GENETIC AND ENVIRONMENTAL INFLUENCES ON CORD BLOOD ATOPIC MARKERS AND ON ATOPIC SENSITISATION IN INFANCY

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DEDICATION

This thesis is dedicated to the mothers and infants who took part in this study. Without their enthusiasm, patience, commitment and trust, important questions would have remained unanswered.

DECLARATION

"I, Matthias Haus, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be* submitted for another degree in this or any other University".

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ABSTRACT

HISTORICAL PERSPECTIVE

It has recently been shown that intensive prophylactic dietary and environmental control measures during early infancy may reduce the incidence and/or postpone the onset of atopic disease.

In order to institute this prophylactic regime, early identification of the infants genetically "at risk" for atopic disease is essential, since sensitisation begins at birth, or even during intra-uterine life.

European and Scandinavian studies have shown that a raised concentration of cord blood serum immunoglobulin E (CBsIgE) is an excellent predictive marker for the subsequent development of atopic disease. Other potential predictive atopic markers such as cord blood eosinophils, platelets and anti-cow's milk serum IgG have also been suggested as having possible predictive relevance for newborns in terms of the development of subsequent atopic disease.

PROBLEM DEFINITION

Most of the work in this field has been done on Caucasian neonates, in Westernised, First World countries. In South Africa, it has been shown that the Black adult ethnic group has serum immunoglobulin E concentrations (sIgE) which are significantly higher than that found in the South African White adult ethnic group. Furthermore, it has been suggested that the elevated sIgE in the adult Blacks may be raised independently of allergic disease. It is, therefore, important to ascertain whether this elevation of sIgE in Black South African adults is evident already at birth in the cord blood sera of Black South African newborns. If so, it is imperative to ascertain whether any such elevation is reflective of a high genetic load for atopy in these Black newborns, and furthermore whether these Black newborns are consequently "high-risk" for the development of subsequent atopic disease, as has been previously reported in the literature for White newborns. Arising from an awareness of these specific South African problems, the following hypothesis was developed.

HYPOTHESIS

The hypothesis states that:

"Black South African newborns without an atopic family history (aFH) have significantly higher CBsIgE values than similar White and Mixed newborns. An aFH does not influence the CBsIgE values in the Black newborns, as it does in the White and Mixed newborns. The CBsIgE values in Black newborns are not, furthermore, predictive for the development of subsequent atopy in infancy, as they are in the other ethnic groups".

A description of the three South African ethnic groups considered in this study is provided in Section IV, (Pg. 74).

AIMS OF THE STUDY

The aims of the study were three-fold:

1. To test the hypothesis.

2. To assess the relevance of alternative cord blood markers (eosinophils, platelets and anti-cow's milk serum IgG) as predictive atopic markers in each of the three ethnic groups.

3. To provide epidemiological information with regard to genetic and environmental influences on CBsIgE, cord blood total eosinophil counts (CBTEC's) cord blood platelet counts (CBPlC's) and cord blood anti-cow's milk serum IgG concentrations (CBacmIgG).

STUDY DESIGN

In order to achieve these aims, a cross-sectional study to investigate the cord blood markers with respect to genetic and environmental influences on their values (Phase I) and a prospective longitudinal follow-up study to document the development of atopic disease in the newborns during infancy and to relate this clinical information back to the cord blood marker values (Phase II) was designed. Fifty three Black, 52 White and 58 Mixed consecutively selected full-term newborns born in the Cape Peninsula of South Africa were admitted to the study.

RESULTS

The study confirmed the hypothesis by proving that the Black newborns without an aFH had significantly higher CBsIgE values than their White and Mixed counterparts. It showed also that, while an aFH significantly influenced the CBsIgE values in the White and Mixed newborns, definitive evidence for this effect on the Black newborns was not possible owing to the small number of Black newborns with an aFH (5 cases). Furthermore, the study showed that the CBsIgE values in the Black newborns who developed atopy during infancy were lower than in those who remained healthy, although this difference was not significant. In the White and Mixed newborns, the CBsIgE in those who developed atopy during infancy were significantly higher than in those who remained healthy, confirming the conclusions of the previously published literature in this field. The study also demonstrated a high degree of atopic sensitisation in the Black infants, thereby contradicting previously published opinion as to the low incidence of atopy in this ethnic group.

This study also provided information as to the atopic and predictive relevance of alternative cord blood markers (CBTEC, CBPIC and CBacmIgG). With the exception of the CBTEC in the Black newborns, no other marker showed any promise as a potential predictive atopic marker in the South African context. Epidemiological data as to the effect of genetic and environmental influences on all of the four cord blood markers studied was provided.

CONCLUSION

In the Black South African ethnic group, it was shown that genetic and environmental factors are at play which prevent the clinical use of CBsIgE as a predictive atopic marker for the development of atopic sensitisation during infancy. Furthermore, among the 47.2% sample of Black infants that were followed for one year, atopic sensitisation occurred in a higher proportion than their White and Mixed counterparts. In the case of the White and Mixed newborns, we confirmed the conclusions of the previously published literature on the relevance of raised concentrations of CBsIgE as a predictive atopic marker. None of the other 3 cord blood markers studied were unequivocally effective in predicting the development of atopic sensitisation during infancy in the Black newborns.

NOTE: The literature quoted in this thesis has been reviewed up until March, 1988.

SCIENTIFIC CONTRIBUTIONS ARISING FROM WORK COMPLETED FOR THIS THESIS

ORIGINAL SCIENTIFIC PUBLICATIONS

1. Haus M, Heese H deV, Weinberg EG, Potter PC, Hall JM, Malherbe D. The influence of ethnicity, an atopic family nistory and maternal Ascariasis on cord blood serum IgE concentrations. J Allergy Clin Immunol. 1988; 82: 179.

2. Haus M, Weinberg EG, Malnerbe D. The development of specific IgE antibodies to Bordetella pertussis following immunisation in infancy. Letter. Lancet. 1988; I : 711.

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1. Haus M, Heese H de V, Weinberg EG, Potter P.C., Hall J.M. "Predictive Markers for Atopy in Paired Cord Sera of Three Ethnic Groups". Thirteentn International Congress of tne European Academy for Allergology and Clinical Immunology. (E.A.A.C.I.) May, 1986. Budapest, Hungary.

2. Haus M, Heese H de V, Weinberg EG, Potter P.C., Hall J.M. "Predictive markers for atopy in 3 ethnic groups". 17th South African Biennial Paediatric Congress. September,1986. Cape Town.

3. Haus M, "Cord blood atopic markers in three ethnic groups". Department of Paediatrics and Child Health, University of Cape Town Researcn Day. October, 1986.

4. Haus M, Potter PC, Hall JM, Bell S." A multi-etnnic study of cord blood anti-bovine milk - specific IgG concentrations". Annual Meeting of tne European Academy of Allergology and Clinical Immunology. (E.A.A.C.I.). April, 1987. Palma de Mallorca, Spain.

5. Bell S, Haus M, Potter P.C. "Inter-ethnic Variations in the expression of milk-specific IgG responses". 3rd International Symposium on Immunological and Clinical Problems of Food Allergy. October, 1986. Taormina, Italy.

6. Haus M, Heese H de V, Weinberg E G, Potter P C Hall J, Malherbe D. "Ethnic variations in cord blood serum IgE concentrations: full-term Black, White and Coloured infants". International Neonatal Intensive Care Collegiate, June, 1987. Sassari (Sardinia), Italy.

7. Haus M, "The prediction and prevention of allergic disease". Paediatric Allergy and Clinical Immunology Refresher Course. University of Cape Town. February, 1987.

8. Haus M, Potter PC, Hall JM, Bell S, Heese H de V. "The relevance of ethno-genetic and environmental influences on cord blood anti-bovine milk-specific IgG concentrations". South African Immunology Society Congress. March, 1988. Durban, South Africa.

9. Haus M, Weinberg EG, Malherbe D. "Specific IgE antibodies to Bordetella pertussis following routine immunization in infancy". The 18th Biennial Congress of The South African Paediatric Association. September, 1988. Pretoria, South Africa.

10. Haus M, Weinberg EG, Malherbe D. "The development of specific IgE to Bordetella pertussis following immunisation in infancy". The XIII International Congress of Allergology and Clinical Immunology. October, 1988. Montreux, Switzerland.

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ABBREVIATIONS

CB	-	cord blood
s	-	serum
IgE	-	Immunoglobulin E
IgG	-	Immunoglobulin G
IgA	-	Immunoglobulin A
IgM	-	Immunoglobulin M
CBsIgE	-	cord blood serum IgE concentration
CBTEC	-	cord blood total eosinophil count
CBPlC	-	cord blood platelet count
CBacmIgG	-	cord blood anti-cows milk serum IgG concentration
sIgE	-	serum IgE concentration
sIgA	-	serum IgA concentration
sIgG	-	serum IgG concentration
PlC	-	platelet count
TEC	-	total eosinophil count
acmIgG	-	anti-cow's milk IgG
RIST	-	Radioimmunosorbent test
PRIST	-	Paper-disc radioimmunosorbent test
ELISA	-	Enzyme-linked immunosorbent assay
RAST	-	Radioallergosorbent test
GM	-	Geometric mean
SD	-	Standard deviation
Ir gene	-	Immune response gene
mg/dl	-	milligrams per decilitre
u/ml or U/ml	-	units per millilitre
IU or iu	-	International Units
PRU/ml	-	Phadebas Rast Units per millilitre
oC	-	degrees centigrade
ml	-	millilitre
g or G	-	gram
vs	-	versus
+ve	-	positive
-ve	-	negative
cont.	-	continued
s or S	-	significant
NS	-	not significant
signif.	-	significance
u or U	-	unit
ng	-	nanogram
no. or NO.	-	number

obs	-	observations
approx.	-	approximate
diff.	-	difference
Fig.	-	Figure
cc	-	clinical categorisation
ic	-	immunological categorisation
RAS	-	Rationalized Atopic Status
CAS	-	Cumulative Adjusted Atopic Status
DPT	-	Diptheria, Pertussis and Tetanus Immunisation
MHC	-	Major Histocompatability Complex
%	-	Percentage
paf-acether	-	platelet activating factor
M.O.U.	-	Midwife Obstetric Unit
cm	-	centimetre
+	-	plus
-	-	minus
kU/l	-	kilounits per litre
FH	-	family history
aFH	-	atopic family history
Inf	-	infant
arith	-	aritnmetic
geom	-	geometric
Max	-	maximum
Min	-	minimum
Stat	-	statistical
excl	-	excluded

Note: kU/l = U/ml = I.U.

GLOSSARY OF TERMS

ethno-genetic	That genetic effect which is not obviously related to the hereditary influence of atopy, but rather to racial factors.
immuno-genetic	That genetic effect which finds expression through immunological mechanisms.

SECTION I

INTRODUCTION, PROBLEM DEFINITION, HYPOTHESIS AND AIMS OF STUDY

CHAPTER I INTRODUCTION

1.1 PRINCIPLES OF THE PREDICTION AND PREVENTION OF ATOPIC DISEASE

Various studies have confirmed the impression that the incidence of atopic disease has increased in recent decades (Glaser, 1966; Kishimoto et al, 1980; Denis et al, 1984; Turner et al, 1984). This trend has focussed much of the current ongoing research effort onto the establishment of methods designed to prevent or modify the development of the allergic response.

During the past decade, researchers from predominantly First World European and Scandinavian countries have produced evidence to suggest that the "high-allergic-risk" newborn may be identified at birth (Michel et al, 1980b; Kjellman, 1982a; Duchateau and Casimir, 1983; Kjellman and Croner, 1984). This concept has encouraged the early application of preventive measures for atopy to the newborn and young infant, before atopic sensitisation can occur (Frick, 1985; Michel et al, 1986; Haus, 1987).

The postponement of allergic disease in infancy per sé appears to be associated with a decrease in the severity of any ensuing disease in adults (Björkstén, 1983). Severe asthma, as well as other forms of allergic disease in childhood, are often preceded by eczema in infancy (Matthew et al, 1977). It has therefore seemed appropriate to suggest that any methods aimed at preventing or even delaying the onset of infantile allergic symptomatology should be encouraged (Businco and Cantoni, 1984).

Since the beginning of this century, both anecdotal and scientific reports began to associate distinctive feeding methods in infancy with the development of infantile atopy. The concept of allergy prevention grew out of a realisation that manipulation of critical

dietary, environmental and non-specific measures in infancy could delay or prevent the onset of subsequent atopic disease. Talbot, in 1918, was one of the first modern-day allergists to appreciate the effect of diet on allergic symptoms in infants. He reported on the apparently causal relationship between the development of eczema in a 3 week old breast fed neonate and the ingestion of a large amount of chocolate by the mother. Subsequently, specific dietary measures in infancy for the prevention of atopic disease have been advocated by numerous workers. These measures include prolonged or exclusive breast feeding (Grulee and Sanford, 1936; Saarinen et al, 1979; Juto et al, 1982), the delayed introduction of potentially allergenic solid foods (Saarinen et al, 1979a; Fergusson et al, 1981) and the avoidance of cow's milk protein (Glaser and Johnstone, 1952; Glaser and Johnstone, 1953; Duchateau and Casimir, 1983).

Environmental manipulation, including the avoidance of aero-allergens (Matthew et al, 1977) and non-specific enhancing factors for allergy such as parental smoking (Kjellman, 1981; Björkstèn and Ahlstedt, 1984) and respiratory viral infections (Frick, 1981; Welliver et al, 1981) has also been advised.

A delay in the administration of the Bordetella Pertussis vaccination in infancy (Frick, 1985; Haus et al, 1988a;) because of its adjuvant effect on the stimulation of the sIgE response (Kjellman, 1982a; Björkstèn and Ahlstedt, 1984), and the avoidance of the pollen or spring season as the projected season of birth for the potentially "high-allergic-risk" newborn (Björkstèn and Suoniemi, 1976; Björkstèn et al, 1980; Saarinen et al, 1982) are additional preventive measures which have received consideration.

Even though the efficacy of some of these preventive measures, such as the role of exclusive breast feeding in infancy have been questioned (Halpern et al, 1973; Lindfors and Enocksson, 1988), Kjellman (1982a), in a comprehensive review of the evidence, reached the conclusion that the effective implementation of preventive measures in infancy reduced the incidence of early developing atopic disease.

It consequently seems justifiable to advocate the rigid implementation of preventive measures for all those newborns who are seen to be "high-risk" for the development of atopy, in spite of the considerable cost in terms of the time and effort involved in the effective implementation of the prophylactic regime. Because of these inherent difficulties, this regime should be reserved only for the "high-allergic-risk" infant (Michel et al, 1980a) and mother.

The fundamental presumption and rationale of both the prediction of the "high-allergic-risk" pregnancy and newborn, as well as the prevention of subsequent atopic symptomatology in this subpopulation is based on immuno-genetic principles. The central concept is one of vigorous and total allergen avoidance in order to prevent atopic sensitisation of the "high-risk", genetically predisposed foetus and infant (Bazaral et al, 1971; Kjellman, 1982a; Lehrer et al, 1982) who experiences a transient, vulnerable period of primary immuno-incompetence (Soothill, 1976; Matthew et al, 1977; Björkstèn, 1983) during the period from conception through early infancy. This immuno-incompetence is associated with a failure of T-suppressor cell function (Björkstèn and Juto, 1983a; Björkstèn and Juto, 1983b; Geha, 1984), which in turn leads to discriminatory effects on various cord blood atopic markers at birth (Michel et al, 1980; Kjellman and Croner 1984).

The identification of the "high-allergic-risk" pregnancy needs to occur before, or as soon as possible after, conception in order to allow the mother to practice adequate ante-natal prophylaxis in terms of the modification of her diet. The human foetus is able to produce IgE from the 11th week of gestation (Miller et al, 1973; Singer et al, 1974). It has, furthermore, been shown that the unborn foetus is able to mount an intra-uterine allergic response to various allergens which are presumed to have crossed the materno-foetal placental barrier (Levine et al, 1971; Kuroume et al, 1976; Michel el at, 1980a). The elimination of highly antigenic foods from the maternal diet before this stage is therefore advisable (Frick, 1985). Since atopic disease has been shown to be hereditary (Cooke and Van der Veer, 1916; Van Arsdel and Motulsky, 1959; Orgel et al, 1975), the

"high-allergic-risk" pregnancy may be identified by an adequate obstetrical history which focuses on the presence or absence of atopy in the prospective mother, her husband and her existing children. Kjellman (1977) has laid down clear probability guidelines for the risk of atopic development in children born with an atopic family history (aFH). By incorporating these probability guidelines into a simple "rule of thumb" known as the Family Score System (Kjellman, 1982), Kjellman allows the identification and prediction of the "high-allergic-risk" pregnancy on the basis of the family history of the prospective newborn. In these index cases, the mothers are advised to practice ante-natal allergy prevention.

During the delivery of the newborns, it has become possible to substantiate the suspicion of the "high-allergic-risk" pregnancy by monitoring the concentration of various cord blood atopic markers (Kjellman, 1982; Kjellman, 1982a; Hattevig et al, 1984; Haus, 1987). The definitive identification of this "high-allergic-risk" subpopulation at birth will further indicate which newborns are candidates for the rigid preventive measures which have been advocated in infancy.

1.2 IDENTIFICATION OF POTENTIAL CORD BLOOD ATOPIC MARKERS

Many different immunological, haematological and biochemical substances inter-react in the cascade of events constituting the Type I anaphylactic or allergic response (Cohen, 1983).

Some of these substances are eminently quantifiable in the cord blood of newborns, and constitute possible prognostic and predictive markers for the subsequent development of atopic disease.

By definition, those markers with a predictive function for the development of future atopic symptomatology should be readily identified in the cord blood of newborn babies (Bousquet and Michel, 1984).

Four cord blood markers with varying degrees of documented atopic and predictive relevance will be assessed in this study. They are:

(i) Immunoglobulin E
(ii) Eosinophils
(iii) Platelets
(iv) Anti-cow's milk Immunoglobulin G

The rationale for the proposal that these cord blood markers have atopic and predictive relevance for the development of subsequent atopic disease is given in Section III.

CHAPTER 2 DEFINITION OF THE SOUTH AFRICAN PROBLEM

2.1 SOCIO-CULTURAL AND ENVIRONMENTAL VARIATIONS IN THE PREVALENCE OF ATOPIC DISEASE

Population variations in respect of the prevalence of atopic disease have been well described. In general, the published studies have suggested that childhood atopy is significantly less prevalent in developing, Third World traditional or rural communities than in Westernised, First World or urban communities.

Wesley et al (1969), in a South African study, found that all 5 Black children admitted to a Durban hospital for asthma were from an urban environment, and were children of professional, higher social class parents who had probably adopted many characteristics of the Western life-style and culture.

Godfrey (1975), in a study in the North-West African state of The Gambia, found no cases of asthma in 231 randomly selected rural school children and adults, while 44 asthmatics were easily identified in the urban environment of the capital town of Banjul.

Merrett et al (1976), in a comparative study between urban and rural African populations in Rhodesia (now Zimbabwe), easily identified 80 asthmatics in the city of Salisbury (Harare). Of the rural sampling of 100 subjects, none, however, presented with evidence of allergic disease, in spite of the fact that their serum IgE mean concentrations were significantly higher, at 1,613 U/ml, than their urban asthmatic counterparts (799 U/ml). This apparent anomaly was ascribed to the high degree of filarial parasitism in the rural population.

Van Niekerk (1979), in a similar study of 671 rural tribal and 694 urban Xhosa children between 6 and 9 years of age in South Africa, reported a rural prevalence rate for asthma of 0,14% (one patient only). The corresponding urban rate was 3,17%.

Further studies reflecting a difference in the prevalence of atopic disease between urban and rural communities have recently been published by an Australian research group doing work in Papua, New Guinea (Dowse and Turner, 1986; Turner et al, 1986).

A socio-cultural and environmental differentiation in the prevalence of atopic disease is therefore evident within similar ethnic groups.

Various explanations for this low rural prevalence rate have been proposed. These suggestions include the protective effects inherent in the persistence of traditional feeding methods such as breast feeding (Van Niekerk, 1979), the relative dearth of environmental aero-pollution together with the absence of the house-dust mite in rural tribal communities (Van Niekerk et al, 1984) and the high incidence of parasitism in rural societies. Parasitisation induces high concentrations of anti-parasite specific serum IgE concentrations in these communities (Godfrey, 1975; Merrett et al 1976; Turner, 1980). It has been suggested by these workers that anti-parasite specific IgE may saturate the mast cell IgE-receptors, thereby blocking these receptors for specific, more ubiquitous, antibody binding. This presupposes that parasite-induced IgE is multispecific and shares common antigenic determinants with the more ubiquitous antigens. Turner et al (1984) go on to suggest that the high incidence of rural parasitosis may also suppress the host's ability to produce more harmful specific IgE antibodies.

Not all authors share consensus on the protective effect of helminthiasis. Joubert et al (1979) for example, are of the opinion that Ascariasis may, in fact, predispose the host to allergic disease. Whatever the reasons, rural populations have been relatively spared from atopic disease. It has, however, been suggested that, once the traditional life-styles of these rural populations become modified, either because of their migration to neighbouring urban environments, or because of the evolutionary Westernisation of their cultural habits, a dramatic increase in the prevalence of atopy may be noted. Merrett et al (1976) found specific IgE antibodies in a fair number of their healthy rural African sampling. They suggest that this would indicate an increased susceptibility for the development of atopic disease if the rural subjects were to move into urban areas. An analogous situation seemed to be at play, he noted, when African and Asian immigrants to Great Britain commonly became readily sensitised to grass pollen or Dermatopygoides pteronyssinus.

2.2 ETHNIC VARIATIONS IN THE PREVALENCE OF ATOPIC DISEASE

Apart from socio-cultural and environmental variations in the prevalence of atopic disease, ethnic differences per sé have been described. This is not unexpected since it seems that both the hereditary nature of atopic propagation (Cooke and Van der Veer, 1916; Van Arsdel and Motulsky, 1959) and the reaginic antibody response (Benacerraf and McDevitt, 1972; Marsh et al, 1981) are under genetic control.

In the South African context, the White and Mixed ethnic groups have the highest prevalence of atopy. The Black ethnic groups have by far the lowest atopic prevalence. These conclusions have been the result of the following studies:

Wesley et al (1969), in a South African study conducted at a Durban hospital between 1963 and 1967, found that Black asthmatics accounted for only 0,02% of the total medical admissions, while White asthmatics accounted for 0,79% of the total medical admissions. During this period there were 24,731 Black and 5,313 White medical admissions to the hospital.

Orren (1974), in an inter-ethnic comparative study of adult blood donors in Cape Town, South Africa, found that, while 17,5% of White subjects gave a positive family history of atopy, only 8% of Mixed subjects and 7,5% of Black subjects gave a similar history.

In a similar inter-ethnic study of serum IgE concentrations in a Cape Town allergy clinic, Orren et al (1975) found 37 White, 62 Mixed but only 7 Black patients.

Walls and Ordman (1983), in a further prospective study of asthmatic patients in a Cape Town allergy clinic, found 148 White, 158 Mixed and only 8 Black patients. The possibility that the low Black representation could have been an artificially low reflection of a realistically higher figure because of extraneous logistical causes such as transport or referral inadequacies was discounted. The other outpatient clinics at the same hospital had a predominance of Black patients during the same period.

In 1984, the statistical analysis of specialist out-patient consultations at the Red Cross War Memorial Children's Hospital in Cape Town (Annual Report, 1984) revealed the following information. Of the 29,764 consultations, 7,087 (23,8%) were for atopic problems. However, only 91 (1,3%) of these atopic consultations were with Black children, even though the total number of Black patients visiting the hospital for all medical conditions in the same period was 25% (38,114 of 152,456 patients).

Significant ethno-genetic influences on the prevalence of atopy in South Africa appear, therefore, to be at play, with the Black group manifesting markedly less atopy than the White group. This fact needs to be taken into consideration in any inter-ethnic allergy study in South Africa, not only to allow for this variable in the formulation of any conclusions, but also to encourage the realisation that individualised guidelines may need to be given for each ethnic group.

2.3 ETHNIC VARIATIONS IN TOTAL SERUM IMMUNOGLOBULIN E CONCENTRATIONS

The normal ranges of immunoglobulins belonging to the classes G, A and M have been shown to vary in different population and ethnic groups in different countries (Buckley et al, 1968; Johansson et al, 1968; Rowe, 1972). Within South Africa, differences in these immunoglobulin classes between the Black and the White ethnic groups have been described in adults (Milner and Calitz, 1971; Shulman et al, 1975), children (Shulman and Gilich, 1976; Roode, 1980) and newborns (Van Rijswijk et al, 1985). In most cases, the Black or Negroid ethnic groups have the highest normal values, when compared to the White or Caucasian groups. Milner and Calitz's study (1971) showed that healthy Black male adults had 40% more IgG, 30% more IgA and 32% more IgM than their White counterparts.

Bazaral et al (1974) have suggested that the inter-ethnic variations described in serum immunoglobulin levels may indicate a genetic effect on the levels of immunoglobulins in general. It has been generally perceived that the relative hypergammaglobulinaemia, as

manifested by the Black races, is an indication of a natural selective immune advantage which they enjoy over other races. It has been brought into play over the centuries as part of their evolutionary development. This function has facilitated their survival against the ravages of microbial, parasitic and protozoan infestations so prevalent in many primitive African societies (Orren and Dowdle, 1975a; Joubert et al, 1979; Lehrer et al, 1982).

Much of this phylogenetic advantage appears, however, to be redundant today, both because of the rapid Westernisation of the traditionally tribal life-style of the Black African races, and because of the effective chemical and pharmaceutical control of many of these hitherto life-threatening infections.

2.3.1. Ethnic variations in adult, childhood and infant serum immunoglobulin E concentrations

Ethnic variations in total sIgE in adults, children and infants have likewise been documented in many parts of the world. This work is summarised in (Table I.1).

Johansson et al, in 1968, showed that Ethiopian children had sIgE concentrations many times higher than White Swedish children.

Orgel et al, in 1974, similarly showed that American-born Filipino children had sIgE values which were significantly higher than their age-matched White counterparts.

Grundbacher (1975) compared sIgE concentrations between American Whites and American Blacks. Mansfield et al (1978) also compared sIgE in these ethnic groups, but included American Japanese. The Blacks and the Japanese had significantly higher sIgE than the Whites in both studies.

Gerrard in 1976, similarly reported on sIgE levels which were significantly higher in the Canadian Metis Indian population than those of the White Saskatchewan population.

TABLE I.1

ETHNIC VARIATIONS (EXCLUDING SOUTH AFRICA): TOTAL SERUM IgE CONCENTRATIONS

AUTHOR	COUNTRY (POPULATION)	ETHNIC GROUP	YEAR	ASSAY METHOD	sIgE (GM) 1 u = 2,3 ng	NO OF OBSERVA-TIONS	SELECTION CRITERIA
Johansson et al (1968)	Sweden	White Swedish	1968	Radio-active radial immuno-diffusion	160 ng/ml	23	Children (Unselected)
	Ethiopia	Black African			4440 ng/ml	25	Children (stool +ve for A. lumbricoides)*
					860 ng/ml	19	Children (stool +ve for A. lumbricoides)*
Orgel et al (1974)	U.S.A. (California)	White American	1974	Competitive inhibition of tagged I^{125} IgE to coupled anti IgE	69 U/ml	24	Children (Unselected 5-17 yrs)
		Filipino American			227 U/ml	27	Children (Unselected 5-17 yrs)
Grundbacher (1975)	U.S.A. (Peoria)	White American	1975	RIST (Pharmacia)	243,9 u/ml	154	Unselected (5-69 yrs)
		Black American			539,1 u/ml	172	Unselected
Gerrard et al (1976)	Canada (Saskatchewan)	White	1976	PRIST (Phadebas)	81,3 u/ml	819	Unselected (1-71 yrs)
		Metis Indian			275,4 u/ml	275	Unselected (1-61 yrs)
Mansfield et al (1978)	U.S.A. (California)	White American	1978	Double antibody radio-immuno-assay	67 u/ml	88	Unselected (2-14 yrs)
		Japanese American			444 u/ml	23	Unselected (6-14 yrs)
		Black American			247 u/ml	6	Unselected

* A. lumbricoides = Ascaris lumbricoides helminth

It should be noted that these studies comprised unselected individuals. They may therefore have included those with atopic disease and helminth infestation, both of which influence sIgE independent of ethnicity. Moreover, the incidence of both of these variables differ markedly in the various ethnic groups compared. In Johansson's study, exclusion of those Ethiopian children with obvious Ascaris Lumbricoides infestation still, however, emphasised a marked ethnic difference between them and their Swedish counterparts (Johansson et al, 1968). It is important to appreciate the fact that direct comparison of the sIgE values between the different studies is not advisable because of the different laboratory methods used for the sIgE immunoassays. This could be the explanation for the marked difference observed in the geometric mean sIgE between the White Canadian and the White American populations in Gerrard's (1976) and Grundbacher's (1975) study (Table I.1). The RIST technique, used by Grundbacher, is well known to produce higher results than the PRIST technique, particularly with levels below 60 u/ml (Johansson et al, 1976).

Ganju et al (1979) are of the opinion that environmental factors are more important than racial factors in explaining the differences in sIgE observed between the ethnic groups. They determined the sIgE in 115 healthy, non-atopic North American Black children living in Chicago, and found that their values approximated that of previously reported American Caucasian values, while being much lower than previously reported African Black values. His study was well designed, in that he excluded any observations which may have been biased by the presence of atopy, helminth infection or a family history of atopy. The environmental influence on sIgE will be addressed again later in this chapter.

In the South African context, clear ethnic differences, both in an unselected sampling of adult blood donors (Orren and Dowdle, 1975), and in a selected sampling of non-allergic adult blood donors (Orren et al, 1975) have been described (Table I.2). In both studies, the Black group had the highest sIgE followed by the Coloured (Mixed) group. The White group had the lowest values. Of interest was their observation that the presence or absence of atopic disease seemed to have no significant effect on the high values of sIgE in the Black group.

In contrast, however, the presence of atopic disease in the White and Coloured (Mixed) groups significantly influenced their sIgE values, tending to obscure the influence of race on this measurement. These observations led the authors to conclude that, while a raised sIgE is of undoubted benefit in terms of the diagnostic benefit for atopic disease in the South African White population, it is of "little value in the assessment of (South African) Black patients for allergic disease".

Heese et al (1984), in a study of 237 unselected Mixed and Black infants in the rural environment of Rehoboth, Namibia, found sIgE values which were significantly higher than previously reported values in age-matched studies reported from Western countries. It was thought that this difference was probably on the basis of ethnicity per sé, since the populations studied were of the well-defined Mixed Baster and Black Nama ethnic groups. Stool samples were, however, not examined for evidence of helminth infestation, nor were other environmental allergens assessed. Environmental influences could, therefore, not be excluded. No statistical difference was, moreover, found in the sIgE concentrations between the Baster and the Nama groups.

The function of this evidently non-specific elevation of sIgE in the Black races is still largely speculative. It has been shown, both in animal studies (Levine and Vaz, 1970; Katz, 1978; Binaghi, 1980; Marsh et al, 1981; Ishizaka, 1982), and in humans (Croner et al, 1982; Kjellman and Croner, 1984) that genetically predetermined "high and low IgE responders" occur in the population. The group of high sIgE responders tend both to maintain a higher level of sIgE throughout life and also to become sensitised more readily than the group of low IgE responders. Kjellman et al, (1976) have suggested that patients with elevated sIgE concentrations, in whom no obvious atopic symptoms could be found, could be good reagin producers who lack an organ target factor, similar to the situation where an increased bronchial hyperactivity exists in asymptomatic asthmatics. Kay (1979; 1980) has suggested that the IgE-mastcell-complement-eosinophil system forms a complex "immunological link-up" in terms of its helminthotoxic function.

TABLE I.2

ETHNIC VARIATIONS (SOUTH AFRICA) : TOTAL SERUM IgE CONCENTRATIONS

AUTHOR	ETHNIC GROUP	YEAR	METHOD	sIgE (u/ml) (MEDIAN)	NO OF PATIENTS	SELECTION CRITERIA
Orren and Dowdle (1975)	White	1975	Radioactive radial immuno-diffusion	85	2159	Unselected adult males
	Cape Coloured (Mixed)			226	773	
	Black			642	144	
	White			70	950	Unselected adult females
	Cape Coloured (Mixed)			180	398	
	Black			289	16	
Orren et al (1975)	White	1975	Radioactive radial immuno-diffusion	42	72	Non-allergic healthy blood donors
	Cape Coloured (Mixed)			201	54	
	Black			739	31	
	White			577	37	Allergy Clinic patients
	Cape Coloured (Mixed)			814	62	
	Black			1636	7	

Mansfield et al (1978), in attempting to rationalise the significant differences in racial elevations of sIgE as found in their study, likewise implicated helminthotoxic mechanisms in their argument. They suggested that populations which were historically resident in areas where parasitic infestation is endemic would experience strong selective pressures over a prolonged period of time for the evolution of an effective anti-parasitic hyper-immune sIgE response. He suggested that the survival-value inherent in such a response could be likened to the phenomenon of the sickle cell gene phylogenetic mechanism. Both of these responses seem to have persisted long after their survival advantage has diminished.

Experimental evidence from work in inbred strains of rats supports the concept of a selective survival advantage inherent in the rats with a high baseline sIgE (Bazin and Pauwels, 1982). Those strains which normally had low baseline sIgE values had weak reaginic responses. In contrast those strains with normally high sIgE values developed powerful reaginic responses on stimulation with most allergens (Rousseaux-Prevost et al, 1977; Pauwels et al, 1979).

The endemic nature of parasitism in Africa could therefore explain the retention of the non-specific high IgE responder phenotype in the phylogeny of the Black African races. This effect, moreover, seems to be unrelated to the pathophysiology of the atopic response. The statement by Kjellman et al (1976) that "a child found to have a high IgE level is very likely to have or soon to develop a clinically significant atopic allergic disorder" is an oversimplification of the status quo in African countries, and may be relevant only for First World White populations.

2.3.2 Ethnic variation in cord blood serum immunoglobulin E concentrations

The documentation of normal reference values for newborn and cord blood sIgE in various population groups has been the subject of many studies (Table I.3).

Direct comparisons of the published CBsIgE geometric mean values are essentially misleading for various reasons. Firstly, it is well-known that a family history of atopy is associated with raised concentrations of cord blood serum IgE concentrations in First World, White newborns (Orgel et al, 1975; Kjellman and Johansson, 1976; Michel et al, 1980; Croner et al, 1982). Secondly, Weil et al (1983) have shown that increased CBsIgE concentrations may be the result of maternal helminth infestation. Differences in both the study design and the inclusion criteria of the various samplings are therefore important reasons for differences in the published values of CBsIgE.

Furthermore, differences in the assay methods employed in the measurement of the samples, as well as differences in laboratory technique and co-efficients of error need to be taken into consideration when assessing such sensitive results. Differences in assay sensitivity and minimum detection limits in the various studies make comparisons equivocal.

Immunoglobulin E is, moreover, present in very low concentrations in the serum (1/50,000 of the IgG concentration in serum - Grundbacher, 1975). Cord blood serum usually contains exceptionally low concentrations of sIgE and requires highly sensitive methods of determination (Kjellman, 1982) such as the PRIST technique (Ceska and Lundkvist, 1972). This method has proved superior to the RIST technique, especially for the low ranges measured in cord-blood (Johansson et al, 1976). Thomas et al (1979) have suggested that more sensitive techniques of assay are needed to establish reliable and accurate ethnic or population differences in epidemiological studies of this nature. Kimpen et al, (1987) have recently reported on a series of newborns where they assayed CBsIgE using a method with a detection limit of 0.01 IU/ml. The method they used for this assay was, however, an ELISA technique, and not the PRIST.

An awareness of the limitations inherent in making a relevant comparison of the CBsIgE concentrations in Table I.3, will give perspective to such a comparison. Relatively higher geometric mean values in the Black CBsIgE were demonstrated in the series reported

TABLE I.3

ETHNIC VARIATION : CORD BLOOD/NEWBORN SERUM IgE CONCENTRATIONS

AUTHOR	COUNTRY	ETHNIC GROUP	YEAR	DETECTION LIMIT	ASSAY METHOD	MEAN (GM)	NO OF OBS	GM + 2SD	SELECTION CRITERIA
Johansson (1968)	Sweden	White	1968	-	RIST	36,3 ng/ml*	37	102 ng/ml	Unselected
Bazaral et al (1971)	U.S.A.	White	1971	-	Compet. inhibition of tagged I^{125} IgE to coupled anti- IgE	2,05 u/ml	33	-	Unselected
Johansson et al (1976)	Sweden	White	1976	-	PRIST	0,39 u/ml	26	-	Unselected
Kjellman et al (1976)	Sweden	White	1976	-	PRIST	0,22 IU	24	1,28	Non-atopic (Normal)
Kjellman and Johansson (1976)	Sweden	White	1976	-	PRIST	1,1 u/ml	30	-	Atopic family history +ve
Kjellman and Johansson (1976)	Sweden	White	1976	-	RIST	1,7 u/ml	30	-	Atopic family history +ve
Thomas et al (1979)	U.S.A.	Black	1979	0,5 u/ml	PRIST	0,35 u/ml	35	-	Unselected
Yoshikawa (1979)	Japan	Japanese	1979	-	-	0,80 u/ml	7	4,96	-

*1 u/ml = 2.3ng (Ganju et al, 1979)

TABLE I.3 (Cont.)

AUTHOR	COUNTRY	ETHNIC GROUP	YEAR	DETECTION LIMIT	ASSAY METHOD	MEAN (GM)	NO OF OBS	GM + 2SD	SELECTION CRITERIA
Ganju et al (1979)	U.S.A.	Black	1979	-	PRIST	1,48 u/ml	21	-	Non-atopic (Normal)
Michel et al (1979a)	France	White	1980	0,5 u/ml	PRIST	0,36 u/ml	107	1,36	Unselected
Michel et al (1980b)	France	White	1980	0,5 u/ml	PRIST	0,32 u/ml	136	1,40	-
Bhalla et al (1982)	U.S.A.	White	1982	-	PRIST	0,53 IU	37	2,05	Non-atopic (Normal)
Magnusson (1984)	Europe	White	1984	0,1 u/ml	PRIST	0,39 u/ml	288	-	Unselected
Magnusson (1984)	Africa	Black	1984	0,1 u/ml	PRIST	1,05 u/ml	60	-	Unselected
Kimpen et al (1987)	Belgium	White Mixed & Black	1987	0,01 lu/ml	ELISA	0,25 IU/ml (Mean) 0.12 IU/ml (Median)	-	-	Unselected

on by Ganju et al (1979) and Magnusson (1984) compared with the White CBsIgE values as reported by Magnusson (1984) and Kjellman et al (1976). These assays all used the PRIST technique. Kjellman's series was selected to include, however, only those newborns who had no family history for atopy and who did not develop any suspected atopic symptomatology at follow-up. Thomas et al's (1979) Black sampling had CBsIgE values similar to Kjellman's White sampling. He admitted, however, that the standard curve of his PRIST assay was insensitive below values of 0,5 u/ml and that assay methods with more sensitive detection limits should be used in studies of this nature. Kimpen et al (1987) have recently published their data on CBsIgE measured in a series of 5353 newborns in Belgium. These newborns had a heterogeneous ethnic parentage. Seventy four percent of these newborns had two Belgian parents, while 15% had two immigrant parents, either from Southern Europe or North Africa. Ten percent had parents of Mixed origin. They found, however, no significant difference in the CBsIgE values between the newborns of the different ethnic origins.

2.4 SUMMARY OF THE SOUTH AFRICAN PROBLEM

In summary, it appears that the Black adult South African ethnic group, in contrast to the White adult South African ethnic group, has inappropriately high concentrations of IgE. These values appear to be non-specifically elevated, and unrelated to the presence of atopic disease.

It is not unreasonable to speculate as to whether a similar non-specific ethno-genetic elevation may occur in the cord blood sIgE of Black newborns. If such an elevation in Black CBsIgE values is shown, furthermore, to be unrelated either to a family history for atopy or to the development of future atopic symptomatology in the newborn, then the use of CBsIgE concentrations has little value as a predictive atopic marker in the Black South African ethnic group. This finding would contradict the statement by Hattevig et al (1984) that "high concentrations of IgE antibodies are almost exclusively seen in infants with atopic disease". This conclusion was reached because all of the newborns in their study with elevated CBsIgE values above 1,2 kU/l subsequently developed atopic disease.

It should be emphasised that the rapid urbanisation and Westernisation of the South African rural Black could enhance the risk of sensitisation in this previously non-atopic population (Merrett et al, 1976). This possibility emphasises the need for the documentation of effective and reliable predictive cord blood markers for Third World multi-ethnic populations. It is possible that alternative cord blood atopic markers, such as eosinophils, platelets or anti-cow's milk IgG may be more reliable than CBsIgE in developing countries where genetic, dietary and environmental influences are appreciably dissimilar to those operative in Western societies.

CHAPTER 3 THE HYPOTHESIS AND AIMS OF THE STUDY

The specific problems intrinsic to South Africa in terms of the ability to institute the concepts of allergy prediction and prevention in this country have been highlighted in Chapter 2. Many of the problems are a result of the complex immuno-genetic influences at play in our Third World, multi-ethnic and multi-cultural population.

It should be obvious from the preceding discussion that, before Western, First World criteria and conclusions be automatically extrapolated to hold true for African Third-World populations, studies need to be done in these populations to determine whether the conclusions and guidelines advocated for First World populations are equally relevant in Africa.

More specifically, since different ethno-genetic and environmental factors are at play in Africa, it is fundamental to assess the effect of these factors on the CBsIgE values in each ethnic group before accepting the widely endorsed conclusion that raised levels of CBsIgE are reflective of an atopic predisposition, both in terms of their association with a family history of atopy, and in terms of being of predictive significance for the development of subsequent atopic disease.

3.1 THE HYPOTHESIS

Arising from an awareness of these specific South African problems, the hypothesis formulated to address these concepts reads as follows:

"Black South African newborns without an aFH have significantly higher CBsIgE values than similar White and Mixed newborns. An aFH does not influence the CBsIgE values in the Black newborns, as it does in the White and Mixed newborns. The CBsIgE values in Black newborns are not, furthermore, predictive for the development of subsequent atopy in infancy, as they are in the other ethnic groups".

3.2 AIMS OF THE STUDY

3.2.1 To test the Hypothesis

Confirmation of the hypothesis will indicate that CBsIgE is of no value as a predictive atopic marker for South African Black newborns, but that it does have this relevance for the South African White and Mixed newborns. The hypothesis would be confirmed by providing data which shows:

i) That the CBsIgE values in the Black newborns without an aFH are higher than those in the White and Mixed newborns, using a highly sensitive modification of the standard PRIST^{R} (Pharmacia) for the immunoassay.

ii) That an aFH in the first degree relatives of the newborns influences the CBsIgE values in the White and Mixed ethnic groups, but not in the Black ethnic group.

iii) That the CBsIgE values in those Black newborns who developed atopic disease during infancy were not higher than the CBsIgE values in those who remained healthy, in contrast to the White and Mixed newborns, in whom the CBsIgE values do demonstrate this difference between the subsequently atopic and the not atopic group.

3.2.2 To assess the atopic and predictive relevance of alternative cord blood markers

Various other cord blood markers have been thought to have atopic relevance (Section III, Ch. 2.3 and 4). They are:

i) Eosinophils
ii) Platelets
iii) Anti-cow's milk IgG

These will individually be assessed in the same way as the CBsIgE concentrations, to determine:

- The influence of an aFH in the first degree relatives of the newborns on their values.

- Whether a significant difference in their cord blood values between those newborns who developed atopic disease during infancy and those who remained healthy can be demonstrated.

3.2.3 To gather epidemiological information on the four cord blood atopic markers

The influence of the following specific genetic and environmental factors on the cord blood atopic markers will be assessed:

i) Ethnicity
ii) An atopic family history
iii) Maternal Ascariasis
iv) The newborn's sex
v) Maternal cigarette smoking

The rationale for examining the influence of these variables is given in Section III, Ch. 1.8. This epidemiological information is not at present available for these cord blood markers in Black, White and Mixed South African newborns.

SECTION II

THE DEVELOPMENT OF THE ATOPIC PHENOTYPE

CHAPTER 1 THE DEVELOPMENT OF THE ATOPIC PHENOTYPE

This study centres around cord blood atopic markers and their role in predicting atopic sensitisation of the infant. It is appropriate that some of the critical events which interact together during the development of the atopic phenotype are addressed.

The pathogenesis of atopic disease is subject to multifactorial influence. Both multigenic hereditary factors and non-genetic factors interact to determine the expression of atopic disease (Soothill, 1976; Marsh et al, 1981; Zeiger et al, 1986).

The genetic factors which control reagin production are considered in Section III, Chapter 1.3. These factors "set the stage", and provide the "fertile soil" for the development of an atopic response (Van Arsdel and Motulsky, 1959). Rapaport (1976) has estimated that a genetic inheritance of "allergic soil" is present in about 80% of those with clinical allergic disease.

Non-genetic factors, in contrast, seem to act as triggers, and are necessary to "switch-on" the allergic response. Soothill (1983) has made the point that, while the mechanisms involved in the genetic transmission of allergic disease are complex and interesting, we have no power to change a patient's tissue type. Various antigen non-specific trigger mechanisms may, however, be influenced by effective environmental manipulation. The development of the atopic phenotype may therefore be prevented in this manner.

Socio-cultural considerations in different populations groups are important factors in the pathogenesis of the allergic response (Joubert et al, 1988). The implication of environmental and other non-genetic factors in the pathogenesis of atopy was suggested after studies in monozygous twin pairs failed to show 100% atopic concordance (Edfors-Lubs, 1971; Bahna, 1984). Bazaral et al (1974) showed, furthermore, that the amount of serum IgE being formed in any

individual, while being of central importance, is not of exclusive importance in the development of the atopic response. Individual variations in effector cell and organ sensitivity are also important. Fundamental to an understanding of the concept of the allergic phenotype, is the principle that clinical atopy is the result of a complex set of interactions between the total individual (hereditary and intrinsic factors) and the total environment (non-genetic and extrinsic factors) (Rapaport, 1971). When these factors interact in such a way as to lower the individual's "allergic threshold" (the individual's constitutional level of resistance), clinical atopy may result.

Katz (1978; 1980; Katz and Fu Tong, 1980) introduced the concept of the "allergic breakthrough" in rationalising the differences between the allergic and the non-allergic phenotype. He noted that the production of serum IgE was usually dampened or suppressed by various non-specific suppressor factors of allergy (SFA). When these damping factors were disturbed by various extrinsic manipulations, the sIgE response was stimulated, and "allergic breakthrough" occurred, with clinical evidence of atopic symptomatology.

Various important intrinsic and extrinsic factors at play in the pathogenesis of the atopic phenotype in infancy merit consideration.

1.1 THE HEREDITARY NATURE OF ATOPIC DISEASE

The hereditary nature of atopy was first reported on by Maimonides in 1190 AD (Maimonides, 1963). It was left, however, to Cooke and Van der Veer (1916) to validate these impressions. Using Mendelian inheritance principles, they suggested that sensitisation was most likely to be inherited as a dominant characteristic.

During the last 50 years, many different theories have been suggested to explain the genetic transmission of the hereditary trait for atopy.

In 1936, Weiner et al proposed a genetic model for the transmission of atopic disease. He envisaged the genotype for atopy to be a single gene which desegregated into two alleles, a recessive "a" and a dominant "A". The "a" determined the atopic phenotype and the "A" the non-atopic phenotype. Homozygous subjects with respect to the allele

"a" would be expected to manifest atopic disease early on in life, while heterozygotes would be expected either to be healthy transmitters of the gene, or to manifest atopic disease later in life. Turner et al (1974) were, however, at variance with this proposal. Their findings, in an inheritance study on hayfever versus asthma, showed that Weiner et al's proposals were not applicable universally to every type of atopic disease complex, even though they may be of relevance for hayfever.

In 1959, Van Arsdel and Motulsky investigated the frequency and hereditability of asthma and allergic rhinitis in 5,818 students at the University of Washington, Seattle. Fifty six point five percent of allergic students had a family history for allergy, as compared with only 22,2% of non-allergic students. A genetic analysis of the data suggested that the transmission of the allergic inheritance was effected by an incomplete recessive gene, although a polygenic system for allergic susceptibility was not excluded.

Kjellman and Johansson (1979) supported the theory of a polygenic transmission for atopic disease. They found, furthermore, that the risk of developing atopy increased as the number of first degree relatives who had atopy increased. If a single parent had atopy, the incidence of atopy in the offspring was 19,8%, in comparison with an incidence of 42,9% in the presence of bilaterally atopic parents. Kjellman also suggested a genetic influence on symptom specificity because of the high incidence (72,2%) of the same atopic symptom in children where their parents both had the equivalent symptom.

The lack of consensus regarding the exact mode of transmission of atopic disease was considered by Edfors-Lubs (1971) to be predictable, in view of the common prevalence of atopy in the population and the variation in symptom severity and expression. She studied a large sampling of 7,000 twin pairs for penetrance and concordance but found low concordance rates in both monozygotic and dizygotic twins. This suggested that environmental factors were additionally of importance in the development of allergic disease. No distinction between Mendelian recessive, dominant or multigenic inheritance could be made.

In spite of the controversy over the mode of the genetic transmission of atopic disease various other clinical studies have confirmed the hereditary nature of atopic disease.

Turner et al (1974) showed that the prevalence of asthma in the children of asthmatic parents was higher than that among children with healthy parents.

Orgel et al (1975) found that 73% (8 of 11) of children who developed atopic disease within the first two years of life had a positive family history of atopy, while Michel et al (1980a) showed that 62% of children with atopy had allergic mothers as compared with only 27% of children with no allergic disease.

In a study of 1,651 6 year old children in Sweden, Kjellman and Croner (1984) found that 42,9% of atopic children had a positive family history for atopy as compared with only 24,7% of non-atopic children. Analysed alternatively, they found that 50,2% of children with a positive family history of atopy developed atopic symptoms within 6 years, compared with only 30,5% of children with no family history of atopy.

Little doubt consequently remains today that atopy and the propensity to produce IgE is inherited as an autosomal dominant characteristic (Cookson and Hopkin, 1988). The high genetic load for atopy present in the newborns with an aFH is the intrinsic soil within which infants subsequently develop atopic sensitisation.

1.2 IMMUNOLOGICAL CONSIDERATIONS

It has been suggested by various authors that infancy represents a transient period of relative immunoincompetence in the hereditarily-predisposed "high-allergic-risk" newborn (Matthew et al, 1977; Suoniemi et al, 1981; Gordon et al, 1982; Björkstén, 1983; Soothill, 1983).

It will be shown in Section III, Chapter 1.3 that the genetic message for an atopic predisposition is transmitted via 2 genetic loci

through their effect on IgE-specific T-suppressor cell and B cell activity.

Other immunological mechanisms are, however, additionally at play in the development of the atopic phenotype. Marsh et al (1981) have noted that statistically significant linear relationships have been demonstrated between log IgE and log IgG antibody responses, and log IgE and log IgA antibody responses. This emphasises the point made by Soothill (1983) that atopy is not recognized by an abnormal IgE response in isolation, but is associated also with abnormal responses in other immunoglobulin classes.

1.2.1 Immunoglobulin E

The role of IgE in the development of the Type I immune response is reviewed in Section III. Ch. 1.

1.2.2 Immunoglobulin A

Taylor et al (1973) were the first investigators to suggest that, because of a transient deficiency of IgA in atopically predisposed infants, excessive allergen was allowed systemic access through the "unguarded" gastro-intestinal and respiratory mucosal "portal of entry". This resulted in IgE hyperstimulation and consequent atopy. It has also been suggested that IgA deficiency could predispose to sensitisation because of the resulting enhancement of adjuvant production in the bowel by endotoxin-producing E.coli (Matthew et al, 1977). They showed also that the IgA deficiency at about 3 months of age is transient, with the atopic infants having developed higher levels of IgA than the non-atopic infants at 1 year of age. Other workers, namely Soothill (1976) and Kjellman (1983) share consensus that IgA deficiency at 3 months of age often precedes the development of eczema in infants of atopic parents, even though the deficiency has usually disappeared by the time the allergy becomes manifest. There are, however, some workers who have supplied data which does not support the contention that a relative IgA deficiency in early infancy predisposes the "high-allergic-risk" infant to develop atopic disease (Saarinen et al, 1979b). This subject therefore remains contentious.

It has recently been shown that anti-IgA antibodies are commonly found in IgA-deficient pregnant mothers (Petty et al, 1985). The offspring of these mothers often themselves have levels of serum IgA which are below the normal age-related mean concentrations. This would suggest that maternal anti-IgA exerts a transplacental effect on the foetal immune system, causing the IgA deficiency described in early infancy. This may be another mechanism whereby the transmission of the genetic message for atopy is translated into the final common immunologic effector pathway for the development of the atopic phenotype.

1.2.3 Immunoglobulin G

Serum IgG crosses the materno-foetal placental barrier, affording the newborn a degree of passive immunisation (Roitt 1984). The concentration of neonatal IgG falls after birth, reaching a nadir at 3 months of age, after which time the infant begins to produce its own IgG.

Jarret and Hall (1983) have produced interesting evidence that, in Hooded Lister rats, the newborn IgE antibody responsiveness to a specific antigen challenge was suppressed if the mothers had been previously immunized with the same antigen while pregnant. The antepartum maternal immunisation resulted in raised levels of antigen-specific IgG in the serum of both the mother and the newborn. These findings led to the concept that stimulation of maternal specific IgG by antepartum immunisation could suppress the newborn IgE response, and therefore prevent atopy in infancy.

Dannaeus et al (1978) documented the above phenomenon in human babies. They found that high concentrations of IgG antibodies to milk and egg proteins in the cord blood of the newborns on the study protected them from developing allergic symptomatology in the first 2 years of life. This finding suggests that antenatal stimulation of the maternal IgG response by the administration of sufficiently high doses of cow's milk or egg protein may be desirable for the preventive manipulation of the atopic response in infancy. This conflicts somewhat with the current recommendation for mothers with a

"high-allergic-risk" pregnancy to avoid or limit allergenic foods such as milk and egg during the last trimester, in order to prevent intrauterine sensitisation of the foetus (Frick, 1985). It has, however, recently been reported that the development of atopic disease in babies whose mothers had abstained from milk and eggs during late pregnancy was no lower than in a control group whose mothers did not eliminate these foods from their diets (Fälth-Magnusson and Kjellman, 1987). Fälth-Magnusson et al (1987) have furthermore shown that maternal abstention from cows-milk and egg in "high-allergic-risk" pregnancies resulted in a significant fall in specific IgG antibodies to cows-milk and egg in these mothers. Seen in the light of the findings of Dannaeus et al above, this would seem to be an undesirable immunological trend. Unequivocal recommendations for mothers of "high-allergic-risk" pregnancies are consequently still unclear.

1.3. FEEDING METHODS IN NEWBORNS AND INFANTS

Ever since Talbot (1918) described the relationship between the ingestion of a pound of chocolate by a breast feeding mother and the development of eczematous symptoms in her baby, much controversy has been generated by the role of feeding methods in the pathogenesis of the atopic phenotype.

Various studies have shown that atopic symptoms in infancy may be prevented or delayed by prolonged and exclusive breast feeding in infancy (Grulee and Sanford, 1936; Saarinen et al, 1979(a); Juto et al, 1982; Duchateau and Casimir, 1983). Grulee and Sanford (1936) reported that the incidence of infantile eczema was seven times higher in artificially fed infants than in exclusively breast fed infants. Saarinen et al (1979a) have shown, furthermore, that serum IgE values in exclusively breast fed infants were lower than those fed cow's milk formulas. This difference disappeared once solid foods were introduced into the diet.

In contrast, Fergusson et al (1981) found that exclusive breast feeding did not convey any protection against the development of eczema in infancy. They found, however, that the incidence of eczema

in infancy increased in direct proportion to the variety of solid foods that the infant had been given in the first 4 months of life. In 1982, Gordon et al reported on the relationship between breast feeding, serum IgE values and the development of atopic symptomatology in 250 infants from atopic families. They, likewise, could find no protective benefit against the development of either infantile atopy or abnormally high serum IgE values in those children who were breast fed if compared with those who were artificially fed.

Björkstén (1983), in an attempt to rationalise this conflicting information, reached the conclusion that much of the confusing data could be due to a non-uniformity of study design. Major differences in the selection criteria for the infants participating in the various studies were evident in a review of the literature. He concluded that breast feeding seemed to be of benefit only for those infants who were "at risk" for atopy by virtue of their inherited genotype. No protective benefit was seen to be gained by breast feeding normal infants. The population as a whole would, therefore, not benefit from this practice.

Some workers have gone even further, and have suggested that breast feeding may, in fact, be contra-indicated for the "high-allergic-risk" infant. Gerrard (1979) has reviewed the literature dealing with the passage of ingested maternal food antigens through her breast milk. The conclusion reached was that foods taken by the mother may find their way into her milk, and subsequently sensitise her infant. Gerrard himself substantiated these findings in 18 infants who became sensitised to various foods ingested by their breast feeding mothers. Cant et al (1985) likewise showed that breast-fed babies who developed eczema could be sensitized by foods ingested by their mothers. In 1986, Cant et al showed, furthermore, that a modification of the maternal diet in breast feeding mothers was of benefit to some breast fed babies who had eczema. Hattevig et al, in 1984, also described specific IgE responses to egg and cow's milk proteins in 9 "high-allergic-risk" infants, before the introduction of these nutrients into their diet.

It seems obvious, from these observations, that mothers who breast feed their infants in the hope that this practice will prevent the onset of atopic symptomatology in their infants should very carefully exclude all potentially allergic nutrients from their own diet (Firer et al, 1981). Failure to do this could, very easily, convert the practice of breast feeding in infancy from a prophylactic factor to an enhancing factor for the development of the atopic phenotype.

To further complicate the issue of the protective role of breast feeding for atopic sensitisation, Björkstén and Saarinen (1978) showed that the very small quantities of cow's milk antigen present in human breast milk are more likely to elicit the production of anti-cow's milk IgE in infants than the large quantities of antigen which are present in cow's milk based formulae. Juto and Björkstén (1980) confirmed this impression in a clinical study of "high-allergic-risk" babies with a positive aFH. They found that those infants who had the longest duration of breast feeding also had the highest sIgE values. They concluded that low antigen doses facilitated the IgE response. Firer et al (1981) also produced evidence to show that the levels of total sIgE and milk specific sIgE were significantly higher in a group of infants who had had minimal exposure to cow's milk as compared to a group of infants who had been fed substantial amounts of cow's milk.

An interesting insight into the inconsequential role of breast feeding in infancy was provided in a study by Saarinen et al (1982). The effect of exclusive breast feeding in infants during the first birch pollen season of their life surprisingly conferred no protective benefit to the infants in terms of the development of birch pollen allergy in later life. Instead, those infants who had been weaned to cow's milk formulae during this period had a significantly reduced incidence of allergy against birch pollen in later life as compared to the exclusively breast fed infants. Björkstén (1983) has suggested that the massive antigen load of cow's milk to which the vulnerable infants are exposed may stimulate and normalise their functionally defective immuno-regulatory systems. This mechanism could account for the protective effect of cow's milk

feeding against the development of birch pollen hypersensitivity in Saarinen's study. Lindfors and Enocksson (1988) have recently published data to suggest that the early feeding of "high-allergic-risk" newborns with a cow's milk formula, before the introduction of breast milk, reduced the chances of them developing atopic symptoms in the first 18 months of life.

These clinical findings have been supported by experimental evidence as to the potent reaginic response resulting from low-dose antigenic sensitisation. In 1970, Levine and Vaz studied the effect of antigen dosage on various inbred strains of mice. Their data suggested that low antigen doses favoured a reaginic rather than an IgG response. They concluded that the atopic response was, in part, a genetically predetermined capacity to respond to minute doses of antigen. Jarrett and Hall, in 1981, came to a similar conclusion when they analysed their data in which high IgE-responder egg albumin-immunized Hooded Lister rats were repeatedly challenged with very small amounts of antigen. Their results showed that extremely small doses of antigen were sufficient to stimulate IgE production. It was also shown, however, that repeated antigenic challenge of these immunised rats abrogated the IgE responsiveness due to the activation of an IgE class-specific suppressor mechanism.

Immunological deficits in the breast milk of lactating mothers with allergic infants have recently been described by Machtinger and Moss (1986). Lower concentrations of both total IgA and specific IgA antibodies to cow's milk proteins were found in the breast milk of mothers with allergic infants when compared to the concentrations of these antibodies in the breast milk of mothers with healthy infants. It seems that sub-optimal concentrations of maternal breast milk IgA antibodies to common nutrient allergens may predispose certain breast fed infants to the development of atopic disease.

It would seem, therefore, that multifactorial considerations hold the key to the rationalisation of the conflicting observations regarding the prophylactic benefit of breast feeding in infancy.

1.4 THE ADJUVANT EFFECT OF THE BORDETELLA PERTUSSIS (DPT) IMMUNISATION IN INFANCY

It has been well documented in animal studies that various adjuvants, such as the Bordetella pertussis bacteria (Mota et al, 1974) and Nippostrongylus brasiliensis (Ishizaka et al, 1980), enhance the IgE antibody response. It has been shown that the Bordetella pertussis vaccine is capaole of inducing an early, high-titre reaginic response in mice (Mitchell, 1976). The lipopolysaccharide content of Bordetella pertussis is the principal active material responsible for this adjuvant effect (Mota et al, 1974).

It is thought that this effect is also operative in humans (Björkstén and Anlstedt, 1984). The development of specific IgE antibodies to Bordetella pertussis following immunisation with the trivalent DPT vaccine during infancy have recently been described by Haus et al (1988a). In another study of IgE concentrations in infancy and early childhood, Orgel et al (1975) noticed that the greatest upward change in the median concentrations of serum IgE occurred between the ages of 6 and 9 months of age. The DPT inoculations in that study were administered at the ages of 2, 4 and 6 months. It is difficult, however, to unequivocally implicate the adjuvant effect of Bordetella pertussis in this reaginic response, particularly since other IgE-stimulating factors, such as the introduction of solid foods, often come into contention at this stage of infancy.

In infancy, the adjuvant effect of the Bordetella pertussis vaccine on the IgE response is particularly relevant because of the routine immunisation of all infants with the trivalent DPT. These inoculations are administered at the ages of approximately 3, 4 and a half and 6 months of age in South Africa. Any epidemiological comparative study of atopic development in infancy should therefore take cognizance of any discrepancy in the incidence and timing of the DPT inoculations in the various samplings.

1.5 THE ROLE OF VIRAL INFECTIONS

It has been suspected for some time that viral infections are potent stimulators of the allergic response. Various authors have confirmed this suspicion by monitoring both clinical and laboratory parameters of the viral and immunological responses during acute infections with various viruses.

Perelmutter et al (1978) presented evidence that sIgE values rose in the acute phase of a viral infection, but fell again in the convalescent phase. They proposed that the viral agent itself had a dampening effect on suppressor T cell function, allowing an increased IgE response by the B cell line of lymphocytes. In another study (Perelmutter et al, 1979), they suggested that the viral agent could possibly stimulate helper T cells in the acute phase of infection and that this could stimulate the increased production of IgE.

Bahna et al (1978), in a study which monitored the IgE response during and after an infection of heterophile-positive infectious mononucleosis, showed an early elevation of sIgE in the acute phase of the illness, followed by a significant drop by the 3rd convalescent month. This indicated that IgE production was very responsive during infectious mononucleosis. It was thought that the drop in sIgE during convalescence could result either from a suppression of IgE production or from an increased catabolism of IgE molecules. Frick, in 1981, furthermore demonstrated immunologic evidence of allergic sensitisation in infants together with, or just after, laboratory documented viral infections. The infants were all from families with a bilateral aFH.

The production of specific IgE antibodies against the respiratory syncytial virus (RSV) in patients who wheezed during an acute RSV infection has been documented by Welliver et al (1981; 1985). They also suggested that the formation of these anti RSV IgE antibodies could be a bad prognostic sign for the development of future episodes of wheezing in certain children (Welliver et al, 1980). It seems, therefore, that RSV infection in infancy and early childhood may be a potent stimulant of both the IgE response and the subsequent development of allergic symptoms.

Without viral and immunological studies in the acute and convalescent phases of these episodes, no definitive cause or effect assumptions can, however, be verified.

1.6 THE EFFECT OF CIGARETTE SMOKE

It has been known for about 2 decades that the children of parents who smoke become ill more often than the children of non-smokers (Cameron et al, 1969). The illnesses described are primarily respiratory illnesses.

Harlap and Davies (1974) showed also that infants of mothers who smoked had significantly more hospital admissions for bronchitis and pneumonia than those infants whose mothers did not smoke. The increased incidence of hospital admissions in those infants whose mothers smoked was moreover directly related to the number of cigarettes which the mother smoked. The acute respiratory effects of sidestream and second hand smoke seem to be related particularly to infancy (Landrigan et al, 1986), after which time this association seems to diminish.

Recently, the issue of "passive smoking" by children has received further attention. Burchfiel et al (1986) have shown that passive exposure to cigarette smoke was associated with both asthmatic and non-asthmatic respiratory symptoms in a large study population of 3,482 children from Tecumseh, Michigan. Pulmonary function tests (FEV_1, FVC and $Vmax_{50}$) in children of parents who were current smokers were significantly worse than in children of parents who had never smoked.

Tager (1986) has carefully reviewed the evidence generated by studies such as these, voicing concern as to the method of assessment in determining exposure to environmental tobacco smoke. It was pointed out that, in all the epidemiological studies to date, the smoking history had been evaluated by means of a questionnaire, which often did not take into consideration factors such as the room size, density of the smokers and ventilation. The evaluation of urinary cotinine has only recently provided a scientific yardstick which

correlates with the number of smokers in any one home. There nevertheless appears to be no reasonable doubt that passive exposure to tobacco smoke in childhood affects short-term respiratory health.

The mechanism by which smoking exerts its detrimental effect on the respiratory system seems to be multi-factorial (Björkstén and Ahlstedt, 1984). Besides being a mucosal irritant, Gerrard et al (1980) showed, in adults, that cigarette smoking is associated with a selective increase in the mean sIgE in spite of a significant simultaneous decrease in both the mean IgG and IgM concentrations when compared to a non-smoking control group. Kjellman (1981) similarly reported that parental smoking gave rise to significantly elevated sIgE values in their infants and young children. Venables et al (1985) have also recently shown that smoking may predispose to the formation of specific IgE antibodies to a hapten protein conjugate, tetrachlorophthalic anhydride human serum albumin. Atopic subjects were more liable to develop this specific IgE response than non-atopic subjects.

These considerations suggest that the effect of passive smoking in infancy is liable to accelerate the development of atopic symptomatology by virtue of its stimulatory effect on IgE synthesis. While the effects of this extrinsic IgE augmenting factor should be recognised in any epidemiological study of IgE synthesis and atopic development, it would seem that reliable quantification of any possible dose-related effect is not feasible without an accurate scientific assessment of the number of smokers in any environment. The estimation of urinary cotinine concentrations remains, at present, the most reliable method for such an assessment, but is outside the scope of this study.

1.7 THE EFFECT OF THE SEASON OF BIRTH

Björkstén and Suoniemi (1976) noticed that a group of male adolescent patients who were allergic to pollens had a month-of-birth clustering in the European Spring months of March, April and May. This birth distribution was significantly different to that of the general

population of Finland. They proposed that the concept of a vulnerable period for atopic sensitisation in early infancy would explain their observations. Certain variable seasonal environmental factors which could influence the development of subsequent atopic symptomatology were thought to be present during one or other of the seasons.

In a later study, Björkstén et al (1980) showed that the risk of immediate hypersensitivity to birch pollen in infancy correlated positively with the number of birch flowers in the environment during the first birch flowering season in Spring. They concluded that an exposure to pollens during the first 6 months of life increased the risk of pollen allergy. Kemp (1979) came to a similar conclusion when he studied the relationship between the season of birth and the development of grass-pollen allergy. He was of the opinion that infants are particularly susceptible to sensitisation at about 3 months of age. A recent study by Croner and Kjellman (1986) has clearly indicated, however, that the seasonal influence of the month of birth on the development of the atopic phenotype is limited to the high-allergic-risk genotype, and not to all newborns. Kimpen et al (1987) have also recently shown a cyclical difference in the CBsIgE in relation to the month of birth in a series of 5353 newborns. The peak CBsIgE was, as expected, seen in the European Spring months of March, April and May, while the trough was in October, in the European Autumn.

1.8 THE EFFECT OF THE NEWBORN'S SEX

The effect of sex on the sIgE in various population groups has been the subject of conflicting reports.

Some workers (Orren et al, 1975; Orren and Dowdle, 1975; Turner et al, 1974) have shown that males tend to have higher sIgE values than females, while others (Nye et al, 1975) have shown no such differences.

It is therefore not certain whether the newborn's sex would preferentially influence the development of the atopic phenotype in any group of subjects.

SECTION III

CORD BLOOD ATOPIC MARKERS - DESCRIPTION AND RATIONALE

CHAPTER 1 SERUM IMMUNOGLOBULIN E

1.1 BIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES

IgE is the World Health Organisation designation for immunoglobulin E, an immunoglobulin antibody which shares general characteristics and antigenic determinants with the other immunoglobulin classes G, A, M and D (Bennich et al, 1968; Johansson and Bennich, 1983).

In 1966, Ishizaka et al described the unique physico-chemical properties of IgE. They discovered also that IgE possessed reaginic activity, a fact which led to the realisation that IgE was the reaginic factor as described by Prausnitz and Küstner in 1921. IgE was therefore confirmed as being the mediator in the Type I anaphylactic reaction (Gell and Coombs, 1964; Johansson and Bennich, 1983a).

IgE is a non complement-binding glycoprotein, with a molecular weight of 190,000, a sedimentation constant of 8 and a carbohydrate concentration of 12% (Roitt, 1984). Its serum concentration is extremely low (0,0001 gram/litre) and it is stable in serum for at least 1 year at -20^{o}C (Lehrer and Bozelka, 1982).

Clinical studies have confirmed experimental evidence that maternal IgE does not cross the placental barrier and that CBsIgE concentrations are foetal in origin (Kjellman and Johansson, 1976; Dannaeus et al, 1978; Section III. Ch. 1.8.1)).

1.2 THE ONTOGENY OF IgE BIOSYNTHESIS

Miller et al (1973) showed that the human foetus may synthesise IgE in the foetal lung and liver from as early on as the 11th week of gestation, and in the foetal spleen by 21 weeks of gestation. Singer et al (1974) confirmed that IgE was present in amniotic fluid from

the 13th week of gestation, often at higher concentrations than those in cord blood samples. These findings suggest that the foetus is capable of intra-uterine sensitisation to antigens which cross the materno-foetal placental barrier. At birth, umbilical serum IgE is present in very small concentrations (Table I.3). Newborns with an atopic predisposition often have raised CBsIgE on the basis of a hereditarily determined IgE-specific T-suppressor cell dysfunction (Björkstén, 1983; Björkstén and Juto, 1983a).

During infancy and childhood, sIgE values rise, along with the other immunoglobulins (Roitt, 1984). Maximal peak concentrations of sIgE in normal, non-atopic populations occur in children at adolescence (Gerrard et al, 1974; Wittig et al, 1980; Bhalla et al, 1982). Kjellman et al (1976) confirmed these trends, finding maximal concentrations of IgE at the age of 10 years in their series of 207 normal neonates, infants and children.

This rise of sIgE in normal individuals is comparable to the rate of rise for sIgA (Johansson and Berg, 1967; Berg and Johansson, 1969) but is markedly slower than the rise in sIgG (Roitt, 1984). Atopic infants and children demonstrate both an earlier and a steeper rise in sIgE in the early years of life, compared with non-atopic controls (Kjellman, 1976a).

A gradual age-dependant decline in sIgE usually occurs from the second decade onwards (Buckley, 1980).

1.3 THE GENETIC CONTROL AND REGULATION OF THE IgE RESPONSE

The ongoing investigation into the exact genetic transmission of atopic disease was greatly facilitated by the discovery of IgE in 1966 (Ishizaka et al, 1966; Johansson, 1967; Bennich et al, 1968). It was soon realised that atopic diseases were mediated by IgE, and that IgE concentrations were elevated in atopic disease (Section III. Ch. 1.4). Oprée et al (1972) analysed the mean IgE levels of three different genotypes in random and allergic patients. Their results supported evidence for a common genetic control mechanism for both an atopic predisposition and the synthesis of IgE. These facts led to

the belief that IgE biosynthesis and regulation may likewise be under genetic control (Buckley, 1980). Many investigators have proposed various theories as to the mechanisms involved in the genetic control and regulation of the sIgE response.

In 1971, Bazaral et al measured IgE levels in normal infants and mothers, and proposed an inheritance hypothesis for the control of sIgE. The sIgE levels in 6 month old infants was substantially higher in those who had an allergic parent than in those who had healthy parents. The frequency distribution of the 35 maternal sIgE values revealed 3 arbitrarily distinct, multimodal groupings arranged in a non-Gaussian manner. If the Hardy-Weinberg law, which mathematically decribes the frequencies of two alleles belonging to one genetic locus in any population, was applied to this distribution, a simple Mendelian hereditable effect on sIgE values was indicated. This correlated with Weiner's proposed genetic model for atopic transmission, where a single gene with 2 alleles constituted the genotypic determinants (Weiner et al, 1936).

Further family studies seemed to confirm a group distribution of IgE levels in normal populations which fitted the Hardy-Weinberg law. Bazaral et al (1974), during a study of sIgE levels in both monozygous and dizygous middle-aged male twin pairs, observed a significant genetic effect on these levels. Monozygous twin pairs were more similar to each other in terms of their respective sIgE levels than dizygous twin pairs. It was re-iterated that the multimodal distribution of sIgE levels suggested that control of these levels was determined by a small number of alleles at one locus. The possibility of a multigenic effect on sIgE levels was not excluded, however. It was also not clear from this study whether sIgE levels were directly under genetic control, or whether they were determined secondarily by other factors which themselves were under genetic control. Turner et al (1974), for example, on the basis of family prevalence studies for atopy and IgE production, proposed a sex-linked factor associated with an inherited capacity for IgE biosynthesis. They interpreted their data to implicate the X-chromosome as the gene carrier for IgE synthesis in man.

The simple Mendelian two allele-one gene inheritance system for the control of IgE biosynthesis as described by Bazaral et al (1971;1974)

was challenged by the discovery of the Histocompatability-linked Immune Response (Ir) gene in animals and in man. Animal studies into the genetic control of murine IgE responses to low antigen doses by Levine and Vaz (1970) stimulated interest in the role of the human HL-A system in the allergic response to complex antigens. Benacerraf and McDevitt (1972) and also Levine et al (1972), first described the Ir gene, which was essential for the generation of ragweed hayfever. This gene was involved in the recognition of low concentrations of specific antigens, and determined specific immune responsiveness. It was mapped within the I (immune) region of the H-2 locus of the Major Histocompatability Complex (MHC). It is believed that the HL-A haplotype system in man is the counterpart of the murine H-2 MHC (Roitt, 1984). It is thought that the Ir gene in man will therefore also be mapped within the D region of the HL-A gene complex on chromosome 6 (Marsn et al, 1981).

Apart from the Ir gene, Levine (1973) definitively described a second genetic factor which controlled the levels and magnitude of the reaginic immune response. This factor exercised its control by more than one locus, had no antigen-specific or dose effect, and was not linked to the MHC. This genotype was responsible for the high and low IgE responder phenotypes in animals (Levine and Vaz, 1970; Katz, 1978; Binaghi, 1980; Marsh et al, 1981; Ishizaka, 1982) and in man (Croner et al, 1982; Kjellman and Croner, 1984). The action of this second genetic IgE control mechanism was possibly through its effect on suppressor T cells (Buckley, 1980). An insufficiency or functional abnormality of suppressor T cells has been shown to lead to increased levels of sIgE (Björkstén and Juto, 1982; Björkstén, 1983; Björkstén and Juto, 1983(a); Björkstén and Ahlstedt, 1984). Kishimoto et al (1980) confirmed, moreover, that the IgE antibody response was regulated by T and B cells which were IgE class specific.

The experimental work of Levine (1973) was substantiated by a clinical study of serum IgE levels in parents and children by Gerrard et al (1974). They concluded that in man, as in the mouse, HL-A linked Ir genes determined specific immune responsiveness per sé but were not related to the actual level or magnitude of the IgE response. Two additional dominant genes determined whether any subject would be a high or a low IgE responder. Low IgE levels were

determined by the presence of both dominant genes, while the absence of one or the other dominant gene resulted in a high IgE responder phenotype.

In 1981, Marsh et al reviewed and summarized the epidemiology and genetics of IgE regulation. Apart from substantiating the role of the HL-A linked Ir gene and the non HL-A linked IgE regulating genes, he provided evidence of an inter-action between these two genetic regulatory systems. His conclusions were that family studies, particularly across ethnic barriers, would be necessary in further determining the relevance of the HL-A system in the epidemiology of atopy.

1.4 CONDITIONS CAUSING RAISED SERUM IgE CONCENTRATIONS

Serum IgE concentrations are raised in various clinical settings (Table III.1).

Many of these conditions, including the atopic diseases, are undesirable and not beneficial to man. It is therefore interesting that the IgE-mediated Type I anaphylactic reaction has survived selective evolutionary pressures for its discontinuation.

The possible helminthotoxic function of IgE could very well have influenced the retention of the IgE-mast cell-eosinophil-complement system in phylogeny (Kay, 1979; Kay, 1980). While there is no universal agreement as to the precise role of IgE in the cytotoxic reaction against parasite infestation, various studies have demonstrated clear associations between metazoan infections in rats and mice and raised levels of sIgE. This association is particularly marked when the parasites involved have prominent tissue or systemic phases in their host life-cycle (Radermecker, 1974). Rousseaux-Prevost et al (1977) documented a rise in sIgE of up to 60 times the basal level, in rats infected with Schistosoma mansoni. Ishizaka et al (1980) described a similar tendency in rats after infection with Nippostrongylus brasiliensis. Capron et al (1980) and Binaghi (1980) in his studies with Trichinella spiralis-infected mice, described the cytotoxic effect of IgE-activated macrophages on the offending parasites.

In developing Third World countries where parasitism is endemic, the beneficial effect of the helminthotoxic function of IgE could therefore be more important to the survival of the indigenous population than the often less life-threatening effects of atopic disease. Kay (1980) has suggested that "natural selection might well tolerate inappropriate allergic reactions which, although irritating, will only rarely be significantly damaging".

The possible protective role of parasitic infection in the prevention of atopic symptomatology has been discussed in Section I. Chapter 2.1.

1.5 THE DIAGNOSTIC VALUE OF SERUM IMMUNOGLOBULIN E CONCENTRATIONS FOR ATOPY

The relationship between atopic disease and raised concentrations of serum IgE was first noted by Johansson (1967), very soon after he co-discovered IgE. He found that 63% of patients with allergic asthma had an elevated sIgE as compared to only 5% of patients with non-allergic asthma. Since then, many studies have proved that the estimation of sIgE concentration is a valuable diagnostic aid for the assessment of atopic disease (Berg and Johansson, 1969; Johansson et al, 1972; Wittig et al, 1980; Zetterström and Johansson, 1981), particularly in allergic asthma (Havnen et al, 1973), atopic dermatitis (eczema) (Church et al, 1976), allergic rhinitis (Seebohm, 1978) food allergy (Björkstén et al, 1983) and contact dermatitis (Bousquet et al, 1978) (Table III.1).

1.6 THE PREDICTIVE VALUE OF SERUM IMMUNOGLOBULIN E CONCENTRATIONS FOR ATOPY IN INFANTS AND CHILDREN

Foucard (1974) was the first investigator to report that a raised sIgE may precede the onset of clinical atopy. He found that the estimation of sIgE in infants and children during an initial episode of wheezy bronchitis could prognosticate or predict which of these patients could be expected to go on to develop asthma. Of the patients who subsequently developed asthma, 44% had a raised sIgE during the initial wheezy illness. Only 7% of the patients who never became asthmatic at the completion of the study had elevated levels of sIgE during the initial attack of wheezing (false positive result).

TABLE III.1

CONDITIONS CAUSING ELEVATED SERUM IMMUNOGLOBULIN E CONCENTRATIONS

CONDITIONS	CATEGORY	EXAMPLES
1. ATOPIC DISEASE	a. Established atopy (Berg and Johansson, 1969; Johansson et al, 1972; Turner et al, 1974; Wittig et al, 1980; Zetterström and Johansson, 1981)	Allergic asthma (Havnen et al, 1973) Allergic rhinitis (Seebohm, 1978) Allergic dermatitis (eczema) (Church et al, 1976) Food allergy (Björkstén et al, 1983) Anaphylactic and urticarial reactions (Knauer and Adkinson, 1983) Contact dermatitis (Bousquet et al, 1978)
	b. The "high-allergic-risk" newborn, infant and child (Kjellman et al, 1976; Kjellman Croner, 1984)	
2. PARASITIC INFECTIONS (i) Metazoan (Knauer and Adkinson, 1983)	a. Nematodes	Toxocara Filariasis Trichinosis Ascariasis Capillariasis Ankylostomiasis Onchocerciasis

TABLE III.1. (cont.)

CONDITIONS	CATEGORY	EXAMPLES
	b. Trematodes	Schistosomiasis
	c. Cestodes	Echinococcus
(ii) Protazoan (Radermerker et al, 1974)		Amoebiasis
3. IMMUNODEFICIENCY AND NEOPLASTIC CONDITIONS (Buckley, 1980; Buckley 1983)		Wiskott-Aldrich syndrome Hyperimmunoglobulin E syndrome Di George syndrome Nezelof syndrome Hodgkins disease IgE myeloma
4. MISCELLANEOUS MEDICAL CONDITIONS (Knauer and Adkinson, 1983)		Drug-induced interstitial nephritis Cystic fibrosis Idiopathic nephrotic syndrome Acute and chronic liver disease Allergic bronchopulmonary aspergillosis

In 1975, Orgel et al supported Foucard's proposal. They stated that "a sIgE of more than 20 U/ml before the end of the first year of life is associated with a substantial risk of developing atopic disease". In their study of 34 infants from both atopic and non-atopic families, all 12 of the infants who had a sIgE of more than 20 U/ml at 1 year of age, had developed definite or possible atopy by the age of 2 years, in comparison with only 6 of the 17 children who had a sIgE of less than 10 U/ml at 1 year. The elevation of the sIgE levels in these patients preceded the manifestations of the atopic diseases in 11 of the 12 infants.

Kjellman, in 1976 and Kjellman et al, in 1977, confirmed the predictive function for atopy of raised sIgE values in children. In the earlier study, Kjellman selected a group of 207 healthy children between 0-14 years of age. He followed these children up for 18 months. Of those infants and children who had raised levels of sIgE (more than GM + 1 SD) at the initial visit, 87,5% had consistently high levels again 18 months later. Of this group, 62,5% had developed atopic symptomatology within this time. In the subgroup of patients between 0-1 year of age at the initial visit, 95% of those who had raised sIgE levels at the initial visit had consistently high levels again 18 months later. Of these patients, 75% developed atopic manifestations within this period. It was similarly shown, therefore, that a rise in sIgE often preceded the onset of atopic symptomatology.

In another publication, Kjellman et al (1976) reported on the examination of 208 apparently normal children between the ages of 0-14 years. Fifty three children had sIgE values of more than GM + 2 SD for their age. Eighteen months later 24 of these had developed atopic disease.

Kjellman concluded that a child with a high sIgE, greater than the normal reference level for his age, is very likely to have, or soon to develop, a clinically significant atopic disorder.

1.7 THE PREDICTIVE VALUE OF CORD BLOOD SERUM IMMUNOGLOBULIN E CONCENTRATIONS IN NEWBORNS

Because of the very low concentrations of serum IgE reported in cord

blood samples (Grundbacher, 1975; Kjellman et al, 1976) (Table I.3), accurate and sensitive laboratory methods for measuring CBsIgE needed to be established before CBsIgE could be assessed in terms of their predictive value for the development of future atopy.

Anticipating this need, Johansson et al (1976) compared 4 methods to quantify CBsIgE. They concluded that the use of the paper-disc RIST (PRIST) direct sandwich radioimmunoassay, as described by Ceska and Lundkvist in 1972, was the most appropriate laboratory method for CBsIgE quantification.

Using this technique, numerous studies in the past decade have confirmed that elevated CBsIgE values are excellent predictive markers for the identification of the 'high-allergic-risk' newborn (Table III.2).

Kjellman and Croner (1984) have the biggest and longest follow-up prediction study to their credit. By 1984, they had followed up 1,651 newborns till the age of 7 years, and had correlated their initial CBsIgE values with the accumulated incidence of atopic disease which they had manifested up to the age of 6 years. They used a detection limit of 0,9 kU/l on the IgE Phadebas PRIST. It was found that the use of this concentration level as a cut-off point for the differentiation between high and low CBsIgE values was a better discriminator for the development of atopic disease over 7 years than the previously suggested cut-off limit of 1,3 kU/l, a level which was equal to the geometric mean + 2 SD for that population group (Kjellman and Johansson, 1976; Croner et al, 1982). Sixty three children (3,8%) developed atopic symptoms at an early stage (before 18 months of age), and persisted with these symptoms up till the age of 6 years. Of this subset, 93,7% had CBIgE values greater than 0,9 kU/l. Only 3,5% of children who never developed any atopic symptomatology over 6 years had CBsIgE values over 0,9 kU/l.

This study did have shortcomings. No routine clinical examinations were performed to confirm a history of clinical atopy. No RAST's were done to confirm specific sensitisation. The histories were, by and large, obtained from a questionnaire filled in by the parents. This study remains, however, the most widely quoted and the most extensive long term follow-up prediction survey.

TABLE III.2

THE PREDICTIVE VALUE OF RAISED CORD BLOOD SERUM IMMUNOGLOBULIN E CONCENTRATIONS FOR ATOPY

AUTHOR, YEAR AND COUNTRY	RADIOIMMUNO-ASSAY METHOD USED ON THE STUDY	NO OF NEWBORNS	FOLLOW-UP PERIOD	CUT-OFF POINT	NO OF INFANTS ABOVE AND BELOW CUT-OFF-POINT	% OF INFANTS ABOVE AND BELOW CUT-OFF-POINT DEVELOPING ATOPY WITHIN FOLLOW-UP PERIOD
Dannaeus et al (1978) Sweden	PRIST	53	2 years	0,4 kU/l	5 above 0,4 48 below 0,4	100% 27%
Michel et al (1980a) France	PRIST	110	12-36 months	0,5 IU/ml	36 above 0,5 74 below 0,5	52% 13%
Michel et al (1980b) France	PRIST	83	9 months	0,5 IU/ml	26 above 0,5 57 below 0,5	65,4% 24,6%
Croner et al (1982) Sweden	PRIST	1701	18 months	1,3 kU/l	90 above 1,3 1611 below 1,3	70% 4,9%
Duchateau & Casimir* (1983) Belgium	PRIST	60	1 month	1,0 U/ml	37 above 1,0 23 below 1,0	67,6% 17,4%
Hattevig et al (1984) Sweden	PRIST	86	4 years	1,3 kU/l	4 above 1,3 82 below 1,3	100% 22%
Magnusson (1984) Belgium	PRIST	190	2 years	1,20 U/ml	36 above 1,20 154 below 1,20	72,2% 7,8%
Kjellman & Croner (1984) Sweden	PRIST	1651	6 years	0,9 kU/l	208 above 0,9 1443 below 0,9	82,2% 30,1%
Kjellman & Croner (1984) (Sweden)	PRIST	1651	18 months	0,9 kU/l	208 above 0,9 1143 below 0,9	52,4% 2,3%

* = Blood taken from the neonate on the 5th day of life to exclude possible maternal contamination of a cord-blood specimen.

Michel et al (1980b), in a study which looked at the influence of maternal factors on CBsIgE values in 83 newborns, found CBsIgE to be a better predictive marker than a family history for atopy. Using a detection level of 0,5 IU/ml, they found that 71% of the 17 infants who were either definitely or possibly atopic after 9 months were born with CBsIgE values greater than 0,5 IU/ml. In contrast, only 21% of the 66 infants who never became clinically atopic within this period of time had CBsIgE values greater than 0,5 IU/ml. Looked at from another perspective, 26 newborns had CBsIgE values greater than 0,5 IU/ml. Of these newborns, 17 (65,4%) developed definite or possible atopic symptomatology within the first 9 months of life.

In another study, Michel et al (1980a) followed-up 110 newborns for 12-36 months. Of the 25 children who became allergic during the follow-up period, 16 (64%) had CBsIgE values greater than 0,5 IU/ml. In contrast, only 20 of the 85 children (24%) who remained non-allergic during the follow-up period had CBsIgE values greater than 0,5 IU/ml.

Croner et al (1982) further substantiated the predictive relevance of CBsIgE estimations. They also intimated that many of the 27 newborns with elevated CBsIgE values (greater or equal to 1,3 kU/l) who never went on to develop atopic symptomatology within the first 18 months of life (false-positive results) actually developed atopy by 4 and a half years of age. The prolonged follow-up period effectively reduced the 30% incidence of false-positive results.

Hattevig et al (1984) showed that every newborn with a CBsIgE values above 1,3 kU/l developed atopic disease within the 4 year follow-up period. This led them to conclude that "high concentrations of IgE antibodies are seen almost exclusively in infants with atopic disease". In the light of the discussion in Section I, Chapter 2, whereby elevated concentrations of serum IgE may be found in the Black races independently of atopic disease, this statement will be challenged by the data presented in this study.

Other studies by Kjellman and Johansson (1976), Dannaeus et al (1978), and Chandra et al (1985) have additionally confirmed the

predictive relevance of CBsIgE estimations. Chandra presented data to show that 44,3% of newborns with a positive family history of atopy had CBsIgE values greater or equal to 0,7 u/ml, in comparison with only 16,0% of newborns with a negative family history. This emphasises the hereditary influence of a family history of atopy on the CBsIgE values, an issue which will also be further examined in this study.

Duchateau and Casimir (1983) likewise confirmed the validity of neonatal sIgE screening for the development of atopic symptomatology in the first few weeks of life. They, however, collected the blood on the 5th day of life, to avoid the possibility of maternal contamination of the specimen. Their results are consequently not reflective of cord blood values.

Magnusson's study (1984), apart from similarly confirming the predictive value of CBsIgE estimations in a sample of 190 Belgian European infants, also described a significant ethnic or racial difference in CBsIgE geometric mean values between his Belgian sampling and an equivalent African-Asian sampling. No follow-up of the African-Asian infants was, however, reported on. The predictive relevance of CBsIgE values in Black ethnic groups has not yet been assessed.

In summary, the estimation of CBsIgE has been shown to provide valuable predictive information in terms of its ability to forecast the development of future atopic illness in the infant and child. All of these studies have, however, been done on White newborns in European Continental and Scandinavian countries, and the conclusions reached from these studies should be confirmed to the population groups in thes countries.

1.8 FACTORS INFLUENCING CORD BLOOD SERUM IMMUNOGLOBULIN E CONCENTRATIONS

Various factors have been thought to influence the CBsIgE found in the umbilical vein of the newborn. While the effects of some of these factors are still controversial, an awareness of these factors is necessary to avoid misinterpretation of the CBsIgE estimations.

1.8.1 The transplacental passage of maternal serum Immunoglobulin E

Most of the clinical evidence reported in the literature supports the contention that CBsIgE is foetal in origin. Johansson and Bennich (1983), co-discoverers of IgE, characterized IgE and confirmed that it is unable to cross the materno-foetal placental barrier.

Many clinical studies in humans have supported this contention, on the following grounds:

a. There is no correlation in sIgE between maternal and newborn serum samples (Bazaral et al, 1971; Kjellman and Johansson, 1976; Dannaeus et al, 1978; Michel et al, 1980a).

b. The very low values of CBsIgE relative to the higher concentrations present in maternal serum seem to negate the existence of a passive equilibrium for sIgE in the materno-foetal circulation, as occurs for serum IgG (Stevenson et al, 1971; Kjellman and Johansson, 1976).

c. Food-specific serum IgE has been demonstrated in cord blood sera without the occurrence of a corresponding food-specific sIgE for the equivalent food in the maternal serum (Michel et al, 1980a).

d. The converse situation, where specific IgE antibodies were found in maternal serum, but not in the cord blood serum of her newborn, has also been described (Kjellman and Johansson, 1976; Croner et al, 1982), further substantiating the foetal origin of CBsIgE.

e. An absence of reaginic activity in the cord blood serum of a newborn, in spite of a high titre of reaginic activity in the maternal serum, has been reported (Stevenson et al, 1971).

f. Mathur et al (1977) have suggested that CBsIgE may be maternal in origin on the basis of the higher mean values of sIgE which they measured in the umbilical vein relative to the sIgE which

they measured in the umbilical artery. Their statistical analysis revealed, however, that this difference was not significant. The assay used for the quantification of the sIgE was, moreover, the Phadebas IgE RIST, a method which has been shown to be inaccurate in the lower ranges below 50 U/ml (Johansson et al, 1976).

On balance, most investigators assume that the CBsIgE represents serum IgE which is foetal in origin.

1.8.2 Intra-partum contamination of the CBsIgE sample by maternal blood

The method which is used for cord blood venesection should be careful and precise, to avoid contamination by the maternal blood which inevitably surrounds the umbilical cord during parturition.

Kjellman and Croner (1984) described maternal contamination of a cord blood sample in one instance. Specific IgE antibodies had been found in a specific cord blood sample. They were, however, not detected again in another sample taken from the same neonate on the 5th day of life. This finding presumed maternal contamination of the cord blood sample, a situation which is largely eliminated by attention to detail during the venesection procedure (Section IV. Ch. 2.2.5).

In order to confirm the foetal origin of any cord blood serum IgE sample, a serum IgA estimation, performed on the cord blood sample, is a reliable indicator of maternal contamination (Björkstén - personal communication, Sweden 1984; Kimpen et al, 1987). Foetal concentrations of serum IgA are very low. Normal cord blood IgA concentrations in the Black, White and Mixed South African ethnic groups have been shown to range between 1,1 and 7,2 mg/dl (Van Rijswikj et al, 1985). Normal female adult levels of serum IgA concentrations in the Black and White ethnic groups in South Africa have been reported to range between 48 and 368 IU/ml (Shulman et al, 1975). Cord blood serum which gives an IgA concentration of less than 7,2 mg/dl is likely therefore to be foetal in origin. Kimpen et al (1987) excluded 157 cord blood samples with an IgA concentration of greater than 32.3 mg/ml from their cord blood IgE study on 5353 newborns, in the belief that those samples were probably contaminated with maternal blood.

This method remains the most reliable to date for the confirmation of the integrity of cord blood samples.

1.8.3 Intra-uterine sensitisation of the foetus

The human foetus has the ability to manufacture IgE from the 11th week of gestation (Miller et al, 1973; Singer et al, 1974). This confers it with the potential to become sensitised in-utero to antigens which have crossed the materno-foetal placental barrier. The source of these maternal antigens are presumed to be from the mother's dietary intake during pregnancy (Kuroume et al, 1976; Katz, 1979).

Clinical studies have confirmed the intra-uterine sensitisation of the foetus. The occurrence of specific IgE antibodies to cow's milk protein (Michel et al, 1980b) and penicillin (Levine et al, 1971) in newborn serum, as well as haemagglutinating antibody titres against lactalbumin, soybean and egg white in human amniotic fluid (Kuroume et al, 1976), have confirmed a foetal immune response to sensitizing maternal antigens.

Further evidence to suggest that a newborn may already be atopic was provided by Kaufman (1971) when he demonstrated a positive Prausnitz-Küstner reaction in a 3 hours old newborn. This finding presumed intra-uterine sensitisation, a factor which was substantiated by the demonstration of a cord blood reaginic IgE concentration of 71 ng/ml in the baby.

Cord blood is normally thought to contain small amounts of IgE which is non-specific (Croner et al, 1982). The demonstration of specific IgE in cord blood samples by RAST indicates intra-uterine sensitisation, and may contribute to an elevation of the total CBsIgE.

1.8.4 The genetic effect of atopy and ethnicity

The genetic control of sIgE is manifest already at birth (Björkstén, 1983). The newborn who is hereditarily predisposed to atopic disease and who carries a high genetic load for atopy may be identified by

having a raised CBsIgE value (Section I. Chapter 1; Michel et al, 1980; Michel et al, 1980b; Croner et al, 1982; Kjellman and Croner, 1984). This high cord blood sIgE responder phenotype is thought to be determined by the non HL-A linked gene (Section III, Ch. 1.3) which exercises its effect by controlling IgE-specific suppressor T cell function (Buckley, 1980; Kishimoto et al, 1980; Björkstén and Juto, 1982; Björkstén and Ahlstedt, 1984).

In a clinical study of 1,701 newborn infants, Croner et al (1982) demonstrated the genetic effect of a positive family history for atopy on CBsIgE. The hereditarily predisposed "high-allergic-risk" genotype was often expressed as a high CBsIgE responder phenotype. They found that a positive family history for atopy significantly influenced the concentration of IgE in the newborn's cord blood. Seven point two percent of newborns (38 of 528) with an immediate family history of atopy had raised CBsIgE values (greater than 1,3 kU/l) in contrast with only 3,8% of newborns (27 of 703) with no family history (p less than 0,01). This genetic effect was perpetuated throughout the 18 month follow-up period, in that those newborns with raised CBsIgE values also had significantly higher IgE concentrations 18 months later, compared to those newborns with low CBsIgE values (less than 1,3 kU/l).

The genetic effect of ethnicity per se on CBsIgE values, independent of a hereditary predisposition for atopy, seems feasible. It has been shown that the adult Black races may manifest high concentrations of IgE in the absence of atopy, and that these "high IgE responder" phenotypes may have resulted from strong evolutionary selective pressures designed as a survival mechanism for the human host defence against endemic parasitism (Section I. Ch. 2.3.1). If the Black ethnic group in South Africa do represent a pool of genetically-coded "high IgE responders", this effect may be observed already at birth, in the cord blood serum. It would seem, furthermore, that the genetic effect of ethnicity on sIgE could be more efficiently assessed in a study of cord blood IgE sera rather than in adults. This would eliminate extra-uterine environmental influences on sIgE (Ganju et al, 1979), although it would not exclude the effect of intra-uterine environmental influences on the foetal production of IgE (Section III. Ch. 1.8.3).

Bazaral et al (1974) have agreed that the genetic effect on sIgE could be more efficiently studied early on in life. They showed that variations and differences in sIgE between individuals with the same genotype (monozygous twins for example) arose largely after childhood. The mean intrapair variance in sIgE of monozygous twin children was significantly less than that of monozygous twin adults.

Magnusson (1984) (Table 1.3) is the only worker to have documented a clear ethnic difference in CBsIgE, using the same radioimmunoassay for the sIgE quantification in a single study of White and Black newborns. The White and Black populations in his study were, however, taken from dissimilar geographic loci, namely Belgium (Europe) and Africa respectively, and the radioimmunoassay method used for the quantification of his samples was sensitive to only 0.1 u/ml. Furthermore, his study did not differentiate between newborns with an atopic or a non-atopic family history, and this factor could obscure the effect of ethnicity per sé.

This is the first controlled study comparing different CBsIgE values in different ethnic groups from a single geographic area using a highly-sensitive radioimmunoassay. The findings should clarify the extent of any ethno-genetic effects acting on CBsIgE values, particularly since the CBsIgE values will be compared in subgroups both with and without an aFH between the various ethnic groups.

1.8.5 Drug administration during pregnancy

Sex steroid hormones such as progesterone have been implicated in the causation of immuno-suppression through their inhibitory effect on cellular immunity. Laboratory studies have described the in-vitro inhibitory effect of progesterone on both phytohaemagglutinin stimulated lymphocytes (PHA) (Mori et al, 1975) and purified protein derivative (PPD) stimulated lymphocytes (Wyle and Kent, 1977). High concentrations of progesterone, which are probably normally only achieved at its biosynthetic site in the foeto-placental unit, are necessary for this immuno-suppressive effect (Weinstein et al, 1977).

It has been shown that IgE-specific B cells are under the direct control of IgE-specific T cells (Kishimoto et al, 1980), and that T-suppressor cell failure may lead to raised concentrations of IgE (Björkstén and Juto, 1982; Björkstén and Ahlstedt, 1984). Immunodeficiency diseases are, moreover, well known for their association with raised IgE concentrations (Table III. 1). It is not unexpected, therefore, that the immuno-suppressive and inhibitory effect of high doses of progesterone on cell-mediated immunity and lymphocyte transformation could be associated with raised levels of CBsIgE, although not all workers share consensus on this issue (Mathur et al, 1977).

In a clinical study, Michel et al (1980b) showed that the administration of progesterone to the pregnant mother tended to increase the mean values of CBsIgE. Fifty three percent of newborn infants whose mothers had received progesterone during gestation had CBsIgE concentrations greater than 0,5 IU/ml as compared with only 24% of newborn infants whose mothers had received no gestational progesterone.

The effect of Salbutamol administration to the pregnant mother was also examined by Michel et al (1980b). The effect of such administration had no significant influence on the CBsIgE.

The effect of oral contraceptives on the CBsIgE was likewise assessed by Michel et al (1980b). They found no significant effect on the CBsIgE values in those mothers who had taken oral contraceptives just before or during the early stages of pregnancy.

1.8.6 The effect of cigarette smoke

The effect of passive smoking on the health and IgE concentrations of infants and children of smoking parents has been discussed in Section II Ch. 1.6.

Whether maternal smoking in pregnancy likewise stimulates foetal IgE production in utero is uncertain. Magnusson (1984) found that mothers who smoked had newborns with significantly higher CBsIgE values than mothers who did not smoke (p less than 0,05).

Michel et al (1980b), in contrast, did not find such a clear differentiation. This was possibly due to the fact that only 16 of their study sample of 136 mothers smoked, making statistical comparison unreliable.

The possibility nevertheless exists that maternal smoking during pregnancy may contribute towards raised CBsIgE values.

1.8.7 Prematurity

It has been suggested by various studies that the effect of prematurity may influence CBsIgE values.

Michel et al (1980b) did not detect IgE in the cord blood of newborns before 36 weeks of gestation. However, the PRIST used for quantification of the CBsIgE had a detection limit of 0,5 IU/ml, and would not register concentrations below this level. A more sensitive method of measuring the CBsIgE may well have detected IgE in those preterm newborns of less than 36 weeks gestation. In another study, Croner et al (1982) did not find CBsIgE values greater than 0,9 IU/ml in any cases where pregnancy lasted less than 37 weeks gestation.

The effect of prematurity on CBsIgE values is, therefore, still equivocal.

1.8.8 Sex

Various studies have shown that male newborn infants have higher CBsIgE values than female newborn infants.

In a study of 1,701 Swedish newborns, Croner et al (1982) showed that 7,3% of boys had CBsIgE values of greater than 1,3 kU/l as compared with only 3,2% of girls (p less than 0,001). Magnusson (1984) also showed a significant sex differentiation in CBsIgE values. In his study, boys more often had raised CBsIgE values than girls (p less than 0,05).

1.8.9 Maternal Ascariasis

Weil et al (1983) have reported on raised total CBsIgE values occurring in Indian newborns whose mothers had immunological evidence of filarial helminth infestation. The cord blood sera of many of these newborns also contained specific IgE antibodies to filarial antigens. This study suggests that intra-uterine sensitisation to maternal parasite antigens could be a contributing factor influencing CBsIgE values.

CHAPTER 2 EOSINOPHILS

2.1 INTRODUCTION

The eosinophil granulocyte was discovered by Paul Ehrlich a little more than a century ago (Ehrlich, 1879).

Since then, an eosinophilia has been widely accepted as being intimately associated with atopic disease (Lowell, 1967; Otteson and Cohen, 1978). Reconfirmation of its role in asthma (Frigas and Gleich, 1986) its role in rhinitis (Mullarkey et al, 1980) and its modulating role in down-regulating the IgE mediated mast-cell dependant reactions (Weller and Goetzl, 1979; Kay, 1980) by degrading various mast-cell mediators (Kater et al, 1976; Henderson et al, 1982) has firmly established this granulocyte as being integrally involved in the allergic Type I anaphylactic response.

It is not surprising, therefore, that its role as a cord blood atopic marker should be addressed (Dry et al, 1980). Its predictive potential has, additionally, stimulated interest since eosinophilia was reported as reflecting a susceptibility or a predisposition for allergy in young boys (Foucard, 1974).

2.2 BIOLOGIC AND PHYSIOLOGIC VARIATIONS IN THE TOTAL EOSINOPHIL COUNT

The consideration of biologic and physiologic variations in the number of eosinophils circulating at any one time is important. Circulating eosinophils represent a balance between the rate of their formation and release from the bone marrow and their disappearance from the blood (Best et al, 1953).

A natural diurnal fluctuation in the level of circulating eosinophils has been well documented (Fisher and Fisner, 1951; Donato and Strumia, 1952; Unrbrand, 1958). This variation seems to be independent of the fluctuation in total leucocyte levels. It appears, however, to be a function of adrenocortical activity, with eosinophil levels being a mirror image of the circulating adrenal cortico-steroids (Otteson and Cohen, 1978).

These hormones seem therefore to have an eosinophilopaenic effect. Minimum TEC's are consequently expected in the mornings, with maximum TEC's occurring late at night.

Various other factors have been shown to cause a peripheral eosinophilopaenia. Stress in general has an eosinophilopaenic effect, which is mediated by an increase in pituitary-adrenal cortical activity (Recant et al, 1950). The administration of oral ephedrine per sé has been shown to cause an eosinophilopaenic effect, if given to normal subjects with an intact anterior pituitary ACTH-adrenocortical axis (Abelson and Moyes, 1950; Best and Samter, 1951). This effect is effectively abolished by propranolol (Koch-Weser, 1968), a beta adrenergic blocker. Trauma (Gabrilove, 1950), surgical shock (Laragh and Almy, 1948; Davis and Hulit, 1949), physical abuse (Koch-Weser, 1968), the intravenous administration of insulin (Laragh and Almy, 1948) emotional stress (Humphreys and Raab, 1950) and acute infections (Weller, 1984) have all been described as causing an eosinophilopaenia.

The stress of labour may also have an influence on the TEC of the mother. Eosinophilopaenia during and after labour has been described by Davis and Hulit (1949), who found that an almost total eosinophilopaenia was present in more than 50% of their patients during the immediate post-partum period following the third stage of labour. The degree of this eosinophilopaenia was thought to be reflective of the degree of labour-induced stress. The TEC's in their study returned to normal only at the end of the third post-partum day.

The effect of physical exercise on the TEC is somewhat contradictory. Both a rise (Davis and Hulit, 1949 in menstruating women) and a fall (Dalton and Selye, 1939 in rats and rabbits) in the TEC following exercise has been reported. It appears that this disparity may be explained because of a bivariate response in the TEC, with a decrease in the TEC directly after strenuous exercise preceding a later increase. Some authors have described a sex difference for TEC's with boys having higher values than girls (Cunningnam, 1975). There is no consensus on this issue, however, since other authors have not reproduced similar findings (Felarca and Lowell, 1967).

To what extent these factors influence the cord blood TEC of the newborn remains unknown. It is, however, probable that some of these biological and physico-chemical variations are at play during labour.

2.3 PATHOLOGIC EOSINOPHILIA

Apart from its association with atopy (Section III. Ch. 2.1), eosinophilia is associated also with a host of pathologic disease-complexes (Otteson and Cohen, 1978). Parasitic infestation of the human host is a common cause of peripheral and tissue eosinophilia (Kay, 1979; Kay, 1980; Weller, 1984). This observation is particularly noticeable for those helminths which do not confine themselves to an intra-luminal gastro-intestinal existence in their life cycle. Metazoan helminths are therefore more commonly associated with eosinophilia than protozoan infections.

2.4 THE CONCEPT OF NORMALITY FOR TOTAL EOSINOPHIL COUNTS

Because of the many biological and physico-chemical influences on the level of circulating eosinophils, normal values have very little meaning, unless they are defined in relation to circumstances operative at the time of venesection.

Normal values reported in the literature consequently range widely. Values of: 0-240 cells/mm^3 (Discombe, 1946), 40-600 cells/mm^3 (Uhrbrand, 1958), 250 cells/mm^3 (Lowell, 1967), 70-450 cells/mm^3 (Best et al, 1953), 34-427 cells/mm^3 (Felarca and Lowell, 1967) have been reported in healthy adults. Values for children are said to be higher, with a mean of 240 cells/mm^3 and a 95% confidence limit of 0-740 cells/mm^3 (Cunningham, 1975).

The terms "eosinophilopaenia" and "eosinophilia" need, therefore, to be seen in context with these widely spread normal values.

2.5 CORD BLOOD TOTAL EOSINOPHIL COUNTS

The rationale for a raised cord blood TEC in those newborns who have a family history for atopy and therefore a predisposition for the development of allergic disease is plausible, for two reasons.

Firstly, the regulation and control of eosinophil production seems to be under immunological control (Weller and Goetzl, 1979) with T lymphocytes actively participating in the eosinophilic response (Basten and Beeson, 1970; Bass et al, 1980). For example, congenitally athymic mice cannot respond with an eosinophilia to a Trichnella spiralis infection (Walls et al, 1971; Ruitenberg et al, 1977), a condition which is usually an adequate antigenic stimulus for an eosinopnilic response in immunlogically intact species. Cord blood eosinophilia may therefore be expected in newborns who have a derangement of their T lymphocyte system, particularly if associated with the relative increase of T-helper cell activity which is a consequence of the imbalance in the OKT_4/OKT_8 T cell system in atopically predisposed neonates (Björkstén and Juto, 1982; Björkstén, 1983; Björkstén and Juto, 1983).

Secondly, Kay (1979; 1980) has suggested that the IgE-mast cell-complement-eosinophil system forms a complex immunological link-up in terms of its helminthotoxic function. It is therefore reasonable to imagine that cord blood eosinophilia could well accompany the elevated CBsIgE values which have been shown to occur in "high-allergic-risk" newborns (Section I. Ch. 1). A further factor favouring the association of cord blood eosinophilia with that of a raised CBsIgE is the fact that eosinophils are motile cells, and are able to migrate and accumulate at the sites where Type I anaphylactic reactions are occurring (Kay, 1980; Weller, 1984). This migration and mobilisation occurs as a result of mast-cell derived eosinophilotactic agents, such as the tetrapeptide "eosinophil chemotactic factor of anaphylaxis" (ECF-A) (Goetzl and Austen, 1975), histamine (Clark et al, 1975), imidazole acetic acid (ImAA) (Turnbull and Kay, 1976) and complement-system fractions such as C5, C5a and C567 (Weller, 1984). Mono-nuclear leukocytes have also been shown to produce the human lymphokine "eosinophil stimulation promoter" (ESP) in response to non-helminthic antigens (Weller et al, 1978).

Dry et al (1980) found a significant difference in cord blood TEC's in his group with a positive family history for atopy relative to the cord blood TEC's in his group with a negative atopic family history (248 and 183 cells/mm^3 respectively) (p less than 0,02).

No further data on the relationship between cord blood TEC's and a family history for atopy, or on the predictive value of cord blood TEC's for the development of future atopic disease has been published.

CHAPTER 3 PLATELETS

The unactivated human platelet is a small, anuclear, smooth-surfaced disc, which has a life-span of 10 days in the peripheral blood circulation (Handin, 1981)

The platelet is formed secondarily to the pnysical fragmentation of megakaryocytes. These megakaryocytes initially arise in the yolk sac, liver and spleen of the foetus, and then ultimately in the bone marrow of the infant (Aster, 1972).

3.1. THE FUNCTION OF PLATELETS

Blood platelets are intrinsically associated with the maintenance of normal haemostasis. This function is effected both by haemostatic and by thromboplastic mechanisms (Handin, 1981)

The haemostatic function is effected by platelet reactions such as agglutination, adhesion, aggregation and cohesion, while the thromboplastic function is effected by a phospholipoprotein fraction known as platelet factor 3 (Williams et al, 1972)

During the course of the last 2 decades, various investigators have provided evidence that the platelet may be implicated in the allergic Type I response. Some of this evidence is summarized below.

3.2. THE ROLE OF PLATELETS IN THE ALLERGIC RESPONSE

About 20 years ago, Barbaro and Zvaifler (1966) described experiments which demonstrated a correlation between antigen-induced histamine release by the platelets of rabbits producing homologous PCA antibody.

The mediator responsible for this reaction was later identified and characterized. It became known as platelet activating factor, or paf-acether. Paf-acether is an alkyl analogue of lysophosphatidylcholine. It is released from IgE-sensitised macrophages, basophils, neutrophils and platelets themselves, and functions as an immune mediator to activate platelet aggregation (Benveniste, 1980).

Various reviews and studies have since provided evidence that the platelet is intimately associated with the allergic response, in particular, that of allergic asthma (Morley et al, 1984; 1985; Battersby et al, 1984; Slater et al, 1985). It was shown that asthmatic symptomatology could be exacerbated as a result of localised pulmonary platelet activation by paf-acether formation in the lung.

Slater et al (1985) furthermore described an increase in pulmonary megakaryocytes in the lung tissue of 3 patients who died of status asthmaticus. In their opinion, this finding suggested that thrombopoeisis was being locally stimulated as a result of the increased pulmonary platelet activation and consumption which occurs after stimulation by paf-acether.

Additional evidence for the mediating role of paf-acether in asthma was supplied by Page et al (1985), who demonstrated that effective suppression of paf-acether-induced asthmatic responses could be realised by the administration of anti-asthma drugs such as ketotifen and theophylline.

Further evidence of the role of platelets in the Type I allergic response was provided by Joseph et al (1986). They demonstrated the in-vitro cytotoxic function of platelets against schistosoma larvae by an IgE-dependant mechanism. This confirmed the presence of Fc receptors for IgE on blood platelets.

Consensus on the role of paf-acether and platelet activation in the pathogenesis of allergic asthma has, however, by no means been reached.

Halonen et al (1985), in a study of the role of platelets and paf-acether in the rabbit, concluded that neither paf-acether nor platelets were important in mediating the pulmonary anaphylactic mechanical alterations in the rabbit experimental model. Ind et al (1985) further questioned the role of platelets in asthma. Using isotopically labelled platelets as markers, they found no evidence of intrapulmonary platelet accumulation in either normal or asthmatic patients who had undergone bronchial challenge with Dermatophygoides pteronyssinus antigen.

In another study, Durham et al (1985) measured circulating concentrations of the platelet-derived proteins, B-thromboglobulin and platelet factor IV during metacholine-induced bronchoconstriction in asthmatics. These platelet products are indicators of platelet activation, and should be raised as a consequence of platelet activation. This feature was, however, not demonstrated in their study.

Greer et al (1984; 1985) similarly found no evidence of platelet activation in asthmatic subjects who had been challenged with either histamine inhalation or house dust mite antigen bronchial provocation. The levels of B-thromboglobulin and thromboxane B_2, a stable metabolite of thromboxane A_2 which is released by activated platelets, were unchanged in these patients. This again contradicted the hypothesis that platelet activation occurred in antigen-induced asthmatic responses in man.

It is clear, therefore, that even though much of the available evidence favours the implication of platelets in the allergic response, the clinical implications and the exact conditions under which this mechanism seems to act, is still a matter of debate.

3.3 CORD BLOOD PLATELET COUNT AS A PREDICTIVE ATOPIC MARKER

The implication of platelets in the allergic response prompted Magnusson and de Weck (1985) to investigate platelet counts in the cord blood of newborns. They found, firstly, that a relative thrombocytopaenia existed in the newborns whose mothers were allergic, if compared to those newborns with non-allergic mothers ($p = 0,014$). Paternal and sibling allergic disease did not, however, influence the cord blood platelet counts. Furthermore, they found that a relative cord blood mean thrombocytopaenia ($196,375/mm^3$) existed in those newborns who went on to develop atopic symptoms within the follow-up period of 18 months, if compared with the cord blood mean platelet count of those newborns who remained healthy ($285,902/mm^3$) ($p = 0,002$). They also found a positive correlation between cord blood IgE concentrations and cord blood platelet counts.

They assumed that the low platelet counts observed in the "high-allergic-risk" newborns could be reflective of intra-uterine sensitisation. The subsequent consumption of foetal platelets, secondary to a paf-acether mediated platelet activation mechanism in this atopically vulnerable foetal population, could be a logical explanation for the relative cord blood thrombocytopaenia. Halonen et al (1980) have shown that profound thrombocytopaenia may accompany paf-acether induced pulmonary anaphylaxis in the rabbit.

Magnusson and de Weck's (1985) study represents the only data on cord blood platelet counts to date. Furthermore, the newborns studied were European ethnics. No inter-ethnic comparative studies for cord blood platelets have been published, either for their potential ethnic variability or for their possible predictive relevance.

In view of the unresolved differences of opinion as to the relevance of platelets in the allergic response, this study will attempt to add further perspective to the predictive relevance of cord blood platelets for atopic disease in different ethnic groups.

3.4. PHYSIOLOGIC VARIATIONS IN NORMAL PLATELET COUNTS

The factors which influence platelet counts in normal subjects are summarised in Table III. 3.

TABLE III.3

PHYSIOLOGICAL VARIATIONS IN NORMAL PLATELET COUNTS
(from Dacie and Lewis, 1984)

FACTORS WHICH INFLUENCE PLATELET COUNTS	Sex	- Platelet count is 20% higher in women
	Menstruation	- Platelet count falls during menstruation. This may be due to a 21-35 day cyclical rhythm effect.
	Diurnal	- This is more obvious on a day-to-day basis rather than during the course of a single day.
	Age (before 6 months)	- At birth, and during early infancy, the platelet count is at the lower level of the adult normal range
FACTORS WHICH DO NOT INFLUENCE PLATELET COUNTS	Oral contraceptives	
	Age (after 6 months)	

CHAPTER 4 ANTI-COW'S MILK IMMUNOGLOBULIN G

4.1 THE ALLERGENIC CAPACITY OF COW'S MILK

Cow's milk is known to contain up to 25 distinct proteins, all of which may induce specific antibody production in the foetus and newborn (Bahna and Heiner, 1980). The more important of these proteins, as well as some of their physical and antigenic characteristics are tabulated in Table III.4.

The prevalence of allergy to cow's milk has been shown to be between 0.3% - 7,5% in various age groups (Bahna and Heiner, 1980). Halpern et al (1973) showed that 1,8% of 1,084 infants who were exposed to cow's milk in the first 6 months of life became allergic to it.

The demonstration of various immunoglobulin classes of antibody directed against specific cow's milk proteins has confirmed the allergenic potential of cow's milk. The presence of precipitating antibodies to cow's milk antigens in the sera of infants have been well documented (Barrett et al, 1979). Specific IgE antibodies to various cow's milk proteins have been similarly described in children with a positive clinical history of cow's milk allergy (Björkstén et al, 1983).

Growing interest has been generated, also, by the suggestion that antibodies of the IgG class may play a role in human allergy. In this respect, Bruynzeel and Berrens (1979) demonstrated both specific IgE and IgG_4 antibodies in the serum of patients with specific allergies. Björkstén et al (1983) substantiated the development of specific IgG_4 antibodies to cow's milk in children with cow's milk allergy. They found, however, that these antibodies did not seem to play a role in the allergic response of these children.

While many of the immunological mechanisms associated with the cow's milk antibody response are still not clearly understood, the above evidence, and the evidence as reviewed by Bahna and Heiner (1980) show unequivocally that both IgE and IgG antibody class production may be stimulated by specific cow's milk antigens.

4.2 THE MODULATING ROLE OF MATERNAL IMMUNITY ON THE INFANT

In 1979, Jarrett and Hall produced experimental evidence that pointed to the existence of antigen specific IgE suppressive factors that were transferred in the milk of previously sensitised lactating rats. In 1983, they provided experimental evidence in rats that maternal IgG antibodies had a suppressive effect on the IgE antibody responsiveness of their offspring.

Maternal immunity was therefore seen, in animal experimental models, as playing an important role in setting the pattern for the development of future allergic responses in their offspring. This subject has been previously discussed in Section II, Ch. 2.2.

These experimental concepts were applied to the study of human mothers and newborns by Dannaeus et al (1978). They showed that the concentration of IgG antibodies to the beta lactoglobulin protein fraction of bovine milk present in the cord blood of newborns played a modulating role in the development of atopic symptomatology during the first 24 months of their lives. Relatively high titres of anti-betalactoglobulin IgG were found in the cord blood of those newborns who remained symptom free during the follow-up period if compared with the cord blood titres of those newborns who developed atopic symptoms during the same period. They suggested that high titres of specific anti-cow's milk IgG antibody in cord blood may confer a protective benefit on the newborns insofar as the development of atopic symptomatology is concerned.

This proposal supports the generally accepted role of passively transferred maternal IgG in the newborn and young infant as a protective immunoglobulin against bacterial toxins and micro-organisms (Roitt, 1984). Confirmation that the concentration of cord blood specific anti-cow's milk IgG was a result of a passive transplacental transfer of a similar maternal titre was also supplied by Dannaeus and his co-workers (1978). They found that the concentrations of the specific anti-cow's milk IgG in the cord blood sera were equivalent to the concentrations of the same antibodies in the relevant paired maternal sera. Furthermore, they observed that, as is the case for IgG in general, the titres of these specific antibodies decreased during the first few months of life, before rising again.

TABLE III.4

CHARACTERISTICS OF SPECIFIC BOVINE MILK PROTEINS

(From Bahna and Heiner, 1980)

PROTEIN FRACTION	PERCENTAGE DISTRIBUTION	MOLECULAR WEIGHT (Daltons)	ALLERGENICITY
CASIENS			
Alpha, Beta, Gamma, Kappa	82%	18,000 - 24,000	++
WHEY PROTEINS			
Lactalbumins	16%		
Beta-lactoglobulin	(10%)	18,000	+++
Alpha-lactalbumin	(5%)	15,000	++
Serum Albumin	(1%)	68,000	+
IMMUNOGLOBULINS	2%		
IgG	(1,8%)	150,000 - 170,000	+
IgM	(0,15%)	900,000 - 1,000,000	+
IgA	(0,05%)	300,000 - 500,000	+

4.3 CORD BLOOD ANTI-COW'S MILK IMMUNOGLOBULIN G CONCENTRATIONS AS A PREDICTIVE ATOPIC MARKER

It follows from the above considerations that low cord blood or maternal concentrations of anti-cow's milk IgG antibody may possibly constitute a predictive marker for the identification of the "high-allergic-risk" newborn. Bousquet and Michel in 1984 and Kjellman in 1985 have similarly endorsed this concept, particularly if low concentrations of maternal anti-betalactoglobulin IgG co-incided with elevated cord blood serum IgE concentrations.

SECTION IV

STUDY DESIGN AND RESEARCH METHODOLOGY

CHAPTER 1 GEOGRAPHIC LOCATION AND POPULATION DESCRIPTION

1.1 GEORGAPHIC LOCATION OF STUDY

The study was executed in the Cape Peninsula of South Africa, an area situated at the South-Western tip of the African continent (Fig IV 1.)

Most of this area is constituted by the greater metropolitan area of the city of Cape Town and its environs. Cape Town has a latitude of 33 55' South and a longitude of 18^{o} 28' East.

Topographically, the peninsula consists of a central mountainous ridge and a flat plain area, the Cape Flats, on its eastern border. This flat plateau lies about 100 feet above sea level. The peninsula is surrounded by two oceans. The relatively cold Atlantic ocean flanks the North-Western shore of the peninsula, while the warmer Indian Ocean flanks the Eastern shore.

The region enjoys a Mediterranean climate, with a winter rainfall and hot, dry summers. The prevailing winds are predominantly South-Easterly in the Summer, and North-Westerly in the Winter. The North West wind often precedes rainfall.

The population is cosmopolitan and multi-ethnic. Commerce, agriculture, industry and tourism are the most important sources of their income.

1.2 POPULATION DESCRIPTION AND DOMICILIARY DISTRIBUTION

The 3 ethnic groups compared in the study were the Black, White and Mixed groups. The domiciliary and residential distribution of these groups in the Cape Peninsula is depicted in Fig. IV. 2.

1.2.1 The Black ethnic group

The Black group was drawn predominantly from the Xhosa nation. The

FIGURE IV. I.

GEOGRAPHIC LOCATION OF STUDY

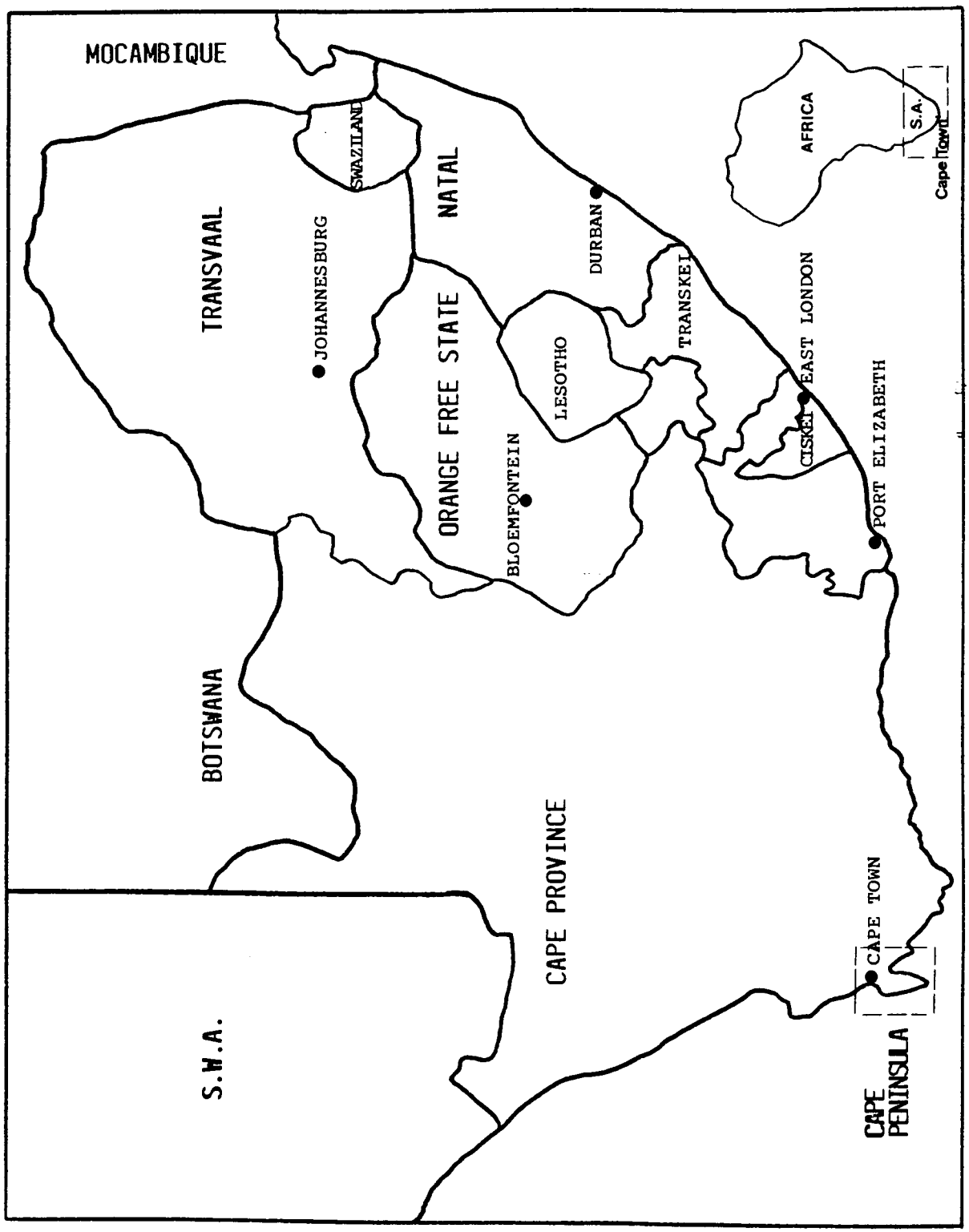
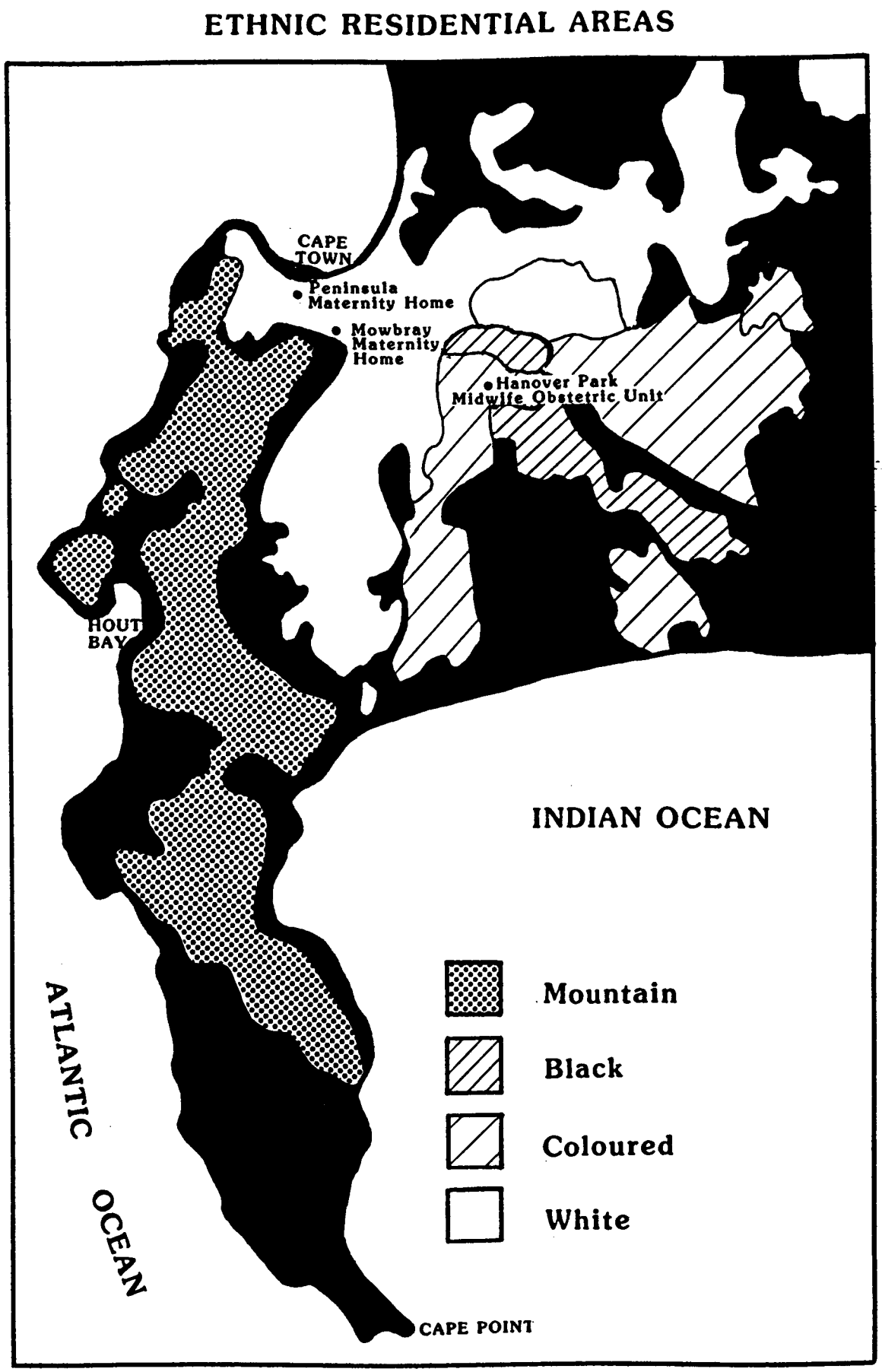


FIGURE IV. 2.


ETHNIC RESIDENTIAL AREAS
CAPE TOWN
Peninsula Maternity Home
Mowbray Maternity Home
Hanover Park Midwife Obstetric Unit
HOUT BAY
INDIAN OCEAN
ATLANTIC OCEAN
CAPE POINT
Mountain
Black
Coloured
White

Xhosa nation is one of the 9 Nguni ethnic tribes, each of which has its own individual socio-cultural, historical, political and liguistic characteristics, (van der Spuy, 1974). Historically, their domicile was in the Eastern Cape homeland states of Transkei and Ciskei. The patients in this study were, however, part of the migrant population to the urban environment of the Western Cape. This tendency to urbanisation has become increasingly prevalent in the last two generations (van Niekerk, 1979). The duration of the mothers' domicile in the Cape Peninsula ranged from 1 year to 37 years in our study group. Some mothers were born in the Cape Peninsula while others migrated there at some later stage of their lives. Rural patients who had come to the Western Cape only temporarily for their confinements were excluded from the study.

The Blacks had the lowest socio-economic standards of the 3 ethnic groups in this study. Most of the families lived in crudely devised squatter accommodation on the Cape Flats. Even though these Blacks are rapidly becoming urbanised, strong tribal customs and folk-lore beliefs are still being exercised in terms of their life style, dietary habits during gestation and infant feeding methods.

1.2.2 The White ethnic group

The White group was drawn predominantly from the stable White established community in the Cape Peninsula. This population is not a homogeneous group, but is divisible into Afrikaans-speaking, English-speaking and a third, miscellaneous group. Botha and Pritchard (1972), in estimating blood group gene frequencies in the White and Coloured (Mixed) populations of the Western Cape, gave an indication of the differing genetic constitutions of these ethnic groups. The Whites were shown to be essentially of Western European stock, but small proportions of Asiatic, Bushman and nomadic Hottentot blood group genes were demonstrated in that study.

Their socio-cultural, lifestyle and dietary practices, while having achieved indigenous characteristics over the 300 years since they first arrived at the Cape of Good Hope in 1652, nevertheless follow a First-World, Western pattern. They enjoy, on the average, a middle-class standard of living, and are domiciled throughout the study area in houses which they usually own themselves.

1.2.3 The Mixed ethnic group

The Mixed group was drawn from the stable, established, so-called Cape-Coloured community. This group has become indigenous to the Western Cape. Originally, they had their origins as a result of intermarriage and temporary relationships between the surplus males of the early Western European Colonists and the indigenous, non-European population of the Cape which included the Hottentot, the Bushmen and South-East Asian stock. Botha and Pritchard (1972) confirmed this historical data by finding the blood group gene frequencies of the Cape Coloured ethnic group being representative of 34% Western European, 36% indigenous Southern African and 30% Asian origin.

The lifestyle of the Cape Coloured, while tending to the Western pattern, has developed a strong ethnic characteristic. They are socio-culturally, economically, linguistically and politically more closely aligned to the local White ethnic group than the Black ethnic group. While the current average standard of living is somewhat lower than that enjoyed by the Whites, socio-political, educational and infra-structural changes are rapidly closing this gap. Most of the study group live in structurally sound rented or subsidized housing on the Cape Flats.

CHAPTER 2 STUDY DESIGN AND EXECUTION

2.1 INTRODUCTION

This study has been executed in two phases in order to satisfy the "Aims of the Study" as specified in Section I. Ch. 3.2.

(i) PHASE I. A cross-sectional study, designed to investigate and compare the specified cord blood markers in each of the three ethnic groups (Section VII. Ch. 1.1).

(ii) PHASE II. A longitudinal, 1 year follow-up investigation of the newborns studied in Phase I, to establish the development of atopic disease in these infants, and to relate any such occurrence back to the values of the cord blood markers at birth. This phase was primarily intended to ascertain the predictive relevance of the cord blood markers for the development of subsequent atopic disease in each ethnic group (Section VIII, Ch. 1.1).

2.2 PHASE I - CORD BLOOD CROSS-SECTIONAL STUDY

2.2.1 Time of execution

This phase of the study was executed in the South African autumn months of March, April and May, 1985. The newborn-maternal pairs which complied to the selection criteria (Section IV, Ch. 2.2.3) were obtained at the listed maternity homes between 9 a.m. and 3 p.m. on consecutive week-days, Mondays to Fridays.

2.2.2 Hospitals of execution

The newborn-maternal pairs on this study were all seen and investigated in maternity homes and a midwife obstetric unit (M.O.U.) which belonged to the University of Cape Town's Peninsula Maternity Hospitals group.

The following institutions were utilized (see Fig. IV. 2 for geographic location).

(i) Peninsula Maternity Hospital. This hospital admitted Black and Mixed mothers for confinement.

(ii) Mowbray Maternity Home. This hospital admitted White and Mixed mothers for confinement.

(iii) Hanover Park M.O.U. This unit admitted Black and Mixed mothers for confinement.

2.2.3 Selection criteria

The newborn-maternal pairs on this study were obtained from random deliveries in the maternity homes provided that the following criteria were met:

(i) Neonatal selection criteria.

(a) A birthweight equal or greater than 2,500g.

(b) A gestational age of 38 weeks or more. This criterion was satisfied if all of the following indicators of gestational age were in agreement.

- A clinical estimation of the gestational age during labour, using the history of the last menstrual period, estimating the expected date of delivery and palpating the mother's abdomen bimanually.
- Ultrasound evidence of gestational age by means of biparietal diameter estimation of the foetus in the first or second trimester of pregnancy.
- A modified Dubowitz score of the newborn (Dubowitz et al, 1970) which was equal to or above, 35 points (a maturity rating of 38 weeks or more - Fig. IV. 3).

FIGURE IV. 3.

MODIFIED DUBOWITZ SCORING SYSTEM FOR NEWBORN GESTATIONAL AGE SCORING

Name : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

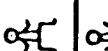
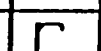
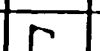
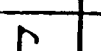
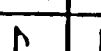
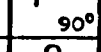
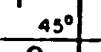
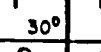
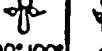
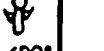
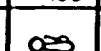
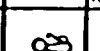
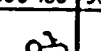
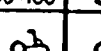
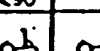
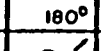
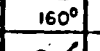
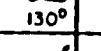
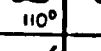
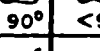
Hospital : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Folder No : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Date of birth : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Age at scoring : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Neuromuscular Maturity

	0	1	2	3	4	5
Posture						
Square Window (wrist)	 90°	 60°	 45°	 30°	 0°	
Arm Recoil	 180°		 100°-180°	 90°-100°	 <90°	
Popliteal Angle	 180°	 160°	 130°	 110°	 90°	<90°
Scarf Sign						
Heel to Ear						

Physical Maturity

Skin	gelatinous red, trans-parent	smooth pink, vis-ible veins	superficial peeling, &/or rash few veins	cracking pale area rare veins	parchment deep cracking no vessels	leathery cracked wrinkled
Lanugo	none	abundant	thinning	bald areas	mostly bald	
Plantar Creases	no crease	faint red marks	anterior transverse crease only	creases ant 2/3	creases cover entire sole	
Breast	barely percept.	flat areola no bud	stippled areola 1-2mm bud	raised areola 3-4mm bud	full areola 5-10mm bud	
Ear	pinna flat, stays folded	sl. curved pinna; soft & slow recoil	well-curv pinna; soft but ready recoil	formed & firm & instant recoil	thick cartilage ear stiff	
Genitals ♂	scrotum empty no rugae		testes descend-ing, few rugae	testes down good rugae	testes pendulous deep rugae	
Genitals ♀	prominent clitoris & labia minora		majora & minora equally prominent	majora large minora small	clitoris & minora completely covered	

MATURITY RATING

Score	Weeks
8	27,2
10	28,0
12	28,8
13	29,2
15	30,0
17	30,8
18	31,2
20	32,0
22	32,8
23	33,2
25	34,0
27	34,8
28	35,2
30	36,0
32	36,8
33	37,2
35	38,0
37	38,8
38	39,2
40	40,0
42	40,8
43	41,2
45	42,0

ASSESSMENT

Weeks

Scoring system for simplified clinical assessment of maturation in newborn infants

(ii) Maternal selection criteria

(a) No progesterone, intravenous insulin, oral epinephrine or propranolol was to have been administered to the mother during pregnancy or labour.

(b) The mothers were to have been resident in Cape Town for at least one year before the delivery of their baby.

(c) Formal maternal consent to participate in the study was mandatory (Section IV, Ch. 2.2.4, Appendix I).

2.2.4. Logistics

The research team consisted of the author, (M.H.) together with a paramedical assistant with a nursing and research background, (Mrs D M Phillips). They established themselves in offices within the relevant hospitals and the midwife obstetric unit (M.O.U.) designated in Section IV, Ch. 2.2.2., between the hours of 09h00 and 15h00. These offices were in close proximity to the labour wards.

On arrival at the hospitals or the M.O.U., the research team visited the labour wards to identify those mothers in labour who would potentially satisfy the selection criteria. These mothers were approached and asked whether they would consent to participation in the study should the babies, likewise, satisfy the selection criteria. If they answered in the affirmative, the consent form was signed (Appendix I) and they were admitted to the study. Arrangements were made with the sister-in-charge of the labour ward to call the team when the identified mothers entered the third stage of labour.

On delivery of the baby, cord blood was obtained, as described in Section IV, Ch. 2.2.5. The baby was then weighed by the labour ward staff using a Salter Scale and checked by the author. The newborn's length was similarly estimated. A modified Dubowitz scoring system (Dubowitz et al, 1970) was used to estimate the gestational age of these newborns (Fig. IV. 3.), and the decision was then made as to whether or not the newborn satisfied the inclusion criteria for the study.

Maternal blood was taken after the delivery of the placenta (Section IV, Ch. 2.2.6). The mother was interviewed in depth during the first 24 hours of the puerperium (Section IV, Ch. 2.2.8). A questionnaire was completed for each maternal-neonatal pair (Section IV, Ch 2.2.9) with the appropriate details of the history and examination of the mother and the newborn entered onto this document. The patients were motivated to return to the Red Cross War Memorial Children's Hospital for their first follow-up visit approximately 3 months later. An appointment card with the date of the follow-up visit was handed to each mother. This card contained an area on which either the mother, the baby's regular doctor or the clinic sister would note down any ailments, symptoms or illnesses the baby may have developed during this time, together with the diagnosis and any treatment which was subsequently prescribed (Section IV, Ch. 2.3.4).

2.2.5 Cord blood venesection - Method

After delivery of the newborn, the umbilical cord was clamped in the standard way and severed. Care was taken to ensure that the cord clamp most proximal to the placenta was applied in such a way as to leave at least 30cm of cord protruding from the vulva.

A large bore needle (no. 12 or 14) was connected to a 20ml disposable plastic syringe. Smaller needles tended to cause haemolysis and increased the time needed for venesection. A 5cm length of cord was swabbed with a dry absorbent swab, which removed any excess maternal blood from the area to be punctured. The umbilical vein was pierced and approximately 15ml of blood was aspirated before the delivery of the placenta.

During Caesarean Section, this procedure was similarly executed directly before the manual removal of the placenta through the uterine incision.

2.2.6 Maternal venesection - Method

Maternal venous blood was obtained from the cubital vein during the immediate post-partum phase of labour, after the delivery of the placenta. If Caesarean Section was required to effect delivery of

the foetus, the maternal sample was taken just prior to the induction of anaesthesia.

Approximately 10ml of blood were aspirated into a 20ml disposable plastic syringe using a standard bore venesection needle (no. 6 or 8).

2.2.7 Processing and storage of the haematological and serum samples

After aspiration, both the cord blood and the maternal blood were immediately transferred from the individual syringes to the following test-tubes in the following quantities:

a) 2ml of each specimen into separate EDTA-primed test-tubes. These were stored in a fridge at $4^{o}C$ for up to 18 hours. These specimens were sent to the Haematology Laboratory at the Red Cross War Memorial Children's Hospital each day, for the estimation of the cord blood and maternal platelet counts.

b) 2ml of each specimen into a further two EDTA - primed test-tubes. These were similarly stored in a fridge at $4^{o}C$ for up to 18 hours. These specimens were delivered to the Allergy Laboratory at the Red Cross War Memorial Children's Hospital each day, for the estimation of the cord blood and maternal total eosinophil counts.

c) The remainder of the blood samples (approximately 10ml of the cord sample and 6ml of the maternal sample) were each transferred to a plain glass test tube, and allowed to clot for 30 minutes. These specimens were then centrifuged at 2,500 revolutions per minute for 10 minutes to separate the serum from the plasma and cellular blood elements. The serum was then separated from the plasma using disposable plastic laboratory pipettes. Each serum sample was transferred into a separate plain plastic test tube and stored at - $20^{o}C$ for up to 3 months until assay.

Once all the required patients had been admitted to Phase I of the study, this batch of serum was transferred to the laboratory of the Department of Clinical Science and Immunology, Medical School, University of Cape Town, for the immunological assays.

2.2.8 Clinical history and examination of the mother and newborn

Within 24 hours of the delivery of the newborn, the mother was interviewed in detail by Mrs Phillips. This interview was supervised by the investigator (M.H.), and appropriate translators were used on the occasions where a communication problem presented itself. This problem was occasionally encountered during the questioning of the Black ethnic group, and every effort was made to ensure that the questions and answers were understood by both the mothers and the interrogators.

The criteria used to define a positive atopic family history for the newborns are considered in Section VII, Ch. 1.3.

2.2.9 Phase I Questionnaire

The information obtained from the mother, together with the relevant details accruing from both the examination of the newborn and the special haematological and immunological laboratory investigations, were entered onto the Phase I Questionnaire in a format which facilitated the transfer of this data onto computer punch cards. This Questionnaire, together with details of the information gathered, is represented in Appendix II.

2.3 PHASE II - LONGITUDINAL FOLLOW-UP STUDY

2.3.1 Duration of study

The Phase II longitudinal follow-up study was designed to run for the first 12 months (1 year) of the newborn's life (ie. during infancy). The motivation and argument for the decision on this time-frame is presented in Section VIII, Ch. 1.2.

2.3.2 Follow-up protocol

The infants were seen and examined at 3, 7, and 12 months of age (+/- 2 weeks). The 3 month visit was planned so that it occured before the infant received the first 3-in-1 Diptheria, Pertussis and Tetanus (DPT) vaccination, usually scheduled for 3 months of age.

2.3.3 Follow-up venue

The mothers and infants were interviewed and examined at the Red Cross War Memorial Children's Hospital in Rondebosch, Cape Town. Three rooms were available to the author and his team in the nurses home of the above-mentioned hospital. One served as a waiting room, the other as the examination room and the third as the procedure and venesection room.

Usually the mothers and their infants made their own way to the hospital. They received a standard travel allowance of R2,00 per visit towards their commuter costs from our research fund. On the occasions when transport was a problem, the research assistant, Mrs Phillips, would either fetch the patients in her car or arrange for a Taxi to facilitate their transport.

2.3.4 Motivation and contact strategy

The success of Phase II depended on the quality of the motivation of the mothers to bring their infants back at the specified intervals for re-examination and assessment. Of note was the fact that the

majority of the babies were healthy. It was, therefore, understandable that some mothers were initially disinclined to return to the hospital for the evaluation of their infant, particularly since the infants venesection procedure usually reduced the infant to vociferate tears. Furthermore, the sight of a needle being inserted into the neck of their baby was an ordeal for those mothers who opted to stay with their infants during this procedure.

Intensive motivation and the establishment of a meaningful, trusting and intimate relationship between the author and the mothers was mandatory.

Before the mothers left the maternity hospitals after their confinement, they were handed a card with the date of their 3 month appointment noted on it. This card also served as a record card for the infant's doctor, paediatrician or the clinic primary care nursing sister. They were encouraged to enter the diagnosis and treatment of any condition or illness which the infant may have developed between the follow-up visits onto this card. This information was used at each follow-up visit to determine the clinical atopic classification for each infant (Section VII, Ch. 1). The mothers were also asked to ensure that the first DPT immunisation was not administered before the first visit, but to ensure that all three immunisations were completed in the 4 months time-period between the 3 and the 7 month follow-up visits.

Full details of the study, with the objectives and the benefits to be gained by their infant from their participation in the study, were carefully explained to them. The early detection of any physical abnormality, as well as the early detection of any possible allergic symptomatology was an obvious benefit to them. The results of the cord blood and subsequent IgE concentration assays, together with the other haematological parameters measured at birth and at each follow-up visit, would be made available to them and their private doctors after each successive visit. They were given our telephone number at the hospital, and were encouraged to call us on any matter which was causing them concern. Finally, the research assistant (Mrs Phillips), promised to telephone them during the 2nd month after the newborns birth, in order to make an appointment to visit their respective homes on a courtesy visit.

Each mother was visited by Mrs Phillips approximately 6 weeks after the birth of the baby. In most cases where the families had telephones, these visits were by appointment. In those cases where no telephone existed (especially relevant in the Black squatter camp population in the Cross Roads area of the Cape Flats), these visits were unannounced. If the mothers were not at home, a message was left and the visit repeated soon afterwards. During these visits, the mothers were again encouraged to return to the hospital for the first 3 month follow-up visit. It was also made clear to the families that treatment for any illness or medical condition the child may develop would be arranged for them, free of charge, should they not have ready access to medical assistance. This was especially relevant for the Black mothers and infants whom did not have access to private medical care. The Red Cross War Memorial Children's Hospital has an enviable reputation among the public as a hospital of excellence, and this facilitated the compliance of the mothers to a large degree.

During each follow-up visit, Polaroid photographs were taken of the babies and given to the mothers. Any co-incidental social problems were attended to, free of charge, and referral to other relevant departments or doctors were facilitated by us if this was necessary. Formal contact reports were sent to the infant's private family physicians where applicable, as a matter of courtesy. This served to enlist the support of those doctors, who then encouraged their patients to continue with the study.

After each visit, the mothers were given a new appointment for the following visit. It was noticed that the opportunity for the mothers to meet each other again after the confinement and to renew the friendship which they had established during their confinement was a great incentive for them to return for each subsequent visit. Great care was subsequently taken to arrange the follow-up visits so that the various groups of mothers who were acquainted could return on the same days.

Each mother was also circulated with a motivational letter about a month before the following appointment, both to act as a reminder to

attend, and also to underpin the personal interest which the investigator (M.H.) had in the welfare of the infants concerned. Appendix III is an example of the letter sent out before the 7 month follow-up visit, and Appendix IV an example of the final letter of thanks sent out after the completion of the study.

Of great concern during the follow-up period was the fact that South Africa was experiencing a time of escalating political, social and economic upheaval. Urban and township violence, intimidation, school and hospital boycotts, security force intervention in the Black townships and confrontation within and across racial barriers became the order of the day during 1985 and 1986. The result of this unstable political climate was the imposition of 2 States of Emergency in the Cape Peninsula during this period. Since much of the unrest was concentrated in the Black townships, many of our Black mothers found it impossible to attend for follow-up in spite of the intensive efforts on our part to locate them. Many returned back to their original homes in Transkei and Ciskei (Fig IV. 1), while others were forced to flee the faction fighting occuring in the squatter areas of Cross Roads and to take refuge in the sprawling relief area of Kyelitsha, 10km east of Cross Roads. This area had no roads or addresses at that time, and was a disaster zone for all intents and purposes.

The drop-out rate for our White and Mixed patients was extremely low, but the events described above lead to a substantial loss of patients belonging to the Black ethnic groups during the 1 year follow-up period.

2.3.5 Logistics of the follow-up visits

Approximately 5 mothers, together with their infants, were booked for each morning during the follow-up periods. Their appointment times were staggered so as to facilitate their processing and to give each mother and infant personalized attention and care. The research assistant (D.P.) welcomed the mothers, gave them a cup of tea, and weighed the infants on a Salter scale. The Polaroid photographs of the infants were taken and distributed amongst the mothers.

The clinical examination and history-taking followed, after which the infants proceeded to the procedure room for venesection. Thereafter, the mothers were given the next appointment and were again motivated to continue with the study.

2.3.6 Clinical history and examination of the infant

Because it was necessary for the author personally to spend some time making social contact with the mothers, and also to do the venesection himself, it was necessary to enlist the help of two Paediatric Medical Officers to elicit the detailed history and to examine the infant. Any undue delay irritated some of the mothers, and jeopardized their further compliance in the study. The Medical Officers were Dr Sehaam Abrahams and Dr Ruth Barruch.

After each examination, the author consulted with these medical officers, and any positive features, either on the history or on the examination, were personally assessed and confirmed by the author.

2.3.7 Infant venesection - external jugular vein puncture

The infants were wrapped in a restraining linen sheet with their head and neck accessible for venesection. The research assistant placed the infant on its side on a padded table top, with the head laterally extended over the edge to expose the external jugular vein. The skin over the vein was cleaned with a disposable PREPTIC swab, and the vein punctured with a scalp vein set needle. Ten ml of blood was aspirated from this vein. If the venesection failed on the one side, for technical reasons, the contralateral external jugular vein was visualized and punctured.

On removal of the needle, a disposable gauze swab was applied to the puncture wound and pressure was exerted on this site by the author's thumb for 2 minutes in order to prevent the formation of a haematoma.

2.3.8 Processing and storage of the haematological and serum samples

The blood specimens obtained from the infants at each follow-up visit were processed and stored in the same manner as was described for the

processing and storage of the cord blood and maternal haematological samples (Section IV, Ch. 2.2.7).

The same haematological and immunologic parameters were measured on these infant specimens as were measured in Phase I of the study on the cord blood with the following exceptions:

(i) Serum IgA concentrations were not measured.

(ii) RAST for Dermatophygoides pteronnysinus was measured, in addition to the RAST for egg white and cows milk.

(iii) RAST for Ascaris lumbricoides was not measured.

2.3.9 Phase II Questionnaire

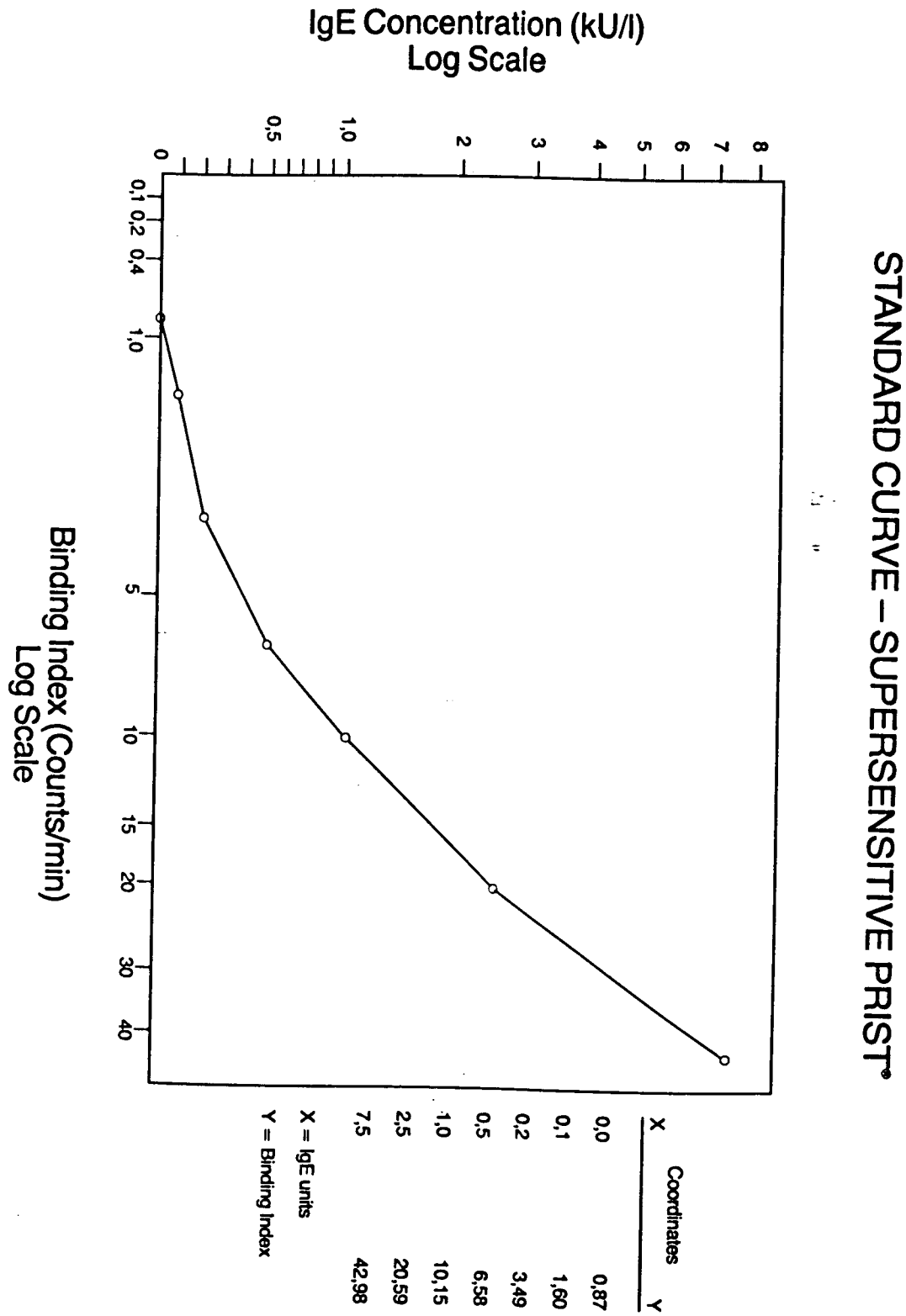
The information obtained from the history and examination of the infants, together with the results of the haematology and serological examinations were entered onto the Phase II Questionnaire (Appendix V).

TABLE IV.I.

SUPERSENSITIVE PRIST : RADIOACTIVITY BINDING INDEX
COUNTS (%) AGAINST STANDARD IgE TEST KIT UNITS.

STANDARD PRIST KIT CONCENTRATIONS IgE (kU/l.)	TIME	COUNTS PER MINUTE (C.P.M.)	BINDING INDEX (%) (REFERENCE)
0.01	1.0	355.0	0.76%
0.01	1.0	404.0	0.99%
			AVERAGE = 0.87%
0.05	1.0	545.0	1.64%
0.05	1.0	530.0	1.57%
			AVERAGE = 1.60%
0.25	1.0	939.0	3.45%
0.25	1.0	955.0	3.53%
			AVERAGE = 3.49%
0.5	1.0	1533.0	6.19%
0.5	1.0	1700.0	6.96%
			AVERAGE = 6.58%
1.0	1.0	2282.0	9.65%
1.0	1.0	2500.0	10.65%
			AVERAGE = 10.15%
2.5	1.0	4697.0	20.78%
2.5	1.0	4612.0	20.39%
			AVERAGE = 20.59%
7.5	1.0	9437.0	42.64%
7.5	1.0	9584.0	43.32%
			AVERAGE = 42.98%

FIGURE IV. 7.


STANDARD CURVE – SUPERSENSITIVE PRIST®
IgE Concentration (kU/l)
Log Scale
0 0,1 0,2 0,4 1,0 0,5 1,0 2 3 4 5 6 7 8
Binding Index (Counts/min)
Log Scale
0 5 10 15 20 30 40
X Coordinates Y
0,0 0,87
0,1 1,60
0,2 3,49
0,5 6,58
1,0 10,15
2,5 20,59
7,5 42,98
X = IgE units
Y = Binding Index

CHAPTER 3 LABORATORY TECHNIQUES AND ASSAY METHODS

3.1 CORD BLOOD AND INFANT FOLLOW-UP SERUM IgE CONCENTRATIONS

CBsIgE was measured using a modification (Haus et al, 1988) of the commercially available IgE PRIST (Pharmacia) (Johannson et al, 1976; Kjellman et al, 1976). Two hundred microlitres of standard serum, control serum or patient serum was incubated with IgE paper discs for 24 hours with continued shaking. After washing three times, 100 microlitres of ^{125}I rabbit anti-human IgE tracer were added and incubated with the disc for 24 hours at room temperature using a Braun Shaker. After washing three times, tubes were capped and the radioactivity counted using a Beckman gamma counter. Counts bound for the standards were expressed as a percentage of the total radioactivity added in the tracer and plotted against the IgE concentration on linear log paper (Table IV, I). A standard curve was constructed by diluting the standards of the Pharmacia IgE PRIST kit with IgE-free diluent at the following concentrations: 7.5, 2.5, 1.0, 0.5, 0.25, 0.05, 0.01 kU/l. IgE-free diluent was used as a negative control and consistently gave readings of less than 0.01 kU/ml. This assay gave predictable and reproducible results with dilutions of standard IgE sera. Since the standard curve reading of IgE from 0.01 - 2.5 ku/l range was piecewise linear, (Fig. IV, 7) the assay was regarded as being sensitive to 0.01 kU/l. Reproducibility was further tested using control sera of known IgE concentration, diluted to fall within the range of the standard curve with IgE-free diluent.

If either a cord blood or an infant follow-up serum IgE concentration was measured above the value of 2.5ku/l using this supersensitive technique, the assay was then repeated on the specimen using the standard overnight incubation Phadebas IgE PRIST ^{R} radioimmunassay (Pharmacia Diagnostics), with a standard curve covering a range of 0.5 - 100kU/l.

Using dilution factors, serum IgE concentrations between 0.5 - 800 kU/l may be measured in this manner.

3.2 MATERNAL SERUM IgE CONCENTRATIONS

The maternal serum IgE concentrations were assayed using the standard overnight Phadebas IgE PRIST radioimmunoassay (Pharmacia Diagnostics) as described in Section IV, Ch. 3.1.

3.3 CORD BLOOD AND INFANT FOLLOW-UP SPECIFIC IgE RAST (PHARMACIA) FOR EGG WHITE, COW'S MILK, ASCARIS LUMBRICOIDES AND DERMATOPHYGOIDES PTERONYSSINUS

The cord blood and infant follow-up specific IgE concentrations for egg white, cow's milk, Ascaris lumbricoides and Dermatophygoides pteronyssinus were measured using a modification of the commercially available, standard single overnight incubation Phadebas RAST radioimmunoassay (Pharmacia Diagnostics). This modification increased the sensitivity of the test.

One hundred microlitres of the patient's serum was incubated at room temperature on a shaker, with the relevant commercial RAST allergen disc, for 24 hours. After washing three times to eliminate any non-specific IgE, 50ml of radioactive anti-IgE ^{125} I tracer was added, and the test tubes were again incubated for a second time at room temperature, on a shaker, for 24 hours. After three more washes, the test tubes were capped and the radio-active specific IgE anti - IgE ^{125} I complex measured using a Beckman Gamma Counter.

The patient counts were compared directly with counts of reference sera run in parallel, as well as with known negative and positive sera.

Calculation of Results:

References A, B, C, D and E were assigned to the Phadebas RAST Unit per ml (PRU/ml) values of 17.50, 3.50, 0.07, 0.35 and 0.11. The mean value of duplicate Gamma Counter counts for each reference serum were plotted against the assigned PRU/ml values in a logarithmic diagram. The unknown patient PRU/ml values for the test samples were then read from this reference. PRU/ml values of less than 0.11 represented absent or undetectable levels of allergen - specific IgE antibodies.

The RAST scoring and evaluation of the patients PRU/ml values are summarised in Table IV. 2. The definition for the corresponding RAST classes as reported in this thesis are also included in this Table.

The reference serum volume was 100 microlitres, together with a Birch reference paper disc, while the control serum volume was also 100 microlitres, together with a cat reference paper disc. The positive control serum was calibrated to contain 2 PRU/ml of specific IgE antibody. The accepted range was +/- 0.1 PRU/ml. The negative control serum contained no allergen - specific antibody.

3.4 MATERNAL SPECIFIC SERUM IgE RAST (PHARMACIA) FOR EGG WHITE, COW'S MILK AND ASCARIS LUMBRICOIDES

The maternal specific serum IgE concentrations for egg white, cow's milk and Ascaris lumbricoides helminth were measured using the standard commercially available single overnight incubation Phadebas RAST (Pharmacia Diagnostics) procedure.

3.5 CORD BLOOD AND MATERNAL SERUM IgA CONCENTRATIONS

Both the cord blood and maternal serum IgA concentrations were estimated by nephelometry, using the Beckman Immunochemistry Analyser. The units used to express the results were mg/dl.

3.6 CORD BLOOD, INFANT FOLLOW-UP AND MATERNAL ANTI-COW'S MILK SERUM IgG CONCENTRATIONS

The cord blood and infant follow-up anti-cow's milk serum IgG concentrations were measured using an ELISA technique.

Ninety six - well microtitres plates (Dynatech Labs) were used for the assay. Fifty microlites of a solution of approximately 20 mg/ml of whole cow's milk were added to each well and incubated at $4^{o}C$ overnignt. This mixture was then washed three times with T.S.T. (Tris Saline Tween - 12.1G Tris, 11.7G Sodium Chloride and 1ml Tween 20 made up to 2 litres and a pH of 8.0). Two hundred and fifty microlitres of a 0.5% gelatin solution was added to each well and incubated at room temperature for 1 1/2 hours. This was followed by

TABLE IV. 2.

RAST SCORING AND EVALUATION

Pnadebas RAST Class	COUNTS OF UNKNOWN (PRU/ml)		Level of allergen specific IgE antibody
	Less than counts of	Greater than counts of	
4	-	Reference A 17.50	Very high
3	Reference A 17.50	Reference B 3.500	High
2	Reference B 3.500	Reference C 0.700	Moderate
1	Reference C 0.700	Reference D 0.350	Low
0	Reference E 0.110	-	Absent or undetectable (Negative)

another two washings with T.S.T. Fifty microlitres of a 1/10th dilution of the patient's serum was then added to each well and again incubated for 1 1/2 hours. This mixture was now washed four times with P.B.S. - 9 (Phosphate Buffered Saline - 9, containing 17.4G Sodium Chloride, 3.56G Sodium Hypophosphate and water added to make the solution up to 2 litres). Fifty microlitres of a 1/2000th solution of peroxidase conjugated sheep anti-human IgG (Cappal Labs) was now added, and again incubated for 1 1/2 hours. Another four washes with P.B.S. - 9 were done before the addition of 100 microlitres of ABTS peroxidase substrate (100 microlitres ABTS, 100 microlitres of a 30% hydrogen peroxide solution and 10ml of citric acid buffer).

Spectrophotometric analysis and reporting units

The spectrophotometer was zeroed against blanks (ie, the components of the assay without the patient serum). The results were reported in units which showed the optical density difference at ± 414 nanometres - Δ O.D. (414nm).

3.7 CORD BLOOD, INFANT FOLLOW-UP AND MATERNAL TOTAL EOSINOPHIL COUNTS

The TEC's were performed within 18 hours of venesection. The method used was a modification of the method as described by Dacie and Lewis, 1984. A mixture of 2% aqueous eosin (5ml), acetone (5ml) and distilled water (90 ml) was used as diluting fluid. The unclotted blood specimen was mixed on a rotator for 5 minutes, after which 0,02 ml of the whole blood was similarly mixed with 0,38 ml of diluting fluid on the rotator for 5 minutes. The improved Neubauer counting chamber was filled with this mixture, which was allowed to settle in a moistened petri dish for 5 minutes. The whole area (3x3 = 9 squares) was then counted. The TEC was obtained by multiplying the count by a correction factor, i.e.

$$\frac{10}{9} \times \frac{20}{1} \times \frac{X}{1}$$

$$= \frac{200}{9} \times \frac{X}{1}$$

$$= 22 \times X \text{ cells/mm}^2$$

3.8 CORD BLOOD, INFANT FOLLOW-UP AND MATERNAL PLATELET COUNTS

The platelet counts were performed using a Model S Plus IV Coulter Counter automated blood cell counter (Coulter Electronics).

An Electrical Voltage Impedence automatic cell counter method was used. The blood sample was not subject to gravitational sedimentation nor was it contrifuged to achieve accelerated sedimentation before the sample was added to the Coulter Counter.

The units reported on were multiplied by a factor of 10^9/litre.

CHAPTER 4 STATISTICAL CONCEPTS AND METHODS

The dependent variables considered for analysis in this thesis are IgE, eosinophils, platelets and anti-cows milk IgG.

The serum concentrations of IgE and IgG are measured on what can be considered a continuous scale. The accuracy to which serum IgE is measured is two decimal places, whilst the accuracy to which anti-cows milk IgG is measured, is to one decimal place. The platelets and eosinophils are discrete count values as they are measured as an integer number. For all variables, the number scale starts at zero and increases. These factors, together with the sample size, are taken into consideration in deciding which statistical test to use to test the hypothesis.

4.1 STUDY DESIGN AND SAMPLE SIZE

This is an exploratory study. The study design is to observe a cohort from eacn of the three races under consideration at birth, and to recall each individual at 3, 7 and 12 months for observation. This gives rise to a cross-sectional study between the races at each observation period (where blood parameters and certain immunoglobulins can be compared) and also to a prospective longitudinal study within each race group.

Since no previous study on the 3 race groups observed here exists, there were no prior estimates of the average values or the standard deviation of the blood parameters under investigation. It was therefore impossible to calculate the necessary sample size for each race required to show a statistically significant difference. The decision as to how many people were included in the study was consequently determined by how many babies qualifying under the inclusion criteria were born during the 3 months of the Autumn season, in hospitals where the study was conducted, during the hours stipulated in Section IV. Ch. 2.2.1.

Any future similar study should include sample size calculations as the required estimates of the statistical parameters needed for these calculations are now available. The contentious issue of whether a statistically significant difference is clinically relevant still, however, remains.

Another issue remains. Can the sample selected be considered representative of the population and, by not specifying prior constraints, will the ratio of individuals in the sample presenting with an aHF to those without an aFH be representative of that ratio in the population? Without prior information, this can lead to unequal numbers in the groups compared.

4.2 STATISTICAL CONCEPTS

The following discussion addresses the concepts required to calculate the power of an hypothesis test, and why this calculation could not be done in this study.

4.2.1 Hypothesis Testing

Hypothesis Testing is the process of making a decision about whether or not to accept a certain statement about parameters from a population based on information contained in the sample drawn from the population. If, however, the sample is not representative of the population, either because the sample nas not been randomly drawn from the population or because the sample size is too small, caution must be exercised in generalizing the results.

The null hypothesis under test is the statement about a statistical parameter cnaracterizing the distribution of some variable of interest. The null hypothesis is always tested against an alternative hypothesis, which is a statement that "the null hypothesis not true".

The decision to accept the null hypothesis is not equivalent to the opinion that "the null hypothesis is true" but rather that "the null hypothesis has not been shown to be false", which could be the result of insufficient evidence.

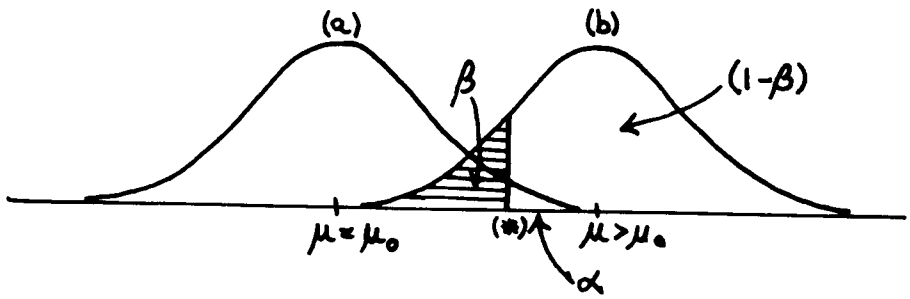
In an exploratory study the alternative hypothesis should always be a directionless statement eg. that the medians of the two groups compared are not equal, giving rise to a two-tailed test.

Testing hypotheses depends upon underlying probability distributional assumptions which will not be considered here.

4.2.2 Components of an Hypothesis Test

Consider that the test statistic (a function of the sample estimate of the population parameter under interest) is given in terms of the population mean, denoted by μ. For simplicity consider a one-tailed test, with null hypothesis $\mu = \mu_0$ and is tested against the simple alternative hypothesis $\mu > \mu_0$.

FIGURE Z



Curve (a) represents the sampling probability distribution of the sample mean, $\overline{X}$, when $\mu = \mu_0$.

Curve (b) represents the sampling probability distribution of the sample mean, $\overline{X}$ when $\mu > \mu_0$.

Here the sample mean, $\bar{X}$, is calculated from the samples drawn and estimates the population mean, μ. The accuracy of this estimate depends upon the "quality" of the data in the selected sample and also upon the sample size.

If the sample parameter differs greatly from the hypothesized value of the population parameter as stated in the null hypothesis, then the null hypothesis would probably not be accepted.

(a) Significance Level

The level of significance, denoted by α, is the maximum probability of not accepting a true null hypothesis. This area is the dark shaded area under curve (a) to the right of (*) in the diagram . In all tests in this study, a 5% significance level, $\alpha = 0.05$, was used.

(b) p-value

The reported p-value is the smallest significance level at which the null hypothesis would not be accepted for the given observation.

This value is compared to the chosen value of the significance level, α. If the p-value is less than 0.05 then, on the information contained in the sample, there is evidence that the null hypothesis cannot be accepted, and hence to accept the alternative hypothesis as being true. This would indicate that the sampling distribution curve (b) in Figure Z would be correct.

The larger the p-value, the stronger the evidence to accept the null hypothesis. When p=1.00, it is accepted that the parameter values being investigated are considered identical.

(c) Errors in Testing Hypotheses

The types of errors which can be made in testing a hypothesis are tabulated below:

		Null Hypothesis	
		Actually true	Actually false
Decision	To accept null hypothesis	Correct	error or type II error
	Not to accept null hypothesis	- error or type I error	Correct

The probability of making a type I error, (not accepting a true null hypothesis) is given by the significance level, denoted by α in Figure Z. The probability of making a type II error, or of accepting the null hypothesis when it is actually false, is denoted by the area β, in Figure Z. This area β, is under cure (b) to the left of (*).

Either error is undesirable and it is desirable to have and small.

The quantities α, β and the sample size are inter-related and generally both α and β can be small only by increasing the sample size. Usually a desirable decrease in the significance level, α, is accompanied by an undesirable increase in β.

(d) The Power of an Hypothesis Test

The power of an hypotnesis test is the ability of an hypothesis test to find a difference (if one exists) in the estimated population parameters, ie. it is the probability, denoted by $1-\beta$, of accepting the alternative hypothesis when it is true.

The power of an hypothesis test depends upon:

- the chosen level of significance α,
- the size of the sample. (small differences will be easier to detect with a large sample, since it will increase the power of the test. Once a statistical difference has been shown, the question then asked is whether it is of practical importance or not).
- the variability of the data. (Generally the greater the variability of the data, the lower the power. The sample size also influences this facet, through the sample variance).
- the distribution of the sample mean, which asymptotically tends to normality ie. as the sample size increases to infinity so the distribution of the sample mean tends to the normal distribution. How quickly this distribution converges to the normal (Gaussian) depends upon the shape of the underlying population distribution from which the sample is drawn. For highly skew distributions it would require large sample sizes, (probably n 100) before the distribution of the sample mean could be considered normal.

Formula for calculating sample size.

Assume that 2 groups are to be compared, and the groups are of equal size, ie. $n = n_1 = n_2$, then:

$$n \geq \frac{(\sigma_1^2 + \sigma_2^2)[z(\frac{\alpha}{2}) + z(\beta)]^2}{(\mu_1 - \mu_2)^2}$$

is the approximate sample size for each group, assuming an underlying normal distribution and that the population variances σ_1^2 and σ_2^2 are known. If they are not known the t-distribution is used and the above formula only provides an approximation, which is good if n_1 and n_2 are large.

If the distribution of the variable under consideration cannot be characterised or normality not assumed, the power of the test cannot be calculated as is the case in this study.

e) Asymptotic Relative Efficiency

For non-parametic tests where the underlying distribution cannot be characterised the Asymptotic Relative Efficiency criterion is used to compare the behaviour of the non-parametic test to its parametic counterpart. More specifically the two-sample parametic test used to detect differences between means is the two sample t-test which assumes that the sample is drawn from a normally distributed population, in addition to other assumptions of the model.

The non-parametic counterpart of the two sample t-test is the Mann-Whitney-U test. The Mann-Whitney-U test is almost as powerful as the t-test if the normality assumptions are true and differs for other distributions. An interesting safety feature of the Mann-Whitney-U test is that the Asymptotic Relative Efficiency is never less than 0.864, which is a valuable property of this non-parametic test.

The calculation of this property depends upon the sample size being very large.

4.3 PROBLEM OF OUTLIER OBSERVATIONS

The arithmetic mean is very sensitive to outlier values in the data set. The standard deviation, being a measure of variation about the mean is similarly sensitive to outlier values in a data set. Neither the median nor its corresponding measure of variation, (the interquartile range) is sensitive to outlier values in a data set, since these statistical parameters depend upon the rank of the observations in a data set. In transforming a data set onto the rank scale information is lost since the distance between the observation values of 0.01 and 0.02 on the untransformed scale is 0.01 and on the rank scale it is 1 unit. Likewise, the distance between 6.77 and 15.00 on the untransformed scale is 8.23 units whilst on the rank scale it is 1 unit since these are the two largest values in the data set.

Using the data set is given in Table IV.3, the transformation to ranks is illustrated in Figure IV.8. The sensitivity of the geometric mean (using the logarithmic transformation of the data), the arithmetic mean, its standard deviation, the median and the inter-quartile range, using the data given in Table IV. 3, is illustrated in Table IV.4.

4.4 DESCRIPTIVE SUMMARY STATISTICS FOR EACH VARIABLE

IgE

The frequency distribution of the values of this variable is highly positively skewed i.e. the majority of the values are found in the lower region of the range with a few very high values which influences the average value or measure of central tendency of the data set. One way to circumvent this problem is to transform the values onto the logarithmic scale to try to create a more symmetric frequency distribution of the data. (This attribute is sought in data sets where parametric tests e.g. the t-test, are used for the statistical analysis).

The arithmetic mean of the untransformed values of IgE is greatly influenced by the high outlying values. In transforming the individual values onto the logarithmic scale, the geometric mean (arithmetic mean of the logarithmic values) is still influenced, though to a lesser extent, by these influential outlying values.

The median, however, remains relatively stable to fluctuations in the outlying values and was thus the preferred measure of the average value of the serum IgE concentration value. The median is that observation such that half the observations exceed it and half fall below it i.e. it is that point of the frequency distribution which divides it into two equal parts. Thus the median is the middle of a frequency distribution but only in the sense of the rank order of the magnitude of the observations. When using the median to describe the average value or the measure of central tendency of a frequency distribution, the magnitude of the distance between observations (i.e. the actual values) is not used but only the ranked value of the observation. Hence information about the individual values in a data set is lost.

TABLE IV 3.

CORD BLOOD SERUM IgE CONCENTRATION LEVELS

VALUE	COUNT	VALUE	COUNT	VALUE	COUNT	VALUE	COUNT
0.01	2	0.12	3	0.33	1	0.96	1
0.02	2	0.13	2	0.37	1	1.02	1
0.03	3	0.14	2	0.38	1	1.54	1
0.04	1	0.16	2	0.44	1	1.92	1
0.05	1	0.17	1	0.46	1	2.03	1
0.06	1	0.18	1	0.77	1	3.18	1
0.07	1	0.19	2	0.79	1	3.38	1
0.08	2	0.23	1	0.84	1	6.77	1
0.09	2	0.24	1	0.86	1	15.00	1
0.10	3	0.28	1	0.90	1		

FIGURE IV 8

TRANSORMATION OF DATA TO NULLIFY INFLUENCE OF OUTLIERS

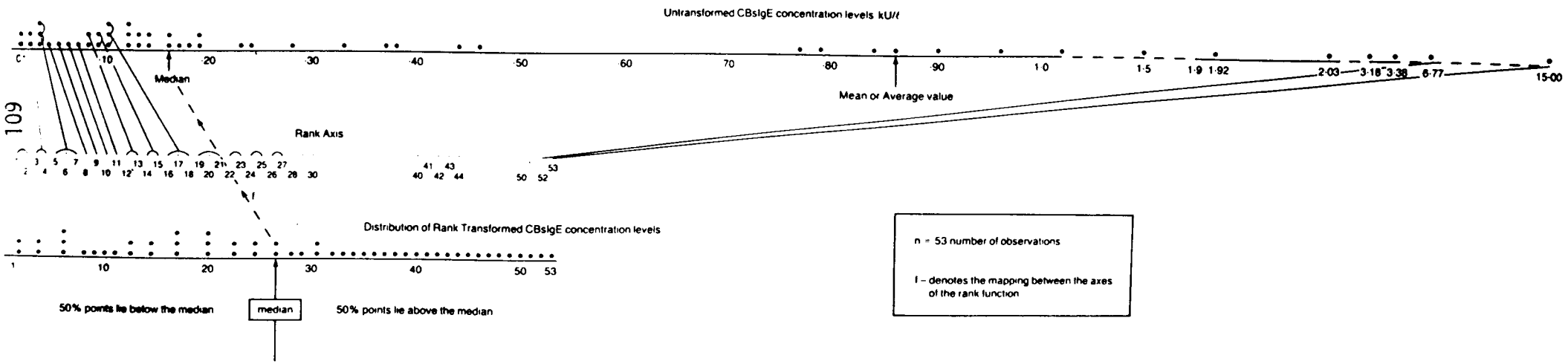


TABLE IV.4.

THE INFLUENCE OF OUTLIERS ON THE DESCRIPTIVE STATISTICS

DATA SET USED	TOTAL NO. OF OBS IN DATA SET	NO. OF OBS EXCLUDED FROM DATA SET	GEOMETRIC MEAN	ARITHMETIC MEAN	MEDIAN	STANDARD DEVIATION	INTERQUARTILE RANGE
Complete	53	0	0.21	0.86	0.16	2.28	0.70
Obs >14.99 excl.	52	1	0.20	0.59	0.16	1.14	0.61
Obs > 6.00 excl.	51	2	0.18	0.47	0.16	0.73	0.38
Obs > 3.30 excl.	50	3	0.17	0.41	0.15	0.62	0.37
Obs > 2.99 excl.	49	4	0.16	0.35	0.14	0.48	0.33

Eosinophils, platlets and anti-cows milk IgG

In order to maintain uniformity, the median was used to describe the average value of the above variables as well.

General comments

The descriptive measure of dispersion if the median is used is the interquartile range or the distance between the 25th (Q1) and the 75th (Q3) percentile. The standard deviation gives a measure of the spread of the observations around the arithmetic mean. The relative position of the mean and the median together with the associated measures of dispersion give an indication of the skewness of the frequency distribution of the variable.

4.5 ANALYTICAL METHODS

4.5.1 The Kruskal-Wallis Test

The Kruskal-Wallis Test was used to test the null hypothesis that there is no difference in the median value of three or more groups against the alternative hypothesis that there is a difference. This test was used when comparing blood parameters for all 3 race groups.

4.5.2 The Mann-Whitney-U Test

The Mann-Whitney-U Test was used to test the hypothesis that there is no difference in the medians (average value) of the two groups compared. The use of a statistical test like the Mann-Whitney-U requires a transformation of the data by ranking. This transformers the values to those with a uniform distribution with not outliers. This test was preferred to the t-test since the number of observations in the groups compared were generally not equal and the underlying distributional assumptions of the t-test were not met (Table IV.5).

TABLE IV 5

COMPARISON OF RESULTS BETWEEN MANN-WHITNEY-U
AND THE t-TEST TO SHOW THE INFLUENCE OF OUTLIERS ON THE p-VALUE

OBSERVATIONS DELETED	NUMBER OF OBSERVATIONS IN DATA SET		MANN-WHITNEY -U TEST. p-VALUE	t-TEST (log transformed) p-VALUE	t-TEST (untransformed) p-VALUE	COMMENTS
	(a)	(b*)				
none	52	53	0.0720	0.0747	0.1821	
Obs >14.99 excl.	52	52	0.0978	0.1182	0.3677	
Obs > 6.00 excl.	52	51	0.1315	0.1752	0.7060	
Obs > 3.30 excl.	51	50	0.1203	0.1417	0.3255	Data set (a): 1 obs. deleted. See note 5
Obs > 2.99 excl.	51	49	0.1612	0.2025	0.5832	

Notes:

1. *: Univariate Descriptive Statistics for Data set (b) given in Table IV.4.
2. The numbers in each Data Set compared are relatively large.
3. The frequency distribution of each Data Set is skewed to the right as can be seen from results tabulated in Table IV. 4.
4. The assumption under question is the distribution of data and its effect on the p-value from Mann-Whitney-U and t-Test, using lo transformed values and untransformed values. It is easy to see how much less sensitive the Mann-Whitney-U test is to the change i the frequency distributions of two Data Sets, (as observations are deleted) than the t-Test using the log transformation or th untransformed values.
5. A marked shift was seen in the middle 50% of the observations in each data set resulting in this sudden decrease in the p-value.

4.5.3 The Fisher's Exact Probability Test

The Fisher's Exact Probability Test is often used when two independent samples (variables) are small in size and placed in two mutually exclusive classifications. The test determines whether the two groups differ in the proportion in which they fall into the two classifications. The level of significance (p-value) of this test is exact as the test does not depend on underlying distributional assumptions.

This test was applied to this data set when the data for each race group was categorized at a cord blood IgE value of 0.50 kU/l and the proportion of individuals above and below this value in the non-atopic and in the atopic family history groups were compared.

4.5.4 The Spearman Rank Correlation Coefficient

A correlation coefficient gives a measure of linear association between the two variables considered. It can also be used to give an indication of the strength of a linear trend in the data.

The Spearman Rank Correlation Coefficient is a non-parametric measure of association and does not require the underlying assumption that the data is normally (Gaussian) distributed. The significance of the value of the correlation coefficient depends on the size of the sample used to calculate it. Caution must be exercised in interpreting the results when testing the hypothesis that the correlation coefficient is zero and hence that there is no linear association between the 2 variables against the alternative hypothesis that the correlation coefficient is not zero and that there is a linear association between the two variables. The results from this hypothesis test are greatly influenced by the sample size of each of the two variables.

SECTION V

RESULTS - PHASE I

CHAPTER I TECHNICAL DETAILS PERTAINING TO THE DATA ANALYSIS AND PRESENTATION

1.1 DEFICIENT DATA

1.1.1 Sex code

One of the Black babies (Patient Code Number 303) had no sex code recorded. This case was omitted from the analysis when the data set was categorized on sex codes.

1.1.2 Birth mass

The following babies had no mass recorded at birth:
White Group:

Patient Code Number	Estimated Gestational Age
402	39 weeks
409	40 weeks

Since one of the exclusion criteria for the newborns was a birth mass of less than 2,500gm, this value was important. It was decided that, since the gestational age of the abovementioned babies was greater than 38 weeks, an assumption could be made that the mass at birth was also acceptable.

1.2 NEWBORNS EXCLUDED FROM THE STUDY

The exclusion criteria were:

a) a gestational age of less than 38 weeks
or b) a mass at birth of less than 2500 gm.
or c) a baby who was born to a mother who had not been resident in the Cape Peninsula for at least one year prior to the birth.

Any one of these criteria needed to be fulfilled for the baby to be excluded. The following newborns were excluded on one or other of these criteria:

ETHNIC GROUP	EXCLUSION CRITERIA	PATIENT CODE NUMBER	RESULT
Black newborns	- 1 on gestational age and on mass at birth	220	35 weeks 2300 gm
	- 1 on mass at birth	230	2150 gm
White newborns	- 1 on mass at birth	415	2360 gm
Mixed newborns	- 2 on mass at birth	51	2300 gm
		54	2060 gm

1.3 CATEGORIZATION OF THE DATA

The data is presented in the form of the following 6 categorizing variables in each ethnic group for each of the four markers.

(i) Ethnic group
(ii) Sex
(iii) Atopic family history
(iv) Maternal Ascaris lumbricoides RAST (Pharmacia)
(v) Combined negative maternal Ascaris lumbricoides RAST (Pharmacia) and negative aFH
(vi) Maternal cigarette-smoking.

The data will, furthermore, be presented in the following formats:

i) Descriptive statistics for each category in each ethnic group.
ii) Comparative statistical analysis for each category between or within the ethnic groups (whichever is the more appropriate to the category concerned).

The definitions used to categorize the data into the six sub-groups are listed below.

1.3.1 Ethnic group categorization

This category was defined according to the common ethnic classification of the newborn's mother.

1.3.2 Sex categorization

This category was defined according to the sex of the newborn (male or female).

1.3.3 Atopic family history categorization

A positive or a negative atopic family history for the newborn was decided on after questioning the mother according to the criteria for a family history of atopy as described in Section VII, Ch. 1.3. Only the first degree relatives of the newborn were considered (mother, father or sibling).

a) Inclusions on a positive atopic family history.

The newborns in this sub-group were those where one or more of the first degree relative had an atopic history.

b) Inclusions on a negative atopic family history.

The newborns in this sub-group were those where none of their first degree relatives had a history of atopic disease. If the allergic status of any of the newborn's first degree relatives was unknown, the atopic status of these family members was also regarded as negative.

1.3.4 Maternal Ascaris lumbricoides RAST (Pharmacia) categorization

All newborns had negative Ascaris RAST results. The maternal Ascaris RAST result was therefore used to divide the data set into two sub-groups, based on newborns who had mothers with either a positive (Phadebas RAST class greater than 0 - see Table IV. 2) or a negative (Phadebas RAST class equal to 0) Ascaris RAST.

1.3.5 Combined negative maternal Ascaris lumbricoides RAST (Pharmacia) and negative atopic family history categorization

For reasons which will be addressed in the discussion of this thesis (Section VII), this category was established by including only those newborns who had neither a positive maternal RAST for Ascaris lumbricoides nor a positive atopic family history.

1.3.6 Maternal cigarette smoking categorization

This category was established to differentiate the newborns into two groups, depending whether or not their mothers smoked cigarettes. This categorization was independent of the number of cigarettes smoked by the mother per day or the smoking habits of the rest of the family.

1.4 THE NUMBER OF NEWBORNS IN EACH SPECIFIED CATEGORY AND SUB-CATEGORY

This data is summarized in Table V.1.

TABLE V. 1.

NUMBER OF NEWBORNS OBSERVED IN EACH RACE GROUP IN THE SPECIFIED CATEGORIES* AND SUB-GROUPS

CATEGORIZING VARIABLE	SUB-GROUPS	NUMBER OF NEWBORNS IN EACH RACE GROUP		
		Black	White	Mixed
Ethnicity		53	52	58
Sex	Male	27	23	34
	Female	25	29	24
Atopic FH	+ve Atopic FH	5	39	18
	-ve Atopic FH	48	13	40
Maternal Ascaris RAST	+ve Ascaris RAST	7	1	7
	-ve Ascaris RAST	46	51	51
Maternal Ascaris RAST and atopic FH	-ve RAST -ve atopic FH	41	13	35
Maternal Smoking	Maternal smoker	3	14	30
	Maternal non-smoker	50	38	28

*The number of observations on each of the blood parameters considered may vary for the various categories above depending upon whether or not the specific measurements could be recorded for each individual.

CHAPTER 2 CORD BLOOD ATOPIC MARKERS

2.1 DESCRIPTIVE STATISTICS

This data is presented for all six categorizations in each ethnic group for the following markers.

2.1.1 Cord blood serum IgE concentrations

This data is presented in Table V.2.

2.1.2 Cord blood total eosinophil counts

This data is presented in Table V.3.

2.1.3 Cord blood platelet counts

This data is presented in Table V.4.

2.1.4 Cord blood anti-cows milk serum IgG concentrations

This data is presented in Table V.5.

TABLE V.2

IgE CONCENTRATION IN CORD BLOOD - DESCRIPTIVE STATISTICS (kU/l)

CATEGORIZING VARIABLE	ETHNIC GROUP	IgE CONCENTRATION IN CORD BLOOD										
		NO.	Arith Mean	SD	Median	GM	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Combined		163	0.54	1.49	0.12	0.13	15.00	0.01	14.99	0.43	0.05	0.38
Race	Black	53	0.86	2.28	0.16	0.21	15.00	0.01	14.99	0.78	0.09	0.69
	White	52	0.41	0.91	0.12	0.12	5.83	0.01	5.82	0.33	0.06	0.27
	Mixed	58	0.37	0.81	0.08	0.10	5.67	0.01	5.66	0.44	0.02	0.42
Sex												
Male	Black	27	1.35	3.10	0.17	0.29	15.00	0.01	14.99	0.96	0.12	0.84
	White	23	0.39	0.61	0.12	0.14	02.20	1.10	2.20	0.43	0.06	0.37
	Mixed	34	0.53	1.02	0.14	0.14	5.67	0.01	5.66	0.80	0.03	0.77
Female	Black	25	0.36	0.50	0.10	0.15	2.03	0.02	2.01	0.45	0.06	0.39
	White	29	0.42	1.10	0.11	0.11	5.83	0.01	5.82	0.23	0.06	0.17
	Mixed	24	0.14	0.21	0.05	0.05	0.81	0.01	0.80	0.15	0.02	0.13
Atopic FH												
Positive	Black	5	0.64	0.80	0.13	0.30	1.92	0.09	1.83	1.44	0.11	1.33
	White	39	0.51	1.03	0.12	0.16	5.83	0.01	5.82	0.49	0.08	0.41
	Mixed	18	0.48	0.53	0.18	0.20	1.68	0.01	1.67	0.89	0.01	0.83
Negative	Black	48	0.88	2.38	0.17	0.21	15.00	0.01	14.99	0.69	0.08	0.61
	White	13	0.09	0.11	0.07	0.05	0.41	0.01	0.40	0.12	0.02	0.10
	Mixed	40	0.32	0.91	0.06	0.07	5.67	0.01	5.66	0.22	0.02	0.20

TABLE V.2 (Cont.)

CATEGORIZING VARIABLE	ETHNIC GROUP	IgE CONCENTRATION IN CORD BLOOD										
		NO.	Arith Mean	SD	Median	GM	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Maternal Ascaris RAST												
Positive	Black	7	0.49	0.69	0.23	0.26	2.03	0.05	1.98	0.46	0.10	0.36
	White	1	(2.21)	-	-	-	-	-	-	-	-	-
	Mixed	7	0.65	0.59	0.49	0.32	1.68	0.02	1.66	1.08	0.06	1.02
Negative	Black	46	0.92	2.43	0.15	0.21	15.00	0.01	14.99	0.80	0.08	0.72
	White	51	0.37	0.88	0.11	0.12	5.83	0.01	5.82	0.25	0.06	0.19
	Mixed	51	0.33	0.84	0.07	0.08	5.67	0.01	5.66	0.23	0.02	0.21
Maternal Ascaris RAST and Atopic FH												
-ve Ascaris -ve FH	Black	41	0.95	2.56	0.16	0.20	15.00	0.01	14.99	0.78	0.08	0.70
	White	13	0.09	0.11	0.07	0.05	0.41	0.01	0.40	0.12	0.02	0.10
	Mixed	35	0.30	0.97	0.05	0.06	5.67	0.01	5.66	0.17	0.01	0.16
Maternal Smoking												
Smoker	Black	3	5.52	8.24	1.54	0.88	15.00	0.03	14.97	15.00	0.03	14.97
	White	14	0.42	0.57	0.20	0.21	2.21	0.02	2.19	0.52	0.10	0.42
	Mixed	30	0.36	0.46	0.16	0.13	1.68	0.01	1.67	0.55	0.04	0.51
Non-Smoker	Black	50	0.58	1.15	0.16	0.20	6.77	0.01	6.76	0.54	0.09	0.45
	White	38	0.40	1.01	0.10	0.10	5.83	0.01	5.82	0.18	0.05	0.13
	Mixed	28	0.38	1.08	0.06	0.07	5.67	0.01	5.66	0.15	0.02	0.13

TABLE V.3

TOTAL EOSINOPHIL COUNT IN CORD BLOOD - DESCRIPTIVE STATISTICS (cells/mm^3)

CATEGORIZING VARIABLE	ETHNIC GROUP	TOTAL EOSINOPHIL COUNT IN CORD BLOOD									
		NO.	Mean	Median	SD	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Combined		162	330	244	272	1888	0	1888	406	163	243
Race	Black	53	269	222	167	777	0	777	366	172	194
	White	52	386	366	252	1155	0	1155	533	183	350
	Mixed	57	337	222	351	1888	0	1888	355	155	200
Sex											
Male	Black	27	292	222	179	711	0	711	400	166	234
	White	23	381	388	246	777	22	755	666	155	511
	Mixed	33	327	200	348	1888	0	1888	355	144	211
Female	Black	25	250	222	154	777	0	777	333	177	156
	White	29	389	355	260	1155	0	1155	511	189	322
	Mixed	24	350	244	362	1711	44	1667	369	155	214
Atopic FH											
Positive	Black	5	242	222	84	377	155	222	318	178	140
	White	39	384	388	226	777	0	777	533	200	333
	Mixed	17	370	244	394	1711	77	1634	344	177	167
Negative	Black	48	272	222	173	777	0	777	372	169	203
	White	13	391	355	327	1155	22	1133	611	133	478
	Mixed	40	322	211	335	1888	0	1888	380	139	241

TABLE V.3 (Cont.)

CATEGORIZING VARIABLE	ETHNIC GROUP	TOTAL EOSINOPHIL COUNT IN CORD BLOOD								
		NO.	Mean	Median	SD	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Maternal Ascaris RAST											
Positive	Black	7	163	166	99	333	0	333	200	133	67
	White	1	(666)	-	-	-	-	-	-	-	-
	Mixed	7	230	166	208	555	0	555	444	44	400
Negative	Black	46	286	233	169	777	0	777	377	177	200
	White	51	380	355	251	1155	0	1155	533	177	356
	Mixed	50	352	233	365	1888	77	1811	355	155	200
Maternal Ascaris Rast and Atopic FH											
-ve Ascaris -ve FH	Black	41	291	244	177	777	0	777	389	177	212
	White	13	391	355	327	1155	22	1133	611	133	478
	Mixed	35	347	244	347	1888	77	1811	388	155	233
Maternal Smoking											
Smoker	Black	3	289	244	-	400	222	-	-	-	-
	White	14	471	477	219	777	22	755	688	328	360
	Mixed	29	268	244	196	888	0	888	311	155	156
Non-Smoker	Black	50	268	222	170	777	0	777	361	163	198
	White	38	354	278	258	1155	0	1155	499	155	344
	Mixed	28	408	211	453	1888	111	1777	433	158	275

TABLE V.4

PLATELET COUNT IN CORD BLOOD - DESCRIPTIVE STATISTICS (x 10^9/l)

CATEGORIZING VARIABLE	RACE GROUP	PLATELET COUNT IN CORD BLOOD									
		NO.	Mean	Median	SD	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Combined		158	343	333	78	575	176	399	393	290	103
Race											
	Black	51	354	336	88	575	176	399	411	296	115
	White	50	356	340	76	543	199	344	417	304	113
	Mixed	57	322	312	67	471	189	282	367	268	99
Sex											
Male											
	Black	27	339	331	90	529	176	353	397	276	121
	White	22	358	340	90	543	199	344	424	302	122
	Mixed	33	319	307	69	471	189	282	362	267	95
Female											
	Black	23	367	342	83	575	266	309	417	313	104
	White	28	355	349	64	484	265	219	409	300	109
	Mixed	24	326	316	66	456	207	249	388	275	113
Atopic FH											
Positive											
	Black	5	359	336	108	529	235	294	451	279	172
	White	38	362	356	80	543	199	344	424	304	120
	Mixed	18	314	306	59	421	214	207	373	267	106
Negative											
	Black	46	353	339	87	575	176	399	413	295	118
	White	12	338	321	59	445	248	197	376	294	82
	Mixed	39	325	319	71	471	189	282	367	268	99

TABLE V.4 (Cont.)

CATEGORIZING VARIABLE	ETHNIC GROUP	PLATELET COUNT IN CORD BLOOD									
		NO.	Mean	Median	SD	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Maternal Ascaris RAST											
Positive	Black	7	325	315	82	492	228	264	342	272	70
	White	1	(418)	-	-	-	-	-	-	-	-
	Mixed	6	353	331	53	426	304	122	416	310	106
Negative	Black	44	359	344	89	575	176	399	416	297	119
	White	49	355	333	76	543	199	344	414	302	112
	Mixed	51	318	307	68	471	189	282	367	267	100
Maternal Ascaris RAST and Atopic FH											
-ve Ascaris -ve atopic FH	Black	39	358	345	88	575	176	399	417	296	121
	White	12	338	321	59	445	248	197	376	294	82
	Mixed	35	320	314	71	471	189	282	356	263	93
Maternal Smoking											
Smoker	Black	3	316	318	-	331	300	-	-	-	-
	White	14	350	340	47	418	265	153	393	315	78
	Mixed	30	325	306	68	471	214	257	381	272	109
Non-Smoker	Black	48	356	342	90	575	176	399	416	292	124
	White	36	359	348	85	543	199	344	440	295	145
	Mixed	27	319	314	68	456	189	267	367	267	100

TABLE V.5.

IgG CONCENTRATION IN CORD BLOOD - DESCRIPTIVE (Δ O.D. 414nm*)

CATEGORIZING VARIABLE	ETHNIC GROUP	IgG CONCENTRATION IN CORD BLOOD									
		NO.	Mean	Median	SD	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Combined		162	58.4	54.5	27.9	147.0	0.0	147.0	78.1	36.7	41.4
Race	Black	53	47.3	41.9	22.1	102.5	14.1	88.4	64.0	31.3	32.7
	White	51	70.6	72.7	30.3	147.0	0.0	147.0	89.9	51.6	38.3
	Mixed	58	57.7	54.4	26.3	118.7	15.7	103.0	72.3	34.0	38.3
Sex											
Male	Black	27	50.1	46.3	19.8	98.2	20.1	78.1	61.2	39.9	21.3
	White	22	71.8	77.5	31.3	147.0	22.0	125.0	91.9	48.7	43.2
	Mixed	34	58.7	55.0	26.7	118.7	15.7	103.0	72.3	33.9	38.4
Female	Black	25	44.5	35.5	24.8	102.5	14.1	88.4	71.2	27.0	44.2
	White	29	69.7	71.0	30.1	127.1	0.0	127.1	87.4	50.1	37.3
	Mixed	24	56.3	52.6	26.3	114.2	17.2	97.0	73.7	35.5	38.2
Atopic FH											
Positive	Black	5	53.1	41.2	32.4	98.2	20.1	78.1	86.4	25.8	60.6
	White	38	76.0	80.1	29.4	147.0	8.2	138.8	95.3	56.4	38.9
	Mixed	18	63.9	65.5	28.6	114.2	15.7	98.5	78.7	38.8	39.9
Negative	Black	48	46.7	42.6	21.2	102.5	14.1	88.4	59.6	31.8	27.8
	White	13	54.9	56.9	28.4	93.2	0.0	93.2	80.7	32.2	48.5
	Mixed	40	54.9	52.6	25.1	118.7	17.2	101.5	70.7	33.1	37.6

*Δ O.D. 414nm = Optical density difference at 414 nanometres.

TABLE V.5 (Cont.)

CATEGORIZING VARIABLE	ETHNIC GROUP	IgG CONCENTRATION IN CORD BLOOD								
		NO.	Mean	Median	SD	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Ascaris RAST											
Positive	Black	7	63.8	68.1	17.5	81.9	34.3	47.6	77.8	47.5	30.3
	White	1	(147.0)	-	-	-	-	-	-	-	-
	Mixed	7	90.6	97.8	19.0	112.5	62.3	50.2	106.0	70.7	35.3
Negative	Black	46	44.8	41.5	21.8	102.5	14.1	88.4	49.4	29.0	20.4
	White	50	69.1	72.3	28.6	127.1	0.0	127.1	89.2	50.7	38.6
	Mixed	51	53.2	51.9	24.0	118.7	15.7	103.0	70.6	33.2	37.4
Maternal Ascaris RAST and Atopic FH											
-ve Ascaris -ve atopic FH	Black	41	43.7	41.7	20.5	102.5	14.1	88.4	47.5	28.7	18.8
	White	13	54.9	56.9	28.4	93.2	0.0	93.2	80.7	32.2	48.5
	Mixed	35	50.9	48.7	23.6	118.7	17.2	101.5	61.1	31.5	29.6
Smoking											
Smoker	Black	3	40.4	45.7	-	47.6	27.9	-	-	-	-
	White	14	63.8	56.6	38.2	147.0	8.2	138.8	89.0	35.6	53.4
	Mixed	30	53.8	53.1	22.6	112.5	15.7	96.8	64.8	38.8	26.0
Non-Smoker	Black	50	47.7	41.8	22.6	102.5	14.1	88.4	67.0	31.3	35.7
	White	37	73.2	76.9	26.9	127.1	0.0	127.1	90.4	56.9	33.5
	Mixed	28	61.9	62.1	29.6	118.7	19.0	99.7	82.0	33.1	48.9

2.1.5 Graphic distribution plot for CBsIgE, relative to a atopic family history

The distribution of CBsIgE values relative to a positive or a negative aFH were plotted. This data is graphically depicted in Fig's. V.1, V.2 and V.3.

The newborns with a positive aFH represented by a solid circle. The newborns with a negative aFH are represented by an open circle.

Fig. V.1. Cord blood serum IgE concentrations (Black newborns).

Fig. V.2. Cord blood serum IgE concentrations (White newborns).

Fig. V. 3. Cord blood serum IgE concentrations (Mixed newborns).

2.2 COMPARATIVE STATISTICS BETWEEN THE ETHNIC GROUPS FOR DIFFERENT CATEGORIZATIONS

The differences in the descriptive data presented in Section V. Ch. 2.1 between each ethnic group, for the following categorizations were tested for statistical significance.

i) Ethnic group categorization (total group).
ii) Atopic family history categorization.
iii) Maternal Ascaris lumbricoides RAST (Pharmacia) categorization. Since the sub-groups with positive maternal Ascaris lumbricoides RAST's were so small (Black 7; White 1; Mixed 7) it was decided only to compare the sub-groups with negative maternal Ascaris lumbricoides RAST's.
iv) Combined negative maternal Ascaris lumbricoides RAST (Pharmacia) and negative aFH categorization.

The results are presented below.

2.2.1 Cord blood serum IgE concentrations

Table V.6.

2.2.2 Cord blood total eosinophil counts

Table V.7.

2.2.3 Cord blood platelet counts

Table V.8.

2.2.4 Cord blood anti-cows milk serum IgG concentrations

Table V.9.

FIGURE. V.1.

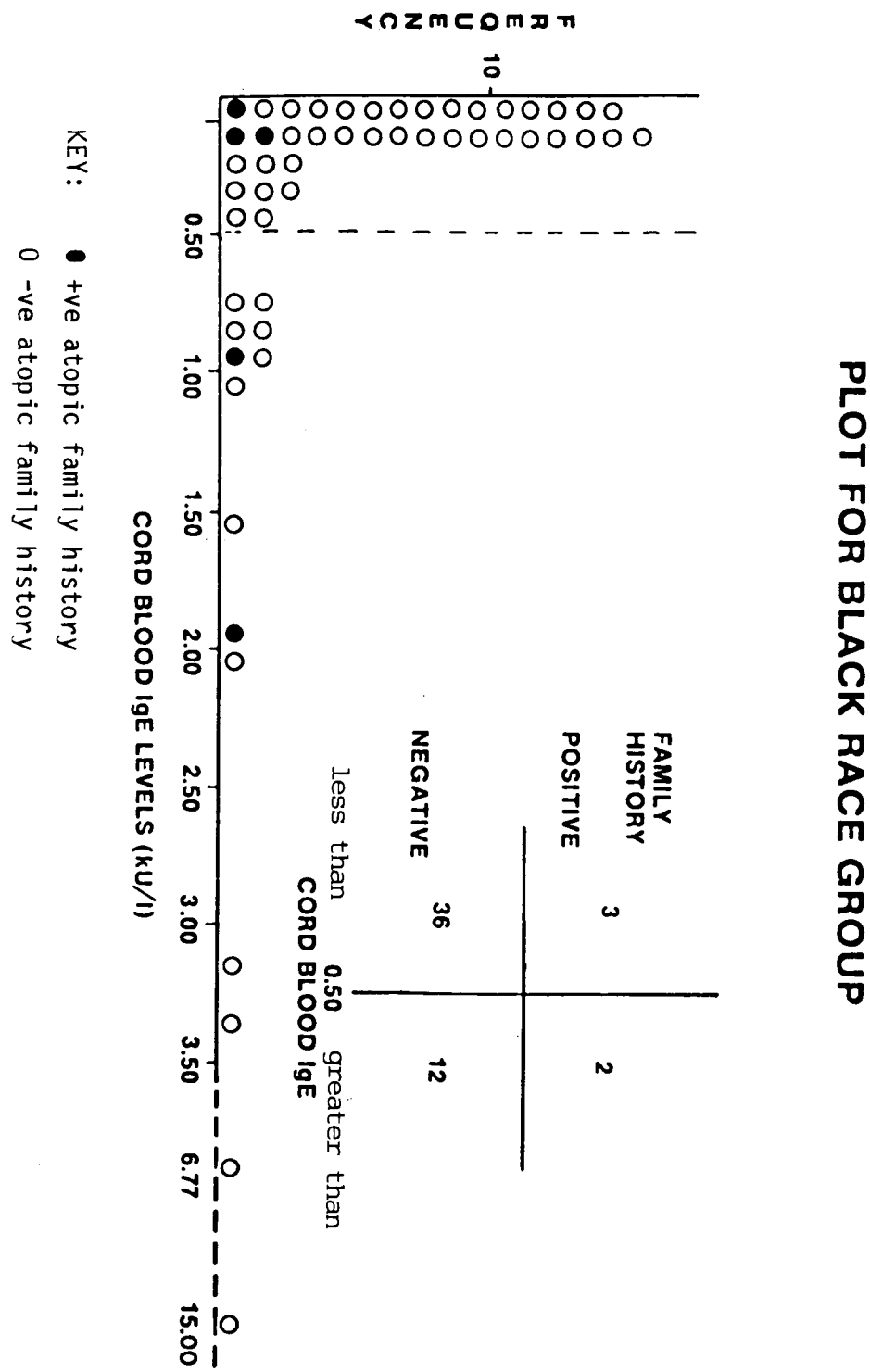

CORD BLOOD IgE:
PLOT FOR BLACK RACE GROUP
FREQUENCY
10
FAMILY HISTORY
POSITIVE
3
2
NEGATIVE
36
12
less than 0.50 greater than
CORD BLOOD IgE
0.50
1.00
1.50
2.00
2.50
3.00
3.50
6.77
15.00
CORD BLOOD IgE LEVELS (kU/l)
KEY: ● +ve atopic family history
O -ve atopic family history

FIGURE. V.2.

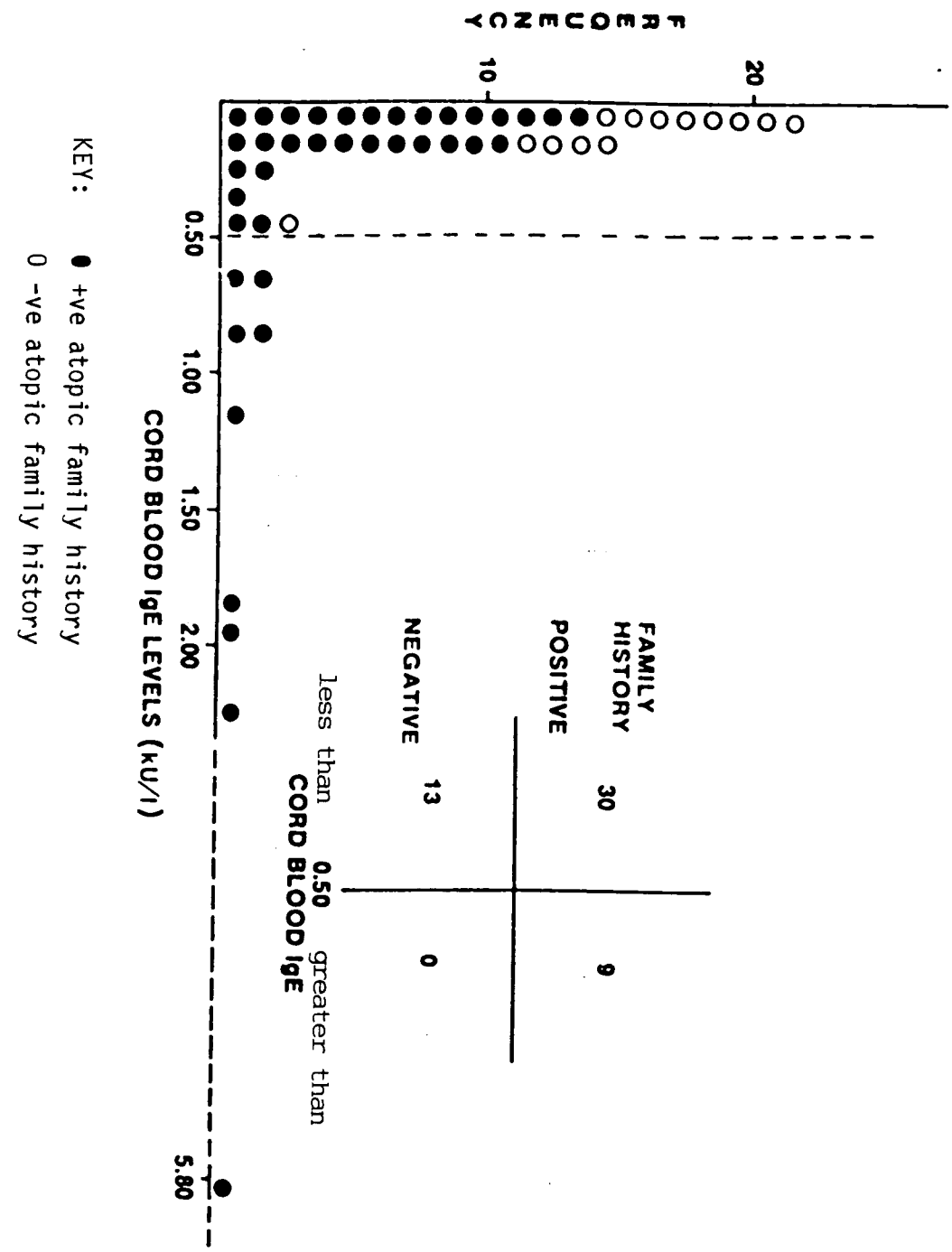

CORD BLOOD IgE:
PLOTS FOR WHITE RACE GROUP
FREQUENCY
10
20
0.50
1.00
1.50
2.00
5.80
CORD BLOOD IgE LEVELS (kU/l)
FAMILY HISTORY
POSITIVE
NEGATIVE
30
13
9
0
less than 0.50 CORD BLOOD IgE
greater than
KEY:
● +ve atopic family history
0 -ve atopic family history

FIGURE. V.3.

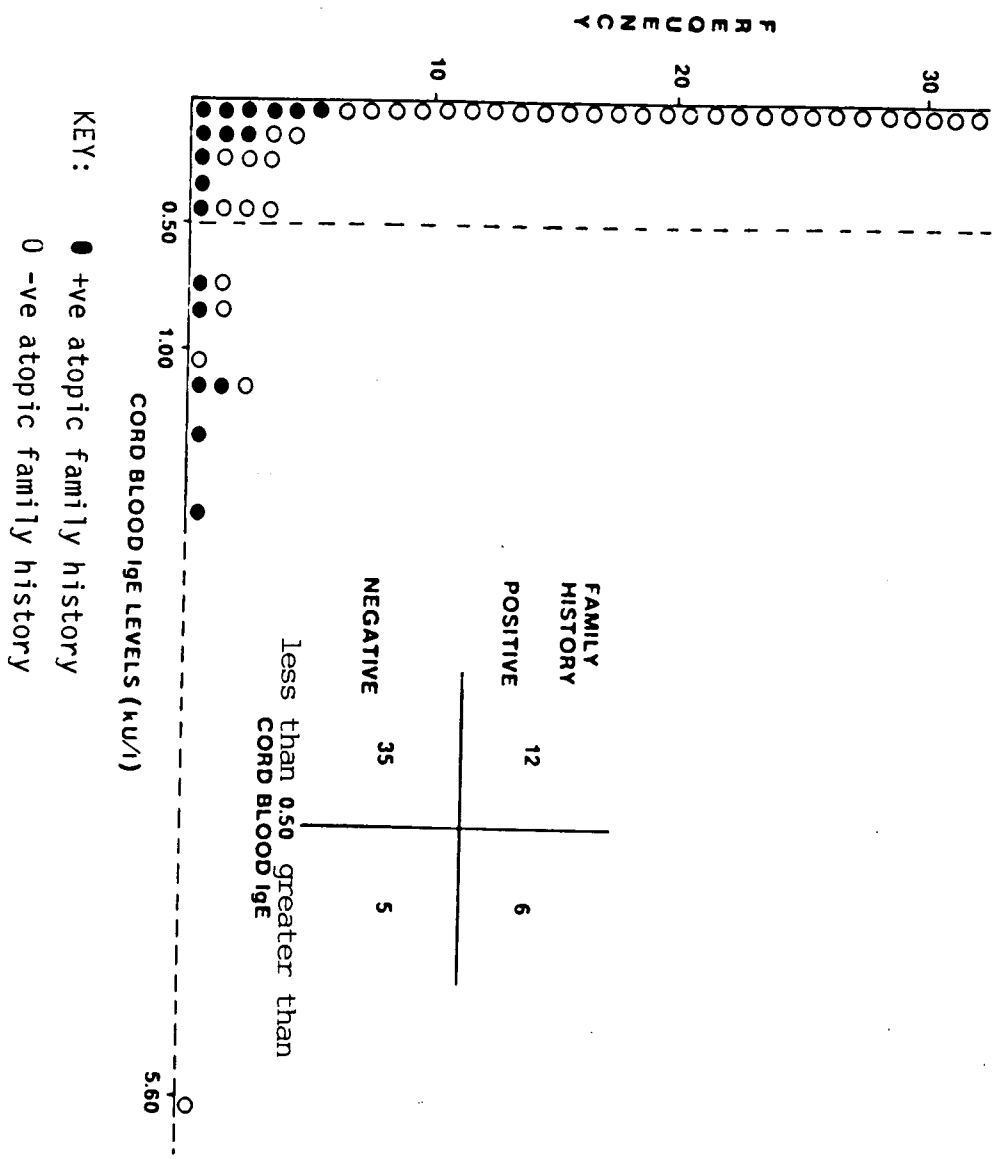

CORD BLOOD IgE:
PLOTS FOR MIXED (COLOURED) RACE GROUP
FREQUENCY
10
20
30
0.50
1.00
5.60
CORD BLOOD IgE LEVELS (kU/l)
FAMILY HISTORY
POSITIVE
NEGATIVE
12
6
35
5
less than 0.50 CORD BLOOD IgE
greater than
KEY:
● +ve atopic family history
0 -ve atopic family history

TABLE V.6.

SERUM IgE CONCENTRATIONS IN CORD BLOOD : COMPARATIVE STATISTICS BETWEEN ETHNIC GROUPS

CATEGORIZING VARIABLE	ETHNIC GROUPS COMPARED	Mann-Whitney-U Test or Kruskal-Wallis Test				
		X^2-approx value	df	p-value	Signif at 5% level	Statistical Decision
Ethnic Group	Combined	6.84	2	0.0327	S	difference
	Black - White	3.24	1	0.0720	?NS *	? no diff.*
	Black - Mixed	6.06	1	0.0138	S	difference
	White - Mixed	0.94	1	0.3311	NS	no diff.
Atopic FH						
Positive	Combined	0.93	2	0.6291	NS	no diff.
	Black - White	0.79	1	0.3472	NS	no diff.
	Black - Mixed	0.14	1	0.7090	NS	no diff.
	White - Mixed	0.34	1	0.5591	NS	no diff.
Negative	Combined	12.76	2	0.0017	S	difference
	Black - White	7.41	1	0.0065	S	difference
	Black - Mixed	9.46	1	0.0021	S	difference
	White - Mixed	0.05	1	0.8270	NS	no diff.
Maternal Ascaris RAST						
Negative	Combined	8.00	2	0.0183	S	difference
	Black - White	3.06	1	0.0801	NS	no diff.
	Black - Mixed	7.24	1	0.0071	S	difference
	White - Mixed	1.81	1	0.1785	NS	no diff.
Maternal Ascaris RAST and atopic FH						
-ve Ascaris RAST -ve atopic FH	Combined	12.42	2	0.0020	S	difference
	Black - White	6.41	1	0.0114	S	difference
	Black - Mixed	9.95	1	0.0016	S	difference
	White - Mixed	0.02	1	0.8976	NS	no diff.

? * no definite statistical decision can be made.

TABLE V.7.

TOTAL EOSINOPHIL COUNTS IN CORD BLOOD : COMPARATIVE STATISTICS BETWEEN ETHNIC GROUPS

CATEGORIZING VARIABLE	ETHNIC GROUPS COMPARED	Mann-Whitney-U Test or Kruskal-Wallis Test				
		X^2-approx value	df	p-value	Signif at 5% level	Statistical Decision
Ethnic Group	Combined	5.62	2	0.0602	? *	? *
	Black - White	4.71	1	0.0300	S	difference
	Black - Mixed	0.00	1	0.9594	NS	no diff.
	White - Mixed	3.80	1	0.0512	? *	? *
Atopic FH						
Positive	Combined	2.25	2	0.3252	NS	no diff.
	Black - White	1.41	1	0.2358	NS	no diff.
	Black - Mixed	0.02	1	0.8751	NS	no diff.
	White - Mixed	1.35	1	0.2461	NS	no diff.
Negative	Combined	0.67	2	0.7166	NS	no diff.
	Black - White	0.60	1	0.4375	NS	no diff.
	Black - Mixed	0.02	1	0.8865	NS	no diff.
	White - Mixed	0.54	1	0.4622	NS	no diff.
Maternal Ascaris RAST						
Negative	Combined	3.35	2	0.1871	NS	no diff.
	Black - White	2.42	1	0.1197	NS	no diff.
	Black - Mixed	0.06	1	0.1137	NS	no diff.
	White - Mixed	2.50	1	0.1137	NS	no diff.
Maternal Ascaris RAST and atopic FH						
-ve Ascaris RAST -ve atopic FH	Combined	0.25	1	0.8834	NS	no diff.
	Black - White	0.23	1	0.6338	NS	no diff.
	Black - Mixed	0.01	1	0.9044	NS	no diff.
	White - Mixed	0.18	1	0.6671	NS	no diff.

? * no definite statistical decision can be made.

TABLE V.8

PLATELET COUNTS IN CORD BLOOD : COMPARATIVE STATISTICS BETWEEN ETHNIC GROUPS

CATEGORIZING VARIABLE	ETHNIC GROUPS COMPARED	Mann-Whitney-U Test or Kruskal-Wallis Test				
		X^2-approx value	df	p-value	Signif at 5% level	Statistical Decision
Ethnic Group	Combined	5.93	2	0.0515	? *	? *
	Black - White	0.09	1	0.7676	NS	no diff.
	Black - Mixed	3.44	1	0.0635	NS	no diff.
	White - Mixed	5.18	1	0.0228	S	difference
Atopic FH						
Positive	Combined	4.86	2	0.0882	NS	no diff.
	Black - White	0.04	1	0.8497	NS	no diff.
	Black - Mixed	0.80	1	0.3710	NS	no diff.
	White - Mixed	4.93	1	0.0264	S	difference
Negative	Combined	1.91	2	0.3843	NS	no diff.
	Black - White	0.22	1	0.6381	NS	no diff.
	Black - Mixed	1.83	1	0.1758	NS	no diff.
	White - Mixed	0.33	1	0.5637	NS	no diff.
Maternal Ascaris RAST						
Negative	Combined	7.34	2	0.0255	S	difference
	Black - White	0.00	1	0.9509	NS	no diff.
	Black - Mixed	5.13	1	0.0235	S	difference
	White - Mixed	5.66	1	0.0174	S	difference
Maternal Ascaris RAST and atopic FH						
-ve Ascaris RAST -ve atopic FH	Combined	3.83	2	0.1473	NS	no diff.
	Black - White	0.41	1	0.5196	NS	no diff.
	Black - Mixed	3.67	1	0.0553	NS	no diff.
	White - Mixed	0.77	1	0.3798	NS	no diff.

? * no definite statistical decision can be made.

TABLE V.9

SERUM IgG CONCENTRATION IN CORD BLOOD : COMPARATIVE STATISTICS BETWEEN ETHNIC GROUPS

CATEGORIZING VARIABLE	ETHNIC GROUPS COMPARED	Mann-Whitney-U Test or Kruskal-Wallis Test				
		X^2-approx value	df	p-value	Signif at 5% level	Statistical Decision
Ethnic Group	Combined	19.08	2	0.0001	S	difference
	Black - White	17.86	1	0.0001	S	difference
	Black - Mixed	4.86	1	0.0274	S	difference
	White - Mixed	6.32	1	0.0119	S	difference
Atopic FH						
Positive	Combined	3.65	2	0.1611	NS	no diff.
	Black - White	2.07	1	0.1500	NS	no diff.
	Black - Mixed	0.36	1	0.5510	NS	no diff.
	White - Mixed	2.22	1	0.1359	NS	no diff.
Negative	Combined	3.45	2	0.1780	NS	no diff.
	Black - White	1.48	1	0.2243	NS	no diff.
	Black - Mixed	2.91	1	0.0881	NS	no diff.
	White - Mixed	0.04	1	0.8362	NS	no diff.
Maternal Ascaris RAST						
Negative	Combined	21.77	2	0.0001	S	difference
	Black - White	18.69	1	0.0001	S	difference
	Black - Mixed	4.12	1	0.0423	S	difference
	White - Mixed	10.06	1	0.0015	S	difference
Maternal Ascaris RAST and atopic FH						
-ve Ascaris RAST -ve atopic FH	Combined	3.93	2	0.1403	NS	no diff.
	Black - White	2.33	1	0.1266	NS	no diff.
	Black - Mixed	2.66	1	0.1029	NS	no diff.
	White - Mixed	0.47	1	0.4937	NS	no diff.

2.3 COMPARATIVE STATISTICS WITHIN EACH ETHNIC GROUP FOR DIFFERENT CATEGORIZATIONS

The differences in the descriptive data presented in Section V. Ch. 2.1 within each ethnic group, for the following categorizations were tested for statistical significance.

i) Sex categorization.

ii) Maternal Ascaris lumbricoides RAST (Pharmacia) categorization.

iii) Atopic family history categorization.

iv) Maternal cigarette-smoking categorization.

The results are presented below.

2.3.1 Cord blood serum IgE concentrations

Table V.10.

2.3.2 Cord blood total eosinophil counts

Table V.11.

2.3.3 Cord blood platelet counts

Table V.12.

2.3.4 Cord blood anti-cows milk IgG concentrations

Table V.13.

2.4 CO-ORDINATED RESULTS FROM STATISTICAL TESTS OF COMPARISONS

The results from Section V, Chapters 2.2 and 2.3 have been co-ordinated and summarized in Table V. 14a and Table V. 14b.

TABLE V.10

SERUM IgE CONCENTRATION IN CORD BLOOD : COMPARATIVE STATISTICS WITHIN ETHNIC GROUPS

ETHNIC GROUP	CATEGORIZING VARIABLE	Mann-Whitney-U Test				
		X^2-approx. value	df	p-value	Signif at 5% level	Statistical decision
	Sex					
Black	Males vs Females	2.54	1	0.1109	NS	No diff.
White	Males vs Females	0.20	1	0.6512	NS	No diff.
Mixed	Males vs Females	4.51	1	0.0338	S	difference
	Maternal Ascaris RAST					
Black	+ve vs -ve	0.33	1	0.5631	NS	no diff.
White	+ve vs -ve	-		-	-	*
Mixed	+ve vs -ve	3.90	1	0.0482	? **	? **
	Atopic FH					
Black	+ve vs -ve	0.21	1	0.6479	NS	no diff.
White	+ve vs -ve	5.62	1	0.0178	S	difference
Mixed	+ve vs -ve	5.26	1	0.0218	S	difference
	Maternal cigarette smoking					
Black	Smoker vs Non-smoker	-	-	-	-	*
White	Smoker vs Non-smoker	3.46	1	0.0629	? **	? **
Mixed	Smoker vs Non-smoker	2.51	1	0.1130	NS	no diff

* not enough observations in one of the groups in this categorization. Comparison not possible.

? ** no definite statistical decision can be made.

TABLE V.11

TOTAL EOSINOPHIL COUNT IN CORD BLOOD : COMPARATIVE STATISTICS WITHIN ETHNIC GROUPS

ETHNIC GROUP COMPARED	CATEGORIZING VARIABLE	Mann-Whitney-U Test				
		X^2-approx. value	df	p-value	Signif at 5% level	Statistical decision
	Sex					
Black	Males vs Females	0.18	1	0.6728	NS	no diff.
White	Males vs Females	0.01	1	0.9192	NS	no diff.
Mixed	Males vs Females	0.04	1	0.8396	NS	no diff.
	Maternal Ascaris RAST					
Black	+ve vs -ve	4.78	1	0.0288	S	difference
White	+ve vs -ve	-	-	-	-	- *
Mixed	+ve vs -ve	1.07	1	0.3006	NS	no diff.
	Atopic FH					
Black	+ve vs -ve	0.00	1	1.0000	NS	no diff.
White	+ve vs -ve	0.15	1	0.6954	NS	no diff.
Mixed	+ve vs -ve	0.34	1	0.5583	NS	no diff.
	Maternal cigarette smoking					
Black	Smoker vs Non-smoker	-	-	-	-	- *
White	Smoker vs Non-smoker	3.01	1	0.0827	NS	no diff.
Mixed	Smoker vs Non-smoker	0.76	1	0.3835	NS	no diff.

* not enough observations in one of the groups in this categorization. Comparison not possible.

TABLE V.12

PLATELET COUNT IN CORD BLOOD : COMPARATIVE STATISTICS WITHIN ETHNIC GROUPS

ETHNIC GROUP COMPARED	CATEGORIZING VARIABLE	Mann-Whitney-U Test				
		X^2-approx. value	df	p-value	Signif at 5% level	Statistical decision
	Sex					
Black	Males vs Females	1.06	1	0.3022	NS	no diff.
White	Males vs Females	0.02	1	0.8989	NS	no diff.
Mixed	Males vs Females	0.24	1	0.6277	NS	no diff.
	Maternal Ascaris RAST					
Black	+ve vs -ve	1.14	1	0.2857	NS	no diff.
White	+ve vs -ve	-	-	-	-	- *
Mixed	+ve vs -ve	1.69	1	0.1935	NS	no diff.
	Atopic FH					
Black	+ve vs -ve	0.02	1	0.8866	NS	no diff.
White	+ve vs -ve	1.02	1	0.3120	NS	no diff.
Mixed	+ve vs -ve	0.26	1	0.6125	NS	no diff.
	Maternal cigarette smoking					
Black	Smoker vs Non-smoker	-	-	-	-	- *
White	Smoker vs Non-smoker	0.01	1	0.9225	NS	no diff.
Mixed	Smoker vs Non-smoker	0.01	1	0.9046	NS	no diff.

* not enough observations in one of the groups in this categorization. Comparison not possible.

TABLE V.13

SERUM acmIgG CONCENTRATION IN CORD BLOOD : COMPARATIVE STATISTICS WITHIN ETHNIC GROUPS

ETHNIC GROUP COMPARED	CATEGORIZING VARIABLE	Mann-Whitney-U Test				
		X^2-approx. value	df	p-value	Signif at 5% level	Statistical decision
	Sex					
Black	Males vs Females	2.26	1	0.1331	NS	no diff.
White	Males vs Females	0.03	1	0.8566	NS	no diff.
Mixed	Males vs Females	0.22	1	0.6414	NS	no diff.
	Maternal Ascaris RAST					
Black	+ve vs -ve	5.59	1	0.0181	S	difference
White	+ve vs -ve	-	-	-	-	- *
Mixed	+ve vs -ve	10.69	1	0.0011	S	difference
	Atopic FH					
Black	+ve vs -ve	0.02	1	0.8791	NS	no diff.
White	+ve vs -ve	4.44	1	0.0351	S	difference
Mixed	+ve vs -ve	1.36	1	0.2427	NS	no diff.
	Maternal cigarette smoking					
Black	Smoker vs Non-smoker	-	-	-	-	- *
White	Smoker vs Non-smoker	1.40	1	0.2372	NS	no diff.
Mixed	Smoker vs Non-smoker	1.12	1	0.2900	NS	no diff.

* not enough observations in one of the groups in this categorization. Comparison not possible

TABLE V.14 (a)

CORD BLOOD PHASE I : CO-ORDINATE RESULTS FROM STATISTICAL TESTS OF COMPARISONS BETWEEN THE ETHNIC GROUPS

CATEGORIZING VARIABLES	ETHNIC GROUPS COMPARED	BLOOD PARAMETERS IN CORD BLOOD			
		IgE	TEC	P1	IgG
Ethnic group	Combined	S	? *	? *	S
	Black - White	NS *	S	NS	S
	Black - Mixed	S	NS	NS	S
	White - Mixed	NS	? *	S	S
Atopic FH					
Positive	Combined	NS	NS	NS	NS
	Black - White	NS	NS	NS	NS
	Black - Mixed	NS	NS	NS	NS
	White - Mixed	NS	NS	S	NS
Negative	Combined	S	NS	NS	NS
	Black - White	S	NS	NS	NS
	Black - Mixed	S	NS	NS	NS
	White - Mixed	NS	NS	NS	NS
Maternal Ascaris RAST					
Negative	Combined	S	NS	S	S
	Black - White	NS	NS	NS	S
	Black - Mixed	S	NS	S	S
	White - Mixed	NS	NS	S	S
Maternal Ascaris RAST and atopic FH					
-ve Ascaris RAST -ve Atopic FH	Combined	S	NS	NS	NS
	Black - White	S	NS	NS	NS
	Black - Mixed	S	NS	NS	NS
	White - Mixed	NS	NS	NS	NS

? * no definite statistical decision can be made.

TABLE V.14 (b)

CORD BLOOD PHASE I : CO-ORDINATED RESULTS FROM STATISTICAL TESTS OF COMPARISON WITHIN THE ETHNIC GROUPS

ETHNIC GROUP COMPARED	CATEGORIZING VARIABLES	BLOOD PARAMETERS IN CORD BLOOD			
		IgE	TEC	Pl	IgG
	Sex				
Black	Males vs Females	NS	NS	NS	NS
White	Males vs Females	NS	NS	NS	NS
Mixed	Males vs Females	S	NS	NS	NS
	Maternal Ascaris RAST				
Black	+ve vs -ve	NS	S	NS	S
White	- - -	-	-	-	- *
Mixed	+ve vs -ve	?**	NS	NS	S
	Atopic FH				
Black	+ve vs -ve	NS	NS	NS	NS
White	+ve vs -ve	S	NS	NS	S
Mixed	+ve vs -ve	S	NS	NS	NS
	Maternal cigarette smoking				
Black	Smoker vs Non-smoker	*	*	*	*
White	Smoker vs Non-smoker	?**	NS	NS	NS
Mixed	Smoker vs Non-smoker	NS	NS	NS	NS

* not enough observations in one of the groups in this categorization. Comparison not possible.

? ** no definite statistical decision can be made.

CHAPTER 3 ANCILLARY INVESTIGATIONS

Various ancillary investigations were done to give perspective to the data presented in Section V. Ch. 2. This data will be presented below.

3.1 THE CONCENTRATION OF THE ATOPIC MARKERS IN MATERNAL BLOOD

The four atopic markers assessed in the cord blood of the newborns were also measured in the maternal blood. These results are presented for each ethnic group in Table V.15.

3.2 THE CORRELATION BETWEEN THE CORD BLOOD AND THE MATERNAL ATOPIC MARKERS

The Spearman Correlation Coefficients for each ethnic group between the cord blood and the maternal values for the four atopic markers are summarized in Table V.16.

3.3 CORD BLOOD AND MATERNAL SERUM IgA CONCENTRATIONS

This data is presented in Table V.17.

3.4 THE CORRELATION BETWEEN CORD BLOOD AND MATERNAL SERUM IgA CONCENTRATIONS

The Spearman Correlation Co-efficients for each ethnic group between the cord blood and the maternal serum IgA concentrations are as follows:

Black ethnic group - 0.26245
White ethnic group - 0.11898
Mixed ethnic group - 0.01535

3.5 CORD BLOOD AND MATERNAL SPECIFIC SERUM IgE CONCENTRATIONS RAST (PHARMACIA)

3.5.1 Cord blood

No newborns developed specific IgE antibodies against egg white,

cow's milk, or Ascaris lumbricoides at birth as defined by a Phadebas RAST score of equal to or greater than Class 1 (equal or greater than 0.110 PRU/ml-Table IV.5).

3.5.2 Maternal blood

Specific IgE antibodies against egg white, cow's milk and Ascaris lumbricoides were measured.

The Black ethnic group included 7 mothers with a positive RAST for Ascaris lumbricoides with a Phadebas RAST Class score of 1 and 2 (between 0.110 and 0.350 PRU/ml).

The White ethnic group included 1 mother with a positive RAST for Ascaris lumbricoides with a Phadebas RAST Class score of 2 (between 0.35 and 0.7 PRU/ml) and 1 mother with a positive RAST for cow's milk with a Phadebas RAST Class score of 1 (between 0.11 and 0.35 PRU/ml).

The Mixed ethnic group included 7 mothers with a positive RAST for Ascaris lumbricoides with a Phadebas RAST Class score of 1, 2 and 3 (between 0.110 and 17.5 PRU/ml).

TABLE V.15

THE CONCENTRATION OF THE ATOPIC MARKERS IN MATERNAL BLOOD

ATOPIC MARKER	ETHNIC GROUP	SERUM IgE CONCENTRATION IN MATERNAL BLOOD										
		NO.	Arith Mean	SD	Median	GM	Max	Min	Range	Q_3 (75%)	Q_1 (25%)	Q_3-Q_1
Serum IgE (kU/l)	Combined	163	215.0	309.7	109.0	94.4	2047.0	0.5	2046.5	240.2	43.0	197.2
	Black	53	270.1	309.8	150.2	137.0	1208.8	6.9	1201.9	359.0	51.8	307.2
	White	52	112.1	166.8	49.0	47.5	897.0	0.5	896.5	119.0	24.3	94.7
	Mixed	58	256.8	382.0	113.4	124.2	2047.0	8.9	2038.1	260.0	52.1	207.9
Total eosinophil count(cells/mm^3)	Combined	158	305	759	177	-	9000	0	9000	311	111	200
	Black	52	250	175	239	-	888	0	888	355	111	244
	White	50	213	144	247	-	1688	22	1666	250	88	162
	Mixed	56	439	177	1238	-	9000	0	9000	285	117	168
Platelet count ($x10^9$/l)	Combined	151	268	263	69	-	484	122	362	319	213	106
	Black	48	261	260	76	-	484	122	362	319	211	108
	White	47	277	271	70	-	427	149	278	319	229	90
	Mixed	56	267	265	61	-	382	138	244	319	209	110
acm IgG (ΔO.D. 414nm)	Combined	162	58.2	50.9	28.8	-	134.8	5.1	129.7	76.4	36.4	40.0
	Black	53	51.2	45.2	23.8	-	123.3	16.2	107.1	62.0	34.7	27.3
	White	51	65.3	63.4	32.4	-	134.8	9.7	125.1	90.5	39.4	51.1
	Mixed	58	58.3	53.1	28.5	-	-120.6	5.1	115.5	75.5	35.9	39.6

TABLE V.16

THE SPEARMAN CORRELATION CO-EFFICIENTS : CORD BLOOD VERSUS MATERNAL ATOPIC MARKER VALUES

ETHNIC GROUP	MATERNAL BLOOD \ CORD BLOOD	IgE	IgG	Total eosinophils	Platelets
Black	IgE	0.34089	-0.07254	0.05828	-0.02815
	IgG	0.02874	0.83802	-0.08002	-0.21383
	Total eosinophils	0.13159	-0.18937	-0.03635	-0.08345
	Platelets	-0.20200	-0.03499	0.08160	0.16938
White	IgE	0.25636	0.22484	0.31645	-0.02978
	IgG	-0.06439	0.88197	0.23807	0.06720
	Total eosinophils	0.00463	-0.19848	0.17647	-0.26806
	Platelets	-0.11875	0.03466	0.19276	0.11889
Mixed	IgE	0.61713	0.10213	-0.23999	-0.16997
	IgG	0.17729	0.83453	-0.02079	-0.03137
	Total eosinophils	0.01427	-0.17079	0.25559	0.18302
	Platelets	-0.13102	0.00832	-0.05734	0.23645

TABLE V. 17

CORD BLOOD AND MATERNAL sIgA CONCENTRATIONS

CATEGORIZING VARIABLE	ETHNIC GROUP	NO.	Mean	Median	SD	Max	Min	Range (75%)	Q_3 (25%)	Q_1	Q_3-Q_1
Cord Blood	Combined	163	2.60	2.51	0.69	5.70	0.83	4.87	2.93	2.16	0.77
	Black	53	2.85	2.89	0.72	5.70	1.16	4.54	3.21	2.47	0.74
	White	52	2.30	2.27	0.42	3.25	0.83	2.42	2.51	2.05	0.46
	Mixed	58	2.64	2.53	0.77	5.65	1.58	4.07	2.95	2.14	0.81
Maternal	Combined	163	214	184	227	2690	35	2655	230	139	91
	Black	53	301	218	371	2690	126	2564	285	179	106
	White	52	135	132	56	288	35	253	168	89	79
	Mixed	58	206	209	68	380	62	318	242	156	86

CHAPTER 4 SUMMARY OF PHASE 1 RESULTS

4.1 CATEGORIZATION ON AN ATOPIC FAMILY HISTORY (Table V.1.)

The White ethnic group had the highest number of newborns with positive aFH (39 = 75%) followed by the Mixed ethnic group (18 = 31%) and then the Black ethnic group (5 = 9.4%).

4.2 CORD BLOOD SERUM IgE CONCENTRATIONS (TABLES V.2, V.6 and V.10)

The key data from this section is summarized in Table V.18.

4.2.1 Ethnic differences

The Black newborns had the hignest geometric mean and median CBsIgE, followed by the White and then the Mixed newborns, In the sub-groups of newborns with a negative aFH, a negative maternal Ascaris RAST and in the sub-group which included only those newborns with both a negative aFH and a negative maternal Ascaris RAST, the Black newborns had the highest values.

Statistical comparison of the CBsIgE between the uncategorized total ethnic group samples revealed significant ethnic differences between the Black-Mixed newborns. These differences persisted when each ethnic group was categorized, and further significant differences between the Black-White newborns were demonstrated in the sub-group with neither an aFH nor immunological evidence of maternal Ascariasis (i.e. -ve maternal RAST for Ascaris lumbricoides).

In the sub-group with a negative aFH, the ethnic differences between the Black-White and Black-Mixed newborns persisted. The effect of a positive aFH obscured this difference, however, and the sub-group with a positive aFH revealed no statistical difference in CBsIgE between any of the ethnic groups.

4.2.2 The influence of an atopic family history

An aFH influenced the CBsIgE in the White and Mixed newborns, but not in the Black newborns. It should be noted, however, that only 5 Black newborns had an aFH. The distribution of the CBsIgE values (Fig's. V.1, V.2 and V.3) with reference to either a positive or a

negative atopic revealed that, in the Black newborns, only 2 (14.3%) with CBsIgE greater than or equal to 0.50kU/l had an aFH. The remaining 12 newborns with CBsIgE of 0.50kU/l or above had no aFH. This included one newborn with a value of 15.00kU/l, the highest recorded value for CBsIgE recorded in the literature. In contrast, the 9 White neonates with CBsIgE greater or equal to 0.50kU/l all (100%) had an aFH. The Mixed group had a ambivalent distribution pattern. Of the 11 newborns with CBsIgE greater than or equal to 0.5kU/l, 6 (58.3%) had an aFH.

4.2.3 The influence of maternal Ascariasis

Maternal Ascariasis had an equivocal influence on the CBsIgE values of the Mixed newborns ($p = 0.05$), but no influence on these values in the Black newborns. Only one White mother had a -ve Ascaris RAST, making statistical comparison impossible for this ethnic group.

4.2.4 The influence of the newborn's sex

The sex of the newborn influenced the CBsIgE values in the Mixed but not in the Black or the White newborns.

4.2.5 The influence of maternal cigarette smoking

Maternal smoking did not influence the CBsIgE values for any of the ethnic groups.

4.3 CORD BLOOD TOTAL EOSINOPHIL COUNTS (TABLES V.3, V.7 and V.11)

The key data from this section is summarized in Table V.19.

4.4 CORD BLOOD PLATELET COUNTS (TABLES V.4, V.8 and V.12)

The key data from this section is summarised in Table V.20.

4.5 CORD BLOOD ANTI-COWS MILK IgG CONCENTRATIONS (TABLES V.5, and V.13)

The dey data from this section is summarized in Table V.21.

TABLE V.18

FACTORS INFLUENCING CORD BLOOD SERUM IgE CONCENTRATIONS WITHIN EACH ETHNIC GROUP

CATEGORIZING VARIABLE	ETHNIC NEWBORNS INFLUENCED BY VARIABLE	ETHNIC NEWBORNS NOT INFLUENCED BY VARIABLE
An atopic FH	White Mixed	Black
Maternal Ascariasis	? Mixed **	Black (*Whites)
Sex of the newborn	Mixed	Black White
Maternal smoking		Black White Mixed

*Only 1 observation with a positive maternal Ascaris RAST in White newborns precluded statistical comparison in this group.

CONVERSELY

1. Black CBsIgE influenced by: - None of the variables.
2. White CBsIgE influenced by: - An aFH.
3. Mixed CBsIgE influenced by: - An aFH.
   - ? Maternal Ascariasis **.
   - Sex of the newborn.
4. Ethnic differences in CBsIgE, independently of Maternal Ascariasis and an aFH, were present between the Black-White and the Black-Mixed newborns.

? ** no definite statistical decision can be made.

TABLE V.19

FACTORS INFLUENCING CORD BLOOD TOTAL EOSINOPHIL COUNTS WITHIN EACH ETHNIC GROUP

CATEGORIZING VARIABLE	ETHNIC NEWBORNS INFLUENCED BY VARIABLE	ETHNIC NEWBORNS NOT INFLUENCED BY VARIABLE
An atopic FH		Black White Mixed
Maternal Ascariasis	Black	Mixed (*Whites)
Sex of the newborn		Black White Mixed
Maternal smoking		Black White Mixed

*Only 1 observation with a positive maternal Ascaris RAST in White newborns precluded statistical comparison in this group.

CONVERSELY

1. Black CBTEC's influenced by: - Maternal Ascariasis.
2. White CBTEC's influenced by: - None of the variables.
3. Mixed CBTEC's influenced by: - None of the variables
4. Ethnic differences in CBTEC's were present in the uncategorized total group samples between the Black-White groups, and marginally, between the White-Mixed groups ($p = 0.05$). These differences disappeared, however, when only the newborns with both a negative aFH and a negative maternal Ascaris RAST were compared.

TABLE V.20

FACTORS INFLUENCING CORD BLOOD PLATELET COUNTS WITHIN

EACH ETHNIC GROUP

CATEGORIZING VARIABLE	ETHNIC NEWBORNS INFLUENCED BY VARIABLE	ETHNIC NEWBORNS NOT INFLUENCED BY VARIABLE
An atopic FH		Black White Mixed
Maternal Ascariasis		Black (*Whites) Mixed
Sex of the newborn		Black White Mixed
Maternal smoking		Black White Mixed

*Only 1 observation with a positive maternal Ascaris RAST in White newborns precluded statistical comparison in this group.

CONVERSELY

1. Black CBPlC's influenced by: - None of the variables.
2. White CBPlC's influenced by: - None of the variables.
3. Mixed CBPlC's influenced by: - None of the variables.
4. Ethnic differences in CBPlC's were present in the uncategorized total group of newborns between the White-Mixed groups. These differences disappeared, however, when only the newborns with both a negative aFH and a negative maternal Ascaris RAST were compared.

TABLE V.21

FACTORS INFLUENCING CORD BLOOD IgG CONCENTRATIONS WITHIN EACH ETHNIC GROUP

CATEGORIZING VARIABLE	ETHNIC NEWBORNS INFLUENCED BY VARIABLE	ETHNIC NEWBORNS NOT INFLUENCED BY VARIABLE
An atopic FH	White	Black Mixed
Maternal Ascariasis	Black Mixed	(*Whites)
Sex of the newborn		Black White Mixed
Maternal smoking		Black White Mixed

*Only 1 observation with a positive maternal Ascaris RAST in White newborns precluded statistical comparison in this group.

CONVERSELY

1. Black CBacmIgG influenced by: - Maternal Ascariasis.
2. White CBacmIgG influenced by: - An aFH.
3. Mixed CBacmIgG influenced by: - Maternal Ascariasis.
4. Ethnic differences in CBacmIgG were present in the uncategorized total group of newborns between the Black-White, and the White-Mixed newborns. These differences disappeared, however, when only the newborns with both a negative aFH and a negative maternal Ascaris RAST were compared.

SECTION VI

RESULTS - PHASE II

CHAPTER I TECHNICAL DETAILS PERTAINING TO THE DATA ANALYSIS

1.1 INTRODUCTION

In order to establish the relevance of the four cord blood atopic markers as predictive markers for the development of atopic sensitisation in infancy for each ethnic group (Aim of Phase II - Section IV, Ch. 2.1), it was essential to establish as far as possible, unambiguous criteria for the classification of the infants at the end of the 1 year study period into either:

(i) the group who developed atopy during infancy
or
(ii) the group who never developed atopy during infancy.

A firm and confident diagnosis of atopy is not always easy in studies of this nature. Firstly, the follow-up period was limited, and did not allow the repeated observation of the recurrent clinical manifestations which are the hallmark of the natural history of atopic disease. Secondly, the availability of special laboratory investigations supporting the diagnosis of atopic disease (Halpern et al, 1980) was not feasible in this study, since the well-documented atopic markers such as the total sIgE or the TEC were the variables under scrutiny in this study. Finally, most of the published prospective studies on atopic disease in infancy have used inconsistent and varied criteria for atopic manifestations (Halpern et al, 1973; Orgel et al, 1975 Kjellman and Johansson, 1976; Matthew et al, 1977; Michel et al, 1980b; Frick, 1981; Kjellman and Croner, 1984; Svensson et al, Cant et al, 1985;). Consequently, no definitive guidelines for the criteria for atopic manifestations in infancy exist.

For the purposes of this study, the criteria used to categorize an infant as either "atopic, or "not atopic" at the end of the 1 year

follow-up period were based on a combination of clinical and immunological parameters. At the end of each follow-up visit these parameters were merged into a Rationalized Atopic Status (RAS) categorization for each infant. At the end of the 1 year study period a Cumulative Adjusted Atopic Status (CAS) categorization of either "atopic" or "not atopic" was given to each infant. The statistical analysis for Phase II was then based on the relationship between the specific cord blood atopic marker value in each newborn and the infant's CAS at the end of the study.

1.2 CRITERIA FOR THE RATIONALISED ATOPIC STATUS (RAS) OF THE INFANT AT EACH FOLLOW-UP VISIT

Table VI. 1. summarises the steps involved in the determination of the RAS for each infant at each follow-up visit. It should be noted that the RAS is derived from both clinical and immunological parameters.

1.2.1 The clinical categorization (cc)

Most of the studies of this nature (Section VI. Ch. 1.1) have used a system whereby infants were categorized clinically as being:

(i) Not atopic
(ii) Possibly or probably atopic
(iii) Definitely or obviously atopic

This study used the same categorizations for the cc. The criteria for the cc of the infants is discussed in Section VIII. Ch. 1.3.

1.2.2 The immunological categorization (ic)

At each follow-up visit, the infants had their serum analysed by RAST (Pharmacia) for the development of specific serum IgE antibodies to the egg white, cow's milk and Dermatophygoides pteronyssinus antigens (Section IV. Ch. 2.3.8). Since the RAST's for egg white and cow's milk were all negative at birth, (Section V. Ch. 3.5.1) a positive RAST for these antigens during the follow-up period suggested subsequent atopic sensitisation.

The addition of the RAST for Dermatophygoides pteronyssinus was included for the reason that the house dust mite is a commonly occurring inhalant allergen on the coastal belt of the Southern African continent, where this study was executed (Ordman, 1971).

The RAST for any of the allergens tested was considered to be positive if it had a Phadebas RAST score of Class 1, 2, 3 or 4 (Table IV. 2). A RAST score of Class 0 was regarded as negative. The ic for each visit was given to each infant after consideration of the three RAST results in the following manner (Table VI. 1.):

(i) If the RAST for one or more of the three allergens was positive, an ic of RAST positive for that particular follow-up visit was given to the infant.

(ii) If the RAST for all of the three allergens tested was negative, an ic of RAST negative for that particular follow-up visit was given to the infant.

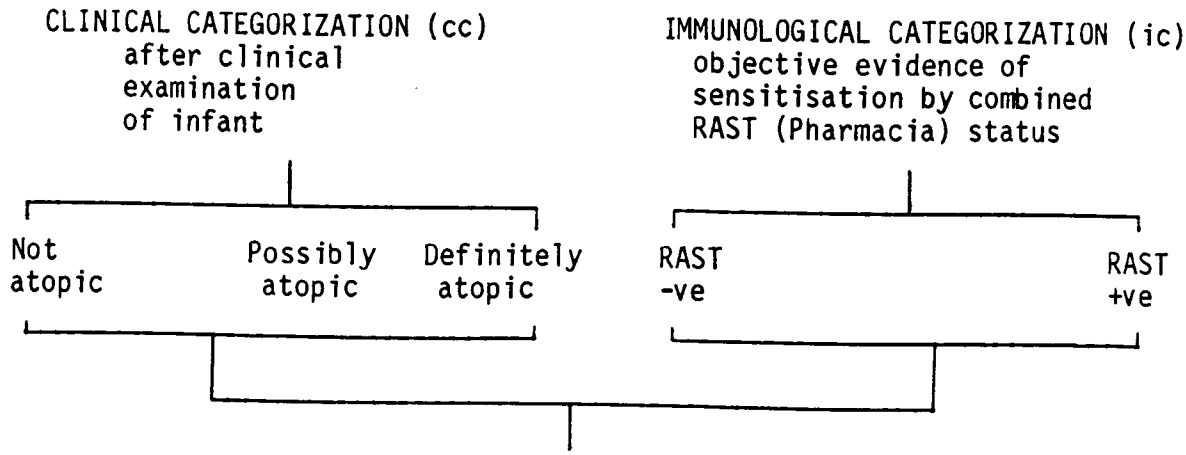
1.2.3 The Rationalized Atopic Status (RAS)

The clinical and the immunological categorizations for each infant were merged to produce a RAS categorization at the end of each follow-up visit (Table VI. I.).

The cc took dominance over the ic in this regard. Consequently a cc of "definitely atopic" gave a RAS of "atopic" irrespective of the outcome of the combined RAST status. Similarly, a cc of "not atopic" gave a RAS of "not atopic" irrespective of the outcome of the combined RAST status. Only where the cc revealed a "possibly atopic" status, were these infants re-distributed into either the "atopic" or the "non-atopic" RAS group depending on their ic. In this group, those with a positive ic acquired the RAS of "atopic" for that particular visit, while those with a negative ic acquired the RAS of "not atopic" for that particular visit.

TABLE VI. I

THE DETERMINATION OF THE RATIONALISED ATOPIC STATUS (RAS) AT EACH FOLLOW-UP VISIT (3, 7 AND 12 MONTHS)



		CLINICAL CATEGORIZATION		
▨ Atopic □ Not Atopic		Definitely atopic	Possibly atopic	Not atopic
IMMUNO-LOGICAL CATEGO-RIZATION	RAST positive	▨	▨	
	RAST negative	▨		

Summary:

Clinical categorization (cc)	Immunological categorization (ic)	Rationalised Atopic Status
Definitely atopic	RAST +ve	Atopic
Definitely atopic	RAST -ve	
Possibly atopic	RAST +ve	
Possibly atopic	RAST -ve	Not atopic
Not atopic	RAST +ve	
Not atopic	RAST -ve	

The rationale for using a positive RAST as a confirmatory objective laboratory indicator for the diagnosis of atopic sensitisation in those infants where the clinical picture was equivocal (i.e. the "possibly atopic" group) was based on the suggestion by Zeiger (1985) that the development of specific IgE to egg and other antigens could be especially helpful in differentiating non-atopic infantile seborrhoeic dermatitis from atopic dermatitis. It seemed reasonable to extrapolate this approach to the infants with other "possibly atopic" clinical disease entities, and to re-classify those infants with positive RAST's into the "definitely allergic" group, and those infants with negative RAST's into the "non-allergic" group. This was, in the author's opinion, the most logical approach to differentiate the clinically categorized "possibly atopic" infants into those who shared objective immunological evidence of atopic sensitisation and those who remained unsensitized. It is accepted that the RAST monitored only two food antigens and one inhalent antigen, but these antigens have been shown, by Michel et al, (1980b); Frick, (1981); Zeiger, (1985) and Croner et al, (1982) to be the most important ones at this age.

1.3 CRITERIA FOR THE CUMULATIVE ADJUSTED ATOPIC STATUS (CAS) CATEGORIZATION FOR EACH INFANT AT THE END OF THE STUDY PERIOD

At the end of the one year study period, the infant's RAS for each of the three follow-up visits were considered in order to allocate the CAS for each infant. If an infant had a RAS of "atopic" for one or more of the follow-up visits, a CAS of "atopic" for the total follow-up period was given, irrespective of whether or not the RAS for any of the other visits were "not atopic" or if the infant dropped out subsequently. If an infant had a RAS of "not atopic" for each and all of the follow-up visits, a CAS of "not atopic" for the total follow-up period was given.

If an infant had a RAS of "not atopic" for the initial follow-up visits, and then did not present for the subsequent visits, the CAS was not computed for that infant, since it is technically feasible that the infant may have acquired an "atopic" RAS for the succeeding visits. Such infants were then excluded from the statistical analysis for Phase II, and were regarded as "drop-outs" or "non-responders".

Alternately, if the infant did not attend for the initial follow-up visits, but attended subsequently until the completion of the study then the infant remained included in the study, and received a CAS for the study period, based on the RAS at the visits which the infant attended (i.e. a CAS of "atopic" if one or more of the RAS categorizations was "atopic" and a CAS of "not atopic" if all of the RAS categorizations which were available for consideration were "not atopic"). The rationale for this decision is that if the infant was "atopic" at 3 months, this would have become evident from the clinical and immunological categorizations at the RAS during the subsequent follow-up visits.

CHAPTER 2 THE RATIONALISED ATOPIC STATUS AND THE CUMULATIVE ADJUSTED ATOPIC STATUS FOR EACH INFANT DURING THE STUDY PERIOD

The data necessary to compute tne RAS at each follow-up visit, and the CAS at the end of the one-year study period is summarised in Taole's VI. 2, VI. 3 and VI. 4 for the Black, White and Mixed infants respectively.

THE CODES USED TO ABBREVIATE THE PARAMETERS PRESENTED ARE AS FOLLOWS:

CODE	CATEGORIZATION	SCORE	INTERPRETATION
cc	clinical categorization (at each follow-up visit)	1 2 3 .	- not atopic - possibly atopic - definitely atopic - missing (infant not present at follow-up)
E	egg white RAST	0 1-4 .	- not sensitised - Phadebas RAST class - missing (result not available)
M	cow's milk RAST	0 1-4 .	- not sensitised - Phadebas RAST class - missing (result not available
D	Dermatophygoides pteronyssinus RAST	0 1-4	- not sensitised - Phadebas RAST class
ic	immunological categorization (at each follow-up visit)	0 1	- not sensitised to E, M and D (RAST -ve) - sensitised to either E or M or D (RAST +ve)
RAS	Rationalised Atopic Status	1 3	- not atopic - atopic
CAS	Cumulative Adjusted Atopic Status	1 3	- not atopic during first year - atopic during first year
Inf.code	infant's code number	.	- drop out (not analysed for Phase II)
NO.	number of infants in eacn race group		

TABLE VI. 2

INDIVIDUAL RATIONALISED ATOPIC STATUS AND CUMULATIVE ADJUSTED ATOPIC STATUS: CLINICAL AND IMMUNOLOGICAL PARAMETERS*

BLACK ETHNIC GROUP. (NO. = 53)

Inf. code	3 month visit						7 month visit						12 month visit						CAS
	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	
200	2	0	2	0	1	3	3	0	2	0	1	3	3	0	1	0	1	3	3
201	1	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
202	1	0	0	0	0	1	2	0	1	0	1	3	3	0	0	0	0	3	3
203	.	.	.	.	.	.	1	0	0	0	0	1	1	0	0	0	0	1	1
204	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
205	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
206	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
207	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
208	2	0	1	0	1	3	.	.	.	.	.	.	.	.	.	.	.	.	3
209	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
210	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
211	2	0	1	0	1	3	3	0	0	0	0	3	3	0	0	0	0	3	3
212	3	0	1	0	1	3	.	.	.	.	.	.	.	.	.	.	.	.	3
213	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
214	3	0	0	0	0	3	3	1	2	3	1	3	.	.	.	.	.	.	3
215	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
216	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
217	2	0	0	0	0	1	3	1	0	0	1	3	3	1	0	3	1	3	3
218	3	1	0	0	1	3	.	.	.	.	.	.	.	.	.	.	.	.	3
219	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
221	1	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
222	1	0	0	0	0	1	1	0	0	0	0	1	2	0	0	0	0	1	1
223	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

* For code abbreviation key and interpretation, see page 161.

TABLE VI. 2 (Cont)

Inf. code	3 month visit						7 month visit						12 month visit						CAS
	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	
224	3	0	0	0	0	3	3	0	1	0	1	3	.	.	.	.	.	.	3
225	2	0	1	0	1	3	3	0	1	0	1	3	.	.	.	.	.	.	3
226	1	0	0	0	0	1	1	0	0	0	0	1	.	.	.	.	.	.	.
227	1	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
228	3	0	1	0	1	3	3	.	.	.	.	3	3	.	.	.	.	3	3
229	2	0	0	0	0	1	2	0	0	0	0	1	.	.	.	.	.	.	.
231	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
232	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
233	2	0	0	0	0	1	2	0	0	0	0	1	.	.	.	.	.	.	.
234	1	0	0	0	0	1	2	0	1	0	1	3	3	.	.	.	.	3	3
235	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
236	3	0	1	0	1	3	3	0	1	0	1	3	3	0	0	0	0	3	3
300	1	0	0	0	0	1	1	0	0	0	0	1	.	.	.	.	.	.	.
301	1	0	0	0	0	1	1	0	0	0	0	1	3	0	0	0	0	3	3
302	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
303	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
304	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
305	1	0	0	0	0	1	1	0	0	0	0	1	.	.	.	.	.	.	.
306	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
307	1	0	0	0	0	1	1	0	0	0	0	1	.	.	.	.	.	.	.
308	1	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
309	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
310	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
311	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
312	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
313	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
314	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
315	1	0	0	0	0	1	1	0	0	0	0	1	.	.	.	.	.	.	.
316	3	0	0	0	0	3	.	.	.	.	.	.	.	.	.	.	.	.	.
317	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TABLE VI. 3

INDIVIDUAL RATIONALISED ATOPIC STATUS AND CUMULATIVE ADJUSTED ATOPIC STATUS: CLINICAL AND IMMUNOLOGICAL PARAMETERS*

WHITE ETHNIC GROUP. (NO. = 52)

Inf. code	3 month visit						7 month visit						12 month visit						CAS
	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	
401	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
402	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
403	3	3	1	0	1	3	3	4	3	0	1	3	3	4	4	1	1	3	3
404	1	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
405	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
406	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
407	2	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
408	1	0	.	0	0	1	1	.	0	.	.	1	.	.	.	.	.	.	.
409	3	2	0	0	1	3	3	1	0	0	1	3	3	0	0	2	1	3	3
411	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
412	2	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
413	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
414	2	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
416	2	0	0	0	0	1	2	0	0	0	0	1	2	0	0	0	0	1	1
417	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
418	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
419	2	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
420	1	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
421	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
422	2	0	.	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
423	1	0	.	0	0	1	1	0	0	0	0	1	2	0	0	0	0	1	1
424	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
425	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
426	2	0	0	0	0	1	2	0	0	0	0	1	2	0	0	0	0	1	1

* For code abbreviation key and interpretation, see page 161.

TABLE VI. 3 (cont)

Inf. code	3 month visit						7 month visit						12 month visit						CAS
	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	
427	1	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
428	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
429	2	0	0	0	0	1	1	0	0	0	0	1	2	0	0	0	0	1	1
430	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
431	3	0	0	0	0	3	3	1	0	0	1	3	3	0	0	0	0	3	3
432	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
433	2	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
434	2	0	0	0	0	1	2	0	0	0	0	1	3	0	0	0	1	3	3
435	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
436	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
437	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
438	2	0	0	0	0	1	3	0	1	0	1	3	3	0	1	0	1	3	3
439	1	0	0	0	0	1	2	0	0	0	0	1	2	0	0	0	0	1	1
440	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
441	3	1	0	0	1	3	3	0	0	0	0	3	3	0	0	0	0	3	3
442	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
443	1	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
445	3	0	0	0	0	3	.	.	.	.	.	.	.	.	.	.	.	.	3
446	3	0	0	0	0	3	3	0	0	0	0	3	.	0	0	0	0	.	3
447	2	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
448	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
449	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
450	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
451	1	0	.	0	0	1	3	2	3	0	1	3	3	2	2	2	1	3	3
452	3	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	3
453	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
454	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
455	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1

TABLE VI. 4

INDIVIDUAL RATIONALISED ATOPIC SCORE AND CUMULATIVE ADJUSTED ATOPIC STATUS: CLINICAL AND IMMUNOLOGICAL PARAMETERS*

MIXED ETHNIC GROUP. (NO. = 58)

Inf. code	3 month visit						7 month visit						12 month visit						CAS
	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	cc	E	M	D	ic	RAS	
2	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
3	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
4	1	0	0	0	0	1	1	0	0	0	0	1	.	.	.	.	.	.	.
5	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3	0	0	0	0	3	.	.	.	.	.	.	.	.	.	.	.	.	3
8	2	0	1	0	1	3	3	0	1	0	1	3	3	0	0	0	0	3	3
9	1	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
10	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
11	2	0	1	0	1	3	.	.	.	.	.	.	.	.	.	.	.	.	3
12	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
13	2	1	1	0	1	3	3	2	2	0	1	3	3	2	2	0	1	3	3
14	2	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
15	3	0	0	0	0	3	3	0	0	0	0	3	3	2	0	0	1	3	3
16	1	0	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	1
17	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
18	1	0	0	0	0	1	1	0	0	0	0	1	2	0	0	0	0	1	1
19	2	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
20	3	0	1	0	1	3	3	0	1	0	1	3	3	1	0	0	1	3	3
21	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
22	1	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
23	1	.	.	.	.	1	1	0	0	0	0	1	1	0	0	0	0	1	1
24	2	0	1	0	1	3	3	0	1	0	1	3	3	0	0	0	0	3	3
25	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
26	1	0	0	0	0	1	3	1	1	2	1	3	3	0	0	3	1	3	3
27	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	2	1	1	1

* For code abbreviation key and interpretation, see page 161.

TABLE VI. 4 (cont)

Inf. code	3 month visit						7 month visit						12 month visit						CAS
	CC	E	M	D	ic	RAS	CC	E	M	D	ic	RAS	CC	E	M	D	ic	RAS	
28	2	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.
29	3	0	0	0	0	3	3	.	.	.	.	3	3	.	.	.	.	3	3
30	1	0	0	0	0	1	.	0	.	.	.	.	.	0	.	.	.	.	.
31	2	0	0	0	1	3	.	.	0	.	.	.	.	.	0	.	.	.	.
32	1	0	0	0	0	1	3	2	3	1	1	3	3	2	2	3	1	3	3
33	3	3	3	0	1	3	.	0	.	.	.	.	.	0	.	.	.	.	.
34	2	0	0	1	0	1	3	0	3	1	1	3	3	0	2	3	1	3	3
35	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
36	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
37	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
38	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
39	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
40	3	3	0	0	1	3	3	1	1	0	1	3	3	1	1	0	1	3	3
41	3	3	1	0	1	3	3	1	1	0	1	3	3	1	1	0	1	3	3
42	3	0	1	0	1	3	2	0	0	0	0	1	1	0	0	0	0	1	1
43	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
44	.	.	.	.	.	.	2	0	0	0	0	1	1	0	0	0	0	1	1
45	.	.	.	.	.	.	1	0	0	0	0	1	1	0	0	0	0	1	1
46	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
47	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
48	.	.	.	.	.	.	1	0	0	0	0	1	1	0	0	0	0	1	1
49	1	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
50	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
52	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
53	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
55	3	0	1	0	1	3	3	1	1	0	1	3	3	1	1	0	1	3	3
56	3	0	1	0	1	3	3	1	1	0	1	3	3	1	1	0	1	3	3
57	1	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
58	2	0	0	0	0	1	3	0	0	0	0	3	3	0	0	0	0	3	3
100	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
101	3	0	0	0	0	3	3	0	0	0	0	3	3	0	0	0	0	3	3
102	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

CHAPTER 3 DESCRIPTIVE STATISTICS OF THE ATOPIC MARKERS DURING THE FOLLOW-UP VISITS

3.1 CATEGORIZATION BY ETHNICITY AND FOLLOW-UP VISIT

The descriptive data for the atopic markers, serum IgE concentrations, total eosinophil counts, platelet counts and serum anti-cow's milk IgG concentrations are presented in Table's VI. 5, VI. 6, VI. 7 and VI. 8, for each ethnic group at each follow-up period.

3.2 CATEGORIZATION BY ETHNICITY, RATIONALISED ATOPIC STATUS AND FOLLOW-UP VISIT

The descriptive data for the atopic markers, serum IgE concentrations, total eosinophil counts, platelet counts and serum anti-cow's milk IgG concentrations for the "atopic" and the "not atopic" RAS categorizations are presented in Tables VI. 9, VI. 10, VI. 11 and VI. 12 for each ethnic group at each follow-up visit.

3.3 GRAPHIC DISTRIBUTION OF CUMULATIVE ADJUSTED ATOPIC STATUS AGAINST CORD BLOOD SERUM IgE CONCENTRATIONS

This data is graphically represented in Fig's VI. 1, VI. 2 and VI. 3.

TABLE VI. 5

DESCRIPTIVE STATISTICS: SERUM IgE CONCENTRATIONS AT 3, 7, AND 12 MONTHS

(kU/l)

STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
NO.	35	27	16	41	36	38	51	43	41
Mean (arith)	12.11	49.81	44.10	7.54	27.69	62.95	19.14	30.59	39.49
SD (arith)	16.67	118.80	47.41	18.59	107.39	303.79	80.77	69.80	64.29
Max	92.40	591.00	179.00	95.00	645.00	1829.99	574.00	425.00	310.00
Q_3	14.80	34.00	68.19	3.30	9.36	10.39	5.86	30.00	39.75
Median	6.86	10.00	41.13	1.53	2.97	5.05	2.45	6.91	15.14
Q_1	3.16	5.79	6.75	0.28	1.23	2.33	1.47	3.05	5.01
Min	0.38	0.90	1.39	0.09	0.24	0.65	0.29	0.60	0.68
GM	6.50	13.62	22.22	1.41	4.03	6.17	3.55	9.42	14.92
SD (geom)	3.24	4.61	3.97	5.85	5.32	4.62	4.20	4.53	4.42

TABLE VI. 6

DESCRIPTIVE STATISTICS: TOTAL EOSINOPHIL COUNTS AT 3, 7, AND 12 MONTHS

(cell/mm^3)

STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
NO.	35	27	16	41	36	35	51	43	39
Mean (arith)	220	285	376	325	270	276	232	300	316
SD (arith)	117	261	355	338	175	205	137	261	184
Max	922	1330	1440	2177	666	811	622	1400	666
Q_3	266	333	333	377	371	333	333	355	466
Median	177	200	300	244	222	200	222	200	244
Q_1	88	133	166	177	139	133	111	133	155
Min	66	66	66	44	44	66	66	66	44

TABLE VI. 7

DESCRIPTIVE STATISTICS: PLATELET COUNTS AT 3, 7, AND 12 MONTHS

($\times 10^9/l$)

STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
NO.	35	27	16	36	36	36	52	42	39
Mean (arith)	601	595	530	612	553	506	615	548	507
SD (arith)	138	121	127	157	191	124	150	119	102
Max	903	866	783	1270	1359	998	1074	887	712
Q_3	695	656	631	622	603	544	709	614	566
Median	565	574	474	599	511	483	593	513	515
Q_1	501	513	450	520	456	435	495	465	435
Min	270	370	317	410	315	315	392	376	296

TABLE VI. 8

DESCRIPTIVE STATISTICS: SERUM ANTI-COW'S MILK IgG CONCENTRATIONS AT 3, 7 AND 12 MONTHS

(Δ O.D. 414nm)

STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
NO.	35	26	16	39	34	34	52	38	39
Mean (arith)	80.0	56.3	131.6	42.4	49.4	85.1	54.7	53.8	100.2
SD (arith)	25.7	15.5	21.2	32.4	30.5	39.4	24.3	12.1	40.8
Max	105.3	78.0	177.2	135.1	119.6	190.7	105.3	71.5	178.7
Q_3	100.5	68.1	145.7	67.4	80.8	114.4	62.3	61.7	134.0
Median	93.5	58.0	132.9	24.1	37.1	89.9	52.4	56.6	101.4
Q_1	58.4	48.2	119.7	18.0	25.2	53.5	37.1	46.2	77.0
Min	19.2	18.9	94.6	5.0	14.6	20.6	13.7	23.1	0.0

TABLE VI. 9

DESCRIPTIVE STATISTICS CATEGORIZED ON RATIONALISED ATOPIC STATUS AT 3, 7 AND 12 MONTHS

SERUM IgE CONCENTRATIONS (kU/l)

RAS	STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
		3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
Not atopic	NO.	23	17	9	32	21	20	31	22	20
	Mean (arith)	5.85	7.79	14.74	3.80	3.03	3.41	2.87	8.75	17.99
	SD (arith)	5.24	5.20	15.83	9.64	3.63	2.13	2.99	12.10	26.28
	Max	24.12	20.00	42.62	50.00	16.00	7.90	16.00	42.00	115.00
	Q_3	7.18	11.00	27.63	2.11	4.27	5.18	4.24	7.02	27.01
	Median	4.22	6.81	6.89	1.00	1.43	3.09	2.12	3.72	7.05
	Q_1	2.46	4.61	4.81	0.21	0.93	1.69	1.10	1.87	2.70
	Min	0.38	0.90	1.39	0.09	0.24	0.65	0.29	0.60	0.68
	GM	3.99	5.85	8.90	0.95	1.76	2.74	1.96	4.20	8.30
	SD (geom)	2.67	2.43	2.99	4.77	2.95	2.06	2.46	3.35	3.70
Atopic	NO.	12	10	7	9	15	16	20	21	21
	Mean (arith)	24.09	121.25	81.86	20.85	62.22	137.36	44.37	53.48	59.98
	SD (arith)	23.79	178.10	48.27	33.35	163.16	452.43	126.70	94.88	81.89
	Max	92.40	591.00	179.00	95.00	645.00	1829.99	574.00	425.00	310.00
	Q_3	31.79	143.50	93.00	36.50	35.00	44.43	26.00	59.00	77.06
	Median	15.95	49.50	75.01	6.65	10.00	11.68	5.65	23.00	32.60
	Q_1	11.42	29.50	44.26	1.08	2.49	4.82	2.66	6.92	11.75
	Min	2.31	5.45	41.00	0.49	1.41	2.64	0.94	1.78	0.97
	GM	16.61	57.32	72.03	5.75	12.88	17.00	8.96	21.99	26.07
	SD (geom)	2.56	3.67	1.70	6.16	5.65	5.39	5.12	3.80	4.28

TABLE VI. 10

DESCRIPTIVE STATISTICS CATEGORIZED ON RATIONALISED ATOPIC STATUS AT 3, 7 AND 12 MONTHS

TOTAL EOSINOPHIL COUNTS (cells/mm^3)

RAS	STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
		3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
Not atopic	NO.	23	17	9	32	21	20	31	22	19
	Mean (arith)	212	227	446	271	209	479	238	234	308
	SD (arith)	148	149	459	164	84	94	151	164	185
	Max	666	622	1440	822	377	756	622	666	644
	Q_3	311	244	655	333	255	535	333	305	466
	Median	155	200	311	222	200	483	200	166	200
	Q_1	111	133	122	177	144	414	111	128	155
	Min	66	66	66	66	77	315	66	66	111
Atopic	NO.	12	10	7	9	15	16	20	21	20
	Mean (arith)	234	384	285	518	356	539	221	370	324
	SD (arith)	230	375	133	643	230	150	115	324	187
	Max	922	1330	555	2177	666	998	466	1400	666
	Q_3	261	516	333	522	622	580	322	511	494
	Median	189	222	266	377	377	682	222	222	355
	Q_1	88	166	200	200	111	443	139	155	158
	Min	66	88	155	44	44	420	66	88	44

TABLE VI. 11

DESCRIPTIVE STATISTICS CATEGORIZED ON RATIONALISED ATOPIC STATUS AT 3, 7, AND 12 MONTHS

PLATELET COUNTS (x 10^9/1)

RAS	STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
		3 Months	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
Not atopic	NO.	23	17	9	28	21	20	32	21	19
	Mean (arith)	622	602	566	599	524	229	625	536	519
	SD (arith)	134	130	157	167	130	195	150	112	101
	Max	903	866	783	1270	815	811	1074	843	712
	Q_3	703	650	697	653	591	222	727	609	597
	Median	565	561	609	578	500	168	594	500	529
	Q_1	513	498	439	513	449	111	497	468	460
	Min	435	423	317	410	315	66	415	376	310
Atopic	NO.	12	10	7	8	15	15	20	21	20
	Mean (arith)	561	584	484	656	593	339	599	559	495
	SD (arith)	143	110	57	115	254	208	152	128	104
	Max	839	762	586	814	1359	755	961	887	699
	Q_3	646	680	542	788	608	477	664	644	563
	Median	591	580	457	620	521	266	593	537	501
	Q_1	471	514	448	549	464	177	489	459	416
	Min	270	370	435	526	370	111	392	378	296

TABLE VI. 12

DESCRIPTIVE STATISTICS CATEGORIZED ON RATIONALISED ATOPIC STATUS AT 3, 7 AND 12 MONTHS

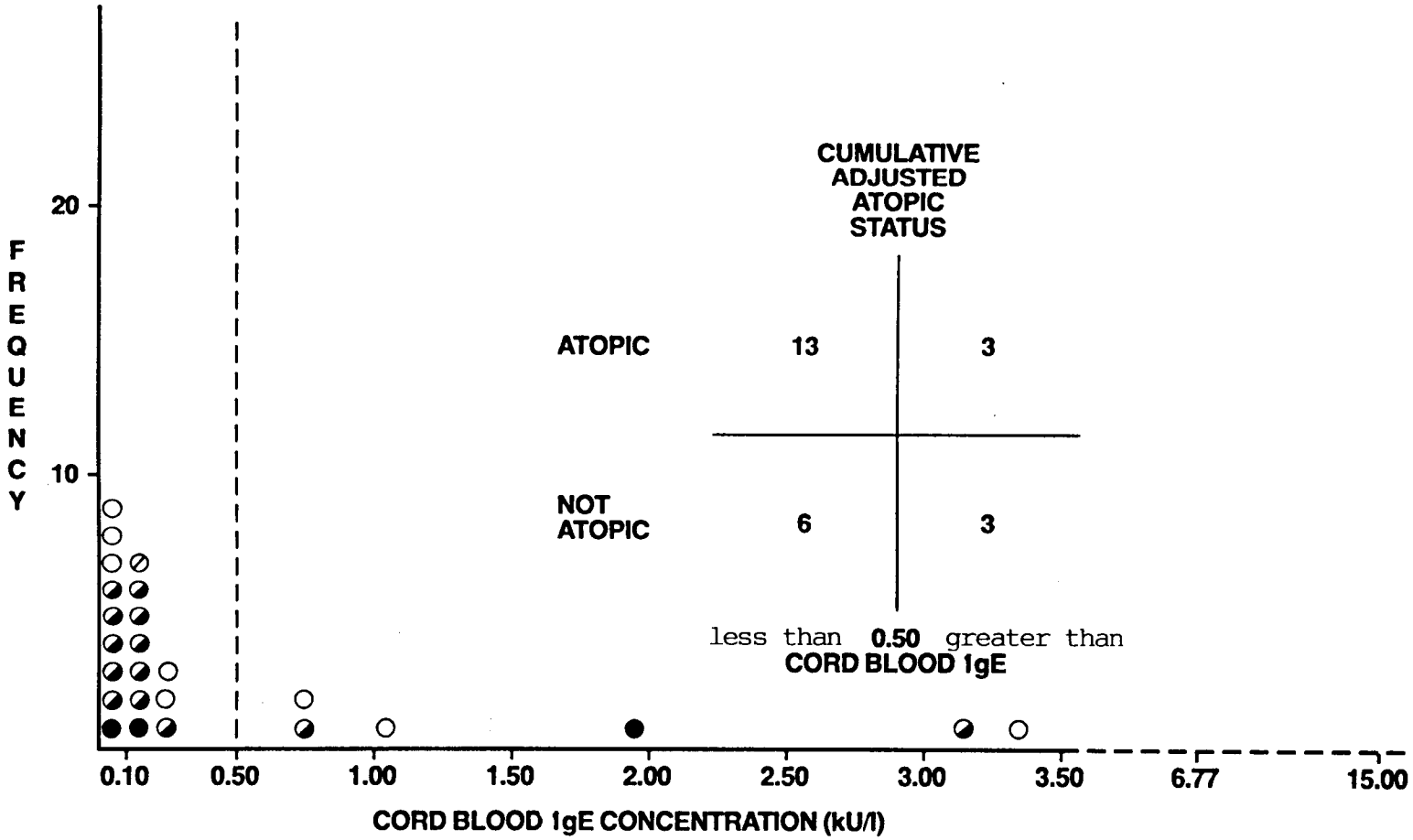
SERUM ANTI-COW'S MILK IgG CONCENTRATIONS (Δ O.D. 414nm*)

RAS	STATISTIC	BLACK INFANTS			WHITE INFANTS			MIXED INFANTS		
		3 Montns	7 Months	12 Months	3 Months	7 Months	12 Months	3 Months	7 Months	12 Months
Not atopic	NO.	23	17	9	30	21	20	32	19	19
	Mean (arith)	77.8	59.7	135.5	40.2	50.3	81.4	49.5	53.8	97.5
	SD (arith)	25.9	13.5	22.2	30.9	31.1	28.9	23.0	12.5	41.1
	Max	104.9	75.2	177.2	135.1	119.6	148.3	99.5	71.5	147.1
	Q_3	100.2	68.8	149.1	64.6	80.9	98.6	61.0	62.0	134.0
	Median	90.7	60.7	137.0	23.7	34.2	83.6	48.4	56.5	105.7
	Q_1	56.5	53.2	119.9	17.9	26.4	56.7	29.7	45.7	79.4
	Min	19.2	22.6	100.0	12.7	14.6	33.7	13.7	23.1	0.0
Atopic	NO.	12	9	7	9	13	14	20	19	20
	Mean (arith)	87.2	49.8	126.6	49.6	47.9	90.3	63.0	53.8	102.7
	SD (arith)	25.2	17.6	20.3	38.1	30.7	51.7	24.7	12.1	41.3
	Max	105.3	78.0	150.4	111.1	111.2	190.7	105.3	70.0	173.7
	Q_3	101.2	61.1	142.4	78.8	70.2	124.5	83.6	61.6	140.7
	Median	94.1	52.0	128.7	62.3	39.0	103.3	58.4	56.7	99.4
	Q_1	90.1	36.5	105.4	12.9	21.6	32.5	44.9	46.4	73.4
	Min	19.7	18.9	94.6	5.0	16.4	20.6	28.2	30.7	27.6

* Δ O.D. 414 nm = Optical density difference at 414 nanometres.

FIGURE VI. 1.

CUMULATIVE ADJUSTED ATOPIC STATUS (CAS) AGAINST CORD BLOOD SERUM IgE CONC. PLOTS FOR BLACK ETHNIC GROUP



KEY:

○ CAS – NOT ATOPIC, NEGATIVE ATOPIC FAMILY HISTORY
⊘ CAS – NOT ATOPIC, POSITIVE ATOPIC FAMILY HISTORY
◪ CAS – ATOPIC, NEGATIVE ATOPIC FAMILY HISTORY
● CAS – ATOPIC, POSITIVE ATOPIC FAMILY HISTORY

FIGURE VI. 2.

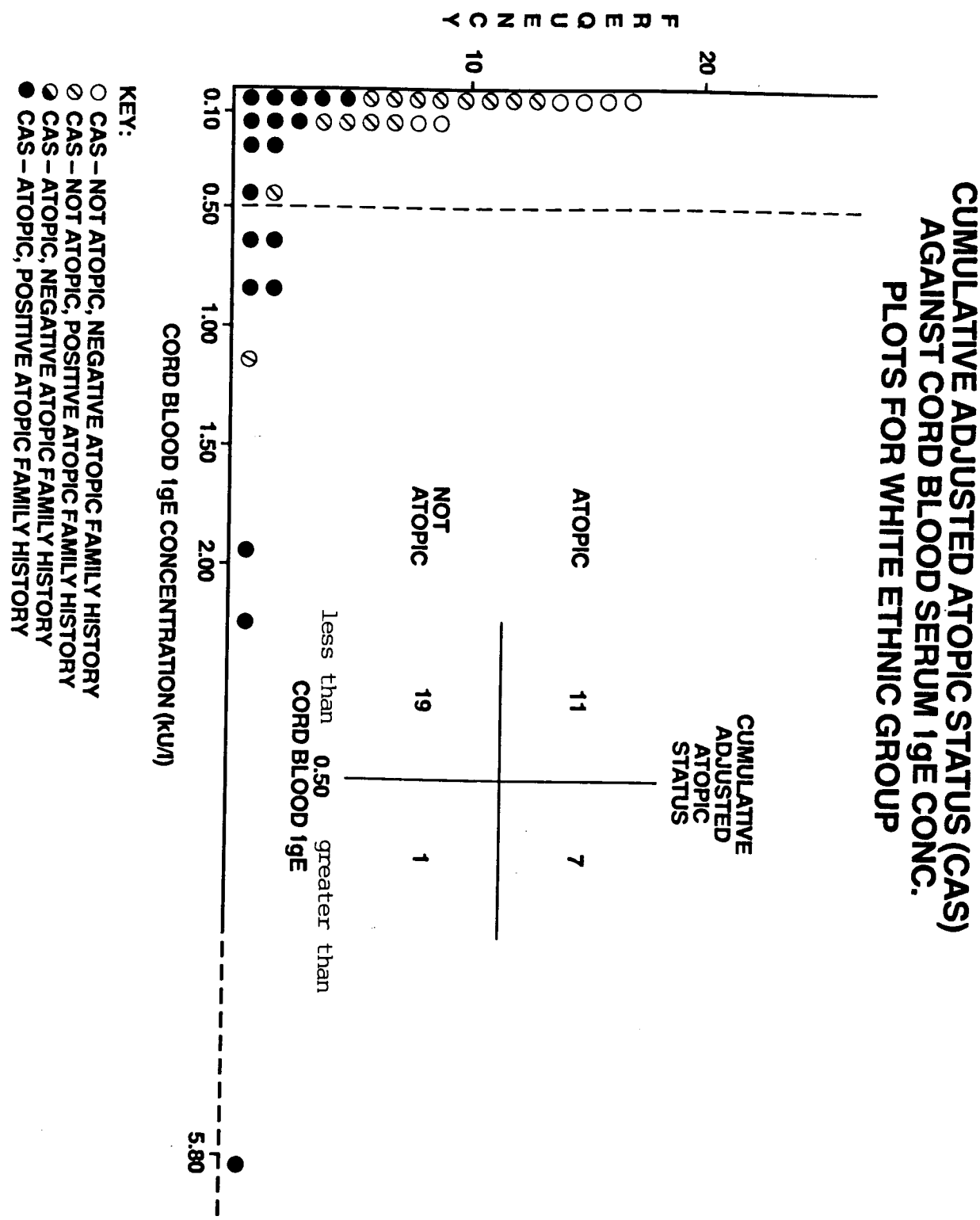
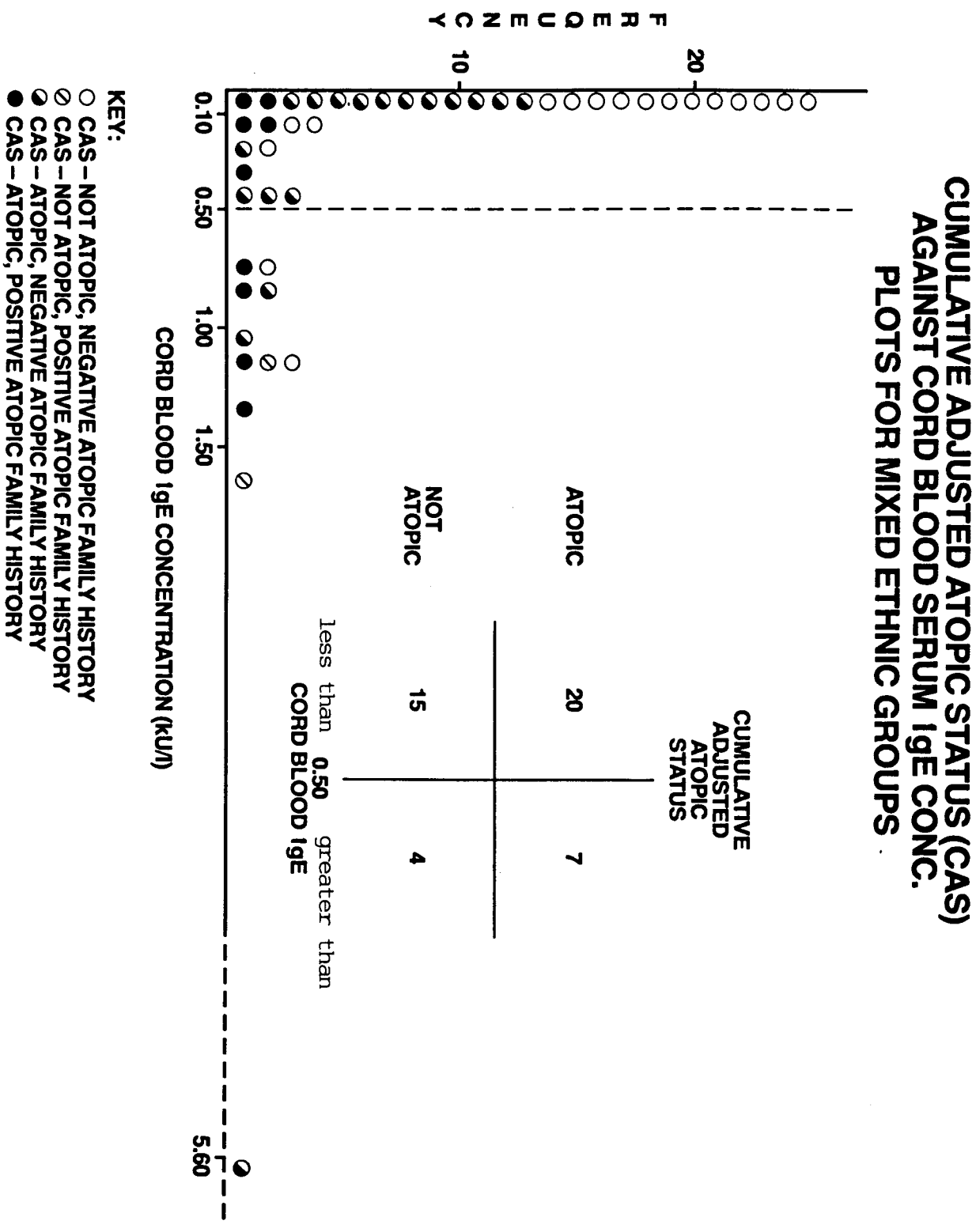

CUMULATIVE ADJUSTED ATOPIC STATUS (CAS) AGAINST CORD BLOOD SERUM IgE CONC. PLOTS FOR WHITE ETHNIC GROUP
FREQUENCY
20
10
0.10
0.50
1.00
1.50
2.00
5.80
CORD BLOOD IgE CONCENTRATION (kU/l)
CUMULATIVE ADJUSTED ATOPIC STATUS
ATOPIC
NOT ATOPIC
11
7
19
1
less than 0.50 CORD BLOOD IgE
greater than
KEY:
○ CAS – NOT ATOPIC, NEGATIVE ATOPIC FAMILY HISTORY
⊘ CAS – NOT ATOPIC, POSITIVE ATOPIC FAMILY HISTORY
◕ CAS – ATOPIC, NEGATIVE ATOPIC FAMILY HISTORY
● CAS – ATOPIC, POSITIVE ATOPIC FAMILY HISTORY

FIGURE VI. 3.


CUMULATIVE ADJUSTED ATOPIC STATUS (CAS) AGAINST CORD BLOOD SERUM IgE CONC. PLOTS FOR MIXED ETHNIC GROUPS
FREQUENCY
20
10
0.10
0.50
1.00
1.50
5.60
CORD BLOOD IgE CONCENTRATION (KU/l)
CUMULATIVE ADJUSTED ATOPIC STATUS
ATOPIC
NOT ATOPIC
20
7
15
4
less than 0.50 greater than
CORD BLOOD IgE
KEY:
○ CAS – NOT ATOPIC, NEGATIVE ATOPIC FAMILY HISTORY
⊘ CAS – NOT ATOPIC, POSITIVE ATOPIC FAMILY HISTORY
◒ CAS – ATOPIC, NEGATIVE ATOPIC FAMILY HISTORY
● CAS – ATOPIC, POSITIVE ATOPIC FAMILY HISTORY

CHAPTER 4 COMPARATIVE STATISTICS OF THE CORD BLOOD ATOPIC MARKERS AND THE CUMULATIVE ADJUSTED ATOPIC STATUS

The cord blood atopic markers in each ethnic group were compared between those newborns with a CAS of "atopic" and those newborns with a CAS of "not-atopic". The p-values resulting from the statistical tests of comparison between these two groups of newborns in each ethnic group as well as their arithmetic means and median values, are summarised in Table VI. 13.

TABLE VI. 13

COMPARATIVE STATISTICS OF THE CORD BLOOD ATOPIC MARKERS AT BIRTH IN RELATION TO THE CUMULATIVE ADJUSTED ATOPIC STATUS CATEGORIZATION IN INFANCY

ETHNIC GROUP	CAS	CORD BLOOD ATOPIC MARKERS											
		CBsIgE (kU/l)			CBTEC (cells/mm^3)			CBP1C counts (x 10^9)			CBacmIgG (O.D. 414nm)		
		NO.	Mean	Median	NO.	Mean	Median	NO.	Mean	Median	NO.	Mean	Median
Black	Atopic	16	0.46	0.13	16	291	251	14	340	352	16	45.0	40.0
	Not atopic	9	0.67	0.23	9	196	166	9	399	397	9	54.7	54.9
	p-value	p = 0.5710			p = 0.0502			p = 0.3776			p = 0.8651		
White	Atopic	18	0.81	0.23	18	478	488	17	378	405	17	73.5	65.2
	Not atopic	20	0.15	0.08	20	361	211	19	347	347	20	73.9	83.3
	p-value	p = 0.0145			p = 0.0693			p = 0.1027			p = 0.8074		
Mixed	Atopic	27	0.55	0.17	26	268	177	27	314	317	27	54.1	54.4
	Not atopic	19	0.27	0.03	19	260	244	19	333	305	19	59.2	60.3
	p-value	p = 0.0238			p = 0.2177			p = 0.5469			p = 0.5469		

SECTION VII

DISCUSSION - PHASE I

CHAPTER I GENERAL CONSIDERATIONS

1.1 INTRODUCTION

The aims of Phase I of this study were centred around the following priorities, as addressed in Section 1. Ch. 3.

(i) To demonstrate ethno-genetic differences in four cord blood atopic markers, independently of an aFH and maternal Ascariasis, between newborns of three defined ethnic groups in South Africa.

(ii) To show that, while raised values of CBsIgE in White and Mixed newborns in South Africa were related to the presence of an aFH in these newborns, the same did not apply to raised concentrations of CBsIgE in the Black newborns.

(iii) To assess the influence of various other genetic and environmental factors on the cord blood atopic markers in each ethnic group.

The presentation and discussion of this data is difficult if one is to avoid confusion or repetition by virtue of the fact that the study attempts to look at 4 markers in 3 ethnic groups each for 5 categorising variables. This leads to a combination of 60 critical observation criteria. For this reason, the data presentation and discussion for Phase I of the study has been considered separately from Phase II.

1.2 AN ATOPIC FAMILY HISTORY AS THE REFERENCE PARAMETER FOR PHASE I

In order to establish the atopic relevance of the four cord blood markers studied in Phase I, it was necessary to establish a

well-documented parameter which is known to reflect an atopic predisposition at birth. This parameter could then become the reference against which the cord blood marker values would be measured, both in terms of elevated concentrations in the case of CBsIgE, or in terms of whether the marker is influenced in any way by the atopic reference parameter or not.

The rationale for using the presence or absence of an aFH as the most reliable pre-natal non-invasive indicator of an atopic predisposition, is as follows:

(i) Atopy has been shown to be hereditary, with the aFH a good marker for the prediction of the atopic genotype. (Section II, Ch. I. 1).

(ii) Between 50% - 70% of atopic patients have an immediate positive aFH in their first degree relatives (Kjellman, 1977; Kjellman, 1982 a).

(iii) Croner et al, (1982) showed that, while an elevated CBsIgE alone (greater than 1.3kU/l) predicted the development of atopic disease by the age of 18 months in 44.4% of cases, the addition of a positive aFH to an elevated CBsIgE identified 85.9% of future atopics. They showed, furthermore, that the stronger the immediacy of an aFH, the greater the number of neonates born with a raised CBsIgE.

(iv) Clinical evidence of a genetic effect on IgE production was provided by Kjellman and Johansson (1976) who showed that neonates born with an aFH tend to have higher levels of CBsIgE.

(v) Orgel et al (1975) and Michel et al (1980a) have confirmed the association between raised CBsIgE values and an aFH.

It should be noted that, with the exception of the study by Orgel et al, all of the studies quoted above were done in Europe and Scandinavia, using what was presumed to be a study population of predominantly First World, Western, Caucasoid newborns.

The rationale for using an aFH as a suitable reference parameter for the assessment of the atopic relevance of the various markers should, therefore, be seen in context as applying only for White, Caucasoid newborns in First World countries. This is precisely the point of reference and motivation for this study, that these conclusions are not of relevance to Black, Third World newborns.

1.3 THE CRITERIA FOR AN ATOPIC FAMILY HISTORY

With the exception of the work by Cant et al (1985), the criteria and definitions used by previous investigators to determine an aFH in studies designed to assess predictive atopic markers are vague, varied and inconsistent (Table VII. 1).

In particular, it should be realized that by asking the mother about an aFH, one is often relying on the diagnosis of a third person, in many cases, the family's own general practitioner. While most general practitioners can be relied on to make an objective and accurate diagnosis for most of the atopic disease complexes, it must, nevertheless, be accepted that an incorrect diagnosis may be a confounding variable in this regard. This fact is unacceptable in this sort of study, but is common to all of the studies of this nature.

Of note, also, is the fact that atopic disease in the Black ethnic group in this country has, historically, been almost unknown (Section I, Ch. 2.2). The exercise of eliciting an aFH from the Black mothers in this study was, subsequently, a difficult task, over and above the limitations imposed by the language barrier (Section IV, Ch. 2.2.8). Within these constraints, every effort was made by the author (M.H.) to take a representative and consistent aFH from the Black mothers.

In some previous studies of this nature, objective in-vivo and in-vitro immunological evidence was used to confirm the presence of a suspected allergic family history in the relevant family members. For example, Micnel et al (1980a) used the collaborative evidence of positive skin tests to aeroallergens, a raised total IgE concentration of greater tnan 250 IU/ml and the demonstration of specific serum IgE antibodies using the RAST to confirm an aFH

TABLE VII.1

CRITERIA FOR AN ATOPIC FAMILY HISTORY - PREVIOUS STUDIES

AUTHOR	CLINICAL ATOPIC SYNDROMES SPECIFIED	DISCUSSION OF CLINICAL SYNDROME CRITERIA	SCIENTIFICALLY CRITICAL
1) Halpern et al (1973)	No	No	No
2) Orgel et al (1975)	Yes	No	No
3) Michel et al (1980a)	No	No	No (Excludes sibling status)
4) Michel et al (1980b)	No	No	No (Excludes sibling status)
5) Croner et al (1982)	No	No	No
6) Van Asperen et al (1984)	No	No	No (Excludes sibling status)
7) Kjellman and Croner (1984)	No	No	No
8) Cant et al (1985)	Yes	Only eczema	Only eczema

in both parents of their study population. Van Asperen et al (1984) similarly considered the patients to be atopic only if there was a positive reaction to one or other allergen in skin testing.

For various reasons, this approach was not practical for or relevant to this study. Firstly, the relevance of serum IgE concentrations as an atopic marker in the Black ethnic group in our country has been questioned (Section I, Ch. 2.3.1) particularly since parasitosis is so common in this population. Secondly, skin tests on dark skins are often difficult to evaluate objectively. Finally, it was not feasible within the framework of this study to access all of the fathers and siblings of the families in order to do these special confirmatory examinations. The logistical implications of such an exercise were unacceptable (Section IV. Ch. 2.3.4).

In order to contain diagnostic bias and eliminate inconsistencies and inaccuracies as far as possible, strict criteria for the various clinical disease complexes in the spectrum of atopic disorders were carefully defined for this study as constituting an aFH in the newborn. An aFH implied disease only in the first degree relatives of the newborn (i.e., the mother, father and siblings). Where the term "medically confirmed" is used in defining these criteria, it should be understood to mean that the relevant clinical disease complexes were diagnosed as such by a medical doctor, or that the individual in question had received treatment for such a diagnosis.

Following below are the criteria which were used to indicate a positive aFH for the newborn in his/her first degree relatives during the questioning of the mother (Phase I Questionnaire, Appendix II. A positive history for any of the atopic disease complexes listed below, in any one or more of the newborn's first degree relatives, constituted an aFH. If there was any doubt as to whether the criteria given were fulfilled or not, the questionnaire was completed reflecting an entry of "unknown or unsure" for that person. In the statistical analysis which followed, these answers were regarded as being negative (i.e., the absence of an aFH).

1.3.1 Asthma

In view of the varied and often impractical definitions used for the diagnosis of asthma, the criteria proposed by an expert panel of the Allergy Foundation of America, chaired by Norman, have been used (Reed and Townley, 1983). Using their definition for a presumptive diagnosis of asthma when pulmonary function tests or other special investigations are not done, (i.e., on history alone), the following criteria applied:

Either

a) Recurrent episodes of wheezing or dyspnoea,

plus

b) A history of episodes of wheezing induced or aggravated on at least two occasions by exposure to a recognised allergenic material; or seasonal appearance or aggravation of symptoms in the spring, summer or fall in at least two years.

or

c) Medically confirmed asthma.

Note: The nature of the term "wheezing" was carefully differentiated by the author (M.H.) from other respiratory symptoms which could confuse the diagnosis in patients who were not familiar with the term.

1.3.2 Allergic rhinitis

The criteria used for the diagnosis of allergic rhinitis on history were:

Either

a) The combined criteria of Murray et al (1969) and Van Asperen et al (1984), namely "nose-rubbing", fits of sneezing, snorting or sniffing, nasal discharge and/or blockage occurring continuously

for at least four weeks and excluding obvious infective rhinitis".

or

b) Medically confirmed allergic rhinitis.

1.3.3 Allergic conjunctivitis

The criteria used for the diagnosis of allergic conjunctivitis on history were:

Either

a) Conjunctivitis occurring in relation to episodes of allergic rhinitis as defined in (1.3.2) above but unrelated to any infective or other pathological conditions of the eyes or conjunctiva.

or

b) Medically confirmed allergic conjunctivitis.

1.3.4 Atopic dermatitis (eczema)

The criteria used for the diagnosis of atopic dermatitis on history were:

Either

a) A combination of the criteria used by Halpern et al (1973) for adults and children and Van Asperen et al (1984), for infants.

In adults and children, "either the acute form of weeping, erythematous, itching, papular vesicular lesions, characteristically distributed (flexural and extensor surfaces), or the typical chronic lesions."

In infants, "clinical evidence of typical atopic dermatitis, i.e., areas of scaly, erythematous, pruritic dermatitis,

primarily involving the flexural folds, face, cheeks or behind the ears."

or

b) Medically confirmed atopic dermatitis.

1.3.5 Allergic urticaria

The criteria used to determine whether allergic urticaria was present on history were:

Either

a) Clinical urticaria with or without angio-oedema if it was confirmed by at least two repeated challenges of the same food or other known allergen and if it was not due to drugs, insects, serum sickness or unknown causes (Halpern et al, 1973; Kjellman and Croner, (1984)).

or

b) Medically confirmed allergic urticaria.

1.3.6 Allergic gastro-intestinal food reactions

The criteria used to determine an gastro-intestinal reactions present on history were:

Either

a) Vomiting, diarrhoea or colic after the ingestion of a specific food at least twice and occurring within the first four hours after the ingestion of the food. (Bahna and Heiner, 1980; Kjellman, 1983; Kjellman and Croner, 1984; Van Asperen et al 1984).

or

b) Medically confirmed gastro-intestinal or food allergy.

A history of anaphylaxis, insect sting reactions or drug sensitivity was considered not to be relevant as constituting an aFH. The reason for this decision was two-fold. Firstly, these events have not commonly been seen as constituting atopic diseases in preceding studies of this nature (Table III. 1). Secondly, Smith (1983), in her review of the possible relationship between the above occurrences and asthma, allergic rhinitis and eczema, showed that the evidence was not convincing enough to warrant their inclusion as constituting atopic disease. Likewise, the inclusion of colic in infancy (unrelated to the definitive challenge of a specific food substance), was not included as constituting positive evidence for an aFH in this study. While many authors have implicated colic as a symptom of the atopic response (Bahna, 1980; Frick, 1981; Kjellman, 1983), it was felt that the remoteness of the history of such a symptom, as remembered by the mother for her entire family as well as for herself, would be an extremely grey area as far as its significance as constituting an aFH is concerned. It will be seen that the diagnosis of colic (cramps and flatus which persisted for longer than two months with occasional vomiting - Frick, 1981) during the follow-up visits of our infants in Phase II was classified as constituting "possible allergy". The reason for the addition of colic to the atopic classification in Phase II of this study and the way in which it affects the clinical categorization of the infants is discussed in Section VIII. Ch. 1.3.

Even though the criteria given above for the diagnosis of atopy in the first degree relatives of the newborn have been described in scientific language, we simplified the description of these symptoms and signs into language which the mothers could understand during our interrogation.

1.4 THE VARYING ETHNIC INCIDENCE OF AN ATOPIC FAMILY HISTORY

The disparity between the incidence of an aFH between the difference ethnic groups was not unexpected if the comparative epidemiology of allergic disease in both Western, Third World Caucasoid and African, First World Black populations is appreciated. Much of the background to this issue has been presented in Section I.

It is feasible, however, that some unintentional and random bias towards either subgroup (with or without an aFH) may have occurred due to the relatively small size of the study sampling in each ethnic group (53 Black, 52 White and 58 Mixed newborns). This fact in no way detracts from the use of this parameter as the reference marker against which the various cord blood markers in each ethnic group are compared regarding their relevance as atopic markers. It must be emphasised that this study is not concerned with incidence or prevalence rates of atopy in different population groups.

The Black newborns had a very low prevalence of an aFH (9.4%). This was anticipated, not because the symptoms or the questioning on atopic disease was not understood by them, or regarded as insignificant (great effort was made by the author to elicit an accurate history in this regard, with the liberal use of interpreters) but rather since it has been shown that Blacks from rural areas have minimal atopic diseases (Van Niekerk, 1979). Furthermore, our figure of 9.4% correlates well with the figure of 7.5% given by Orren (1974) for the Black subjects on her study who had an aFH. The Black newborns in our study, even though born in an urban environment, historically had a rural heritage, (Section IV. Ch. 1.2.1), with many newborns having first degree relatives who had only later on in their lives moved to the metropolitan urban environment of Cape Town.

The White newborns had a 75% prevalence of an aFH. This was undeniably high, but not excessively more so than some other series. Orgel et al (1975) had a series of newborns with an aFH of 53%. The incidence of a positive aFH should always be far higher than the incidence or prevalence of atopic disease in any stable, influx-controlled population, and these two variables should not be confused.

The incidence of an aFH in the Mixed newborns was, as expected, somewhere between that demonstrated in the Black and the White newborns (31%). The genetic inheritance which the Mixed group share with both the White and the Black group (Botha and Pritchard, 1972) make this finding both rational and predictable.

1.5 RATIONALISATION FOR THE CATEGORIZATION CRITERIA OF THE DATA

In order to fulfil the aims of the study, the data in Phase I was categorized into 6 sub-groups of newborns for analysis and discussion (Section V. Ch. 1.3). The scientific rationalisation for this categorization follows below.

1.5.1 Ethnic categorization

From the descriptive statistics in Table V.2. it can be seen that CBsIgE's are higher in the Black South African newborns than in either the White or the Mixed South African newborns. In order to demonstrate that CBsIgE values in the Black South African newborns are significantly higher than those of the White or Mixed South African group of newborns, the combined group of newborns in each ethnic group were compared for this categorization. The other 3 cord blood markers being assessed were also compared for this ethnic categorization.

1.5.2 Ethnic categorisation, independently of an atopic family history and maternal Ascariasis

Since both an aFH and maternal Ascaris infestation have been described as causing raised CBsIgE values in newborns (Section I. Ch. 2) the subgroup of newborns which excluded those with these variables were compared in order to establish the fact that any ethnic differences observed in CBsIgE values were not reflective of atopy or obvious Maternal Ascariasis, but were reflective rather of other ethno-genetic factors. The other cord blood markers were also compared for this categorization.

1.5.3 An atopic family history

The newborns were divided into subgroups with either a positive or a negative aFH. This categorization was necessary, not only to assess the validity of ethnic differences in the potential atopic markers independently of an aFH, but also to determine the influence of an aFH per sê on these markers. A marker which was influenced by an aFH would be considered as having some validity as an atopic marker.

1.5.4 Maternal Ascariasis

In order to prove or disprove the influence of maternal Ascariasis on the various cord blood markers, this categorization was used on the basis of the maternal RAST for the Ascaris lumbricoides helminth. In this regard, it was decided that a positive maternal RAST for the Ascaris lumbricoides helminth would be regarded as reliable immunological evidence that specific serum IgE antibodies were produced in response to maternal exposure to the Ascaris antigen (De Filippi et al, 1981). This was the most practical diagnostic procedure available to assess maternal Ascaris infestation in this study, and was thought to be preferable to analysing maternal stool samples for the presence of Ascaris ova. For the purposes of this study, a positive maternal RAST for Ascaris lumbricoides was therefore equated with a diagnosis of maternal Ascariasis.

1.5.5 Maternal cigarette smoking

The evidence for the effect of maternal cigarette smoking on CBsIgE values was reviewed in Section III. Ch. 1.8.6. This categorization was used to assess each marker in each ethnic group for this environmental variable, since this study provided a unique opportunity to make this observation. It should be emphasised, however, that neither the amount of cigarettes smoked by the mother, or the smoking habits of other members of the family sharing the home environment (the effect of passive or side-stream smoking) were taken into account in the statistical analysis.

1.5.6 The sex of the newborn

The evidence for the effect of newborn's sex on CBsIgE values was reviewed in Section III. Ch. 1.8. This study provided an excellent opportunity to add to the epidemiological body of knowledge regarding the effect of other genetic factors such as sex on the cord blood markers considered.

1.6 THE INTEGRITY OF THE CORD BLOOD SPECIMENS

This study is based on intimate materno-foetal and materno-neonatal relationships. Of fundamental importance to the credibility of the data generated is the integrity of the cord blood specimen, in the sense that it is not contaminated or influenced by maternal blood. This is particularly relevant since the venesection of the cord occurs seconds after the newborn is delivered, while the placenta is still in-situ, with a considerable amount of maternal haemorrhage distributed over a wide area of her perineum and surrounding area.

Great care was taken, therefore, to ensure that the cord blood specimens were carefully aspirated from a cord which had been adequately cleaned (Section IV. Ch. 2.2.5). The rationalé for assuming that an uncontaminated specimen of cord blood is foetal in origin was given in Section III. Ch. 1.8.1. In this study, the general principles indicating the foetal origin of the cord blood samples were confirmed by our data, for the following reasons:

i) The very poor correlation co-efficients between the cord blood and the maternal specimens for serum IgE concentrations, the total eosinophil counts and the platelet counts indicate that the two specimens reflected two separate haematological systems (Table V.16). The correlation co-efficients for serum IgG concentrations between the cord blood and maternal specimens were, however, uniformly good. This was expected because the low molecular weight of IgG allows free transplacental passage of this immunoglobulin from the mother to the foetus.

ii) The very low concentrations of serum IgE in the cord blood relative to the higher concentrations present in the maternal serum (Table V.15) negate the possibility of a passive equilibuium of maternal serum IgE across the materno-foetal placental barrier.

iii) Various specific IgE antibodies were demonstrated in some of the maternal blood specimens, by the RAST (Section V. Ch. 3.5.2). In none of the cord blood specimens were any specific IgE

antibodies demonstrated even though a supersensitive, double incubation RAST method (Section IV. Ch. 3.3) was used for the immunoassay. This further indicated that placental transfer of these antibodies did not occur, and that the cord blood samples were foetal in origin.

To exclude intra-partum maternal contamination of the cord blood specimens each cord blood and maternal specimen was assayed for the serum IgA concentration. It has been shown that the concentration of serum IgA in cord blood is very low in comparison to that in maternal serum (Section III. Ch. 1.8.2). Any contamination of the cord blood specimen by maternal blood would result in concentrations of cord blood IgA which are appreciably higher than the normal values for newborns.

In this study, none of the newborns had raised concentrations of IgA above the quoted normal values (Section III. Ch. 1.8.2). When compared to their corresponding maternal concentrations, they were furthermore of a different order (Table V.17). The maximum cord blood serum IgA concentrations in the Black, White and Mixed newborns were 5.70, 3.25 and 5.65 mg/dl respectively. These values are all lower than the quoted upper limit of normal for these South African ethnic groups of 5.80, 5.00 and 7.20 mg/dl respectively (Van Rijswikj et al, 1985). Contamination of our cord blood specimens was subsequently excluded.

The evidence to suggest that the data attributable to the cord blood specimens in this study was of foetal origin, and reflective of an uncontaminated haematological specimen, is therefore scientifically credible.

CHAPTER 2 CORD BLOOD SERUM IgE CONCENTRATIONS

2.1 THE DEFINITION OF A RAISED CORD BLOOD SERUM IgE CONCENTRATION

Normal reference values have not yet been established for CBsIgE values in any of the South African ethnic groups. The definition of what constitutes a "raised level" has subsequently not yet been established in this country.

Kjellman states that a "knowledge of serum IgE variations in age-matched subjects of similar ethnic backgrounds is a prerequisite for understanding the predictive capacity of a certain serum IgE concentration" (Kjellman, 1982). He contends also that the "use of serum IgE as a parameter for the prediction of atopy implies prior knowledge of the relevant IgE values in healthy (non-atopic) subjects" (Kjellman et al, 1976).

An acceptable definition of the upper limits of normality in terms of sIgE is the geometric mean plus or minus two standard deviations (GM + 2 SD) (Kjellman, 1982). Based on this definition, a cut-off level greater than 1,3 u/ml was used by Croner et al (1982) and Kjellman and Croner (1984) and in their prediction studies to define the upper limit of normal for CBsIgE. This corresponded to the GM + 2 SD for a normal Swedish newborn population in a previous study (Kjellman et al, 1976).

Lower CBsIgE cut-off points of 1,20 u/ml (Magnusson, 1984), 0,9 u/ml (Kjellman and Croner, 1984), 0,7 u/ml (Chandra et al, 1985) and 0,5 u/ml (Michel et al, 1980a) have been subsequently suggested by various authors to include a greater percentage of potentially "high-allergic-risk" newborns and to increase the predictive specificity of the CBsIgE estimations.

For the purpose of this study, an arbitrary CBsIgE value of 0,5 kU/l was decided on to represent the point at which any value equal to or greater than this point was considered to be raised.

This "cut-off point" was especially relevant when assessing the relationship between an aFH and raised levels of CBsIgE.

2.2 THE RELEVANCE OF THE SUPERSENSITIVE SERUM IgE PRIST LABORATORY IMMUNOASSAY

In Section I. Ch. 2.3.2., it was noted that IgE is present in very low concentrations in cord blood . Highly sensitive immunoassays are therefore essential in epidemiological studies of this nature, particularly when small differences could be crucial to demonstrate comparative differences between ethnic groups, and in assessing the influence of various factors on CBsIgE.

For this study we used a modification of the standard IgE PRIST (Pharmacia) immunoassay for the estimation of the cord blood serum IgE concentrations (Haus et al, 1988), which differentiated between CBsIgE values of 0.01 kU/l (Section IV. Ch. 3.1). In previous CBsIgE studies, the detection limits for CBsIgE were of the order of 0.5 kU/l (Table 1.3). This is not a sensitive enough immunoassay for comparative studies of this nature.

The detection limit of 0.01 kU/l achieved for our PRIST immunoassay is the lowest yet described in the literature. The previous most sensitive PRIST reported on was by Magnusson (1984) who described a detection limit of 0.1 u/ml. Kimpen et al (1987) reported on a detection limit of 0.01 iu/ml, but the method used for the immunoassay was an ELISA technique and not a PRIST.

The standard curve for the supersensitive PRIST technique used in this study (Fig. IV. 7) emphasises the accuracy and reliability with which serum IgE concentrations of 0.01 kU/l can be differentiated.

2.3 THE INFLUENCE OF ETHNICITY

Phase I of this study has shown that the median CBsIgE values in a random selection of Black South African full-term newborns is significantly higher than the corresponding values in a similar sampling of both White and the Mixed newborns, in those newborns who had neither a family history of atopy nor immunological evidence of maternal Ascariasis (Section V. Ch. 4.2.1). The fact that no significant ethnic differences were demonstrated in this

sub-group between the White-Mixed newborns suggests that genetically, as well as socio-culturally, the Mixed ethnic group share a greater heritage with the White group than with the Black group. This confirms Botha's findings on the blood group gene frequencies as an indicator of the genetic constitution of some ethnic groups in Cape Town (Botha and Pritchard, 1972).

2.4 THE INFLUENCE OF AN ATOPIC FAMILY HISTORY

In this study, an analysis of the CBsIgE values in the White newborns in relation to an aFH reflected the previously published work in terms of their mutual interdependence (Section III. Ch. 1.8.4). All of the White newborns with a CBsIgE value of 0.5kU/l or greater had an aFH (Fig. V. 2.).

The Black newborns did not show the same relationship. The high CBsIgE values in the Black group were not related to an aFH. It should be stressed, however, that we were unable to provide definitive evidence of the effect of an aFH on the CBsIgE values in the Black newborns due to the fact that only 5 of these newborns had an aFH. They had the lowest incidence (9%) of an aFH of all the 3 ethnic groups (Table V. 1) in spite of having the highest CBsIgE values. This could possibly be explained because of their rural heritage as discussed in Section VII. Ch. I.4. The distribution of the Black CBsIgE values over 0.50 kU/l (Fig. V. 1) showed that most were unrelated to an aFH including the newborn with the very high value of 15.00 kU/l.

The Mixed newborn's findings were inconclusive, in that the CBsIgE values over 0.50 kU/l were representative of both those with and those without an aFH (Fig. V. 3). Statistically, the influence of an aFH did, however, affect the CBsIgE values ($p < 0.05$). This clinical ambivalence may again be the result of the genetic inheritance with the Mixed group share with both the White and the Black group (Botha and Pritchard, 1972).

Some of the statistical calculations leading up to these conclusions could be open to criticism. For example, only 9 of the 39 White newborns born with an aFH had CBsIgE values elevated above 0.5 kU/l. Based on this information from the White newborns, since

only 5 Black newborns were born with an aFH, it follows that only 23% of Black newborns with an aFH (i.e. 1 newborn) might have been expected to have a CBsIgE values of greater than 0.5 kU/l. It may, therefore, be concluded that, since 2 of 5 such Black newborns with an aFH had CBsIgE concentrations above 0.5 kU/l, it makes their data at least as significant as that of the White newborn's. Even though this argument is based on the same unsubstantiated premise which this thesis is designed to contest, that is, that the same reasoning and rationale which is applicable to the relevance of raised CBsIgE values in White newborns may be extrapolated and be applicable to the relevance of raised values in Black newborns, it is nevertheless of interest to follow the statistical reasoning leading to the rejection of this hypothesis.

The reasoning here is:

39 White babies were born to atopic families.
9 of these 39 had CBsIgE values greater than 0.5 kU/l.
i.e. 9/39 = 23% of this sample, had CBsIgE levels greater than 0.5 ku/l.

If one considers what is happening in the Black group, using the White group as the baseline, one finds that 5 Black infants were born to atopic families. Working with the ratio calculated for the White newborns of atopic families with CBsIgE values greater than 0.5 kU/l one would expect 23% of 5 = 1 Black infant to have CBsIgE values greater than 0.5 kU/l.

In the sample selected for this study, 2 of the 5 Black newborns of atopic families had CBsIgE values greater than 0.5 kU/l. Hence the results for the Black group cannot be less significant than the results from the White group.

The statistical significance of these ratios can be investigated using Fishers's Exact Test. The hypothesis which this test tests is:

Ho: the proportion of infants from atopic families with CBsIgE values greater than 0.50 kU/l is the same as the proportion of infants from non-atopic families with CBsIgE values greater than or equal to 0.50 kU/l.

against the alternative hypothesis:

Ha: the proportion (described in Ho) differs. (It may be larger or smaller, but the direction of magnitude is not specified).

Details of the frequencies on which this test is based follows:

	WHITE CBsIgE			BLACK CBsIgE		
	less than 0.50 kU/l	greater than or = 0.50 kU/l	Row Total	less than 0.50 kU/l	greater than or = 0.50 kU/l	Row Total
Atopic FH						
+ve	30	9	39	3	2	5
-ve	13	0	13	36	12	48
Column Total	43	9	52	39	14	53
	p-value: 0.091			p-value: 0.599		

Now using the following logic:

The ratio between the 2 race groups was statistically compared in the following table for a positive aFH only:

RACE	CBsIgE		Row Total
	less than 0.50 kU/l	equal or greater than 0.50 kU/l	
White	30	9	39
Black	3	2	5
Column total	33	11	44
p-value: 0.586			

The conclusion to draw here is that no statistical difference could be shown in the ratio of the number of infants of atopic families with CBsIgE values less than 0.50 kU/l in the Black and the White race groups, when compared to the ratio of those with CBsIgE values equal to or greater than 0.5 kU/l. The inability to show such a difference could, however, be accounted for by the small numbers in each cell in the Black group.

2.5 THE INFLUENCE OF MATERNAL ASCARIASIS

It has been shown that increased CBsIgE values may be the result of maternal helminth infection (Weil et al, 1983). This phenomenon is thought to be due to a foetal immune response to the parasite antigens rather than due to transplacental transfer of the serum IgE from mother to foetus. The foetus has been shown to be capable of IgE synthesis in utero by the 11th week of gestation (Miller et al, 1973), and intrauterine sensitisation of the foetus has been well

documented (Michel et al, 1980b; Levine et al, 1971; Kuroume et al 1976). In this study, it was therefore important to ascertain whether the relatively high CBsIgE values in the Black newborns as well as the ethnic differences in CBsIgE values between the Black-White and the Black-Mixed newborns were not possibly a resultof maternal Ascariasis. It was felt that a maternal immunological response to the Ascaris lumbricoides antigen as demonstrated by the formation of specific IgE antibodies to this helminth using the RAST was a logical way to assess maternal Ascariasis. This consideration was particularly relevant to this study since many more Black mothers had a positive RAST for Ascaris lumbricoides (7 mothers) than White mothers (1 mother).

The fact that the ethnic differences between the Black-White and the Black-Mixed groups persisted when the statistical analysis was performed on the subgroups which included only those newborns with a negative aFH together with a negative maternal RAST for Ascaris lumbricoides suggests that these differences are predominantly a reflection of ethno-genetic factors. No specific IgE to Ascaris lumbricoides was demonstrated in the cord blood specimens of any of the newborns.

2.6 THE INFLUENCE OF THE NEWBORN'S SEX

The influence of the newborn's sex on their CBsIgE values was apparent only in the Mixed ethnic group. This variable did not impact in any way on the Black CBsIgE values, and subsequently did not need to be taken into consideration when assessing the significance of the raised values in the Black newborns.

2.7 THE INFLUENCE OF MATERNAL CIGARETTE SMOKING

This variable did not impact on the CBsIgE values in any of the ethnic groups. The data published by Magnusson (1984), where smoking mothers gave birth to newborns with elevated CBsIgE concentrations relative to those newborns whose mothers never smoked (Section III. Ch. 1.8.6) was not reproduced by this study. The data generated by this study supports the findings of Michel et al (1980a) who could not find any differentiation in CBsIgE values for this variable.

2.8 GENERAL COMMENT

Animal studies in defined, inbred strains of rats have shown that there is a correlation between the baseline total IgE concentrations and the magnitude of the resulting specific IgE responses (Pauwels et al, 1979). Inbred strains of mice with low basal total IgE concentrations have weak specific sIgE responses following antigen stimulation (low-responder phenotypes), whereas strains with high basal total sIgE concentrations develop powerful specific IgE responses (high-responder phenotypes). The high CBsIgE values found in the Black group in this study probably likewise represent a pool of genetic IgE 'high-responder' phenotypes (Croner et al, 1982; Kjellman and Croner, 1984). Furthermore, the high CBsIgE concentrations in the Black group is in line with the generalized hypergammaglobulinaemia which has been reported in various Black populations (Section 1. Ch. 2.3). It has been suggested that this immunological hyperactivity may represent the evolution of a natural phylogenetic advantage mechanism against the parasitic and microbial infections which are often endemic in Third World underdeveloped countries (Kay, 1979; Kay, 1980). The statement often made that "high concentrations of IgE antibodies are almost exclusively seen in infants with an atopic disease" (Molknou and Metayer, 1987) would therefore seem to be an oversimplification of the evidence as regards the Black ethnic groups in Africa.

Whether these high levels reflect a propensity for the development of atopic sensitisation later in infancy and childhood will become clear once these newborns have been followed up and assessed over a period of time. The outcome of Phase II of this study will indicate whether those who did develop atopic disease in infancy had higher values of CBsIgE at birth.

When considering the clinical significance of the high CBsIgE values other potential causes for the raised concentrations needed to be excluded. In this study, there was no immunological evidence of intrauterine sensitisation in the newborns (all the cord blood RAST's for the development of specific sIgE to egg white, cows milk,

and Ascaris lumbricoides antigen were negative). Croner et al (1982) have suggested, however, that CBsIgE is probably non-specific in nature. This study supports their views. The possibility of maternal contamination of the cord blood samples was largely excluded (Section VII. Ch. 1.6). Phase I of this study has supported that part of the hypothesis, (Section I. Ch. 3.2) which states that "the CBsIgE values in a randomly selected sampling of South African full-term Black newborns without an aFH are significantly higher than the CBsIgE values of a similar sampling of South African White and Mixed newborns. An aFH does not, furthermore, influence the CBsIgE in the Black newborns (as it does in the White and Mixed newborns).

These differences illustrate the fact that immunogenetic parameters vary in different ethnic populations. Black newborns in Third World African communities may represent a pool of high IgE-responder phenotypes, with the high levels of non-specific CBsIgE possibly reflective of ethno-genetic influences, unrelated to environmental factors.

These considerations may render the use of CBsIgE values of limited value in Black populations as a predictive marker for the development of atopic disease during infancy. Phase II of this study addresses this aspect of the hypothesis.

CHAPTER 3 CORD BLOOD TOTAL EOSINOPHIL COUNTS

3.1 BIOLOGIC AND PHYSIOLOGICAL VARIATIONS IN CORD BLOOD TOTAL EOSINOPHIL COUNTS

Section III. Ch. 2.2 reviewed the various factors which have been described as influencing the number of circulating peripheral eosinophils at any one time, in children and in adults.

Whether these factors impact on newborns remains to be proved. The exact relevance of raised or lowered CBTEC's should consequently be considered with circumspection and caution. The abstract by Dry et al, (1980) did not mention that any factors were adjusted for in attempting to standardise the TEC's of the newborns they studied.

In this study, various of tne biological and physiological variants which have been shown to impact on TEC's in general were taken into consideration when planning tnis study, even thougn their possible influence on CBTEC's have not been unequivocally documented.

The possibility of the influence of a diurnal fluctuation on the level of circulating cord blood eosinophils was addressed in tnis study by ensuring that all haematological specimens were taken during the 6-hour period between 09H00 and 15H00 (Section IV. Ch. 2.2.5).

The eosinophilopaenic effect of the stress of labour on the mother, as described by Davis and Hulet (1949), was also demonstrated in this study (Table VII. 2). Seven mothers had an absolute eosinophilopaenia (0 cells/mm^3), while 20 mothers had a relative eosinophilopaenia of 88 cells/mm^3 or less. Of interest is the fact that 26% of the White mothers had TEC's below 88 cells/mm^3 in contrast to the Black and Mixed motners, of wnom only 13.4% and 12.5% had corresponding values. This observation encourages speculation on a socio-cultural level as to whether White mothers do not possibly suffer more intra-partum stress than Black or Mixed mothers, leading to an increase in pituitary-adrenocortical activity (Recant et al, 1950) and resultant eosinophilopaenia. Trauma and surgical shock

have been described as having an eosinophilopaenic effect (Laragh and Almy, 1948; Davis and Hulet, 1949; Gabrilove,1950). In our study, the White mothers did not undergo more Caesarean Sections than the Black mothers (Table VII. 3). The stress of intra-partum surgery was, therefore, not obviously responsible for their trend to develop eosinophilopaenia more readily than their Black counterparts.

In order to ascertain whether Caesarean Section per sê had a statistically significant effect on the CBTEC's in the newborns, the newborns were divided into 2 subgroups, depending on whether or not they were delivered by Caesarean Section (Table VII.4). The differences in the CBTEC median values for each subgroup were then compared for statistical significance in each ethnic group. A similar statistical exercise was done for the maternal TEC's for comparison and interest. In no ethnic group did delivery by Caesarean Section influence the TEC's in either the maternal or the newborn specimens (Table VII. 5). It was, therefore, not necessary to adjust for the method of birth in assessing the CBTEC's for the other variables.

Various drugs have also been shown to influence TEC's. During the questioning of the mother (Questionnaire, Section IV. Ch. 2.2.9), it was ensured that none of the mothers on the study had been exposed to oral epinephrine, propranolol or intravenous insulin during pregnancy or labour, all of which have been reported to influence TEC's (Section III. Ch. 2.2). Whether these pharmacologically active substances have similar effects on cord blood TEC's is unclear. The precaution to exclude mothers exposed to these substances was nevertheless taken.

The effect of physical exercise on TEC's (Dalton and Selye, 1939; Davis and Hulet, 1949) was not adjusted for in this study, since labour itself is a fairly active form of muscular activity, and quantitative assessment of this variable was not appropriate within the boundaries of this study.

TABLE VII. 2

POST-PARTUM MATERNAL EOSINOPHILOPAENIA

ETHNIC GROUP	NO.	ABSOLUTE EOSINOPHILOPAENIA (0 cells/mm^3)	% AGE OF TOTAL GROUP	RELATIVE EOSINOPHILOPAENIA (less than 88 cells/mm^3)	% AGE OF TOTAL GROUP	TOTAL GROUP OF MOTHERS WITH TEC'S (less than 88 cells mm^3)
Black	52	5	9,6%	2	1,8%	13,4
White	50	0	0,0%	13	26,0%	26,0%
Mixed	56	2	3,6%	5	8,9%	12,5%
Combined	158	7	4,4%	20	12,7%	17,1%

TABLE VII. 3

THE RELATIVE INCIDENCE OF CAESAREAN SECTION VERSUS VAGINAL DELIVERY IN THE MATERNAL STUDY COHORT

ETHNIC GROUP	NO. OF PATIENTS	%
VAGINAL DELIVERY		
Black	32	60.4%
White	34	65.4%
Mixed	41	71.9%
CAESAREAN SECTION		
Black	21	39.6%
White	18	34.6%
Mixed	16	28.1%

TABLE VII. 4

TOTAL EOSINOPHIL COUNTS CATEGORISED ON DELIVERY BY CAESAREAN SECTION

DESCRIPTIVE DATA (cells/mm^3)

SPECIMEN	CATEGORIZING Variable	ETHNIC	NO.	Mean	SD	Median	Max	Min	Range (75%)	Q^3 (25%)	Q^1	Q^3-Q^1
Maternal	Birth by Caesarean Section	Black	20	272	151	289	577	0	577	355	119	236
		White	18	281	361	200	1688	66	1622	277	133	144
		Mixed	16	222	132	172	466	0	466	372	133	239
	Normal Vertex Delivery	Black	32	236	190	200	888	0	888	328	111	217
		White	32	175	145	122	667	22	645	244	88	156
		Mixed	40	526	1459	177	9000	0	9000	274	111	163
Cord Blood	Birth by Caesarean Section	Black	21	293	183	244	777	77	700	389	144	245
		White	18	368	233	355	800	0	800	538	189	349
		Mixed	16	278	223	200	1020	111	909	344	139	205
	Normal Vertex Delivery	Black	32	254	156	200	711	0	711	322	177	145
		White	34	395	264	366	1155	22	1133	550	172	378
		Mixed	41	359	390	244	1888	0	1888	372	155	217

TABLE VII. 5

MATERNAL AND CORD BLOOD TOTAL EOSINOPHIL COUNTS CATEGORISED ON CAESAREAN SECTION VERSUS NORMAL VERTEX DELIVERY

SPECIMEN	CATEGORIZING VARIABLES	ETHNIC GROUP	Mann-Whitney-U Test				
			X^2-approx value	df	p-value	Signif. at 5% level	Statistical Decision
MATERNAL	Caesarean Section	Black	1.35	1	0.2457	NS	no diff.
	vs	White	3.26	1	0.0710	NS	no diff.
	Normal Vertex Delivery	Mixed	0.11	1	0.7432	NS	no diff.
CORD BLOOD	Caesarean Section	Black	0.67	1	0.4121	NS	no diff.
	vs	White	0.03	1	0.8624	NS	no diff.
	Normal Vertex Delivery	Mixed	0.25	1	0.3835	NS	no diff.

Apart from the considerations addressed above no other potentially modifying factors were taken into consideration when assessing the effect of the other variables on CBTEC's.

3.2 THE CONCEPT OF NORMALITY FOR CORD BLOOD TOTAL EOSINOPHIL COUNTS

No reference value (or range of values) for normality was suggested for the assessment of the CBTEC's in this study for the following reasons:

(i) The reference values for normality reported in adults and children for TEC's range widely (Section III. Ch. 2.4.).

(ii) No normal reference values for cord blood TEC's have been published.

(iii) It is not precisely known which biological and physico-chemical factors influence the CBTEC's in newborns.

Because of these problems, the conclusions of Dry et al (1980) regarding the significance of raised CBTEC's were reached by the consideration of a comparative statistical assessment between those newborns with and those without an aFH. No absolute CBTEC value was established to indicate an eosinophilophilia. They reported, rather, on a "p" value of less than 0.02 between the CBTEC's of those newborns who had an aFH and those who did not. In their series, the mean CBTEC for the newborns with an aFH was 248 cells/mm^3, while the mean for newborns without such a history was 183 cells/mm^3.

The CBTEC's in this study were consequently analysed in a similar manner, with the main emphasis being on the demonstration of statistical differences occuring on comparison between the categorising variables considered, and not relative to an absolute reference value for normality, (as was done for the assessment of the CBsIgE values).

3.3 THE INFLUENCE OF ETHNICITY

Phase I of this study has demonstrated clear ethnic differences in

the CBTEC's between the total uncategorized groups of Black-White newborns, and a marginal ethnic differences between the White-Mixed newborns (Table V.7). These differences disappeared, however, when only the subgroups with both a negative aFH and the absence of maternal Ascariasis were compared.

It will be seen below (Ch. 3.4 and Ch. 3.5) that, while an aFH did not influence the CBTEC's in any ethnic group, maternal Ascaris infestation did affect the CBTEC's in the Black newborns. It is possible, therefore, that the ethnic differences in CBTEC's demonstrated between the total uncategorized Black-White newborns could be related to maternal Ascariasis rather than to ethno-genetic factors, making this marker even more susceptible to Third World environmental factors than CBsIgE values.

3.4 THE INFLUENCE OF AN ATOPIC FAMILY HISTORY

An aFH did not influence the CBTEC's in any of the three ethnic groups of newborns. This contradicted the findings of Dry et al (1979), and compromised the speculation that atopically predisposed newborns could be identified at birth using this cord blood marker.

Phase II of this study will indicate conclusively whether there was any statistical differentiation in CBTEC's between those newborns who developed atopic symptoms during infancy and those who remained healthy.

3.5 THE INFLUENCE OF MATERNAL ASCARIASIS

Maternal Ascariasis was shown to influence the CBTEC's in the Black newborns only. Of interest, however, is the unexpected finding that the median CBTEC's of the 7 Black newborns whose mothers did have immunological evidence of Ascaris infestation was, in fact, lower (166 cells/mm^3) than the corresponding value for the subgroup whose mothers did not have maternal Ascariasis (233 cells/mm^3) (Table V.3). Ascariasis and other helminth infestation of the human host is reported to result in an eosinophilia rather than an eosinophilopaenia (Section III. Ch. 2.3). It is feasible, however, that the demonstration of a relative eosinophilopaenia in those

newborns whose mothers had a positive Ascaris RAST when compared with the newborns whose mothers were not parasitised could be the result of the paucity of observations for the group of newborns from the parasitised mothers.

It is significant, also, that the apparent ethnic differences in CBTEC's referred to in Section VII. Ch. 3.3 disappeared when those newborns with an aFH and maternal Ascariasis were excluded from the statistical calculations. This suggests that maternal Ascariasis obscured the effect of ethnicity on the CBTEC's, whatever the reason, since an aFH on its own was shown to have no effect on the CBTEC's in any of the ethnic groups.

3.6 THE INFLUENCE OF THE NEWBORNS SEX

No significant sex variation was found for CBTEC's in any of the ethnic groups when the data was categorized into male and female newborns.

Our results conflict, therefore, with those of Cunningham (1975), who found boys to have higher TEC's than age-matched girls. His results were, however, obtained from the examination of infants and children, and not for newborns. Furthermore, his study population was unselected, and included both atopic and non-atopic subjects. Felarca et al (1967), like us, showed no sex difference in their study of selected non-atopic subjects but, like Cunningham's series, his study population did not include newborns.

3.7 THE INFLUENCE OF MATERNAL CIGARETTE SMOKING

Maternal cigarette smoking did not influence the CBTEC's in any of the ethnic groups.

No data has been previously published on this subject in relation to CBTEC's. In view of the equivocal reports published on the effects of maternal cigarette smoking on CBsIgE values (Section III. Ch. 1.8.6) this data is, nevertheless, of interest.

3.8 GENERAL COMMENT

The only variable which had an unambiguous statistically significant influence on the CBTEC's in the various ethnic groups was the effect of maternal Ascariasis in the Black newborns (Table V.19). Even this effect was clouded by the contradictory finding of a relative eosinophilopaenia in those Black newborns who had mothers with immunological evidence of Ascariasis. If this effect is indeed spurious, it may be due to tne paucity of observations (7) relating to mothers with immunological evidence of Ascariasis.

The influence of ethnicity on the CBTEC's in the total uncategorized group of Black-White and White-Mixed newborns was similarly abrogated when an aFH and maternal Ascariasis were adjusted for.

An aFH clearly had no influence on the CBTEC's in any ethnic group. Unless a clear statistical difference can be demonstrated for this marker between those newborns who developed atopic symptoms during infancy and those who did not (Phase II), this cord blood marker would not seem to hold any promise as a predictive atopic marker during infancy.

CHAPTER 4 CORD BLOOD PLATELET COUNTS

4.1 THE CONCEPT OF NORMALITY FOR CORD BLOOD PLATELET COUNTS

The normal range of platelet counts in healthy humans is 150-400 x 10^9 platelets per litre of blood, with counts in individual subjects being relatively constant (Dacie and Lewis, 1984). Recent data published on a series of 564 normal patients in South Africa (Lamparelli et al, 1988) reflected a mean platelet count of 283 x 10^9/l for the South African population. Unfortunately they did not mention the sex or the ethnicity of the study sampling, nor the age of the patients. This study did, however, reflect the fact that a progressive decrease in platelet counts occurred in pregnant women with advancing gestation, a parameter which was not mentioned in the review of variations in platelet counts by Dacie and Lewis (1984) (Table III. 3).

The large variation in normal values reported in the literature is thought, by Dacie and Lewis, to be due to differences in measurement and counting techniques. They have estimated that the co-efficient of variation (CV) using a visual method of counting is of the order of 10%, whereas electronic counting may lower the CV to 3-4%.

Because of this large variation in normal values, and because no data is available for cord blood values in the different ethnic groups examined in this study, it was decided to structure the analysis of the CBPIC data around comparative statistical methods, (as we did for CBTEC's) rather than use an arbitrary reference figure for thrombocytopaenia or thrombocytosis, as we did for the CBsIgE evaluations.

Magnussen and de Weck (1985) used a similar statistical method to describe their data. Their definition of thrombocytopaenia was not referable to any absolute standard figure, but was instead that statistical mean value obtained from a subgroup of newborns who developed atopy by 18 months of age which was statistically significantly lower than the corresponding value from the sub-group of newborns who remained healthy.

This study has used the same principle to define relative differences between subgroups, and did not attempt to define normality for the CBPlC for the newborns.

4.2 PHYSIOLOGICAL VARIATIONS IN CORD BLOOD PLATELET COUNTS

The documented physiological variations in platelet counts have been summarized in Table III. 3. As was the case for the CBTEC's it is not known whether these variations apply also for CBPlC's.

What is known, is that age affects the platelet count, and that at birth and during infancy, the counts are at the lower levels of the adult normal range. Since our newborns were all full-term babies (a gestational age of 38 weeks or older), we are confident that we did not have to adjust the data further for age.

The diurnal variations described for platelet counts have been thought to occur on a day to day basis, rather than during the course of a single day. Our specimens, as described in Section IV. Ch. 2.2.1, were in any event taken during the 6-hour period between 09H00 and 15H00.

The effect of menstruation on platelet counts was obviously not an issue in this study, and the influence of the newborn's sex was considered as part of the protocol for this study.

4.3 THE INFLUENCE OF ETHNICITY

The interpretation and relevance of the ethnic differences shown for CBPlC's (Table V. 8) are difficult to assess. It will be seen below that neither an aFH nor Maternal Ascariasis had any significant statistical effect on the CBPLC's in any of the ethnic groups (Table V. 12). It is difficult to rationalise, therefore, the effect which the non-consideration of these variables had in obscuring the effect of ethnicity on the CBPLC.

It is tempting to speculate that other unknown factors which may be associated with those newborns with an aFH may be responsible

for the apparent ethnic differences observed when these categorizing variables are included in the sampling of newborns compared. Until any additional influencing factors are identified, it is important to appreciate that ethnic differences for CBPLC's, as described in this study, do exist. Whether these are due to ethno-genetic or environmental influences is still unclear.

4.4 THE INFLUENCE OF AN ATOPIC FAMILY HISTORY

As mentioned in Section VII. Ch. 4.3, an aFH per sê did not have any significant effect on the CBPlC's in any ethnic group (Table V.12). This finding contradicts the findings published by Magnussen and de Weck (1985), and suggests that CBPlC's are probably not of value in the identification of the "high-allergic-risk" newborn.

Unequivocal evidence to this effect will be generated by Phase II of the study, where a lack of differentiation in CBPlC's between those newborns who developed atopic symptoms during infancy and those who remained healthy confirm its redundancy as a cord blood atopic marker.

4.5 THE INFLUENCE OF MATERNAL ASCARIASIS

Maternal Ascariasis did not directly affect the CBPlC's in any of the ethnic groups. It should be empnasised that only 1 White mother had a positive RAST for Ascaris, making comparative statistical analysis for this ethnic group impossible.

4.6 THE INFLUENCE OF THE NEWBORN'S SEX

The newborn's sex did not influence the CBPLC's significantly in any of the ethnic groups. It seems, therefore, that the sex differentiation documented by Dacie and Lewis (1984), where females had platelet counts 20% higher than males, is operative only later in life, and not in newborns. It is interesting to note, however, that the female newborns in each ethnic group did have marginally higher median CBPlC values than the male newborns. This difference was not, however, statistically significant.

4.7 THE INFLUENCE OF MATERNAL CIGARETTE SMOKING

Maternal cigarette smoking did not influence the CBPlC in any of the ethnic groups. Tne literature does not suggest that this variable has been ever assessed in relation to CBPlC's. In view of the recent discovery of the role of platelets in the allergic response, (Section III. Ch. 3.2) and the reported effect of cigarette smoking on CBsIgE values in some series (Section III. Ch. 1.8.6), the relationship between maternal cigarette smoking and CBPlC's needed to be established.

4.8 GENERAL COMMENT

Phase I of this study suggests that CBPlC's are of no value as a cord blood atopic marker to predict the "high-allergic-risk" newborn. Phase II of this study will confirm this impression.

Furthermore, none of the four variables investigated in Phase I of this study (ethnicity had an effect before exclusion of the positive aFH and positive maternal Ascariasis RAST sub-group) had any definitive effect on tne CBPlC's. The contribution of tnis section of the study is, therefore, predominantly to challenge the data of Magnussen and de Weck (1985) in terms of their suggestion that CBPlC's may differentiate between those newborns with and those without an aFH.

CHAPTER 5 CORD BLOOD ANTI COW'S MILK SERUM IgG CONCENTRATIONS

5.1 NORMAL REFERENCE VALUES FOR CORD BLOOD ANTI-COWS MILK IgG CONCENTRATIONS

No normal reference values for CBacmIgG have yet been published. There are various reasons for this.

Firstly, the estimation of the specific IgG antibody concentration as a potential predictive marker for atopy is of contemporary interest. Epidemiological studies to define and establish the range of normality for either maternal or CBacmIgG have not yet been done. In particular, long-term predictive follow-up studies are necessary before retrospective analyses of which cord blood concentrations at birth constitute "normality" for the development of atopic symptomatology is possible for this marker.

Secondly, in the previously published studies concerned with specific IgG antibody concentrations, the investigators have used different laboratory techniques for the assay methodology as well as different descriptive methods for reporting on the data. Dannaeus et al (1978) used an IgG antibody assay method based on the binding of IgG to Staphylococcus protein A-coated Sepharose, using ^{125}I-labelled allergen. They expressed their results as a percentage of a reference value, which was the geometric mean +/- 1 SD. Jarrett and Hall (1983), by comparison, estimated serum IgG concentrations in their test serum fractions by using a single radial immunodiffusion method, and used normal rat serum with a known immunoglobulin G concentration as the standard.

Finally, since the cord blood concentration of specific IgG antibody is dependant on the immune reactivity of the mother, varying maternal immunological and dietary influences will result in a wide quantitative range for normal reference values.

This study will, as did the study of Dannaeus et al (1978), interpret the results of the CBacmIgG values as a relative comparison between the different categorizing variables.

5.2 THE INVERSE RELATIONSHIP BETWEEN CORD BLOOD ANTI-COW'S MILK IgG AND CORD BLOOD SERUM IgE CONCENTRATIONS

A review of the modulating role of maternal immunity on the newborn and infant was presented in Section III. Ch. 4.2. In particular, the animal studies of Jarrett and Hall (1979) suggested that an inverse relationship existed between maternal sIgG antibodies and cord blood sIgE antibodies. By corollary, since antibodies of the IgG class cross the materno foetal placental barrier by virtue of their low molecular weight (Spiegelberg, 1974), the cord blood IgG concentration should closely mirror the maternal IgG concentration. This was demonstrated in our study, by the fact that the correlation co-efficients of maternal versus cord blood anti-cows milk serum IgG concentrations were very high for each ethnic group (Table V.16).

The extrapolation of these dynamics to the relationship between cord blood serum IgG and IgE should therefore suggest that an inverse relationship in the quantitative concentrations of these two markers should also exist, irrespective of the clinical relevance of such a relationship.

The data arising from this study did, in fact, demonstrate this relationship for the Black newborns. It was shown in Table V.2 that the median CBsIgE values in the Black newborns were significantly higher than those of the White and Mixed newborns, while the median CBacmIgG values in the Black newborns were significantly lower than those of either the White or the Mixed newborns (Table V. 5) Whether this indicates that the Black newborns would develop relatively more atopic disease in infancy will be ascertained in Phase II. These ethnic differences and their implications will be addressed more fully below.

5.3 THE INFLUENCE OF ETHNICITY

Ethnic differences in CBacmIgG values between all of the total uncategorized groups of newborns were demonstrated in this study (Table V.9).

The fact that these differences persisted when those newborns who had evidence of maternal Ascariasis were excluded from the study,

indicates that the varying incidence of maternal Ascariasis in the different ethnic groups was not responsible for these ethnic differences.

Alternatively, the fact that these differences disappeared when the newborns were split into subgroups depending on the presence or absence of an aFH strongly suggests that an aFH impacts differentially on the CBacmIgG values in the different ethnic group. The ethnic differences were still absent when only those newborns with neither an aFH nor evidence of maternal Ascariasis were compared between the ethnic groups. This suggests that, as for CBsIgE, the effect of atopy obscures the effect of ethnicity on CBacmIgG. Alternatively, the ethnic differences in CBacmIgG concentrations in the total group of newborns could reflect the unique maternal dietary customs in the different ethnic groups. Since dietary factors are associated with atopic sensitisation (Section II. Ch. 2.3), it could possibly be the influence of these associated dietary factors which are responsible for the abrogation of the ethnic differences when the sampling is categorised on an aFH.

5.4 THE EFFECT OF AN ATOPIC FAMILY HISTORY

Only the White newborn CBacmIgG values were influenced by an aFH.

This indicates that this marker could have atopic relevance in terms of its predictive capacity, and this will be further addressed in Phase II.

The fact that the Black newborn CBacmIgG values were not influenced by an aFH (as was the case for the Black CBsIgE values) indicates once again that the expression of certain immunological markers in this ethnic group are not readily reflective of an atopic genetic inheritance, but are rather more sensitive to environmental stimuli (see Ch. 5.5).

5.5 THE EFFECT OF MATERNAL ASCARIASIS

The subgroups of newborns with maternal Ascariasis had significantly

higher CBacmIgG values than those without maternal helminth infestation. Since none of the newborns had a positive Ascaris RAST (even though this immunoassay was highly sensitive) suggests that transplacental passage of the Ascaris antigen was not operative.

Statistically, maternal Ascariasis affected the CBacmIgG values in both the Black and the Mixed newborns. Ascariasis per sê has been shown by Godfrey (1975) and Turner (1980) to protect against the development of atopy by the saturation of mast cell IgE receptors with specific Ascaris IgE antibodies.

The raised CBacmIgG values in the newborns with maternal Ascariasis, together with the evidence that raised CBacmIgG values are associated with the abrogation of the allergic response (Dannaeus et al, 1978) could be one of the immunological effector mechanisms responsible for the observation that Ascariasis seems to protect against atopic sensitisation. This immunologicol rationale needs to be investigated more closely. It could, however, be one of the mechanisms at play which relates the low incidence of atopic disease in the rural Black ethnic groups to the high prevalence of Ascaris infestation in Third World, developing countries.

5.6 THE EFFECT OF THE NEWBORN'S SEX

From an epidemiological point of view, it is interesting to note that the sex of the newborn did not influence the CBacmIgE values in any ethnic group. It is relevant, however, to appreciate that the influence of an aFH, maternal Ascariasis and perhaps other ethno-genetic factors on this marker could obscure any minor sex differentiation at birth. A larger study would be needed to make such a distinction.

5.7 THE EFFECT OF MATERNAL CIGARETTE SMOKING

While this variable did not have any influence on the CBacmIgG values in any ethnic group, the same principle applies to the possible effect of this marker as was made above, namely that the effect of the other variables could possibly have obscured the effect of maternal smoking on this cord blood marker.

5.8 GENERAL COMMENT

It seems evident that CBacmIgG values are influenced by both environmental and ethno-genetic factors which could impact on the development of the atopic phenotype. It is the only cord blood marker other than CBsIgE values which is influenced by an aFH in the White ethnic group, and by maternal Ascaris infestation in each of the ethnic groups. This finding may further clarify the role of the Ascaris helminth in the development of atopic sensitisation in Black populations in developing Third World countries (Phase II).

SECTION VIII

DISCUSSION - PHASE 2

CHAPTER I GENERAL CONSIDERATIONS

1.1 INTRODUCTION

The objectives of Phase II of this study were twofold.

Firstly, the newborns were closely monitored, both clinically and immunologically, for the development of atopic symptoms and sensitisation during the first year of their lives. At the end of the study period, they were classified on their Cumulative Adjusted Atopic Status (CAS) (Section VI. Ch. 1.3) as being either "atopic" or "not atopic".

Secondly, the median values of the cord blood atopic markers for the "atopic" and the "not atopic" group were then statistically compared for each ethnic group in order to determine whether the infants who developed atopy had cord blood marker values which differed from the cord blood marker values of the infants who remained healthy. This would give an indication as to a possible predictive relevance of each marker for the ethnic group.

The rationale for approaching the analysis of the Phase II data in terms of identifying a possible statistically significant difference in the cord blood markers between the "atopic" and the "not atopic" groups (rather than using a specific "cut-off-point" for each marker) is based on the fact that no "normal values" for any of the cord blood markers exist for the South African ethnic groups studied (Section VII.). Kjellman et al (1976) made the point that, for the establishment of normal values of IgE at different ages, a number of criteria for the selection of a healthy, non-atopic study population was mandatory. The same criteria would also apply for the establishment of normal values for the other cord blood markers assessed during this study. The newborns in this study were unselected as far as an aFH was concerned (Section IV. Ch. 2.2.3). This study was, furthermore, not designed to establish normal values, but was designed rather to establish the need for such reference values.

For this reason the demonstration of statistical differences in the cord blood markers between the "atopic" and the "not atopic" group was considered to be the best method to establisn whether the specific cord blood markers could possibly prospectively differentiate between the two groups of infants at birth.

1.2 MOTIVATION FOR THE 1 YEAR FOLLOW-UP PERIOD

Previous studies examining the predictive relevance of various atopic markers in newborns, infants and children have varied significantly in design as far as the duration of the follow-up period is concerned.

Halpern et al (1973) and Kjellman and Croner (1984) followed up newborns for 7 years, in order to assess the development of atopic disease in these children. Kjellman's study is still ongoing, and is the longest follow-up study in this field reported in the literature.

Orgel et al (1975), Michel et al (1980b) and Croner et al (1982) followed their newborns up for much shorter periods of time, ranging from a 9 month follow-up period to 2 years.

In order to decide on a practical time frame for the duration of our study, it was important that enough time was allowed during the follow-up period to observe the development of atopic symptomatology. In this regard, the natural history of atopic disease is of importance. Halpern et al (1973) studied the development of childhood allergy over 7 years, and found that, of the study group who developed atopic symptoms over this time, 64% had developed their symptoms by 1 year of age. Furthermore, 93% of those who would develop gastro-intestinal allergy and 80% of those who would develop atopic dermatitis within the 7 year follow-up period, had already developed their symptoms by one year of age. Van Asperen et al (1984), who prospectively followed up a group of infants with an aFH from birth up to 20 months of age, similarly found that the majority of infants who developed either food allergy or atopic dermatitis during this period, did so in the first 12 months of age. Of those infants who developed allergic rhinitis, most had developed these symptoms by 12 months of age. Furthermore, tne prevalence of

atopic dermatitis and allergic rhinitis increased significantly during the first year of life but decreased markedly thereafter. The only potentially atopic symptom to increase its prevalence after 8 months of age was wheezing.

Arising from the consideration of the preceding arguments, it was decided that a follow-up period of 1 year after birth would give us a reasonable opportunity to make a balanced assessment of those infants who were predisposed to the development of atopic symptomatology. A longer follow-up period would certainly have provided a few more cases of atopy which would develop after the first year of life, particularly as regards the development of asthma. The advantages accrueable in extending the follow-up period were, however, considered to be outweighed by the fact that such an extension would inevitably result in a far greater drop-out rate than would have been acceptable. Some of the difficulties encountered in ensuring adequate compliance from the mothers on the study have been addressed in Section IV. Ch. 2.3.4. Of note, also, is the fact that, in contrast to some of the preceding studies in which most of the follow-up information was provided by means of a questionnaire and not a visit, (Croner et al, 1982; Kjellman and Croner, 1984), our mothers and infants were physically required to come into the hospital on 3 occasions during the year, with essentially healthy infants. This required a commitment on their part which was not easy to justify for a period beyond 12 months, particularly since the external jugular venepuncture (Section IV. Ch. 2.3.7) was an unpleasant occurrence which the mother had to come to terms with on every visit.

1.3 CRITERIA FOR THE CLINICAL CATEGORISATION OF THE INFANTS AT EACH FOLLOW-UP VISIT

In order to determine the cc applicable to each infant at the follow-up visits, (Section VI. Ch. 1.2.1) the author (M.H.) considered the information available on the infant from three different sources:

(i) The infant's medical history record card (Secton IV. Ch. 2.3.4).

(ii) The mother's history of the infant's health and medical status.

(iii) The findings on the physical examination of the infant by the author (M.H.).

Note:
If the infant's own physician made a firm diagnosis of asthma, eczema or any other of our defined atopic manifestations according to his own criteria, and entered this onto the infant's medical history record card, the author (M.H.) accepted this diagnosis as contributing to a cc of "definitely atopic" unless the findings of the author (M.H.) during the history and examination of the infant at the follow-up visit caused the author to change his opinion of the physicians' findings, in which case the author's decision overrode the diagnosis of the private physician. If, however, the physician or a primary care nursing sister at the clinic entered data onto the infant's medical history record card which was equivocal as far as the author's (M.H.) criteria for the classification of the atopic manifestations was concerned, a cc of "possibly atopic" was made. As in the previous example, any information which accrued to the author (M.H.) during the history and examination of the infant at the follow-up visits overrode any such diagnosis of "possibly atopic".

The specific clinical criteria for the cc of the infants at each follow-up visit into the three groups

a) Definitely atopic
b) Possibly atopic
c) Not atopic

are given below.

1.3.1 Definite atopic signs or symptoms: (definitely atopic)

(i) Definite asthma

Two or more attacks of wheezing (Van Asperen et al, 1984;

Murray, personal communication), with at least one attack documented by the author (M.H.) and the other attacks documented by the infant's personal doctor or clinic sister (from infant's medical history record card).

(ii) Definite atopic dermatitis (eczema)

The criteria of Van Asperen et al (1984) were used, namely "clinical evidence of typical atopic dermatitis, i.e., areas of scaly, erythematous, pruritic dermatitis, primarily involving the flexural folds, face, cheeks or behind the ears". The pruritic element makes "scratching" a valid additional criterion, as babies with eczema are said to scratch from the age of three months. (Murray, personal communication).

iii) Definite allergic gastro-intestinal food reactions

A combination of the criteria used by Bahna and Heiner (1980) Kjellman (1983) Kjellman and Croner (1984) and Van Asperen et al (1984) were used, namely; vomiting, diarrhoea or colic after the ingestion of a specific food at least twice and occurring within the first four hours after the ingestion of the food.

(iv) Definite allergic urticaria

Clinical urticaria, with or without angio-oedema, was diagnosed if it was confirmed by at least two repeated challenges from the same food or other human allergen, and if it was not due to drugs, insects, serum sickness or unknown causes. (Halpern et al, 1973; Kjellman and Croner, 1984).

1.3.2 Possible atopic signs or symptoms: (possibly atopic)

Infants were classified into tne "possibly atopic" group in the following instances:

(i) If the criteria given above for definite asthma, atopic dermatitis, allergic gastro-intestinal food reactions and allergic urticaria were not personally determined by the author (M.H.), but were rather based only on the infant's private physician's diagnosis or the diagnosis of the primary health clinic sister as documented on the infant's medical record card.

(ii) If any of the following additional clinical criteria applied:

a) Clinical evidence of seborrhoeic dermatitis

The possible relationship of this clinical manifestation to the atopic state was suggested by Kjellman (1983), who reported on data where 53.6% of children who developed atopic dermatitis during a prospective study involving 144 infants had a provisional diagnosis of seborrhoeic dermatitis made during infancy.

b) Clinical evidence of possible allergic rhinitis

The combined criteria of Murray et al (1969) and Van Asperen et al (1984) were used, namely, "nose-rubbing, fits of sneezing, snorting or sniffing, nasal discharge and/or blockage occurring continuously for at least four weeks and excluding obvious infective rhinitis". The reason for classifying this symptom complex under the "possibly atopic" and not the "definitely atopic" category is two-fold. Firstly, many of the criteria mentioned above are subjective and open to bias in the maternal perception of her infant's health. Secondly, Van Asperen et al (1984) concluded that, based on the findings of their prospective study designed to define the role of atopy and IgE related mechanisms for many commonly occurring, so-called "allergic" symptoms in infancy, rhinitis was not shown to be definitively related to atopy.

c) A miserable and irritable infant who does not sleep well at night (Crook et al, 1961; Weinberg, personal communication).

d) Recurrent otitis media (two or more attacks) Kjellman (1976a).

e) Moderate to severe colic, continuously for more than one month, unrelated to the ingestion of any particular food (Bahna and Heiner, 1980).

1.3.3 No atopic signs or symptoms: (Not atopic)

The infants were classified into this category at each follow-up visit if none of the criteria listed under Ch. 1.3.1 (definitely atopic) or Ch. 1.3.2 (possibly atopic) applied.

1.4 INVESTIGATION OF A POSSIBLE NON-RESPONDER BIAS

During the course of the 1 year follow-up period, some of the infants failed to attend at 3, 7 or 12 months, for one or other reason. These infants were regarded as drop-outs or non-responders, and were excluded from the data pool for that particular visit.

In order to assess whether these non-responders introduced an element of bias to the results obtained for the various markers at each follow-up visit, we assessed the statistical effect of their drop-out at each visit with reference back to their cord blood birth data, since this was the reference point which was used to analyse the data for Phase II. If significant statistical differences were found in the cord blood marker values between the responder and the non-responder groups at the various follow-up visits, then the non-responder factor would be seen to have introduced an element of bias into the analysis of the Phase II data. The fact that no such differences were found in the cord blood marker concentrations between the responder and the non-responder groups at each follow-up visit in this study (Table VIII. 1) indicates that the non-responders dropped out at random, and were not responsible for the introduction of any statistical bias to the analysis of the Phase II data.

1.5 THE INFLUENCE OF SENSITISING VARIABLES DURING THE FOLLOW-UP PERIOD ON THE CUMULATIVE ADJUSTED ATOPIC SCORE OF THE INFANTS

Phase II of this study was designed so that a confident diagnosis of either "atopic" or "not atopic" could be made for each infant completing the study (CAS). It was therefore important to ensure that, as far as possible, any variables having a sensitising effect on the infants during the follow-up period should impact equally and uniformly on all of the infants. To a large extent, this was not within the scope of this study.

In Section II. Ch. 1 the intrinsic and extrinsic factors which play a role in the sensitisation of infants were reviewed. The development of atopic sensitisation during infancy is clearly dependant on the extent, duration and timing of an infant's exposure to the extrinsic factors.

Since this study was not designed to assess the effect of these factors on the development of atopic sensitisation in infancy and because the limited size of our study groups precluded sub-categorization dependant on the relevance of each of these factors, we did not adjust our data specifically for the impact of all of these factors in each infant. It was, for example, practically impossible to standardise these culturally, socio-economically and ethnically diverse families in terms of their feeding methods during infancy. We have shown, moreover, that much of the evidence available on the beneficial effect of breast-feeding as far as its protective effect against the development of atopic disease is concerned is contradictory and conflicting (Section II. Ch. 1.3). It was also outside the scope of this study to quantify objectively the dose-related effect of passive smoking by measuring the infant's urinary cotinine (Section II. Ch. 1.6).

We specifically resisted the temptation to manipulate the infants environment by suggesting or prescribing any preventive measure to the mothers during the follow-up visits. This would inevitably have introduced bias into the follow-up study by influencing the CAS at the end of the study period for some of the infants, depending on how closely they would have followed any such proposals.

TABLE V III. 1

INVESTIGATION OF NON-RESPONDER BIAS

Comparison of the average value of each of the cord blood marker at birth between the group of responders and the group of non-responders' at each follow-up visit.

FOLLOW UP VISIT (months)	RACE	NUMBER IN GROUP AT BIRTH	NUMBER OF RESPONDERS AT FOLLOW-UP	NUMBER OF NON-RESPONDERS AT FOLLOW-UP	CORD BLOOD MARKERS							
					IgE		EOSINOPHILS		PLATELETS		CBacmIgE	
					p-value	Stat. decision	p-value	Stat. decision	p-value	Stat. decision	p-value	Stat. decision
3	Black	53	35	18	0.1853	NS	0.4861	NS	0.7750	NS	0.7459	NS
	White	52	44	8	0.5676	NS	0.5090	NS	0.1607	NS	0.7169	NS
	Mixed	58	52	6	0.7879	NS	0.1121	NS	0.8656	NS	0.7593	NS
7	Black	53	27	26	0.4988	NS	0.6429	NS	0.4342	NS	0.6059	NS
	White	52	37	15	0.9034	NS	0.2929	NS	0.9662	NS	0.1858	NS
	Mixed	58	44	14	0.9057	NS	0.4354	NS	0.6412	NS	0.2099	NS
12	Black	53	16	37	0.5224	NS	0.4488	NS	0.6945	NS	0.4326	NS
	White	52	36	16	0.8660	NS	0.1239	NS	0.8925	NS	0.1738	NS
	Mixed	58	41	17	0.9795	NS	0.1196	NS	0.9222	NS	0.5671	NS

NOTE: The statistical decision is made at the 5% significance level.

Our role during the follow-up visits was, therefore, confined to observing and investigating the infants as dictated by the protocol. If any specific problem areas were identified during these visits, the infants were referred back to their own physician or the appropriate referral centres for independent treatment and advice.

Notwithstanding these comments, we did design the protocol in such a way so as to standardize for a few of the extrinsic sensitising factors impacting on the infants.

In particular, this was of relevance in two areas.

(i) The standardisation of the season of birth.

The study was designed so that the newborns on the study were all delivered in the South African autumn months of March, April and May. This precluded any possible influence which the high pollen counts prevalent in the spring months could have exerted in selectively influencing the development of atopic sensitisation in newborns if some were to have been born in the autumn and some in the spring months (Section II. Ch. 2.7).

(ii) The standardisation of the three-in-one DPT inoculations.

Good evidence exists that the administration of the DPT immunisation in infancy could enhance the development of atopic sensitisation by acting as an adjuvant for the production of IgE and by stimulating the development of specific IgE antibodies to the Bordetella pertussis antigen (Haus et al, 1988a). For this reason, it was considered essential that all of the infants uniformly received all of the three immunisations, at more or less the same time. The mothers were therefore instructed to ensure that their infants have the first DPT immunisation after the 3 month follow-up visit, and that all three immunisations were to be completed before the 7 month follow-up visit (Section IV. Ch. 2.3.4). In this way, the potential of the Pertussis vaccine to enhance atopic sensitisation impacted equally on all of the infants in this study.

1.6 ETHNIC VARIATIONS IN THE DEVELOPMENT OF ATOPY DURING INFANCY

Ethnic variations in the prevalence of atopic disease have been well described in the literature. This concept has been reviewed in Section I. Ch. 2.2. Whether these variations are a result of socio-cultural and environmental factors (Section I. Ch. 2.1) or intrinsic ethno-genetic and immuno-genetic factors (Section II. Ch. 2) has not yet been resolved. In all probability, the conclusions of Soothill (1976), Marsh et al (1981) and Zeiger et al (1986), who suggest that the expression of atopic disease is a complex interaction between multigenic, intrinsic hereditary factors and extrinsic non-genetic factors, is correct.

The conclusion of most of the literature reviewed (Section I. Ch. 2.2) is that the prevalence of atopic disease in Black populations is far less than in other ethnic groups. The results of Phase I of this study, which demonstrated a far lower incidence of an aFH in the first degree relatives of the Black newborns (9.4%) on this study relative to the incidence of an aFH in the White newborns (75%) seemed to endorse this perception.

The results of Phase II of this study challenge the contention that atopic sensitisation is rare in the Black ethnic groups. Table VIII. 2 shows that the percentage of Black infants with a CAS of "atopic" was greater than that of the White or the Mixed infants (64.0%, 47.4% and 58.7% respectively). Furthermore, 52.0% of the Black infants who were assessed at the end of the study period had developed specific IgE antibodies to at least one of the allergens tested for (cow's milk, egg white or Dermatophygoides pteronyssinus) at some stage during the first 12 months of their lives, in contrast to only 18.4% of the White and 37.0% of the Mixed infants.

The incidence of the development of atopy in the White and Mixed infants, while lower than that found in the Black infants, is still high. This is not unexpected, however, when seen in the light of the relatively high incidence of a positive aFH in these White and Mixed infants (Section V. Ch. 4.1), indicating a high genetic load for atopy at play in these population cohorts.

TABLE VIII. 2.

THE INCIDENCE OF A POSITIVE CUMULATIVE ADJUSTED ATOPIC STATUS (CAS) AND A POSITIVE RAST IN INFANCY FOR EACH ETHNIC GROUP

ETHNIC GROUP	TOTAL NO. OF INFANTS ANALYSED AT 1 YEAR	CAS		RAST	
		ATOPIC	PERCENTAGE OF TOTAL NO.	POSITIVE	PERCENTAGE OF TOTAL NO.
Black	25	16	64.0%	13	52.0%
White	38	18	47.4%	7	18.4%
Mixed	46	27	58.7%	17	37.0%

What is unexpected, is the finding that the extremely high percentage of Black infants who developed atopy is, paradoxically, at variance with their very low incidence of a positive aFH. The genetic load for the development of atopy in this ethnic group should have been low, and contradicts the predictive nature of an aFH on the development of atopic sensitisation as reviewed in Section II. Ch. 1.1. In the White infants, all who developed atopy also had an aFH (Fig VI. 2) whereas a large number of the Black infants without an aFH developed atopy (Fig VI.1).

Of interest also was the finding that the Black infants with a CAS of "atopic" had a lower median CBsIgE concentration than their healthy counterparts with a CAS of "not-atopic" (Table VI. 13). This contrasts with the data for the White and the Mixed infants, where the median CBsIgE concentrations in the groups with a CAS of "atopic" were significantly higher than in the groups with a CAS of "not atopic".

The factors at play in the development of atopy in the Black South African infants seem, therefore, to be complex, and do not follow the generally accepted patterns relating to the relationship between an aFH, raised CBsIgE concentrations and subsequent atopic disease (Section II. Ch. 2.1).

1.7 THE RATIONALE FOR THE HIGH INCIDENCE OF ATOPIC SENSITISATION IN THE BLACK INFANTS

Van Niekerk (1979) has shown that rural Black South African children have a far lesser incidence of asthma than their urban counterparts. It was pointed out in Section VII Ch. 1.4 that the Black newborns on our study had a rural heritage, even though the study was executed in an urban environment.

The fact that such a large number of the Black infants on our study developed atopy during infancy, often with objective immunological evidence of atopic sensitisation, (Table VIII. 2), indicates that environmental, extrinsic factors which are prevalent in the urban milieu are potent catalysts for atopic sensitisation in this ethnic group. The Black newborns on this study, almost invariably, were the first generation to be born in an urban environment.

Their parents had spent most of their lives in the rural environment of the Eastern Cape (Section IV. Ch. 1.2.1). This fact was mirrored by the extremely low incidence of an aFH in the first degree relatives of the newborns (9%), concording with Van Niekerk's conclusions regarding the relatively low incidence of atopic disease in rural communities.

It seems, from this epidemiologic evidence, that modulating factors associated with urbanisation may constitute potent stimuli for atopic sensitisation in Black infants, and that these stimuli often result in objective evidence of sensitisation almost immediately after exposure to urban environments. The reasons for this phenomenon is still, at present, speculative. Animal studies done by Rousseaux-Prevost et al (1977) and Pauwels et al (1979) have, however, provided a model which could be paralleled in man. Inbred strains of rats with high basal IgE concentrations (the high IgE responder phenotypes) developed powerful serum IgE responses subsequent to antigen exposure, in contrast to inbred strains of rats with low basal IgE concentrations (low IgE responder phenotypes) who subsequently developed very weak reaginic responses after antigen exposure.

Phase I of this study showed that some of the Black newborn cohort had extremely high CBsIgE values at birth (greater than 6.00 kU/l). Furthermore, the median CBsIgE values in the Black cohort were significantly higher than that found in the White and Mixed cohorts, in the sup-groups without an aFH. This IgE was non-specific and polyclonal to the best of our knowledge (the specific IgE antibodies tested for on the cord blood were all negative on the supersensitive RAST), and confirmed the impression held by Croner et al (1982) on the non-specific nature of CBsIgE. It is reasonable to assume that our Black newborn cohort could therefore represent the human counterpart of the inbred rat high IgE responder phenotypes (Haus et al, 1988). If this assumption is correct, the subsequent apparent clinical hyper-responsiveness of our Black infants to the urban environment, where both the allergen profile as well as the cultural feeding habits and practices are so fundamentally different to that found in the rural areas, is not difficult to appreciate.

One confounding aspect of this situation remains. The Black infants who developed atopy were, statistically, not necessarily those with the highest CBsIgE values at birth. This observation was discussed above, in Ch. 1.6, and will be addressed again in the following Chapter (Ch. 2.1).

CHAPTER 2 THE PREDICTIVE RELEVANCE OF THE CORD BLOOD ATOPIC MARKERS IN EACH ETHNIC GROUP

Table VI. 13 summarises the statistical comparative analysis of the four cord blood atopic markers in relation to the infants who became "atopic" during infancy and those who remained "not atopic" in each ethnic group.

2.1 CORD BLOOD SERUM IgE CONCENTRATIONS

This marker discriminated significantly at birth for the White and the Mixed newborns between the "atopic" and the "not atopic" infants (p less than 0.05 in both ethnic groups). The median CBsIgE values of the infants who developed atopy were moreover significantly higher than the CBsIgE values of those who remained healthy. This finding is in line with the previously published data in this field as regards the predictive value of CBsIgE concentrations in predominantly First World, Western populations (Section III. Ch. 1.7). Figure VI. 2 shows that, of the White infants who completed the study, all but one with CBsIgE values greater than 0.50 kU/l developed atopic disease (87.5%). Figure VI. 3 shows that, of the Mixed infants who completed the study, seven of eleven with CBsIgE values greater than 0.50 kU/l developed atopic disease (63.6%).

In the Black newborns, this situation was reversed. The median CBsIgE values of the "atopic" infants were not statistically significantly different from the median CBsIgE values of those who remained "not atopic" (p = 0.57). Furthermore, the median CBsIgE values of the "atopic" infants were lower than the CBsIgE values of those who remained "not atopic". Figure VI. 1 shows that, of the Black infants who completed the study, only 50% with CBsIgE values greater than 0.50 kU/l developed atopy. Most of the infants who developed atopy had CBsIgE values below 0.2 kU/l.

The results for our Black infants did not show statistically significant associations between CBsIgE and the subsequent development of atopy. This part of the analysis was, however, based on only 16 Black infants who developed atopy during the follow-up period. We were therefore unable to comment as to the true predictive relevance of CBsIGE. Noenetheless, it appears unlikely

that CBsIgE as a predictor of atopy would be clinically useful among South African urban Blacks. These findings confirm the second part of the hypothesis on which this thesis is based.

2.2 CORD BLOOD TOTAL EOSINOPHIL COUNTS

The values of this marker did not clearly differ between those infants who became "atopic" and those who remained "not atopic" in any of the ethnic groups.

In tne Black infants, the statistical test of comparison was, however, equivocal ($p = 0.05$) with the infants who became "atopic" having a median CBTEC of 291 cells/mm^3 and the infants who remained "not atopic" having a median CBTEC of 196 cells/mm^3.

Even tnough Phase I of tnis study did not confirm the work of Dry et al (1980) who showed that CBTEC's were significantly higher in newborns with an aFH than in newborns with no such history, it is possible that a larger study could indicate that, of the four markers studied, the CBTEC's in Black, South African newborns is the most useful atopic marker in terms of its predictive capacity for the development of future atopic sensitisation.

2.3 CORD BLOOD PLATELET COUNTS

Since the values of this marker did not differ at birth between those infants who became "atopic" and those who remained "not atopic" in any of the ethnic groups, its relevance as a predictive cord blood atopic marker seems to be limited. The results of this study did not support the rnetoric of Magnusson and de Weck (1985) on the possible predictive function of cord blood thrombocytopaenia.

2.4 CORD BLOOD ANTI-COW'S MILK SERUM IgG CONCENTRATIONS

This marker similarly did not differ at birtn between those infants who became "atopic" and those who remained "not atopic" any of the

ethnic groups. In spite of the outcome of Phase I of this study, where it was shown that an aFH influenced the CBacmIgG values in the White newborns, a possible predictive relevance for this marker regarding the development of atopic disease in infancy was not demonstrated for any ethnic group in Phase II. Of interest, however, is the proposal by Dannaeus et al (1978) Bousquet and Michel (1984), and Kjellman (1985), that relatively high titres of CBacmIgG may afford the newborns a measure of protection insofar as the development of atopic symptomatology is concerned (Section III. Ch. 4.2 and Ch. 4.3). In all of the infants who completed Phase II of this study, irrespective of the ethnic groups to which they belonged, those that became "atopic" had lower values of CBacmIgG at birth than those who remained "not atopic" (Table VI. 13). This difference was not, however, statistically significant (p greater than 0.5 in each ethnic group).

SECTION IX

CONCLUSION AND SUGGESTIONS FOR FUTURE STUDY

CHAPTER I CONCLUSION

This study has tested and confirmed certain aspects of the hypothesis, shed light on the remainder, and has satisfied the aims as detailed in Section I. Ch. 3. It has provided an insight into possible genetic and environmental mechanisms operative in Black, Third World populations, and has shown tnat these are profoundly different to those mechanisms operative in White, First World populations.

More specifically, this study has provided new information in the following areas:

1.1 THE RELEVANCE OF GENETIC AND ENVIRONMENTAL INFLUENCES ON CORD BLOOD SERUM IgE CONCENTRATIONS IN SOUTH AFRICAN NEWBORNS AND INFANTS

(i) This study has shown that, in a randomly selected sampling of full-term Soutn African Black, White and Mixed newborns, the Black group had the highest CBsIgE values. This ethnic difference was statistically significant if the newborns with an aFH were excluded from the analysis, indicating an ethno-genetic influence on this atopic marker, independent of the influence of a high genetic load for atopy.

(ii) This study set out to challenge previously published literature which suggests that an aFH is reflected in newborns by raised levels of CBsIgE. Because of the small number of newborns witn an aFH in our Black cohort (5 cases) we were unable to provide definitive evidence to this effect in the Black ethnic group. As expected, however, the aFH did influence the CBsIgE in our White and Mixed newborns.

(iii) This study has shown that CBsIgE values in the Black newborns did not differ at birth between those who became "atopic" and those who remained "not atopic" during infancy. Paradoxically, those Black infants who developed atopy during

infancy had lower median CBsIgE values at birth than those who remained "not atopic", although this difference was not statistically significant. The conclusion to be drawn from this is that raised CBsIgE values at birth are probably clinically not relevant as predictive markers for the development of atopic disease in infancy for the Black South African population. This contradicts the previously published literature which suggests that a raised CBsIgE in a newborn is almost invariably associated with the development of subsequent atopy in that newborn. The White and Mixed newborns on our study did, however, reflect the previously published literature on this subject, by showing clear statistical differentiation in their CBsIgE values between those who became "atopic" in infancy and those who remained "not atopic". This indicates that CBsIgE has been confirmed as being of probable value as a predictive atopic marker for our White and Mixed South African newborns.

(iv) This study has refuted the suggestions made in the literature that Black, underdeveloped populations do not readily develop atopy. We have clearly shown that Black infants precipitously and vigorously develop atopic sensitisation if born into an urban environment, to a greater degree than White and Mixed newborns in the same environment. The high percentage of Black infants who developed atopy occured in spite of their very low incidence of a positive aFH, indicating that the dynamics operative in the transition from a rural to an urban environment for our Black populations have profound effects on the epidemiology of atopic sensitisation. The implications of this situation are not yet clear, particularly since the levels of CBsIgE in the Black newborns did not seem to be related in any way to the subsequent development of atopic disease in these infants.

(v) This study contributed to the current knowledge on the epidemiology of CBsIgE. More specifically, the effects of ethnicity, an aFH, maternal Ascariasis, the newborn's sex and maternal cigarette smoking on the CBsIgE values in the three ethnic groups under consideration, were assessed. Even though the size of some of the sub-groups assessed were small, our statistical methods of analysis were, in our opinion, reliable enough to draw our conclusions.

1.2 THE RELEVANCE OF GENETIC AND ENVIRONMENTAL INFLUENCES ON ALTERNATIVE CORD BLOOD MARKERS IN SOUTH AFRICAN NEWBORNS AND INFANTS

(i) This study has assessed cord blood eosinophils, cord blood platelets and cord blood anti-cow's milk IgG for their possible relevance as predictive atopic markers for the development of atopy in infancy in three South African ethnic groups.

The cord blood total eosinophil count was not influenced by an aFH in any of the ethnic groups, but was possibly of value in predicting the "high-allergic-risk" Black newborn who goes on to develop atopic disease in infancy. This cord blood marker was the only one to suggest that the "high-allergic-risk" Black newborn can possibly be identified at birth.

The cord blood platelet count was not influenced by an aFH in any ethnic group nor did it differ between those infants who became "atopic" and those who remained "not atopic" in any ethnic group. Its possible relevance as a cord blood predictive atopic marker in South African populations was, therefore, not supported by this study.

The cord blood anti-cow's milk IgG concentration was influenced by an aFH in White newborns only. It did, however, not differ between the infants who became "atopic" and those who remained "not atopic" in any ethnic group. Its possible relevance as a predictive atopic marker in South African populations was, therefore, similarly not supported by this study.

(ii) This study has contributed to the current knowledge on the epidemiology of cord blood total eosinophil counts, cord blood platelet counts and cord blood anti-cow's milk IgG concentrations by considering the effects of ethnicity, an aFH, maternal Ascariasis, the newborn's sex and maternal cigarette smoking on their values.

CHAPTER 2 SUGGESTIONS FOR FUTURE STUDY

As is the case with most studies of this nature, the new questions posed by the data presented in the study invariably outnumber the questions answered.

This study was no exception to the rule. Quite clearly, the future challenge in this field lies in attempting to understand more fully the immunogenetic and environmental mechanisms responsible for the rapid development of atopic sensitisation in the urbanised Black infants in the South African environment. Only when these are understood, will we be able to devise methods whereby the "high-allergic-risk" Black newborn can be identified and manipulated in terms of the prevention of future atopic disease.

More specifically, future studies could include investigation into the following areas:

(i) The establishment of normal reference values for CBsIgE in all of the ethnic groups in this country. This is imperative before clear guidelines can be given as to any deviations from normality. This study has shown that international data and values are not always applicable to local populations. "Normal value" studies in this context imply that large numbers of normal, non-atopic newborns need to be assessed.

(ii) The identification of alternative predictive atopic markers for the identification of the "high-allergic-risk" South African Black newborns should be a priority now that the predictive relevance of CBsIgE values in this ethnic group has been disputed. In this regard, the further assessment of the cord blood total eosinophil count in this ethnic group seems to hold the most promise.

(iii) The extremely high basal values of CBsIgE in some of the Black newborns merit further study. The influence of the mother's diet and environment during her pregnancy on the CBsIgE values could be assessed and compared to that operative in White mothers. The question also arises whether the high CBsIgE values in the Black newborns are truly polyclonal, or whether

a significant degree of intrauterine sensitisation has not already occured by birth. The opportunity exists for the intensive screening of cord blood with a far larger panel of RAST antigens, using very sensitive techniques to detect the development of any possible specific IgE antibodies.

Intra-amniotic fluid, obtained by amniocentesis, could also be studied to clarify the ontogeny and development of the intrauterine IgE response in these Black, high IgE-responder phenotypes.

(iv) The effect of urbanisation in enhancing the development of atopic disease in Black infants needs to be clarified. Specific environmental, socio-cultural and epidemiological influences acting differentially on rural and urban Black infants need to be identified and compared.

APPENDIX

APPENDIX I

CONSENT FORM

PATIENT STUDY CODE NO._____

MATERNAL CONSENT FORM FOR CHILD AND SELF TO PARTICIPATE IN CORD BLOOD AND 1 YEAR FOLLOW-UP IgE STUDY

I, _____

of (address) _____

_____

_____

confirm that the details of the study have been explained to me satisfactorily and I am willing to give my consent for myself and my son/daughter to participate in the above study.

NAME OF SON/DAUGHTER: _____

I also understand that my child and myself may withdraw from this clinical trial at any time if I should wish to do so.

Signature of Parent _____

I confirm that I have explained to the mother the full extent and nature of this clinical study.

Signature of Investigator _____

Date _____

APPENDIX II

PHASE 1 QUESTIONNAIRE

GENERAL DETAILS

Patient code number

Ethnic group

Black = 1
White = 2
Mixed = 3

Surname (Mother) ----------

Christian name (Mother) ----------

Christian name (Baby) ----------

Address ----------

Postal code ----------

Telephone number Home ----------

Work ----------

Other ----------

Hospital of Delivery

Peninsula Maternity Home = 1
Mowbray Maternity Home = 2
Midwife Obstetric Unit = 3
(Hanover Park)

INFORMATION FROM MOTHER

Date of interview of mother

Year
Month
Day

Age of mother in years

Number of years domiciled in Cape Town

Family status

Married	= 1
Stable relationship but unmarried	= 2
No permanent relationship	= 3

Occupation

Mother ----------

Father ----------

Smoking habits of mother

does smoke	= 1
does not smoke	= 2
other members of family smoke	= 3

describe type of
smoking & frequency: ----------

Drugs taken during pregnancy and labour

Insulin I.V.	Yes	=	1	No	=	2	Unknown or Unsure	= 9
Progesterone	Yes	=	1	No	=	2	Unknown or Unsure	= 9
Propranolol	Yes	=	1	No	=	2	Unknown or Unsure	= 9
H.C.G. (Pregnyl)	Yes	=	1	No	=	2	Unknown or Unsure	= 9
Oral Epinephrine	Yes	=	1	No	=	2	Unknown or Unsure	= 9

Medical problems during pregnancy

Yes = 1 No = 2 Unknown or Unsure = 9

Specify: ----------

FAMILY HISTORY OF ATOPIC DISEASE

Relationships

Mother	Yes	=	1	No	=	2	Unknown (or unsure)	= 9
Father	Yes	=	1	No	=	2	Unknown (or unsure)	= 9
Siblings of Infants							Yes = 1 No	= 2
(Brothers & Sisters)							Unknown (or unsure	= 9

PHYSICAL EXAMINATION OF THE INFANT

Date of birth of infant

Year
Month
Day

Sex of infant

Male = 1
Female = 2

Birthweight (grams)
Head circumference (cms)
Length (cms)

Estimated gestational age of infant (weeks)

From date of last menstrual period
From bimanual abdominal palpation
From modified Dubowitz score
From estimation by early ultrasound

Selection criteria satisfied (Yes = 1 No = 2)

Newborn
1. Full-term (38 weeks or more by above criteria)
2. Birthweight (2,500 grams or more)

Maternal
1. No specified medication in pregnancy
2. Resident for 1 year or more in Cape Town

LABORATORY INVESTIGATION OF CORD BLOOD

Total eosinophil count (cells/m^3)
Platelet count ($\times 10^9$ L)
IgE (kU/l)
IgA (mg/dl)
IgG (anti-bovine milk-specific) Δ.O.D.
(414mm)

RAST (specific IgE antibodies)

	Phadebas RAST Class Score Code
cows milk	Neg = 0, 1 = 1, 2 = 2, 3 = 3, 4 = 4
egg white	Neg = 0, 1 = 1, 2 = 2, 3 = 3, 4 = 4
Ascaris lumbricoides	Neg = 0, 1 = 1, 2 = 2, 3 = 3, 4 = 4

LABORATORY INVESTIGATION OF MATERNAL BLOOD

Total eosinophil count (cells/m^3)
Platelet count ($\times 10^9$ L)
IgE (kU/l)
IgA (mg/dl)
IgG (anti-bovine milk-specific) Δ.O.D.
(414mm)

RAST (specific IgE antibodies)

	Phadebas RAST Class Score Code
cows milk	Neg = 0, 1 = 1, 2 = 2, 3 = 3, 4 = 4
egg white	Neg = 0, 1 = 1, 2 = 2, 3 = 3, 4 = 4
Ascaris lumbricoides	Neg = 0, 1 = 1, 2 = 2, 3 = 3, 4 = 4

APPENDIX III

MOTIVATIONAL LETTER FROM AUTHOR (M.H.) TO PARENTS FOR 7 MONTH FOLLOW-UP VISIT

UNIVERSITY OF CAPE TOWN

Department of Paediatrics & Child Health

Institute of Child Health
Red Cross War Memorial Children's Hospital
Rondebosch, Cape 7700
Telephone 65-6529
Telegraphic Address: Hosfil
Head: Prof. H. de V. Heese, M.D., B.Sc., F.R.C.P., D.C.H.

21st October 1985

Dear

We would like to thank you most sincerely for being so co-operative and enthusiastic regarding compliance with our cord blood IgE - Allergy Study.

We know it is not pleasant to hear the little infants cry while blood is being taken but somehow, they soon forget, and the information obtained from the results will ensure that any abnormality is picked up at the earliest possible stage.

Since the study is not yet finished, the conclusions drawn from the results will obviously not be to the immediate benefit of this infant.

However, we have some exciting preliminary results, but without your continued support, our study will not realise its full potential, and a golden opportunity will be lost to successfully predict and prevent allergic disease.

May we therefore ask you, if at all possible, to keep your 7 month follow-up appointment - be assured it is much appreciated, and you will be contributing in a very positive way to ongoing medical research.

Yours sincerely

Signed

DR MATT HAUS

PROFESSOR H. DE. V. HEESE

APPENDIX IV

LETTER OF THANKS FROM AUTHOR (M.H.) TO PARENTS ON COMPLETION OF THE STUDY

UNIVERSITY OF CAPE TOWN

Department of Paediatrics & Child Health

Institute of Child Health
Red Cross War Memorial Children's Hospital
Rondebosch, Cape 7700
Telephone 65-6529
Telegraphic Address: Hosfil
Head: **Prof. H. de V. Heese, M.D., B.Sc., F.R.C.P., D.C.H.**

16 JUNE 1986

Dear

Once again, I would like to thank you most sincerely for the continued support and interest you showed during the year in which I followed up your babies on the Allergy Study.

I want you all to know that the study is a great success, and I trust that it will go a long way towards helping the successful prevention of allergic disease in the country.

As promised, here are the final 1 year results for your baby:

Total IgE	_____	iu (Normal - up to about 10)
RAST - milk	_____	
egg	_____	
housedust mite	_____	
Ascaris	_____	
IgA	_____	
Hb	_____	
MCV	_____	

Thank you once more for all your co-operation. I hope that we will not lose contact altogether. If you have any queries, you may phone me at Cape Town 65-5011 ext 519. I will still be here for a few months, after which Mrs Heuer will give you my forwarding address.

Keep well

Signed

MATT HAUS

APPENDIX V

PHASE II QUESTIONNAIRE

GENERAL DETAILS

Follow-up study

$^{3}/_{12}$ = 3
$^{7}/_{12}$ = 7
$^{12}/_{12}$ = 12

Patient code number

Ethnic group

Black = 1
White = 2
Mixed = 3

Surname (Mother) ----------

Christian name (Mother) ----------

Christian name (Baby) ----------

Address ----------

Postal code ----------

Date of interview and examination

Year 19
Month
Day

INFORMATION FROM MOTHER

Medical history of infant

1. Feeding

Breast only	= 1
Breast and artificial	= 2
Artificial only	= 3

2. Illnesses

Has child been ill? Yes = 1 No = 2

If YES,

Unwell, but no health care	Yes = 1	No = 2
Visit to clinic	Yes = 1	No = 2
Visit to doctor	Yes = 1	No = 2
Admission to hospital	Yes = 1	No = 2

Specify_____

3. Allergic history

General

- Irritable, miserable, wakeful child	yes = 1
	no = 2
	unsure = 3
- Failure to thrive	yes = 1
	no = 2
	unsure = 3

Dermatological

- Facial eczema	yes = 1
	no = 2
	unsure = 3

- Flexural eczema

yes = 1
no = 2
unsure = 3

- Seborrhoea/cradle cap

yes = 1
no = 2
unsure = 3

- Itchy rash

yes = 1
no = 2
unsure = 3

- Dry skin

yes = 1
no = 2
unsure = 3

- Napkin dermatitis

yes = 1
no = 2
unsure = 3

- Other e.g. "suur", heat rash

Specify_____

yes = 1
no = 2
unsure = 3

Occular

- Recurrent, itchy, discharging eyes:

Ongoing, possibly allergic	Yes = 1	No = 2
Treatable bacterial/viral	Yes = 1	No = 2
Blocked tear duct	Yes = 1	No = 2

ENT

- stuffy nose, constant cold/watery nasal discharge

yes = 1
no = 2
unsure = 3

- Otitis media/ear infection/ discharging ear — yes = 1; no = 2; unsure = 3

- Tonsillitis — yes = 1; no = 2; unsure = 3

Respiratory

- wheezing/LAO (lower airways obstruction) — yes = 1; no = 2; unsure = 3

- if YES - how many "attacks" — one = 1; two = 2; three = 3; more than 3 = 4

  were these "attacks" associated with an illness and a temperature — yes = 1; no = 2

  were these "attacks" with hyperventilation/ tachypnoeic or shortness of breath — yes = 1; no = 2

Gastrointestinal

- vomiting soon after ingestion of certain foods — yes = 1; no = 2; unsure = 3

- if YES how many times has this occurred? — once = 1; twice = 2; thrice = 3; more than thrice = 4

  is it always the same food? — yes = 1; no = 2; unsure = 3

  Colic and abdominal cramps — yes = 1; no = 2; unsure = 3

PHYSICAL EXAMINATION OF THE INFANT

Weight of infant (grams)

Head circumference (cm)
Length (cm)

General examination

Clinically anaemic	Yes	=	1	No	=	2	Unsure	= 3
Clubbing	Yes	=	1	No	=	2	Unsure	= 3

Lymphadenopathy

Generalized	Yes	=	1	No	=	2
Local - Cervical	Yes	=	1	No	=	2
- Axillary	Yes	=	1	No	=	2
- Inguinal	Yes	=	1	No	=	2

General Condition/Nutritional Status	Good	= 1
	Bad	= 2
	Intermediate	= 3
Irritable, miserable child	Yes = 1	No = 2

Skin

Facial eczema	Yes	=	1	No	=	2
Flexural eczema	Yes	=	1	No	=	2
Cradle cap/seborrhoea	Yes	=	1	No	=	2
Dry skin	Yes	=	1	No	=	2
Papular urticaria	Yes	=	1	No	=	2
Nutritional lesions	Yes	=	1	No	=	2

Occular

Recurrent, Itchy, discharging eyes:

- Ongoing allergic	Yes	=	1	No	=	2
- Treatable bacterial/viral	Yes	=	1	No	=	2
- Blocked tear duct	Yes	=	1	No	=	2

ENT

- Stuffy nose, watery nasal discharge — Yes = 1 No = 2
- Swollen nasal mucous membrane (Turbinates) — Yes = 1 No = 2

- Ear Drums
  - normal = 1
  - dullness/red injected = 2
  - discharging = 3
  - perforated = 4
  - perforated & discharging = 5

- High arched palate — Yes = 1 No = 2

- Posterior pharangeal lymphoid hyperplasia — Yes = 1 No = 2

- Tonsillitis (viral, bacterial) — Yes = 1 No = 2

Abdominal
(G.I.T.)

Hepatomegaly — Yes = 1 No = 2

If YES, size in cms_____

Splenomegaly — Yes = 1 No = 2

If YES, size in cms_____

Other — Yes = 1 No = 3

Specify_____

Respiratory

Inspection

- Chest movements	Normal	=	1	Abnormal	=	2
- Anatomical defects	Yes	=	1	No	=	2
- Respiratory rate/min						
- Use of accessary respiratory muscles	Yes	=	1	No	=	2

Palpation

- Trachea central	Yes	=	1	No	=	2
- Cardiac apex	Normal position	=	1	Abnormal	=	2

Specify_____

Percussion

- Hyperresonant	Yes	=	1	No	=	2
- Liver pushed down	Yes	=	1	No	=	2
- Abnormal dullness	Yes	=	1	No	=	2

Auscultation

- Rhonchi	Yes	=	1	No	=	2
- Crepitations	Yes	=	1	No	=	2
- Transmitted sounds	Yes	=	1	No	=	2
- Wheeze/lower airways obstruction	Yes	=	1	No	=	2

If YES....	Unilateral	=	1	Bilateral	=	2
	Expiratory	=	1	Inspiratory	=	2

Cardiovascular System

Pulse Rate (per minute)

- Regular	Yes	=	1	No	=	2

Heart

- Cardiomegaly	Yes	=	1	No	=	2
- Heart sounds	Abnormal	=	1	Normal	=	2
- Murmurs	Yes	=	1	No	=	2

Specify_____

Clinical atopic classification of infant

No atopic signs or symptoms	=	1
Possible atopic signs or symptoms	=	2
Definite atopic signs or symptoms	=	3

Immunization programme

B.C.G. (Bacillus Calmette Guerien Vaccine)

	Yes	=	1	No	=	2
	Unknown	=	9			

If yes, give date: Year 19
Month
Day

D.P.T. (Diptheria, Pertussis and Tetanus)

no 1. + Polio	Yes	=	1	No	=	2
	Unknwon	=	9			

If yes, give date: Year 19
Month
Day

no 2. + Polio Yes = 1 No = 2
Unknown = 9

If yes, give date: Year 19
Month
Day

no 3. + Polio Yes = 1 No = 2
Unknown = 9

If yes, give date: Year 19
Month
Day

- Measles Yes = 1 No = 2
Unknown = 9

If yes, give date: Year 19
Month
Day

LABORATORY INVESTIGATION OF THE INFANT

Total eosinophil count (cells/m^3)
Platelet count (x 10^9/L)
IgE (kU/l)
IgG (anti-bovine milk-specific) .O.D. (414mm)

RAST (specific IgE antibodies)

	Phadebas RAST Class Score Code															
cows milk	0	=	0	1	=	1	2	=	2	3	=	3	4	=	4	
egg white	0	=	0	1	=	1	2	=	2	3	=	3	4	=	4	
Ascaris lumbricoides	0	=	0	1	=	1	2	=	2	3	=	3	4	=	4	
Dermatophygoides Pteronyssinus	0	=	0	1	=	1	2	=	2	3	=	3	4	=	4	

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