INFANTILE HYPOPHOSPHATASIA:
CARRIER MANIFESTATIONS

A thesis submitted

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THE FACULTY OF GRADUATE STUDIES

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In partial fulfillment of the requirements for the

DEGREE OF MASTER OF SCIENCE

by

© BERNARD CHODIRKER

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TABLE OF CONTENTS

	Page
Dedication	1
Acknowledgements	2
Abstract	3
List of abbreviations used	4
Units used in tables and figures	5
List of figures	6
List of tables	7
Introduction	8
Literature Review	
History	10
Clinical classification	11
Clinical features - Infantile type	13
Clinical features - Childhood type	15
Clinical features - Adult type	16
Dental manifestations	17
Biochemical data	18
Inheritance	22
Infantile hypophosphatasia - Carrier manifestations	24
Project Outline	26
Method	
Ascertainment	28
Family contact	29
Data collection	30
Historical data	31
Physical examination and anthropometric data	33
Biochemical data - Serum	36
Biochemical data - Urine	38
Dental radiographs	39
Carrier assignment - Logistic regression analysis	47
Descriptive and comparative statistics	48
Results	
Ascertainment	52
Clinical features of infantile cases	52
Alkaline phosphatase and phosphoethanolamine in controls and carriers	60
Carrier assignment	60

Comparisons between carriers and non-carriers 66
Logistic regression analysis 73

Discussion
Birth prevalence, gene frequency and carrier frequency 93
Inheritance 96
Carrier assignment 97
Comparisons between carriers and non-carriers 101
Significance of hyperphosphatemia 110

Conclusions 116

References 117

Appendix I
Letters sent to doctors, dentists, medical record departments and chemistry laboratories 122
Appendix II
Consent form 127
Appendix III
Data collection sheet 129

TO AARON AND CINDY
WHOSE GOOD HEALTH HAS MADE ME TRULY FORTUNATE

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ABSTRACT

Infantile hypophosphatasia is a severe, often fatal, inherited metabolic bone disorder. It is generally assumed that the inheritance of this disorder is autosomal recessive. Carriers for this condition show a decrease in serum alkaline phosphatase activity and an increase in urine phosphoethanolamine excretion. With the exception of these biochemical markers, little is known about the phenotype of carriers. Logistic regression analysis was used to compare 20 obligate carriers and 33 controls with regard to their serum alkaline phosphatase activity and urine phosphoethanolamine excretion. The derived equation was used to classify first degree relatives of the obligate carriers. Fifty - three relatives were classified as carriers and 45 as non-carriers. Carriers were then compared to non-carriers with regard to various criteria from history and physical examination, dental radiographs and biochemical analysis. Mean outer canthal distance, height/span ratio and span - height distance were different in females of the two groups ($0.01<p<0.05$). Serum phosphate was significantly higher in carriers of both sexes ($p\leq 0.0001$). The implications of these findings for future studies including the development of a screening program are discussed.

LIST OF ABBREVIATIONS USED

ALPL.....gene for the liver/bone/kidney isozyme of alkaline phoshatase

AP.....serum alkaline phosphatase activity

Ca.....serum calcium

CI.....cephalic index

DMF.....diseased plus missing plus filled teeth

DT.....deciduous teeth

FDR.....first degree relative

HC.....head circumference

HOPS.....gene for infantile hypophosphatasia

H/S.....height/span ratio

HT.....height

IC.....inner canthal distance

IP.....interpupillary distance

LRA.....logistic regression analysis

LS.....lower segment

MRP.....mucous retention phenomenon

OC.....outer canthal distance

PAP.....pedigree analysis package

PEA.....urine phosphoethanolamine excretion

Ph.....serum phosphate

S-H.....(arm)span minus height

US.....upper segment

WT.....weight

UNITS USED IN TABLES AND FIGURES

Alkaline Phosphatase.....U/L

Phosphoethanolamine.....umol/mmol creatinine

Calcium.....mmol/L

Phosphate.....mmol/L

Age walked.....months

Age D.T. lost.....years

Age.....years

IC.....cm

IP.....cm

OC.....cm

HT.....cm

WT.....kg

HC.....cm

CI.....%

S-H.....cm

Gonial angle.....degrees

LIST OF FIGURES

		page
1	Dental radiograph: Gonial angle	42
2	" " Osteosclerosis	42
3	" " MRP	43
4	" " Antrolith	43
5	" " Bifid canal	44
6	" " Tooth structures	44
7	" " Hypercementosis	45
8	" " Soft tissue calcification	45
9	" " Zygomatico-temporal suture	46
10	" " Eminence pneumatization	46
11	Flow diagram of study protocol	49
12	Radiographs of an infantile case	
	a) skull radiograph	57
	b) body radiograph	58
	c) lower extremities radiograph	59
13	Pedigree of family showing consanguinity	61
14	Serum alkaline phosphatase distribution	64
15	Urine phosphoethanolamine distribution	65
16	Pedigree of family showing carrier assignment	69
17	Scatter diagram: AP VS. PEA	71
18	Outer canthal distance distribution (in female first degree relatives)	87
19	Height/span ratio distribution (in female first degree relatives)	88
20	Span - height distribution (in female first degree relatives)	89
21	Serum phosphate distribution	111

LIST OF TABLES

		page
I	Igbokwe's model of inheritance	23
II	Normal ranges for chemistry laboratories which tested blood samples	37
III	Ascertainment of probands	53
IV	Summary of infantile cases	54
V	Alkaline phosphatase - Obligate carriers vs. controls	62
VI	Alkaline phosphatase - Distribution by sex	62
VII	Phosphoethanolamine - Obligate carriers vs. controls	62
VIII	Phosphoethanolamine - Distribution by sex	63
IX	Sex Distribution - Carriers vs. controls	63
X	Age Distribution - Carriers vs. controls	63
XI	Characteristics of controls who appear to be carriers	67
XII	Predictions according to logistic regression analysis	68
XIII	Participants who stated health was not good	74
XIV	Categorical historical data	75
XV	Non-categorical historical data	77
XVI	Physical examination categorical data	78
XVII	Physical examination (both sexes) non-categorical data	79
XVIII	Physical examination (males only) non-categorical data	80
XIX	Physical examination (females only) non-categorical data	80
XX	Dental categorical data	81
XXI	Dental non-categorical data	84
XXII	Alkaline phosphatase distribution (first degree relatives)	85
XXIII	Alkaline phosphatase - Distribution by sex (first degree relatives)	85
XXIV	Phosphoethanolamine distribution (first degree relatives)	85
XXV	Phosphoethanolamine - Distribution by sex (first degree relatives)	85
XXVI	Biochemical data	86
XXVII	Logistic regression analysis predictions (methods 1-4)	91
XXVIII	Discrepancies between carrier assignments	92
XXIX	Serum phosphate - Comparisons to control group	109

INTRODUCTION

Infantile hypophosphatasia is a severe, often fatal, inherited metabolic bone disease. It is generally considered that this form of hypophosphatasia is an autosomal recessive disorder. In contrast, the inheritance patterns for the milder adult and juvenile forms are less clear. One hypothesis states that patients with these milder forms represent heterozygotes for infantile hypophosphatasia.

The biochemical hallmarks of hypophosphatasia are a decreased level of serum alkaline phosphatase and a increased excretion of phosphoethanolamine in the urine. Carriers for the infantile form often show similar but less striking biochemical abnormalities. Aside from this, the clinical phenotype of these carriers has not been well described.

The purpose of this project was to investigate the phenotype of carriers of infantile hypophosphatasia. This may in the future aid clinicians in deciding which individuals are at an increased risk of having an affected child. Increased knowledge of the carrier phenotype would also be particularly valuable in developing a screening program for this disease. This information may also prove helpful in understanding the confusing inheritance of hypophosphatasia in total.

MacPherson et al. first noted the relatively high incidence of hypophosphatasia in the Mennonite communities

of Manitoba and Saskatchewan (1). The experience of the clinical geneticists in Winnipeg supported their impression. It was therefore felt that Manitoba would be an ideal place to undertake a study on the genetics of hypophosphatasia.

LITERATURE REVIEW

HISTORY

The first case report of hypophosphatasia in the English language literature was in 1935 by Dr. Bruce Chown of Winnipeg (2). Two sisters with "birth deformities like those of severe rickets" were born to consanguineous parents of English-Welsh descent. These cases, which were investigated prior to the assay for measuring serum alkaline phosphatase being routinely available, were described as representing renal rickets and dwarfism. Others have since reviewed these cases and concluded that they were in fact true cases of infantile hypophosphatasia (1).

The assay for measuring alkaline phosphatase activity was developed in 1934 by King and Armstrong of Toronto (3). Dr Rathbun, also of Toronto, recognized the association between the clinical findings and the low serum level of alkaline phosphatase (4). He was the first to use the term hypophosphatasia. In 1957, Dr. Donald Fraser of Toronto published a major review on this disorder (5). He described a clinical classification based on age of onset of symptoms. According to his classification, there were three major forms of hypoposphatasia, i.e. infantile, juvenile and adult forms. Dr. Fraser and Dr. McCance from England share the honour of being the first to recognize that patients with hypophosphatasia excrete increased amounts of phosphoethanolamine in their urine (5,6).

CLINICAL CLASSIFICATION

The clinical classification developed by Fraser, or modifications of it, is still in use today. The first category is the infantile form. In this form, lesions are present at birth or develop within the first six months of life. Currarino chose to subdivide this category i.e. into neonatal and later infantile forms (7). In his neonatal category, signs and symptoms were present within the first month. The infants in the later category first showed abnomalities between one and six months of age. For those in whom symptoms were present at birth, this was essentially a lethal disorder. The prognosis for the late infantile cases, although better, was still poor.

Fraser's second class represented the juvenile or childhood cases. These were the children in whom lesions gradually became apparent after the age of six months. The final major category was the adult form of the disease.

The most significant problem with this classification is that it is too arbitrary. The time of onset of symptoms cannot always be established accurately. Also, the age of diagnosis does not always reflect the severity of the disease. For example, patients with adult hypophosphatasia may have had symptoms in childhood that went undiagnosed. As Currarino pointed out, there is much overlap between the various classes (7). He stated that gradually patients in his infantile group became indistinguishable from those of the next group in most clinical features and course of their

disease.

Other types of hypophosphatasia have been described. Fraser did discuss a fourth class which he called "atypical hypophosphatasia" (5). This was based on a family originally described by Klein et al. in which a male infant presented with hypoglycemia, renal glycosuria, marked skin pigmentation, low serum alkaline phosphatase activity and increased phosphoethanolaminuria but with normal bones (8). An older brother apparently had the same disorder. Fraser felt that it would be unreasonable to include these patients with the hypophosphatasia cases.

A form of hypophosphatasia without skeletal manifestations has been described by Bixler et al. (9). Affected individuals had decreased serum alkaline phosphatase activity and premature loss of their deciduous incisors. This form appeared to be inherited in an autosomal dominant fashion.

Scriver and Cameron reported a three month old girl with what they termed "pseudohypophosphatasia" (10). This girl had all the hallmarks of classic hypophosphatasia including increased urinary excretion of phosphoethanolamine but with one exception. She had a normal level of serum alkaline phosphatase activity.

Despite this classification into groups the symptoms of hypophosphatasia can be viewed as being on a spectrum of severity. The most severe are the neonatal lethal cases, while the mildest are those with only dental involvement.

CLINICAL FEATURES - INFANTILE TYPE

The earliest presentation of hypophosphatasia is in the prenatal period. Suspicion of the diagnosis can arise in one of two ways. A prenatal ultrasound examination can suspect this diagnosis in the absence of a positive family history or when a pregnancy is followed because of the birth of a previously affected infant. In normal pregnancies an easily defined fetal head is seen on ultrasound examination by 16 weeks gestation. The first clue to the diagnosis can be failure or difficulty in visualizing the fetal head at this time (11). Measurement of amniotic fluid or maternal serum alpha-fetoprotein can be used to rule out the alternative diagnosis of anencephaly. More recent ultrasound reports describe the visualization of a faint, low echodense skull outline (12). Other prenatal features seen with ultrasound include reduced echogenicity of the fetal bones with gross shortening, bowing and fractures of the fetal long bones (12,13). Metaphyseal flaring has also been detected on a prenatal ultrasound examination (14).

Skeletal hypomineralization can also be detected radiographically in the prenatal period (14). Prenatal diagnosis can also be achieved by measurement of alkaline phosphatase activity in cultured amniotic fluid cells (15). More recently, the diagnosis has been made on a chorion villus sample with the use of specific monoclonal antibodies (13).

The neonatal form of hypophosphatasia is essentially a lethal disorder. Affected babies are usually stillborn or die soon after birth (16). Survival is possible if symptoms do present later. Of the 16 cases originally classified as infantile hypophosphatasia by Fraser, eight had symptoms at birth. All eight cases died within one year. Five of the other eight cases (i.e. gradual onset prior to six months) survived the first year of life (5). Five of Currarino's nine neonatal cases died within one hour of birth (7). The longest survivor in this group was six months. Of his 14 "late infantile" cases, six died before one year of age and one died at 29 months. Survival into adulthood has been reported by Ish-Shalom and others including Fraser (17,18). Clinical and radiographic manifestations of the disease gradually improved without therapy in five patients diagnosed in infancy who were moderately or severely affected.

The appearance of an infantile form is that of a "short-limbed" skeletal dysplasia syndrome such as osteogenesis imperfecta or thanatophoric dwarfism (1). Symptoms include anorexia, irritability, vomiting, fever, seizures and high pitched cries (1,5). Cyanosis and often fatal respiratory distress, secondary to an excessively compliant chest wall, are seen (2). Failure to thrive can be seen in infants who survive the first few days (1). A severely affected infant has a globular and "boneless" skull, a soft skeleton and severe deformities of extremities

(7). Radiologically there is marked osseous undermineralization with rachitic-like changes most pronounced at the metaphysis (1). In most cases a rachitic rosary was present (7). The skull bones exhibit ossification of the central portions only.

According to Currarino, those in this category that survived had symptoms indistinguishable from those with the childhood form (7).

CLINICAL FEATURES - CHILDHOOD TYPE

The prognosis for affected patients with this form is much better. Most of the children in this group were quite healthy on the whole (5). None of the patients described by Fraser or by Currarino died of complications of their disease (5,7). Clinical findings in these patients included early loss of the deciduous teeth, marked dental caries, growth retardation, craniosynostosis, brachycephaly, proptosis, rachitic rosary, delayed onset of walking, painful extremities, fractures, bone deformities and rachitic-like enlargements of the wrists, knees and ankles (1,5,7). Spontaneous improvement of the rachitic skeletal defects is possible (19). Dr. Robinow has been able to follow a girl with juvenile hypophosphatasia for 20 years (20). She presented to him at the age of 20 months having lost four teeth in the previous six weeks. She had remained at the third percentile for height with this short stature being primarily due to short lower extremities. Her

skeletal deformities (rachitic rosary, Harrison's grooves, knobby wrists and knock knees) had largely corrected themselves. Her only remaining defects were mild knock knees, a minor chest deformity and a waddling gait due to a unilateral coxa vara. Craniosynostosis was present but asymptomatic. Intelligence was normal. This improvement was felt to be spontaneous and unrelated to various therapies which were briefly tried.

CLINICAL FEATURES - ADULT HYPOPHOSPHATASIA

Adult hypophosphatasia is the mildest form of the disease. The purely dental form is milder but this in fact may be a less severe expression of adult hypophosphatasia. Affected individuals may give a history of early loss of the primary teeth or of rachitic deformities in childhood. Usually the presentation is of early loss of the adult teeth or of recurrent fractures secondary to "osteomalacia" (19). Delayed walking, childhood rickets, recurrent or pathologic fractures, slow healing of fractures, dolicocephaly, prominent eyes, skeletal deformities, backache, leg bowing, bone tenderness and loose joints have all been described in case reports of adult hypophosphatasia (21).

In a study of a large adult hypophosphatasia family, Whyte et al. showed that an individual could have a subnormal serum level of alkaline phosphatase activity (presumably because they inherited the gene for adult hypophosphatasia) and still be asymptomatic (21). However,

of eight relatives (over 18 years of age) of an adult hypophosphatasia proband who had low levels of alkaline phosphatase activity, five had dental symptoms including premature loss of the permanent dentition and a inability of the teeth to hold fillings. One individual gave a history of poor fracture healing. Neither osteopenia or radiologic features of a metabolic bone disorder were found on a limited skeletal survey done on these individuals. It was reported that no family member with a normal alkaline phosphatase activity had an unusual skeletal history. Although the dental histories in the "normal" family members were variable, they were "not as strikingly abnormal".

The radiologic features of adult hypophosphatasia are non-specific. The major abnormalities seen are osteopenia and fractures of several types. More common than traumatic fractures are stress fractures. A pseudofracture or Looser zone located in the subtrochanteric femur is also characteristic. Chondrocalcinosis, periarticular calcification and vertebral ligament calcification have also been described (21).

DENTAL MANIFESTATIONS

A wide variety of dental abnormalities have been reported in the juvenile and adult forms of hypophosphatasia. Premature loss of the deciduous teeth is frequently the first manifestation (22). Early loss or extraction of the permanent dentition occurs in adult life

(21). This early dental loss is not associated with significant gingival or peridontal disease (22). Other dental features which can be seen include hypoplasia or aplasia of the cementum, loss of alveolar bone, enamel hypoplasia, enlargment of the pulp chamber, irregular calcification, alveolar bone lesions, premature root resorption and an inabilty of the teeth to hold fillings (21,22).

BIOCHEMICAL ASPECTS

The biochemical hallmark of all forms of hypophosphatasia is deficient circulating and tissue activity of the liver/bone/kidney or tissue non-specific isozyme of alkaline phosphatase (23). The gene which codes for this enzyme has been mapped to the short arm of chromosome one (24). The other alkaline phosphatase isozymes (i.e. placental and intestinal) are coded for by separate genes (23). Post-translational modification accounts for the biochemical differences between the bone, liver and kidney forms (23).

Alkaline phosphatase activity has been reported in a wide variety of units with a wide range of normal values due to the large number of methods used in assaying its activity (25). It is therefore difficult to apply the results of one study directly to another. The "normal" reference interval for each laboratory should be taken into consideration.

Children have higher mean serum alkaline phosphatase

activities than do adults (25). A steady decrease towards adult values is seen in late adolescence of both sexes. This process is usually complete by the twentieth year in females but may continue into the third decade in males (26). Eastman and Bixler used 18 and 19 years as their "adult-age" cut-off for males and females respectively in their study of the inheritance of hypophosphatasia (26).

Although the age related changes in adults are smaller than in children, it is generally agreed that males up to age 50 do have a higher mean serum alkaline phosphatase activity than do females (25). Eastman and Bixler found that for serum alkaline phosphatase activity measured in International Union of Biochemistry units,the normal adult range for males was 62-176 and for females was 56-155 (26). The means for males and females were 111 +/-34 and 100 +/-33 respectively.

The isozymes of alkaline phosphatase seen in sera of patients with certain disases have the characteristics of the specific form present in bone, liver, intestinal, placental and renal tissues depending on which organ is involved by the disease (27). For example, the placental isozyme is increased in women who are pregnant. The predominant forms in normal sera are the liver and bone varieties (27). A small amount of intestinal alkaline phosphatase activity may also be demonstrated usually after a fatty meal, particularily in sera of individuals who are of blood groups B or O and who are secretor positive (27).

In a study of a family with adult hypophosphatasia, Millan found that of nine individuals with a low total alkaline phosphatase activity, the bone isozyme was low in all nine, while the hepatic isozyme was low in four (28). Danovitch had shown that intestinal alkaline phosphatase was not decreased in hypophosphatasia (29).

Other conditions associated with a low level of serum alkaline phosphatase activity include starvation, antilipidemic drugs, steroids, estrogen-progesterone combination pills, hypothyroidism and deficiencies of protein, zinc, vitamin B_{12} and vitamin C (25). The mean alkaline phosphatase activity in women on an estrogen-progesterone pill was 88% of controls. Patients who received replacement therapy for hypothyroidism had levels which were normal for age.

The other major biochemical feature of hypophosphatasia is an increased excretion of phosphoethanolamine in the urine. This was originally shown independently by Fraser and by McCance (5,6). Eastman and Bixler showed that phosphoethanolamine urine excretion is higher in childhood and relatively stable after the time of skeletal maturity (18 years in their study) (30). Their normal range (5th-95th percentile) for phosphoethanolamine excretion in adults was 15 - 84 umoles/g creatinine (26). This translates to 1.7 - 9.6 umoles/mmol creatinine. They used a Beckman 121 M amino acid analyzer to measure phosphoethanolamine excretion.

There are other causes for an increased excretion of phosphoethanolamine. These include hypertension, other bone diseases and certain endocrine disorders (31).

Hypercalcemia has been described in infantile cases but serum calcium is usually normal in older children and adults (32). Serum phosphate has usually been reported in the normal range (5,32). A few case reports have documented elevated or high normal levels of serum phosphate (33,34,35). Recently Whyte and Rettinger reported on 27 patients with various forms of hypophosphatasia (36). All had above normal levels of inorganic phosphate. Fifteen were frankly hyperphosphatemic. They felt that this was due to enhanced renal reclamation of phosphate.

Although it is known that patients with hypophosphatasia have a decreased activity of the bone isozyme of alkaline phosphatase and have symptoms similar to rickets with abnormal bone calcification, it is not known how alkaline phosphatase functions in bone metabolism. Increased plasma concentration and urine excretion of pyrophosphate have been documented in hypophosphatasia (37). Pyrophosphate is, therefore, thought to be a natural substrate for alkaline phosphatase (23). Pyrophosphate has also been shown to to be an inhibitor of bone mineral deposition and dissolution (23).

Recently Whyte et al. have documented increased levels of the vitamin B_6 metabolite pyridoxal-5'-phosphate in patients with hypophosphatasia (23). Alkaline phosphatase

is thought to act in the metabolism of this compound.

INHERITANCE

The inheritance for hypophosphatasia in general has not been clearly elucidated. There is, however, a consensus on the autosomal recessive inheritance of the infantile form (38). The precise modes of inheritance for the juvenile and adult forms remain unclear as do their relationship to the infantile disease.

Some investigators have suggested that the juvenile disease is a recessive disorder while others favor a dominant mode of inheritance (38). The same discrepancy of opinions can be seen in articles on adult hypophosphatasia (38).

Eastman and Bixler used segregation analysis to conclude that hypophosphatasia was an autosomal dominant disorder with homozygous lethality (39). In their model, 100% of heterozygotes showed significantly lower alkaline phosphatase activity and significantly higher phosphoethanolamine excretion. Eighty five percent had clinical signs of premature dental loss.

Igbokwe proposed a system of three codominant alleles to explain the inheritance of hypophosphatasia (38). His model is illustrated in Table I. In this model, an individual who inherits two infantile hypophosphatasia alleles (II), has infantile hypophosphatsia. Inheriting two

TABLE I

IGBOKWE'S MODEL OF INHERITANCE

PHENOTYPE	PROPOSED MODEL (GENOTYPE)
INFANTILE HYPOPHOSPHATASIA	II
CHILDHOOD HYPOPHOSPHATASIA	CC, CI
ADULT HYPOPHOSPHATASIA	NC, NI
ABSENCE OF HYPOPHOSPHATASIA	NN

Adapted from reference (38).

normal alleles (NN) results in a normal individual. One normal (N) and one abnormal allele (I or C) causes adult hypophosphatasia while two abnormal alleles providing both are not I's cause childhood disease. A two-loci model has recently been proposed by Timmons et al. (40).

INFANTILE HYPOPHOSPHATASIA - CARRIER MANIFESTATIONS

As previously stated low levels of serum alkaline phosphatase activity and increased phosphoethanolaminuria are seen in carriers of hypophosphatasia. Eastman and Bixler felt than 100% of carriers show this biochemical abnormality (39). Using paper chromatography, Harris was able to demonstrate in 1959, abnormal phosphoethanolamine excretion in 58% of carriers (41). Timmons et al. were able to discriminate between carriers and non-carriers by using the log of the phosphoethanolamine/alkaline phosphatase ratio (40).

Aside from these biochemical abnormalities little is known about the phenotype of carriers. The medical literature can be used in two ways to determine this phenotype. One can look at the information gathered on parents of affected infants. Unfortunately there is little non-biochemical data available on such parents. The data that are present in case reports do not include normal control data for comparison. The alternative method of studying carriers is to assume that the adult and juvenile patients do represent individuals heterozygous for the gene

for infantile hyopophosphatasia. There are, however, problems with this approach. This theory of inheritance has not been universally accepted. Another problem lies in the description of the typical patient. Most case reports describe patients with severe or interesting symptoms. More formalized family studies such as those done by Eastman and Bixler and by Whyte et al. are necessary to look at the "average" heterozygote (21,39). Eastman and Bixler stated that 85% of carriers of hypophosphatasia had clinical signs of premature dental loss. As stated previously Whyte et al. have described eight non-proband adult cases of which five had dental symptoms and one had problems with poor fracture healing.

PROJECT OUTLINE

The objectives of this study were to test the following hypotheses:

1) There is an increased incidence of infantile hypophosphatasia in the Mennonite population of Manitoba.

2) Carriers can be differentiated from non-carriers by using a combination of their serum alkaline phosphatase activity and their urine phosphoethanolamine excretion.

3) Carriers differ from non-carriers in ways that can be tested by history and physical examination, dental radiographs and routine biochemical analysis.

We also hoped to collect data which would support the hypothesis that infantile hypophosphatasia is an autosomal recessive disorder. A long-term goal is to develop a screening program to detect carriers of infantile hypophosphatasia in a high risk population such as the Mennonites in Manitoba.

This investigation was part of a larger study on the genetics of hypophosphatasia. The purpose of this specific project was to better describe the carrier phenotype of infantile hypophosphatasia. This was done by collecting clinical, biochemical and dental radiologic data from families in which a child had died from infantile hypophosphatasia. Other data have been collected for future analysis, including data from families of juvenile and adult hypophosphatasia probands. These data will only be

mentioned when appropriate. Hand radiographs on the family members have been obtained but have not yet been analyzed and therefore will not be discussed. Blood samples have already been sent to Dr. S. Coburn in Fort Wayne, Indiana for measurement of vitamin B_6 vitamers including pyridoxal-5'-phosphate. Blood was drawn before and after a loading dose of vitamin B_6. These studies are still in progress. Some of the data have already been used to show linkage between the genes for infantile hypophosphatasia (HOPS) and the Rh blood group and also between HOPS and ALPL (the gene for the liver/bone/kidney isozyme of alkaline phosphatase) (42,43).

METHOD

ASCERTAINMENT

Multiple sources of ascertainment were used to locate as many probands with any form of hypophosphatasia as possible. The starting point for this search was individuals and families known to the Section of Clinical Genetics and Metabolism at the Children's Hospital of Winnipeg. The medical records at the Health Sciences Centre and St. Boniface Hospital in Winnipeg were reviewed for the years 1976-1986 inclusive. Search criteria were chondrodysplasias or disorders of phosphate metabolism. Although hypophosphatasia is not a primary disorder of phosphate, for the purposes of medical records it is classified as such. All charts obtained using these search criteria were personally reviewed. As computerization of records were not available at the pathology departments of these two hospitals, autopsy summmary reports were manually reviewed from 1964 until 1986 at the St. Boniface Hospital and from 1967 until 1986 at the Health Sciences Centre. Although incomplete, the patient diagnosis files at the Fetal Assessment Unit at the Health Sciences Centre were also reviewed. Dr. J. McGuire had previously searched for all cases of skeletal dysplasias including infantile hypophosphatasia, diagnosed antenatally in Manitoba (14). The results of his search were kindly made available to us. The Congenital Anomalies Registry was also reviewed for the

years 1979-1986.

Letters requesting referral of patients and families were sent to all dentists in Manitoba with the assistance of The Manitoba Dental Association. Similar letters were also sent to all pediatricians, radiologists, orthopedic surgeons and hospital medical records departments in south-central Manitoba including Winnipeg. This geographic area was chosen as it is the region where the majority of Manitoba Mennonites live. Letters were also sent to general practioners and clinical chemistry laboratories in this area excluding Winnipeg. Samples of these letters are included in Appendix I.

The birth prevalence of infantile hypophosphatasia was calculated by dividing the number of cases ascertained by the number of Mennonite births in Manitoba over a given time period. The number of Mennonite births was estimated from information supplied by the Department of Vital Statistics of the Government of Manitoba.

FAMILY CONTACT

After ascertainment of the probands, the parents (of infantile and juvenile cases) or patients (adult cases) were contacted with permission of their doctor. They were then invited to participate in our study with as many of their first degree relatives as possible. This would include all their children, parents and siblings. Some more

distant relatives were asked to participate primarily for the linkage part of the study. Unrelated spouses were also invited to participate. These individuals would comprise the control group.

Informed consent was obtained prior to entry into the study. A sample consent form is shown in Apppendix II.

DATA COLLECTION

Study participants were subjected to a history and physical examination. In the majority of cases this data was collected personally. Other individuals who assisted in this data collection were Dr. Cheryl Greenberg, Dr. Jane Evans, Leonie Stranc, Steve McKendry-Smith, Bohdan Bybel and Shawna Redekopp. The historical data were originally recorded as given by the participant. Later the data was coded into specific categories. A copy of the data collection sheet is included in Appendix III.

All data were collected without knowledge of the biochemical results. It was, however, impossible to examine the obligate carriers (i.e. parents of an affected infant) or controls (i.e. unrelated spouses) without being biased to the carrier status. Evaluation of the first degree relatives was, however, done blind to their carrier status.

Our original goal was to collect complete information on each participant. Unfortunately, several factors prevented this. Most participants were seen at the Children's Hospital of Winnipeg or at their local hospital

or medical clinic. A few individuals were seen at their homes or at their places of employment. Time, space and equipment constraints made obtaining complete information on all participants impossible. Consent was not obtained from some individuals for certain aspects of the study.

On five individuals, historical data including height and weight were collected by telephone or letter and their blood and urine samples were sent by mail. The routine serum biochemical tests were done at their local laboratory. In two of these individuals, carrier assignment could not be made and their data were not included for analysis.

HISTORICAL DATA

Participants were questioned in regard to a history of:

1) RICKETS: i.e. was there a history of rickets during childhood? Responses were coded as positive or negative.

2) CRANIOSYNOSTOSIS: Responses were coded as positive or negative.

3) CHEST DEFORMITY: Responses were coded as positive or negative.

4) DENTAL FILLINGS (problems with): specifically did any fall out? Responses were coded as positive or negative.

5) BONE OR JOINT PAIN: Responses were coded as positive, negative or unrelated. For a response to be coded as unrelated, a specific non-hypophosphatasia explanation (e.g. gout) had to be given for the pain.

6) BONE FRACTURES: Responses were coded as positive,

negative or unrelated. Positive responses implied that a fracture occurred spontaneously or with minimal trauma. Unrelated fractures occurred with significant trauma as judged by the participant. Examples of severe trauma included motor vehicle accidents and falling off a roof.

Participants were also asked about the following:

7) AGE WALKED: i.e. at what age in months did they first walk.

8) AGE D.T. LOST: i.e when did they first lose their deciduous teeth (D.T.). Responses were coded in years respectively.

9) ADULT TEETH: Information regarding the number of adult (permanent) teeth lost and the timing of such loss was requested. Responses were classified as follows:

a: EARLY - $\geq$ 10 teeth lost $\leq$ age 35 (years)
or all teeth lost $\leq$ age 40 .

b: INTERMEDIATE - > 6 but < 10 teeth lost $\leq$ age 35 or $\geq$ 10 but <28 teeth lost after age 35

c: NORMAL - $\leq$ 6 teeth lost.

Wisdom teeth or teeth removed for orthodontic reasons were not included.

10) CARIES: The number of caries that the individual had (whether filled or untreated) was coded as follows:

a: FEW - 0-5

b: AVERAGE - 6-9

c: MANY - > 10

NOTE: All the above information was coded according to the response of the participant. Independent verification was not obtained. Unsure or unknown responses were left out of any analysis.

PHYSICAL EXAMINATION AND ANTHROPOMETRIC DATA

1) HEIGHT (HT): When possible this was measured on a clinic or hospital scale. If such a scale was unavailable, participants were simply asked to state their height. Results were coded in centimeters (cm.).

2) WEIGHT (WT): When possible this was measured directly on a hospital or clinic scale and recorded in kilograms. If not, again participants were simply asked to state their weight.

3) HEAD CIRCUMFERENCE (HC): This was measured with a cloth tape measure as the maximum cranial circumference and recorded in cm. The normal adult (18 year old) range is 53-58.5 cm. for males and 52-58 cm. for females (44).

4) CEPHALIC INDEX (CI): This was calculated by dividing the head width by the head length and multiplying by 100. The head length was measured with obstetric calipers as the greatest anterio-posterior diameter of the head. The head width was measured with obstetric calipers as the greatest transverse diameter of the head. The normal range is 75-80 (45).

5) INNER CANTHAL DISTANCE (IC): This was measured in cm. with a plastic ruler as the distance between the inner

canthi. The normal adult (14 year old) range is 2.5-3.5 cm (44).

6) OUTER CANTHAL DISTANCE (OC): This was measured in cm. with a plastic ruler as the distance between the outer canthi. The normal adult (14 year old) range is 7.8 - 9.9 cm (44).

7) INTERPUPILLARY DISTANCE (IP): This was calculated according to the formula described in Merloff et al (41). This formula states that IP = 0.7 + 0.59 IC + 0.4 OC. The normal adult (14 year old) range is 5.0-6.3 cm (44).

8) UPPER SEGMENT/LOWER SEGMENT RATIO (US/LS): This was calculated by dividing the upper segment distance in cm. by the lower segment distance in cm. The lower segment (LS) was measured with a cloth tape measure as the distance from the symphysis pubis to the bottom of the heels. The upper segment (US) was calculated as the difference between the height and the LS. The normal adult (15 year old) 50th percentile standards are 1.01 for females and 0.98 for males (47).

9) SPAN MINUS HEIGHT (S-H): This was calculated by subtracting the HT from the armspan. The armspan was measured with a paper tape measure in cm. as the maximum distance from one third fingertip to the contralateral third fingertip with the arms extended. The normal adult (15 year old) 50th percentile standards are 1.2 cm. for females and 4.3 cm. for males (47).

10) HEIGHT/SPAN RATIO (H/S): This was calculated by

dividing the HT by the armspan.

The following criteria were subjectively assessed by the examiner:

11) ABNORMAL SKULL SHAPE: Results were coded as positive or negative.

12) EXCESS LORDOSIS: Results were coded as positive or negative.

13) CLINODACTYLY: Results were coded as positive or negative.

14) BOWED LEGS: Results were coded as positive or negative.

14) JOINT LAXITY: This was assessed as being normal, increased or decreased.

BIOCHEMICAL DATA - SERUM

All but four blood samples were analyzed at the clinical chemistry department of the Health Sciences Centre in Winnipeg. The normal adult ranges for this laboratory and the other laboratories which tested blood samples are shown in Table II. The serum assays (Ca, Ph, AP) measured at the Health Sciences Centre were done using established methods (48,49,50) adapted for automated analysis on the Hitachi System 737 at 37°C. Serum phosphate measurements were not included in the analysis if the sample was grossly hemolyzed. All participants were instructed to fast overnight prior to the blood sample being drawn. The vast majority of participants complied with this request. A few stated they had toast or coffee for breakfast. None admitted to eating a fatty meal. No individual was excluded from analysis based on their dietary history. Blood samples were drawn in the morning and after serum separation of the appropriate sample, the blood was transported to the laboratory within one to six hours. One woman was pregnant but her alkaline phosphatase level had been previously assayed at the Health Sciences Centre and so this result was used.

One obligate carrier had her serum sample analyzed at the Thompson General Hospital. Two other obligate carriers (a male and a female) had their serum sample analyzed at the MDS laboratories in St. Catherines, Ontario. These

TABLE II

NORMAL RANGES FOR CHEMISTRY LABORATORIES

WHICH TESTED BLOOD SAMPLES

LABORATORY	Ca	Ph	AP
Health Sciences Centre, Winnipeg, MB	2.10 - 2.60	0.81 - 1.45	30 - 125
Thompson General Hospital, Thompson, MB	2.0 - 2.6	0.8 - 1.3	25 - 100
MDS, St. Catherines, ON	2.12 - 2.62	0.80 - 1.45	35 - 110
Southern Alberta Regional Hospital, Lethbridge, AB	2.12 - 2.62	0.82 - 1.45	80 - 270

alkaline phosphatase results for these two people were reported as < 25 U/L. The mean activity for those obligate carriers whose alkaline phosphatase activity was < 25 U/L, was 18.5 U/L. Therefore, for the purposes of analysis, arbitrary values of 18 U/L and 19 U/L were assigned to the male and female respectively. One first degree relative had her sample analyzed at the Southern Alberta Regional Hospitals laboratory in Lethbridge, Alberta. As the normal range for the alkaline phosphatase activity for this laboratory was so different from that of the Health Sciences Center, her results were not used in the analysis. No carrier assignment was, therefore, made.

No Ca, Ph or AP results were available from the other first degree relative on whom information was collected by letter. Consequently no carrier assignment was made.

BIOCHEMICAL DATA - URINE

All urinary phosphoethanolamine excretions were measured at the Health Sciences Centre Department of Clinical Chemistry. Five samples were sent to Winnipeg by mail. The rest were delivered to the laboratory within one to six hours of collection. Urines were collected as single void morning samples. Phosphoethanolamine levels were quantitated by amino acid analysis according to the method of Spackman on the LKB Amino Acid Analyzer Alpha Plus 4151 equipped with a high resolution column and a lithium based eluent system using computerized data processing (51). The

normal urinary phosphoethanolamine excretion for this laboratory is less than 10 ummol/mmol creatinine.

DENTAL RADIOGRAPHS

Panoramic dental radiographs were obtained primarily in one of two locations, i.e. the Dental College of the University of Manitoba in Winnipeg or at the Morden Dental Center. Two participants had these radiographs taken prior to the study and copies were sent to us for assessment. Initially all participants were requested to have a dental radiologic examination. However, after three months of data collection no further dental radiographs were requested. This decision was made for two reasons. First, the cost of these radiographs was becoming prohibitive. Secondly, a preliminary analysis indicated that this would not be a useful technique to distinguish carriers from non-carriers.

All radiographs were independently analyzed by a pathologist (Dr. C. Lavelle) and a oral pathologist (Dr. S. Ahing). Both are also dentists in the Faculty of Dentistry at the University of Manitoba. Both were unaware of the carrier status of the individual.

The following criteria were assessed by Dr. Lavelle as being either present or absent:

1) ENLARGED PULP CHAMBER:

2) ROOT RESORPTION: i.e. loss of definition of the root apex.

3) HYPERCEMENTOSIS: i.e. obliteration of the peridontal

ligament space due to hypercementosis.

4) LAMINA DURA THICKENING: i.e. significant thickening not reflecting periodontal disease.

5) THINNNED ENAMEL: i.e. enamel hypoplasia.

6) UNUSUAL TRABECULATION: i.e. a disturbance in the normal trabecular arrangement of the alveolar bone.

7) GROSS PERIODONTAL DISEASE: i.e. significant loss of alveolar bone.

8) ABNORMAL CALCIFICATION: i.e. focal regions of alveolar bone showing sclerotic or lytic areas.

Criteria 1,2,3,4,5 and 7 are only meaningful if teeth are present and were, therefore, not scored in edentulous individuals.

The following criteria were assessed by Dr. Ahing as being either present or absent:

9) WIDENING OF THE ZYGOMATIC SUTURE:

10) ABNORMAL SOFT TISSUE CALCIFICATION:

11) MRP: i.e. a mucous retention phenomenon or an accumulation of submucosal secretions in the maxillary sinus or antrum.

12) EMINENCE PNEUMATIZATION: i.e. air cells present in the eminence of the temporomandibular joint and not just limited to the mastoid.

13) BIFID CANALS: i.e. a bifid inferior dental canal instead of the normal single canal.

14) CALCIFICATION OF THE FACIAL ARTERY:

15) OSTEOSCLEROSIS: i.e condensation or sclerosis of

the mandibular bone.

16) ANTROLITH: i.e. a calcification in the maxillary sinus.

The following quantative data were calculated from the panoramic radiographs by Dr. Ahing:

17) TEETH: i.e the total number of teeth present excluding wisdom teeth.

18) DMF SCORE: i.e. the sum of the number of decayed plus missing plus filled teeth. Wisdom teeth were not included.

19) AVERAGE GONIAL ANGLE: This was measured according to the technique of Mattila et al. (52). The mean adult (15 -20 year old) average gonial angle is 127.8 (52) . If the quality of the radiograph was too poor, the angle was not calculated. If only one angle could be calculated, this was taken to be the average angle.

Examples of the dental parameters studied are illustrated in figures 1-10.

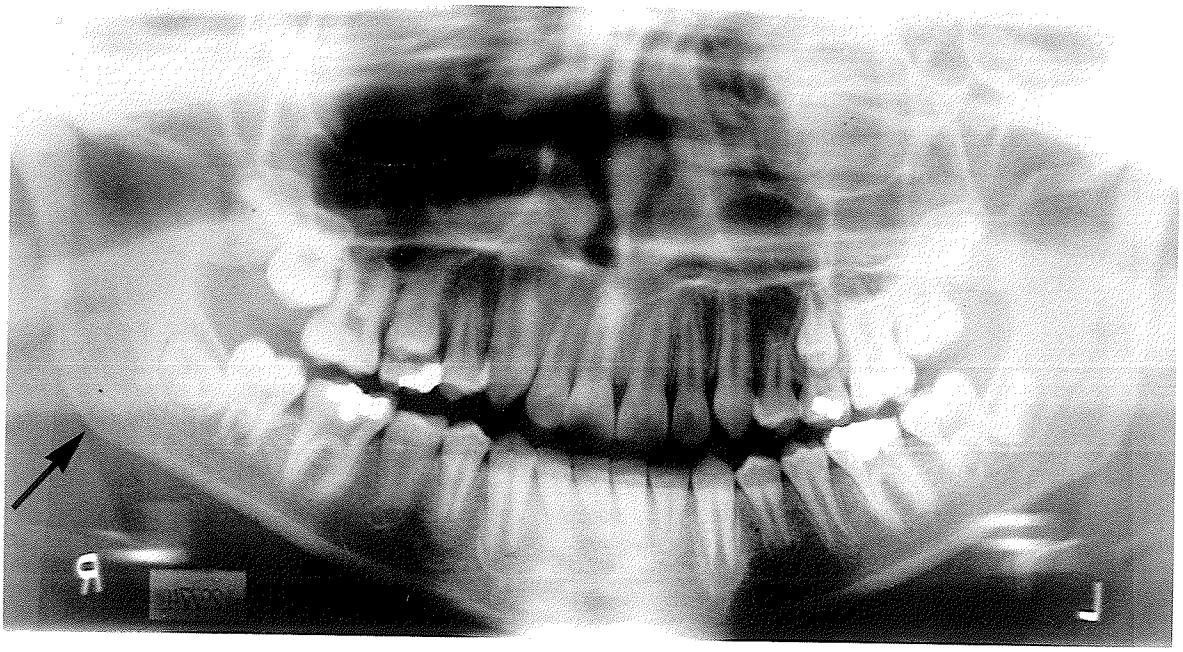


Fig. 1: Panoramic Radiograph showing gonial angle

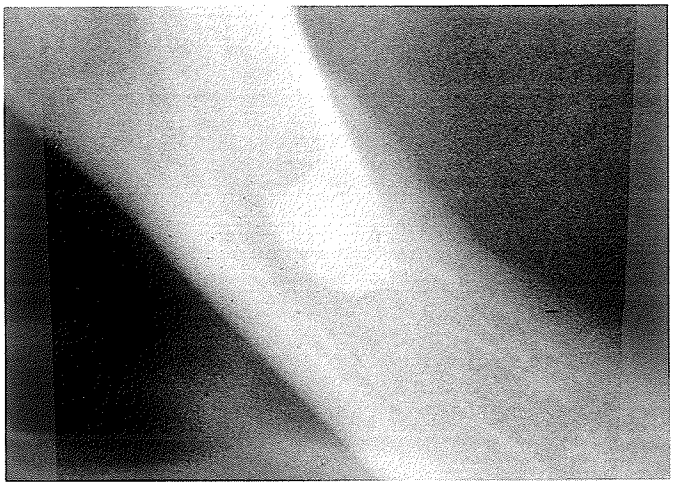


Fig. 2: Osteosclerosis

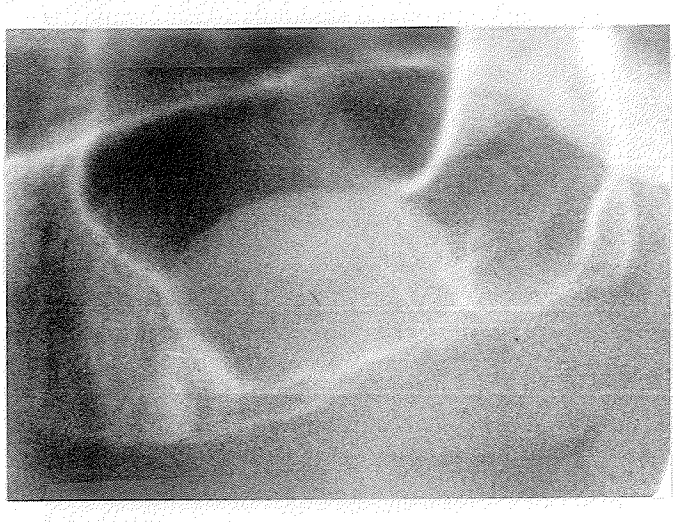


Fig. 3: Mucous retention phenomenon of antrum

Fig. 4: Antrolith

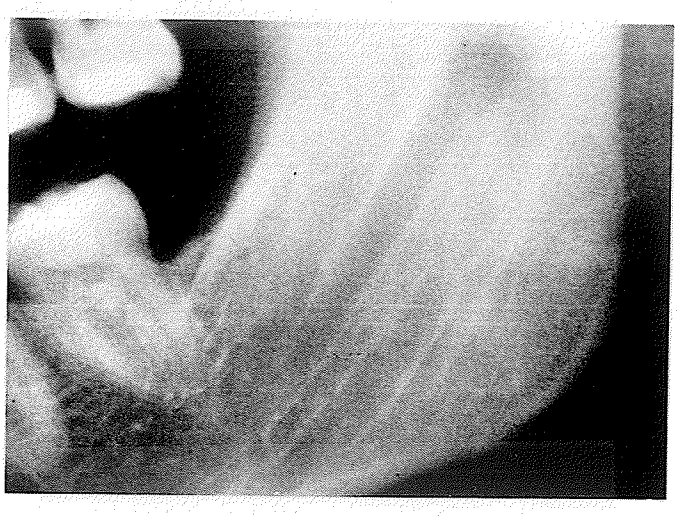


Fig. 5: Bifid Canal

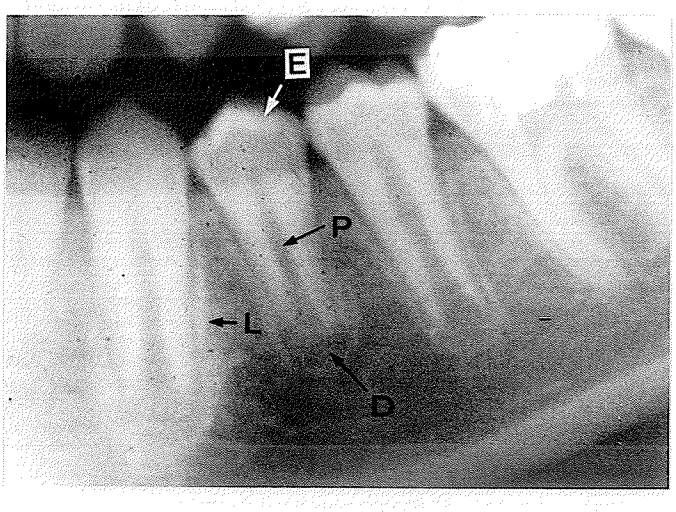


Fig. 6: Tooth Structures
D = Dental Papilla
L = Lamina Dura
E = Enamel
P = Pulp

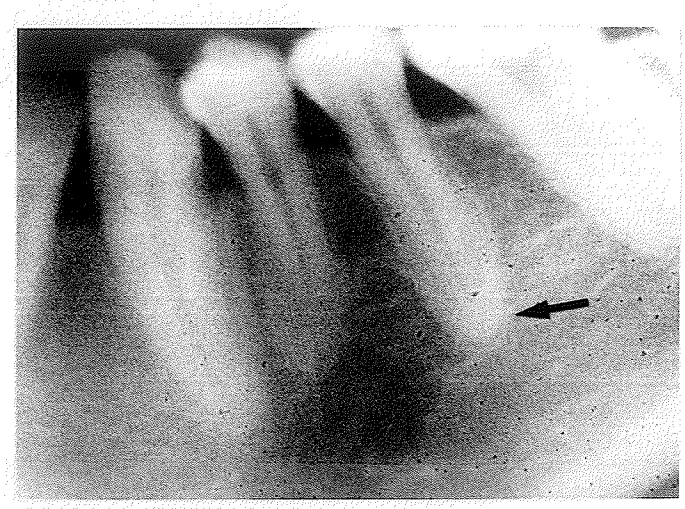


Fig. 7: Hypercementosis

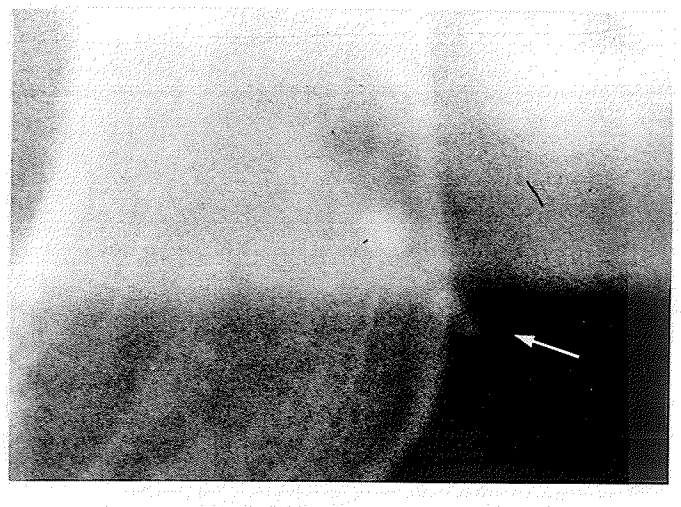


Fig. 8: Soft Tissue Calcification

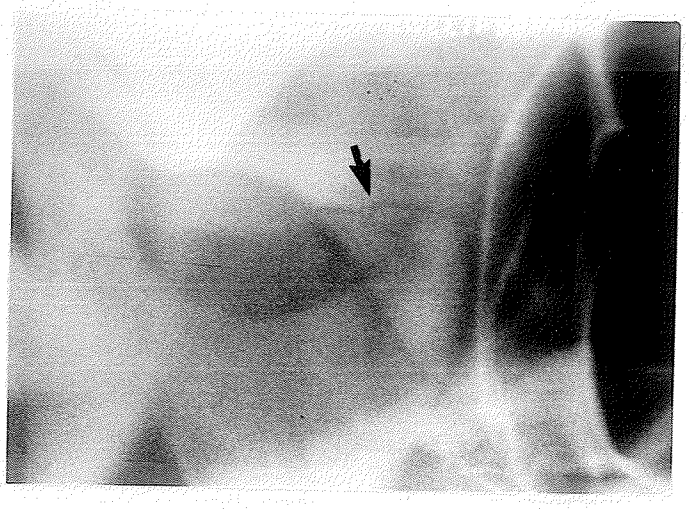


Fig. 9: Zygomatico-Temporal Suture

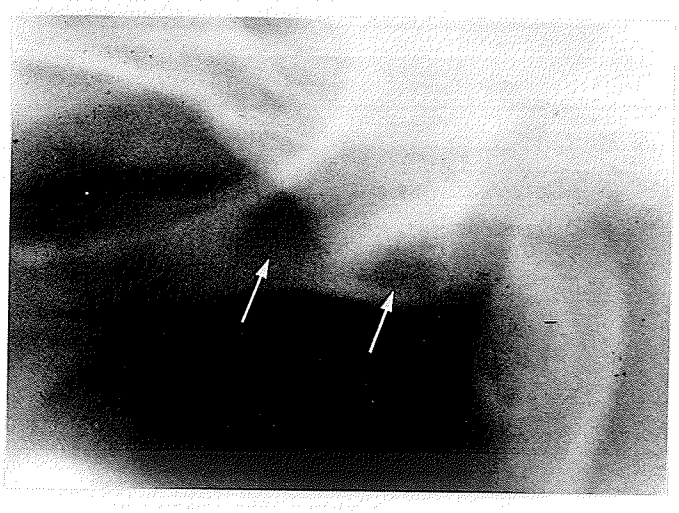


Fig. 10: Eminence Pneumatization

CARRIER ASSIGNMENT - LOGISTIC REGRESSION ANALYSIS

Logistic regression analysis using SAS (Statistical Analysis System) was done with the assistance of Mary Cheang of the Department of Biostatistics (53). This was done as a two-stage procedure. Controls were first compared to obligate carriers in regard to their alkaline phosphatase activity. Controls who appeared to be carriers at this stage were excluded from further analysis. This involved three controls of which two also had a urine phosphoethanolamine result available. For step two, both serum alkaline phophatase and urine phosphoethanolamine were compared between obligate carriers and the new control group. Logistic regression analysis was then used to develop an equation which could predict the probability of an individual belonging to the carrier group.

This equation was then applied to the first degree relatives to determine their carrier status. Only individuals over 18 years of age were included in these analyses. This equation could only be applied to participants on whom both alkaline phosphatase and phosphoethanolamine results were available. For the purposes of classification, carriers were defined as those individuals having a greater than 99% chance of being a carrier. Non-carriers were defined as those having a less than 1% chance. Those with intermediate results (or those

with incomplete alkaline phosphatase and phosphoethanolamine data) were excluded from further analysis. The only exception was that two parents of affected infants were deemed to be carriers even though no urine result was available. As stated previously, the three controls who appeared to be carriers were excluded.

The purpose of this analysis was to establish groups of carriers and non-carriers that could be used in comparisons of the various criteria measured. The development of the groups is illustrated schematically by a flow diagram (Fig. 11).

Logistic regression analysis was later used to separate obligate carriers from non-carrier controls based on 1) alkaline phosphatase alone, 2) alkaline phosphatase and serum phosphate, 3) alkaline phosphatase, phosphate and phosphoethanolamine. The derived equations were then applied as above to the first degree relatives.

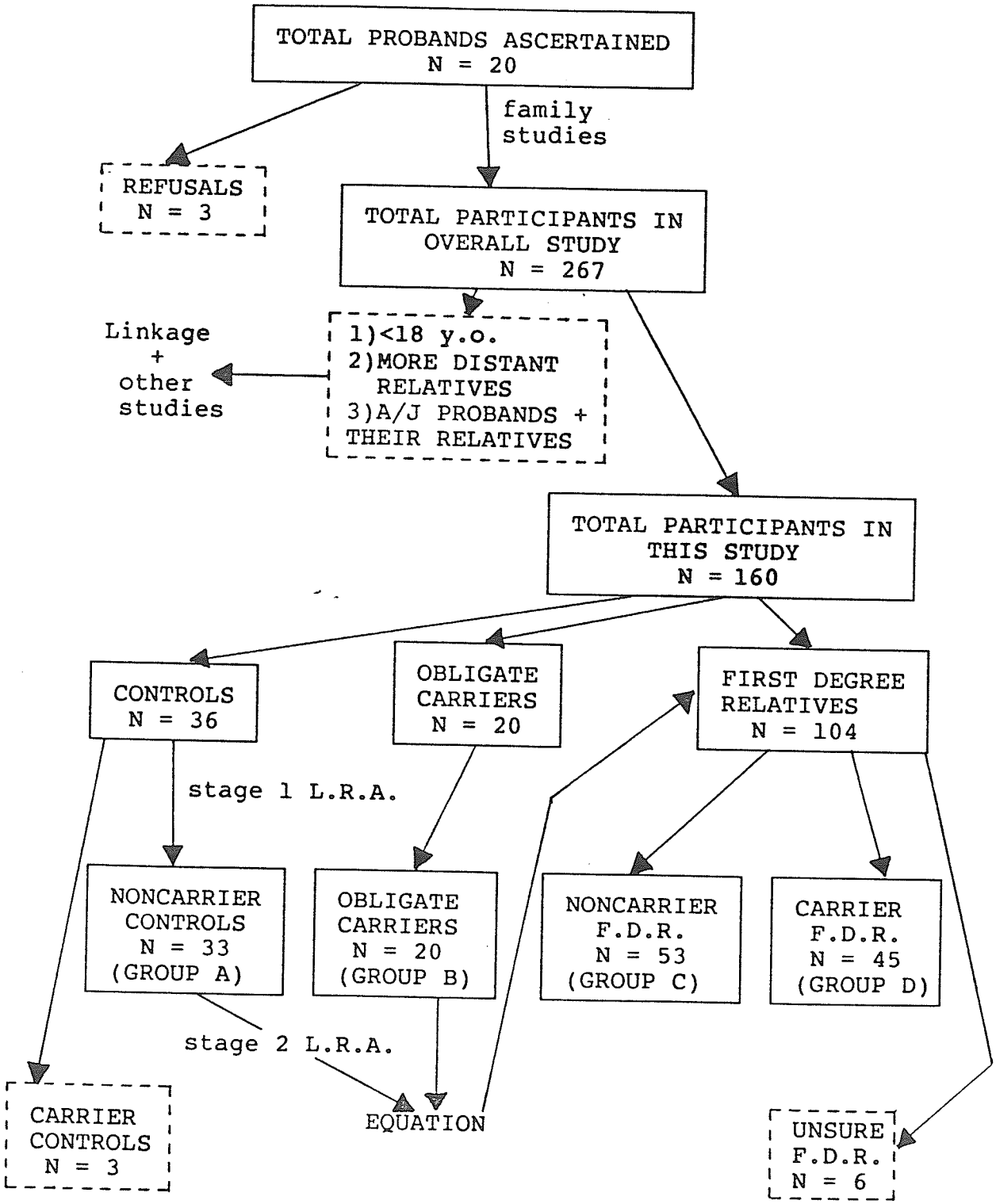
DESCRIPTIVE AND COMPARATIVE STATISTICS

All calculations were done using the Number Cruncher Statistical System (54). Chi-squared analyses were used to compare categorical data. T-tests were used for non-categorical or interval data. Carriers were compared to non-carriers as previously defined with respect to the various study criteria. Only individuals over 18 years of age were included in these analyses.

In order to reduce bias, only carrier first degree

FIGURE 11

FLOW DIAGRAM OF STUDY PROTOCOL



KEY

- excluded from study

- included in study at this point

A/J - adult and juvenile

F.D.R. - first degree relatives

L.R.A. - logistic regression analysis

relatives were compared to non-carrier first degree relatives with respect to the data obtained on the history and physical examination i.e. group C was compared to group D. The non-carrier controls (group A) and the obligate carriers (group B) were not included as their genotype could be known or presumed prior to examination. The control and obligate carrier groups were, however, included with the first degree relatives for analysis of the dental studies i.e. groups A + C were compared to groups B + D. This was done as these tests were done blind to the individuals carrier status. As calcium and phosphate were measured simultaneously with the alkaline phosphatase by an automated analysis which was set up by a technician unaware of the carrier assignment, these criteria were evaluated with respect to this entire group i.e groups A + C were compared to groups B + D. In addition, group C was compared to group D for calcium and phosphate.

HT, WT, HC, CI, US/LS, S-H and H/S were analyzed separately for males and females.

RESULTS

ASCERTAINMENT

Twenty hypophosphatasia probands were identified through a variety of means. Table III shows the method by which each proband was detected and the specific form of hypophosphatasia that they had. The results reported below are limited to the analysis of infantile hypophosphatasia.

CLINICAL FEATURES OF INFANTILE HYPOPHOSPHATASIA

The clinical features and family data of the infantile cases are summarized on Table IV. All had typical radiologic features of infantile hypophosphatasia. Patients G.L. and J.L. were the two original patients described by Chown (2). Alkaline phosphatase results were not available on either of these infants or on their parents. In all other cases the parents of an infantile case had documented low or low-normal serum alkaline phosphatase activity. Where available, the alkaline phosphatase activity of the infantile cases are recorded on the table. A "normal" activity was found in one case (B.T.) This was from a fetal blood sample drawn at 31 weeks gestation. Meconium peritonitis was present. As marked elevations in intestinal alkaline phosphatase can be seen in intestinal disorders such as obstruction with strangulation (55), it is possible that this apparently normal value was due to an increased activity of intestinal alkaline phosphatase. The family

TABLE III

ASCERTAINMENT OF PROBANDS

PROBAND #	INITIALS	DISEASE TYPE	ASCERTAINMENT
1	A.P.	I	A, C, G, Pa
2	R.H.	I	G, Pa
3	J.Q.	I	A, G, H, Mc, Pa
4	C.T.	I	A, C, F, G, H
5	B.K.	I	C, G, Pa
6	L.D.	I	C, Pa, R
7	P.F.	I	A, C, F, Mc
8	B.T.	I	C, F, Pa, R
9	B.M.	I	C
10	A.W.	I	A, G, Pa, R
11	G.L.	I	C
12	B.H.	I	C, G
13	C.H.	J	C, G, L
14	J.B.	J	G, H
15	C.S.	J	C, D, G, H
16	J.H.	J	C, Pe
17	C.J.	J	G, O
18	M.D.	A	O, R
19	J.K.	A	M, O
20	J.Z.	A	R

KEY:

DISEASE TYPE:
- I = Infantile hypophosphatasia
- J = Juvenile hypophosphatasia
- A = Adult hypophosphatasia

ASCERTAINMENT:
- A = Congenital anomalies registry
- C = Children's Hospital of Winnipeg Department of Radiology (including cases described by Macpherson et al.(1))
- D = Dentists
- F = Family practioners and general practioners
- G = Department of Genetics and Metabolic Diseases Children's Hospital of Winnipeg
- H = Health Sciences Center of Winnipeg Medical Records
- L = Chemistry Laboratories
- M = Peripheral hospitals Medical Records Departments
- Mc = Dr. J McGuire
- Pa = Pathology Departments
- Pe = Pediatricians
- R = Radiologists

TABLE IV

SUMMARY OF INFANTILE CASES

PATIENT	SEX	Y.O.B.	A.O.P.	AGE AT DEATH	ETHNICITY	FAMILY HX	AP
1) A.P.	M	1986	Prenatal	Stillborn	Mennonite		<5 U/L
2) R.H.	F	1975	1 month*	9 months	Mennonite		5-8 U/L
3) J.Q.	M	1985	Birth	< 24 hours	Mennonite	3rd cousin = C.T.	"absent"
4) C.T.	F	1980	12 hours	6 days	Mennonite	3rd cousin = J.Q.	12 U/L
5) B.K.	M	1970	1 day	3 days	Mennonite	older sister N.N.D. likely hypophosphatasia	1.0 U/L
6) L.D.	F	1985	Birth	5 days	Mennonite	2nd cousin = B.T.	10 U/L
7) P.F.	M	1984	Prenatal	< 1 hour	Mennonite	product of incest i.e. parents were sibs	?
8) B.T.	F	1986	Prenatal	Stillborn	Mennonite	2nd cousin = L.D.	101 U/L°
9) B.M.	F	1959	Birth	2 hours	Non-Mennonite		?
10) A.W.	M	1986	Birth	1 day	Mennonite	1st cousin once removed = B.H. 2nd cousin once removed = J.B.+	5 U/L
11) B.H.	F	1963	"Shortly after birth"	13 days	Mennonite	1st cousin once removed = A.W. 2nd cousin = J.B.+	"absent"
12) G.L.	F	1932	Day 10	3 months	Eng./Welsh	parents were 1st cousins sister = J.L.	?
13) J.L.	F	1934	Day 10	6 months	Eng./Welsh	parents were 1st cousins sister = G.L.	?

TABLE IV (con't)

SUMMARY OF INFANTILE CASES

KEY: Y.O.B. = year of birth
A.O.P. = age of presentation
N.N.D. = neonatal death
* = self limited seizures noted on day 1
+ = patient with juvenile hypophosphatasia
° = alkaline phosphatase measured on a fetal blood sample. Meconium peritonitis present.

NOTE: Patient #3 (J.Q.) = patient #4 ref. (14)
Patient #5 (B.K.) = case #2 ref. (1)
Patient #7 (P.F.) = patient #3 ref. (14)
Patient #11 (B.H.) = case #3 ref. (1)
Patient #12 (G.L.) = case #4 ref. (1)
Patient #13 (J.L.) = case #5 ref. (1)

histories were unremarkable except where indicated. No attempt was made to contact the parents of G.L. and J.L. The parents of B.M. were contacted but refused to participate in the study.

The clinical data on patient A.P. is presented as an example of the phenotype of an infantile case. A prenatal ultrasound examination at 38 weeks gestation showed absent ossification of the skull, decreased mineralization of the skeleton and decreased length of the long bones. A.P. was subsequently delivered stillborn. Grossly the upper and lower limbs showed rhizomelic shortening and the chest appeared shortened and compressed. Radiographs (Fig. 12) showed very poor ossification of the skull bones especially the cranial vault. The ribs were thin. The metaphyses, especially of the femur and tibia were irregular with a moth-eaten appearance. Samples of the right and left sixth rib were taken for histology. No long bone samples were taken. The ribs were thinner than normal with slightly swollen costochondral junctions. There was irregular ossification of the cartilagenous plates as well as isolated islands of irregularily ossified residual chondroid material seen in sections of the chostochondral junction. Histologic examination of the lungs showed a moderate degree of pulmonary hypoplasia. No evidence of nephrocalcinosis was seen. Serum alkaline phosphatase was less than 5 U/L. The bone derived isozyme of alkaline phosphatase was absent in a skin-derived fibroblast culture.

Figure 12a

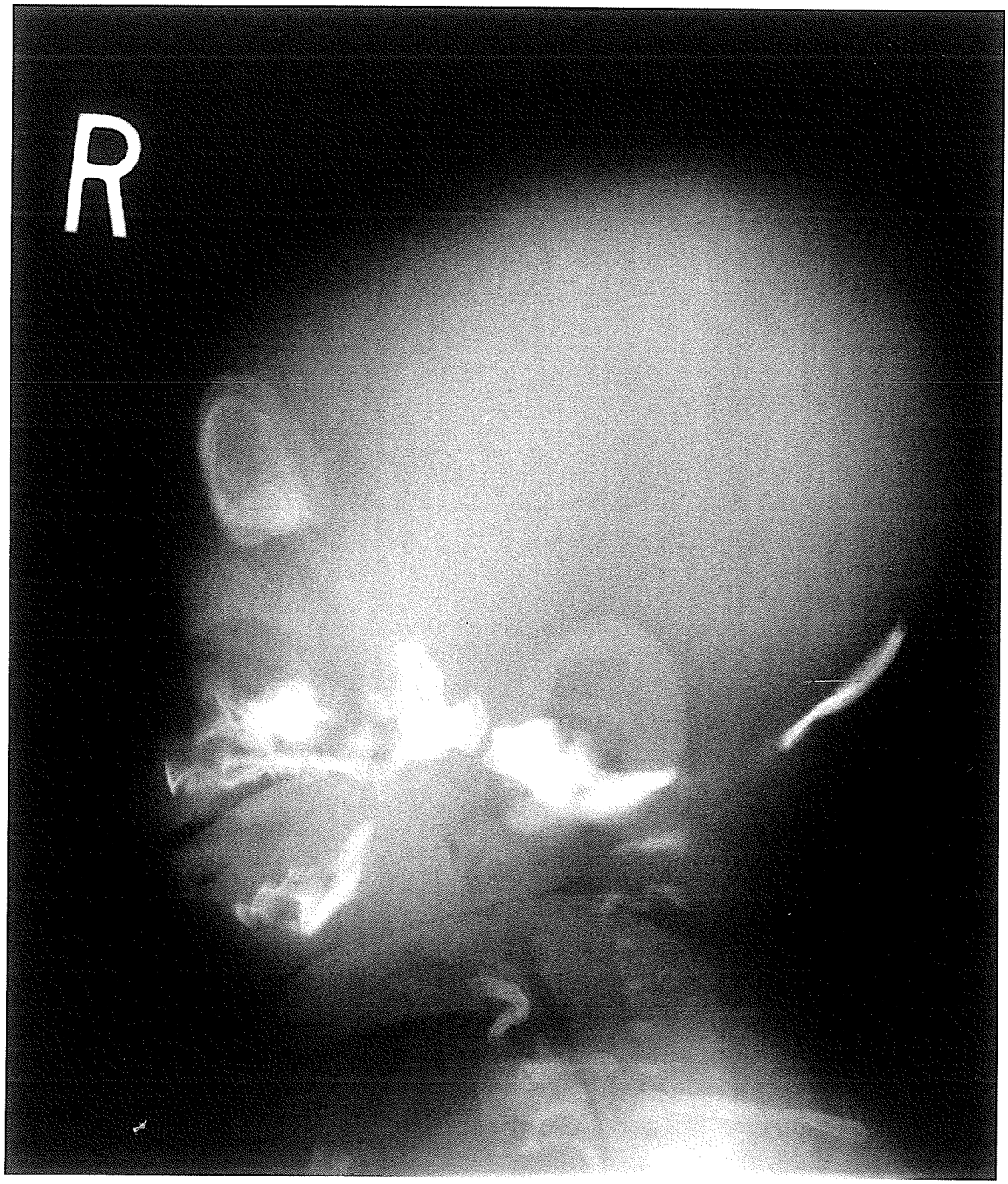


Figure 12b

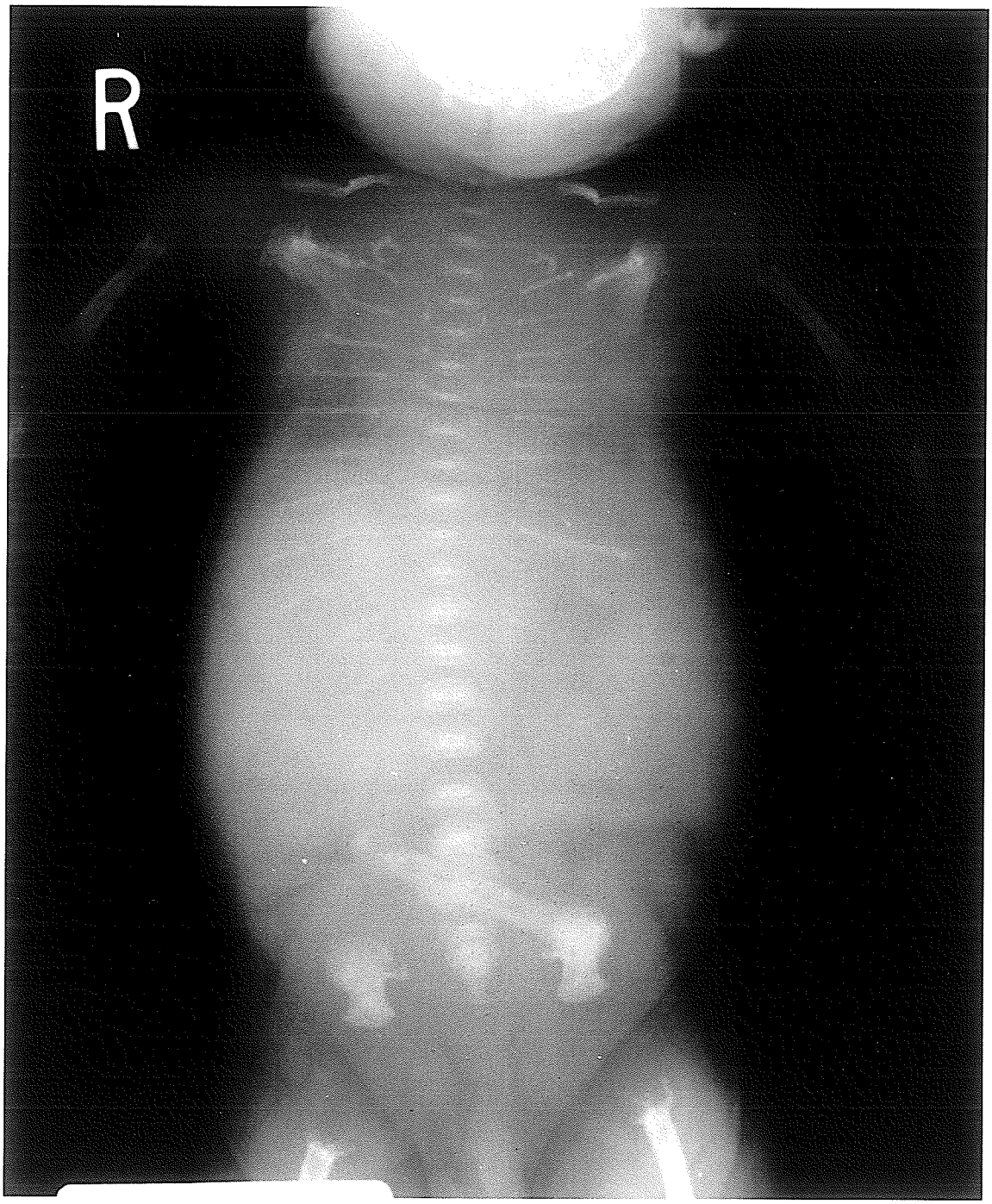
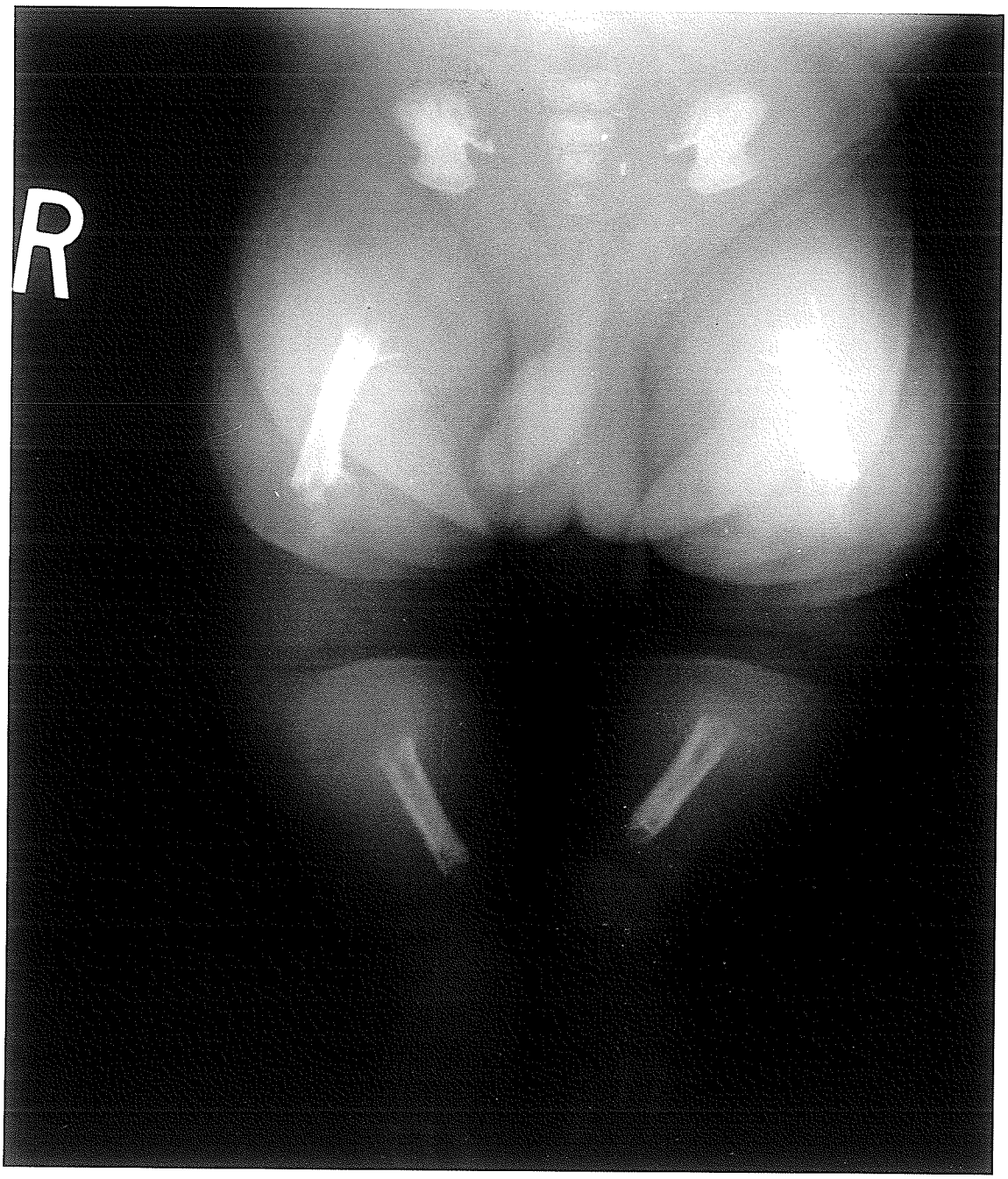


Figure 12c



Twenty obligate carriers (parents of an affected child), 104 first degree relatives of the obligate carriers and 36 unrelated spouses (controls) were seen. Other less closely related individuals were seen for the linkage study. The families of the adult and juvenile probands were also seen for a separate aspect of the study. Fig. 13 shows the family tree of one infantile hypophosphatasia family (family 7) as an example where consanguinity was noted.

ALKALINE PHOSPHATASE AND PHOSPHOETHANOLAMINE IN CARRIERS AND CONTROLS

Tables V and VII show the comparison of alkaline phosphatase results between obligate carriers and controls with regard to alkaline phosphatase activity and phosphoethanolamine excretion. The breakdown by sex in each group is shown in Tables VI and VIII. Table IX illustrates the chi-square analysis of the sex distribution between carriers and controls. Table X gives the distribution by ages. The frequency distribution of alkaline phosphatase activities and urinary phosphoethanolamine excretion for carriers, first degree relatives and controls is shown in Fig. 14 and 15.

CARRIER ASSIGNMENTS

Preliminary or first stage logistic regression analysis

Figure 13

PEDIGREE OF FAMILY SHOWING CONSANGUINITY

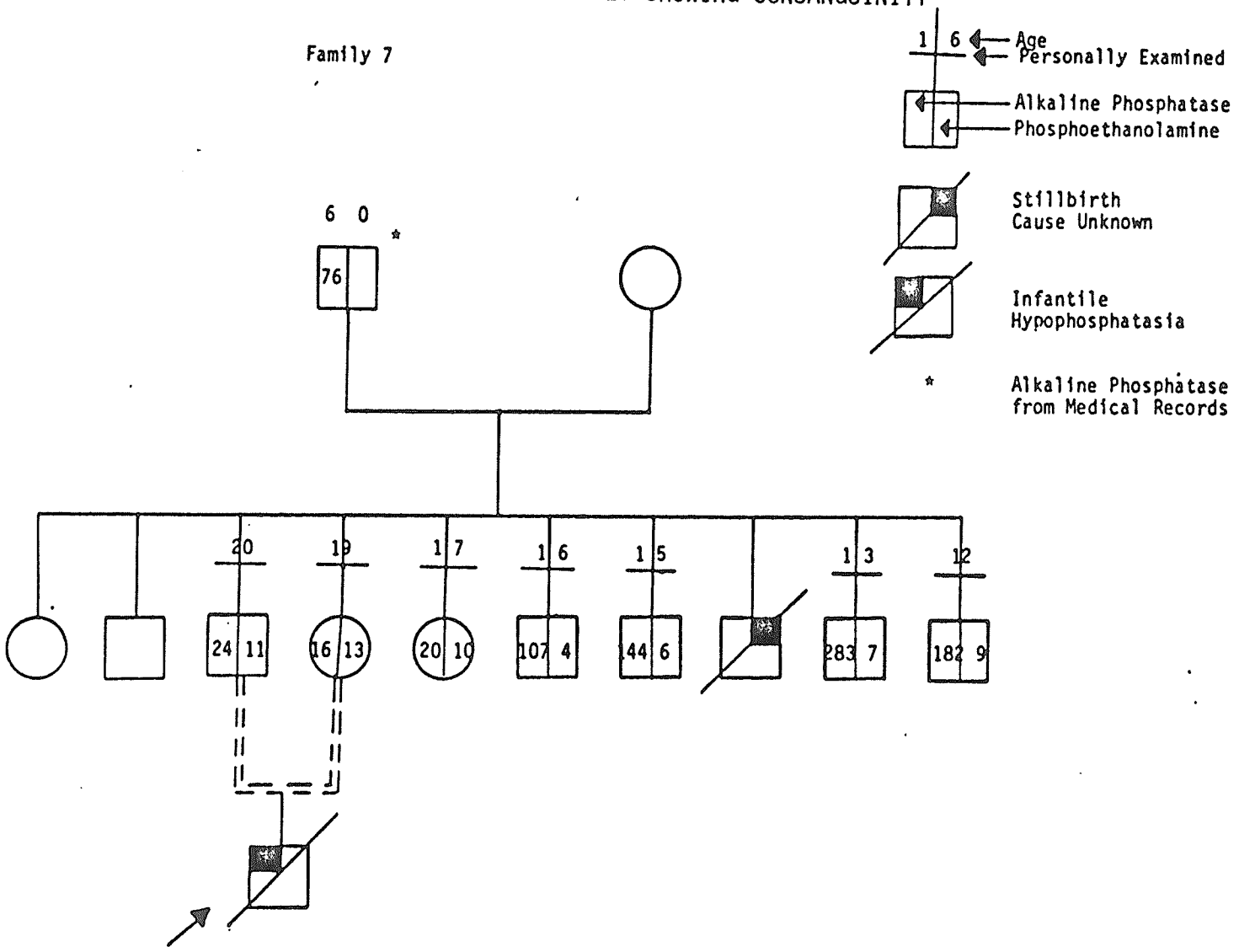


TABLE V

ALKALINE PHOSPHATASE

OBLIGATE CARRIERS VS. CONTROLS

GROUP	N	MEAN	S.D.	RANGE	p
CARRIERS	20	25.7	7.5	15 - 43	<0.0001
CONTROLS	36	64.6	20.1	26 - 113	

TABLE VI

ALKALINE PHOSPHATASE

DISTRIBUTION BY SEX

GROUP	TOTAL	# MALES	MEAN	(S.D.)	# FEMALES	MEAN	(S.D.)	p
CARRIERS	20	10	26.3	(9.0)	10	25.0	(6.2)	0.71
CONTROLS	36	11	71.5	(19.6)	25	61.5	(19.9)	0.17

TABLE VII

PHOSPHOETHANOLAMINE

OBLIGATE CARRIERS VS. CONTROLS

GROUP	TOTAL	MEAN	S.D.	RANGE	p
CARRIERS	18	9.4	3.2	5 - 17	0.004
CONTROL	35	6.2	4.6	3 - 30	

TABLE VIII

PHOSPHOETHANOLAMINE

DISTRIBUTION BY SEX

GROUP	TOTAL #	# MALES	MEAN	(S.D.)	# FEMALES	MEAN	(S.D.)	p
CARRIER	18	10	8.7	(2.3)	8	10.4	(4.0)	0.31
CONTROLS	35	11	5.3	(1.8)	24	6.6	(5.4)	0.28

TABLE IX

SEX DISTRIBUTION

CARRIERS VS. CONTROLS

	# CONTROL	# CARRIER	
MALE	11	10	
FEMALE	25	10	
			p = 0.15

TABLE X

AGE DISTRIBUTION

CARRIERS VS. CONTROLS

GROUP	TOTAL	MEAN (S.D.)	p
CARRIERS	20	32.1 (10.9)	
CONTROLS	36	36.8 (13.4)	0.16

Figure 14

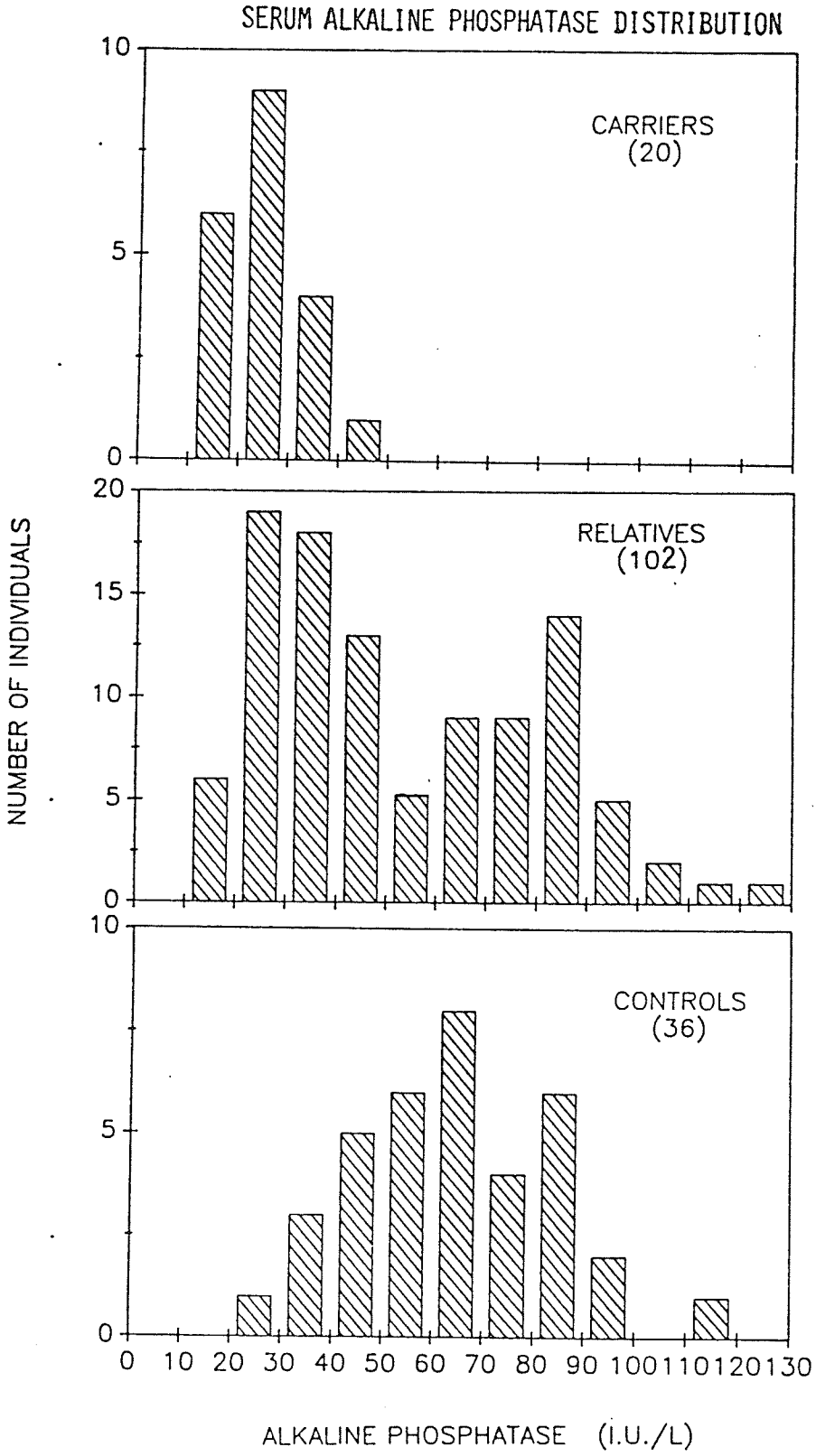
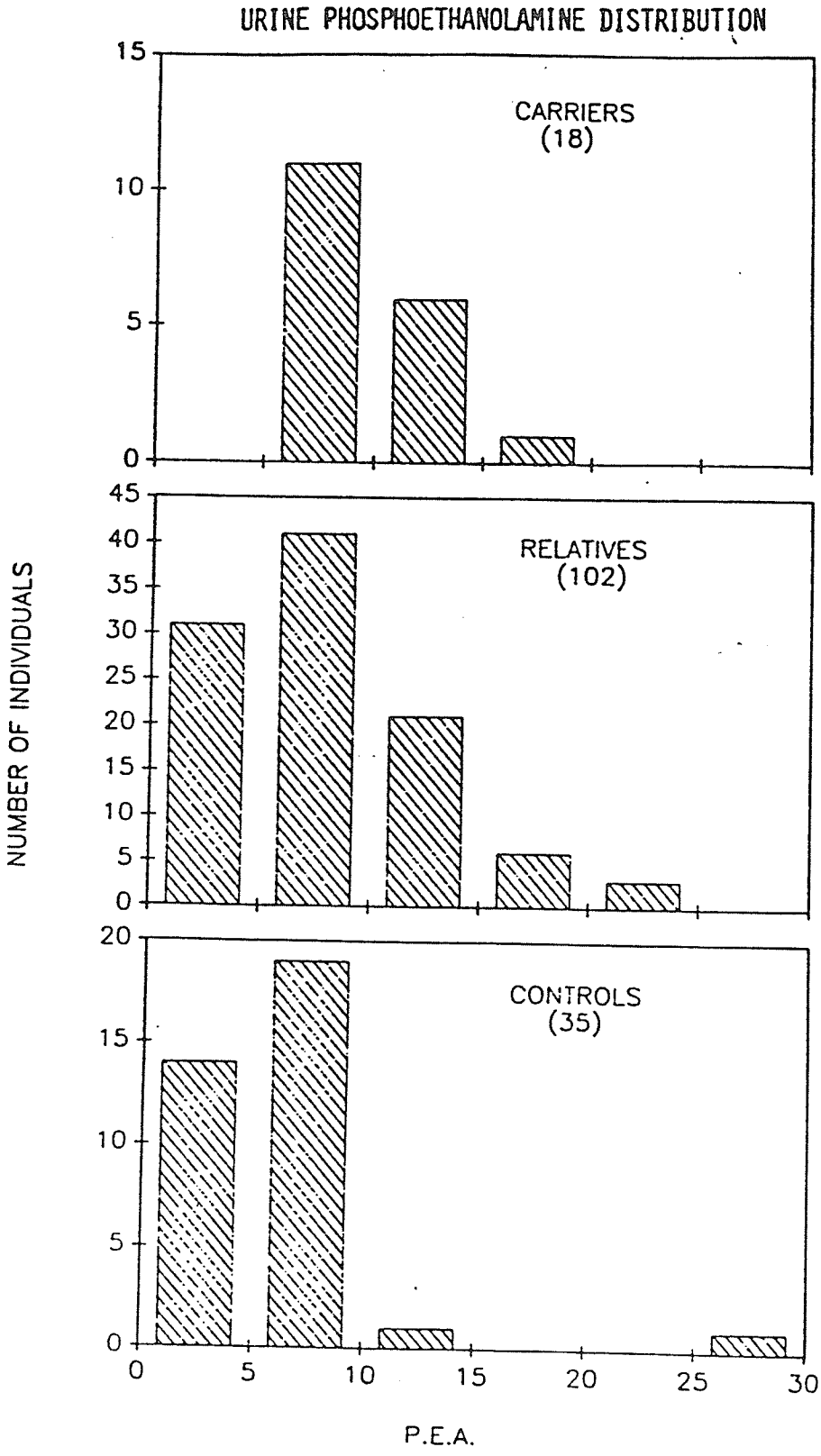

SERUM ALKALINE PHOSPHATASE DISTRIBUTION
CARRIERS
(20)
RELATIVES
(102)
CONTROLS
(36)
NUMBER OF INDIVIDUALS
ALKALINE PHOSPHATASE (I.U./L)
0 10 20 30 40 50 60 70 80 90 100 110 120 130

Figure 15


URINE PHOSPHOETHANOLAMINE DISTRIBUTION
CARRIERS
(18)
RELATIVES
(102)
CONTROLS
(35)
NUMBER OF INDIVIDUALS
P.E.A.
0
5
10
15
20
25
30
35
40
45

which was based on alkaline phosphatase alone, suggested three controls were carriers. Characteristics of these individuals are shown in Table XI. Initially phosphoethanolamine excretion was not a helpful adjunct to alkaline phosphatase for discriminating carriers from non-carriers. This was mainly because one result (i.e. A.K.'s) was so much higher than the mean that it negated the discriminatory ability of the test. Consequently, these three "carrier" controls were excluded from step two of the analysis. One obligate carrier was given only a 13% probability of being a carrier based on his alkaline phosphatase results. He was not excluded from stage two.

The results of the second stage logistic regression analysis are shown in Table XII. The results are depicted for both the original participant type (i.e. obligate carrier, control or first degree relative) and the family of the proband. Fig. 16 shows the pedigree of Family 1, and the carrier status of the family members illustrating how carrier and non-carriers were divided in a family. Fig. 17 shows a scatter diagram where alkaline phosphatase is plotted against phosphoethanolamine for individuals on whom a prediction of carrier status was made.

COMPARISONS BETWEEN CARRIERS AND NON-CARRIERS

The results of the comparisons done using the criteria from the history and physical examination, dental radiographs and biochemistry are displayed in tabular form.

TABLE XI

CHARACTERISTICS OF CONTROLS WHO APPEARED TO BE CARRIERS

INITIALS	SEX	AGE	Ca	Ph	AP	PEA
A.K.	F	38.2	2.14	1.60	33	30
S.W.	F	21.5	2.26	1.09	33	10
S.P.	F	50.0	2.10	1.17	26	?

KEY: F = female

TABLE XII

PREDICTIONS ACCORDING TO LOGISTIC REGRESSION ANALYSIS

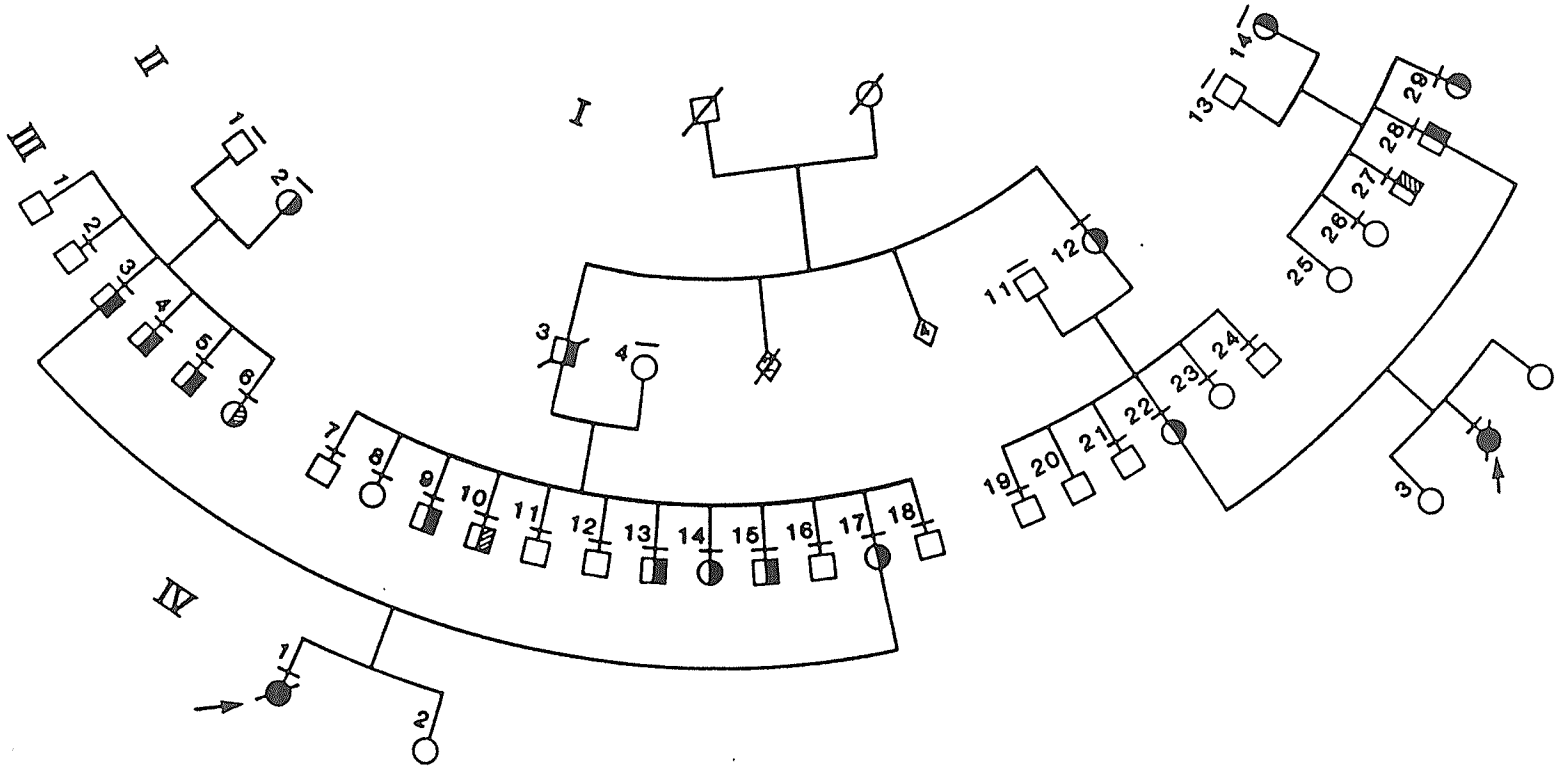
Family	Proband	OBLIGATE CARRIER # Carrier	# Incomplete	CONTROL # Carrier	# Non-Carrier	# Incomplete	1ST DEGREE RELATIVE # Carrier	# Non-Carrier	# Unsure	# Incomplete
1	L.D., B.T.	4		1*	7		10	16	2*	1*
2	M.D.§				3					
3	A.W., B.H.	3	1†	1*	3		7	9		
4	J.K.	2			5	1*	6	6		
5	A.P.	2			3		7	9		
6	J.K.§				1					
7	P.F.	2								
8	J.Q., C.T.	4			9		9	9		1*
9	R.H.	1	1†		2		6	4		2*
Subtotal		18	2	2	33	1	45	53	2	4
TOTAL		20			36			104		

KEY: † = counted as carrier for future analysis, alk phos <30
* = excluded from further analysis
§ = adult patient

Figure 16

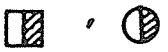
PEDIGREE OF FAMILY SHOWING CARRIER ASSIGNMENT

Family 1



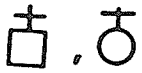
KEY

= CARRIER



= UNSURE CARRIER STATUS

= AFFECTED (INFANTILE HYPOPHOSPHATASIA)

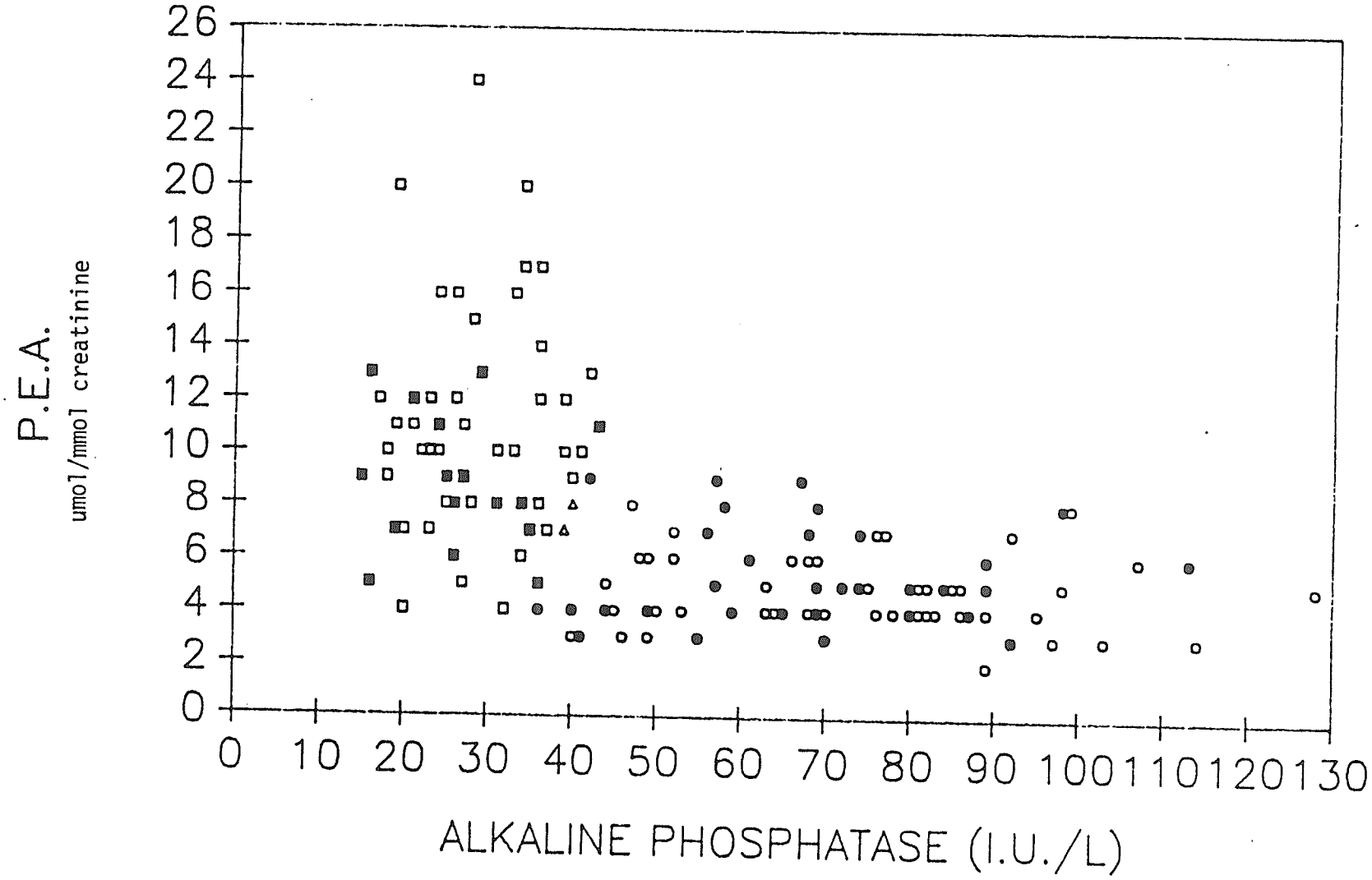


= ← CARRIER TESTING DONE

← NON-CARRIER

Figure 17

SCATTER DIAGRAM: AP vs. PEA



KEY

- Oligate Carriers
- Non-Carrier - Controls
- First Degree Relatives - Carriers
- First Degree Relatives - Non-Carriers
- First Degree Relatives - Uncertain Carrier Assignment

Table XIII shows the individuals who stated their health was either not good or "abnormal" and the particular problem. Three women admitted to taking the birth control pill (a non-carrier control, a carrier first degree relative and a first degree relative whose carrier status was uncertain). Tables XIV - XXVI show the results of the comparisons between carriers and non-carriers. Figures 18-20 illustrate the distribution of outer canthal distances, height to span ratio and span - height difference in female first degree relatives according to carrier status. Figure 21 shows the distribution of phosphate levels for the study participants also according to carrier assignment. For these four figures only individuals on whom a carrier assignment was made were included.

LOGISTIC REGRESSION ANALYSIS

The equations derived for predicting carrier status based on Method 1 (alkaline phosphatase alone), Method 2 (alkaline phosphatase and phosphoethanolamine), Method 3 (alkaline phosphatase and phosphate) and Method 4 (alkaline phosphatase, phosphoethanolamine and phosphate) are as follows:

Method 1: $\text{Probability } (P) = 1/1 + e^{-15.61 + 0.407\ AP}$

Method 2: $P = 1/1 + e^{-396.68 + 12.94\ AP - 15.35\ PEA}$

Method 3: $P = 1/1 + e^{9.54 + 27.908\ AP - 1048.9\ Ph}$

TABLE XIII

PARTICIPANTS WHO STATED HEALTH WAS NOT "GOOD"

CARRIERS

CLASSIFICATION	HEALTH
OC	Hypertension on meds
FDR	Hypertension mild no meds
FDR	Hypertension on meds
FDR	"
FDR	"
FDR	Asthma on meds
FDR	Coronary artery disease on med
FDR	Epilepsy on meds
FDR	Cleft lip repaired
FDR	Fungal skin infection on meds
FDR	Kidney stones, coronary artery disease on meds
FDR	Diabetic on insulin
FDR	Past history thyroid disease

NON-CARRIERS

CLASSIFICATION	HEALTH
CON	Hypertension on meds
CON	Hypetension mild no meds
CON	Venous edema, glaucoma on meds
CON	Past history jejuenal bypass
FDR	Hypertension on meds
FDR	"
FDR	"
FDR	Epilepsy on meds
FDR	Pregnant*
FDR	Asthma on meds
FDR	Epilepsy not on meds
FDR	Past history partial gastrectomy re ulcers

KEY: OC - obligate carier
FDR - first degree relative
CON - control
* - blood drawn prior to pregnancy, weight estimated as prepregnancy weight

TABLE XIV

CATEGORICAL HISTORICAL DATA

CRITERIA		% of CARRIERS (N=45)	% OF NON-CARRIERS (N-53)	p
Rickets	+	2.2	3.8	0.66
	-	97.8	96.2	
Craniosynostosis	+	0	0	*
	-	100	100	
B/J pain	+	51.1	41.5	0.62
	-	46.7	56.6	
	Unrelated	2.2	1.9	
Fractures°	Traumatic	37.8	22.6	0.10
	None	62.2	77.4	
Chest Deformity	+	8.9	5.7	0.54
	-	91.1	94.3	
Bone Deformity	+	0	3.8	0.19
	-	100	96.2	

TABLE XIV con't

CRITERIA		% OF CARRIERS (N=45)	% OF NON-CARRIERS (N=53)	p
Fillings (problems with)	+	15.6	13.2	0.74
	-	84.4	86.8	
Adult Teeth	1	51.1	35.8	0.31
	2	11.1	15.1	
	3	37.8	49.1	
Caries	Few	24.4	17.0	0.57
	Average	35.6	34.0	
	Many	40.0	49.0	
Sex	Male	42.2	62.3	0.048
	Female	57.8	37.7	

KEY: + = criteria present
- = criteria absent
B/J = bone or joint
Adult teeth 1,2,3 refer to number and age that adult teeth were lost according to classification described in Method
* Chi-squared analysis was not possible in this category as no individual gave a positive history of craniosynostosis
° No individual gave a history of fracture without significant trauma

TABLE XV

NON-CATEGORICAL HISTORICAL DATA

CRITERIA	CARRIERS	MEAN (S.D.)	NONCARRIERS	MEAN (S.D.)	p
Age walked°	14	12.0 (1.7)	24	12.4 (2.1)	0.57
Age D.T. lost°	10	6.3 (0.8)	21	6.1 (0.9)	0.58
Age°	45	41.6 (15.2)	53	40.1 (15.4)	0.62
Age (Females)°	26	43.8 (14.1)	20	39.0 (16.1)	0.28
Age (Males)°	19	38.6 (16.4)	33	40.7 (15.1)	0.63
Age (Females*)°	24	44.3 (13.8)	15	40.0 (15.3)	0.37
Age (Males*)°	16	38.8 (14.5)	29	41.9 (15.6)	0.20
Age (Panorex)°	30	39.2 (15.0)	35	40.2 (13.8)	0.78
Age $	65	38.7 (14.6)	86	38.7 (14.7)	0.95

KEY: Panorex - individuals on whom a panorex was taken
D.T. - deciduous teeth
° - results of first degree relatives
$ - results of controls, obligate carriers and first degree relatives
* - individuals on whom a span and a height measurement are available

TABLE XVI

PHYSICAL EXAMINATION CATEGORIAL DATA

CRITERIA		% OF CARRIERS (N=41)	% OF NONCARRIERS (N=46)	p
	+	9.8	6.5	
Clinodactyly				0.58
	-	90.2	93.5	
	+	0	4.3	
Lordosis				0.18
	-	100	95.7	
	+	9.8	21.7	
Bowlegs				0.13
	-	90.2	78.3	
	+	7.3	17.4	
Abnormal Skull				0.16
	-	92.7	82.6	
	Normal	87.8	91.3	
Joint Laxity	Increased	9.8	6.5	0.87
	Decreased	2.4	2.2	

KEY: + = criteria present
- = criteria absent

TABLE XVII

PHYSICAL EXAMINATION (BOTH SEXES)

NON CATEGORICAL DATA

CRITERIA	# CARRIER	MEAN	(S.D.)	# NONCARRIER	MEAN	(S.D.)	P
CI	41	82.5	(3.7)	46	81.4	(4.7)	0.21
IC	41	2.9	(0.4)	46	3.0	(0.4)	0.20
IP	41	5.9	(0.5)	46	6.1	(0.4)	0.06
OC	41	8.4	(0.8)	46	8.7	(0.5)	0.04

TABLE XVIII

PHYSICAL EXAMINATION (MALES ONLY)

NON-CATEGORICAL DATA

CRITERIA	# CARRIER	MEAN	(S.D)	# NONCARRIER	MEAN	(S.D)	p
HT	18	175.4	(5.2)	31	175.9	(5.0)	0.74
WT	19	80.2	(10.3)	33	80.4	(9.5)	0.93
HC	16	57.6	(1.6)	28	58.4	(1.9)	0.13
CI	16	82.4	(3.5)	28	81.6	(4.3)	0.48
S-H	16	8.8	(3.8)	29	10.2	(4.8)	0.31
H/S	16	0.953	(0.02)	29	0.946	(0.02)	0.34
US/LS	16	0.90	(0.06)	29	0.91	(0.07)	0.39
OC	16	8.9	(0.5)	29	8.8	(0.69)	0.69

TABLE XIX

PHYSICAL EXAMINATION (FEMALES ONLY)

NON-CATEGORICAL DATA

CRITERIA	# CARRIER	MEAN	(S.D.)	# NONCARRIER	MEAN	(S.D.)	p
HT	26	160.5	(5.1)	16	161.5	(5.9)	0.58
WT	25	69.4	(16.6)	20	66.9	(13.0)	0.57
HC	25	55.6	(1.6)	16	55.2	(1.6)	0.40
CI	24	82.9	(3.6)	17	81.4	(5.4)	0.33
S-H	24	9.9	(5.9)	15	5.6	(4.3)	0.014
H/S	24	0.943	(0.03)	15	0.967	(0.03)	0.014
US/LS	25	0.82	(0.19)	15	0.89	(0.09)	0.12
OC	25	8.1	(0.8)	17	8.6	(0.5)	0.03

TABLE XX

DENTAL CATEGORICAL DATA

CRITERIA		% OF CARRIERS	% OF NONCARRIERS	p
Wide Zygomatic Suture*	+	13.3	2.9	0.11
	-	86.7	97.1	
Soft Tissue Calcification*	+	10.0	14.3	0.60
	-	90.0	85.7	
M.R.P.*	+	33.3	17.1	0.13
	-	66.7	82.9	
Eminence Pneumatization*	+	3.3	11.4	0.22
	-	96.7	88.6	
Bifid Canal*	+	0	8.6	0.10
	-	100	91.4	

TABLE XX con't

CRITERIA		% OF CARRIERS	% OF NONCARRIERS	p
Antrolith*	+	3.3	5.7	0.65
	-	96.7	94.3	
Calcified Facial Artery*	+	3.3	0	0.28
	-	96.7	100	
Osteosclerosis*	+	50.0	34.3	0.20
	-	50.0	65.7	
M.R.P. and Wide Zygomatic Suture*	+ +	6.7	0	0.11
	+ -	33.3	20.0	
	- -	60.0	80.0	
Enlarged Pulp°	+	16.7	7.1	0.28
	-	83.3	92.9	
Root Resortion°	+	16.7	10.7	0.53
	-	83.3	89.3	

TABLE XX con't

CRITERIA		% OF CARRIERS	% OF NONCARRIERS	p
Hypercementosis°	+	8.3	7.1	0.87
	-	91.7	92.9	
Thick Lamina Dura°	+	29.2	39.3	0.44
	-	70.8	60.7	
Thin Enamel°	+	25.0	28.6	0.77
	-	75.0	71.4	
Trabeculations*	+	13.3	22.9	0.32
	-	86.7	77.1	
Abnormal Calcification*	+	23.3	17.1	0.752
	-	76.7	82.9	
Gross Periodontal Disease°	+	4.2	7.1	0.64
	-	95.8	92.9	

KEY: + criteria present; - criteria absent;
* Total Carriers = 30; Total Noncarriers = 35;
° Total Carriers = 24; Total Noncarriers = 28

TABLE XXI

DENTAL NON-CATEGORICAL DATA

CRITERIA	# CARRIER	MEAN (S.D.)	# NONCARRIER	MEAN (S.D.)	P
Teeth	30	15.3 (10.7)	35	17.4 (10.9)	0.42
DMF	30	17.5 (7.5)	35	15.9 (8.1)	0.41
Gonial Angle	21	124.1 (8.5)	29	126.6 (9.0)	0.31

TABLE XXII

ALKALINE PHOSPHATASE DISTRIBUTION
FIRST DEGREE RELATIVES

STATUS	TOTAL	MEAN	S.D.	RANGE	p
CARRIER	45	28.7	7.27	17 - 42	<0.0001
NON-CARRIER	53	73.8	19.55	40 - 128	

TABLE XXIII

ALKALINE PHOSPHATASE DISTRIBUTION BY SEX
FIRST DEGREE RELATIVES

STATUS	TOTAL	# MALES	MEAN	(S.D.)	# FEMALES	MEAN	(S.D.)	p
CARRIER	45	19	30.7	(6.9)	26	27.3	(7.4)	0.13
NON-CARRIER	53	33	75.2	(21.8)	20	71.4	(15.3)	0.45

TABLE XXIV

PHOSPHOETHANOLAMINE DISTRIBUTION
FIRST DEGREE RELATIVES

STATUS	TOTAL	MEAN	S.D.	RANGE	p
CARRIER	45	10.9	4.3	4 - 24	<0.0001
NON-CARRIER	53	4.6	1.4	2 - 8	

TABLE XXV

PHOSPHOETHANOLAMINE DISTRIBUTION BY SEX
FIRST DEGREE RELATIVES

STATUS	TOTAL	# MALE	MEAN	(S.D.)	# FEMALE	MEAN	(S.D.)	p
CARRIER	45	19	10.4	(4.0)	26	11.3	(4.5)	0.46
NON-CARRIER	53	33	4.5	(1.5)	20	4.9	(1.6)	0.27

TABLE XXVI

BIOCHEMICAL DATA

CRITERIA	# CARRIERS	MEAN (S.D.)	# NONCARRIERS	MEAN (S.D.)	p
Calcium*	65	2.30 (0.10)	86	2.28 (0.11)	0.28
Calcium°	45	2.29 (0.09)	53	2.27 (0.09)	0.22
Phosphate*	60	1.30 (0.17)	85	1.08 (0.16)	<0.0001
Phosphate°	41	1.29 (0.17)	52	1.08 (0.14)	<0.0001
Phosphate° (Males)	17	1.26 (0.18)	32	1.05 (0.15)	0.0001
Phosphate° (Females)	24	1.31 (0.15)	20	1.13 (0.11)	<0.0001

KEY: * = results of controls, carriers as well as first degree relatives

° = results of first degree relatives

Figure 18

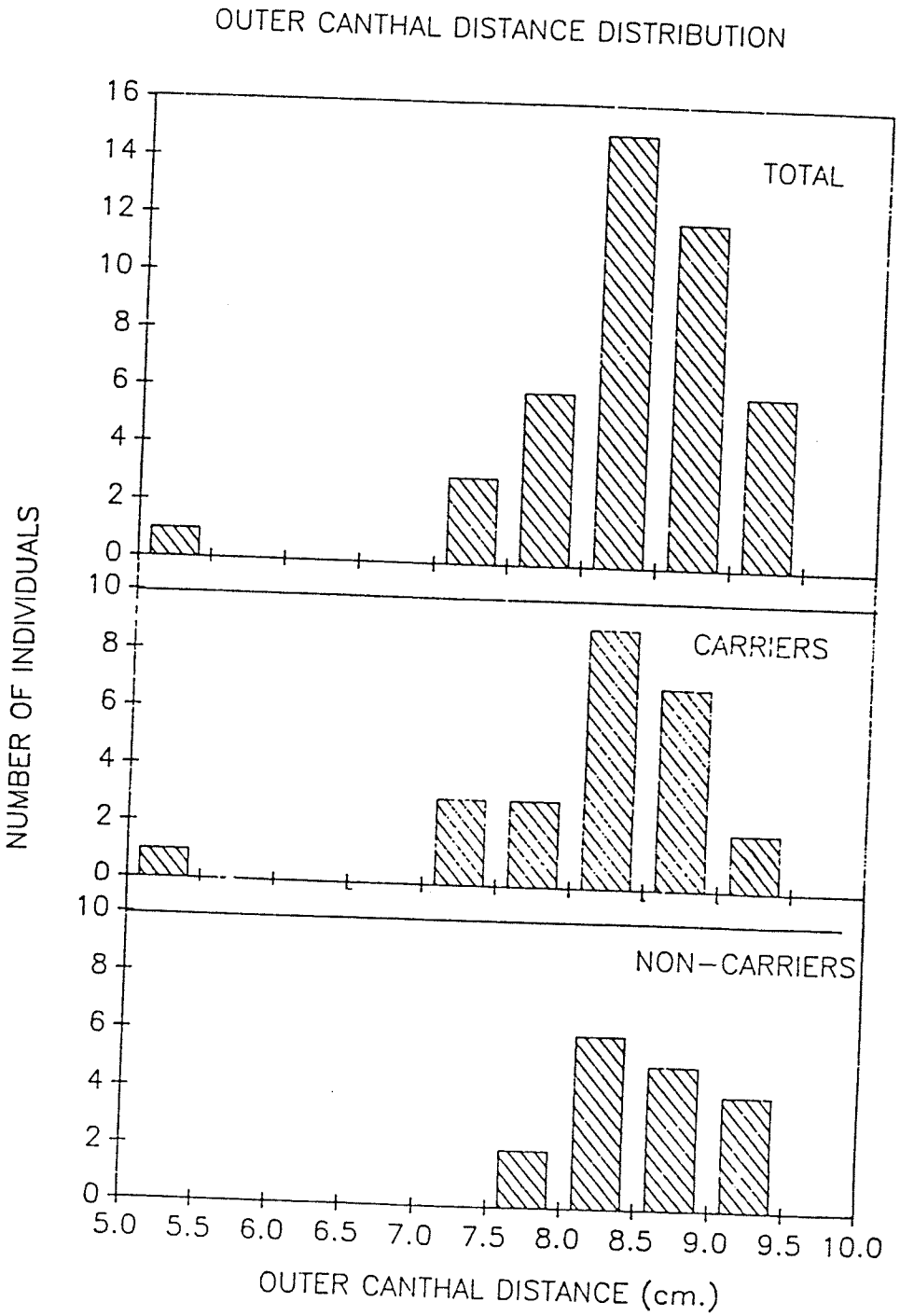

OUTER CANTHAL DISTANCE DISTRIBUTION
TOTAL
CARRIERS
NON-CARRIERS
NUMBER OF INDIVIDUALS
0
2
4
6
8
10
12
14
16
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
OUTER CANTHAL DISTANCE (cm.)

Figure 19

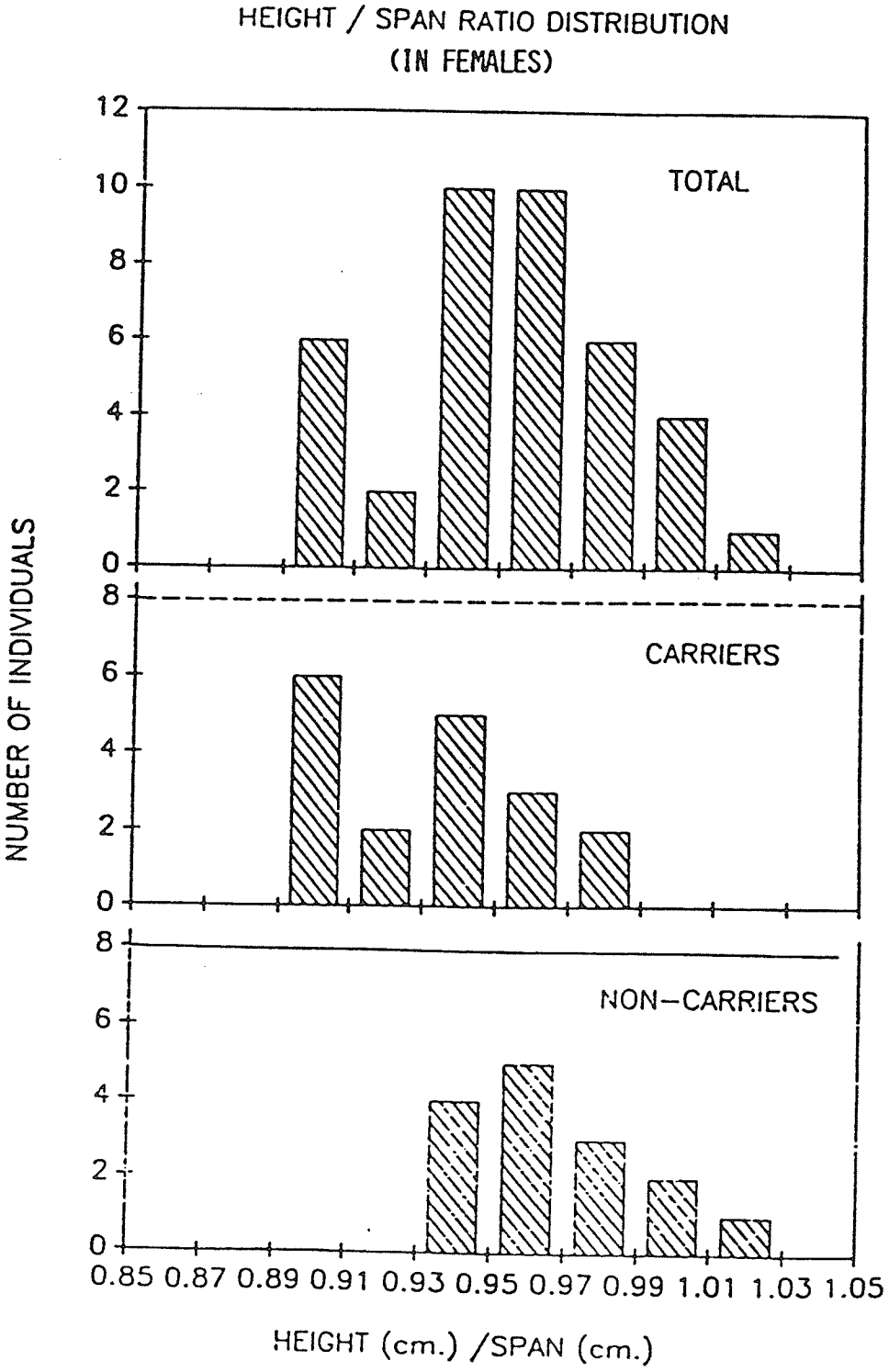
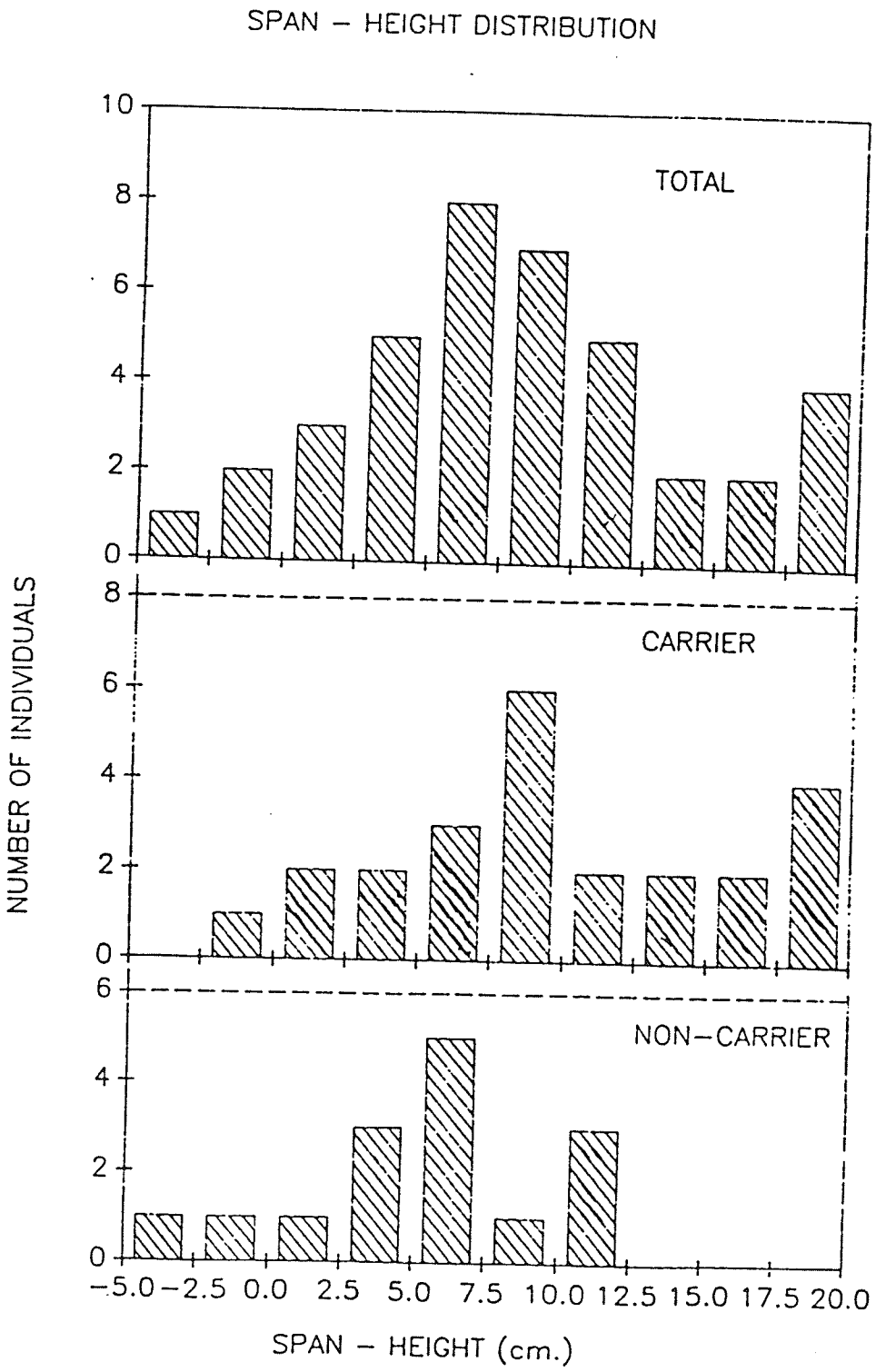

HEIGHT / SPAN RATIO DISTRIBUTION
(IN FEMALES)
TOTAL
CARRIERS
NON-CARRIERS
NUMBER OF INDIVIDUALS
0
2
4
6
8
10
12
0.85 0.87 0.89 0.91 0.93 0.95 0.97 0.99 1.01 1.03 1.05
HEIGHT (cm.) /SPAN (cm.)

Figure 20


SPAN – HEIGHT DISTRIBUTION
TOTAL
CARRIER
NON–CARRIER
NUMBER OF INDIVIDUALS
SPAN – HEIGHT (cm.)
0
2
4
6
8
10
–5.0 –2.5 0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0

Method 4: $P = 1/1 + e^{83.605 + 8.02\ AP - 4.06\ PEA - 336.83\ Ph}$

where AP = alkaline phosphatase activity in U/L, PEA = urinary phosphoethanolamine excretion in umol/mmol creatinine and Ph = serum phosphate in mmol/L.

The results of these four methods of predicting carrier status are shown in Table XXVII. Table XXVIII illustrates the four individuals on whom there was a discrepancy between methods 2 - 4.

TABLE XXVII

LOGISTIC REGRESSION ANALYSIS PREDICTION

BY METHODS 1 - 4

FIRST DEGREE RELATIVES

PREDICTION	METHOD 1	2	3	4
Carrier	22	45	45	41
Noncarrier	46	53	52	52
Unsure	34	2	0	2
Incomplete*	0	2	5	7

CONTROLS AND OBLIGATE CARRIERS

PREDICTION	METHOD 1	2	3	4
Carrier§	14	18	19	17
Noncarrier	27	33	33	33
Unsure	12	0	0	0
Incomplete	0	2	1	3

KEY: Method 1 = prediction on AP result alone
Method 2 = prediction on both AP and PEA
Method 3 = prediction on both AP and Ph
Method 4 = prediction based on all three
* = 2 first degree relatives without AP results were left out of Table
§ = three controls who appeared to be carriers after the first stage analysis were not included.

TABLE XXVIII

DISCREPANCIES BETWEEN CARRIER ASSIGNMENTS

RESULTS

INDIVIDUAL	AP	PEA	Ph
H.D.	34	8	0.97
J.D.	40	8	1.19
D.H.	39	7	1.13
E.K.	40	3	1.16

PREDICTIONS - PROBABILITY OF BEING A CARRIER

INDIVIDUAL	DNA*	Rh*	METHOD 1	2	3	4
H.D.	Carrier	Carrier	0.86	>0.99	>0.99	0.95
J.D.	Carrier	Non-Carrier	0.34	0.87	>0.99	>0.99
D.H.	Non-Carrier	Non-Carrier	0.44	0.37	>0.99	>0.99
E.K.	Non-Carrier	Not Informative	0.34	<0.01	>0.99	0.19

KEY:
AP = serum alkaline phosphatase activity
PEA = urine phosphoethanolamine excretion
Ph = serum phosphate
DNA = prediction according to DNA analysis
Rh = prediction according to Rh blood type
* = assuming individual is a non-recombinant
Method 1 = prediction based on AP alone
Method 2 = prediction based on AP and PEA
Method 3 = prediction based on AP and Ph
Method 4 = prediction based on all three

DISCUSSION

BIRTH PREVALENCE, GENE FREQUENCY AND CARRIER FREQUENCY

The findings of this study support the impression that there is a increased incidence (and birth prevalence) of infantile hypophosphatasia in the Mennonite population of Manitoba. All but three infantile hypophosphatasia cases were of Mennonite descent on both the maternal and paternal sides. Two of these cases were in one family. These were the sisters first reported by Dr. Chown (2). It is, however, difficult to determine an exact incidence figure from our data. Although we used multiple sources, our ascertainment cannot be considered complete. The milder cases of hypophosphatasia i.e. adults without bone symptoms would likely not have come to our attention unless there was a positive family history. Similarily, the severe lethal cases may have been undiagnosed and simply labelled as stillbirths. Calculations based on our data would, therefore, only reflect minimum frequencies.

There are two methods that can be used to calculate the gene frequency of hypophosphatasia in the Mennonite community of Manitoba. An indirect method is to calculate the birth prevalence and use this calculation to estimate the gene frequency. This may underestimate the gene frequency due to underascertainment of cases. Alternatively one can estimate the gene frequency directly by calculating the number of carriers in a representative population such

as our control group. The frequency of affected individuals (i.e. q^2) calculated from this figure would tend to overestimate the birth prevalence if "affected" conceptions were less likely to survive to a point in gestation where they would be recognized.

In order to calculate a birth incidence among Mennonites in Manitoba, one first needs to know the number of Mennonite births in Manitoba per year. This data is not easily available as statistics relating to religious affiliation are not collected by Statistics Canada. The number of births by geographic region is, however, known. According to the Department of Vital Statistics of the Government of Manitoba, there were a total of 7,578 births in the Central Region of Manitoba from 1981 - 1985 inclusive. This translates to an average of approximately 1,500 births per year. As a large proportion of people in that region are Mennonite, a large but unknown percentage of these births would be Mennonite. Similarily, these births would represent a large but again unknown percentage of the total Mennonite births in Manitoba. There were a total of six infantile cases born in the years 1984-1986 inclusive. Assuming the number of Mennonite births in those years in Manitoba was 3 X 1,500 or 4,500, the birth prevalence would be 6/4,500 or 1.3 per thousand. The gene frequency (q) would be 0.04 and the carrier frequency (2pq) would then be 0.071 (1/14.1) according to Hardy-Weinberg principles. If these calculations were done for the years 1977-1987

instead, we would find that there were 7 cases out of an estimated 15,000 births. The birth prevalence, gene frequency and carrier frequency would then be 0.5 per thousand, 0.02 and 0.042 (1/24) respectively.

An alternative method of estimating the frequency of affected individuals to look at the control group. Thirty-two of the 36 controls were of Mennonite background. Of these 32, three appeared to be carriers. Assuming that all three were in fact carriers for infantile hypophosphatasia, the carrier frequency would be 3/32 or 0.094 (1/10.7). According to Hardy-Weinberg theory, q^2 would be 2.2 per thousand. This figure would likely represent an overestimate of the birth prevalence for the reasons stated above. The inaccuracies of these methods for determining frequencies are self evident. It is dangerous to try to derive an incidence based on such small numbers even if one did not have to make assumptions. It would appear, however, that that these results do suggest a much greater incidence than the 1 in 100,000 quoted by Fraser (5). Swift et al. calculated the incidence and gene frequencies of ataxia-telangiectasia, another autosomal recessive disorder by using segregation analysis techniques and looking at the incidence of the disease in cousins of affected individuals (56). Their calculations were done using the computer program Pedigree Analysis Package or PAP (57). A more precise estimate of the incidence of infantile hypophosphatasia can perhaps be derived by using these

methods.

INHERITANCE

Our data provide further support for the theory that infantile hypophophatasia is an autosomal recessive disorder. Finding an increased incidence in an inbred population such as the Mennonites is very consistent with such a mode of inheritance. Other examples of autosomal recessive diseases with an increased frequency in different inbred ethnic populations are sickle cell disease in Blacks and Tay-Sachs disease in Ashkenazi Jews. We also identified two families with parental consanguinity. The first such family was originally described by Chown (2). In this family, the non-Mennonite parents were first cousins. The second example of consanguinity was in the case of P.F. In this case the parents were siblings. Both parents appear to be carriers biochemically and the grandfather appears to be a non-carrier according to historical data (see Fig. 13). The most likely explanation is that the grandmother was a carrier and the two sibling parents inherited the abnormal gene from her with P.F. in turn inheriting one abnormal gene from each parent resulting in the homozygous lethal state.

Further evidence for recessive inheritance comes from the following: All parents who were tested had biochemical evidence of being a carrier. In the families where both grandparents were tested, one out of every grandparental pair also appeared to be a carrier. The other grandparent

from the pair had normal biochemistry. Of the 98 first degree relatives on whom a decision was made regarding their carrier status, 53 were determined to be carriers and 45 non-carriers. This is not statistically significantly different from the 49:49 expected distribution. Although our data stongly supports infantile hypophosphatasia being an autosomal recessive condition, we cannot yet comment on the inheritance of the other forms of hypophosphatasia or on their relationship to the infantile disease. Segregation analysis using the computer program PAP may be useful in answering these questions (57).

CARRIER ASSIGNMENT

We have shown that alkaline phosphatase activity is significantly lower in obligate carriers compared to controls (Table V). Similarily urine phosphoethanolamine is significantly higher in obligate carriers (Table VII). This is consistent with what has been reported by others (39,41). These differences remained significant even though three of the controls were probably carriers. Within each category, males tended to have a slightly higher alkaline phosphatase activity, although the differences were not statistically significant (Table (VI). Again, this is consistant with the findings of others (25). Although the sex distibution among obligate carriers and controls was not significantly different from expected, there were over twice as many female as male controls. This would have tended to

lower the mean alkaline phophatase of the controls compared to what would have been expected with a 50:50 sex ratio. Consequently had we had equal numbers of male and female controls, the difference between carriers and controls would have been even more striking. The mean ages of the two groups were also similar.

The distribution of the alkaline phosphatase results among controls, carriers and first degree relatives is illustrated in figure 14. The control and obligate carrier graphs are only partially overlapping with each showing a single modal peak. The distribution of results in the first degree relatives is bimodal suggesting there are in fact two distinct populations i.e. carriers and non-carriers. In fact, the graph for the first degree relatives almost appears to be a composite of the other two graphs. A similar phenomenon is seen in the distribution of phospho-ethanolamine results (fig. 15). Although a bimodal distribution is not seen for the first degree relatives the graph obtained is similar to the graph that would be created by adding the graphs for the obligate carriers and controls together.

I did feel it was justified to exclude three individuals from the control group after stage one of the logistic regression analysis. Although I could assume in advance that parents of an affected infant were carriers (given autosomal recessive inheritance and biologic parenthood) I could not be sure that any control group

would not contain carriers. As our controls were predominantly Mennonite, it is not surprising to find carriers among them.

Using the equation derived from the second stage logistic regression analysis which incorporated the alkaline phosphatase and phosphoethanolamine results, we were very easily able to separate carriers from non-carriers in our population. Fig. 17 shows a scatter plot obtained when alkaline phophatase is plotted against phosphoethanolamine. All carriers are found on the upper left part of the diagram and all non-carriers on the lower right. It is interesting to note that no individual who was labelled a non-carrier had a phosphoethanolamine value greater than 9 ummols/mmol creatinine. We were able to classify all but two of 100 first degree relatives on whom complete results were available with a greater than 99% probability. Using this equation, all obligate carriers including the one who was initially only given a 13% probability, were calculated to have a greater than 99% chance of being carriers. All 33 second stage controls were given less than a 1% chance of carrier status. Of the three carriers who were excluded from the second stage, two had a greater than 99% chance of being a carrier based on the combined results. The other control excluded did not have a urine result available, therefore, no second stage prediction could be made.

It is theoretically possible that we have merely

separated these individuals into two groups based on their biochemistry in a manner unrelated to their carrier status. This, however, would be unlikely. There is precedent in the literature for using a similar discriminant to separate carriers from non-carriers (40). On an intuitive level, the use of such an equation makes sense. Patients with hypophosphatasia and to a lesser extent carriers do show decreased alkaline phosphatase activity and increased phosphoethanolaminuria. Therefore, it is not surprising that an equation which incorporates both results should be useful as a discriminant. The fact that close to 50% of first degree relatives were predicted to be carriers also argues for the validity of the method.

Additional support comes from the linkage studies which have been done on this population using this method to assign the genotype for hypophosphatasia (42,43 and unpublished data). Linkage between the genes for hypophosphatasia (HOPS) and the Rh blood group (RH) has been established at a recombinant fraction of 0.08 with a peak combined Lod score of 6.36. We have also shown extremely tight linkage between HOPS and the gene for the liver/bone/kidney isozyme of alkaline phosphatase (ALPL) with a peak combined Lod score of over 13. No recombinants have been seen to date in 53 informative meioses between ALPL and the genotype predicted by the logistic regression analysis. Weiss et al. had previously demonstrated linkage between ALPL and RH with a recombinant fraction of 0.1 (24).

The consistency of these linkage studies provides additional evidence of the validity of this method.

Further proof will require development of a better test for carrier status that is independant of alkaline phosphatase and phosphoethanolamine results. Markedly increased levels of pyridoxal-5'-phosphate have been recently reported in patients with hypophosphatasia. We are in the process of analyzing blood samples from our study participants in regard to measuring their vitamin B_6 vitamers including pyridoxal-5'-phosphate. This work is being done in collaboration with Dr. S. Coburn of Fort Wayne, Indiana and Dr. M. Whyte of St. Louis, Missouri.

We should emphasize that this particular equation was derived for a specific population with results essentially from one laboratory. We would not recommend applying the equation directly to individuals from other populations tested with different methods. We have deliberately confined our analysis to persons over 18 years of age to avoid the problems relating to different normal ranges. Consequently the equations derived can not be applied to individuals younger than 18.

COMPARISONS BETWEEN CARRIERS AND NON-CARRIERS

Having been able to so clearly separate carriers from non-carriers provided us with the perfect population to examine the carrier phenotype. In order to avoid bias, only data derived blind to the hypophosphatasia genotype was used

in our comparisons. The non-carriers were used as a "control" group for the carrier group. Only by using such a control group was it possible to determine if the phenotype of a carrier was different from an average normal individual.

Many methodologic flaws were unfortunately present in our data collection. This study was done at the same time that we were collecting data for the linkage and other studies. Consequently five blood tubes, one urine sample, dental and hand radiographs and numerous historical and physical data had to be collected. Given that up to 40 participants were sometimes seen in a single morning it was not possible to obtain complete information on everyone. As the dental radiographs were taken at a separate location, there was even greater difficulty in obtaining these. Another problem was recall bias. For example, many individuals could not remember at what age they lost their deciduous teeth. Fortunately, as the interviews for first degree relatives were done with both the interviewer and the participant unaware of their carrier status, this problem should have been equally present in the two groups. Another problem we encountered was the inability to use the same technique for all measurements. In some settings there was no scale available to measure height or weight. We were, therefore, forced to rely on the estimated values supplied by the participant. Technical problems were also evident in the dental radiographs. Although the radiographs were

primarily taken at two centers, there was a wide range in the technical quality. For example on many films, the gonial angle could not be reliably measured. Bone density could not be estimated due to variations in technique. Again due to the blind nature of the study these problems should not affect the interpretation of the comparisons between the two groups. The actual values obtained for various measurements may be suspect but the comparisons should be valid as the methodologic flaws would not be influenced by carrier status.

There was one advantage in being forced to evaluate participants in a less than optimum environment. We were trying to determine if the average physician or dentist could distinguish carriers from non-carriers with routinely available methods. If a factor that required a specialized test was found to be discriminatory, its applicability would be limited by the difficulty in doing the test. Dentists could not easily obtain panoramic radiographs of sufficiently consistent quality to determine bone density. Therefore, it would not be extremely helpful to measure bone density on panorexes with the hope of it becoming a useful tool for dentists.

None of the criteria assessed by interview differed between carriers and non-carriers with a p value less than 0.05. It is interesting to note that the sex distibution among first degree relatives was different than expected with a p value just under 0.05. There were 33 non-carrier

males compared to only 19 carriers. When one does multiple comparisons one expects 1 in 20 factors to be significant at the 5% level simply on the basis of chance. This unequal sex ratio may simply represent this phenomenon. Alternatively, as males do have a higher alkaline phosphatase activity and possibly a lower phosphoethanolamine excretion than females (see Tables VI, VIII, XXIII and XXV), it is possible that the equation derived from a combined analysis misclassified a few male carriers as non-carriers. This would unlikely represent a large number as the difference between male and female was much less than the difference between carrier and non-carrier. As there were a total of only 20 obligate carriers, it would not have been possible to do the logistic regression analysis separately for males and females.

There were three tests on physical examination whose results were significant at the 5% level. The difference in outer canthal distances although significant for males and females combined, was primarily due to the difference among females. In females, the mean outer canthal distance for carriers was 8.1 cm. compared to 8.6 cm. for non-carriers. The p value was 0.03. It is difficult to accept this result as being truly significant however. As stated earlier, one of the problems with doing multiple comparisons is that 5% of tests will have a p value less than 0.05 simply on the basis of chance. One then has the option of demanding a greater significance level (i.e. $p < 0.01$)

before accepting a result as real. The difference in outer canthal distances would then no longer be considered significant. When one looks at the distribution of outer canthal distances in female carriers versus non-carriers (Fig. 18), one is more inclined to dismiss the difference as being insignificant. Except for one individual, the two graphs are very similar. That particular carrier had an outer canthal distance much less than anyone else suggesting either an error was made in obtaining her measurement or that some other factor was responsible for this abnormality.

Both the height/span ratio (H/S) and the span minus height difference (S-H) were significantly different in carrier females versus non-carrier females ($p = 0.014$). These tests are two different ways of looking at the same data. The fact that the H/S ratio was lower in carrier (0.943 vs.0.967) and the S-H was greater in carriers (9.9 vs 5.6 cm.) both imply that the height is less than the span. A possible explanation is that women who are carriers develop vertebral compression similar to women with osteoporosis. Dequecker et al. studied women with osteoporosis using women with osteoarthritis as a control (58). The osteoporotic women had a mean age of 62 years and the osteoarthritic women 63 years. In our study the mean ages for women who had this test were 44.3 and 40.0 years for carriers and non-carriers respectively. The difference between the mean span and mean height was 9.8 cm. for

osteoporotic women and 4.2 cm. for osteoarthritic women. The mean H/S ratio were 0.939 and 0.973 for osteoporotic and osteoarthritic women respectively. Our results suggest that women who are carriers for infantile hypophosphatasia are similar to older women with osteoporosis. We are still left with the problem of how significant these results are. Although close, the p value is not less than the arbritrary rigid cutoff of 0.01. Some clarification can be obtained by looking at the distributions of these results (Fig. 19 and 20). Although there is much overlap between the carrier and non-carrier graphs, there are eight carriers whose results do not overlap with either method. This suggests that some women who are carriers do have vertebral shortening to a greater degree than non-carriers. The average age of the eight women is 47.9 years so age alone is not an explanation. I, therefore, suggest that women who have advanced signs suggestive of osteoporosis (i.e. a decrease in height vs. armspan) may possibly be carriers for hypophosphatasia. Physicians who are involved in the care of Mennonite women may want to consider this possibility in evaluating such women. It is difficult to imagine that this test would be useful in screening either older women or women from the general population because osteoporosis would be so much more common than hypophosphatasia. It would be interesting to speculate on the degree to which hypophosphatasia contributes to osteoporosis in general. Unfortunately we have no data to answer this question.

Prospective evaluation of young women found to be carriers for hypophosphatasia may help in resolving this issue.

None of the criteria tested on the dental radiographs were significantly different between carriers and non-carriers. Even combining two criteria (i.e. M.R.P. and widening of the zygomatic suture) did not produce significant differences. Part of the reason may be the poor sensitivity of this technique to detect changes related to an underlying bone disease. Social and environmental factors may also have hidden the effects due to genetic (i.e. hypophosphatasia) factors. The best example of this can be seen by looking at the number of teeth present on the dental radiograph. Carriers (average age = 40.2 years) had an average of 15.3 teeth. Non carriers (average age 39.2 years) had an average of 17.4 teeth. Many participants responded that they had several teeth removed simply because that was the easiest and cheapest way to deal with a problem.

It is possible that had we studied larger numbers of panoramic radiographs and had we demanded a higher quality radiologic study that a statistically significant difference between the two groups could have been found. It is doubtful however that this difference would be clinically significant.

It is not surprising to see that alkaline phosphatase and phosphoethanolamine are significantly different between carrier first degree relatives and non-carriers (Tables XX

and XXII). This is because these two groups were defined according to these results. The fact that serum calcium is not different between the two groups is also consistent with what was expected. The finding of a very significant difference in the serum phosphate value is most interesting. As most reports had indicated that serum phosphate was normal in hypophosphatasia, this test (and the serum calcium) were only done as a crude screen for other bone diseases. I only recently became aware of the report by Whyte and Rettinger which showed relative hyperphosphatemia in patients with hypophosphatasia (36). This is now the first evidence of such a finding in carriers for the infantile disease.

The very significant difference ($p \leq 0.0001$) in serum phosphate levels was seen if one compared carriers to non-carriers in controls, obligate carriers and first degree relatives (i.e groups A + C vs. B + D in Fig. 11) or just in first degree relatives alone (i.e. group C vs. D). The significance was still preserved if one looked at male and female first degree relatives separately.

I also compared the original control group against the obligate carriers of infantile hypophosphatasia with virtually identical results. I then compared this control group to the parents of juvenile case and to the children of adult cases (Table XXIX). Data on these people had been collected as part of the overall study. A similar result was seen although the p value was not as low as the number

TABLE XXIX

SERUM PHOSPHATE: COMPARISON TO CONTROL GROUP

GROUP	GROUP TOTAL	GROUP MEAN (S.D.)	CONTROLS TOTAL	CONTROLS MEAN (S.D.)	p
Parents of Infantile Cases	19	1.33 (0.17)	36	1.10 (0.19)	<0.0001
Parents of Juvenile Cases	6	1.33 (0.14)	36	1.10 (0.19)	0.009
Children of Adult Cases	7	1.28 (0.15)	36	1.10 (0.19)	0.02

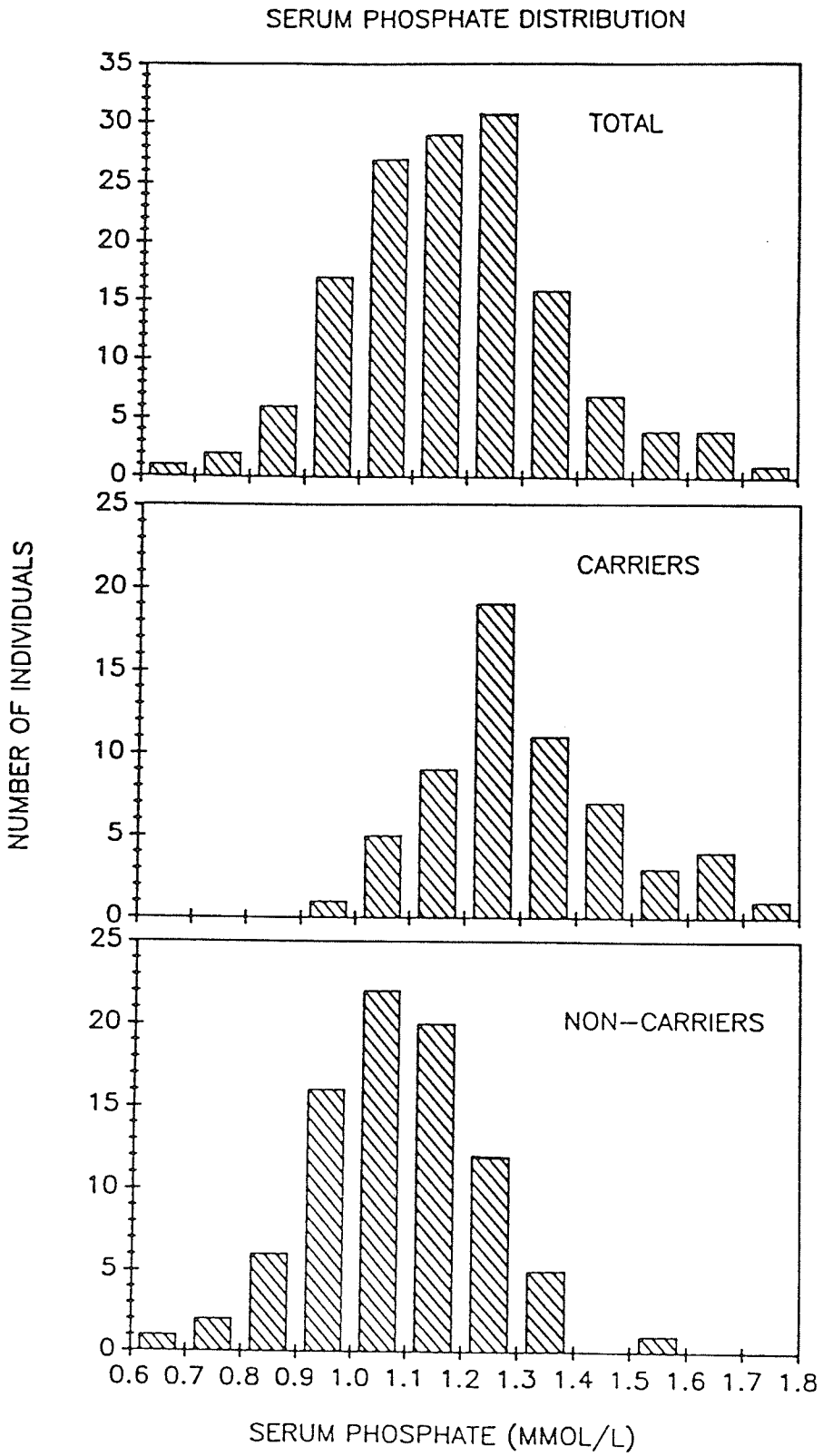
in those groups were smaller. The mean phosphate level of the three controls excluded after step one of the logistic regression analysis was 1.29 mmol/L. Therefore, had we excluded these "carriers" from the control group. the control mean would have been lower and consequently the p value obtained in the comparisons would also have been more significant.

Both mean values (1.30 mmol/L for carriers and 1.08 mmol/L for non-carriers) lie within the normal range for our laboratory. If we had only looked at individual results we would merely have stated that most results are within the normal range. The relative hyperphosphatemia of the carriers only became apparent when the two groups were compared. This may explain why most case reports have stated that the serum phosphate was normal. If we look at the distribution of phosphate results (Fig. 21), we see that most carriers do have phosphate levels in the normal range with a few having slightly elevated values. The distribution in both the carrier and non-carrier groups approximate a normal distribution. The distibution of the total group is obviously not normal as there is a broadening of the central portion of the graph.

SIGNIFICANCE OF HYPERPHOSPHATEMIA

The exact mechanism for this relative hyperphosphatemia is not clear. Whyte and Rettinger have postulated that increased renal reabsorbtion of phosphate is the explanation

Figure 21


SERUM PHOSPHATE DISTRIBUTION
TOTAL
CARRIERS
NON–CARRIERS
NUMBER OF INDIVIDUALS
SERUM PHOSPHATE (MMOL/L)
0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8

(36). The population we have described would be an excellent one to test this hypothesis and therefore to explore the role of alkaline phosphatase in bone metabolism.

This new finding of a relative hyperphosphatemia in carriers should prove helpful in solving the puzzling inheritance of hypophosphatasia. This is now the third criteria along with alkaline phosphatase activity and phosphoethanolaminuria, that can be used in segregation analysis. Vitamin B_6 studies may soon contribute further useful criteria.

A major goal of this project was to develop better methods for screening for carriers of hypophosphatasia. Couples in which both members are found to be carriers for infantile hypophosphatasia could then receive appropriate genetic counselling. Prenatal diagnosis would be one option available to them. We believe that this finding of relative hyperphosphatemia is a major contribution towards the development of such a program. As shown in Table XXVII, alkaline phosphatase activity alone (method 1) can not sufficiently separate carriers from non-carriers. Too many (46 of 155) individuals had intermediate results and therefore could not be conclusively classified. That is, their probability of being a carrier was between 1 and 99%. In six of these 46 cases, method 1 determined the probability of being a carrier to be between 1 and 49%. In all six cases, the other three methods calculated the probability of being a carrier as > 99%. One of these six

individuals was in fact an obligate carrier. These six carriers would therefore all have been missed if one relied only on the alkaline phosphatase activity.

By combining these results with the phosphoethanolamine result (method 2), a much better separation was obtained. Only two individuals out of 151 had an intermediate result. An equally good separation was seen using a combination of alkaline phosphatase activity and the serum phosphate level (method 3). Combining all three variable (method 4) gave similar results.

There were only four cases where the predictions based on the latter three methods did not agree (Table XXVIII). In the cases of H.D. and J.D. this disagreement was merely a matter of degree. Two methods predicted the individual was a carrier with > 99% probability while one method made the same prediction but with a lower degree of certainty (i.e. 86 or 95%). In the other two examples methods 3 and 4 stated that the individuals were carriers contrary to the prediction of method 2. In one case (E.K.), method 2 gave a conclusive result (i.e. probability of carrier <1%) while in the other case (D.H.) the result was intermediate (i.e. probability of carrier = 37%). The predictions based on DNA linkage studies agreed with method 2 in all cases.

The reason for these discrepencies is unclear. Serum phosphate may be artificially appear elevated if the blood sample is hemolyzed. If this is undetected a falsely elevated phosphate may be reported. In cases where the

alkaline phosphatase is in the borderline region an inaccurate carrier prediction may result.

The fact that method 3 may overdiagnose an occasional person as being a carrier does not detract from its value in a screening program. A screening program based on two serum tests that can be done on one machine would certainly be cheaper and more practical than one which requires a urine sample to be collected in addition to a blood sample especially if the urine was to be tested by an expensive method such as amino acid analysis. In a screening program one wishes a test with the maximum sensitivity with a reasonable specificity. Using both alkaline phosphatase and phosphate together seems to meet the needs for such a program. If one excludes intermediate or incomplete results and assumes that method 2 is 100% accurate in determining carrier status, then the sensitivity of method 3 is 100% and the specificity is 98.8% The exact details regarding the practical aspects of establishing such a program still require further analysis. Individuals detected by a low activity of serum alkaline phosphatase and a relative hyperphosphatemia could also be patients with (or possibly carriers for) the other milder forms of hypophosphatasia. It is not known at present whether or not these people are at risk for having a child with the infantile form of the disease. Therefore, prior to developing such a screening program, the overall inheritance of hypophosphatasia and the relationship between the various

forms would need to be better understood.

CONCLUSIONS

1. Infantile hypophosphatasia is an autosomal recessive disorder with an increased frequency in the Mennonite population of Manitoba.

2. Logistic regression analysis using a combination of the serum alkaline phosphatase activity and the phosphoethanolamine excretion can reliably separate carriers from non-carriers.

3. Routine history and physical examination and dental radiographs do not appear to reliably distinguish carriers from non-carriers.

4. Women who are carriers for infantile hypophosphatasia may show abnormalities similar to women with osteoporosis i.e a decrease in height relative to arm span. Physicians should consider this possibilty in younger women, especially if they are of Mennonite descent.

5. Carriers for hypophosphatasia are relatively hyperphosphatemic compared to non-carriers. This fact can be important in future studies on hypophosphatasia or on the role of alkaline phosphatase. This fact also has important implications for the development of a screening program for hypophosphatasia.

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APPENDIX I

LETTER SENT TO DOCTORS, DENTISTS,
MEDICAL RECORD DEPARTMENTS AND
CLINICAL CHEMISTRY LABORATORIES.

THE UNIVERSITY OF MANITOBA

FACULTY OF MEDICINE
Department of Human Genetics

250 Old Basic Sciences Building
770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3

(204) 788-6392

Dear Dr.

I am conducting research on a condition called hypophosphatasia for the Department of Human Genetics at the University of Manitoba. There are three forms of this disease. The infantile form which has an increased incidence in the Mennonite population is characterized by skeletal deformities and growth failure and is usually fatal. The childhood form is characterized by early loss of the deciduous teeth and bony changes similar to rickets. Craniostenosis may also be present. The adult type is characterized by early loss of the deciduous and permanent teeth and possibly repeated bony fractures. Biochemically these conditions are characterized by low serum levels of alkaline phosphatase and excretion of phosphoethanolamine in the urine.

The goals of this research project are to better elucidate the genetics of this condition with the hopes of developing a preventative strategy for the severe neonatal form. I would be most interested in contacting any of your patients who might have this condition or families in which the disease has occurred. We would then like to do detailed family studies which would involve, in addition to a history and physical examination, certain radiologic and biochemical tests. Any help you could give me in the above matter would be greatly appreciated.

Sincerely yours,

Bernie Chodirker, M.D.
Research Fellow
Department of Human Genetics

kmc-22

THE UNIVERSITY OF MANITOBA

FACULTY OF MEDICINE
Department of Human Genetics

250 Old Basic Sciences Building
770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3

(204) 788-6392

Dear Dr.

I am conducting research on a condition called hypophosphatasia for the Department of Human Genetics at the University of Manitoba. This is an inherited condition which appears to have an increased incidence in the Mennonite population. The severe form of this disease is characterized by early neonatal death and skeletal deformities. The childhood form is characterized by bony changes similar to rickets. In the adult form, repeated fractures of the long bones may be present.

The dental manifestations of the childhood and adult forms are as follows: There is premature exfoliation of the deciduous teeth and possibly permanent teeth. This exfoliation occurs spontaneously or as a result of slight trauma. The teeth may be overerrupted and loose in the alveolar bone. The roots show little signs of resorption. There typically is absence severe clinical gingival inflammation. Both sets of teeth are hypoplastic with thin enamel. Dental X-rays may show enlarged pulp chambers and root canals, minimal root resorption and advanced loss of alveolar bone.

One hallmark of this condition is a low level of serum alkaline phosphatase. There are, however, other biochemical manifestations of this disorder. We would be most interested in contacting any of your patients who you feel may have a form of hypophosphatasia. We hope to gather enough data with this study in order to design an effective preventative program in regard to the severe neonatal type. Any help you could give us would be greatly appreciated. Should you require any more information, please feel free to contact us.

Sincerely yours,

Bernié Chodirker, M.D.
Research Fellow
Department of Human Genetics

kmc-21

THE UNIVERSITY OF MANITOBA

FACULTY OF MEDICINE
Department of Human Genetics

250 Old Basic Sciences Building
770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3

(204) 788-6392

May 14, 1987

Medical Records Departments

Dear Sir/Madame:

I am conducting research on a condition called hypophosphatasia for the Department of Human Genetics at the University of Manitoba. There are three forms of the disease. The infantile form, which has an increased incidence in the Mennonite population, is characterized by skeletal deformities and growth failure and is usually fatal. The childhood form is usually characterized by early loss of the deciduous teeth and bony changes similar to rickets. The adult type is characterized by early loss of deciduous and permanent teeth and possibly repeated bone fractures. One hallmark of this disease is a low serum level of alkaline phosphatase. It is unclear what is the relationship of one form to another.

The goals of this research project are to better elucidate the genetics of this condition with hopes of developing a preventative strategy for the severe neonatal form.

I would be most interested in reviewing your medical records, looking for individuals with this condition. I would then want to contact them through their doctor in order to arrange for the testing.

As I do not know the coding system used in your department, I am unsure exactly how to select these charts. At the Health Science Centre many, but not all, cases were coded under "perinatal deaths" or "disorders of phosphate metabolism". Any help you could give me would be most appreciated. If you require more information please do not hesitate to contact me.

Sincerely yours.

Bernie Chodirker, M.D.
Research Fellow
Department of Human Genetics

kmc-21

THE UNIVERSITY OF MANITOBA

FACULTY OF MEDICINE
Department of Human Genetics

250 Old Basic Sciences Building
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Canada R3E 0W3

(204) 788-6392

May 12, 1987

Clinical Chemistry Laboratories

Dear Sir/Madame:

I am conducting research on a condition called hypophosphatasia for the Department of Human Genetics at the University of Manitoba. Some manifestations of this disease can range from early teeth loss in adults to neonatal deaths. The goals of the research project are to better elucidate the genetics of this condition with hopes of developing a preventative strategy for the severe neonatal form. We do know that there is an increase incidence of the neonatal form in the Mennonite communities. One hallmark of this disease is a low serum level of alkaline phosphatase. I would, therefore, be most interested in reviewing your laboratory records, looking for low alkaline phosphatase values. If such levels were found I would then want to contact these individuals through their doctor in order to arrange further testing.

Any help you could give in the above matter would be greatly appreciated. Please do not hesitate to contact me should you require any further information.

Sincerely yours,

Bernie Chodirker, M.D.
Research Fellow
Department of Human Genetics

kmc-21

APPENDIX II

CONSENT FORM

GENETIC ANALYSIS IN HYPOPHOSPHATASIA

(A) CONSENT FOR VENOUS BLOOD SAMPLING

I, _____ give consent for Dr. _____, or one of his/her colleagues, to take a sample of approximately 25 mL (1.5-2 tablespoonfuls) venous blood for research purposes. I understand that DNA, the genetic material present in all cells in the body, will be extracted from the white cells in the blood. As well the levels of certain minerals (calcium, phosphorus), certain proteins (alkaline phosphatase), Vitamin B_6 and certain blood groups will be studied.

(B) CONSENT FOR URINE SAMPLING

I, _____, consent to have Dr. _____ or one of his/her colleagues, test a 24 hour urine sample for certain chemical compounds that may be important in hypophosphatasia.

(C) CONSENT FOR X-RAYS

I, _____, consent to have an x-ray of my _____ (hand/teeth) taken as part of the research project on hypophosphatasia. It has been explained that this will involve a very small amount of radiation exposure. (For females - To my knowlege I am not pregnant.)

(D) CONSENT FOR PYRIDOXINE (VITAMIN B_6) TESTING

I, _____, agree to participate in this part of the hypophosphatasia study. The role of Vitamin B_6 testing in the diagnosis of carriers of the gene for hypophosphatasia has been explained to me and I understand that the risks are very minimal. I agree to take _____ mg of pyridoxine orally once a day in the morning for six days and to have a blood sample of approximately 1-2 teaspoonsful drawn on the seventh morning. (For females - To my knowledge I am not pregnant.)

APPENDIX III

DATA COLLECTION SHEET

FAMILY # _____

PERSONAL

NAME: _____
D.O.B.: _____ D.O. EXAM: _____
SEX: M F ETHNIC BACKGROUND: _____
MARITAL STATUS: _____ PLACE OF BIRTH: _____
NAME/D.O.B. PARENTS: _____

PARENTAL CONSANGUINITY: NO YES
NAME/D.O.B. CHILDREN: _____

M.D. NAME: _____ DENTIST NAME: _____

HISTORY

GENERAL HEALTH: _____
CHRONIC DIARRHEA: NO YES _____
THYROID DISEASE: NO YES _____
CURRENT MEDICATIONS: NO YES _____
DIET - ABNORMAL: NO YES _____
AVERAGE FRUIT INTAKE/DAY: _____
PREVIOUS X-RAYS: NO YES _____

CHILDHOOD

RICKETS: NO YES _____
CRANIOSTENOSIS: NO YES _____
FRACTURES: NO YES _____
BONE/JOINT PAIN: NO YES _____
CHEST DEFORMITY: NO YES _____
OTHER BONE DEFORMITY: NO YES _____
AGE FIRST WALKED: _____

DENTAL

AGE DECIDUOUS TEETH LOST: _____
AGE PERMANENT TEETH LOST: _____
CARIES: NONE FEW MANY
PROBLEMS WITH FILLINGS: NO YES _____
OTHER DENTAL PROBLEMS: NO YES _____

PHYSICAI

HEIGHT: _____ %tile _____
WEIGHT: _____ %tile _____
H.C.: _____ %tile _____
HEAD IENGTH: _____ %tile _____
HEAD WIDTH: _____ %tile _____
I.C.: _____ %tile _____
I.P.: _____ %tile _____
O.C.: _____ %tile _____
UPPER SEGMENT: _____
LOWER SEGMENT: _____
ARM SPAN: _____
SKUI I SHAPE: NORMAI ABNORMAI _____
SCIERA: WHITE BI UE _____
PROPTOSIS: NO YES _____
TEETH: NUMBER - _____
COIOR - _____
CARIES - _____
JOINT IAXITY: EIBOWS/FINGERS - NORMAI ABNORMAI
PAIMAR CREASES: _____
SINGIE DIGITAI CREASE: NORMAI ABNORMAI
EXCESS LORDOSIS: NO YES
BOWED IEGS: NO YES _____
_____

LAB

DAY 1

BASEIINE BIOOD _____ (Ca, PO4, AIK PHOS, VITAMIN B6 VITAMERS, DNA BIOOD GROUP MARKERS)

24 HOUR URINE _____ (PEA)

PANOREX _____

X-RAY RIGHT HAND _____

PYRIDOXINE IOAD _____ (0.3 mg kg p.o. in A.M. x 6d)

DAY 7 (POST IOAD)

BLOOD _____ VITAMIN B6 VITAMERS
WBC STUDIES
RBC AST