COMPLEMENT FIXING PLATELET ANTIGENS

AND ANTIBODIES IN MAN

by

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CONTENTS

BIBLIOGRAPHY

PUBLISHED PAPER: Complement–fixing platelet antibodies in the
    serum of a pregnant woman suffering from transfusion reactions.

SUMMARY

Platelet antigen polymorphisms in Man have been des-
cribed by a number of workers using agglutination and complement
fixation techniques. A number of these antigens may be genetically
independent of each other and some are almost certainly related to
the leukocyte antigens. Shulman et al (1964) and Aster et al (1964)
have described a number of complement fixing antigens shared by
platelets and leukocytes. Both Shulman and Aster have indicated
that they have not been able to establish the specificity of all
the antibodies that they have discovered.

Since it appeared that there was scope for further
investigation of complement fixing platelet antibodies, efforts
were made to find these antibodies in the sera of normal persons,
patients with idiopathic thrombocytopenic purpura (I.T.P.), mothers
of infants with I.T.P., and polytransfused persons. As a result of
these studies, complement fixing antibodies were found in one of
three mothers of infants with I.T.P., and fifteen of 119 poly-
transfused persons, but not in normal persons.

Sera from Adelaide containing antibodies were sent to
Dr. R.H. Aster of Boston, who found that three sera contained
anti-PlB1. A study of antigen distribution in 432 platelet donors
showed that five sera contained an antibody against an antigen

controlled by an allele of PlB1. This antigen was designated by
PlB2 (present in 20% of Caucasian population), and the corresponding
gene by P1B2 (gene frequency 0.10). Another serum was found to
contain an antibody against an antigen controlled by a third allele
of this locus (denoted P1B3). The frequency of anti-P1B3 positives
was 33% (gene frequency 0.18). The distribution of the PlB antigens
in the population was not as expected in a random mating population
in Hardy-Weinberg equilibrium. It has not yet been determined
whether this deviation is due to a systematic serological error or
an incomplete genetic hypothesis. A comparison of the distribution
of a number of other antigens with the PlB antigens was also made.
The results of typing 67 Australian Aborigines and 128 New Zealand
Maoris indicated that the antigens are distributed differently in
different racial groups.

Studies on 17 Caucasian families with 47 children are
in agreement with the hypothesis that P1B1, P1B2, and P1B3 are
alleles at one locus. The distribution of the PlB1 and PlB3 antigens
in the Caucasian population of South Australia is not significantly
different from the distribution of LA2 and LA1 antigens reported by
Payne and Bodmer (1964). Similarly the distribution of the antigens
P1B2 and PLB3 is not significantly different from the distribution
of the antigens 7d and 7c reported by van Rood et al (1966). These
comparisons suggest that PlB2 corresponds with 7d and P1B3 with 7c

and LA1. The suggested association between complement fixing platelet antibodies and leukocyte agglutinins, indicates that the complement fixation test could be of value in the matching of donor and recipient pairs for homotransplantation.

DECLARATION

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that, to the best of my knowledge and belief, the thesis contains no material published or written by another person, except when due reference is made in the text of the thesis.

Bronte W. Gabb

April, 1968.

INTRODUCTION

In 1964 Aster, Cooper and Singer described a simplified compliment fixation test for the detection of platelet antibodies in man. These authors indicated that it was possible to screen large numbers of sera against multiple platelet antigens, using only a few drops of serum from each patient. Aster et al indicated that they had recognized six platelet antigens and that there were other antigens that they had found antibodies against, but had not characterized. Similar results were found by Shulman et al (1964). These groups of workers showed that some of the antigens they had found were present on leukocytes as well as platelets.

These results indicated that the detection of additional complement fixing platelet antibodies and a study of their activity, could produce results of interest in the field of human genetics. Accordingly, a search for complement fixing platelet antibodies, and a study of the distribution of the corresponding antigens in the human populations was undertaken. The methods, results and conclusions of this endeavour are recorded in this thesis.

LITERATURE REVIEW

A. STUDIES ON PLATELET AND LEUKOCYTE ANTIGENICITY IN ANIMALS

The occurrence of antigenic differences between the red cells of humans has been known since Landsteiner (1900) showed that sera of some persons agglutinated the washed red cells of others. Information on the antigenic differences between individuals for the other cells in the blood was not readily obtainable early in the century as these cells constitute a smaller proportion of the blood volume and were not easily separated from the red cells with a high degree of purity. These other cells in the blood were shown to have antigenic properties by hetero-immunization experiments. White cells of various morphological types and/or platelets from one species, were injected into animals of a different species to produce an "immune serum" in the recipient animal. When this serum was injected into the circulation of an animal of the donor species, it was possible to show that a profound fall in the number of cells of the appropriate type could be induced in the circulating blood.

Anti-platelet sera were first produced in 1905 by Marino. The results of Bedson (1921, 1922) showed that separate platelet and leukocyte (polymorph) antisera could be produced in the rabbit, against the respective cells of the guinea pig. When antisera prepared by injecting guinea pig platelets into rabbits were injected into guinea pigs, the platelet count fell. Anti-leukocyte serum caused the

leukocyte count to fall, while the platelet count remained normal.
Bedson was able to show that in his experiments, only guinea pigs
treated with antiplatelet antibody, developed purpuric disease, while
those treated with anti-leukocyte, anti red cell and antiserum anti-
bodies did not. Chew et al (1936) injected neutrophils from a sterile
guinea pig exudate, into rabbits. The rabbit serum was subsequently
found to contain an antibody which caused a neutropenia when injected
into guinea pigs. In 1937 it was shown that anti-lymphocyte serum
could also be produced. With the methods then available, these
workers were not able to show that the species they studied were
polymorphic for any leukocyte or platelet antigens.

Recently Balner et al (1966) have worked with leukocytes of
rhesus monkeys (Macacus mulatta) and have shown that the leukocyte
antigen differences between individual monkeys are demonstrable by
the common serological techniques. Monkeys immunized with skin,
blood and leukocytes from members of the same species, usually prod-
uced leuko-agglutinins four to eight weeks after commencing an
immunization programme.

The heterologous antigenicity of platelets was demonstrated
by de Nicola et al (1955), using the complement fixation technique.
These workers also used the rabbit anti-guinea pig system, referred
to earlier. Although de Nicola et al looked for complement fixing
isoantibodies in humans their search proved unproductive.

B. <u>NOMENCLATURE OF PLATELET AND LEUKOCYTE GROUPS IN MAN</u>

The nomenclature of red cell antigens and their genes has grown in an awkward and sometimes inconsistent manner, (e.g. the use of the letters M and N for antigens controlled by alleles at one locus). Even now considerable effort is expended in an attempt to unify the terminology of the Rh system. In spite of the suggestion by Ford (1955) that a systematic nomenclature for new systems could avert much controversy, abbreviations of proper names have continued to pepper the literature.

Two systematic nomenclatures have been introduced in the literature on leukocyte and platelet antigens. The first by Shulman <u>et al</u> (1961) used the letters Pl, Gr, Ly combined or singly to indicate the cell type (platelets, lymphocytes or granulocytes respectively) on which the antigen occurred. Each locus was given a letter – alphabetically as discovered, A, B, C etc. and each allele at the locus was given a number 1, 2 etc. The letters and numbers of each symbol were written as superscripts in the usual way. An antigen was denoted by $PlGrLy^{B1}$ and the corresponding gene by $\underline{PlGrLy}^{B1}$. The null allele was denoted by $\underline{PlGrLy}^{B}$.

Payne <u>et al</u> (1964) introduced a similar nomenclature for the system of leukocyte antibodies they described. The letter 'L' was used for leukocyte, A for the locus, (B C etc. being reserved for new loci) and the numbers 1, 2 and 3 were used for the antigens. Payne

et al deleted the use of superscripts which they suggested are "cumbersome for the printer and incompatible with automatic data processing equipment". Their symbols were written as LA1, LA2 etc. for antigens, LA1, LA2 for genes. The symbol LA3 was used for an assumed antigen – the absence of LA1 and LA2. Had LAo been introduced initially for the null allele the nomenclature could have been extended without altering the meaning of previously introduced symbols. Antibodies are denoted by the symbol for the corresponding antigen prefixed by anti-, e.g. anti-LA2, anti-P1B1.

Other workers Dausset et al (1965), Terasaki et al (1965) have used integers to designate leukocyte antigens. As no systematic nomenclature has been introduced by these authors it is necessary to include the authors name in all references to antigens they have described. The unique system of antigen symbols used by van Rood et al (1965) is referred to in section C. 2. (a) of this chapter.

During this project attention has been focused on antibodies related to the P1GrLy^{B1} system described by Shulman et al (1962). In this thesis the superscript has been dropped to the line (after Payne et al, 1964) so that the symbol used for the antigen is P1B1 and the gene P1B1. Codominant alleles in the system are numbered 1, 2, 3 etc. The recessive (serologically undetectable) allele in the system is denoted by the symbol P1Bo.

In certain tables it has been necessary to summarize P1B antigenic types for individuals or groups of individuals. It has been

found convenient to do this using the numbers only.

P1B Antigens		Anti P1B1	Anti P1B2	Anti P1B3	Presumed Genotype
120	denotes	+	+	−	P1B1/P1B2
103		+	−	+	P1B1/P1B3
023		−	+	+	P1B2/P1B3
100		+	−	−	P1B1/P1B1 or P1B1/P1Bo
020		−	+	−	P1B2/P1B2 or P1B2/P1Bo
003		−	−	+	P1B3/P1B3 or P1B3/P1Bo
000		−	−	−	P1Bo/P1Bo

The 0 in the respective position denotes that a test has been made for that antigen and that the test was negative. The 0 must be distinguished from the dash − which when used in this system denotes that no test has been made for the appropriate antigen.

e.g. 100 denotes antigens 2 and 3 are absent

1−0 denotes that antigen 3 is absent, no test for antigen 2.

For identification in this thesis antibodies which are not yet sufficiently investigated to include or exclude from already known systems will be denoted by the initials of the person forming them prefixed by anti, e.g. anti-Si. The corresponding antigen and gene will be denoted by Si and Si respectively.

C. STUDIES ON PLATELET AND LEUKOCYTE ANTIGENICITY IN HUMANS

Leukocyte and platelet antigen differences between human individuals, were demonstrated in the early 1950's. An understanding of these antigenic differences has been developed by different workers concurrently. It is therefore more convenient to survey the initial literature dealing with each of these cell types separately.

(1) Platelet Antibodies and Antigens

The suggestion that platelet antigen differences existed in humans, was made by Harrington et al (1953). These workers found platelet agglutinins in the serum of normal mothers whose infants had neonatal thrombocytopenic purpura, and also in the serum of repeatedly transfused patients in whom survival of homologous platelets was shortened. Stephanini et al/found the platelets of 350 individuals could be divided into 5 groups, but no family studies were reported. These groups were defined by agglutinins found in the serum of immunized subjects.
(1953)

The incidence of agglutinins reported by Stephanini et al was high as shown in the table drawn up from their data (Table 1:1). It appears that this high incidence of agglutinins and the occurrence of naturally occurring agglutinins, has not been confirmed by others (van Loghem et al 1959).

	Patients trans-fused with platelet rich material	Multi-transfused patients	Idiopathic thrombocyto-penic patients	Normal sub-jects (un-transfused never pregnant)
Number tested	26	295	78	215
Number with agglutinins	9	9	21	13
Percentage	24.6	3.1	26.9	6.0

Table 1:1 Incidence of platelet agglutinins reported by Stephanini et al (1953)

Using naturally occurring agglutinins, Stephanini et al described four platelet groups which they suggested were analagous to the ABO groups of the red cells. It is clear from the last two lines of Table 1:2 that their observations do not agree with this hypothesis.

Platelet groups	I	II	III	IV (0)	Total
Reaction with Platelet agglutinin					
Anti I	+	−	+	−	
Anti II	−	+	+	−	
Incidence %	10.2	4.6	3.7	81.5	
Number observed	29	13	11	232	285
Number expected if antigens controlled by allelic genes	38.2	22.2	1.8	222.8	285

Table 1:2 Designation of platelet groups and their incidence among the general population (after Stephanini et al 1953)

Moulinier (1957) reported a specific platelet antigen defined by an incomplete antibody which developed during pregnancy and which was detected by the Coomb's consumption test. The antigen was named DUZO after the donor of the serum, which defined the antigen. The antigen was present in 22% of 82 donors at the Blood Transfusion Service of Bordeaux.

A simplified agglutination technique described by Dausset and Maulinvaud (1954), was used by van Loghem et al (1959), who found the serum of a transfused patient (Mrs Zw) contained an antibody which strongly agglutinated the platelets of 280 of 287 persons tested. Results of typing three families with ten complete matings and 40 children agreed with the hypothesis that the antigen was controlled by a simple Mendelian dominant autosomal gene. The system was denoted by Zw. Subsequently van der Weerdt et al found another antibody by the same technique, which led to the recognition of an antigen controlled by an allele of the gene above. These genes have since been denoted by Zw^a and Zw^b, and no other allele at this locus has as yet been found.

A third platelet antigen, named Ko, was found by van der Weerdt et al and by Dausset and Berg (1963), using the same technique. This antigen was present in 21.3 and 16.9% of the Dutch and French populations respectively, and was independent of the antigen Zw^b. The gene Ko is a Mendelian dominant.

Following his use of the complement fixation test to study the immunology of quinidine induced thrombocytopenic purpura (1958), Shulman turned his attention to the serum of patients who had suffered from post-transfusion purpura and febrile transfusion reactions. Shulman also investigated the serum of mothers whose infants had developed neonatal thrombocytopenic purpura. In each of these groups, Shulman et al (1964), were able to find complement fixing antibodies which defined various antigenic specificities for which the human population is polymorphic. In two sera from patients with post-transfusion purpura, Shulman et al (1961), found an antibody which reacted with the platelets of 203 of 206 randomly selected normal individuals and which was inherited in a simple Mendelian manner. The antigen was named Pl^{A1}. With the quantitative complement fixation test, Shulman et al were able to show that positive reactors with the post-transfusion sera referred to above could be divided into two non-overlapping groups, according to the number of units of complement fixed by a given number of their platelets. Results from random population and from selected family studies agreed with the hypothesis that homozygous reactors with anti PlA1, fixed 1.7 - 2.4 times more complement than did heterozygous reactors.

Shulman et al (1964) exchanged sera with Dr. van Loghem, and found that anti PlA1 reacted only with Zw^a positive platelets. Therefore PlA1 was the same as Zw^a. The agglutinating antibody anti - Zw^b found by van der Weerdt et al was tested by Shulman et al and

reacted with all anti-P1A1 negative platelet specimens as well as all
of 15 P1A1 heterozygotes. Furthermore, anti-$\underline{Zw}^b$ did not react with
platelets from any of 55 individuals whose cells had high activity
with anti P1A1. Thus it appears that the Zw and P1A platelet antigen
systems are identical.

Following the discovery of anti P1A1, the platelets of
mothers of infants with neonatal purpura, where a maternal platelet
antibody was suspected but not initially demonstrable, were tested with
the antibody. Of 27 such tested women, 13 were shown to lack the P1A1
antigen, whereas the platelets of their husbands and infants contained
the antigen. Using the elegant quantitative complement fixation test,
Shulman et al (1964) were able to demonstrate the presence of an anti-
body in the serum of the 13 women referred to above, which had the
capacity to prevent the fixation of complement by anti P1A1 in the
presence of P1A1 positive platelets. This type of antibody has been
referred to by Shulman as a blocking antibody. This blocking anti-
body appears analogous to the incomplete red cell antibodies which have
previously been shown to interfere with red cell agglutination, by an
agglutinating antibody (Race and Sanger 1962). Thus it became apparent
that P1A1 was involved in a proportion of cases of neonatal thrombo-
cytopenic purpura as well as in post transfusion purpura. It would
seem possible that the neonatal purpura is probably produced by a
mechanism similar to that involved in haemolytic disease of the new-
born. The mechanism of production of post-transfusion purpura

(previously classified as idiopathic and possibly "autoimmune"), is less obvious. Shulman et al (1961) have suggested that in vivo, PlA1 antigen-antibody complexes are transferred to the recipients own platelets, which are then destroyed.

Shulman et al examined further sera from polytransfused patients and mothers of thrombocytopenic infants, and found additional platelet antibodies (1962). These antibodies have been used to define a total of six platelet antigens and one lymphocyte antigen. Three of the antigens have been shown to be common to lymphocytes and granulocytes, as well as being present on platelets. The outstanding merit of Shulman's technique, is that it enabled him to find sera which gave unequivocal positive or negative reactions and to find multiple examples of sera behaving identically, against a large panel of cells (1965). As will be seen later, these important sero-logical advantages have so far not been found by those using other techniques with leukocytes. Shulman et al (1964) have also reported at length on certain of the physiological and clinical character-istics of the platelet and leukocyte antigens and antibodies which they have described. It is not planned to review this aspect of the subject here. Pearson et al (1964) have reported clinical and serological findings in nine infants with neonatal thrombocytopenic purpura of isoimmune etiology. The description given provides a rational basis for the anticipation and treatment of this rare but sometimes serious disorder.

Aster et al (1964) described a simplified qualitative complement-fixation test for the detection of platelet antibodies in human serum. The principle of this test is similar to the quantitative complement fixation technique outlined by Shulman et al (1964). The qualitative test can be set up in larger numbers by one technician. With their simplified method, Aster et al, examined the sera of 172 patients repeatedly transfused with blood or platelets, and found antibodies in 15 (8.7%). These antibodies reacted with at least six different platelet antigens. In their second paper on platelet antibodies, Aster et al (1964) indicated that eight of their 15 sera contained antibodies additional to those detecting the six platelet antigens which they described earlier. This indicates that some contribution to the understanding of human platelet polymorphisms would follow further research using the complement-fixation test.

Aster et al (1964) stated that "Red cell phenotypes were determined in 49 persons whose platelet phenotypes were known. There was no distinct association between the erythrocyte antigens tested and platelet antigens II - VII". It is disappointing that the data on which these conclusions were based have not been published. Even more disappointing, is the absence of published data on the relationships between the platelet antigens themselves in the reports of Aster et al (1964 a and b) and in the reports of Shulman et al (1962 a, b, c 1964, 1965), except for the comparisons of P1A1 with P1A2 and P1E1 with P1E2.

The absence of comparative data from the literature presents a challenge to determine whether any of the antigens are related as alleles or pseudo-alleles, and if so how many loci are involved. It is of interest that Shulman et al (1965) imply the existence of six loci, presumably not closely linked, whereas Aster et al (1964 a and b) make no comment on the relationships between their antigens II - VII.

An estimate of the frequency of occurrence of complement-fixing platelet antibodies in pregnant women has been made by Klemperer et al (1966), who tested the sera of 6592 such women against a panel of platelet specimens from 10 - 15 random donors. These workers used the technique described by Aster et al (1964). In their series 109 (1.65%) of the pregnant women were found to have complement fixing antibodies, whereas no complement fixing antibodies were found in the 714 normal sera without a history of pregnancy or transfusion.

(2) Leukocyte Antigens and Antibodies

(a) Detected by Agglutination

Leukocyte isoantigens and isoantibodies in man were studied early in the last decade by various agglutination techniques. This early work has been reviewed by Killmann in his monograph, Leukocyte Agglutinins (1960). A number of difficulties beset those attempting to understand the place of leukocyte antigens in biological science. These difficulties were in part conceptual and in part technical.

Leukocyte antibodies were first found in patients with leukopenia.
As stated by Killmann in the introduction to his monograph, "the
prevailing concept was that these leukocyte agglutinins had the
character of antibodies, which in vivo caused leukopenia by destroy-
ing the patients' leukocytes." This autoimmune conception has been
replaced by that of iso-immunity, that is, antibodies are produced
following stimulation of a subject by a foreign antigen, as may occur
during pregnancy or a blood transfusion. These antibodies react with
foreign antigens rather than the patients' own antigens.

The difficulties in technique, are considered at length
in Killmanns book. He indicates two possible errors and gives some
indications as to how these may be avoided. The errors he considers
are:-

i. false negative reactions attributable to one or more of
the following factors:-

(a) Presence of various anticoagulants

(b) Failure to inactivate the agglutination inhibitor.

(c) Destruction of leukocyte agglutinin during inactivation

(d) Incubation for an inadequate time or at a sub-optimal temperature

(e) Prozone phenomenon

(f) Titration in untreated normal serum (because of agglutination
         inhibition

(g) Storage of leukocytes for too long after blood has been drawn

(h) Siliconized glassware

(i) Inadequate size of leukocyte panel.

ii. false positive reactions attributable to one or more of the following factors:-

(a) Incompatibility with respect to ABO blood groups

(b) Foreign bodies

(c) Bacterial contamination

(d) Non-specific aggregation

(e) Rouleaux formation of leukocytes.

It is not proposed to discuss the relative importance of each of the factors in the above list. The list is given to indicate that the technical difficulties of leukocyte agglutination are not inconsiderable. It is not difficult to appreciate that reproducibility may sometimes be less than one hundred percent. In spite of all the above problems, Dausset was able to describe a leukocyte group in 1958, which he called MAC. The frequency of the antigen in the French population was 0.58 (11 positive out of 19 - 95% confidence limit 0.34 - 0.82). It is apparent from the thesis of van Rood (1962, page 41), that in the interval 1958 - 1962, Dausset had not developed the MAC system, as no reliable anti MAC sera were then available. Subsequently the MAC group description has been criticized (Shulman 1965), as being "so vague as to resemble any number of antigen systems that have been described since".

Preliminary studies on identical twins suggested that the leukocyte antigens are genetically controlled characters (Dausset and

Brecy (1957), Lalezari and Spalt (1959). Foetomaternal leukocyte
incompatibility was investigated by Payne and Rolfs (1958), who
showed that leukocyte isoantibodies were formed during pregnancy,
and that these antibodies agglutinated the white cells of the child
and his father, but not those of the mother. These results suggest
that the child had inherited a gene controlling leukocyte antigenicity
from his father. Further evidence of inheritance of human leukocyte
antigens was presented by Payne and Hackel (1961) who studied eight
antigens in 30 families with 70 children, and in a population of 99
random individuals. It was apparent from Payne and Hackel's data,
that the antigens detected by each serum, behaved as simple dominant
characters. Further, the reactions with some sera were certainly not
unrelated, but no simple relationship between the sera could be
recognized. It was later realized (Payne et al 1964), that conclus-
ions concerning the independence of antigens described in 1961, were
not strictly valid.

In 1963 van Rood and van Leeuwen published results of
agglutination tests on 100 leukocyte specimens with 66 selected
maternal sera, containing leukocyte agglutinins. The results with the
sera were analysed by computer to determine if any significant
association existed. It was apparent that five of the sera recognized
one antigen while seven other sera recognized a second antigen. The
distribution of these antigens in the panel of 100 unrelated individ-
uals and in 33 families with 140 children, was in agreement with the

hypothesis that the antigens were controlled by two alleles at one
locus. By selection of sera which gave only strongly positive results,
a reliability of antigen determination of over 90% was obtained.
Combining many such sera and ignoring the occasional discrepancy, this
figure could be improved. "The fact that even with the precautions
taken, the results of the grouping of two leukocyte samples out of
the 100 used in the panel, could not be reproduced implies that
leukocyte grouping is still not completely reliable. Nevertheless
the reliability of the grouping with a number of strongly reacting
sera of similar specificity is considerably better than results
obtained with a random serum, reproducibility in the series reported
here being as good as 98%. Even better reproducibility has been
claimed by Shulman, who used the complement fixation test" (van Rood
and van Leeuwen 1963). Since 1963 van Rood and others have continued
to test maternal sera for leukocyte antibodies and have described
ten additional antigens, designated by 5a, 5b, 6a, 6c, 7a, 7b, 7d, 8a,
9a (van Rood et al 1965). The numbers 4 - 9 indicate separate groups
presumably thought initially to be independent. The letters a, b, c
etc. indicate alleles within each group. The use of lower case letters
for dominant characters is not conventional practice in genetic
symbolism. The relationships of these antigens to each other and to
other antigens will be discussed later. Payne and Bodmer (1965)
described two antigens 4c and 4d which they showed were associated
with the 4 system described by van Rood et al (1962). The antigen 4c

was strongly associated with 4a and 4d with 4b.

Payne et al (1964) used an agglutination test to divide selected maternal sera into two groups, recognising two antigens which they designated LA1 and LA2. These antigens behaved as if controlled by two alleles at one locus, with gene frequencies of 0.1617 and 0.2811 respectively. An amorph LA3 with a gene frequency of 0.5572 made the antigen and genetic situation analogous to that of the ABO blood groups. Population data (135 individuals) and family data (40 families with 70 children) were in agreement with the genetical hypothesis suggested above. The LA system has since been complicated by the discovery of a third antigen. This antigen is denoted by LA3, is controlled by a gene LA3 and is present in a sample of the American population with a frequency of 0.2091 Bodmer et al (1966). Tests on 110 individuals, show that there is a negative association between the activities of anti LA1, 2 and 3 respectively, as would be expected if LA1, LA2 and LA3 were alleles. However the agreement between expectations and observations on the basis of this simple hypothesis is not good. $(\chi^2_3 = 12.3 \qquad p < 0.01)$ The discrepancy is attributed by Bodmer et al to difficulties in typing with presently available anti LA3 sera.

(b) Detected by Lymphocyte Cytotoxicity

It has long been known (Pappenheimer 1917) that lymphocytes damaged by antibodies, will not exclude a dye (such as trypan blue)

from their cytoplasm, when they are incubated with the dye. It has been shown that these changes are associated with a change in the permeability of the cell membrane, when cells are incubated with antibody and complement (Green & Goldberg 1960). Effects of antibodies on lymphocyte motility, in the presence of complement, has been studied by Terasaki et al (1960), who used time-lapse cinematography. These workers showed that iso-antibodies would reduce the motility of incubated lymphocytes and induce morphological changes characteristic of cell death (i.e. swelling and clearing of cytoplasm and pynkosis of the nucleus). Techniques have been developed to detect these cytotoxic changes in lymphocytes treated with iso-antibodies.

Recently, Terasaki et al (1964) described a cytotoxic test for the demonstration of lymphocyte antibodies, using very small quantities of serum. With this technique, Terasaki et al (1965) were able to divide 154 sera into 6 groups according to their cytotoxic activity with lymphocytes, using 294 donors. In no case however, were two sera found to have identical activity. It would seem from the observations of Terasaki et al, that some of the difficulties of the agglutination technique are present also when the cytotoxic technique is used.

Dausset et al (1965) have worked with both leukocyte agglutination, and cytotoxicity. These workers have found that their results are compatible with the hypothesis that most of their sera

are detecting antigens, controlled by a complex locus which has been called Hu-1, and controls at least 10 antigens. Although there is no doubt that the work of Dausset et al (1965) is very valuable, it is difficult to decide how many of their conclusions can be accepted because no identical sera were found or produced by absorption, nor were family studies included.

(c) Detected by Complement Fixation

In addition to the work reported by Shulman et al (1964), Wilson et al (1962) described a complement fixation test which they used to study antibodies produced by ten volunteers who were immunized with leukocytes. Six volunteers produced complement-fixing antibodies. All volunteers produced leukocyte agglutinating antibodies. The antisera were used to study the leukocytes of 43 donors. The data obtained (but not presented), suggested that most of the induced antisera contained antibodies against a single leukocyte antigen.

The detection of leukocyte and platelet antibodies, by a complement consumption test has been described by Chudomel et al (1959). This procedure differs slightly from the classical complement fixation in that the patient's own serum is used to lyse the indicator haemolytic system, instead of being inactivated by heat, and complement from another source being used. As the sera are not heated, heat labile antibodies, if these exist, could be detected. A comparison of the data of Chudomel et al with data of other workers is difficult,

because important information on exposure of patients to the antigenic stimulus of pregnancy or transfusion has not been given. The procedure as presented by Chudomel et al, does not appear immediately useful to students of human genetics because of the large amount of work necessary to type a few specimens.

D. PRESENCE OF RED CELL ANTIGENS ON LEUKOCYTES AND PLATELETS

The occurrence of red cell antigens on leukocytes and platelets, has been the subject of many papers. These have been reviewed by Race and Sanger (1962), and by Dausset and Tangun (1965). It appears that every red cell blood group that has been studied by more than one group of workers, has been found to be present and absent on leukocytes and platelets. It is not intended to discuss this dichotomy of scientific opinion beyond the ABO groups. The ABO groups are of particular importance in a number of fields, because of the natural occurrence of anti-A and anti-B in humans lacking the respective antigens. Dausset and Tangun (1965) affirm "the presence of ABO antigens on leukocytes and platelets is no longer a matter of doubt", whereas Shulman et al (1964) conclude "In view of conflicting reports and the fact that techniques used are susceptible to false positive reactions, it seems likely that erythrocyte iso-antigens, either are not present on leukocytes and platelets or are present in relatively insignificant amounts." The in vivo experiments

of Aster (1965) are not good support for those who suggest that the
ABO antigens are present on platelets. It is difficult to appreciate
how platelets could survive for a normal period in vivo, if the blood
contains an antibody against an antigen on the surface of the
platelet. Incompatible A or B red cells have been shown to survive
in circulation for only a few minutes (Jandl and Tomlinson 1958).

E. PRESENCE OF LEUKOCYTE AND PLATELET ANTIGENS ON RED CELLS

All reports to date indicate that leukocyte and platelet
antigens are not present on the surface of red cells. This
observation is supported by many theraputic red cell transfusions
given to patients with leukocyte antibodies, in whom transfused red
cells have normal survival times. (Chaplin et al 1959)

F. RELATIONS BETWEEN ANTIGENS COMMON TO LEUKOCYTES AND PLATELETS

In 1965 at Leiden, leukocytes and platelets from 45 individ-
uals already typed by van Rood et al, were typed by a number of other
workers with some of their best sera. The most important results of
this Leiden workship were reported in Histocompatibility Testing 1965.
These results were:

i. that most of the leukocyte antigens recognized by van
Rood et al (1965) by the defibrination agglutination procedure could
also be detected with the EDTA-agglutination and cytotoxicity tests.
Four of the antigens could also be recognized by the complement

fixation test.

ii. that the antigens Mac, B1, LA2, Group 2 of Terasaki, and 8^a if not identical, are very much alike. The antigens C1 and 7^a are probably similarly related.

At the Leiden meeting, Dausset et al (1965) suggested that the leukocyte antigens they had studied by agglutination and cyto-toxicity, were controlled by genes at a complex locus which they denoted by Hu-1. Other workers have now also realised the possibility that all or most of the antigens they are studying, are in fact controlled by one locus (presumably the same one). Van Rood et al (1966) have adopted the working hypothesis that their groups four, six, seven, eight and nine are closely linked, while group five might be an independent system. Bodmer et al (1966) have presented a pedigree showing the independent segregation of the LA and the 4 genes. Repeat testing of this family did not verify that independent segregation of the genes controlling the two systems had taken place (Bodmer 1967 personal communication). This implies that the leukocyte antigens of the LA system and of the groups four, six, seven, eight and nine groups may all be controlled by the same genetic locus.

It appears that the study of leukocyte antigens is now entering a phase of consolidation. Van Rood et al (1966) and Vredevoe et al (1966) have both published tables indicating that leukocyte and platelet antibodies described independently by various

workers in this field may be identical. The relationships
suggested by van Rood and Vredevoe will be discussed later in this
thesis after the presentation of the results.

G. IMPORTANCE OF LEUKOCYTE AND PLATELET ANTIGENS

(1) Clinical Importance in Transfusion

The physiological and pathological effects of leukocyte
and platelet antibodies in vivo in humans, have been reviewed by
Shulman et al (1964). The experiments reviewed indicate clearly
that infusion of antibody into persons carrying the corresponding
antigen, will cause a precipitous fall in the count of cells in the
blood carrying that antigen. However cells not carrying the antigen
are not so affected. Aster et al/(1964) have shown that platelet and
leukocyte antibodies are an important factor in the etiology of
"fever-chill" transfusion reactions in situations where there is no
red cell incompatibility between donor and recipient. In a group
of 15 patients with platelet and leukocyte antibodies, ten had
suffered transfusion reactions presumably due to receiving cells
containing incompatible platelet and/or leukocyte antigens.

(2) Clinical Importance in Neonatal Thrombocytopenic Purpura

The importance of platelet antibodies in idiopathic
neonatal thrombocytopenic purpura, has been reviewed by Shulman et al
(1964) and by Pearson et al (1964). It appears that a detailed

knowledge of platelet groups is essential before a rational approach can be made to determine the best therapy in individual cases of the disease. Pearson et al have begun this task, but it would seem that in view of the diversity of antigens which may be involved in this disease, a large number of cases will require full documentation before firm conclusions can be drawn.

(3) Possible Clinical Importance of Leukocyte and Platelet

Groups in Organ Transplantation

The investigation of organ transplantation in humans, has intensified in recent years, particularly since the description of the leukocyte groups. The studies recently reported in the Annals of the New York Academy of Sciences by Dausset and Rapaport (1966) and by Ceppellini et al (1966), are the beginning of the experimental elucidation of the importance of blood group differences in skin grafts between individuals. Ceppellini et al have shown that antigenic incompatibilities at the ABO locus, reduce the survival time of skin grafts in unrelated individuals and in sibs, compared with survival time of grafts between compatible persons. Ceppellini et al have suggested that the "strength" of an allele may be only relative to the donor recipient combination. An A_1 allele is probably much stronger in an O, than in an AB recipient, while it may have little effect at all in an A_2 recipient. Incompatibility with respect to the P blood groups (particularly P_1 donors on P_2 recipients) also leads to a reduction in mean graft survival time,

whereas the MN and Rh(D) groups have shown no relation to skin grafts, Ceppellini et al were able to show by analysis of variance, that the mean skin graft survival time for sib-sib grafts, was significantly greater than the time for parent-child grafts, which in turn was significantly greater than skin graft survival times between un-related individuals. (Mean survival times for these three groups were 16.67, 14.63 and 12.12 days respectively). Analysis of variance of the data of Ceppillini et al (1966) was performed after the removal of the P incompatible grafts as well as the ABO incompatible grafts. The differences in mean survival time of grafts between sibs, parent-child and unrelated donors were still all significant.

The results reported by Ceppellini et al with skin graft survival, parallel closely the report of Gleason and Murray (1966), showing that ABO incompatibility increases the chance that a kidney homograft will never function, and that incompatibility with respect to the P groups may lead to a decrease in the duration of function. It is well known that the proportion of survivors after renal trans-plantation is higher when a graft from a related, rather than an unrelated donor has been made (Starzl et al 1966). The work of

Dausset and Rapaport (1966) suggests that the genetical factors disclosed by Ceppellini et al as influencing skin graft survival, may be detectable by leukocyte grouping. Van Rood et al (1966) have shown by leukocyte grouping the donors and recipients in Ceppellini's

experiments, that skin graft survival time is significantly reduced
by an increase in the number of incompatibilities between individ-
uals (particularly for sib-sib pairs).

The parallel between skin and kidney graft results referred
to above, suggests that study of the relation between organ graft
survival (particularly kidney), and leukocyte groups may be
particularly fruitful. Terasaki et al (1966) consider the results
on kidney transplantation of selection (where possible) of a donor
having a low number of incompatibilities with the recipient.
Results with selected donors were better than previous results with
random donors, but the differences have not achieved statistical
significance. However the number of recipients receiving selected
organs was not large (32 cases). In a recent review The Relevance
of Leukocyte Antigens for Allogenic Renal Transplantation, van Rood
et al (1967) conclude: "Too few data are available at the moment to
allow us to make a statement on the importance of leukocyte groups
for kidney graft survival using random donors. However, in view of
the data in the sib-sib group it is likely that leukocyte group
matching will improve the prognosis for renal graft from a random
donor." The suggestion that the leukocyte antigens thought to be
important in transplantation are also present on the platelets,
implies that platelets could also be used in grouping for trans-
plantation.

(4) Other Facets of Biological Importance of Leukocyte and Platelet Antigens

If the leukocyte and platelet antigens are in fact transplantation antigens as appears likely, their study will involve consideration of other matters of fundamental biological importance,

i.e.

i. the chemical nature of the antigens and their normal role in the organism

ii. the selective pressures which influence the polymorphic structure of natural populations.

The selective importance of a transplantation antigen has been studied in the rat by Michie and Anderson (1966). The maintenance of polymorphism in the face of intensive inbreeding indicates the strong selective effect of the genes involved. The occurrence of transplantation antigens in the walls of bacteria, Rapaport, et al (1966) is a further point of fundamental significance. Further study of these antigens should lead to a better understanding of their function in the individual and in the population.

CHAPTER TWO

MATERIALS AND METHODS

The methods and materials used in the investigation of platelet and leukocyte groups are described in this chapter in order to avoid subsequent repetition and breaks in the continuity of presentation. This chapter is divided into three parts:-

A. Collection and preservation of specimens

B. Serological procedures

C. Statistical Design of Experiments.

A. COLLECTION AND PRESERVATION OF MATERIAL

(1) Serum: Human serum to be tested for platelet anti-bodies was obtained from venous blood which had been collected by clean venipuncture and allowed to clot in a plain glass container for 1 to 24 hours. After separation from the clot, serum was some-times stored at $4^{\circ}C$ for up to three days. Longer storage was continued in a deep freeze at $-30^{\circ}C$. No preservatives were added to the sera collected. Before use in a complement fixation test, serum was heated at $56^{\circ}C$ for 30 minutes to inactivate the complement present. Sera for screening for platelet antibodies were obtained from:-

    i. normal donors of the Red Cross Blood Transfusion Service

    ii. maternity patients presenting for anti-natal blood tests at the Red Cross Blood Transfusion Service

     iii. hospital patients at the Royal Adelaide Hospital who
         were being cross matched for blood transfusion and who
         had received previous blood transfusions

     iv. patients with idiopathic thrombocytopenic purpura
         investigated by Dr. Woodliff in the Haemotology
         Department of Royal Perth Hospital.

     v. mothers of patients suffering from neonatal thrombo-
         cytopenic purpura who were treated or referred to the
         Adelaide Children's Hospital

     vi. patients of the renal unit at the the Queen Elizabeth
         Hospital who were being treated on dialysis programme.

A number of sera containing complement fixing platelet antibodies were obtained from Dr. M. Berah of Melbourne. These were treated as above.

(2) <u>Platelets</u>: Platelet preparations were made from whole blood collected in siliconized glassware, using disodium ethylenediamine tetraacetate (EDTA) 5% in 0.85% NaCl solution. One ml. of this solution was added to a one ounce universal container and was sufficient to prevent the coagulation of up to 30 ml. of blood. Platelet rich plasma was obtained from this blood by sedimentation at 500g (2000 rpm) for $5\frac{1}{2}$ minutes in a swing out head MSE 'Super-Multex" Centrifuge and then aspirating the supernatant plasma. Platelet buttons were prepared from this plasma by sedimentation at 3000g (5000rpm) for 10 minutes in a Servall refrigerated centrifuge at 4°C. Platelets were washed once by resuspending in 1% ammonium oxalate and twice in 0.85% NaCl containing 0.1% sodium azide.

The wash in ammonium oxalate caused the lysis of any contaminating red cells. After the second saline wash the platelets were re-suspended in 0.85% NaCl containing 0.1% sodium azide and stored in a cold room at 4°C or in a refrigerator with a temperature range from 2 - 10°C. The yield of platelets from 30ml of blood was usually sufficient to make 3 to 7ml of platelet suspension at a concentration of 300,000 platelets per cubic mm. Initially platelet counts were performed by the method of Brecher and Cronkite (1950). Later platelets were diluted by eye, in order to facilitate the preparation of larger numbers of specimens. Usually 30ml of blood gave a platelet button several millimeters in diameter, which could be resuspended to give a platelet suspension similar in appearance to that prepared by counting. Platelets prepared as above were also used in the quantitative complement fixation test and in absorption and elution experiments outlined later in this chapter. On those occasions when it was necessary to prepare platelets from quantities of blood smaller than 30mls (i.e. 4 - 10ml) then the initial centrifugation was performed as specified above in a test tube of smaller diameter than the one ounce bottle, referred to above, so that the same depth of blood (about 5cm) could be spun. Platelets prepared and stored as above could be resuspended and used as a source of antigen in the complement fixation test several months after they had been prepared.

The method of platelet separation described above is very

similar to the methods used by Aster et al (1964) and by Shulman et al (1964).

(3) Reagents for Complement Fixation Test

i. Diluent (Veronal Buffer): The diluting fluid used in the complement fixation test was that described in Kabat and Meyer's Experimental Immunochemistry (1961). A stock solution was prepared by dissolving:-

      85.0 gram of NaCl

      3.75 gram of Na-5, 5-diethyl barbiturate (Barbitone Sodium) in about 1400ml of distilled water.

      5.75 gram 5, 5-diethyl barbituric acid (Barbitone) was dissolved separately in 500ml of hot distilled water and the solutions mixed.

      After allowing to cool to room temperature 5.0ml of a solution containing 1.00 M $MgCl_2$ and 0.30 M $CaCl_2$ were added and distilled water was added to 2000m.

This solution was diluted five fold with distilled water and the pH checked before use, with a Phillips PR 9400 continuous reading pH meter. The usual pH of buffer prepared as above was 7.5 to 7.6. This was adjusted to be within the range 7.3 - 7.5 with a small quantity (about 1ml per 250ml of final buffer) of N/10 HCl.

ii. Haemolysin: Anti-sheep red cell rabbit serum was purchased from Commonwealth Serum Laboratories, Melbourne, Australia. This serum was titrated as described below to determine the strength that should

be used to sensitize sheep red cells for the complement fixation test.

iii. <u>Sheep Red Cells</u>:   Sheep red cells in Alsever's solution were purchased from Commonwealth Serum Laboratories.   These red cells were usually at least several days old when received and could be kept for several weeks before the presence of excessive spontaneous haemolysis rendered them unsatisfactory.   Red cells obtained from this source did not appear to vary greatly in their sensitivity to complement, but titres were usually performed on each batch of sensitized sheep red cells to ensure that standard amounts of complement were used in the complement fixation test.

iv. <u>Complement</u>:   Guinea pig serum as a source of complement was obtained by collecting blood from the jugular veins and carotid arteries of large guinea pigs.   Blood was collected separately from each animal and after clotting and standing for one hour at room temperature the specimen was centrifuged and the serum asperated. Sera from 12 or more animals were pooled and stored in aliquots of one or two ml. at -30°C until required.   Under these conditions it was found that complement activity did not alter significantly over a six month period.   When a quantity of guinea pig serum less than that in one aliquot was required, the remaining serum was immediately re-frozen.   This was done up to six times without noticeable alteration of complement activity.

B. QUALITATIVE COMPLEMENT FIXATION SEROLOGICAL PROCEDURES

  In all qualitative procedures described in this thesis where quantities of reagents were dispensed in drops, the drops were delivered from disposable glass Pasteur pipettes ("TransPets" manufactured by Clay Adams New York U.S.A.)

  The size of drops from these pipettes was tested for variation by weighing single drops of distilled water delivered by a sample of the pipettes. Mean drop weight was 0.0269 grams $\pm$ 0.0014 i.e. 37 $\pm$ 2 drops to 1ml of distilled water.

  (1) Titration of Haemolysin: Haemolysin was titrated by preparing dilutions in veronal buffer from 1/400 to 1/12800 and adding 0.1ml of each dilution to a row of tubes (in quadruplicate). Following this 0.2ml of guinea pig serum diluted 1/20 and 0.5ml of a 1% sheep red cell suspension washed four times and finally suspended in veronal buffer, were added separately to each tube and the mixture incubated for 30 minutes at 37°C with occasional shaking to ensure that the red cells remained in suspension. After incubation the tubes were centrifuged at 500g for 2 minutes. The end point was read as the highest dilution of haemolysin which gave complete haemolysis of the sheep's red cells. Usually a 1/4000 dilution of haemolysin was sufficient to cause complete haemolysis.

  (2) Sensitization of Sheep Red Cells: Sheep red cells were sensitized by incubation with haemolysin for 30 minutes at 37°C.

Equal volumes of 5% red cell suspension washed four times in veronal buffer and haemolysin at four times the titre determined in the titration of haemolysin (i.e. usually 1/1000), were mixed slowly with stirring and were incubated as above. After incubation, red cells could usually be stored about one week before excessive spontaneous haemolysis occurred.

Dilution of sheep red cells for titration of haemolysin and for sensitization was carried out photometrically using a Shimadzu Spectrophotometer type ZV-50. A 1/15 dilution in distilled water of a 5% red cell suspension gives an optical density of 0.760 (Shulman et al 1964).

(3) <u>Titration of Complement</u>: Dilutions of guinea pig serum in veronal buffer from 1/10 to 1/80 were prepared and one drop of each dilution added to a 10 x 75mm test tube. Titrations were usually performed in quadruplicate. After one hour incubation at 37°C, 0.6ml of sensitized sheep cells diluted one to five with veronal buffer were added using a Becton Dickinson 2ml dispensing syringe. (Becton, Dickinson and Company Rutherford, N.J., U.S.A.) Tubes were then incubated for a further 40 minutes at 37°C and transferred to ice cold water until they could be conveniently centrifuged at 2000rpm for a few minutes to settle any unlyzed red cells. Tubes were shaken once during the incubation period to reduce settling which may affect the proportion of red cells lyzed.

Tubes were examined by viewing them from above while they were
supported in a rack with a perspex bottom. This rack was placed on
a locally made viewing box 12" x 9" illuminated by one circular
25 watt fluorescent tube. Comparisons between tubes could be made
quickly by moving only the eye of the viewer. Up to 80 tubes could
be examined at one time.

Results typical of those obtained from such a titration
are shown in Table 2:1 below.

Dilution of C'	29/6/67			
1/10	–	–	–	–
1/15	+w	+w	+w	+w
1/17.5	+w	+w	+w	+w
1/20	+w	+w	+w	+w
1/22.5	+w	+w	+w	+w
1/25	+w	+w	+w	+w
1/30	+	+	+	+
1/40	2+	2+	2+	2+
1/60	4+	4+	4+	4+

Table 2:1   Titration of C' in guinea pig serum (12/12/66) with sheep
            red cells sensitized 29/6/67.

Where 4+ denoted that there has been no lysis of red cell
The supernatant contains no detectable haemoglob

3+ (not observed in this titration) denotes that there is a trace of haemoglobin liberated but that there is still a substantial proportion of red cells remaining,

2+ more than a trace of haemoglobin and slightly less unlyzed red cells,

+ large proportion of cells lyzed but still an obvious deposit in the bottom of the tube,

+w almost 100% haemolysis, but some red cells in the bottom of the tube,

− no detectable sheep red cells unlyzed.

The results of the titration did not appear significantly different when an extra two drops of diluent were added to each tube so that the volume in each tube would then be identical with that used in the complement fixation test. On some occasions the incubation period prior to the addition of sheep red cells was shortened or omitted. Results obtained suggested slightly greater activity of complement than if incubation had been one hour.

Titration of complement was used as a guide to ascertain the quantity of complement which should be used in the complement fixation test. Usually the quantity of complement used was sufficient to cause almost complete lysis of 0.6ml of sheep red cells in 40 minutes at 37°C. On the basis of the results in Table 2:1 it was decided to use 1/22 dilution of guinea pig serum

(12/12/66). This was in good agreement with previous experience with this serum. It was found that if extra units of haemolysin were added when the sheep red cells were sensitized then the quantity of guinea pig serum required to produce a given degree of lysis was reduced.

(4) <u>The Qualitative Complement Fixation Test</u>: These tests were carried out in 10 x 75mm test tubes. One drop of the appropriate dilution of guinea pig serum was added to each tube. This was followed by one drop of serum or serum dilution, and finally one drop of platelet suspension prepared as outlined above. Usually it was convenient to perform up to 480 tests at one time. The same serum or serum dilution was added to each tube in a column (columns labelled from A - J). Platelets from one person were added to each row of tubes labelled numerically from 1 to n (integers). All tubes used in large qualitative complement fixation tests were labelled with a letter (indicating column) and a number (indicating row) so that the possibility of inadvertant mixing of tubes was very low. The last two rows and columns were kept for controls, containing serum only and platelets only respectively.

	Platelets	Serum					
		A	B	C	D . . . I	J	
	1						
	2						
	3						
	4						
	5						
	6						
Control[1]	7						
Control[2]	8						

Figure 2:1 Usual arrangement of tubes for the qualitative complement fixation test

The serum controls contained one and two drops of serum respectively. Occasional occurrence of anticomplementary sera made it advisable to have two controls as recommended in Kabat and Meyer (1961). Usually only one platelet control was included, for seldom was it found that platelets were anticomplementary. As seen in Figure 2:1, there were usually at least two tubes containing only complement which served as a control of complement activity. In order to reduce the deterioration which could occur in the complement while standing at room temperature, racks of partially prepared experimental material which were not required, were stored in the

freezing compartment of a refrigerator (-15 to +2°C). It was not usual to leave tubes in the freezing compartment for more than one hour. Immediately after platelets were added to a set of tubes, they were incubated at 37°C for 1 hour. While incubation continued, 0.60ml of sensitized sheep red cells diluted one to five with veronal buffer were added and incubation continued for 40 minutes. Red cells could be added to each of 80 tubes in a rack in less than two minutes using a Becton-Dickenson 2ml dispensing syringe referred to previously. Red cells were agitated by shaking the tubes at least once during the incubation period. After incubation, tubes were removed and chilled to 4°C until they were centrifuged at 2000rpm for a few minutes. The results were then read using the method described for the reading the Qualitative Titration of Complement. Results were recorded as 4+, 3+, 2+, +, +w on the basis of the criteria referred to previously. Negative results (no remaining red cells) occurred infrequently. In this thesis the results are recorded in tables and appendices as 4, 3, 2, 1, and 0 where 0 corresponds to combined - and +w reactions, 1 to 1+ etc. These results were then interpreted in the light of appropriate controls as either positive, negative or doubtful. Any tube giving two pluses greater than controls was considered positive, one plus greater than controls doubtful positive, and same as controls, negative. Most of the sera used to obtain the genetical data presented in this thesis were used at a dilution such that a positive platelet specimen gave a 3+ or 4+ reaction while a negative specimen gave a +w reaction.

(5) <u>Absorption and Elution of Antibody</u>:   Antibodies were absorbed from serum and eluted from platelets using the method described by Shulman <u>et al</u>.(1964). Platelet suspensions to be used in an absorption experiment were centrifuged at 5000g for 10 minutes to give a button of approximately 10^9 platelets. These platelets were then suspended in about 1ml of serum and the mixture incubated at 37° for one hour with occasional mixing, to ensure that platelets remained in suspension. After incubation, the platelet serum mixture was centrifuged at 20,000g at 4^oC for 15 minutes. The serum was then removed and after washing the inside of the tube once or twice with cold saline, the platelets were then resuspended in cold saline, and this suspension was acidified to pH3 with N/10 HCl added dropwise, with constant mixing. The pH level was controlled using the Phillips Pr 9400 continuous reading pH meter referred to previously. The acidified platelet suspension was centrifuged (3000g for 10 minutes) after standing at room temperature (about 20^oC) for 10 minutes. The supernatant was removed and neutralized with N/10 NaOH (to pH 5.5) and N/50 NaOH (up to pH 6.5 - 7.0). The alkali was added dropwise and the solution mixed with a pasteur pipette to ensure an even distribution of reagent. The pH level was controlled as above. Care was taken to stop the neutralization of antibody on the acid side of pH7, as excessive alkali is said to denature antibody (Shulman <u>et al</u> 1964).

During the neutralization of eluate, a white fluffy

precipitate often formed. This was removed by centrifugation at 500g
for 5 minutes before storage of the eluate at -30°C. Eluates were
then used as antibody. Absorbed serum was also titrated to determine
the change in antibody level.

C. STATISTICAL DESIGN OF EXPERIMENTS

(1) <u>Number of Platelet Specimens Used to Test Sera for
Platelet Antibodies</u>: While considering the design of experiments to
detect platelet antibodies, it was felt that those antigens for which
the population was polymorphic would be more easily studied than those
antigens which were either very common, or very rare. Detecting an
antibody against a very common antigen (present in say 90% or more
of a population) does not require a large sample size. If the
frequency of the antigen is 0.9, then the probability of detecting the
corresponding antibody using cells from two random donors is 0.99. It
was decided that antibodies which reacted with less than 20% of the
population would be less easily studied than those which reacted with
more than this proportion. Table 2:2 shows that screening sera against
a panel of platelets from 14 donors gives 95% certainty of detecting
antibodies which react with 20% of the population and reasonable
opportunity (from 78% up) of detecting antibodies reacting with 10
to 20% of the population. After certain platelet antigens had been
defined it became possible to exclude the presence of certain complement
fixing platelet antibodies by testing sera against platelets of known
antigen composition.

Frequency of Antigen in a population	Probability of detecting corresponding antibody with sample of 14 random platelet specimens
0.50 or more	0.999939
0.40	0.9992
0.30	0.994
0.20	0.956
0.19	0.947
0.18	0.938
0.15	0.897
0.10	0.772
0.05	0.522
0.01	0.131

Table 2:2

(2) <u>Minimum Number of Cell Suspension to be Tested to Determine the Specificity of Sera Containing Platelet Antibodies</u>:

i. <u>Where two sera contain the same antibody</u>: A number of possible relationships may exist between two sera containing antibodies. The simplest possible relationship is that each serum contains the same antibody. When this relationship exists between two sera, then all persons tested will be either positive or negative with both sera. The result of testing 'n' persons with such sera is as shown in Table 2:3.

	+	−	
+	a	o	a
−	o	d	d
	a	d	n

Table 2:3

Race and Sanger (1962) have shown that the number of specimens required to show that the antibodies in two sera are the same is quite small. At 95% level $a = d = 3$ $(n = 6)$ would occur by chance only once in twenty times if the two sera used were unrelated.

ii. Where one serum contains two antibodies and other serum contains one of these antibodies: A second possible relationship is that one serum contains two antibodies, only one of which is found in the second serum. Results expected from a classification of individuals with two such sera are shown in Table 2:4.

	+	−	
+	a	o	a
−	b	d	b + d
	a + b	d	n

Table 2:4

The number required to obtain the 95% significance level under these circumstances is somewhat higher than the minimum number above. For example the probability of the results in Table 2:5 being obtained with unrelated antibodies is 7/99 (Fisher's Exact Method, Fisher 1958).

	+	−	
+	5	0	5
−	3	4	7
	8	4	12

Table 2:5

iii. **Sera with independent antibodies:** A third relationship which may exist between two sera is that the two sera may contain independent antibodies. The sera may have one or more antibodies each. When these antibodies are independent entries should appear in all four cells of the two way classification and should be proportional to the products of the marginal totals (Table 2:6).

	+	−	
+	a	c	a + c
−	b	d	b + d
	a + b	c + d	n

Table 2:6

The χ^2_1 test can be used to show whether data obtained agree with this hypothesis of independence of reactions produced by two sera, provided that the smallest number of entries expected in any cell is greater than five.

iv. <u>Sera with many antibodies where some are shared</u>: Where two sera have one or more antibodies in common, and each serum has other antibodies not shared, it is expected that there will be a positive association between the reactions with the sera so that 'ad' will be greater than 'bc'. The magnitude of this association will depend on the frequency of reaction with the antibodies present in common compared with the frequency of reaction with the unshared antibodies in each serum.

v. <u>Antibodies against associated antigens</u>: Detection of antigens which are associated in a population may give the appearance of an association between two antibodies. An example of such an association can be seen when looking at the distribution of positives with anti-C and anti-D of the Rhesus system on red blood cells. The strength of association observed depends on the distribution of the antigens in the population studied.

vi. <u>Antibodies against antigens controlled by alleles</u>: Where two antibodies are detecting antigens which are the product of allelic genes, the association will be in the opposite direction to (i) above i.e. 'bc' will be greater than 'ad'. The magnitude of the

association will depend on the frequency and distribution of the alleles in the population. For 'd' $\geq$ 0 there must be at least three alleles in the population, two producing antigens and the third producing nothing reacting with the two antisera being considered. This situation is not unknown in blood group systems; perhaps the best known example is the ABO system of red cell antigens.

(3) <u>Number of Cell Suspensions to be Tested to Distinguish on Population Data Between the Hypothesis that Two Antigens are Controlled by Independent or Allelic Genes</u>: When a group of n persons is tested with two different antibodies, the results may be set down in a 2 x 2 table as shown in Table 2:6 above. It is well known that once the marginal totals are fixed only one of a, b, c or d can be varied independently. The table can be rewritten as shown below, Table 2:7.

	$+$	$-$	
$+$	a	$a_2 - a$	a_2
$-$	$a_1 - a$	$n - a_1 - a_2 + a$	$n - a_2$
	a_1	$n - a_1$	n

<u>Table 2:7</u>

χ^2_1 testing independence of the two antigens detected

is $\chi^2_1 = \dfrac{\left[(a_1 - a)(a_2 - a) - a(n - a_2 - a_1 + a)\right]^2 n}{a_1(n - a_2)\, a_2(n - a_1)}$

Now $(a_1 - a)(a_2 - a) - a(n - a_2 - a_1 + a) = a_1 a_2 - na$

$\therefore \chi^2_1 = \dfrac{(a_1 a_2 - an)^2 n}{a_1(n - a_2)\, a_2(n - a_1)}$

If the antigens (+) represented in Table 2:8 are controlled by allelic genes at the same locus present in a population with a frequency of p and q while other alleles are present with a total frequency r then $p + q + r = 1$. The proportion of observations expected in each class on the basis of this hypothesis is shown in Table 2:8.

	+	−	
+	$2pq$	$q^2 + 2qr$	$1 - (p + r)^2$
−	$p^2 + 2pr$	r^2	$(p + r)^2$
	$1 - (q + r)^2$	$(q + r)^2$	1

Table 2:8

When estimates of gene frequency which agree with the marginal total are used, then it may be seen that $1 - (q + r)^2$ of Table 2:8 is equivalent to a_1/n in Table 2:7. If Table 2:7 is taken to represent observations, then χ_1^2 to test whether the data agree with the hypothesis that the antigens are controlled by alleles is:

$$\chi_1^2 = \frac{(a - 2pqn)^2}{2pqn} + \frac{(a_1 - a - (p^2 + 2pr)n)^2}{(p^2 + 2pr)n} + \frac{(a_2 - a - (q^2 + 2qr)n)^2}{(q^2 + 2qr)n}$$

$$+ \frac{(n - a_1 - a_2 + a - r^2 n)^2}{r^2 n}$$

$$= \frac{(a - 2pqn)^2}{n} \left[\frac{1}{2pq} + \frac{1}{p^2 + 2qr} + \frac{1}{q^2 + 2qr} + \frac{1}{r^2} \right]$$

since each of the numerators above are equal to $(a - 2pqn)^2$.

These equations for χ_1^2 can be used to determine the population size (n) to be examined in order to distinguish between the hypotheses above at a certain probability level. This is similar to the method of planning family size in order to distinguish two segregations as outlined by Mather (1957). The number of specimens required to distinguish between these hypotheses at the 97.5% level for various gene frequencies is shown in Table 2:9. It will be noted that the entries in Table 2:9 are larger than those which could be calculated

from the formula given by Bodmer and Payne (1965). This difference
arises as Bodmer and Payne have calculated n for a situation where
the data agree exactly with one hypothesis, whereas here account is
taken of the possibility that observations may deviate by chance
from expectations based on a correct hypothesis.

Proportion of population with antigen	0.51	0.36	0.19
Corresponding gene frequency	0.3	0.2	0.1
0.3	147		
0.2	209	305	
0.1	410	594	1158

Table 2:9 Size of population to be sampled to distinguish
hypotheses.

INCIDENCE OF COMPLEMENT FIXING

PLATELET ANTIBODIES

Platelets from 14 random donors were used to screen
sera for complement fixing platelet antibodies. As indicated in
Chapter 2 a panel of cells from 14 donors provides reasonable
certainty that antibodies against common antigens in the population
will be detected. When platelets from one batch had been used or
became more than three weeks old then these platelets were replaced
by a fresh batch from different individuals.

Maternal sera were examined in the initial part of this
study by testing them against platelets from random blood donors.
In order to eliminate reactions attributable to the ABO blood group
system sera were subsequently screened against platelets from random
group O donors only.

A. COMPLEMENT FIXING PLATELET ANTIBODIES IN MATERNAL SERA

Serum was obtained from expectant mothers gravida two
or more who presented at the Red-Cross Blood Transfusion Centre during
the last three months of their pregnancy for routine red cell antibody
tests. This method of selection resulted in a large proportion of
Rh-ve sera being tested (171/273 = 63%). The distribution of the ABO
and Rh groups of a series of 273 women studied is given in Table 3:1.

Rh groups

ABO Groups		+	−	
	A	39	77	116
	O	50	72	122
	B	12	19	31
	AB	1	3	4
		102	171	273

Table 3:1 ABO and Rh groups of 273 women screened for complement

fixing platelet antibodies

Complement fixing activity was obtained with sera from 28 of these women. The distribution of the ABO and Rh groups in those subjects with antibodies is shown in Table 3:2.

Rh groups

	+	−	
A	1	3	4
O	11	9	20
B	1	3	4
AB	−	−	−
	13	15	28

Table 3:2 ABO and Rh groups of 28 women with complement fixing

platelet antibodies

A comparison of the Rh and ABO groups of those with and those without antibodies (Table 3:3) indicates that there is no association between the Rh group of the women and the presence of platelet antibodies ($\chi^2_1 = 0.71$, $p > 0.30$). However there was a significantly higher incidence of antibodies in group O women compared with group A women. (Table 3:3 $\chi^2_1 = 9.61$, $p > 0.01$). Groups B and AB have not been included in this comparison because of the smaller number tested.

	Rh +	Rh −	
anti- +	13	15	28
bodies −	89	156	245
	102	171	273

	A	O	
+	4	20	24
−	112	102	214
	116	122	238

Table 3:3 Comparison of Rh and ABO blood groups of women with and without complement fixing activity against platelets in their sera.

Examination of the ABO groups of panel members showed that 21 sera had reacted only with platelets from ABO incompatible donors. Mainly sera from group O women reacted with platelets from group A blood donors. This relationship indicated that the complement fixation observed with these sera could be attributed to interaction between ABO antibody and antigen, Table 3:4.

ABO Groups	Reaction with platelets from ABO compatible donors	Complement Fixation with platelets from ABO incompatible donors only
O	2	18
A	3	1
B	2	2
	7	21

Table 3:4   ABO blood groups of women whose serum fixed complement with ABO compatible and incompatible platelets.

As the antibodies detected in this series were all weak (active in neat serum but not at a dilution of 1/8) and were available only in a small volume no further attempt has been made to elucidate their nature. No details of the specificity of the 7 sera giving reactions not attributable to the ABO system are available.

B. COMPLEMENT FIXING ANTIBODIES IN POLYTRANSFUSED PATIENTS

Two hundred and five sera from 119 transfused patients from the Royal Adelaide Hospital were screened for complement fixing antibodies against a panel of 14 random group O donors. The ABO and Rh groups of these patients is given in Table 3:5. Antibodies were found in 15 sera. No association is present between platelet antibodies and the ABO or Rh group of the serum donor Table 3:6.

Red cell groups	Rh +	Rh −	
C	52	9	61
A	34	11	45
B	6	3	9
AB	4	−	4
	96	23	119

<u>Table 3:5</u> ABO and Rh groups of transfused patients tested for complement fixing platelet antibodies.

	Rh groups +	Rh groups −		ABO groups A	ABO groups O	
Complement Fixing antibodies +	11	4	15	5	8	13
Complement Fixing antibodies −	85	19	104	40	53	93
	96	23	119	45	61	106

$$\chi^2_1 = 0.18, \; p > 0.50 \qquad \chi^2_1 = 0.01, \; p > 0.9$$

<u>Table 3:6</u> Comparison of Rh and ABO groups of transfused persons with and without complement fixing platelet antibodies.

Brief clinical details of the patients with serum containing platelet antibodies are shown in Table 3:7. Clinical details for all patients tested in this series are recorded in Appendix One. The incidence of complement fixing platelet antibodies in this series of patients $(15/119 = 12.6\%)$ is not significantly different from the results of Aster <u>et al</u> (1964) who found antibodies in 15 of 172 patients (8.7%).

Name	Sex	Blood group	Age	Diagnosis	No. Units Trans- fused blood	No. Sera Tested
CL	M	O+	65	Myelofibrosis	227	1
CO	M	O+	57	Hypoplastic anaemia	10	1
CA	F	O+	63	Ca pancreas	18	1
CA	F	A+	28	Choriocarcinoma	28	3
GO	M	B-	63	Chronic Renal Failure	10	2
NO	F	A+	45	Renal Failure, Cirrhosis	14	1
PA	M	O-	44	Fracture neck femur	1	1
JE	F	O-	70	Aplastic Anaemia	66	5
KL	F	AB+	42	Ulcerative Colitis	50	1
MA	F	A+	45	Aplastic Anaemia	79	1
SA	F	A+	36	Acute Leukaemia	114*	6
SZ	F	O+	36	Antipartum Haemor. .	2	1
VO	F	O+	79	ChronicMyeloid Leukaemia	14	1
WH	F	O+	63	Multiple hereditary telangiectasis	50	6
WE	F	A-	54	Acute Leukaemia	5	1

Table 3:7 Clinical details of transfused patients with complement fixing platelet antibodies.

(1) Relationship to number of Transfusions Given

Approximately half of the patients of Aster et al were treated with whole blood and the remainder with whole blood and platelet concentrates. In the series reported here only three patients received platelet concentrates. In Table 3:7 and Appendix one these individuals have been marked with an asterisk.

The transfusion histories of the patients tested for platelet antibodies in Adelaide and in Boston are compared in Table 3:8, a and b respectively.

	Number	Total number units transfused	Mean number units per patient
(a)			
Patients without antibodies	104	2111	20.3 ± 1.9
Patients with antibodies	15	688	45.9 ± 15.5
(b)			
Patients without antibodies	157	5361	34.1
Patients with antibodies	15	621	41.4 ± 7.5

Table 3:8 Comparison of the transfusion histories of patients with and those without platelet antibodies,

(a) from Adelaide   (b) from Boston (Aster et al 1964)

It will be noted that the mean number of transfusions given to patients who developed platelet antibodies is approximately the same in both series. The mean number of transfusions received by patients with antibodies is greater than the average for patients without antibodies in both series. In neither series however is the mean difference between the group with antibodies and the group without antibodies significant at the 5% level. For the Adelaide series $(d = 1.6, p = 0.10)$.

It appears that one exposure to foreign antigen may be sufficient to produce platelet antibodies in a recipient. Usually however repeated transfusions are necessary before antibody can be detected.

(2) Relationship to Transfusion Rections

Complement fixing platelet antibodies were found frequently $(10/29 = 34.5\%)$ in patients who suffered pyrexial reactions associated with blood transfusion. In this series no attempt was made to distinguish between "febrile" and "fever-chill" reactions as Aster et al did in 1964. The series reported here is similar to that of Aster et al as the incidence of platelet antibodies is significantly higher in patients with a febrile reaction compared with patients with no reaction. $(\chi^2_1 = 13.3, \quad p < 0.001 \quad \text{Table 3:9})$

| | (a) | | (b) | |
Reaction	Number with antibody	Number without antibody	Number with antibody	Number without antibody
Urticarial	0	4	0	28
Pyrexial	10	19	10	28
No reaction	5	81	5	135

Table 3:9 Frequency of Platelet Antibodies in Patients with and
without Transfusion Reactions,

(a) in Adelaide   (b) in Boston (from Aster et al 1964)

(3) Relationship to Sex

The incidence of complement fixing platelet antibodies and
transfusion reactions in males and females is compared in Table 3:10.
No significant differences in incidence in different sexes are apparent.

	(a) Antibodies			(b) Transfusion Reactions		
	Present	Absent	Total	Present	Absent	Total
Males	4	53	57	12	45	57
Females	11	51	62	21	41	62
	15	104	119	33	86	119

$$\chi^2_1 = 2.2, \; p > 0.1 \qquad \chi^2_1 = 1.8, \; p > 0.1$$

Table 3:10 Incidence of (a) complement fixing platelet antibodies
(b) transfusion reactions in different sexes.

(4) Relation to Diagnosis

A summary of the diagnoses of transfused patients tested
for antibodies is given in Table 3:11. A comparison of this summary
with that given by Aster et al (1964) indicates that:

i. a wider range of diagnoses were found in the patients in this
   series who produced antibodies than was the case in the series
   of Aster et al. In Aster's series 14/15 patients with
   antibodies had haematological diseases whereas in this series
   only 7/15 had this type of disease.

ii. the incidence of platelet antibodies in this series was not
    significantly lower in leukaemics than in aplastic anaemics.
    In Aster's series the incidence of platelet antibodies was
    significantly lower in leukaemic patients than in aplastic
    anaemic patients $(\chi^2_1 = 9.2, p < 0.001)$

C. IDIOPATHIC THROMBOCYTOPENIC PURPURA

Twenty sera from patients with idiopathic thrombocytopenic
purpura (I.T.P) were examined from complement fixing platelet anti-
bodies. These sera had been forwarded for examination by Dr. Woodliff
of the Royal Perth Hospital. Antibodies were found in 2 sera SP and
DO.

Name	Sex	Blood Group	Age	Diagnosis	Times Tested	Number Pregnancies	Transfusion Units
SP	F	A+	17	I.T.P.	1	nil	4
DO	F	A-	47	I.T.P.	1	5	3

Diagnosis	Number with Antibodies	Number without Antibodies
Leukaemia	3	9
Aplastic anaemia	2	8
Myelofibrosis	1	2
Other Anaemias	1	11
Chronic Renal Disease	2	8
Carcinoma (various sites)	2	15
Ulcerative Colitis	1	2
Duodenal and Peptic Ulceration	-	13
Cirrhosis	-	6
Other Surgical Diseases	1	10
Other Medical Diseases	2	20
	15	104

Table 3:11 Incidence of complement fixing antibodies in transfused patients according to diagnosis.

Both of these patients had been transfused before their serum was
tested for platelet antibodies. Clinical details of the patients
whose serum did not contain complement fixing platelet antibodies
are not available. Complement fixing antibodies against platelets
were found in the serum of one patient in the Royal Adelaide Hospital
with I.T.P. This woman's history included two pregnancies and one
recent blood transfusion with a pyrexial reaction. The specificity
of her antibody has not yet been identified.

D. COMPLEMENT FIXING PLATELET ANTIBODIES IN THE SERUM OF MOTHERS OF
   INFANTS WITH NEONATAL THROMBOCYTOPENIC PURPURA

The sera of three mothers with infants who developed purpura
shortly after birth were tested for platelet antibodies. One serum
(SI) was found to contain a strong complement fixing antibody, the
other two were negative. Examination of these sera for blocking
antibody activity was not conducted.

E. COMPLEMENT FIXING PLATELET ANTIBODIES IN THE SERUM OF PATIENTS ON A
   CHRONIC DIALYSIS PROGRAMME

Patients receiving dialysis on a renal unit could be included
in the polytransfused series. However because of the special interest
in this group who are potential recipients of kidney grafts they will
be considered separately. Sera from nine dialysed patients at the

Queen Elizabeth Hospital were tested for complement fixing antibodies. Three positive sera were found. Clinical details of these patients are shown in Table 3:12. At each dialysis patients were exposed to three pints of fresh blood.

Name	Sex	Blood Group	Age	Number of Pregnancies	Dialyses	Antibodies
SC	M	0+	26	–	93	no
WE	F	0+	39	3	38	yes
LA	M	0	25	–	194	no
WI	F	0	43	1	119	yes
MA	F	0	23	–	74	no
FO	M	0+	40	–	129	yes
GO	M	A+	48	–	4	no
MC	M	0+	34	–	71	no
KN	F	0+	23	1	3	no

Table 3:12   Clinical details of dialysis patients tested for complement fixing platelet antibodies.

F. COMPLEMENT FIXING PLATELET ANTIBODIES IN THE SERUM OF NORMAL BLOOD

DONORS

Serum from 138 random normal blood donors was screened for platelet antibodies but none was found. This result is in agreement with the results of Aster et al (1964) and Klemperer et al (1966) who studied 55 and 714 normal sera respectively without finding a

"naturally occurring" complement-fixing platelet antibody. The difference between the normal and transfused series reported here is significant, $p < 10^{-5}$. The antibody specificity of the sera considered in this chapter will be summarized in chapter 10.

PRELIMINARY ATTEMPTS TO IDENTIFY COMPLEMENT
FIXING PLATELET ANTIBODY SPECIFICITY

A. A PRELIMINARY COMPARISON OF THE COMPLEMENT FIXING PLATELET ANTIBODY ACTIVITY IN EIGHT SERA

As a preliminary step in the resolution of the platelet antibodies, a group of eight sera available in a sufficient quantity and reacting with not less than 10% of the population were tested against a panel of up to 98 different platelet suspensions. The platelets were prepared from random Red Cross blood donors, in eight batches. Tests were completed within 10 days of blood collection. The sera were tested at various dilutions as had been indicated by preliminary titration experiments (i.e. CL and MA) or the serum was used neat. The result of each test was recorded in accordance with the criteria set out for reading the qualitative complement fixation test in Chapter 2, and can be seen in Appendix 2.

The activity of each serum or serum dilution is recorded in Table 4:1. As these experiments were conducted with a minimal quantity of complement, the difference between a + and +w is not great. In fact some of the control tubes gave + reactions, others +w. When these two classes are grouped it is clear that most sera (WH excepted) divided people into two main classes, those whose platelets gave 4+ and 3+ reactions and those whose platelets gave +, +w, and −

reactions. The proportion of specimens giving 2+ reactions was less than 5% (CO 13% and VO 10% excepted).

Serum	Strength	4+	3+	2+	+	+w	-	
NO	Neat	49	15	1	10	23	-	98
CO	Neat	18	6	13	18	38	5	98
VO	Neat	38	4	10	9	36	1	98
VO	1/8	26	5	7	16	44	-	98
SI	Neat	10	-	2	4	45	15	76
CL	1/16	68	2	2	9	4	-	85
MA	Neat	65	4	3	6	19	1	98
MA	1/8	56	1	5	12	23	1	98
WH	Neat	-	-	9	11	32	-	52
KL	Neat	20	2	3	27	45	1	98

Table 4:1 Reactions of eight sera with a panel of up to 98 platelet suspensions from unrelated adult blood donors.

A comparison of the activities of the sera taken pairwise from the results of the experiment reported above, was made by setting down in a 6 x 6 table, the numbers of platelet specimens giving particular strength reactions with the pair of sera being compared. Table 4:2 is an example of such a comparison. To facilitate

the preparation of these tables, the data were transferred to punched cards and these were then sorted to obtain the information required. In order to analyse the serum comparisons, the data were reduced to 2 x 2 tables by pooling classes 4+ and 3+ and classes 2+ or less (except for WH, where the 2+ and + classes were pooled). Table 4:2 was thereby reduced to Table 4:3.

NO (neat)

		4+	3+	2+	+	+w	−		
	4+	13	3	1	1	−	−		18
	3+	3	2	−	1	−	−		6
CO (neat)	2+	9	2	−	−	2	−		13
	+	9	−	−	4	5	−		18
	+w	14	8	−	4	12	−		38
	−	1	−	−	−	4	−		5
		49	15	1	10	23	−		98

Table 4:2 Comparison of activities of NO and CO with a panel of 98 platelet suspensions.

CO (neat)		NO		
		4+ or 3+	2+ or less	
	4+ or 3+	21	3	24
	2+ or less	43	31	74
		64	34	98

$$\chi_1^2 = 5.67, \ 0.02 > p > 0.01$$

Table 4:3 Comparison of activities of NO and CO with a panel of 98 platelet suspensions.

Table 4:4 shows all the 32 comparisons that were made in this way. χ_1^2 values testing proportionately (Yates correction applied) for each 2 x 2 comparison were calculated regardless of whether or not the smallest expectation was less than five. These χ_1^2 values were used only as a guide to formulate the series of working hypotheses set out below. The significant χ_1^2 values calculated from the comparisons are shown in Table 4:5. An examination of these χ_1^2 values suggest the following hypotheses were worth further investigation:

1. MA and NO have an antibody in common

2. the antibody in 1. may also be in CL 1/16

3. CO, NO, MA may share an antibody

4. CO and VO may share an antibody

5. VO and SI may share an antibody

6. VO and MA may share an antibody

Table 4:4 Comparison of various sera considered pair-wise with a panel of 98 platelet suspensions.

Reaction with 1st serum		3 or 4+	3 or 4+	2+ + +w –	2+ + +w –	Total	χ^2_1
2nd serum		3 or 4+	2+ + +w –	3 or 4+	2+ + +w –		
Sera							
1st	2nd						
NO	CO	21	43	3	31	98	5.7
NO	VO(neat)	30	34	12	22	98	n.s
	SI	6	43	4	23	76	n.s
	CL 1/16	51	4	19	10	84	9.6
	MA	59	5	10	24	98	39.4
	MA 1/8	51	12	6	28	97	34.0
	KL	11	53	11	23	98	2.93
	WH	11	21	9	11	52	n.s
CO	VO	16	8	26	48	98	6.0
	SI	1	19	9	47	76	n.s
	CL 1/16	17	3	52	12	84	n.s
	KL	4	20	18	56	98	n.s
	MA	23	1	47	27	98	7.8
	MA 1/8	19	6	41	31	97	n.s
	WH	2	9	18	23	52	n.s

Reaction with 1st serum		3 or 4+	3 or 4+	2+ + +w −	2+ + +w −	Total	χ^2_1
2nd serum		3 or 4+	2+ + +w −	3 or 4+	2+ + +w −		
Sera							
1st	2nd						
VO	SI	9	25	1	40	75	7.3
	CL 1/16	32	3	38	12	85	n.s
	KL	4	38	18	38	98	5.8
	MA	35	7	34	22	98	4.8
	MA 1/8	26	15	32	24	97	n.s
	WH	8	13	12	19	52	n.s
SI	CL 1/16	9	0	45	10	64	n.s
	KL	1	9	17	49	76	n.s
	MA	7	3	54	21	85	n.s
	WH	4	2	8	17	31	n.s
CL 1/16	KL	11	57	8	9	85	5.8
	MA	56	14	4	11	85	14.5
	MA 1/8	50	20	0	15	85	23.2
	WH	15	23	5	9	52	n.s
KL	MA	12	10	58	18	98	3.9
	WH	3	7	17	25	52	n.s
WH	MA	12	8	24	8	52	n.s

n.s. = not significant

$$\chi^2_1 < 3.84$$

Sera	NO	CO	VO	SI	CL1/16	KL	MA
CO	5.7						
VO	n.s.	6.0					
SI	n.s.	n.s.	7.3				
CL 1/16	9.6	n.s.	n.s.	n.s.			
MA	39.4	7.8	4.8	n.s.	14.5	(-)3.9	
KL	n.s.	n.s.	(-)5.8	n.s.	(-)5.8		
WH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant

Table 4:5 χ^2_1 values obtained from 2 x 2 comparison of 8 sera tested against a panel of 98 platelet suspensions.

The results with SI and CL 1/16 are compatible with the hypothesis
that the antibody in SI is also in CL at a titre greater than 1/16.
As the exact probability equals 0.193, these results are clearly
not statistically significant. In all of the above hypotheses,
the plural antibodies could be used instead of the singular. The
χ^2_1 values with the (-) sign before them indicate that there is
a significant negative correlation between the sera compared.
These results are compatible with the hypothesis that the anti-
bodies are detecting the products of allelic genes.

Examination of Table 4:4 indicates that in no case were
two sera observed to have identical activity. In only two cases
(CL 1/16 v SI and CL 1/16 v MA 1/8) could the antibody(s) in one
serum be said to be included in the second serum. The minimum
number of "exceptions" from Table 4:4 to each of the six hypotheses
concerning mixed antibody specificity referred to above are:

1.	NO	MA	5/98
2.	NO	CL 1/16	4/84
	MA	CL 1/16	4/85
	MA 1/8	CL 1/16	0/85
3.	CO	NO	3/98
	CO	MA	1/98
4.	CO	VO	8/98
5.	VO	SI	1/75
6.	VO	MA	7/98

Some of these "exceptions" could have arisen as a result of the presence of at least two antibodies in each of the sera compared, one of these antibodies being common to both sera while the second antibody was in each case not shared (i.e. as in CO - VO comparison). The exceptions noted at this stage are probably errors in typing, e.g. in the NO, MA, CL, CO comparisons in 1, 2 or 3 above. These errors could have arisen as a result of either:

i. prozone phenomena resulting in false negatives,

ii. technical incompetance manifesting itself in the occasional omission of a drop of reagent from a tube. (Tests were set up in batches of 400 - 500).

It was not possible to determine which of the reasons suggested above were responsible for the exceptional reactions, although work reported in Chapter 7 with VO clearly suggests that i. could have occurred during this series of experiments. The exceptions or errors found during the analysis of the data presented in this chapter did not defeat the purpose of the experiment, i.e. to begin the elucidation of the antibodies in the sera studied.

B. <u>RESULTS OBTAINED BY DR. R.H. ASTER WITH COMPLEMENT FIXING PLATELET</u>

<u>ANTISERA FOUND IN ADELAIDE</u>

A number of the sera used in experiments reported in this chapter were sent to Dr. R.H. Aster, Harvard Medical School, Boston, U.S.A., for testing to determine if they contained any antibody previously recognized by him. His results reported in two letters June 27, 1966, and May 24, 1967 are set out below:

<u>June 27. 1966</u>

MA: has anti PlB1 strongly positive at 1/30 dilution plus at least one other weaker antibody reacting at about 1/10;

SI: very weak reaction at 1/3 with 20% of panel;

KL: strongly positive at 1/15 with 30% of panel. Not tested at higher concentration;

VO: positive at 1/10 with 20% of panel, has prozone;

CL: very strong anti-PlB1 antibody reacting at dilution 1/150. Has 1 or possibly 2 other antibodies which titre out at 1/60.

<u>May 24, 1967</u>

VO: 1/7/66 several weak antibodies positive at 1/3, reactivity same as MA at 1/8 dilution, 0'G 9/8/66 appears to have the same antibody;

NO 10/6/66 probably PlB1 positive at 1/10 but additional antibody at 1/3;

HA: did not react with panel of 20 donors;
& MA:

MA: 17/6/66 has anti PlB1 at 1/30 as before, plus
weaker activity;

SI: 4/6/66 now has very strong reactivity with 4 of 20
at 1/20 dilution, specificity unknown;

KL: 3/5/66 positive at 1/5 with only 1 of 20;

CL: 12/7/66 very strong anti PlB1 as before, positive at
1/80 plus several weaker antibodies positive at 1/20;

WH: 23/7/66 gave weak positive reaction with only 1 of 20.

The results reported by Dr. Aster explain the significant
χ_1^2 between MA − NO, MA − CL 1/16 and NO and CL 1/16. These
associations were due to each of the sera containing anti-PlB1.
The other associations reported in Chapter 4 were no further
resolved by the reports from Dr. Aster. However his report did
confirm that most of the sera sent to him showed complement fixing
activity with some platelet specimens. The proportion of cells
showing activity with sera tested by Dr. Aster is not significantly
different from the proportion positive when the same sera were
tested in Adelaide. It was unfortunate that no indication was
given of the panel size used to test the first batch of antisera
referred to Dr. Aster.

IDENTIFICATION OF PLB1 AND

PLB2 ANTIGENS

A. COMPARISON OF THE SERA NO AND SZ

During an experiment to detect new platelet antibodies it became apparent that the serum NO could contain the antibody(s) present in the serum SZ. This hypothesis was based on results shown in Table 5:1.

	Strength of Reaction	NO				
		4+	3+	2+	1+	0
SZ	3+	1				
	2+	3				
	1+	5				
	0	2	1			4
	Total	11	1			4

Table 5:1   Results of Typing Platelets from 15 random group 0 donors with sera NO 28/1/67 and SZ 29/1/67 (neat).

The difference between 3+ and 4+ reactions is slight. As it is convenient to reduce the data in Table 5:1 to a 2 x 2 table for analysis these two classes were grouped together in Table 5:2.

NO 28/1/67 (1/8)

		+	−	
SZ 29/1/67	+	9	0	9
	−	3	4	7
		12	4	16

p = 1/52

Table 5:2 Reduction of Table 5:1 to a 2 x 2 table.

In this experiment slightly more complement was used than was desirable (see section of Methods) so that negative rather than +w reactions were obtained in controls and non-complement fixing serum-platelet mixtures. Accordingly all the positive reactors with SZ were grouped together. The probability of obtaining the results as grouped in Table 5:2 is 1/52.

Serum from SZ obtained on 8/2/67, showed a more avid antibody at a higher titre. This serum gave 4+ reactions at a dilution of 1/5 to 1/8 with most positive specimens. A more extensive comparison of the sera of NO 8/2/67 and SZ 8/2/67 was made by testing both sera against platelets from 289 random group O donors. The result of this series of experiments is shown in Table 5:3. This table was drawn up from Appendix 3.

NO 1/8, 8/2/67

		4+	3+	2+	1+	0		
	4+	113						113
	3+	17						17
SZ	2+	5						5
8/2/67	1+	8			11	1		20
1/5-1/8	0	26	5	3		100		134
		169	5	3	11	101		289

Table 5:3   Results of typing platelets from 289 donors with the
            sera NO 8/2/67 and SZ 8/2/67.

In this series of experiments and in those that follow excessive

quantities of complement were avoided so that control tubes usually

contained a small deposit of red cells with almost complete

haemolysis (+w). In some batches of experiments control reactions

were as strong as 1+. Those specimens giving 1+ and +w reactions

(scored as 1+ and 0 in tables and appendices) will be grouped

together when data are reduced to a 2 x 2 table because of the

variability in standardization of negative reactions and because of

the subjective nature of the difference between + and +w reactions.

Those specimens which gave 2+ reactions will be grouped with 3+

and 4+ reactors when data similar to the results recorded in Table 5:3

are reduced to a 2 x 2 table. This convention is similar to that used for most of the comparisons made in Chapter 4. The data in Table 5:4 shows that NO 8/2/67 at 1/8 contains the antibody (or antibodies) in SZ 8/2/67 together with another antibody not active in SZ 8/2/67 at the dilution used in these experiments. $(p < 10^{-43})$

NOR 8/2/67 at 1/8

		+	−	
SZ 8/2/67 1/5-1/8	+	135	0	135
	−	42	112	154
		177	112	289

B. ELUATES PREPARED FROM NO AND SZ

Absorption and elution experiments with selected platelet specimens were conducted to determine whether the antibodies in NO 8/2/67 could be separated into two or more groups. Eluates were prepared from NO 8/2/67 using NO+ SZ− platelets. Each of these eluates was tested against selected platelet specimens to determine whether the eluate contained specific platelet antibody activity. Each eluate was titrated against NO+ SZ+ and NO+ SZ− cells to determine the highest dilution at which the eluates could be used. It was found that a $\frac{1}{2}$ dilution of all eluates (except

that prepared with 8157 or 98726) would reliably produce 3 or 4+

positives when used as antibody, where reactions were expected on

the basis of the activity with the sera NO and SZ. The pH of

eluates from NO with 8157 and 98726 was accidentally raised to 9

for a brief period during neutralization. This probably accounts

for these eluates not reacting with 6 and 4 specimens respectively

where reactions were expected on the basis of the theory out-

lined below. The other eluates prepared from NO with NO+ SZ-

platelet specimens fixed complement with all NO+ SZ- platelet

specimens as well as with some NO+ SZ+ specimens. Typing of

platelets with more than one NO+ SZ- eluate was in agreement, apart

from the exceptions with the eluates prepared with 8157 and 98726

referred to above.

Five eluates were prepared from NO 8/2/67 using platelets

which were NO+ SZ+ but negative with the eluates prepared from NO

with NO+ SZ- platelets. These eluates were also titrated to

determine the maximum dilution at which they could be used. Each

of the five NO+ SZ+ eluates referred to above, was found to fix

complement (3+ or 4+) with all SZ+ platelet specimens. These

eluates were negative with all but three SZ- platelet specimens.

Two of these specimens (98731 and 98770) gave only weak (2+)

reactions with NO serum and with NO+ SZ+ eluates. Specimen 98756

gave a 4+ reaction with NO and with 3 of 4 eluates prepared with

NO+ SZ- cells.

One eluate was prepared from NO 8/2/67 using platelets 94915 which were SZ+ and positive with the eluate from NO+ SZ- platelets. The eluate with 94915 fixed complement with all NO+ platelets against which it was tested, but not with any NO- platelets.

An eluate from SZ 13/4/67 with SZ+ platelets fixed complement with fifteen SZ+ specimens, but not with nine SZ negative specimens. The comparison of NO 1/8 with SZ and the results from the absorption and elution experiments, suggest that at suitable dilution NO 8/2/67 could show the same activity as SZ 8/2/67. Table 5:5 shows that with one exception NO 8/2/67 at 1/24 dilution has the same specificity as SZ 8/2/67 at 1/5. It is thought that this aberrant reaction with platelet No. 23955 was due to a reaction between the platelets and the weaker antibody in NO.

		NO 8/2/67 at 1/24					
		4	3	2	1	0	
	4	29	1	1			31
SZ	3			1			1
8/2/67	2	1					1
	1			1	1		2
	0				5	43	48
		30	1	3	6	43	83

Table 5:5 Result of Typing 104 platelet specimens with NO 8/2/67 at 1/21, 1/24 and SZ 8/2/67 at 1/5.

C. FURTHER EXAMPLES OF THE ANTIBODY COMMON TO NO+ SZ (ANTI PLB1)

As a result of the dilution and elution experiments described above, it was decided that NO 8/2/67 contained two anti-bodies. The higher titre antibody was present also in the serum of SZ and was probably anti-PlB1 (see Chapter 4). Other sera were found which also contained this antibody. Results presented in Table 5:6 show concordant results with six sera when tested against 18 or more random platelet specimens. Four of these sera contained other antibodies of unknown specificity. The serum CL also contained anti-PlB1 as well as other complement fixing platelet antibodies. Some experiments with this serum will be described in Chapter 7 of this thesis.

	MA 1/30		MU 1/8		MA 1/16		DU 1/16		KL		DE	
	+	−	+	−	+	−	+	−	+	−	+	−
NO +	17	0	19	0	19	0	9	0	9	0	9	0
1/24 −	0	24	1	23	1	23	4	5	4	5	0	9

Table 5:6   Comparison of a number of sera containing anti-PlB1.

D. FURTHER EXAMPLES OF THE SECOND ANTIBODY IN NO

The lower titre antibody in NO 8/2/67 was not present in SZ.
Two other sera AD from Melbourne 2/6/67 at 1/2 and CO 3/3/66 at
1/24, showed identical activity with 89 platelet preparations,
Table 5:7.

	+	−	
+	27	0	27
−	0	62	62
	27	62	89

$p < 10^{-22}$

Table 5:7 Result of typing 89 platelet specimens with CO 3/3/66
at 1/24 and AD ex Melbourne 2/6/67 at 1/2 − 1/3.

CO 3/3/66 and AD were positive with those platelet
specimens which fixed complement with the eluate prepared from NO
8/2/67 with platelet specimen number 16152, (NO+ SZ−) and were
negative with the specimens which did not fix complement with this
eluate. The antibody in CO and AD apparently corresponds to the
weaker antibody in NO. $p < 10^{-6}$, Table 5:8.

	+	−	
+	9	0	9
−	0	15	15
	9	15	24

Table 5:8 Result of typing 25 platelet specimens with CO 3/3/66 (1/24)
AD ½ and with eluate from NO prepared with platelets number
16152.

CO 3/3/66 at 1/24 and AD at 1/2 were also positive with all specimens
which fixed complement with NO 8/2/67 at 1/8 but did not fix
complement with NO at 1/24 or with SZ. Another serum SP obtained
by courtesy of Dr. Woodliff of Royal Perth Hospital, W.A., showed
the same activity as CO 1/24 and AD 1/2. As shown in Table 5:9
$p < 0.001$. SP also contains a second antibody but its specificity
has not been determined.

CO 1/24

		+	−	
SP	+	7	3	10
	−	0	14	14
		7	17	24

$p < 0.001$

Table 5:9 Comparison of the activity of SP with CO.

The results with CO 3/3/66 at 1/24, with AD 1/2, with
NO 8/2/67 at 1/8 and with the eluate of NO with 16152 agree with
the suggestion that each of these sera contain the same antibody.
It seems reasonable to suggest that this antibody is recognizing
a single antigen. The nine negative reactions with eluates prepared
from NO 8/2/67 with platelet numbers 8157 and 98726 are probably due
to excessive dilution of these two reagents. The hypothesis that
CO etc. react with a compound antigen whose components sometimes

occur separately in the population, cannot be excluded until about
thirty absorptions have been completed, Troup et al (1966).

E. POPULATION DISTRIBUTION OF TWO ANTIGENS PLB1 AND PLB2 AND THE
PROBABLE RELATIONSHIP OF THESE ANTIGENS TO EACH OTHER

The results with NO 8/2/67, SZ 8/2/67 and 13/4/67, CO
AD and the various eluates prepared with NO, have been used to
determine whether the platelet specimens from the donors listed
in Appendix 3 contained the PlB1 antigen and the other antigen
defined by NO 8/2/67. The distribution of the two antigens in
412 random group O donors typed is shown in Table 5:10.

Anti-PlB1

		+	−	
CO etc.	+	28	55	83
	−	170	159	329
		198	214	412

$\chi^2_1 = 7.838$, $p < 0.01$

Table 5:10 Distribution of the two antigens described in this
chapter in the general population.

It is clear from Table 5:10 that the antigens detected
by anti-PlB1 and the other antibody are not independent
$(\chi^2_1 = 7.8$, $p < 0.01)$. As shown below, the distribution of the
two antigens in the population typed agrees with the hypothesis

that the antigens are the products of allelic genes. It is therefore proposed to call the second antigen P1B2, the corresponding antibody anti-P1B2 and the gene P1B2. A first approximation to the frequency of the genes P1B1 and P1B2 may be calculated using the formula $1 - p_1 = \sqrt{a_1/n}$ where p_1 is the frequency of the gene P1B1 and a_1 is the number of specimens negative with anti-P1B1 $(p_1 = 0.279)$. Similarly we may calculate the frequency of P1B2 $(p_2 = 0.107)$. With these two estimates we can calculate the expected distribution of the antigens P1B1 and P1B2 in the population. The frequency p of the null allele or alleles denoted by P1B- is 0.614. The hypothesis that the genes controlling the two antigens described in this chapter are alleles is supported by their population distribution (Table 5:11),

P1B1	P1B2	Observed numbers	Expected numbers	Formula
+	+	28	24.6	$2p_1p_2n$
+	−	170	173.2	$(p_1^2 + 2p_1p_0)n$
−	+	55	58.9	$(p_2^2 + 2p_2p_0)n$
−	−	159	155.3	p_0^2
		412	412	

Table 5:11   Observed and expected distribution of P1B1 and P1B2 antigens if they are controlled by alleles.

$$\chi_1^2 = 0.88, \ p > 0.30$$

F. EFFECT OF ABSORPTION ON ANTIBODY LEVEL IN SERUM

The results of titration of NO 8/2/67 against two platelet specimens containing the antigen PlB1 and two platelet specimens containing the PlB2 antigen are shown in Table 5:12.

Dilution of serum	1/8	1/12	1/16	1/20	1/24	1/30	1/32	1/40	Control
Unabsorbed	4+	4+	4+	4+	4+	3+	3+	+	+w
Absorbed once with PlB1 platelets 96767	4+	2+	+	NT	NT	NT	NT	NT	+w
Absorbed once with PlB2 platelets 8149	4+	4+	4+	4+	4+	NT	NT	NT	+w

Table 5:12 (a) Titration of NO 8/2/67 against platelets containing PlB1 antigen (14059).

Dilution of serum	1/2	1/4	1/6	1/8	1/12	1/16	1/20	1/24	Control
Unabsorbed	NT	NT	4+	4+	3+	2+	+	+w	+w
Absorbed once with PlB1 platelets 96767	NT	NT	4+	3+	+	+w	NT	NT	+w
Absorbed once with PlB2 platelets 8149	2+	+	+w	NT	NT	NT	NT	NT	NT

Table 5:12 (b) Titration of NO 8/2/67 with platelets containing PlB2 antigen. NT = not tested

Platelets with identical antigens gave the same results. It can be seen that after absorption with a platelet specimen containing an

antigen, the level of the corresponding antibody was reduced, whereas the level of the other antibody was unaltered. One absorption with SZ 13/4/67 with P1B1 containing platelets, removed all complement fixing antibody activity from this low titre (1/3) serum.

G. THREE UNUSUAL PLATELET SPECIMENS

During the early part of the investigation of the antibodies in the sera of NO and SZ two platelet specimens 98731 and 98770 were found which gave weaker reactions (2+ compared with 4+) than is usual for specimens positive with NO 1/8. Both specimens also gave 2+ reactions with the NO+ SZ+ eluates that they were tested against.

Both specimens were repeatedly negative with SZ 8/2/67 using various serum dilutions (1/3 to 1/8). Platelet specimen 98756 was also negative with SZ but positive with NO and with both types of NO eluate.

Eluates prepared with 98756 and 98770 reacted as shown in Table 5:13. Both eluates show anti-P1B1 activity and eluate with 98756 showed anti-P1B2 activity also. These results suggest that 98756, 98731 and 98770 have P1B1 P1B2, P1B1 and P1B1 antigens respectively.

Eluate from   N0   8/2/67

	with 98756			with 98770			Presence of Antigens	
	Neat	$\frac{1}{2}$	$\frac{1}{4}$	Neat	$\frac{1}{2}$	$\frac{1}{4}$	P1B1	P1B2
98770	4	1	1	4	1	1		
98756	4	4	3	4	1	1		
98731	4	2	1	3	1	1		
93257	4	4	2	4	3	4	+	+
M.H.	4	3	2	4	4	4	+	−
94933	4	4	4	1	1	1	−	+
P.S.	1	1	1	1	1	1	−	−
93250	4	4	4	1	1	1	−	+
93253	4	4	4	1	1	1	−	+
93271	4	4	2	1	1	1	−	+
98730	4	1	1	4	4	3	+	−
98732	4	2	1	4	4	3	+	−
98735	4	4	1	4	4	4	+	+
I.G.	1	1	1	2	1	1	−	−
G.P.	1	1	1	1	1	1	−	−
98768	1	1	1	1	1	1	−	−
98734	1	1	1	1	1	1	−	−
98739	1	1	1	1	1	1	−	−
Control	1	1	1	1	1	1	−	−

Table 5:13   Activity of eluates prepared from N0 with platelets
98756 and 98770.

No further examples of this anomally were found although platelets from several hundred more donors were typed. Donor 98731 returned as 9975 and was on that occasion negative with NO and SZ. Detailed investigation was not carried out on the second specimen from this donor as the information that 98731 and 9975 were from the same person was not available until much later. Second specimens from 98756 and 98770 were not obtained. Whether the results presented in this section are due to a real phenomenon or technical artifact has not been resolved because of the limited quantities of platelets available for the investigation of this problem.

H. REPRODUCIBILITY OF PLATELET GROUPING

After 412 platelet specimens had been grouped a survey of donors was conducted to ascertain whether any repeat specimens had been obtained. It was found that specimens from 37 donors had been obtained on two or more occasions. With the exception of the specimens from the donor 98731 referred to in section G. of this chapter,, all repeat specimens gave the same grouping result with anti-PlB1 and anti-PlB2 as the first specimen. These repeat tests are listed in Appendix 4.

IDENTIFICATION OF THE PLB3 ANTIGEN

A. COMPARISON OF THE SERA BR AND WE

A small quantity of the serum BR was obtained by courtesy
of Dr. M. Berah of Melbourne.   This serum had been supplied to Dr.
Berah by Professor J. Dausset of Paris who said that it contained
complement fixing anti-LA1.   A summary of the result of typing 42
platelet specimens with BR at 1/60 dilution and with WE 21/3/67
at a 1/8 dilution is shown in Table 6:1.   This table may be condensed
to a 2 x 2 table (Table 6:2) by grouping 2, 3 and 4+ reactions
together.   After this simplification it is apparent that at the
dilutions employed these sera had the same specificity.

	BR (1/60)					
Strength of reaction	4	3	2	1	0	
4	8	4	2			14
3		1				1
2						
1						
0				2	25	27
	8	5	2	2	25	42

(WE 21/8/67 1/8)

Table 6:1   Results of Typing 42 platelet specimens with BR 1/60
(anti-LA1) and WE 21/3/67 at 1/8.

	+	−	
+	15	0	15
−	0	27	27
	15	27	42

Table 6:2   Results in Table 6:1 reduced to 2 x 2 table.

Following Mrs WE's second blood transfusion the titre of antibodies in her serum showed a considerable rise in activity with 4+ complement fixation at a dilution of 1/300 with some platelet specimens. Sera obtained from Mrs WE on 21/3/67 and 4/8/67 were titrated against 74 platelet specimens. It was found that those preparations which gave 4+ reactions with this serum at high dilution (1/128 or greater) were also positive with the serum 21/3/67 (1/8). This suggests that the antibody specificity of the sera obtained in March and August is the same when the sera are used at dilutions of 1/8 and 1/128 respectively, Table 6:3.

	+	−	
+	24	0	
−	0	50	
	24	50	74

$p < 10^{-19}$

Table 6:3   Result of typing with WE 21/3/67 at 1/8 and WE 4/8/67 at 1/128

B. <u>COMPARISON OF THE REACTIONS OF THE SERUM WE WITH THE REACTIONS</u>
<u>OF ANTI-PlB1</u>

It has been shown (Payne & Bodmer 1964) that the antigens
LA1 and LA2 are distributed in the population as would be expected
if they were controlled by alleles at one locus. Anti-PlB1 is
thought to be the same as, or closely related to anti-LA2.,
(<u>Histocompatibility Testing 1965</u>, Vredevoe <u>et al</u> 1966). These two
reports and the suggestion by Dausset that BR contained anti-LA1
prompted a comparison of the activity of anti-PlB1 sera and the
serum WE.   This comparison is made in Table 6:4 which shows that
the activities of anti-PlB1 and WE (21/3/67 at 1/8, 4/8/67 at 1/128)
are not statistically independent, $(\chi^2_1 = 33.8, p < 0.001)$. When
gene frequencies are calculated by the method used in Chapter 5 the
data in Table 6:4 give gene frequencies of 0.279 for PlB1, 0.185 for
the antigen detected by WE and 0.536 for the remaining alleles.
These gene frequencies were used to calculate the number of specimens
expected in each of the 4 classes. These expectations and the
corresponding observations are shown in Table 6:5. It can be seen
from this table that the results obtained with anti-PlB1 and WE
at high dilution are in agreement with the hypothesis that the
antigens detected with these sera are controlled by allelic genes,
$(\chi^2_1 = 0.97, p > 0.30)$. The antibody in WE at high titre was there-
fore called anti-PlB3, the antigen detected PlB3 and the
corresponding allelomorph <u>PlB3</u>.

anti-PlB1

		+	−	
WE	+	38	100	138
	−	160	114	274
		198	214	412

$$\chi^2_1 = 33.78, \; p < 0.001$$

Table 6:4   Comparison of anti-PlB1 and WE high titre activity.

PLB1	PLB3	Observed Numbers	Expected Numbers
+	+	38	42.5
+	−	160	155.3
−	+	100	95.8
−	−	114	118.4
		412	412.0

$$\chi^2_1 = 0.967$$
$$p > 0.30$$

Table 6:5   Observed and expected distribution of PlB1 and PlB3 antigens if they are controlled by alleles.

C, OTHER ANTIBODIES IN WE 4/8/67

The serum of WE showed complement fixation with other platelet specimens apart from those referred to above. The result of testing 144 platelet specimens with various dilutions of WE

shown in Table 6:6. The titres in Table 6:6 indicate the last tube in the titration which gave a 4, 3 or 2+ reaction with the platelet specimens. The distribution of the end point of complement fixation is clearly different for the class having the P1B1 antigen and the class lacking P1B1 and P1B2. This difference can be most easily demonstrated by grouping those positive at a dilution of 1/8 or more to form a 2 x 2 table as shown in Table 6:7.

The 34 specimens negative with WE 4/8/67 at 1/8 shown in Table 6:7 were re-tested with WE 4/8/67 at 1/4. The four specimens negative with anti-P1B1 fixed complement with WE 1/4 whereas only one of the 30 which carried the P1B1 antigen reacted in this way. A statistical test for the independence of the lower titre antibodies of WE 4/8/67 from the P1B1 antigen indicates that these antibodies are also related to the P1B system. A test for heterogeneity between the first two rows of Table 6:6 (after combining 1/128 dilution with 1/64 because of the small numbers reacting at these dilutions) gives $\chi^2_4 = 28.85$ (p < 0.001). This also indicates that the additional antibody(s) in WE are related to the P1B system.

Titre		less than 1/8	1/8	1/16	1/32	1/64	1/128	
P1B1	P1B2							
−	−	4	6	14	14	8		46
+	−	30	14	15	8	1	1	69
−	+	1	6	6	1			14
+	+	3	12					15
		38	38	35	23	9	1	144

Table 6:6 Results of titrating 144 platelet specimens against WE 4/8/67.

P1B1	P1B2	Reaction with WE 4/8/67 at 1/8 dilution		
		+	−	
+	−	39	30	69
−	−	42	4	46
		81	34	115

$$X_1^2 = 14.44$$
$$p < 0.001$$

Table 6:7 Comparison of activity of WE with anti-P1B1 positive and negative platelet specimens.

D. ELUTION EXPERIMENTS

Absorption and elution experiments as extensive as those outlined in Chapter 5 with the serum of NO were not carried out with WE. An eluate prepared with the platelets of one high titre reactor (GA) fixed complement with all other platelet specimens which reacted to a titre of 1/128 or more. This eluate did not fix complement with any negative, low or intermediate titre platelets.

A second eluate was prepared using a platelet specimen which gave a 4+ complement fixation reaction with WE 4/8/67 at a dilution of 1/16, (30108). This eluate was tested against 23 P1B3 platelet specimens and 4+ results were obtained with all of them. The activity of this eluate with thirty-five platelet specimens lacking P1B3 is shown in Table 6:8. These results suggest that at least one of the antibodies in WE in addition to anti-P1B3 has been removed from the serum by absorption with platelets from donor 30108.

As the eluate from WE with donor 30108 reacted with all platelet specimens carrying the P1B3 antigen as well as those specimens shown in Table 6:8 some consideration should be given to hypotheses that could account for this result.

These are:

    i.   that the eluate was contaminated with a small amount of anti-PlB3

    ii. that the antigen detected by the eluate is sometimes present alone but is always (or almost always) present when PlB3 is present.

To distinguish between these hypotheses it would be necessary to:

    1. resuspend (and thereby wash) platelets which have absorbed antibody. This should remove any contaminating antibody from the platelets. However it has also been shown to reduce slightly the titre of absorbed antibody (Shulman 1964).

    2. absorb WE 4/8/67 repeatedly with PlB3 positive platelets until no anti-PlB3 activity remained. Then test the absorbed serum for activity similar to that of the eluate prepared with 30108. This absorption may have to be repeated with platelets from several individuals. The platelets selected for absorption may have antigens which will remove anti-PlB3 and the activity corresponding to the eluate 30108.

No attempt was made to distinguish between these hypotheses as time did not permit further investigation on the lines suggested above. Further investigation of the antibodies in WE 4/8/67 has not been conducted.

Cells with PlB1 antigen only		Titre of activity with WE 4/8/67						
		<1/4	1/4	1/8	1/16	1/32	1/64	Total
Reaction with eluate	4+				2	1		3
	0	11	1	2	1			15
Cells lacking PlB antigens								
Reaction with eluate	4+			1	2	3	1	7
	0	2	2	5	1			10

Table 6:8   Activity of eluate from WE 4/8/67 with platelet number
30108.

E. REPEATABILITY OF PLB3 TYPING

Repeatability of PlB3 typing was assessed in the same way as the repeatability of PlB1 and PlB2 typing. Thirty-two people were tested twice for this antigen and concordant results were obtained in 31 cases. The two discordant repeat specimens are shown in Table 6:9. It appears reasonable to conclude from Table 6:9 that the donors of both of these specimens lack the PlB3 antigen but that they carry an antigen which reacts with the intermediate titre antibody in WE.

Fifty-six platelet specimens up to three months old with PlB3 antigen were titrated against WE 9/8/67 and all gave 4+ complement fixation with the serum at a dilution of 1/128 or more.

		Initial typing	Titration with WE 4/8/67			
			1/32	1/64	1/96	1/128
same donor	10029	–ve with WE 21/3/67 1/8	4+	+	+	+
	34380	4+ with WE Aug 67, pooled 1/50	nt.	+	+	+
same donor	8169	4+ with WE 25/1/67 at unknown dilution	nt.	nt.	nt.	+
	20230	–ve with WE 21/3/67 1/8	nt.	4+	2+	+

Table 6:9 Discrepant PlB3 typings. At time of titrations all specimens thought to carry the PlB3 antigen gave a 4+ with WE at 1/128 or greater dilution.

F. STABILITY OF THE PLB ANTIGENS DURING STORAGE

Results selected from the titration of WE 4/8/67 using platelets with the PlB3 antigen show that this antigen deteriorated gradually with regard to complement fixing activity with the serum of WE (see Table 6:10). All the tests shown in Table 6:10 were performed at the same time using the same dilutions of serum and complement. Similar tests for the deterioration of the PlB1 and PlB2 antigens during storage are shown in Tables 6:11 and 6:12.

Time of platelet storage	Platelet No.	Titre with WE 4/8/67				Platelet Control
		1/64	1/128	1/256	1/320	
3 days	36463	4+	4+	4+	4+	1
	36466	4	4	4	4	1
2 weeks	34341	4	4	2	2	1
	34374	4	4	4	2	0
4 weeks	32137	4	4	3	1	0
	32139	4	4	1	1	0
2 months	29029	4	4	1	1	0
	28034	4	4	1	1	0
4 months	18160	4	4	1	1	1
	18179	4	3	1	1	1
6 months	09986	4	2	1	1	1
	10023	4	2	1	1	1
9 months	96774	1	1	1	1	1
	96775	2	1	1	1	1

Table 6:10   Deterioration in complement fixing activity of P1B3 platelets stored for up to nine months.

Time of platelet storage	Platelet No.	Titre with NO 8/2/67					Platelet Control
		1/8	1/16	1/24	1/32	1/40	
2 weeks	36498	4	4	4	4	3	0
	36496	4	4	4	4	4	0
4 months	20283	4	4	3	0	0	0
	20281	4	4	4	2	2	0
8 months	00698	4	4	1	0	0	0
	00697	4	4	1	0	0	0
10 months	93258	4	3	3	1	0	1
	93254	4	3	1	0	0	1

<u>Table 6:11</u> Deterioration in complement fixing activity of PlB1 platelets stored for up to 10 months.

Time of storage	Platelet No.	Titre with NO 8/2/67						Platelet Control
		1/4	1/6	1/8	1/12	1/16	1/24	
1 day	38528	nt.	nt.	4	4	1	0	0
2 weeks	36433	nt.	nt.	4	3	0	0	0
	36434	nt.	nt.	4	4	1	0	0
4 months	18179	4	2	2	1	0	0	0
	18217	4	4	4	3	1	0	0
6 months	9986	4	3	2	1	0	0	0
	10016	4	4	2	1	0	0	0
7 months	8196	3	3	2	1	0	0	0
	8199	4	3	2	0	0	0	0

<u>Table 6:12</u> Deterioration of the PlB2 antigen during storage.

G. THE POPULATION DISTRIBUTION OF THE PLB ANTIGENS

As the series of blood donors typed for their PlB antigens approached 400 it was noted that the distribution of the antigens in the population departed significantly from that expected on the basis of the Hardy-Weinberg equilibrium. At this stage an additional series of 203 individuals from the Royal Adelaide Hospital outpatients department were typed to determine whether this trend would be found in that data also. Table 6:13(a) shows that there is no significant heterogeneity between the two series. Comparison of the PlB platelet groups and the ABO red cell groups shows that these systems are independent Table 6:13(b). The PlB platelet groups are also distributed independently of sex (Table 6:13(c)).

Gene frequencies of the alleles at the PlB locus were calculated from the combined data of the hospital and blood donor series using the maximum likelihood method outlined by Mather (1957). The frequencies obtained were:

PlB1	p	.2790	$\pm$ .0138
PlB2	q	.1023	$\pm$ .0088
PlB3	r	.1809	$\pm$ .0115
PlB-	s	.4378	$\pm$ .0150

	(a)		
Antigens present	Hospital patients	Blood donors	Total
120	18	30	48
103	18	40	58
023	4	26	30
100	62	138	200
020	16	31	47
003	41	80	121
000	44	87	131
	203	432	635

$$\chi^2_6 = 6.0, \ p > 0.30$$

(b)	
Group O	other groups A, B, AB
36	12
43	15
27	3
167	33
37	10
94	27
104	27
508	127

$$\chi^2_6 = 5.9, \ p > 0.30$$

Table 6:13   Comparison of the ABB groups of hospital patients and blood donors classified according to:
(a) mode of ascertainment
(b) ABO blood groups
(c) sex (group O blood donors only).

	(c)		
	Male	Female	
120	15	13	28
103	27	11	38
023	18	7	25
100	94	38	132
020	19	11	30
003	51	24	75
000	56	28	84
	280	132	412

$$\chi^2_6 = 4.0, \ p > 0.50$$

The numbers expected with each combination of antigens on the basis of these gene frequencies are shown in Table 6:14. They deviate significantly from the Hardy Weinberg expectations, $\chi^2_3 = 11.30$, p = 0.01. It appears that this χ^2 value is due mainly to the relative deficiency of individuals with P1B2 only and an excess of those with P1B2 and P1B1 or P1B3 together. Two possible reasons for the misfit of the data with the hypothesis are:-

  i. serological, due to occasional misclassification

  ii. genetical, due to a more complex situation existing than three detectable alleles at one locus. If for instance the "alleles" occasionally occur in "coupling" (cf the Rh system) and if in particular P1B2 combines with P1B1 or P1B3 in this way then we would expect the distribution of the antigen to be altered in this direction. On this hypothesis an occasional triple positive having all P1B antigens would also be expected. No such individual has yet been found. If the former suggestion explains the deviation then it is unusual that no misclassification of the P1B2 antigen has been observed in the series of repeated specimens. The cause of this significant deviation of the data from expectation remains an enigma.

P1B1	P1B2	P1B3	Observed number	Expected number	Deviation
+	+	−	48	36.25	11.75
+	−	+	58	64.10	6.10
−	+	+	30	23.50	6.50
+	−	−	200	204.56	4.56
−	+	−	47	63.52	16.52
−	−	+	121	121.36	0.36
−	−	−	131	121.71	9.29
			635	635	

Table 6:14   The distribution of the P1B antigens in the South Australian Caucasian population.

$$\chi^2_3 = 11.30, \quad p = 0.01$$

OTHER ANTIBODIES AND ANTIGENS

A THE SERUM CL

1. Anti-PlB1, Anti-PlB2 and Antibody in SI Demonstrated by

Titration Experiments: This serum was studied during the preliminary analysis recorded in Chapter 4. In that chapter it was suggested that the serum contained anti-PlB1 at high titre together with other antibodies. To elucidate the details of the antibodies in this serum two series of titrations with various dilutions were conducted. In the first series the end point with each specimen was taken as the last tube with a 2+ reaction (or greater). In the second series the last tube giving a 4+ reaction was taken as the end point. A summary of the titres of platelet preparations having the various PlB antigens described in Chapters 5 and 6 is shown in Tables 7:1 and 7:2. The data in Tables 7:1 and 7:2 are reduced to two 2 x 2 tables (7:3) showing that anti-PlB1 is present in the serum CL (15/7/66).

PlB Antigen Phenotype	1/8	1/40	1/80	Total
12			4	4
13			4	4
23		1	1	2
1		6	14	20
2		2	1	3
3	4	4	7	15
0	10	2	2	14
	14	15	33	62

Table 7:1 First series of titrations with CL 15/7/66. Number of specimens of each PlB1 phenotype giving an end point at dilutions of 1/8, 1/40, 1/80. No specimens were found negative at 1/8 dilution.

PlB antigen Phenotype	<1/24	1/24	1/32	1/48	1/64	1/96	Total
120			1	4	1		6
103		1	1	4	4		10
023				2	1		3
100		1	1	10	5	4	21
020			1				1
003	3	1	4	7	1	1	17
000	10	1	3	7	1		22
							80

Table 7:2 Second series of titrations with CL 15/7/66. Number of specimens of each PlB phenotype with an end point at the appropriate dilution are shown in each cell.

Serum of CL at 1/40	PlB1 present	Antigen absent	
+	28	20	48
–	0	14	14
	28	34	62

$$p < 10^{-3}$$

Serum of CL at 1/24	PlB1 present	Antigen absent	
4+	37	30	67
not 4+	0	13	13
	37	43	80

$$p < 10^{-3}$$

Table 7:3 Presence of anti-PlB1 in CL

The data remaining after removal of the platelet preparations carrying PlB1 has been summarized with respect to the PlB2 antigen in Table 7:4. It appears that anti-PlB2 is probably also present in the serum CL. The antibody anti-PlB3 is probably not present in this serum at a titre higher than 1/8 as there are several platelet specimens in both series of titrations with the PlB3 antigen that do not give strong complement fixation reactions with the diluted serum of CL.

Reaction with CL at dilution 1/24 or greater	PlB2 antigen		
	Present	Absent	
+	9	41	50
–	0	27	27
	9	68	77

$$p = 0.016$$

Table 7:4 Presence of anti-PlB2 in CL. Data obtained by pooling results from Table 7:1 and 7:2.

It was suggested in Chapter 4 that the antibody in SI was also present in CL although the data agreeing with this hypothesis were not significant at the 5% level. Further information on the relationship between the sera CL and SI is shown in Table 7:5 where the results of the two titrations are pooled and platelet specimens with the PlB1 and PlB2 antigens are omitted. It is clear that the antibody in SI is present also in CL and that this antibody is active to a dilution of CL of slightly greater than 1/32.

	SI			
	+	−		
Reaction with CL at dilution of 1/32 or greater +	12	28	40	
−	0	28	28	
	12	56	68	$p < 10^{-3}$

Table 7:5 Presence of antibody   SI in CL.

2. Results of Elution of Antibodies from CL:

The other antibodies in CL have not been systematically investigated. Since a wide range in end point titres with different platelet specimens still remains after allowing for the three antibodies already demonstrated in CL, it is presumed that this serum contains more than one additional antibody. It is possible to separate the antibodies in CL using selected platelets to prepare eluates. The result of a preliminary separation of antibodies

from this serum by the acid elution method is shown in Table 7:6.

The platelets used were donated by departmental colleagues

selected only for accessibility. It is clear from Table 7:6 that

the eluates prepared with GA and MA do not contain anti-PlB1 and

that the eluates prepared with HO and HY do not contain the anti-

body in the eluate prepared with MA. Further absorption studies

with the serum CL have not been performed as it seems that more

can be learned at the moment from this serum by comparison of the

results of titration experiments with results from other less

complex sera. It is clear from Tables 7:5 and 6 that there are

more interesting antibodies in CL to be investigated.

Platelet antigen structure			Name	Reaction with CL			Reaction with eluate from CL prepared with platelets of:				
PlB											
1	2	3		1/16	1/32	1/64	BA	HO	HY	GA	MA
1	0	0	BA	4	4	4	4	4	4	1	4
1	0	–	HO	4	4	4	4	4	4	4	0
1	0	–	HY	4	4	4	4	4	4	4	0
0	0	3	GA	4	4	3	4	0	2	4	0
0	0	–	MA	4	1	0	4	0	0	1	4

Table 7:6   Activity of Five Eluates Prepared from CL.

All platelets negative with SI
1, 2 or 3 indicates the presence of the respective antigen;
0 absence of antigen;
– no test for antigen;
0 .... 4 strength of reaction between platelets and antibody.

B. THE SERUM SI

The serum of Mrs. SI was found to contain complement fixing platelet antibody after her infant developed thrombocytopenic purpura a few hours after birth. It has already been shown that this antibody is also present in the serum CL.

1. The Frequency of Positives:

The frequency of positive reactors with this serum is 0.125 (Table 7:7). The data in Table 7:7 shows that there is no heterogeneity in the frequency of positives found during the different time intervals that this antiserum was studied.

	+	−	Total
Series reported in Chap. 4	10	66	76
Blood donors tested in 1966	20	164	184
Blood donors tested in 1967	48	384	432
Hospital patients tested 1967 R.A.H & Q.E.H.	34	169	203
	112	783	895

$\chi^2_3 = 6.4$

$0.10 > p > 0.05$

Table 7:7   Frequency of SI positives.

2. Elution and Absorption Studies:

Three eluates were prepared from the serum SI with SI positive platelets and one eluate with SI negative platelets. No complement fixing activity was found in the eluate prepared with SI negative platelets on testing it against 18 SI positive platelet

preparations and two SI negative platelet preparations. The three eluates prepared with SI positive platelets fixed complement with the 18 platelet specimens from SI positive donors but not with the two from SI negative donors. Although this work is not sufficient to prove that the serum SI is detecting only one antigen it will be presumed that this situation exists during the remainder of this thesis.

The absorption of antibody from the serum with reactive platelets is shown to lower the antibody titre whereas absorption with non-reactive platelets does not have this effect (Table 7:8).

Serum dilution	1/8	1/16	1/32	1/64
Absorbed with SI positive platelets	4+	+	-	-
Absorbed with SI negative platelets	nt	4+	2+	+w
Unabsorbed	nt	nt	4+	-

Table 7:8   Reduction in titre of complement fixing activity in serum SI after absorption with reactive platelets. (nt = not tested)

3. Time Required for Complement Fixation:

During the experimental work reported in this thesis the incubation time of 1 hour prior to the addition of the haemolytic system   was   adhered to carefully. On one occasion an experiment

to show whether complement fixation would occur in less time than one hour was conducted. SI positive platelets 93249 were added to a 1/32 dilution of SI and complement 1/20. The haemolytic system was added after 0, 19, 33 and 40 minutes incubation at $37^{o}C$. Each experiment was performed in duplicate. The results were:-

Incubation period	Strength of Reaction	
0 minutes	+w	+w
19	+	+
33	+	2+
40	4+	4+

These results show that under certain conditions incubation time may be reduced. This could be of some value when results of platelet typing are required urgently.

4. <u>Relation of the SI Antigen to the PlB Antigens</u>:

The distribution of SI+ and SI- in the various PlB phenotypes is shown in Table 7:9. This population data supports the hypothesis that the PlB antigens and the antigen detected by SI are independent, $\chi^2_6 = 6.2$, $p > 0.30$. Whether the antigens are genetically independent or not will be discussed in Chapter 9.

5. <u>Other Sera Containing an Antibody Related to SI</u>:

Three other sera containing an antibody related to SI were received from Dr. M. Berah of Melbourne. The serum BE was obtained by Dr. Berah from Professor Dausset of Paris. A comparison of the

activity of BE and SI is shown in Table 7:10. Whether the three
SI+ BE- reactions observed were due to overdilution of BE or a
second antibody in SI has not been investigated. The other two
sera KA and HE gave only weak reactions and were not available in
a sufficient quantity for extensive investigation. Table 7:10
also indicates that the antibodies in KA and HE are probably related
to the antibody in SI.

	SI		
P1B Antigens	+	−	
120	10	38	48
103	8	50	58
023	6	24	30
100	23	177	200
020	8	39	47
003	12	109	121
000	15	116	131
	82	553	635

$\chi^2_6 = 6.2,\ p > 0.30$

Table 7:9 Relation of SI+ to the P1B system.

	BE		KA		HE	
	+	−	+	−	+	−
SI+	11	3	4	0	3	1
−	0	49	0	5	0	5
	11	52	4	5	3	6

$p < 10^{-9}$ $p = 1/126$ $p = 1/21$

Table 7:10 Comparison of BE, KA and HE with SI.

C. THE ANTIBODIES IN JE, KL and NO

The complement fixing activity of platelet preparations
from 42 random donors with the sera KL, JE and SA is shown in
Table 7:11. It is clear from the first half of the table that the
antibodies in NO, JE and KL are the same, (Table 7:11(a)).

	+	−
+	4	0
−	0	17
		21

$p = 1/11,970$

Table 7:11(a)

The antibody activity in the serum JE did not give strong complement
fixing activity with reactive platelets even when diluted less than
1/4.

The antibody activity activity in NO, JE and KL is present
with other antibodies in the serum SA. This is shown in Table 7:12.
It is assumed that the sera NO, JE and KL have an antibody against
a single antigen. Table 7:13 shows that there is no obvious
association between this antigen and the P1B antigens. The data
presented in Table 7:14 are not sufficiently large to determine
whether the antigens detected by the sera KL and SI are controlled
by independent loci or not. The expected number of double positive
individuals in this series is 4.5 for independent loci and 2.3 for
a single locus. It is clear from Table 2:9 that a much larger
series is required to determine the relation between these two antigens.

Table 7:11   Activity of NO, JE, KL SA with 42 random
platelet preparations.

Donor No.	NO		JE 27/7/67	KL 3/5/66	SA 23/3/67	P1B antigens
	1/4	1/6	1/4	1/2	1/8	
20205	1	1	0	0	1	000
10	4	3	2	4	4	100
18	1	1	0	0	4	003
21	1	1	0	0	1	100
26	1	1	0	0	0	023
30	1	1	0	0	2	000
31	4	3	2	4	3	100
36	1	1	0	0	3	100
37	1	1	0	0	0	100
39	1	1	0	0	4	003
41	4	4	3	4	4	003
42	1	1	0	0	3	003
44	1	1	0	0	4	120
49	1	1	0	0	4	120
50	1	1	0	0	1	103
63	4	3	2	4	4	103
64	1	1	0	1	1	100
81	1	1	0	1	3	100
82	1	1	0	0	0	000
83	1	1	0	0	3	100

Table 7:11 (ctd)

Donor No.	NO		JE 27/7/67	KL 3/5/66	SA 23/3/67	PlB antigens
	1/4	1/6	1/4	1/2	1/8	
85	0	0	0	0	1	003
Control	1	1	0	0	1	
22113			0	1	1	003
17			2	4	4	000
21			2	4	2	000
23			2	4	2	100
31			0	1	4	000
34			1	1	4	003
38			0	1	1	000
41			0	1	1	020
46			2	4	2	003
48			2	4	2	100
52			2	4	4	103
53			0	1	2	003
62			0	1	1	120
64			0	1	2	000
69			2	4	4	100
72			0	1	4	003
73			0	0	2	000
74			2	4	2	000
76			1	4	2	120
79			0	1	0	000
82			0	1	0	100
Control			0	1	0	

	SA +	SA −	
KL +	13	0	13
KL −	16	13	29
	29	13	42

$p < 0.003$

Table 7:12 A comparison of the activity of the sera KL and SA (from Table 7:11).

KL

	+	−	Total
120	3	6	9
103	3	7	10
023	1	3	4
100	9	31	40
020	3	6	9
003	3	18	21
000	10	22	32
	32	93	125

$\chi^2_6 = 2.819, \; p > 0.8$

Table 7:13 The distribution of NO, JE and KL reactions in the various P1B antigen types.

	SI +	SI −	
KL (etc) +	4	28	32
KL (etc) −	16	77	93
	20	105	125

$\chi^2_1 = 0.17, \; p > 0.50$

Table 7:14 Comparison of the distribution of the antigens detected by the sera SI and KL.

D. THE SERUM VO

1. Difficulties which Prevented Detailed Elucidation of VO:

The serum VO was found to fix complement with 38 of 98 specimens tested with a 1/8 dilution in the preliminary series of experiments. The proportion of weak reactions (2+, +) neat (19%) and diluted 1/8 (23%) combined with the prozone this serum showed with some specimens (25% or 10/42) made this serum more difficult to work with than those described in early sections of this thesis.

The first series of platelet specimens tested with this serum had been stored for one to two weeks before the complement fixation tests were carried out. A subsequent series of platelet specimens tested less than 24 hours after the blood had been collected gave a higher frequency of positive reactors.

VO (1/8) 30/6/66

	+	−	
Tested 1 – 2 weeks after platelets collected	38	60	98
Tested within 24 hours of platelet collection	53	31	84
	91	91	182

$$\chi^2_1 = 8.4$$

$$p < 0.001$$

These difficulties encountered early in the study of this serum resulted in a postponement of a detailed investigation of the serum's activity until details of the more stable antigens described in earlier chapters had been resolved. The work on VO has not yet

been completed. The incomplete details included here are of some
interest because they reveal certain features not found with other
sera.

2. Comparison of VO with Other Antibodies:

The result of testing 42 fresh specimens with VO and O'G
(both diluted 1/8) is set out in Table 7:15 (a) and (b).

<div align="center">O'G 1/8</div>

reaction with VO 1/8	4	3	2	1	0	
4	15	1		2	4	22
3					3	3
2				1	2	3
1	1	1		1	4	7
0			1		6	7
	16	2	1	4	19	42

Table 7:15   Results with O'G and VO
     (a)

<div align="center">O'G</div>

	+	−	
+	16	12	28
−	3	11	14
	19	23	42

$$\chi^2_1 = 3.6,$$
$$0.10 > p > 0.05$$

Table 7:15 (b)   Above table reduced to 2 x 2 comparison.

The association between these two antisera is not quite significant at the 5% level ($\chi^2_1 = 3.6$, $0.10 > p > 0.05$). These results are compatible with the hypothesis suggested in the report of Dr. R.H. Aster (Chapter 4) that O'G and VO share an antibody but further tests are necessary to substantiate this.

A series of stored specimens tested with VO and MA (from Dr. R.H. Aster of Boston) indicated that an antibody(s) present in MA was also present in VO, ($p = 0.019$) Table 7:16. Unfortunately no specimens were tested with all three of VO, MA and O'G.

		MA +	MA −	
VO	+	4	7	11
	−	0	16	16
		4	23	27

$p = 0.019$

Table 7:16 Result of testing 27 specimens with MA and VO.

The series of 42 platelet preparations typed with O'G and VO had also been typed for the PlB antigens. The results in Table 7:17 suggest there may be an antibody in VO which reacts more frequently with those platelets having the PlB3 antigen and lacking all PlB antigens than with the group having the PlB1 antigen only.

P1B Antigen structure	Reaction with 0'G 1/8		Reaction with VO 1/8	
	+	−	+	−
120	3	1	3	1
103	1	2	3	0
023	1	0	1	0
100	5	8	3	10
020	1	0	1	0
003	3	7	8	2
000	5	5	9	1
	19	23	28	14

Table 7:17   Comparison of 0'G and VO activity with the
P1B antigens.

3.   The Transfusion of VO and three other Patients whose Serum

Contained Complement Fixing Platelet Antibodies:

The donor of the serum VO was a patient with
chronic Myeloid Leukaemia who had been transfused with 14 units of
blood prior to the demonstration of complement fixing platelet
antibodies. She had not been pregnant. She had suffered severe
nausea, vomiting headache and pyrexia (104°F) during and following
several blood transfusions prior to the first demonstration of
platelet antibodies in her serum. Her serum was examined for red

cell antibodies

by the transfusion service but none could be demonstrated by the two stage papain or Coomb's techniques.

Mr. Vincent of the Red Cross Blood Centre found that the serum contained leukocyte agglutinins as well as complement fixing antibodies. Since the patient required a further two units of blood attempts to find non-reacting donors were made. Six donors were tested for their reactions with this serum by the platelet complement fixation and leukocyte agglutination tests. The results are shown below.

Donor Number	VO Serum Complement Fixation Test							VO Serum Leukocyte Agglutination Test (Mr. Vincent)
	2 drops neat	1 drop neat	$\frac{1}{2}$	$\frac{1}{4}$	1/8	1/16	1/32	
'47	+	+w	−	−	−	+w	+w	2+
'55	3+	3+	3+	3+	+	+w	+w	4+
56	+w	+	3+	4+	4+	3+	+	3+
68	2+	+	+w	+w	+w	−	−	4+
72	2+	3+	3+	+	+	+w	+w	4+
74	+	+w	−	−	−	+w	+w	4+
serum control	+	+w	−	−	−	−	+w	

The next day the patient was transfused with packed red cells
from donor number '47 without incident. She was asymptomatic
and her temperature remained below $99^{\circ}F$ during the transfusion
and the two hour interval allowed before commencing the second
unit of packed cells. The red cells from donor number '56
were used for the second unit. Withinan half-hour of commencing
this unit the patient developed another symptomatically severe
transfusion reaction. Her temperature rose to $101^{\circ}F$ and the
transfusion was discontinued after only 50ml had been given.
Subsequently this patient was given another five units of packed
cells selected from 42 donors by the complement fixation test
(with titration as above) on the same basis as donor number
'47 above, i.e. no complement fixation at any dilution tested.
Leukocyte agglutination tests were not carried out on these
donors. During the transfusion of these five units of packed
cells the patient suffered no transfusion reaction. After the
fourth unit her temperature rose to $102^{\circ}F$ but this appeared to
be part of a febrile pattern that is sometimes a part of chronic
myeloid leukaemia. No symptomatic ill effects were attributed to
the transfusion by the patient. In spite of a satisfactory rise
in haemoglobin to 11.9gms per 100mls, following this trans-
fusion the patient collapsed suddenly and died six days after

her last transfusion. The _in vivo_ and _in vitro_ results of the last 7 units of blood given her are shown in the table below.

	in vivo		
	+ (reaction)	− (no reaction)	
+ (complement fixation)	1	0	1
in vitro − (no complement fixation)	0	6	6
	1	6	7

$p = 1/7$

While not significant at the 5% level the results with this patient indicate that her complement fixing antibodies could be used to help her avoid transfusion reactions. Unfortunately no specimen was available for the investigation of the platelet antigens of the donor whose blood had given a transfusion reaction in this patient before her platelet antibodies were found.

Similar attempts to make transfusion more comfortable for two patients with aplastic anaemia MA and JE were not successful. Febrile responses and symptoms were produced by units of blood negative with the complement fixation test. The patient MA was transfused with saline washed packed red cells. Blood was

centrifuged at 1500rpm, the supernatant fluid removed and after saline was added cells were resuspended and sedimented. Blood was washed twice in this way but even treatment of blood by this procedure did not ameliorate the patient's symptoms. Finally the double dextran sedimentation procedure for removal of white cells from red cells for transfusion as described by Chaplin et al (1959) was performed. MA, JE and two other patients KL and M9 were given red cells prepared in this way without febrile or symptomatic response. The procedures on blood for transfusion into patients were all conducted by the Red Cross Blood Transfusion Service at Pirie St., Adelaide. From the experience outlined above it would seem that dextran sedimented red cells are a good therapy for those rare patients who suffer recurrent transfusion reactions due to the non-red cell components of blood. The sera of these patients were not examined for antibodies against serum proteins.

PLATELET ANTIGENS IN AUSTRALIAN AEORIGINES AND

NEW ZEALAND MAORIS

A. PLATELET ANTIGENS IN AUSTRALIAN ABORIGINES

Blood specimens from 81 Australian Aborigines were

obtained by Dr. B. Boettcher at Yundamu (160 miles north-west of

Alice Springs). This blood was forwarded by airfreight to Adelaide

and the platelets were separated using the techniques described in

Chapter 2. Platelet separation was performed within three days of

the collection of the blood specimens and complement fixation

tests were completed a few days later. Fourteen specimens were

found to be anticomplementary and have been deleted from the record.

The results on the remaining 67 individuals are shown in Appendix 5.

It is clear that the PlB3 antigen is uncommon in the

Australian Aborigines as no positive reaction was obtained with the

serum WE (21/3/67) diluted 1/8. However the antigens PlB1 and

PlB2 are frequently present as is indicated by the positive

reactions with NO (8/2/67) and SZ (8/2/67 at 1/5). A platelet

specimen positive with both NO and SZ is considered to have the

PlB1 antigen whereas a specimen positive with NO but negative with

SZ is thought to have the PlB2 antigen. The combination of PlB1

and PlB2 antigens could not be distinguished from PlB1 alone as the platelet suspensions were not tested with antisera which permitted this difference to be determined. The distribution of the PlB antigens in the 67 individuals studied is shown in Table 8:1.

Platelet Antigens	Wailbri	Pintubi	Total
1_0	15	6	21
020	22	3	25
000	18	3	21
	55	12	67

Table 8:1   Distribution of the PlB antigens in two Australian aboriginal tribes.

Three specimens were positive with SZ but not with NO (Y 96, 121, 344 each 2+). These reactions have been ignored as the three platelet donors were group A and some of the platelet preparations were contaminated with red cells (SZ is group 0). These results with SZ suggest that some group A PlB2 specimens may have been misclassified as PlB1. The data in Table 8:2 indicate that this error (if it has occurred at all) has not introduced a significant bias in the direction suggested.

The 67 individuals typed for platelet antigens were from 2 tribes, Pintubi and Wailbri. No significant difference in the distribution of the antigens in these tribes was detected in this

survey (Table 8.1). However the number of specimens tested so far is very small so that very little weight can be attributed to the data.

	Group 0	Group A	
P1B1	12	9	21
P1B2	12	13	25
	24	22	46

Table 8:2   Distribution of the P1B antigens in Group 0 and
Group A Aborigines.

In Table 8:3 the distribution of the P1B antigens in Central Australian Aborigines and South Australian Caucasians is compared. It is clear that there are significant frequency differences between the two races. In particular there is an absence of the P1B3 antigen in the Aborigine. P1B1 is present at a lower frequency and P1B2 at a higher frequency than the corresponding antigens in the Caucasian series.

	Caucasian	Aborigine
103	58	0
023	30	0
1-0	248	21
020	47	25
003	121	0
000	131	21
	635	67

Table 8:3 Distribution of the PlB antigens in Caucasians and
Aborigines.

The reactions of the 67 aboriginal platelet specimens
with two dilutions (1/8 and 1/40) of the serum CL 15/7/66 are
shown in Table 8:4 where the specimens are classified according
to their PlB groups and their strength of reaction with CL. These
results are concordant with those of Chapter 7 which suggest that
CL contains anti-PlB1 and anti-PlB2. The reactions with CL 1/8
indicate that the Aborigines are probably polymorphic for other
platelet antigens. This statement presumes that CL contains a
mixture of platelet antibodies in addition to anti-PlB1 and
anti-PlB2.

	PlB Antigens Present		
	PlB1	PlB2	nil
4+	21	25	17
3+			1
2+			2
1+			
0			1
	21	25	21

Strength of reaction with CL 1/8 15/7/66

	PlB1	PlB2	nil
4+	15	7	
3+	3	5	
2+	1	4	
1+	1	2	3
0	1	7	18
	21	25	21

Strength of reaction with CL 1/40 15/7/66

Table 8:4 Strength of reaction of 67 aboriginal platelet specimens with CL (1/8 and 1/40). Platelet donors classified according to their PlB antigens.

Two series of 7 and 16 specimens prepared earlier in this study will not be reported here in detail as the antibodies in the sera used have not yet been resolved in simple serological terms. In the second series of specimens tested no positive reactions were obtained with the sera SI, MA or KL.

B. PLATELET ANTIGENS IN THE NEW ZEALAND MAORI

Blood specimens from 128 Maoris were collected in the North Island of New Zealand. Maoris were bled at the Waikato Hospital Hamilton, at the Rotorua Hospital and at the Waikeria Youth Centre. The platelets were separated within 24 hours of collection of the blood and kept under refrigeration until they were tested about one month later. The result of testing the Maori platelet specimens with NO, SZ, CO, WE and SI are set out in Appendix 6. The ABO and RH blood group of 114 specimens are also recorded. The distribution of positive reactions with the sera NO, SZ, CO and WE agreed with the observations on the Caucasian series with regard to the activity of the sera. One platelet specimen from a group A donor (number 64) was positive with SZ but not with NO, presumably due to contamination of the specimen with red cell antigen. The distribution of the PlB antigens in this series is shown in Table 8:5. The frequencies of the genes controlling the PlB antigens calculated from this data by the maximum liklihood method are:

$$P1B1 \quad 0.306 \pm .032$$

$$P1B2 \quad 0.339 \pm .034$$

$$P1B3 \quad 0.057 \pm .015$$

$$P1Bo \quad 0.298 \pm .042$$

Antigens present	Number observed	Number expected	
120	27	26.56	
103	3	4.46	
023	4	4.95	
100	36	35.33	
020	41	40.57	
003	7	4.76	
000	10	11.37	$\chi^2_3 = 1.90$
	128	128.00	$p > 0.50$

Table 8:5   Distribution of the P1B antigens in a sample of
New Zealand Maoris.

The distribution of the antigens in the Maori population is in
agreement with the genetical model of three codominant alleles and
a null allele as postulated in Chapter 6, $\chi^2_1 = 1.90$, $p > 0.50$.

In Table 8:6 red cell and platelet typings uncommon in
the Maori are recorded. The frequencies of these phenotypes can be
used to estimate the frequency of the appropriate gene in the Maori
population. On the assumption that these genes were absent from
the Maori before the Caucasian population arrived in New Zealand it
is possible to estimate the proportion of European admixture in the
present sample of the Maori population from the ratio of the
respective genes in the two populations. Estimates of the proportion
of European admixture from data reported here are set out in Table 8:6,

and range from 0.148 - 0.315. Each of these estimates has a large standard error so that little weight can be placed on any one of them. However they do show reasonable consistency. Whether or not the genes responsible for these antigens were really absent from the Maori population in New Zealand before the arrival of the Europeans will not be defended in this thesis. The data presented here are not inconsistent with the hypothesis that the population sampled contained 20 - 30% European admixture. This estimate is consistent with the views of Staveley, Director of the Blood Transfusion Service, Auckland Hospital, New Zealand (personal communication 1968), that there are now very few full-blood Maoris.

	No.	Sample size	Prop- ortion	Estimated gene freq. A	Estimated gene freq. in Eng. population B	Estimate of proportion of European admixture in Maori A/B
Anti-B(+)	3	114	0.026	.013	0.061	0.216
Rh-ve	1	114	0.009	.095	0.3886	0.244
P1B3	14	128	0.1093	.057	0.1809	0.315
SI+	2	128	0.018	.009	0.061	0.148

Table 8:6 Observed distribution of some antigens uncommon in New Zealand Maoris.

FAMILY STUDIES

While the population studies outlined in earlier chapters
of this thesis were in progress platelets were obtained from the
parents and children of 17 families. These platelets were typed
for the PlB antigens described earlier in this thesis. The red
cells of these families were typed for the ABO, Rhesus and MN
antigens. The results of these typings are set out in Appendix 7.
No exclusion of parentage was suggested by the results of the
typings. A number of positive reactions with the sera SZ and AD
which are not in agreement with the interpretations suggested in
earlier chapters have been marked with an asterisk. These
positives have been ignored as they can be attributed to complement
fixation by contaminating red cell antigen and anti-A in the two
sera concerned.

A. INHERITANCE OF THE PLB ANTIGENS

There are seven possible phenotypes in a diploid organism
polymorphic for an autosomal locus with three codominant alleles
and an amorph. The number of phenotypically different matings
is 28. In view of the small number of families typed so far the
data have been summarized in Table 9:1 for each antigen separately.

The data are in agreement with the hypothesis that each antigen
is controlled by an autosomal dominant gene. The expected number
of families of each type of mating has been calculated from the
Caucasian population data by multiplying together the appropriate
phenotype frequencies and the total number of families tested,
e.g. number of families with P1B1 + x + $= (306/635)^2$ x 17. The
proportion of children expected to be negative from each type of
mating was calculated by multiplying the proportion of matings
expected to involve a heterozygote (in the case of + x -) or two
heterozygotes (in the case of + x +) by $\frac{1}{2}$ or $\frac{1}{4}$ respectively.
This proportion was then multiplied by the total number of
children observed from the particular mating type to give the
expected number of negatives. The family data available are in
good agreement with the suggested model.

Three families were found where one parent carried two
P1B antigens and the other parent lacked these antigens. The
eight children from these families contained only one of the
antigens present in the heterozygous parent (Figure 9:1).

	Parental Types			Number of Families Observed	Expected	Offspring +	−	Expectation	
	+	x	+	3	3.9	7	1	6.6	1.4
P1B1	+	x	−	8	8.5	17	13	17.4	12.6
	−	x	−	6	4.6	0	9	0	9
	+	x	+	0	0.7	0	0	0	0
P1B2	+	x	−	3	5.4	5	7	6.3	5.7
	−	x	−	14	10.9	0	35	0	35
	+	x	+	2	1.8	3	0	2.4	0.6
P1B3	+	x	−	7	7.5	10	9	10.4	8.6
	−	x	−	8	7.7	0	25	0	25

Table 9:1 Observed and expected segregation of the P1B antigens in 17 families with 47 children.

Figure 9:1 Segregation of the PIB antigens in 3 families.
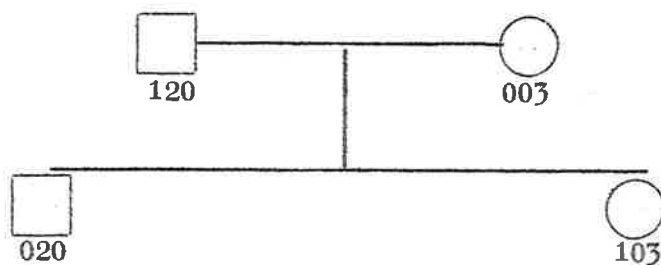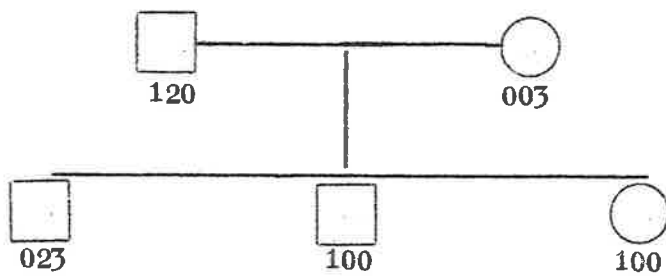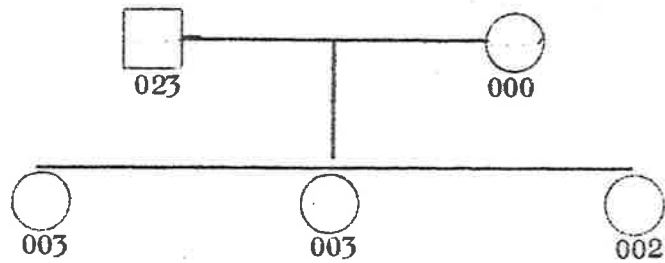
The numbers 1, 2, 3 under the genetic symbol representing each
individual indicate the presence of the respective antigen whereas
the symbol 0 indicates the absence of an antigen or antigens.

In Figure 9:1 the numbers 1, 2, 3 under the genetic symbol

representing each individual indicate the presence of the respective

antigen whereas the symbol 0 indicates the absence of an antigen or

antigens. These results are in agreement with the hypothesis that

the antigens are controlled by a series of alleles at one locus.

The probability of obtaining the antigen distribution observed in

these families if the antigens were controlled by genetically

independent loci is $\left(\frac{1}{2}\right)^8 = 1/256$.

B. RELATION OF THE PLB ANTIGENS TO THE RED CELL ANTIGENS (ABO, RH, MN)

Five families (numbers 5, 8, 14, 15, 16 segregate for the

PlB groups and at least one of the red cell groups. Families 8 and

16 exclude close linkage between the PlB and ABO loci. Families

14 and 15 exclude close linkage between the PlB and MN loci. Family

5 excludes the possibility of close linkage between the Rh locus

and the PlB locus.

C. INHERITANCE OF THE ANTIGEN DETECTED BY THE SERUM SI

Sixteen families with 46 children were typed with the

serum SI. The distribution of this antigen in the families is

shown in Table 9:2. These results are in agreement with a simple

mendelian model for the inheritance of this antigen. It was noted

in Chapter seven that the antigen detected by SI and the PlB antigens

are distributed independently in the population sampled. Only
family number sixteen provides critical evidence of the relation-
ship between the P1B antigens and this additional antigen. This
family does not provide evidence against the hypothesis that these
antigens are controlled by closely linked genes. However further
family studies are required as the observed distribution of these
antigens in the family concerned could have occurred by chance
(with probability of 1/8) if the antigens are controlled by
independent genetic loci.

Parental Types			Number of Families Observed	Expected	Offspring +	−	Expectation	
+	x	+	0	0.25	−	−		
+	x	−	4	3.5	5	6	5.7	5.3
−	x	−	12	12.25	−	35	0	35

Table 9:2   Observed and expected segregation of the antigen
detected by the serum SI in 16 families with 46
children.

DISCUSSION AND CONCLUSIONS

A. A RESUME OF PLATELET COMPLEMENT FIXING ANTIBODY SPECIFICITIES

The sera with antibodies studied during the course of this project are listed in Table 10:1. The antibodies identified are summarized in Table 10:2. The two antibodies which have not yet been identified with a particular system have been labelled SI and KL (after the initials of the donor first producing the antibody) until their relationship to the P1B system can be clearly determined. Without extensive exchange of antisera and cells between laboratories it is not possible to be certain that specificities of sera which seem to be related are identical. So far only anti-P1B1 has been definitely identified by Aster in sera he has received, namely CL, MA, and NO. Table 10:2 suggests that some other antibodies discovered in Adelaide may correspond to those described by Aster (1964). It seems likely on the basis of antigen frequency in the population and the frequency of antibody formation in the two series that P1B2 and Aster group V may be the same. Similarly P1B3 and Aster group VI may be the same. The postulated correspondence between Aster III and IV and KL and SI respectively is even less certain than the two associations suggested above.

| Serum donor | anti- P1B1 | Antibody Specificity | | | | |
		-P1B2	P1B3	SI	KL	Unknown
Polytransfused Patients						
CL	+	+		+		+
CO		+				+
CA						+
CAR						+
GO						+
NO	+	+				
PA						+
JE					+	
KL	+				+	+
MA	+					+
SA					+	+
SZ	+					
VO						+
WH						+
WE			+			+
Dialysis Patients						
WI	+	+				+
WE			+			+
FO						+
Mother of Purpuric Infant						
SI				+		
Sera Received from Melbourne						
AD		+				
MU	+					+
MA	+					+
DE	+					
NO					+	
KA				+		+
HE				+		+
Sera Received from Perth						
SP		+				+
DO						+
Other Sera						
HO						+
DU	+					+
OG						+

Table 10:1   Antisera found and antibodies identified during this project.

	(a)			(b)	
	Number of times identified	Proportion of population with antigen		Number of times identified	Proportion of population with antigen
Anti-PlB1	10	.482 ± .020	II	5	.489 ± .038
B2	6	.197 ± .016	V	7	.226 ± .032
B3	2	.329 ± .019	VI	2	.373 ± .044
Anti-SI	4	.125 ± .011	IV	2	.089 ± .023
Anti-KL	4	.256 ± .039	III	2	.237 ± .032
			VII	1	.024 ± .016

Table 10:2 Summary of antibodies identified:
  (a) in this project
  (b) by Aster et al (1964)

B. PROBABLE RELATIONSHIP BETWEEN CERTAIN PLATELET ANTIGENS AND THE LEUKOCYTE ANTIGENS

Recent publications by van Rood et al (1966) and by Vredevoe et al (1966) have suggested that the leukocyte and platelet antigens described by different workers are the same or similar. Table 10:3 constructed from the above publications summarizes the relationships now thought to exist between antigens described independently.

Antigens Recognised by:-

Van Rood	4a	4b	5a	5b	6a	6b	7a	7a	7c	7d	8a	9a
Dausset	3	7				9			10	8	1,2	
Payne										LA1	LA2	
Shulman								C1			B1	
Terasaki					4	5				1	2	6

Table 10:3 Suggested correspondence between leukocyte and platelet antigens described by various workers from van Rood et al (1966) and Vredevoe et al (1966).

Since LA2 is thought to correspond to PlB1 and since the serum here denoted by PlB3 has shown the same activity as an anti-LA1 from Dausset a comparison has been made in Table 10:4 of the data from Bodmer et al (1966) with the PlB data presented here. There is no evidence of significant heterogeneity between the two series ($\chi^2_3 = 6.3$, $0.10 > p > 0.05$). Since the gene frequencies of 7c and 7d are not significantly different from PlB3 and PlB2 respectively a comparison of the distribution of the corresponding antigens in the populations studied has been made in Table 10:5. There is no evidence of heterogeneity between the series. ($\chi^2_3 = 4.8$, $p > 0.10$).

anti-			anti-			Total
LA2	LA1		P1B1	P1B3		Total
+	+	36	+	+	58	94
+	–	112	+	–	248	360
–	+	69	–	+	151	220
–	–	115	–	–	178	293
		332			635	967

Table 10:4   A comparison of the distribution of the P1B and
LA antigens, LA antigen data from Bodmer et al (1966).

anti-			anti-			Total
7c	7d		P1B3	P1B2		Total
+	+	10	+	+	30	40
+	–	76	+	–	179	255
–	+	42	–	+	95	137
–	–	101	–	–	331	432
		229			635	864

Table 10:5   A comparison of the P1B and '7' antigens.
'7' antigen data from van Rood et al (1966).

These data combined are in agreement with the hypothesis that PlB1
corresponds to LA2, PlB3 corresponds to LA1 and 7c and that PlB2
could correspond to 7d. This hypothesis is contrary to the
suggestion by Vredevoe et al that LA1 corresponds to 7d. The
frequencies of the antigens involved in this difference of opinion
are shown in Table 10:6. No firm decision can yet be reached.

	Antigen	Frequency in Population	Antigen	Frequency in Population
Daussett	10	$0.308 \pm .040$	8	$0.180 \pm .033$
van Rood	7c	$0.375 \pm .032$	7d	$0.227 \pm .028$
Payne	LA1 $0.316 \pm .026$ (1966)			
	LA1 $0.296 \pm .039$ (1964)			
PlB	3	$0.329 \pm .019$	2	$0.197 \pm .016$

Table 10:6 A comparison of certain leukocyte and platelet antigen
frequencies. There is conflicting evidence on the
position of LA1 in this scheme.

C. THE RACIAL DISTRIBUTION OF LEUKOCYTE AND PLATELET ANTIGENS

The distribution of leukocyte and platelet antigens in different racial groups has so far received little attention. The only figures presently available are those of Dausset et al (1965). The figures of Dausset for Mac, 4a and 4b indicate that there are differences between racial groups. The figures presented in Chapter 8 of this thesis substantiate the hypothesis that leukocyte and platelet polymorphisms may be expected to contribute to our knowledge of the differences between races.

D. PRESENT STATUS OF THE RELATIONSHIP BETWEEN LEUKOCYTE AND PLATELET ANTIGENS AND ANTIBODIES AND HOMOTRANSPLANTATION

The relationship between leukocyte and platelet antigens and homotransplantation in humans is not yet clear. Ceppellini et al (1966) have shown that genetic factors in addition to the ABO and P red cell groups have an important influence on the time of rejection of grafted skin in non-immunized untreated subjects. Van Rood et al (1966) typed the leukocytes of the subjects who participated in Ceppellini's experiments. Their results are in agreement with the hypothesis that leukocyte antigen incompatibility will reduce the time of survival of grafted skin. An experiment of Dausset and Rapaport (1966) has shown that skin from a donor lacking a number of common leukocyte antigens (8, 9, 10)

survived much longer when transplanted to his brother than is usual, (43 days compared with 10 - 20 days). Further experiments are necessary to determine the importance of individual leukocyte antigens.

Observations on renal homotransplants in humans have not yet yielded clear results. Although the difference in survival between matched and mis-matched renal homotransplants reported by Terasaki et al (1966) has not reached the usually accepted significance levels the results are not incompatible with the hypothesis that matching could improve the results. Demonstration of the importance of particular antigens will no doubt be easier in the experimental environment (skin grafting) than in the therapeutic environment of renal homotransplantation.

So far little has been published on the survival of renal transplants in patients with pre-formed platelet and leukocyte antibodies. Kissmeyer-Neilsen et al (1966) have described two patients with pre-formed antibodies who rapidly rejected kidney transplants. As there is no report of a kidney from a donor with specific leukocyte and platelet antigens surviving in the presence of antibodies of the same specificity it is not yet possible to say that leukocyte and platelet antibody antigen systems are

unimportant in kidney transplantation. Two Adelaide patients with complement-fixing platelet antibodies reacting with the platelets of a large proportion of the population both had a transplanted kidney removed shortly after they had received the organ. Although these events prove little they do underline the importance of collecting all the possible available information on the antigens of the donor and recipient so that proper analysis can be carried out as soon as possible.

TRANSFUSED PATIENTS TESTED FOR COMPLEMENT

FIXING PLATELET ANTIBODIES

Name & age	Sex	Blood	Diagnosis	Times tested	Number preg- nancies	Trans- fusions	Anti- bodies	Reactions
AL34	M	O+	Peptic Ulcer	4	–	76	No	No
AN85	M	A+	Anaemia	1	–	3	No	No
AL38	F	O–	Haemolytic Anaemia	1	?	14	No	No
BL83	F	A–	Megakaryocytic leukaemia	2	?	30	No	No
BR75	M	A+	Chronic Renal Failure	1	–	25	No	No
BI74	F	A+	Sideroblastic Anaemia	4	nil	48	No	Skin itching
BI58	M	A+	Porphyria	1	–	27	No	Mild pyrexia
BR60	M	O–	Bleeding stomach ulcer	2	–	22	No	No
BA74	F	O+	Leukaemia	1	?	5	No	No
BR69	F	O+	Myelofibrosis	2	1	35	No	No
BR57	M	O+	Bleeding peptic ulcer	1	–	7	No	No
BE50	M	O+	Duodenal ulcer	1	–	18	No	No
BA66	M	A+	secondary ca	1	–	35	No	No
BL55	F	O–	Ca ampulla Vater	1	?	4	No	Urticaria
BA23	M	O+	Leukaemia	1	–	17 *	No	No

Name & Age	Sex	Blood Group	Diagnosis	Times tested	Number preg-nancies	Trans-fusions	Anti-bodies	Reactions
BU70	M	0+	Uraemia	1	–	10	No	No
BR84	F	A+	Sideroblastic Anaemia	1	5	18	No	Mild Pyrexia
CA37	F	0+	Lymphosarcoma	1	–	5	No	No
CH56	F	0+	Chronic renal failure	3		62	No	No
CL65	M	0+	Myelofibrosis	1	–	227	Yes	Febrile
CO57	M	0+	Hypoplastic anaemia	1	–	10	Yes	Febrile
CO72	M	A+	Visico-colic fist-ula	1	–	8	No	Urticaria
CR25	M	A+	Fracture amputation	1	–	15	No	No
CA63	F	0+	Ca pancreas	1	11	18	Yes	No
CA66	M	A+	Leukaemia	3	–	17	No	No
CL55	F	0–	Post-traumatic	1	–	3	No	No
CH51	F	0+	Ca breast	1	yes	16	No	No
CA24	F	A+	Choriocarcinoma	3	1	28	Yes	Pyrexia
CO73	F	0+	Multiple Myeloma	1	4	8	No	No
CL83	M	0+	Chronic Blood loss anaemia	1	–	10	No	No
DA35	F	A–	Fracture tibia	1	yes	24	No	No
DA76	M	0+	Chronic blood loss Abdominal mass	1	–	7	No	No
DE61	F	0+	Epistaxsis	1	?	10	No	No
DI77	M	0+	Anaemia	2	–	15	No	No

Name & Age	Sex	Blood Group	Diagnosis	Times tested	Number preg- nancies	Trans- fusions	Anti- bodies	Reactions
DO50	M	B+	Malignant hyper- tension	1	−	7	No	No
ED54	F	A+	Aplastic anaemia	1	5	28	No	Rigor once
FL65	F	A−	Aplastic Anaemia	2	nil	3	No	No
FL85	F	A+	Haematemesis & Malena	3	5	49	No	Pyrexia mania
GO60	F	O+	Aplastic Anaemia	2	?	22	No	No
GR54	F	O+	Multiple Myeloma	2	−	10	No	No
GI23	F	A+	Bleeding following Hydatidiform mole	1	nil	17	No	urticaria
GO27	F	A−	Hodgkin's disease Anaemia	1	yes	8	No	No
GO63	M	B−	Chronic Renal failure	2	−	10	Yes	No
GR78	F	A+	Aplastic anaemia	3	2	40	No	No
GO14	F	A+	osteosarcoma	1	nil	2+	No	Pyrexia
HO41	F	O+	Bleeding gastric ulcer	1	9	17	No	No
HU23	M	A+	Aplastic anaemia	1	−	28	No	No
HA60	M	A+	Ca bladder	1	−	5	No	No
HA65	M	A+	Corpulmonale	1	−	5	No	Pyrexia
ME60	M	O+	Myelofibrosis	3	−	31	No	No
MA24	F	B+	Cirrhosis & port. hypertension	2	nil	35	No	No

Name & Age	Sex	Blood Group	Diagnosis	Times tested	Number pregnancies	Trans-fusions	Anti-bodies	Reactions
MI37	M	O-	Craniotomy	1	–	5	No	No
MA32	M	O+	Acute renal failure	1	–	3	No	yes
MA48	F	A+	Ca of ovary	1	4	5	No	No
MA53	M	A+	Post gastrectomy syndrome	1	–	10	No	No
MA63	F	A+	Chronic lymphatic leukaemia	1	–	7	No	No
NO72	F	O+	Oesophagal varices cirrhosis	1	yes	22	No	No
NO45	F	A+	Renal failure cirrhosis	1	2	14	yes	No
OD70	M	O+	Ca Bladder	2	–	13	No	No
OW15	F	O+	Acute Myeloid leukaemia	8	–	24	No	No
PA30	F		Hogkins disease	1	2	7	No	Pyrexia
PO34	F	A+	Acute myeloid leukaemia	1	nil	23	No	No
PE67	F	A-	Carcinomatosis	1	?	17	No	No
PA44	M	O-	Frac. neck femur	1	–	1	yes	Rigor
QU54	F	A-	Fibroids, blood loss at theatre	1	–	28	No	No
RA69	F	B-	Aplastic Anae.	6	1	117	No	Pyrexia
RI28	M	A-	Acute Leukaemia	5	–	57	No	No
RE29	F	A+	Pelvic Abcess	1	1	5	No	No
RU23	M	O-	Ca of Parotid	1	–	18	No	No

Name & Age	Sex	Blood Group	Diagnosis	Times tested	Number pregnancies	Trans-fusions	Anti-bodies	Reactions
HA62	F	A+	Rheumatoid Arth.	1	?	8	No	No
RA70	M	A+	Ca colon	11	–	13	No	No
SC24	F	A–	Incomplete M/C road accident	1	yes	17	No	No
SM75	M	A+	Hodgkin's disease	7	–	50	No	No
ST54	F	O+	Post Gastrectomy anaemia	1	?	8	No	No
SE20	M	B+	Gastric Ulcer	1	–	8	No	No
SM36	F	O+	Haematemasis	1	yes	6	No	No
HA53	M	O+	Bleeding Varices Cirrhosis	1	–	18	No	No
HO79	M	O+	Ca prostrate	1	–	32	No	No
HO76	F	B+	Ulcerative colitis	1	–	12	No	Jaundice
HA81	M	A–	Sideroblastic Meg. Anaemia	1	–	9	No	No
HA42	F	O+	Anaemia & Pyelitis Ulcerative colitis	1	1	76	No	fever nausea
IA50	F	AB+	Pelvic Abscess Menorrhagia	1	1	31	No	No
JE70	F	O–	Aplastic anaemia	5	1	66	Yes	Pyrexia nausea
JO57	M	B+	Gastric fistula	1	–	43	No	pyrexia once
JE16	M	O+	Malena	1	–	4	No	No
KL42	F	AB+	Ulcerative colitis	1	2	50+	yes	pyrexia
KA71	M	O+	Chronic Renal fail.	1	–	11	No	No

Name & Age	Sex	Blood Group	Diagnosis	Times tested	Number preg-nancies	Trans-fusions	Anti-bodies	Reactions
L067	M	O+	Prostatectomy Uraemia	1	–	9	No	Pyrexia Urticaria
LA60	F	O+	Anaemia Invest.	1	1	7	No	No
LY57	M	O+	Hepatic Cirrhosis	1	–	41	No	No
L014	M	O+	Aplastic Anaemia	9	0	65	No	Pyrexia
L075	M	O+	Atheromatous Arterial disease	1	–	10	No	No
LA28	F	A+	Caesarian Section	1	1	2	No	Yes
LI74	F	O+	Ca breast	1	–	19	No	No
LU55	F	O+	Hydronephrosis	1	1	7	No	No
MU58	M	O+	Ca breast	1	–	9	No	Yes ?
MI74	F	AB+	Ca bladder	1	nil	15	No	No
MA87	F	O+	Chronic renal fail.	1	–	6	No	No
MA45	F	A+	Aplastic Anaemia	1	1	79	Yes	pyrexia
MA72	F	A–	Osteomyelitis	2	yes	8	No	No
MA47	F	O+	Multiple myeloma	5	2	31	No	Rigors
MC61	M	O+	Duodenal Ulcer	1	–	14	No	No
MI38	M	O+	Thalassaemia minor	1	–	7	No	No
M039	M	O–	Uraemia renal T.B.	5	–	66	No	No
SZ41	M	B–	Paraplegia – Bleeding d. ulcer	2	–	45	No	No
SA36	F	A+	Acute Leukaemia	6	1	114*	Yes	Yes
ST40	M	O+	Ca Colon	1	–	15	No	No

Name & Age	Sex	Blood Group	Diagnosis	Times tested	Number preg-nancies	Trans-fusions	Anti-bodies	Reactions
SE79	M	A+	Bleeding	1	–	2	No	No
SH54	M	0+	Chronic Lymphatic leukaemia	2	–	37*	No	yes
SZ36	F	0+	Anti-partum haemorrhage	1	3	2	yes	Rigor
V079	F	0+	Chronic Myeloid leukaemia	1	nil	14	yes	pyrexia
T056	M	A+	Duodenal ulcer	1	–	23	No	No
WH63	F	0+	Multiple hereditary telangectasis	6	–	50+	Yes	No
WH40	F	A+	Liver failure	1	–	6	No	Rigor
WH39	M	0+	Alcoholic Cirrhosis	3	–	37	No	No
WI72	M	0+	Aplastic Anaemia	2	–	11	No	No
WE54	F	A–	Acute Leukaemia	1	5	5	Yes	No
WI47	M	B+	Haematemesis & Malena	1	–	3	No	No
WA77	M	A+	Ca Stomach	1	–	8	No	No

* includes transfusions of platelet concentrates as well as whole blood or packed red cells.

PRELIMINARY SURVEY OF PLATELET ANTIBODY

SPECIFICITY

An analysis of this data is presented in Chapter 4.

Donor	NO Neat 6/66	CO Neat 3/66	VO Neat 5/66	VO 1/8 5/66	SI Neat 6/66	CL 1/16 5/66	MA Neat 2/66	MA 1/8 2/66	WH Neat 5/66	KL Neat 12/65
1	0	0	0	0	0	4	1	2		0
2	0	0	0	0	0	1	0	0		0
3	4	0	4	4	0	4	2	3		0
4	4	4	0	0	0	4	4	4		2
5	4	1	4	1	4	4	4	4		1
6	4	4	4	0	0	4	4	4		0
7	0	0	0	0	0	3	0	0		4
8	4	2	4	4	0	4	4	4		4
9	0	0	4	3	0	4	3	4		1
10	1	1	3	2	0	4	1	1		0
11	4	1	2	2	4	4	4	4		4
12	0	0	0	0	0		0	0		4
13	0	0	0	0	0		1	1		4
14	4	0	0	0	0		4	4		0
15	0	0	4	1	0		4	0		0
16	0	0	0	0	0		0	0		0
17	4	2	0	0	0		4	4		4
18	4	0	0	0	0		4	4		0
19	4	0	4	1	4		4	4		0
20	4	4	4	4	0		4	3		0
21	4	4	4	4	0		4	4		0
22	4	4	4	0	0		4	4		0
23	4	4	4	0	0		4	3		0
24	4	2	4	4	0	4	4	4		1
25	0	0	0	0	0	4	2	1		0
26	4	0	0	0	0	4	4	4		0
27	3	4	4	0	0	4	4	4		4
28	3	4	3	1	0	4	4	4		1
29	4	1	1	0	0	4	4	4		1
30	4	2	4	0	0	4	4	4		1
31	4	1	0	0	0	4	4	4		1
32	4	2	0	0	0	4	4	4		1
33	0	2	0	0	0	4	1	1		4
34	4	2	2	0	1	4	4	4		0

Donor	NO Neat 6/66	CO Neat 3/66	VO Neat 3/66	VO 1/8 5/66	SI Neat 6/66	CL 1/16 5/66	MA Neat 2/66	MA 1/8 2/66	WH Neat 5/66	KL Neat 12/65
35	0	2	0	0	0	4	0	0		1
36	0	1	4	4	4	4	2	1	2	0
37	0	1	4	4	0	4	0	0	0	0
38	4	0	4	0	4	4	4	4	2	1
39	4	0	0	0	0	2	0	0	2	4
40	0	0	4	4	0	1	0	0	0	0
41	0	0	4	4	4	4	4	1	2	0
42	4	1	4	1	0	4	4	4	0	4
43	4	1	2	0	0	4	4	4	0	1
44	3	2	0	0	0	4	4	4	0	1
45	3	0	4	4	0	1	0	0	0	0
46	3	0	0	0	0	4	0	1	2	1
47	3	0	0	0	0	4	4	4	0	1
48	2	4	4	0		0	4	0	0	1
49	4	3	0	0		4	4	4	0	2
50	4	1	0	0		4	4	4	0	0
51	4	0	3	2		4	4	4	1	0
52	4	0	4	0		4	4	4	0	0
53	0	0	0	0		1	3	1	0	4
54	0	0	0	0		4	4	4	1	0
55	1	4	2	2	0	4	4	4	1	0
56	3	2	2	1	0	4	3	1	1	0
57	0	0	4	4	4	4	0	0	0	0
58	4	0	0	1		4	4	4	1	0
59	4	0	0	0		4	4	4	0	0
60	0	0	4	4		4	3	0	1	0
61	3	3	1	0		1	4	0	0	0
62	0	1	0	1		2	0	0	1	4
63	4	3	4	4		4	4	1	1	1
64	4	0	4	4			4			1
65	4	0	4	4		4	4	4	1	1
66	1	1	1	1		4	4	4	0	3
67	1	0	0	0		0	0	0	0	0
68	3	0	0	0		4	4	4	0	0
69	3	0	0	0		4	4	4	0	0
70	4	0	0	0		4	4	4	0	0
71	3	0	0	2		4	2	2	0	0
72	4	2	4	3		4	4	4	0	3

Donor	NO Neat 6/66	CO Neat 3/66	VO Neat 3/66	VO 1/8 5/66	SI Neat 6/66	CL 1/16 5/66	MA Neat 2/66	MA 1/8 2/66	WH Neat 5/66	KL Neat 12/65
73	4	2	1	1	0	4	4	4	0	4
74	1	1	4	4	4	4	1	2	2	1
75	1	3	2	1	0	1	0	0	0	4
76	1	0	0	1	0	1	4	2	0	1
77	4	1	1	2	0	4	4	4	2	0
78	4	4	2	1	0	4	4	4	0	1
79	1	0	4	4	1	4	3	4	2	0
80	4	4	4	1	3	4	4	4	0	1
81	3	0	1	3	1	1	0	0	1	1
82	3	4	4	4	2	4	4	1	0	0
83	4	1	1	2	0	4	4	4	1	1
84	0	1	1	4	0	1	0	1	2	0
85	4	4	3	3	0	4	4	4	0	0
86	1	1	0	1	0	0	0	0	0	4
87	0	1	0	4	0	0	0	1	2	4
88	4	4	4	4	4	4	4	2	0	1
89	1	0	0	3	0	3	1	3	0	4
90	4	3	2	0	0	4	4	4		4
91	4	2	0	0	0	4	4	4		4
92	0	0	2	4	0	4	0	0		0
93	4	4	4	4	0	4	4	4		2
94	4	0	4	4	4	4	4	4		1
95	4	4	4	4	0	4	4	4		0
96	4	4	4	4	0	4	4	4		1
97	3	0	1	1	0	4	4	4		1
98	3	3	2	0	0	4	4	4		4

REACTIONS OF PLATELET SPECIMENS WITH

VARIOUS PLATELET ANTIBODIES

Donor No	Sex	Blood Group	NO 1/8 2/67	NO 1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
93238	M	A	0		0			4	0	003
40	F	0	4		4			0	0	100
41	M	A	0		0			4	0	003
42	M	A	4		4			4	0	103
44	M	A	0		0			0	0	000
45	M	0	0		0			4	0	003
47	M	0	0		0			4	0	003
50	M	0	4		0			4	0	023
53	M	A	4		0			3	0	023
54	F	0	4		4			2	0	100
55	M	0	0		0			0	0	000
57	F	0	4		0/2			0	0	120
58	M	0	4		4			1	0	100
59	M	A	0		0				0	00-
66	F	A	4		4				0	10-
71	M	A	4		0				0	02-
78	M	A	4		0				0	02-
81	M	0	4		4				0	10-
84	M	A	4		4				0	10-
46	F	0	4		4			0	4	100
49	M	0	0		0				4	00-
94911	M	0	1		1			0	0	000
15	F	0	4		4			0	0	120
19	M	0	4		4			4	4	103
22	M	0	4		4			4	0	103
24	M	0	4		4			3	0	103
25	M	0	4		4			0	0	100
26	M	0	4		1			4	0	023
28	M	0	4		4			0	0	100
32	F	0	1		1			4	0	003
33	F	0	4/4		1/0			4	0	023
35	M	0	1		1			0	0	000
37	M	0	4		4			0	0	100
39	M	0	1		1			0	0	000
40	M	0	4		4			4	4	103

Donor NO	Sex	Blood Group	NO 1/8 2/67	1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
94950	F	0	4/4		1/0			2	0	020
60	M	0	4		4			0	0	100
96725	F	0	3/4		0/0			1	0	020
27	M	0	0		0			4	0	003
30	F	0	0		0			4	0	003
33	M	0	0/0		0/0			4	0	003
37	M	0	4/4		2/2			0	0	100
42	M	0	4/4		2/3			0	0	100
46	F	0	4/4		2/4			4	0	103
52	M	0	4/4		3/1			0	0	100
53	M	0	4/4		3/2			4	0	103
57	M	0	4/4		3/4			0	4	120
58	M	0	4/4/4		2/4/4			4	0	103
59	F	0	4/4		3/3			0	4	100
61	M	0	0		0			4	0	003
62	F	0	4/4		3/3			0	0	100
67	M	0	4/4		4/4			0	0	100
70	F	0	0		0			0	0	000
74	M	0	3/3		0/0			4	0	023
75	F	0	0		0			4	0	003
96831	M	0	4/4		0/0			4	0	023
98726	M	0	4		0				0	02-
29	M	0	4		4				0	12-
30	M	0	4		1/4				1	10-
31	M	0	2		0/0				4	10-
32	M	0	4		4				1	10-
34	M	0	0		0				1	00-
35	M	0	4		4				1	12-
36	M	0	4		3				1	12-
39	M	0	0		0				1	00-
41	M	0	0		0				1	00-
45	M	0	4		3				1	10-
46	M	0	4		3				0	10-
48	F	0	4		2/4				4	10-
49	F	0	0		0				0	00-
54	M	0	0		0				1	00-
56	M	0	4		0/0				4	12-
58	F	0	4		2/4				0	10-
61	M	0	0		0				1	00-
66	F	0	4		3				0	10-
68	M	0	0		0				0	00-
70	M	0	2		0/0				0	10-
00643	F	0	4/4		0			1	0	020

Donor No	Sex	Blood Group	NO 1/8 2/67	1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
C0656	F	O	4/4		4			0	0	100
62	M	O	0/0		0			0	0	000
67	M	O	4/4		4			4	0	103
68	M	O	4/4		4			1	0	100
70	F	O	4/4		4			0	0	100
74	M	O	4/4		0			0	0	020
75	F	O	0/0		0			4	0	003
78	F	O	4/4		4			0	0	100
79	F	O	4/4		3			0	4	100
86	M	O	4/4		4			0	4	100
90	M	O	0/0		0			3	0	003
91	M	O	0/0		0			0	0	000
95	F	O	4/4		4			0	1	100
96	M	O	4/4		0			0	4	020
97	F	O	4/4		4			4	0	100
98	F	O	4/4		4			0	0	100
04311	F	O	0		0			0	0	000
12	F	O	0		0			4	0	003
15	M	O	0		0			0	0	000
19	M	O	4		4			0	0	100
20	M	O	4		4			0	0	100
22	F	O	0		0			0	0	000
26	M	O	0		0			3	0	003
37	M	O	0		0			0	0	000
42	M	A	4		4			0	0	100
45	M	O	4		0			0	4	020
54	M	B	4		4			0	4	120
57	M	A	4		4			0	0	100
60	M	O	0		0			0	0	000
64	M	A	4		4			0	0	100
65	M	O	0		0			4	0	003
68	F	A	4		4			0	1	100
72	M	O	4		3			0	1	100
74	M	O	4		4			1	1	100
78	F	A	0		0			4	1	003
93	M	O	4		4			0	1	100
96	M	O	0		0			3	4	003
06188	M	O	4		4			0	0	120
98	F	O	4		1			0	0	020
06207	F	O	4		4			0	0	120
08	M	O	4		4			0	0	100

Donor No	Sex	Blood Group	NO 1/8 1/24 2/67	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
06209	M	0	4	4			0	0	100
11	F	0	1	1			4	0	003
22	M	0	4	4			0	0	100
24	M	0	1	1			4	0	003
27	F	0	4	1			4	0	023
33	M	0	4	1			0	0	020
34	M	0	1	1			0	0	000
38	M	0	4	4			0	0	100
43	F	0	4	4			0	0	100
44	F	0	1	1			0	0	000
54	M	0	4	4			0	0	100
56	M	0	1	1			4	0	003
57	F	0	1	1			4	0	003
59	F	0	4	4			0	0	120
62	F	0	4	1			4	0	023
69	M	0	4	4			0	0	120
81	F	0	1	1			0	0	000
08149	M	0	4	0			2	0	020
51	F	0	4	4			2	0	120
53	M	0	0	0			2	0	000
54	F	0	4	4			2	0	100
56	M	0	4	4			4	0	103
57	M	0	4	0			2	0	020
58	M	0	4	4			2	1	100
62	M	0	0	0			4	0	003
66	M	0	0	0			2	1	000
67	M	0	4	4			2	1	100
70	M	0	4	4			2	1	100
71	F	0	4	4			4	1	103
82	F	0	3	0			4	0	023
83	M	0	4	4			4	4	103
86	F	0	0	0			4	0	003
96	F	0	4	0			2	0	020
99	M	0	4	0			2	0	020
09973	M	0	0	0			0	1	000
75	M	0	0	0			0	4	000
77	M	0	4	4			4	1	103
78	F	0	4	2			0	1	100
83	M	0	4	4			0	1	100
85	M	0	4	3			0	0	100
86	F	0	4	0			4	0	023
87	M	0	4	3			0	1	100
88	M	0	0	0			4	0	003
90	M	0	4	4			0	1	100

WE with 8149 – 99 slightly anticomplementary, serum control gave 2+ activity.

Donor No	Sex	Blood Group	NO 1/8 2/67	NO 1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
09991	M	O	4		4			0	1	100
93	M	O	0		0			0	1	000
95	M	O	4		3			0	1	100
10004	F	O	0		0			0	4	000
07	M	O	0		0			4	1	003
16	M	O	2		0			0	0	020
22	M	O	0		0			4	0	003
29	M	O	4		2			0	4	100
11918	F	O	0	0	0	0		0	1	000
19	M	O	4/3	4	4	0		0	4	100
25	M	O	4/3	4	4	0		4	4	103
26	M	O	0	0	0	0		0	1	000
28	M	O	0	0	0	0		0	4	000
31	M	O	0	0	0	0		4	1	003
32	F	O	4	4	4	0		4	1	103
35	F	O	4	4	4	0		0	1	100
39	M	O	4/3	1	0	3/3	4	0	1	020
43	M	O	4/3	0	0	1/2	4	4	0	023
45	F	O	4	4	4	4/2	4	0	0	120
48	M	O	4	4	4	0		0	0	100
14029	M	O	4	0	0	2	4	4	0	023
30	M	O	4	4	4	2	4	0	0	120
31	M	O	0	0	0	0		0	0	000
35	M	O	0	0	0	0		0	0	000
39	M	O	0	0	0	0		0	0	000
42	M	O	4	0	0	2	4	4	2	023
48	M	O	4	4	4	0		0	0	100
37	M	O	3	0	0	3		0	0	020
49	M	O	0	0	0	0		0	0	000
50	M	O	0	0	0	0	0	4	0	003
51	M	O	4	0	0	4	4	4	4	023
52	F	O	0	0	0	0		0	0	000
53	M	O	4	1	0	4	4	4	0	023
55	M	O	4	4	2	3	4	0	0	120
56	M	O	0	0	0	0		4	4	003
58	M	O	4	4	4	0		0	0	100
59	M	O	4	4	4	0		0	4	100
61	M	O	0	0	0	0		4	0	003
62	M	O	0	0	0	0		4	0	003
64	M	O	4	4	4	0		0	0	100
16074	M	O	4	4	2	0		0	0	100
82	F	O	0	0	0	0		0	0	000

Donor No	Sex	Blood Group	NC 1/8 2/67	1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
16084	M	0	4	4	2	0		0	4	100
86	F	0	4	2	2	0		0	0	100
93	M	0	0	0	0	0		4	2	003
98	M	0	0	0	0	0		0	0	000
99	M	0	0	0	0	0		4	0	003
16103	M	0	4	4	2	0		0	0	100
04	F	0	0	0	0	0		4	0	003
05	M	0	4	4	2	0		0	4	100
12	M	0	4	4	2	0		0	0	100
14	F	0	0	0	0	0		4	3	003
16	F	0	4	2	2	0		4	0	103
22	M	0	4	0	0	4	4	4	4	023
23	M	0	4	4	2	0		0	0	100
24	F	0	4	4	1	0		0	0	100
27	M	0	0	0	0	0		0	0	000
28	F	0	4	4	2	0		0	0	100
35	M	0	0	0	0	0	0	0	0	000
52	F	0	4	0	0	4		0	4	020
18151	F	0	4	4	4	0	0	0	0	100
59	M	0	0	0				0	0	000
60	M	0	4	4	2	0	0	4	4	103
66	M	0	4	0	0	4	4	0	4	020
69	M	0	0	0	0	0	1	0	0	000
79	M	0	3	0			4	4	0	023
82	M	0	4	4	3	0	0	4	0	103
90	M	0	4	4	3	0	0	0	0	100
91	M	0	4	4	4	4	4	0	0	120
94	M	0	4	4	3	0		0	0	100
96	F	0	4	4	3	0	0	0	0	100
18201	M	0	0	0				0	0	000
02	M	0	4	4	3	0	0	0	0	100
04	M	0	4	4	3	4	4	0	2	120
05	M	0	0	0				4	0	003
15	F	0	0	0				0	0	000
17	M	0	4	0	0	4	4	0	0	020
20	M	0	2	0	0	4	4	0	0	020
30	M	0	0	0				0	0	000
31	F	0	4	3	1	0	0	0	0	100
53	M	0	4	4	3	0	0	0	0	100
20205	M	0	0	0	0	0	0	0	0	000
18	M	0	0	0	0	0	0	3	0	003
21	M	0	4	4	4	0	0	0	0	100
26	M	0	3	0	0	4	2	4	0	023
30	M	0	0	0	0	0	0	0	0	000

Donor No	Sex	Blood Group	NO 1/8 2/67	1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	PlB typing
20237	M	0	4	3	4	0	0	0	0	100
39	F	0	0	0	0	0	0	4	0	003
49	M	0	0	0	0	0	0	4	0	003
42	F	0	0	0	0	0	0	4	0	003
44	F	0	4	4	3	4	2	0	4	120
49	F	0	4	2	4	4	2	0	0	120
50	F	0	4	2	4	0	0	4	0	103
63	F	0	4	4	4	0	0	4	0	103
64	F	0	4	1	4	0	0	0	0	100
81	M	0	4	2	3	0	0	0	0	100
82	F	0	0	0	0	0	0	0	0	000
83	M	0	4	1	4	0	0	0	0	100
85	M	0	0	0	0	0	0	4	0	003
22113	M	0	0	0	0	0	0	4	0	003
23	M	0	4	2	3	0	0	0	0	100
34	F	0	0	0	0	0	0	4	0	003
38	M	0	0	0	0	0	0	0	0	000
44	M	0	4	0	0	4	3	0	0	020
46	F	0	0	0	0	0	0	4	0	003
48	M	0	4	3	4	0	0	0	0	100
52	M	0	4	4	4	0	0	4	0	103
62	M	0	4	4	4	4	4	0	0	120
64	M	0	0	0	0	0	0	0	0	000
69	M	0	4	2	4	0	0	0	4	100
72	M	0	0	0	0	0	0	4	0	003
73	F	0	0	1	0	0	0	0	0	000
74	M	0	0	0	0	0	0	0	0	000
76	F	0	4	4	4	4	4	0	0	120
79	M	0	0	0	0	0	0	0	0	000
82	M	0	4	4	4	0	0	0	4	100
23942	M	0	4	4	4		2	1	4	100
46	M	0	4	4	4		2	1	0	100
48	M	0	4	4	4		2	4	0/0	103
55	M	0	4	2	1		4	1	0/0	020
64	F	0	0	1	1		1	0	0	000
66	M	0	4	4	4		1	4	0	103
71	M	0	4	4	4		1	0	0	100
72	F	0	0	0	0		0	0	0	000
75	F	0	●	1	0		1	0	0	000
79	M	0	0	0	0		0	0	0	000

Donor No	Sex	Blood Group	NO 1/8 2/67	NO 1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
23980	F	0	0	0	0		0	0	0	000
81	F	0	0	0	0		0	0	4/4	000
82	M	0	0	0	0		0	0	4	000
88	F	0	0	0	0		0	4	0	003
24006	F	0	4	4	4		0	0	0	100
08	M	0	0	0	0		0	4	0	003
25864	M	0	3	3			0	0	1	100
75	M	0	0	0			0	3	1	003
81	M	0	0	0			0	3	1	003
89	F	A	3	0			3	0	3	020
92	F	0	3	3			1	3	1	103
25915	M	0	3	1			3	3	1	023
22	F	0	3	3			0	0	1	100
29	M	0	3	1			3	3	0	023
34	M	0	0	0			0	0	0	000
37	M	0	3	3			0	0	3	100
40	M	0	0	0			0	3	0	003
44	M	0	3	3			3	0	0	120
46	F	0	3	3			0	0	0	100
49	M	0	0	0			0	3	3	003
51	F	0	0	0			0	3	3	003
56	M	0	0	0			0	0	0	000
58	M	0	3	3			0	3	0	103
67	M	0	0	0			0	0	3	000
84	M	0	3	3			0	0	0	100
28029	M	0	4	0	0		4	4	0	023
32	M	0	4	4	4		0	0	0	100
34	M	0	4	4	4		0	4	0	103
39	M	0	4	0	0		4	4	0	023
65	F	0	0	0	0		0	0	0	000
68	M	0	0	0	0		0	0	4	000
70	F	0	0	0	0		0	0	0	000
72	F	0	4	2	4		0	0	0	100
83	F	0	4	0	0		2	0	0	020
85	M	0	0	0	0		0	0	4	000
86	M	0	0	0	0		0	4	0	003
91	F	0	4	0	0		4	4	0	023
92	M	0	0	0	0		0	0	0	000
96	F	0	0	0	0		0	4	0	003
28101	M	0	0	0	0		0	2	0	000
10	M	0	4	3	2		0	4	0	103
11	M	0	4	4	3		0	0	0	100

Donor No	Sex	Blood Group	NO 1/8 2/67	NO 1/24 2/67	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
30053	M	0	4	0	0		4	4	0	023
59	M	0	4	1/2	4		0	0	0	100
75	F	0	0	0	0		0	4	0	003
77	M	0	4	4	4		0	4	0	103
79	M	0	0	0	0		0	4	0	003
81	F	0	4	4	4		0	0	0	100
89	M	0	4	4	3		0	0	0	100
93	M	A	4	4	4		0	0	4	100
94	M	A	0	0	0		0	4	0	003
96	M	A	4	4	4		4	0	0	120
99	M	A	0	0	0		0	4	0	003
30100	M	A	0	0	0		0	0	0	000
01	M	0	4	4	3		2	0	0	120
02	M	A	4	4			0	0	0	100
04	M	0	0	0			0	4	0	003
06	M	0	4	4			3	0	1	120
07	M	A	0	0			0	0	0	000
08	F	0	0	0			0	1	0	000
09	M	A	0	0			0	4	4	003
32137	M	0	0	0	0	0	0	4	0	003
39	M	0	4	4	3	0	0	4	0	103
42	F	0	0	0	0	0	0	0	0	000
46	M	0	4	4	3	0	0	0	0	100
53	F	0	4	4	4	4	4	0	0	120
56	M	0	4	4	3	0	0	4	0	103
59	F	0	4	4	4	2	4	0	0	120
63	M	0	4	4	3	0	0	2	0	100
64	M	0	4	4	4	0	0	0	0	100
66	F	0	4	4	4	0	0	1	0	100
82	M	0	0	0	0	0	0	0	0	000
89	M	0	0	0	0	0	0	2	0	000
91	F	0	4	2	3	0	0	0	0	100
32195	F	0	2/0	0	0	0	0	0	0	000
32204	M	0	4	4	3	0	0	0	0	100
08	F	0	4	4	4	0	0	0	0	100
21	F	0	4	4	3	0	0	0	0	100
31	M	0	4	2	3	0	0	0	0	100
35	M	0	4	3	4	0	0	0	0	100
36	F	0	4	0	0	2	3	4	4	023

Donor No	Sex	Blood Group	NO 1/8 2/67	NO 1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
33485	M	0	4	1	0	4	4	0	0	020
90	M	0	4	0	0	3	4	0	0	020
91	M	0	4	4	4	4	4	0	0	120
95	M	0	4	2	4	0	0	0	0	100
98	F	0	2	0	0	3	4	0	0	020
33503	F	0	0	0	0	0	0	4	0	003
06	M	0	4	0	0	4	4	4	0	023
07	M	0	4	2	4	1	0	0	0	100
14	M	0	0	0	0	0	0	4	0	003
15	F	0	4	3	3	0	0	4	0	103
22	M	0	4	0	0	4	4	0	0	020
34341	M	0	4	4	4		0	4	0	103
58	M	0	4	4	4		0	1	0	100
65	F	0	1	1	1		0	1	0	000
70	M	0	4	4	4		0	0	0	100
74	F	0	4	4	4		0	4	0	103
76	F	0	4	4	4		4	1	0	120
79	M	0	4	4	4		0	2	0	100
82	M	0	1	1	1		0	2	0	000
83	M	0	1	1	1		0	1	0	000
84	M	0	1	1	1		0	4	0	003
94	F	0	0	0	0		0	4	0	003
96	F	0	4	4	4		0	1	0	100
34402	M	0	4	4	4		0	4	0	103
04	M	0	2	2	2		0	4	0	103
07	F	0	4	4	4		0	1	0	100
08	F	0	1	1	1		0	4	0	003
14	M	0	4	4	4		0	1	0	100
16	M	0	4	4	4		0	1	0	100
18	F	0	1	1	1		0	4	0	003
19	M	0	4	4	4		0	3	0	100
36432	M	0	3	3	2		0	0	0	100
34	F	0	4	2	0	3	4	0	0	020
38	M	0	4	4	3		0	0	0	100
39	M	0	0	0	0		0	0	0	000
50	M	0	0	0	0		0	2	0	000
63	M	0	4	4	3		0	4	0	103
66	M	0	0	0	0		0	4	0	003
68	M	0	4	4	4		0	0	0	100
72	M	0	4	4	3	3	3	0	0	120
74	F	0	4	4	3		0	0	0	100
87	M	0	0	0	0	0	0	4	0	003

Donor No	Sex	Blood Group	NO		SZ	AD	CO	WE	SI	P1B typing
			1/8 2/67	1/24	1/6 2/67	1/2 6/67	1/24 3.66	1/8 3/67	1/24 6/66	
36491	F	0	0	0	0	0	0	4	0	003
92	M	0	4	4	4	0	0	1	0	100
98	M	0	4	4	3		0	0	1	100
36511	F	0	0	0	0		0	4	0	003
38527	M	0	0	0	0		0	0	0	000
28	M	0	4	0	0		4	0	0	020
29	M	0	4	4	3		0	0	0	100
31	M	0	4	4	3		0	0	0	100
43	F	0	4	4	3		2	0	4/4	120
54	M	0	4	4	3		0	4	0	103
55	M	0	4	4	4		4	0	0	120
56	M	0	0	0	0		0	0	0	000
60	M	0	0	0	0		0	4	0	003
65	M	0	4	4	2		0	0	0	100
67	F	0	4	0	0		4	0	0	020
72	M	0	0	0	0		0	4	0	003
76	F	0	0	0	0		0	4	0	003
80	M	0	0	0	0		0	0	0	000
95	M	0	0	0	0		0	0	0	000
38604	F	0	4	4	4		0	0	0	100
23	M	0	4	4	4		0	0	0	100
40694	M	0	0	0	0		1	4	0	003
719	M	0	4	4	3		1	0	0	100
25	M	0	0	0	0		1	4	0	003
26	F	0	4	4	3		1	0	0	100
29	F	0	0	0	0		1	0	0	000
34	M	0	0	0	0		0	0	0	000
37	M	0	0	0	0		0	4	0	003
39	F	0	0	0	0		0	0	0	000
44	F	0	0	0	0		0	0	0	000
48	M	0	0	0	0		0	0	0	000
49	F	0	4	4	3		0	0	0	100
50	F	0	3	4	3		0	4	0	103
57	M	0	0	0	0		1	3	0	003
63	M	0	0	0	0		0	4	0	003
67	M	0	4	4	2		0	0	0	100
69	F	0	4	4	2		0	4	0	103
70	F	0	0	0	0		0	0	0	000
801	M	0	4	4	3		4	0	0	120

Donor No	Sex	Blood Group	NO 1/8 2/67	NO 1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
96732	M	0	4		4			1	0	100
96	F	0	4		0			2	0	020
00688	M	0	0		0			4	0	003
081 63	M	0	0					4	1	003
69	M	0	0		0			4	1	003
98	F	0	4		0			2	0	020
11914	F	0	4	1	0	3	4	0	1	020
21	M	0	4	4	4	0		4	1	103
27	M	0	4	4	4	0		0	1	100
29	M	0	0	0	0	0		4	1	003
36	M	0	4	4	4	0		0	1	100
38	M	0	0	0	0	0		0	1	000
41	M	0	3	0	0	4	4	0	4	020
46	M	0	4	4	4	0	0	0	0	100
53	F	0	0	0	0	0	0	4	0	003
14063	M	0	4	4	4	0		4	0	103
20210	M	0	4	4	4	0	0	0	0	100
31	M	0	4	4	4	0	0	0	0	100
36	M	0	4	4	4	0	0	0	0	100
22117	M	0	0	0	0	0	0	0	0	000
21	F	0	0	0	0	0	0	4	0	003
31	F	0	0	0	0	0	0	0	4	000
53	M	0	0	0	0	0	0	4	0	003
23931	M	0	4	4	4		2	1	4	100
32	M	0	4	4	4		2	1	0	100
34	F	0	4	4	4		4	1	0	120
50	M	0	0	0	0		2	1	0	000
25879	M	0	3	3			0	3	1	103
28080	M	0	0	0	0		0	0	0	000
30054	F	0	4	2	3		0	0	0	100
34378	M	0	1	1	1		0	4	0	003
80	M	0	4	4	4		0	0	4	100
36433	M	0	4	1	0	3	4	4	1	023
45	M	0	0	0	0		0	1	4	000
46	F	0	4	4	4		4	1	0	120
79	F	0	0	0	0	0	0	0	0	000
96	M	0	4	4	3	0	0	1	1	100
38533	M	0	4	3	2		0	0	4	100
46	F	0	0	0	0		0	0	0	000
74	M	0	4	0	0		3	4	0	023
40692	M	0	4	0	0		4	0	0	020

Donor No	Sex	Blood Group	NO 1/8 2/67	1/24	SZ 1/6 2/67	AD 1/2 6/67	CO 1/24 3/66	WE 1/8 3/67	SI 1/24 6/66	P1B typing
BA	M		4		4					10-
HO	M		4		4					10-
MA	M		1		1					00-
RO	M		4		4					12-
GO	M		1		1			0		00-
PS	F		0		0					^0-
MA	F		1		0			4		003
BY	M		4		3					10-
SH	M		1		1					00-
HA	M		4		3			4	0	023
WA	M		4	4	4	2		0	0	120
SU	M		0	0	0		0	0	4	000
BO	M		0	0	0		0	0	0	000
GA	M		0	0			0	4	0	003
MA	M		4		4			4	0	103
SM	M		4		4			0	0	100
MA	M		0		0			4	4	003
LE	F		0		0			4	0	003
00666	M	0	0		0			0	0	000
00673	M	0	4		4			0	0	100
09974	M	0	4		3			0	0	100
10023	F	0	0		0			4	0	003

REACTIONS OF ELUATES FROM NO WITH

PLATELETS FROM VARIOUS BLOOD DONORS

NO 8/2/67 eluted with

Donor No	94915 (120)	96762 (100)	96732 (100)	96737 (100)	96767 (100)	96831 (020)	96796 (020)	96725 (020)	98726 (020)	P1B Grouping
94911			1				1			000
15	4	4	4			4	4			120
19			4				1			103
22			4				1			103
24			4				1			103
25			4				1			100
26			1				4			023
28			4				1			100
32			1				1			003
33	4	0	1		0	4	4	3	4	023
35			1				1			000
37			4				1			100
39			1				1			000
40			4				1			103
50	4	0	1		0	4	4		4	020
60			4				1			100
BA			4				1			10-
HO			4				1			10-
MA			1				1			00-
RO	4	4	4	4		2	2	3		12-
GO				1	1			1	0	00-
FS				0				0		00-
MA				1				1		00-
HY				4				1	1	10-
SH				1	0			1	0	00-
HA					0				4	023
MA					4				0	103
SM					4				0	100
MA					0				0	003
LE					0				0	003
96725	4	1	0			4	3			020
32	4	4	4			0	0			100
33	0	0	0			0	0			003
37			3				0			100
42	4	4	4			0	0			100
46			4				0			103

Donor No	94915 (120)	96762 (100)	96732 (100)	96737 (100)	96767 (100)	96831 (020)	96796 (020)	96725 (020)	98726 (020)	P1B Grouping
93238	0	0				0				003
40	4	3			4	0			0	100
41	0	0				0				003
42	4	4			4	0			0	103
44	0	0				0				000
45	0	0				0				003
47	0	0				0				003
50	4	0			0	4			4	023
53	3	0				4				023
54	4	4			4	0			0	100
55	0	0				0				000
57	4	4		4	4	4		4	4	120
58	4	4				1				100
59	0	0				0				00-
66	4	4				0				10-
71	4	0				4				02-
78	4	0				4				02-
81	4	2				0				10-
84	4	4				0				10-
46	4	3				0				100
49	0	0				0				00-

Donor No	94915 (120)	96762 (100)	96732 (100)	96737 (100)	96767 (100)	96831 (020)	96796 (020)	96725 (020)	98726 (020)	PIB Grouping
96752			4				0			100
53			4				0			103
57	4	4	4		4	4	4		0	120
58	4	4	4		4	1	1		0	103
59			4				1			100
62	4	4	4			0	1			100
67			4				1			100
74			0				4			023
75	0	0				0				003
96	3	0	0		0	4	4		1	020
831	3	0	0			4	4			023
98726		0		0	0	4		4	4	02-
29				4	4		2	4	2	12-
30				4	4		0	1	1	10-
31				2	2			1	1	10-
32		4		4	4	0		0	0	10-
.4				1				0		00-
35				4	4			4	0	12-
36				4	4			3	0	12-
39				0				0		00-
41				1				0		00-
45		4		4	4	0		0	0	10-
46		4		4	4	0		0	0	10-
48		4		4	4	0		1	0	10-
49				0				0		00-
54				0				0		00-
56		0		4	3	4		4	4	12-
58				4	4			2	:	10-
61				1				1		00-
66				4	4			2	1	10-
68				0				0		00-
70				2	2			2	1	10-
00643					0				4	020
56					4				0	100
62					0				0	000
66					0				0	000
67					4				0	103
68					4				0	100
70					4				0	100
73					4				0	100
74					0				4	020
75					0				0	003
78					4				0	100

Donor No	96767 (100)	8149 (020)	8157 (020)	96725 (020)	98726 (020)	P1B Grouping
00679	4				0	100
86	4				0	100
88	0				0	003
90	0				0	003
91	0				0	000
95	4				0	100
96	0				2	020
97	4				0	100
98	4				0	100
04319		0	1			100
20		0	1			100
42		1	1			100
45		3	3			020
54		3	3			120
57		0	0			100
64		0	1			100
65		0	0			003
68		0	1			100
72		0	1			100
74		0	1			100
93		0	1			100
06188		2	3	3		120
98		3	3			020
207		3	3	3		120
08		0	1			100
09		0	1			100
22		1	1			100
27		3	3			023
33		3	3			020
38		1	1			100
43		0	0			100
54		0	0			100
59		3	3			120
62		3	2			023
69		3	3			120
08149		4	0			020

Donor No		8149 (020)	8157 (020)	96725 (020)	96796 (020)	P1B Grouping
08151		4	4		3	120
54		0	0		0	100
56		0	0			103
57		4	4			020
58		0	0		0	100
67		0	0	3		100
70		0	0	2		100
71		0	0	2		103
82		4	2			023
83		0	0			103
96		3	1			020
98		3	1			020
99		4	0			020
09974		1	2			100
77		1	2			103
78		1	1			100
83		2	2			100
86		4	4			023
87		2	2			100
90		1	2			100
91		2	1			100
95		2	1			100
10016		4	2			020
29		2	2			100

Platelets in series from 09974 gave 1+ and 2+ reactions in controls.

Donor No	SZ with 16123 (100)	NO with 16128 (100)	NO with 16152 (020)	P1B Grouping
14029	0	0	4	023
31	0	0	0	000
35	0	0	0	000
42	0	0	4	023
48	4	4	0	100
51	0	0	4	023
58	4	4	0	100
59	4	4	0	100
16123	2			100
28		4		100
52			4	020
18151	3	4	0	100
60	4	4	0	103
66	0	0	4	020
69	0	0	0	000
82	4	4	0	103
90	3	4	1	100
91	4	4	4	120
94	3	4	0	100
96	3	4	0	100
18202	4	4	0	100
04	3	4	2	12₁
17	0	0	4	020
20	0	0	4	020
31	1	4	0	100
53	2	4	0	100

ABO RED CELL GROUPS AND PlB PLATELET

GROUPS OF 203 HOSPITAL OUT-PATIENTS

No	ABO Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	DE 1/24	PlB Grouping
H1	A	4	0	0	4	0	0		020
2	A	0	0	0	0	0	4		000
3	O	0	0	0	0	0	0		000
4	B	0	0	0	0	0	0		000
5	O	0	0	0	0	0	0		000
6	A	4	4	4	0	0	0		100
7	A	4	4	4	1	4	0		103
8	A	4	1	1	4	0	0		020
9	A	4	4	4	4	0	0		120
10	A	1	1	1	1	4	0		003
11	A	4	4	4	4	0	0		120
12	O	4	4	4	0	4	4		103
13	A	1	1	1	1	4	0		003
14	B	4	4	4	4	0	0		120
16	A	0	0	0	0	4	0		003
17	O	4	4	2	0	0	0		100
18	A	4	4	4	1	4	0		103
19	B	4	4	4	1	0	0		100
20	AB	4	4	4	1	0	0		100
21	B	4	4	4	1	0	0		100
22	O	0	0	0	1	0	0		000
23	A	4	0	1	4	0	0		020
24	O	0	0	0	0	0	0	0	000
25	A	4	4	2	0	3	0	4	103
26	A	0	0	0	0	0	0	0	000
27	B	4	4	2	0	0	4	4	100
28	O	0	0	0	0	0	0	0	000
29	A	4	4	2	0	4	0	4	103
30	O	0	0	0	0	4	0	0	003
31	O	0	0	0	0	0	0	0	000
32	A	0	0	0	0	4	0	0	003
33	O	4	4	2	4	0	4	4	120
34	O	4	4	3	0	0	0	4	100
35	O	4	4	2	0	4	4	4	103
36	O	0	0	0	0	4	0	0	003
37	A	0	0	0	0	0	4	0	000
38	A	4	4	2	0	0	0	4	100

No	ABO Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	DE 1/24	P1B Grouping
39	0	4	4	3	0	0	2	4	100
40	A	0	0	0	0	0	0	0	000
41	0	4	4	1	0	0	0	4	100
42	0	0	0	0	0	4	4	0	003
43	A	0	0	1	0	4	0	0	003
44	A	3	1	0	4	0	0	0	020
45	0	4	4	3	0	0	0	4	100
46	A	0	0	0	0	4	0	0	003
47	0	0	0	0	0	0	0	0	000
48	A	4	0	3*	3	4	4	0	023
49	0	0	0	0	0	4	0	0	003
50	0	4	0	0	4	0	4	0	020
51	0	0	0	0	0	4	0	0	003
52	B	1	0	0	0	0	0	0	000
53	A	4	4	3	0	0	0	4	100
54	0	0	0	0	0	4	0	0	003
55	0	4	4	3	4	0	4	0	120
56	A	4	4	3	0	4	0	4	103
57	A	4	0	0	4	0	0	0	020
58	A	0	0	0	0	0	0	0	000
59	A	0	0	0	0	0	0	0	000
60	0	0	0	0	0	4	4	0	003
61	B	0	0	0	0	0	0	0	000
62	B	0	0	0	0	4	0	0	003
63	0	4	4	3	0	0	0	4	100
64	0	4	1	0	4	3	4	0	023
65	0	4	4	3	3	0	0	4	120
66	0	4	4	3	0	0	0	4	100
67	B	0	0	0	0	4	0	0	003
68	A	4	4	3	0	0	3	4	100
69	A	4	0	0	4	0	0	0	020
70	A	0	0	0	0	4	0	0	003
71	0	0	0	0	0	4	0	0	003
72	A	0	0	1	0	4	0	0	003
73	0	4	3	2	0	0	0	4	100
74	A	4	4	3	0	0	0	4	100
75	A	0	0	0	0	0	0	0	000
76	A	0	0	0	0	0	0	0	000
77	A	4	4	3	3	0	0	4	120
78	0	4	4	3	0	0	0	4	100
79	0	0	0	0	0	4	0	0	003
80	A	4	4	3	0	4	0	4	103
81	A	4	1	3*	4	0	0	0	020
82	0	4	4	3	0	0	0	4	100

No	ABO Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	DE 1/24	P1B Grouping
83	A	4	4	4	4	0	4	4	120
84	A	4	4	3	0	0	0	4	100
85	0	4	4	0**	0	0	0	4	100
86	A	0	0	0	0	4	0	0	003
87	A	0	0	2*	0	1	0	0	000
88	B	0	0	0	0	4	0	0	003
89	0	0	0	0	0	4	0	0	003
90	0	0	0	0	0	4	0	0	003
91	A	4	4	3	0	4	0	4	103
92	0	4	4	3	0	4	0	4	103
93	0	4	4	3	0	0	0	4	100
94	A	0	0	0	0	0	0	0	000
95	A	0	0	0	0	4	4	0	003
96	0	0	0	0	0	0	0		000
97	0	4	4	3	1	0	0	2	100
98	B	4	4	3	1	0	0	4	100
99	A	4	4	4	1	0	0	4	100
100	A	0	0	1	1	4	0	0	003
101	A	4	4	4	0	4	0	4	103
102	0	4	4	4	0	0	4	4	100
103	0	0	0	0	0	4	0	0	003
104	0	0	0	1	0	4	0	0	003
105	A	4	4	4	0	0	0	4	100
106	AB	4	4	4	0	4	0	4	103
107	A	0	0	1	0	4	0	0	003
108	0	4	0	0	4	4	0	0	023
109	A	0	0	1	0	0	0	0	000
110	0	4	4	3	0	0	4	4	100
111	0	4	1	2	0	0	0	2	100
112	A	0	0	1	0	4	0	0	003
113	0	4	0	0	4	0	0	0	020
114	0	4	4	2	1	1	0	4	100
115	B	4	4	4	4	0	0	4	120
116	B	0	0	0	0	0	0	0	000
117	B	4	1	2	0	0	0	4	100
118	A	4	4	4	0	0	0	4	100
119	0	4	4	4	0	0	4	4	100
120	A	4	1	3	0	0	0	4	100
121	0	0	0	0	0	4	0	0	003
122	0	0	0	0	0	0	4	0	000
124	0	4	2	4	0	0	0	3	100
125	A	4	4	4	0	0	0	4	100
126	A	4	4	3	0	0	0	4	100
127	0	4	4	4	0	0	0	4	100
128	A	4	4	3	0	0	0	4	100

No	ABO Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	DE 1/24	P1B Grouping
129	AB	4	4	4	0	0	0	4	100
130	O	4	4	4	0	0	4	4	100
131	A	4	4	3	1	4	0	4	103
132	O	4	4	2	1	0	0	3	100
133	O	4	4	4	4	0	0	4	120
134	O	0	0		0	0	0	0	000
135	O	4	4		0	0	0	4	100
136	A	4	4		0	0	0	4	100
137	A	0	0		0	4	0	0	003
138	A	0	0		0	0	0	0	000
139	A	0	0		0	4	2	0	003
140	O	4	2	0	3	0	0	0	020
141	O	4	2	0	3	0	0	0	020
142	O	4	4	2	4	0	4	4	120
143	A	0	0	2	0	0	0	0	000
144	O	4	4	2	0	0	0	4	100
145	O	0	0	0	0	0	4	1	000
146	O	0	0	0	0	4	0	1	003
147	B	0	0	0	0	0	0	1	000
148	A	4	4	3	0	0	0	4	100
149	O	4	4	3	0	0	0	4	100
150	A	0	0	0	0	0	0	1	000
151	O	0	0	0	0	4	0	0	003
152	O	0	0	0	0	4	0	0	003
153	O	4	4	3	0	4	0	4	103
154	A	0	0	1	0	0	0	0	000
155	O	0	0	0	0	0	4	0	000
156	A	4	4	3	1	4	0	4	103
157	O	4	4	4	0	0	4	4	100
158	A	4	3	3	3	0	0	0**	120
159	O	0	0	0	0	0	0	0	000
160	O	4	4	2	0	0	0	3	100
161	O	4	4	3	0	0	4	4	100
162	O	0	0	0	0	0	0	0	000
163	A	4	4	4	4	0	0	4	120
164	O	4	4	4	0	0	0	4	100
165	O	4	4	3	0	0	0	4	100
166	O	4	4	2	4	0	0	3	120
167	O	0	0	0	0	4	0	0	003
169	O	0	0	2	0	4	4	0	003
170	O	4	4	0	0	0	4	4	100
171	O	4	0	0	4	0	0	0	020

No	ABO Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	DE 1/24	P1B Grouping
172	A	0	0	0	0	4	4	0	003
173	A	2	0	0	4	0	0	0	020
174	0	4	0	0	4	0	0	0	020
175	0	0	0	0	0	0	4	0	000
176	A	4	1	4	0	0	0	4	100
177	0	0	0	0	0	0	0	0	000
178	0	0	0	0	0	0	0	0	000
179	AB	0	0	0	0	4	4	0	003
180	A	0	0	0	0	4	0	0	003
181	0	4	4	3	0	0	0	4	100
182	0	4	2	3	0	0	0	0**	100
183	0	4	3	3	0	4	0	4	100
184	A	0	0	0	0	4	0	0	003
185	A	4	4	4	0	0	0	4	100
186	0	4	0	0	4	0	4	0	020
187	0	0	0	0	0	0	0	0	000
188	B	0	0	0	0	0	4	0	000
189	A	4	2	2	0	0	0	4	100'
190	0	4	0	0	4	0	0	0	020
191	A	0	0	1	0	0	0	1	000
192	0	4	4	4	0	0	0	4	100
193	AB	4	4	3	0	4	0	4	103
194	0	4	2	3	0	0	0	4	100
195	B	4	4	4	0	4	0	4	103
196	A	4	4	2	0	4	0	4	103
197	AB	4	4	4	4	0	0	4	120
198	0	4	4	2	4	0	4	4	120
199	A	4	0	1	4	4	0	0	023
200	0	0	0	0	0	0	0	0	000
201	AB	0	0	0	0	0	0	0	000
202	AB	4	4	3	4	0	0	4	120
203	0	4	4	2	0	4	0	4	103
204	0	4	4	3	4	0	0	4	120
205	0	0	0	0	0	0	0	0	000
206	A	0	0	0	0	0	0	0	000

Numbers 15, 123, 168 have been omitted due to insufficient platelet specimens available.

* Unexpected positive, probably due to contamination with red cell antigen.

** Unexpected negative, insufficient specimen for further test.

REPEATED PLATELET SPECIMENS

Donor numbers on the same line originated from the same individual.

Donor No	Donor No	Donor No
20236	8170	
36433	14042	
20231	96742	
8163	96727	
28080	14049	
23931	11919	
22113	98741	
34378	22153	10022
34380	10029	
11921	667	
11941	696	
20210	8158	
9993	22117	
36446	23934	11945
22131	10004	
11953	675	
36479	23964	
30054	16086	
11914	643	

Donor No	Donor No	Donor No
8198	96796	
22121	10023	
11927	96732	
9974	98730	
9995	98746	
10007	98754	
36496	11936	
25879	14036	
20230	8169	
36445	11928	
11929	688	
666	11938	
23932	11946	673
23950	691	
25937	38533	
96770	38546	
25915	38574	
8149	40692	

PLATELET AND RED CELL REACTIONS OF BLOOD

from

67 AUSTRALIAN ABORIGINES

| No | Sex | Tribe | ABO Group | Reaction with | | | | Platelet Control | PlB Antigen Grouping |
				CL 7/66 1/8	CL 7/66 1/40	NO 2/67 1/8	SZ 2/67 1/5		
Y66	M	W	0	4	4	4	4	0	1-0
Y67	M	W	A	4	4	4	0	0	020
Y69	M	W	0	4	1	0	0	0	000
Y72	M	W	A	4	4	4	0	0	020
Y73	M	W	A	4	0	0	0	0	000
Y74	M	F	A	4	0	4	1	0	020
Y75	M	W	0	4	4	4	4	0	1-0
Y76	M	W	0	4	1	0	0	0	000
Y81	M	W	0	2	0	0	0	0	000
Y82	F	W	0	4	0	4	0	0	020
Y83	F	W	0	4	4	4	0	0	020
Y84	F	W	A	4	0	0	0	0	000
Y85	F	W	0	4	3	4	0	0	020
Y86	F	W	A	4	0	0	0	0	000
Y87	F	W	A	4	2	4	1	0	020
Y89	F	W	A	4	3	4	0	0	020
Y93	F	P	0	4	0	0	0	0	000
Y94	F	P	A	4	1	4	1	0	020
Y95	F	W	A	4	0	0	1	0	000

No	Sex	Tribe	ABO Group	Reaction with				Platelet Control	P1B Antigen Grouping
				CL 7/66 1/8	CL 7/66 1/40	NO 2/67 1/8	SZ 2/67 1/5		
Y96	F	W	A	4	1	0	2	0	000
Y97	F	W	O	4	0	4	0	0	020
Y98	F	P	O	4	4	4	4	0	1-0
Y99	F	W	O	4	4	4	0	0	020
Y90	F	W	O	4	0	3	0	0	020
Y91	F	W	A	4	0	1	0	0	000
Y92	F	W	A	4	0	0	0	0	000
Y101	M	W	O	4	0	4	0	0	020
Y102	M	W	A	4	4	4	4	0	1-0
Y103	M	W	A	4	0	1	1	0	000
Y104	M	W	A	4	4	4	4	0	1-0
Y105	M	W	A	4	3	4	0	0	020
Y106	M	W	O	4	4	4	4	0	1-0
Y107	M	W	A	4	2	4	1	0	020
Y108	M	W	O	4	0	3	0	0	020
Y109	M	W	A	4	1	4	0	0	020
Y110	M	W	O	4	3	4	4	0	1-0
Y111	M	W	A	4	2	4	0	0	020
Y113	M	W	O	4	2	4	4	0	1-0
Y115	M	W	A	4	3	3	0	0	020
Y116	F	W	O	4	0	4	4	0	1-0

| No | Sex | Tribe | ABO Group | reaction with | | | | Platelet Control | P1B Antigen Grouping |
				CL 7/66 1/8	CL 7/66 1/40	NO 2/67 1/8	SZ 2/67 1/5		
Y117	M	W	0	2	0	0	0	0	000
Y118	F	W	0	4	0	2	0	0	020
Y119	F	W	0	4	4	4	0	1	020
Y120	F	P	A	4	3	4	4	0	1-0
Y121	F	W	A	4	0	0	2	0	000
Y320	F	W	0	3	1	0	0	0	000
Y322	F	W	0	4	4	4	4	1	1-0
Y323	F	W	A	4	4	4	2	1	1-0
Y324	F	W	A	4	3	2	2	1	1-0
Y332	F	W	A	4	4	4	1	1	020
Y336	F	P	A	4	2	4	1	0	020
Y339	M	P	A	4	4	4	2	1	1-0
Y340	M	P	A	4	4	4	4	0	1-0
Y341	M	W	0	4	2	3	0	0	020
Y342	M	W	0	4	0	0	0	0	000
Y343	M	W	A	4	4	4	4	0	1-0
Y344	M	W	A	4	1	0	2	0	000
Y346	M	W	0	4	4	4	0	0	020
Y347	M	W	A	4	0	0	1	1	000
Y348	M	W	0	4	4	4	4	0	1-0

| No | Sex | Tribe | ABC Group | Reaction with | | | | Platelet Control | PlB Antigen Grouping |
				CL 7/66 1/8	CL 7/66 1/40	NO 2/67 1/8	SZ 2/67 1/5		
Y349	M	P	O	4	4	4	4	0	1-0
Y122	F	P	O	0	0	0	0	0	000
Y123	F	W	O	4	4	4	4	0	1-0
Y124	F	P	A	4	1	4	4	0	1-0
Y125	F	W	O	4	4	4	4	0	1-0
Y126	F	P	A	4	0	1	0	0	000
Y127	F	W	A	4	0	1	1	0	000
Serum Controls				0	0	0	0		
				0	0	0	0		

All specimens were tested with WE 21/3/67 at 1/8 and were negative.
A European control specimen gave a 4+ reaction.

W = Wailbri

P = Pintubi

PLATELET AND RED CELL REACTIONS OF BLOOD
FROM 128 NEW ZEALAND MAORIS

No	Sex	Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	P1R Antigen
1	M	O+	4	4	4	0	1	1	100
2	M	O+	4	4	4	4	1	1	120
3	F	A+	4	4	4	4	1	1	120
4	M	O+	4	1	1	4	1	1	020
5	M	A+	4	4	4	1	1	1	100
6	M	O+	4	1	1	4	1	1	020
7	M	A+	4	4	4	1	1	1	100
8	M	O+	4	4	4	1	1	1	100
9		A+	4	1	1	4	1	1	020
10		O+	4	4	4	4	1	1	120
11		A+	4	1	0	4	1	1	020
12		A+	4	1	1	4	0	1	020
13		A+	4	1	1	4	4	1	023
14		O+	4	4	1	2	1	1	020
15		A+	4	1	1	4	1	1	020
16		O+	4	4	4	4	1	1	120
17		A+	4	4	4	1	1	1	100
18		A+	4	1	1	4	1	1	020
19		O+	0	0	1	1	4	1	003
20		A+	4	4	4	1	1	1	100
21		O+	4	4	4	1	1	1	100
22	M	A+	4	4	3	1	2	1	103
23	M	O+	4	4	4	1	1	1	100
24	M	O+	4	4	4	1	1	1	100
25	M	A+	4	4	3	1	1	1	100
26	M	A+	3	1	0	3	0	0	020
27	M	A+	4	1	0	4	0	0	020
28	M	A+	4	1	0	4	0	0	020
29	M	O+	4	4	3	4	0	0	120
30	M	A+	4	4	4	0	0	0	100
31	M	A+	4	4	4	4	0	0	120
32	M	A+	4	4	4	4	0	0	120
33	M	O+	4	1	0	3	0	0	020
41	M	O+	4	4	2	0	0	0	100
42	M	A+	0	0	0	0	0	0	000
43	M	O+	4	4	3	0	0	0	100
44	M	A+	4	4	4	0	0	0	100
45	M	A+	4	4	3	4	0	0	120
46	M	O+	4	0	0	4	0	0	020
47	M	A+	4	4	4	4	0	0	120
48	M	O+	4	2	0	4	0	0	020

No	Sex	Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	P1B Antigen
49	M	A+	4	4	3	0	0	0	100
50	M	A+	4	2	1	3	0	0	020
51	M	O+	4	4	4	4	0	0	120
52	M	A+	0	0	0	0	4	0	003
53	M		0	0	0	0	4	0	003
54	M		4	4	3	0	0	0	100
55	M		4	1	0	4	4	0	023
56	M		4	2	0	3	0	0	020
57	M		4	4	1	3	0	0	020
58	M		4	2	0	4	0	0	020
59	M		4	4	3	0	0	0	100
61	M	O+	4	4	3	0	0	0	100
62	M	O+	4	2	0	4	0	0	020
63	M	A+	0	0	0	0	4	0	003
64	M	A+	1	1	3*	1	1	0	000
65	M	A+	4	2	1	4	1	0	020
66	M	A+	4	4	3	0	4	0	103
67	M	A+	0	0	1	1	1	0	000
68	M	O+	0	0	0	0	0	0	000
69	M	O—	1	1	1	1	1	0	000
70	M	O+	4	2	0	4	1	0	020
72	M	O+	4	2	0	4	1	0	020
73	M		4	4	3	4	0	0	120
74	M		4	4	3	4	0	0	120
75	M	O+	4	2	0	4	0	0	020
76	M	A+	4	2	0	4	4	0	023
77	M	A+	4	4	4	0	0	0	100
78	M	B+	4	2	0	3	0	0	020
79	M	O+	4	4	4	0	0	0	100
101	M	O+	0	0	0	0	0	0	000
102	M		4	4	3	0	0	0	100
103	M		4	4	4	1	1	0	100
104	M		4	4	4	1	0	0	100
105	M	A+	4	4	4	1	1	0	100
106	M	A+	0	0	0	0	0	0	000
107	M	A+	4	4	4	0	0	0	100
108	M	A+	4	0	0	4	0	0	020
109	M	O+	4	2	0	4	4	0	023
110	M	A+	4	4	4	0	1	0	100
111	M	O+	4	2	0	4	1	0	020
112	M	O+	4	4	4	0	0	0	100
113	M	A+	4	4	4	4	0	0	120
114	M	A+	4	4	4	0	0	4	100

No	Sex	Group	NO 1/8	NO 1/24	SZ 1/3	CO 1/24	WE 1/128	SI 1/24	P1B Antigen
115	M	0+	4	4	2	4	0	0	120
116	M	0+	4	4	3	4	0	0	120
117	M		4	4	4	4	1	0	120
121	F	0+	4	4	3	4	0	0	120
122	F	A+	4	2	0	4	1	0	020
123	F	AB+	4	4	4	4	1	0	120
124	F	A+	4	4	4	4	1	0	120
125	F	A+	4	2	0	4	1	0	020
126	F	A+	4	2	0	4	1	0	020
127	F	0+	0	0	0	0	4	0	003
128	F	A+	1	1	1	1	1	1	000
129	F	A+	4	1	1	4	1	1	020
130	F	A+	1	1	1	1	4	4	003
131	F	0+	4	2	1	2	1	1	020
132	F	0+	4	2	0	4	1	1	020
133	F	0+	4	4	3	1	1	1	100
134	M	A+	4	1	1	3	1	1	020
135	F	A+	4	1	1	4	1	1	020
136	F	A+	4	4	3	0	1	1	100
137	F	B+	4	1	1	4	0	0	020
138	F	A+	4	4	4	4	1	1	120
139	F	A+	4	4	3	0	1	1	100
140	F	A+	4	4	4	4	1	1	120
141	F	A+	4	4	4	3	1	1	120
142	F	A+	4	1	1	4	1	1	020
143	F	0+	0	0	0	0	0	0	000
144	F	0+	0	0	0	⊛	4	0	003
145	F	A+	4	0	0	3	0	0	020
146	F	A+	4	4	4	0	0	0	100
147	F	A+	4	4	4	0	0	0	100
148	F	A+	4	0	0	4	0	0	020
149	F	A+	3	2	2	2	0	0	120
150	M	0+	4	4	3	1	0	0	100
151	M	A+	0	0	0	0	0	0	000
152	M	0+	4	4	3	1	0	0	100
153	M	A+	4	4	4	4	0	0	120
154	M	0+	4	4	3	0	4	0	103
156	M	0+	4	4	4	4	0	0	120
157	F	0+	4	1	3	0	0	0	100
159	F	0+	4	2	0	4	0	0	020
160	F	A+	4	4	4	4	0	0	120
161	F	0+	4	4	3	4	0	0	120
162	F	A+	4	2	0	3	1	1	020
164	F	A+	4	2	0	3	0	1	020

* Unexpected positive, probably due to contamination with red cell antigen.

FAMILY STUDIES - RED CELL GROUPS

Donor	Sex	Anti A	B	Ulex	Dolichos	Anti M	N	C	c	D	E	Presumed Genotype	P1B Antigens
Family 1.													
Father	M	−	−	+	−	+	+	−	+	−	−	0 MN cde/cde	100
Mother	F	−	−	+	−	+	+	−	+	−	−	0 MN cde/cde	020
Child 1	M	−	−	+	−	−	+	−	+	−	−	0 NN cde/cde	120
Family 2.													
Father	M	+	−	−	+	+	−	−	+	−	−	A MM cde/cde	103
Mother	F	−	+	−	−	+	+	+	+	+	−	B MN CDe/cde	100
Child 1	M	+	+	−	+	+	+	+	+	+	−	AB MN CDe/cde	100
Child 2	M	+	+	−	+	+	−	+	+	+	−	AB MM CDe/cde	103
Child 3	M	+	+	−	+	+	−	−	+	−	−	AB MM cde/cde	100
Family 3.													
Father	M	+	−	−	+	+	+	+	+	+	−	A MN CDe/cde	103
Mother	F	−	−	+	−	+	−	−	+	+	+	0 MM cDE/cde	023
Child 1	M	+	−	−	+	+	−	−	+	−	−	A MM cde/cde	003
Child 2	M	+	−	+	−	+	−	+	+	+	−	A MM CDe/cde	120
Family 4.													
Father	M	+	−	−	+	+	−	+	+	+	+	A MM CDe/cDE	003
Mother	F	−	+	+	−	+	+	+	−	+	−	B MN CDe/CDe	003
Child 1	M	−	−	+	−	+	+	+	−	+	−	0 MN CDe/CDe	003
Child 2	F	+	+	−	+	+	−	+	−	+	−	AB MM CDe/cDe	003
Family 5.													
Father	M	+	−	+	+	+	+	+	+	+	−	A MN CDe/cde	100
Mother	F	−	−	+	−	+	−	−	+	+	+	0 MM cDE/cde	100
Child 1	F	+	−	+	−	+	+	+	+	+	−	A MN CDe/cde	100
Child 2	M	+	−	+	−	+	−	+	+	+	−	A MM CDe/cde	100
Child 3	M	+	−	+	−	+	−	+	+	+	−	A MN CDe/cde	100
Child 4	F	−	−	+	−	+	−	+	+	+	+	0 MM CDe/cDE	100

Donor	Sex	Anti A	Anti B	Ulex	Dolichos	M	N	C	c	D	E	Presumed Genotype	P1B Antigens
Family 6.													
Father	M	+	−	−	+	+	+	+	+	+	+	A MN CDe/cDE	000
Mother	F	+	−	−	−	+	−	−	+	−	−	A MM cde/cde	100
Child 1	F	+	−	−	−	+	+	−	+	+	+	A MN cDE/cde	100
Child 2	M	+	−	−	−	+	+	+	+	+	−	A MN CDe/cde	000
Child 3	F	+	−	−	+	+	−	−	+	+	+	A MM cDE/cde	000
Family 7.													
Father	M	+	−	−	+	+	−	+	+	+	−	A MM CDe/cde	120
Mother	F	+	+	−	−	+	−	−	+	−	−	AB MM cde/cde	100
Child 1	F	−	+	−	−	+	−	+	+	+	−	B MM CDe/cde	100
Family 8.													
Father	M	−	+	−	−	+	+	−	+	−	−	B MN cde/cde	100
Mother	F	−	−	+	−	+	+	+	+	+	−	O MN CDe/cde	000
Child 1	F	−	+	−	−	+	+	+	+	+	−	B MN CDe/cde	000
Child 2	M	−	−	+	−	+	+	+	+	+	−	O MN CDe/cde	000
Child 3	M	−	−	+	−	+	+	+	+	+	−	O MN CDe/cde	000
Child 4	M	−	+	−	−	+	+	−	+	−	−	B MN cde/cde	100
Child 5	F	−	+	−	−	+	+	+	+	+	−	B MN CDe/cde	000
Family 9.													
Father	M	+	−	−	+	−	+	−	+	−	−	A NN cde/cde	100
Mother	F	+	−	−	+	+	−	+	+	+	−	A MM CDe/cde	000
Child 1	M	+	−	−	+	+	+	+	+	+	−	A MN CDe/cde	100
Child 2	M	+	−	−	+	+	+	+	+	+	−	A MN CDe/cde	000
Child 3	M	+	−	−	+	+	+	+	+	+	−	A MN CDe/cde	000
Family 10.													
Father	M	−	−	+		+	−	+	+	+	−	O MM CDe/cde	000
Mother	F	−	+			+	+	+	−	+	−	B MN CDe/CDe	100
Child 1	M	−	+			+	−	+	−	+	−	B MM CDe/CDe	100
Child 2	M	−	−	+		+	+	+	+	+	−	O MN CDe/cde	100
Child 3	M	−	+			+	−	+	+	+	−	B MM CDe/cde	100
Child 4	F	−	+			+	−	+	−	+	−	B MM CDe/CDe	100
Family 11.													
Father	M	+	−	−	+	+	+	+	+	+	−	A MN Cde/cDe	
Mother	F	−	−	+	−	+	+	+	+	+	−	O MN CDe/cde	003
Child 1	F	−	−	+	−	+	−	+	+	−	−	O MM Cde/cde	000
Child 2	M	−	−	+	−	+	+	+	+	+	−	O MN CDe/cDe	003
Child 3	M	+	−	−	+	+	−	+	+	+	−	A MM CDe/cDe	003
Child 4	M	−	−	+	−	+	+	+	+	+	−	O MN CDe/cDe	003

Donor	Sex	Anti A	B	Ulex	Dolichos	Anti M	N	C	c	D	E	Presumed Genotype	P1B Antigens
Family 12.													
Father	M	−	−	+	−	−	+	+	−	+	−	0 NN CDe/CDe	100
Mother	F	−	−	+	−	+	+	+	+	+	−	0 MN CDe/cde	000
Child 1	F	−	−	+	−	−	+	+	−	+	−	0 NN CDe/CDe	100
Child 2	M	−	−	+	−	−	+	+	−	+	−	0 NN CDe/CDe	100
Child 3	M	−	−	+	−	−	+	+	−	+	−	0 NN CDe/CDe	100
Child 4	M	−	−	+	−	−	+	+	−	+	−	0 NN CDe/CDe	100
Family 14.													
Father	M	+	−	−	+	+	+	+	−	+	−	A MN CDe/CDe	023
Mother	F	+	−	−	+	−	+	+	−	+	−	A NN CDe/CDe	000
Child 1	F	+	−	−	+	+	+	+	−	+	−	A MN CDe/CDe	003
Child 2	F	+	−	−	+	+	+	+	−	+	−	A MN CDe/CDe	003
Child 3	F	+	−	−	+	+	+	+	−	+	−	A MN CDe/CDe	020
Family 15.													
Father	M	−	−	+	−	+	+	+	+	+	−	0 MN CDe/CDe	120
Mother	F	+	−	−	+	−	+	−	+	−	−	A NN cde/cde	003
Child 1	M	+	−	−	+	+	+	+	+	+	−	A MN CDe/cde	020
Child 2	F	+	−	−	+	+	+	+	+	+	−	A MN CDe/cde	103
Family 16.													
Father	M	−	−	+	−	+	−	+	+	+	−	0 MM CDe/cde	120
Mother	F	+	−	−	+	−	+	−	+	−	−	A NN cde/cde	003
Child 1	M	−	−	+	−	+	+	+	+	+	−	0 MN CDe/cde	023
Child 2	F	−	−	+	−	+	+	−	+	−	−	0 MN cde/cde	100
Child 3	M	+	−	−	+	+	+	−	+	−	−	A MN cde/cde	100
Family 17.													
Father	M	+	−	−	+	+	−	−	+	+	−	A MM cDe/cde	003
Mother	F	+	−	−	+	+	+	−	+	+	+	A MN cDE/cde	100
Child 1	F	+	−	−	+	+	−	−	+	+	+	A MM cDE/cde	100
Child 2	F	+	−	−	+	+	+	−	+	+	+	A MN cDE/c e	100

FAMILY STUDIES - PLATELET GROUPS

Donor	PlB Antigens Present	NO 1/8	NO 1/24	SZ 1/3	AD 1/2	CO 1/24	WE 1/8	SI 1/24	NO eluted with 16128	NO eluted with 16152	SZ eluted with 16123
Family 1											
Father	100	4	3	4	0		2	0			
Mother	020	4	1	0	4		0	0			
Child 1	120	4	4	4	3		0	0			
Family 2.											
Father	103	4	4	4	1		4	0	2	0	
Mother	100	4	4	4	0		0	0	4	0	
Child 1	100	4	2	4	2*		0	0	1	0	
Child 2	103	4	4	3	1		4	0	1	0	
Child 3	100	4	4	4	4*		0	0	3	0	
Family 3.											
Father	103	4	4	4	0		4	0	4	0	
Mother	023	4	0	0	4		4	0	0	4	
Child 1	003	0	0	0	1		4	0	0	0	
Child 2	120	4	4	4	4		0	0	3	4	
Family 4.											
Father	003	0	0	0			4	0			
Mother	003	0	0	0			4	0			
Child 1	003	0	0	0			4	0			
Child 2	003	0	0	0			4	0			
Family 5.											
Father	100	4	4	3	0		0	0			
Mother	100	4	4	3	0		0	0			
Child 1	100	4	4	3	0		0	0			
Child 2	100	4		4	1		0	0			
Child 3	000	0	0	0			0	0			
Child 4	100	4	4	4		0	0	0			

Donor	P1B Antigens Present	NO 1/8	NO 1/24	SZ 1/3	AD 1/2	CO 1/24	WE 1/8	SI 1/24	NO eluted with 16128	NO eluted with 16152	SZ eluted with 16123
Family 6.											
Father	000	0	0	4*	4*		0	0	0	0	0
Mother	100	4	4	4	4*		0	0	1	0	2
Child 1	100	4	4	4	4*		0	0	4	0	4
Child 2	000	0	0	0	4*		0	0	0	0	0
Child 3	000	0	0	0		0	0	0			
Family 7.											
Father	120	4	4	4	4		0	4	4	4	
Mother	100	4	4	4	0		0	0	4	0	
Child 1	100	4	4	4	1		0	4	4	0	
Family 8.											
Father	100	4	4	3	0		0	0			
Mother	000	0	0	0			0	2			
Child 1	000	0	0	0			0	0			
Child 2	000	0	0	0			0	0			
Child 3	000	0	0	0			0	0			
Child 4	100	4	3	3	1		0	0			
Child 5	000	0	0	0			0	0			
Family 9.											
Father	100	4	4	3	1		0	0			
Mother	000	0	0	0			0	0			
Child 1	100	4	4	3	1		0	0			
Child 2	000	0	0	0			0	0			
Child 3	000	0	0	0			0	0			
Family 10.											
Father	000	0	0	0	0		0	0			
Mother	100	4	4	4	0		0	0			
Child 1	100	4	4	3	0		0	0			
Child 2	100	4	4	4	0		0	0			
Child 3	100	4	4	4	0		0	0			
Child 4	100	4	3	4	1		0	0			
Family 11											
Father	003	0	0	1		0	4	0			
Mother	000	0	0	0		0	0	0			
Child 1	003	0	0	0		0	4	0			
Child 2	003	0	0	0		0	4	0			
Child 3	003	0	0	2		0	4	0			
Child 4	003	0	0	0		0	4	0			

Donor	P1B Antigens Present	NO 1/8	NO 1/24	SZ 1/3	AD 1/2	CO 1/24	WE 1/8	SI 1/24	NO eluted with 16128	NO 16152	SZ 16123
Family 12.											
Father	100	4	4	4		0	0	0			
Mother	000	0	0	0		0	0	0			
Child 1	100	4	4	4		0	0	0			
Child 2	100	4	4	3		0	0	0			
Child 3	100	4	4	4		0	0	0			
Child 4	100	4	4	4	0	0	0	0			
Family 13.											
Father	103	4	4	1		0	4				
Mother	003	0	0	0		0	4				
Child 1	003	0	0	0		0	4				
Family 14.											
Father	023	4	0	0	4	4	4	0			
Mother	000	0	0	1	4*	0	0	0			
Child 1	003	0	0	0	4*	0	4	0			
Child 2	003	0	0	0	2*	0	4	0			
Child 3	020	4	1	0	4	4	0	0			
Family 15.											
Father	120	4	4	0		2	0	0			
Mother	003	0	0	0		0	4	0			
Child 1	020	4	1			4	0	0			
Child 2	103	4	4			0	4	0			
Family 16.											
Father	120	4	4	4	2	4	0	0			
Mother	003	0	0	1	4*	0	4	4			
Child 1	023	4	1	0	4	4	4	0			
Child 2	100	4	3	4	0	0	0	4			
Child 3	100	4	4	3		0	0	3			
Family 17.											
Father	003	0	0	1		0	4	0			
Mother	100	4	4	3		0	0	4			
Child 1	100	4	4	3		0	0	3			
Child 2	100	4	4	3		0	0	4			

* indicates reactions attributed to contamination of platelets with red cells.

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