2	R_1R_2
3312	2	R_or	R_or	3316	2	R_1r	R_1r
3317	2	rr	rr	3319	1	R_or	R_or
3321	0	R_1r		3322	1	R_1r	
3224	2	rr	rr	3325	2	R_or	R_or
3327	2	rr	rr	3328	2	R_or	R_or
3329	2	R_1R_1		3332	2	R_1r	
3333	2	R_1r		3334	2	R_1r	
3335	2	R_1R_1		3336	2	rr	rr
3337	2	rr	rr	3338	1	R_1r	R_1r
3340	2	rr	rr	3342	2	rr	rr
3343	1	R_1r	R_1r	3344	1	R_1r	
3347	1	R_1r		3349	0	R_or	R_or
3352	2	rr	rr	3354	1	R_or	R_or

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3355	1	$R_o r$	$R_o r$	3356	2	$R_1 r$	
3360	0	$R_1 r$	$R_1 r$	3361	1	$R_1 r$	$R_1 r$
3362	1	rr	rr	3363	1	$R_1 r$	$R_1 r$
3364	2	$R_1 r$		3365	2	$R_1 r$	$R_1 r$
3367	2	$R_1 R_2$	$R_1 R_2$	3369	1	rr	rr
3370	2	rr	rr	3371	2	rr	rr
3372	2	$R_1 R_1$		3373	1	$R_1 R_1$	
3374	2	$R_1 r$		3375	0	$R_1 R_2$	
3377	2	rr	rr	3378	1	$R_2 r$	
3379	1	$R_1 r$		3384	2	$R_o r$	$R_o r$
3385	2	$R_1 r$		3386*	1	$R_2 R_2$	
3387	0	$R_1 R_2$		3399	1	$R_1 r$	
3390	1	$R_2 r$		3392	1	rr	rr
3395	1	$R_o r$	$R_o r$	3396	1	rr	rr
3397	0	$R_2 r$		3398	1	$R_1 r$	
3399	2	$R_1 r$	$R_1 r$	3400	1	$R_2 r$	
3401*	1	rr	rr	3402	0	$R_2 r$	$R_2 r$
3403	1	$R_2 r$	$R_2 R_o$	3404	1	$R_2 r$	
3405	1	$R_1 r$		3406*	0	$R_1 R_2$	
3407	1	$R_1 r$		3409	1	$R_o r$	$R_o r$
3410	2	$R_1 r$	$R_1 r$	3411	2	$R_1 r$	
3412	2	$R_1 r$		3414	2	$R_o r$	$R_o r$
3416*	2	$R_2 r$		3417	2	$R_1 r$	
3418	2	$R_1 r$		3420	2	$R_1 r$	
3421	2	$R_1 r$	$R_1 r$	3423	1	rr	rr
3424	1	rr	rr	3425	0	$R_1 r$	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3427	1	R_1r		3428	1	R_1r	
3429	1	rr	rr	3430	2	R_1r	R_1r
3431	2	R_1r	R_1r	3432	2	rr	rr
3433	2	rr	rr	3434	2	R_1r	
3435	2	R_1R_2		3435	2	R_1r	
3437	2	R_1r		3439	2	R_1R_1	
3440	0	R_1r		3441	2	R_1r	
3442	2	R_1r	R_1r	3443	2	rr	rr
3444	1	R_1r	R_1r	3443	0	R_1R_1	R_1R_1
3446	1	R_1R_1	R_1R_1	3448	1	R_1r	R_1r
3449	1	R_1r	R_1r	3451	1	R_1r	R_1r
3452	1	R_1R_1	R_1R_1	3453	2	R_or	R_or
3454	0	R_2r		3455	2	R_1r	R_1r
3456	2	R_or	R_or	3457	1	R_1R_1	
3458	1	R_1r		3460	1	R_1R_2	
3461	1	R_1r	R_1r	3462	1	R_or	R_or
3463	1	R_1R_2		3466	1	R_1r	
3468	2	R_1r		3469	2	R_or	R_or
3471	2	R_or	R_or	3472	2	R_or	R_or
3473	2	R_1r		3475	2	rr	rr
3476	2	rr	rr	3477	2	rr	rr
3478	2	R_2r		3479	2	R_2r	
3480	0	R_2R_2		3481	1	R_2R_2	
3482	1	R_2r		3483	1	R_2R_2	
3485	2	R_2r		3486	0	R_2r	
3487	2	R_1r		3489	2	rr	rr

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3490	2	R_2r	R_2r	3491	2	rr	rr
3492	2	rr	rr	3493	0	R_1r	
3495	1	R_2r		3496	0	R_1R_1	
3497	0	R_1r		3499	2	R_2r	R_2r
3500	2	R_2r	R_2r	3501	2	rr	rr
3505	2	R_1r	R_1r	3506	2	R_1r	R_1r
3509	2	rr	rr	3510	2	rr	rr
3511	2	rr	rr	3512	2	rr	rr
3514	2	rr	rr	3516	1	R_1R_2	R_1R_2
3517	1	R_2r	R_2r	3518	1	R_1R_2	R_1R_2
3519	1	R_2r	R_2r	3520	1	R_2r	R_2r
3522	1	R_1r	R_1r	3525	2	R_1r	R_1r
3527	1	rr	rr	3528	2	rr	rr
3529	2	R_2r	R_2r	3530	2	R_1R_1	R_1R_1
3531	0	R_2r		3532	2	R_1r	R_1r
3533	0	R_or	R_or	3534	0	R_2r	
3535	2	R_1R_1	R_1R_1	3537	2	R_1R_2	R_1R_2
3538	2	R_1R_2	R_1R_2	3539	2	R_1r	R_1r
3540	0	R_2r	R_2r	3541	2	R_1r	R_1r
3543	2	R_1r		3545*	1	R_1r	
3546	0	R_1R_1		3547	0	R_2r	
3548*	1	R_1R_1		3553	2	rr	rr
3554	1	R_1r	R_1r	3555	1	R_1r	R_1r
3556	2	rr	rr	3557	1	R_1r	R_1r
3559	0	R_2R_2		3560	2	rr	rr
3561	0	R_2r		3562	0	R_1R_1	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3563	2	R_2r		3564	2	R_or	R_or
3565	2	rr	rr	3566	2	R_2r	
3567	2	R_2r	R_2R_o	3568	0	R_1R_1	
3569	0	R_1r		3570	2	R_1r	R_1r
3571	2	R_1R_2	R_1R_2	3572	2	rr	rr
3573	1	R_1r		3575	1	rr	rr
3577	1	R_1r		3580	1	rr	rr
3581	0	R_1R_2		3582	1	R_1r	R_1r
3583	2	R_1R_1		3584	2	R_1r	
3585	1	R_1r		3587	2	R_1r	
3588	2	R_1R_1		3589	1	rr	rr
3590	0	rr	rr	3591	1	rr	rr
3592	2	R_1r		3593	2	R_1r	
3594	2	R_1r		3595	2	R_1r	R_1r
3597	0	R_1r		3598	2	rr	rr
3599	1	R_2r	R_2r	3601	2	R_1r	R_1r
3605	2	rr	rr	3606	2	rr	rr
3607	2	rr	rr	3609	2	rr	rr
3612	1	R_1r	R_1r	3614	1	R_1r	R_1r
3615	1	R_1R_2		3617*	1	R_1R_1	
3618	1	R_1r		3620	2	R_1r	
3629	1	R_1r		3630	1	R_1r	
3631	1	R_1r		3632	2	R_1R_1	
3633	0	R_1R_2		3634	2	rr	rr
3637	0	R_1R_1		3638	1	R_1r	
3639	0	R_1r		3640	2	R_1r	R_1r

Sample		Phentoype	Genotype	Sample		Phenotype	Genotype
3641	2	rr	rr	3642	2	rr	rr
3644	0	R_1R_2		3645	1	R_1R_2	
3646	1	$R_o r$	$R_o r$	3647	1	rr	rr
3648	0	$R_1 r$		3649	0	R_1R_2	
3658	1	$R_1 r$		3659	0	$R_1 r$	
3660	1	R_1R_1		3661	1	R_1R_1	
3662	1	R_1R_2		3663	0	$R_1 r$	
3664	1	$R_2 r$		3667	2	rr	rr
3668	2	$R_1 r$	$R_1 r$	3669	2	$R_1 r$	$R_1 r$
3671	0	R_1R_2		3675	2	$R_2 r$	
3676	2	$R_o r$	$R_o r$	3677	2	$R_2 r$	
3679	2	rr	rr	3680*	2	$R_1 r$	
3681	2	rr	rr	3683	2	rr	rr
3684	2	rr	rr	3686	2	rr	rr
3687	2	rr	rr	3689	1	$R_2 r$	
3690	2	$R_1 r$	$R_1 r$	3691	2	$R_1 r$	$R_1 r$
3692	2	R_1R_1		3693	2	R_1R_1	
3694	2	rr	rr	3695	2	rr	rr
3697	1	$R_2 r$	$R_2 r$	3698	2	$R_2 r$	
3699	1	$R_1 r$		3700	2	$R_o r$	$R_o r$
3701	2	$R_2 r$		3702	2	rr	rr
3703	2	$R_2 r$		3704	2	$R_2 r$	
3708	2	rr	rr	3709	0	$R_1 r$	$R_1 r$
3710	1	rr	rr	3711	1	rr	rr
3712	0	$R_1 r$		3715	0	R_1R_2	
3717	1	$R_1 r$	$R_1 r$	3718	2	$R_1 r$	$R_1 r$

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3719	0	R_1r	R_1r	3721	1	R_1r	R_1r
3722	1	R_1r	R_1r	3723	1	R_1r	R_1r
3724	1	rr	rr	3725	0	R_1R_2	
3726	1	rr	rr	3728	2	R_1r	R_1r
3729	0	R_1r		3730	0	R_1R_1	
3731	0	R_1R_1		3732	0	R_1r	
3734	2	R_1r		3736	1	R_1r	
3737	1	rr	rr	3738	1	R_or	R_or
3739	1	R_1r		3740	2	R_1r	
3742	2	R_1r	R_1r	3743	0	R_2r	
3744	1	R_or	R_or	3745	1	R_1R_2	
3748	2	R_1r	R_1r	3750	2	rr	rr
3751*	1	R_1r		3752	2	R_1r	R_1r
3753	1	R_1r		3754	2	R_1R_1	
3756	2	R_1r	R_1r	3758	2	R_or	R_or
3759	1	R_1R_1		3761	2	R_1r	
3766	2	R_1r	R_1r	3768	1	rr	rr
3769	1	rr	rr	3770*	2	rr	rr
3772	0	R_2r		3774	1	R_1r	R_1r
3777	2	R_1r	R_1r	3778	2	R_1r	R_1r
3779	2	rr	rr	3780	2	rr	rr
3781	2	R_1r	R_1r	3783	0	R_1r	
3786	2	R_or	R_or	3789	0	R_1r	
3790	2	rr	rr	3791	1	R_1R_1	
3792	1	R_1r		3793	2	R_1r	R_1r
3795	2	rr	rr	3796	2	R_1R_1	R_1R_1

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3798	2	rr	rr	3799	1	R_1r	
3801	1	R_1R_1		3802	0	R_2r	
3804	2	R_1r	R_1r	3805	1	R_1r	R_1r
3806	1	R_1R_1		3808	2	R_1r	R_1r
3809	0	R_2r	R_2r	3811	1	rr	rr
3812	2	rr	rr	3813	0	rr	rr
3814	1	rr	rr	3816	2	rr	rr
3817	0	R_or	R_or	3818	1	rr	rr
3810	1	rr	rr	3820	1	rr	rr
3821	2	R_1r	R_1r	3822	2	R_1r	R_1r
3825	0	R_1r		3826	1	R_1r	
3827	1	R_1R_1		3828	2	R_or	R_or
3829	0	R_1r		3830	1	R_1r	
3832	1	R_1r	R_1r	3833	2	R_2r	
3834	2	R_1r	R_1r	3835	1	R_1r	R_1r
3836	1	rr	rr	3837	1	R_1r	R_1r
3838	1	rr	rr	3839	0	rr	rr
3840	2	R_1r	R_1r	3841	2	R_2r	R_2r
3842	2	R_1R_2	R_1R_2	3843	2	R_1R_2	R_1R_2
3844	0	R_2r	R_2r	3847	2	R_2r	R_2r
3848	1	R_1r	R_1r	3849	1	R_1R_2	
3850	1	R_1r		3851	1	R_1r	R_1r
3852	1	R_1r	R_1r	3853	1	R_1r	R_1r
3854	0	R_1r	R_1r	3855	1	R_1r	R_1r
3856	0	R_2r		3858	2	rr	rr
3859	2	R_1r	R_1r	3860	2	R_1r	R_1r

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3861*	1	R_1r		3862	0	rr	rr
3963	1	rr	rr	3865	2	rr	rr
3866	2	R_1r	R_1r	3867	1	R_1r	R_1r
3868	2	rr	rr	3869	2	R_1r	R_1r
3870	2	rr	rr	3871	2	rr	rr
3873	2	R_or	R_or	3874	1	rr	rr
3875	1	R_2r		3876	1	R_2r	
3878	1	R_2r		3882	2	rr	rr
3883	2	rr	rr	3888	1	R_1r	R_1r
3889	1	R_1R_2	R_1r''	3890	1	R_1R_2	R_1r''
3891	1	R_1R_2	R_1r''	3892	2	rr	rr
3895	2	rr	rr	3897	1	R_1r	
3898	1	R_1r	R_1r	3900	1	R_1R_1	
3901	0	R_2r	R_2r	3902	1	R_1r	R_1r
3903	0	rr	rr	3905	2	R_1r	
3906	0	R_2r	R_2r	3907	1	R_2r	R_2r
3908	2	R_1r	R_1r	3909	1	R_1r	R_1r
3910	1	R_1r	R_1r	3911	1	R_2r	
3912	2	R_1r	R_1r	3913	0	R_1R_2	R_1R_2
3915	1	rr	rr	3916	1	R_1R_2	
3918	2	R_1r	R_1r	3920	1	R_1R_2	R_1R_2
3922	2	R_1R_1	R_1R_1	3923	2	R_1r	R_1r
3924	2	rr	rr	3925	2	R_1r	R_1r
3926	1	R_1r	R_1r	3927	2	R_1r	R_1r
3928	1	R_1R_1	R_1R_1	3930	0	R_1r	R_1r
3931	2	R_1r		3932	2	R_1R_1	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3933	2	R_1R_2		3924	1	R_2r	R_2r
3935	1	rr	rr	3937	2	R_1R_1	
3938	2	R_2r		3939*	0	R_2R_2	
3940*	1	R_1r		3942	1	R_1r	
3943	1	R_1r		3944	1	rr	rr
3945	1	rr	rr	3946	1	R_1r	R_1r
3947	1	R_1r	R_1r	3950	2	rr	rr
3953	2	R_1r		3954	2	R_1r	
3955	2	R_2r	R_2r	3957	0	R_1r	R_1r
3958	1	R_1R_2	R_1R_2	3959	2	rr	rr
3960	1	rr	rr	3961	1	rr	rr
3962	2	rr	rr	3963*	1	R_1R_2	
3964*	1	R_1r		3965	2	R_0r	R_0r
3966	0	rr	rr	3967	1	R_1r	R_1r
3969	0	rr	rr	3970	2	rr	rr
3971	0	R_1R_2	R_1R_2	3972	2	R_1R_1	
3973*	0	R_1R_2		3974	1	R_1R_2	
3975	0	R_1R_2		3976	2	rr	rr
3977	2	R_1r	R_1r	3978	2	rr	rr
3979	2	R_1R_1	R_1R_1	3980	2	R_0r	R_0r
3982*	1	R_2r		3983	2	R_2r	
3985	2	rr	rr	3986	2	R_1R_1	
3987	2	r"r	r"r	3988	2	r"r	r"r
3989	2	R_1r		3990	2	R_1r	
3991	1	R_0r	R_0r	3992	0	R_2R_2	
3993	2	rr	rr	3994	2	R_0r	R_0r

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
3996	2	R_1r		3997	0	R_1R_2	
3998	1	R_1r		3999	1	R_2r	
4000	1	R_or	R_or	4001	1	R_1r	
4002	1	R_1r		4003	2	R_2r	
4097	2	R_1r	R_1r	4135	2	R_1R_2	R_1R_2
4141	1	R_1R_2		4242	1	R_2r	R_2r
4200	1	rr	rr	4240	1	R_or	R_or
4311	0	rr	rr	4454	1	R_1r	R_1r
4493	0	rr	rr	4495*	2	R_2r	
4701	2	rr	rr	5001	2	R_1r	
5004	1	rr	rr	5006	2	R_1r	
5009*	2	R_1R_2		5017	2	R_1r	R_1r
5032	2	R_2r		5034	1	rr	rr
5037	2	R_2r		5039	1	rr	rr
5053	2	R_1r		5097	2	R_1r	
5108	2	R_1R_1		5112	1	rr	rr
5139	2	r"r	r"r	5140*	1	R_1r	
5142	1	rr	rr	5148	2	rr	rr
5149	0	R_1r		5157	2	rr	rr
5167	2	R_1r		5170	2	rr	rr
5177	2	rr	rr	5182	2	rr	rr
5189	1	rr	rr	5190	2	rr	rr
5191	2	R_1r		5195	2	R_1r	
5198	2	R_1r		5199	2	R_1r	
5200	2	R_1r		5202	2	R_1r	
5204	1	rr	rr	5208	1	R_1r	

Sample		Phenotype	Genotype	Sample		Phenotype	Genotype
5239	2	rr	rr	5244	2	R_1R_1	
5246	2	rr	rr	5280	2	rr	rr
5291	0	R_2r		5304	1	R_1r	
5305	2	R_1R_2		5309	2	R_1R_2	
5330	0	R_1R_2		5413	1	rr	rr
5439	0	R_1R_1		5450	0	R_1r	
5452	1	R_1R_2		5468	2	rr	rr
5469	2	R_1R_1		5515	2	R_2R_2	
5516	0	R_1r		5518	0	R_1r	
5519	1	R_1R_1		5520	0	R_1R_2	
5521	2	R_1r		5522	2	R_1r	
5523	1	R_1r		6212	1	rr	rr
6641	2	R_2r	R_2r	6642	0	R_1r	
6648	1	R_or	R_or	6649	1	R_or	R_or
6651	1	R_1R_2		6652	1	R_1R_2	R_1R_2
6733	2	rr	rr	6832	1	rr	rr
7156	2	rr	rr	7219	1	rr	rr
7220	0	rr	rr	7225	2	R_1r	
7244	1	R_1R_2	R_1R_2	7700	1	R_1R_1	
8074	0	rr	rr	8101	0	r"r	r"r
8212	0	R_1R_1					

Parental Exclusions

Sample	Blood Group Systems Involved	Parent Excluded
1069	Rhesus	Father
2001	ABO, Rhesus, Kell, Duffy & Kidd	Father
	ABO	Mother
2002	Rhesus	Father
2009	ABO	Father
2100	ABO, Rhesus & MNSs	Father
2198	Rhesus	Father
3013	ABO	Mother
3064	Rhesus	Mother
3091	Rhesus & Duffy	Mother
3093	ABO & Rhesus	Mother
3123	Rhesus & Kidd	Father
3148	Rhesus	Mother
3152	Rhesus	Mother
3179	Rhesus	Father
3180	Rhesus	Father
3188	Rhesus & MNSs	Father
3250	Rhesus	Mother
3252	ABO & Kell	Father
3278	Rhesus & MNSs	Mother
3279	Rhesus	Father
3287	Rhesus	Father
3288	Rhesus	Father
	Rhesus	Mother
3296	Rhesus	Mother

Parental Exclusions

Sample	Blood Group Systems Involved	Parent Excluded
3298	ABO	Father
3304	ABO	Father
3386	Rhesus	Mother
3401	Rhesus	Mother
3406	Rhesus & Duffy	Mother
3416	Rhesus	Father
3545	Rhesus	Father
3548	ABO	Father
3617	Rhesus	Mother
3680	Rhesus & MNSs	Father
3751	Rhesus & MNSs	Father
3770	Rhesus	Father
3861	Rhesus	Father
3939	Rhesus	Father
3940	Rhesus	Father
3963	ABO	Father
3964	ABO	Father
3973	Rhesus	Father
3982	Rhesus	Mother
4495	Rhesus	Father
5009	Rhesus	Father
5140	ABO & Rhesus	Father

There are 47 exclusions in a total of 1133 individuals, a rate of 4.1%. Of these 31 are paternal and 16 are maternal exclusions, in to cases both parents are excluded.

APPENDIX II

APPENDIX II

THE RHESUS PHENOTYPES & GENOGYPES OF THE FIRST, SECOND AND THIRD DEGREE RELATIVES OF THE THREE GROUPS OF PATIENTS: HODGKIN'S DISEASE; IMMUNODEFICIENCY & "EMBRYONIC TUMOUR".

Hodgkin's Disease			First Degree Relatives.		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1003	R_1r		1004	R_2r	
1006	R_1r		1008	R_1r	
1012		rr	1013		R_1r
1023		rr	1030		R_1r
1042		rr	1043		R_2r
1045		R_2r	1141		rr
1147		R_1R_1	1149		R_1r
1150		R_2r	1151	R_1R_1	
1152		R_1R_1	1180	R_1r	
1186		R_1r	1218		rr
1259		R_2r	5001	R_1r	
5148		rr	5170		rr
5190		rr	5191	R_1r	
5202	R_1r		6733		rr

N = 28

Known Genotypes = 18 = 64.3%

Hodgkin's Disease			Second Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1009	R_1R_2		1011	R_1r	
1016	R_1R_1		1018		rr
1024		rr	1027		R_1R_1
1028		rr	1037		R_1r
1038		R_1r	1040		rr
1055		rr	1064		R_1r
1065		R_1r	1066		rr
1067		rr	1068		R_1r
1070	R_1R_2		1078	R_1R_1	
1079		R_1r	1080		rr
1083	R_1r		1095	R_1r	
1101		rr	1102		R_1r
1104	R_1r		1105	R_1r	
1133		R_1r	1139		rr
1142		rr	1153		R_1r
1161		R_1r	1164		rr
1173		rr	1182	R_1r	
1245	R_1r		2039		R_1r
2081	R_2r		2082	R_2r	
2090		R_1r	2105		R_2R_2
2195		R_1r	2197		rr
2199	R_1r		3301		R_1R_1
3790		rr	5006	R_1r	
5009	R_1R_2		5017		R_1r
5142		rr	5189		rr

Hodgkin's Disease			Second Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
5204		rr	5208	R_1r	
5239		rr			

N = 53

Known Genotypes = 36 = 67.9%

Hodgkin's Disease			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1015		R_2r	1029		R_1r
1036		R_2r	1039		rr
1047	R_2R_2		1049	R_1R_2	
1053		R_2r	1056		R_2r
1060		R_2r	1069	R_1r	
1084		R_1r	1086		rr
1087		R_2r	1090		R_1r
1091		rr	1094		rr
1096	R_1R_2		1097	R_1R_2	
1099		rr	1116		R_2r
1117		rr	1118		R_1r
1119	R_1R_1		1123		R_2r
1124		R_1r	1125		R_1r
1126		R_2r	1127		R_2r
1143		rr	1148		rr
1157		R_1r	1162		R_1r
1163		R_1r	1165	R_1R_1	
1166	R_1R_1		1168	R_1R_1	
1175		R_1r	1191	R_1r	
1206		rr	1225		R_1r
1237		rr	1238		rr
1247	R_1r		1253	R_1r	
1289		rr	2003		R_2r
2005		R_1r	2006		R_1r
2007		R_1r	2008		R_1R_1

Hodgkin's Disease			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
2009	R_1R_1		2010	R_1r	
2011		rr	2012		rr
2013		rr	2026		R_1R_1
2028	R_2r		2029		rr
2031	R_1r		2032		rr
2033		rr	2034		R_1r
2035	R_1r		2036		rr
2037		R_1r	2040	R_1r	
2041	R_1R_2		2044		R_1r
2052		R_1r	2053		R_1r
2054		R_1r	2055		R_1r
2058		R_2r	2060		R_2r
2063		rr	2065		rr
2066	R_1r		2068		R_1r
2069	R_1r		2070	R_1r	
2071		rr	2072	R_1r	
2073	R_1r		2075		R_1r
2076		rr	2077		R_1r
2078		rr	2080		rr
2088		rr	2089		R_1r
2096		rr	2103		rr
2104		R_1r	2107		R_1r
2108	R_1r		2109		rr
2198		rr	3045		rr
3056	R_1R_2		3060		R_1r

Hodgkin's Disease			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
3080		rr	3084		R_1r
3221	R_1r		3242	R_1R_2	
3243	R_1R_2		3251	R_1r	
3257	R_1R_2		3260	R_1R_2	
3338		R_1r	3344	R_1r	
3352		rr	3399		R_1r
3403		R_2R_o	3439	R_1R_1	
3477		rr	3490		R_2r
3511		rr	3512		rr
3514		rr	3563	R_2r	
3564		R_or	3565		rr
3566	R_2r		3592	R_1r	
3632	R_1R_1		3641		rr
3647		rr	3726		rr
3734	R_1r		3736	R_1r	
3737		rr	3738		R_or
3793	R_1r		3795		rr
3801	R_1R_1		3804		R_1r
3812		rr	3841		R_2r
3842		R_1R_2	3843		R_1R_2
3859		R_1r	3950		rr
3993		rr	4003	R_2r	
5157		rr	5167	R_1r	
5177		rr	5195	R_1r	
5198	R_1r		5199	R_1r	

Hodgkin's Disease			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
5200	R_1r		5244	R_1R_1	
5304	R_1r		6212		rr
6641		R_2r	7225		R_1r

N = 156

Known Genotypes = 106 = 67.9%

Immunodeficiency			First Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1029	R_1r		1084		R_1r
1117		rr	1118		R_1r
1119	R_1R_1		1139		rr
1142		rr	1161		R_1r
1162		R_1r	1163		R_1r
1165	R_1R_1		1166	R_1R_1	
1168	R_1R_1		1203	R_1r	
1218		rr	5148		rr
5244	R_1R_1		7225	R_1r	

N = 18

Known Genotypes = 10 = 55.6%

Immunodeficiency			Second Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1027		R_1R_1	1028		rr
1078	R_1R_1		1079		R_1r
1086		rr	1104	R_1r	
1105	R_1r		1120	R_1R_2	
1133		R_1r	1149		R_1r
1154	R_1R_1		1164		rr
1237		rr	1238		rr
1245	R_1r		3035	R_1r	
3221	R_1r		3294	R_1R_1	
3790		rr	5142		rr
5177		rr			

N = 21

Known Genotypes = 11 = 52.4%

Immunodeficiency			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1063		R_2r	1072		rr
1077		rr	1080		rr
1081		R_2r	1082		R_2r
1100		rr	1112		rr
1131		R_1r	1134		R_2r
1135		R_2r	1147		R_1R_1
1148		rr	1150		R_2r
1151	R_1R_1		1152		R_1R_1
1186		R_1r	1202		R_2r
1225		R_1r	1239		rr
1247	R_1r		1259		R_2r
2006		R_1r	2007		R_1r
2040	R_1r		2041	R_1R_2	
2063		rr	2065		rr
2067		R_1r	2073	R_1r	
2074		rr	2077		R_1r
2078		rr	2088		rr
2092	R_2r		2096		rr
2102		rr	2103		rr
2104		R_1r	2107		R_1r
2196		R_1r	2198		rr
3250	R_1R_2		3288	R_1R_1	
3477		rr	3490		R_2r
3641		rr	3695		rr
3793	R_1r		3795		rr

Immunodeficiency			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
3859		R_1r	3961		rr
5001	R_1r		5004		rr
5053	R_1r		5195	R_1r	
5198	R_1r		5199	R_1r	
5200	R_1r		7219		rr

N = 60

Known Genotypes = 45 = 75.0%

"Embryonic Tumours"			First Degree Relatives		
Sample	Phenotype	Genotype	Sample	PHenotype	Genotype
1144		rr	1169		rr
1170		rr	1171		rr
1177		R_1r	1178		R_or''
1217		R_1r	1226		r''r
1229	R_1r		1230		rr
1231		rr	1232		r''r
1235		R_or''	1255		R_1r
1256		R_or	1349		r''r
3598		rr			

N = 17

Known Genotypes = 16 = 94.1 %

"Embryonic Tumour"			Second Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1001		R_1r	1014	R_1r	
1020		R_or	1051	R_1r	
1052		R_1r	1071		rr
1146		rr	1159	R_2R_2	
1185		rr	1204	R_1R_1	
1207		R_1r	1221	R_1r	
1236		R_1R_o	1241		R_1r
1257		r"r	1258		r"r
1345		R_1r"	1347		rr
1348		r"r	1350		rr
3385	R_1r		3421		R_1r
3689	R_2r				

N = 23

Known Genotypes = 16 = 69.6%

"Embryonic Tumour"			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
1087		R_2r	1138		rr
1160	R_1r		1205		rr
1209	R_1r		1210		R_1r
1211		R_1r	1212		R_1r
1213		R_1R_1	1222		rr
1233		R_1r	1243		rr
1250		rr	2001	R_2r	
2002		R_2r	2004		R_1r
2014	R_1r		2015	R_1r	
2016	R_1r		2017	R_1r	
2018		R_or	2019		R_1r
2020		R_1r	2021		rr
2025		rr	2030	R_1r	
2064	R_1r		2084		R_or
2085		R_or	2086		R_or
2093		rr	2094	R_1r	
2100	R_2R_2		2101		R_1R_2
2111	R_2r		2112		R_1R_2
3006		rr	3073		R_or
3099		rr	3116		rr
3175		R_or	3247	R_1R_2	
3252	R_1r		3377		rr
3386	R_2R_2		3423		rr
3424		rr	3439	R_1R_1	
3527		rr	3592	R_1r	

"Embryonic Tumour"			Third Degree Relatives		
Sample	Phenotype	Genotype	Sample	Phenotype	Genotype
3638	R_1r		3721		R_1r
3722		R_1r	3723		R_1r
3724		rr	3739	R_1r	
3774		R_1r	3833	R_2r	
3915		rr	4240		R_or
5006	R_1r		5034		rr
5097	R_1r		5112		rr
5142		rr	5280		rr

N = 66

Known Genotypes = 44 = 66.7%

APPENDIX III

Figure 6.

Hodgkin's disease: patient 6731, minimal pedigree.

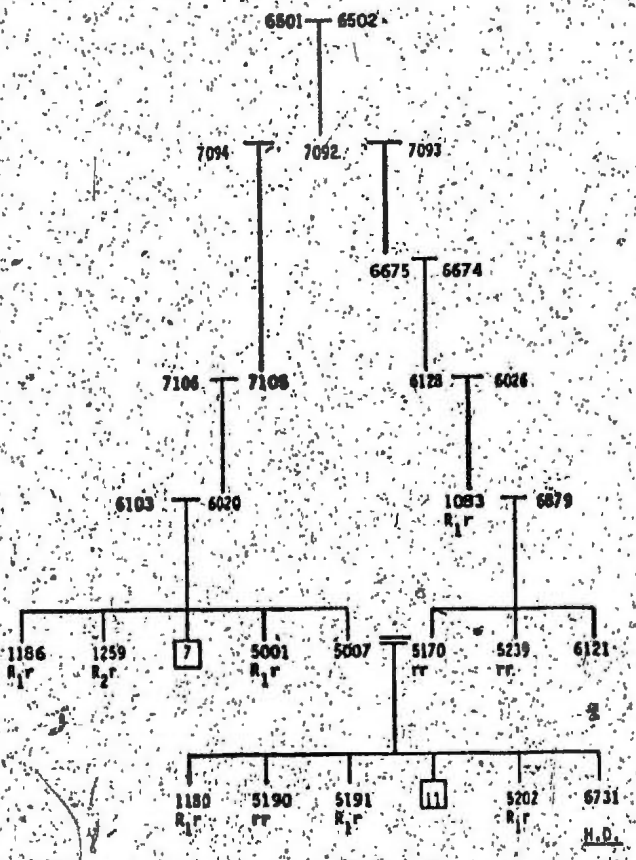

Figure 7.

Hodgkin's disease: patient 6790 and immunodeficiency: patient 1144, minimal pedigree.

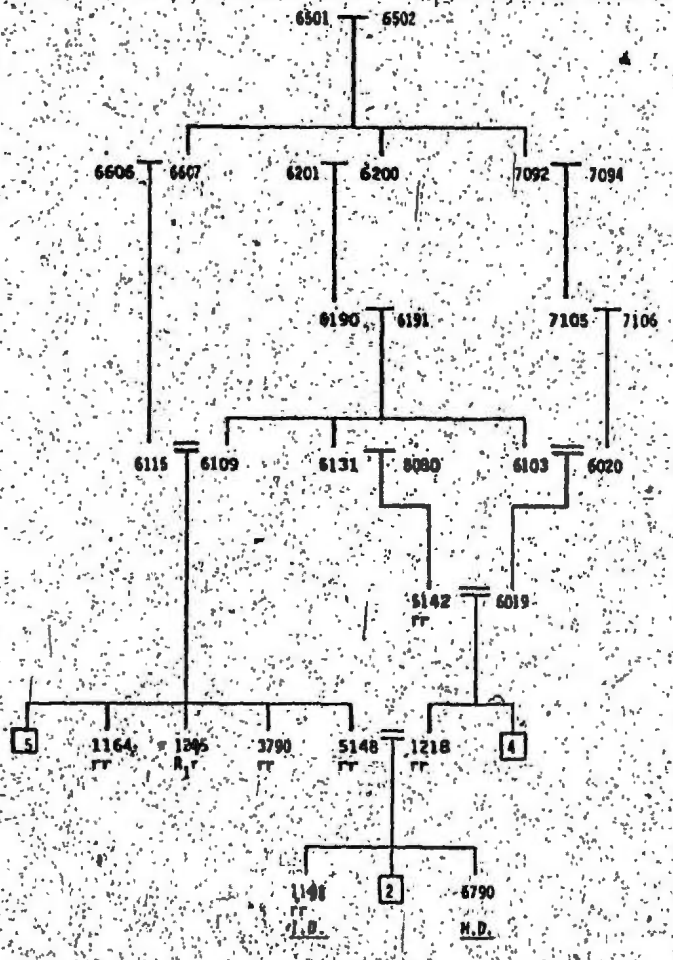

Figure 8.

Hodgkin's disease; patient 6800, minimal pedigree.

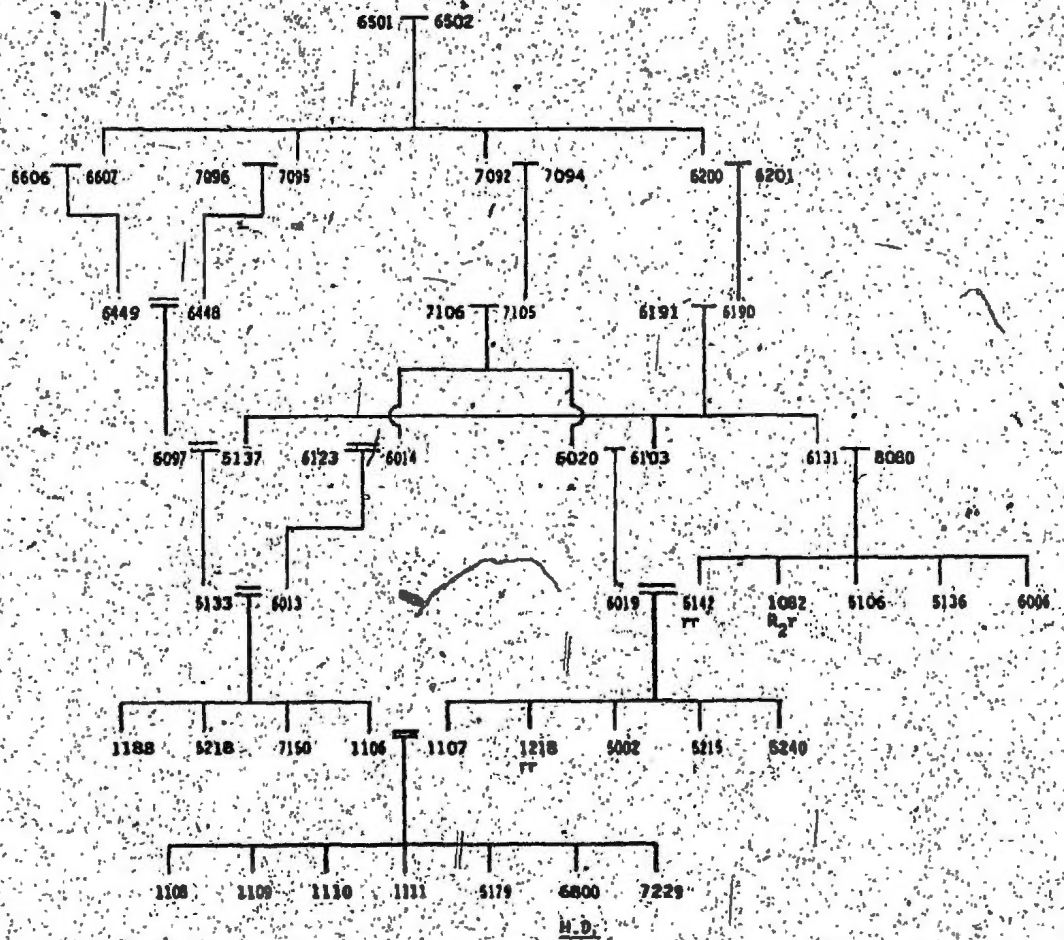

Figure 9.

Hodgkin's disease, patients 1005 & 6019, minimal pedigree.

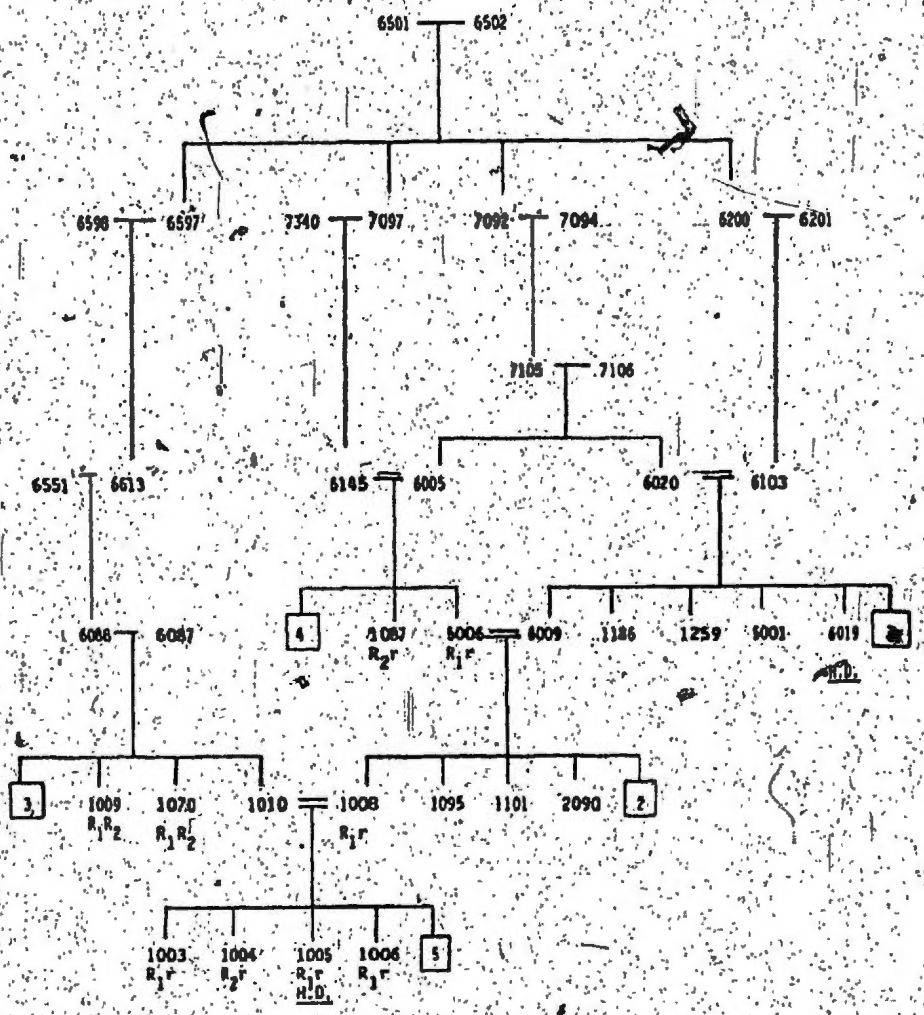

Figure 10.

Hodgkin's disease; patient 6765 & Immunodeficiency; patients 1148 & 6500;

minimal pedigree.

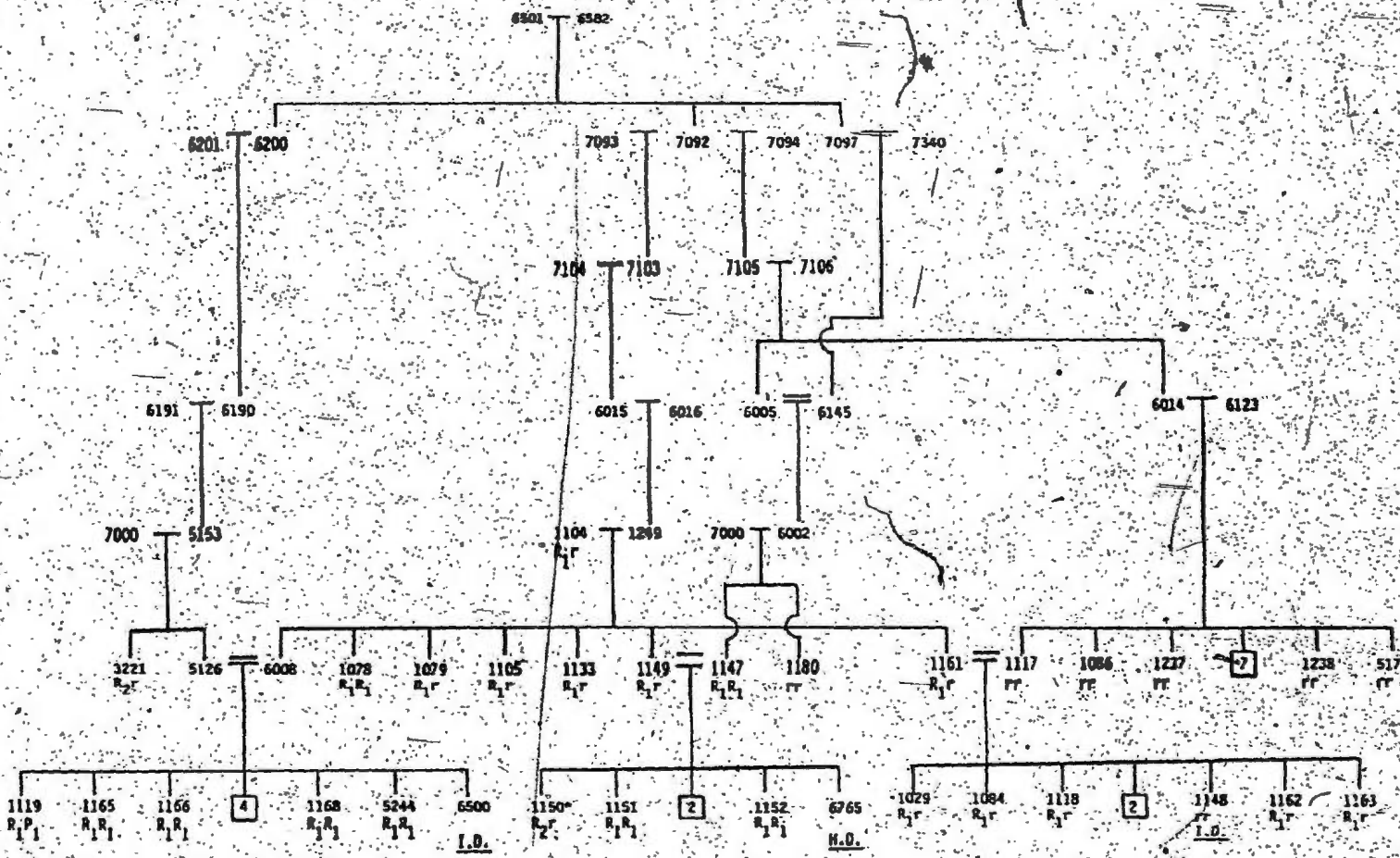

Figure 11.

Hodgkin's disease: patient 6086, minimal pedigree.

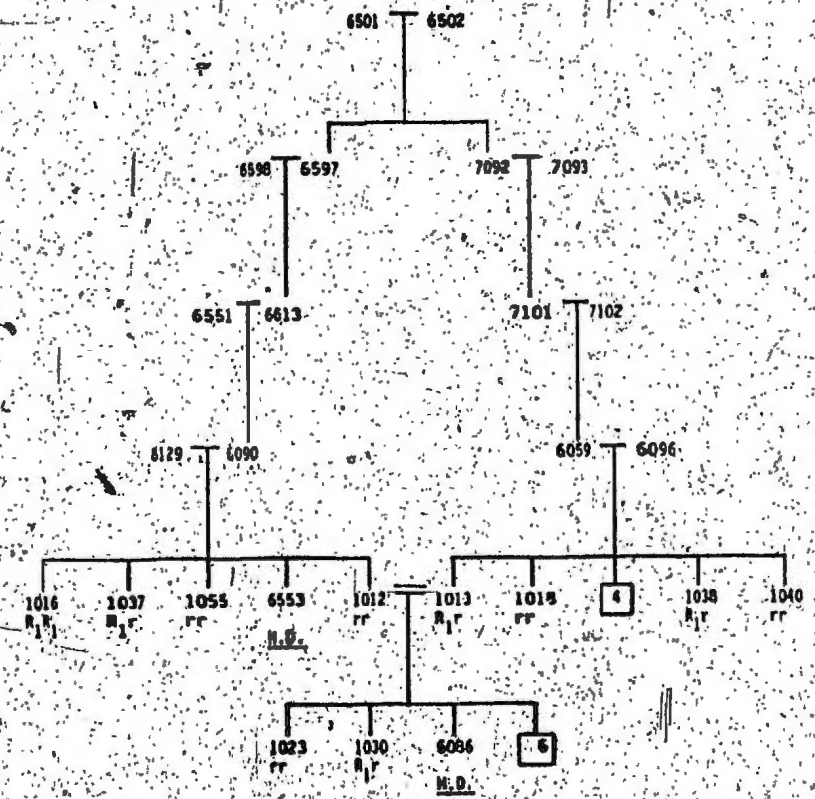

Figure 12.

Embryonic tumours; patients 6184, 6589 & 7238, minimal pedigree.