IMMUNOGLOBULIN MEASUREMENTS IN A GENETIC ISOLATE

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IMMUNOGLOBULIN MEASUREMENTS IN A GENETIC ISOLATE

A Thesis Proposal

Presented to

the Faculty of Medicine

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Master of Science

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Lekan Samusa Salimonu

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To my *late* mother

RABIAT AYOKA SALIMONU

ABSTRACT

In three geographically and genetically close Newfoundland communities, there was an aggregate of 19 cases of Hodgkins disease, embryonic tumour, lymphosarcoma, leukaemia, thymoma and immunodeficiency. In this study, 939 sera from community members and control samples from 321 blood donors and healthy children were tested for their concentrations of immunoglobulins G, A, M and D by immunodiffusion. The results were submitted to a multifactorial analysis of variance.

The main findings were that (i) significant differences in immunoglobulin concentrations associated with age and sex; (ii) no significant association between variations in tonsil size and the mean concentrations of the 4 immunoglobulin classes; (iii) the mean concentrations of IgG, IgA and IgM were elevated in the first and second degree relatives of the patients particularly relatives of those with embryonic tumour, lymphosarcoma, leukaemia and thymoma, and of those with immunodeficiency, and to a lesser extent in relatives of patients with Hodgkins disease; (iv) the relatives of patients with Hodgkins disease had a significantly elevated mean IgD level compared with the mean IgD levels found for other groups; (v) many relatives of the patients showed immunoglobulin deficiencies of various grades and

types including one case each of hypogammaglobulinaemia and isolated IgA deficiency.

Elevated immunoglobulins in relatives of patients with lymphoreticular malignancies and immunodeficiencies may result from increased antigenic stimulation of the immune system, perhaps by an infective agent. A subtle form of immunodeficiency which permits the entry of antigens into these individuals more easily than in healthy people, may be a predisposing factor. It is also possible that the closely associated immunodeficiency and malignancy could both result from the same cause. The peculiar genetic make up of this community with a high incidence of inbreeding raises the possibility of an inherited disposition to both conditions. Continued exposure of the community to an infective agent (Virus(es)) may lead to raised immunoglobulin levels in many people, and to overt disease such as malignancy or severe immunodeficiency in a few. Since the functional state of the immune system may be inherited, it is likely that the predisposition to virus carriage is genetically determined.

It is suggested that both genetic and environmental factors may be contributing significantly to immunopathology in these communities.

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INTRODUCTION

Vertebrates have evolved an elaborate system of defence to overcome the effects of parasitism with viruses, unicellular microbes and multicellular brganisms. Chief among this system of defences is the specific immune system which can produce cells and synthesize proteins with the ability to attack specifically individual variety of invaders. In the mammals, the immune system has developed to the point where a first infection generates immunological "memory" so that subsequent infection may be more easily overcome. The cells which undertake the specific immune responses are found in the blood and in the lymphoreticular tissues. Synergistic mechanisms exist between specific immune reactants, and the non-specific components of the defence system such as the phagocytes and the complement system. The specific and non specific reactants work together to destroy infectious organisms.

By far the best studied part of the specific immune system is that which ultimately produces antibody molecules. The antibody molecules are of great diversity and due to repeated gene duplication and fusion, are of many different molecular classes, each of which has different functional properties. Some of the molecular, biological and clinical details of antibodies will now be reviewed.

REVIEW OF LITERATURE

Major Classes of Immunoglobulins and Their Roles

The gamma globulins were first recognized as a distinct group of serum proteins by Tiselius (1937) who performed electrophoresis on normal and immune sera. He found that a major group of proteins migrated towards the anode more slowly than did other major groups. He gave the name gamma globulin to this group of slow moving proteins. Tiselius and Kabat in 1938 hyperimmunized rabbits with pneumococcal polysaccharide and produced a serum with a high concentration of specific antibody. They absorbed this serum with the antigen and ran electrophoresis on the absorbed as well as the unabsorbed serum. They found that only the gamma globulin fraction was significantly reduced after the absorption, thus showing that the antibodies of serum are present in the gamma globulin fraction.

It was later found through several studies, that the blood of man and some other animals contains a variety of globulins consisting of at least 5 distinct classes with antibody activity. These globulins or immunoglobulins as they are now called, are capable of combining more or less specifically with, and inhibiting foreign substances which gain entrance into the body. In man the 5 major classes of immunoglobulins present are designated IgG, IgA, IgM,

IgD and IgE.

Methods which included polypeptide chain separation by cysteine reduction followed by aklylation, and analysis of N-terminal and C-terminal amino acids led Porter (1963) to propose a 4 chain model for rabbit IgG. This model showed two pairs of polypeptide chains, one pair of long (heavy) and one pair of short (light) chains arranged symmetrically, the chains being linked covalently by disulphide bonds. The current model does not differ very much from Porter's original description except in the positioning and the number of disulphide bonds (Marchalonis and Edelman 1965 and 1966).

It is generally accepted that each immunoglobulin is a protein with the same basic structure as described for IgG. IgM for example, is relatively easily broken down to five 7S subunits by mild reduction with 2-mercapto ethanol (Lammer et al., 1966) and further breakdown shows that these are built on the same general pattern as IgG.

There are 2 types of light chains namely the Kappa or κ and the Lambda or λ chains. Each class or subclass of heavy chain may combine with either κ or λ. These light chains show major variations among themselves in the amino acid pattern of the amino terminal halves called the variable regions. On the other hand, only minor variations exist in the carboxyl terminal halves called the constant regions (Gray et al., 1967). Each light chain has a molecular

weight of about 22,000 and consists of about 214 amino acid residues (Putnam et al., 1966).

The heavy chains differ from one class or subclass to the other in amino acid sequence as well as a few other properties. The heavy chain of IgG and probably that of IgA contains one variable region at the amino terminal end and a constant portion containing three homology regions or domains at the carboxyl terminal end of the polypeptide (Edelman and Cunningham 1969). That of IgM and probably those of IgD and IgE each contains one variable and four constant region domains (Putnam et al., 1973).

Hydrolysis by the enzyme papain separates the IgG, IgD, IgE and subunits of IgA and IgM into three roughly equal portions (Bernier et al., 1965; Bennich and Johansson 1967). Two of these 3 fractions are identical and each of the two contains the antigen combining site; these are called the Fab ('fragment antigen binding'). The Fab produced by this digestion is monovalent. Though it is capable of combining with the antigen it cannot form precipitates. The third fragment consists of two heavy chains of the C terminal halves of two heavy chains joined by a disulphide bond. It is termed the Fc ('fragment crystallizable') because it readily crystallizes in water in the cold. It is known that in man the passage of maternal IgG through the placenta to the foetus, the complement fixing activity, and cytophilic activities including IgE

binding to most cells depend on the Fc fragment.

Use of pepsin at acid pH however splits the immunoglobulin molecule into two fragments, one of which is a divalent antibody fragment $(Fab)_2$ of molecular weight of about 10,000 which is capable of precipitating antigen, and the other is the Fc fragment (Wilson et al., 1969).

HEAVY CHAIN SUBCLASSES

IgG could be separated into 4 subclasses due to differences in the antigenic properties of the gamma (γ) chain. There are also differences in the biological properties and the number of disulphide bonds joining the heavy chains. For example there are 2 each for IgG1 and IgG4; 4 for IgG2 and 5 for IgG3 (Milstein, 1969). There are differences in the IgG subclass concentrations in normal adult serum. Yount et al. (1970) quantified IgG subclass concentrations in 145 adult caucasians and reported a mean percentage concentration of 66 percent for IgG1; 23 percent for IgG2, about 7 percent for IgG3, and about 4 percent for IgG4.

The IgG subclass concentrations are determined both by the synthetic and catabolic rates of the different subclasses. The half life of IgG1, IgG2 and IgG4 are reported to be in the range of 11 to 21 days whereas that of IgG3 is much shorter (Spiegelberg and Weigle 1968).

In the IgA class there are 2 subclasses--IgA1 and

IgA2 present in the ratio of 9 to 1 in serum but present in equal amounts in saliva (Vaermann et al., 1966).

GENETICS

It is generally believed that one gene codes for the constant region and a separate one codes for the variable region of each heavy or light chain (Day, 1972). The variable regions of both the heavy and the light chains are responsible for the great diversities in structure which account for the specific antigen binding properties of the different classes of immunoglobulins. This specificity of antibody for antigen is of an immense survival value to the host animal.

Immunoglobulins contain genetic markers which may be present on the light chains (κ or λ) and on the heavy chains (e.g. gamma and alpha chains). These genetic markers may or may not be allelic.

The Kappa Light Chain

The Kappa chain contains 3 allelic genetic markers, which are subject to simple Mendelian heredity. These markers which are in the constant region are called Inv^1 (km^1); $Inv^{1,2}$ ($km^{1,2}$); and Inv^3 (km^3) and were originally thought to be associated with a single amino acid interchange at position 191 of the amino acid sequence (Milstein, 1966). It is now known (E. Van Loghem, personal communications 1975) that the situation is more complex. Two positions, 153 and

191 are involved with 3 different amino acids combinations as shown in the table below.

AMINO ACID POSITIONS

	#153	#191
Inv^1 (very rare)	Val	Leu
$Inv^{1,2}$ (less rare)	Ala	Leu
Inv^3 (common)	Ala	Val

The Lambda Light Chain

The lambda chain also has amino acid interchanges in the constant region. There is lysine in position 190 for $O_z(+)$ and arginine in this position for $O_z(-)$ λ chains. However these are not allelic i.e. $O_z(+)$ and $O_z(-)$ chains are both present in normal human serum.

Three of the IgG Subclass Heavy Chains

Three of the IgG subclass heavy chains bear allelic genetic markers in the constant region of the heavy chains. The genetic markers (Gm allotypes) associated with IgG1 subclasses are a, x, f, e, p, z, Rouen 2, San Francisco 2 and y); those for IgG2 are GM (e and n); and for IgG3 are Gm (b, b^3, b^4, b^5, c^3, c^5, s, t and g). IgG4 has not yet been shown to bear a regular genetic marker. Gm (4a or 4b) present on IgG4 are also shared by other IgG subclasses. For example Gm (4a) is also present in IgG1 and IgG3 whilst Gm (4b) is also present in IgG2 (Kunkel et al., 1970).

The IgA2 has genetic markers (A2m1+ or A2m2+) which is inherited as a Mendelian dominant trait in strict allotypic fashion; IgA_1 has not yet been shown to have any (Natvig et al., 1973).

No genetic markers have been discovered in association with the IgM, IgD and IgE classes.

Biological Properties of Immunoglobulins (Table 1a)

In the following table are given the important biological and immunochemical properties of the various immunoglobulins, some of which have not been mentioned in the text.

Factors Influencing Immunoglobulin Levels in Health

Several factors influence the levels of immunoglobulins in health. These factors include age, sex and other genetic factors, environmental conditions, vascular volume and haemoconcentration, rate of synthesis and catabolism and losses from the body. Of these, age and environment-related differences are much more clearly defined and universal than any of the others.

AGE EFFECTS

IgG

An infant possesses a considerable amount of IgG at birth, the majority of which is of maternal origin, having been selectively transported through the placenta to the foetus. The placenta evidently recognizes some specific

TABLE 1a

MAJOR PROPERTIES OF THE 5 CLASSES OF HUMAN IMMUNOGLOBULINS

Properties	IgG	IgA	IgM	IgD	IgE
Sedimentation Coefficient	7S	7S (9S, 11S, 13S)	19S	7S	8S
Molecular Weight	150,000 (monomer)	170,000 (monomer) and 390,000 (dimer)	870,000-970,000 (Pentamere)	185,000 (monomer)	188,000 (monomer)
Molecular weight of the heavy chain	53,000 (γ)	60,000 (α)	60,000- 70,000 (μ)	60,000 (δ)	75,000 (ε)
Known number of subclasses	4	2	2	1	1
Distribution (percent intravascular)	40%	40%	75%	75%	50%
Carbohydrate content	2.8%	10.5%	12.2%	13%	10.7%
Half life	23 days	6 days	5 days	2.8 days	2.5 days
Synthetic rate (per kg body weight per day)	30.0 mg	*24.0 mg	7.0 mg	0.5 mg	0.02 mg
Fractional catabolic rate (percent intravascular pool per day)	6.5%	25.0%	18.0%	37.0%	89.0%
Complement fixation	+	- (only via the alternative pathway)	++	-	-

*Synthetic rate is for serum IgA only.

TABLE 1a (continued)

MAJOR PROPERTIES OF THE 5 CLASSES OF HUMAN IMMUNOGLOBULINS

Properties	IgG	IgA	IgM	IgD	IgE
Heterologuous (species) skin sensitization	+	-	-	-	-
Homologous (species) skin sensitization	?	-	-	-	++
Placental Transmission	+	-	-	-	-
Antibody activity	The only antibody capable of cytophilic binding to macrophages; major antibacterial antitoxin and antiviral activities in serum. Major line of defence in the first few weeks of life.	Several antiviral, antibacterial and antitoxins activities.	Several anti lipopolysaccharides; cold agglutinins; isohaemagglutins and cytolytic antibodies.	Antibodies to diphtheria toxoid and penicillin.	Reagin antibodies against many helminthic infections and related antigens.

structural attributes of the Fc fragment of the IgG class. It is not known if this is an energy-dependent active process (Holland et al., 1966). The rate of maternal-foetal tranfer of IgG is a function of the maternal-foetal IgG levels as well as the age of the placenta. At low levels of maternal IgG, there is little IgG transfer; at higher levels of maternal IgG, transfer occurs in proportion to the maternal IgG level. This is also true of specific antibody transfer (Chandra et al., 1970). Because of the reduced time available for transfer, pre-term infants at birth have low serum levels of IgG in direct proportion to the gestational age (Gitlin, 1971). The correlation between cord IgG and gestational age is very high and has led some authors to suggest that the cord level of IgG can be used to estimate the gestational age of the foetus (Yeung and Hobbs, 1968). The selective transfer of IgG is supported by the fact that foetuses and newborn children of mothers having hyper gamma globulinaemia or hypo gammaglobulinaemia are known to contain elevated or diminished levels of IgG respectively. Bridges et al., (1959) for example, reported a very low level of IgG in utero and at birth in a normal baby born of a hypo gammaglobulinaemic mother. The baby's level remained low until the first year of life when it reached a normal level for its age group. Such low levels of IgG were not found in foetuses, nor at birth in children born of mothers with normal IgG levels. Cord blood IgG levels higher than maternal IgG

concentrations have been reported by some authors--Chandra et al., 1970.

It has also been shown that the human placenta towards the end of pregnancy is permeable to specific antibodies. At birth the maternal level approximately equals that of the infant (Osborn et al., 1952).

In the studies of Fudenberg and Fudenberg (1964) a Gm(a-) mother married to a Gm(a+ve) father produced three Gm(a-) children. The fourth pregnancy resulted in a Gm(a+) foetus. The foetus evidently synthesized Gm(a+) in utero, because the mothers immune system was stimulated to produce anti Gm(a+) agglutinating antibody. Such maternal antibodies against genetic (Gm) determinants may be one of the major causes of transient hypo gammaglobulinaemia of infancy. Further evidence of synthesis of immunoglobulins in utero comes from the work of Gitlin and Biasucci (1969) and that of Lawton et al., (1972a) who have shown that B cells bearing IgG surface determinants can be detected in bone marrow, liver, spleen and in the blood circulation at about 10½ weeks to 11½ weeks of foetal life.

After birth the IgG level falls rapidly in the first few months of life due to high catabolism of maternal IgG and relatively low synthesis by the baby. At about one year of age the level rises rapidly at first and slowly later, till it reaches the adult level around the 7th year of life (Allansmith et al., 1968).

There are several reports on further effects of age on IgG levels. Kalff (1970) measured immunoglobulins on 278 subjects in a Dutch population over 5 years of age up to 70 years and over. He found that IgG increased gradually with age. Stoop et al., (1969) confirmed such gradual increases in IgG with age in another Dutch community. However, not all workers agree, for example, Norberg (1967) observed no age differences in IgG, IgA and IgM levels of 370 Dutch people consisting of 100 students aged 18-30 years; 200 blood donors aged 18-56 years; and 70 elderly people 65-92 years of age.

Chandra et al., (1972a) quantitated immunoglobulins G, A and M in 800 apparently healthy children in India whose ages ranged from birth to 16 years. They reported lower IgG values than in other countries with less infection. In their studies the mean IgG levels were high at birth. The levels decreased markedly in the first few months of life reaching its lowest level in the 4th month of life. From then on, it gradually increased till the 16th year of life.

Toshkov et al., (1974) measured immunoglobulin concentrations on 927 Bulgarian blood donors (consisting of 757 men and 170 women) aged 18-60 years and reported no significant age differences in the IgG, IgA and IgM levels. Veys (1973) in Sweden reported no age differences in the IgG and IgM of 415 subjects aged 20-65 years whose immunoglobulin levels were evaluated.

Blanco et al., (1974) quantified immunoglobulins

(G, A, M) in 204 normal subjects from birth to adulthood in Spain. They reported age-related differences in IgG, IgA and IgM. IgA was not detected in any of the cord sera.

Fokina et al., (1974) in Russia measured immunoglobulins (IgG, IgA, IgM) levels in 104 cord sera and 474 children in Moscow from birth to 16 years of age. They found high levels of IgG at birth which fell in the first 4 months. The level then rose gradually till about the second year of life. It reached an adult level by about the seventh to eighth year of life. IgA and IgM on the other hand were present in very low levels at birth and increased with age till the 16th year of life.

In Canada, Collins-Williams et al., (1967) quantified immunoglobulins G, A and M on 200 sera from infants and children ranging from 2 months to 15 years. These were separated into 6 age groups (i.e. 2-6 months, 7-12 months, 13-23 months, 2-5 years, 5-10 years and 10-15 years). The samples were not separated into different sexes. They observed increases in IgG and IgA with age up to 5-10 years whilstIgM steadily increased with age up till 10-15 years.

Lichtman et al., (1967) reported no significant age differences in the IgG and IgM levels in 112 caucasians and 109 negroes (between the ages of 15 and 74 years) studied in Evans County, Georgia. Stiehm et al., (1966) estimated immunoglobulins of hospital employees and of parents and their infants and children who were attending Well Baby

Clinic at the University of California Medical Centre. The samples estimated consisted of 22 newborn children, 274 children (aged between 1 month and 16 years) and 30 adults. They reported that IgG, IgA and IgM increased with age and reached adult levels at 16 years of age for IgG and IgM, whereas IgA continued to increase throughout early adulthood. Buckley and Dorsey (1970) in their studies in North Carolina reported a gradual increase in IgG level up to the second decade of life. They also observed that the level starts declining appreciably from the third decade of life until the sixth decade.

With regards to the developing countries the IgG (as well as IgM) level usually reaches adult level earlier than in the developed countries. This is in part related to the more frequent and wider variety of infections which populations of the developing countries experience. For example Rowe and McGregor et al., (1968) observed in a Gambian community (West Africa) that the adult level of IgG was reached at about 5 years of age and IgM at 15 years, after which the levels only rose slightly. Similar observations have been made in the underpriviliged segments of the society in industrialized countries.

There are also differences in the turnover rates of human gammaglobulins (including IgG, IgA and IgM) which is age dependent. It is found that the turnover rate declines with age in adults (McFarlane, 1957).

There are several reports with regards to the shape of the frequency distribution (histogram of population frequencies against a set of increasing non-overlapping concentration intervals) of IgG. There seems to be no agreement in the different findings. Some authors (Kalff in The Netherlands, 1970; Rowe and McGregor in Gambia, 1968 and Grundbacher in the United States, 1974) reported unimodal frequency distributions skewed to the right, other authors (Johansson in Sweden, 1967; Veys in Sweden, 1973; Allansmith et al., in the United States, 1968; and Lichtman in the United States in 1967) showed that following log transformation, the values plotted with a normal distribution. Others deny a normal distribution of IgG levels even after log transformation (Clamman and Merril in the United States, 1964).

Summary. It appears from these surveys that (i) IgG is derived from maternal serum in the newborn; (ii) after an initial drop it gradually increases till about the 16th year of life when adult levels are reached; (iii) there are conflicting reports however on age-related differences after the 16th year of age.

IgA

In healthy infants IgA is either not detectable or is present in very low concentration at birth. There is very little evidence that IgA is produced in a normal foetus (Van Furth et al., 1965; Stiehm et al., 1966). Cord blood contains

less than one percent of maternal blood level of IgA (Rosen, 1971). IgA usually appears a few days after birth and increases gradually to reach about 25 percent of adult level by the end of the first year of life; 50 percent of adult level at about 4 years of age and the adult level at the age of puberty (Hitzig, 1957; Allansmith et al., 1968). Cassidy et al., (1974) quantitated immunoglobulins on 3213 sera from the whites in Michigan (U.S.A.). IgA estimation was carried out on 1523 males and 1687 females 5 to 94 years of age. They observed that IgA levels increased continuously with age till the end of life. They also observed that the increase with age is greater for IgA than for IgG i.e. the slope of the regression of IgA concentrations on age is steeper than that for IgG.

Very high levels of IgA are usually reported in gastro-intestinal tract and respiratory infections (Thompson et al., 1969). For example patients with coeliac disease, regional enteritis or ulcerative colitis are known to have higher levels of IgA than in normal subjects (Asquith et al., 1969; Kraft et al., 1968). Walman et al., (1970) reported markedly elevated IgA levels in patients convalescing from choleraic diarrhoea.

In normal adults, a broad range of IgA levels has been reported, with high levels becoming more frequent with increasing age (Rowe, Boyle and Buchanan, 1968).

There are conflicting reports on the frequency

distribution of IgA. Van Munster et al., (1965) reported that IgA follows a normal (bell-shaped) frequency distribution. On the other hand Clamman and Merril (1964) in their IgA measurements of 54 normal adults consisting of 10 negroes and 44 caucasians in Denver (U.S.A.) reported a log normal distribution. Other workers have also reported a log normal distribution. These include Johansson (1967); Goldman et al., (1967); Lichtman et al., (1967); Allansmith et al., (1968) and Veys (1973). A study originating from Gambia showed that IgA does not follow a normal distribution (Rowe and McGregor, 1968).

Summary. It appears that (i) the level of IgA at birth is very low; (ii) the IgA is manufactured in utero by the foetus; (iii) the level increases continuously with age throughout life.

IgM

The IgM level is low at birth. IgM is synthesized in utero by the infant and is present in all normal neonates (Van Furth et al., 1965). It is always present in a detectable amount at birth (Haworth et al., 1965; Fulginiti et al., 1966 and Stiehm et al., 1966). Several studies have shown that the IgM present at birth is manufactured by the foetus. The cord blood contains less than 10 percent of maternal level of IgM (Rosen, 1971). IgM does not cross the placenta. Cord blood IgM levels of more than 20 mg. per 100 ml. are indicative

of intra-uterine antigenic stimulation e.g. by infection, or of materno-foetal transfusion. Experiments have shown that radioactively labelled IgM injected into the mother does not flow into the baby (Gitlin et al., 1964). The studies by Kochwa (1961) revealed that maternal IgM antibody of known specificity is not found in the cord serum.

Studies by Moore and Owen (1965) on chickens show that haematopoietic stem cells derived from the yolk sac start entering the bursa of Fabricius about the 13th day of embryonic life. Immunofluorescent studies have shown that IgM producing cells are detectable after the 14th day of embryonic life in the chicken (Kincade and Cooper, 1971; Lawton and Cooper, 1973). In man B cells bearing IgM surface determinants in peripheral blood, bone marrow, liver, and spleen have been detected at about 10½ and 11½ weeks of foetal life (Gitlin and Biasucci, 1969; Lawton et al., 1972).

The IgM level increases sharply after the first few days of life. This increase is most probably due to the response of the infant's system to antigenic stimulation in the new environments soon after birth. IgM is the chief immunoglobulin synthesized by the neonate. The increase then continues but at a less rapid rate until it reaches adult level at about the beginning of the 2nd decade of life (Buckley and Dorsey, 1971). Buckley and Dorsey (1970) measured IgG, IgA and IgM from 811 subjects of different races whose ages ranged from birth to 92 years. They reported

that the IgM level increases from birth to about the beginning of 2nd decade of life. They also found that IgM reaches maximum level and maximum variance also at about the 5th decade of life. In their results the level declines somewhat after the 5th decade of life. The onset of the increase in IgM level in childhood is usually earlier than that of IgG but they both usually reach maximum level about the 2nd decade of life (West et al., 1962; Stiehm and Fudenberg, 1966).

However, not all workers agree, for example Allansmith et al., (1968) quantitated immunoglobulins G, A and M in sera ranging from cord blood to adults and reported that the adult level of IgM is reached after the 1st or the 2nd year of life. This is much earlier than that reported by Buckley and Dorsey (1970). Other workers have found no age differences in IgM after the 5th year of life. Cassidy et al., (1974) reported no age differences in IgG, IgA and IgM. They reached these conclusions after analysing 3213 samples aged 5-94 years. Norberg (1967) also reported no age differences in the IgG, IgA and IgM after analysing 370 samples from apparently healthy subjects aged 18-92 years.

As with the other immunoglobulins, there are several reports on the shape of the frequency distribution of IgM. Some workers report a normal frequency distribution (Van Munster and Stoelinga, 1965 and Cwynarski, 1968).

Kalff (1970) analysed immunoglobulin contents of 252 subjects over 5 years of age from 3 villages in the southern

part of The Netherlands. He reported a log normal frequency distribution for IgM. Allansmith et al. (1968) collected samples from 946 apparently healthy individuals from cord sera to adults in two communities in San Francisco. They reported that their IgM follows a normal frequency distribution. Similar findings were reported by Clamman and Merril 1964 and Goldman et al. (1967).

Summary. It appears that (i) the IgM present at birth is synthesized by the foetus; (ii) the level rises slowly with age till about the 16th year of life when the maximum level is reached.

IgD

IgD is not normally present at birth. It is only very rarely found in cord serum. It is not secreted into serum in utero and does not readily cross the placenta (Johansson 1967; Rowe and Crabbe et al., 1968). It becomes detectable between the 3rd and the 10th month of life. The level increases progressively with age reaching a maximum at about the 10th year of life. The level then falls slowly till it reaches adult level at about 15-20 years of age after which higher levels are observed less frequently (Rowe and McGregor, 1968).

Wide ranges of IgD levels are found in health (from zero to very high values) due to differences in rates of synthesis and catabolism (Rogentine et al., 1966).

Geny et al., (1974) quantitated IgD in 214 apparently healthy children aged 10 months to 15 years in Paris, France. They reported that IgD increases progressively with age up to about the 5th year when there is a slight decline. The level later rises from the 6th year up to the 15th year when the adult level is reached.

Markedly elevated levels are observed in pregnancy especially during labour. Leslie (1973) studied IgD levels at different stages of pregnancy in New Orleans (U.S.A.). His latest samples consisted of 38 women in early pregnancy (up to 15 weeks); 49 women in intermediate stage of pregnancy (16-28 weeks); and 42 women in late pregnancy (29 weeks and over); and 27 samples from women in labour. His controls which were not age matched consisted of 29 nulliparous women, 44 women with no previous pregnancy and 84 with one or more previous pregnancies. His results showed that IgD rises at the onset of pregnancy. It progressively increases up to the later part of the intermediate stage of pregnancy. It then decreases slightly during late pregnancy, after which there is a sharp rise during labour. Similar findings were reported by Klapper and Mendenhall (1971) and Geny et al., (1974).

Summary. From this survey it appears that (i) IgD is not present at birth; (ii) it is only detectable in serum after the second month of life; (iii) the level increases

gradually reaching a maximum at about the age of puberty. after which there is a decline; (iv) not all normal serum contain detectable levels of IgD; (v) there is a physiological rise during pregnancy.

IgE

At birth IgE is either absent or is present in the serum in very low concentration. The IgE present at birth is synthesized by the foetus, as the IgE does not seem to cross the placenta. This conclusion is supported by the similarity in the levels of IgE in the sera of newborns of allergic and non-allergic mothers (Johansson, 1968b).

Bazaral et al., (1971) used competitive inhibition radio immunoassay to analyse the IgE levels of 35 post partum mothers and 33 newborn cord sera. They reported low level of serum IgE at birth, and no placental transfer of IgE. In their studies, about one-third of the 6-week old infants had no detectable serum IgE and three 6-month old infants had adult levels of IgE.

The IgE rises very rapidly in the first two months of life and slowly afterwards reaching 75 percent of adult level by the 5th year of life. The adult level is reached at the age of 7. From about the 15th year the level decreases very slowly throughout life.

The IgE level is low even in adults when compared with the other immunoglobulin levels; its concentration

being about 1/50,000 of the normal serum IgG level. There is wide variation in health.

IgE levels are raised in allergic diseases such as hay fever, asthma and atopic eczema and in parasitic infections notably helminth infections (Johansson et al., 1967b; Rosenberg et al., 1970). Gleich et al., (1970) measured IgE in 80 blood donors, 32 non-allergic healthy subjects, 32 patients with previous allergic hay fever or asthma and positive wheal and flare skin tests who had been treated, and 30 allergic patients not treated. They reported a wide range of values in the normal subjects and significantly higher ranges in allergic patients. The untreated patients had significantly higher IgE levels than treated patients, and much higher still than the normal controls.

Johansson (1967) found 63 percent of patients with allergic asthma with markedly elevated IgE levels but only 5 percent of non-allergic patients had elevated IgE levels.

IgE frequency distribution is reported to be bimodal by Bazaral et al., (1971).

Relationship of IgE to T cell function. There appears to be a T cell modulation of IgE production. Patients with a demonstrable defect of cellular immunity defect generally have high serum IgE levels (Berglund et al., 1968). The main exception to this is ataxiatelangiectasia in which both T cell function as well as IgE production are impaired.

A similar association between T cell dysfunction and IgE elevation is also seen in malnutrition; however the situation gets complicated here because of the frequent presence of parasites in such individuals with nutritional deficiency.

Summary. It appears that (i) IgE is only present in serum in very low concentration at birth; (ii) it increases gradually till about the 7th year of life when the adult level is reached; (iii) IgE concentration in serum in health is very much lower than any of the other classes of immunoglobulins throughout life; (iv) the levels are high in parasitic infestation and in allergic states.

SEX EFFECT

The influence of sex on the levels of different immunoglobulins is well documented. Sex differences are found to be more marked in IgM concentrations than in any other immunoglobulin class.

IgG

Several reports show that IgG levels are usually higher in females than in males (Stoop et al., 1969). In the immunoglobulin measurements in childhood (2-15 years) Berg et al., 1969 reported a higher level of IgG in females in all the (2-year interval) age groups. Boys have been reported to be more susceptible to infections than girls by several authors (Childs, 1965). This sex difference in

susceptibility to infections has been partly attributed to sex differences in IgG and IgM levels (Washburn et al., 1965). These sex differences are most probably caused by differences in hormonal levels between males and females.

Quintiliani et al., (1974) in Rome analysed serum Immunoglobulins (G, A and M) levels in 773 apparently normal adults (408 males and 365 females) aged 21-55 years. They reported higher IgG in females than in males. Some reports however indicate no significant differences in IgG levels between the sexes in some populations (Rowe and McGregor et al., 1968; Rhodes et al., 1969).

In pregnancy, according to Leslie, (1973) the IgG level falls progressively through pregnancy reaching its lowest level at about the 10th week. It then rises gradually till it reaches the normal post-partum level. The IgA and IgM however remains unchanged, whereas IgD is elevated during pregnancy. Maroulis et al., (1971) in North Carolina quantified IgG, IgA and IgM in 33 black and white pregnant women at different stages of pregnancy. They concluded from their findings that there is a diminution in IgG level but no significant differences in IgA and IgM in age and race matched controls. No such decrease was observed in IgG by Mendenhall (1970). On the other hand, Godson (1969) reported elevated levels of IgG during pregnancy. Generally most reports identified lowered IgG level in pregnancy. These differences in IgG levels in pregnant and non-pregnant

women are probably due to differences in the hormonal levels at the different periods of pregnancy.

Summary. It appears from this survey that (i) IgG levels are usually higher in females than in males. This is most probably due to differences in hormonal balance between the two sexes; (ii) there are changes in IgG levels during pregnancy.

IgA

Reports on the sex differences in IgA levels are conflicting. Kalff et al., (1970) reported no sex differences after analysing samples from 290 subjects representing 4 communities (aged 5 years to over 70 years). Similar findings were reported by Berg et al., (1969) who quantified IgA on 219 Swedish children (127 boys and 92 girls) aged 2-15 years. Rhodes et al., (1969) in England estimated IgA levels in 56 apparently healthy subjects; and in 38 women with chromosomal abberations. They also reported no sex-related differences in IgA levels. No clear-cut sex-related differences were found by Stoop et al., (1969) who estimated IgA levels on 270 apparently healthy children in the Netherlands aged 4-13 years and 30 adults.

On the other hand Cassidy et al., (1974) reported slightly but significantly more serum IgA levels in males than in females. Also in Grundbacher's (1974) studies, IgA levels were analysed on 444 apparently normal subjects

belonging to 64 families in Virginia. He found sex-related differences with significantly more elevated Mean IgA levels in males than in the females in both the black and white populations studied. Similar findings of significantly raised IgA levels in males than in females have been reported by Buckley and Dorsey, (1971) in Durham, U.S.A. after quantifying IgA in 819 apparently healthy individuals aged 1-92 years.

Summary. It seems IgA levels are slightly more elevated in males than in females.

IgM

Mean concentrations of IgM are usually higher in females than in males in all age groups (Allansmith, McClellan and Butterworth, 1967; Buckley and Dorsey et al., 1970). IgM in females has been found to be as much as one-third higher than the male of the same age group (Grundbacher, 1972a). The sex differences are more pronounced in the reproductive age and are most probably due to differences in sex linked inheritance or differences in hormonal balance between the two sexes.

The effect of the X chromosome on IgM has been reported by several workers. Grundbacher, (1972a) has reviewed evidence from family studies of both black and white races that the X chromosome of man carries (gene(s) affecting the concentration of IgM. For example Wood et al., (1969) have

suggested that the level of IgM is influenced by the number of X chromosomes present, and have carried out immunoglobulin measurements on normal men with (46 X Y) chromosomes; normal women with (46 X X) chromosomes; and in women with dysgenetic ovaries (Turner's Syndrome) having (45 X 0) chromosomes. They found that whereas the levels of IgM were higher in women with (X X) chromosomes, the levels in men (X Y) and X 0 women were similar. Rhodes et al., (1969) quantitated IgM on 28 women aged 20-74 years; 10 men with other chromosome aberrations 7 with (XXY) and 3 with (XXXY); and in age matched controls consisting of 28 normal healthy men with (XY) chromosomes and 28 normal healthy women with (XX) chromosomes. They observed significantly higher levels of IgM in females with 46 XXX chromosomes than in normal females with 46 XX chromosomes; and the lowest levels were found in males with 46 XY chromosomes. The Mean IgM level of (XXY) men was similar to those of (XX) women, whilst those of XXXY group were found to be similar to those of XXX group. They concluded that the level of IgM is influenced by the number of X chromosomes, and not by the presence or absence of Y chromosomes. No such linkage was observed for IgA and IgG levels.

It was also observed by Garvie et al., (1961) that certain types of agammaglobulinaemia (affecting IgM as well as IgG and IgA) might be sex linked with the agammaglobulinaemia occurring in boys. This also points to an

association between immunoglobulin and the X chromosome.

Summary. Thus it appears that IgM is usually higher in females than in males. This might be because of differences in hormonal levels between the two sexes and/or the X chromosome might probably carry quantitative genes for IgM as suggested by Grundbacher, (1972a).

IgD and IgE

No sex differences have been associated with IgD and IgE levels (Rowe, Crabbe and Turner, 1968; Johansson, 1968b; Berg et al., 1969).

AUTOSOMAL GENETIC FACTORS

There is abundant evidence that autosomal genetic factors are involved in the control of levels of the different immunoglobulins in health and disease. Such evidence could be found in the work of Rowe, Boyle and Buchanan, (1968) who studied immunoglobulin levels in monozygotic and dizygotic twins. They observed more concordance in IgG, IgA and IgM levels in monozygous than in dizygous adolescent twins (both males and females). Among adults the male monozygotic twins had significantly smaller intertwin differences in IgG levels than dizygotic twins. No differences were observed in IgA or IgM in adult twin pairs. The study provides suggestive evidence of genetic regulation of IgG, IgA and IgM, but the effect gets masked during adulthood, especially in the case of IgA and IgM. Similar studies

by Allansmith et al., 1969 showed a possible genetic influence on IgG and IgA levels but not on IgM. Also Biozzi's (1970) breeding studies in mice are conclusive on this point.

Leonhardt et al., 1962 measured total proteins and quantitative electrophoretic protein fractions. They observed closer agreement between monozygotic than between dizygotic twin pairs. The dizygotic twins in turn showed closer agreement than unrelated pairs of the same age and sex. Similar findings have been reported by Frey Näntö and Kulonen (1968) and Kalff et al., (1969).

It is observed that people with certain chromosomal abnormalities (autosomal syndromes) show departure from normal in their immunoglobulin levels. These altered levels may be from childhood or in adulthood. For example Stiehm and Fudenberg (1966) studied the immunoglobulin (IgG, IgA and IgM) levels in 15 adult Mongols. These adult Mongols had elevated levels of IgG and IgA and a dimunition in IgM levels. Levels found in Mongol children under 5 years were reported normal.

Racial differences in immunoglobulin levels have been suggested as providing supportive evidence for a genetic influence. Such differences have been reported for IgG levels by Rowe and McGregor et al., (1968); Lichtman, Vaughn and Hames (1967); Turner and Voller (1966). In these several studies the IgG or both IgG and IgM levels have been found to be more elevated in blacks than in whites. Buckley and Dorsey (1971) reported higher IgG levels in blacks than in

whites. White males had higher levels of IgA than black males whereas white females had lower levels than black females. IgM levels were similar in the males, but white females had higher values than the black females.

Comparative studies of Johansson, Mellbin and Valquist, (1968) revealed more elevated levels of the IgG, IgD and IgE classes in pre-school children of an Ethiopean community than in children of the same age group in a Swedish community, but these differences were more likely to be due to nutritional and infective disorders in the Ethiopean population.

Racial differences in immunoglobulin levels have also been observed in New Guinea where the Watut aborigines are known to have significantly higher levels of IgG and IgM than the non-Watut aborigines (Wells, 1968).

The several reports on variation in immunoglobulin levels due to race show that IgG is more elevated in blacks than in whites. Reports on the other immunoglobulin classes are not consistent. It seems most likely that the several reported differences in immunoglobulin levels depend more on the differences in the social and economic status, nutrition, and the environment (especially the frequency and severity of infective antigenic challenge) than on the genetic make up of the different races.

ROLE OF ENVIRONMENT

Environmental factors include infections, nutrition,

climatic conditions, geographical location, drugs, steroids and administration of immunosuppressives.

Infection

The major function of the immunoglobulins is to provide immunity. It is to be expected that their levels in a population would be highly dependent on the wide range of antigenic stimuli provided by the variety of infections to which the population is exposed. Such elevations in immunoglobulins due to infections are well documented. For example IgG is raised in leishmania and malarial infections (Holmes et al., 1955; McGregor and Gilles, 1956; McGregor, 1972). IgA on the other hand is raised by infections of the respiratory tract and gastrointestinal tract. In the Middle East, infections of the gastrointestinal tract (with elevated IgA) have been implicated in alpha chain disease (Seligmann et al., 1968). IgM levels are markedly elevated following continuous and direct exposure to such antigens as Trypanosomiasis (Mattern et al., 1961; Hobbs, 1970). IgD is known to be raised after tetanus and diphtheria immunization (Heiner et al., 1970). IgE is increased after the entry of allergens, and after infection with certain parasites especially the helminth (Ishizaka et al., 1967b).

Studies by several workers have shown that the rate of synthesis of the different classes of immunoglobulin molecules in mice raised in a germ-free environment have been

found to vary from less than 1/300 to 1/50 of normal (Sell and Fahey, 1964; Fahey and Sell, 1965). On the other hand mice which are hyperimmunised with haemocyanin or raised in an environment with high bacterial content synthesize immunoglobulins of all classes at rates which are about 5-10 times higher than are seen in normal animals.

Nutrition

It would seem logical to expect that antibody formation and immunoglobulin levels would be greatly impaired by starvation. Studies made on prisoners of war and cachetic hospital patients however showed considerable depletion of serum albumin whereas the gammaglobulins were normal (Humphrey and White, 1970). Similarly children with protein-calorie malnutrition (P.C.M.) were found to have decreased albumin levels, but no significantly low levels of immunoglobulins (Gomez et al., 1955).

In children with kwashiokor, the mean absolute concentrations of total proteins, albumins, α_2-globulins and β globulins were significantly diminished, but there were no significant changes in the mean gammaglobulin concentrations Edozien (1960).

However there are some studies which have shown that malnutrition affects the immunoglobulin levels. In studies in Egyptian children with kwashiokor, diminished levels of IgG, IgA and IgM have been reported in children who had

kwashiokor very early in life. Those children who had kwashiokor much later in life had raised IgG and reduced IgM and variable levels of IgA. This study indicates that the period of life in which kwashiokor appears, may be important in determining the levels of the different immunoglobulins (Aref et al., 1970). Studies have been carried out to find the effect of feeding on the immunoglobulin levels of children with kwashiokor. Children with severe and moderate degree of malnutrition were fed for a long period of time with high protein food. They showed no significant changes in the immunoglobulin levels except for those with severe malnutrition who later had higher IgA (McFarlane, Reddy, Cooke, Longe, Onabamiro and Houba 1970).

Studies on experimental animals show that deficiencies of several different nutrients can lower immunoglobulin levels. For example rats fed on diets lacking either Vitamin A or any of the vitamins in the Vitamin B complex have been shown to synthesise low immunoglobulin levels.

Low Birth Weights and Gestational Age

Studies done on infants with low birth weights show that their IgG levels at birth are always lower than those with normal birth weights. The IgM on the other hand is reported to be similar for both low and normal birth weight infants. Some workers have reported lower IgA levels for low birth weight whilst others have claimed similarities

in IgA levels at birth (Haworth et al., 1965; Berg, 1968).

In Berg's study immunoglobulins G, A, M and D were analysed in 65 infants whose birth weights were lower than 2500 gms. These children were separated into 3 groups according to their gestational age; and into another 3 groups depending on their birth weights. Those whose birth weights were less than 1500 gms. were in one group. The other groups were those weighing 1500-2000 gms; and 2001-2500 gms. No normal birth weight children were included as controls. They found that IgG levels progressively increased with increasing gestational age and with increasing birth weight. No significant differences were found in the IgM levels at birth between low birth weight and normal birth weight infants. They observed that IgM started to increase from about the 1st to the 3rd day of life to about the 3rd week, the increase being more pronounced in the lowest birth weight infants. No IgA was detected in the serum of the low birth weight infants until after the 3rd week of life. IgD was reported to be present in one out of the 65 infants after 3 months. They concluded that IgD pattern in normal, and low birth weight infants are the same.

Geographical Location

Influence of the environment has been studied by several workers. For example higher levels of IgG, IgA and IgM (but similar IgD levels) have been reported in the

adult Gambian community than in the British and North American adults whose environmental conditions are different from the Gambians (Rowe and McGregor, 1968; Fahey and McKelvey, 1965; Clamman and Merril, 1964). Similar IgG and IgA (but lower IgM) levels as in Gambia have been reported in other communities (Nigeria and Congo) possessing the same type of environmental conditions (Turner and Voller, 1966; Michaux, 1966). This shows that environmental conditions are most likely responsible for the differences found in these communities.

A revealing longitudinal study was performed by Schofield (1957) who followed the gammaglobulin concentrations of West African students after they left Africa. He showed that West Africans resident in Britain for less than 2 years had average gammaglobulin levels of 2.2 gm per 100 ml whereas those who had resided for 2 to 4 years had an average of 2.0 gm per 100 ml and those who were resident for 5-8 years had an average of 1.6 gm per 100 ml. Thus in a different environment the immunoglobulins in these students gradually dropped; it is suggested that the absence of malaria may be a major environmental factor allowing these changes to occur.

Increased gammaglobulin levels observed in Pygmies and Bantus have been attributed to their environmental conditions (Simbeye, 1970).

Seasonal Changes

Seasonal changes have also been documented as factors influencing immunoglobulin levels in health. In Nigerian adults, the mean IgG and IgM concentrations are higher in the rainy than in the dry season, whereas the IgA and IgM levels are raised in infants in the wet season (McFarlane, 1966). In young Gambian children IgG and IgM levels are subject to seasonal variations. The IgA and IgD levels are not affected by this seasonal change (McGregor, Rowe et al., 1967; McGregor, Rowe and Wilson, 1970) which would make associated gastrointestinal infections unlikely causative factors.

Altitude

Effect of altitude and climate on immunoglobulin levels have been studied by Alarcon-Segovia and Fishbein (1970). They reported lower IgG and IgM levels in residents of Mexico City (2240 meters above sea level) when compared with those who reside by the Pacific Coast of Mexico (at Acapulco). There are several possibilities for the diminution in IgG and IgM levels in people living in high altitudes. The differences might probably be due to their increased plasma volume - a mechanism to avoid excessive blood viscosity when erythrocytosis tends to occur because of high altitude. It might be a direct effect of the altitude or due to hormonal differences. It is also known that infants

in high altitudes generally have a dimunition in birth weights and in foetal thymus weight. All these may be contributory to the lower level of IgG and IgM in adulthood reported in the studies.

Drug Effects

Drugs like chloropromazine, phenacetin, sedormid, quinidine, amidopyrin, stibophen, or phenolphthalein occasionally give rise to special types of hypersensitivity reactions due to reactions of antibodies (immunoglobulins). The drug forms a complex with the surface of a formed element of the blood and this complex causes the production of antibodies which are cytotoxic for the cell-drug complex resulting in purpura, haemolytic anaemia and agranulocytosis. In these cases there are markedly raised immunoglobulins which may return to normal after the causative drug is no longer being taken. Despite the fact that many people take these drugs only few produce antibodies (Cluff et al., 1964; B.M.J., 1970).

Adrenal Corticosteroids. Adrenal corticosteroids are known to suppress immune responses and to result in lower immunoglobulin levels. The immunoglobulin level is known to be influenced by the level of corticosteroids physiologically present or administered to the individual. These corticosteroids may act by suppressing DNA and RNA synthesis and cell mitosis. They are also reported to be capable of

destroying lymphocytes (Kidson, 1967).

Non-Steroid Immunosuppressive Drugs. There are other immunosuppressive drugs which can reduce immunoglobulin levels in health. These include antimetabolites and antibiotics. The effects of the antimetabolites and antibiotics include inhibition of purine and pyrimidine synthesis and the inhibition of RNA or protein synthesis (Prussoff, 1968; Coutsogeorgopoulos, 1966; Waring, 1968). Their administration into an animal usually leads to a low immune response and low protein (including immunoglobulins) biosynthesis (Rowley et al., 1973).

X-Irradiation and Antilymphocyte Serum

Both x-irradiation and antilymphocyte serum produce damage to lymphatic tissue with death of lymphoid cells. It is not surprising therefore to find that there may be *profound, dose related, suppression of immunoglobulin* synthesis (Fakete, 1973; Pierce et al., 1972a and b).

BLOOD VASCULAR VOLUME

The blood vascular volume could influence the immunoglobulin level. The blood vascular volume may be reduced as in dehydration. In such cases, elevated levels of Albumin and globulins could result due to diminution in the water content of plasma and consequent increased concentration of all the non-diffusible components (including the

immunoglobulins). However, in most cases in which dehydration is a prominent feature, such complicating factors as malnutrition, diarrhoea and vomiting exert opposing influences. Elevation of only the IgM class of immunoglobulins was reported in children with diarrhoea (Haider, 1971). Also Waldmann, Bencie et al., (1971) reported elevated IgA in choleraic diarrhoea patients, during the acute phase of the illness, which increased further during convalescence; an elevated IgM in non-choleraic and a more elevated IgM than normal in choleraic patients with diarrhoea was also noted.

Reduced blood volume can be caused by shock, burns, diabetic acidosis, Addison's disease, intestinal obstruction, intestinal fistula, pyloric obstruction, rigid restriction of fluid intake, heat exhaustion. Each of these can influence serum immunoglobulin concentration. For example Ritzmann et al., (1973) reported the following immunoglobulin patterns in thermal burns. The IgG level dropped significantly in the first few days after the burns. It then gradually rose reaching a level higher than normal in the adults. The IgA and IgM fell only slightly a few days after the burns and then rose until they reached values higher than the normal. Other workers reported a marked decrease in IgG level immediately after burns which very slowly returned to normal. The decreases in IgA and IgM following the burns were not significant, and returned to normal level more quickly (Arturson et al., 1969; Muster et al., 1970).

These immunoglobulin changes in patients with burns may represent losses through the injured skin as well as changes in the synthetic and catabolic rates; both are complicated by the state of hydration of the patient and the degree of infection.

In a number of conditions there may be an increased plasma volume due to plasma dilution. In these conditions there is usually a generalised hypoproteinaemia which also affects the immunoglobulins. Such conditions include the period after acute massive haemorrhage, or following a sudden recovery from severe dehydration such as in malnourished subjects or after diabetic coma.

RATE OF SYNTHESIS AND CATABOLISM

The primary factors controlling the concentrations of the different immunoglobulins in serum are the rates of synthesis and catabolism of these proteins in the body. Isotope techniques suggest that the concentration of gamma globulins, like the other protein fractions, is maintained by a process of balanced synthetic and catabolic rates. Immunoglobulin from fresh serum is purified and labelled with I^{131} or I^{125}; this radio-labelled protein is administered in tracer amounts (20-50 microcuries of Iodine) to the study subject. At the same time a saturated solution of potassium iodide is given to prevent thyroid uptake of any of the radioiodine. The radioiodine released after protein

catabolism is rapidly excreted largely into the urine and to some extent in the stool. Its rate of disappearance from the serum as well as from the whole body and its rate of excretion in urine and stool are measured. These are used to calculate the half life of the immunoglobulin in the circulation, the total body pool, the intravascular and extravascular distribution, the synthetic and catabolic rates.

Using such a technique it was found that the total body gamma globulins in a healthy adult is approximately 80 gm. About 25 percent of the circulating gamma globulin passes across capillaries into the tissue fluid per day; and roughly the same amount is returned to the blood stream through the main lymphatic ducts (Cohen and Freeman, 1960). The normal Mean synthetic rate is about 2.3 gm. per day (Solomon, Waldmann and Fahey, 1963).

The biological half life of IgG in an adult is about 23 days. Its synthetic rate is about 40 mg/kg bodyweight per day. The half lives of IgA and IgM on the other hand, are about 4-5 days each. Differences between the levels of IgA and IgM in serum are due to the differences in their synthetic rates. The synthetic rate for IgA is approximately 20 mg/kg bodyweight, whilst that of IgM is only 4 mg/kg bodyweight. The synthetic rate of IgM is thus one-fifth that of IgA. The rate for IgG is 80 times and 1000 times greater than those of IgD and IgE respectively. The half

life of IgD is 2.8 days and that of IgE is 2.5 days (Solomon, Waldmann and Fahey, 1963; Solomon and Tomasi, 1964).

The rates of synthesis however differ from one individual to another for other reasons such as different experiences with infections. In malaria, for example, there could be up to a 7-fold increase in the synthetic rate of IgG (Cohen and McGregor, 1963).

As regards catabolism, part of the catabolism of immunoglobulin takes place in the liver (Cohen, Gordon et al., 1962). Denatured immunoglobulins and those which have former complexes with antigens are most probably taken up and degraded by the cells of the reticulo endothelial system (Benacerraf, Sebestyen and Cooper, 1959). In man and mouse, the fractional catabolic rate of IgG is directly related to its serum level (Fahey and Robinson, 1963). This could be due to a feed-back mechanism. Hence those with high serum concentrations of IgG due to infections or hyper immunization usually have increased catabolic rates of IgG (Humphrey et al., 1961; Waldmann et al., 1969), whereas some patients with hypogamma globulinaemia may have decreased catabolic rates (Waldmann et al., 1965).

The serum levels of IgA and IgM however do not influence their catabolic rates. For example the catabolic rate of IgM is the same in normal subjects as in hypogammaglobulinaemic patients with reduced IgM, and in patients with macroglobulinaemia with increased IgM concentrations

(Rogentine, Rowe, Bradley, Waldmann and Fahey, 1966).

In the case of IgD the normal synthetic rate is from zero to about 1.5 mg per kg bodyweight per day. The biological half life is from about 58 hours to 139 hours. The level of IgD depends mainly on the synthetic rate since this can be up to about 15 fold more than the catabolic rate. High serum levels of IgD are usually associated with low catabolic rates, and low levels with high catabolic rates. The fractional catabolic rate of IgD is significantly higher than that of IgG and IgM; it is reported to be similar to or slightly higher than that of IgA (Barth et al., 1964; Solomon and Tomasi, 1964).

LOSS FROM THE BODY

Immunoglobulin destruction occurs inside phagocytes (which have engulfed bacteria and foreign particles coated with the antibody) present in the cells of the reticulo endothelial system. Immunoglobulin can also be lost into the urinary or gastrointestinal tract. Loss through damaged glomeruli of the kidney is selective and significant in patients with nephrotic syndrome. In these cases the relatively small molecular weight immunoglobulins like IgG are removed in the urine much more rapidly than those with high molecular weight like IgM (Johachim et al., 1964).

Several studies using labelled proteins have shown that excessive protein loss through the gastrointestinal

tract is one of the major factors leading to hypogammaglobulinaemia (including low immunoglobulins) in protein-losing enteropathy. For example, Strober et al., (1967) reported low immunoglobulin levels of all the major classes in 19 patients with intestinal lymphangiectasia.

EFFECTS OF Gm ALLOTYPES

The concentrations of certain immunoglobulins notably IgG appear to be influenced by the constant region genes. The evidence for this is an association of concentration differences with different allotypic markers (Gm allotypes). These markers are present in the constant region of the γ (IgG heavy) chains. In IgG3 subclass for example homozygous Gm(b) individuals have significantly higher levels of IgG3 than do persons who are homozygous Gm(g) (Yount et al., 1967).

In the case of IgG2, homozygous Gm(e) individuals have lower levels of IgG2 than do people who are homozygous Gm(n). The type of Gm allotype in IgG1 is also found to influence the IgG1 subclass concentration (Litwin and Balaban, 1972).

Preliminary findings by Van Loghem, (1971) on the IgG4 subclass also indicates a marked effect of homozygous allotype of IgG2 on the level of IgG4.

Whether the actual Gm marker is responsible for the effects or whether it simply acts as a marker for a particular complex of C region structural genes is not clear. However

the latter explanation seems to be the most plausible.

The Gm allotype has also been claimed to influence the level of IgD. Walzer et al., (1974) typed the Gm allotype on 700 blood donors in New York. People homozygous for Gm(f) with Gm(b) haplotypes were found to have significantly lower IgD levels than those who were homozygous for Gm(a) plus Gm(g) haplotypes. The authors took this to mean that δ chain constant region structure was important in determining the half life and that the constant region genes for δ were so closely placed to the γ chain genes containing the allotype sequences, that the γ allotypes could act as markers for high, or low IgD haplotypes. It appears from a study of Gm and Am allotypic markers that crossing over in this region is an exceedingly rare event.

INTRODUCTION AND OBJECTIVES

The investigation which lead to this study began with the observation of a high incidence of cases of lymphoreticular malignancy and immunodeficiency in an extended family living in 3 neighbouring communities on the west coast of Newfoundland. The occurrence of lymphoreticular malignancy and immunodeficiency together in a family suggested the idea that immunodeficiency, may be in a subtle form, could be present in members of the family and predispose them to develop lymphoma. This predisposition could be because of a breakdown in immunological surveillance--tumour cells not being destroyed by the immune system as efficiently as they should be. It could also be because the defect allows an oncogenic virus to gain entry to the body and to be incompletely destroyed by the defective immune system.

In 1974, a team visited the 3 communities for a voluntarily attended "Health Survey" in which the residents were offered a full physical check up and a blood test. Blood was taken in excess of that required for a routine blood count and a collection of serum was stored frozen. A multidisciplinary investigation of the situation was launched at that time, which included genetic, immunological, viral, clinical and epidemiological studies. The work reported in this thesis represents one segment of this larger study.

The aim of this work was to see if measurements of the immunoglobulins (G, A, M and D) in this collection of sera would throw any light on the pathogenesis of immunodeficiency or lymphoreticular malignancies, or help to explain why there has been such a high incidence of these disorders in the communities. For example the hypothesis quoted above might predict the presence of either low or may be high immunoglobulin concentration in some family members. Specific objectives were as follows: (1) to measure immunoglobulins (G, A, M and D) in members of these 3 communities; (2) for comparison, to measure immunoglobulins on a suitable control group; (3) to perform statistical analysis of the results; (4) in a separate analysis, to compare immunoglobulin concentrations (G, A, M and D) in relation to different tonsil sizes, looking for association and using the tonsil size data already available; (5) to interpret the results of the analysis, as far as possible, in both biological and clinical terms; (6) to delineate the next step to be undertaken for continuation of the investigation of pathogenesis of immunodeficiency and malignancy in this extended family.

POPULATION, MATERIALS AND METHODS

Description of the Population Studied

People in the study population reside in three communities situated very close together in St. Barbe South, in Newfoundland, Canada. The ancestors of these three communities originated mainly from either the South West Coast of England or from the Channel Islands, and a few were of the French or Acadian descent.

The total number of individuals in the three communities were 345 (165 males and 180 females); 490 (260 males and 230 females); and 575 (315 males and 260 females) respectively (Statistics Canada 1971 census). The occupation of most of the adults is fishing (cod, salmon and herring). There is also some lumbering and zinc mining. Some workers of the community are on social welfare because of the declining fishery coupled with the reluctance to move away to areas where other job opportunities may exist. Those who move away from the communities often return for visits or stay in the area during the summer fishing season.

Rearing of large families is very common (Fig. 1). Most of the children remain in the communities and very few outsiders have moved in thus favouring increasing intermarriage between close relations (Figs. 2-4). The unusually high level of intermarriage is noted in the common family names. Six such names account for about 70 percent of the population.

Figure 1*

A simplified pedigree of the family which contains multiple cases of lymphoreticular and other tumours and 10 malignancies. The pedigree only represents a minimum number of people and lines to show the relationship of the cases to the common ancestors (John and Mary).

 = Male  = Female

 = Hodgkin's disease

 = Other lymphatic malignancies

 = "Embryonal Tumours"

 = Immunodeficiency

*This pedigree was compiled by Ms. S. K. Buehler and is reproduced here with her permission.

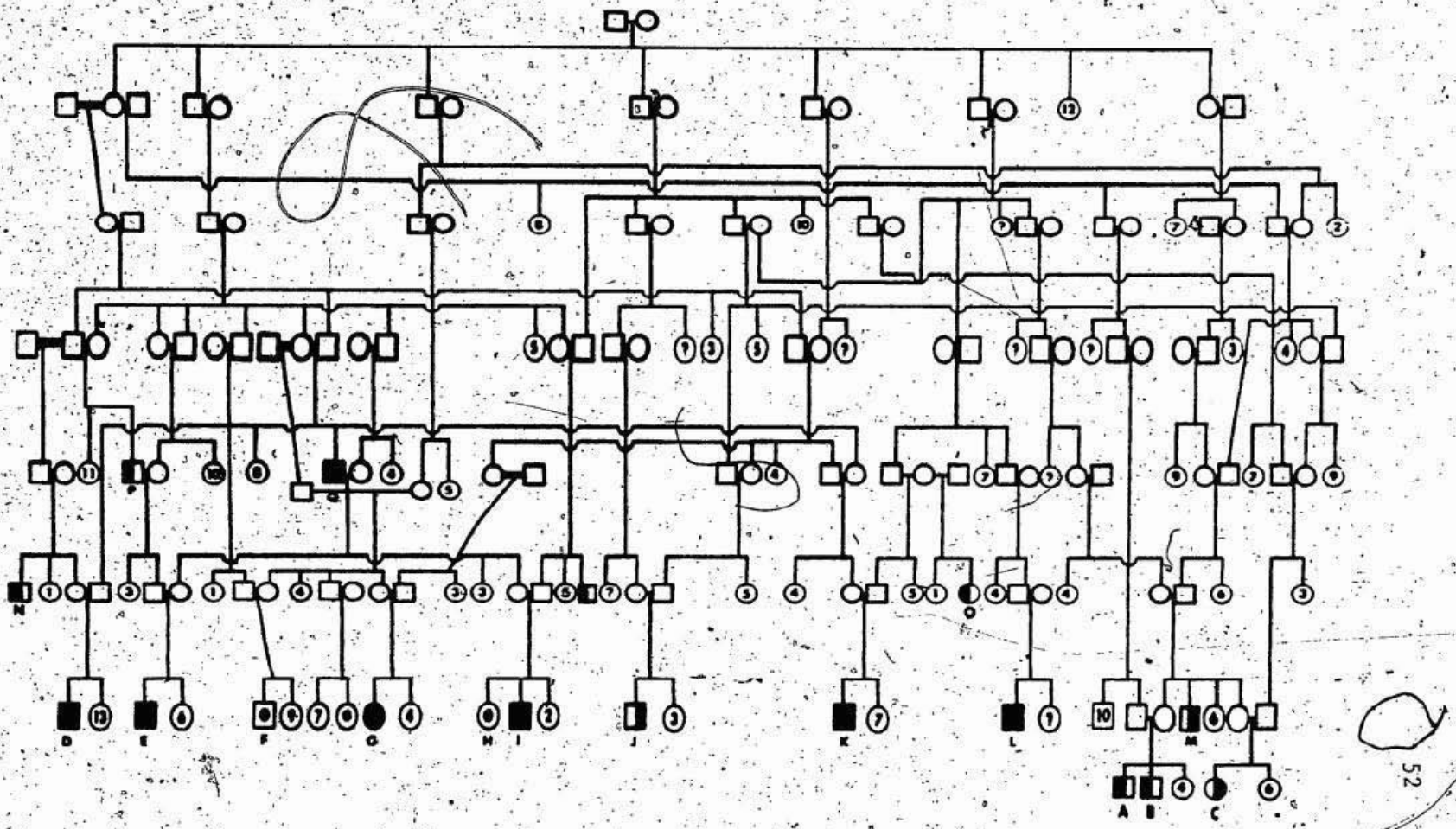

D
E
F
G
H
I
J
K
L
M
A
B
C

Figure 2

Pedigree of a patient (#1261 chronic lymphocytic leukaemia) showing his relationship to the common ancestors of the big pedigree, John and Mary. Patient A's parents were first cousins once removed.
(This pedigree was compiled by Ms. S.K. Buehler and is reproduced here with her permission).

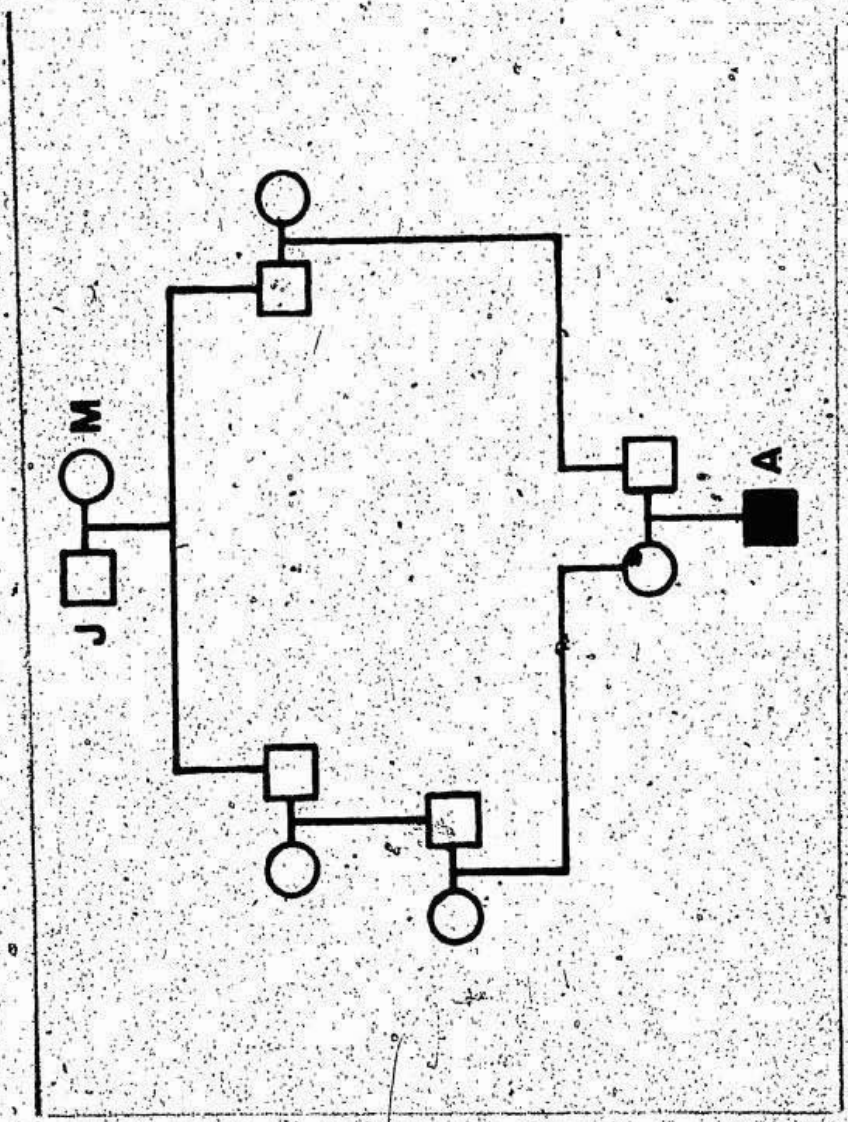

M
J
A

Figure 3

Pedigree of patient (# 6189 Retinoblastoma). This shows the many pathways by which the patient may receive genes from the common ancestors from both her parents.
(This pedigree was compiled by Ms. S.K. Buehler and is reproduced here with her permission).

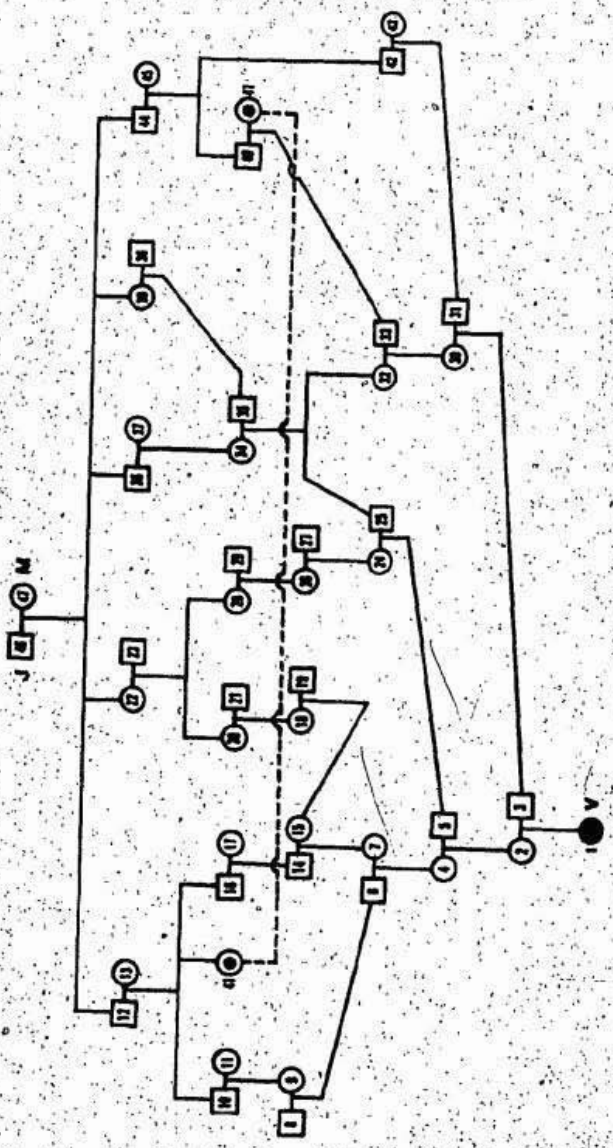
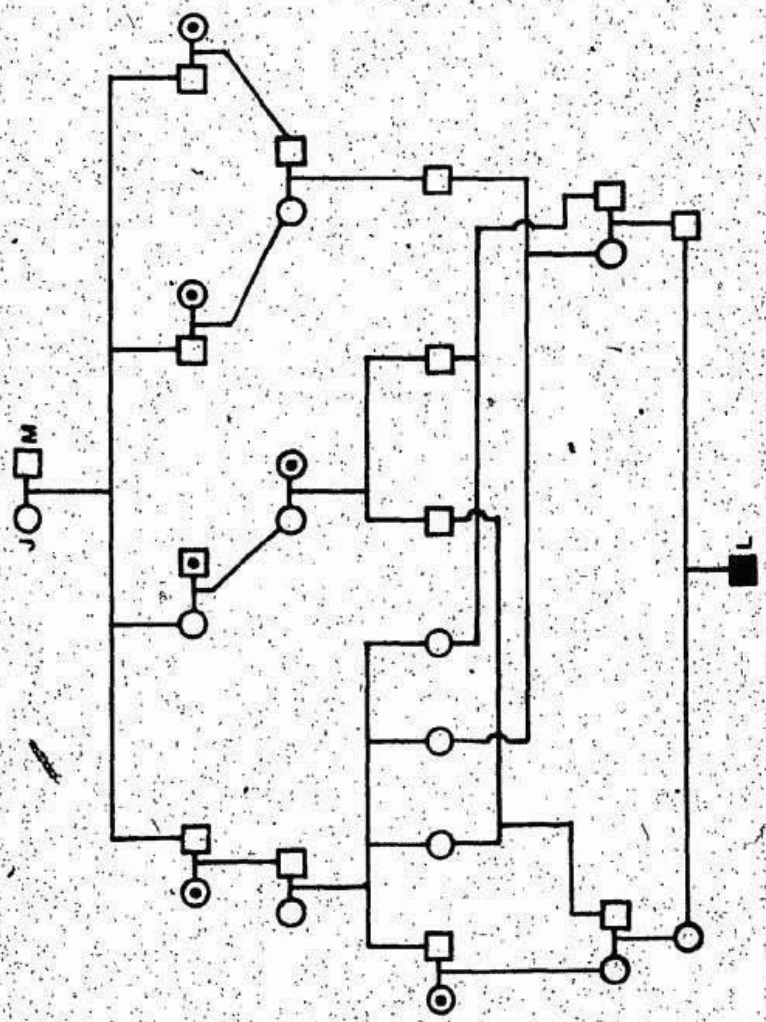


Figure 4

Pedigree of patient (#6800 Hodgkins disease). This shows a pathway intermediate, in complexity when compared to Figures 2 and 3, by which the patient may receive genes from the common ancestors through both his parents. (This pedigree was compiled by Ms. S.K. Buehler and is reproduced here with her permission).



Also the total number of descendants of one founder couple (who migrated to the West Coast from South West of England in 1817) in the 3 communities account for 85 percent, 81 percent and 83 percent respectively of the populations (S. K. Buehler, personal communication)(Fig. 5).

Until 1963, the main approach to the communities was by sea. At that time the first road linking them with the large towns to the south was built. It is a dirt and gravel road that had not been completely paved at the time of this study. The cottage hospital serving the communities is 30 miles from the closest of the 3 communities.

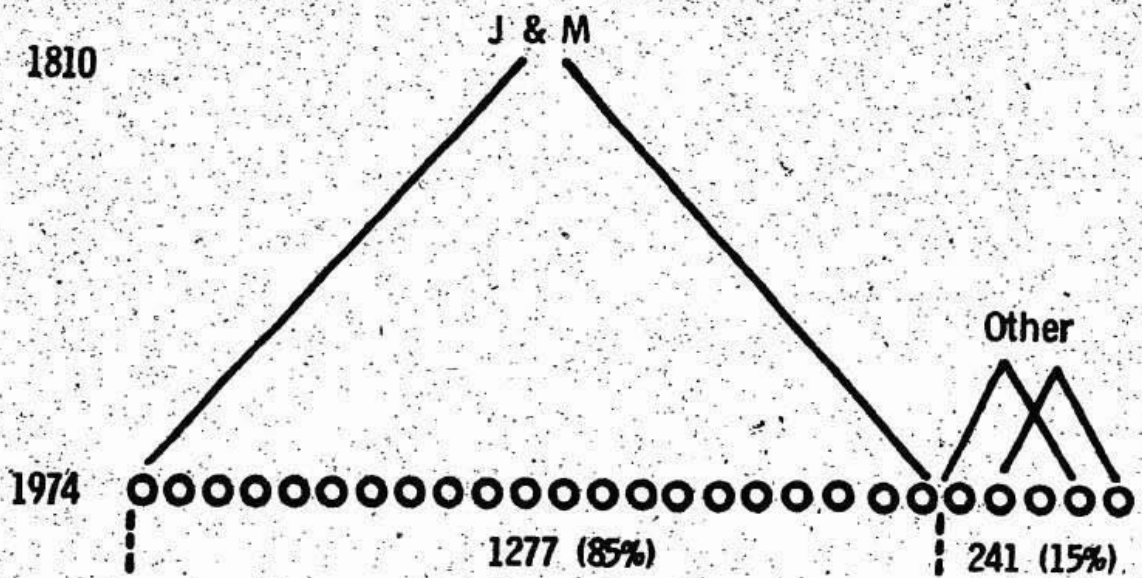
The immunopathological significance of these unique genealogic characteristics is illustrated by the report of Buehler et al., (1975a). She described seven cases of Hodgkin's disease, three of lymphosarcoma, two of thymoma, three common variable immunodeficiency, and single cases of retinoblastoma, neuroblastoma and rhabdomyosarcoma. The recorded deaths due to neoplasms of lymphatic and haemopoietic tissue in a 10-year period 1964-1973 reveal a 5-8 fold higher incidence in these three communities when compared with either Newfoundland as a whole, or with Canada (Buehler et al., 1975b).

Blood Collection

Blood from 939 members of the communities of St. Barbe

Figure 5

A diagram to show the structure of the population. "J & M" are the common ancestors who remained in 1810 and whose family in the community now numbers 1277 people. People not descended from "J & M" labelled here "Other" account for only 15 percent of the present community.



STRUCTURE OF WEST COAST COMMUNITY (POP 1518)

South was collected during a "Health Survey" by venepuncture using vacutainer. Serum was separated after the clot had retracted at room temperature and was stored thereafter at -20°C.

Control serum samples were obtained from 185 apparently healthy Red Cross blood donors (age range 18-65 years) by separation from the pilot tubes; by venepuncture from 71 children (aged 2-17 years) attending the Janeway Child Health Centre, St. John's, for conditions known not to alter immunoglobulin levels; and by deep finger prick, allowing free unassisted flow of blood from 65 apparently healthy school children (aged 6-14 years).

Immunoglobulin Estimations

PRINCIPLE

The method employed depends on antigen-antibody precipitation in agar gel. A monospecific antibody (anti IgG, anti IgA or anti IgM) is incorporated into the agar before it solidifies. The standards and test sera are then allowed to diffuse from circular wells cut in the agar. If the antibody is monspecific and in the right concentration for the range of antigen levels to be determined, a sharply defined precipitin ring forms around the antigen well. After a sufficient time has been allowed for diffusion, the size of the precipitin ring is a function of the initial concentration fo the antigen placed in the well. Measurements

are made of diameters of precipitin rings formed by a series of known concentrations of antigen (Standard solutions) and of the unknown solutions to be tested. A plot of $\log_{10}$ concentration of the standards containing known concentrations of the antigen (ordinate) versus the diameters of precipitate ring (abscissa) produces a straight line. From this standard curve the values of the antigen concentrations in an unknown sample can be determined.

MATERIALS

(a) Phosphate Buffer pH 8.0; 0.3N

Weigh 88.7 gm K_2HPO_4 (anhydrous)

4.42 gm KH_2PO_4 (anhydrous)

Add 18 mls 1M NaN_3 (Sodium Azide) Preservative

Make up to 1800 mls with distilled water.

(b) Noble Agar (Difco) or Agarose (Difco Inc., Detroit).

(c) 1M Sodium Azide.

(d) Hamilton microlitre syringe (50µl)(Hamilton Company, California).

(e) Glass tubes 15 cm X 2.0 cm and 7.5 cm X 1.5 cm.

(f) Circular metal punch for punching holes in agar (2.4 mm. external diameter).

(g) Hyland viewer with micrometer eye piece (Fisher Scientific Co.).

(h) Normal Saline:- 9 gm of NaCl made up to 1 litre with distilled water.

(i) Test antisera:- anti IgG (Behringwerke Batch No. 2622D)

anti IgA (Behringwerke Batch No. 2537T)

anti IgM (Behringwerke Batch No. 2716A)

(j) W.H.O. Reference Preparation No.97/67 (Lausanne).

(k) British Research Standard Solution No. 67/37 (W.H.O. Reference Centre Lausanne).

(l) IgD plates Behringwerke Batch Numbers 3014 and 3066.

(m) Behringwerke IgD Standard Batch No. 674A.

(n) Magnetic Stirrer (Pyro Magnestir, Labline Instruments Inc., Chicago, Ill.).

(o) Ponceau S Dye:- 3.0 gm Trichloroacetic acid was dissolved in 100 ml water. 0.2 gm of Ponceau S was dissolved in the trichloroacetic acid solution.

(p) Coated microscope slides:- 7.5 cm X 2.5 cm microscope slides were cleaned in methanol and dried. They were then coated with agar by being immersed in hot 0.2 percent molten noble agar made up in phosphate buffer pH 8.0, removed and allowed to dry standing upright in a drying rack.

(q) Coated Photographic glass plates:- 8.2 cm X 10.2 cm photographic glass plates (Eastman Kodak Co., Rochester, New York) were boiled in water until all the photographic emulsion was removed. The plates were allowed to cool. They were washed in tap water and rinsed several times in distilled water. When dry, the plates were coated with 0.2 percent noble agar in the same manner as had been used for microscope slides.

(r) Determination of Monospecificity of Antisera:- Immunoelectrophoreses were run with each test anti-serum against whole human serum. The single precipitin arcs produced (Figs. 6) show that each antiserum is monospecific.

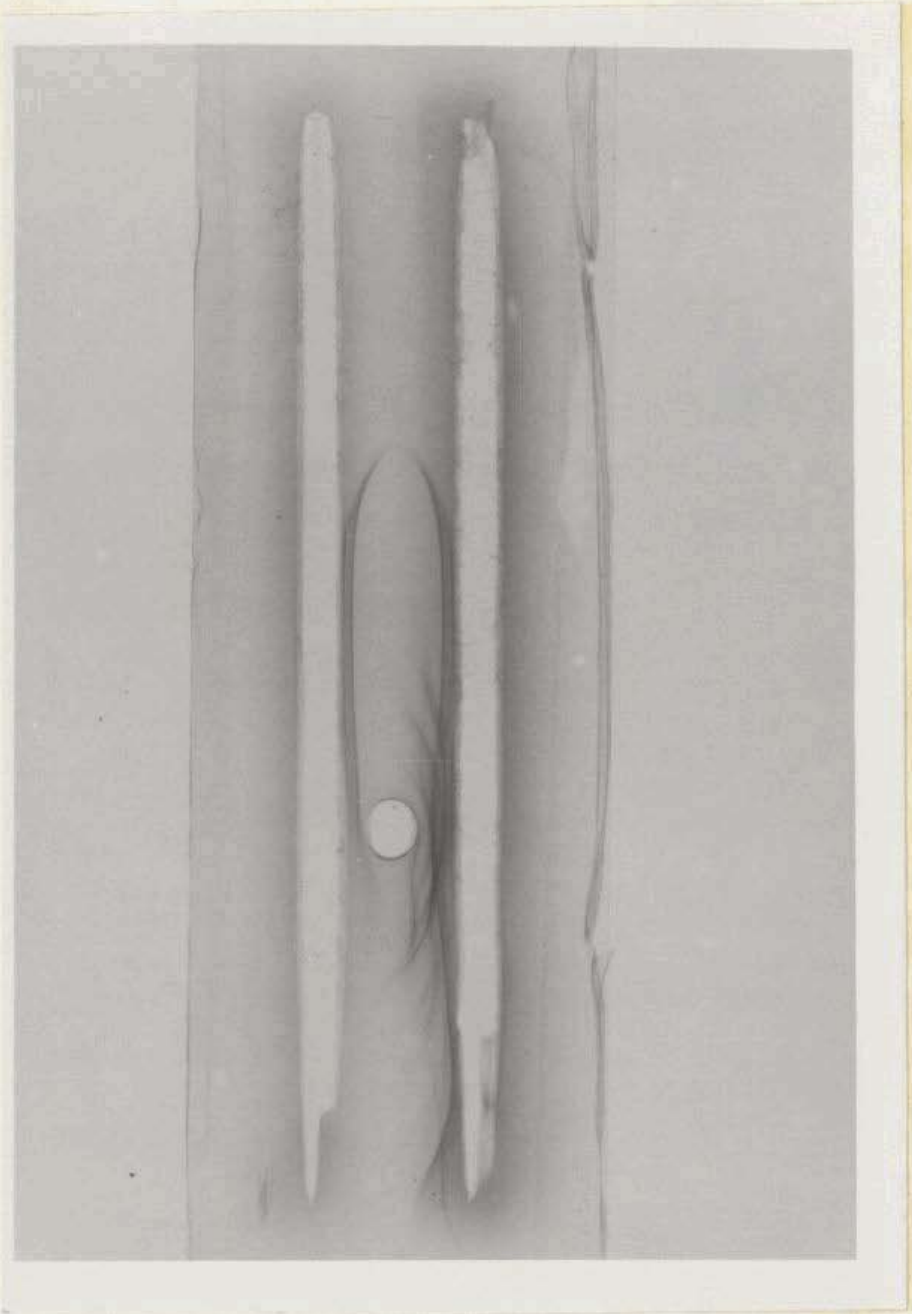
METHOD

The method is a slight modification of the single radial diffusion technique of Fahey and McKelvey (1965).

Figure 6

This Figure shows immunoelectrophoretic pattern of anti IgG used in the study. Top trough contained test antiserum (Behringwerke). Botton trough contained anti whole human serum (Behringwerke as control). The well contained whole Human serum diluted 1 in 5. The single arc confirms monospecificity of the serum.

The monospecificity of antisera against IgA and IgM was similarly shown. The same batch of antiserum was used for all samples.



Preparation of Agar

297 ml cold phosphate buffer pH 8.0 plus 3 ml of 1M sodium azide were added to 7.2 gm noble agar in a 1 litre conical flask to make 2.4 percent agar suspension. The level of the agar suspension was marked on the conical flask. The agar suspension was constantly stirred on a magnetic stirrer with heat until the agar was completely dissolved. If, at the end of this time, the level was less than the original level due to evaporation, more phosphate buffer was added until the original level was reached. The agar solution was distributed in 8 ml aliquots in 15 cm X 2.0 cm tubes. The agar was allowed to cool and solidify on the bench. The tubes were covered with rubber stoppers and stored in the refrigerator at 4°C until needed.

Preparation of Plates

The solidified agar (2.4 percent) was placed in a boiling water bath to melt, it was distributed in aliquots (1.5 ml for microscope slides, 8 ml for the photographic plate) in 7.5 cm X 1.5 cm tubes and placed in 56°C water bath for 5-10 mins. A volume (1.5 ml for microscope slides and 8 ml for photographic plates) of the appropriate anti-serum in an optimal dilution (see later) was placed in 56°C water bath in 7.5 cm X 1.5 cm tubes for at least 10 mins. The agar was then quickly and thoroughly mixed with the particular antiserum and poured on coated microscope slides or photographic plates placed on a levelled surface. For the

latter 6-10 inversions of the tube were routinely made in order to mix the reagents. Formation of air bubbles was totally avoided as they interfere with the precipitin rings.

A series of wells was cut in the agar plate with the metal punch spaced at 12 mm between centres for the IgG, IgA and IgM. Agar was carefully removed from wells with a smooth edged pasteur pipette attached to a vacuum pump, taking care not to damage the sides of the well. The plates were now ready for use.

Use of the Plates

The wells were each filled with a measured volume (see later) of test or standard serum using a Hamilton micro-litre syringe which was rinsed three times in saline in between samples. The plates were placed in humid boxes. The IgG plates were placed in a 37°C incubator whilst the IgA and IgM plates were placed in the refrigerator at 4°C. The time of incubation was determined by preliminary tests which are detailed later.

The diameters of the precipitin rings were measured in two directions at right angles to the nearest 0.1 mm using a Hyland viewer with a micrometer eye piece (Figs. 7-10).

For permanent keeping, the plates were stained as follows: The plates were placed in normal saline. The saline was changed at least 4 times in 24 hours. The plates were then placed in tap water. The water was changed twice in 2-3 hrs. Plates were removed and wet filter paper was

Figure 7

This Figure shows a plate used for IgM quantitation. Sixty-three wells can be seen, each with a precipitin ring surrounding it; some are for test sera and others for standards (see text).

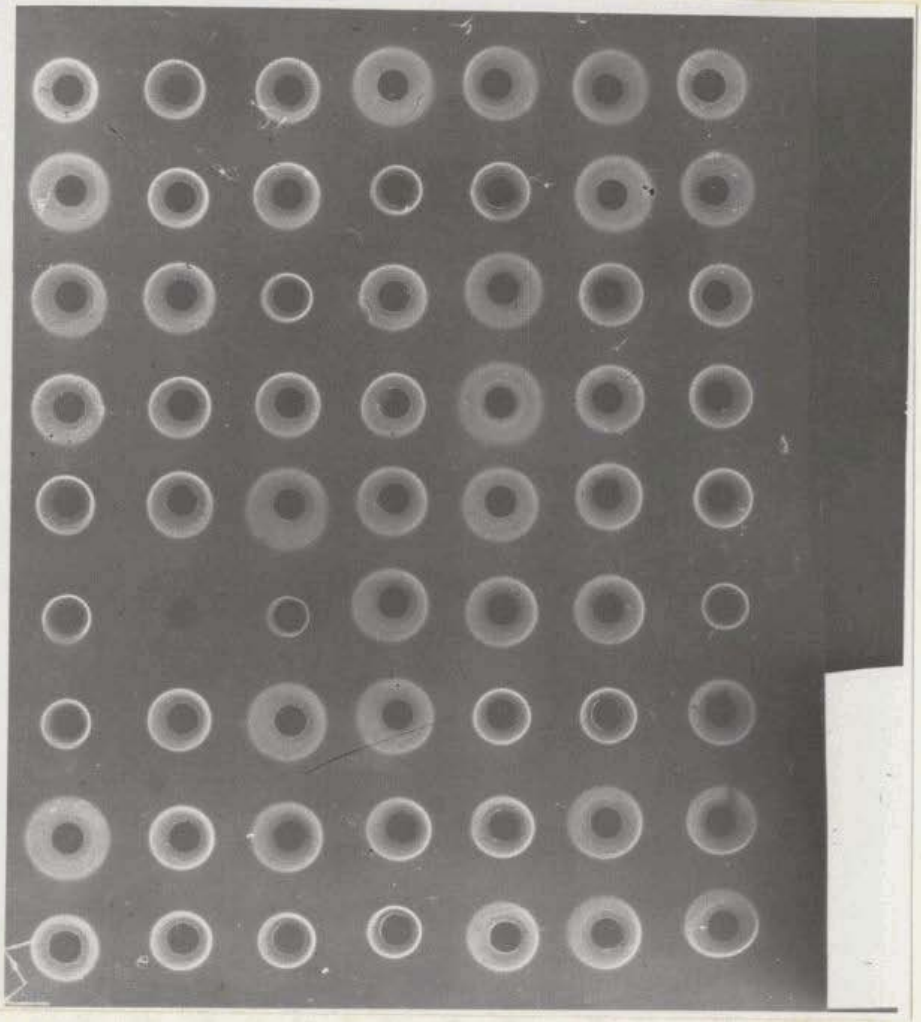


Figure 8

This figure shows the IgG standard graph. Log_{10} concentrations (ordinate) were plotted against the diameters (mm) of the precipitin rings.

IgG STANDARD CURVE

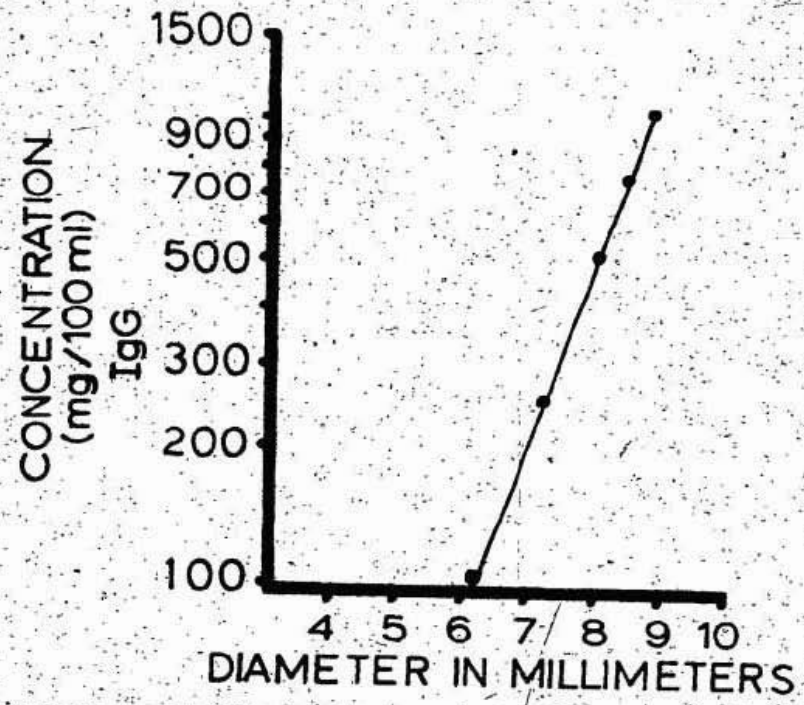


Figure 9

This figure shows the IgA standard graph. Log_{10} concentrations (ordinate) were plotted against the diameters (mm) of the precipitin rings.

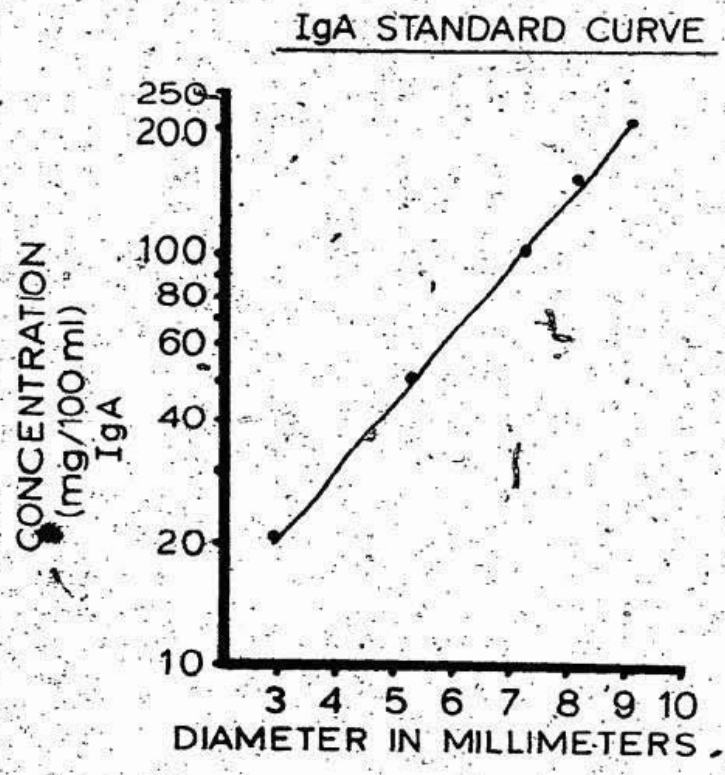
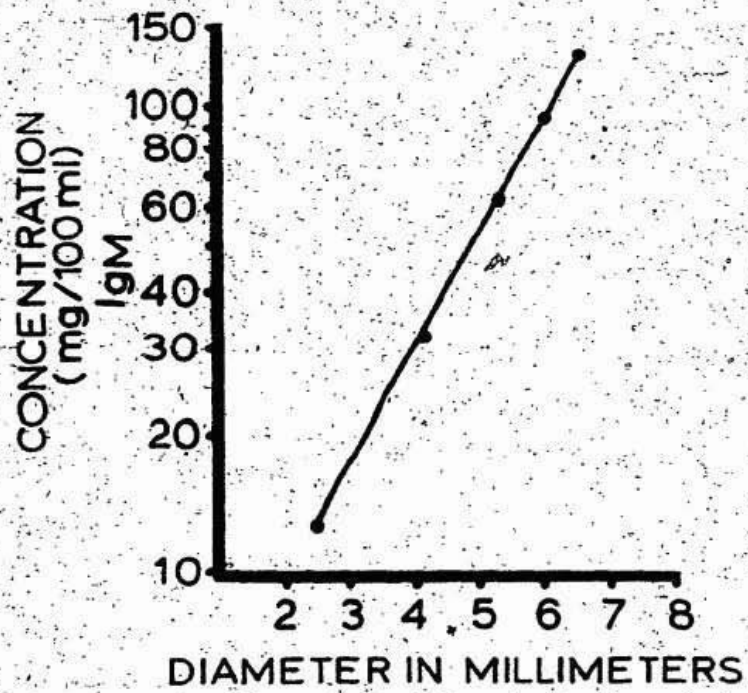

IgA STANDARD CURVE
CONCENTRATION (mg/100 ml) IgA
250
200
100
80
60
40
20
10
3 4 5 6 7 8 9 10
DIAMETER IN MILLIMETERS

Figure 10

This figure shows IgM standard graph. Log_{10} concentrations (ordinate) were plotted against the diameters (mm) of precipitin rings.

IgM STANDARD CURVE



applied on the agar surface. They were left on the bench with the filter papers on them for a day or two until dry. The filter papers were removed. Plates were then stained in Ponceau S for 20 mins. Excess stain was washed off in several changes of 5 percent acetic acid. Wet filter papers were applied on the surface of the plates. The plates were left on the bench overnight to dry.

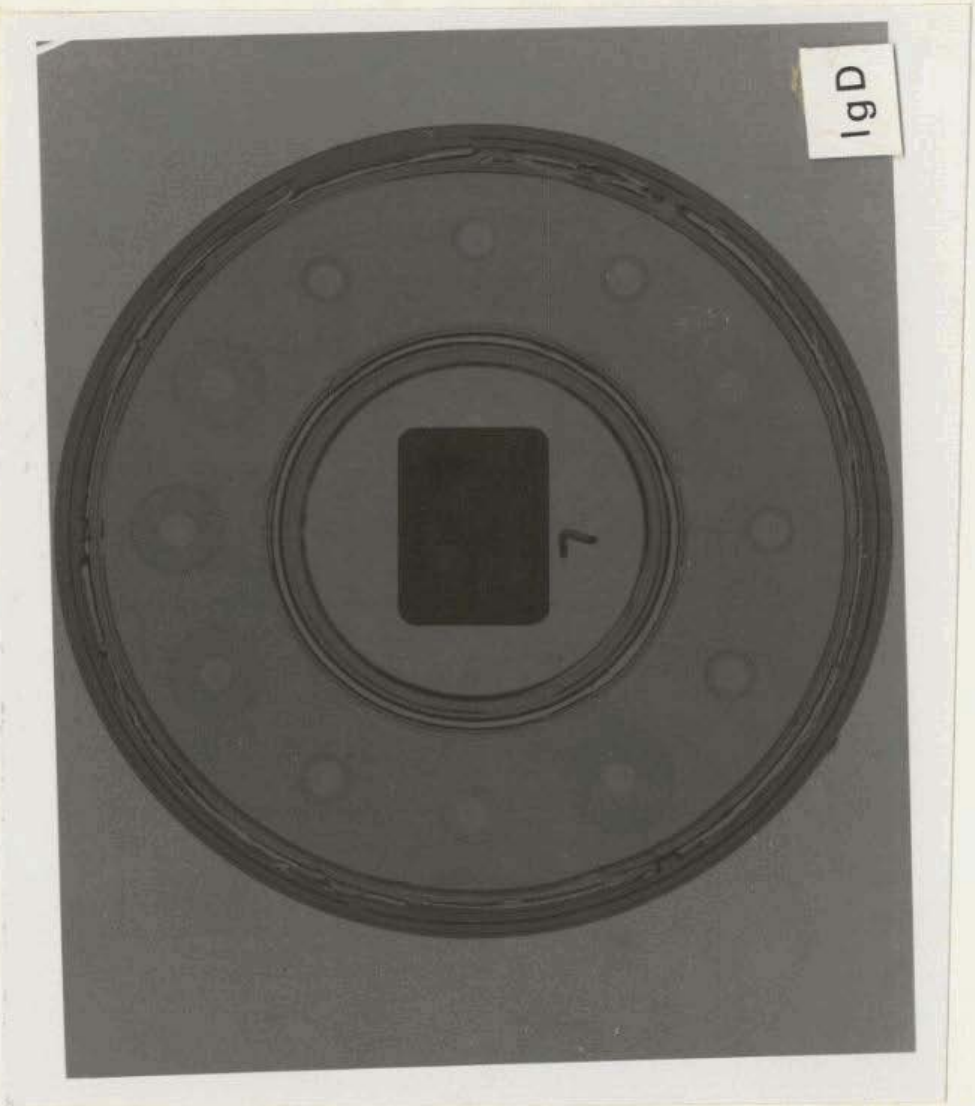
IgD Estimations

Serum IgD levels were measured using commercially prepared monospecific IgD plates and standards which were stored at 4°C. Before use, each place was opened and left at room temperature for 5-10 mins. The IgD standard containing 208 International Units of IgD per ml was diluted 1 in 2, 1 in 4 and 1 in 8 to give 104 units, 52 units, and 26 units per ml respectively. 20 microlitres (0.02 ml) of the test sample or standard solution was placed in each of the wells using a Hamilton mirco-syringe. Wells 1, 4, 8 and 11 of the first plate of every batch of estimations were filled with the standards. Each of the other plates contained at least one Behringwerke standard solution. In addition, IgD standard from the WHO Reference Centre was included in each batch of measurements.

After all the wells were filled, the plates were closed tightly and allowed to diffuse for 3 days at room temperature (Fig.11). The diameters of the precipitin rings were measured in two directions at right angles to the

Figure 11

Photograph of a plate used for IgD quantitation. There are 12 wells and around each can be seen precipitin ring produced by test serum. One well is occupied by a standard serum of known IgD concentration.


IgD
7

nearest 0.1 mm using a Hyland viewer with micrometer eye piece and the average values were taken.

Plots were made on ordinary millimeter graph paper, with the squared ring diameters on the ordinates and the concentration of the standards on the abscissa. These gave straight line graphs intercepting the ordinate at 20 (Fig.12). This was confirmed on communication with Behringwerke. Concentrations of the test solutions were obtained from the Standard Curve.

PRELIMINARY TRIALS

It was necessary initially to find the optimum concentration of each antiserum and the optimum time for reading the results. Experiments were conducted using various concentrations of antiserum and various times for reading the results as shown. Duplicate wells were filled for each dilution of antiserum and for each of 5 concentrations of a standard pool of normal serum. In addition various volumes of serum were tried for filling the wells.

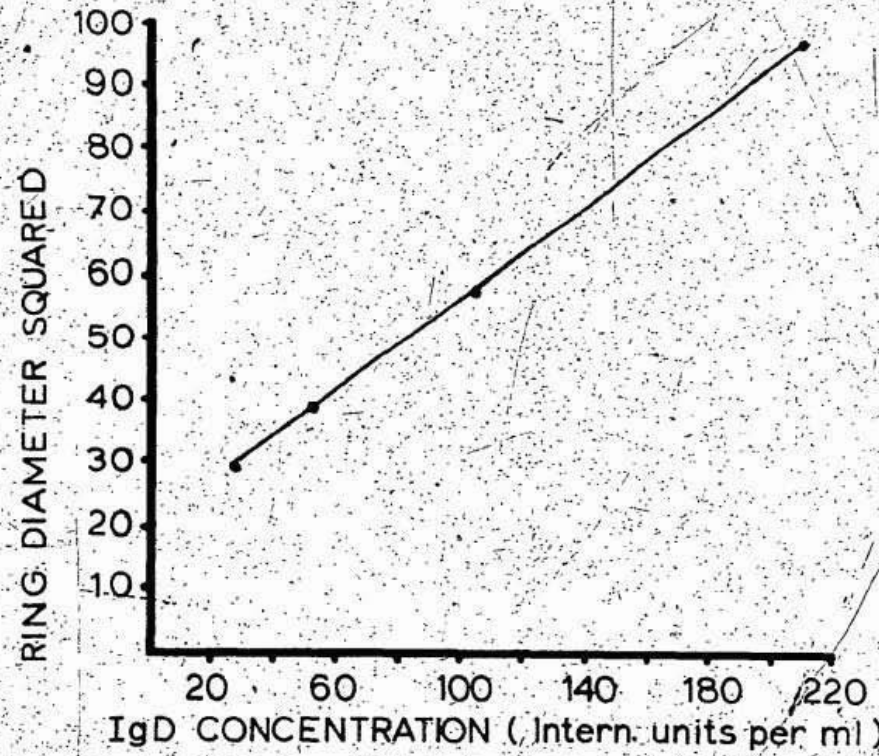
Dilution of antisera 1 in 5, 1 in 6, 1 in 8, 1 in 10, 1 in 12.5 1 in 20, 1 in 30, and 1 in 40.

Times of reading ring sizes:- IgG: 2½ hrs, 3 hrs, 3½ hrs, 4 hrs, 4½ hrs, and 5 hrs.

IgA and IgM: 16 hrs, 18 hrs, 20 hrs, 22 hrs, 24 hrs, 26 hrs, 28 hrs, 30 hrs.

Figure 12

This figure shows the IgD standard graph. The precipitin ring diameters, squared, (ordinate) were plotted against IgD concentrations (abcissa).


RING DIAMETER SQUARED
100
90
80
70
60
50
40
30
20
10
20
60
100
140
180
220
IgD CONCENTRATION (Intern. units per ml)

RESULTS

It was observed that at high antiserum concentrations the ring precipitates of all the standards were relatively small and intense. With progressively lower antiserum concentrations the precipitates became less distinct and their diameters increased. The results of these observations and the subsequent plots of the ring diameters of the 5 concentrations of standard serum (100%, 75%, 50%, 25%, 10%) are given in the Tables which follow. The most appropriate antiserum dilutions giving well defined precipitin rings were 1 in 5 for anti IgG; 1 in 6 for anti IgA and 1 in 10 for anti IgM. The best volumes for filling the wells was found to be 6µl (0.06 ml) for IgG as well as IgA, and 8µl (0.08 ml) for IgM. The optimal times for reading the ring diameters under these conditions and which gave a straight line plot for the standards were found to be 3-5 hrs. at 37°C for IgG, 18-30 hrs. for IgA, and 20-30 hrs. for IgM. (Tables 1 - 3)

Table 1b

Using 4µl Volume for Each Standard

Immuno-globulin	Incubation Time	Diameter Readings		
		Highest Standard (100 Percent)	Lowest Standard (10 Percent)	Comment
IgG	2½ hrs.	7.0 mm	4.0 mm	Low Reading
	3 hrs.	7.3 mm	4.0 mm	Low Reading
	3½ hrs.	7.5 mm	4.1 mm	Low Reading
	4 hrs.	7.8 mm	4.3 mm	Low Reading
	4½ hrs.	7.9 mm	4.3 mm	Low Reading
	5 hrs.	8.0 mm	4.3 mm	Low Reading
IgA	16 hrs.	7.8 mm	2.5 mm	Low Reading
	18 hrs.	7.8 mm	2.5 mm	Low Reading
	20 hrs.	7.9 mm	2.5 mm	Low Reading
	22 hrs.	8.0 mm	2.5 mm	Low Reading
	24 hrs.	8.1 mm	2.5 mm	Low Reading
	26 hrs.	8.1 mm	2.5 mm	Low Reading
	28 hrs.	8.1 mm	2.5 mm	Low Reading
	30 hrs.	8.2 mm	2.5 mm	Low Reading
IgM	16 hrs.	4.5 mm	<2.5 mm	Low Reading
	18 hrs.	4.6 mm	<2.5 mm	Low Reading
	20 hrs.	4.7 mm	<2.5 mm	Low Reading
	22 hrs.	4.7 mm	<2.5 mm	Low Reading
	24 hrs.	4.8 mm	<2.5 mm	Low Reading
	26 hrs.	5.0 mm	<2.5 mm	Low Reading
	28 hrs.	5.0 mm	<2.5 mm	Low Reading
	30 hrs.	5.1 mm	<2.5 mm	Low Reading

Table 2

Using 8µl Volume for Each Standard

Immuno-globulin	Incubation Time	Diameter Readings		Comment
		Highest Standard (100 Percent)	Lowest Standard (10 Percent)	
IgG	2½ hrs.	9.9 mm	6.9 mm	Rings overlapping
	3 hrs.	10.0 mm	7.2 mm	Rings overlapping
	3½ hrs.	>10.0 mm	7.4 mm	Rings overlapping
	4 hrs.	>10.0 mm	7.5 mm	Rings overlapping
	4½ hrs.	>10.0 mm	7.5 mm	Rings overlapping
	5 hrs.	>10.0 mm	7.5 mm	Rings overlapping
IgA	16 hrs.	>10.0 mm	3.6 mm	Rings overlapping
	18 hrs.	>10.0 mm	3.7 mm	Rings overlapping
	20 hrs.	>10.0 mm	3.7 mm	Rings overlapping
	22 hrs.	>10.0 mm	3.8 mm	Rings overlapping
	24 hrs.	>10.0 mm	3.8 mm	Rings overlapping
	26 hrs.	>10.0 mm	3.9 mm	Rings overlapping
	28 hrs.	>10.0 mm	3.9 mm	Rings overlapping
	30 hrs.	>10.0 mm	3.9 mm	Rings overlapping
IgM	16 hrs.	6.2 mm	2.5 mm	Adequate ring diameters
	18 hrs.	6.3 mm	2.5 mm	Adequate ring diameters
	20 hrs.	6.4 mm	2.6 mm	Adequate ring diameters
	22 hrs.	6.5 mm	2.6 mm	Adequate ring diameters
	24 hrs.	6.6 mm	2.6 mm	Adequate ring diameters
	26 hrs.	6.7 mm	2.6 mm	Adequate ring diameters
	28 hrs.	6.7 mm	2.7 mm	Adequate ring diameters
	30 hrs.	6.8 mm	2.7 mm	Adequate ring diameters

Table 3 —

Using 6µl Volume for Each Standard

Immuno-globulin	Incu-bation Time	Diameter Readings		
		Highest Standard (100 Percent)	Lowest Standard (10 Percent)	Comment
IgG	2½ hrs.	7.9 mm	6.1 mm	Adequate ring diameters
	3 hrs.	8.1 mm	6.3 mm	Adequate ring diameters
	3½ hrs.	8.4 mm	6.4 mm	Adequate ring diameters
	4 hrs.	8.8 mm	6.6 mm	Adequate ring diameters
	4½ hrs.	9.0 mm	6.6 mm	Overlapping
	5 hrs.	9.1 mm	6.7 mm	Overlapping
IgA	16 hrs.	8.6 mm	2.7 mm	Adequate ring diameters
	18 hrs.	8.8 mm	2.7 mm	Adequate ring diameters
	20 hrs.	9.0 mm	2.7 mm	Adequate ring diameters
	22 hrs.	9.1 mm	2.8 mm	Adequate ring diameters
	24 hrs.	9.2 mm	2.8 mm	Adequate ring diameters
	26 hrs.	9.3 mm	2.9 mm	Overlapping

STANDARDS

The 100 percent standard consisted of pooled sera from 16 members of the staff (aged 19-24 years) of the Immunology Department of the Memorial University. Duplicate 4µl volumes of those standards were placed in the wells of one plate; and duplicate 8µl volumes were placed in the second plates both for IgG, IgA, or IgM.

The immunoglobulin content of these standards was later converted to mg per 100 ml by setting them up with the World Health Organization Reference Preparation (No. 97/67) containing 96.2 international units of IgG per ml corresponding to 8.2 mg IgG per ml; 95.3 international units of IgA per ml corresponding to 1.43 mg IgA per ml; and 96.2 international units IgM per ml corresponding to 0.86 mg IgM per ml (Rowe et al., 1970a). The 100 percent pooled standard sera were found to contain 10.25 mg per ml IgG; 20.4 mg per ml IgA; and 1.27 mg per ml IgM by this conversion.

FINAL DESIGN

The collection of sera was tested on quantitative plates made on 43 glass photographic plates. There were 63 wells per plate; 50 of these were filled with test samples and 13 were filled with standards. All the test sera whose ring diameters lay outside the standard range 10 percent to 100 percent were repeated. Values higher

than 130 percent were verified by repeated examination of the undiluted as well as a 1 in 4 dilution of the test samples in phosphate buffer. Values obtained for the latter were multiplied by the dilution factor (4).

IgD was quantitated on 132 IgD plates. There were 12 wells per plate; 10 of these at most were filled with test samples and at least two with standards. No test ring diameter was higher than the highest standard reading.

REPRODUCIBILITY OF THE TECHNIQUE (FOR IgG, IgA AND IgM)

The coefficient of variation for repeated measurement of the same sample which was carried out throughout the experiment was computed using the formula $100t_{n-1}(SD/Mean)$ to find the 95 percent confidence limits for each assay. The values were ± 6 percent for IgG; ± 9 percent for IgA and ± 8 percent for IgM. The Standard Errors of the Mean in these repeated sample measurements were also computed to be 7.2, 2.3 and 1.0 respectively for IgG, IgA and IgM as shown in Table 4 which follows.

Table 4

Repeated Estimations of Test Sample No. 3693

		IgG mg/100 ml	IgA mg/100 ml	IgM mg/100 ml
	1	953	135	89
	2	1025	131	92
	3	974	129	89
	4	1025	139	96
	5	974	131	89
	6	943	131	91
	7	974	139	94
	8	1004	141	99
	9	974	147	89
	10	984	133	96
	11	974	143	89
	12	1004	129	95
	13	974	139	91
	14	943	129	87
	15	1025	141	89
Σ (sum of)		14750	2037	1375
$\bar{x}$ (Mean)		983	135.8	91.6
SD^2 (Variance)		769	34.2	12.5
SD (Standard Deviation)		27.7	5.8	3.5
SED (Standard Error of the Mean)		7.2	2.3	1
Coefficient of Variation ($100t_{n-1}$ (SD/Mean)		±6%	±9%	±8%

Table 5

Repeated Quantitations of Test Sample No. 3693

		µg per ml IgD
	1	50.76
	2	42.30
	3	42.30
	4	50.76
	5	42.30
	6	42.30
	7	50.76
	8	42.30
	9	42.30
	10	50.76
	11	42.30
	12	42.30
	13	54.99
	14	42.30
	15	42.30
Σ (sum of)		681.03
$\bar{\chi}$ (Mean)		45.02
SD^2 (Variance)		21.80
SD (Standard Deviation)		4.70
S.E.M.		1.20
Coefficient of variation ($100t_{n-1}$ (SD/Mean)		±22%

REPRODUCIBILITY OF THE TECHNIQUE FOR IgD

The coefficient of variation for repeated measurements of IgD on the same sample which were carried out throughout the experiment was computed using the formula $100t_{n-1}$(SD/Mean) to find the 95 percent confidence limits for each assay. The results are as shown in Table 5 which follows. This value for IgD was found to be ± 22 percent. The standard error of the mean was found to be 1.2.

D. Storage and Computer Handling of Results

Each member in the survey was assigned a number on arrival at the clinic. All blood samples were labelled with that number. Forms were filled for the people studied which contained all the relevant clinical information. This was later key punched in a master file on magnetic tape.

Immunoglobulin results were copied from laboratory note books on to transfer sheets together with the patient identification numbers. Data from the transfer sheets were key punched and added to the master file.

The relevant information for the analysis reported in this thesis which are contained in the computer master file are as follows: (1) Patient identification numbers which run from 1001 to 4002; (2) Sex; (3) Age; (4) IgG concentration; (5) IgA concentration; (6) IgM concentration; (7) IgD concentration; (8) Tonsil size [(i) Absent or

vestigial, (ii) Normal, (iii) Enlarged, (iv) Tonsilectomy, N.B. all those individuals who had absent tonsils due to tonsillectomy are in group (iv); those of group (i) are naturally absent or vestigial]. The tonsil sizes are reported as (a) Vestigial, if only a small tag of lymphoid tissue was present in the tonsillar bed. (b) Normal, if tonsil was easily visible but did not project beyond the anterior faucial pillars. (c) Enlarged, if tonsil projected beyond the anterior faucial pillars. This classification was done by physicians.

For the analysis it was necessary to extract this information from the master file and to create a subfile where group identifications were added. The groups to be studied were as follows:

(a) Group 1: 1st and 2nd degree relatives of Hodgkins Disease patients plus direct line descendants.*

(b) Group 2: 1st and 2nd degree relatives of patients with lymphosarcoma and embryonic tumours.

(c) Group 3: 1st and 2nd degree relatives of patients with immunodeficiency,** leukaemia and thymoma.

(d) Group 4: Controls from elsewhere (Healthy blood bank donors plus children in St. John's).

---

*These are all people in the Line of Direct Descent from John and Mary (Identification Numbers 6501 and 6502) to the patients.

**The number of propositi with immunodeficiency and their first degree relatives was too small for being analysed as a separate group.

(e) Group 5: Non-descendants of John and Mary (founder couple) who live in the study community.

(f) Group 6: Remainder of the community not in (a) to (e) above.

The subfile was prepared using a specially designed programme and a deck of cards with the appropriate identification numbers and group identifications punched on to them. The numbers themselves were obtained either from pedigree charts, or a print-out from the pedigree file, or else were available in a work book provided to the collaborators in this multidisciplinary study. The whole procedure is summarised in Figure 14 which follows on page 97.

E. Statistical Analysis

THE RAW DATA

Sera from 939 people were available which had been collected in a Health Survey carried out in 1974. IgG, IgA, IgM and IgD concentrations were measured as detailed in the Methods Section. These raw data are given as an appendix (Appendix 1). The control samples were collected from blood donors across the Province and healthy school children in St. John's (see under "Population Materials and Methods").

POPULATION STRUCTURE BY AGE AND SEX

The population whose immunoglobulin concentrations were studied represent about 70 percent of the total population of the 3 communities. Attendance by children as well as the mothers who brought them was high. This is apparent

from the age and sex structure of the population who gave blood for immunoglobulin measurements (Fig. 13 below). Except for 35-40 year age group, more females of child bearing age attended the clinic than their male counterparts.

After the age of 70 years, the number of people who gave blood was relatively small, hence my using 70 years of age as the cut off point in my estimations.

GROUPS FOR COMPARISON

I chose three main groups of relatives and three groups of controls for my study, (referred to collectively as clinical groups). These are (1) 1st and 2nd degree relatives of Hodgkin's disease patients plus direct line descendants connecting the common ancestors John and Mary to the 19 patients (210 people); (2) 1st and 2nd degree relatives of patients with lymphosarcoma or embryonic tumours (74 people); (3) 1st and 2nd degree relatives of patients with immunodeficiency, leukaemia or thymoma (116 people).

The three control groups are (4) blood transfusion donors and healthy school children from elsewhere (321 people); (5) Non-descendants of the founder couple who live in the study community (116 people); (6) Remainder of the community not included in any of the above groups (559 people).

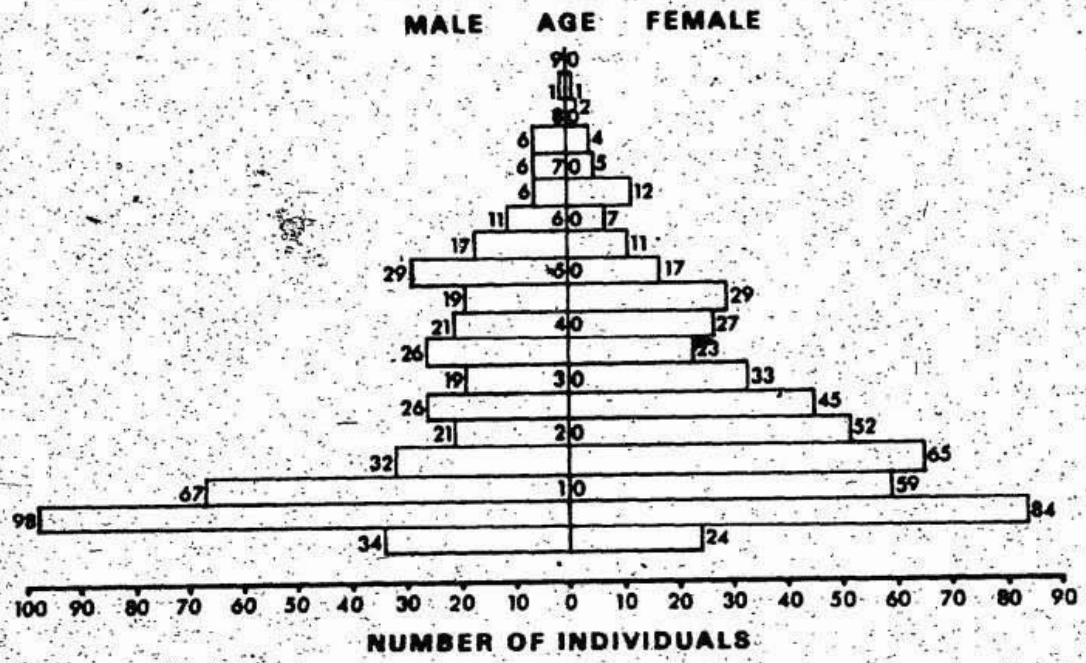
The study population and controls were divided into seven age groups: Group 1 (1-5 years), Group 2 (6-9 years), Group 3 (10-14 years), Group 4 (15-19 years), Group 5 (20-36 years), Group 6 (37-52 years); and Group 7 (53-70 years).

The tonsil groups were as follows: (1) Absent;

Figure 13

This figure shows age and sex structure of the study population whose immunoglobulin concentrations were measured.

AGE AND SEX STRUCTURE OF POPULATION WHOSE IMMUNOGLOBULIN CONCENTRATIONS WERE MEASURED



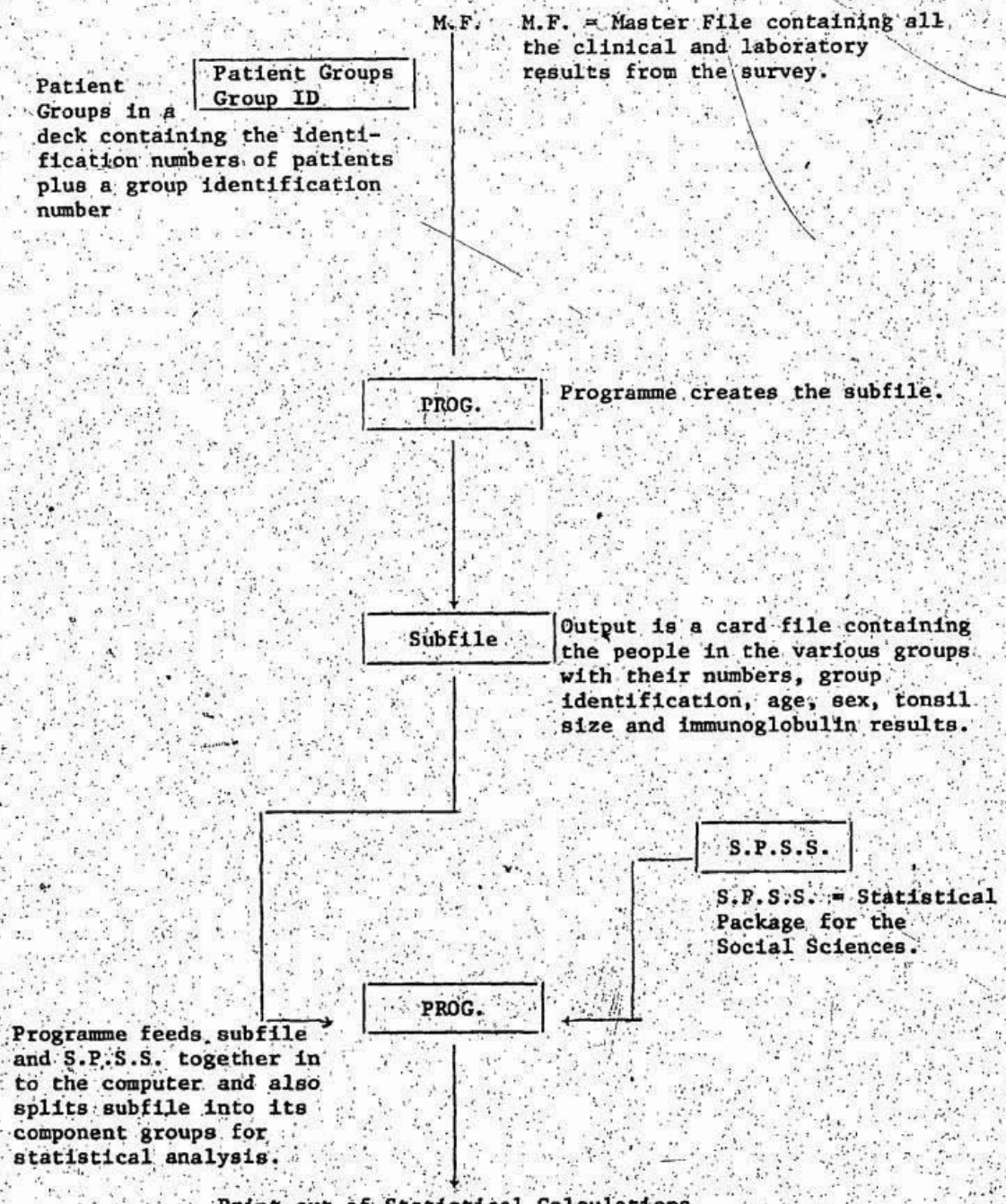

M.F.
M.F. = Master File containing all the clinical and laboratory results from the survey.
Patient Groups
Group ID
Patient Groups in a deck containing the identification numbers of patients plus a group identification number
PROG.
Programme creates the subfile.
Subfile
Output is a card file containing the people in the various groups with their numbers, group identification, age, sex, tonsil size and immunoglobulin results.
S.P.S.S.
S.P.S.S. = Statistical Package for the Social Sciences.
PROG.
Programme feeds subfile and S.P.S.S. together in to the computer and also splits subfile into its component groups for statistical analysis.
Print out of Statistical Calculations.

Figure 14

(2) Vestigial; (3) Normal; (4) Enlarged (5) Tonsillectomy. People with data on tonsil size numbered 1049 and all of them come from the three study communities. None of the controls had been examined in this way.

It was considered that a Multiple Factorial Analysis of variance would be appropriate for the investigation of the data. As stated in the Methods' Section, the data was prepared for analysis by computer using the S.P.S.S. statistical package (Kim and Kohout, 1975). It was decided to undertake three separate analyses (1) Analysis of variance of four major classes of immunoglobulins (IgG, IgA, IgM and IgD) by sex, age and the clinical groupings; (2) Analysis of variance of these immunoglobulins by sex, age and tonsil sizes. The second analysis was carried out only for the community population as tonsil sizes were not measured in the blood donors and St. John's School children. This approach of carrying out two separate analyses instead of a combined one was because the latter process demanded such a large amount of computer storage space and C.P.U. (Central Processing Unit) time that the cost would have been prohibitive; (3) Calculate Mean ± 2SD of IgG, IgA and IgM of controls from other parts of Newfoundland, and compare the number of abnormal individuals.

MULTIFACTORIAL ANALYSIS

Multifactorial analysis of variance (after Log transformation) between five sub-groups of the population (groups 1, 2, 3, 5 and 6) and the control data from elsewhere (group 4) were performed. This analysis of variance

by computer was a collaborative affair; my role in this, and in particular my analysis by hand of a small sample of the data are given below.

ANALYSIS OF VARIANCE

For this analysis, I was given statistical advice and guidance by Dr. David Bryant, and was helped by Mr. Larry Crumley for the data processing since he wrote all the programmes necessary for marrying the data subfile with statistical analysis programme of the S.P.S.S.(Statistical Package for the Social Sciences). My role in this work was (a) to define the various groups of individuals to be compared (see previous section); (b) to assist with the preparation of programme cards by keypunching them and to run, correct and re-run the programme many times until it was satisfactory. I also calculated by hand a small data sample (see below).

CONTROL SAMPLE

In order to be certain that the computer was handling the data correctly and providing an accurate print out, I calculated analysis of variance on a small sample of the data by hand (Tables 6 and 7). When this sample of cards was run with the S.P.S.S. and produced the same results on the print out (Table 8) as I had calculated manually, I knew the results of the analysis of the whole file of data should be accurate.

THE CALCULATION BY HAND

The correction factor "C" for the Total Sum of Squares (SS_{Total}) and Treatment Sum of Squares ($SS_{Treatments}$) =

$$\left(\sum_{i=1}^{A} \sum_{j=1}^{B} \sum_{k=1}^{n} x_{ijk} \right)^2 / ABn$$

A & B are the factors (ID & sex)

i is the number of observations in each group

j is the number of ID groups

n is the number of sexes.

$$C = \frac{(70.131846)^2}{24} = 204.93649$$

$SS_{Total} = (2.816904^2 + 2.92993^2 + 2.940018^2 + 2.950365^2 + 2.885926^2 + \text{-----} + 2.940018^2 + 3.071514^2) - 204.93649$

$= \underline{0.18197}$

$SS_{Treatments} = (5.746834^2 + 5.8145^2 + 5.890383^2 + \text{---} + 5.691297^2 + 6.011532^2) - 204.93649 = \underline{0.10787}$

$SS_A = (11.561334^2 + 11.991012^2 + 11.660129^2 + \text{----} + 11.702829^2) - 204.93649 = \underline{0.03411}$

$$SS_B = \frac{35.005774^2 + 35.126072^2}{12} - 204.93649 = \underline{0.0006}$$

$SS_{AB} = 0.10787 - 0.03411 - 0.0006 = \underline{0.07316}$

$SS_{Residual} = 0.18197 - 0.10787 = \underline{0.0741}$

TABLE 6

ANALYSIS OF VARIANCE CALCULATION BY HAND
Log_{10} IgG by Sex and ID

Factor A (ID)	Factor B (Sex)		Total	Mean
	B1 (Male)	B2 (Female)		
A1 (Group 1)	2.816904 2.929930	2.940018 2.874482	11.561334	2.890334
A2 (Group 2)	2.940018 2.950365	3.010724 3.089905	11.991012	2.997753
A3 (Group 3)	2.885926 3.07154	2.788875 2,913814	11.660129	2.9150322
A4 (Group 4)	2.836957 2.897627	2.836957 2.950365	11.521906	2.880477
A5 (Group 5)	2.974512 3.010724	2.885926 2.823474	11.694636	2.923659
A6 (Group 6)	2.940018 2.751279	2.940018 3.071514	11.702829	2.925707
Totals	35.005774	35.126072	70.131846	
Means	2.917148	2.927173		2.922160

TABLE 6 (CONTINUED)

Cell	A1B1	A1B2	A2B1	A2B2
Total Sum	5.746834	5.8145	5.890383	6.100629
Cell	A3B1	A3B2	A4B1	A4B2
Total Sum	5.95744	5.702689	5.734584	5.787322
Cell	A5B1	A5B2	A6B1	A6B2
Total Sum	5.985236	5.7094	5.691297	6.011532

TABLE 7

ANOVA TABLE

Source of Variation	Corrected Sum of Squares	Degrees of Freedom	Mean Square	F***
B (sex)	0.00060	1	0.0006	0.0971659
A (ID)	0.03410	5	0.006822	1.1047773
AB (ID Sex)	0.07316	5	0.014632	2.3695546
Explained	0.10787	11	0.0098063	1.5880647
Residual	0.07410	12	0.006175	
Total	0.18197	23	0.0079117	

***F is $\frac{\text{Factor Mean Square}}{\text{Residual Mean Square}}$

TABLE 8

COMPUTER PRINT OUT OF THE SAME ANALYSIS

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F
Main Effects	0.0347419	6	0.0057903	0.938
Sex	0.0006056	1	0.0006056	0.098
ID	0.0341363	5	0.0068273	1.106
2-way Interactions	0.073178	5	0.0146356	2.371
Sex ID	0.073178	5	0.0146356	2.371
Explained	0.1079199	11	0.0098109	1.590
Residual	0.0740628	12	0.0061719	
Total	0.1819827	23	0.0079123	

RESULTS

Analysis of Variance of Immunoglobulins by Sex, Age and Clinical Groupings

For all the analyses the level of significance taken was $P \leq 0.05$. The results for each immunoglobulin will be described in turn.

IgG

The analysis (Table 9) shows that there are sex related differences in the immunoglobulin G levels. The data in Table 10 show that females have higher Mean values than males.

There are age related differences (Table 9) and it can be seen in Table 10 where the means are displayed together with the results of Scheffé's S- test that the mean IgG level increases with age. It reaches a peak in the 37-52 year age group after which it drops to puberty mean levels.

Of particular interest is the finding that there are significant differences between the clinical groups (Table 9). In Table 10 the groups have been placed in rank order; from the results of Scheffé's S- test it can be seen that the three groups of relatives of patients have higher Mean IgG concentrations than the three control groups. Furthermore the rank order in the control groups is, from highest to lowest; Group 6 (remainder of the John and Mary pedigree);

TABLE 9

ANALYSIS OF VARIANCE FOR IMMUNOGLOBULINS FROM SPSS
Log_{10} IgG by SEX, AGE AND GROUP IDENTIFICATION

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main effects	1.9857629	12	0.1654802	10.814	0.001
Sex	0.148948	1	0.148948	9.734	0.002
Age	1.2437996	6	0.2072999	13.547	0.001
ID	0.3999942	5	0.0799988	5.228	0.001
2-way interactions	1.2810863	41	0.031246	2.042	0.001
Sex Age	0.2596306	6	0.432718	2.828	0.010
Sex ID	0.1102425	5	0.0220485	1.441	0.206
Age ID	0.9071852	30	0.0302395	1.976	0.002
3-way interactions	0.5834332	24	0.0243097	1.589	0.035
Sex Age ID	0.5834328	24	0.0243097	1.589	0.035
Explained	3.8502875	77	0.0500037	3.268	0.001
Residual	20.1679625	1318	0.0153019		
Total	24.01825	1395	0.0172174		

TABLE 10

Log_{10} IgG

Sex							
	Male	Female					
N	459	590					
Mean	2.9179	2.9506					
**Scheffé's S-test	df = 1.	M.S. = 0.0153019					
Age							
	1	2	3	4	5	6	7
N	95	197	192	135	371	260	146
Mean	2.8466	2.895	2.9233	2.9526	2.9552	2.9588	2.9318
**Scheffé's S-test	df = 6.	M.S. = 0.0153019					
ID (Clinical Groups)							
	2	3	1	6	4	5	
N	74	116	210	559	321	116	
Mean	2.9861	2.9593	2.9429	2.9306	2.9223	2.8949	
**Scheffé's S-test	df = 5.	M.S. = 0.0153019					

Multiple R squared for IgG is 0.083;

**Underlined subset not significantly ($P \leq 0.05$) different; Scheffé's S-test.

Group 4 (blood bank and St. John's children control); Group 5 (community members not in the John and Mary pedigree). The Scheffé's S- test shows that the first 3 groups are not significantly different from each other as a group. They are quite different from the last two (Groups 4 and 5). Comparison between the middle groupings show some overlap at this point.

When the two way interactions are examined, it is seen that there are significant interactions between sex and age as well as between age and clinical group. Both of these, and particularly the latter interaction, should eventually be analysed in detail. In addition there is significant three way interaction between sex, age and clinical group. To pursue these two way and three way interactions further will require the generation of some 14 and 84 Means respectively for Scheffé's S- test.

In Summary

In summary, for IgG, apart from well known sex and age differences, there is good statistical evidence that relatives of the three patient groups had elevated Mean IgG concentrations.

Tonsil Size

In the subsidiary analysis on Tonsil Size in the study community, age and sex factors were also included. Sex and age related differences reported in the previous

analysis were also noted here for IgG, IgA and IgM. In the case of the IgD the study population this time showed no significant age differences whereas in the previous analysis significant variations in IgD levels due to age were found in the combined figures from the study population plus the controls from elsewhere (blood bank and St. John's controls). This might be due to differences in the proportions of the two populations with no detectable IgD levels. About 23 percent of the population in the study community had no detectable IgD levels whereas nearly double this proportion (about 40 percent) had none in the controls from elsewhere.

IgG

The analysis of variance (Table 11) shows that there are differences in the mean IgG levels and the people with vestigeal tonsils have the lowest. However these differences in IgG levels due to variations in tonsil sizes are not significant when Scheffé's S- test was used (Table 12). This test is a more stringent test of probability than the multiple factorial analysis of variance.

When the two-way interactions are examined it is found that there are significant interactions between age and tonsil sizes. This needs further analysis in future work. There is also a significant three-way interaction between sex, age and tonsil sizes which also require further

TABLE 11

LOG_{10} IgG BY SEX, AGE AND TONSIL

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main effects	0.992618	9	0.1102909	7.561	0.001
Sex	0.1572965	1	0.1572965	10.784	0.001
Age	0.5564371	4	0.1391093	9.537	0.001
Tonsil	0.1870611	4	0.0467653	3.206	0.013
2-way Interactions	1.62635	24	0.0677646	4.646	0.001
Sex Age	0.256001	4	0.0640002	4.388	0.002
Sex Tonsil	0.1195431	4	0.0298858	2.049	0.084
Age Tonsil	1.2956719	16	0.0809795	5.552	0.001
3-way Interactions	1.9412766	16	0.1213298	8.318	0.001
Sex Age Tonsil	1.9412766	16	0.1213298	8.318	0.001
Explained	4.56025	49	0.0930663	6.380	0.001
Residual	14.5720125	999	0.0145866		
Total	19.1322625	1048	0.018256		

TABLE 12

LOG_{10} IgG TONSIL SIZE

	1	2	3	4	5
N	43	100	720	132	54
Mean	2.9624	2.9131	2.9329	2.9586	2.9329

**Scheffé's S-test

df = 4 M.S. 0.0145886

Multiple R Squared = 0.052

**underlined subset not significant ($P \leq 0.05$)

different; Scheffé's S-test

analysis.

Amount of the total variation which can be related to tonsil size must be small since only 5 percent could be accounted for by sex, age, and tonsil size combined. Thus it appears that there are significant differences in the IgG that are caused by unequal tonsil sizes. People with absent tonsil sizes tend to have the highest mean IgG concentrations whilst those with vestigeal tonsil sizes have the lowest. These differences are however not significant by the Scheffé's comparison test.

IGA

For the IgA measurements (Table 13) there are no significant sex related differences.

There are age related differences in the IgA concentrations (Table 13). The Mean IgA level rises with age (Table 14) to a maximum level in the 53-70 years age group.

There are significant differences of IgA concentration between the clinical groups (Table 13). Table 14 shows a rank order arrangement of the six groups in relation to their Mean IgA concentrations. From Scheffé's S- test which are also displayed in this table it is seen that the 3 groups of patients' relatives are statistically similar to each other but differ significantly from 2 of the control groups (6 and 4). There is an overlap between group 1 (1st

Table 13

LOG_{10} IgA BY SEX, AGE AND GROUP IDENTIFICATION

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main Effects	13.1440312	12	1.0953359	28.186	0.001
Sex	0.1333671	1	0.1333671	3.432	0.061
Age	10.441562	6	1.7406926	44.793	0.001
ID	0.5929609	5	0.1185922	3.052	0.010
2-way Interactions	2.0621375	41	0.0502960	1.294	0.103
Sex Age	0.3212142	6	0.535357	1.378	0.219
Sex ID	0.0368376	5	0.0073675	0.190	0.999
Age ID	1.7375824	30	0.0579194	1.490	0.043
3-way Interactions	0.7696687	24	0.0320695	0.825	0.999
Sex Age ID	0.7696660	24	0.0320694	0.825	0.999
Explained	15.9758375	77	0.2074784	5.339	0.001
Residual	51.21895	1318	0.0388611		
Total	67.1947875	1395	0.0481683		

TABLE 14

Log_{10} IgA

Sex

	Male	Female
N	647	749
Mean	2.1958	2.1917

**Scheffé's S-test

df = 1. M.S. = 0.0388611

Age

	1	2	3	4	5	6	7
N	95	197	192	135	371	260	146
Mean	1.9547	2.0721	2.1583	2.2289	2.2263	2.2822	2.288

**Scheffé's S-test

df = 6. M.S. = 0.0388611

ID

	3	2	1	5	6	4
N	116	74	210	116	559	321
Mean	2.2780	2.2622	2.2513	2.1761	2.1688	2.1592

**Scheffé's S-test

df = 5 M.S. = 0.038861

Multiple R squared for IgA is 0.196.

**Underlined subset not significantly ($P \leq 0.05$) different; Scheffé's S-test.

and 2nd degree relatives of Hodgkin's disease patients) and group 5 (community members not in John and Mary pedigree). The 3 control groups are similar to each other. The group 3 relatives of patients (immunodeficiency, leukaemia and thymoma) had the highest Mean IgA levels whereas group 1 relatives (Hodgkin's disease patients and direct line descendants of John and Mary) had slightly lower values than either group 3 or group 2. In the control groups, the highest Mean IgA concentrations are found in group 5 (community members not in John and Mary pedigree), whilst group 4 (controls from elsewhere) have the lowest.

With regards to the two way interactions, there are significant two way interactions between age and clinical groups (Table 13). This should be analysed in detail in future work.

The amount of the total variations in IgA which can be accounted for by sex, age and clinical groups in this analysis is 19.6 percent.

In Summary

For IgA there are age related differences. The relatives of patients showed significantly higher IgA than the people in the three control groups.

IGA AND TONSIL SIZE

The analysis (Table 15) shows that there are statistical differences in the Mean IgA levels in relation to

Table 15

LOG_{10} IgA BY SEX, AGE AND TONSIL

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main Effects	9.0727625	9	1.0080844	25.601	0.001
Sex	0.1452007	1	0.1452007	3.687	0.052
Age	8.657375	4	2.1643437	54.964	0.001
Tonsil	0.6326117	4	0.1581529	4.016	0.003
2-way Interactions	1.4823562	24	0.0617648	1.569	0.040
Sex Age	0.2173328	4	0.0543332	1.380	0.238
Sex Tonsil	0.2622981	4	0.0655745	1.665	0.155
Age Tonsil	0.8775727	16	0.0548483	1.393	0.137
3-way Interactions	0.9930062	16	0.0620629	1.576	0.068
Sex Age Tonsil	0.9930027	16	0.0620627	1.576	0.068
Explained	11.548125	49	0.235676	5.985	0.001
Residual	39.3378	999	0.0393772		
Total	50.885925	1048	0.0485552		

variations in tonsil sizes. Individuals with vestigial tonsil sizes have the highest Mean IgA levels whereas those who had tonsillectomy have the lowest. However these differences in IgA levels due to variations in tonsil sizes are not significant when Scheffé's S-test was applied (Table 16).

TABLE 16

LOG_{10} IgA TONSIL SIZE

	1	2	3	4	5
N	43	100	720	132	54
Mean	2.185	2.2348	2.2045	2.2187	2.2324

**Scheffé's S-test

df = 4 M.S. = 0.0393772

Multiple R Squared 0.178

**Underlined subset not significantly ($P \leq 0/05$) different; Scheffé's S-test.

Amount of the total variations in IgA measurements which can be accounted for by sex, age and tonsil size is higher than it is for IgG. About 18 percent could be accounted for by sex, age and tonsil size whereas in the IgG only 5 percent could be accounted for by these three factors.

In Summary

Although variations exist in the Mean IgA levels which are related to differences in tonsil sizes, these differences did not reach significance when the Scheffé's S-test was used.

IGM

The initial analysis (Table 17) indicates that the IgM

TABLE 17

LOG_{10} IgM BY SEX, AGE AND GROUP IDENTIFICATION

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Mean Effects	7.8881625	12	0.6573469	19.756	0.001
Sex	4.0326309	1	4.0326309	121.197	0.001
Age	1.5846254	6	0.2641042	7.937	0.001
ID	0.8325016	5	0.1665003	5.004	0.001
2-way Interactions	1.6671812	41	0.0406629	1.222	0.161
Sex Age	0.2746023	6	0.045767	1.375	0.220
Sex ID	0.3399523	5	0.0679905	2.0433	0.069
Age ID	0.9464141	30	0.0315471	0.948	0.999
3-way Interactions	0.9433437	24	0.0393060	1.181	0.248
Sex Age ID	0.9433453	24	0.0393061	1.181	0.248
Explained	10.4986875	77	0.1363466	4.098	0.001
Residual	43.8542937	1318	0.0332733		
Total	54.3529812	1395	0.0389627		

levels show sex related differences. Females (Table 18) have significantly higher Mean concentrations than the males.

There are differences in IgM concentrations related to age (Table 17). The concentration increases with age till a maximum is reached at puberty, thereafter the small fluctuations between age groups do not reach statistical significance when Scheffé's test is applied (Table 18).

Significant differences exist between the clinical groups (Table 17). In Table 18 where the Means are arranged in order of magnitude, starting from the highest, it is seen that the 3 groups of patients' relatives are not statistically different from each other, and show the highest Mean values. They are, as a group, statistically different from the three control groups. When Scheffé's S-tests are done between groups in the middle of the rank it is found that there is no significant difference between group 1 (Hodgkin's disease relatives and direct line descendants of John and Mary) and groups 5 and 6 (community members not in John and Mary pedigree and the remainder of the John and Mary pedigree). The 3 control groups are shown to be statistically similar by Scheffé's S-test. Group 2 (relatives of patients with embryonic tumours and lymphosarcoma) had the highest Mean IgM concentrations whereas group 4 (controls from elsewhere) had the lowest.

The amount of the total variations in IgM which can be accounted for by sex, age and clinical groupings is 14.5 percent.

TABLE 18

Log_{10} IgM

Sex		
	Male	Female
N	647	749
Mean	1.9665	2.0875
**Scheffé's S-test	df = 1	M.S. = 0.0332733

Age							
	1	2	3	4	5	6	7
N	97	197	192	135	371	260	146
Mean	1.9344	1.9649	2.0372	2.0797	2.0568	2.0583	2.0196
**Scheffé's S-test							
	df = 6.	M.S. = 0.0332733					

ID						
	2	3	1	6	5	4
N	74	116	210	559	116	321
Mean	2.0993	2.0981	2.0665	2.0234	2.0107	1.9858
**Scheffé's S-test						
	df = 5.	M.S. = 0.0332733				

Multiple R Squared for IgM is 0.145;
**Underlined subset not significantly ($P \leq 0.05$) different; Scheffé's S-test.

In Summary

Apart from sex and age differences in the IgM concentrations, it is evident from this statistical analysis that the relatives of the three patient groups had increased Mean IgM concentrations.

IGM AND TONSIL SIZE

The analyses (Tables 19 and 20) show that differences in tonsil sizes do not influence the Mean immunoglobulin levels.

IGD

There are no sex related differences for the IgD measurements (Tables 21 and 22).

There are age related differences in the IgD concentrations as shown by the analysis of variance (Table 21). The IgD Mean concentration increases with age up to the 10-14 year age group after which there is a continuous decline. These are, however, not significant, using Scheffé's S-test (Table 22).

There are significant differences between the clinical groups (Table 21). From Table 22 it is seen that 2 groups of patients' relatives (group 1, Hodgkin's disease and direct line descendants of John and Mary and group 2, embryonic tumour and lymphosarcoma patients) are statistically similar. Group 1 is different from the remaining 4 groups (the 3 control groups and the immunodeficiency, leukaemia and thymoma

TABLE 19

LOG_{10} IgM BY SEX, AGE AND TONSIL

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main Effects	6.0976652	9	0.6775184	19.123	0.001
Sex	3.7273465	1	3.7273465	105.204	0.001
Age	1.3973621	4	0.3493405	9.860	0.001
Tonsil	0.2021501	4	0.0505375	1.426	0.222
2-way Interactions	1.5870223	24	0.0661259	1.866	0.007
Sex Age	0.2640053	4	0.0660013	1.863	0.114
Sex Tonsil	0.2829094	4	0.0707273	1.996	0.092
Age Tonsil	1.0498594	16	0.0656162	1.852	0.021
3-way Interactions	1.8898875	16	0.1181180	3.334	0.001
Sex Age Tonsil	1.8898848	16	0.1181178	3.334	0.001
Explained	9.574575	49	0.1953995	5.515	0.001
Residual	35.3942937	999	0.0354297		
Total	44.9688687	1048	0.0429092		

TABLE 20

LOG_{10} IgM TONSIL SIZE

	1	2	3	4	5
N	43	100	720	132	54
Mean	2.185	2.2348	2.2045	2.2187	2.1324

**Scheffé's S-test

df = 4 M.S. = 0.0354297

Multiple R Squared = 0.136

**Underlined subset not significantly ($P \leq 0.05$) different; Scheffé's S-test.

TABLE 21

LOG_{10} IgD BY SEX, AGE AND GROUP IDENTIFICATION

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main Effects	366.9063	12	30.575525	13.656	0.001
Sex	8.1038250	1	8.103825	3.619	0.054
Age	60.0483562	6	10.0080562	4.470	0.001
ID	322.6992	5	64.5398375	28.825	0.001
2-way Interactions	93.5827	41	2.2825047	1.019	0.438
Sex Age	25.255925	6	4.2093207	1.880	0.080
Sex ID	3.7138652	5	0.742773	0.332	0.999
Age ID	59.0015562	30	1.9667184	0.878	0.999
3-way Interactions	35.4545	24	1.4772707	0.660	0.999
Sex Age ID	35.4544062	24	1.4772668	0.660	0.999
Explained	495.9440	77	6.4408309	2.877	0.001
Residual	2951.0672	1318	2.2390492		
Total	3447.0112	1395	2.4709754		

TABLE 22

Log_{10} IgD

Sex

	Male	Female
N	647	749
Mean	2.3077	2.2415

**Scheffé's S-test

df = 1. M.S. = 2.2390492

Age

	1	2	3	4	5	6	7
N	95	197	192	135	371	260	146
Mean	1.9716	2.3432	2.625	2.4026	2.2049	2.1889	2.107

**Scheffé's S-test

df = 6. M.S. = 2.2390492

ID

	1	2	3	6	5	4
N	210	74	116	559	116	321
Mean	2.850	2.5615	2.5303	2.4267	2.2351	1.4785

**Scheffé's S-test

df = 5. M.S. = 2.2390492

Multiple R Squared = 0.106

**Underlined subset not significantly ($P \leq 0.05$) different; Scheffé's S-test.

patients' relatives), whilst group 2 is only different from controls from elsewhere (group 4). Group 1 had the highest Mean IgD concentrations whilst group 4 had the lowest (Table 22).

The amount of the total variation in IgD which could be accounted for by sex, age and clinical groupings is 10.6 percent.

In Summary

There were no differences due to sex and very little differences due to age in the IgD concentrations. The relatives of patients in groups 1 and 2 had significantly elevated IgD concentrations.

IGD AND TONSIL SIZE

There are no tonsil size related differences in the Mean IgD levels (Tables 23 and 24).

The mean $\pm$ 2SD of IgG, IgA and IgM of blood donors and apparently healthy school children (controls from other parts of Newfoundland) were calculated after log transformation on a Wang 600 desk calculator (Table 25).

Individuals whose immunoglobulin concentrations were outside the mean $\pm$ 2SD for their age group and sex in the study population as well as in the controls from elsewhere were manually sorted out and are shown in Table 26. Tables 27 and 28a, b and c show that results from various groups are significantly different. Examination of the Tables shows

TABLE 23

LOG_{10} IgD BY SEX, AGE AND TONSIL

Source of Variation	Corrected Sum of Squares	Degree of Freedom	Mean Square	F	Significance of F
Main Effects	34.4469562	9	3.8274395	1.742	0.075
Sex	3.6154742	1	3.6154742	1.646	0.197
Age	19.4819687	4	4.8704922	2.217	0.064
Tonsil	9.1793812	4	2.2948453	1.045	0.383
2-way Interactions	94.0331437	24	3.9180477	1.784	0.012
Sex Age	23.941375	4	5.9853437	2.725	0.028
Sex Tonsil	14.4872437	4	3.6218109	1.649	0.159
Age Tonsil	53.7438812	16	3.3589926	1.529	0.082
3-way Interactions	25.5067	16	1.5941687	0.726	0.999
Sex Age Tonsil	25.5067125	16	1.5941695	0.726	0.999
Explained	153.9872	49	3.1425957	1.431	0.029
Residual	2194.6160	999	2.1968125		
Total	2348.6032	1048	2.2410332		

TABLE 24

LOG_{10} IgD TONSIL SIZE

	1	2	3	4	5
N	43	100	720	132	54
Mean	2.1348	2.6112	2.4981	2.6326	2.3209

**Scheffé's S-test

df = 4 M.S. = 2.1968125

Multiple R Squared = 0.015

**Underlined subset not significantly ($P \leq 0.05$) different; Scheffé's S-test.

TABLE 25

MEAN $\pm$ 2SD RANGE OF CONTROL FROM OTHER PARTS OF NEWFOUNDLAND

	Age	IgG	IgA	IgM
Male	0 - 7 yrs.	279 - 1121	16 - 212	11 - 149
	8 - 12 yrs.	317 - 1263	49 - 225	29 - 155
	13 - 17 yrs.	371 - 1303	31 - 275	29 - 157
	18 - 30 yrs.	563 - 1219	74 - 264	50 - 146
	31 - 40 yrs.	512 - 1332	106 - 286	43 - 155
	41 - 70 yrs.	557 - 1253	84 - 280	50 - 156
Female	0 - 7 yrs.	277 - 1229	29 - 181	26 - 170
	8 - 12 yrs.	310 - 1276	42 - 214	26 - 190
	13 - 17 yrs.	488 - 1344	53 - 249	41 - 187
	18 - 30 yrs.	561 - 1381	80 - 264	56 - 172
	31 - 40 yrs.	555 - 1335	81 - 245	61 - 193
	41 - 70 yrs.	542 - 1342	87 - 259	59 - 165

TABLE 26

IDENTIFICATION NUMBERS OF INDIVIDUALS OUTSIDE MEAN $\pm$ 2SD

IgG	Individuals with Immunoglobulin Values > Mean + 2SD	Individuals with Immunoglobulin Values < Mean - 2SD
GP 1	1231, 2044, 3288, 1009, 1239, 1238.	2108, 2088, 1148, 3490, 2196.
GP 2	1231, 1239, 1238, 1241.	1148, 2196.
GP 3	1241, 1238, 1239.	2108, 2088, 3490, 2196.
GP 4	CD 07 1, CD 11 1, CO 23 1, CO 23 1, CO 33 1, CD 06 2, CD 03 2, CD 11 2.	CO 30 1, CO 21 1, CO 64 1, CO 22 2.
GP 5	3307, 3534, 3550	1085, 3230, 3445, 3569, 3725, 3743.
GP 6	3054, 3093, 3096, 3116, 3151, 3243, 3248, 3251, 3276, 3281, 3365, 3371, 3372, 3373, 3379, 3384, 3428.	3229, 3231, 3310, 3388, 3435, 3640, 3721, 3922.
IgA		
GP 1	1053, 2003, 1212, 1231, 1153, 1210, 1177, 2005, 2013, 1009, 1037, 1055, 1082, 1220, 2082, 3632, 3812, 1060, 1211, 2052, 2094, 1202, 2090, 2105, 1208, 3793, 1112, 1134, 1143, 1238, 2029, 2066, 2069, 1070.	2039, 1064, 1148, 2102.
GP 2	1212, 1231, 1210, 1177, 1220, 2094, 1208, 1238, 1134, 1112, 1349, 3385, 1146.	1148, 2102.

TABLE 26 (continued)

	Individuals with Immunoglobulin Values > Mean + 2SD	Individuals with Immunoglobulin Values < Mean - 2SD
GP 3	1170, 1144, 2049, 3099, 1208, 1143, 1238, 1344, 3385, 3993, 1146, 2002, 2118, 1210, 2025, 2064, 1082, 1220, 3632, 3812, 1202, 3793, 1112, 1134.	3195, 2102.
GP 4	CD 12 1, CO 33 1, CO 25 1, CO 42 1, CD 04 2, CD 05 2, CD 09 2, CO 23 2, CO 53 2.	CD 11 1, CO 25 2.
GP 5	1129, 3174, 3236, 3332, 3639, 3684, 3809, 3829, 3907, 3909, 3973, 3992.	3235, 3590.
GP 6	1045, 1058, 1061, 1087, 1137, 1138, 1185, 1198, 2022, 2036, 2048, 2049, 2074, 2100, 2199, 3001, 3011, 3017, 3019, 3061, 3096, 3102, 3116, 3135, 3212, 3232, 3238, 3261, 3265, 3275, 3286, 3287, 3373, 3374, 3483, 3510, 3518, 3519, 3529, 3537, 3541, 3543, 3553, 3572, 3580, 3638, 3646, 3768, 3777, 3816, 3819, 3833, 3849, 3918, 3934, 3943, 3974, 4002.	1158, 1172, 1183, 3054, 3274, 3416, 3437, 3495, 3584, 3676, 3680, 3700, 3721.
IgM		
GP 1	1004, 1006, 2041, 1105, 1117, 1133, 1186, 3632, 3961, 1003, 1180, 1008, 1149, 2089, 1229, 1119, 1163, 1162, 1165, 2052, 2094, 3077, 3080, 3793, 1081, 1112, 3695, 1245, 3598.	1012, 1015, 1141, 1148, 1256, 2109.

TABLE 26 (continued)

	Individuals with Immunoglobulin Values > Mean + 2SD	Individuals with Immunoglobulin Values < Mean - 2SD
GP 2	1212, 1231, 1117, 3961, 3077, 1162, 1163, 2094, 1112, 3695, 1229, 3598, 1230, 1169, 1345, 3385.	1148, 3421, 1256.
GP 3	1229, 1144, 2019, 2094, 3099, 3077, 1345, 3385, 3993, 1245, 1117, 1163, 1165, 1119, 1150, 3793, 1112, 1149, 3695, 1245, 1105, 1133, 3961.	2109.
GP 4	CD 03 1, CD 05 1, CD 10 1, CO 19 1, CO 18 1, CO 37 1, CO 55 1, CD 08 2, CD 14 2, CO 23 2.	
GP 5	3009, 3046, 3058, 3235, 3480, 3550, 3559, 3659, 3684, 3687, 3725, 3825, 3854.	1085, 3454.
GP 6	1137, 1185, 1233, 1248, 1346, 2093, 2111, 2199, 3004, 3006, 3019, 3027, 3048, 3049, 3064, 3069, 3083, 3106, 3112, 3204, 3209, 3210, 3294, 3325, 3327, 3364, 3476, 3481, 3543, 3545, 3548, 3565, 3570, 3571, 3580, 3589, 3633, 3664, 3667, 3668, 3690, 3698, 3701, 3722, 3724, 3766, 3777, 3778, 3850, 3851, 3853, 3855, 3927, 3931, 3933, 3937, 3940, 3977.	1047, 3618.

TABLE 27

PROPORTION OF PEOPLE OUTSIDE
MEAN ± 2SD*

IgG	High	Low	High + Low	Total No. in the group	Proportion of people with high values	Proportion of people with low values
GP 1	6	5	11	210	0.0286	0.0238
GP 2	4	2	6	74	0.0541	0.0271
GP 3	3	4	7	116	0.0259	0.0345
GP 4	8	4	12	321	0.0249	0.0125
GP 5	3	6	9	116	0.0259	0.0517
GP 6	17	8	25	559	0.0304	0.0143
IgA						
GP 1	34	4	38	210	0.1619	0.019
GP 2	13	2	15	74	0.1757	0.0270
GP 3	24	2	26	116	0.2069	0.0172
GP 4	9	2	11	321	0.028	0.0062
GP 5	12	2	14	116	0.1034	0.0172
GP 6	58	13	71	559	0.1038	0.0233
IgM						
GP 1	29	6	35	210	0.1381	0.0286
GP 2	16	3	19	74	0.2162	0.0405
GP 3	23	1	24	116	0.1983	0.0086
GP 4	10	0	10	321	0.0312	0
GP 5	13	2	15	116	0.1121	0.0172
GP 6	58	2	60	559	0.1038	0.0036

*The normal values thus derived were compiled from various age groups which had been split also by sex. See Table 25.

TABLE 28a

NUMBER OF INDIVIDUALS WITH HIGH IgG LEVELS AMONG PATIENTS' RELATIVES AND OTHER CONTROLS

IgG	High		Not High (Rest)		Total
	Observed	(Expected)	Observed	(Expected)	
GP 1	6	(6.1676)	204	(203.832)	210
GP 2	4	(2.1733)	70	(71.8244)	74
GP 3	3	(3.4069)	113	(112.5896)	116
GP 4	8	(9.4278)	313	(311.5626)	321
GP 5	3	(3.4069)	113	(112.5896)	116
GP 6	17	(16.4178)	542	(542.5654)	559
Total	41		1355		1396

$$\chi^2 = \frac{(6 - 6.1676)^2}{6.1676} + \frac{(4 - 2.1733)^2}{2.1733} + \cdots + \frac{(542 - 542.5654)^2}{542.5654}$$

$$= 1.93055 \qquad df = (6 - 1)(2 - 1) = 5$$

$P > 0.2$

TABLE 28b

NUMBER OF INDIVIDUALS WITH HIGH IgA LEVELS AMONG PATIENTS' RELATIVES AND OTHER CONTROLS

IgA	High		Not High (Rest)		Total
	Observed	(Expected)	Observed	(Expected)	
GP 1	34	(22.5645)	176	(187.4355)	210
GP 2	13	(7.9476)	61	(66.0524)	74
GP 3	24	(12.4584)	92	(103.5416)	116
GP 4	9	(34.4754)	312	(286.5246)	321
GP 5	12	(12.4584)	104	(103.5416)	116
GP 6	58	(60.0366)	501	(498.9634)	559
Total	150		1246		1396

$\chi^2 = 43.2566$ $(P < 0.005)$

TABLE 28c

NUMBER OF INDIVIDUALS WITH HIGH IgM LEVELS AMONG PATIENTS' RELATIVES AND OTHER CONTROLS

IgM	High		Not High (Rest)		Total
	Observed	(Expected)	Observed	(Expected)	
GP 1	29	(22.414)	181	(187.593)	210
GP 2	16	(7.8958)	58	(66.1042)	74
GP 3	23	(12.3772)	93	(103.6228)	116
GP 4	10	(34.2507)	311	(286.7493)	321
GP 5	13	(12.3772)	103	(103.6228)	116
GP 6	58	(59.6)	501	(499.3547)	559
Total	149		1247		1396

$\chi^2 = 40.9893$ $P < 0.005$

there were higher proportions of individuals with elevated IgA and elevated IgM in the three groups of patient relatives than in the control groups; this is in accord with the analysis of variance results. The proportions of individuals with elevated IgG concentrations were not significantly different between the 6 groups.

Comparison of the study population with the control from elsewhere (Tables 29a, b and c) showed that the study population had a higher proportion of people with immunoglobulin deficiencies than in the controls. These are however not significant (Table 30) for IgG ($P>0.2$), IgA ($P>0.1$), or IgM ($P>0.1$).

Table 30 is a breakdown of the number of people with abnormal immunoglobulin concentrations in the study population as well as in the controls from elsewhere.

Table 31 shows the tonsil sizes together with the immunoglobulin results of individuals with immunoglobulin deficiencies of one or more classes.

TABLE 29a

COMPARISON OF THE PROPORTION OF PEOPLE WITH LOW IgG LEVELS IN THE STUDY POPULATION WITH CONTROLS FROM OTHER PARTS OF NEWFOUNDLAND

IgG	Low	Not Low (Rest)	Total
Study community	18 (a)	921 (b)	939 (a+b)
Controls	4 (c)	317 (d)	321 (c+d)
Total	22 (a+c)	1238 (b+d)	1260 (n)

$$\chi^2 = \frac{n(ad - bc)^2}{(a+c)(b+d)(a+b)(c+d)} = 0.6275 \qquad (P > 0.2)$$

TABLE 29b

LOW IgA LEVELS

IgA	Low	Not Low (Rest)	Total
Study community	20	919	939
Controls	2	319	321
Total	22	1238	1260

$\chi^2 = 3.1663$ $\qquad (P < 0.1)$

TABLE 29c

LOW IgM LEVELS

IgM	Low	Not Low (Rest)	Total
Study community	9	930	939
Controls	0	321	321
Total	9	1251	1260

$\chi^2 = 1.4248$ $\qquad (P > 0.1)$

TABLE 30

NUMBER OF INDIVIDUALS OUTSIDE THE NORMAL RANGE
IN STUDY POPULATION AND CONTROLS

	Study Population	Controls From Province of Newfoundland
Combined elevation of IgG, IgA & IgM	1 (No 1231)	None
Combined elevation of IgG and IgA	3	None
Combined elevation of IgA and IgM	18	None
Isolated elevation of IgG	21	8
Isolated elevation of IgA	91	9
Isolated elevation of IgM	89	10
Combined IgG, IgA, and IgM Deficiencies	1 (No 1148)	None
Combined IgG and IgA Deficiencies	1 (No 3721)	None
Combined IgG and IgM Deficiencies	1 (No 1085)	None
Isolated IgG Deficiencies	15	4
Isolated IgA Deficiencies	17	2
No Detectable IgA level	1 (No 3590)	None
Isolated IgM Deficiencies	7	None
Total Number of Abnormal Individuals	265	33
Total Number of Subjects in the Study	939	321

TABLE 31

TONSIL SIZE RELATED TO THE PRESENCE OF LOW IMMUNOGLOBULINS* IN THE STUDY POPULATION

Identification Numbers	IgG mg/100 ml		IgA mg/100 ml		IgM mg/100 ml		Tonsil Size
3231	440	Low	184	N	89	N	Absent
2102	1025	N	59	Low	67	N	Absent
3235	1025	N	86	Low	202	High	Absent
3274	718	N	43	Low	48	N	Absent
3437	769	N	61	Low	108	N	Absent
1012	666	N	255	N	48	Low	Absent
3388	461	Low	192	N	78	N	Vestigeal
3584	1025	N	57	Low	108	N	Vestigeal
3700	666	N	47	Low	89	N	Vestigeal
3421	943	N	168	N	62	Low	Vestigeal
3454	1025	N	116	N	47	Low	Vestigeal
1148	492	Low	27	Low	47	Low	Normal
2108	513	Low	137	N	92	N	Normal
2088	554	Low	108	N	154	N	Normal
2196	461	Low	178	N	94	N	Normal
3445	482	Low	157	N	71	N	Normal
3569	543	Low	178	N	110		Normal
3725	533	Low	196	N	304	High	Normal
3743	543	Low	147	N	139	N	Normal
3229	461	Low	168	N	75	N	Normal
3435	513	Low	215	N	137	N	Normal
3640	513	N	215	N	78	N	Normal
3721	461	Low	41	Low	158	N	Normal
3922	513	Low	204	N	68	N	Normal
2039	769	N	84	Low	81	N	Normal
1064	749	N	24	Low	77	N	Normal
3915	871	N	74	Low	139	N	Normal

TABLE 31 (Continued)

Identification Numbers	IgG mg/100 ml		IgA mg/100 ml		IgM mg/100 ml		Tonsil Size
1158	513	N	27	Low	83	N	Normal
3054	1640	N	20	Low	116	N	Normal
3416	615	N	31	Low	43	N	Normal
3676	923	N	61	Low	111	N	Normal
1015	830	N	235	N	34	Low	Normal
1256	1025	N	118	N	57	Low	Normal
2109	1179	N	194	N	44	Low	Normal
1085	461	Low	106	N	49	Low	Enlarged
3230	502	Low	168	N	52	N	Enlarged
1183	1076	N	82	Low	101		Enlarged
3495	1025	N	47	Low	127		Enlarged
3680	810	N	41	Low	85	N	Enlarged
3310	513	Low	184	N	92	N	Tonsillectomy
1172	615	N	63	Low	152	N	Tonsillectomy

*The normal and abnormal values thus derived were compiled from various age groups which had been split also by sex. See Table 25.

N = Normal.

DISCUSSION

The communities studied in this work are genetically and geographically isolated. The increased incidence of intermarriage between close relatives (Figs. 2 - 4) coupled with the findings of high occurrence of lymphoreticular malignancies and immunodeficiency make this an interesting community to study immunologically.

This study has shown that in the extended family of about 1000 people examined, the relatives of patients with embryonic tumour, lymphosarcoma, immunodeficiency, leukaemia and thymoma, had significantly elevated mean concentrations of IgG, IgA and IgM. The relatives of patients with Hodgkins disease showed a similar though less pronounced trend.

The relatives of patients with Hodgkins disease showed markedly elevated mean serum IgD levels, whilst relatives of those with other tumours and immunodeficiency showed a mild elevation. There are no previous published reports on IgD measurements in such families.

Other workers have examined serum immunoglobulin levels in families of patients suffering from lymphoreticular malignancies. For example, Till et al., (1975) in their studies of close relatives of 6 children with acute leukaemia found significant elevation of IgA in all the fathers. In addition, 2 of the fathers had higher and one

had lower levels of IgM than the controls. They also reported significantly elevated mean IgM concentrations in the parents and parent's sibs, which was attributed to the contribution of a single large sibship with markedly altered IgM levels. The findings by Till et al. of elevated IgA and IgM are similar to our findings.

Similarly Chandra (1972b) found increased IgA and IgM in the mothers and a significant decrease in IgG in the sibs of children with acute lymphoblastic leukaemia.

Sutton, Bishun and Soothill (1969) observed a significant dimunition in IgA in siblings of children with acute lymphoblastic leukaemia, and a significantly higher IgM concentration in the patients' mothers than in matched controls.

Twomey et al., (1967) observed mildly elevated levels of IgA in the sibs and a significantly raised IgA in the aunt of a patient with acute leukaemia. The IgM levels were slightly lowered in the patient's first and second degree relatives.

Snyder et al., (1970) reported low IgG and IgA concentrations in the father of 2 children with "reticuloendothelial malignancy".

Fraumeni et al., (1969) observed decreased levels of IgG, IgA and IgM in the sibs of a patient who had chronic lymphocytic leukaemia.

Fraumeni et al., (1975) studied a family with

multiple lymphoreticular malignancies including Hodgkins disease and found that 3 of the 9 relatives of the patient with Hodgkins disease had elevated IgM. One had a monoclonal IgM spike.

Zorballa-Mallios and Sutton (1974) found elevated E-B virus antibody of the IgM class in the mothers and siblings of children with acute leukaemia.

Previous studies have been mainly confined to relatives of patients with acute leukaemia. In most reports, a small number of individuals have been examined. Except for Fraumeni's (1975) report on relatives of one patient, there is no documentation of immunoglobulin levels in relatives of Hodgkins disease patients. Thus the present study which includes data on 264 first and second degree relatives of 19 patients with immunopathological diseases (183 are relatives of 7 Hodgkins disease patients) with its built in internal control population of 675 people from the same community is unique in many respects. This is the largest number of patients with Hodgkins disease relatives and matched controls reported in any single study to date. The data shows a familial pattern in the occurrence of Hodgkins disease, other malignancies and immunodeficiency.

A most striking finding in the Hodgkins disease relatives is the elevation of the IgD. However, even though it is elevated in diphtheria and tetanus infections (see literature review), the pathophysiologic role of this

immunoglobulin is not established.

Apart from these findings, sex related differences were found in the levels of IgG and IgM, the females having higher mean concentrations than the males. Such sex related differences, possibly due to hormonal variations, are expected and have been reported by others (see literature review).

Age related differences in the IgG, IgA, IgM and IgD concentrations found in this study also correlate with the findings of other workers (see literature review).

Analysis of the data for a possible relationship of immunoglobulin concentrations and tonsillar size failed to reveal significant correlation when examined by a stringent statistical test--Scheffé's S-test.

In the studies by Donovan and Soothill (1973) lower IgA concentrations were found in children undergoing tonsillectomy for recurrent throat infections than in control children. They reported an association between the immunological findings and incidence of infections after operations but not before. For example, they found that the patients' IgA concentrations were not related to the incidence of sore throats in the previous 6 months before tonsillectomy. In the present study also, the lowest mean IgA concentration was found in the group who had undergone tonsillectomy. However, studies by Veltri et al., (1972) showed elevated IgG and IgM but normal IgA in patients with

recurrent tonsillitis before tonsillectomy. They reported a dimunition in the IgG levels while the IgM, IgA and IgD remained unchanged a few months after tonsillectomy.

One possible explanation for the observed elevation in immunoglobulin concentrations of the relatives of the patients in this study is that it is due to increased antigenic stimulation of the immune system. This could be due to chronic infection. The occurrence of chronic infection and consequent increased antigenic stimulation could be predisposed by the presence of a subtle form of immunodeficiency which permits the entry of antigens more easily than in healthy people. A number of individuals among the relatives of the patients showed various grades and type of immunoglobulin deficiencies. A total of three patients with hypogammaglobulinaemia and another with isolated IgA absence were found.

Other possible causes of this elevation in immunoglobulin concentrations in these relatives include (i) climatic conditions (see literature review): it is very unlikely however that climatic conditions are responsible for this increase, since controls from the same part of the province did not show such elevations; (ii) hormone changes. There is no reason to suspect that hormonal changes contribute significantly to the presently observed differences; (iii) use of drugs: this again seems unlikely as a cause since very few people in this study population were on any

kind of medication; (iv) the primary factors which control immunoglobulin concentrations are their rates of synthesis, catabolism and loss. There are several reports of increased catabolism as in myotonic dystrophy, or of loss, as in various renal and gastro-intestinal disorders, which result in low immunoglobulin concentrations, especially of the IgG class. On the other hand, reduced catabolism which would result in elevated immunoglobulin levels, has not yet been reported. It is therefore very unlikely that the elevated immunoglobulins reported in this study are due to reduced catabolism. It is therefore concluded that the higher concentrations of the immunoglobulins are probably caused by increased antigenic stimulation of the immune system in these individuals.

An explanation for the association in this study of immunodeficiency and malignancy could be that both result from the same cause. The peculiar genetic make-up of this community, with a high incidence of inbreeding raises the possibility of an inherited predisposition to both conditions.

It is possible that this community is living in a peculiar kind of relationship with a certain infective agent (virus(es)) chronic exposure to which leads to raised immunoglobulins in many people, and may be to overt disease such as malignancy or severe immunodeficiency in a few. Since the functional state of the immune system may be

inherited (McDevitt and Benacerraf, 1968; Soothill et al., 1971), it is likely that the predisposition to virus carriage is genetically determined. Thus there could be both genetic and environmental factors operating in these cases.

Since there is a close relationship between immunodeficiency and the development of malignancy (based on epidemiologic information and experimental data) especially for malignancy of the lymphoreticular system, these ideas on aetio-pathogenesis have a logic basis.

It is apparent that further studies are required to throw more light on the elevated levels of immunoglobulins in relatives of patients with immunodeficiency and lymphoreticular malignancies reported in this study.

Individuals whose immunoglobulin concentrations make major contributions to the significant differences between the groups should be further investigated. These investigations should include a look at their clinical records for history of past infections, other genetic markers, virus antibody titres and if possible epidemiological study of their contacts with each other and with the patients.

It would be worthwhile to do some correlation studies within the immunoglobulin classes in a given individual. In this study, a preliminary analysis indicates that undetectable IgD concentrations may be more common in people with low IgG levels than in people with normal

levels of IgG.

IgE measurements should be carried out especially in relatives of Hodgkins disease patients who in this study showed elevated mean IgG, IgA, IgM and IgD concentrations.

This study has focussed attention on only a part of the immune system. It is essential to look into the other specific and non-specific immunity mechanisms to investigate if these aspects of host defense are altered in the patients and their relatives.

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APPENDIX

Computer Print Out of the Immunoglobulin (G, A, M & D) Levels of the 6 Clinical Groupings (See Text)

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
1	HA	10	1	1004	1,128	131	183	20,825
1	HA	11	1	1043	933	125	66	1,675
1	HA	11	1	1124	923	139	81	2,525
1	HA	11	1	1348	769	215	91	4,450
1	HA	16	1	1006	1,025	188	158	3,600
1	HA	14	1	1042	830	112	70	4,450
1	HA	16	1	1099	820	186	85	526
1	HA	60	1	1012	666	255	48	1,400
1	HA	43	1	1015	830	235	34	526
1	HA	42	1	1023	749	104	66	1,400
1	HA	46	1	1053	595	388	62	13,500
1	HA	51	1	1147	718	139	70	5,800
1	HA	71	1	1259	1,230	204	190	4,125
1	HA	49	2	2003	800	430	87	16,290
1	HA	05	1	1096	646	85	72	3,000
1	HA	07	1	1212	1,025	266	152	1,675
1	HA	07	1	1231	1,538	337	152	2,225
1	HA	05	1	1236	584	55	49	
1	HA	07	1	3477	1,025	112	118	1,920
1	HA	07	1	3689	718	112	54	72
1	HA	03	1	1153	820	286	152	3,000
1	HA	07	1	1028	933	118	80	72
1	HA	09	1	1097	728	92	120	3,000
1	HA	11	1	1232	1,025	118	102	6,125
1	HA	11	1	2053	656	155	133	
1	HA	13	1	2055	851	141	119	
1	HA	11	1	2078	656	82	72	7,700
1	HA	11	1	2199	625	215	215	
1	HA	10	1	3338	1,025	165	120	3,900
1	HA	10	1	1065	666	102	54	1,920
1	HA	14	1	1210	892	286	119	6,900
1	HA	13	1	1222	871	194	127	2,785
1	HA	15	1	1230	1,076	177	152	15,790
1	HA	16	1	2041	851	225	164	6,700
1	HA	15	1	2058	718	112	95	
1	HA	16	1	2195	513	168	86	4,125
1	HA	17	1	1206	1,076	163	90	4,775
1	HA	17	1	2054	554	155	101	
1	HA	17	1	2103	769	98	139	
1	HA	13	1	1067	666	176	58	2,225
1	HA	06	2	1003	933	102	158	7,200
1	HA	07	2	1127	1,025	86	101	2,785
1	HA	27	2	1141	677	112	35	1,920
1	HA	04	2	1347	871	90	152	1,400
1	HA	07	2	1360	933	157	165	1,920
1	HA	07	2	1139	1,025	65	139	10,775
1	HA	05	2	1142	871	59	145	6,700
1	HA	12	2	1123	1,128	100	152	7,200
1	HA	10	2	1125	1,025	139	127	5,075
1	HA	08	2	1126	718	104	110	6,900
1	HA	11	2	1011	666	102	70	3,000

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
1	HA	26	2	1060	830	327	70	6,900
1	HA	27	2	1094	1,025	204	137	5,075
1	HA	23	2	1116	615	204	109	7,200
1	HA	25	2	1180	923	245	215	10,775
1	HA	18	2	3736	820	100	99	4,125
1	HA	45	2	1008	933	194	190	2,225
1	HA	49	2	1149	1,128	204	254	2,525
1	HA	60	2	1013	749	145	145	4,450
1	HA	12	2	1161	923	196	127	
1	HA	11	2	1211	1,179	490	127	1,675
1	HA	12	2	2011	871	215	152	6,125
1	HA	08	2	2040	851	82	133	8,375
1	HA	08	2	2060	605	204	127	
1	HA	09	2	3737	923	155	152	11,300
1	HA	11	2	1098	728	104	109	
1	HA	11	2	1066	749	159	80	72
1	HA	09	2	1064	749	24	77	
1	HA	15	2	1071	1,230	186	165	4,125
1	HA	13	2	1118	1,025	86	183	
1	HA	14	2	2089	800	180	202	17,900
1	HA	17	2	1223	1,230	215	152	2,785
1	HA	17	2	1229	1,230	235	265	526
1	HA	17	2	2012	738	204	202	8,800
1	HA	14	2	1068	1,128	106	116	2,225
1	HA	28	2	1029	830	145	145	1,400
1	HA	26	2	1084	820	92	99	3,000
1	HA	25	2	1119	1,128	178	329	5,800
1	HA	18	2	1150	1,025	196	221	1,115
1	HA	22	2	1152	1,025	178	165	2,525
1	HA	18	2	1162	615	196	183	2,785
1	HA	18	2	1163	820	245	234	2,525
1	HA	24	2	1165	820	196	316	
1	HA	21	2	1166	615	215	165	
1	HA	26	2	1217	1,128	102	63	5,075
1	HA	24	2	1235	1,025	159	78	72
1	HA	18	2	2008	933	108	114	
1	HA	21	2	2009	1,076	157	165	2,785
1	HA	19	2	2052	718	358	468	
1	HA	28	2	2080	1,025	131	152	5,800
1	HA	27	2	2081	892	204	95	7,980
1	HA	20	2	2088	554	108	154	3,000
1	HA	21	2	2094	677	358	329	2,225
1	HA	21	2	2096	851	127	67	18,400
1	HA	25	2	2104	871	157	108	6,900
1	HA	29	2	2107	677	255	102	2,525
1	HA	18	2	2197	605	204	127	2,785
1	HA	20	2	3352	800	190	109	325
1	HA	22	2	3641	892	104	127	
1	HA	30	2	3077	1,025	215	215	2,225

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
								100
1	HB	23	1	1039	830	198	111	5,500
1	HB	28	1	1135	871	225	152	5,075
1	HB	19	1	1177	1,128	317	70	1,675
1	HB	22	1	2010	1,025	215	139	11,300
1	HB	25	1	2044	1,230	255	90	3,000
1	HB	23	1	2063	1,794	100	90	2,785
1	HB	19	2	3045	902	196	116	2,525
1	HB	28	1	3060	1,025	235	95	2,225
1	HB	28	1	3301	1,025	139	145	72
1	HB	27	1	3795	923	170	115	
1	HB	30	1	2115	769	194	89	526
1	HB	36	1	2005	933	644	87	4,450
1	HB	33	1	2006	1,128	84	114	3,900
1	HB	31	1	2013	1,025	481	97	21,400
1	HB	39	1	2026	933	204	154	1,115
1	HB	37	1	2039	769	84	81	72
1	HB	35	2	3288	1,558	114	127	850
1	HB	31	1	3399	1,025	184	158	13,500
1	HB	53	1	1009	1,794	388	97	5,800
1	HB	63	1	1037	933	409	111	8,800
1	HB	68	1	1055	933	480	66	4,450
1	HB	58	1	1072	728	245	127	17,900
1	HB	55	1	1080	646	245	109	
1	HB	53	1	1082	871	327	75	14,100
1	HB	55	1	1101	820	255	103	2,225
1	HB	46	1	1105	923	245	254	6,700
1	HB	57	2	1117	1,128	147	278	1,675
1	HB	47	1	1131	1,025	204	116	2,225
1	HB	44	1	1133	923	127	221	9,975
1	HB	53	1	1161	718	178	101	2,785
1	HB	41	1	1078	1,025	127	133	72
1	HB	52	1	1164	574	168	119	
1	HB	52	1	1175	1,025	176	119	1,400
1	HB	61	1	1186	1,025	215	582	3,900
1	HB	48	1	1220	1,025	306	102	4,775
1	HB	42	1	1239	2,050	225	89	1,920
1	HB	49	2	2003	800	430	87	16,290
1	HB	41	1	2034	718	149	95	325
1	HB	53	1	2067	718	141	119	
1	HB	48	1	2082	1,179	358	51	9,600
1	HB	58	1	2092	605	92	61	1,920
1	HB	62	1	2108	513	137	92	1,400
1	HB	64	1	3632	718	368	165	1,400
1	HB	55	1	3738	1,128	151	127	16,290
1	HB	62	1	3812	1,025	510	63	325
1	HB	49	1	3961	636	123	608	
1	HB	68	1	1055	933	480	66	4,450
1	HB	76	1	1104	1,230	317	265	72

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
1	HB	75	1	1254	1,076	654	80	2,225
1	HB	72	1	1018	666	131	97	1,675
1	HB	38	2	1077	923	133	103	72
1	HB	22	2	1148	492	27	47	
1	HB	33	2	1202	820	347	95	6,125
1	HB	33	2	1226	1,128	204	171	7,200
1	HB	37	2	2007	1,076	133	78	
1	HB	39	2	2073	605	163	110	5,500
1	HB	39	2	2075	605	184	86	526
1	HB	39	2	2090	1,025	262	86	8,800
1	HB	35	2	2105	800	276	97	3,000
1	HB	38	2	3080	902	157	329	72
1	HB	34	2	3084	1,025	235	89	2,525
1	HB	40	2	1208	1,179	358	127	2,525
1	HB	39	2	3250	1,025	204	120	526
1	HB	31	2	3490	554	139	71	5,800
1	HB	33	2	3793	1,128	490	240	5,075
1	HB	35	2	3858	1,025	204	118	72
1	HB	62	2	1038	749	198	97	526
1	HB	51	2	1040	666	184	91	5,075
1	HB	45	2	1063	820	163	133	
1	HB	55	2	1079	923	143	127	4,125
1	HB	44	2	1081	1,076	245	165	1,920
1	HB	48	1	1095	820	172	103	15,790
1	HB	46	2	1100	1,128	172	152	1,675
1	HB	53	2	1102	1,025	172	99	3,600
1	HB	48	2	1112	923	408	316	3,900
1	HB	42	2	1134	1,128	572	86	1,920
1	HB	44	2	1143	677	266	119	5,500
1	HB	61	2	1238	1,948	441	119	1,115
1	HB	42	2	1252	1,025	184	102	8,800
1	HB	47	2	1253	871	204	152	72
1	HB	55	2	1256	1,025	118	57	2,785
1	HB	49	2	2029	656	368	101	4,775
1	HB	42	2	2032	769	255	81	
1	HB	50	2	2065	851	174	127	
1	HB	42	2	2066	574	337	106	72
1	HB	51	2	2068	718	204	95	5,800
1	HB	45	2	2069	851	368	64	
1	HB	51	2	2102	1,025	59	67	72
1	HB	46	2	2109	1,179	194	44	72
1	HB	58	2	2196	461	178	94	526
1	HB	59	2	3403	1,025	168	71	4,450
1	HB	50	2	3695	790	147	265	72
1	HB	42	2	3804	820	147	89	72
1	HB	66	2	1016	850	131	111	
1	HB	74	2	1237	1,076	327	86	
1	HB	67	2	1245	892	137	291	
1	HB	66	2	1086	1,025	172	137	
1	HB	60	2	1070	923	429	145	7,700
1	HB	51	2	1040	666	184	91	5,075

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
								99
1	G1	45	2	1008	933	194	190	2,225
1	G1	60	1	1012	666	255	48	1,400
1	G1	60	2	1013	749	145	145	4,450
1	G1	77	2	1014	666	225	66	1,115
1	G1	76	1	1104	1,230	317	265	72
1	G1	57	2	1117	1,128	147	278	1,675
1	G1	67	2	1146	1,076	347	132	1,115
1	G1	51	1	1147	718	139	70	5,800
1	G1	49	2	1149	1,128	204	254	2,525
1	G1	53	1	1161	718	178	101	2,785
1	G1	44	1	1171	1,025	215	95	5,500
1	G1	68	2	1204	933	92	72	
1	G1	33	2	1226	1,128	204	171	7,200
1	G1	75	1	1254	1,076	654	80	2,225
1	G1	37	1	1255	1,076	157	177	325
1	G1	55	2	1256	1,025	118	57	2,785
1	G1	81	2	1257	1,025	510	119	2,225
1	G1	37	2	1549	933	368	87	6,125
1	G1	39	1	1351	1,025	296	114	1,115
1	G1	40	2	3598	1,128	155	278	
								26
2	LB	07	1	1212	1,025	266	152	1,675
2	LB	07	1	1231	1,538	337	152	2,225
2	LB	05	1	1236	584	55	49	
2	LB	07	1	3689	718	112	54	72
2	LB	13	1	1222	871	194	127	2,785
2	LB	14	1	1210	892	286	119	6,900
2	LB	15	1	1230	1,076	177	152	15,790
2	LB	19	1	1177	1,128	317	70	1,675
2	LB	30	1	2115	769	194	89	526
2	LB	28	1	1135	871	225	152	5,075
2	LB	48	1	1220	1,025	306	102	4,775
2	LB	57	2	1117	1,128	147	278	1,675
2	LB	49	1	3961	636	123	608	
2	LB	47	1	1131	1,025	204	116	2,225
2	LB	58	1	2092	606	92	61	1,920
2	LB	53	1	2067	718	141	119	
2	LB	58	1	1072	728	245	127	17,900
2	LB	42	1	1239	2,050	225	89	1,920
2	LB	24	2	1235	1,025	159	78	72
2	LB	26	2	1217	1,128	102	63	5,075
2	LB	30	2	3077	1,025	215	215	2,225
2	LB	26	2	1084	820	92	99	3,000
2	LB	28	2	1029	830	145	145	1,400
2	LB	18	2	1162	615	196	183	2,785
2	LB	18	2	1163	820	245	234	2,525

SET	GR	AGE	SEX	PAT#	IGG	IGA	IGM	IGD
2	LB	21	2	2094	677	358	329	2,225
2	LB	40	2	1208	1,179	358	127	2,525
2	LB	33	2	1226	1,128	204	171	7,200
2	LB	35	2	3858	1,025	204	118	72
2	LB	35	2	3288	1,558	114	127	850
2	LB	39	2	3250	1,025	204	120	526
2	LB	22	2	1148	492	27	47	
2	LB	38	2	1077	923	133	103	72
2	LB	66	2	1086	1,025	172	137	
2	LB	74	2	1237	1,076	327	86	
2	LB	61	2	1238	1,948	441	119	1,115
2	LB	42	2	1134	1,128	572	86	1,920
2	LB	44	2	1081	1,076	245	165	1,920
2	LB	51	2	2102	1,025	59	67	72
2	LB	48	2	1112	923	408	316	3,900
2	LB	56	2	3695	790	147	265	72
2	LB	46	2	1100	1,128	172	152	1,675
2	LB	45	2	1063	820	163	133	
2	LB	58	2	2196	461	178	94	526
2	LB	42	2	1252	1,025	184	102	8,800
2	LB	17	2	1223	1,230	215	152	2,785
2	LB	17	2	1229	1,230	235	265	526
2	LB	13	2	1116	1,025	86	183	
2	LB	15	2	1071	1,230	186	165	4,125
								49
2	EA	40	2	3598	1,128	155	278	
2	EA	37	2	1349	933	368	87	6,125
2	EA	33	2	1226	1,128	204	171	7,200
								3
2	EB	17	2	1229	1,230	235	265	526
2	EB	26	2	3247	800	190	114	
2	EB	14	2	1169	1,025	215	216	9,600
2	EB	36	1	3421	943	168	62	850
2	EB	40	2	3598	1,128	155	278	
2	EB	37	2	1349	933	368	87	6,125
2	EB	39	2	1241	1,691	225	145	72
2	EB	55	2	1256	1,025	118	57	2,785
2	EB	47	2	3719	790	135	139	
2	EB	42	2	1252	1,025	184	102	8,800
2	EB	49	1	1345	1,076	245	330	325
2	EB	44	2	1001	666	255	59	1,115
2	EB	52	2	1020	933	92	104	
2	EB	54	2	1051	933	159	65	
2	EB	46	2	3385	1,179	296	177	3,600
2	EB	46	2	1221	1,128	225	132	
2	EB	67	2	1146	1,076	347	132	1,115
2	EB	77	2	1014	666	225	66	1,115

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
2	EB	68	2	1204	933	92	72	
2	EB	44	1	1171	1,025	215	95	5,500
2	EB	52	1	1258	1,128	155	75	4,450
2	EB	47	1	1052	666	169	80	1,115
2	EB	11	1	1232	1,025	118	102	6,125
2	EB	42	1	1207	1,025	176	102	1,675
								24
3	OA	18	2	1170	1,179	266	162	850
3	OA	26	2	1217	1,128	102	63	5,075
3	OA	17	2	1229	1,230	235	265	526
3	OA	17	2	1144	513	286	291	
3	OA	24	2	1235	1,025	159	78	72
								5
3	OB	18	2	2019	677	133	183	
3	OB	19	2	2030	738	96	87	72
3	OB	23	2	2084	871	108	152	4,125
3	OB	24	2	2085	677	106	127	4,450
3	OB	20	1	2086	871	215	127	18,400
3	OB	21	2	2094	677	358	329	2,225
3	OB	28	2	3099	902	460	221	5,075
3	OB	26	2	3247	800	190	114	
3	OB	21	2	3915	871	74	139	1,115
3	OB	30	2	3077	1,025	215	215	2,225
3	OB	39	2	1241	1,691	225	145	72
3	OB	32	2	2004	1,025	114	97	
3	OB	34	2	3175	1,025	106	89	
3	OB	35	2	3858	1,025	204	118	72
3	OB	40	2	1208	1,179	358	127	2,525
3	OB	44	2	1001	666	255	59	1,115
3	OB	52	2	1020	933	92	104	
3	OB	54	2	1051	933	159	65	
3	OB	44	2	1143	677	266	119	5,500
3	OB	46	2	1221	1,128	225	132	
3	OB	61	2	1238	1,948	441	119	1,115
3	OB	42	2	1252	1,025	184	102	8,800
3	OB	47	2	1253	871	204	152	72
3	OB	42	2	1344	759	388	110	850
3	OB	49	1	1345	1,076	245	330	325
3	OB	46	2	3385	1,179	296	177	3,600
3	OB	59	2	3403	1,025	168	71	4,450
3	OB	47	2	3719	790	135	139	
3	OB	50	2	3993	1,025	358	228	6,900
3	OB	66	2	1086	1,025	172	137	
3	OB	67	2	1146	1,076	347	132	1,115
3	OB	68	2	1204	933	92	72	
3	OB	74	2	1237	1,076	327	86	
3	OB	67	2	1245	892	137	291	

SET	GR	AGE	SEX	PAT#	IGG	IGA	IGM	IGD
3	OB	81	2	1257	1,025	510	119	2,225
3	OB	77	2	1014	666	225	66	1,115
3	OB	11	1	1348	769	215	91	4,450
3	OB	10	1	2002	738	235	114	9,975
3	OB	13	1	2014	1,179	180	127	72
3	OB	09	1	2017	1,025	96	87	2,785
3	OB	12	1	2118	513	368	75	10,500
3	OB	09	1	3592	1,128	149	101	1,400
3	OB	14	1	1210	892	286	119	6,900
3	OB	13	1	1222	871	194	127	2,785
3	OB	15	1	1230	1,076	177	152	15,790
3	OB	13	1	2001	933	204	114	3,000
3	OB	16	1	2015	1,025	90	51	7,980
3	OB	16	1	2020	1,025	192	102	
3	OB	21	1	2021	933	96	120	
3	OB	19	1	2025	800	715	53	8,800
3	OB	21	1	2064	1,025	368	90	
3	OB	23	1	3252	1,179	114	73	6,125
3	OB	24	1	3377	1,025	184	67	1,920
3	OB	30	1	2115	769	194	89	526
3	OB	77	2	1014	666	225	66	1,115
3	OB	47	1	1052	666	159	80	1,115
3	OB	53	2	1082	871	327	75	14,100
3	OB	57	2	1117	1,128	147	278	1,675
3	OB	52	1	1164	574	168	119	
3	OB	52	1	1175	1,025	176	119	1,400
3	OB	42	1	1207	1,025	175	102	1,675
3	OB	48	1	1220	1,025	306	102	4,775
3	OB	52	1	1258	1,128	155	75	4,450
3	OB	62	1	2108	513	137	92	1,400
3	OB	64	1	3632	718	368	165	1,400
3	OB	55	1	3738	1,128	151	127	16,290
3	OB	59	1	3790	820	245	82	325
3	OB	62	1	3812	1,025	510	63	325
3	OB	76	1	2109	1,230	317	265	72
3	OB	75	1	1254	1,076	654	80	2,225
3	OB	71	1	1259	1,230	204	190	4,125
								71
3	IA	18	2	1162	615	196	183	2,785
3	IA	18	2	1163	820	245	234	2,525
3	IA	21	2	1166	615	215	165	
3	IA	24	2	1165	820	196	316	
3	IA	25	2	1119	1,128	178	329	5,600
3	IA	26	2	1084	820	92	99	3,000
3	IA	26	2	1029	630	145	145	1,400
								7
3	IB	18	2	1150	1,025	196	221	1,115

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
3	IB	22	2	1152	1,025	178	165	2,525
3	IB	20	2	2088	554	108	154	3,000
3	IB	21	2	2096	851	127	67	18,400
3	IB	25	2	2104	871	157	108	6,900
3	IB	22	2	3641	892	104	127	
3	IB	38	2	1077	923	133	103	72
3	IB	33	2	1202	820	347	95	6,125
3	IB	37	2	2007	1,076	133	78	
3	IB	39	2	2073	605	163	110	5,500
3	IB	39	2	3250	1,025	204	120	526
3	IB	31	2	3490	554	139	71	5,800
3	IB	33	2	3793	1,128	490	240	5,075
3	IB	45	2	1063	820	163	133	
3	IB	55	2	1079	923	143	127	4,125
3	IB	44	2	1081	1,076	245	165	1,920
3	IB	46	2	1100	1,128	172	152	1,675
3	IB	48	2	1112	923	408	316	3,900
3	IB	42	2	1134	1,128	572	86	1,920
3	IB	49	2	1149	1,128	204	254	2,525
3	IB	61	2	1238	1,948	441	119	1,115
3	IB	42	2	1252	1,025	184	102	8,800
3	IB	50	2	2065	851	174	127	
3	IB	05	2	2077	851	241	145	7,200
3	IB	51	2	2102	1,025	59	67	72
3	IB	58	2	2196	461	178	94	526
3	IB	50	2	3695	790	147	265	72
3	IB	46	2	2109	1,179	194	44	72
3	IB	66	2	1088	1,025	172	137	
3	IB	74	2	1237	1,076	327	86	
3	IB	67	2	1245	892	137	291	
3	IB	58	1	1072	728	245	127	17,900
3	IB	41	1	1078	1,025	127	133	72
3	IB	46	1	1105	923	245	254	6,700
3	IB	47	1	1131	1,025	204	116	2,225
3	IB	44	1	1133	923	127	221	9,975
3	IB	42	1	1239	2,050	225	89	1,920
3	IB	53	1	2067	718	141	119	
3	IB	58	1	2092	605	92	61	1,920
3	IB	59	1	3790	820	245	82	325
3	IB	49	1	3961	636	123	608	
3	IB	76	1	1104	1,230	317	265	72
								42
4	CO	18	1	1	1,025	220	66	
4	CO	23	1	2	933	235	76	3,300
4	CO	29	1	3	841	129	116	
4	CO	28	1	4	687	210	95	1,500
4	CO	27	1	5	1,179	188	82	
4	CO	27	1	6	769	220	82	
4	CO	21	1	7	841	129	76	1,850

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
4	CO	24	1	8	933	106	61	72
4	CO	25	1	9	841	255	89	7,700
4	CO	22	1	10	933	163	89	
4	CO	21	1	11	769	170	66	1,500
4	CO	23	1	12	1,735	276	70	3,300
4	CO	21	1	13	902	153	85	72
4	CO	26	1	14	902	121	101	
4	CO	19	1	15	902	182	165	
4	CO	22	1	16	1,025	153	89	72
4	CO	30	1	17	513	153	78	
4	CO	22	1	18	800	98	85	
4	CO	21	1	19	513	110	72	
4	CO	27	1	20	800	174	78	2,300
4	CO	22	1	21	871	139	132	1,850
4	CO	24	1	22	943	180	145	1,500
4	CO	24	1	23	871	196	89	
4	CO	26	1	24	871	225	145	
4	CO	18	1	25	871	145	102	3,300
4	CO	18	1	26	943	163	114	
4	CO	23	1	27	871	82	80	3,300
4	CO	27	1	28	1,128	255	102	1,500
4	CO	26	1	29	1,025	204	102	2,300
4	CO	25	1	30	666	196	89	1,850
4	CO	26	1	31	749	145	118	72
4	CO	25	1	32	810	123	77	3,300
4	CO	25	1	33	943	266	77	
4	CO	18	1	34	871	157	152	
4	CO	24	1	35	871	145	102	
4	CO	29	1	36	871	129	102	
4	CO	22	1	37	749	235	102	3,000
4	CO	24	1	38	871	194	70	
4	CO	23	1	39	1,332	215	96	
4	CO	23	1	40	974	133	118	
4	CO	23	1	41	871	184	110	72
4	CO	25	1	42	718	147	102	
4	CO	18	1	43	1,128	133	91	3,300
4	CO	28	1	44	800	123	118	
4	CO	22	1	45	800	141	127	
4	CO	23	1	46	1,025	123	110	3,300
4	CO	30	1	47	871	118	102	9,175
4	CO	39	1	1	841	177	76	
4	CO	31	1	2	933	235	82	5,500
4	CO	34	1	3	1,025	129	68	850
4	CO	37	1	4	933	177	145	
4	CO	39	1	5	841	255	89	2,225
4	CO	37	1	6	841	177	158	72
4	CO	40	1	7	769	147	68	
4	CO	31	1	8	1,179	153	108	
4	CO	32	1	9	800	143	108	1,675
4	CO	34	1	10	851	204	92	
4	CO	31	1	11	1,025	159	57	

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
4	CO	34	1	12	646	168	92	
4	CO	38	1	13	564	204	78	1,400
4	CO	33	1	14	1,281	215	139	7,700
4	CO	31	1	15	1,025	266	102	
4	CO	38	1	16	943	276	110	4,100
4	CO	39	1	17	943	145	70	1,850
4	CO	36	1	18	1,025	180	89	850
4	CO	33	1	19	943	129	89	2,300
4	CO	40	1	20	810	180	118	
4	CO	40	1	21	871	245	139	
4	CO	40	1	22	718	204	89	
4	CO	36	1	23	1,128	174	70	
4	CO	33	1	24	974	204	152	
4	CO	40	1	25	666	184	102	3,000
4	CO	36	1	26	718	225	89	1,115
4	CO	34	1	27	666	174	70	
4	CO	36	1	28	1,332	255	142	72
4	CO	33	1	29	1,435	266	70	72
4	CO	51	1	1	1,025	177	68	3,300
4	CO	55	1	2	1,179	163	76	
4	CO	57	1	3	841	129	101	
4	CO	59	1	4	1,025	255	127	7,700
4	CO	43	1	5	933	194	57	
4	CO	60	1	6	902	137	152	
4	CO	42	1	7	718	168	62	3,000
4	CO	48	1	8	646	255	72	
4	CO	46	1	9	718	88	108	1,850
4	CO	42	1	10	1,179	306	137	
4	CO	44	1	11	1,179	235	127	1,200
4	CO	45	1	12	1,128	180	127	
4	CO	56	1	13	871	215	89	
4	CO	44	1	14	943	100	102	
4	CO	45	1	15	871	225	118	
4	CO	61	1	16	810	255	89	72
4	CO	48	1	17	943	225	59	
4	CO	49	1	18	810	188	110	
4	CO	47	1	19	1,230	151	89	
4	CO	41	1	20	871	174	132	
4	CO	45	1	21	953	174	132	
4	CO	64	1	22	513	129	110	
4	CO	46	1	23	1,025	194	89	72
4	CO	45	1	24	871	151	89	
4	CO	65	1	25	871	215	114	
4	CO	43	1	26	800	155	114	72
4	CO	55	1	27	953	174	165	
4	CO	56	1	28	636	106	70	72
4	CO	49	1	29	800	170	89	1,300
4	CO	24	2	1	841	163	66	
4	CO	24	2	2	1,025	170	101	
4	CO	24	2	3	933	194	158	
4	CO	22	2	4	933	170	82	1,650

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
4	CO	29	2	5	1,025	106	137	1,300
4	CO	23	2	6	933	125	132	72
4	CO	28	2	7	1,179	163	127	
4	CO	28	2	8	841	204	101	1,850
4	CO	28	2	9	1,025	194	101	
4	CO	24	2	10	841	188	61	
4	CO	21	2	11	769	235	89	6,100
4	CO	24	2	12	933	163	109	3,300
4	CO	19	2	13	1,025	174	96	
4	CO	29	2	14	902	121	127	
4	CO	22	2	15	943	137	89	1,850
4	CO	26	2	16	800	168	165	
4	CO	26	2	17	1,025	116	127	
4	CO	23	2	18	943	188	177	
4	CO	25	2	19	943	235	108	
4	CO	28	2	20	1,281	159	67	
4	CO	26	2	21	851	110	92	3,300
4	CO	27	2	22	1,179	139	142	
4	CO	26	2	23	1,179	123	158	
4	CO	23	2	24	1,025	255	142	
4	CO	23	2	25	943	276	110	3,700
4	CO	23	2	26	943	180	110	
4	CO	26	2	27	943	215	89	6,400
4	CO	22	2	28	513	255	127	72
4	CO	22	2	29	1,281	215	110	72
4	CO	23	2	30	749	174	132	72
4	CO	26	2	31	1,281	118	127	72
4	CO	22	2	32	1,128	194	127	
4	CO	27	2	33	953	235	110	
4	CO	23	2	34	1,230	215	165	6,000
4	CO	22	2	35	953	184	65	72
4	CO	19	2	36	1,352	215	78	4,800
4	CO	22	2	37	1,128	194	85	72
4	CO	24	2	38	1,025	129	108	
4	CO	24	2	39	1,281	174	132	1,350
4	CO	23	2	40	871	112	110	72
4	CO	26	2	41	513	194	142	2,300
4	CO	25	2	42	718	129	110	72
4	CO	25	2	43	1,332	133	127	
4	CO	22	2	44	666	147	152	
4	CO	27	2	45	800	102	152	
4	CO	25	2	46	1,179	72	110	
4	CO	23	2	47	974	255	102	
4	CO	19	2	48	615	147	110	
4	CO	26	2	49	1,128	194	96	1,800
4	CO	21	2	50	666	174	85	1,500
4	CO	20	2	51	1,025	118	65	
4	CO	33	2	1	1,179	170	171	
4	CO	31	2	2	933	155	145	526
4	CO	35	2	3	554	235	89	72
4	CO	36	2	4	1,025	168	108	

SET	GR	AGE	SEX	PAT#	IGG	IGA	IGM	IGD
4	CO	33	2	5	902	143	132	72
4	CO	39	2	6	1,179	215	165	72
4	CO	40	2	7	800	92	92	
4	CO	31	2	8	1,025	174	165	
4	CO	34	2	9	1,025	174	62	72
4	CO	32	2	10	943	215	127	5,200
4	CO	33	2	11	1,230	196	139	1,350
4	CO	34	2	12	716	147	142	
4	CO	33	2	13	1,128	133	165	
4	CO	35	2	14	666	102	118	72
4	CO	33	2	15	871	123	85	72
4	CO	56	2	1	1,179	151	76	
4	CO	41	2	2	933	155	82	72
4	CO	53	2	3	841	210	101	2,025
4	CO	53	2	4	1,179	266	177	72
4	CO	59	2	5	1,025	168	127	
4	CO	41	2	6	800	137	85	
4	CO	52	2	7	646	188	108	
4	CO	44	2	8	871	157	139	7,300
4	CO	46	2	9	871	106	102	72
4	CO	61	2	10	687	129	127	
4	CO	52	2	11	1,128	184	127	
4	CO	59	2	12	1,025	184	102	72
4	CO	44	2	13	718	155	127	
4	CO	49	2	14	1,281	235	91	
								185
4	CD	02	2	4401	1,025	127	121	
4	CD	02	2	4402	615	41	72	
4	CD	04	2	4403	923	204	101	7,500
4	CD	02	2	4404	390	33	89	
4	CD	04	2	4405	564	110	54	
4	CD	05	2	4406	615	74	81	72
4	CD	03	2	4407	564	147	121	
4	CD	04	2	4408	892	147	94	
4	CD	02	2	4409	820	78	89	
4	CD	04	2	4410	790	131	104	7,300
4	CD	05	2	4411	492	67	38	
4	CD	04	2	4412	697	92	145	72
4	CD	03	2	4413	1,230	63	38	
4	CD	05	2	4414	492	92	165	72
4	CD	07	2	4415	994	159	121	1,500
4	CD	05	2	4416	1,199	225	165	1,200
4	CD	06	2	4417	790	51	57	72
4	CD	07	2	4418	595	82	75	
4	CD	07	2	4419	390	174	101	
4	CD	05	2	4420	697	102	145	
4	CD	05	2	4421	790	88	70	
4	CD	04	2	4422	994	102	108	1,700
4	CD	06	2	4423	595	67	165	

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
4	CD	07	2	4424	790	112	57	
4	CD	06	2	4425	1,332	82	101	4,900
4	CD	06	2	4426	492	112	63	
4	CD	06	2	4427	697	102	108	1,300
4	CD	07	2	4428	892	82	145	72
4	CD	05	2	4429	697	112	70	
4	CD	05	2	4430	595	102	101	5,500
4	CD	05	2	4431	697	102	70	
4	CD	09	2	4501	943	108	77	8,600
4	CD	09	2	4502	790	51	121	
4	CD	09	2	4503	564	245	139	1,700
4	CD	09	2	4504	1,025	108	132	
4	CD	08	2	4505	492	108	38	
4	CD	10	2	4506	390	88	57	6,200
4	CD	08	2	4507	790	108	89	7,300
4	CD	08	2	4508	687	127	108	9,000
4	CD	08	2	4509	790	147	89	1,500
4	CD	08	2	4510	687	123	108	
4	CD	10	2	4511	1,230	88	145	1,700
4	CD	08	2	4512	892	127	108	
4	CD	08	2	4513	1,179	163	202	500
4	CD	09	2	4514	564	147	145	72
4	CD	08	2	4515	892	123	44	
4	CD	08	2	4516	687	204	100	72
4	CD	11	2	4517	687	63	89	2,900
4	CD	11	2	4518	892	159	89	
4	CD	11	2	4519	1,025	139	94	2,200
4	CD	11	2	4520	943	127	82	
4	CD	12	2	4521	790	180	116	
4	CD	12	2	4522	461	147	190	2,000
4	CD	12	2	4523	595	88	177	8,100
4	CD	12	2	4524	492	174	101	
4	CD	12	2	4525	790	108	63	72
4	CD	11	2	4526	1,332	88	101	
4	CD	13	2	4601	994	225	132	8,100
4	CD	13	2	4602	1,076	184	101	72
4	CD	14	2	4603	892	127	94	
4	CD	17	2	4604	1,199	159	121	
4	CD	14	2	4605	892	225	132	1,300
4	CD	16	2	4606	1,281	196	121	1,700
4	CD	17	2	4607	687	147	89	
4	CD	15	2	4608	790	147	70	
4	CD	14	2	4609	994	67	101	2,500
4	CD	14	2	4610	543	88	202	4,900
4	CD	15	2	4611	790	108	57	
4	CD	14	2	4612	1,076	163	140	72
4	CD	15	2	4613	697	123	127	
4	CD	04	1	3601	943	159	132	2,000
4	CD	04	1	3602	687	127	66	500
4	CD	03	1	3603	615	63	54	
4	CD	02	1	3604	820	90	59	72

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
4	CD	02	1	3605	759	31	110	
4	CD	03	1	3606	605	82	152	72
4	CD	03	1	3607	410	194	76	
4	CD	04	1	3608	574	133	38	
4	CD	04	1	3609	461	108	67	
4	CD	04	1	3610	595	102	38	72
4	CD	04	1	3611	1,056	143	82	72
4	CD	04	1	3612	318	51	63	200
4	CD	05	1	3613	994	63	165	
4	CD	06	1	3614	892	255	145	
4	CD	05	1	3615	580	88	38	
4	CD	06	1	3616	728	41	59	700
4	CD	05	1	3617	595	129	76	2,800
4	CD	07	1	3618	892	82	82	
4	CD	07	1	3619	400	108	101	1,500
4	CD	06	1	3620	790	143	76	72
4	CD	07	1	3621	1,199	82	101	
4	CD	07	1	3622	584	102	38	
4	CD	07	1	3623	554	133	76	7,500
4	CD	06	1	3624	697	108	59	72
4	CD	06	1	3625	595	129	82	
4	CD	06	1	3626	666	153	76	
4	CD	07	1	3627	892	174	59	1,200
4	CD	09	1	3701	615	61	101	2,200
4	CD	08	1	3702	718	147	89	2,500
4	CD	10	1	3703	535	127	94	1,000
4	CD	08	1	3704	1,025	159	77	
4	CD	10	1	3705	718	196	121	6,800
4	CD	10	1	3706	1,230	180	81	500
4	CD	10	1	3707	1,004	184	165	2,500
4	CD	09	1	3708	892	194	77	5,500
4	CD	09	1	3709	892	170	77	
4	CD	09	1	3710	687	61	57	
4	CD	09	1	3711	697	82	101	3,100
4	CD	10	1	3712	595	102	76	500
4	CD	10	1	3713	687	88	57	72
4	CD	09	1	3714	451	72	77	
4	CD	11	1	3715	687	127	108	2,900
4	CD	12	1	3716	687	90	51	
4	CD	11	1	3717	1,128	159	77	8,600
4	CD	11	1	3718	492	47	101	3,900
4	CD	11	1	3719	923	147	59	
4	CD	11	1	3720	390	108	145	10,200
4	CD	11	1	3721	1,300	210	95	3,300
4	CD	12	1	3722	697	163	120	72
4	CD	11	1	3723	1,025	108	38	
4	CD	12	1	3724	882	225	120	7,300
4	CD	12	1	3725	790	230	152	
4	CD	13	1	3801	994	102	73	
4	CD	13	1	3802	769	123	70	72
4	CD	14	1	3803	892	147	94	5,500

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
4	CD	14	1	3804	564	127	54	
4	CD	14	1	3805	584	67	114	
4	CD	17	1	3806	790	88	127	5,500
4	CD	15	1	3807	697	159	110	1,500
4	CD	16	1	3808	1,281	184	25	72
4	CD	14	1	3809	790	108	145	10,900
4	CD	14	1	3810	595	245	95	4,900
4	CD	15	1	3811	1,300	266	82	
4	CD	14	1	3812	974	108	97	5,500
4	CD	16	1	3813	697	204	85	
4	CD	17	1	3814	790	215	132	
								136
5	ND	34	1	1062	666	176	80	3,600
5	ND	68	1	1085	461	106	49	
5	ND	54	1	1103	728	245	57	3,900
5	ND	43	1	1129	1,076	296	92	3,600
5	ND	07	1	1155	543	110	81	5,800
5	ND	51	2	2061	687	184	95	1,400
5	ND	31	2	3009	625	180	304	7,700
5	ND	37	2	3034	851	188	108	
5	ND	24	1	3039	1,128	165	82	4,450
5	ND	26	2	3040	902	188	90	72
5	ND	18	2	3042	1,332	135	95	
5	ND	26	2	3046	769	84	392	1,920
5	ND	39	2	3057	769	125	95	
5	ND	25	2	3058	902	245	215	8,375
5	ND	74	1	3114	1,179	429	89	3,600
5	ND	27	2	3125	718	94	94	7,980
5	ND	60	1	3157	574	94	113	
5	ND	27	2	3171	615	145	114	
5	ND	38	1	3173	636	255	120	72
5	ND	09	1	3174	800	266	83	5,800
5	ND	15	1	3176	718	139	104	4,450
5	ND	13	2	3177	902	61	127	1,400
5	ND	38	1	3203	574	153	63	2,785
5	ND	50	1	3205	923	253	100	
5	ND	43	1	3208	574	229	127	1,920
5	ND	09	2	3213	574	116	71	526
5	ND	55	1	3214	820	159	95	12,700
5	ND	08	1	3217	513	118	152	
5	ND	30	2	3220	646	112	120	
5	ND	32	1	3230	502	168	52	526
5	ND	54	2	3235	1,025	86	202	
5	ND	18	2	3236	1,025	300	116	526
5	ND	26	1	3237	1,025	192	68	2,525
5	ND	20	2	3255	902	180	108	3,000
5	ND	41	2	3270	800	123	139	
5	ND	59	1	3271	1,025	143	61	2,525
5	ND	45	2	3297	564	127	71	72

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
5	ND	24	2	3300	636	123	75	1,400
5	ND	35	2	3309	1,025	143	149	
5	ND	07	2	3306	851	76	95	5,075
5	ND	07	1	3307	1,230	159	66	6,900
5	ND	49	2	3308	1,179	106	75	72
5	ND	22	2	3332	902	358	86	4,450
5	ND	39	1	3347	943	184	58	72
5	ND	26	1	3348	1,128	139	172	5,500
5	ND	21	2	3356	1,128	153	82	7,200
5	ND	44	2	3359	1,025	159	127	2,785
5	ND	24	2	3375	1,025	102	133	5,500
5	ND	56	2	3387	666	149	145	5,500
5	ND	24	2	3397	1,025	204	82	
5	ND	63	1	3402	769	153	58	526
5	ND	29	2	3406	1,025	184	82	
5	ND	34	2	3425	1,025	108	146	3,600
5	ND	43	1	3448	482	157	71	4,450
5	ND	49	2	3454	1,025	116	47	
5	ND	39	1	3462	666	180	120	
5	ND	29	2	3480	902	215	221	19,500
5	ND	49	2	3486	943	129	82	526
5	ND	66	2	3493	943	159	82	
5	ND	51	1	3496	687	235	101	5,500
5	ND	49	1	3497	851	178	63	9,175
5	ND	35	1	3531	1,128	88	101	3,600
5	ND	30	2	3533	943	153	95	72
5	ND	12	2	3534	1,281	168	177	6,125
5	ND	09	2	3536	841	72	132	
5	ND	05	2	3540	1,128	88	132	2,525
5	ND	27	1	3546	1,025	133	77	
5	ND	18	2	3550	1,845	192	304	2,225
5	ND	24	2	3559	797	121	297	72
5	ND	66	1	3561	1,025	225	113	72
5	ND	74	2	3562	841	155	115	72
5	ND	38	1	3568	923	153	61	
5	ND	33	2	3569	543	178	110	9,600
5	ND	23	1	3581	923	163	145	
5	ND	44	1	3590	1,128		118	3,600
5	ND	49	2	3637	1,025	204	115	72
5	ND	69	1	3639	677	449	152	1,675
5	ND	27	1	3649	1,025	127	78	325
5	ND	30	2	3659	1,128	170	290	72
5	ND	78	1	3663	790	204	120	850
5	ND	50	2	3671	820	255	127	1,115
5	ND	04	2	3683	871	135	110	72
5	ND	12	1	3684	615	306	209	6,700
5	ND	09	2	3686	718	155	145	526
5	ND	08	2	3687	790	196	290	1,920
5	ND	29	1	3709	718	177	61	850
5	ND	47	2	3719	790	135	139	
5	ND	27	2	3725	333	196	304	526

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
5	ND	34	1	3729	646	155	109	850
5	ND	12	2	3730	820	133	133	3,000
5	ND	33	2	3731	728	186	137	72
5	ND	09	1	3732	728	118	127	325
5	ND	28	2	3743	543	147	139	1,675
5	ND	27	2	3772	871	170	115	
5	ND	48	2	3783	923	155	137	
5	ND	40	1	3809	1,230	634	58	8,375
5	ND	59	2	3813	749	266	63	1,675
5	ND	31	2	3817	749	102	127	6,900
5	ND	29	2	3829	820	255	354	
5	ND	36	2	3829	820	337	165	
5	ND	33	2	3839	923	215	165	5,500
5	ND	67	1	3844	769	139	85	
5	ND	44	2	3854	871	188	247	1,920
5	ND	13	1	3856	871	59	61	
5	ND	28	2	3862	666	180	152	72
5	ND	12	1	3888	1,025	102	96	
5	ND	05	2	3889	1,025	188	101	850
5	ND	10	1	3890	769	196	127	72
5	ND	07	2	3891	666	177	96	
5	ND	46	1	3906	769	145	73	1,400
5	ND	15	2	3907	769	266	110	
5	ND	13	2	3909	666	266	110	1,400
5	ND	19	1	3910	769	86	73	5,075
5	ND	39	2	3912	871	106	110	5,800
5	ND	17	1	3973	1,025	368	96	
5	ND	15	1	3992	943	317	145	72
5	ND	17	2	3997	943	70	83	15,400
5	ND	08	2	3998	943	106	83	526
5	ND	06	2	3999	513	47	78	
5	ND	07	1	4000	697	76	90	
								120
6	G6	20	2	1021	749	151	76	2,785
6	G6	19	2	1022	595	143	65	8,800
6	G6	02	1	1024	595	59	48	
6	G6	05	2	1027	749	125	120	72
6	G6	35	1	1030	933	245	91	72
6	G6	11	1	1032	595	131	37	7,200
6	G6	15	1	1033	728	70	53	9,175
6	G6	17	2	1034	749	215	91	4,125
6	G6	18	1	1035	820	100	85	3,000
6	G6	37	2	1036	666	245	58	526
6	G6	62	1	1041	933	266	80	2,525
6	G6	36	2	1044	749	198	91	4,450
6	G6	18	1	1045	595	266	53	4,450
6	G6	21	2	1046	666	204	97	4,125
6	G6	69	1	1047	830	245	44	72
6	G6	26	1	1049	830	245	91	1,115

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	08	1	1054	1,025	125	72	3,900
6	G6	27	1	1056	830	204	62	12,125
6	G6	09	2	1057	871	129	80	7,200
6	G6	26	2	1058	1,025	327	93	4,775
6	G6	06	2	1061	933	286	80	14,390
6	G6	21	2	1069	1,025	204	109	10,775
6	G6	78	1	1087	728	388	76	2,225
6	G6	04	2	1090	574	70	109	526
6	G6	02	2	1091	728	53	66	
6	G6	07	2	1093	820	151	94	
6	G6	05	1	1113	492	100	75	3,900
6	G6	04	1	1115	718	92	43	
6	G6	08	1	1120	1,025	133	152	9,175
6	G6	10	1	1128	1,025	163	109	
6	G6	18	1	1130	1,128	123	57	
6	G6	15	1	1132	871	141	86	1,400
6	G6	39	1	1136	871	163	92	72
6	G6	45	2	1137	871	347	265	325
6	G6	65	2	1138	871	449	145	72
6	G6	38	2	1145	1,025	255	190	
6	G6	04	2	1154	543	41	110	850
6	G6	22	2	1157	871	135	165	4,775
6	G6	02	2	1158	513	27	83	2,225
6	G6	76	1	1160	1,076	266	101	1,920
6	G6	25	2	1172	615	63	152	2,525
6	G6	04	1	1173	718	59	101	2,225
6	G6	06	2	1174	543	86	95	1,400
6	G6	01	2	1182	718	57	158	4,125
6	G6	43	2	1183	1,076	82	101	
6	G6	71	2	1184	1,076	409	183	72
6	G6	58	1	1185	1,076	327	171	
6	G6	05	2	1196	543	72	70	2,785
6	G6	03	2	1197	513	45	80	4,450
6	G6	11	2	1198	615	245	81	5,500
6	G6	12	1	1199	441	127	54	3,600
6	G6	08	1	1200	615	168	47	6,700
6	G6	09	1	1201	523	110	46	1,400
6	G6	06	1	1203	543	47	139	6,900
6	G6	60	1	1213	923	141	63	72
6	G6	06	2	1214	933	108	72	
6	G6	44	1	1225	871	204	92	526
6	G6	68	2	1233	851	241	297	1,400
6	G6	89	2	1234	1,076	163	109	
6	G6	80	2	1240	1,076	612	177	
6	G6	58	1	1242	1,025	194	152	
6	G6	76	1	1247	871	194	228	1,675
6	G6	69	2	1248	1,025	129	171	
6	G6	60	2	1250	769	194	139	72
6	G6	45	2	1343	1,179	265	162	
6	G6	23	2	1346	1,025	155	196	
6	G6	80	1	1603	1,076	510	59	1,400

SET	GR	AGE	SEX	PAT#	IGG	IGA	IGM	IGD
6	G6	18	2	2016	1,076	215	70	8,800
6	G6	12	2	2018	933	57	120	72
6	G6	56	1	2022	1,179	592	127	2,225
6	G6	35	2	2035	851	184	86	1,920
6	G6	40	1	2036	933	368	97	10,500
6	G6	45	2	2037	1,078	245	95	3,600
6	G6	06	1	2045	656	102	68	5,075
6	G6	08	1	2046	892	163	72	6,900
6	G6	09	2	2047	1,076	141	67	13,500
6	G6	11	1	2048	1,128	286	86	20,825
6	G6	13	1	2049	851	317	76	6,125
6	G6	33	2	2070	656	131	72	9,175
6	G6	43	1	2072	769	225	72	72
6	G6	40	1	2074	605	490	63	2,525
6	G6	46	2	2076	851	184	101	72
6	G6	10	1	2083	718	241	101	
6	G6	16	2	2091	892	159	127	3,900
6	G6	26	2	2093	800	180	196	
6	G6	14	2	2097	605	90	95	9,600
6	G6	46	2	2100	1,332	306	139	1,115
6	G6	53	1	2101	584	235	89	2,225
6	G6	08	1	2106	584	102	102	
6	G6	44	2	2111	1,025	235	316	
6	G6	17	1	2112	1,179	65	78	
6	G6	18	2	2114	892	151	127	4,125
6	G6	05	2	2116	574	147	152	850
6	G6	29	2	2199	625	276	215	3,000
6	G6	51	1	3001	677	430	152	
6	G6	71	2	3002	800	108	196	
6	G6	05	2	3003	677	84	152	
6	G6	09	2	3004	625	51	202	
6	G6	21	2	3006	800	180	404	
6	G6	13	1	3007	677	204	165	6,900
6	G6	81	2	3010	625	317	196	
6	G6	24	2	3011	677	276	165	11,300
6	G6	07	1	3012	902	157	78	526
6	G6	17	1	3013	748	177	95	1,115
6	G6	44	1	3015	625	204	114	850
6	G6	07	1	3016	677	215	114	72
6	G6	59	1	3017	625	327	87	16,290
6	G6	77	2	3018	800	215	152	72
6	G6	31	2	3019	738	673	202	526
6	G6	70	2	3020	1,640	204	108	72
6	G6	10	2	3022	1,128	90	132	
6	G6	54	1	3023	769	204	127	1,920
6	G6	06	1	3025	1,128	157	152	2,225
6	G6	08	1	3026	769	114	116	
6	G6	10	1	3027	1,128	204	240	8,800
6	G6	12	1	3029	902	110	108	
6	G6	14	1	3031	902	74	152	72
6	G6	35	2	3032	902	110	152	1,115

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	08	2	3035	851	153	116	4,125
6	G6	06	1	3037	1,025	98	61	6,900
6	G6	24	2	3038	1,332	153	90	526
6	G6	40	1	3043	769	114	61	72
6	G6	36	2	3048	1,025	80	215	
6	G6	18	2	3049	1,025	157	196	1,400
6	G6	11	1	3050	1,128	90	63	3,600
6	G6	33	2	3054	1,640	20	116	
6	G6	25	1	3055	1,128	188	76	1,400
6	G6	31	2	3056	1,128	215	127	9,975
6	G6	08	1	3061	902	255	108	5,500
6	G6	22	2	3063	1,128	125	158	
6	G6	31	1	3064	1,128	215	202	
6	G6	29	1	3065	902	125	70	72
6	G6	69	2	3066	1,025	141	63	
6	G6	55	1	3067	564	114	90	72
6	G6	29	1	3068	769	114	70	3,900
6	G6	47	1	3069	1,128	215	329	1,400
6	G6	09	1	3072	902	84	53	325
6	G6	29	2	3073	1,332	141	158	3,900
6	G6	06	1	3074	902	125	76	
6	G6	09	1	3075	902	168	82	1,400
6	G6	35	1	3076	1,025	165	44	72
6	G6	11	1	3078	902	153	76	
6	G6	09	1	3081	1,128	174	82	3,900
6	G6	16	1	3082	1,025	168	120	9,975
6	G6	15	2	3083	1,128	159	215	14,900
6	G6	19	2	3086	656	215	139	6,125
6	G6	24	1	3088	1,128	215	116	1,920
6	G6	28	1	3092	1,332	196	127	
6	G6	22	1	3093	1,384	125	152	850
6	G6	39	1	3095	1,025	176	70	2,785
6	G6	11	2	3096	1,332	266	158	3,600
6	G6	04	1	3101	513	125	63	13,500
6	G6	30	2	3102	1,025	347	83	4,125
6	G6	32	1	3103	1,025	188	133	14,100
6	G6	48	1	3105	1,025	139	94	
6	G6	32	2	3106	1,332	200	234	526
6	G6	04	2	3110	718	67	120	3,600
6	G6	09	2	3112	1,025	139	196	
6	G6	68	2	3116	1,384	511	145	526
6	G6	71	2	3117	1,179	68	132	
6	G6	05	1	3120	718	133	78	10,775
6	G6	06	2	3122	636	94	132	5,500
6	G6	09	2	3124	1,128	159	118	1,115
6	G6	15	1	3126	820	159	127	2,785
6	G6	08	1	3127	800	106	75	8,800
6	G6	17	1	3129	1,261	214	67	2,785
6	G6	52	1	3130	718	153	78	526
6	G6	95	1	3131	820	118	84	3,600
6	G6	46	2	3138	1,179	388	158	

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	46	1	3143	1,025	204	132	72
6	G6	14	1	3144	1,025	127	104	
6	G6	18	2	3146	615	235	145	1,400
6	G6	10	1	3147	943	149	127	2,785
6	G6	10	2	3149	923	72	106	2,785
6	G6	11	1	3150	574	102	106	4,450
6	G6	11	2	3151	1,281	125	142	4,450
6	G6	18	1	3152	820	153	89	
6	G6	13	1	3155	923	131	95	13,500
6	G6	60	1	3156	820	241	100	
6	G6	18	2	3159	646	131	142	72
6	G6	09	2	3164	513	131	133	526
6	G6	14	1	3172	564	153	63	
6	G6	17	1	3178	1,179	96	109	72
6	G6	15	2	3179	902	182	109	
6	G6	15	2	3180	800	153	127	2,525
6	G6	13	2	3181	820	153	106	3,600
6	G6	40	1	3183	646	145	100	72
6	G6	22	2	3186	902	159	132	6,125
6	G6	15	2	3188	820	94	114	6,900
6	G6	07	1	3189	646	104	66	5,500
6	G6	19	2	3190	646	118	84	2,225
6	G6	14	2	3191	513	137	84	6,900
6	G6	09	2	3194	646	155	106	6,700
6	G6	07	1	3195	923	163	53	5,075
6	G6	10	1	3197	738	204	84	20,000
6	G6	11	1	3199	646	145	113	5,500
6	G6	05	1	3202	574	159	63	3,000
6	G6	19	2	3204	820	215	254	9,975
6	G6	49	1	3206	615	204	95	72
6	G6	12	2	3209	646	153	254	1,115
6	G6	35	2	3210	574	166	304	4,775
6	G6	47	2	3212	902	388	132	72
6	G6	04	1	3219	430	80	84	
6	G6	61	2	3221	533	229	130	1,115
6	G6	07	1	3222	461	102	84	8,375
6	G6	08	2	3223	574	159	80	4,775
6	G6	51	1	3224	923	215	84	9,975
6	G6	11	2	3226	646	159	114	72
6	G6	10	2	3227	543	102	70	72
6	G6	12	2	3228	574	168	60	5,075
6	G6	34	2	3229	461	168	75	
6	G6	53	1	3231	440	184	89	12,990
6	G6	46	2	3232	1,025	337	101	5,500
6	G6	22	2	3233	902	157	92	
6	G6	26	1	3234	1,179	262	127	3,600
6	G6	14	2	3238	584	337	92	11,000
6	G6	06	1	3242	871	143	61	
6	G6	08	1	3243	1,558	27	89	
6	G6	06	1	3246	851	83	54	4,775
6	G6	05	1	3248	1,261	139	63	4,450

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	60	2	3249	902	190	83	
6	G6	10	2	3251	1,691	151	127	3,000
6	G6	17	2	3256	1,230	176	89	
6	G6	12	1	3257	595	76	58	72
6	G6	41	1	3258	902	139	86	1,400
6	G6	09	1	3259	1,179	123	63	2,785
6	G6	01	1	3260	1,025	157	72	3,600
6	G6	42	2	3261	902	409	145	2,525
6	G6	05	2	3262	636	45	109	
6	G6	61	2	3263	871	96	102	4,775
6	G6	43	1	3265	1,025	358	157	
6	G6	08	2	3266	902	131	78	5,500
6	G6	18	2	3267	902	225	108	5,075
6	G6	12	1	3269	1,025	135	77	8,000
6	G6	08	1	3272	1,025	143	95	6,125
6	G6	07	2	3273	800	41	61	72
6	G6	38	1	3274	716	43	48	
6	G6	55	1	3275	800	409	57	9,175
6	G6	12	1	3276	1,332	190	120	7,200
6	G6	05	1	3278	810	65	61	72
6	G6	27	2	3279	902	84	89	4,125
6	G6	14	1	3281	1,538	153	83	2,000
6	G6	52	1	3286	1,025	490	104	72
6	G6	25	1	3287	636	276	89	72
6	G6	08	2	3289	636	153	63	72
6	G6	07	1	3294	666	67	215	325
6	G6	27	1	3296	769	245	108	72
6	G6	14	2	3298	718	78	104	1,920
6	G6	29	1	3302	902	106	67	72
6	G6	16	2	3304	1,025	153	104	3,000
6	G6	65	1	3310	513	184	92	72
6	G6	16	1	3312	1,025	98	158	9,600
6	G6	06	1	3316	554	104	35	3,600
6	G6	27	2	3317	851	214	67	2,525
6	G6	52	2	3319	1,332	139	83	
6	G6	05	2	3324	769	67	108	3,900
6	G6	12	2	3325	584	123	215	72
6	G6	09	2	3327	666	92	196	2,225
6	G6	06	2	3328	769	131	92	
6	G6	12	2	3329	759	198	158	72
6	G6	04	2	3334	759	45	139	
6	G6	08	1	3335	851	149	127	
6	G6	05	1	3336	759	80	77	1,115
6	G6	08	2	3337	943	139	89	5,500
6	G6	28	2	3340	1,261	219	67	
6	G6	06	2	3342	1,025	198	95	4,450
6	G6	22	2	3343	759	172	72	
6	G6	23	2	3349	1,128	184	110	4,775
6	G6	23	2	3354	1,261	194	152	
6	G6	12	1	3360	851	153	102	3,900
6	G6	07	1	3361	851	139	63	3,900

SET	GR	AGE	SEX	PAT#	IGG	IGA	IGM	IGD
6	G6	62	1	3362	1,025	245	66	7,700
6	G6	22	2	3363	1,025	123	101	7,200
6	G6	18	2	3364	769	245	254	8,800
6	G6	24	1	3365	1,384	159	102	13,500
6	G6	61	2	3367	1,179	127	89	
6	G6	04	1	3369	1,179	139	102	3,000
6	G6	18	2	3370	1,261	184	82	5,500
6	G6	26	2	3371	1,486	131	120	
6	G6	16	2	3372	1,538	190	109	8,800
6	G6	46	1	3373	1,896	419	91	9,600
6	G6	14	1	3374	1,025	368	158	10,500
6	G6	12	2	3378	1,025	204	158	3,000
6	G6	06	2	3379	1,230	121	77	1,920
6	G6	24	2	3384	1,845	114	82	7,200
6	G6	02	1	3386	1,025	114	137	12,990
6	G6	57	1	3388	461	192	78	1,920
6	G6	58	1	3389	584	149	58	325
6	G6	26	1	3390	974	176	71	325
6	G6	22	2	3392	564	123	158	1,920
6	G6	05	1	3395	871	72	49	
6	G6	06	1	3396	718	70	77	
6	G6	10	2	3398	769	127	51	850
6	G6	06	1	3400	871	133	89	850
6	G6	07	2	3401	1,025	149	49	3,000
6	G6	16	1	3404	1,025	133	101	
6	G6	14	2	3405	943	204	95	72
6	G6	31	1	3407	1,025	159	95	72
6	G6	31	2	3409	943	215	118	
6	G6	19	2	3410	943	176	152	5,500
6	G6	08	1	3411	769	86	66	72
6	G6	06	1	3412	841	86	89	
6	G6	33	1	3414	769	94	132	
6	G6	09	1	3416	615	31	43	
6	G6	14	2	3417	718	194	146	
6	G6	07	1	3418	718	76	61	3,900
6	G6	10	2	3420	1,025	204	82	
6	G6	08	2	3427	871	67	102	4,450
6	G6	06	2	3428	1,230	143	120	2,225
6	G6	08	1	3429	1,025	165	67	
6	G6	19	1	3430	1,025	176	127	7,980
6	G6	14	2	3431	1,025	176	171	13,500
6	G6	26	2	3432	718	165	171	4,450
6	G6	12	1	3433	718	184	71	7,200
6	G6	08	2	3434	666	102	111	2,225
6	G6	28	2	3435	513	215	137	9,175
6	G6	05	2	3436	584	100	116	6,125
6	G6	20	2	3437	769	61	108	526
6	G6	12	2	3439	1,025	225	95	72
6	G6	13	2	3441	1,025	184	102	9,975
6	G6	17	2	3442	871	149	158	
6	G6	06	2	3443	1,025	114	82	850

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	17	1	3444	595	184	115	2,785
6	G6	09	2	3446	718	121	111	2,785
6	G6	12	2	3448	1,025	133	111	5,075
6	G6	16	1	3449	492	143	43	1,400
6	G6	13	1	3451	718	121	43	3,600
6	G6	14	2	3452	1,128	135	77	5,500
6	G6	06	1	3453	851	67	51	72
6	G6	17	2	3456	943	159	66	2,785
6	G6	13	1	3457	1,025	123	57	5,500
6	G6	16	2	3458	871	118	67	
6	G6	14	1	3460	759	116	67	72
6	G6	46	2	3461	851	108	72	72
6	G6	26	1	3463	759	235	57	1,400
6	G6	31	2	3466	1,025	153	110	2,785
6	G6	08	1	3468	759	149	63	3,600
6	G6	12	2	3469	1,230	88	127	526
6	G6	10	2	3471	769	139	196	1,400
6	G6	06	1	3472	554	116	57	72
6	G6	04	2	3473	759	108	82	72
6	G6	11	2	3475	943	127	127	6,125
6	G6	41	2	3476	1,281	184	304	72
6	G6	29	1	3478	1,025	141	165	72
6	G6	40	1	3479	584	204	116	6,125
6	G6	10	2	3481	902	149	215	15,400
6	G6	09	2	3482	902	149	116	16,800
6	G6	07	2	3483	584	266	145	8,800
6	G6	33	1	3489	759	184	89	2,525
6	G6	08	2	3491	871	90	67	1,115
6	G6	05	1	3492	707	90	82	2,225
6	G6	53	2	3495	1,025	47	127	
6	G6	07	2	3505	769	102	190	15,500
6	G6	06	1	3506	769	45	171	12,990
6	G6	36	1	3509	1,025	175	118	16,775
6	G6	08	2	3510	1,128	235	127	13,500
6	G6	04	1	3511	615	82	66	6,900
6	G6	06	1	3512	1,025	141	145	4,125
6	G6	05	1	3514	943	78	118	1,675
6	G6	08	2	3516	1,128	153	118	72
6	G6	20	2	3517	902	245	158	
6	G6	18	1	3518	1,281	347	132	2,785
6	G6	15	1	3519	1,025	368	127	
6	G6	13	1	3520	943	215	127	5,800
6	G6	22	1	3522	769	245	142	3,900
6	G6	07	1	3528	1,128	235	127	6,125
6	G6	06	1	3529	1,128	266	165	3,000
6	G6	10	1	3532	543	104	81	5,075
6	G6	31	2	3535	1,128	225	145	1,920
6	G6	11	1	3537	943	327	118	526
6	G6	14	2	3539	1,025	159	118	5,075
6	G6	13	2	3541	1,332	409	158	3,900
6	G6	10	1	3543	1,128	235	215	72

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	06	1	3545	492	78	177	1,115
6	G6	28	2	3548	584	139	177	72
6	G6	24	2	3553	1,179	266	165	2,785
6	G6	64	2	3554	707	255	83	72
6	G6	74	1	3555	1,025	184	329	5,800
6	G6	05	1	3556	841	149	83	7,980
6	G6	71	1	3557	1,025	409	354	2,525
6	G6	31	1	3560	1,025	200	127	526
6	G6	05	1	3563	841	163	70	
6	G6	10	2	3564	769	163	83	4,125
6	G6	11	2	3565	1,025	155	215	1,920
6	G6	17	2	3566	1,025	137	95	
6	G6	42	2	3567	1,025	110	70	
6	G6	13	2	3570	1,025	149	234	
6	G6	57	2	3571	1,025	184	278	
6	G6	56	1	3572	769	368	95	
6	G6	10	1	3573	492	225	78	1,400
6	G6	33	2	3575	820	215	85	5,500
6	G6	46	2	3577	1,025	204	152	2,225
6	G6	20	2	3580	1,128	327	215	
6	G6	03	1	3582	513	41	83	72
6	G6	16	2	3583	923	72	121	3,900
6	G6	54	1	3584	1,025	57	108	
6	G6	53	2	3585	923	143	145	72
6	G6	18	2	3587	923	137	127	
6	G6	13	1	3588	841	63	101	
6	G6	14	2	3589	923	98	215	2,785
6	G6	19	2	3591	1,025	184	145	8,800
6	G6	07	1	3592	841	78	78	
6	G6	12	1	3594	1,025	121	127	3,900
6	G6	36	2	3596	923	121	165	650
6	G6	62	1	3597	625	204	127	850
6	G6	16	2	3599	923	100	114	4,129
6	G6	28	2	3601	923	118	133	526
6	G6	55	2	3612	923	225	99	
6	G6	21	1	3615	769	215	68	
6	G6	20	2	3617	923	184	83	1,675
6	G6	24	1	3618	1,025	102	44	3,600
6	G6	06	2	3629	841	72	95	
6	G6	10	2	3630	1,179	163	152	526
6	G6	09	1	3631	1,179	143	152	
6	G6	23	2	3633	1,025	184	304	526
6	G6	25	1	3634	871	204	101	
6	G6	66	2	3638	1,025	327	73	
6	G6	64	2	3640	513	215	78	
6	G6	44	2	3642	1,025	123	127	
6	G6	39	2	3645	1,179	121	89	72
6	G6	39	1	3646	1,281	266	70	14,100
6	G6	06	2	3647	1,128	63	57	
6	G6	33	1	3648	1,025	184	63	4,775
6	G6	98	2	3658	1,179	96	152	

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	07	2	3660	1,025	57	115	
6	G6	09	1	3661	666	57	71	
6	G6	49	2	3664	1,025	155	290	
6	G6	16	2	3667	790	123	228	10,775
6	G6	21	2	3668	1,025	255	177	15,400
6	G6	28	2	3676	923	61	111	
6	G6	04	1	3677	923	53	63	4,125
6	G6	10	2	3679	1,076	65	145	1,400
6	G6	09	2	3680	810	41	65	5,500
6	G6	42	2	3690	790	147	215	
6	G6	14	1	3691	718	129	47	1,400
6	G6	20	2	3692	790	143	145	1,400
6	G6	45	1	3693	953	135	89	3,600
6	G6	13	2	3698	790	186	221	5,500
6	G6	06	2	3699	666	143	102	7,200
6	G6	10	1	3700	666	47	89	72
6	G6	22	2	3701	953	215	265	1,920
6	G6	14	1	3703	666	98	75	3,000
6	G6	18	1	3704	718	155	71	1,400
6	G6	24	2	3708	790	204	71	
6	G6	04	1	3710	718	98	76	72
6	G6	04	2	3711	871	94	71	
6	G6	42	1	3712	718	196	127	72
6	G6	05	1	3717	543	90	92	
6	G6	28	2	3718	718	163	145	
6	G6	19	1	3721	461	41	158	
6	G6	13	2	3722	595	94	304	72
6	G6	17	1	3723	718	196	62	
6	G6	11	2	3724	790	67	278	
6	G6	16	2	3726	595	94	127	526
6	G6	30	1	3728	718	135	120	4,450
6	G6	06	2	3734	1,025	151	127	2,225
6	G6	59	2	3739	666	235	101	72
6	G6	04	2	3740	923	92	127	6,125
6	G6	38	1	3742	543	129	54	3,900
6	G6	10	2	3744	533	63	158	72
6	G6	07	1	3745	492	92	158	72
6	G6	28	1	3751	1,025	106	61	72
6	G6	34	2	3758	974	123	82	526
6	G6	36	1	3759	1,128	225	165	
6	G6	48	2	3766	974	170	190	
6	G6	13	2	3768	1,025	266	81	3,600
6	G6	07	1	3769	646	118	81	
6	G6	08	1	3770	871	110	89	
6	G6	31	2	3774	820	163	85	
6	G6	39	2	3777	1,230	439	240	
6	G6	18	2	3778	1,025	123	221	4,125
6	G6	16	2	3779	923	170	165	850
6	G6	14	1	3780	871	153	115	
6	G6	07	2	3781	1,025	170	106	850
6	G6	19	2	3786	1,025	245	127	3,000

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	10	1	3789	871	170	59	14,100
6	G6	09	2	3791	1,128	153	76	10,500
6	G6	07	1	3792	820	215	82	850
6	G6	28	2	3796	1,128	225	82	3,000
6	G6	04	1	3798	666	76	82	72
6	G6	36	1	3799	749	102	106	
6	G6	05	1	3801	666	106	82	1,920
6	G6	69	1	3802	820	170	76	2,785
6	G6	12	1	3805	820	133	115	
6	G6	11	1	3806	749	63	58	
6	G6	28	1	3808	923	170	76	
6	G6	34	1	3816	820	510	97	7,700
6	G6	05	1	3818	820	147	127	3,900
6	G6	11	1	3819	1,128	716	145	
6	G6	10	2	3820	820	116	97	72
6	G6	24	1	3821	923	110	70	
6	G6	20	2	3822	923	225	127	1,675
6	G6	10	2	3826	1,025	225	115	2,525
6	G6	07	1	3827	820	225	145	4,125
6	G6	58	1	3828	820	102	76	
6	G6	15	2	3830	923	225	115	
6	G6	21	2	3832	749	147	115	2,525
6	G6	62	1	3833	749	358	97	
6	G6	33	1	3834	820	225	97	72
6	G6	09	1	3835	749	225	63	7,200
6	G6	05	1	3836	533	137	139	
6	G6	06	1	3837	749	143	63	10,775
6	G6	08	2	3838	1,025	235	145	5,075
6	G6	35	1	3840	923	235	58	1,400
6	G6	10	2	3841	1,025	186	139	10,775
6	G6	11	1	3842	1,128	196	66	1,400
6	G6	04	1	3843	718	94	82	1,400
6	G6	06	1	3847	871	245	63	
6	G6	26	2	3848	1,128	245	118	1,920
6	G6	28	1	3849	1,025	378	90	2,525
6	G6	44	2	3850	1,128	180	240	
6	G6	13	2	3851	1,025	86	254	72
6	G6	15	2	3853	1,025	86	240	72
6	G6	47	1	3855	1,025	133	254	
6	G6	41	1	3859	1,025	225	96	72
6	G6	21	2	3860	871	116	127	72
6	G6	09	1	3861	513	84	68	526
6	G6	20	2	3863	871	204	132	
6	G6	44	1	3865	1,025	266	165	6,700
6	G6	12	2	3869	871	174	118	526
6	G6	29	2	3873	1,128	102	118	2,785
6	G6	09	1	3874	871	116	57	
6	G6	06	1	3875	666	53	54	
6	G6	07	2	3876	1,025	53	85	2,225
6	G6	04	1	3878	769	49	54	325
6	G6	08	1	3882	1,025	116	85	72

SET	GR	AGE	SEX	PAT.	IGG	IGA	IGM	IGD
6	G6	11	1	3883	871	196	120	526
6	G6	50	1	3892	666	180	78	72
6	G6	12	1	3895	666	86	106	1,400
6	G6	20	2	3901	871	215	183	1,675
6	G6	60	1	3908	871	225	78	526
6	G6	28	2	3911	871	153	68	72
6	G6	27	1	3916	666	215	127	7,700
6	G6	08	2	3918	666	215	110	3,600
6	G6	25	2	3920	769	180	127	325
6	G6	47	2	3922	513	204	68	850
6	G6	11	1	3923	871	157	190	
6	G6	54	1	3924	871	204	132	1,675
6	G6	29	2	3927	769	86	202	72
6	G6	08	1	3928	769	204	165	
6	G6	53	2	3931	871	127	259	72
6	G6	12	1	3932	564	94	127	3,000
6	G6	09	1	3933	1,025	94	265	72
6	G6	08	2	3934	851	245	177	4,450
6	G6	09	1	3935	851	218	127	2,785
6	G6	21	2	3937	1,025	172	310	
6	G6	18	1	3938	851	151	110	1,675
6	G6	38	1	3939	943	266	145	3,600
6	G6	12	2	3940	1,179	157	316	1,400
6	G6	07	2	3942	851	78	110	5,500
6	G6	37	2	3943	851	449	145	4,775
6	G6	06	1	3944	769	98	68	7,200
6	G6	07	1	3945	943	123	177	9,975
6	G6	08	2	3946	943	196	90	16,290
6	G6	10	1	3947	851	172	91	6,900
6	G6	19	2	3959	851	123	102	
6	G6	78	2	3960	871	306	68	1,675
6	G6	53	2	3962	697	94	132	7,980
6	G6	24	2	3967	943	255	165	
6	G6	17	2	3974	923	296	177	4,450
6	G6	16	2	3977	1,230	215	329	6,900
6	G6	23	2	3982	943	245	165	526
6	G6	06	2	3983	851	116	78	72
6	G6	51	1	3986	851	180	73	72
6	G6	11	1	3987	943	78	119	72
6	G6	08	1	3988	666	196	119	9,975
6	G6	09	1	3989	697	145	132	325
6	G6	06	2	3990	943	133	110	2,225
6	G6	33	2	3991	943	98	139	2,225
6	G6	53	1	3994	1,025	172	53	72
6	G6	21	1	3996	851	196	110	5,075
6	G6	54	1	4001	769	276	66	20,000
6	G6	51	1	4002	943	306	83	6,125

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