LYMPHOCYTES AND RHEUMATOID ARTHRITIS

ROBERT PARNELL MANDEVILLE

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

LYMPHOCYTES AND RHEUMATOID ARTHRITIS

by

© Robert Parnell Mandeville, M.B., B.S.

A Thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Medicine
Memorial University of Newfoundland
October 1978

St. John's Newfoundland

ABSTRACT

This thesis describes research into the immunological aspects of Rheumatoid Arthritis. The work uses as its model the immune-mediated reactions between graft and host which have been investigated intensively by immunologists in animals. The only natural situation in which these reactions might be precipitated are those that arise during pregnancy when the immune systems of mother and offspring are in close contiguity. Accordingly pregnancy is the focal point with concentration upon aetiological agents that might interact with the genetic make-up of mother and child to give rise to the disease manifestation of Rheumatoid Arthritis in the later life of the offspring.

In order to see if specific immunological sensitivity or tolerance could be passed from mother to offspring experiments were performed on rats in which the skin sensitizing agent Dinitrochlorobenzene was put on females prior to mating and the offspring were tested for hypersensitivity using a popliteal lymph node weight assay. Within the limits of this assay there was no detectable effect of maternal priming on the reaction of the offspring to this antigen. However, there was a pronounced effect of maternal priming on the spleen weights of the offspring in spite of the removal of the antigen in the skin slough by the time of conception. The spleen weights had been determined crudely to detect immunological runting in the offspring but retrospective analysis revealed that in all experiments in which this protocol was followed the spleen effect was significant in spite of the standard error introduced by the assay.

Five Juvenile Rheumatoid Arthritis families were investigated using the mixed leucocyte culture reactions to detect genetically determined factors that might predispose to maternal-offspring immune interaction. Two and possibly

three of the probands were homozygotes at the HLA-D locus and in two of the families there was some evidence of genotypic or phenotypic interference not related to the HLA region of chromosome six. These results are discussed in the light of the initial model and some possible immunological mechanisms outlined in an appendix.

ACKNOWLEDGEMENTS

It is easy to pick the person having the most influence upon me in the production of this thesis — Dr. William H. Marshall, my supervisor, advisor, financial arranger and friend in times of trouble.

Without the Faculty of Medicine at Memorial University of Newfoundland I would have been discouraged in my pursuit of this research at a very early stage. In the event the place and the people such as Dr. Ian Rusted, Dr. Al. Cox, and Dr. Ken Roberts have been a tremendous source of support in the five years it took me to find my way to the last page.

The Immunology Group in the Faculty of Medicine are a united, friendly and stimulating company. The thesis has been materially advanced by Dr. Bodil Larsen, Dr. Ranjit Changra and Dr. Len English by their ideas and criticism, and by John Barnard and Sheila Drover for their technical advise and the tissue-typing of the patients, and by Pauline Gulliver for her excellent typing of the final manuscript.

The Rheumatology Unit at St. Clare's run by Dr. John Martin with Dr. John Lochead have been very helpful in supplying co-operative families. The animal quarters run by Dick Campbell have been turned into a first class unit against considerable odds at times and have generously treated my demands and foibles.

I should like to thank Dr. Keith Pettingale of King's College Hospital, London for encouraging me and discussing some of the ideas behind this work in 1968.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Rheumatoid Arthritis is a chronic inflammatory disease affecting mainly the synovial lining of the joints but also many other tissues of the body. Despite intensive scientific investigation during this century, the aetiology of this relatively common condition remains obscure while other arthritides, such as Rheumatic Fever and Haemochromatosis, have yielded some aetiological mechanisms to bacteriological and biochemical genetic investigation (respectively). It is the intention of this thesis to explore the very early phases of growth and development, the foetal stage, for possible aetiological and pathogenic mechanisms that predispose to disease expression in later life, concentrating particularly upon the interactions of the immune systems of mother and child that are placed in such close apposition.

AN "AUTOIMMUNE DISEASE" The disease has been labelled "Autoimmune", that is to say "A condition in which structural or functional damage is produced by the action of immunologically competent cells or antibodies against normal components of the body." MacFarlane Burnet who was responsible for the definition quoted above as well as being largely responsible for the recognition of "Autoimmune Disease" as a specific entity found the following evidence that Rheumatoid Arthritis is one of that group (Mackay, 1973):

1. Hypergammaglobulinaemia is commonly present indicating an overactive immune system (antibodies = Immunoglobulins = Ig's being the largest component of the gammaglobulin component of serum proteins).
2. Circulating autoreactive antibodies (in the form "Rheumatoid Factor or Factors" which react with autologous and heterologous Immunoglobulin

"G" = IgG making them anti-antibodies) are usually demonstrable in patients with the disease.

3. There is deposition of the antigen (IgG) in the lesions.

4. Lymphocytic infiltration is seen in the affected areas (the lymphocyte is the central cell of the immune system).

5. The disease responds to steroids and other immune suppressing compounds (and to Thoracic Duct drainage that depletes the body of recirculating lymphocytes).

6. The disease occurs in association with various other autoimmune conditions.

N.B. The comments and evidence in brackets () are additions to Burnet.

Fulfillment of all criteria for the classification of a disease as "Autoimmune" does not exclude an external factor in the initiation of the condition as can be seen readily by the cases of Rheumatic Fever and Syphilis that also satisfy most of the criteria above, though it does indicate that the immune system is a highly significant factor in disease expression. There is some evidence that neither the auto-antibody (Rheumatoid Factor) nor the antigen (IgG) are necessary for disease expression:

1. Rheumatoid Factor is not demonstrable in early cases.

2. The Factor may be entirely lacking in up to 10% of undoubted cases.

3. The disease occurs in the absence of immunoglobulin (Agammaglobulinaemia).

4. The Factor can be present in the absence of disease.

5. Rheumatoid Serum can be given to volunteers with no ill-effects

according to Vaughan and Harris, 1959.

There can be little doubt that the disease has a significant autoimmune component and in the continuing absence of a readily identifiable precipitating agent the disease can be classified as a Primary Autoimmune Disease by default.

CURRENT HYPOTHESES There still remain investigators prepared to countenance the vicissitudes of microbiological research in the disease in spite of the repeated history of misleading false-positive identification of agents from Streptococcal Agglutinins (Cecil, 1931) to *Mycoplasma Fermentans* (Williams, 1970), to Diphtheroids (Stewart, 1969), to the "Active Agent" of Warren, 1969, that resisted heating to 121°C for 40 minutes, to name but a few of those chastened by the experience. There are probably many more that believe that a new microbiological agent akin to the recently isolated "Legionnaires' Disease Bacillus" will prove to be the answer though they wisely stay away from risking an otherwise sound reputation.

Another fertile ground for hypothesis has been the role of Rheumatoid Factor in the disease in spite of the limitations of the Factor in defining the disease enumerated above. The ultimate expression of this has been the development of the Immune-Complex hypothesis (Hollander, 1965; Zvaifler, 1965) in which a genetically determined propensity to develop low-affinity autoantibodies, suitably but non-specifically stimulated, will cause the production of Rheumatoid Factor that will complex with autologous IgG and fix complement and, being concentrated in the joints,

will cause the ingress of polymorphonuclear leucocytes and with the release of their enzymes cause the dissolution of the joint. This has been the springboard for many recent investigations but does appear to have stalled without yielding clues to aetiology.

Burnet has pursued the concept of autoimmunity in an *a posteriori* hypothesis that during the physiological process of lymphocytic clonal expansion by somatic mutation in early foetal life there could arise auto-reactive clones that if not eliminated could produce the manifestations of autoimmune disease in later life. This hypothesis places the aetiological mechanism so close to the normal physiology of the foetus that it has not been possible to devise any definitive test for the concept nor yet would such proof supply an immediate remedy for the condition.

Burnet has concentrated the attention of the scientific community on the process of clonal elimination, and since most of this activity is accomplished in the foetus he has thereby drawn attention to the intra-uterine period of development in the pathogenesis of the disease, which is far removed temporally from the clinical onset. This thesis will explore the complexity of the intra-uterine period of development, but will not be primarily concerned with physiological events but rather underline the definable aetiological mechanisms that would leave a permanent imprint on the immune system of the foetus.

The initial part of this thesis will be an introduction which will be complex of necessity as the areas covered are broad and have been the subject of much scientific and clinical curiosity in the past. These include:

1. The definition of the disease (and the imprecision of criteria). The epidemiology of the disease (using the criteria) and the genetic/familial pattern of the disease.
2. The pathology of the disease
3. Maternal influences on the developing immune system

DEFINITION

Rheumatoid Arthritis is a protean disease, though the major and primary sites of pathological damage are the synovial linings of the joints and the contiguous tissues such as the articular cartilage and the juxta-articular bone, frequently accompanied by effusion in the synovial cavity of a proteinaceous and leukocyte-rich fluid (that probably gave the "rheum" to the name). Classically it causes pain, swelling, redness and heat, (inflammation), and loss of function notably after rest as "morning stiffness" in multiple symetrical peripheral joints in maximal use, e.g. the metacarpophalangeal, that slowly progresses to cause erosion of the articular cartilage in contact with the inflamed synovial excrescences called "pannus" with eventual destruction of the joint and subluxation. An auto-reactive antibody already alluded to, Rheumatoid Factor, is detectable in many cases and when associated with the presence of "Rheumatoid nodules" which are fibrous nodules lying in the subcutaneous tissues exposed to trauma (though they may involve all manner of deeper connective structures such as the bursae, periosteum, pericardium, tendon sheaths and tendons) is generally found with severe, erosive and systemic disease. The onset of the condition is generally insidious with marked malaise and tiredness which as symptoms are real but difficult to quantify.

Research workers undertaking population and genetic studies of Rheumatoid Arthritis are faced with a problem of definition. Although it is easy to recognise well-established Rheumatoid Arthritis, it is very difficult to be certain of the diagnosis in the early phases of the disease as the condition may be remittent. The American Rheumatism Association criteria

for diagnosis (see Table 1), in which satisfaction of three or four criteria gives the diagnosis as "probable" Rheumatoid Arthritis and five or more criteria as "definite", have been found by O'Sullivan and Cathcart (1972) to indicate a prevalence in Sudbury, Massachusetts, of 3.8% for women and 1.3% for men. When these investigators used the much more restrictive New York criteria (see Table 1), however, the prevalence dropped to 0.5% for women and 0.1% for men. In following up the patients originally diagnosed as having "definite" Rheumatoid Arthritis by the A.R.A. criteria only one third were still "definite" three to five years later and only 15% of the patients in the "probable" category were later still "probable". On the other hand, 65% to 70% of those originally diagnosed as having the disease by the New York criteria still carried the diagnosis. The authors of this report questioned whether the patients diagnosed by the A.R.A. criteria that were not detectable by the New York criteria had that disease in a remittent form, i.e. a benign non-deforming condition, or had some other malady. Glynn (1968) regards the benign form of polyarthritis as the form of the disease to be expected in the absence of any autoimmune component to perpetuate the response.

The previous paragraph and Table 1 are adapted from an account given by Johnson and Vaughan (1973) in a review of the Rheumatoid Arthritis literature for the years 1970-1972, but it echoes succinctly a problem raised in every review of the epidemiology of the disease. From an epidemiological point of view this is seen in the most easily interpretable form as the "Rheumatoid Range" of mountains with the most

TABLE 1

AMERICAN RHEUMATISM ASSOCIATION CRITERIA	NEW YORK CRITERIA
1. Morning stiffness. 2. Joint tenderness or pain on motion. 3. Soft-tissue swelling of one joint. 4. Soft-tissue swelling of a second joint (within three months). 5. Soft-tissue swelling of symetrical joints (excludes distal interphalangeal joint). 6. Subcutaneous nodules. 7. X-ray changes. 8. Serum positive for Rheumatoid factors. --- 9. Poor mucin precipitate from synovial fluid. 10. Characteristic histological changes in synovial membrane. 11. Characteristic histological changes in nodules.	1. History of episode of three painful limb joint groups. 2. Swelling, limitation, subluxation, or ankylosing of three limb joints (must include a hand, wrist or foot), and symetry of one joint pair and must exclude distal and fifth proximal interphalangeal and first metatarsophalangeal joints, and hips. 3. X-ray changes. 4. Serum positive for Rheumatoid factors.

severe cases being the "peaks of diagnostic clarity and valleys of indecision" being at the base with the vast mass of unclassifiable arthritis (Cobb, 1971). However, it may well prove to be the case that the "definite" disease that satisfies the restrictive New York criteria may be governed by factors far removed from the more benign, less-easily-classifiable cases. With that caveat to the fore, the following epidemiological information seems to be well-established concerning the artificial groups defined by these criteria:

1. A disease primarily of middle-aged women. The disease seems to be two to four times more common in females except in juveniles in whom there may be a male predominance (partly dependent upon the inclusion of cases that may be a juvenile form of Ankylosing Spondylitis) and in early adult-onset Rheumatoid Arthritis (i.e. 25-34 years) and in severe erosive arthritis diagnosed radiologically (Engel, 1966). The highest prevalence rates in Western European populations are found in the elderly (though this is not found in the Slovaks and Bulgarians - Tzonchev, 1968; Sitaj, 1968), but the peak rates of onset of new cases are found in females between the ages of 48-54 years of age (Lawrence and Ball, 1969).

2. An atypical disease in juveniles (and the elderly). The disease pattern in juveniles shows greater signs of systemic disease such as malaise, fever, weight loss, splenomegaly, lymphadenopathy, skin rashes and iritis. There are also usually fewer joint signs since the entheses (or attachment sites of ligaments and tendons to the bone) as well as the synovia are more often the primary sites for pathological damage with an axial, ankylosing, pauciarticular presentation in some cases. This pat-

tern, which is different from adult Rheumatoid Arthritis, has led to the disease being split from the main body of Rheumatoid Arthritis, although the adult form of the disease is seen in children and vice versa. Juvenile Rheumatoid Arthritis, or Still's disease, is also characterised by an absence of Rheumatoid Factor detected by conventional tests (though there may be non-agglutinating Rheumatoid Factors of the IgG and IgA classes present). Bywaters (1967) has made a case that the disease is, in fact, at least three separate syndromes: a) a juvenile form of Ankylosing Spondylitis and other miscellaneous axial arthritides; b) Rheumatoid Arthritis in the young; c) true Stills disease (though Still, 1897, described three different types of arthritis) that can present in the male up to the age of thirty-five. If the disease is further split into "probable", "definite", and "benign" categories there are reasonable grounds to believe that the splitting process has gone too far for ready comprehension. There is some suspicion that the disease in the elderly may be a separate form because of its clinical behaviour which can be benign in spite of a rapid onset of severe symptoms and its geographical distribution (see previous paragraph).

3. A universal disease of mankind. There is evidence that no population is completely spared. The genetic incidence, that is, the proportion of the population that will develop the disease sometime in their lifetime, is disproportionately low in Japan (0.2% "definite" in women, 0% in men - Shichikawa, 1968), in Puerto Rico (Mendez-Bryan, 1964), in the criminally insane, batchelors and people in higher income brackets in North America (Engel, 1968), while Jamaicans (Lawrence, 1966), and

the Yakima Indians (Beasley, 1971) have a notably increased incidence. The differences do not seem amenable to simple interpretation and there are many pitfalls in comparing rates between populations with different lifestyles; for instance, the wearing of shoes seems to prevent bony erosions in the feet detectable on X-ray (Lawrence, 1977).

4. An endemic disease. It is a sporadic disease without temporal or spatial clustering apart from a slight decrease in prevalence in large urban communities detected in Holland (de Graaf, 1959), England (Kellgren, 1966) and the U.S.A. (Engel, 1968). There appear to be significant seasonal effects in exacerbations of Rheumatoid Arthritis (Short, 1957) which can also be seen with the monthly variation of prevalence of episodic or "benign" arthritis from 2% in May to 7% in October (Valkenburg, 1968). There is one report of an increased incidence of the disease in the spouses of probands (using an interview technique only - Schull, 1969) but this has not been confirmed as a statistically significant effect by others who clinically examined the spouses (Bennett, 1968; Hellgren, 1969; Dalakos, 1969). Bennett (1968) reported that the less severe forms of arthritis were more common than expected in large families when compared with small families though this effect was not found in England (Lawrence, 1977).

There are significant differences in point prevalences in Rheumatoid Factor in age cohorts (Lawrence, 1960) that could implicate an epidemic environmental factor, but these variations in prevalence had little association with the prevalence of arthritis in that cohort, indeed, there was an almost inverse relationship. Although Rheumatoid Factor is associated with

severe disease the Factor is found at various titres in healthy people and when a group of them were followed-up for five years by Ball and Lawrence (1961), the incidence in the high titre group of overt disease was 19% (age adjusted), and only 11% in the negative group; the suprising finding in this study was that the incidence in the low titre group was only 8% which was significantly less than those with a negative Sensitised Sheep Cell Test (SCAT) which was the particular technique employed. Bennett (1968) in his study of the Blackfeet and Pima Indians found evidence of concordance of Rheumatoid Factor among siblings in the cohort in one of the tribes aged 55-64 years but less in younger and older cohorts which could implicate an epidemic environmental factor of early life in this cohort, particularly as this tendency was not transmitted between generations. From these data it can be seen that the disease *per se* shows little evidence of an epidemic environmental agent in its aetiology although the possible epiphenomenon, Rheumatoid Factor, does appear to have been provoked by such an agent in certain cases.

5. A Non-Mendelian disease. Family studies have demonstrated that first degree relations of seropositive, severe erosive or severe clinical arthritics have a significantly increased risk of having a similar grade of arthritis compared with the surrounding population (6 and 2.6 times respectively according to Lawrence, 1970). This aggregation does not seem to be apparent in lesser degrees of arthritis or in seronegative arthritis.

A Mendelian recessive mode of inheritance is unlikely from the equality between the genetic incidence in the parents (8.8 times the expected) and siblings of the affected (7 times according to Lawrence, 1977). A Men-

delian dominant mode of inheritance is unlikely *per se* given the large difference between the concordance rates in monozygotic twins (34%) and dizygotic twins (7%, Harvald and Hauge, 1965; Lawrence, 1970) though a large component of variance due to environmental differences or to genic/environmental interaction could account for the lack of penetrance in the dizygotes or "overpenetrance" in the monozygotes. An example of "overpenetrance" produced during the intra-uterine period of development would be the development of a maternal immune response in response to cells of one of the monozygotes, this response could be expected to cause changes in the tissues of the other twin as its cells carry the same antigen determinants. However, there are many other situations in which the very presence of the twin will augment expression beyond that of the surrounding family and population.

Juvenile Rheumatoid Arthritis seems to fall into at least three groups on analysis of family data; the seropositive cases with erosions show the same family aggregates as the adult-onset disease though the affected relations are all <u>seronegative</u> which does not ordinarily cluster in families! There is a notable involvement of mothers (<u>16.7</u> times the expected incidence) and even grandmothers when compared with fathers (3 times expected) and this matrilineal inheritance suggests a cytoplasmic inheritance that has been previously postulated in anencaphaly and spina bifida (Nance, 1969) though there is obvious relevance for the type of maternal-foetal immune interaction that is later postulated in this thesis. There is little data for twins, with two out of five

monozygotes concordant and one out of eleven dizygotes concordant which are both close to the expected derived from the adult-onset disease (Ansell, 1962; 1969). The seronegative cases with both peripheral and axial involvement seem to be a separate juvenile entity though they may present into adult life, while other cases with a mainly axial presentation (and perhaps HLA-B27) have a strong aggregation of Ankylosing Spondylitis (16% of brothers affected and 7% of fathers though the probands can be of either sex).

When concordance rates in siblings and offspring are quoted it should be noted that there seems to be a lower fertility in Rheumatoid patients even in those who develop the disease after the menopause (Hargreaves, 1958; Kay, 1965) and a diminished survival in siblings of probands (Dixon, 1964). These data support the hypothesis that there is a lethal factor present in "Rheumatoid families" operating in both the pre- and postnatal phases so that the incidence rates in the survivors need to be interpreted with caution.

The disease appears to be multifactorial with a probable underlying polygenic mode of inheritance in severe disease but with no evidence of any familial basis for disease susceptibility in the less severe cases though a documented lethal factor may tend to mask incidence rates in relations.

6. A heritable disease. In view of the cayeats expressed above it is of questionable value to go through the exercise of calculating degrees of heritability (h^2) which is a measure of additive genic variance compared with total variance and makes the assumption that dom-

inance genic variance is negligible and that environmental variances (particularly familial variances) do not interact in a complex manner. Nevertheless, Lawrence (1977) quotes a heritability of 60% calculated from first degree relations of severely affected probands, 58% using like-sex dizygotes, 61% using unlike-sex dizygotes, and 70% for monozygotes by the method of Falconer (1960; 1965). The data from the monozygotes has also been processed by the method of Smith (1970) based on Edwards' method (1969) which is said to be more accurate with high degrees of heritability tending to show a greater additive genic effect. This gives an h^2 of 74% (Cavalli-Sforza, 1971) which is within a standard error of the heritability for Paralytic Polio (based on data from Herndon, 1951) and Tuberculosis (based on data from Harvald, 1965). To quote Edwards (1969):- "....there is a grave danger of assuming that high heritability, which was once demonstrable in Tuberculosis, will be misunderstood to mean that environmental control is unlikely to be effective, or that environmental research is unlikely to be rewarding."

7. A disease with genetic markers. Attempts to delineate genetic factors have revealed an association with Haptoglobin 2-2 in seronegative cases with a diminished frequency of Hp 1-1 (Nettelbladt, 1965). Within the last year it has been shown by Stastny (1977) and others (McMichael, 1977) that there is a significant association with the allele HLA DRw4 (that segregates with the major histocompatibility region) in patients with adult Rheumatoid Arthritis and DRw3 in the juvenile disease. Previously there have been reports of associations

with Rhesus D-ve (Stoia, 1967) and an α_1 antitrypsin Pi type z allele (Cox, 1976) though these have not been confirmed definitively.

PATHOLOGY

It is the gross appearance of the joints at operation that has emphasised the role of the synovium in the destruction of the joint and this description has changed little in the years between that of Stockman (1920), Fisher (1929), Klinge (1933; 1934), Collins (1949), Gardner (1965), and Solokoff (1966). The following description is adapted from Gardner (1972) and includes most of the relevant points:

"In the earliest stages of the disease, the synovial tissues are found to be swollen and reddish-pink in colour and to protrude from the divided capsule as the joint is opened. With time, the congestion, redness, and swelling become more pronounced until a seaweed-like mass of hyperplastic tissue appears to occupy more than the normal joint space. Synovial villous processes are seen to be loosely adherent to marginal articular cartilaginous surfaces with which they apparently come into random contact. Adhesion to the cartilage margin is light and the adherent superficial synovial villous processes can easily be raised from the articular surface.

In time the adhesions merge with, and become coextensive with, the granulation tissue that comprises 'Pannus.' At the cartilage margins where villous adhesions are most common the inflamed synovia extend onto and insidiously replace the edge of the articular cartilage and the rough, dull, red-pink zone formed in this way is termed a 'Pannus' (Latin: Cloth). This cannot be detached from the cartilage surface into which it extends actively thereby causing the bony erosions characteristic of the disease radiographically.

In joints that have been inflamed for some weeks or months the opposing, roughened synovial and marginal cartilaginous surfaces are often seen to be loosely joined by strands of fibrin. These strands become organised and more extensive and increasing volumes of the joint space are obliterated by the process of fibrous ankylosis, though this seems to be delayed or prevented by the presence of persistent, large synovial effusions (Collins, 1949)."

The lining cell layer of the normal joint is only one to four cells thick on examination under the microscope and covers all the free surfaces of the joint apart from the articular cartilage itself. The tissues it covers vary from the fibrous capsule to loose areolar tissue to the fat pads of the joints. It is arranged upon a delicate network of fine collagen fibres having a pericellular reticular pattern including capillaries and connective tissue cells with the occasional mast cell (Castor, 1960; Barnett, 1961). The main lining cells are of two distinct types; the macrophage-like "A(M)" cells with numerous finger-like projections that interdigitate with the extracellular matrix and contain cytoplasmic vacuoles and lysosomes as well as mitochondria; and the fibroblast-like "B(F)" cells that are less numerous and have abundant endoplasmic reticulum (ergastoplasm) and so are very likely to be involved in the active synthesis and export of material, presumably synovial fluid components (Barland, 1962). The cellular nomenclature was devised by Barland though others have since detected the existence of an intermediate cell type called, naturally, the "C" cell but this has not appreciably altered understanding of the normal or diseased joint.

In the Rheumatoid joint the synovial layer becomes more extensive as the villous fronds expand into the joint cavity carrying with them the covering, and the layer becomes thicker (up to ten cells deep - Schumacher, 1972) though it is somewhat controversial as to which cell type predominates. Electron microscopic examination of this layer by Norton (1966) did not reveal any evidence that this was the focus of any attack by immune mechanisms and the cells seem to be remarkably healthy and robust in view of the serious metabolic disturbances of the milieu (*vide infra*). Occasionally in surgical specimens the lining layer can be seen to have been stripped away and there is often a fibrinous exudate over this area (Solokoff, 1972). The presence of fibrin-like material in the inflamed joint led to the term "Fibrinoid Necrosis" (Neumann, 1880) and similarities to the staining in Rheumatic Fever and experimental arthritis in rabbits suggested an identity of origin (Klinge, 1929), but there is sufficient evidence now that the Fibrinoid seen in the variety of disease states is heterogeneous in composition and cannot form the basis for discussion of pathogenetic mechanisms (Gardner, 1972).

Multinucleated giant cells are seen occasionally and they seem to be of two types; one being in close apposition to the synovial layer and is probably derived from the "A(M)" cells, and the other being in close contact with the destruction of bone and is probably a "foreign body" response of the macrophages (Solokoff, 1972) and neither are of the type associated with granulomatous infections such as Mycobacteria.

The main pathological changes are found in the tissues deep to the syno-

vial lining layer and many observers have drawn attention to the relatively unaffected interlayer between the lining and the subsynovial inflammatory infiltrate (e.g. Ziff, 1971). From this evidence and that of Norton (1966) one could make a case that the lining layer hyperplasia is only secondary to the intense immunological reaction beneath in a manner similar to the hyperplasia of the dermis above a chronic cell-mediated hypersensitivity reaction (Turk, 1975). The initial changes in the subsynovium are characterised by a diffuse lymphocytic infiltrate that becomes polarised around the blood vessels particularly the venules (Gardner, 1972) which some have found to be dilated with hypertrophy and hyperplasia of the endothelial cells with "holes" that allow leakage of erythrocytes and plasma proteins, and with prominent platelet plugging and thrombosis (Kilka, 1966) though there is not unanimity of opinion on the existence of all these changes. These changes have led some observers to suspect that the target for immune attack lies in the blood vessel wall, but for the immunopathologist the phenomenon of vascular changes particularly in the post-capillary venule is familiar being seen in hypersensitivity reactions to exogenous antigens and the physiological reaction in the lymph-nodes and spleen to antigenic stimulation (Turk, 1975) in which one supposes that the vessel wall changes are not due to immune attack *per se* but a bystander effect in a high traffic area for leucocyte migration and pharmacologically active factor production.

Examination of the Rheumatoid synovial villi under the microscope would seem to be the first element in understanding the disease process, yet

thisexercise can prove to be misleading unless great care is taken to quantitate the cells present in a large number of fields in a similarly large number of sections as the pathological changes within one joint can be extremely variable (Gardner, 1972). When the Electron Microscope is used the possible sampling errors and artefacts are legion, nevertheless it has proved to be a useful tool in the hands of experts looking for known structures (e.g. cell morphology) with a rigid system for quantifying the results.

One of the better reports using this technique (although uncomfortably containing observations such as ".....lymphocytes passing through the endothelial cytoplasm of the venules") is that of Kobayashi and Ziff (1973) in which attempts were made to quantitate the cellular components in the deep sub-synovium. These authors noted that in the predominantly lymphocytic areas with 87% lymphocytes there were also 4.6% lymphoblasts, 6.6% fibroblasts, 1.9% macrophages, just 0.5% polymorphs and 0.25% plasma cells; whereas in the plasma cell rich accumulations with 67% plasma cells there were also 18.4% macrophages, 10.9% fibroblasts, 2.3% plasmablasts and just 0.8% of both lymphoblasts and polymorphs.

In this report there is the interesting observation that a number of the lymphocytes and some of the nearby fibroblasts showed evidence of degeneration with condensed nuclei depleted of chromatin and with large perinuclear vacuoles in the cytoplasm which in the end stage led to only the naked nuclei remaining to be seen of the cell. The comment

from the authors was: "...these degenerate cells were seen both at random or in groups which on occasion appeared with the degenerated fibroblasts as necrotic areas in the lymphocytic collections." If this evidence is added to Ziff's previous work with Norton (1966) the only cells in the primary tissue affected by the disease, i.e. the synovium, that show signs of immunologically mediated damage are the lymphocytes themselves (and nearby fibroblasts) quite removed from the synovial lining layer of the joint. Although there has been little or no comment on these observations since they were published the most obvious reaction would be to place these as an "innocent bystander" effect of a strong immunological reaction to antigenic components of the joint, however, one can postulate that the converse could be the case with the joint as an "innocent bystander" to a lymphocyte war in the sub-synovium.

Rheumatoid Arthritis is a chronic inflammatory disease and it is not surprising that the predominating cell is the lymphocyte (and its progeny the plasma cell) as this is the central cell of the immune system. There are many lymphocyte populations that have been delineated or postulated, but the main division seems to be between the "T" cells that have been processed in the Thymus and are associated with cell-mediated immune responses and the inductive phases and control of antibody responses, and the "B" cells that carry antibody and can differentiate into plasma cells specialised for export of the antibody. Although both types of lymphocyte can be found in the Rheumatoid synovium there is more than ample evidence that the predominating cell is the "T" lymphocyte at all stages of the disease (Frøland, 1973; van Boxel, 1975;

Abrahamsen, 1975; 1977; Williams, 1975; Tannenbaum, 1975; Bankhurst, 1976; Wangel, 1977). The most concrete evidence that these lymphocytes are important in disease expression has been the complete regression of symptoms in patients that have had chronic Thoracic Duct drainage in an effort to control rampant disease. The benefit occurred within 14 days of the institution of the regimen with disappearance of Rheumatoid nodules within 35 days (Paulus, 1973; Pearson, 1976). Experimental animal work has repeatedly demonstrated that nearly all the cells removed in the early phases of Thoracic Duct drainage are "T" lymphocytes while the remainder are usually "B" lymphocytes (Howard, 1970; 1970b), though the proportion of "B" lymphocytes and other cells increases once the "T" lymphocyte population has been depleted.

This important work has thrown a new light upon the pathogenetic mechanisms in operation and reinforces the concept of a strong (auto)immune component of the disease. The "T" lymphocyte population is conceived by contemporary immunologists as having a major division into helper and suppressor populations and it is not clear from the Thoracic Duct drainage experiments whether the amelioration of the disease is acheived primarily by the depletion of the antigen reactive helper cells (or effector cells) or the suppressor cells that prevent elimination of the antigenic stimulus. Peripheral blood lymphocytes isolated and injected into the autologous knee have no effect on the disease whereas the Thoracic Duct lymphocytes during early drainage cause a violent flare of the disease lasting for weeks afterward but those obtained late in the drainage when there is clear evidence of reduced cellular immunity (though

little evidence of impairment of antibody levels to common antigens) cause only transient, mild synovitis (Paulus, 1973; Pearson, 1976). When the lymphocytes causing the flare of arthritis in the injected joint were labelled with Chromium-51 less than 1% were found in the Thoracic Duct effluent per day suggesting that either the cells had lost their label due to intensive blastic activity or that they had been trapped by the antigenic milieu of the joint. This evidence tends to favour the concept of pathogenic helper "T" cells, but it does not constitute direct definitive evidence against a suppressor cell deficiency allowing these to function pathogenically.

There can be little doubt that the Rheumatoid synovium is the site for intense metabolic activity mainly involving the lymphoid cells. The plasma cells perform their function of secreting immunoglobulin that continues *in vitro* at a rate rivalling that of the spleen (Smiley, 1968); some of the antibody being anti-immunoglobulin-immunoglobulin (McCormick, 1963) and some part of that being Rheumatoid Factor (e.g. IgM anti IgG). Complement components are also secreted *in vitro* with local production of C3, C4, C2 and C5 (Ruddy, 1974) and it is highly likely that the macrophages are largely responsible. The lymphocytes themselves are highly active as can be seen by the 4.6% lymphoblast population quoted above and by the production of lymphokines that attract and stabilise a cell-mediated response (Rothenberger, 1970). Zvaifler (1973) quotes Ziff as using the blast evidence as indicating only a low level of activity, but in a chronic, unsynchronised reaction even this level of mitotic activity is considerable when multiplied over the course

of the illness.

Page-Thomas (1955; 1957) and Dingle (1956) performed a series of investigations that showed that the Rheumatoid synovial villous processes have a high rate of metabolism, particularly aerobic metabolism of carbohydrate, compared with Osteoarthritic and traumatic arthritic synovia. One of the criteria used was the index $q_{O_2}^{O_2}$ of oxidative metabolism which was assessed as less than 1 in the controls but up to 37 in the affected tissue depending upon the cellular infiltrate. They showed that the glucose utilization of Rheumatoid tissues is inhibited by hydrocortisone under both aerobic and anaerobic conditions and was greatest in those tissues with the highest rates of oxygen uptake (a linear relationship), i.e. those with the greatest cellular infiltrate; its effect could be mimicked using spleen fragments.

The blood supply to the inflamed joint increases markedly so that radioactive Xenon-133 is removed three times as fast as from an unaffected joint (Goetzl, 1971 based on the technique pioneered by W.C. Dick) and this has been shown many times using many different materials (Ahlström, 1956; Harris, 1958; Nakamura, 1967; St. Onge, 1968). If there is rapid transit of Albumin, Sodium, Phenosulphthalein and Xenon from these joints there seems to be a very efficient blockade to the egress of the products of metabolism with pH reduced (6.84 in one patient), pCO_2 increased (up to 98mm Hg) and lactate 6.4mM/l. (Falchuk, 1970). Similarly there appear to be blockades to the ingress of Glucose (Ropes, 1960) and Oxygen so that a partial pressure for this gas of zero has been recorded in one inflamed Rheumatoid joint (although this was exceptionally low) and

other inflamed joints had very low levels when compared with those found in traumatic effusions (Lund-Olesen, 1970). The reduction in pO_2 and the other metabolic imbalances were found to be strongly correlated with the proliferation of the synovial cells and the inflammatory infiltrate (Falchuk, 1970; Goetzl, 1971) though not with the synovial fluid leucocyte count. These same authors found that although there was a degree of consistency in the results of repeated tests on the same joint there was little correlation between contralateral joints. All these metabolic disturbances correlated with the increased metabolic activity of the joint demonstrated by the fall in pO_2 in the irrigating saline of an ischaemic joint compared with controls and were reversed by synovectomey and steroid injection.

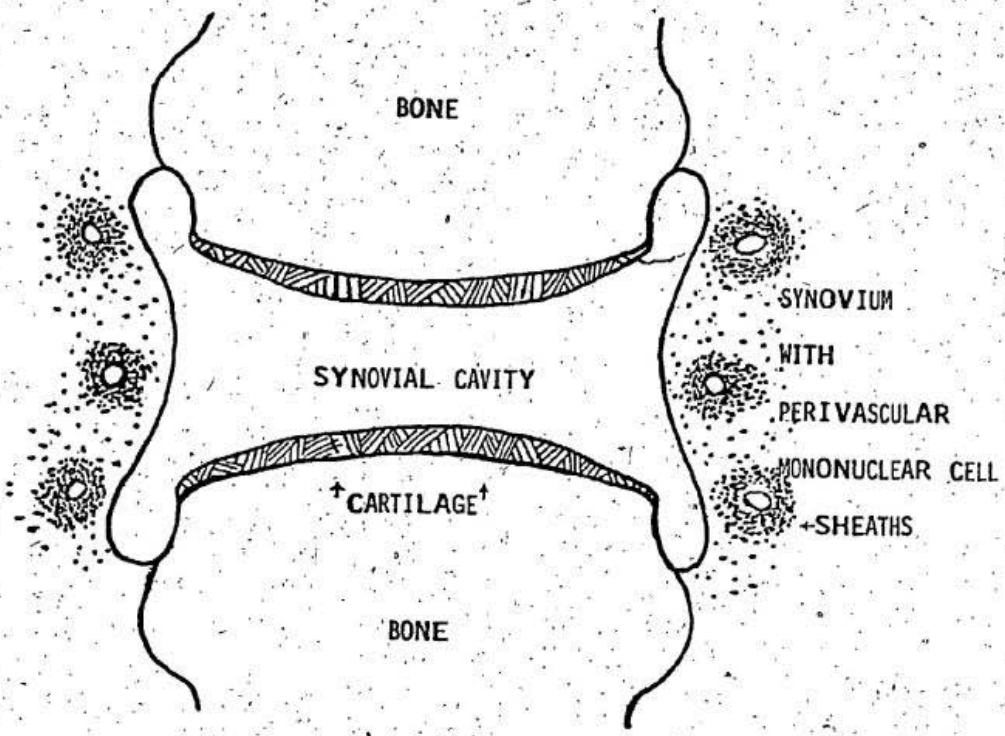
These data seem to be definitive and any theory of pathogenesis would have to account for the strange behaviour of the Rheumatoid metabolism observed. The main anomaly is that the blood supply has risen to meet the demands of the inflamed joint but seems to be incapable of overcoming the block in the pathway of the metabolically important molecules. Ropes (1960) from the evidence of the transmission rates of glucose infused intravenously into calves and patients concluded that there was a selective blockade that could be inhanced by simultaneous infusion of galactose suggesting that a competition for receptors existed. However, this conclusion was somewhat tentative and has not been confirmed by subsequent studies. Falchuk (1970) laid some emphasis upon the focal microangiopathy observable in severe disease with marked metabolic upset but was unable to offer any explanation for the relative

increase in transport for Xenon and other metabolically unimportant substances. This explanation which could be extended to include an immune-complex mediated vasculitis is also unable to account for the marked differences in derangement between contralateral joints which one would suppose to be equally affected by circulating complexes. There is little doubt that immune complex vasculitis is a complication of Rheumatoid Arthritis (Bywaters, 1957) producing insufficient circulation particularly to peripheral areas but one would expect that in these cases the Xenon clearances would be lowered rather than raised as has been observed though one could invoke an increase in the micro vasculature in the same manner as the lung perfusion studies but immediately one would expect an increase in transport of Glucose, Oxygen, and Carbon Dioxide and that has definitively been excluded.

A possible sequence of events can be extrapolated from this evidence:

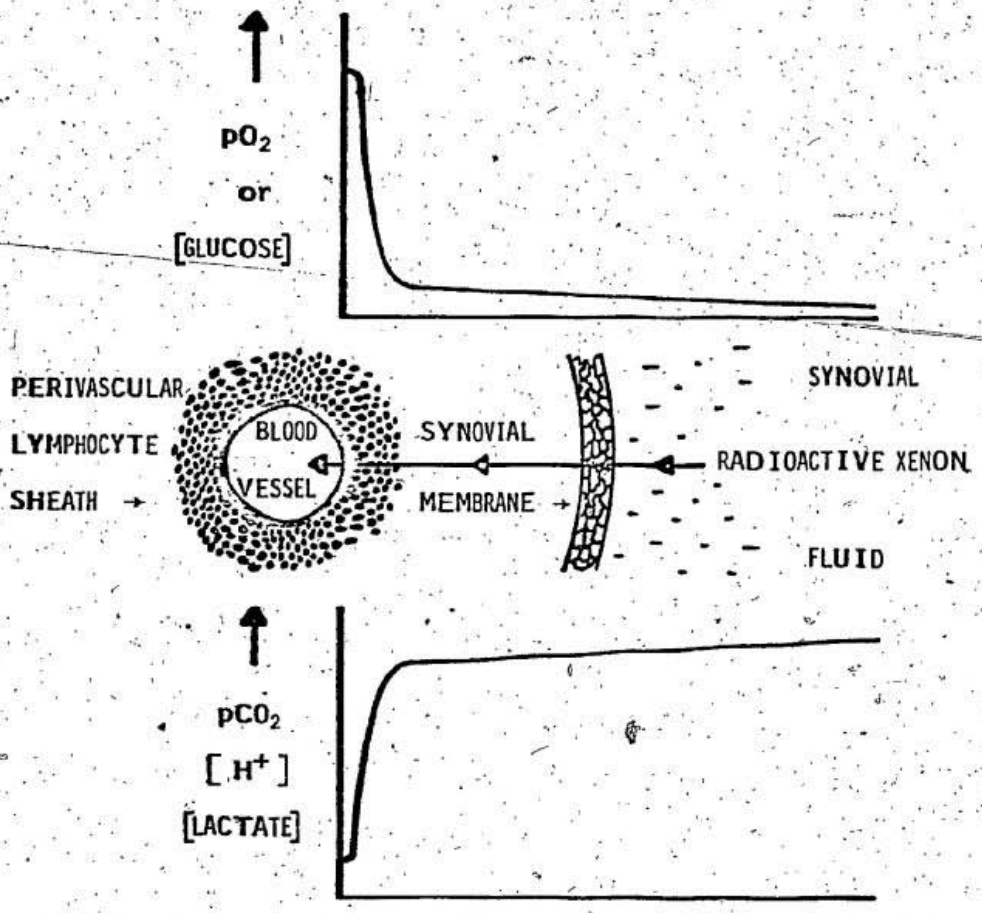
1. The initial lesion is a perivascular inflitration of mononuclear cells in the subsynovium with a typical inflammatory transudate of serum proteins that wash into the synovial cavity overcoming the molecular sieving effect of the Hyaluronate in the ground substance and fluid (*vide infra*) and carrying a limited number of mononuclear cells (Fig. 1).

2. The perivascular collections of mononuclear cells are metabolically very active and set up very steep gradients for glucose, ozygen, carbon dioxide, lactate and pH so that there is little or no gradient between the joint cavity and the outer layers of the infiltrate and therefore a relative blockade (Fig. 2).



EARLY RHEUMATOID ARTHRITIS WITH THE SYNOVIUM ENLARGED TO SHOW PERIVASCULAR COLLECTIONS OF LYMPHOCYTES, MONOCYTES AND PLASMA CELLS

FIGURE 1



THE FORMATION OF THE METABOLIC BLOCKADE IN RHEUMATOID ARTHRITIS DUE TO THE HYPERMETABOLISM OF THE PERIVASCULAR LYMPHOID SHEATH

The radioactive Xenon being metabolically inert is not prevented from leaving the joint whereas CO_2 is, by the flat diffusion gradient

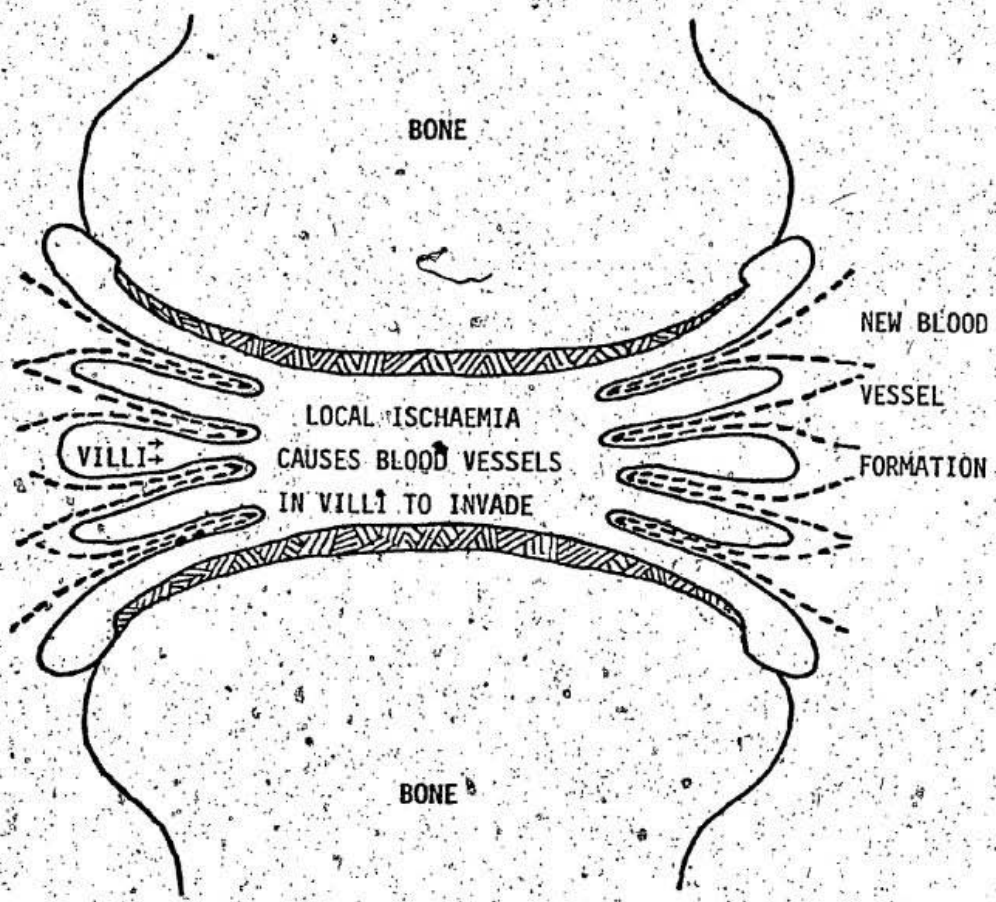
FIGURE 2

3. There is ischaemic damage to the cartilage surface and this causes the release of compounds specific for neovasculization which causes the blood vessels in the synovium to multiply and invade the joint cavity as villous fronds in a manner analogous to the formation of intestinal adhesions with intra-abdominal ischaemia. Necrotic debris attract large numbers of neutrophil leucocytes into the cavity mimicking the mechanisms found in pustules and abscesses and these cells die rapidly from multiple insults such as anoxia liberating many tissue-damaging enzymes (Fig. 3).

4. Around each new villous blood vessel there form perivascular inflammatory infiltrates which perpetuate the metabolic blockade in spite of the increased blood supply.

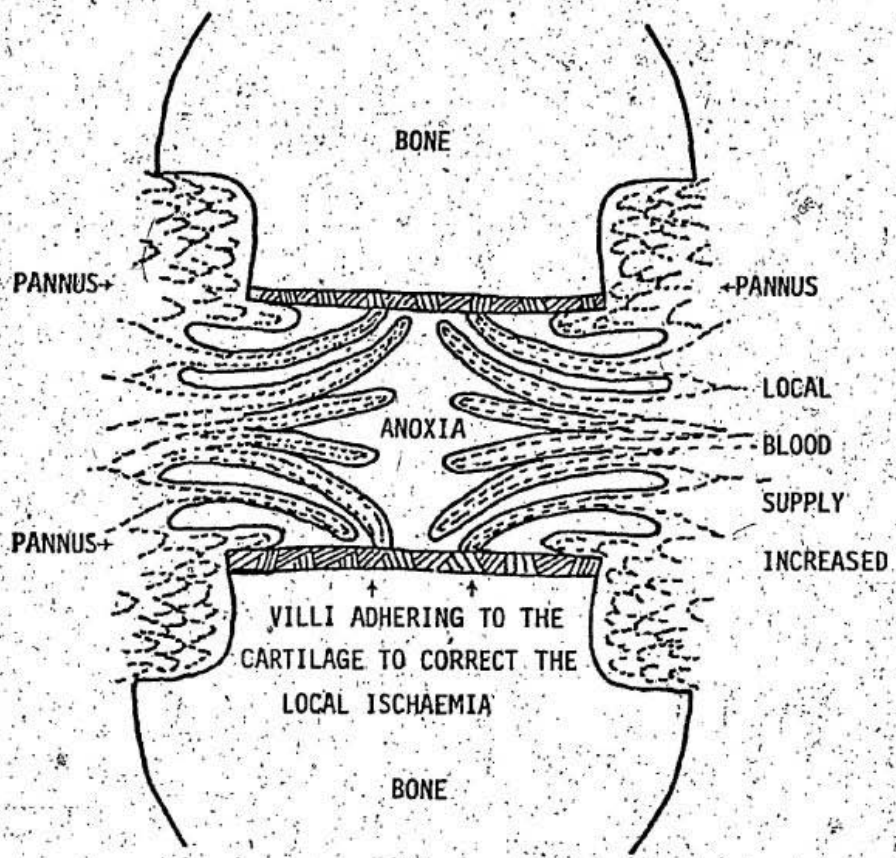
5. The villous fronds if allowed to attach firmly to the joint surface (for instance due to joint immobilisation from pain) burrow into that surface in an attempt to correct the metabolic upset and in doing so dissolve the matrix at the periphery of the surface, and when amalgamated form a granulation tissue (pannus) that causes bony erosions (Fig. 4).

IMMUNE COMPLEXES, RHEUMATOID FACTOR AND COMPLEMENT. In the sequence above the emphasis is plainly upon the role of cell mediated immune responses rather than Rheumatoid Factor or of immune complexes in spite of a wealth of literature that assumes a pivotal rôle in the pathogenesis for these immunological mechanisms. This is not meant to imply that the humoral aspect does not have very important manifestations and does not contain clues as to the aetiology of the disease, but it is inferred from the presence of villous synovitis in the absence of a humoral component in agammaglobulinaemia and seronegative arthritis that this cannot repre-



RHEUMATOID ARTHRITIS SHOWING THE SYNOVIAL VILLI CONTAINING BLOOD VESSELS BEING STIMULATED TO ENCROACH ON THE JOINT CAVITY BY ISCHAEMIA

FIGURE 3



THE INVASION OF BONE AND CARTILAGE BY SYNOVIAL VILLI AND PANNUS IN RESPONSE TO ISCHAEMIC CHANGES IN THOSE TISSUES

FIGURE 4

sent a *sine qua non* in Rheumatoid synovitis. It is part of the central core of this thesis that it is the absence of immunoglobulin that determines disease susceptibility but this will be elaborated as the evidence is further reviewed. However, it is probably better if the evidence concerning synovial fluid is first reviewed since it was from this work that the immune complex theory elaborated and promulgated by Hollander (1965) and Zvaifler (1965) and Ziff (1965) arose and has contributed enormously to the understanding of the disease, particularly its many complications.

SYNOVIAL FLUID. The synovial fluid of the normal joint is a highly viscid, clear liquid consisting of Hyaluronate (3.5 mg/ml - Hamerman, 1962) which is a copolymer of glycosamine and glucuronic acid in long linear chains of 2500 units randomly coiled and kinked so that the molecule has the shape of a sphere (Hamerman, 1962) and has a molecular weight of 6.5 to 10.9 x 10^6 Daltons (Balazs, 1967), and proteins of around 17.2 mg/ml which is mainly Albumin (60-75%) with only 6-7% α_2-globulin (Sandson, 1962). The peculiar distribution of proteins compared with serum reflects a relative exclusion of high molecular weight and highly asymetrical molecules such as Fibrinogen and Complement components which may reflect the molecular sieving effect of Hyaluronate (Ogston, 1961) which is itself covalently bound to protein and makes up much of the intercellular matrix of the synovial lining layer (Gardner, E. 1972).

The synovial fluid in the inflamed joint shows many changes from that found in normal joints but only a few of these changes are specific for Rheumatoid Arthritis. The mucin clot formed in normal synovial fluid

from the acid precipitation of Hyaluronate becomes poor in the Rheumatoid joint probably reflecting the change in structure of this component which seems to be of a lower molecular weight ($1.4 \times 2.1 \times 10^6$ Daltons; Balazs, 1967) possibly due to enzymatic degredation. The fluid becomes less viscid but the protein content rises toward that of serum, gaining globulins and also the ability to clot as fibrinogen and other clotting factors gain access. The formation of fibrin clot and fibrin degradation products in the joint is very florid; on the surface of the synovium, in the intercellular matrix of the synovial membrane, as fibrin masses in the cytoplasm of polymorphs, and as "Rice bodies" in the synovial fluid (Schur, 1963; Caughey, 1967; Barnhart, 1967; Gormsen, 1971); but none of these are unique to Rheumatoid disease and they are regularly seen in many different joint conditions (Zvaifler, 1973).

There is little or no complement in the normal joint whereas in synovitis of many different causes (including the early Rheumatoid) the complement components can be detected in amounts approaching those found in serum. However, in the later stages of Rheumatoid Arthritis there is a fall in the level of synovial fluid complement that correlates well with the presence of Rheumatoid Factor, subcutaneous nodules, an unremitting course, severe joint involvement and vasculitis (Schur, 1975; previous evidence fully reviewed by Zvaifler, 1973).

The Rheumatoid synovial fluid is turbid which is a sign of the ingress of significant numbers of leucocytes. The predominant leucocyte in early disease is the lymphocyte (Schumacher, 1972; Gatter, 1973) but

this predominance is lost rapidly to the neutrophil polymorph, and in late disease the numbers of these cells becomes very marked (15,000 to 20,000 per mm^3) even though they have a short half-life in the joint (four hours according to Bodel and Hollingsworth, 1966). Hollingsworth went on to estimate that in a moderate effusion of the knee joint of 20 ml there would be a turnover of more than 10^9 polymorphs during a 24 hour period with the probable destruction and release of their enzymes into the joint.

The polymorphs differ from those seen in blood due to their striking loss of granules and presence of lyso-phagosomes (Hollander, 1964; Zucker-Franklin, 1966) within which are degenerating subcellular fragments, such as mitochondria and nuclear material as well as fibrin and lipid-like material. In these polymorphs as well as in the phagocytic "A(M)" lining cells Rheumatoid Factor, IgG, Complement components, Fibrin and cellular debris were found in varying combinations. This topic has been reviewed in depth by Zvaifler (1973) who summarised the findings of a number of investigators (Hollander, 1964, being an important source) as follows:

1. Anti-γ-globulin inclusions are correlated with Rheumatoid Factor in the serum or synovial fluid and inversely with joint fluid complement.

2. The quantity of a complement component within cells is inversely proportional to the concentration of the same component in the same fluid.

3. Normal polymorphs exposed to seropositive synovial fluids will develop cytoplasmic inclusions containing IgG, IgM, and C_3, but not with seronegative fluid unless IgM Rheumatoid Factor is added.

4. IgG isolated from Rheumatoid serum injected into unaffected joints of a patient will precipitate inflammation with polymorphs with inclusions.

This is strong evidence that the inflammatory process can involve the complexing of Rheumatoid Factor and IgG with the consumption of complement components and phagocytosis of the whole by polymorphs, which by inference could be chemotactically drawn to the site by active biproducts of the complement cascade and whose enzymes could be largely responsible for the dissolution of the joint. The former part of that statement seems solidly based upon evidence so that the only areas for major dissention are the inferences drawn in the latter half and the generalisations that have tended to become dogma in the numerous uncritical reviews.

The presence of complement depletion accompanying antigen (IgG) and antibody (Rheumatoid Factor) would appear at first sight to be definitive evidence of immune complex involvement with the activation of the complement cascade by the classical pathway. However, some note of caution must be introduced by the excellent investigation by Munthe (1975) of patients with hypogammaglobulinaemia and arthritis resembling the Rheumatoid pattern. In these four patients, two with Bruton type, one adult with severe hypogammaglobulinaemia, and one with common variable immunodeficiency there was a consistency in the serum C_3 levels which were normal or significantly increased but the synovial fluid C_3 levels were significantly depressed in spite of the virtual absence of the other components of immune complex formation. This evidence could be

construed as implicating the alternative pathway of complement activation which is supported by the evidence that there are significant deposits of properdin and C_3a (in the lining cell layer) in the absence of detectable C_1q and C_4 in those sites or in any other sites in the synovium. This evidence indicates that complement activation and depletion can be present in Rheumatoid Arthritis without the (detectable) presence of immune complexes.

We have now re-encountered Cobb's "Peaks of Clarity and Valleys of Indecision" in which the seropositive individual with aggressive disease and vasculitis displays undoubted evidence of circulating immune complexes with depressed serum complement levels and extra-ordinarily increased levels of classic Rheumatoid Factor (IgM anti-IgG) whereas the majority of the patients lack some of these features/complications and have normal or increased levels of serum complement and little evidence of immune complexes likely to cause pathological damage.

MATERNAL INFLUENCES UPON THE DEVELOPING IMMUNE SYSTEM

Events in recent years have drawn attention to the intra-uterine environment in the pathogenesis of major abnormalities; the most dramatic example being the association of Phocomelia with the drug Thalidomide; other examples being the severe deformities associated with minor infections such as Rubella, Toxoplasmosis, and Cytomegalovirus.

There are manifest advantages in allowing an advanced stage of development of the young to take place within the protection of the womb, but there may be a "price to pay" for these advantages in the necessity for fundamental modification and elaboration of the immune system to allow the mutually foreign tissues of mother and child to be closely contiguous without inextricable intermingling. The price exacted could be the congenital abnormalities cited above, as well as an increased likelihood for developing cancer and autoimmune disease exploiting the gaps in a compromised immune system.

Transplacental traffic of lymphocytes between mother and child has been repeatedly suggested as a cause of human disease. Oliner (1961) quoted Billingham as speculating about maternal lymphocytes causing autoimmune disease in the offspring as early as 1959, but at that time there was no evidence that lymphocytes could cross the placenta. Desai (1963) suggested that the maternal lymphocytes that he had found in human cord blood might be the cause of autoimmune disease in later life. Field (1971) amplified these speculations by suggesting that there might be an interim "benign lymphocyte chimerism' before the onset of the Graft-Versus-Host mediated autoimmune disease. Kaplan (1959) noted the similarity of

Hodgkin's disease to allogeneic disease (chronic Graft-Versus-Host disease in experimental animals), and Green (1960) proposed a maternal origin for the lymphocytes causing the Graft-Versus-Host disease with the lymphoma being the sequela. Hard (1973) suggested that maternal lymphocytes could cause a Host-Versus-Graft disease in humans manifest as S.L.E. from the similarities in the renal histology seen in experimental mice.

There is a natural scepticism that arises when the possibility of maternal to foetal passage of lymphocytes is discussed. This partly stems from the belief that if this were possible it would invariably cause severe disease in the offspring and this would have been eliminated by Darwinian selection in the evolution of the viviparous animals. However, the maternal effect upon the immunologic endowment of the offspring may also carry a selective advantage which can be appreciated in the example of the transplacental passage of immunoglobulin "G" from mother to child, so that one can postulate that a similar benefit in cell mediated immunity, such as resistance to Tuberculosis or Smallpox, might cancel the selective disadvantage of Graft-Versus-Host disease providing the latter was an infrequent complication.

There is the danger that the mother might immunologically reject her own child and this too had tended to be assumed to be so disadvantageous that any possibility of this happening should have been removed by selection.. If there is any cross-reaction with foetal antigens the close proximity of the maternal immune system represents an everpresent hazard for the im-

mature immune system of the child that may magnify the dangers of some normally benign infections such as Rubella. For these reasons the recent demonstration by Beer and Billingham (1971; 1972; 1974; 1976) that female rats can be manipulated immunologically to effect pathological changes in their offspring attains some considerable degree of significance. This work does not stand in isolation since haemolytic disease of the newborn clearly implicates the maternal immune system in the pathogenesis of life-threatening pathology in the offspring, but in Beer and Billingham's work there was the clear inference that maternal lymphocytes had crossed into the foetus to bring about the pathological changes. However, the Rhesus story does help to give a concrete example to the idea of the potential penalties that the offspring may incur in receiving immunological "help" from the mother.

EVIDENCE DERIVED FROM EXPERIMENTAL ANIMAL INVESTIGATIONS The experiment that Beer and Billingham finally put together was elegant and disarmingly simple: they skin grafted the mother (rat) with father's skin so that it was actively rejected by immunological mechanisims during the early part of pregnancy which caused immunological damage to the offspring presumably due to the same (paternal) antigens being on the foetus. The damage that they noted was the delayed evolution of immunological "runting" following parturition that sometimes led to the death of the animal but sometimes resolved spontaneously. Previously Billingham, Brent and Medawar (1957) had looked for signs of runting following such a maternal stimulus but had been unable to observe any effect. It is now believed by Billingham that the offpsring were not observed for long enough in the

earlier work and there also seems to be a requirement for skin-grafting one to two weeks before mating which was probably not fulfilled. From the latest work with rats and anecdotally with other animals, the syndrome of immunological runt disease could be provoked in the offspring by maternal priming which by inference from the considerable literature on disease must have caused maternal "T" lymphocytes to cross the placenta.

GRAFT-VERSUS-HOST DISEASE The effects of Graft-Versus-Host disease form a characteristic syndrome (runt disease) in rats and mice, with splenomegaly (Simonsen, 1957), cachexia, diarrhoea, weight loss, a "hunched look", skin and hair changes (Billingham, 1957; 1959); haemolytic anaemia (Porter, 1960; Oliner, 1961), carditis and arthritis (Stastny, 1965) have also been documented in the chronic disease. It is usually provoked by the administration of (parental) immune-competent cells into the neonate (rodent) or other immunologically immature semiallogeneic host. Classically the parent is a pure strain animal homozygous at every genetic locus and the offspring is a cross-breed (F_1) and therefore an obligatory heterozygote at a number of loci. If there is heterozygosity at the major histocompatibility loci the effect of this combination is to achieve a genetically restricted one-way reaction in which the offspring is unable to reject the parental tissue as it carries the same antigens itself while the parental lymphocytes have no such restriction and can reject the offspring (Graft-Versus-Host) causing the syndrome (immunological runting) characterised above.

The destructive changes seen in the host are not caused by antibodies but are due to the lymphocytes that come from stem cells in the bone marrow (McGregor, 1968) and are processed in the Thymus (Good, 1962) and join the lymphocyte recirculation to be found in the Thoracic duct (Gowans, 1962) and the peripheral blood (Hildemann, 1962) and so in contemporary terms are "T" cells. Recently there has been evidence in rodents that two subpopulations of the "T" cell, T_1 and T_2, act in a synergistic fashion both in the afferent or recognition phase and the efferent or effector phase of the Graft-Versus-Host reaction (Tigelaar, 1972; 1973; Hayry, 1974). T_1 cells which are found primarily in the Thymus are spleen seeking in the irradiated rodent and are thought to be the initiators of the reaction while T_2 are lymph-node seeking in the irradiated rodent and are thought to be only amplifiers of the recognition phase but are the primary effectors in the cytolysis of the host.

Atrophy of the Thymus and peripheral lymphoid tissues is a prominent feature of systemic Graft-Versus-Host disease and there is a caveat concerning the specificity of the syndrome as the same effects, both symptomatic and pathologic, with the exception of splenomegaly (Simonsen, 1962), can be produced in neonatal mice by the surgical ablation of the Thymus (Miller, 1962) unless the animals are kept germ-free. In G.V.H. the main sites for attack by the grafted "T" cells seem to be those populated by lymphocytes with no changes to be seen in muscle, for example, (Elkins, 1971) so that Miller (1962) may be correct in his speculation thant G.V.H. is an immunological means of achieving a Thymectomy and a secondary immunodeficiency. The splenomegaly in this model would be completely separate from the main

attack and be due to the migratory properties of the T_1 cell.

The "Graft-Versus-Host" reaction sounds as though there is but one active population of cells, that from the donor, which is responsible for all the effects, but it is clearly recognised now that both the putative target as well as the effector are actively contributing to the final outcome and that the concepts involved transcend the simplistic one-way model inferred from the genetic pattern. The ultimate expression of this is the recovery from G.V.H. that has been observed even in some of the most lethal combinations of phenotypes in graft and host. In addition, one would expect *a priori* that all strains of animal should show qualitatively the same effects, but even in the rodents that supplied the confirmatory evidence for Classical Graft-Versus-Host effects there are strains, such as the C57Bl mouse, that have not caused runting when lymphoid cells were implanted into neonatal F_1 offspring (Cudkowicz, 1964; Goodman, 1965). In order to explain this effect upon genetic lines it has to be postulated that in these strains (only) there are recessively expressed gene products of histocompatibility loci that are recognised as "non-self" by the heterozygote (reviewed by Elkins, 1971).

The term "Graft-Versus-Host" also suffers from containing two separate concepts, defining not only the genetic restrictions discussed earlier but also the pathology produced in the host by grafts. This dichotomy is most clearly illustrated by the Host-Versus-Graft reaction in which F_1 lymphoid tissue is implanted into parental(P) strain neonates and can produce a lethal condition in those animals even though there is no genetic restriction upon the hosts to prevent rejection. Billingham and Brent

(1959) found that 5 out of 15 A strain mice developed pronounced lympho-splenomegaly within 50 days of neonatal intravenous injection of as few as 10^7 F_1 hybrid (C57 x A) spleen cells, which should be a Host-Versus-Graft reaction, with the long latent interval presumably due to some form of immunological inertia in the immature animals.

More recently Hard (1970; 1973) has determined that the following factors are essential for the production of Host-Versus-Graft disease in mice: a) the hosts must be newborn or immunologically immature, b) the grafted tissues must be administered intravenously and contain immune-competent cells, c) there must be the genetic restriction that the graft cannot attack the host (*though he has not presented the evidence upon which this inference was made*), and d) only low initial doses of injected cells cause the effect (*unlike the G.V.H. reaction that seems to be dose dependent*). With all of these conditions fulfilled the injected animals can remain healthy and grow at a rate only slightly below normal until there is a dramatic onset of ill-health, with lethargy, a marked pallor of the mucous membranes and paws with autopsy evidence of colitis and intestinal blood loss with enlargement of the spleen and lymph nodes (mean weight five times the match control animals) and there was gross Thymic atrophy in some of them. Thus the pattern of the disease is very different from those suffering from Graft-Versus-Host disease and the prominent lymphoid hyperplasia would also not be typical of that condition nor the prolonged latent interval before disease expression. Nonetheless, the pathology is aetiologically related to the graft and at that level could be classed as a Graft-Versus-Host disease.

The two patterns of disease with G.V.H. characterised by an immediate and steady downhill course and H.V.G. characterised by outward health until the sudden terminal event has been mirrored in the breeding experiments of Palm (1970; 1974). These experiments arose from observations of a normal breeding colony of rats that had failed to produce homozygotes at the major histocompatibility loci after many generations of brother/sister matings. The experimental analogue of this was the reciprocal backcross mating of rat strains (for instance BN/DA x BN) to produce the F_2 generation that could be tissue typed for the major histocompatibility antigens to assess whether there was a selective loss when the mother was a P (parental) strain or F_1 hybrid of homozygous offspring at the MHR. Palm found that in the offspring of the pure strain mothers there was a selective morbidity and mortality of the offspring from runting, particularly of the males and the homozygotes at the MHR:

Thus out of 233 offspring:

66 were female heterozygotes, 69 homozygotes

54 were male heterozygotes, 34 homozygotes.

When the mothers were the F_1 hybrids, there were fewer offspring suggesting an increased mortality which from observation arose from healthy looking F_2 offspring that suddenly collapsed without any prior signs of runting:

Thus out of 174 offspring:

48 were female heterozygotes, 46 homozygotes

40 were male heterozygotes, 40 homozygotes. In this latter group there was no appreciable selective loss except that both groups of males were considerably reduced in numbers compared with the females.

This experiment demonstrated that in a normal rat colony in which there was semiallogeneic matings, there was an appreciable mortality from two separate disease conditions, one of which resembled Graft-Versus-Host disease in the male AgB homozygous offspring of pure strain mothers. Palm interpreted this data to suggest that the MHR loci confer some protection from this pattern of disease which by inference has been produced by maternal lymphocytes attacking minor histocompatibility antigens including perhaps the "Y" (male) antigen. The data from the offspring of the F_1 mothers is more difficult to interpret except if the inference is made that the mortality was due to a Host-Versus-Graft disease that did not depend upon homozygosity at the MHR to cause catastrophic reactions.

The breeding experiments of Palm lead back naturally to the work of Beer as the protocols for the induction of Graft-Versus-Host disease can be directly translated into normal hazards of pregnancy certainly in inbreeding rats and less certainly in the outbred pregnancy in man. The results of Palm's experiments seem clearcut though later experiments by Palm to confirm the effect showed a much lower level of mortality in the offspring suggesting that there was additional environmental effects in the earlier work that were augmenting the mortality of the offspring from the maternal influence.

There is still room for doubt that the effects seen by Palm and Beer were due to maternal "T" cells though Graft-Versus-Host disease investigated before has been proved to have been caused by "T" cells to most peoples' satisfaction since isolated cells cause the effects and are present in the diseased animals and can be transferred to other animal producing disease and the disease can be abrogated by specific anti-T antibody. The runt disease seen in the experiments of Palm and Beer had all the hallmarks of Graft-Versus-Host disease and so the inference is strong that it had a similar aetiology by the necessary confirmatory investigations as outlined for G.V.H. have not been performed. It was one of the major aims of this thesis to demonstrate experimentally that maternal "T" cells are causing the pathological changes directly, or indirectly with the synovia acting as "innocent bystanders" to a "lymphocyte war" as seen in cutaneous GVH reactions (Ramseier, 1966).

In the experimental animal work that defined Graft-Versus-Host disease the cells that were administered were usually of more than one population, for instance crude spleen suspensions with "T" cells, "B" cells, plasma cells and macrophages. However, if we accept that G.V.H. disease affected the runted rat offspring in Palm's experiments and that this implicated maternal "T" cells we must also accept that probably two "T" cell populations are co-operating, the T_1 and T_2 cells of Tigelaar and Asofsky (1972) that have been proved to be necessary for the complete expression of recognition and cytolysis. Either this had been accomplished in the mother with only the T_2 cytolytic effector cells crossing the placenta, or both T_1 and T_2 cells have crossed the placenta, or the T_1 cells have

crossed and co-opted the help of the offsprings' T_2 cells. Considering the delay in the action of the disease states it seems somewhat unlikely that the cytolytic T_2 cells could maintain their activity, and by the same reasoning, diluted in the overall population of the hosts' cells, it also seems unlikely that a powerful collaboration of two separate populations of "T" cells would be possible after such a time interval, and so the possibility of cell collaboration between the cells of mother and offspring must be considered.

ALLOGENEIC LYMPHOCYTE CO-OPERATION. The presence or absence of allogeneic barriers to lymphocyte co-operation defined chiefly by the major histocompatibility loci is under intensive study at this time so that information in the literature is outdated by the time it is published. This work has been concentrated of late on the mouse with the well defined histocompatibility loci (H-2) and recombinant backcrosses readily available, though some of the early work was performed on guinea pigs since there were strains already characterised for immune responses to defined antigens that behaved in a Mendelian (single gene) fashion (Rosenthal, 1973). The picture is still far from clear concerning the limitations to cellular collaboration but the following seems to be in the main stream of the current work.

1. Cytotoxic effector "T" cells directed against non-H-2 antigens on the surface of cells will kill target cells carrying these antigens only if they also carry the same K or D surface antigens (products of alleles of loci in the Major Histocompatibility Region = MHR) that were

present on the sensitizing cell (Shearer, 1975; Bevan, 1975; Zinkernagel, 1976; Blank, 1976) and that if effector and target differ in the H-2 region cytotoxicity is only possible if the effector has been processed in the Thymus syngeneic with the target (Zinkernagel, 1978). Translated into the maternal/foetal situation the T_2 cells of the mother are only able to directly lyse the cells of the offspring in response to viral antigens on the surfaces if there is identity at the MHR or if they have matured from the precursor cell in the environment of the offspring's Thymus and are able to recognise that as "self".

2. Antigen primed "T" cells can only collaborate with "B" cells or be restimulated by macrophages if the "B" cells or macrophages carry the same I-region (part of the mouse MHR containing loci with alleles defining immune response status to certain antigens) alleles present on the "B" cell or macrophage during priming (Katz, 1976; Pierce, 1976).

3. For responses to Immune-response-gene restricted antigens, the relevent gene must be carried on the same haplotype as the genes involved in a compatible collaboration (Katz, 1973; Shevach, 1973).

The definitions of the current position are taken from Swain (1977) who took issue with the experimental bases for the conclusions in paragraphs 2 and 3. By careful elimination of the allogeneic stimulated lymphocytes, Swain and his co-workers were able to achieve good collaboration in a Hapten-Carrier type of protocol with histoincompatible "T" and "B" cells. However, the conclusions in the first paragraph concerning cytotoxicity seem beyond doubt at this time though they have not been sub-

jected to the procedures necessary to completely eliminate the allogeneic effect.

The experimental basis for this work remains the mouse and can only be translated into human terms with some reservations. It seems unlikely that mature maternal cells could be processed in the foetal Thymus in the normal pregnancy and so this inability to cause cytolysis of the host could be one mechanism involving the Major Histocompatibility Antigens that protect the foetus from the harmful effects of the mother attacking antigens defined by minor loci which was the mechanism proposed by Palm (1974).

Although this work seems to have nothing to contribute to the delineation of aetiological mechanisms in Rheumatoid Arthritis it has been introduced without apology to demonstrate that there are at present no definitive data to deny the hypothesis that maternal "T" cells can collaborate with the offspring's "B" cells if suitably filtered to remove alloreactive cells. One could postulate that the placenta would be the ideal organ to hold and destroy such cells.

EVIDENCE OF *IN VIVO* CELL CO-OPERATION Beer and Billingham's work stemmed from an observation by Stastny (1965) that Sprague Dawley rats, when suitably primed with Lewis strain skin grafts in early pregnancy, gave birth to offspring that also showed evidence of prior sensitization by the premature or "second set" rejection of Lewis skin graft challenge which was specific for that strain. The experiment was repeated by Beer (1972) who confirmed the observation and then went on to use immuno-

logically tolerant rats bearing Lewis skin grafts through pregnancy that produced offspring that displayed a normal pattern of rejection to Lewis skin graft challenge even though exposed *in utero* to the relevant antigens.

Thjs latter observation makes it extremely unlikely that the effect upon the offspring in the original experiments was due to the transplacental passage of antigen and it seems more likely that cellular immunity exemplified by the "T" lymphocyte has been passed into the offspring via the placenta or in the breast milk. The only alternative explanation would be the passage of an informational humoral factor such as Lawrence's Transfer Factor (Lawrence, 1956) instead of cells but if this were the case it is difficult to see how such a factor could cause the Graft-Versus-Host disease seen in the later experiments of Beer and Billingham.

Since Stastny's observations with third party skin grafts are now amply confirmed his observations in the same manner with Tuberculin hypersensitivity used essentially the same protocol and so by inference may involve the same mechanism of maternal "T" lymphocytes crossing the placenta. The whole work seems to make it likely that in his rats the maternal "T" cells were co-operating with the offspring's "T" cells in second set skin-graft rejection and Tuberculin hypersensitivity even though the Sprague Dawley rat is not totally inbred and so should have some histocompatibility differences between mother and child, that is, collaboration across potential allogeneic differences.

DIRECT ANTIGEN EFFECTS UPON THE FOETUS Not every investigator working with experimental animals is as sanguine about maternal lymphocytes as the mediators of the maternal effect upon the immune responses of the offspring. The alternative explanation of the transplacental passage of antigen has been reported by Gill (1971 in the F344 strain of rat using radioactive ^{125}Iodine labelled poly(Glu^{52}Lys^{33}Tyr^{15}) antigen aggregated with methylated bovine serum albumin administered in Freund's complete adjuvant in two divided doses to the female thirty days before mating, with the radioactivity being detected in the bone marrow, spleen and thymuses removed from the subsequent offspring when aged 8 to 10 weeks. This protocol has been the subject of some attack; Stern (1976) has pointed out that there is a well documented avidity of the foetus for Iodine (Pickering, 1961) which could easily explain the presence of the radioisotope in the absence of its coupled antigen; and from the discussion of a paper by Gill (1977) at a recent conference it was suggested that the immunological data that he had subsequently used to further his assertion of the role of antigen could equally be used by those advocating the role of cells.

Kindred (1974) has clearly documented the presence of a clonally restricted immunoglobulin response as detected by isoelectric focussing to nitrophenyl haptens in the offspring from immunised CBA/J female mice. The protocol that Kindred followed was essentially that of Gill (1971) with Trinitrophenyl and Dinitrophenyl derivatives coupled with bovine gammaglobulin used as the antigens which were not, however, radioactive. Although Kindred does briefly consider maternal suppressor "T" cells and

maternal helper "T" cells the interpretation favoured was that the antigen itself was the foetal priming agent.

MATERNAL ANTIBODY Ono (1974) published a report of experiments performed using C_3H/HE female mice primed with either Sheep red blood cells or denatured calf-Thymus-DNA in Freund's adjuvant and mated. The offspring of these mice were bled out at three weeks of age and the spleens removed, broken up and the cells injected into another mouse that is a normal syngeneic but X-irradiated mouse which was then challenged with the antigen and killed after eight days and the splenic plaque forming cells assayed by Jerne's (1963) technique. Ono found that the offspring of mice primed with Sheep red blood cells did not show evidence of priming, but those offspring from DNA primed mice showed many more plaque forming cells derived from their spleens compared with control animals. This latter observation was confirmed directly upon the offspring at six weeks of age after the maternal antibody had been metabolised, by antigen challenge with the plaque forming cells assayed directly from their spleens after four days using DNA-coupled Sheep red blood cells. Ono interpreted this evidence to suggest that maternal antibody was the factor causing the observed maternal effect, but this appears to be the weakest part of an otherwise excellent presentation.

EVIDENCE SUPPORTING THE CELLULAR HYPOTHESIS The evidence is reasonably strong that maternal cellular immunity may influence the offsprings' subsequent responses from the work of Stastny and Beer. A recent contribution to this has been the hapten-carrier work of Stern (1976)

who examined the effect of maternal priming with Bovine Serum Albumin (BSA) administered with *Bordetella pertussis* in CBA mice three months before syngeneic mating. The offspring of these mice and those born to unimmunized control animals were primed with Dinitrophenyl conjugated Chicken Gammaglobulin (DNP-CGG) with *Pertussis* at three months of age and six weeks later were challenged with DNP-CGG or DNP-PPD (Purified Protein Derivative of Tuberculin antigen), or DNP-BSA.

The mice were then serially bled and the sera assayed for anti-DNP antibodies, using DNP coated Ox erythrocytes and guinea pig complement, in serial dilutions for lytic activity. This protocol was designed to test the presence of carrier (BSA) primed "T" lymphocytes from the mother in an offspring primed with Hapten (DNP) coupled with a heterologous carrier (CGG). The offspring of primed and unprimed mothers showed a maximal response to DNP-CGG naturally as they had already been primed to that hapten-carrier combination and they both showed a low-grade response to PPD-DNP illustrating the specificity of the carrier effect but the offspring of primed (BSA) mothers showed a much greater response to DNP-BSA than did the offspring of unprimed mothers. The only weakness in this rather complicated protocol was the *Bordetella pertussis* itself which does not seem to have been administered to the control mothers though the absence of a difference to CGG-DNP between the groups suggest that this did not cause a "maternal effect" (*i.e. the pertussis acting as a carrier*).

Other work that has tended to confirm the maternal influence upon the

cellular immunity of the offspring that must be included is that of Uphoff (1970a; 1970b; 1972; 1973; 1976) who has used tumour resistance as well as the Graft-Versus-Host effects to demonstrate the influence of the uterine milieu upon the antigenicity of the offspring and the sensitivity to transplanted tumours. Although the results confirmed a major influence of the mother the experiments reported used rather complicated protocols or were subject to interpretative ambiguities (such as what constitutes tumour resistance and the antigenicity detected in the Graft-Versus-Host assay) and so the details of this work will not be discussed further in this thesis except in the context of the immunological component of breast milk.

Other experimental animal workers in this field are as follows: Barnes (1969) who reported that normal CFW mouse embryos nurtured in the NZB/Bl hybrid womb had a propensity for developing autoimmune haemolytic anaemia due to a "maternal agent" most probably a virus but possibly abberant lymphocyte clones; Minden (1976) who reported that BCG-associated tumour resistance had been vertically transmitted from female strain 2 guinea pigs to their offspring (*though subject to the same difficulty of interpretation as Uphoff*); Turakulov (1974) who reported lymphocyte accumulations in the Thyroids of offspring of female chinchilla (presumably outbred) rabbits immunised with homologous Thyroid gland extracts in adjuvant (*though he interpreted the pathology as due to maternal antibody*); Dukova (1976) who reported the presence of spontaneous lymphomata in the F_2 offspring of hybrid female mice (CBAxC57Bl/6) that were suffering from Graft-Versus-Host disease induced by the injection

of C57Bl/6 lymphocytes prior to mating.

BREAST MILK AS A SOURCE OF LYMPHOCYTES It has been known by farmers that breast milk is very important in the prevention of disease in animals, for instance neonatal piglets very rarely survive if given milk from other species. The influence of humoral components of breast milk such as immunoglobulin A and now including a host of non-immunoglobulin factors has been documented in recent years, but in the course of these investigations in rats it has been realised that the cellular contribution of colostrum in the form of lymphocytes, plasma cells and monocytes seems to have a beneficial influence in protecting the offspring from infection particularly of the gut. If the cells can survive in the gut it can be postulated that they might gain access to the circulation and tissues of the offspring possibly causing pathology.

Once again Beer and Billingham (1974; 1975) have been in the van with the reports that F_1 hybrid rat offspring (Fischer x DA for instance) developed skin lesions when suckled by a mother that had a skin graft upon it and that this effect could be imitated by the intra-gastric infusion of lymphoid cells from adult Fischer donors sensitized to DA antigens but not using serum from the same source. The skin lesions resembled those seen in the early phases of runt disease but the rats did not appear to be suffering from Graft-Versus-Host disease as they remained healthy otherwise. The effect was most marked if the skin graft was put on the mother between the seventh and tenth day post-partum but did not appear to be related to histocompatibility antigens as the graft could be syngeneic with the mother and still caused the skin changes in the offspring

Foster nursing on allogeneic mothers immediately after birth did cause a high mortality in the offspring following skin lesions and appearances similar to Graft-Versus-Host disease. However, it should be noted that the time sequence in these experiments for skin graft challenge of the mothers was entirely different from that used for the *in utero* induction of Graft-Versus-Host disease by the same authors.

SUMMARY OF THE EVIDENCE FROM ANIMAL INVESTIGATIONS There can be little remaining doubt that in rats and mice specific sensitivity can be passed into the foetus during the early *in utero* phases of development. There is the strong inference from the Graft-Versus-Host disease produced that maternal "T" cells can cross the placenta though there is still no definitive evidence that these cells continue to exist in the offspring and are at the nidus of pathological changes in later life. These effects seem separate from the effects due to direct antigen challenge of the foetus though such effects should not be ignored in human investigations. Although inflammatory arthritis is a recognised feature of experimental chronic allogeneic disease it is not known whether this is a direct attack or whether it is an "innocent bystander" in a lymphocyte "war".

EVIDENCE DERIVED FROM HUMAN INVESTIGATIONS

This thesis is primarily concerned with a human disease without a direct parallel in other species so that without some confirmatory evidence derived from human investigation the experimental animal data could be accused of being irrelevant. Most of the reports cited in the last section have concerned rodent investigations but these animals have markedly different gestational patterns compared with humans with the young being born at a comparatively immature stage of development with parturition only three weeks after implantation, and the data should only be extrapolated to the human condition with caution. The data examined has been solely physiological concerned with the changes in the function of the immune system of the offspring rather than the morphological identification of the maternal cells. There is a body of data that has been accumulated concerning such investigations in rodents particularly mice (reviewed by Schröder, 1975; Beer, 1971) but it is somewhat controversial and has not been reviewed here as it is of doubtful significance in the human context, but human investigations are well decumented and have a more immediate relevance and will be briefly reviewed here before the physiological data.

EVIDENCE THAT THE HUMAN PLACENTA IS PERMEABLE TO CELLS There is overwhelming evidence that foetal to maternal passage of cells is not at all uncommon, witnessed by the Rhesus isoimmunization of pregnant women by erythrocytes derived from their offspring that have crossed the placenta (reviewed by Schröder, 1975). It is unusual for a primigravid Rhesus negative woman to generate high titres of antibody except after parturition and this would be consistent with the hypothesis that sig-

nificant leakages of erythrocytes are usually due to trauma to the placenta during labour.

There are good grounds for the belief that leakage from the foetus to the mother is more likely than from the mother to the foetus in the haemochorial placenta. The structure of the human placenta with the foetal circulation splitting into small vessels enclosed in villi dipping into the maternal lakes of blood and the relatively higher pressure of the foetal vascular bed compared with the maternal side (45 mm Hg. versus 10 mm Hg. lying and 35 mm Hg. standing erect - Page, 1960) are two factors that have been documented (Macris, 1958).

In spite of this evidence Naeslund (1946) using radioactive phosphorus ^{32}P labelled erythrocytes injected into women at term demonstrated the presence of tagged cells in one out of six offspring post-partum. Later with Hedenstedt (1946) using pregnant women injected with elliptocytes he demonstrated these characteristic erythrocytes in one infant that had come from a toxaemic pregnancy but in none in another child from a normal pregnancy. Although there have been many investigations since these (Mengert, 1955; Duhring, 1959; Lee, 1962; Zarou, 1964; Cohen, 1965; Donovan, 1966; Fischer, 1967; Eimer, 1969; Wong, 1972; Fujikura, 1975) using ^{59}Fe and ^{51}Cr tagged erythrocytes or sickle-cells or blood group immunofluorescence, the evidence has not changed from these first simple observations that blood can leak into the foetus after a complicated late pregnancy but the occurence of this is exceptional and usually of small volume.

Many of the references cited above purport to demonstrate the commonness of the entrance of maternal erythrocytes into the foetal circulation. However, careful work by Cohen (1965) revealed only one neonatal child out of 154 with recognisable maternal cells by immunofluorescence in blood taken from a heel prick compared to between 11 and 37% of the corresponding cord blood depending upon the care taken to minimise contamination. Similarly, Donovan (1965) used an almost obsessionally clean technique and thereby showed that ^{51}CR tagged erythrocytes could not be detected in cord blood from eight mid-term terminations, but that the slightest deviation from perfect technique caused artefactual detection of radioactivity. These two reports have emphasized that most of the techniques are too sensitive compared to the general contamination of the field with maternal blood in every delivery so that even the smallest amount of maternal blood on the instruments (<0.0001 ml. - Donovan) used in the collection of blood will give artefactual data on the transmission of maternal red blood cells into the foetus. It would be difficult to justify the use of the previous evidence gathered by such a technique to support a hypothesis that such leakages can cause modification of the whole immune apparatus of the offspring from the million or so unselected lymphocytes that occasionally wash in with the erythrocytes.

The lymphocyte does not need an anatomical break in the integrity of the placental circulation in order to cross from mother to child as it is a highly motile cell that can invade every tissue of the body apart from solid bone and cartilage if stimulated to do so. Using an *in vitro*

technique Russell (1975) demonstrated that human lymphocytes could be induced to cross membranes (in that instance Millipore filters) by activation with antigen *in vitro* or *in vivo* with the presence of a chemotactic gradient across the membrane to give direction to the migration (Wilkinson, 1977), but only in the presence of Albumin which seemed to act in a "chemokinetic" fashion to promote migration. From the evidence of Beer and that of Wilkinson one would not suppose that lymphocytes would cross the placenta in significant numbers under normal conditions but that lymphocyte activation by antigen as well as an antigenic gradient across the placenta (perhaps only in the absence of major histocompatibility differences from Palm's evidence) plus albumin would be needed in order to produce a measurable effect upon the offspring. To some extent this *a priori* reasoning has been confirmed by the evidence available which has been recently reviewed by Schröder (1975).

The evidence from the many studies of erythrocytes is useful to put into perspective the infrequent reports of maternal lymphocytes found in the circulation of the offspring. The most elegant technique used was that employing *in vitro* Quinacrine labelled leukocytes injected into women at term and subsequently detected in the cord blood of the foetus after parturition (Desai, 1963; Rigby, 1964). Both of these investigations employed techniques using cord blood that Donovan and Cohen have attacked as giving artefactual data with erythrocytes, and only in the Rigby report was there any attempt to document circulating labelled cells in the offspring some hours after parturition. There was brief mention in the Rigby report that in smears made from the infant's blood there were

rare but definitely labelled immature mononuclear leukocytes in an offspring of a woman with a myeloblastic leukaemia with other forms being detected in the cord blood which are probably better ignored as possible artefacts. Nevertheless the positive identification of the maternal cells in the peripheral blood of this offspring (of a highly abnormal mother) constitutes the only report above criticism of a healthy child with this phenomenon.

Identification of maternal cells using chromosomal analysis has been attempted (Turner, 1966; Olding, 1972) but two factors at least make this data meaningless: the use of cord blood as already criticized, and the presence in the foetus of lymphocytes suppressing mitosis of maternal lymphocytes (Olding, 1974; Oldstone, 1977) and soluble factors also having that effect such as Human Chorionic Gonadotrophin (Adcock, 1973). These two factors have the effect that if maternal mitoses were detected this could be due to artefact and if they were not detected this could be due to suppressor mechanisms. This has meant that the data has no quantitative or qualitative meaning. However, there are two reasonably well documented maternal-foetal chimaeras. The male infant with hypospadiasis reported by El-Alfi (1969) showed 20 out of 48 metaphases with the XX configuration soon after birth but five months later only XY metaphases could be found on leukocyte culture. In this case there was an interesting experiment performed of culturing the offspring's leukocytes in the presence of maternal and paternal antigens which showed hyporesponsiveness to the mother, but since a "maternal and paternal leukocyte extract" was used this evidently does not correspond to a mixed lymphocyte

reaction which depends upon the presence of living stimulating cells.

Kadowaki (1965) reported a phenotypically male infant that appeared normal up to one month of age when it developed "croup" and subsequently severe respiratory tract infection, cachexia, malabsorption, an extensive skin rash, alopecia and lymphadenopathy, with death at 16 months. Metaphase spreads prepared from culturing peripheral blood were obtained with a poor yield in spite of normal lymphocyte counts on three occasions and always showed a mixed population of XX and XY karyotypes in the lymphocytes while evidence from the buccal mucosa, granulocytes and erythrocytes demonstrated that no other cell type was chimaeric. There had been no evidence of a twin pregnancy so the almost inescapable conclusion was the the infant displayed maternal lymphocyte chimaerism which was causing a Graft-Versus-Host type of reaction and a human "runt disease" syndrome. Necropsy revealed a very small thymus, grossly atrophic lymphoid tissue except in the spleen and abdominal and thoracic lymph nodes, villous atrophy in the intestines and lymphocytic infiltrations in the liver, and prominent lymphocyte and plasma cell infiltrations of the salivary glands, and perivascular mononuclear infiltrates in the skin with hyperkeratosis. In all other sites lymphocytes and particularly plasma cells were rare which was in accord with the clinical picture of hypogammaglobulin "G".

GRAFT-VERSUS-HOST DISEASE IN MAN This picture of human Graft-Versus-Host disease seen in Kadowaki's case has been mirrored recently in two reports of a similar disease pattern seen after bone marrow transplan-

tation for Acute Myelogenous Leukaemia (Lawley, 1977) and Aplastic Anaemia and Burkitt's lymphoma (Gratwhol, 1977). The Graft-Versus-Host changes in the skin with lymphoid infiltration into the epithelia associated with focal coagulative necrosis (Slavin, 1973) were noted but there were also the gross changes of Sjögrens syndrome (dry eyes and mouth from chronic inflammation of the salivary and lacrimal glands) and Scleroderma (diffuse fibrosis of the connective tissues of the skin associated with lymphocyte infiltration of the early lesions) and Discoid Lupus Erythematosus, all of which conditions seem to overlap with Rheumatoid Arthritis in the spectrum of "autoimmune" diseases having in many cases hypergammaglobulinaemia and circulating Rheumatoid Factors. Of four long-term survivors of severe Graft-Versus-Host disease followed at the NIH (Lawley, 1977) all have been found to have clinical, laboratory and histopathologic evidence of Sjögren's syndrome. However, the direct translation of this to Rheumatoid Arthritis is not possible as the disease states of the patients reported by Kadowaki and Lawley and Gratwhol are far more severe than that usually seen in Rheumatoid Arthritis though one could speculate what might have arisen from smaller numbers of lymphocytes that were clonally restricted in their activity.

THE MATERNAL INFLUENCE UPON THE IMMUNE RESPONSES OF THE CHILD

In man, the changes induced in the physiological responses of the immune system of the offspring by "maternal factors" are more difficult to obtain adequate control for the test population than in experimental animals and the results are much more difficult to interpret since it can never be determined whether external environmental factors interacted and by which mechanism the "maternal effect" was achieved. An example of good data but with difficulties in interpretation is the report by Aase (1972) in which he followed up twelve Eskimo children after intrauterine exposure to a Mumps virus epidemic. None of the children investigated had Mumps antibodies but 10 out of the 12 had positive skin tests using Mumps viral antigens whereas in a control population of children born after the epidemic only six out of 32 had positive skin tests.

The difficulties of interpretation start with the question of whether this pronounced effect is due to direct exposure of the foetus to the antigen or to informational factors including maternal cells crossing the placenta. It can be postulated that if the infant had been infected it would have developed antibodies (of the IgM class) though there is no proof that such antibodies would persist for ten years. If it is accepted that cellular immunity has crossed into the child, there is no evidence to test whether this was due to a humoral factor or transmission of cells, though Lawrence's Transfer Factor (Lawrence, 1971) is probably unable to prime virgin lymphocytes but reawakes dormant responses. If it is accepted that cells have crossed into the offspring there is no evidence whether this was accomplished in the breast milk or during gestation. Aase interpreted his own data to suggest that it

was the Mumps virus itself in small amounts in early pregnancy that produced the effect and consequently it would be very suspect to adduce this evidence to support the hypothesis of transmission of maternal "T" cells across the placenta.

In another investigation of virus infection in pregnancy Ruben (1975) found raised IgM antibody titres to Influenza A virus in the serum of the offspring of mothers with serological evidence of recent infection in an epidemic of Influenza A/England/42/72. In seven cases in which cord blood lymphocytes were cultured *in vitro* with Influenza viral antigens low-grade proliferative responses were observed in three out of the seven but these were all in children who did not have demonstrable IgM antibodies whereas all of the other children whose lymphocytes did not respond had detectable antibodies. There was one child out of eight in a separate experiment that had such a proliferative response and this was in a child exposed in the first trimester of pregnancy. Again the limitations of the data do not allow one to interpret the data as to the mechanism of this lymphocytes sensitization.

Mohr (1972) reported that using tuberculin (PPD) skin testing he had found that out of 30 mothers five had been skin tested positive and five out of nine of their infants had shown sensitization (all breast fed) whereas none out of 78 tuberculin negative mothers showed evidence of sensitization. Mohr interpreted his own data to suggest that maternal cell-mediated immunity had crossed in the breast milk, but the data does not allow definition of the mechanism of sensitization which could even have been from the bacillus in the post-partum period.

Other work in this field includes that from Field (1971) using his controversial macrophage electrophoretic technique who demonstrated a concordance of sensitization of mothers and children to a battery of antigens compared with the fathers. The mothers included four healthy, three with sarcoidosis, one with Hodgkin's disease and one with Brill-Symmers lymphoma. There is no reason to disregard the data but the interpretation again remains difficult. Similarly others have found a concordance of lymphocyte responsiveness detected by tritiated thymidine incorporation in mother and child pairs to antigens derived from *Mycobacterium Leprae* (Barnetson, 1976) and Dental Plaque (Horton, 1976).

SYNTHESIS

The introduction has been broken up into two components, the review of the characteristics of Rheumatoid Arthritis, and the review of the influence of the maternal milieu on the immune system of the offspring. In the process of presenting the information in this highly compressed form the underlying theme may have been lost in the quicksand of the surrounding data. I will now briefly outline the position of each component with an indication of how firm the data has been from which it sprung.

It is postulated that Rheumatoid Arthritis is an aberrent immune response to a common antigen - 'common' because of the universality of the disease - 'aberrent immune response' because of the very prominent immune mechanisms in operation that seem ineffective at disposing of the primary aetiological agent. It is postulated that the immune response may be the lack of an antibody response in the presence of cell mediated immune responses as this dichotomy has been observed in infants after maternal virus infection in early pregnancy and would lead to ineffective clearing of the antigen in a manner similar to that seen in Chronic Granulomatous disease and Agammaglobulinaemia.

It is postulated that maternal "T" lymphocytes are responsible for the aberrent immune response since they would be able to transfer cell-mediated immunity from mother to child in mice and rats, less certainly in man. It is postulated that the entire immunological changes observed in patients with the disease are related to the ensuing bilateral war between the two lymphocyte populations, almost certainly a "T" cell war less certainly a maternal-host war. A definable aetiological agent such as a virus or

bacterium may provoke this situation and if partially protected by antigenically resembling components of the hosts tissues such as the synovium may cause the subsequent immunological war to localise in these tissues. There is little doubt that the proponderant cell in the RA synovium is the "T" lymphocyte, but there is little or no evidence for or against an allogeneic or autologous lymphocyte war in that site.

The only evidence that allows the possibility of some of these mechanisms to be put forward comes by analogy with the various 'autoimmune' phenomena seen in graft-versus-host situations in experimental animals in which the defined aetiological agent is the presence of two incompatible semi-allogeneic lymphocyte populations. The evidence from rodents seems clear at this time that there can be interference with the immune system of the offspring by suitable manipulations of the mother; but the evidence in humans is anything but clearcut. There is no formal proof that even the effects seen in rodents are due to the transplacental passage of lymphocytes.

Putting together the data concerning the pathological changes in the joint allows a correlation of the lymphocyte infiltration with the increased metabolism with the degree of inflammation with the metabolic upset observed with the gross morphological alterations including synovial villus and pannus formation. The only single entity that could theoretically cater for these well-founded correlations has to be the metabolic blockade of inflammation first hinted at in an antithetical fashion nearly twenty years ago by Marion Ropes.

OBJECTIVES

The experimental work to be described falls into two completely separate categories: experimental animal (rat) to test the specificity of the maternal effect and to try to identify maternal cells in the offspring, and human investigation utilising the nuclear families of patients with Juvenile Rheumatoid Arthritis to test whether there are identifiable immunogenetic reasons that would favour a maternally mediated pathology.

INTRODUCTION

The most direct and satisfactory experiment to prove that Rheumatoid Arthritis is caused by maternal lymphocytes would be to identify them chromosomally in the affected tissues of the disease, to isolate such lymphocytes using histocompatibility markers, to use such lymphocytes to demonstrate some of the mechanisms postulated *in vitro*, to challenge unaffected synovia in the patient with such cells to demonstrate pathogenesis *in vivo*, and to effect a cure by the administration of antibodies to maternal cells. In order to accomplish this there is a requirement for many fresh synovia from patients either males in whom the female karyotype of the maternal lymphocyte could be recognised simply, or that have HLA typed mothers so that the maternal lymphocytes could be recognised by the difference in cell surface antigens.

The protocol outlined above was the original plan for the experimental work for this thesis, unfortunately the requirements listed below the protocol could never be fulfilled here. There were three synovectomies in the first eighteen months of this research and in every case the mother

was either dead or the patient thought she was too old to be troubled. Only one synovium was obtained from a male and in this case it had been removed as part of a joint replacement and the tissues were extremely fibrotic. PHA culture of lymphocytes from this synovium produced very few metaphase spreads and after staining with quinacrine to identify the X and Y chromosomes by a banding technique (Caspersson, 1969) there were only four spreads in which any kind of karyotype could be achieved and of which there was only one in which there was any suspicion of the XX configuration and this would not bear defence.

In the meantime, synovial fluid from male patients was obtained with the co-operation of Dr. John Martin and the lymphocytes recovered from that and from autologous blood. Griffiths (1974) had reported that synovial fluid lymphocytes respond in mixed lymphocyte culture with peripheral blood lymphocytes unilaterally. There seemed to be a chance that the reaction she was observing was that of the maternal lymphocytes responding to host antigens and I thought that karyotyping such responders might demonstrate the maternal source in male patients. However, the response observed from nine fluids (six were male) proved to be very small compared with PHA culture (stimulation index of 1 to 1.5 compared with 2 to 10 for synovial fluid PHA and 10 to 100 for peripheral blood PHA culture of lymphocytes with ^{14}C-Thymidine incorporation) and in spite of careful screening only two poor metaphase spreads were obtained from such cultures both probably XY karyotype.

If the locale did not seem to favour a direct assault upon the problem it seemed to have the requirements for an indirect approach as Newfoundland

is blessed with a stable population isolated in numerous outports with large nuclear families which have allowed other investigators to obtain genetic information unobtainable elsewhere (Larsen, 1977 for example), and the university had extensive animal quarters, newly completed, capable of sustaining rat investigations to follow those of Beer and Billingham.

I knew from the effort to collect synovia that the parents of patients seen in the hospital with Rheumatoid Arthritis were difficult to find and so I decided to concentrate my efforts to the collection of the nuclear families of patients with Juvenile Rheumatoid Arthritis. This had the advantage of the ease of obtaining relatively young parents and I was able to find a group of patients that had had severe disease that had largely "burnt out" so that they were now on minimal drugs and had adolescent and adult siblings capable of volunteering blood. With these families I planned to find out by HLA typing and mixed lymphocyte culture (and latterly cell-mediated cytolysis) whether there was any special relationship between mother and proband, and using HLA matched siblings whether there was sensitization to loci outside the major histocompatibility supergene in the patients as might be predicted from the models discussed earlier.

In the rat work I decided to determine if antigens other than those carried by the placenta could stimulate "T" lymphocytes to cross into the foetus as Stastny (1965) had demonstrated using Tuberculin hypersensitivity as an index. I would then try to identify such lymphocytes by karyotyping in the sites of inflammation in the offspring. The potent skin sensitizer 1-Chloro-2.4.dinitrobenzene (DNCB) was used as this would

not produce the kinds of difficulty of karyotypic identification to be expected if one used skin (or lymphocytes) from third party animals as the source of antigen in the same manner as Stastny (1965). The popliteal lymph node of the rat seemed to be an obvious site to look for maternal lymphocytes during a local response to DNCB challenge to the hind paw, as the antigen could be expected to be concentrated at that site and the maternal "T" lymphocytes subsequently recruited from the circulating pool through the walls of the post-capillary venules of the node. The metaphase spreads could be obtained directly from the nodes by the intravenous administration of colchicine shortly before killing the animal and extracting the lymph node and subsequently teasing out the lymphocytes. The initial experiments were designed to quantitate the differences between primary and secondary responses to DNCB, and the subsequent experiments were designed to detect differences between the lymph node responses of the offspring of primed and unprimed mothers. The final phase of karyotypic classification of the lymphocytes was never implemented for reasons that will be obvious when the data is examined, but some preliminary work using the *in vivo* method for obtaining metaphase spreads was started.

The next two chapters document the data obtained from firstly the rat work and subsequently the human work. As the two sets of experiments were entirely different they will be produced with separate Introductions, Materials, Methods, and Results sections.

RAT WORK

INTRODUCTION *In Vivo* measurement of delayed hypersensitivity in man has a long history starting with the early use of tuberculin skin testing for hypersensitivity to tuberculosis pioneered by Robert Koch, to patch testing for hypersensitivity to contact irritants such as nickel. Similar demonstration and quantitation of delayed hypersensitivity of rats has been fraught with difficulty, for example, for many years it was supposed that rats did not develop skin hypersensitivity to tuberculin following priming with the tubercle bacilli (reviewed by Lefford, 1974).

Wessels (1974) first demonstrated dermal tuberculin sensitivity and since that time both intradermal and footpad test have been described (Flax, 1962; Gray, 1961), but since measurements depend upon the area of erythema or of gross swelling of the foot (Willoughby, 1966; Axelrad, 1968; Rowley, 1961) they become difficult or impossible to quantitate with lesser degrees of hypersensitivity. In order to overcome this limitation Lefford (1974) developed a method of *in vivo* labelling of monocytes with radioactive Thymidine and then using the isolated monocytes injected intravenously following antigenic challenge to the pinna of the rats' ears. Dermal hypersensitivity was detected by the local accumulation of radioactivity in the inflamed pinna removed in the biopsy punch, and the assay proved to be accurate and sensitive though complex. Previously Lefford had worked in collaboration with D.B. McGregor and G.B. Mackaness using the radioactivity detected in the draining lymph node that swells in size more quickly in the sensitized animal due to the recruitment of specific (and non-specific) mononuclear cells brought into the paracortical region

by the lymphokines secreted by those lymphocytes.

In the meanwhile, a very reproducible and simple process has been developed for measuring local graft-versus-host reactions by means of the simple weighing of the draining popliteal lymph nodes of challenged hind paws of the rat, which depends for its reliability on the constancy in the rat of the draining lymphatics that drain to a single node in the popliteal fossa from an area that includes the whole of the footpad as well as the dorsum of the foot and a variable amount of the lower leg. Levine (1968) first documented the great enlargement of the popliteal node in F_1 rats in response to suitable challenge with parental lymphoid cells injected into the footpad. This model system has been successfully employed to gain insight into the types and numbers of cells responsible and responding (Ford, 1970; 1971; Yoshida, 1971a; 1971b; Bonney, 1973).

It was realised from the start of the following experiments that there would not be the massive enlargements of the nodes that are seen in local graft-versus-host reactions, however by inference from the work of Lefford it was expected that the kinetics of the lymph node enlargement would clearly differentiate between primary and secondary responses. The popliteal node assay in rats is efficient as there is usually the single node which drains the entire area of the foot so that, unless there is antigen excess, the node can be expected to carry the major part of the total response of the rat during the early part of that reaction.

MATERIALS

RATS All rats were ultimately obtained from the Canadian Breeding Laboratories, Ontario, and they were subsequently kept in the animal quarters here. Three types of rat were utilised: -Sprague Dawley that were outbred and maintained in that condition by avoidance of inbreeding - Fischer strain that were a well characterised totally inbred stock - Hooded (Agouti) Long Evans that are an inbred strain. Colonies of all three kinds of rat had been well established here though the Fischer strain rats became infected with conjunctivitis with upper respiratory tract involvement during the course of these experiments and the entire breeding stock was replaced.

CAGES The rats were housed in wood-chip bedded polyethylene cages with four adults or one litter to a cage. They were fed with Purina Rat Chow and water *ad libitum*. After antigenic challenge to the footpads the rats were placed in multiple single wire-bottomed cages alone or in pairs in order to restrict their activity and interactions that might cause variation in the popliteal node size.

INSTRUMENTS A standard dissecting kit was used to obtain the nodes though it was found that with a scalpel blade (#11), sharp scissors for the skin, and two pairs of fine forceps the rat could quickly be dissected. The rats were anaesthetized with ether in a wire mesh lined glass desiccator for all procedures and killed by cervical dislocation. The nodes were weighed, using a H18 Mettler (Zurich) balance, to the nearest 0.1 mg, the spleens using a DLT5 (The Torsion Balance Co., Montreal) balance to the nearest 25 mg, and the rats were weighed with a Dialogram (Ohaus Corp.) to the nearest gram. Antigen challenge was administered using a Hamilton

dispenser (Hamilton Co. Inc., California) with a standard disposable plastic 1 ml syringe to give 20 µl aliquots through a 26 gauge needle.

ANTIGEN 1-Chloro-2:4-dinitrobenzene, Organic Analytic Standard (BDH Chemicals Ltd., England). For antigen priming 50 gram/100 ml of acetone was made up freshly; for antigen challenge 1 gram/100 ml of solution consisting of 90 ml of acetone and 10 ml of Dimethyl Sulphoxide was made up freshly.

METHODS

Some care was taken with antigenic priming of the mothers as it had been found in the earlier experiments that if they had fur still contaminated by the priming dose of DNCB the offspring showed evidence of sensitization which possibly could be attributed to this cause. The final method was to depilate an area approximately 2 cm x 2 cm in the interscapular area of the upper thoracic spine and carefully dispense 60 µl of the solution over this area so that it dried in a 1 cm^2 in the centre. The rats were immediately wrapped with gauze and elastoplast bandages around the thorax, making sure that the rat could still breathe. After one week the bandages were generally in poor condition and could be easily removed without anaesthesia if they had not been removed already by the rat. By the time the bandages had come off the area (if primed with DNCB) showed marked changes with local erythema around a central area of yellow stained patently unhealthy liquified skin that dried to a hard scab and which then dropped off within a few days to leave a raw area of granulation tissue that healed quickly but thereafter had few

hairs upon it.

Some care was also taken with the antigen challenge as variations in the dose administered had caused unnecessary variation in the lymph node size in earlier experiments. The final method devised was to carefully dispense three 20 µl aliquots on the dorsum of the foot, blowing dry after each and then wiping the whole foot with gauze soaked in the diluent to remove surface antigen to prevent the rat from ingesting it by licking. Dimethyl Sulphoxide was included in the diluent in order to carry the DNCB through to the dermis though it is questionable whether it was strictly necessary. Both hind paws were used with the left paw always receiving the antigen and the right paw always receiving the diluent only as this prevented confusion at the time of harvest (though the challenged foot had a distinctly yellow hew). All these procedures unless explicitly stated were accomplished on fully anaesthetised animals.

A week prior to challenge the rats were placed in the small wire-bottomed cages in order that the lymph node variation due to the new environment would be minimised, and they were replaced in these cages after challenge and subsequently randomised into the four different days of harvest and ideally with members of each litter represented on every day and their group labels replaced with a harvest number only, so that the measurements could be made blind. After killing, the rats were weighed to the nearest gram; they were completely dissected and the popliteal lymph nodes were found, removed and freed of fat and weighed to the nearest 100 µ gram within 15 seconds of removal to prevent the effect

of drying. The spleens were also found, removed and freed of fat and weighed to the nearest 25 milligrams. The data was entered upon a card and only decoded at the conclusion of the days weighing. The data was entered by hand into a Wang 600 programmable calculator for regrouping and tabulating the results. The results were also entered by hand into a Hewlett Packard 9815A programmable calculator for graphical presentations using the 9883A printer/plotter and small programs developed by myself, and, in Experiment II, for a Three Way Analysis of Variance using the Statistics, Volume 2 package tape cassette supplied by the manufacturer. In order to adequately analyse the complex interactions of Experiment III the ANOVA program from the Statistical Package For the Social Sciences was used for the Analyses of Variance (Nie, 1975) using an IBM 370 computer; Optional changes for the format for these analyses included exclusion of interactions after early analyses revealed no significant interactions (Option 3), and a multiple regression approach to analyse the significance of the covariate (rat weight) simultaneously (Option 9).

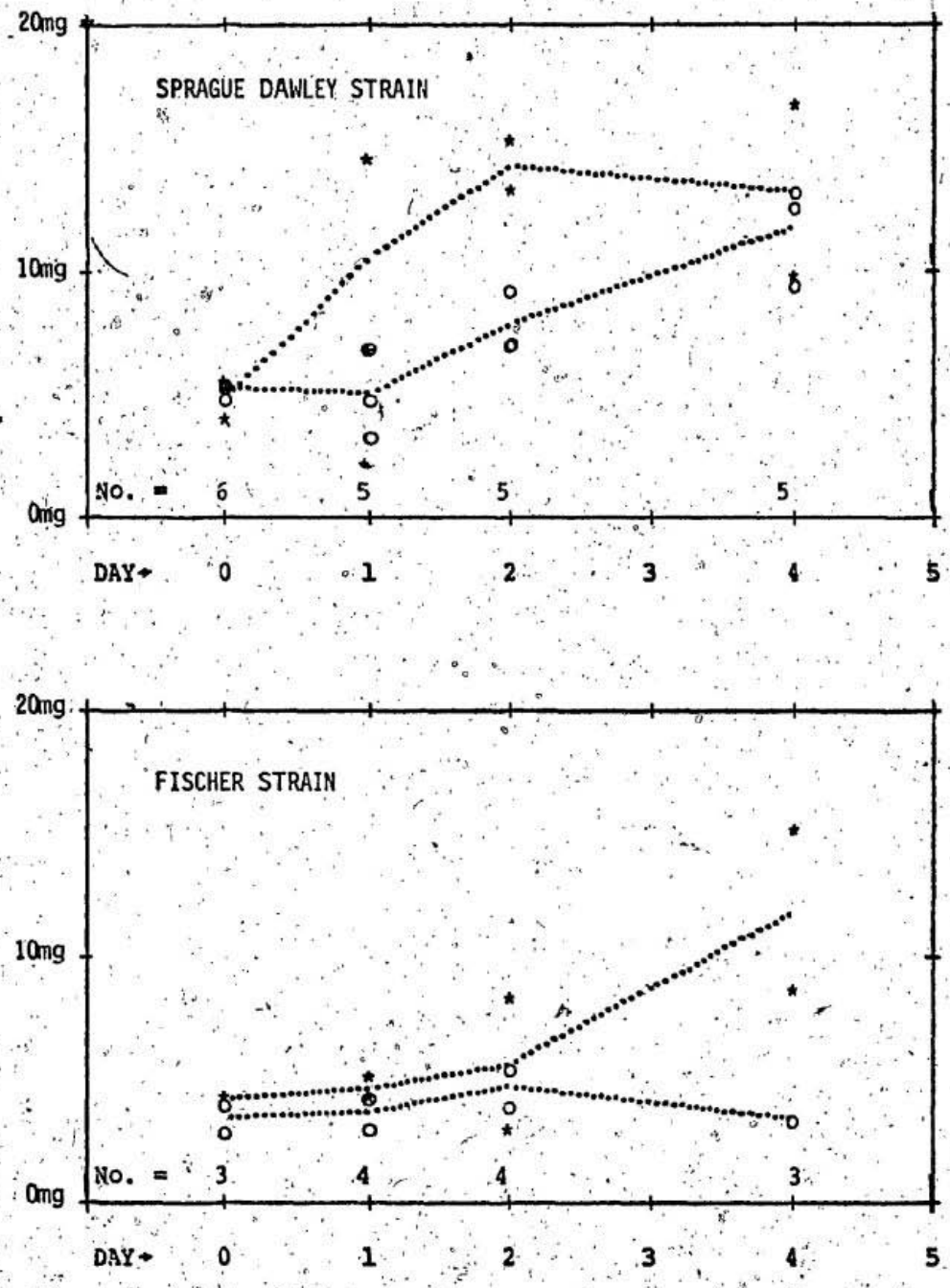
EXPERIMENT I. Female rats aged 90 days, 12 Sprague Dawley and 16 Fischer, were paired off and primed by the technique described before with one of each pair receiving the DNCB and the other receiving the diluent only and both groups earpunched for ready identification. After 10 days they were mated with the reciprocal male, that is Sprague Dawley with Fischer and *vice versa*, with three or four females to a male (that had already sired litters in earlier experiments), and with the control and primed females deliberately mixed together so that the conditions of

breeding should be identical. Only three out of the females failed to bear a litter after being with the male for one week (see Appendix B). When these females were approximately 180 days old they were challenged with DNCB to the Left paw and the rats were killed and dissected for the Popliteal nodes and Spleens after 0, 1, 2, and 4 days (though the zero day animals were unchallenged and harvested between those of day 2 and 4). For convenience the females were divided into two groups, mated one week apart so that the same males were used.

EXPERIMENT II. 12 female Hooded Long Evans strain rats aged 180 days and 12 F_1 hybrids (Hooded Long Evans x Fischer), the mothers of which were littermates of the first group of Hooded rats, aged 70 days were paired off within the groups and primed with DNCB or diluent. After the bandages had been removed at one week they were left undisturbed for 90 days and then all were challenged on the Left foot with DNCB and killed and dissected on day 0 and 3 (actually at 66 hours) only.

EXPERIMENT III. The offspring of Experiment I. were suckled for twenty-five days in individual litter boxes to prevent the groups mixing and were then sexed before being placed in litter boxes of their siblings of the same sex, (to prevent any possibility of accidental pregnancy), for three weeks. They were then transferred to wire-bottomed individual cages and left for one week to acclimatise. They were challenged with DNCB to the Left paws when aged 51-56 days and randomly allotted into the groups for various days (0, 1, 2, and 4) making sure that all the parallel day groups received members from each appropriate litter/sex group. By this means there were created 32 groups of genetically similar rats (the products of Sprague x Fischer matings differentiated by the 4 days of

FIGURE 5 Experiment I Graphs Showing Expansion of Popliteal Lymph Node Weights in Response to Antigen (DNCB)



Graphs showing the popliteal node weights of female 180 day old rats after foot pad challenge with DNCB. The individual weights are shown adjusted for rat weight. o....o are control rats with the dotted line showing the trend of the arithmetic means, *.....* those primed with DNCB aet. 90 days

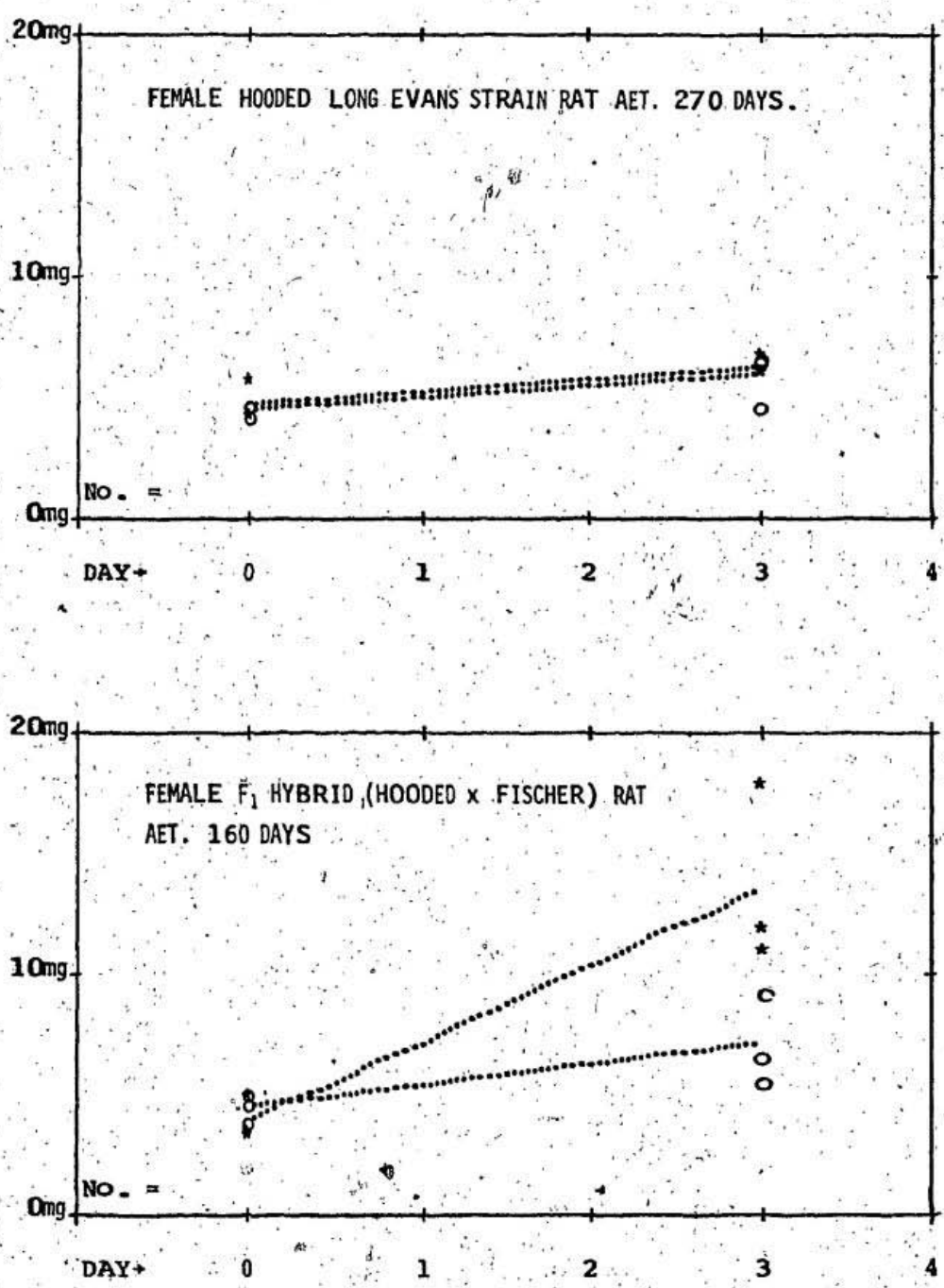
harvest, by sex, by the strain of the mother, and finally by whether the mother had received priming with DNCB (the Primed or Test group) or had just received the diluent (the Control group). All groups of rats had 7 rats per group to assist the Analyses of Variance though the Sprague Dawley mothers were fewer in number and so had too few offspring to completely fill all of the groups so that some had only 6 rats. The rats were all housed together in one room and a careful check was made every day for signs of illhealth. Nevertheless a few offspring disappeared presumably eaten by their mother, and the surplus offspring were destroyed at the conclusion of the experiment.

RESULTS

EXPERIMENT I. Figure 5 shows the graphical representation of the time course of the Left Popliteal lymph node weight in mg indexed to the overall rat weight using the formula Lymph node weight (mg) $\times \frac{200}{\text{rat weight (gm)}}$ after DNCB challenge on Day 0.

Although relatively few animals were used, the clear separation of the node weights from primed and unprimed animals particularly on Day 2 is obvious (and also statistically distinguishable with a Student t value of 5.234 with four degrees of freedom, $p < 0.005$) with the Sprague Dawley rats only. The Fischer rats seem to have a completely different pattern of node enlargement in the primed animals with a very late rise in the overall node weight on Day 4 only. This result is in accord with the recent observation that Fischer rats have defective monocyte function since much of the node weight gain is due to the ingress of these cells.

FIGURE 6 Experiment II Graphs Showing the Effect of Age on Lymph Node Response to Antigen



Graphs showing the weight indexed popliteal node weights of female rats after challenge with DNCB on footpad. o.....o are control rats, *.....* are rats primed 90 days earlier with DNCB.

The main purpose of this experiment was to indicate the kind of pattern that might be seen in the offspring of the primed rats in the later experiment (III).

EXPERIMENT II. Figure 6 shows the weight indexed Popliteal Lymph node weights in mg using the formula used for the previous experiment after DNCB challenge on Day 0.

The top graph illustrates the very flat response in the draining lymph node in these elderly Hooded female rats and shows that there was no evidence that priming causes a "second set" or early response to the challenge with DNCB. The bottom graph illustrates the comparatively brisk response in the younger Hybrid rats and the indications that priming has induced a long term memory (for three months at least) with early weight gain of the lymph node upon restimulation.

Tables 2 and 3 represent the Three Way Analyses of Variance of the unconverted lymph node weights in mg with the Factor 1 being Time of Harvest (Day 0 or 3), Factor 2 being Group of animal (whether control or Primed), and Factor 3 being the Side of the popliteal lymph node (Left = Test, Right = Control side that had received only diluent). In the case of the elderly Hooded females, the only factor that seems to be significant ($p < 0.01$) is whether the node was draining the challenged (Left) side, and this also interacts with the Time ($p < 0.05$), but there are no effects from whether the animal had been primed in the past. In the case of the Young Hybrids all the factors are significant

EXPERIMENT II

MAIN EFFECT MEANS

Day	74.1667	83.0000	= Day 0 and Day 1
Group	78.4167	78.7500	= Control and Test
Side of Node	87.0000	70.1667	= L Node (Test) and R Node (Control)

TWO WAY MEANS

1 x 2 means	75.5000	72.8333	81.3333	84.6667
1 x 3 means	76.1667	72.1667	97.8333	68.1667
2 x 3 means	82.5000	74.3333	91.5000	66.0000

OVERALL MEAN 78.5833

ANALYSIS OF VARIANCE TABLE

SOURCE	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUE
Total		154994.0000		
Mean		148208.1667		
Total Adjusted		6785.8333		
Factor 1 = Day	1	468.1667	468.1667	2.4231 p=0.1391
Factor 2 = Group	1	0.6667	0.6667	0.0035 p>0.2
Factor 3 = Side	1	1700.1667	1700.1667	8.7997 p=0.0091
1 x 2 Interaction	1	54.0000	54.0000	0.2795 p>0.2
1 x 3 Interaction	1	988.1667	988.1667	5.1145 p=0.038
2 x 3 Interaction	1	450.6667	450.6667	2.3325 p=0.1462
1x2x3 Interaction	1	32.6667	32.6667	0.1691 p>0.2
Error	16	3091.3333	193.2083	

TABLE 2

Hooded Long-Evans Female Rats AET. 270 Days, Popliteal Lymph Node Weights Mg/10

THREE WAY ANALYSIS OF VARIANCE

EXPERIMENT II

MAIN EFFECT MEANS

Day	54.4167	105.2500	= Day 0 and Day 1
Group	68.7500	90.9167	= Control or Test
Side of Node	96.5833	63.0833	= L Node (Test) and R Node (Control)

TWO WAY MEANS

1 x 2 means	58.8333	50.0000	78.6667	131.8333
1 x 3 means	55.8333	53.0000	137.3333	73.1667
2 x 3 means	75.3333	62.1667	117.8333	64.0000

OVERALL MEAN 79.8333

ANALYSIS OF VARIANCE TABLE

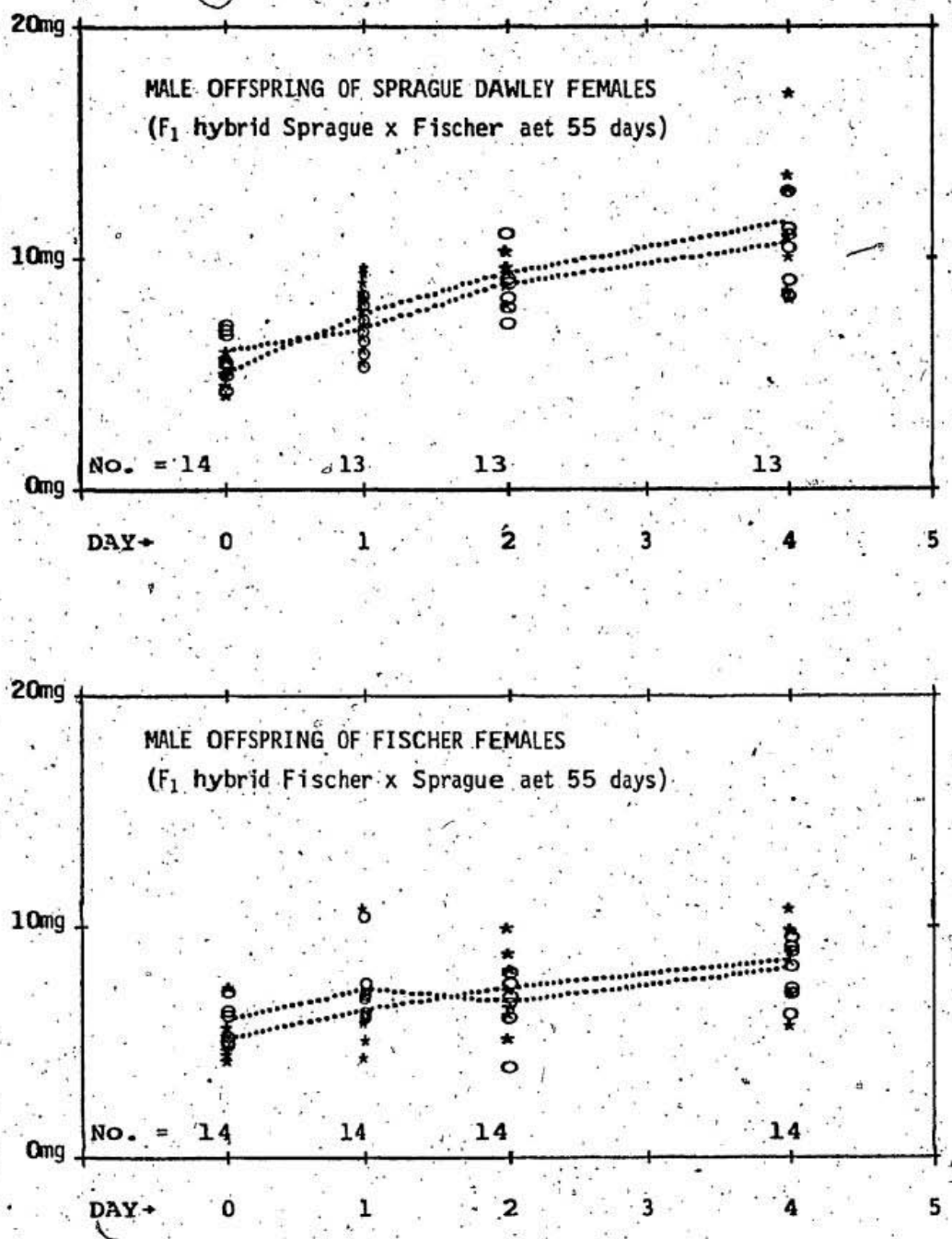
	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F VALUE
Total		202426.0000		
Mean		152960.6667		
Total Adjusted		49464.3333		
Factor 1 = Day	1	15504.1667	15504.1667	29.2462 p<0.0001
Factor 2 = Group	1	2948.1667	2948.1667	5.5613 p=0.0314
Factor 3 = Side	1	6733.5000	6733.5000	12.7017 p=0.0026
1 x 2 Interaction	1	5766.0000	5766.000	10.8767 p=0.0045
1 x 3 Interaction	1	5642.6667	5642.6667	10.6440 p=0.0049
2 x 3 Interaction	1	2480.6667	2480.6667	4.6794 p=0.046
1x2x3 Interaction	1	1908.1667	1908.1667	3.5995 p=0.076
Error	16	8482.0000	530.1250	

TABLE 3

F_1 Hooded Long Evans x Fischer Female Rats AET. 160 Days, Popliteal Lymph Node Weights Mg/10

THREE WAY ANALYSIS OF VARIANCE

FIGURE 7 Experiment III Graphs of Lymph Node Response in Male Progeny



Graphs showing the weight indexed popliteal nodes after foot pad challenge with DNCB. o.....o are those of offspring of control mothers, *.....* from mothers primed with DNCB 10 days prior to mating.

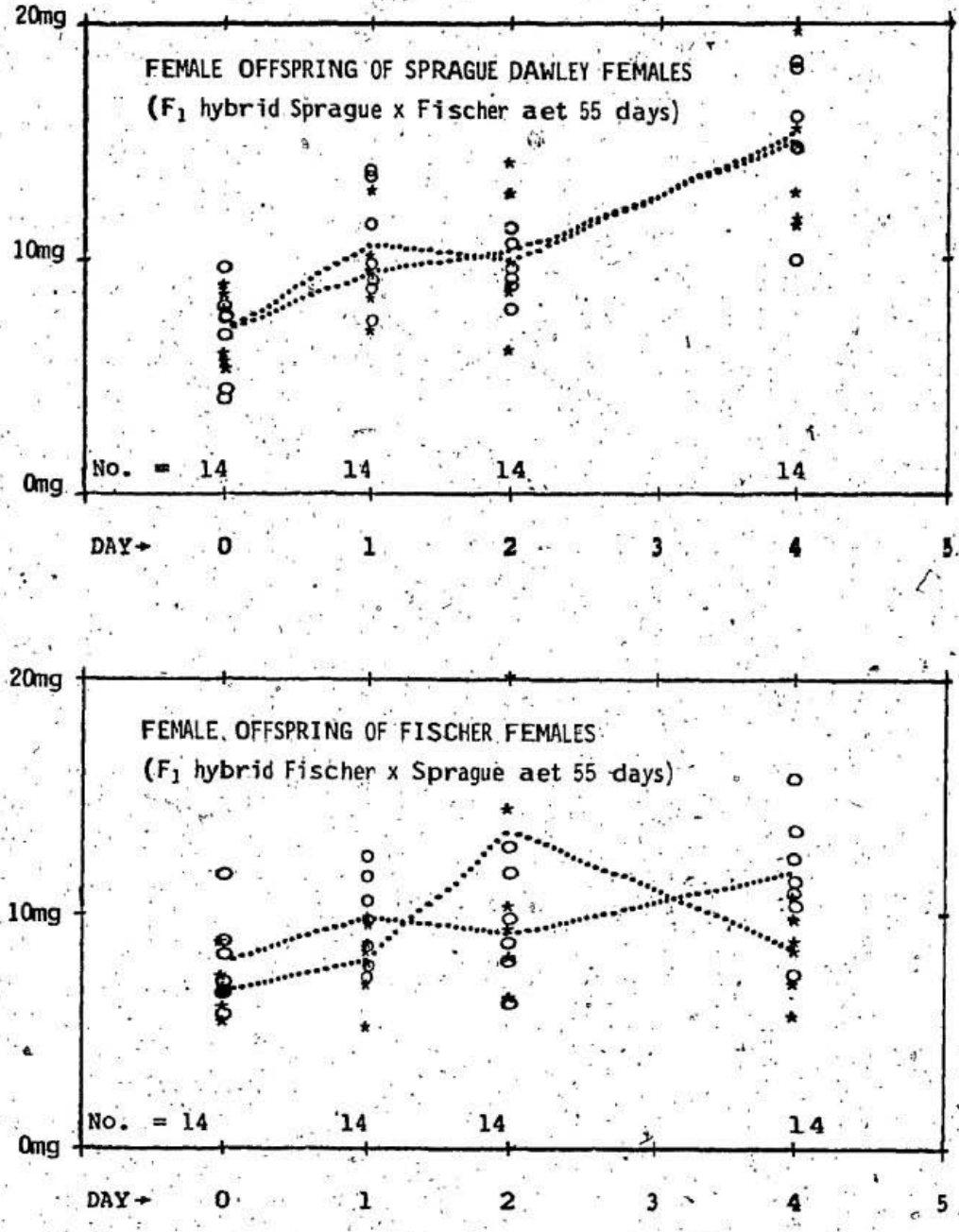
($p < 0.0001$, $p < 0.05$, and $p < 0.005$ respectively) and it should be noted that there is a very highly significant interaction between the Time factor of the lymph node enlargement and both the Group ($p < 0.005$) and the Side ($p < 0.005$).

The crude data from this experiment can be found in Appendix B and illustrates that there is an appreciable increase in the contralateral node (Right) even though it has received no direct stimulation from the DNCB in the Hybrid animals.

EXPERIMENT III. Figures 7 and 8 illustrate the Left or Test Popliteal lymph node enlargement in response to DNCB indexed to weight according to the formula used in Experiment I. in the male (Fig. 11) and the female (Fig. 12) offspring of Sprague (Top Graphs) or Fischer (Bottom Graphs) mothers. It can be seen at a glance that there were no differences between the offspring born to primed mothers compared with unprimed mothers.

This observation has been born out by the Analysis of Variance of the data (see Appendix C) in which the Right and Left lymph node weights can be seen to have been under the influence of the Day of harvest, and the Sex of the offspring and the Strain of the mother ($F = 14.284$; $p < 0.001$) but not by the Group which was whether there had been maternal priming. However, in performing further analyses it became obvious that the spleen weight though only comparatively crudely weighed in order to be able to spot obvious immunological runts showed highly significant changes mainly in the offspring of Fischer females ($p < 0.001$) though

FIGURE 8 Experiment III, Graphs of Lymph Node Response in Female Progeny of Test and Control Mothers



Graphs showing the weight indexed popliteal node weights after challenge with DNCB. o.....o are those offspring of control mothers, *.....* from mothers primed with DNCB 10 days prior to mating.
(N.B. outlier in Fischer female offspring harvested on day 2)

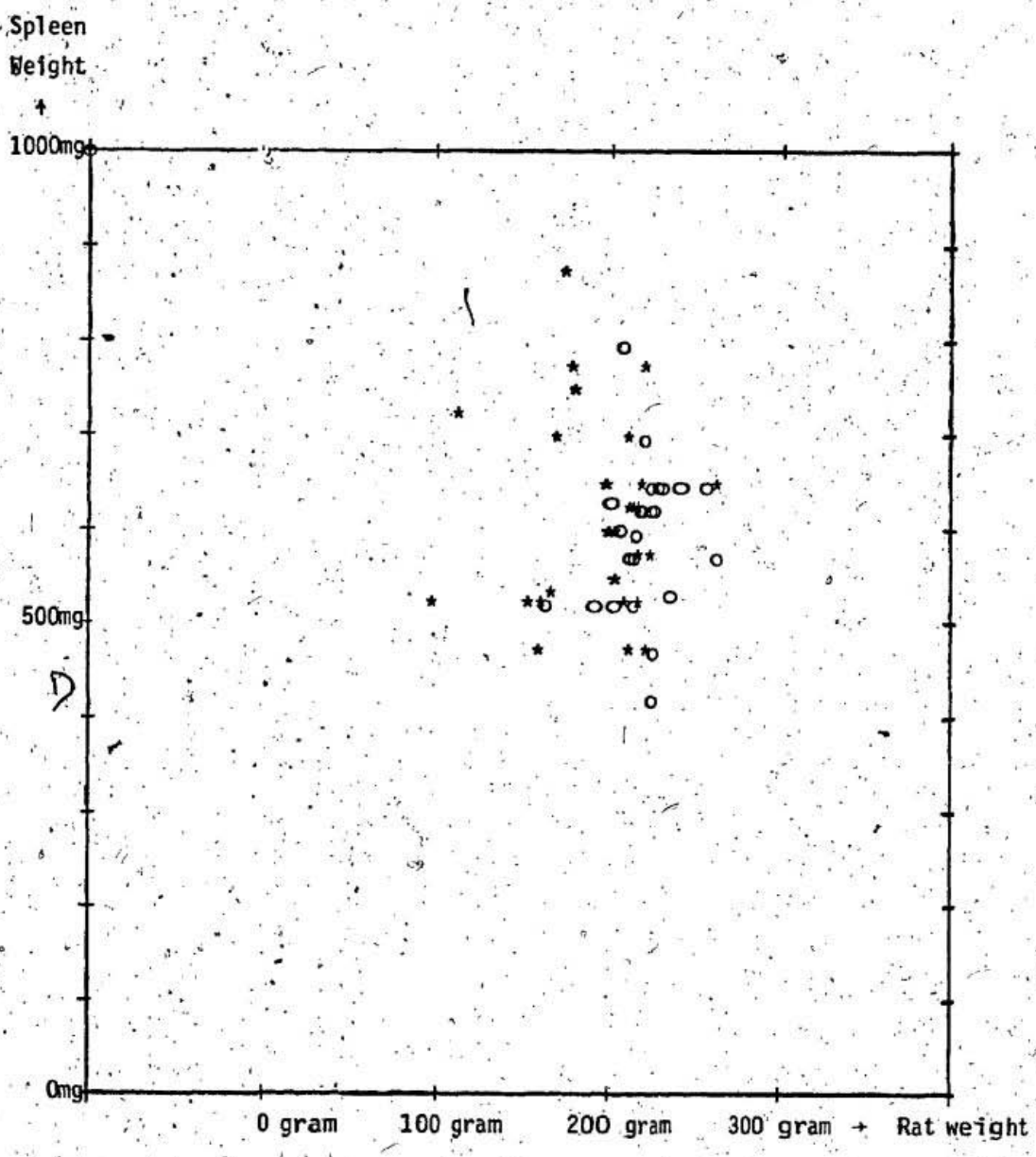
some effect was seen in the offspring of Sprague females ($F = 3.779$, $p = 0.055$) that only just does not reach significance. (See Appendix C).

This would have been easier to interpret had the two observations been in the same sense, but in fact the offspring of Fischer mothers had smaller spleens in the primed (mother) group whereas the offspring of the Sprague mothers had larger spleens in the primed (mother) group.

In order that the immunological runts defined by their (lack of) body weight and (large) spleen weights could be easily spotted these two parameters were used to create scattergrams of the individual rats in Figures 9 to 12. These show where the differences between the control and primed groups have occurred but have not really helped in the delineation of the runts though the males have a much greater scatter which would be expected if a certain number of them were runted. The nutritional runts would be expected to have both low body and spleen weights (lower left quadrant) and the immunological runts would be expected to have low body but high spleen weights (upper left quadrants). Two examples of the latter can be seen in Figure 14, clearly separated from the others, and with the exception of Figure 16. (female offspring of Fischer mothers) immunological runts appear commoner in the offspring of DNCB primed mothers.

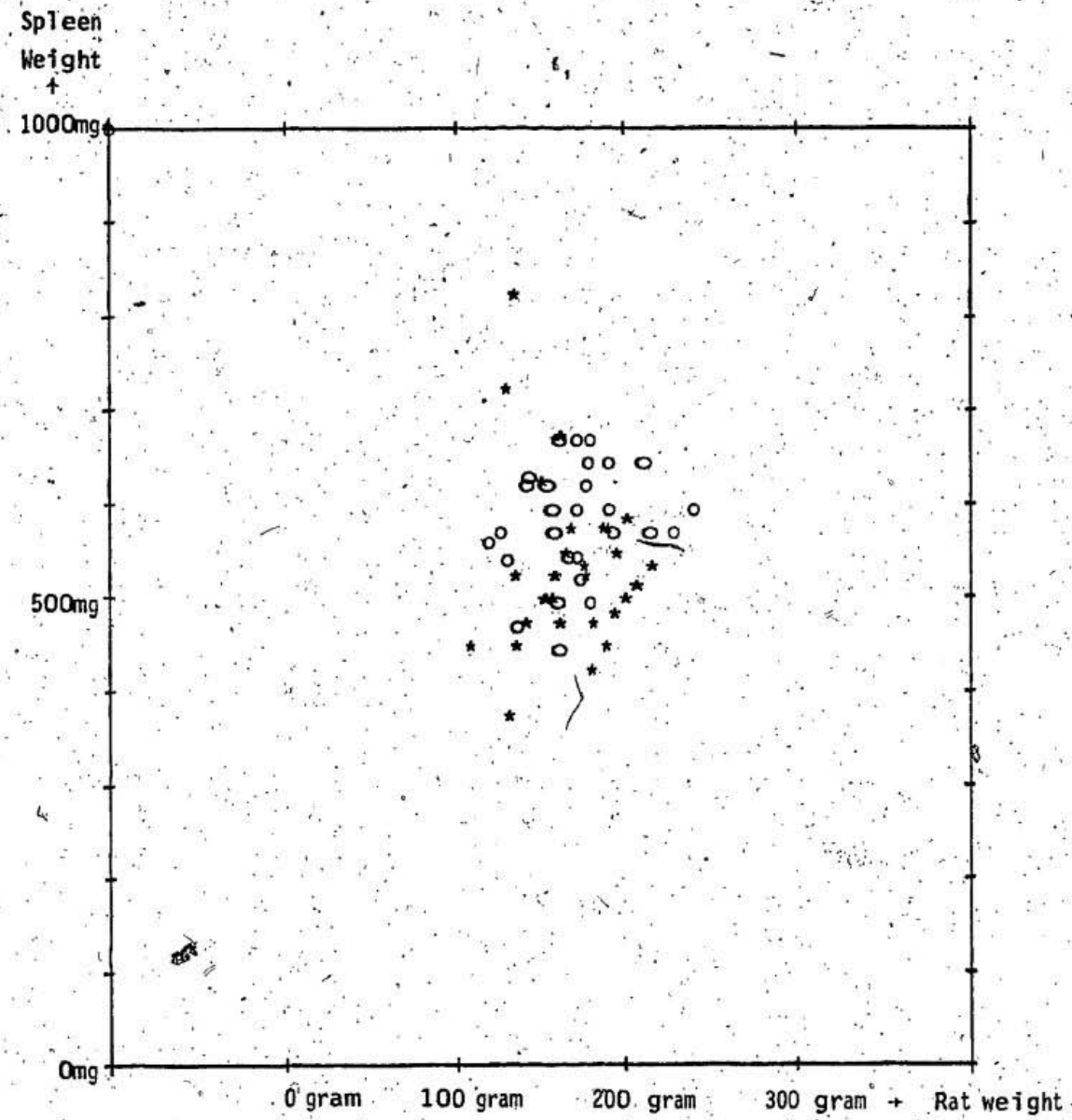
FURTHER EXPERIMENTS A series of other experiments were carried out using syngeneic matings, BCG as the antigen, and attempts to prime the mothers closer to the time of mating. These experiments (five in all) although all carried through to the final analysis were technical failures

FIGURE 9. Experiment III Scattergram of Rat Weights and Spleen Weights



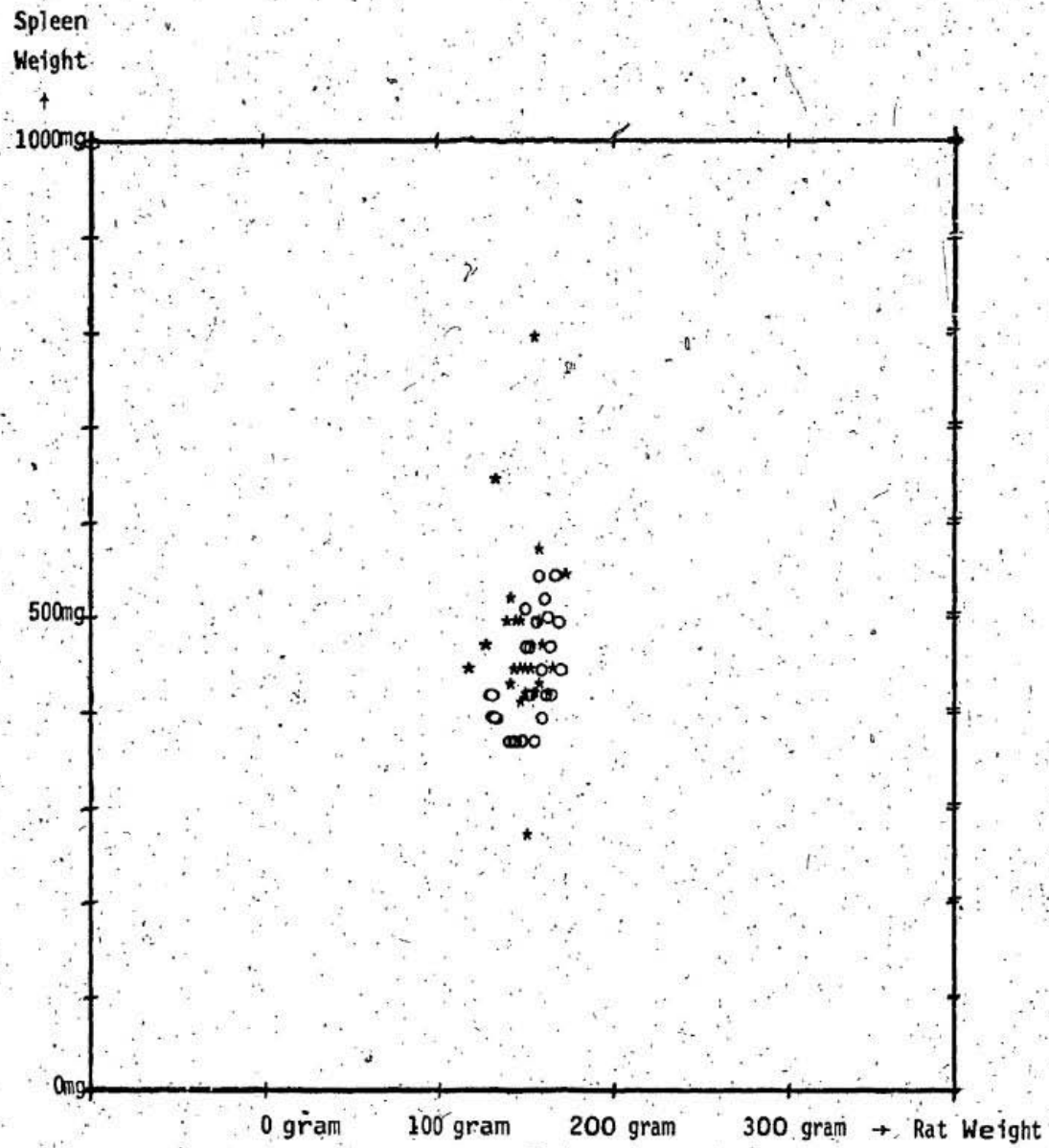
Scattergram of Rat weight v Spleen weight in the male offspring aet 55 days of control (o) and primed (*) Sprague female rats (mated x Fischer).

FIGURE 10 Experiment III Scattergram of Rat and Spleen Weights



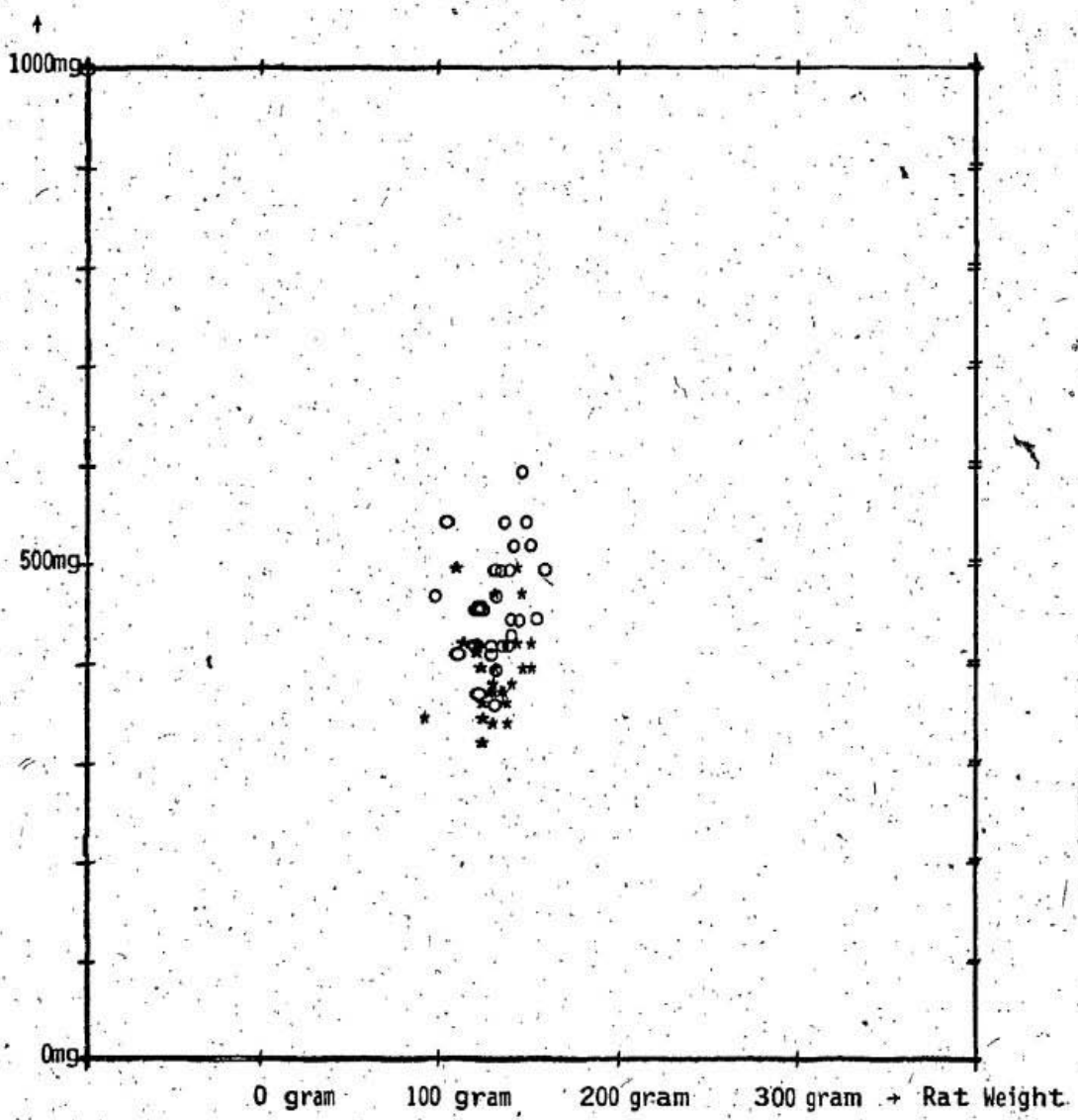
Scattergram of Rat weights v Spleen weights in the male offspring aet 55 days of control (o) and primed (*) Fischer female rats (mated x Sprague)

FIGURE 11 Experiment III Scattergram of Rat and Spleen Weights



Scattergram of Rat weight v Spleen weight in the female offspring aet 55 days of control (o) and primed (*) Sprague female rats (mated x Fischer)

FIGURE 12 Experiment III Scattergram of Rat and Spleen Weights



Scattergram of Rat weight v Spleen weight in the female offspring aet. 55 days of control (o) and prime (*) Fischer female rats (mated x Sprague)

mainly due to breeding problems that limited the numbers of offspring of the same age in the various experimental and control groups. This effect was due to the discouraging effect of bandages upon mating in two experiments as the only rats to become pregnant according to schedule had accidentally lost these. Another significant factor that is well recognised by rodent breeders is the effect of anaesthesia upon the oestrus cycle and this may well have delayed conception in some of the rats primed near the time of mating. However, in no case was there any suggestion of the transmission of specific hypersensitivity as detected by the popliteal lymph node assay.

DISCUSSION

The results did not match the *a priori* expectations at all. There was no appreciable excess Popliteal lymph node swelling in the challenged side due to a maternal effect and so it proved unrewarding to try to obtain metaphase spreads of lymphocytes from these sites to check if these contained a subpopulation of female (= maternal) cells in male offspring. However, there were some very significant effects noted in these experiments which are as follows:

1. Fischer rats showed a much reduced and delayed secondary response to DNCB as witnessed by popliteal lymph node swelling, and this seemed to be passed in a matrilineal fashion to the offspring. These rats were poor mothers in the sense that they tended to ignore their off-

spring allowing them to become very dirty and fail to gain weight as fast as genetically identical rats reared by Sprague Dawley mothers so that the effect upon the immune response might be due to malnutrition in early extra-uterine development leading to a permanent deficit in "T" cell responses. In Experiment III. the spleens were slightly smaller in the offspring of Fischer females (500 v 525 mg., $F = 4.272$, $p = 0.04$) which might be used as an indication of nutritional as against immunological runting.

2. The offspring of Fischer (female) x Spraque (male) matings showed a highly significant decrease in the size of their spleens when the mothers had been primed with DNCB 10 days prior to mating. The offspring of Spraque x Fischer matings showed an increase in the size of their spleens after the mothers had been similarly primed though this effect just failed to reach statistical significance.

3. The popliteal lymph node assay seemed to be a reasonable sensitive technique for detecting secondary immune responses to DNCB using a total of 0.6 mg of antigen applied to the skin of the foot. However, this assay was unable to detect any influence from maternal priming with the agent either of hypersensitivity or tolerance.

4. Old female rats seemed to be unable to develop secondary cell-mediated hypersensitivity as detected by the popliteal lymph node assay to the doses of DNCB used. These rats were still able to bear litters so that this might be used to determine whether the spleen effect was due to antigen or immunological mediators in future experiments using old

rats that had been sensitized in youth and those sensitized at the time of challenge.

CONCLUSIONS. Within the limitations of the experiments performed there was ample evidence of very profound maternal effects upon the immune responses of the offspring determining the size of the spleens and the time course of the primary reactions in the lymph nodes. However, there was no evidence of any more specific effect concerned with the antigen employed.

HUMAN INVESTIGATIONS

INTRODUCTION The *in vitro* testing for the recognition and effector phases of human allogeneic immune responses have been extraordinarily advanced by the needs of the transplantation programme in recent years. Bain (1963) was the first to show that the mixture of lymphocytes from different individuals *in vitro* resulted in the production of blastlike cells labelled with ^{3}H-Thymidine in radioautographic smears except when those cells were from monozygotic twins (Bain, 1964).

Bach (1964) suggested that this might be a useful measure of histocompatibility which was best seen in the segregation of stimulation seen in mixtures of lymphocytes from sibling pairs (Bach, 1964) that indicated that genetic, even Mendelian, factors were involved. The dissection of the reactions seen in the Mixed Lymphocyte Culture (MLC) test was rendered easier by the treatment of one cell population with Mitomycin-C (Kasakura, 1965) or X-rays (Bach, 1966) to cause the treated cells to be unable to divide but to be able to live long enough to stimulate the other cell population to produce a one-way reaction. The possibility for doing large numbers of simultaneous cultures from relatively small samples of blood became a reality with the invention of the microculture system (Hartzman, 1971) and the cell harvesting machines (for instance - Hartzman, 1972) that automated the collection of the cells labelled with radioactive Thymidine on glass filter papers for easy counting in a scintillation counter.

The Mixed Lymphocyte Culture test is a measure of the recognition or proliferative phase of the allogeneic response of lymphocytes, but the

effector or cytotoxic phase of that response can now be assessed by the use of Chromium-51 labelled target cells that release their label after the integrity of their cell membranes has been lost during the attack by the reacting effector cells. The Chromium label is released into the surrounding culture fluid and the vigour of the cytotoxicity can be directly assessed by the radioactivity of the supernatant fluid after centrifugation and removal of the intact cells. This has been made easier to accomplish in the last two years by the development of simple multisample harvesters that gather the supernatant through a glass-fibre filter that prevents the intact cells from reaching the collecting swabs which can then be directly counted in the gamma counter (Hirschberg, 1977).

Using this technique the cytotoxic activity of peripheral blood mononuclear cells can be measured as Lymphocyte Mediated Cytolysis or LMC. If antibody is added then it is called a Antibody Dependent Cell-Mediated lympholysis (ADCC or ABCIL) and may include some effect from the monocytes as well as the T lymphocytes. Finally the lymphocytes primed after a six day MLC may be used and probably involve a different population of T lymphocyte (the human equivalent of the T_2 cells of Tigelaar, 1972) and then it is called Cell Mediated Lympholysis (CML).

Investigators have used the Mixed Lymphocyte Culture test using lymphocytes from patients with Rheumatoid Arthritis in order to test for signs of disorder of the general function of the immune system, usually in parallel and to complement cultures with Phytohaemagglutinin (PHA).

The difficulty in interpreting the data from these experiments stems from the relatively large differences in the multitudinous protocols. The main areas that seem to cause variation in the results when compared to a "control" population can be broken down under the following headings:

1. Non-response to fellow Rheumatoid Arthritics though not to pools of normal cells (Astorga, 1969) may be due to increase in the HLA-D locus antigen Dw4 and the increased incidence of homozygosity for this in the patients (Stastny, 1978).

2. Serum factors seem to be a potent cause for variation, Caperton (1974) implicating Foetal Calf Serum as the cause for hyporesponsiveness, while Keystone (1976) demonstrated that autologous RA serum had a similar depressing effect when compared with pooled human serum, which observation had been previously reported by Maclaurin (1971).

3. In the hands of some experimenters (for instance Lloyd, 1977) the lymphocytes from RA peripheral blood had a relatively high background incorporation of tritiated thymidine suggesting that a pre-existing activation that would cause a relatively low stimulation index in MLC when the background is corrected for was present.

4. Jennings (1971) reminded the scientific community that many Rheumatoid patients were generally very debilitated and that this *per se* could cause impairment of the immune response *in vivo* and *in vitro*.

5. There is evidence that Rheumatoid lymphocytes respond *in vitro* to aggregated IgG (especially in the presence of complement, Kinsella, 1974). This means that there may be false positive reactions to allogeneic

stimulation due to IgG-antigen complexes on their surfaces.

6. The majority of Rheumatoid patients are taking large quantities of drugs, many of which are "anti-inflammatory" and thus capable of at least some interference with immune function, the most obvious candidates being the adrenocorticosteroids.

The patients that I chose to investigate were the late-adolescent and early adults who had had the diagnosis of Juvenile Rheumatoid Arthritis made in the Rheumatology Unit at St. Clare's Hospital, St. John's, by Drs John Martin and John Lochead. They were chosen because they were members of large, nearly adult sibships that could give informed consent for the use of their blood for this study. The patients had severe disease (except for one patient) that was in remission and therefore their need for drugs was minimal (though one patient had been unable to be completely weaned from his steroid and another developed pericarditis requiring the use of steroids between two investigations of his family).

Stastny (1974, 1975) had demonstrated that Rheumatoid patients had an increased incidence for the HLA-D allele now called HLA-Dw4 and that there was a very marked increase in homozygosity of this allele (though in Juvenile Rheumatoid Arthritis this allele was not involved). With the assumption that maternal-foetal interactions might underly the disease this observation might seem to be an important pointer to an unusual maternal-offspring immunologic relationship with selective impairment (or enhancement) of the recognition or effector phases of the Mixed Lymphocyte reaction *in utero*; on the other hand the homozygosity

detected might be due to the influence of the disease on the phenotypic performance of the lymphocytes as stimulators.

Accordingly, by taking large sibship groups with the presence of the mother (and where possible the father and an unrelated control) and performing Mixed Lymphocyte Culture experiments testing every individual against every individual (a statistical 'complete block') any genetic predisposition or phenotypic modulation of the patient/mother relationship should be able to be directly compared with HLA identical and non-identical siblings. The influence of preformed antibodies in the sera of all the individuals was to be assessed by setting up identical MLC matrices with autologous, i.e. from the source of the responding lymphocytes, and heterologous, i.e. from the source of the stimulating lymphocytes, sera.

The kinds of 'special' immunological relationship between mother and patient that were to be looked for were as follows:

1. HLA-D homozygosity of the patients might be even more common than Stastny (1975) had found and might predispose to Host-Versus-Graft.
2. Evidence of presensitization of the patient to the mother due to the lymphocyte war.
3. Dominant effects from minor histocompatibility loci that have been implicated in the rat breeding experiments of Joy Palm (1970).
4. Phenotypic modulation of the patient either by clonal elimination or by blocking antibody suppressing or enhancing the reaction to mother.

5. The effector phase (cell killing) of the MLC might be deficient in the patients allowing maternal colonization and could be determined by Chromium release Cell Mediated Lympholysis studies in the absence and presence of autologous serum.

MATERIALS AND METHODS

The blood was taken from the patients by venepuncture into "Vacutainers" (10 ml Sodium Heparin 3200KA, Becton Dickinson, Cat. No. 4716) through 20G multisample needles (Cat. No. 7225). Transport Medium, 3 ml per tube, was added if the blood had to travel further than 100 miles and this consisted of 1% foetal calf serum (GIBCO, Batch C665721, Cat. No. 614) in Medium 199 with 25mmol/l Hepes buffer with antibiotic/antimycotic 10 ml/l added (Penicillin 10K units/ml, Streptomycin 10K mcgm/ml, Fungizone 25 mcgm/ml Cat. No. 524L GIBCO).

The Mixed Lymphocyte Culture Microtechnique used was only slightly modified from the method of Helgesen, Hirschberg and Thorsby (1974). Essentially the blood was diluted one part in one and a half parts of "normal" saline using a 50 ml disposable syringe and with a 16G needle this was carefully layered over 3.3 ml of Ficoll/Hypaque (24 parts of 8% Ficoll mixed with 33 parts of 22.9% Hypaque) in a sterile capped 17 ml screw topped glass culture tube (Kimax). The syringe was rinsed five times with saline betwen specimens and reused though discarded at the end of the experiment. The tubes were centrifuged to give a centrifugal force of 800G at the fluid interface according to the protocol of Boyum (1968) for 40 minutes. The mononuclear cells at the fluid interface were aspirated using a Pasteur pipette and added to another 17 ml sterile capped glass tube and diluted at least 50% with transport medium and centrifuged for 10 mins at 800G. Thereafter the supernatant was poured off carefully and the cells resuspended in the drop remaining and 5 ml of medium was added and centrifuged at 150G and the procedure repeated twice. After the final wash the cells were resuspended in 4 ml

of medium and 2 ml was taken from this and placed in another sterile tube with 0.2 ml of a 1 mg/ml solution of Mitomycin C (Sigma, Cat. No. M-0502) and incubated at 37°C for 30 minutes and then washed as before three times, the last in parallel with the original cells and both sets were resuspended in RPMI-1640 (GIBCO, Cat. No. 240 with Glutamine and 25 mM Hepes buffer), and a differential white cell count made of each using a Model F Coulter Counter with a particle size analyser. In the early experiments the cell suspensions were made up to give 2×10^6 per ml but latterly this was decreased to 1×10^6 per ml. In the experiments in which Cell Mediated Lympholysis was performed 2 ml aliquots of cell suspension were removed from the unblocked cells and incubated with 20% foetal calf serum in 7 ml sterile screw topped culture tubes in a 5% CO_2 humidified atmosphere at 37°C, Phytohaemagglutinin 0.1 ml of 1:10 dilution (Burroughs-Wellcome) added after 72 hours and harvested at 144 hours for labelling with Chromium. The two-way MLC experiments were identical to the one-way though set up with unblocked stimulators.

The MLC cells were dispensed in the microtitre plates (Polystyrene "V" and "U" bottomed, Cooke Laboratory Products, Cat. Nos. 1-220-25A and 1-220-24A respectively, the former for MLCs and the latter for CMLs) in 50 λ aliquots per well using a Hamilton repeating dispenser (Hamilton Corp. Cat. No. PB-600) with a 2.5 ml glass syringe that was stored with 70% ethanol and rinsed with saline before use and between specimens. The stimulating (blocked) cells were added vertically in triplicates per row and the responding cells horizontally in the plates and the sera were diluted three parts to two of medium and 50 λ added on top of the aliquots of cells. The plates were covered and placed in the CO_2 incubator

to equilibrate before wrapping with polythene until harvest after 120 hours. At the termination of the incubation for the MLC 25 λ of a 1:30 dilution in Hank's Basic Salt solution of Methyl-^{3}H-Thymidine 21 Ci/nmol (The Radio-Chemical Centre, Amersham, Cat. No. TRK 120 Batch 118) was added using a dropping pipette and the incubation was continued for 4 hours. At the end of this time the wells were harvested using a Skatron Multisample Harvesting Machine (Skatron, Norway distributed in Canada by Flow Laboratories) and by this means the cells were washed from the plate wells on to circular pads of glass fibre filter paper which were then dried and placed into vials of scintillation fluid (PPO, Amersham-Searle, diluted 38.5 ml in 800 ml of Toluene) and counted in a liquid scintillation counter (Beckman, California), and the results were put onto paper tape by the Teletypewriter output. The paper tape was processed in a Wang 614 programmable calculator and the final Tables were produced by the Hewlett Packard 9815A using the 0883A printer/plotter which were programmed to accept triplicates and calculate the arithmetic mean of the counts per minute and coefficient of variation of the samples and store the matrix upon magnetic tape cassettes from which another program could extract the data and perform stimulation indices or ratios.

The relatively high activity of the Thymidine and the short pulse for the labelling was derived from a protocol of Dr. Len English from our group and the aim of which was to minimise the variances due to "pool" size of the cold Thymidine in the well while harvesting before the cells could be killed by the high specific activity. The technique had worked ex-

tremely satisfactorily in a prior investigation of an Ankylosing Spondylitic family and so was continued into this experiment. There had been some batch variation in his experiments so that only one batch of isotope was used. The Cell Mediated Lympholysis assay was only slightly modified from the method of Hirschberg, Skare and Thorsby (1977). The PHA blasts produced in the 2 ml tubes were amalgamated into one 13 ml disposable sterile culture tube (Falcon) per subject and centrifuged down and washed twice in medium. The cell pellet was then resuspended in a drop at the bottom of the tube and Sodium Chromate 1mC:ml Chromium-51 (New England Nuclear, Cat. No. NEZ-030S) 0.1 ml was added and the solution was incubated in a water bath at 37°C for one hour (this protocol was derived from Dr. Calvin Stiller). At the end of the incubation 4 ml of medium was then added and the cells were then carefully washed (centrifuging at 150G for three minutes) three further times. The tubes were then checked in the gamma counter for the total radioactivity of the cells and the concentration of the cells was adjusted to 2.5×10^4 per ml unless the radioactive label was insufficient to give adequate counts (i.e. $<10^4$ cpms per ml) in which case a compromise with larger concentrations of cells was attempted.

One tenth of a ml of the appropriate cell suspension was then added to each of the microtitre wells using a Hamilton Dispenser with a disposable 1 ml syringe to give 20 λ per click, i.e. 5 clicks per well, to insure that the cell button at the bottom had been freed by the impulses, and the microtitre plate was then incubated at 37°C for six hours. The plate was then centrifuged at 400 rpm for five minutes at room temper-

ature (though this was not strictly necessary in the protocol) and then harvested in the disposable supernatant harvest system designed by Hirschberg in which the press squeezes a swab into the well and the fluid enters the swab for collection but the intact cells are prevented from entering by a glass fibre filter paper pushed before (the system marketed commercially in Canada by Flow Laboratories). Six wells containing solely the target cell aliquots per subject were set aside and 0.1 ml of 5% Cetavlon (ICI) solution was added and freeze-thawed and harvested to give a maximal release value for the cells. The sample swabs were placed into labelled disposable tubes and counted in the gamma counter and the output from the Teletypewriter converted to paper tape for processing as for the MLC though with the preceeding maximal release values. The specific release was calculated from the (sample CPMs - autologous release CPMs) ÷ (Maximum release CPMs - autologous release CPMs).

The programmable calculators stored at the end of every matrix the HLA-A and B haplotypes determined by tissue typing a small aliquot of the cells in the Clinical Immunology Diagnostic Laboratory. These results were entered by hand into the memory and the whole store of memory when accessed by the interpretative programmes would then put these and the subject number at the top and sides of the table. Various different combinations of mean (arithmetric, geometric) and index (stimulation index and ratio and logarithmic index) were tried and each had some virtues for the initiated, but it was found that arithmetric mean in the main did not differ markedly from the geometric and that the art-

ificial indices were a cause of confusion when presented and the stimulation index of sample mean divided by the autologous stimulated mean was the least artificial. When the degree of aggregation of the variances with the means was investigated using the data from these and other mixed lymphocyte cultures performed at the same time it was found that the most appropriate transformation varied between the cube and fifth root. In this situation with the rather arbitrary choice of a uniform transformation the median can be used though not without the danger that with obvious experimental failure the variance about that median is difficult to express. The use of the arithmetic mean in the results section tends to show as a larger coefficient of variation so that the bias is against significance so that obvious differences that remain are significant.

RESULTS

Table 4, page 124 shows the matrix dose response of responding lymphocytes of one person in rows 1 to 4 (150000, 100000, 66000, and 44000 cells per well respectively) to stimulation by blocked lymphocytes of another person in columns 1 to 4 (150000, 100000, 66000, and 44000 cells per well), in a "V" bottomed microtitre plate. In row 1 column 1, abbreviated to (1,1), and row 2 column 1 (2,1) it can be appreciated at a glance that the counts are not as high as in the adjacent matrix. This can be interpreted to suggest that with this configuration of plate, overcrowding particularly with stimulators actually decreases the counts (per minute) seen. The other parts of the matrix, for instance row 3 or 4 or column 3 or 4, show a clear dose dependent response. Columns 5 and 6 represent the counts of the unblocked but unstimulated cells with column 6 being a double dose of those cells, i.e. 300000, 200000, 132000, and 88000 per well.

Table 5, page 125 shows the same matrix with the same cells but placed in a "U" bottomed microtitre plate. It can be seen on comparing this with the previous table that there were no longer any effects from stimulator overcrowding in (1,1) and (2,1) but that the middle elements of the table, e.g. (2,2), (2,3) and (3,2) and (3,3) were approximately equivalent, but that lower levels of response were seen in the "U" bottomed plate than the "V" bottomed, e.g. (4,3) and (4,4), with the lower numbers of responding cells. Since it was likely that lymphocytes were going to be in short supply in some families for all the wells it was decided to use the "V" bottomed plates and to keep the cell numbers down.

Figure 13, page 126, shows the "Malone" nuclear family with some clinical details about the patient (No. 1) and his mother (No. 3). The ages of the members of the family are typed above each member and the HLA-A and B types arranged in haplotypes beside that figure. The family was unusual in having only two distinct haplotypes (A2, B21) and A1, B8) which was simplistically interpreted initially to suggest that there might exist a Graft-Versus-Host situation between mother and child *in utero*, with the patient being unable to reject the maternal homozygotic cells. However, the Mixed Lymphocyte Culture results of Tables 6-11 and DR typing of the "B" cells showed that the mother was heterozygotic in the HLA-D region. Column 6 in all these tables was a pooled panel of mitomycin treated cells from three people unrelated to the family, and row 6 was a control with cells from another unrelated person.

It can be seen (for instance in Table 9, (2,6) versus (2,4) or (2,5) that the family were stimulated far more by the pooled panel lymphocytes than by their own members, and this effect extended to the mother (3) and the father (2) who were unrelated except by marriage. This could be interpreted to suggest that (2) and (3) shared a common HLA-D determinant, and this suggestion has received some confirmation by later DR typing that they both shared the common allele HLA-DRw3 (with A1, B8 haplotype) while the mother had DRw6 and the father DRw4 with the other haplotypes. Even so the very low level of stimulation suggest that these two haplotypes containing the DRw4 and DRw6 were not as reactive as those in the pool unless there is a very marked gene dose

effect.

There was an HLA identical sibling to the patient in (5) and when (1,5) is compared with the autologous (1,1), and when (5,1) is compared with the autologous (5,5) in all three matrices it can be demonstrated that they did indeed react as if having a common set of haplotypes. Having this sibling (and the father who also had reactions of identity) shows that there was little to be seen of phenotypic variation in the responses of the patient due to his illness.

Comparing the matrices (Tables 6, 8, and 10) for the effects of serum there are a number of significant differences. In (2,2) in Table 6 versus Table 8 it can be seen that the autologous stimulation was greater in the Foetal Calf Serum than in Autologous Serum, and this effect can be found almost universally in the many following matrices possibly due to some mitogenic effect of the Foetal Calf Serum. The same tables (3,1)and (3,2) and (3,5) show diminution of stimulation in autologous serum which may be related to the mother (3) having a strong anti-HLA B21 antibody. In Tables 8 and 10 there was a marked effect of serum on (4,1) which might be showing that (1), the patient, seems to have a blocking factor operating in the Heterologous system (Table 10) though there was a high coefficient of variation. It is difficult to account for the low value of the control serum inhibiting autologously the stimulation (see row 6, Table 8 compared with Tables 6 and 10).

Figure 14, page 133, shows the "Sparrow" nuclear family, and Tables 12

to 19 show the MLC results of this family. Comparison between the matrices again demonstrated the influence of the patient's serum (column 1 in Table 16 on depressing the responses of a non-idential sibling and depressing the responses of the patient in row 1 in Table 14, (1,2) (1,4) (1,5) while seemingly stimulating (1,3) in the same row. A more important effect in this family however was the one-way reaction between mother and patient with the patient responding to the mother (1,3) but the mother unable to respond to the patient (3,1) in all three matrices, so that there in absence of other measures she would be labelled a HLA-D homozygote similar to those detected by Stastny. However, the sibling (2) shares a paternal haplotype with the patient (A9, B40) and therefore should have the common HLA-D allele but she reacted to the patient in Tables 12 and 14 (2,1) so that by that measure the patient was not a homozygote unless there had been HLA-B-D recombination in (2) which is unlikely in view of the stimulation seen between (2) and (5). In this situation an HLA-identical sibling would have helped to delineate if the effects were to be genotypic or phenotypic.

Table 18, page 140, shows the result of early termination of the MLC at 72 hours instead of the usual 120 hours to check that there was no peak of premature activity due to presensitization, but there was no effect to be found there. At the bottom of this table can be seen the result of stimulation with the potent Streptococcal antigens Streptokinase/ Streptodornase (SK/SD) on the unblocked cells and the incorporation of isotope into the unstimulated cells and the mitomycin treated cells.

This has been repeated in two of the other matrices but there is little of interest in the normal stimulation levels seen in any of these.

Figure 15, page 142, shows the "Meany" nuclear family and it should be noted that the patient (2) had an HLA identical sibling (3). Tables 20 - 25 show the results of MLC testing and that demonstrated that the identical siblings were non-stimulatory in culture in all three matrices. Perhaps the most important feature about this matrix was the lack of response of the mother to the patient and his HLA identical sib seen in all three matrices (though Table 20, (5,2) shows mild stimulation this was not seen in (5,3) or the other matrices). However, using the major histocompatibility markers (4) and (6) also have the A3, B14 haplotype so that unless there has been recombination between the HLA-B and D loci in both (as they are mutually nonstimulatory) they should both react to (2) and (3) as though they were homozygotes, i.e. one-way stimulation of (2) and (3) by (4) and (6) but not the other way round. This is patently not the case though (3) does not seem to be a very good stimulator to any of the cells. The only satisfactory way of interpreting this data would be to assume that histocompatibility loci removed from the major locus are responsible for the effect though it could be argued that due to the deficiency of sib (3) as a stimulator phenotypic modulation of the mother/patient relationship had destroyed what would otherwise have been a perfectly normal two-way response. This matrix like that of the "Sparrow" family matrix did not contain an unrelated control which was regretable but unavoidable in the circumstances and it was perhaps fortunate that sibling (1)

having none of the involved haplotypes reacted vigorously and stimulated strongly thereby acting as a positive control.

Figure 16, page 149, shows the "Hoben" nuclear family and it should be noted that the siblings 2, 5, 6, 7 were HLA identical and that in spite of the large numbers of siblings there were no HLA identical sibs to the patient (4). In this experiment it was possible to obtain an unrelated control for the matrix and that person has been labelled (8).

In the large matrices derived from this family (plus the control) it becomes very difficult to perform the multiple comparisons that need to be performed, but the first feature that became apparent was that the stimulation between family members was very much less than that seen when those same family members were stimulated by the outside control (see Tables 26 to 31, pages 150 to 155, columns (8), which was similar to the findings in the "Malone" matrix. Much more significantly, perhaps, there appears to be a lack of mutual stimulation between the patient and the mother specifically, for instance (1,4) and (4,1) in all three matrices compared with the autologous (1,1) and (4,4).

There was one important feature of the matrix Table 26, page 150, that was missed by the calculator in printing the table and that was that the serum used was a pool of human serum (from male donors but not of the AB blood group) which included serum from the control (8). It would appear likely from this table and that of Table 28 compared with Table 30, row (8) that this pool contained a mild inhibitor of lympho-

cyte stimulation in the MLC. The control seemed to have a very high incorporation of Thymidine in the autologous stimulation of (8,8) in both pool serum and autologous serum which meant that there was little or no stimulation by the family of his cells in comparison. It is of anecdotal interest only that both he and the sib (2) were complaining of mild head colds at the time of venepuncture which might account for the high autologous incorporations seen in (2,2) and (8,8),

Table 32, page 156, "Hoben Two-Way MLC 4" was an exploration that using a two-way MLC (i.e. the stimulators were not treated with Mitomycin) might reveal interactions (or lack of interaction) not seen in the one-way cultures. The matrix was set up in the pooled serum of which there is now some suspicion from the previous matrices and it was hardly surprising that the results were uninterpretable with the high autologous stimulations (for instance (1,1)) swamping the stimulations seen with allogeneic cells (for instance (4,4)), however it can be seen that of all the combinations the mother and patient were the least reactive of the maternal combinations which was not simply due to the low input of the patient since two of the other siblings had similar levels of auto-stimulation (3,3) and (7,7), but nevertheless stimulated with the mother (1,3) and (1,6) to a much greater extent than did the patient (1,4) or (4,1).

Table 33, page 157, "Hoben One-Way CML (Propositus) 6" shows the result of overlaying the whole MLC with Chromium labelled PHA-treated lymphoblasts from the patient after six days in culture. The maximum release, 453 cpms, compared with the autologous level (4,4) of spontan-

eous release of 290 cpms means that small variations could cause seemingly large changes in the specific releases recorded in the following table (Table 34). The table is manifestly indigestible although certain features such as the somewhat higher releases associated with wells containing the control cells in the underlying MLC, but the lympholysis in these wells seems to be non-specific since the counts are high in both row and column (8). This suggests that some of the effects seen must be due to non-specific, possibly humoral, factors. Only the wells in column (4) had been primed with the patient's own cells so it is of interest that the mother's lymphocytes in row (1) seem to be stimulated by all her children to lyse the patient's targets though (4,1) which was the mother/patient combination performed poorly (6.1% specific release), perhaps reflecting the non-stimulation in MLC.

The next table (35), page 159, is the same experiment only performed with the mother's PHA lymphoblasts as target cells. In this case there has been adequate labelling with Chromium-51 to give 1603 cpms against the spontaneous release in the autologous culture (1,1) of 511 cpms so that more faith can be placed in the percentage specific releases quoted. Most of the points that have been raised in the previous table apply in this table but a caveat in interpreting the column 1 data which were the maternal stimulated wells is that there seems to be a high coefficient of variation in this column which may be related to the manner in which the labelled cells were added, or to the original MLC, or to the completeness of the final supernatant har-

vest.

The next table (37), page 161, was an attempt to obtain a control using the sibling with the common haplotype (A11, B18) to the patient. With the notable exception of the results in the first column the specific releases in the next table (38) were remarkably similar to those seen with the patient's cells. Following this table there are four sets of tables using the same pattern of target cells as the stimulators in the columns of the original MLCs. The data contain at least three major flaws: the first row in every row was accidentally inoculated with maternal cells labelled with Chromium; the sibling (7) cells have failed to take up the label indicating that they were probably dead; and finally, that the PHA lymphoblasts from siblings (5) and (6) became hopelessly contaminated with yeasts and had to be discarded so that the overlying cells in those columns were actually the same as those in column (2) which were of the same HLA pattern. The tables include one straightforward CML (Table 39, page 182), one overlay on a two-way MLC that certainly seems to have produced some very convincing specific(?) releases, and two overlays on MLCs set up with autologous and heterologous serum to form a modified form of antibody-dependent cell-mediated cytotoxicity (ADCC) mentioned in the introduction. From these last two tables (43 and 45) it can be readily seen that the patient's serum in row (4) of Table 43 and column (4) of Table 45 showed a marked inhibitory effect on the cytolysis recorded by the Chromium release. This observation is in accord with Chihara (1976) and Austin (1976) and Isturiz (1976) who proposed

from similar observations that the protective humoral agent was the Rheumatoid Factor which could be tested by the addition of purified Rheumatoid Factor to normal serum (the patient was seropositive).

Figure 17, page 171, shows the "Best" family and it can be seen from the brief clinical notes that the patient suffered from severe, intractable diarrhoea as an infant which becomes of interest when this is compared with the proband in the "Hoben" family who had had a very similar story (see Figure 20, page 168). This family was complete and the matrix satisfactory with the presence of both parents and one unrelated control but it should be noted that the patient was being treated with steroids at the time of the investigation, though the last dose had been 36 hours prior to venepuncture. There were two other siblings with identical HLA haplotypes and two with totally dissimilar haplotypes.

Examination of Table 47, page 172, showed that the propositus and the two HLA identical siblings, (6) (2) and (4) respectively, seem to be difficult to obtain adequate stimulation even with the outside control (for example:- (4,8) or (2,8) or (6,8)) and they all resolutely refuse to be stimulated by other family members (for example:- the mother in column (7) or the father in column (1) as well as all the other siblings). This finding was in sharp contrast to the HLA non-identical siblings 3 and 5 who responded quite well to the outside control (for example:- the stimulation index of 11.4 in (5.8) in Table 50 or (8.9) in Table 48). This presents one with a problem of interpretation as the patient and his identical HLA siblings behave in MLC as if both

mother and father were homozygotes, but from the data with the other siblings it is known that the parents cannot be homozygotes for all the MLC loci so that one is reduced to appeal to minor loci or phenotypic modulation of the stimulator cells in order to explain the reactions seen. It was unfortunate that the control should have relatively high autologous stimulation as this prevents this from being of use in the assessment of the stimulating cells' ability to stimulate (column (8) in both Tables 47 and 49).

The "Two-Way MLC" (Table 51, page 176), of the "Best" family was set up, but as with the "Hoben" family it remains difficult if not impossible to interpret. One can add geometrically the counts of the autologous stimulations (for example:- (1,1) and (6,6)) and divide by two to reach a geometric mean and then compare this with the observed means seen in (6,1) and (1,6) to create a stimulation index, or a more stringent test of stimulation would be to see if the mixed cells had a higher count than the highest of the autologous stimulations. Looking at the block defined by (6,6), (6,7), (7,6), and (7,7), it can be seen that (6,7) was appreciably higher than either of the autologous cultures (3589 v 2773 or 494 cpms) which was supported by (7,6) counts of 2597 cpms to confirm that the mother and patient stimulated in the two-way system though there was no indication here which way the reaction was proceeding. It should be noted that the same cell suspensions were used in the rows and in the columns so that 3589 and 2597 cpms should be regarded as aspects of the same reaction and illustrate the variation intrinsic to the experimental technique.

The following table (Table 52, page 177) shows the results from using autologous serum in the Two-Way protocol and is notable only for the similarity of the responses seen with the pooled serum.

The following table (Table 53, page 178), though rather unsatisfactory as there was an insufficiency of cells from sibling (2) and the mother and control columns were inadvertently exchanged, records an attempt to see if early termination of the two-way MLC would bring out features not apparent in the normal 120 hour incubation, and accordingly the patient (6) and an HLA non-identical sibling were harvested at 36 hours and at 80 hours (the bottom table). All that can be said about these tables are that they show little or no stimulation for either sibling by the mother ((6,7) and 5,7)) compared with a reasonable if low response to the control ((6,8) and 5,8)). The final table in the "Best" series is Table 54 on page 179, which was a repetition of the ADCC in which Chromium labelled target cells were laid over the Two-Way MLC set up in Autologous serum and harvested four hours later to detect the direction of the reaction. The maternal cells do not seem to produce much cytolysis compared with sibling (3) for instance, but otherwise there is much data but little to comment upon.

The final family in this series was the nuclear family "Freake" (Figure 18, page 181) in which the proband (5) had had an atypical monoarticular arthritis for seven years in his right knee. Tissue typing the family revealed that the patient had HLA-B27 that has been associated with the disease Ankylosing Spondylitis, and taken with the history of the

patient's disease raises the possibility that he might have been suffering from a Juvenile form (*fruste*) of this disease. The boy was completely well at the time of the blood taking with no clinical evidence of any disease, peripheral or axial.

Tables 56 to 61, pages 182 to 187, record the disappointing MLC results that merely demonstrate the complete identity of the four children who were HLA haplo-identical, and the complete dissimilarity of the parents. However, the results from this family are in sharp contrast to the other families in the series because of the clear and unequivocal typing responses (with the exception of the patient himself who seems somewhat poorly recognised by the mother in (2,5)). To augment this aspect I have introduced Tables 62 to 67, pages 188 to 193, which represent the results with an extended family with Ankylosing Spondylitis, the "Brown" family, in which (1) and (2) had the disease, (3) was a related cousin who was homozygous for the offending HLA haplotype (Aw31, B27), and (5) was the mother. This family was investigated in the middle of the investigations of the Rheumatoid Families and shows the same technique used but in this case there are the clear typing responses present that allow Two-Way reactions, One-Way reactions with the homozygotes (1) and (3), and reactions of identity to be easily spotted. A number of other MLCs have been set up with this extended family and there have been uniformity of the vigour of the responses in culture that parallels the results in the "Freake" family and is in contrast to the difficult evaluation of the Rheumatoid Families which may be due to the HLA-D types involved which in the Anky-

losing Spondylitis families seem to be of the DRw1 on "B" cell typing, and not the DRw3 and DRw4 types found in the Juvenile Rheumatoid Families. However, the evidence from the "Meany" family would support the idea that other loci are involved in these families that are definitely not linked to the HLA region of chromosome 6, and this interesting possibility is further enhanced by the results of the "Best" MLCs which are not explicable by conventional immunogenetic theory.

I have presented the data from five JRA families (four seronegative, and one seropositive) with the contrasting data from a condition that often confused in the child namely Ankylosing Spondylitis. If there were not already the HLA B27 marker for the latter condition the results of the MLC tests in the families would seem on this preliminary survey to be seperable from those obtained from JRA families and of diagnostic use. I have tried to interpret the data in as rigorously conservative fashion as possible with full revellation of the data not to confuse the readers but to alert them to the real possibility of over-interpreting and narrowly defining a real phenomenon, namely that the Mixed Lymphocyte Culture reactions of Juvenile Rheumatoid Arthritis families show lower levels of response than one would expect particularly the patient/mother combinations. The addition of various combinations of serum only caused modulation of particular reactions when there was a cytotoxic antibody directed at an HLA B antigen in the presence of human complement; JRA sera had no effect on the level of allogenic reaction.

						CELLS	CELLS * 2
		1	2	3	4	5	6
		150000	100000	66000	44000	0 1	0 2
1	150000	2709 ± 90%	6916 ± 12%	5740 ± 20%	6263 ± 7%	469 ± 41%	1184 ± 4%
2	100000	4629 ± 23%	6318 ± 14%	5391 ± 11%	3675 ± 1%	308 ± 45%	409 ± 17%
3	66000	5056 ± 10%	4151 ± 23%	3784 ± 21%	2119 ± 51%	217 ± 28%	336 ± 16%
4	44000	2701 ± 18%	3133 ± 10%	3476 ± 23%	2192 ± 16%	141 ± 23%	244 ± 12%

MALONE MLC (DOSE RESPONSE "V" PLATE)

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 4

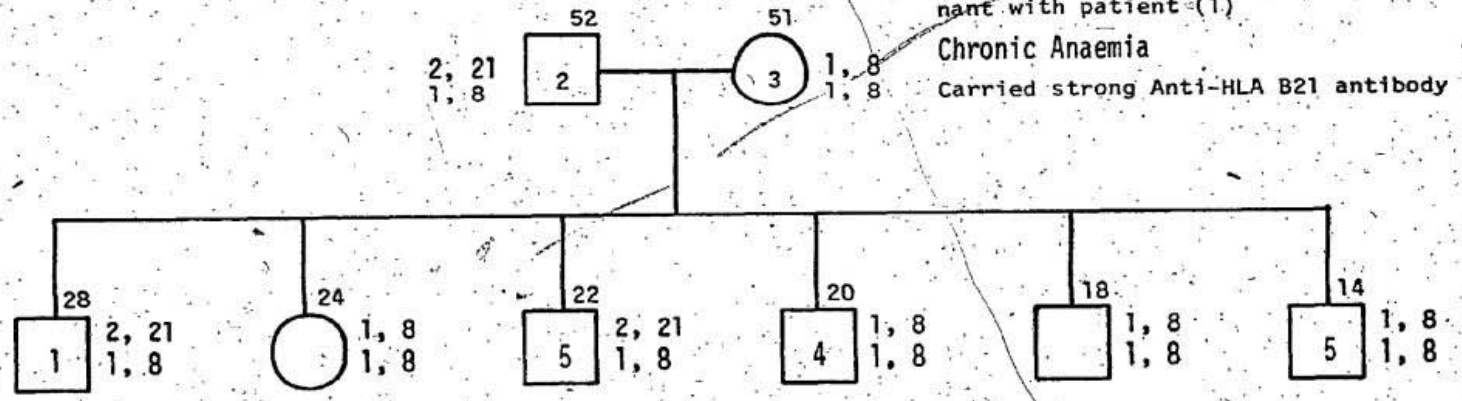
					CELLS	CELLS * 2
	1	2	3	4	5	6
	150000	100000	66000	44000	0 1	0 2
1 150000	8248 ± 56%	8203 ± 19%	7027 ± 9%	4683 ± 25%	357 ± 23%	754 ± 15%
2 100000	5246 ± 19%	6248 ± 32%	4410 ± 19%	3503 ± 11%	266 ± 27%	681 ± 12%
3 66000	3998 ± 18%	3450 ± 18%	2516 ± 20%	2418 ± 22%	169 ± 26%	311 ± 13%
4 44000	2400 ± 10%	3873 ± 14%	1675 ± 15%	1197 ± 52%	120 ± 19%	184 ± 32%

MALONE MLC (DOSE RESPONSE "U" PLATE)

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 5

MALONE FAMILY



Episode of Acute Arthritis while pregnant with patient (1)

Chronic Anaemia

Carried strong Anti-HLA B21 antibody

Severe seronegative unremitting Non-nodular JRA - onset aet 12

Surgery to hands + feet

Recent Pericarditis

Rx Butazolidin + Aspirin
(Nil on day of M.L.C)

FIGURE 18

HLA TYPE		1 2 21 1 8	2 2 21 1 8	3 1 8 1 8	4 1 8 1 8	5 2 21 1 8	6 99 99 99 99
1	2 21 1 8	2604 ±104%	3786 ± 22%	6253 ± 15%	4527 ± 21%	1914 ± 34%	20528 ± 75%
2	2 21 1 8	1696 ± 3%	1009 ± 12%	4192 ± 15%	1519 ± 9%	1186 ± 15%	15292 ± 6%
3	1 8 1 8	8932 ± 23%	7153 ± 12%	3885 ± 7%	4617 ± 16%	8122 ± 30%	22513 ± 22%
4	1 8 1 8	3477 ± 50%	4092 ± 20%	1772 ± 15%	590 ± 21%	5363 ± 10%	14079 ± 13%
5	2 21 1 8	1139 ± 10%	1372 ± 40%	2905 ± 26%	4500 ± 99%	603 ± 61%	13501 ± 4%
6	99 99 99 99	10550 ± 3%	8013 ± 19%	9341 ± 5%	7445 ± 8%	9400 ± 10%	9720 ± 17%

MALONE MLC (FOETAL CALF SERUM) 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 6

HLA TYPE		1 2 21 1 8	2 2 21 1 8	3 1 8 1 8	4 1 8 1 8	5 2 21 1 8	6 99 99 99 99
1	2 21 1 8	1.00	1.45	2.40	1.74	0.74	7.88
2	2 21 1 8	1.68	1.00	4.15	1.51	1.18	15.16
3	1 8 1 8	2.30	1.84	1.00	1.19	2.09	5.79
4	1 8 1 8	5.89	6.94	3.00	1.00	9.09	23.86
5	2 21 1 8	1.89	2.28	4.82	7.46	1.00	22.39
6	99 99 99 99	1.09	0.82	0.96	0.77	0.97	1.00

MALONE MLC (FOETAL CALF SERUM) 1

STIMULATION INDEX

TABLE 7

HLA TYPE		1 2 21 1 8	2 2 21 1 8	3 1 8 1 8	4 1 8 1 8	5 2 21 1 8	6 99 99 99 99
1	2 21 1 8	1228 ± 54%	1879 ± 18%	4704 ± 12%	3525 ± 17%	1577 ± 37%	20358 ± 11%
2	2 21 1 8	486 ± 13%	290 ± 14%	2554 ± 15%	560 ± 29%	338 ± 16%	13478 ± 5%
3	1 8 1 8	2877 ±103%	8335 ± 26%	2833 ± 69%	6074 ± 9%	4797 ± 23%	17810 ± 9%
4	1 8 1 8	5214 ± 52%	7338 ± 24%	2169 ± 30%	440 ± 52%	5449 ± 13%	16092 ± 10%
5	2 21 1 8	633 ± 43%	653 ± 29%	2068 ± 33%	1746 ± 38%	457 ± 25%	12902 ± 17%
6	99 99 99 99	322 ± 32%	3393 ± 67%	4986 ± 94%	6660 ± 8%	8158 ± 18%	9869 ± 49%

MALONE MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 8

HLA TYPE		1 2 21 1 8	2 2 21 1 8	3 1 8 1 8	4 1 8 1 8	5 2 21 1 8	6 99 99 99 99
1	2 21 1 8	1.00	1.53	3.83	2.87	1.28	16.58
2	2 21 1 8	1.68	1.00	8.81	1.93	1.17	46.47
3	1 8 1 8	1.02	2.94	1.00	2.14	1.69	6.29
4	1 8 1 8	11.85	16.68	4.93	1.00	12.38	36.57
5	2 21 1 8	1.39	1.43	4.52	3.82	1.00	28.23
6	99 99 99 99	0.03	0.34	0.51	0.67	0.83	1.00

MALONE MLC (AUTOLOGOUS SERUM) 2

STIMULATION INDEX

TABLE 9

HLA TYPE		1 2 21 1 8	2 2 21 1 8	3 1 8 1 8	4 1 8 1 8	5 2 21 1 8	6 99 99 99 99
1	2 21 1 8	673 ± 46%	1768 ± 27%	2711 ± 41%	4582 ± 33%	1129 ± 19%	24237 ± 18%
2	2 21 1 8	438 ± 17%	238 ± 36%	2185 ± 11%	703 ± 35%	355 ± 24%	14006 ± 2%
3	1 8 1 8	7650 ± 21%	9937 ± 3%	2717 ± 48%	5597 ± 18%	8042 ± 5%	20802 ± 4%
4	1 8 1 8	722 ± 82%	4721 ± 35%	1533 ± 35%	279 ± 41%	5158 ± 29%	18976 ± 3%
5	2 21 1 8	909 ±123%	566 ± 21%	1314 ± 51%	1832 ± 75%	351 ± 17%	15139 ± 6%
6	99 99 99 99	9126 ± 10%	10976 ± 16%	8998 ± 17%	10686 ± 8%	8140 ± 8%	11909 ± 8%

MALONE MLC (HETEROLOGOUS SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 10

HLA TYPE		1 2 21 1 8	2 2 21 1 8	3 1 8 1 8	4 1 8 1 8	5 2 21 1 8	6 99 99 99 99
1	2 21 1 8	1.00	2.63	4.03	6.81	1.68	36.01
2	2 21 1 8	1.84	1.00	9.18	2.95	1.49	58.84
3	1 8 1 8	2.82	3.66	1.00	2.06	2.96	7.66
4	1 8 1 8	2.59	16.92	5.49	1.00	18.48	68.00
5	2 21 1 8	2.59	1.61	3.74	5.22	1.00	43.13
6	99 99 99 99	0.77	0.92	0.76	0.90	0.68	1.00

MALONE MLC (HETEROLOGOUS SERUM) 3

STIMULATION INDEX

TABLE 11

SPARROW FAMILY

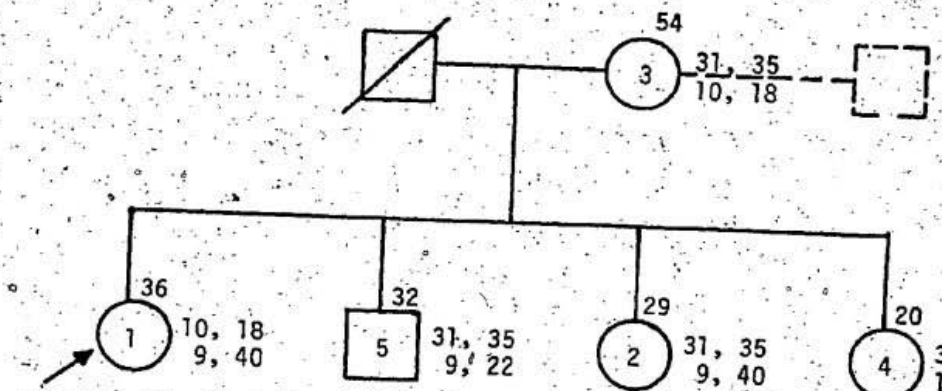


FIGURE 14

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	808 ± 83%	13212 ± 18%	13288 ± 11%	11077 ± 24%	19241 ± 12%
2 31 35 9 40	15167 ± 83%	6454 ± 30%	21730 ± 12%	19628 ± 14%	20643 ± 7%
3 31 35 10 18	3826 ±160%	16137 ± 8%	4864 ± 52%	1418 ± 29%	11387 ± 76%
4 31 35 11 7	14621 ± 77%	25237 ± 19%	10279 ± 12%	5700 ± 40%	19640 ± 20%
5 31 35 9 22	12405 ±110%	24288 ± 18%	20548 ± 16%	19749 ± 3%	9492 ± 7%

SPARROW MLC (FOETAL CALF SERUM) 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 12

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	1.00	16.35	16.44	13.71	23.81
2 31 35 9 40	2.35	1.00	3.37	3.04	3.20
3 31 35 10 18	0.79	3.32	1.00	0.29	2.34
4 31 35 11 7	2.57	4.43	1.80	1.00	3.45
5 31 35 9 22	1.31	2.56	2.16	2.08	1.00

SPARROW MLC (FOETAL CALF SERUM) 1
STIMULATION INDEX

TABLE 13

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	404 ± 25%	10213 ± 31%	9663 ± 16%	6013 ± 11%	18652 ± 26%
2 31 35 9 40	17273 ± 27%	2126 ± 35%	11910 ± 12%	10797 ± 4%	21098 ± 11%
3 31 35 10 18	347 ± 32%	929 ± 40%	334 ± 92%	740 ± 25%	3475 ± 42%
4 31 35 11 7	13220 ± 24%	13790 ± 34%	8342 ± 21%	2889 ±130%	15776 ± 36%
5 31 35 9 22	33672 ± 8%	29892 ± 11%	28491 ± 34%	15635 ± 16%	4102 ± 70%

SPARROW MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 14

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	1.00	25.28	23.92	14.88	46.17
2 31 35 9 40	8.12	1.00	5.60	5.08	9.92
3 31 35 10 18	1.04	2.78	1.00	2.22	10.40
4 31 35 11 7	4.58	4.77	2.89	1.00	5.46
5 31 35 9 22	8.21	7.29	6.95	3.81	1.00

SPARROW MLC (AUTOLOGOUS SERUM) 2 %

STIMULATION INDEX

TABLE 15

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	260 ± 33%	16067 ± 34%	2757 ± 30%	11064 ± 54%	32705 ± 12%
2 31 35 9 40	2694 ± 82%	2522 ±117%	5799 ± 42%	12523 ± 12%	34539 ± 7%
3 31 35 10 18	328 ± 46%	15308 ± 35%	990 ± 40%	1947 ± 98%	19347 ± 21%
4 31 35 11 7	1770 ±123%	23409 ± 23%	7003 ± 14%	2768 ± 83%	25663 ± 15%
5 31 35 9 22	3105 ±157%	12576 ±110%	12086 ± 35%	15783 ± 28%	6970 ± 12%

SPARROW MLC (HETEROLOGOUS SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 16

SPARROW MLC (HETEROLOGOUS SERUM) 3

STIMULATION INDEX

TABLE 17

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	1.00	61.79	10.60	42.55	125.77
2 31 35 9 40	1.07	1.00	2.30	4.97	13.69
3 31 35 10 18	0.33	15.46	1.00	1.97	19.54
4 31 35 11 7	0.64	8.46	2.53	1.00	9.27
5 31 35 9 22	0.45	1.80	1.73	2.26	1.00

HLA TYPE		1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22	
1	10 18 9 40	296 ± 21%	1890 ± 3%	1020 ± 2%	1468 ± 15%	4257 ± 4%	
2	31 35 9 40	3424 ± 88%	429 ± 4%	3486 ± 35%	2782 ± 19%	5347 ± 11%	
3	31 35 10 18	287 ± 7%	673 ± 51%	302 ± 11%	357 ± 44%	972 ± 46%	
4	31 35 11 7	3935 ± 18%	4892 ± 8%	883 ± 16%	313 ± 34%	4239 ± 13%	
5	31 35 9 22	8359 ± 14%	6073 ± 3%	6050 ± 29%	3885 ± 7%	997 ± 11%	
6	0 0 0 0	369 ± 7%	1605 ± 67%	568 ±100%	451 ± 27%	1728 ±112%	CELLS +SK-SD
7	0 0 0 0	156 ± 20%	150 ± 11%	143 ± 7%	152 ± 6%	348 ±138%	CELLS [NOT BLOCKED]111
8	0 0 0 0	91 ± 52%	49 ± 49%	54 ± 33%	55 ± 28%	61 ± 33%	CELLS [BLOCKED]

SPARROW MLC 72HR. (AUTOLOGOUS SERUM) 4

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 18

HLA TYPE	1 10 18 9 40	2 31 35 9 40	3 31 35 10 18	4 31 35 11 7	5 31 35 9 22
1 10 18 9 40	1.00	6.38	3.45	4.96	14.38
2 31 35 9 40	7.98	1.00	8.13	6.48	12.46
3 31 35 10 18	0.95	2.23	1.00	1.18	3.22
4 31 35 11 7	12.57	15.63	2.82	1.00	13.54
5 31 35 9 22	8.38	6.09	6.07	3.90	1.00

SPARROW MLC 72HR. (AUTOLOGOUS SERUM) 4
STIMULATION INDEX

TABLE 19.

MEANY FAMILY

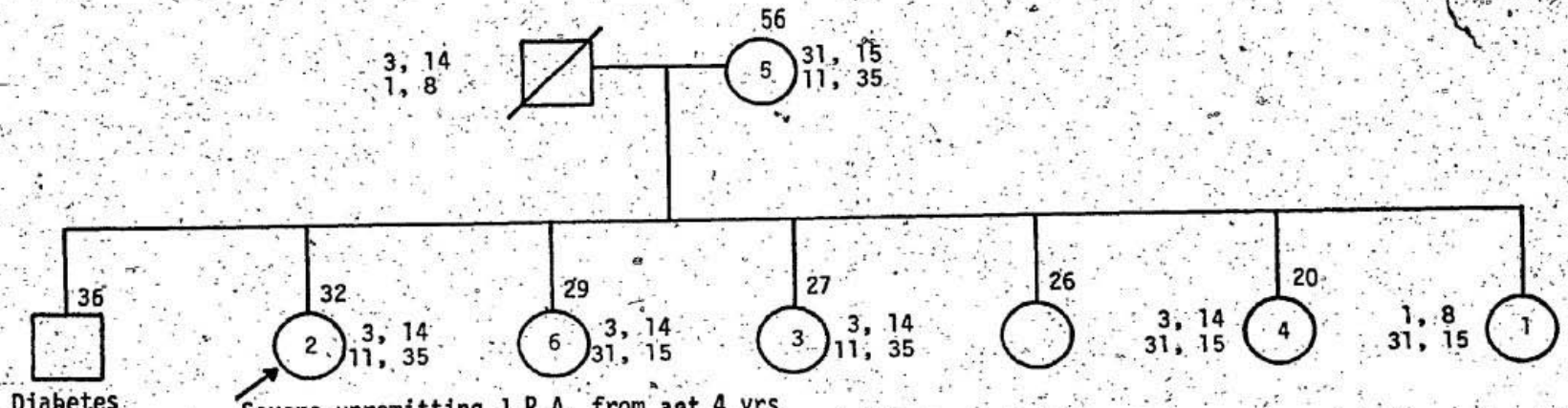


FIGURE 15

HLA TYPE		1 1 8 32 15	2 3 14 11 35	3 3 14 11 35	4 3 14 31 15	5 31 15 11 35	6 3 14 31 15
1	1 8 32 15	261 ± 21%	1416 ± 15%	220 ± 34%	801 ± 25%	2168 ± 22%	1200 ± 22%
2	3 14 11 35	1171 ± 24%	405 ± 16%	238 ± 28%	1000 ± 15%	1391 ± 43%	1797 ± 32%
3	3 14 11 35	2709 ± 15%	377 ± 22%	212 ± 36%	1402 ± 13%	2558 ± 20%	2537 ± 24%
4	3 14 31 15	5633 ± 29%	7924 ± 21%	1120 ± 12%	1439 ± 35%	11771 ± 9%	1520 ± 16%
5	31 15 11 35	1041 ± 47%	1041 ± 41%	306 ± 26%	899 ± 9%	473 ± 34%	1383 ± 40%
6	3 14 31 15	1158 ± 29%	1289 ± 6%	297 ± 29%	302 ± 12%	1600 ± 3%	423 ± 9%

MEANY MLC (FOETAL CALF SERUM) 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 20

HLA TYPE	1 1 8 32 15	2 3 14 11 35	3 3 14 11 35	4 3 14 31 15	5 31 15 11 35	6 3 14 31 15
1 1 8 32 15	1.00	5.42	0.84	3.07	8.31	4.60
2 3 14 11 35	2.89	1.00	0.59	2.47	3.43	4.44
3 3 14 11 35	12.78	1.78	1.00	6.61	12.06	11.97
4 3 14 31 15	3.91	5.51	0.78	1.00	8.18	1.06
5 31 15 11 35	2.20	2.20	0.65	1.90	1.00	2.92
6 3 14 31 15	2.74	3.05	0.70	0.71	3.78	1.00

MEANY MLC (FOETAL CALF SERUM) 1

STIMULATION INDEX

TABLE 21

	1	2	3	4	5	6
HLA TYPE	1 8 32 15	3 14 11 35	3 14 11 35	3 14 31 15	31 15 11 35	3 14 31 15
1 1 8 32 15	508 ± 45%	13688 ± 24%	4675 ± 14%	5011 ± 7%	18238 ± 3%	5664 ± 16%
2 3 14 11 35	16886 ± 17%	463 ± 30%	246 ± 23%	6818 ± 29%	7445 ± 11%	13756 ± 10%
3 3 14 11 35	14743 ± 22%	472 ± 8%	222 ± 39%	7024 ± 26%	10165 ± 23%	11902 ± 13%
4 3 14 31 15	14176 ± 6%	14502 ± 6%	5189 ± 42%	805 ± 58%	17351 ± 5%	720 ± 42%
5 31 15 11 35	9760 ± 12%	2859 ± 76%	1363 ± 76%	4408 ± 26%	1530 ± 56%	7487 ± 47%
6 3 14 31 15	7701 ± 29%	8261 ± 31%	1850 ± 33%	439 ± 11%	11404 ± 14%	754 ± 46%

MEANY MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 22

HLA TYPE	1 1 8 32 15	2 3 14 11 35	3 3 14 11 35	4 3 14 31 15	5 31 15 11 35	6 3 14 31 15
1 1 8 32 15	1.00	26.94	9.20	9.86	35.90	11.15
2 3 14 11 35	36.47	1.00	0.53	14.72	16.08	29.71
3 3 14 11 35	66.40	2.13	1.00	31.63	45.78	53.60
4 3 14 31 15	17.61	18.01	6.45	1.00	21.55	0.89
5 31 15 11 35	6.38	1.87	0.89	2.88	1.00	4.89
6 3 14 31 15	10.21	10.96	2.45	0.58	15.12	1.00

MEANY MLC (AUTOLOGOUS SERUM) 2

STIMULATION INDEX

TABLE 23

HLA TYPE		1 1 8 32 15	2 3 14 11 35	3 3 14 11 35	4 3 14 31 15	5 31 15 11 35	6 3 14 31 15	
1	1 8 32 15	2333 ±152%	15074 ± 14%	3050 ± 40%	2690 ± 10%	17853 ± 14%	4819 ± 34%	
2	3 14 11 35	16292 ± 11%	593 ± 37%	212 ± 22%	5667 ± 26%	10122 ± 10%	8471 ± 11%	
3	3 14 11 35	13399 ± 15%	612 ± 32%	828 ± 18%	7160 ± 9%	9882 ± 16%	8981 ± 13%	
4	3 14 31 15	15247 ± 4%	23112 ± 10%	6257 ± 22%	887 ± 57%	22937 ± 6%	2375 ± 41%	
5	31 15 11 35	9240 ± 19%	2772 ± 23%	448 ± 54%	4010 ± 20%	2524 ± 45%	5066 ± 34%	
6	3 14 31 15	9476 ± 16%	10728 ± 3%	1451 ± 11%	1051 ±108%	15558 ± 9%	1029 ± 27%	
7	0 0 0 0	3883 ± 11%	8321 ± 18%	1769 ± 30%	9807 ± 22%	4502 ± 5%	3231 ± 19%	CELLS +SK-SD
8	0 0 0 0	385 ± 42%	420 ± 60%	246 ± 38%	228 ± 29%	293 ± 45%	296 ± 43%	CELLS [NOT BLOCKED]

MEANY MLC (HETEROLOGOUS SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 24

HLA TYPE	1 1 8 32 15	2 3 14 11 35	3 3 14 11 35	4 3 14 31 15	5 31 15 11 35	6 3 14 31 15
1 1 8 32 15	1.00	6.46	1.31	1.15	7.65	2.07
2 3 14 11 35	27.47	1.00	0.36	9.56	17.07	14.28
3 3 14 11 35	58.76	2.68	1.00	31.40	43.34	39.39
4 3 14 31 15	17.19	26.05	7.05	1.00	25.86	2.68
5 31 15 11 35	3.66	1.10	0.18	1.59	1.00	2.01
6 3 14 31 15	9.21	10.43	1.41	1.02	15.12	1.00

MEANY MLC (HETEROLOGOUS SERUM) 3

STIMULATION INDEX

TABLE 25

HOBEN FAMILY

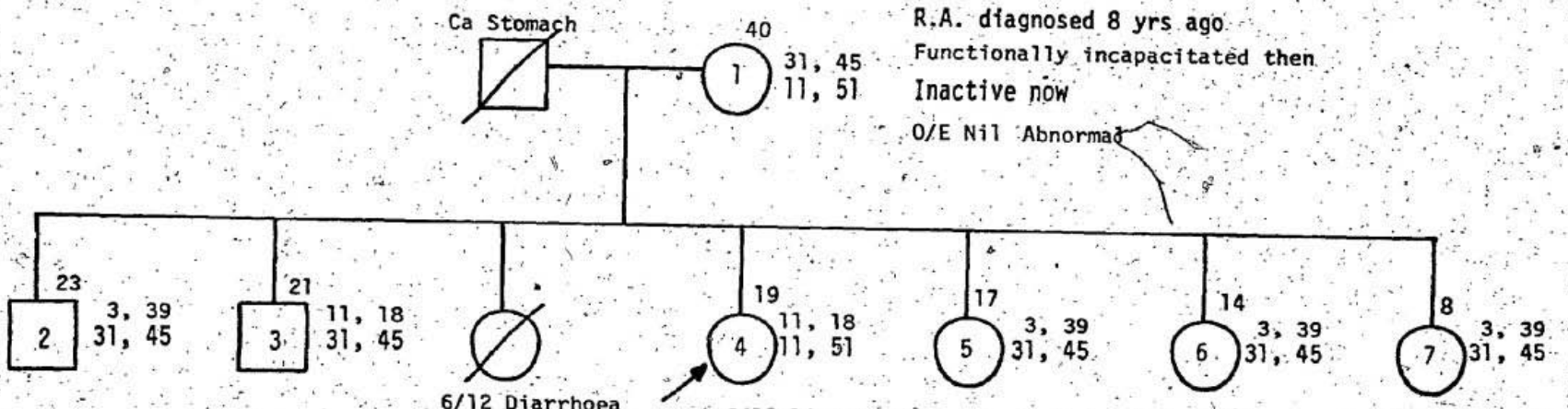


FIGURE 16

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	1512 ± 51%	6810 ± 17%	2196 ± 74%	1815 ± 30%	6697 ± 21%	5969 ± 9%	3669 ± 5%	7242 ± 13%
2 3 39 31 45	2661 ± 36%	3992 ± 18%	3960 ± 15%	2740 ± 56%	3027 ± 36%	3636 ± 20%	2716 ± 11%	5832 ± 62%
3 11 18 31 45	311 ± 9%	1776 ± 14%	193 ± 2%	119 ± 21%	540 ± 77%	859 ± 35%	353 ± 27%	1325 ± 27%
4 11 18 11 51	235 ± 18%	1228 ± 29%	242 ± 20%	128 ± 37%	255 ± 38%	313 ± 58%	255 ± 20%	1018 ± 25%
5 3 39 31 45	805 ± 23%	291 ± 26%	866 ± 38%	885 ± 46%	527 ± 73%	213 ± 17%	255 ± 30%	3596 ± 18%
6 3 39 31 45	2088 ± 7%	1186 ±107%	1238 ± 38%	329 ± 29%	1378 ± 64%	560 ± 59%	394 ± 36%	6743 ± 8%
7 3 39 31 45	584 ± 50%	339 ± 53%	446 ± 43%	286 ± 81%	351 ± 34%	129 ± 17%	176 ± 26%	4159 ± 15%
8 2 14 9 35	5870 ± 15%	8503 ± 9%	6553 ± 25%	4675 ± 11%	7751 ± 10%	7788 ± 7%	4777 ± 17%	3796 ± 7%

HOBEN ONE-WAY MLC 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 26

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	1.00	4.50	1.45	1.20	4.43	3.95	2.43	4.79
2 3 39 31 45	0.67	1.00	0.99	0.69	0.76	0.91	0.68	1.46
3 11 18 31 45	1.61	9.20	1.00	0.62	2.80	4.45	1.83	6.87
4 11 18 11 51	1.84	9.59	1.89	1.00	1.99	2.45	1.99	7.95
5 3 39 31 45	1.53	0.55	1.64	1.68	1.00	0.40	0.48	6.82
6 3 39 31 45	3.73	2.12	2.21	0.59	2.46	1.00	0.70	12.04
7 3 39 31 45	3.32	1.93	2.53	1.63	1.99	0.73	1.00	23.63
8 2 14 9 35	1.55	2.24	1.73	1.23	2.04	2.05	1.26	1.00

HOBEN ONE-WAY MLC 1

STIMULATION INDEX

TABLE 27

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	1071 ± 48%	6065 ± 6%	1927 ± 53%	1299 ± 14%	5582 ± 12%	5192 ± 57%	2847 ± 3%	8316 ± 8%
2 3 39 31 45	4167 ± 23%	4018 ± 18%	4214 ± 7%	4319 ± 7%	3137 ± 14%	3479 ± 20%	2862 ± 23%	10874 ± 12%
3 11 18 31 45	2087 ± 9%	5306 ± 18%	234 ± 31%	471 ± 45%	3999 ± 12%	2377 ± 12%	1042 ± 16%	9135 ± 7%
4 11 18 11 51	308 ± 29%	1161 ± 46%	241 ± 26%	134 ± 31%	753 ± 59%	433 ± 88%	139 ± 21%	1529 ± 34%
5 3 39 31 45	1561 ± 28%	965 ± 74%	1552 ± 43%	869 ± 43%	600 ± 99%	895 ± 91%	720 ± 76%	5911 ± 32%
6 3 39 31 45	2605 ± 74%	1848 ± 35%	3024 ± 11%	631 ± 48%	1346 ± 28%	1109 ± 22%	1046 ±114%	8376 ± 10%
7 3 39 31 45	1170 ± 11%	506 ± 33%	917 ± 10%	559 ± 57%	518 ± 44%	507 ± 28%	274 ± 6%	3234 ± 26%
8 2 14 9 35	6558 ± 4%	11201 ± 3%	9234 ± 4%	6762 ± 4%	9199 ± 4%	8070 ± 7%	6037 ± 15%	4364 ± 32%

HOBEN ONE-WAY MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 28

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	1.00	5.66	1.80	1.21	5.21	4.85	2.66	7.76
2 3 39 31 45	1.04	1.00	1.05	1.07	0.78	0.87	0.71	2.71
3 11 18 31 45	8.92	22.67	1.00	2.01	17.09	10.16	4.45	39.03
4 11 18 11 51	2.30	8.66	1.80	1.00	5.62	3.23	1.04	11.41
5 3 39 31 45	2.60	1.61	2.59	1.45	1.00	1.49	1.20	9.85
6 3 39 31 45	2.35	1.67	2.73	0.57	1.21	1.00	0.94	7.55
7 3 39 31 45	4.27	1.85	3.35	2.04	1.89	1.85	1.00	11.80
8 2 14 9 35	1.50	2.57	2.12	1.55	2.11	1.85	1.38	1.00

HOBEN ONE-WAY MLC (AUTOLOGOUS SERUM) 2

STIMULATION INDEX

TABLE 29

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	1413 ± 28%	8553 ± 16%	4721 ± 18%	1393 ± 30%	7181 ± 17%	2324 ± 17%	4252 ± 20%	11310 ± 22%
2 3 39 31 45	3459 ± 38%	4890 ± 8%	7321 ± 6%	4384 ± 12%	3472 ± 29%	748 ± 36%	1884 ± 52%	10244 ± 11%
3 11 18 31 45	1075 ± 16%	3037 ± 14%	373 ± 26%	266 ± 11%	1974 ± 24%	437 ± 29%	907 ± 34%	6654 ± 34%
4 11 18 11 51	351 ± 18%	1574 ± 43%	815 ± 17%	211 ± 20%	912 ± 35%	379 ± 53%	509 ± 49%	2199 ± 12%
5 3 39 31 45	896 ± 3%	504 ± 68%	1713 ± 63%	779 ± 87%	186 ± 12%	144 ± 11%	190 ± 42%	3828 ± 28%
6 3 39 31 45	2177 ± 10%	2409 ± 68%	3765 ± 55%	1455 ±100%	803 ± 70%	232 ± 4%	438 ± 36%	7518 ± 11%
7 3 39 31 45	716 ± 39%	362 ± 35%	1414 ± 40%	169 ± 14%	179 ± 41%	120 ± 21%	201 ± 50%	2145 ± 31%
8 2 14 9 35	6569 ± 19%	11025 ± 8%	8728 ± 8%	3897 ± 19%	7550 ± 7%	4948 ± 9%	4912 ± 22%	1063 ± 64%

HOBEN ONE-WAY MLC (HETEROLOGOUS SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 30

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	1.00	6.05	3.34	0.99	5.08	1.64	3.01	8.00
2 3 39 31 45	0.71	1.00	1.50	0.90	0.71	0.15	0.39	2.09
3 11 18 31 45	2.88	8.14	1.00	0.71	5.29	1.17	2.43	17.84
4 11 18 11 51	1.66	7.46	3.86	1.00	4.32	1.80	2.41	10.42
5 3 39 31 45	4.82	2.71	9.21	4.19	1.00	0.77	1.02	20.58
6 3 39 31 45	9.38	10.38	16.23	6.27	3.46	1.00	1.89	32.40
7 3 39 31 45	3.56	1.80	7.03	0.84	0.89	0.60	1.00	10.67
8 2 14 9 35	6.18	10.37	8.21	3.67	7.10	4.65	4.62	1.00

HOBEN ONE-WAY MLC (HETEROLOGOUS SERUM) 3

STIMULATION INDEX

TABLE 31

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
1 11 51 31 45	12857 ± 25%	16446 ± 13%	8061 ± 9%	5901 ± 16%	16774 ± 20%	17153 ± 19%	10865 ± 9%	21811 ± 31%
2 3 39 31 45	18135 ± 11%	15437 ± 15%	12778 ± 11%	9422 ± 5%	11752 ± 12%	13737 ± 13%	8195 ± 25%	18586 ± 9%
3 11 18 31 45	8881 ± 15%	10932 ± 11%	400 ± 19%	2533 ± 12%	9329 ± 9%	10989 ± 7%	3912 ± 11%	17021 ± 7%
4 11 18 11 51	4786 ± 4%	8221 ± 20%	1438 ± 66%	642 ± 24%	4962 ± 19%	7978 ± 13%	3857 ± 6%	11292 ± 26%
5 3 39 31 45	14110 ± 18%	7241 ± 4%	7369 ± 6%	5778 ± 34%	1730 ± 112%	2945 ± 72%	1223 ± 15%	19527 ± 3%
6 3 39 31 45	13964 ± 28%	6620 ± 4%	9006 ± 26%	8416 ± 5%	2829 ± 28%	4279 ± 51%	1441 ± 47%	17547 ± 6%
7 3 39 31 45	10139 ± 16%	7721 ± 12%	5459 ± 32%	2860 ± 5%	1416 ± 84%	1959 ± 39%	423 ± 26%	12021 ± 41%
8 2 14 9 35	20858 ± 12%	6782 ± 32%	14942 ± 39%	13271 ± 22%	17858 ± 7%	19816 ± 4%	14678 ± 26%	7497 ± 21%

HOBEN TWO-WAY MLC 4

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 32

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%
1 11 51 31 45	291 ± 11%	334 ± 4%	330 ± 2%	300 ± 4%	319 ± 5%	323 ± 5%	318 ± 3%	349 ± 1%
2 3 39 31 45	282 ± 33%	332 ± 1%	328 ± 10%	329 ± 5%	307 ± 2%	304 ± 3%	302 ± 8%	332 ± 8%
3 11 18 31 45	280 ± 27%	296 ± 10%	265 ± 7%	279 ± 5%	276 ± 5%	278 ± 3%	265 ± 5%	298 ± 15%
4 11 18 11 51	235 ± 21%	275 ± 9%	286 ± 4%	290 ± 9%	262 ± 3%	275 ± 5%	271 ± 4%	271 ± 6%
5 3 39 31 45	270 ± 20%	275 ± 9%	285 ± 2%	280 ± 4%	277 ± 10%	282 ± 5%	268 ± 6%	327 ± 5%
6 3 39 31 45	260 ± 25%	288 ± 5%	293 ± 2%	297 ± 5%	280 ± 4%	279 ± 3%	273 ± 9%	307 ± 6%
7 3 39 31 45	268 ± 25%	265 ± 10%	284 ± 6%	298 ± 2%	290 ± 5%	288 ± 1%	280 ± 6%	318 ± 7%
8 2 14 9 35	280 ± 20%	325 ± 3%	345 ± 3%	328 ± 8%	317 ± 7%	310 ± 2%	281 ± 10%	283 ± 4%

HOBEN ONE-WAY CML (PROPOSITUS) 6.

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 33

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
MAXIMUM RELEASE	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%	453 ± 19%
1 11 51 31 45	0.6%	27.0%	24.5%	6.1%	17.8%	20.2%	17.2%	36.2%
2 3 39 31 45	-4.9%	25.8%	23.3%	23.9%	10.4%	8.6%	7.4%	25.8%
3 11 18 31 45	-6.1%	3.7%	-15.3%	-6.8%	-8.6%	-7.4%	-15.3%	4.9%
4 11 18 11 51	-33.7%	-9.2%	-2.5%	0.0%	-17.2%	-9.2%	-11.7%	-11.7%
5 3 39 31 45	-12.3%	-9.2%	-3.1%	-6.1%	-8.0%	-4.9%	-13.5%	22.7%
6 3 39 31 45	-18.4%	-1.2%	1.8%	4.3%	-6.1%	-6.8%	-10.4%	10.4%
7 3 39 31 45	-13.5%	-15.3%	-3.7%	4.9%	-0.0%	-1.2%	-6.1%	17.2%
8 2 14 9 35	-6.1%	21.5%	33.7%	23.3%	16.6%	12.3%	-5.5%	-4.3%

HOBEN ONE-WAY CML (PROPOSITUS) 6

SPECIFIC RELEASE%

TABLE 34

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%
1 11 51 31 45	511 ± 12%	582 ± 7%	625 ± 7%	519 ± 3%	525 ± 11%	597 ± 3%	551 ± 6%	641 ± 10%
2 3 39 31 45	582 ± 31%	601 ± 5%	616 ± 14%	575 ± 3%	517 ± 8%	567 ± 5%	509 ± 4%	877 ± 38%
3 11 18 31 45	536 ± 17%	578 ± 7%	476 ± 3%	475 ± 3%	525 ± 8%	478 ± 9%	463 ± 2%	579 ± 9%
4 11 18 11 51	471 ± 40%	764 ± 17%	497 ± 7%	502 ± 9%	597 ± 4%	601 ± 22%	543 ± 14%	694 ± 6%
5 3 39 31 45	609 ± 23%	489 ± 4%	491 ± 3%	472 ± 6%	473 ± 8%	467 ± 5%	442 ± 14%	610 ± 15%
6 3 39 31 45	434 ± 27%	485 ± 2%	508 ± 3%	523 ± 21%	484 ± 5%	468 ± 6%	501 ± 12%	530 ± 5%
7 3 39 31 45	488 ± 20%	457 ± 10%	488 ± 6%	520 ± 2%	497 ± 3%	458 ± 3%	474 ± 8%	531 ± 3%
8 2 14 9 35	616 ± 42%	830 ± 7%	834 ± 17%	617 ± 6%	710 ± 30%	647 ± 1%	557 ± 2%	465 ± 2%

HOBEN ONE-WAY CML (MOTHER) 6

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 35

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
MAXIMUM RELEASE	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%	1603 ± 13%
1 11 51 31 45	0.0%	6.5%	10.4%	0.7%	1.3%	7.9%	3.7%	11.9%
2 3 39 31 45	6.5%	8.2%	9.6%	5.9%	0.5%	5.1%	-0.2%	33.5%
3 11 18 31 45	2.3%	6.1%	-3.2%	-3.3%	1.3%	-3.0%	-4.4%	6.2%
4 11 18 11 51	-3.7%	23.2%	-1.3%	-0.8%	7.9%	8.2%	2.9%	16.8%
5 3 39 31 45	9.0%	-3.8%	-1.8%	-3.6%	-3.5%	-4.0%	-6.3%	9.1%
6 3 39 31 45	-7.0%	-2.4%	-0.3%	1.1%	-2.5%	-3.9%	-0.9%	1.7%
7 3 39 31 45	-2.1%	-4.9%	-2.1%	0.8%	-1.3%	-4.9%	-3.4%	1.8%
8 2 14 9 35	9.6%	29.2%	29.6%	9.7%	18.2%	12.5%	4.2%	-4.2%

HOBEN ONE-WAY CML (MOTHER) 6

SPECIFIC RELEASE%

TABLE 36

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%
1 11 51 31 45	284 ± 20%	341 ± 12%	310 ± 3%	300 ± 1%	325 ± 3%	326 ± 11%	309 ± 2%	369 ± 3%
2 3 39 31 45	292 ± 22%	335 ± 5%	352 ± 5%	356 ± 1%	318 ± 4%	315 ± 4%	297 ± 2%	352 ± 8%
3 11 18 31 45	286 ± 19%	320 ± 7%	279 ± 3%	295 ± 4%	332 ± 15%	278 ± 2%	307 ± 3%	375 ± 12%
4 11 18 11 51	288 ± 21%	359 ± 10%	310 ± 9%	304 ± 5%	272 ± 17%	285 ± 4%	307 ± 4%	343 ± 6%
5 3 39 31 45	315 ± 24%	273 ± 7%	314 ± 2%	298 ± 8%	291 ± 5%	289 ± 1%	291 ± 4%	315 ± 11%
6 3 39 31 45	252 ± 24%	287 ± 2%	281 ± 4%	289 ± 1%	285 ± 2%	271 ± 2%	282 ± 1%	285 ± 4%
7 3 39 31 45	326 ± 33%	257 ± 5%	265 ± 3%	280 ± 2%	253 ± 4%	265 ± 10%	255 ± 3%	288 ± 5%
8 2 14 9 35	323 ± 5%	334 ± 4%	276 ± 32%	301 ± 2%	314 ± 3%	310 ± 7%	278 ± 6%	234 ± 6%

HOBEN ONE-WAY CMI (SIB 3) 8

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 37

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
MAXIMUM RELEASE	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%	456 ± 17%
1 11 51 31 45	2.8%	35.0%	17.5%	11.9%	26.0%	26.6%	16.9%	50.8%
2 3 39 31 45	7.4%	31.6%	41.2%	43.5%	22.0%	20.3%	10.2%	41.2%
3 11 18 31 45	4.0%	23.2%	0.0%	9.0%	29.9%	-0.6%	15.8%	54.2%
4 11 18 11 51	-5.1%	45.2%	17.5%	14.1%	-3.9%	3.4%	15.8%	36.2%
5 3 39 31 45	20.3%	-3.4%	19.8%	10.7%	6.8%	5.6%	6.8%	20.3%
6 3 39 31 45	-15.2%	4.5%	1.1%	5.6%	3.4%	-4.5%	1.7%	3.4%
7 3 39 31 45	26.6%	-12.4%	-7.9%	0.6%	-14.7%	-7.9%	-13.6%	5.1%
8 2 14 9 35	24.9%	31.1%	-1.7%	12.4%	19.8%	17.5%	-0.6%	-25.4%

HOBEN ONE-WAY CML (SIB 3) 8
SPECIFIC RELEASE%

TABLE 38

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	1644 ± 13%	1055 ± 6%	492 ± 17%	477 ± 19%	1059 ± 6%	1066 ± .5%	37 ± 15%	1198 ± 13%
1 11 51 31 45	1278 ± 21%	1484 ± 15%	956 ± 15%	1147 ± 7%	1297 ± 15%	1401 ± 13%	753 ± 3%	1842 ± 8%
2 3 39 31 45	631 ± 11%	479 ± 5%	403 ± 18%	329 ± 11%	472 ± 12%	342 ± 11%	32 ± 28%	1032 ± 7%
3 11 18 31 45	603 ± 6%	589 ± 22%	302 ± 1%	326 ± 1%	473 ± 7%	370 ± 4%	32 ± 4%	986 ± 10%
4 11 18 11 51	595 ± 4%	613 ± 16%	323 ± 5%	328 ± 3%	462 ± 8%	357 ± 8%	36 ± 5%	980 ± 3%
5 3 39 31 45	562 ± 3%	423 ± 7%	326 ± 4%	319 ± 7%	437 ± 3%	316 ± 1%	25 ± 13%	1015 ± 1%
6 3 39 31 45	593 ± 1%	413 ± 3%	305 ± 2%	326 ± 9%	426 ± 3%	304 ± 4%	31 ± 22%	910 ± 6%
7 3 39 31 45	597 ± 2%	437 ± 6%	317 ± 3%	332 ± 2%	438 ± 3%	318 ± 8%	33 ± 12%	922 ± 1%
8 2 14 9 35	835 ± 4%	655 ± 10%	404 ± 8%	373 ± 4%	648 ± 4%	454 ± 20%	30 ± 8%	873 ± 3%

HOBEN ONE-WAY CML 5

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 39

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
MAXIMUM RELEASE	1644 ± 13%	1055 ± 6%	492 ± 17%	477 ± 19%	1059 ± 6%	1066 ± 5%	37 ± 15%	1198 ± 13%
1 11 51 31 45	0.0%	174.5%	344.2%	549.6%	138.3%	144.0%	17987.4%	298.1%
2 3 39 31 45	-0.8%	0.0%	53.2%	0.7%	5.6%	5.0%	-24.6%	48.9%
3 11 18 31 45	-3.6%	19.1%	0.0%	-1.3%	5.8%	8.7%	-25.2%	34.8%
4 11 18 11 51	-4.4%	23.3%	11.1%	0.0%	4.0%	7.0%	-74.8%	32.9%
5 3 39 31 45	-7.7%	-9.7%	12.6%	-6.0%	0.0%	1.6%	-199.8%	43.7%
6 3 39 31 45	-4.6%	-11.5%	1.6%	-1.3%	-1.8%	0.0%	-49.7%	11.4%
7 3 39 31 45	-4.2%	-7.3%	7.9%	2.7%	0.2%	1.8%	0.0%	15.1%
8 2 14 9 35	19.5%	30.6%	53.7%	30.2%	33.9%	19.7%	-75.1%	0.0%

HOBEN ONE-WAY CML 5

SPECIFIC RELEASE%

TABLE 40

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	1488 ± 13%	955 ± 6%	445 ± 17%	432 ± 19%	959 ± 6%	965 ± 5%	34 ± 15%	1084 ± 13%
1 11 51 31 45	1282 ± 11%	1277 ± 3%	1076 ± 10%	982 ± 7%	1447 ± 12%	1225 ± 13%	733 ± 4%	1823 ± 15%
2 3 39 31 45	678 ± 10%	542 ± 10%	373 ± 1%	340 ± 3%	710 ± 8%	341 ± 2%	33 ± 9%	855 ± 25%
3 11 18 31 45	649 ± 3%	612 ± 8%	272 ± 4%	295 ± 3%	831 ± 2%	414 ± 12%	39 ± 49%	928 ± 13%
4 11 18 11 51	573 ± 1%	483 ± 3%	289 ± 8%	278 ± 5%	734 ± 9%	334 ± 23%	33 ± 17%	887 ± 8%
5 3 39 31 45	624 ± 6%	488 ± 1%	321 ± 6%	303 ± 6%	429 ± 7%	317 ± 4%	36 ± 20%	826 ± 6%
6 3 39 31 45	621 ± 3%	462 ± 3%	304 ± 3%	296 ± 4%	436 ± 2%	293 ± 4%	25 ± 29%	851 ± 4%
7 3 39 31 45	612 ± 3%	494 ± 7%	304 ± 10%	290 ± 10%	455 ± 11%	300 ± 3%	39 ± 44%	863 ± 5%
8 2 14 9 35	550 ± 28%	471 ± 31%	301 ± 25%	267 ± 31%	453 ± 39%	379 ± 36%	28 ± 39%	658 ± 25%

HOBEN TWO-WAY CML 9

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 41

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
MAXIMUM RELEASE	1488 ± 13%	955 ± 6%	445 ± 17%	432 ± 19%	959 ± 6%	965 ± 5%	34 ± 15%	1084 ± 13%
1 11 51 31 45	0.0%	178.0%	464.7%	457.1%	192.1%	138.7%	-13799.9%	273.5%
2 3 39 31 45	4.4%	0.0%	58.4%	40.3%	53.0%	7.1%	120.0%	46.2%
3 11 18 31 45	0.9%	16.9%	0.0%	11.0%	75.8%	18.0%	-0.1%	63.4%
4 11 18 11 51	-8.0%	-14.3%	9.8%	0.0%	57.5%	6.1%	119.8%	53.8%
5 3 39 31 45	-2.0%	-13.1%	28.3%	16.2%	0.0%	3.6%	60.1%	39.4%
6 3 39 31 45	-2.4%	-19.4%	18.5%	11.7%	1.3%	0.0%	278.7%	45.3%
7 3 39 31 45	-3.4%	-11.6%	18.5%	7.8%	4.9%	1.0%	0.0%	48.1%
8 2 14 9 35	-10.7%	-17.2%	16.8%	-7.1%	4.5%	12.8%	218.8%	0.0%

HOBEN TWO-WAY CML 9

SPECIFIC RELEASE%

TABLE 42

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	1451 ± 13%	931 ± 6%	434 ± 17%	421 ± 19%	935 ± 6%	941 ± 5%	33 ± 15%	1058 ± 13%
1 11 51 31 45	1214 ± 18%	1405 ± 9%	1021 ± 7%	993 ± 6%	1090 ± 25%	1433 ± 23%	765 ± 2%	1843 ± 6%
2 3 39 31 45	618 ± 13%	436 ± 6%	312 ± 11%	309 ± 8%	326 ± 18%	346 ± 11%	27 ± 12%	866 ± 12%
3 11 18 31 45	513 ± 6%	492 ± 9%	271 ± 4%	251 ± 3%	315 ± 13%	343 ± 9%	26 ± 14%	845 ± 8%
4 11 18 11 51	504 ± 6%	413 ± 5%	269 ± 8%	274 ± 4%	268 ± 8%	324 ± 10%	39 ± 40%	815 ± 17%
5 3 39 31 45	529 ± 5%	373 ± 6%	279 ± 2%	277 ± 2%	274 ± 6%	318 ± 10%	34 ± 13%	869 ± 4%
6 3 39 31 45	477 ± 2%	371 ± 9%	272 ± 5%	270 ± 5%	261 ± 4%	294 ± 4%	27 ± 25%	802 ± 4%
7 3 39 31 45	478 ± 2%	386 ± 6%	288 ± 5%	252 ± 9%	259 ± 11%	322 ± 13%	30 ± 11%	875 ± 9%
8 2 14 9 35	793 ± 10%	687 ± 8%	372 ± 5%	307 ± 6%	393 ± 1%	538 ± 4%	31 ± 29%	700 ± 4%

HOBEN AUTOLOGOUS ADCC (CML) 10

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 43

HLA TYPE	1 11 51 31 45	2 3 39 31 45	3 11 18 31 45	4 11 18 11 51	5 3 39 31 45	6 3 39 31 45	7 3 39 31 45	8 2 14 9 35
MAXIMUM RELEASE	1451 ± 13%	931 ± 6%	434 ± 17%	421 ± 19%	935 ± 6%	941 ± 5%	33 ± 15%	1058 ± 13%
1 11 51 31 45	0.0%	195.8%	460.1%	489.1%	123.5%	176.0%	24465.8%	319.3%
2 3 39 31 45	1.3%	0.0%	25.2%	23.8%	7.9%	8.0%	-99.8%	46.4%
3 11 18 31 45	-11.1%	11.3%	0.0%	-15.6%	6.2%	7.6%	-133.0%	40.5%
4 11 18 11 51	-12.2%	-4.6%	-1.2%	0.0%	-0.9%	4.6%	300.5%	32.1%
5 3 39 31 45	-9.2%	-12.7%	4.9%	2.0%	0.0%	3.7%	133.2%	47.2%
6 3 39 31 45	-15.4%	-13.1%	0.6%	-2.7%	-2.0%	0.0%	-99.4%	28.5%
7 3 39 31 45	-15.3%	-10.1%	10.4%	-15.0%	-2.3%	4.3%	0.0%	48.9%
8 2 14 9 35	22.0%	50.7%	62.0%	22.4%	18.0%	37.7%	33.9%	0.0%

HOBEN AUTOLOGOUS ADCC (CML) 10
SPECIFIC RELEASE%

TABLE 44

	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	1415 ± 13%	908 ± 6%	423 ± 17%	411 ± 19%	912 ± 6%	918 ± 5%	32 ± 15%	1032 ± 13%
1 11 51 31 45	1063 ± 1%	1363 ± 14%	831 ± 18%	875 ± 7%	1037 ± 12%	1117 ± 15%	660 ± 2%	1527 ± 13%
2 3 39 31 45	724 ± 14%	385 ± 3%	280 ± 14%	271 ± 4%	319 ± 19%	318 ± 7%	31 ± 10%	906 ± 3%
3 11 18 31 45	550 ± 6%	442 ± 5%	264 ± 6%	268 ± 2%	312 ± 6%	309 ± 6%	34 ± 20%	815 ± 6%
4 11 18 11 51	538 ± 3%	447 ± 10%	279 ± 9%	267 ± 4%	313 ± 6%	323 ± 2%	30 ± 11%	830 ± 4%
5 3 39 31 45	540 ± 6%	403 ± 6%	275 ± 7%	274 ± 11%	266 ± 4%	303 ± 2%	26 ± 15%	788 ± 3%
6 3 39 31 45	541 ± 6%	392 ± 4%	256 ± 5%	260 ± 3%	261 ± 2%	301 ± 5%	25 ± 8%	764 ± 6%
7 3 39 31 45	549 ± 5%	388 ± 4%	256 ± 4%	269 ± 3%	268 ± 10%	298 ± 3%	30 ± 14%	763 ± 3%
8 2 14 9 35	750 ± 32%	514 ± 27%	294 ± 35%	240 ± 26%	290 ± 51%	405 ± 17%	22 ± 34%	617 ± 23%

HOBEN HETEROLOGOUS ADCC (CML) 11

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 45

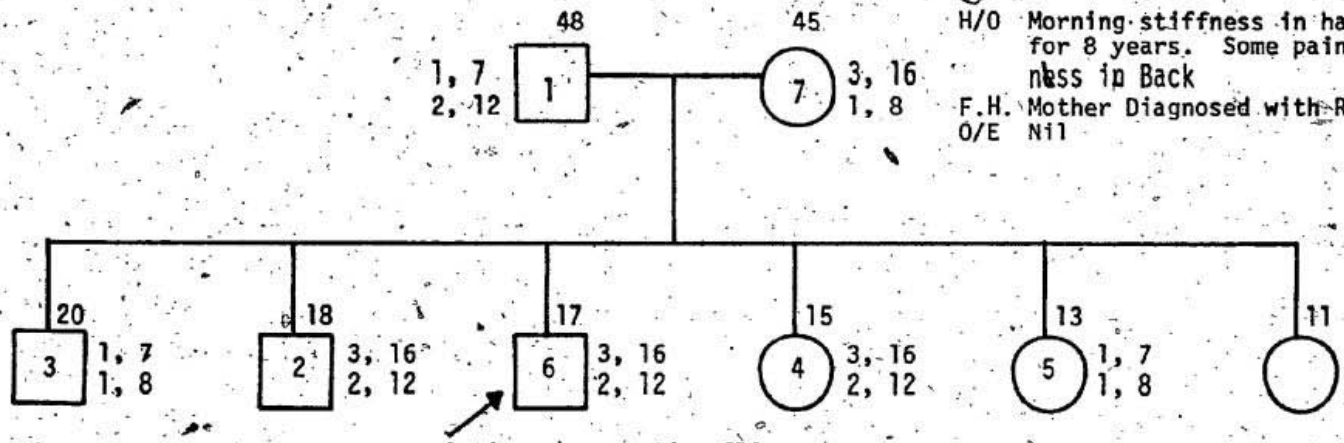
	1	2	3	4	5	6	7	8
HLA TYPE	11 51 31 45	3 39 31 45	11 18 31 45	11 18 11 51	3 39 31 45	3 39 31 45	3 39 31 45	2 14 9 35
MAXIMUM RELEASE	1415 ± 13%	908 ± 6%	423 ± 17%	411 ± 19%	912 ± 6%	918 ± 5%	32 ± 15%	1022 ± 13%
1 11 51 31 45	0.0%	187.0%	356.6%	422.2%	119.4%	132.3%	31481.1%	219.3%
2 3 39 31 45	21.7%	0.0%	10.1%	2.8%	8.2%	2.8%	49.8%	69.6%
3 11 18 31 45	2.0%	10.9%	0.0%	0.7%	7.1%	1.3%	200.2%	47.7%
4 11 18 11 51	0.7%	11.9%	9.4%	0.0%	7.3%	3.6%	-0.1%	51.3%
5 3 39 31 45	0.9%	3.4%	6.9%	4.9%	0.0%	0.3%	-199.8%	41.2%
6 3 39 31 45	1.0%	1.3%	-5.0%	-4.9%	-0.8%	0.0%	-250.2%	35.4%
7 3 39 31 45	1.9%	0.6%	-5.0%	1.4%	0.3%	-0.5%	0.0%	35.2%
8 2 14 9 35	24.7%	24.7%	18.9%	-18.7%	3.7%	16.9%	-398.8%	0.0%

HCBEN HETEROLOGOUS ADCC (CML) 11

SPECIFIC RELEASE%

TABLE 46

BEST FAMILY



H/O Morning stiffness in hands for 8 years. Some pain/stiffness in Back
F.H. Mother Diagnosed with R.A.
O/E Nil

Active seronegative JRA
Controlled by Steroids (3 yrs ago 2.5 mgs Prenisone - alternate days)
Onset aet 3 years - Polyarticular
FTND but Diarrhoea +++ in Infancy
O/E Cushingoid
Restricted mobility - cervical spine, shoulders, elbows, wrists, forefeet
No evidence of clinically active R.A.
He has proved very difficult to tail Steroid dose

FIGURE 17

HLA TYPE		1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
1	2 12 1 7	1503 ± 31%	5843 ± 12%	1878 ± 30%	1054 ± 46%	1456 ± 22%	6035 ± 12%	3327 ± 6%	8302 ± 26%
2	2 12 3 16	4317 ± 27%	3373 ± 24%	4654 ± 12%	770 ± 19%	2517 ± 16%	1693 ± 15%	3602 ± 17%	7381 ± 15%
3	1 8 1 7	1613 ± 33%	2502 ± 9%	803 ± 5%	527 ± 9%	944 ± 23%	3841 ± 4%	1621 ± 16%	3947 ± 26%
4	2 12 3 16	421 ± 31%	306 ± 15%	392 ± 18%	409 ± 25%	326 ± 23%	315 ± 22%	272 ± 33%	567 ± 2%
5	1 8 1 7	2251 ± 4%	4245 ± 3%	470 ± 6%	618 ± 34%	357 ± 2%	3570 ± 8%	852 ± 9%	3177 ± 13%
6	2 12 3 16	2602 ± 4%	2036 ± 18%	2734 ± 27%	736 ± 33%	2260 ± 6%	1695 ± 11%	1677 ± 20%	6301 ± 8%
7	1 8 3 16	1515 ± 25%	1577 ± 15%	828 ± 14%	476 ± 19%	445 ± 16%	1086 ± 10%	426 ± 13%	1422 ± 21%
8	1 17 28 35	2044 ± 27%	3004 ± 14%	1831 ± 13%	1176 ± 31%	1707 ± 15%	2872 ± 13%	2336 ± 18%	1923 ± 35%

BEST MLC (PCOLED SERUM) 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 47

HLA TYPE		1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
1	2 12 1 7	1.00	3.89	1.25	0.70	0.97	4.02	2.21	5.52
2	2 12 3 16	1.28	1.00	1.38	0.23	0.75	0.50	1.07	2.19
3	1 8 1 7	2.01	3.12	1.00	0.66	1.18	4.78	2.02	4.92
4	2 12 3 16	1.03	0.75	0.96	1.00	0.80	0.77	0.67	1.39
5	1 8 1 7	6.31	11.89	1.32	1.73	1.00	10.00	2.39	8.90
6	2 12 3 16	1.54	1.20	1.61	0.43	1.33	1.00	0.99	3.72
7	1 8 3 16	3.56	3.70	1.94	1.12	1.04	2.55	1.00	3.34
8	1 17 28 35	1.06	1.56	0.95	0.61	0.89	1.49	1.21	1.00

BEST MLC (POOLED SERUM) 1

STIMULATION INDEX

TABLE 48

HLA TYPE		1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
1	2 12 1 7	973 ± 87%	5274 ± 9%	1541 ± 3%	1261 ± 11%	2269 ± 13%	7395 ± 20%	4131 ± 20%	10347 ± 9%
2	2 12 3 16	4059 ± 14%	3145 ± 13%	3275 ± 17%	867 ± 19%	4043 ± 12%	1994 ± 24%	3379 ± 17%	5920 ± 11%
3	1 8 1 7	2006 ± 13%	2644 ± 8%	712 ± 3%	499 ± 4%	1317 ± 25%	3228 ± 26%	2739 ± 14%	3430 ± 13%
4	2 12 3 16	384 ± 14%	361 ± 13%	323 ± 14%	390 ± 17%	288 ± 14%	307 ± 4%	253 ± 17%	682 ± 11%
5	1 8 1 7	2357 ± 12%	5100 ± 9%	529 ± 14%	532 ± 26%	412 ± 19%	5379 ± 5%	1473 ± 11%	4699 ± 11%
6	2 12 3 16	3127 ± 17%	1566 ± 10%	3305 ± 9%	668 ± 16%	3810 ± 17%	1624 ± 25%	2727 ± 18%	7471 ± 6%
7	1 8 3 16	526 ± 14%	836 ± 1%	716 ± 91%	380 ± 14%	362 ± 14%	819 ± 7%	310 ± 17%	899 ± 20%
8	1 17 28 35	2301 ± 15%	2352 ± 12%	2445 ± 10%	1222 ± 7%	3272 ± 24%	3404 ± 34%	3612 ± 15%	2377 ± 7%

BEST MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 49

HLA TYPE		1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
1	2 12 1 7	1.00	5.42	1.58	1.30	2.33	7.60	4.25	10.63
2	2 12 3 16	1.29	1.00	1.04	0.28	1.29	0.63	1.07	1.88
3	1 8 1 7	2.82	3.71	1.00	0.70	1.85	4.53	3.85	4.82
4	2 12 3 16	0.98	0.93	0.83	1.00	0.74	0.79	0.65	1.75
5	1 8 1 7	5.72	12.38	1.28	1.29	1.00	13.06	3.58	11.40
6	2 12 3 16	1.93	0.96	2.04	0.41	2.35	1.00	1.68	4.60
7	1 8 3 16	1.70	2.70	2.31	1.23	1.17	2.64	1.00	2.90
8	1 17 28 35	0.97	0.99	1.03	0.51	1.38	1.43	1.52	1.00

BEST MLC (AUTOLOGOUS SERUM) 2

STIMULATION INDEX

TABLE 50

	HLA TYPE	1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
1	2 12 1 7	5037 ± 9%	8995 ± 13%	5555 ± 26%	1840 ± 15%	4710 ± 11%	8865 ± 9%	5030 ± 43%	10670 ± 7%
2	2 12 3 16	6499 ± 2%	4477 ± 21%	6975 ± 15%	1005 ± 20%	7567 ± 18%	3488 ± 4%	3242 ± 21%	8058 ± 7%
3	1 8 1 7	5277 ± 19%	6568 ± 13%	1066 ± 16%	644 ± 25%	1201 ± 12%	6966 ± 8%	3145 ± 5%	9508 ± 8%
4	2 12 3 16	1807 ± 31%	1226 ± 32%	448 ± 11%	304 ± 11%	700 ± 8%	621 ± 32%	470 ± 18%	2982 ± 20%
5	1 8 1 7	3730 ± 11%	5201 ± 1%	1473 ± 16%	687 ± 23%	493 ± 14%	5747 ± 4%	1463 ± 19%	5643 ± 19%
6	2 12 3 16	6689 ± 6%	4508 ± 26%	7316 ± 3%	719 ± 7%	5974 ± 8%	2773 ± 4%	3589 ± 11%	7319 ± 11%
7	1 8 3 16	4906 ± 23%	5196 ± 4%	2658 ± 1%	460 ± 11%	1672 ± 14%	2597 ± 9%	494 ± 19%	3570 ± 18%
8	1 17 28 35	7676 ± 16%	8832 ± 18%	10122 ± 0%	2426 ± 32%	6266 ± 16%	9244 ± 14%	5349 ± 23%	3717 ± 34%

BEST TWO-WAY MLC (POOLED SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 51

HLA TYPE		1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
1	2 12 1 7	5024 ± 5%	10296 ± 4%	5628 ± 22%	1553 ± 3%	5320 ± 12%	11101 ± 9%	5251 ± 25%	8975 ± 21%
2	2 12 3 16	7075 ± 8%	8347 ± 11%	6602 ± 26%	765 ± 20%	8288 ± 9%	5147 ± 24%	5758 ± 2%	8341 ± 4%
3	1 8 1 7	5593 ± 24%	5995 ± 22%	1922 ± 11%	739 ± 18%	2474 ± 15%	7827 ± 9%	3977 ± 22%	7175 ± 14%
4	2 12 3 16	2140 ± 19%	1615 ± 32%	675 ± 26%	325 ± 3%	854 ± 11%	1103 ± 43%	544 ± 17%	3526 ± 16%
5	1 8 1 7	5163 ± 2%	7236 ± 8%	2214 ± 13%	1064 ± 17%	937 ± 28%	8168 ± 7%	2780 ± 7%	7762 ± 12%
6	2 12 3 16	6423 ± 15%	4846 ± 23%	6308 ± 23%	801 ± 41%	6763 ± 22%	4416 ± 22%	3568 ± 8%	6931 ± 7%
7	1 8 3 16	2505 ± 9%	3134 ± 10%	1433 ± 5%	357 ± 21%	892 ± 10%	2106 ± 13%	361 ± 13%	3047 ± 22%
8	1 17 28 35	6129 ± 16%	6549 ± 16%	8207 ± 13%	2267 ± 35%	5591 ± 32%	3833 ± 1%	4922 ± 30%	5989 ± 20%

BEST TWO-WAY MLC (AUTOLOGOUS SERUM) 4

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 52

HLA TYPE	1 2 12 1 7	2 1 8 1 7	3 2 12 3 16	4 1 8 1 7	5 2 12 3 16	6 1 17 28 35	7 1 8 3 16
1 2 12 3 16	488 ± 10%	504 ± 8%	363 ± 6%	413 ± 9%	405 ± 14%	507 ± 7%	360 ± 7%
2 1 8 1 7	443 ± 14%	395 ± 43%	428 ± 12%	450 ± 28%	441 ± 6%	541 ± 0%	360 ± 5%

36 HOURS

3 2 12 3 16	1738 ± 8%	2709 ± 8%	418 ± 18%	1827 ± 13%	735 ± 7%	2662 ± 9%	968 ± 23%
4 1 8 1 7	1184 ± 14%	546 ± 15%	359 ± 22%	382 ± 2%	1858 ± 17%	1878 ± 9%	475 ± 3%

BEST TWO-WAY MLC (36 - 80HR.) 5

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 53

HLA TYPE	1 2 12 1 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 1 16	8 1 17 28 35
MAXIMUM RELEASE	612 ± 4%	210 ± 8%	216 ± 5%	36 ± 13%	310 ± 5%	932 ± 3%	214 ± 5%	183 ± 4%
1 2 12 1 7	225 ± 4%	119 ± 20%	137 ± 10%	23 ± 10%	99 ± 12%	530 ± 14%	82 ± 9%	88 ± 18%
2 2 12 3 16	218 ± 5%	76 ± 5%	109 ± 8%	17 ± 11%	96 ± 8%	325 ± 4%	76 ± 9%	75 ± 20%
3 1 8 1 7	580 ± 10%	202 ± 9%	81 ± 10%	30 ± 14%	113 ± 4%	825 ± 3%	110 ± 16%	171 ± 4%
4 2 12 3 16	232 ± 5%	70 ± 14%	92 ± 19%	20 ± 14%	115 ± 4%	362 ± 3%	65 ± 17%	74 ± 6%
5 1 8 1 7	248 ± 12%	69 ± 10%	80 ± 4%	40 ± 55%	90 ± 9%	344 ± 7%	71 ± 14%	67 ± 7%
6 2 12 3 16	200 ± 2%	63 ± 16%	124 ± 6%	17 ± 51%	89 ± 12%	316 ± 3%	68 ± 11%	68 ± 6%
7 1 8 3 16	227 ± 8%	80 ± 14%	81 ± 12%	15 ± 11%	97 ± 12%	345 ± 10%	64 ± 7%	68 ± 5%
8 1 17 28 35	332 ± 1%	93 ± 5%	123 ± 9%	22 ± 40%	110 ± 4%	454 ± 12%	91 ± 15%	74 ± 9%

BEST TWO-WAY CML (ADCC) 6

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 54

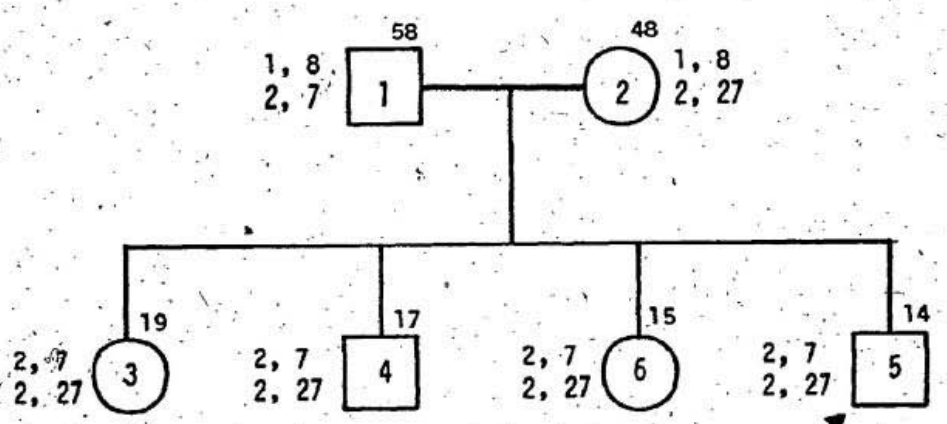
	HLA TYPE	1 2 12 2 7	2 2 12 3 16	3 1 8 1 7	4 2 12 3 16	5 1 8 1 7	6 2 12 3 16	7 1 8 3 16	8 1 17 28 35
	MAXIMUM RELEASE	612 ± 4%	210 ± 8%	216 ± 5%	36 ± 13%	310 ± 5%	932 ± 3%	214 ± 5%	183 ± 4%
1	2 12 1 7	0.0%	32.1%	41.5%	18.7%	4.1%	34.7%	12.0%	12.9%
2	2 12 3 16	-1.8%	0.0%	20.7%	-18.8%	2.7%	1.5%	8.0%	0.9%
3	1 8 1 7	91.7%	94.0%	0.0%	62.5%	10.5%	82.6%	30.7%	89.0%
4	2 12 3 16	1.8%	-4.5%	8.2%	0.0%	11.4%	7.5%	0.7%	-0.0%
5	1 8 1 7	5.9%	-5.2%	-0.7%	125.3%	0.0%	4.5%	4.7%	-6.4%
6	2 12 3 16	-6.5%	-9.7%	31.9%	-18.5%	-0.5%	0.0%	2.7%	-5.5%
7	1 8 3 16	0.5%	3.0%	0.0%	-31.3%	3.2%	4.7%	0.0%	-5.5%
8	1 17 28 35	27.6%	12.7%	31.1%	12.7%	9.1%	22.4%	18.1%	0.0%

BEST TWO-WAY CML (ADCC) 6

SPECIFIC RELEASE%

TABLE 55

FREAKE FAMILY



7 yr H/O Painful + Swollen
R knee
Seronegative, non-nodular
Now in remission following
Synovectomy

Δ Pauci-Articular Presentation
In view of HLA; ?Juvenile Aky-
losing Spondylitis.

FIGURE 18

HLA TYPE	1 2 7 1 8	2 2 27 1 8	3 2 27 2 7	4 2 27 2 7	5 2 27 2 7	6 2 27 2 7
1 2 7 1 8	3890 ± 16%	14660 ± 7%	11453 ± 17%	10917 ± 14%	9365 ± 14%	8558 ± 12%
2 2 27 1 8	17550 ± 4%	7233 ± 11%	15609 ± 9%	16525 ± 10%	12220 ± 1%	9154 ± 86%
3 2 27 2 7	12129 ± 3%	11017 ± 4%	2961 ± 8%	3675 ± 37%	3180 ± 23%	2601 ± 21%
4 2 27 2 7	4755 ± 76%	10370 ± 6%	2705 ± 4%	2912 ± 7%	3168 ± 15¢	2799 ± 26%
5 2 27 2 7	8587 ± 8%	9098 ± 23%	3827 ± 7%	4030 ± 12%	3093 ± 4%	3225 ± 9%
6 2 27 2 7	6472 ± 3%	5294 ± 3%	1656 ± 23%	1964 ± 29%	1181 ± 17%	935 ± 77%

FREAKE MLC (FOETAL CALF SERUM) 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 56

HLA TYPE	1 2 7 1 8	2 2 27 1 8	3 3 27 2 7	4 2 27 2 7	5 2 27 2 7	6 2 27 2 7
1 2 7 1 8	1.00	3.77	2.94	2.81	2.41	2.20
2 2 27 1 8	2.43	1.00	2.16	2.28	1.69	1.27
3 2 27 2 7	4.10	3.72	1.00	1.24	1.07	0.88
4 2 27 2 7	1.63	3.56	0.93	1.00	1.09	0.96
5 2 27 2 7	2.78	2.94	1.24	1.30	1.00	1.04
6 2 27 2 7	6.92	5.66	1.77	2.10	1.26	1.00

FREAKE MLC (FOETAL CALF SERUM) 1

STIMULATION INDEX

TABLE 57

HLA TYPE	1 2 7 1 8	2 2 27 1 8	3 2 27 2 7	4 2 27 2 7	5 2 27 2 7	6 2 27 2 7
1 2 7 1 8	1.00	4.56	3.69	4.30	3.07	3.22
2 2 27 1 8	2.49	1.00	2.25	2.46	1.91	1.94
3 2 27 2 7	4.37	4.39	1.00	1.15	0.55	0.98
4 2 27 2 7	7.14	6.65	1.27	1.00	0.86	1.03
5 2 27 2 7	8.84	6.72	1.98	1.03	1.00	1.14
6 2 27 2 7	7.61	7.50	0.99	1.63	0.90	1.00

FREAKE MLC (AUTOLOGOUS SERUM) 2

STIMULATION INDEX

TABLE 59

HLA TYPE		1 2 7 1 8	2 2 27 1 8	3 2 27 2 7	4 2 27 2 7	5 2 27 2 7	6 2 27 2 7
1	2 7 1 8	2846 ± 30%	12969 ± 10%	10509 ± 13%	12232 ± 7%	8743 ± 12%	9167 ± 11%
2	2 27 1 8	12159 ± 4%	4884 ± 10%	10989 ± 10%	12005 ± 11%	9310 ± 9%	9468 ± 21%
3	2 27 2 7	8435 ± 9%	8457 ± 9%	1928 ± 21%	2223 ± 34%	1051 ± 30%	1883 ± 50%
4	2 27 2 7	7579 ± 8%	7059 ± 14%	1349 ± 16%	1062 ± 62%	909 ± 36%	1096 ± 69%
5	2 27 2 7	7794 ± 6%	5931 ± 7%	1746 ± 44%	911 ± 18%	882 ± 32%	1004 ± 30%
6	2 27 2 7	5896 ± 7%	5814 ± 24%	768 ± 34%	1261 ± 40%	701 ± 19%	775 ± 38%

FREAKE MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 58.

HLA TYPE	1 2 7 1 8	2 2 27 1 8	3 2 27 2 7	4 2 27 2 7	5 2 27 2 7	6 2 27 2 7
1 2 7 1 8	3552 ± 23%	10743 ± 11%	10455 ± 13%	12936 ± 9%	6729 ± 5%	9416 ± 18%
2 2 27 1 8	13808 ± 14%	3505 ± 17%	10518 ± 5%	12559 ± 13%	7293 ± 13%	10978 ± 7%
3 2 27 2 7	8911 ± 10%	5305 ± 12%	1794 ± 30%	1515 ± 27%	1186 ± 45%	1498 ± 13%
4 2 27 2 7	8310 ± 19%	6179 ± 6%	1626 ± 23%	1641 ± 42%	716 ± 32%	1624 ± 17%
5 2 27 2 7	8974 ± 8%	6663 ± 26%	1222 ± 27%	2002 ± 33%	805 ± 16%	1253 ± 40%
6 2 27 2 7	6311 ± 7%	4099 ± 10%	751 ± 15%	1134 ± 37%	416 ± 51%	818 ± 52%

FREAKE MLC (HETEROLOGOUS SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 60

HLA TYPE		1 2 7 1 8	2 2 27 1 8	3 2 27 2 7	4 2 27 2 7	5 2 27 2 7	6 2 27 2 7
1	2 7 1 8	1.00	3.02	2.94	3.64	1.89	2.65
2	2 27 1 8	3.94	1.00	3.00	3.58	2.08	3.13
3	2 27 2 7	4.97	2.96	1.00	0.84	0.66	0.83
4	2 27 2 7	5.06	3.77	0.99	1.00	0.44	0.99
5	2 27 2 7	11.15	8.28	1.52	2.49	1.00	1.56
6	2 27 2 7	7.71	5.01	0.92	1.39	0.51	1.00

FREAKE MLC (HETEROLOGOUS SERUM) 3

STIMULATION INDEX

TABLE 61

HLA TYPE		1 31 27 3 35	2 31 27 28 40	3 31 27 31 27	4 2 40 2 40	5 2 12 28 40	6 31 27 2 40
1	31 27 3 35	366 ± 20%	6035 ± 20%	777 ± 21%	11411 ± 2%	2969 ± 14%	6431 ± 26%
2	31 27 28 40	448 ± 47%	586 ± 40%	746 ± 30%	5912 ± 13%	943 ± 37%	3003 ± 9%
3	31 27 31 27	519 ± 63%	2913 ± 16%	314 ± 31%	3303 ± 9%	835 ± 31%	2757 ± 9%
4	2 40 2 40	1999 ± 74%	3625 ± 16%	2474 ± 6%	340 ± 31%	538 ± 8%	2446 ± 7%
5	2 12 28 40	827 ± 41%	837 ± 28%	1053 ± 17%	722 ± 33%	185 ± 32%	1220 ± 48%
6	31 27 2 40	624 ± 38%	1947 ± 20%	342 ± 38%	1606 ± 26%	734 ± 25%	483 ± 29%

BROWN MLC (FOETAL CALF SERUM) 1

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 62

HLA TYPE	1 31 27 3 35	2 31 27 28 40	3 31 27 31 27	4 2 40 2 40	5 2 12 28 40	6 31 27 2 40
1 31 27 3 35	1.00	16.49	2.12	31.18	8.11	17.57
2 31 27 28 40	0.76	1.00	1.27	10.09	1.61	5.12
3 31 27 31 27	1.65	9.28	1.00	10.52	2.66	8.78
4 2 40 2 40	5.88	10.66	7.28	1.00	1.58	7.19
5 2 12 28 40	4.47	4.52	5.69	3.90	1.00	6.59
6 31 27 2 40	1.29	4.03	0.71	3.32	1.52	1.00

BROWN MLC (FOETAL CALF SERUM) 1

STIMULATION INDEX

TABLE 63

HLA TYPE		1 31 27 3 35	2 31 27 28 40	3 31 27 31 27	4 2 40 2 40	5 2 12 28 40	6 31 27 2 40
1	31 27 3 35	269 ± 76%	11916 ± 21%	121 ± 25%	19951 ± 16%	8255 ± 20%	15254 ± 8%
2	31 27 28 40	374 ± 22%	132 ± 28%	95 ± 16%	4085 ± 15%	655 ± 35%	2709 ± 27%
3	31 27 31 27	302 ± 27%	2928 ± 32%	266 ± 37%	6520 ± 31%	2121 ± 13%	3061 ± 43%
4	2 40 2 40	2755 ± 27%	3903 ± 15%	2563 ± 35%	144 ± 39%	502 ± 42%	3705 ± 18%
5	2 12 28 40	400 ±104%	422 ± 61%	258 ± 97%	335 ± 36%	58 ± 43%	378 ± 66%
6	31 27 2 40	124 ± 36%	461 ± 19%	48 ± 43%	697 ± 44%	202 ± 31%	87 ± 76%

BROWN MLC (AUTOLOGOUS SERUM) 2

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 64

HLA TYPE	1 31 27 3 35	2 31 27 28 40	3 31 27 31 27	4 2 40 2 40	5 2 12 28 40	6 31 27 2 40
1 31 27 3 35	1.00	44.28	0.45	74.15	30.68	56.69
2 31 27 28 40	2.83	1.00	0.72	30.94	4.96	20.52
3 31 27 31 27	1.14	11.01	1.00	24.51	7.97	11.51
4 2 40 2 40	19.13	27.10	17.79	1.00	3.49	25.72
5 2 12 28 40	6.89	7.27	4.45	5.77	1.00	6.51
6 31 27 2 40	1.42	5.29	0.55	8.00	2.32	1.00

BROWN MLC (AUTOLOGOUS SERUM) 2

STIMULATION INDEX

TABLE 65

HLA TYPE	1 31 27 3 35	2 31 27 28 40	3 31 27 31 27	4 2 40 2 40	5 2 12 28 40	6 31 27 2 40
1 31 27 3 35	1267 ± 66%	12053 ± 28%	1707 ± 75%	9356 ± 99%	6034 ± 19%	16596 ± 27%
2 31 27 28 40	476 ± 78%	220 ± 26%	276 ± 50%	9007 ± 10%	505 ± 70%	6296 ± 25%
3 31 27 31 27	171 ± 13%	1773 ± 35%	163 ± 34%	3892 ± 65%	1558 ± 42%	2358 ± 4%
4 2 40 2 40	2190 ± 46%	3281 ± 41%	5464 ± 21%	133 ± 37%	424 ± 16%	3175 ± 53%
5 2 12 28 40	292 ± 62%	443 ± 12%	857 ± 12%	714 ± 26%	88 ± 41%	810 ± 43%
6 31 27 2 40	111 ± 24%	520 ± 63%	88 ± 14%	1051 ± 58%	71 ± 21%	75 ± 56%

BROWN MLC (HETEROLOGOUS SERUM) 3

RAW DATA (CPMs) ± COEFF. OF VARIATION

TABLE 66

	1	2	3	4	5	6
HLA TYPE	31 27 3 35	31 27 28 40	31 27 31 27	2 40 2 40	2 12 28 40	31 27 2 40
1 31 27 3 35	1.00	9.51	1.35	7.38	4.76	13.10
2 31 27 28 40	2.16	1.00	1.25	40.94	2.30	28.61
3 31 27 31 27	1.05	10.88	1.00	23.87	9.56	14.46
4 2 40 2 40	16.46	24.66	41.07	1.00	3.19	23.87
5 2 12 28 40	3.32	5.03	9.73	8.11	1.00	9.20
6 31 27 2 40	1.48	6.93	1.17	14.00	0.95	1.00

BROWN MLC (HETEROLOGOUS SERUM) 3

STIMULATION INDEX

TABLE 67

DISCUSSION

The results of both rat and human experiments have certainly not been in accord with the predictions made at the outset of either. However, the results have some interesting features and do not altogether run counter to the hypothesis that maternal immunologic factors can interfere with the immune system of the offspring, nor that there may be immunogenetic abnormalities in the Rheumatoid patient in relation to the mother.

In the rat experiments the *a priori* hypothesis was that maternal "T" lymphocytes (or arguable the antigen) would cross the placenta and cause the rat to demonstrate some evidence of presensitization on rechallenge with the same antigen. The spleen effect was known for its use by Simonsen and others to demonstrate runting in the Graft-Versus-Host experiments in chickens and rodents but unless there were to be polyclonal G.V.H. activation it was not predicted to happen in these experiments. However, there is little doubt in my mind that the spleen effects were real and related to the antigen administered to the mother before mating, and that with the precautions taken there seems little possibility of the antigen itself crossing the placenta as the eschar had been thrown off before the foetus was 10 days old in the womb due to an intense inflammatory response.

Although the lymph node assay remains unknown in terms of sensitivity it proved to be sensitive enough to detect hypersensitivity many months after the initial priming and if the foetus had received such a stimulus as would be necessary for the spleen effects one would hope to be able

to demonstrate either cellular hypersensitivity or tolerance, but in the event the lymph node expansion was identical in the offspring of primed and unprimed mothers. The spleen effect seen with the Fischer/Sprague Dawley combination may have been influenced by intercurrent upper respiratory tract infections in the mother but in all subsequent experiments it has been retrospectively realised that spleen effects were seen and at statistically highly significant levels.

Two interesting aspects in the results of the rat experiments were: a) the inability to elicit recall hypersensitivity to DNCB in the Fischer strain of rat that may be due to it having defective macrophage function as has recently been suggested; b) the inability to demonstrate recall hypersensitivity to DNCB in middle-aged rats while having no such inability with rats under the age of 100 days. It is also worthy of note that the offspring of Fischer mothers have the same time course of popliteal node expansion as their mothers whereas the offspring of the Sprague Dawley rats have the same time course as their mothers even though they are genetically more similar to the offspring of the Fischer (i.e. they were both Fischer/Sprague cross breeds). This is yet more evidence that the mother influences the phenotypic expression of the inherited immune response genes though there was no evidence in these experiments to be able to speculate whether this was due to allelic exclusion or to direct interference by antibodies or lymphocytes. If this effect were to be due to macrophage dysfunction it would seem unlikely that this would be linked to the major histocompatibility loci.

In the human experiments the *a priori* prediction was that the mothers

would all be HLA-D homozygotes leading to a Graft-Versus-Host situation in the offspring in later life. It soon became apparent from this work and that of Stastny that there was an excess of homozygosity in the patients themselves which would only be consistent in classical terms with Host-Versus-Graft disease. In two and possibly three of the patients suffering from Juvenile Rheumatoid Arthritis in these families could a case be made out for the patients being HLA-D homozygotes (this, of course, is far more than would be expected even in Newfoundland), but it is the exceptions that test the rule and in at least two families there is evidence that the mother and patient respond bilaterally in Mixed Lymphocyte Culture. However, apart from the one family with the child with possible Juvenile Ankylosing Spondylitis there was some evidence that the patient/mother immunological mediated interactions were very marginal compared to the responses seen in other families studied, e.g. Ankylosing Spondylitis.

A valid criticism of this work is that no evidence has been adduced from "normal" families to compare the offsprings' reaction with the mothers so that any comment about the suboptimal stimulations seen must have that caveat to the fore. However, if this were to be the general pattern of intrafamilial reactions then the reactions seen universally in the large Ankylosing Spondylitis Family (of which only one but of three large MLCs has been placed in the tables) would have to be considered abnormal in their ability to respond to each other. It may be speculated that there are weak and strong HLA-D alleles which in the case of Ankylosing Spondylitis family involves the Dw1 allele whereas in the Juven-

ile Rheumatoids the Dw3 and Dw4 alleles have been found in the few that have been typed (though more properly they should be put as DRw alleles until these have been firmly linked to the Dw alleles).

In the Cell-Mediated-Lympholysis experiments there were some confirmations for the minimal responses between proband and mother and occasional dichotomies between the results of the MLCs and the CMLs but in general terms the test does not seem to have helped in coming to some understanding of the families. One important finding, though only a confirmation of an already recognised phenomenon, was that Rheumatoid serum (in this case seropositive by conventional testing) was markedly inhibitory for cytolysis and this effect was not specifically directed at the patient's effector cells but had the same effects with the other members of the family as well as the control. Two-way mixed lymphocyte cultures seemed to be very difficult to interpret though the overall impression did not differ radically from the one-way parallel cultures.

Although the *a priori* expectations for the results of the experiments proved to be mistaken in the light of the actual data nevertheless there is considerable interest to be derived from both rat and human data. The remarkable effect upon the spleens of the rats born to immunised mothers raises the possibility of some similar findings in humans though such extrapolation should not be assumed to be correct until the relevant method of testing has been tried. In the context of Juvenile Rheumatoid Arthritis the common finding of splenomegaly in the condition is a very tempting parallel, but again this can be found in overt infection so that it must remain a nonspecific effect and without too much

REFERENCES

Aase, J.M., G.R. Noren, D.V. Reddy, J.W. St. Geme, Jr. Mumps-Virus infection in pregnant women and the immunologic response of their offspring. The New England Journal of Medicine, Vol. 286, No. 26, Pages 1379-1382, 1972.

Abrahamsen, T.G., S.S. Froland, J.B. Natvig, J. Pahle. Elution and Characterization of lymphocytes from Rheumatoid inflammatory tissue. Scandinavian Journal of Immunology, Vol. 4 (8), Pages 823-830, 1975.

Abrahamsen, T.G., S.S. Froland, J.B. Natvig, J. Pahle. Lymphocytes eluted from synovial tissue of Juvenile Rheumatoid Arthritis patients. Arthritis and Rheumatism, Vol. 20, Pages 772-778, 1977.

Adcock, E.W., III. Maternal lymphocytes: Suppression by human chorionic gonadotropin. Gynecol. Invest., Pages 913-914, 1974.

Ahlström, S., P.O. Gedda, H. Hedberg. Disappearance of radioactive serum albumin from joints in Rheumatoid Arthritis. Acta Rheumatologica Scandinavia, Vol. 2, Pages 129-136, 1956.

Ansell, B.M., E.G.L. Bywaters, J.S. Lawrence. Familial aggregation and twin studies in Still's Disease. Population Studies and Genetics. Rheumatology (Basel), Vol. 2, Page 37, 1969.

Ammann, A.J., D.W. Wara, Pediatric Immunology, University of California. Maternal-Fetal Graft Vs. Host reaction. The Journal of Pediatrics, Vol. 87, No. 2, Letters to the Editor, Pages 329-330, 1975.

Apice, A.J.F., P.J. Morris. The role of antibody-dependent cell-med-

weight in assessing the influence of maternal factors. The absence of any confirmatory data of transplacental passage of specific immunity is in retrospect not the disaster it was thought at the first assessment of the data as it would have been difficult to test whether this was due to maternal lymphocytes or antigen as the methods for karyotyping the lymph node cells proved to be more difficult than expected. There was still that uncertainty in the spleen effects particularly as these appeared to be equal in the intra-strain progeny, but it may prove to be possible using anti-maternal strain sera to test for the presence of the mother's cells in the offspring although this would have to be done in an F_2 situation in order to distinguish the intruding cells. The effect could yet be due to antigen but it then seems strange that the popliteal lymph node assay did not demonstrate either enhancement or tolerance to the antigenic stimulus.

The human data confirms the findings of Stastny but the concept of weak MLC reactions in the nuclear families seems as yet pure supposition without an adequate data base. The direct assault on the synovial membrane in order to demonstrate maternal cells, or treatment of the patient with anti-maternal serum seems to be a much more satisfactory approach though the protocol would have to include very careful briefing of the patients and families in order that informed consent could be obtained.

iated cytotoxicity in renal allograft rejection. Transplantation, Vol. 18 (1), Pages 20-26, 1974.

Astorga, G.P., R.C. Williams. Altered reactivity in mixed lymphocyte culture of lymphocytes from patients with rheumatoid arthritis. Arthritis and Rheumatism, Vol. 12, Pages 547-554, 1969.

Auerback, R., S. Clark. Immunological tolerance: Transmission from mother to offspring. Science, Vol. 189, Pages 811-812, 1975.

Austin, R.M., C.A. Daniels. Inhibition by rheumatoid factor, anti-Fc, and staphylococcal protein A of antibody-dependent cell-mediated cytolysis against herpes simplex virus-infected cells. Journal of Immunology, Vol. 117, Pages 602-607, 1976.

Axelrad, M.A. Suppression of delayed hypersensivity by antigen and antibody. Immunology, Vol. 15, Pages 159-171, 1968.

Bach, F.H., K. Hirschhorn. Lymphocyte interaction; a potential histocompatibility test *in vitro*. Science, Vol. 143, Pages 813-814, 1964.

Bach, F.H., N.K. Voynow. One-way stimulation in mixed leukocyte cultures. Science, Vol. 153, Pages 545-547, 1966.

Bain, B., M. Vas, L. Lowenstein. A reaction between leukocytes in mixed peripheral blood cultures. Federation Proceedings (Federation of the American Societies of Experimental Biology), Vol. 22, Page 428, Abstr. 1597, 1963.

Bain, B., L. Lowenstein. Genetic studies on the mixed leukocyte reaction. Science, Vol. 145, Pages 1315-1316, 1964.

Bain, B., M.R. Vas, L. Lowenstein. The development of large immature mononuclear cells in mixed leukocyte cultures. Blood, Vol. 23, Pages 108-116, 1964.

Balazs, E.A., D. Watson, I.F. Duff, S. Roseman. Hyaluronic acid in synovial fluid. 1. Molecular parameters of hyaluronic acid in normal and arthritic human fluids. Arthritis and Rheumatism, Vol. 10, Pages 357-376, 1967.

Ball, J., J.S. Lawrence. Epidemiology of the sheep cell agglutination test. Annals of the Rheumatic Diseases, Vol. 20, Pages 235-243, 1961.

Bankhurst, A.D., G. Husby, R.C. Williams. Predominance of T cells in the lymphocytic infiltrates of synovial tissues in rheumatoid arthritis. Arthritis and Rheumatism, Vol. 19, Pages 555-562, 1976.

Barland, P., A.B. Nivikoff, D. Hamerman. Electron microscopy of the human synovial membrane. Journal of Cell Biology, Vol. 14, Pages 207-220, 1962.

Barnes, R.D., M.A. Tuffrey. A transplacental factor in the disease of the autoimmune NZB/BL mouse. The Lancet, Vol. 1, Pages 1240-1242, 1969.

Barnett, C.H., D.V. Davies, M.A. Macconaill. Synovial joints, their structure and mechanics. Thomas, Springfield, Illinois, 1961.

Barnetson, R. StC., G. Bjune, M.E. Duncan. Evidence for a soluble lymphocyte factor in the transplacental transmission of T-lymphocyte responses to *mycobacterium laprae*. Nature, Vol. 260, Pages 150-151, 1976.

Batchelor, J.R., I.K. Welsh, H. Burgos. Transplantation Antigens *per se* are poor immunogens within a species. Nature, Vol. 273, Pages 54-56, 1978.

Beasley, R.P., R.F. Willkens. High prevalence of RA in Yakima Indians. Arthritis and Rheumatism, Vol. 14, Page 150, 1971.

Beer, A.E., R.E. Billingham. Immunobiology of mammalian reproduction. Advances in Immunology, Vol. 14, Pages 1-84, 1971.

Beer, A.E., R.E. Billingham, S.L. Yang. Maternally induced transplantation immunity, tolerance, and runt disease in rats. The Journal of Experimental Medicine, Vol. 135, Pages 808-826, 1972.

Beer, A.E., R.E. Billingham. Maternally acquired runt disease. Science, Vol. 179, Pages 240-243, 1973.

Beer, A.E., R.E. Billingham. Procurement of runt disease of maternal origin. Transplantation Proceedings, Vol. 5, Pages 887-891, 1973.

Beer, A.E., R.E. Billingham. The embryo as a transplant. Scientific American, Vol. 230, Pages 36-46, 1974.

Beer. A.E., R.E. Billingham, J. Head. The immunologic significance of the mammary gland. The Journal of Investigative Dermatology, Vol. 63, (1), Pages 65-74, 1974.

Beer, A.E., R.E. Billingham. Immunologic benefits and hazards of milk in maternal-perinatal relationship. Annals of Internal Medicine, Vol. 83, Pages 865-871, 1975.

Beer, A.E., R.E. Billingham, J.R. Head, M.J. Parmely. Possible influence of maternal-to-perinatal cell transfer on the development of host defences. In: Development of host defenses Ed. M.D. Cooper, D.H. Dayton,

Pages 251-271, 1976.

Bennett, P.H., T.A. Burch. The distribution of rheumatoid factor and rheumatoid arthritis in the families of Blackfeet and Pima Indians. Arthritis and Rheumatism, Vol. 11, Pages 546-553, 1966.

Bevan, M.J. The major histocompatibility complex determines susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. Journal of Experimental Medicine, Vol. 142, Pages 1349-1364, 1975.

Billingham, R.E., L. Brent. Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. Philosophical Transactions B, Vol. 242, Pages 439-477, 1959.

Billingham, R.E. Reactions of grafts against their hosts. Transplantation immunity works both ways - Hosts destroy grafts and grafts may harm hosts. Science, Vol. 130, Pages 947-953, 1959.

Billingham, R.E., W.K. Silvers. Transplantation and cutaneous genetics. The Journal of Investigative Dermatology, Vol. 60, (6), Pages 509-515, 1970.

Blank, K.J., H.A. Freedman, F. Lilly. T-Lymphocyte response to Friend virus-induced turned tumour cell lines in mice of strains congenic at H-2. Nature, Vol. 260, Issue 5548, Pages 250-252, 1976.

Bodel, P.T., J.W. Hollingsworth. Comparative morphology, respiration, and phagocyte function of leukocytes from blood and joint fluid in rheumatoid arthritis. Journal of Clinical Investigation, Vol. 45, Pages 580-589, 1966.

Bonney, W.W., T.L. Feldbush, Graft versus host reaction in the rat popliteal lymph node. Transplantation 15, Vol. 15, Pages 215-220, 1973.

Boyum, A. Separation of leucocytes from blood and bone marrow. Scandinavian Journal of Clinical Laboratory Investigation. Vol. 21, Supp. 97, Page 1, 1968.

Bywaters, E.G.L. Peripheral vascular obstruction in rheumatoid arthritis and its relationship to other vascular lesions. Annals of the Rheumatic Diseases, Vol. 16, Pages 84-103, 1957.

Bywaters, E.G.L., Heberden Oration, 1966. Categorization in Medicine: A survey of still's disease. Annals of the Rheumatic Diseases, Vol. 26, Pages 185-193, 1967.

Caperton, E.M., D.R. Baker, R.A. King. Lymphocyte reactivity in rheumatoid arthritis. Annals of the Rheumatic Diseases, Vol. 34, Pages 231-234, 1975.

Castor, C.W. The microscopic structure of normal human synovial tissue. Arthritis and Rheumatism, Vol. 3, Pages 140-151, 1960.

Caughey, D.E., T.C. Higton. Components of the fibrinolytic system in synovial joints: Normal bovine compared with normal and abnormal human synovial joints. Annals of the Rheumatic Diseases, Vol. 26, Pages 297-305, 1967.

Cavalli-Sforza, L.L., W.J. Bodmer. The genetics of human populations. Freeman, San Francisco, 1971.

Cecil, R.L., E.E. Nichols, W.J. Stainsby. The etiology of rheumatoid arthritis. American Journal of Medical Science, Vol. 181, Pages 12-25, 1931.

Chihara, T., J.H. Vaughan. The influence of rheumatoid factors (RF) on antibody-dependent cell-mediated cytotoxicity (ADCC). Federation Proceedings Vol. 35, Page 790, 1976.

Cobb, S. The frequency of the rheumatic diseases. A.P.H.A. Vital and Health Statistics Monographs. Harvard University Press, 1971.

Cohen, F., Z.W. Wolf. The transplacental passage of maternal erythocytes into the fetus. Am. J. Obst. and Gynec., Vol. 93, No. 4, Pages 566-569, 1965.

Collins, D.H. The pathology of articular and spinal diseases. Edward Arnold, London, 1949.

Cox, D.W., O. Huber, Rheumatoid arthritis and alpha-1-antitrypsin. Lancet i, Pages 1216-1217, 1976.

Cramer, D.V., H.W. Kunz, T.J. Gill, III. Immunologic sensitization prior to birth. Am. J. Obst. and Gynec., Vol. 120, Pages 431-439, 1974.

Cudkowicz, G., J.H. Stimpfling. Induction of immunity and of unresponsiveness to parental marrow grafts in adult F_1 hybrid mice. Nature, Vol. 204, Pages 450-453, 1964.

Dalakos, T.B., R.N.M. McSween, K. Dick, W.C. Boyle, M.K. Jasani, E. Wilson, W.W. Buchanan, R.B. Goudie. Conjugal prevalence of rheumatoid arthritis, rheumatoid factor and other autoantibodies in RA. Clinical and Experimental Immunology, Vol. 3, Pages 761-769, 1968.

tion and immunology in the rheumatic diseases. Ed. D.C. Dumond, Pages 375-403, Blackwell Scientific Publications, 1976.

Dupont, B., J.A. Hansen, E.J. Yunis. Human mixed lymphocyte culture reaction: Genetics, specificity, and biological implications. Advances in Immunology, Vol. 23, Pages 108-202, 1976.

Edwards, J.H. Familial predisposition in man. British Medical Bulletin, Vol. 25, Pages 58-64, 1969.

El-Alfi, O.S., H. Hathout. Maternofetal transfusion: Immunologic and cytogenetic evidence. Am. J. Obst. and Gynec., Vol. 103, No. 4, Pages 599-600, 1969.

Elkins, W.L. Cellular immunology and the pathogenesis of graft versus host reactions. Progress in Allergy, Vol. 15, Pages 78-187, 1971.

Engel, A., J. Roberts, T.A. Burch. RA in adults. United States 1960-2. Public Health Service Publication, No. 1000, Series 11, No. 17, 1966.

Falchuk, K., E. Goetzl, J. Kulka. Respiratory gases of synovial fluids. An approach to synovial tissue circulatory-metabolic imbalance in rheumatoid arthritis. American Journal of Medicine, Vol. 49, Pages 223-231, 1970.

Falconer, D.S. Introduction to quantitative genetics. Ronald Press Company, 1960.

Falconer, D.S. The inheritance of liability to certain diseases, estimated from incidence among relatives. Annals of Human Genetics, Vol. 29,

DeHoratius, R.J., R.P. Messner. Lymphocytotoxic antibodies in family members of patients with systemic lupus erythematosus. The Journal of Clinical Investigation, Vol. 55 (6), Pages 1254-1258, 1975.

Desai, R.G., W.P. Creger. Maternofetal passage of leukocytes and platelets in man. The Journal of Hematology, Blood Vol. 21, No. 6, Pages 665-673, 1963.

Dingle, J.T., D.P. Page-Thomas. *In vitro* studies on human synovial membrane. A metabolic comparison of normal and rheumatoid tissue. British Journal of Pathology, Vol. 37, Pages 318-323, 1956.

Dixon, A. St. J., A. Kay. Arthritis and Rheumatism Council, Annual Report, 1964.

Donovan, J.C., C.J. Lund. Transplacental passage of maternal erythrocytes. Am. J. Obst. and Gynec., Vol. 95, No. 6, Pages 834-837, 1966.

Duhring, J.L., K. Smith, J.W. Greene, Jr., D.B. Rochlin, W.S. Blakemore. Placental transfer of maternal erythrocytes into the fetal circulation. Gynecology and Obstetrics, Surgical Forum, Vol. 10, Pages 720-722.

Dukova, V.S., V.V. Zarudin, A.S. Shovelev. Malignization of lymphoid tissue in offspring of mice-hybrids with induces (before pregnancy) reaction transplant against host. Doklady Adademii Nauk SSSR, Vol. 230 (1), Pages 237-239, 1976.

Dumonde, D.C., R.H. Kelly, J. Morley. Lymphoid and microvascular dysfunction in experimental models of rheumatoid inflammation. In: Infec-

Pages 51-76, 1965.

Field, E.J., E.A. Caspary. Is maternal lymphocyte sensitisation passed to the child? The Lancet, Pages 337-342, 1971.

Fisher, A.G.T. Chronic (Non-Tuberculous) arthritis. H.K. Lewis, London, 1929.

Flax, M.H., B.H. Waksman. Delayed cutaneous reactions in the rat. Journal of Immunology, Vol. 89, Pages 496-504, 1962.

Ford, W.L., M. Simonsen. The factor of immunization in the rat. J. Exp. Med., Vol. 133, Pages 938-949, 1971.

Ford, W.L., W. Burr, M. Simonsen. A lymph node weight assay for the graft versus host activity of rat lymphoid cells. Transplantation, Vol. 10, Pages 258-266, 1970.

Froland, S.S., J.B. Natvig, G. Husby. Immunological characterization of lymphocytes in synovial fluid from patients with rheumatoid arthritis. Scandinavian Journal of Immunology, Vol. 2, Pages 67-73, 1973.

Fujikura, T., B. Klionsky. Transplacental passage of maternal erythrocytes with sickling. The Journal of Pediatrics, Vol. 87, No. 5, Pages 781-783, 1975.

Gardner, D.L. The pathology of rheumatoid arthritis. Edward Arnold, London, 1965 and 1972.

Gardner, E. The structure and function of joints. In: Arthritis and Allied Conditions. Ed. J.L. Hollander, D.J. McCarty, Jr., A Textbook of Rheumatology, 8th Edition, Pages 32-50, Lea and Febiger, Philadelphia, 1972.

Gatter, R.A., J.D. Richmond. The predominance of synovial fluid lymphocytes in early rheumatoid arthritis. Abstract, Arthritis and Rheumatism, Vol. 16, Page 544, 1973.

Gershon, R.K. T-cell control of antibody production. In: Contemporary Topics in Immunobiology, Vol. 3, Pages 1-40, 1974.

Gill, T.J., III, H.W. Kunz, C.F. Bernard. Maternal-fetal interaction and immunological memory. Science, Vol. 172, Pages 1346-1348, 1971.

Gill, T.J., III. Maternal/fetal interactions and the immune response. Lancet, Vol. 1, Pages 133-135, 1973.

Glynn, L.E. The chronicity of inflammation and its significance in rheumatoid arthritis. Annals of the Rheumatic Diseases, Vol. 27, Pages 105-121, 1968.

Goetzl, E.J., K.H. Falchuk, L.S. Zeiger, A.L. Sullivan, C.L. Hebert, J.P. Adams, J.L. Decker. A physiological approach to the assessment of disease activity in rheumatoid arthritis. The Journal of Clincial Investigation, Vol. 50, Pages 1167-1180, 1971.

Good, R.A., A.P. Dalmasso, C. Martinez, O.K. Archer, J.C. Pierce, B.W. Papermaster. The role of the thymus in development of the immunologic

capacity in rabbits and mice. Journal of Experimental Medicine, Vol. 116, Pages 773-796, 1962.

Goodman, J.W. A search for parental antigens not expressed in F_1 hybrid mice. Transplantation, Vol. 3, Pages 190-201, 1965.

Gormsen, J., R. Bach-Anderson, C. Feddersen. Fibrin-fibrinogen breakdown products in pathological synovial fluids. Arthritis and Rheumatism, Vol. 14, Pages 503-509, 1971.

Gowans, J.L. The fate of parental strain small lymphocytes in F_1 hybrid rats. Annals of the New York Academies of Science, Vol. 99, Pages 432-455, 1962.

De Graaf, F. Proceedings of the ISRA Symposium on the social aspects of chronic rheumatic joint affections. International Congress Series No. 23, Excerpta Medica Foundation, 1959.

Gratwhol, A.A., H.M. Moutsopoulos, T.M. Chused, M. Akiquki, R.O. Wolf, J.B. Sweet, A.B. Deisseroth. Sjogren-type syndrome after allogeneic bone-marrow transplantation. Annals of Internal Medicine, Vol. 87, No. 6, Pages 703-706, 1977.

Gray, D.F., J.L. Noble, M. O'Hara. Allergy in experimental rat tuberculosis. J. Hygiene (London), Vol. 59, Pages 427-436, 1961.

Green, I., M. Inkelas, L.B. Allen. Hodgkin's Disease: A maternal-to-foetal lymphocyte chimaera? Lancet, Vol. i, Pages 30-32, 1960.

Griffiths, M.M., R.C. Williams. *In vitro* peripheral blood and synovial fluid lymphocyte interactions. Arthritis and Rheumatism, Vol. 17, Pages 111-120, 1964.

Hamerman, D., M. Schubert. Diathrodial joints. An essay, American Journal of Medicine, Vol. 33, Pages 555-590, 1962.

Hard, R.C., Jr., B. Kullgen. Etiology, pathogenesis, and prevention of a fatal Host-Verus-Graft syndrome in parent/F1 mouse chimeras. American Journal of Pathology, Vol. 59, Pages 203-224, (2), 1970.

Hard, R.C., C.W. Moncure, W.J.S. Still. Renal lesions with organized deposits and lipid as part of the Host-Versus-Graft syndrome in parent/F1 mouse chimeras. Laboratory Investigation, Vol. 28, No. 4, 1973.

Hargreaves, E.R. A survey of Rheumatoid Arthritis in West Cornwall. Annals of the Rheumatic Diseases, Vol. 17, Pages 61-75, 1958.

Harris, J., J.H. Vaughan. Transfusion studies in RA. Arthritis and Rheumatism, Vol. 4, Pages 47-55, 1961.

Harris, R., J.B. Millard, S.K. Banerjee. Radiosodium clearance from the knee joint in Rheumatoid Arthritis. Annals of the Rheumatic Diseases, Vol. 17, Pages 189-195, 1958.

Hartzman, R.J., M. Segall, M.L. Bach, F.H. Bach. Histocompatibility matching. VI. Miniaturization of the Mixed Leukocyte Culture test: A preliminary report. Transplantation, Vol. 11, Pages 268-273, 1971.

38-47, 1964.

Hirschberg, H., H. Skare, E. Thorsby. Cell mediated lympholysis: CML. A microplate technique requiring few target cells and employing a new method of supernatant collection. Journal of Immunological Methods, Vol. 16, Pages 131-141, 1977.

Hollander, J.L., D.J. McCarty, G. Astorga, E. Castro-Murillo. Studies on the pathogenesis of Rheumatoid joint inflammation. Annals of Internal Medicine, Vol. 62, Pages 271-280, 1965.

Horton, J.E., J.J. Oppenhein, S.P. Chan, J.J. Baker. Relationship of transformation of newborn human lymphocytes by dental plaque antigen to the degree of maternal periodontal disease. Cellular Immunology, Vol. 21, Pages 153-160, 1976.

Howard, J.C., S.V. Hunt, J.L. Gowans. Identification of marrow-derived and thymus derived small lymphocytes in the rat lymphoid tissue and thoracic duct lymph of normal rats. Journal of Experimental Medicine, Vol. 135, Pages 200-219, 1972b.

Howard, J.C. The lifespan and recirculation of marrow-derived small lymphocytes from the rat thoracic duct. Journal of Experimental Medicine, Vol. 135, Pages 185-199, 1972a.

Humphrey, J.H., H.U. Keller. Some evidence for specific interaction between immunologically competent cells and antigens. In: Developmental aspects of antibody formation and structure. Ed. J. Sterzl, I. Riha, Page 485, Academia Prague, 1970.

Hartzman, R.J., M.L. Bach, F.H. Bach. Precipitation of radioactively labelled samples: A semi-automatic multiple sample processor. Cellular Immunology, Vol. 4, Pages 182-186, 1972.

Harvald, B., M. Hauge. Hereditary factors eluciated by twin studies. Genetics and the Epidemiology of Chronic Diseases, U.S. Public Health Service, 1965.

Hayry, P., L.C. Andersson. T-cell synergy in Mixed Lymphocyte Culture-induced cytolysis. European Journal of Immunology, Vol. 4, Pages 145-147, 1974.

Hedenstedt, S., J. Naeslund. Investigations of the permeability of the placenta with the help of elliptocytes. Acta Medica Scand., Vol. 170, Pages 126-134, 1939.

Helgesen, A., H. Hirschberg, E. Thorsby. Mixed Lymphocyte Culture micro-technique. Mannual of Tissue Typing Techniques, Pages 93-95, National Institute of Allergy and Infectious Diseases, Pb-236 916, 1974.

Hellgren, L. Marital status in RA. Acta Rheumatologica Scandinavia, Vol. 15, Pages 271-276, 1969.

Herndon, C.N., R.G. Jennings: A twin-family study of susceptibility to poliomyelitis. American Journal of Human Genetics, Vol. 3, Pages 17-46, 1951.

Hildemann, W.H. Immunological properties of small blood lymphocytes in the graft-versus-host reaction in mice. Transplantation, Vol. 2, Pages

Irby, R., D.M. Hume. Joint changes observed following renal transplants. Clinical Orthopaedics and Related Research, No. 57, Pages 101-114, 1968.

Isturiz, M.A., M.M. de Bracco, A.M. Pizzi, J.A. Manni. Antibody-dependent cell-mediated cytotoxicity in rheumatoid arthritis. Arthritis and Rheumatism, Vol. 19, Pages 725-730, 1976.

Jennings, J.F. Lymphocyte responsiveness in Rheumatoid Arthritis. Lancet, Vol. i, Page 1239, 1971.

Johnson, J.S., J.H. Vaughan, P.K. Hench, S.E. Blomgren. Rheumatoid Arthritis, 1970-1972. Annals of Internal Medicine, Vol. 78, Pages 937-953, 1973.

Kadwaki, J., W.W. Zeulzer, A.J. Brough, R.I. Thompson, R.V. Woolley, Jr., D. Gruber. XX/XY lymphoid chimaerism in congenital immunological deficiency syndrome with thymic alymphoplasia. The lancet, Vol. ii, Pages 1152-1156, 1965.

Kaplan, H.S., D.W. Smithers. Auto-immunity in man and homologous disease in mice in relation to the malignant lymphomas. Lancet, Vol. ii, Pages 1-4, 1959.

Kasakura, S., L. Lowenstein. The effect of irradiation *in vitro* on mixed leukocyte cultures and on leukocyte cultures with phytohaemagglutinin. Histocomaptibility Testing 1965, Pages 211-212, Eds. H. Balner, F.J. Cleton, J.G. Eernisse, Munksgaard, Copenhagen, 1965.

Katz, D.H., T. Hamaoka, M.E. Dorf, B. Benacerraf. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (Ir) gene in the control of lymphocyte interactions in responses controlled by

the gene. Journal of Experimental Medicine, Vol. 137, Pages 1405-1418, 1973.

Katz, D.H. The role of the histocompatibility gene complex in lymphocyte differentiation. Cold Spring Harbor Symposia on Quantitative Biology, Vol. 41, Part 2, Pages 611-624, 1977.

Kay, A., F. Bach. Subfertility before and after the development of Rheumatoid Arthritis in women. Annals of the Rheumatic Diseases, Vol. 24, Pages 169-173, 1965.

Kellgren, J.H. Epidemiology of Rheumatoid Arthritis. Arthritis and Rheumatism, Vol. 9, Pages 658-674, 1966.

Keystone, E.C., D.G. Gladman, M.B. Urowitz, D.A. Clarke, J.A. Falk, D. Osoba, D.A. Gordon. Mixed leukocyte reaction in Rheumatoid Arthritis. Arthritis and Rheumatism, Vol. 19, Pages 532-538, 1974.

Kindred, B., G.E. Roelants. Restricted clonal response to DNP in adult offspring of immunized mice: A maternal effect. The Journal of Immunology, Vol. 113, Pages 445-448, 1974.

Kinsella, T.D. Enhancement of human lymphocyte transformation by aggregated human immunoglobulin. The Journal of Clinical Investigation, Vol. 53, Pages 1108-1114, 1974.

Klinge, F. Die eiweissuberempfindlichkeit (Gewebs anaphylaxie) der gelenke. Experimentelle pathologische-anatomische. Studie zur pathogenese des gelenkrheumatismus. Beitrig Zur Pathologische Und Anatomie, Vol. 83, Pages 185-216, 1929.

Klinge, F. Der rheumatismus: Pathologisch-anatomische und experimentell-pathologische tataschen und ihre auswertung fur das arztliche-rheumaproblem. Bergman, Munich, 1933.

Klinge, F. Handbuk der speziellen pathologischen anatomie und histologie. Vol. 9, Part III, Pages 107-251, Eds. O. Lubarsch und F. Henke, Springer, Berlin, 1934.

Kobayashi, I., M. Ziff. Electron microscopic studies of lymphoid cells in the Rheumatoid synovial membrane. Arthritis and Rheumatism, Vol. 16, Pages 471-486, 1973.

Kulka, J.P. Modern trends in Rheumatology. Ed. A.G.S. Hill, Appleton, New York, 1966.

Larsen, B., A. Arnason, J.M. Barnard, S.K. Buehler, J.H. Edwards, W.H. Marshall. Bf types of HLA haplotyped individuals in an isolated Newfoundland population. Tissue Antigens, Vol. 10, Pages 403-409, 1977.

Lawley, T.J., G.L. Peck, H.M. Moutsopoulos, A.A. Gratwohl, A.B. Deisseroth. Scleroderma, Sjogren-like Syndrome, and Chronic Graft-Versus-Host Disease. Annals of Internal Medicine, Vol. 87, No. 6, Pages 707-709, 1977.

Lawrence, H.S. The transfer in humans of skin sensitivity of the tuberculin type with components of disrupted leucocytes. Bulletin of the New York Academy of Medicine, Vol. 32, Pages 236-238, 1956.

Lawrence, J.S. Methods of assessing RA in population studies and initial findings. Proceedings of the ISRA Symposium on the Social Aspects of Chronic Joint Affections. Excerpta Medica Foundation, 1960.

Lawrence, J.S. Geographical studies of Rheumatoid Arthritis. Population Studies of the Rheumatic Diseases. Eds. P.H. Bennett and P.H.N. Wood. Excerpta Medica Foundation, 1968.

Lawrence, J.S. Heberden oration, 1969. Rheumatoid Arthritis - nature or nurture? Annals of the Rheumatic Diseases, Vol. 29, Pages 357-379, 1970.

Lawrence, J.S. Rheumatism in populations. William Heineman Medical Books, 1977.

Lee, R.E., J.J. Vazquez. Immunocytochemical evidence for transplacental passage of erythrocytes. Laboratory Investigation, Vol. 11, Pages 580-584, 1962.

Lefford, M.J. The measurement of tuberculin hypersensitivity in rats. Int. Arch. Allergy, Vol. 47, Pages 570-585, 1974.

Levine, S. Local and regional forms of Graft-Versus-Host Disease in lymph nodes. Transplantation, Vol. 6, Pages 799-802, 1968.

Lloyd, T.M., R.S. Panush. Cell mediated immunity in Rheumatoid Arthritis. Journal of Rheumatology, Vol. 4, Pages 231-244, 1977.

Lund-Olesen, K. Oxygen tension in synovial fluids. Arthritis and Rheumatism, Vol. 13, Pages 769-776, 1970.

Mackay, I.R., F.M. Burnet. "Autoimmune Diseases, Pathogenesis, Chemistry and Therapy." Charles C. Thomas, American Lecture Series 518, 1963.

MacLaurin, B.P. Lymphocyte reactivity against lymphoma cells in Rheumatoid Arthritis. Lancet, Vol. i, Pages 1070-1071, 1971.

Macris, N.T., L.M. Hellman, R.J. Watson. The transmission of transfused Sickle-Trait cells from mother to fetus. Am. J. Obst. and Gynec., Vol. 76, No. 6, Pages 1214-1218, 1958.

Mayer, S., A. Falkenrodt, M.M. Tongio. Cold lymphocytotoxins in infections and parasitic infestations. Tissue Antigens, Vol. 3, Pages 431-438, 1973.

McGregor, D.D. Bone marrow origin of immunologically competent lymphocytes in the rat. Journal of Experimental Medicine, Vol. 127, Pages 953-966, 1968.

McCormick, J.N. An immunofluorescence study of Rheumatoid Factor. Annals of the Rheumatic Diseases, Vol. 22, Pages 1-10, 1963.

McMichael, A.J., I. Sasazuki, H.O. McDevitt, R.O. Payne. Increased frequency of HLA-Cw3 and HLA-Dw4 in Rheumatoid Arthritis. Arthritis and Rheumatism, Vol. 20 (5), Pages 1037-1042, 1977.

Mendez-Bryan, R., R. Gonzales-Alcover, L. Roger. RA: Prevalence in a tropical area. Arthritis and Rheumatism, Vol. 7, Pages 171-176, 1964.

Mengert, W.F., C.S. Rights, C.R. Bates, A.F. Reid, G.R. Wolf, C. Nabors. Placental transmission of erythrocytes. Am. J. Obst. and Gynec., Vol. 69, Pages 678-683, 1955.

Miller, J.F.A.P., J.G. Howard. Some similarities between the neonatal-thymectomy syndrome and Graft-Versus-Host Disease. Journal of Reticuloendothelial Society, Vol. 1, Pages 369-392, 1964.

Minden, P., J. Sandridge, M.A. Wainberg, J.K. McClatchy. Transmission of BCG-associated tumor resistance from maternal to newborn Guinea Pigs. Journal of the National Cancer Institute, Vol. 56, No. 1, Pages 153-157, 1976.

Mitchison, N.A. The dosage requirements for immunological paralysis of soluble proteins. Immunology, Vol. 15, Pages 509-530, 1968.

Mittal, K.K., R.D. Rossen, J.T. Sharp, M.D. Lidsky, W.T. Butler. Lymphocyte cytotoxic antibodies in Systemic Lupus Erthematosus. Nature, Vol. 225, Pages 1255-1256, 1970.

Mohr, J.A. Lymphocyte sensitisation passed to the child from the mother. The Lancet, Vol. i, Page 688, 1972.

Moretta, L. Active thymus derived suppressor lymphocytes in human cord blood. Nature, Vol. 269, Pages 333-335, 1977.

Morse, H.C., III, M.R. Harrison, R. Asofsky. Graft-Vs.-Host reactions in reciprocal hybrid mice. The Journal of Experimental Medicine, Vol. 139, Pages 721-731, 1974.

Munthe, E., J.B. Natvig. Immunoglobulin classes, subclasses and complexes of IgG Rheumatoid Factor in Rheumatoid plasma cells. Clinical and Experimental Immunology, Vol. 12, Pages 55-70, 1972.

Naeslund, J. Undersokningar over placentas permeabilitet med tillhjalp av bl.a. blodgrupps-bestamingar, radioaktivt markta blodkroppar och elliptocyter. Bank 108, Hafte 11, Pages 589-592, 1945.

Naeslund, J., G. Hylin. Investigations on the permeability of the placenta with the aid of red blood corpuscles tagged with radio-active phosphorus. Acta Medica Scand. Supp., Vol. 170, Pages 390-398, 1946.

Nakamura, R., H. Asai, H. Sonozaki, M. Nagano. Phenosulphonphthalein clearance from the knee joint in normal and pathological states. Annals of the Rheumatic Diseases, Vol. 26, Pages 246-250, 1967.

Nance, W.E. Anencephaly and Spina Bifida: A possible example of cytoplasmic inheritance in man. Nature, Vol. 224, Pages 373-375, 1969.

Nettelbladt, E., L. Sundblad. Haptoglobins in serum and synovial fluid. Acta Rheumatologica Scandinavia, Vol. 11, Pages 11-14, 1965.

Neumann, E. Die picrocarminfarbung und ihre anwendung auf die entzundungslehre. Archivs Mikroskopie und Anatomie, Vol. 18, Pages 130-150, 1880.

Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner, D.H. Bent. Statistical package for the social sciences, second edition. McGraw-Hill Book Company, New York, 1975.

Norton, W.L., M. Ziff. The ultra-structure of Rheumatoid synovium and subcutaneous nodule. Arthritis and Rheumatism, Vol. 7, Page 335, 1964.

Nosanchuk, J.S., B. Schnitzer. Follicular hyperplasia in lymph nodes from patients with Rheumatoid Arthritis. A clinicopathologic study. Cancer (Philadelphia), Vol. 24, Pages 343-354, 1969.

Ogston, A.G., C.F. Phelps. The partition of solutes between buffer solutions and solutions containing Hyaluronic Acid. Biochemical Journal, Vol. 78, Pages 827-833, 1961.

Olding, L. Materno-foetal transfer of lymphocytes in human subjects. Acta path. microbiol. scand., Vol. 76, Pages 150-160, 1969.

Olding, L. The possibility of materno-foetal transfer of lymphocytes in man. Acta Paediat. Scand., Vol. 61, Pages 73-75, 1972.

Olding, L.B., K. Benirschke, M.B.A. Oldstone. Inhibition of mitosis of lymphocytes from human adults by lymphocytes from human newborns. Clinical Immunology and Immunopathology, Vol. 3, Pages 79-89, 1974.

Oliner, H., R. Schwartz, W. Dameshek. Studies in experimental autoimmune disorders. I. Clinical and laboratory features of autoimmunization (Runt Disease) in the mouse. Blood, Vol. 12, Pages 20-44, 1961.

St. Onge, R.A., W.C. Dick, G. Bell, J.A. Boyle. Radioactive Xenon (^{133}Xe) disappearance rates from the synovial cavity of the human knee joint in normal and arthritic subjects. Annals of the Rheumatic Diseases, Vol. 27, Pages 163-166, 1968.

Ono, Y., T. Sasaki, N. Ishida. Regulation of clonal development of immune responding cells by antibody of maternal origin. Nature, Vol. 250,

Pages 593-594, 1974.

O'Sullivan, J.B., E.S. Cathcart. The prevalence of Rheumatoid Arthritis. Annals of Internal Medicine, Vol. 76, Pages 573-577, 1972.

Page, E.W. Physiology of the human placenta at term. Clin. Obs. and Gynec., Pages 279-285, 1960.

Page-Thomas, D.P., J.T. Dingle. *In Vitro* studies of Rheumatoid synovium. Preliminary metabolic comparison between synovial membrane and villi. British Journal of Experimental Pathology, Vol. 36, Pages 195-198, 1955.

Page-Thomas, D.P., J.T. Dingle. Studies on human synovial membrane *in vitro*. The metabolism of normal and Rheumatoid synovia and the effect of Hydrocortisone. Biochemical Journal, Vol. 68, Pages 231-238, 1958.

Palm, J. Association of maternal genotype and excess heterozygosity for Ag-B histocompatibility antigens among male rats. Transplantation Proceedings, Vol. 1, Pages 82-84, 1969.

Palm, J. Maternal-fatal histoincompatibility in rats: An escape from adversity . Cancer Research 34, Pages 2061-2065, 1970.

Palm, J. Maternal-fetal interactions and histocompatibility antigen polymorphisms. Transplantation Proceedings, Vol. 2, No. 1, Pages 162-173, 1970.

Paulus, H.E., H.I. Machleder, R. Banger, J.A. Stratton, L. Goldberg, M. W. Whitehouse, D. Yu, C.M. Pearson. A case report: Thoracic duct lymphocyte drainage in Rheumatoid Arthritis. Clinical Immunology and Immunopathology, Vol. 1, Pages 173-181, 1973.

Pickering, D.E., N.E. Kontaxis. Thyroid function in the foetus of the Macaque Monkey (*Macaca Mulatta*). II. Chemical and morphological characteristics of the foetal thyroid gland. Journal of Endocrinology, Vol. 23, Pages 267-275, 1961.

Pierce, C.W., J.A. Kapp, B. Benacerraf. Regulation by the H-2 gene complex of macrophage-lymphoid cell interactions in secondary antibody responses *in vitro*. Journal of Experimental Medicine, Vol. 144, Pages 371-381, 1976.

Porter, K.A. Immune hemolysis: A feature of Secondary Disease and Runt Disease in the rabbit. Annal of the New York Academies of Science, Vol. 87, Pages 391-402, 1960.

Ramseier, H., R.E. Billingham. Studies on Delayed Cutaneous Inflammatory Reactions Elicited by Inoculation of Homologous Cells into Hamsters' Skins. Journal of Experimental Medicine, Vol. 123, Pages 629-656, 1966.

Rigby, Captain P.G., Major T.A. Hanson, Captain R.S. Smith. Passage of leukemic cells across the placenta. The New England Journal of Medicine, Vol. 271, No. 3, Pages 124-127, 1964.

Robertson, M.D.J., F.D. Hart, W.F. White, G. Nuki, P.L. Boardman, Rheumatoid lymphadenophaty. Annals of the Rheumatic Diseases, Vol. 27, Pages 253-260, 1968.

Ropes, M.W., A.F. Muller, W. Bauer. The entrance of glucose and sugars into joints. Arthritis and Rheumatism, Vol. 3, Pages 496-514, 1960.

Rosenthal, A.S., E.M. Shevach. The function of macrophages in antigen recognition by Guinea Pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. Journal of Experimental Medicine, Vol. 138, Pages 1194-1212, 1973.

Rothenberger, W., H.G. Thiele. In vitro-studie zur pathogenese der primärchronischen polyarthritis mittels des migration-inhibitionstests. Klinische Worschren, Voo. 48, Pages 1308-1311, 1970.

Rowley, D.A., J. Chutkow, C. Attig. Severe active cutaneous hypersensitivity in the rat produced by hemophilus pertussis vaccine. J. Exp. Med., Vol. 110, Pages 751-770, 1959.

Ruben, F.L., A. Winkelstein, R.E. Sabbagha. In utero sensitization with influenza virus in man (38918). Proceedings of the Society for Experimental Biology and Medicine, Vol. 149, Pages 881-883, 1975.

Ruddy, S., H.R. Colton. Rheumatoid Arthritis. Biosynthesis of complement proteins by synovial tissues. New England Journal of Medicine, Vol. 290, Pages 1284-1288, 1974.

Russell, R.J., P.C. Wilkinson, F. Sless, D.M.W. Parrott. Chemotaxis of lymphoblasts. Nature, Vol. 256, Pages 646-648, 1975.

Sandson, J., D. Hamerman. Isolation of hyaluronate proteins from human synovial fluid. Journal of Clinical Investigation, Vol. 41, Pages 1817-1830, 1962.

Schroder, J. Passage of leukocytes from mother to fetus. Scan. J. Immunol., Vol. 3, Pages 369-375, 1974.

Schroder, J. Transplacental passage of blood cells. Journal of Medical Genetics, Vol. 12, No. 230, Pages 230-242, 1975.

Schull, W.J., S. Cobb. The intrafamilial transmission of RA. Journal of Chronic Diseases, Vol. 22, Pages 217-222, 1969.

Schwartz, R.S. Trojan-horse lymphocytes. The New England Journal of Medicine, Vol. 290, No. 7, Pages 397-398, 1974.

Schumacher, H.R., R.C. Kitridou. Synovitis of recent onset. A clinicopathologic study during the first month of disease. Arthritis and Rheumatism, Vol. 15, Pages 465-485, 1972.

Schur, P.H., J. Sandson. Immunologic studies of the proteins of synovial fluid. Arthritis and Rheumatism, Voo. 6, Pages 115-129, 1963.

Schur, P.H., M.C. Britton, A.E. Franco, J.M. Corson, J.L. Sosman, S. Ruddy. Rheumatoid Synovitis: Complement and immune complexes. Immunological Aspects of Rheumatoid Arthritis, Rheumatology, Vol. 6, Pages 34-42, 1975.

Shearer, G.M., T.G. Rehn, C.A. Garbarino. Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. Effector cell specificity to modify cell surface components controlled by the H-2K and H-2D serological regions of the murine major histocompatibility complex. Journal of Experimental Medicine, Vol. 141, Pages 1348-1364, 1975.

Shevach, E.M., A.S. Rosenthal. The function of macrophages in antigen recognition by Guinea Pig T lymphocytes. II. Role of the macrophage in the regulation of genetic control of the immune response. Journal of Experimental Medicine, Vol. 138, Pages 1213-1229, 1973.

Shichikawa, K. Prevalence of Rheumatic Disease in Japan. Population Studies of the Rheumatic Diseases. Eds. P.H. Bennett, P.H.N. Wood, Excerpta Medica Foundation, 1968.

Short, C.L., W. Bauer, W.E. Reynolds. Rheumatoid Arthritis. Harvard University Press, 1957.

Siimes, M.A., E. Johansson, J. Rapola. Scleroderma-like Graft-Versus-Host Disease as late consequence of bone-marrow grafting. The Lancet, Vol. ii, Pages 831-832, 1977.

Sitaj, S., M. Sebo. Rheumatoid Arthritis and Ankylosing Spondylitis in Czechoslovakia. Population Studies of the Rheumatic Diseases. Eds. P.H. Bennett, P.H.N. Wood, Excerpta Medica Foundation, 1968.

Simonsen, M. The impact on the developing embryo and newborn animal of adult hologous cells. Acata Pathologica Scandinavia, Vol. 40, Pages 480-500, 1957.

Simonsen, M. Graft-Versus-Host reactions. Their natural history and applicability as tools of research. Progress in Allergy, Vol. 6, Pages 349-467, 1962.

Smiley, J.D., C. Sachs, M. Ziff. *In vitro* synthesis of immunoglobulin by Rheumatoid synovial membrane. Journal of Clinical Investigation, Vol. 47, Pages 624-632, 1968.

Smith, C. Heritability of liability and concordance in monozygous twins. Annals of Human Genetics, Vol. 34, Pages 85-91, 1970.

Solokoff, L. The pathology of Rheumatoid Arthritis and allied disorders. Arthritis and Allied Conditions, 7th and 8th Editions, A Textbook of Rheumatology. Ed. J.L. Hollander, Lea and Febiger, Philadelphia, Pages 309-332, 1966 and 1972.

Sprent, J., H. von Boehmer, M. Nabholz. Association of immunity and tolerance to host H-2 determinants in irradiated F_1 hybrid mice reconstituted with bone marrow cells from one parental strain. The Journal of Experimental Medicine, Vol. 142, Pages 321-331, 1975.

Stastny, P., V.A. Stembridge, M. Ziff. Homologous Disease, a model for Human Autoimmune Disease. Arthritis and Rheumatism, Vol. 6, No. 1, Pages 64-69, 1963.

Stastny, P. Accelerated graft rejection in the offspring of immunised mothers. Journal of Immunology, Vol. 95, Pages 929-936, 1965.

Stastny, P., C.W. Fink. HLA-Dw4 in adult and juvenile Rheumatoid Arthritis. Transplantation Proceedings, Vol. 9, Pages 1863-1866, 1977.

Stastny, P. Mixed Lymphocyte Culture typing cells from patients with Rheumatoid Arthritis. Tissue Antigens, Vol. 4, Pages 571-579, 1974.

Stern, C.M.M. The materno-foetal transfer of carrier protein sensitivity in the mouse. Immunology, Vol. 30, Pages 443-448, 1975.

Stewart, S.M., W.R.M. Alexander, J.J.R. Duthie. Isolation of Diphtheroid Bacilli from synovial membrane and fluid in Rheumatoid Arthritis. Annals of the Rheumatic Diseases, Vol. 28, Pages 477-487, 1969.

Still, G.F. On a form of Chronic Joint Disease in children. Med. Chir. Transactions, London, Vol. 80, Pages 47-59, 1897.

Stockman, R. Rheumatism and Arthritis. Green and Sons, Edinburgh, 1920.

Stoia, I., R. Ramneantu, M. Poitas. Blood groups ABO and Rh(D) factor in the Rheumatic Diseases. Annals of the Rheumatic Diseases, Vol. 26, Pages 332-333, 1967.

Swain, S.L., P.E. Trefts, H.Y.S. Tse, R.W. Dutton. The significance of T-B collaboration across haplotype barriers. Cold Spring Harbor Symposia on Qunatitative Biology, Vol. 41, Part 2, Pages 597-609, 1977.

Tannenbaum, H., G.S. Pinkus, L.G. Anderson, P.H. Schur. Immunological characterization of the mononuclear cell infiltrates in Rheumatoid synovia, in Rheumatoid nodules and in lip biopsies from patients with Sjögrens Syndrome. Arthritis and Rheumatism, Vol. 18, Pages 305-314, 1975.

Terasaki, P.I., J.D. McClelland. Microdroplet assay of human serum cytotoxins. Nature, Vol. 204, Pages 998-1000, 1964.

Terasaki, P.I., V.D. Mottironi, E.V. Barnett. Cytotoxins in disease. Autocytotoxins in Lupus. The New England Journal of Medicine, Vol. 283, Pages 724-728, 1970.

Tigelaar, R.E., R. Asofsky. Synergy among lymphoid cells mediating the Graft-Versus-Host response IV. Journal of Experimental Medicine, Vol. 135, Pages 1059-1070, 1972.

Tigelaar, R.E., R. Asofsky. Synergy among lymphoid cells mediating the Graft-Versus-Host response V. Journal of Experimental Medicine, Vol. 137, Pages 239-253, 1973.

Turakulov, Ya. Kh., E.P. Artemova, T.P. Tashkhodzhaeva. A study of the first generation of rabbits from mothers immunized with homologous thyroid gland plus adjuvant. Bulletin of Experimental Biology and Medicine, Vol. 77, No. 2, Pages 161-164, 1974.

Turk, J.L. Delayed hypersensitivity. Frontiers of Biology. Eds. A. Neuberger, E.L. Tatum. North-Holland Publishing Company, 1975.

Turner, J.H., N. Wald, W.L.G. Quinlivan. Cytogenetic evidence concerning possible transplacental transfer of leukocytes in pregnant women. Am. J. Obst. and Gynec., Vol. 95, No. 6, Pages 831-833, 1966.

Turner, M.W. Current Review: The specificity of IgM Rheumatoid factors. Current Titles in Immunology, Transplantation and Allergy, January 1975.

Tzonchev, V.T., T. Pilossoff, K. Kanev. Prevalence of inflammatory Arthritis in Bulgaria. Population Studies of the Rheumatic Diseases. Eds.

P.H. Bennett, P.H.N. Wood, Excerpta Medica Foundation, 1968.

Uphoff, D.E. Maternal influences on the immune response. Biomedicine, Vol. 10, Pages 13-22, 1973.

Valkenberg, H.A. Observer variance and prevalence of Rheumatoid Arthritis and Osteoarthritis in a longitudinal population study in the Netherlands. Population Studies of the Rheumatic Diseases. Eds. P.H. Bennett, P.H.N. Wood, Excerpta Medica Foundation, 1968.

Van Boxel, J.A., S.A. Paget. Predominantly T-cell infiltrate in Rheumatoid synovial membranes. The New England Journal of Medicine, Vol. 293, Pages 517-520, 1975.

Waller, M., R. Irby, F. Mullinax, E.C. Toone. Connective-tissue Disease and Rheumatoid factors in patients with Renal Transplants. The New England Journal of Medicine, Vol. 273, Pages 12-18, 1965.

Wangel, A., M. Klockars. Lymphocyte subpopulations in Rheumatoid synovial tissue. Annals of the Rheumatic Diseases, Vol. 36, Pages 176-180, 1977.

Warren, S.L., L. Marmor, D.M. Liebes, H.M. Rosenblatt. An active agent from human Rheumatoid Arthritis which is transmissible in mice. Archives of Internal Medicine, Vol. 124, Pages 629-634, 1969.

Wessels, C.C. Tuberculosis in rats; gross-organ changes and tuberculin sensitivity in rats infected with Tubercle Bacilli. Amer. Rev. Tuberc., Vol. 43, Pages 449-458, 1941.

Wilkinson, P.C., D.M.V. Parrott, R.J. Russell, F. Sless. Antigen-induced locomoter responses in lymphocytes. The Journal of Experimental Medicine, Vol. 145, Pages 1158-1169, 1977.

Williams, M.H., J. Brostoff, I.M. Roitt. Possible role of *Mycoplasma Fermentans* in pathogenesis of Rheumatoid Arthritis. Lancet Vol. ii, Pages 277-280, 1970.

Williams, R.C., Jr., J.D. Emmons, G.P. Astorga. Studies of antilymphocyte antisera produced against normal and Rheumatoid Arthritis lymphocytes. Arthritis and Rheumatism, Vol. 14, Pages 697-705, 1971.

Williams, R.C., Jr., R.B. Lies, R.P. Messner. Inhibition of Mixed Leukocyte Culture responses by serum and γ-globulin fractions from certain patients with connective tissue diseases. Arthritis and Rheumatism, Vol. 16, Pages 597-605, 1973.

Willoughby, D.A. The mechanism of cutaneous hypersensitivity in the rat and its suppression by immunological methods. J. Path. Bact., Vol. 92, Pages 139-150, 1966.

Winchester, R.J., J.B. Winfield, F. Siegal, P. Wernet, Z. Bentwich, H.G. Kunkel. Analyses of lymphocytes from patients with Rheumatoid Arthritis and Systemic Lupus Erythematosus. The Journal of Clinical Investigation, Vol. 54(5), Pages 1082-1092, 1974.

Winfield, J.B., R.J. Winchester, P. Wernet, S.M. Fu, H.G. Kunkel. Nature of cold-reactive antibodies to lymphocyte surface determinants in Systemic Lupus Erythematosus. Arthritis and Rheumatism, Vol. 18, Pages 1-8,

1975.

Wong, T.T.T., M.C.K. Chan. Transfusion reaction following ABO-incompatible maternofetal transfusion. The Journal of Pediatrics, Vol. 80, Pages 479-483, 1972.

Yoshida, Y., D.G. Osmond. Graft-Versus-Host activity of bone marrow lymphocytes. Experimentia, Vol. 27, Pages 834-835, 1971a.

Yoshida, Y., D.G. Osmond. Graft-Versus-Host activity of rat bone marrow, marrow fractions, and lymphoid tissues quantitated by a popliteal lymph node weight assay. Transplantation, Vol. 12, Pages 121-129, 1971b.

Zarou, D.M., H.C. Lichtman, L.M. Hellman. The transmission of chromium-51 tagged maternal erythrocytes from mother to fetus. Am. J. Obst. and Gynec., Vol. 88, No. 5, Pages 565-571, 1964.

Ziff, M. Heberden Oration, 1964. Some immunologic aspects of the connective tissue diseases. Annals of the Rheumatic Diseases, Vol. 24, Pages 103-115, 1965.

Ziff, M. Reported by Zvaifler (1973) from the Western Regional Conference on the Rheumatic Diseases, San Francisco, 1971.

Zinkernagel, R.M., P.C. Doherty. Major transplantation antigens, viruses and specificity of surveillance T cells. The "Altered Self" hypothesis. Comtemporary Topics in Immunobiology, Vol. 7, Pages 179-220, 1976.

Zinkernagel, R.M., G.N. Callahan, J. Klein, G. Dennert. Cytotoxic T cells learn specificity for self H-2 during differentiation on the thymus. Nature, Vol. 271, Pages 251-253, 1978.

Zucker-Franklin, D. The phagosomes in Rheumatoid synovial fluid leukocytes: A light, fluorescence, and electron microscope study. Arthritis and Rheumatism, Vol. 9, Pages 24-36, 1966.

Zvaifler, N.J. A speculation on the pathogenesis of joint inflammation in Rheumatoid Arthritis. Arthritis and Rheumatism, Vol. 8, Pages 289-293, 1965.

Zvaifler, N.J. The immunopathology of joint inflammation in Rheumatoid Arthritis. Advances in Immunology. Eds. F.J. Dixon, H.G. Kunkel, Vol. 16, Pages 265-336, 1973.

APPENDIX A

EXPERIMENT II · CRUDE DATA

	270 Day Old Hooded Long-Evans Female Rats				180 Day Old F_1 Hood x Fischer Female Rats			
	Weight	L Node	R Node	Spleen	Weight	L Node	R Node	Spleen
Control	331	7.7	7.7	620	279	6.4	5.9	545
	335	7.0	8.0	540	241	6.1	5.9	480
Day 0	324	7.6	7.3	650	269	5.2	5.8	480
Mean	330.0	7.43	7.67	603.3	263.0	263.0	5.87	501.7
S.D.	5.6	.83	.35	56.9	19.7	19.7	.06	37.5
Primed	331	8.0	7.7	745	298	7.3	4.5	580
	369	7.7	6.4	770	253	4.2	4.7	530
Day 0	328	6.9	6.2	580	252	4.3	5.0	460
Mean	336.0	7.80	6.76	698.3	267.6	5.26	4.73	523.3
S.D.	29.8	.95	.81	103.2	26.3	1.76	.25	60.3
Control	328	10.7	8.1	610	242	7.9	6.1	445
	302	10.7	8.4	810	279	7.7	5.5	380
Day 3	280	6.5	5.1	730	258	11.9	8.1	545
Mean	303.3	9.07	7.20	716.7	259.7	9.17	6.57	456.7
S.D.	24.0	2.25	1.82	100.7	18.6	2.37	1.36	83.1
Primed	342	10.3	5.2	595	247	13.5	7.0	395
	332	11.1	5.1	500	290	17.2	7.6	480
Day 3	338	10.1	9.0	800	272	24.2	9.6	580
Mean	337.3	10.50	6.43	187.1	269.6	18.30	8.07	485.0
S.D.	5.0	.53	2.22	44.5	21.6	5.43	1.36	92.6

*Rat weight in grams, L and R node weights in mg, spleen weight in mg.

APPENDIX B

EXPERIMENT III SHOWING THE BREEDING PATTERNS

Fischer Mothers

CONTROL	TEST
C1 Litter born 13.5.75 Number 5, 1 died early Weaned 4, 2 male 2 female	D1 Litter born 13.5.75 Number 12 Weaned 12, 5 male 7 female
C2 Litter born 11.5.75 Number 11, 1 died early Weaned 10, 4 male 6 female	D2 Litter born 9.5.75 Number 12, 1 runt, 2 died *after weaning* Weaned 12, 4 male 6 female
C3 Litter born 11.5.75 Number 12, 2 died after weaning Weaned 12, 4 male 6 female	D3 Litter none
C4 Litter born 17.5.75 Number 11, 2 died after weaning Weaned 11, 2 male 7 female	D4 Litter born 18.5.75 Number 10, 1 died later Weaned 9, 3 male 6 female
C5 Litter born 17.5.75 Number 13, 2 died Day 1 Weaned 11, 5 male 6 female	D5 Litter born 18.5.75 Number 12 Weaned 12, 5 male 7 female
C6 Litter born 23.5.75 Number 13, 1 died early 1 died late Weaned 11, 7 male 4 female	D6 Litter born 20.5.75 Number 10, 1 died after weaning Weaned 10, 4 male 5 female
C7 Litter born 22.5.75 Number 12 Weaned 12, 8 male 4 female	D7 Litter born 17.5.75 Number 13 Weaned 13, 7 male 6 female
C8 Litter born 18.5.75 Number 2, killed by mother Weaned 0	D8 Litter born 18.5.75 Number 5 Weaned 4, all male
Total 78 born, 70 weaned, 4 died later	Total 74 born, 72 weaned, 3 died later

APPENDIX B

EXPERIMENT III SHOWING THE BREEDING PATTERNS

Sprague Mothers

CONTROL	TEST
C1 Litter born 12.5.75 Number 14, 1 died early Weaned 13, 8 male 5 female	D1 Litter born 12.5.75 Number 13 Weaned 13, 5 male 8 female
C2 Litter born 9.5.75 Number 15, 2 runts Weaned 14, 8 male 4 female	D2 Litter born 11.5.75 Number 13, 1 died early Weaned 12, 7 male 5 female
C3 Litter born 9.5.75 Number 13, 1 runt Weaned 13, 2 male 10 female	D3 Litter born 13.5.75 Number 13 Weaned 13, 8 male 5 female
C4 Litter born 17.5.75 Number 14, 1 died early 4 died later Weaned 9, 0 male 8 female	D4 Litter born 23.5.75 Number 14, 1 died early 1 died late Weaned 12, 8 male 3 female
C5 Litter born 19.5.75 Number 10 Weaned 10, 5 male 5 female	D5 Litter none
C6 Litter born 17.5.75 Number 14, 4 died first day Weaned 10, 1 male 5 female	D6 Litter born 23.5.75 Number 4 Weaned 4, 2 male 2 female
Total 80 born, 66 weaned, 8 died later	Total 57 born, 54 weaned, 1 died later

Note that the number weaned does not necessarily add up to the males plus the females since those represent the animals still alive at Day 55.

APPENDIX C

RECIPROCAL CROSSBREEDING
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 86/02/18) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

B6/

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

LNODE	LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP	WHETHER MOTHER WAS CONTROL OR PRIMED
DAY	TIME AFTER ONCB CHALLENGE TO LEFT PAW
SEX	OFFSPRINGS SEX
WITH WEIGHT	OF RAT IN GRAMS

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	6584.102	1	6584.102	20.221	0.000
WEIGHT	6584.102	1	6584.102	20.221	0.000
MAIN EFFECTS	59812.648	5	11962.527	36.739	0.000
GROUP	66.385	1	66.385	0.204	0.653
DAY	54120.531	3	18040.176	55.405	0.000
SEX	3447.704	1	3447.704	10.589	0.002
EXPLAINED	66396.750	6	11066.125	33.986	0.000
RESIDUAL	32560.688	100	325.607		
TOTAL	98957.438	106	933.561		

COVARIATE - RAW REGRESSION COEFFICIENT

WEIGHT	0.224

107 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING 86/
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 86/02/18) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * M U L T I P L E C L A S S I F I C A T I O N A N A L Y S I S * * *

BY LNODE LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

GRAND MEAN = 83.71

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
GROUP							
1 CONTROL	54	1.25				-0.82	
2 PRIMED	53	-1.28	0.04			0.84	0.03
DAY							
0 ZERO	27	-27.75				-29.34	
1 ONE	26	-8.29				-6.86	
2 TWO	27	-0.88				1.75	
4 FOUR	27	35.81	0.76			33.43	0.74
SEX							
1 MALE	53	1.23				-0.87	
2 FEMALE	54	-1.21	0.04			0.90	0.30
MULTIPLE R SQUARED							0.671
MULTIPLE R							0.819

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT
OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL
INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT
IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN SPRAGUE-DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

RNODE RIGHT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	4034.075	1	4034.075	26.730	0.000
WEIGHT	4034.075	1	4034.075	26.730	0.000
MAIN EFFECTS	1185.203	5	237.041	1.571	0.175
GROUP	38.610	1	38.610	0.256	0.614
DAY	1078.169	3	359.390	2.381	0.074
SEX	80.011	1	80.011	0.530	0.468
EXPLAINED	5219.277	6	869.879	5.764	0.000
RESIDUAL	15092.063	100	150.921		
TOTAL	20311.340	106	191.616		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.176

107 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING.
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * MULTIPLE CLASSIFICATION ANALYSIS * * *

RNODE RIGHT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

GRAND MEAN = 58.61

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
GROUP							
1 CONTROL	54	1.97				0.63	
2 PRIMED	53	-2.00	0.14			-0.64	0.05
DAY							
0 ZERO	27	-1.72				-2.73	
1 ONE	26	0.16				1.22	
2 TWO	27	3.65				4.74	
4 FOUR	27	-2.09	0.17			-3.19	0.23
SEX							
1 MALE	53	4.22				-1.38	
2 FEMALE	54	-4.14	0.30			1.36	0.10
MULTIPLE R SQUARED							0.257
MULTIPLE R							0.507

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

→ THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING 06/
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	342768.750	1	342768.750	48.610	0.000
WEIGHT	342768.750	1	342768.750	48.610	0.000
MAIN EFFECTS	256314.688	5	51262.938	7.270	0.000
GROUP	26649.367	1	26649.367	3.779	0.055
DAY	14522.105	3	4840.699	0.686	0.562
SEX	151431.188	1	151431.188	21.475	0.000
EXPLAINED	599083.438	6	99847.188	14.160	0.000
RESIDUAL	705145.563	100	7051.453		
TOTAL	1304229.000	106	12304.047		

COVARIATE RAW REGRESSION COEFFICIENT

WEIGHT 1.618

107 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT
OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL
INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT
IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * MULTIPLE CLASSIFICATION ANALYSIS * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

GRAND MEAN = 528.78

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
GROUP							
1 CONTROL	54	-14.90				-16.48	
2 PRIMED	53	15.14				16.74	
			0.14				0.15
DAY							
0 ZERO	27	-5.08				-9.70	
1 ONE	26	15.83				18.78	
2 TWO	27	-0.64				2.65	
4 FOUR	27	-9.53				-11.04	
			0.09				0.11
SEX							
1 MALE	53	73.01				60.12	
2 FEMALE	54	-71.66				-59.01	
			0.66				0.54
MULTIPLE R SQUARED							0.459
MULTIPLE R							0.678

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER) THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS 06/02

* * * * * * * * * * ANALYSIS OF VARIANCE * * * * * * * * * *

	LNODE	LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
BY	LNOUP	WHETHER MOTHER WAS CONTROL OR PRIMED
	DAY	TIME AFTER DNCB CHALLENGE TO LEFT PAW
	SEX	OFFSPRINGS SEX
WITH	WEIGHT	OF RAT IN GRAMS

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	1292.113	1	1292.113	2.595	0.110
WEIGHT	1292.113	1	1292.113	2.595	0.110
MAIN EFFECTS	12345.129	5	2469.026	4.958	0.000
GROUP	134.364	1	134.364	0.270	0.625
DAY	9481.453	3	3163.818	6.353	0.001
SEX	2059.732	1	2059.732	4.136	0.045
EXPLAINED	13637.242	6	2272.874	4.564	0.000
RESIDUAL	52290.820	105	498.008		
TOTAL	65928.063	111	593.946		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.119

112 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 86/02/28) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * MULTIPLE CLASSIFICATION ANALYSIS * * *

	INODE	LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
BY	GROUP	WHETHER MOTHER WAS CONTROL OR PRIMED
	DAY	TIME AFTER ONCB CHALLENGE TO LEFT PAW
	SEX	OFFSPRINGS SEX
WITH	WEIGHT	OF RAT IN GRAMS

GRAND MEAN = 68.63

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
GROUP							
1 CONTROL	56	1.27				1.10	
2 PRIMED	56	-1.27				-1.10	
			0.05				0.05
DAY							
0 ZERO	28	-12.02				-13.02	
1 ONE	28	-6.73				-4.11	
2 TWO	28	6.45				6.55	
4 FOUR	28	12.30				10.58	
			0.40				0.38
SEX							
1 MALE	56	-1.43				-2.89	
2 FEMALE	56	1.43				2.89	
			0.06				0.24
MULTIPLE R SQUARED							0.207
MULTIPLE R							0.455

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

ERROR: RIGHT POPLITEAL NODE WEIGHT IN TENTHS
BY GROUP EXPTHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	1247.745	1	1247.745	10.893	0.001
WEIGHT	1247.745	1	1247.745	10.893	0.001
MAIN EFFECTS	699.713	5	139.943	1.222	0.304
GROUP	465.497	1	465.497	4.064	0.046
DAY	293.940	5	67.980	0.593	0.621
SEX	39.314	1	39.314	0.343	0.559
EXPLAINED	1947.461	6	324.577	2.834	0.013
RESIDUAL	12027.480	105	114.547		
TOTAL	13974.941	111	125.900		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.117

112 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING 867
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * MULTIPLE CLASSIFICATION ANALYSIS * * *

BY RNODE RIGHT POPLITEAL NODE WEIGHT IN TENTH MGS
GROUP WHETHER MOTHER HAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

GRAND MEAN = 45.16

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
GROUP							
1 CONTROL	56	2.14				2.04	
2 PRIMED	56	-2.14				-2.04	
			0.19				0.18
DAY							
0 ZERO	28	-1.66				-2.26	
1 ONE	28	-0.13				1.44	
2 TWO	28	0.24				0.29	
4 FOUR	28	1.55				0.52	
			0.10				0.12
SEX							
1 MALE	56	1.86				-0.01	
2 FEMALE	56	-1.86				0.01	
			0.17				0.07
MULTIPLE R SQUARED							0.139
MULTIPLE R							0.373

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	250725.625	1	250725.625	55.919	0.000
WEIGHT	250725.625	1	250725.625	55.919	0.000
MAIN EFFECTS	312898.438	5	62579.688	13.957	0.000
GROUP	99329.813	1	99329.813	22.153	0.000
DAY	4140.101	3	1380.380	0.308	0.819
SEX	203419.750	1	203419.750	45.369	0.000
EXPLAINED	563624.063	6	93937.313	20.951	0.000
RESIDUAL	470790.563	105	4483.719		
TOTAL	1034414.625	111	9319.047		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	1.653

112 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * MULTIPLE CLASSIFICATION ANALYSIS * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

GRAND MEAN = 491.96

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
GROUP							
1 CONTROL	56	30.00				29.80	
2 PRIMED	56	-30.00				-29.80	
			0.31				0.31
DAY							
0 ZERO	28	10.36				9.19	
1 ONE	28	-1.25				1.00	
2 TWO	28	-5.36				-5.24	
4 FOUR	28	-3.75				-5.76	
			0.06				0.06
SEX							
1 MALE	56	63.75				58.35	
2 FEMALE	56	-63.75				-58.56	
			0.66				0.61
MULTIPLE R SQUARED							0.545
MULTIPLE R							0.738

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH ALL INTERACTIONS SUPPRESSED SINCE THEY WERE INSIGNIFICANT IN PRIOR ANALYSES

RECIPROCAL CROSSBREEDING
STRAIN SPRAGUE-DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * *

LNODE LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER UNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	9647.816	1	9647.816	29.257	0.000
WEIGHT	9647.816	1	9647.816	29.257	0.000
MAIN EFFECTS	60867.410	5	12013.480	36.434	0.000
GROUP	156.512	1	156.512	0.475	0.493
DAY	53084.387	3	17694.795	53.664	0.000
SEX	4358.926	1	4358.926	13.220	0.000
2-WAY INTERACTIONS	2020.375	7	288.625	0.875	0.529
GROUP DAY	298.268	3	99.423	0.302	0.824
GROUP SEX	386.446	1	386.446	1.172	0.282
DAY SEX	1365.578	3	455.193	1.380	0.254
3-WAY INTERACTIONS	923.406	3	307.802	0.933	0.428
GROUP DAY SEX	923.406	3	307.802	0.933	0.428
EXPLAINED	69281.438	16	4330.090	13.132	0.000
RESIDUAL	29676.000	90	329.733		
TOTAL	98957.438	106	933.561		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.470

107 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING 067
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * ANALYSIS OF VARIANCE * * * * * * * * *

ENCODE RIGHT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	1899.192	1	1899.192	12.086	0.001
WEIGHT	1899.192	1	1899.192	12.086	0.001
MAIN EFFECTS	1195.737	5	239.147	1.522	0.191
GROUP	45.262	1	45.262	0.288	0.593
DAY	1093.706	3	364.585	2.320	0.081
SEX	55.288	1	55.288	0.352	0.555
2-WAY INTERACTIONS	788.646	7	112.664	0.717	0.656
GROUP DAY	446.095	3	148.698	0.946	0.422
GROUP SEX	17.006	1	17.006	0.108	0.743
DAY SEX	330.521	3	110.174	0.701	0.554
3-WAY INTERACTIONS	148.842	3	49.614	0.316	0.814
GROUP DAY SEX	148.842	3	49.614	0.316	0.814
EXPLAINED	6168.881	16	385.550	2.454	0.004
RESIDUAL	14142.539	90	157.139		
TOTAL	20311.348	106	191.616		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.289

107 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

06/

RECIPROCAL CROSSBREEDING
STRAIN SPRAGUE DAWLEY
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	10622.852	1	10622.852	1.404	0.239
WEIGHT	10622.852	1	10622.852	1.404	0.239
MAIN EFFECTS	247601.938	5	49520.387	6.544	0.000
GROUP	26710.234	1	26710.234	3.530	0.064
DAY	15151.219	3	5050.406	0.667	0.574
SEX	137296.313	1	137296.313	18.144	0.000
2-WAY INTERACTIONS	22758.625	7	3251.232	0.430	0.881
GROUP DAY	6415.168	3	2138.389	0.283	0.838
GROUP SEX	265.980	1	265.980	0.035	0.852
DAY SEX	16051.066	3	5350.355	0.707	0.550
3-WAY INTERACTIONS	1198.738	3	399.579	0.053	0.984
GROUP DAY SEX	1198.738	3	399.579	0.053	0.984
EXPLAINED	623179.813	16	38948.738	5.147	0.000
RESIDUAL	681049.188	90	7567.211		
TOTAL	1304229.000	106	12304.047		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.493

107 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE PATERNAL (CREATION DATE = 86/02/18) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * *

LNODE LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER ONCH CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	2477.501	1	2477.501	5.269	0.024
WEIGHT	2477.501	1	2477.501	5.269	0.024
MAIN EFFECTS	12363.395	5	2472.679	5.259	0.000
GROUP	133.423	1	133.423	0.284	0.596
DAY	9471.359	3	3157.120	6.714	0.000
SEX	2071.021	1	2071.021	4.404	0.038
2-WAY INTERACTIONS	4951.668	7	707.381	1.504	0.175
GROUP DAY	3145.900	3	1048.646	2.230	0.090
GROUP SEX	74.244	1	74.244	0.158	0.692
DAY SEX	1680.268	3	560.089	1.191	0.317
3-WAY INTERACTIONS	2645.566	3	881.855	1.875	0.139
GROUP DAY SEX	2645.566	3	881.855	1.875	0.139
EXPLAINED	21257.465	16	1328.592	2.825	0.001
RESIDUAL	44670.598	95	470.217		
TOTAL	65928.063	111	593.946		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.240

112 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER) THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

ANODE RIGHT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	871.464	1	871.464	7.618	0.007
WEIGHT	871.464	1	871.464	7.618	0.007
MAIN EFFECTS	702.132	5	140.426	1.228	0.302
GROUP	464.851	1	464.851	4.064	0.047
DAY	205.567	3	68.519	0.599	0.617
SEX	41.566	1	41.566	0.363	0.548
2-WAY INTERACTIONS	751.773	7	107.396	0.939	0.481
GROUP DAY	268.810	3	89.937	0.781	0.507
GROUP SEX	13.073	1	13.073	0.114	0.736
DAY SEX	470.337	3	156.779	1.371	0.257
3-WAY INTERACTIONS	407.757	3	135.919	1.188	0.318
GROUP DAY SEX	407.757	3	135.919	1.188	0.318
EXPLAINED	3107.715	16	194.232	1.698	0.060
RESIDUAL	10867.227	95	114.392		
TOTAL	13974.941	111	125.900		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.142

112 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING
STRAINS COMBINED

86/6

FILE MATERNAL (CREATION DATE = 86/02/18) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

	1 NOVE	LEFT POPLITEAL NODE WEIGHT IN TENTH MGS
	BY GROUP	WHETHER MOTHER WAS CONTROL OR PRIMED
	DAY	TIME AFTER DNCB CHALLENGE TO LEFT PAW
	SEX	OFFSPRINGS SEX
	STRAIN	MATERNAL STRAIN
	WITH WEIGHT	OF RAT IN GRAMS

* *

SOURCE OF VARIATION			SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES			10974,980	1	10974,980	27,040	0,000
WEIGHT			10974,980	1	10974,980	27,040	0,000
MAIN EFFECTS			75076,750	6	12512,789	30,829	0,000
GROUP			15,357	1	15,357	0,038	0,846
DAY			52356,004	3	17452,001	42,999	0,000
SEX			5854,379	1	5854,379	14,424	0,000
STRAIN			5797,352	1	5797,352	14,284	0,000
2-WAY INTERACTIONS			13995,941	12	1166,328	2,874	0,001
GROUP	DAY		2083,417	3	694,472	1,711	0,166
GROUP	SEX		255,222	1	255,222	0,629	0,429
GROUP	STRAIN		115,559	1	115,559	0,285	0,594
DAY	SEX		28,983	3	9,661	0,024	0,995
DAY	STRAIN		11620,070	3	3873,357	9,543	0,000
SEX	STRAIN		27,732	1	27,732	0,068	0,794
3-WAY INTERACTIONS			6303,145	10	630,314	1,553	0,121
GROUP	DAY	SEX	2150,484	3	716,161	1,769	0,155
GROUP	DAY	STRAIN	1635,894	3	545,298	1,344	0,262
GROUP	SEX	STRAIN	13,134	1	13,134	0,032	0,857
DAY	SEX	STRAIN	2732,588	3	910,863	2,244	0,085
4-WAY INTERACTIONS			1108,359	3	369,453	0,910	0,437
GROUP	DAY	SEX	1108,359	3	369,453	0,910	0,437
	STRAIN						
EXPLAINED			118552,863	32	3704,752	9,128	0,000
RESIDUAL			75492,750	186	405,875		
TOTAL			194044,613	218	890,114		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0,356

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER) THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING 06/
STRAIN FISCHER
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER ONCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
WITH WEIGHT OF RAT IN GRAMS

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES	2830.672	1	2830.672	0.613	0.436
WEIGHT	2830.672	1	2830.672	0.613	0.436
MAIN EFFECTS	309893.813	5	61978.762	13.413	0.000
GROUP	99403.688	1	99403.688	21.512	0.000
DAY	4127.930	3	1375.977	0.298	0.827
SEX	200000.750	1	200000.750	43.283	0.000
2-WAY INTERACTIONS	16674.484	7	2382.069	0.516	0.821
GROUP DAY	5900.793	3	1966.931	0.426	0.735
GROUP SEX	2123.214	1	2123.214	0.459	0.500
DAY SEX	8509.996	3	2836.665	0.614	0.608
3-WAY INTERACTIONS	15106.000	3	5035.332	1.090	0.357
GROUP DAY SEX	15106.000	3	5035.332	1.090	0.357
EXPLAINED	595441.375	16	37215.086	8.054	0.000
RESIDUAL	438973.250	95	4620.770		
TOTAL	1034414.625	111	9319.047		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.256

112 CASES WERE PROCESSED.
0 CASES (0.0 PCT) WERE MISSING.

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING
STRAINS COMBINED

FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * *

SPLEEN WEIGHT IN MILLIGRAMS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER DNCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
STRAIN MATERNAL STRAIN
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION			SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES			12236.672	1	12236.672	2.030	0.156
WEIGHT			12236.672	1	12236.672	2.030	0.156
MAIN EFFECTS			393273.936	6	65545.625	10.874	0.000
GROUP			9949.336	1	9949.336	1.651	0.200
DAY			9686.621	3	3228.874	0.536	0.658
SEX			334601.500	1	334601.500	55.509	0.000
STRAIN			25746.422	1	25746.422	4.272	0.040
2-WAY INTERACTIONS			134554.688	12	11212.891	1.860	0.042
GROUP	DAY		3297.761	3	1099.253	0.182	0.908
GROUP	SEX		234.103	1	234.103	0.039	0.844
GROUP	STRAIN		111016.625	1	111016.625	18.417	0.000
DAY	SEX		7771.366	3	2590.455	0.430	0.732
DAY	STRAIN		8863.609	3	2954.536	0.490	0.690
SEX	STRAIN		1440.843	1	1440.843	0.239	0.625
3-WAY INTERACTIONS			38543.258	10	3854.326	0.639	0.779
GROUP	DAY	SEX	10411.777	3	3470.592	0.576	0.632
GROUP	DAY	STRAIN	9272.789	3	3090.930	0.513	0.674
GROUP	SEX	STRAIN	2392.934	1	2392.934	0.397	0.529
DAY	SEX	STRAIN	15997.512	3	5332.504	0.885	0.450
4-WAY INTERACTIONS			5944.961	3	1981.654	0.329	0.805
GROUP	DAY	SEX STRAIN	5944.961	3	1981.654	0.329	0.805
EXPLAINED			1291594.000	32	40362.313	6.696	0.000
RESIDUAL			1121188.000	186	6027.891		
TOTAL			2412782.000	218	11067.805		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.376

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

RECIPROCAL CROSSBREEDING
STRAINS COMBINED
FILE MATERNAL (CREATION DATE = 06/02/78) EFFECTS UPON THE IMMUNE SYSTEM OF RATS

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

PNODE RIGHT POPLITEAL NODE WEIGHT IN TENTH MGS
BY GROUP WHETHER MOTHER WAS CONTROL OR PRIMED
DAY TIME AFTER ONCB CHALLENGE TO LEFT PAW
SEX OFFSPRINGS SEX
STRAIN MATERNAL STRAIN
WITH WEIGHT OF RAT IN GRAMS

* *

SOURCE OF VARIATION			SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
COVARIATES			2675.441	1	2675.441	19.822	0.000
WEIGHT			2675.441	1	2675.441	19.822	0.000
MAIN EFFECTS			5271.934	6	878.656	6.510	0.000
GROUP			454.531	1	454.531	3.368	0.068
DAY			862.298	3	287.433	2.130	0.098
SEX			77.245	1	77.245	0.572	0.450
STRAIN			2583.195	1	2583.195	19.139	0.000
2-WAY INTERACTIONS			1622.336	12	135.195	1.002	0.449
GROUP	DAY		535.414	3	178.471	1.322	0.268
GROUP	SEX		3.257	1	3.257	0.024	0.877
GROUP	STRAIN		62.767	1	62.767	0.465	0.496
DAY	SEX		540.891	3	180.297	1.336	0.264
DAY	STRAIN		417.127	3	139.042	1.030	0.380
SEX	STRAIN		72.063	1	72.063	0.534	0.466
3-WAY INTERACTIONS			610.755	10	61.076	0.453	0.918
GROUP	DAY	SEX	122.122	3	40.707	0.302	0.824
GROUP	DAY	STRAIN	205.062	3	68.354	0.506	0.678
GROUP	SEX	STRAIN	48.215	1	48.215	0.357	0.551
DAY	SEX	STRAIN	232.190	3	77.397	0.573	0.633
4-WAY INTERACTIONS			442.208	3	147.403	1.092	0.354
GROUP	DAY	SEX STRAIN	442.208	3	147.403	1.092	0.354
EXPLAINED			19075.781	32	596.118	4.417	0.000
RESIDUAL			25104.934	186	134.973		
TOTAL			44180.715	218	202.664		

COVARIATE	RAW REGRESSION COEFFICIENT
WEIGHT	0.176

N.B. THESE RATS WERE ALL F_1 HYBRIDS (SPRAGUE X FISCHER)
THE STRAIN INDICATED AT THE TOP OF THE PAGE IS THAT OF THE MOTHER

THE ANALYSIS OF VARIANCE WAS PERFORMED WITH THE REGRESSION APPROACH SO THAT THE COVARIANCE OF WEIGHT WAS ASSESSED SIMULTANEOUSLY

APPENDIX D

HYPOTHESES FOR THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

RHEUMATOID FACTOR The presence of IgM anti-IgG in high titres in the majority of patients with Rheumatoid Arthritis is strong evidence of a common immunological mechanism and would seem to offer an insight into the actual processes involved, but this confident prediction has been thwarted by the heterogeneous collection of low affinity antibodies actually found in these patients. It has been accepted for some time that the major specificity for these antibodies seems to be antigens located upon the constant region of altered (e.g. aggregated or combined with antigen) IgG. Turner (1974) has made a case for there not being any hidden antigen uncovered by such a process but that the Factors are of such a low affinity that it is only in the cases in which multiple antigenic determinants are brought into close proximity that the combined affinity of the ten binding sites gives a stability to the complex and the opportunity to crosslink the antigens. If this proves to be the case it is difficult to see how Rheumatoid Factor would be capable of acting *in vivo* in the absence of an IgG aggregating factor though it is not difficult to see how *in vitro* damage to native IgG could cause artefactual binding of Rheumatoid Factor and fixation of complement. As for complement fixation whether the Factor acts to block fixation on aggregated IgG or fixes complement itself *in vitro* seems to depend upon the exact technique, but certainly no investigator could possibly claim that it has anything approaching the levels seen with the experimental analogue, Rabbit anti-human-Ig (Coombs' reagent).

For these reasons Rheumatoid Factor appears to be a deliberately and perversely functionless collection of antibodies that only exceptionally

has recognisable and/or strong affinities for immunoglobulin allotypes. Turner (1974) has made a good case for these recognisable affinities being directed against IgG's no longer present in the body namely the maternally derived, placentally transmitted immunoglobulins that had been catabolised in early infancy though not before causing an immune response with production of anti-allotypes which are commonly detected in young children. Here again there is evidence of a tangential response in the form of a restimulated secondary response to a non-existent antigen in patients with joint disease.

Another piece of evidence originates from Munthe (1972) concerning the presumed site of production for most of these Rheumatoid Factors in the plasma cell infiltrate of the synovium. Most of the plasma cells could be shown by immunofluorescent techniques to be producing immunoglobulin but relatively few could be shown to be producing anti-immunoglobulin until the cells were treated with pepsin which is an enzyme that cleaves immunoglobulin G and by so doing destroys the antigen for most Rheumatoid Factors. After pepsin treatment of the tissue sections, the majority of plasma cells could be shown to bind aggregated IgG which indicates the presence of "hidden" Rheumatoid Factor already bound to its antigen IgG. These authors were also able to demonstrate that if the sections were overlaid by fresh human complement C_3 and C_1q could be detected inside many of the plasma cells (>50%) indicating again the presence of preformed complexes inside the cells. Finally, they noted that in using double staining techniques both IgG and IgM could be detected in 1-2% of the plasma cells. All these shreds of evidence are concordant with

the idea that immune complexes are forming inside these cells from a combination of endogenous IgG and exogenous anti-IgG (and vice versa) which in most instances is of the IgG class itself and therefore not the IgM Rheumatoid Factor detected by the routine tests in serum.

Another line of evidence that pertains to the production of Rheumatoid Factor stems from investigations into a seemingly different problem using an animal model of the anatomical changes observed in Rheumatoid lymph nodes (Dumonde, 1976). The lymph nodes of patients with Rheumatoid Arthritis are very often enlarged with 29% v 9% of controls reported in one series (Robertson, 1968), and 82% v 52% of controls in another series (Short, 1957). These observations tend to lessen the differences claimed by some for Felty's syndrome and Stills' disease though there seem to be wide differences of opinion as to what constitutes lymphadenopathy as witnessed by the two series quoted above. The nodes most often claimed to be enlarged are those draining lymph from joints with active disease and were mainly in seropositive patients (Robertson, 1968).

Nosanchuk (1969) wrote of the histologic change found in Rheumatoid lymph nodes: "The outstanding histologic features (*of Rheumatoid lymph nodes*) were: 1. follicular hyperplasia, and 2. plasmacytosis. The follicles were generally diffuse throughout both Cortex and Medulla of the node.... were characterised by large germinal centres well demarcated from the surrounding mantle of mature lymphocytes, which, in turn, were discrete from the adjacent interfollicular areas.....were frequently observed

to have mitotic activity and phagocytosis of debris ("starry sky" pattern)....usually contained a mixture of cells consisting of immature and mature lymphocytes, histiocytes and occasional plasma cells. The interfollicular zone had a characteristic appearance with small and large aggregates or sheets of plasma cells being prominent.....the plasma cell was often the only cell type present.....capillary endothelial hyperplasia was a consistent finding. Sinus histiocytic hyperplasia (*was*) present in all cases. In our material 3 of 21 patients had been incorrectly diagnosed as having follicular lymphoma and were treated with irradiation and cancer chemotherapy."

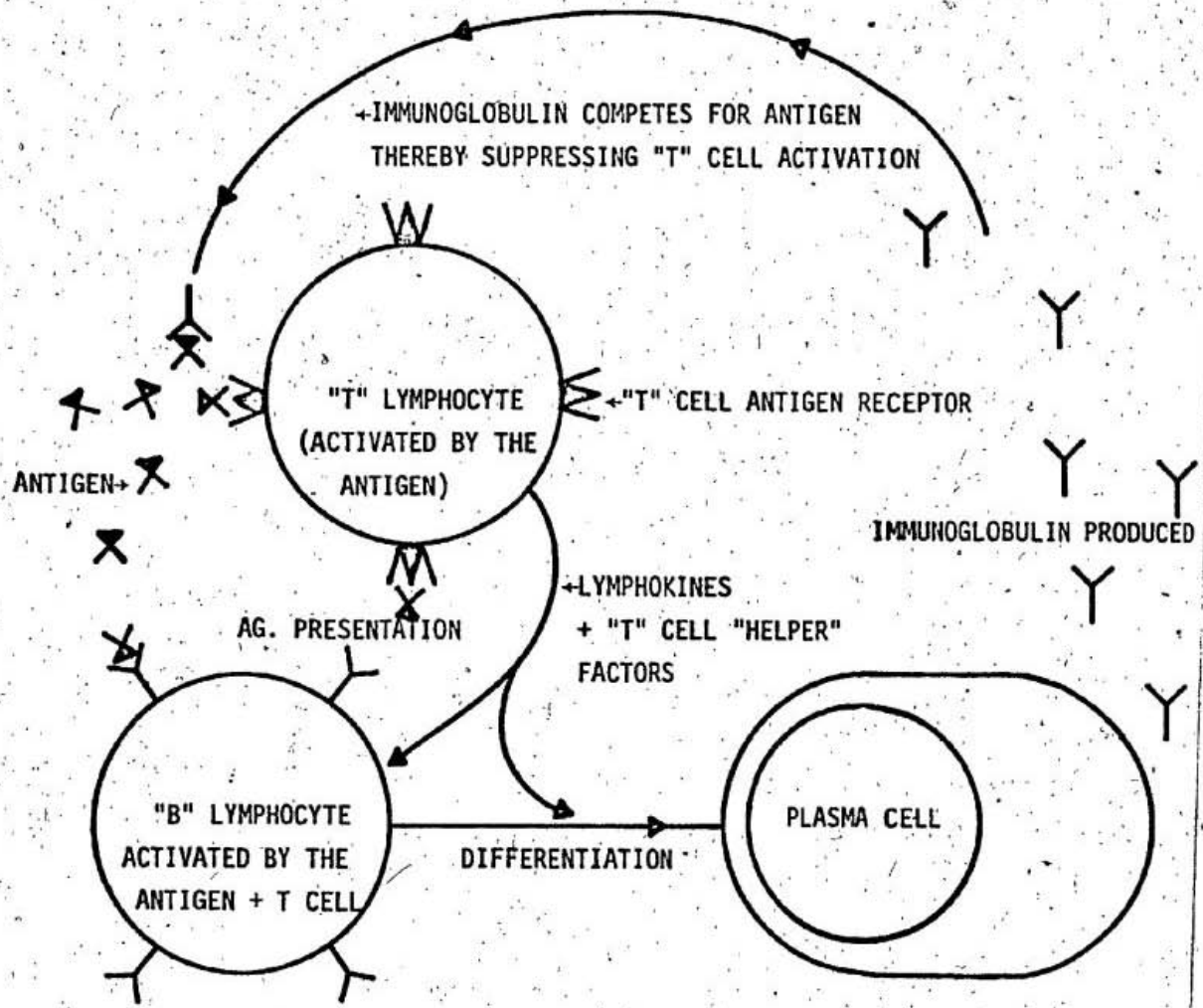
This picture of intense germinal centre formation coupled with the sustained early differentiation of plasma cells as witnessed by their presence in the lymph node cortical region is unique in human pathology and until recently without parallel in experimental animals. As Dumonde (1976) comments, this picture combines many of the features found in acute high-antigen-dose stimulation of rodent lymph nodes together with features commonly found in chronic antigen stimulation. However, the two states are not compatible as there is morphological degradation of the node if stimulated chronically by high antigen doses.

Dumonde (1976) reported that some of the features of the Rheumatoid node could be mimicked in experimental mice by the administration of mitogenic lymphokines (pharmacologically active products of lymphocytes) into the afferent lymph ducts of the lymph node. This caused paracortical hyperplasia ("T" lymphocyte region) followed by intense germinal centre formation. However, there was no infiltration by plasma cells and this

model is not sufficiently similar to the Rheumatoid node for that reason.

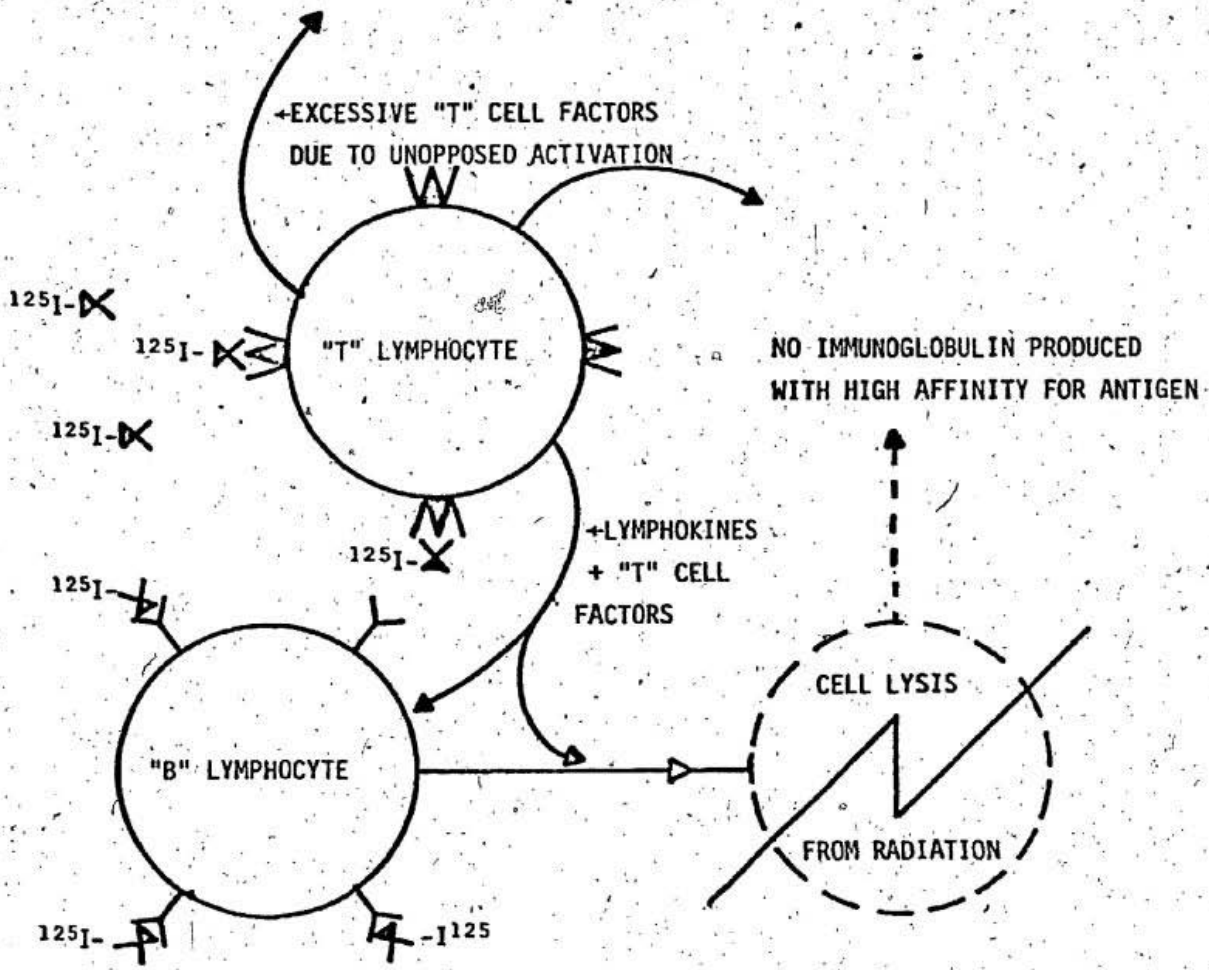
The next model described by Dumonde was based upon the previously published experiments of Humphrey (1970) involving the suicide of antigen binding "B" cells after union with radioactive antigen. It had been observed that mice injected with Iodine-125 Keyhole-Limpet-Haemocyanogen (which is a potent "T" lymphocyte dependent antigen) responded with a depression or total abrogation of the specific antibody response. This appeared to be due to the lethal irradiation of the antigen-binding "B" cells, with a seemingly smaller lethal effect upon the "T" cells. The subsequent morphologic changes seen in the node could be expected to show the effects of "T" mediated stimulation in the absence of antibody mediated feedback limitation (see Figures 5 and 6).

The lymph nodes were two or three times the size of the contralateral control nodes that had been stimulated with normal KLH and showed striking hyperplasia of both paracortex (a "T" lymphocyte area) and cortex (a "B" lymphocyte area) with multiple rows of germinal centres in the cortex (compared with the normal single row, but not in the medulla as reported for the Rheumatoid node). Plasma cells were present in markedly increased numbers and in atypical sites; (in the germinal centres, and in the interfollicular cortex) and the picture was completed by the presence of capillary endothelial hyperplasia and sinus histiocytosis. The similarity to the pathological anatomy of the Rheumatoid node is very close and the unusual nature of these observations overcomes some of the natural scepticism that should be applied



THE NORMAL HOMEOSTASIS OF THE IMMUNE RESPONSE BY THE REMOVAL OF ANTIGEN BY ANTIBODY

Figure 19



ANTIGEN INDUCED SUICIDE OF "B" CELLS WITH HIGH AFFINITY ANTIBODY BY THE RADIOACTIVE ANTIGEN WITH THE CONSEQUENT OVERACTIVITY OF THE "T" CELLS (AFTER J.H. HUMPHREY 1970)

Figure 20

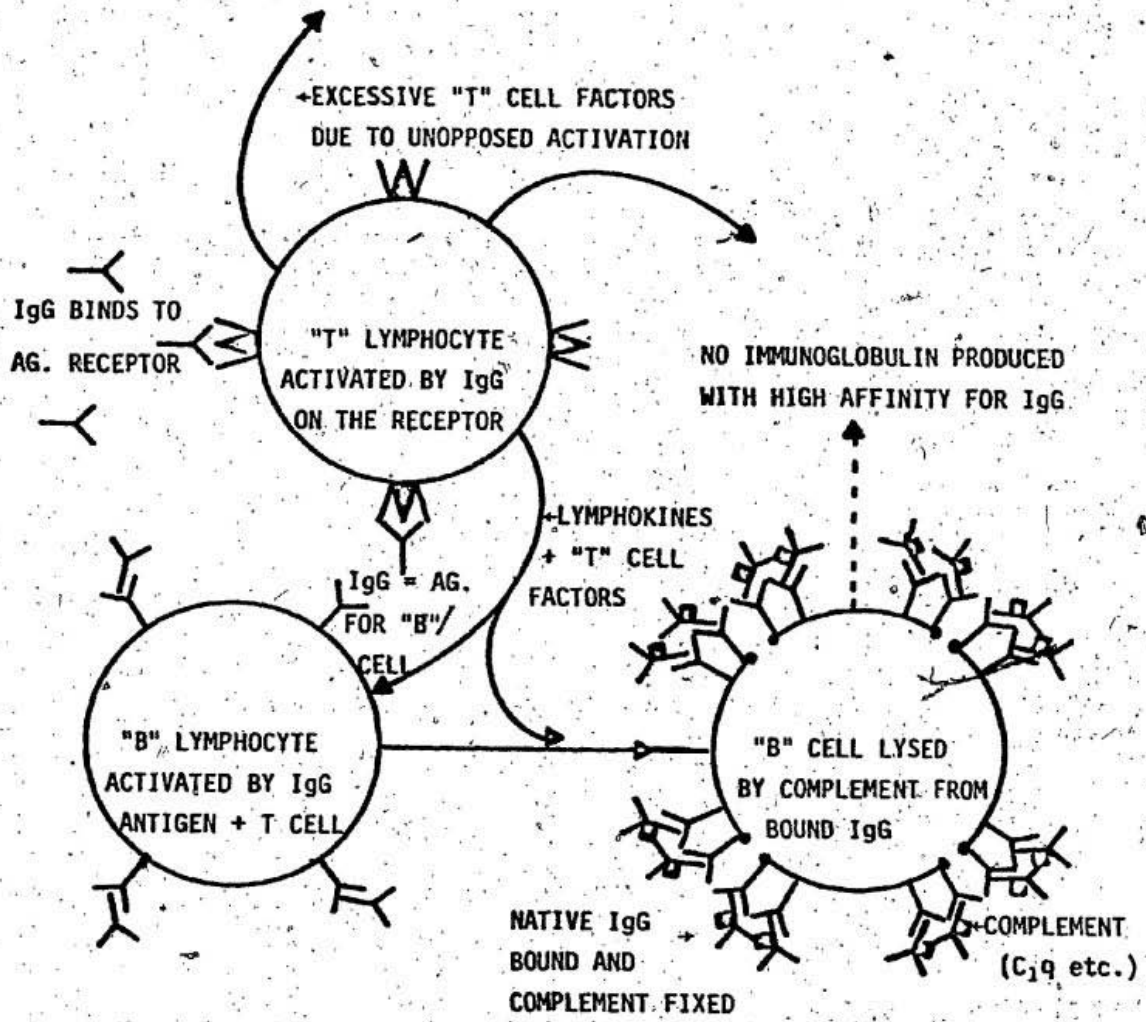
in correlating experimental animal and human pathology.

None of the lines of investigation presented above fall into the main stream of evidence of the nature, function and natural history of Rheumatoid Factor, but they are well documented and from reliable research workers. If the data is synthesised, the following inference appears consistent with those findings:

HYPOTHESIS:

RHEUMATOID FACTOR APPEARS TO BE PREVENTED FROM FORMING HIGH AFFINITY ANTIBODY TO ITS PRESUMED ANTIGEN, IgG, AND THE HISTOLOGY OF ONE OF THE SITES OF PRODUCTION (THE LYMPH NODE) WOULD BE CONSISTENT WITH ANTIGEN INDUCED SUICIDE OF THE HIGH AFFINITY CLONES OF PLASMA CELLS (AND PRESUMABLY THEIR PRECURSOR CELL THE "B" LYMPHOCYTE).

One does not need to look far for a cause for the antigen induced suicide; high affinity anti-IgG will bind that antigen as soon as it meets the extracellular milieu, possibly even sooner if the IgG can gain access to the intracellular cysterns. This means that while still close to its parent cell, it will bind to native IgG and by cross-linking the molecules it will then aggregate it causing the fixation of complement to the bound IgG and thereby the secreting cell will by lysed or rendered functionally incompetent. The destruction or disarming of the high affinity clones of cells will tend to leave the antigen (IgG) in the environment to further stimulate the "T" cell clones that initiated the reaction and the resulting "T" cell factors will cause the low affinity "B" cell clones to be maximally stimulated and helped to differentiate to form



THE PRODUCTION OF RHEUMATOID FACTOR

ANTIGEN INDUCED SUICIDE OF "B" CELLS WITH HIGH AFFINITY FOR IgG DUE TO THEIR DESTRUCTION BY COMPLEMENT FIXATION TO THE AGGREGATED IgG ON THEIR SURFACES

Figure 21

plasma cells (See Fig. 7).

This line of thought involves "T" lymphocytes as the central cells in the pathogenesis of Rheumatoid Factor initiating and continuing the response to native IgG. Immediately it becomes difficult to conceive how such reactive "T" lymphocytes could survive the clonal elimination of the foetal period without a self-destructive response when first formed. Mitchison (1968) has obtained clear evidence in mice that clonal elimination may not be present for all self antigens and in his example of "low zone tolerance" there may be "B" cells capable of responding to the antigen but with the absence of helper "T" cells and in the presence of suppressor "T" lymphocytes (Gershon, 1973) the body prevents self destruction.

The evidence from Rheumatoid Arthritis suggests that there are indeed reactive "B" cells to native IgG and the suppressor "T" cells are somehow overriden. However, there is evidence in some of the milder cases that the process is not systemic, and that only the local synovium shows pathological changes. This would be consistent with the presence of normal numbers of suppressor "T" cells in the rest of the lymphatic system. In the context of "autoimmune" disease the pathogenesis of Rheumatoid Arthritis has been thought by many to be due to the failure of suppressor "T" cells to prevent a suicidal attack by the immune system against "self" antigens, particularly in the local environment of the synovium but it has been difficult to see why a helper "T" cell should become sensitized to a "self" component (IgG) that is a very poor immunogen in its native state.

HYPOTHESIS:

THE "T" LYMPHOCYTE HELPING THE RESPONSE TO IgG IS NOT ITSELF RESPONDING TO THAT AS AN ANTIGEN BUT THE IgG IS ALREADY BOUND TO THE CELL CLOSE TO THE ANTIGENIC BINDING SITE AND IS INCIDENTALLY PRESENTED TO AN APPROPRIATE "B" CELL AS AN ANTIGEN.

Rather than pursue the idea that there may be distortions of the normal homeostatic mechanisms of the immune system which account for the escape of self reactive clones of "T" cells I have considered instead the ways in which normal "T" cells responding to an antigen might initiate the production of Rheumatoid Factor. The diagram (Fig. 7) represents the effect of IgG parasitizing a "T" cell responding to a completely different antigen and thereby converting the "B" cell response from the "T" cells antigen to that of an anti-IgG response particularly to determinants on the Fc part of the molecule. Batchelor (1978) has reported that like IgG, the antigens of the major histocompatibility systems are poor immunogens within the species unless presented upon viable lymphocytes which seem to provide a "second signal" that stimulates production of antibodies by the host. This evidence could be directly translated in the context of cell surface IgG; the poorly immunogenic IgG being attached by its binding sites to antigens on the surface of the "T" cell (closely associated with the antigen binding sites of that cell) could cause the recruitment and stimulation of the appropriate anti-IgG "B" cell clones by helper factors and lymphokines secreted by that "T" cell. The "B" cell clones provoked by such a mechanism will tend to bind native IgG and the antigenic suicide of these will lead to the histological

picture seen in the draining lymph nodes and the "hidden Rheumatoid Factors" demonstrated in the plasma cells of the synovium by Munthe (1975).

The immunologic mechanism inferred from the presence of Rheumatoid Factor could only operate if there were IgG anti-"T" cell antibodies present at some stage of the disease. That this is a likely situation has been demonstrated by Terasaki (1970). He reported the presence of lymphocytotoxic antibodies in a variety of "auto-immune" diseases and showed that in Rheumatoid Arthritis 30 out of 53 patients' sera contained such antibodies. Unlike the lymphocytotoxic antibodies found after pregnancy, these "autocytotoxins" or Lymphocytotoxins" seemed to be maximally active at 4-15^{o}C rather than the physiological 37^{o}C. They resembled those found in Systemic Lupus Erthyematosus in specificities when tested against lymphocytes from a panel of people. However, in contrast to those found in S.L.E. there was a lower range of lysis, 10-50% versus 50-100% of the panel as assessed by the microdroplet cytotoxicity assay of Terasaki (1964).

The degree of cytotoxicity observed depends upon the exact methodology and Mittal (1970) who first published evidence for these antibodies detected only four positive sera out of a panel of 28 Rheumatoid sera and made the comment that the specificities detected using lymphocytes from a panel of people did not correlate with defined HLA (the lymphocyte antigens coded for by genes in the human major histocompatibility supergene) antisera, and this observation has been confirmed by other investigators subsequently. The cytotoxicity test for these antibodies is probably

insensitive as one can predict with a degree of confidence that there will be non-complement fixing antibodies produced, and the first hints that this might be the case came from the work of Williams (1971). He found that the lymphocytes from Rheumatoid patients were less able to provoke the production of anti-human-lymphocyte antibodies when injected into Rabbits (in adjuvant) than those from normal people, and he interpreted this to suggest that these lymphocytes could be coated with a serum component that blocked their antigenicity (he absorbed all the Rabbit sera with human serum before use). Further work by Williams (1973) help to define that human IgG was in fact the serum component found on Rheumatoid lymphocytes.

Probably the most complete investigation of these antibodies has been the publications of Winchester (1974) and Winfield (1975) reporting the results of an assay using immunofluorescence with fluorochrome tagged Fab fragments (antigen combining part of immunoglobulins) of anti-human-immunoglobulin antibody on the isolated lymphocytes of patients. They demonstrated that freshly isolated cooled Rheumatoid lymphocytes (at $4^{o}C$) had an excess of immunoglobulin bearing cells compared with controls. The excess was so marked that when this proportion of the cells which would normally be taken for "B" lymphocytes was added to the "T" cells enumerated by Sheep red cell rosetting it exceeded 100% of the cells in the majority of the patients studied. This can only be interpreted to mean than many of the "T" cells so enumerated also carried immunoglobulin on their surfaces. In all 11 cases in which they incubated the cells overnight in normal human AB serum the proportion of immunoglobulin bearing

cells fell markedly and this effect was immediately reversible by exposure to autologous plasma. This confirms the cytophilic immunoglobulin serum component that Williams (1973) had demonstrated and they went on to show that this was not necessarily cytotoxic and some would not have been detected by Terasaki (1970).

Winchester (1974) also found that when these cells were enumerated for aggregated IgG receptors (usually found on "B" but not "T" lymphocytes) there was an excess of these compared with lymphocytes obtained from controls. When these cells were incubated overnight in normal human serum the excess disappeared, which effect was immediately reversible by exposure to autologous plasma. Winchester interpreted this to mean that the Rheumatoid Factor was binding to the cytophilic antibody on the surface of the lymphocytes and in that position binding aggregated IgG. If that is the case, the cytophilic antibody must be of the IgG class as the Rheumatoid Factor does not bind to other classes.

Winchester found that if the initial enumeration of "T" cells in freshly isolated lymphocytes was performed in the cold ($4^{o}C$) in the absence of normal human serum there was a demonstrable shortfall from the rosette forming cells detected conventionally. This effect was reversible by short incubation with normal human serum at $37^{o}C$ even if the enumeration was once again carried out at $4^{o}C$. This can most simply be interpreted to suggest that the cytophilic antibody is binding to antigenic sites close to the Sheep red blood cell receptors.

Although the temperature for cytolysis with these antibodies seems to be incompatible with *in vivo* activation Winchester remarks of his immunofluorescent studies: "Some evidence was obtained in the present studies that low concentrations of the antibodies, particularly of the IgG type, might be absorbed (*by the lymphocytes*) even at body temperatures." The overall picture of the "T" lymphocytophilic antibody binding to sites near the S.R.B.C. receptors and in turn being the target for Rheumatoid Factor closely resembles that extrapolated from the evidence presented earlier. If Rheumatoid Factor represents the antibody that escapes the suicidal response to IgG then lymphocytophilic antibody may represent the antibody that escapes the blocked response to lymphocyte antigens (perhaps blocked by locally effective anti-IgG antibodies in the synovium).

A certain note of caution is appropriate at this point as the evidence from Systemic Lupus Erthymatosus strongly suggests an environmental agent in the origin of the potent lymphocytotoxins found in that disease. There is a notable clustering of lymphocytotoxins detected in the sera of people in close contact with cases of S.L.E. which includes the non-consanguinous household members and the clustering does not include close relations that are not in close daily contact with the probands (DeHoratius, 1975). Mottironi and Terasaki (1970) had reported the occurence of high levels of lymphocytotoxic antibodies from the sera of patients with viral infections such as Measles, Rubella, and Infectious Mononucleosis, and since that time Mayer (1973) reported similar activity in Mycoplasma Pneumonia, Mumps, Influenza A and B, and even parasitic infestations. From

this evidence people have tended to dismiss these antibodies as a non-specific epiphenomenon found in the immune responses to many infections.

One can defend the thesis that lymphocytotoxins are not an epiphenomenon of Rheumatoid Arthritis on two separate planes:

1. On *a priori* grounds one could not expect the body to have sufficient survival ability to indulge in the production of epiphenomena in serious viral infections, *ergo* such people would be at a selective disadvantage if they showed such a reaction. It might be that these infections are serious precisely because they can divert the bodies immune system to self-destruction but the lymphocytotoxins are thereby rendered anything but trivial epiphenomena.

2. On *a posteriori* grounds one could draw the distinctions between the lymphocytotoxins found in Rheumatoid Arthritis which are IgG and are poorly cytolytic and require "T" cell help for their generation, and the lymphocytotoxins found in the other conditions which are almost universally of the IgM class and are strongly cytolytic and might not require "T" cells for their generation. This would tend to separate the lymphocytophilic antibodies of Rheumatoid Arthritis from consideration as lymphocytotoxins.

In this hypothesis there appears to be a requirment for an explanation for the appearance of low titred Rheumatoid Factors in Sub-acute-bacterial-endocarditis and kidney transplant recipients (Waller, 1965; Irby, 1968). In the case of the kidney transplants, a direct quotation from Irby (1968)

concerning "autoimmune phenomena" in their patients is in full concordance with the mechanisms postulated for Rheumatoid Arthritis: "...the demonstration that anti-transplant antibodies may attach to the host's own tissues raises the possibility that these auto-antibodies may be a factor in the joint changes noted. The presence of Rheumatoid Factor appearing in the blood following transplantation adds further evidence to this assumption". In bacterial endocarditis the theory for Rheumatoid Factor formation that is most widely quoted is that the presence of large amounts of antigen-antibody complexes with IgG bound to the *Streptococci* could lead to the incidental manufacture of Rheumatoid Factor. However, the post-Rheumatic-Fever patient seems to have increased susceptibility to chronic infection particularly due to *Streptococci* and one could postulate (in the complete absence of evidence) that these people readily develop lymphocytotoxins stimulated (or controlled) by the infectious organisms.

From the evidence that Rheumatoid lymphocytophilic antibodies are IgG, I have already inferred that a "T" lymphocyte is needed to collaborate with a "B" cell in the production of anti-"T"-lymphocyte antibody as this class of antibody requires such help. This means that the proposed causal mechanism for the disease is a direct "T" lymphocyte/"T" lymphocyte war which at first sight seems more unlikely than "T" lymphocytes sensitized to IgG which does fit into the frame of the main body of this thesis.