Familial Hypophosphatemia with Vitamin D-Resistant Rickets. II:

Three Additional Kindreds of the Sex-Linked Dominant Type with a Genetic Analysis of Four Such Families[1]

JOHN B. GRAHAM,[2] VERNON W. McFALLS,[3] AND ROBERT W. WINTERS[4]

From the Departments of Pathology, Pediatrics, and Medicine, The University of North Carolina School of Medicine, Chapel Hill

INTRODUCTION

THE WIDESPREAD PROPHYLACTIC USE of vitamin D in infant nutrition has virtually eliminated rickets due to deficiency of vitamin D from the United States. One consequence of this achievement has been the recognition of rickets which develops in spite of vitamin D prophylaxis. Several types of rickets of this sort have been delineated, and each type appears to result from a congenital or acquired metabolic defect (2–4, 7, 8, 10–12).

The most common form of "endogenous" rickets is an entity usually referred to as "vitamin D resistant (or refractory) rickets" (1). The clinical picture of this disorder is quite similar to that of deficiency rickets, except that large, even massive, doses of vitamin D are required for healing of the bone lesions. The only clearly defined metabolic defect is hypophosphatemia which has been attributed in large part to deficient reabsorption of phosphate from the glomerular filtrate by the renal tubular cells. It is not clear whether the latter abnormality results from an intrinsic defect in the kidney itself, or whether it is the consequence of excessive parathyroid stimulation secondary to deficient absorption of calcium from the gastro-intestinal tract. The clinical, pathological and biochemical features of this disease and the accumulated literature have been reviewed by us recently (14).

Many authors have recognized the frequent familial occurrence of "vitamin D resistant rickets". Our review of the previously published genetic data suggested that the generally accepted idea of autosomal dominant transmission might be erroneous. In the first kindred reported by our group ("E") clear evidence of a sex-linked dominant mode of inheritance was found. This result was obtained by using serum inorganic phosphorus concentration rather than skeletal abnormality to indicate presence of the abnormal gene and by analyzing the progeny of affected persons

Received April 13, 1959.

[1] Supported (in part) by research grants (H-1333 and H-3140) from the Public Health Service and a grant from the United Medical Fund of North Carolina.

[2] Professor of Pathology, University of North Carolina.

[3] Intern in Pediatrics, University of Arkansas Medical Center; formerly Student Fellow in Pathology, University of North Carolina.

[4] Assistant Professor of Physiology, University of Pennsylvania; formerly Fellow of the National Foundation for Infantile Paralysis, University of North Carolina.

separately by sex of affected parent. Penetrance was found to be high (possibly complete) for hypophosphatemia, but considerably reduced for overt bone lesions (13, 14).

Many hypophosphatemic persons in the "E" kindred lacked clinical, radiographic or historical evidence of skeletal disease. This dissociation of hypophosphatemia and bone lesions usually occurred in females. Hypophosphatemic males, on the other hand, almost invariably had skeletal involvement ranging from moderate to marked rickets, post-rachitic deformities, or osteomalacia.

We have studied three similar kindreds of the sex-linked dominant variety ("B", "H", and "L") since our original kindred was reported. The results of these later studies are presented in this paper. An additional kindred in which the disease appears to have been due to a different mechanism will be reported separately (15).

METHODS

The clinical and radiographic diagnoses of active rickets or post-rachitic deformities were made using criteria outlined previously (14). Briefly, there are three major ones: (a) typical onset and progression of characteristic skeletal deformities,

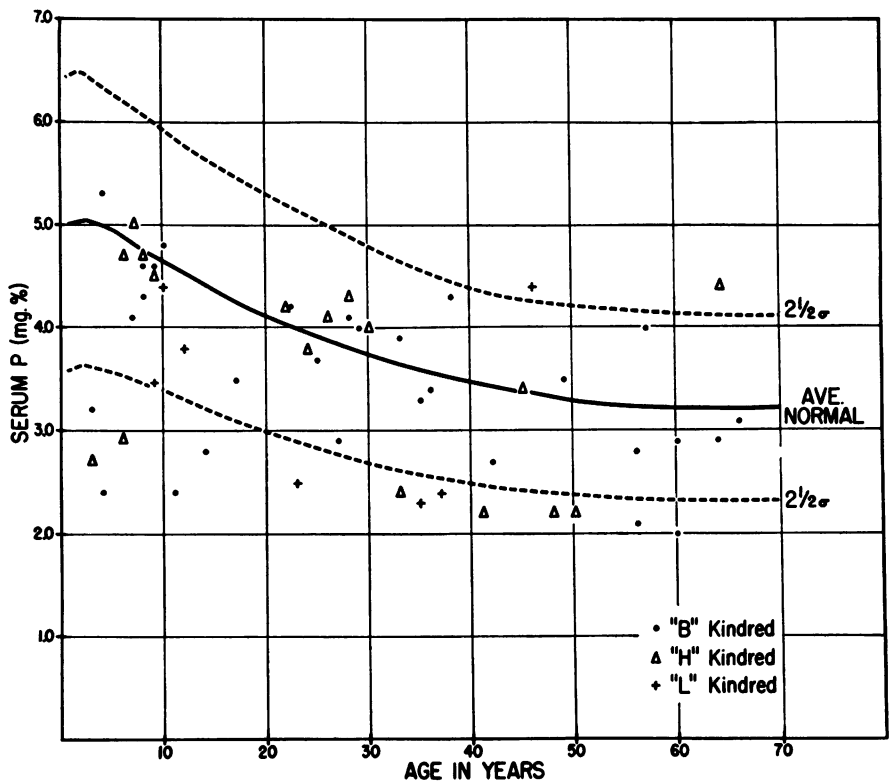

FIG. 1. The normal range of serum inorganic phosphorus for males, with 99% confidence limits. The values plotted represent tested members from the three new kindreds.

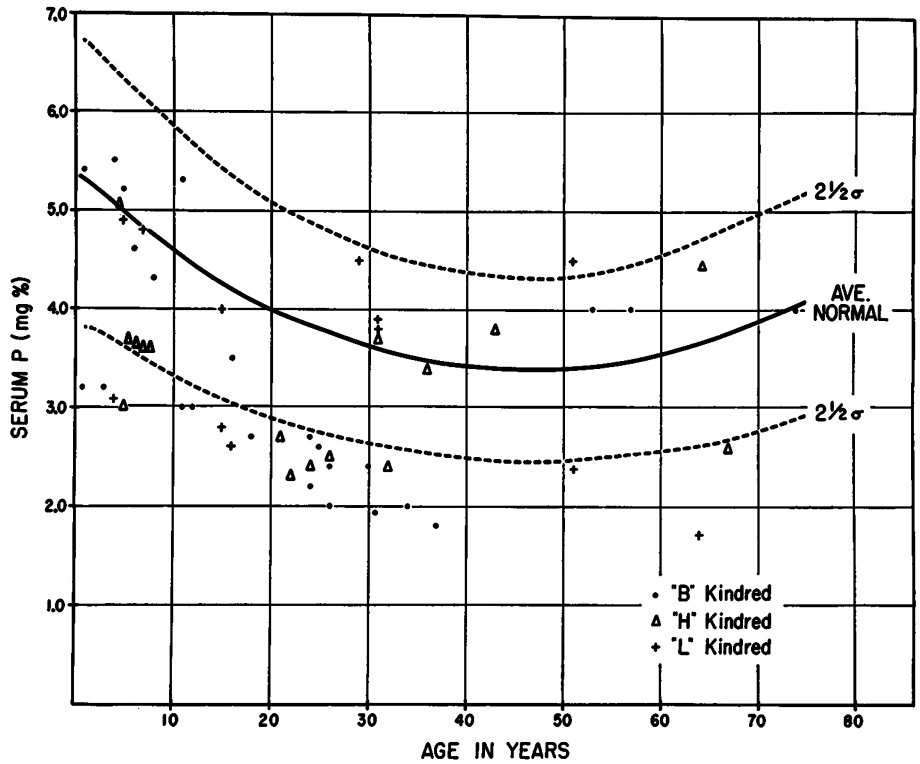

FIG. 2. The normal range of serum inorganic phosphorus for females, with 99% confidence limits The values plotted represent tested members of the three new kindreds.

despite amounts of vitamin D adequate to prevent or cure deficiency rickets, (b) typical physical signs of active rickets or of post-rachitic deformities—most often *genu valgum* or *genu varum*—usually accompanied by short stature, (c) typical radiographic signs of rickets in children or post-rachitic deformities in adults.

Nearly all of the chemical data reported in the present paper were performed on blood samples obtained before breakfast. In the few instances when this was not possible, blood samples were taken at least 4 hours after the last meal. The technique for handling blood samples and our chemical methods have been described elsewhere (5, 14). The individual determinations recorded in the tables represent averages of all values on a given patient for a particular determination. The number of such determinations ranges from one to five.

The discriminant functions used in the diagnosis of familial hypophosphatemia have been described elsewhere (5, 14). They were devised by fitting regression curves of serum inorganic phosphorus on age separately by sexes to a large normal population. An individual is considered hypophosphatemic for our purposes if his serum inorganic phosphorus falls below the lower 99% confidence limit of the normal function for his sex. Mathematical analysis has shown that this lower confidence limit has *average* operating characteristics of no more than about 0.5% false positives and 6%

false negatives, even if the frequency of familial hypophosphatemia in the general population is assumed to be as great as 5% (5). If, as is more likely, the incidence is 1% or less, the discriminant is considerably more efficient. It should be emphasized, however, that the discriminant is not uniformly efficient, being more efficient with adults than children and least efficient with prepubertal girls (5).

In Figures 1 (males) and 2 (females) we have plotted the normal regression curves for serum inorganic phosphorus with their 99% confidence limits. Against these backgrounds have been plotted the serum phosphorus values of all tested members of the three kindreds to be described below. Those persons whose values were below the lower broken curve (lower 99% confidence limit of the normal population) have been scored as hypophosphatemic. Those whose levels were above this lower curve have been scored as normophosphatemic.

EXPERIMENTAL OBSERVATIONS

"B" Kindred

History of the Proband: The proband (IV-45 in Figure 3) was a 29 year old white female brought to our attention by an associate who knew of our interest in familial rickets. She was the product of a normal full-term pregnancy and delivery and was considered entirely normal until a *genu varum* deformity developed when she began to stand and walk alone. This deformity persisted despite a total of approximately 60 ml. of natural cod-liver oil ingested over a period of several years. Radiographic signs of active rickets were present at the knees at 5 years of age. Corrective osteotomies were attempted at this time with only partial success, but the deformities stabilized following surgery. She had had two pregnancies, both deliveries requiring Caesarian section because of a rachitic pelvic deformity.

Physical examination (Table A) revealed a well-developed woman, 57 inches tall. The only significant deformity was a moderate *genu varum*. Skeletal x-rays showed a coarse trabecular pattern in the long bones, particularly the legs. Pseudofractures and generalized osteomalacia were absent, but there was moderate contraction of the pelvic inlet.

Urinalysis was negative. Chemical examination of her serum revealed only one abnormality, hypophosphatemia (see Table B).

Description of the Kindred: Figure 3 is a pedigree chart summarizing the data on 208 members of the "B" kindred. The great majority of these persons are farmers and live in Pitt County on the Coastal Plain of North Carolina. One hundred eighty-nine persons were examined by one of us, and the serum inorganic phosphorus was measured on 53.

The clinical information on 20 members of the "B" kindred found to be hypophosphatemic is tabulated in Table A and the chemical information in Table B of the Appendix. Information on 33 normophosphatemic members of the kindred is contained in Table C. A total of 11 individuals, 6 male and 5 female, had active rickets or post-rachitic deformities on clinical and radiographic grounds. In addition, histories highly suggestive of typical deformities were obtained on III-2, III-3 and III-11 who are dead. Equivocal or sometimes conflicting histories of deformities were obtained on II-1 and II-2.

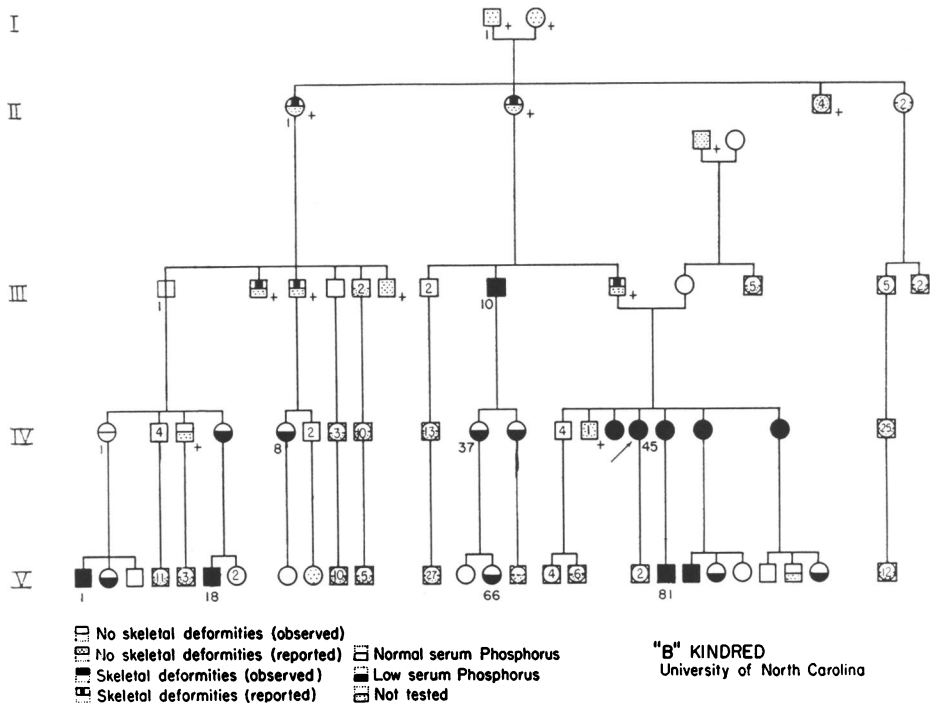

FIG. 3. The "B" kindred with sex-linked dominant hypophosphatemia.

All of the 11 persons with bone disease still living had serum inorganic phosphorus levels which were abnormally low, judged by our discriminant. In addition, 9 other persons (all women) closely related to those with bone lesions had unequivocal hypophosphatemia without clinical, radiographic, or historical evidence suggesting bone disease.

From the point of view of bone disease alone, penetrance is considerably reduced in this kindred. However, when the pedigree chart is scrutinized for hypophosphatemia, every hypophosphatemic person in generations IV and V is seen to have had either a hypophosphatemic parent or one with bone disease.

It is noteworthy that in this kindred, as in the one previously reported (13, 14), all 6 of the hypophosphatemic males had clinically recognizable rachitic bone disease, whereas only 5 of 14 hypophosphatemic females had recognizable skeletal abnormality (see Table A). It is of interest that all 5 of the hypophosphatemic women *with* bone disease were in the sibship of the proband, a female, and that their affected parent was the father. All had had moderately severe rickets as children (verified in 4 instances by examination of old roentgenograms) and were observed to have post-rachitic deformities as adults.

"H" Kindred

History of the Proband: The proband (IV-2, Figure 4) was a four year old white boy referred because of *genu varum* refractory to treatment with vitamin D. He

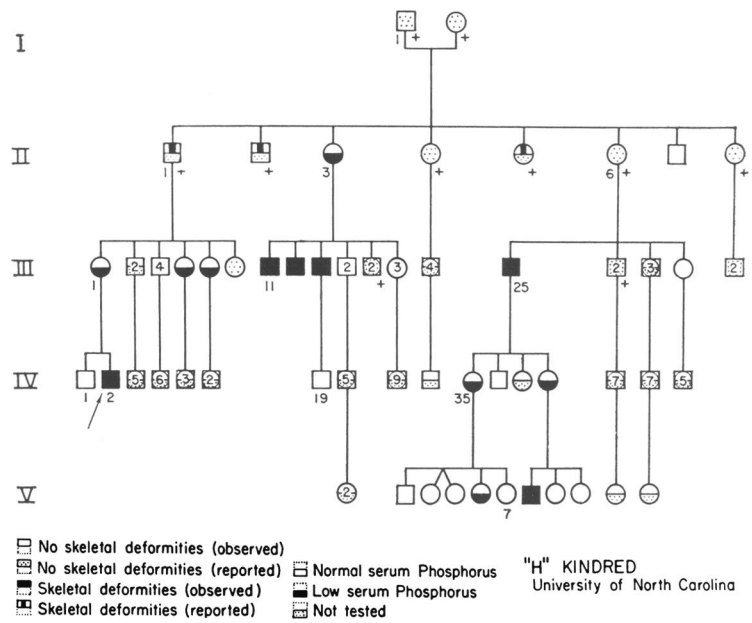

FIG. 4. The "H" kindred with sex-linked dominant hypophosphatemia.

was born after a normal, full term pregnancy and delivery, and his growth and development during early life had been entirely normal. His legs were noted to be bowed when he began walking at the age of 14 months. He had been given 5000 I.U. of vitamin D per day and liberal exposure to sunlight after the diagnosis of rickets had been made. At the age of three years his parents noted frontal enlargement of the head and a deformity of the sternum. The *genu varum* deformity progressed for 3 years despite heavy vitamin D therapy.

On physical examination at age 4 years, he weighed 37 pounds (50th percentile) and was 38¼ inches tall (<3rd percentile). Prominent frontal, parietal and occipital bossing of the head were present, but the teeth were normal. A prominent "pigeon breast" deformity of the chest was present along with Harrison's grooves and slight enlargement of the costochondral junctions. There was marked *genu varum* deformity and slight anterior bowing of both tibiae. The epiphyses were enlarged at the knees.

Radiographic studies revealed moderately severe rickets, particularly at the junctions of the lower femoral metaphyses and epiphyses, but only minimal evidence of rickets was observed at the wrists and ankles. The femoral shafts showed coarse trabeculation.

The hemogram and urinalysis were normal, and the Sulkowitch test was negative. Clinical data on the patient are shown in Table D while chemical determinations on his serum are shown in Table E. The only chemical abnormalities were the hypophosphatemia and the slightly elevated serum alkaline phosphatase.

Description of the Kindred: This patient and his kin are shown in Figure 4. Most of

the members of the family reside in Guilford and Alamance Counties, in the central Piedmont region of North Carolina, and are employed either in farming or industry. The pedigree chart shows 112 members of the kindred, 97 of whom were examined. On clinical grounds alone, 6 persons (all male) were diagnosed as having bone disease. Highly suggestive histories were obtained on three persons now dead (II-1, II-2 and II-5). The serum inorganic phosphorus was measured one or more times on 33 members of this kindred, 13 of whom proved to be hypophosphatemic. Clinical data on the 13 hypophosphatemic relatives are tabulated in Table D and blood chemical data in Table E. Data on 20 normophosphatemic relatives can be found in Table F.

As in the "B" kindred, all persons with clinical bone disease were hypophosphatemic. In the "H" kindred, however, *all* persons with clinical bone disease were males. There were 7 individuals, all female, found to be hypophosphatemic without skeletal deformities. As in the "B" family, penetrance for bone disease was incomplete, but every hypophosphatemic person was found to have a hypophosphatemic parent when this information was available.

"L" Kindred

History of the Proband: The proband (IV-1, Figure 5), a 37 year old white male, was referred because of a family history of "bowed legs". As far as could be determined, he had had a normal infancy. However, during early childhood, the *genu varum* deformity had been noted. This had progressed until adolescence when slight improvement had occurred. He had not received deliberate anti-rachitic prophylaxis or therapy, but had always been generously exposed to sunlight.

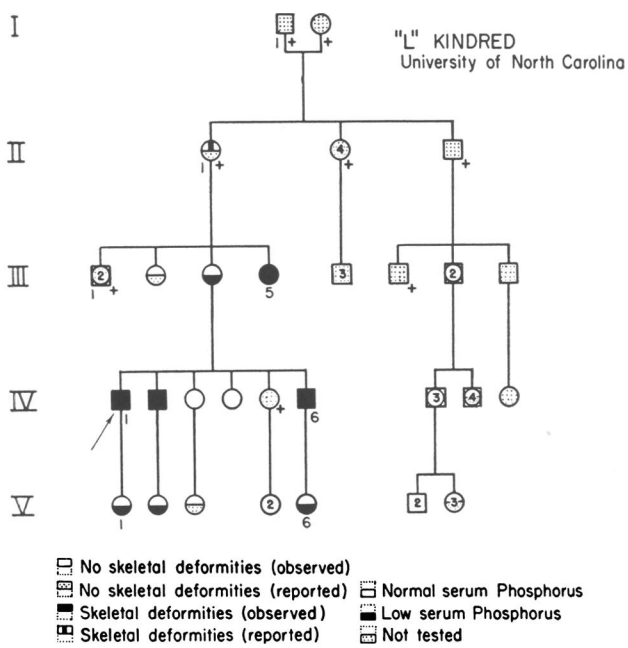

FIG. 5. The "L" kindred with sex-linked dominant hypophosphatemia.

Physical examination (Table G) revealed a short but well developed man, only 63 inches tall. There were no deformities of the head, chest or upper extremities. The teeth were markedly carious, and many were missing. The only discernible abnormality of the skeleton was a moderate degree of *genu varum.*

Urinalysis was normal. Hypophosphatemia was the only abnormality noted in his serum (Table H).

Description of the Kindred: Some information was obtained on 45 members of the kindred (Figure 5). Nearly all of these persons live in Edgecombe and Wilson Counties on the Coastal Plain of North Carolina and are farmers. Physical examination of 28 individuals in this family uncovered 3 additional cases of bone disease, 2 males and 1 female. Serum chemical studies of 19 individuals closely related to the proband showed 7 additional instances of hypophosphatemia. The 8 hypophosphatemics consisted of the 4 chemically abnormal persons, plus 4 females without clinical evidence of deformities. The clinical and chemical information on the members of the kindred are tabulated in Tables G, H and I. Once again it can be seen from the symbols on the pedigree chart that each hypophosphatemic child had a hypophosphatemic parent, but that there was "skipping" with respect to bone disease.

ANALYSIS OF THE FAMILY DATA

These three new kindreds add 15 affected males and 26 affected females to those previously reported. The amount of new information added by each of the new kindreds is not as great as by the original "E" kindred, because the new kindreds were not studied as exhaustively. It should be emphasized that the same excess of normal

TABLE 1. CLASSIFIED CHILDREN OF HYPOPHOSPHATEMIC PARENTS, SIBSHIPS COMPLETELY CLASSIFIED

Line No.	Kindred	Number of Sibships	Children						Total Children
			Males			Females			
			Affected	Normal	Total	Affected	Normal	Total	
			Affected Fathers						
1	"B"	0	0	0	0	2	0	2	2
2	"H"	1	0	1	1	0	0	0	1
3	"L"	3	0	0	0	3	0	3	3
4	Subtotal	4	0	1	1	5	0	5	6
5	"E"	5	0	9*	9	10*	0	10	19
6	Total	9	0	10	10	15	0	15	25
			Affected Mothers						
7	"B"	7	4	2	6	3	6	9	15
8	"H"	3	2	2	4	1	5	6	10
9	"L"	0	0	0	0	0	0	0	0
10	Subtotal	10	6	4	10	4	11	15	25
11	"E"	9	8	6	14	5†	9†	14	28
12	Total	19	14	10	24	9	20	29	53
Grand Totals		28	14	20	34	24	20	44	78

* The baby daughter of V-153 has been classified completing this sibship which was incomplete when "E" kindred was published. The sibship thus consists of three normal sons and two affected daughters in the birth order NNNAA.

† Number VI-69, "E" kindred originally classified as normal has been reclassified as hypophosphatemic.

TABLE 2. CLASSIFIED CHILDREN OF HYPOPHOSPHATEMIC PARENTS, *both* COMPLETELY CLASSIFIED AND INCOMPLETELY CLASSIFIED SIBSHIPS INCLUDED

Line No.	Kindred	Number of Sibships	Children						Total Children
			Males			Females			
			Affected	Normal	Total	Affected	Normal	Total	
			Affected Fathers						
1	"B"	2	0	4	4	4	0	4	8
2	"H"	2	0	2	2	2	0	2	4
3	"L"	3	0	0	0	3	0	3	3
4	Subtotal	7	0	6	6	9	0	9	15
5	"E"	7	0	10	10	12*	0	12	22
6	Total	14	0	16	16	21	0	21	37
			Affected Mothers						
7	"B"	8	4	3	7	4	6	10	17
8	"H"	4	5	4	9	1	8	9	18
9	"L"	1	3	0	3	0	2	2	5
10	Subtotal	13	12	7	19	5	16	21	40
11	"E"	9	8	6	14	5†	9†	14	28
12	Total	22	20	13	33	10	25	35	68
Grand Totals		36	20	29	49	31	25	56	105

* † See footnotes at bottom of Table 1.

males and affected females as in the "E" kindred (14) is also present in the new kindreds. In the analysis which follows, the new kindreds have been combined with the "E" kindred to give maximum numbers for the various tests.

Mode of Inheritance: The classified children of hypophosphatemic parents are tabulated in Tables 1 and 2. Table 1 includes only sibships in which all children were classified, while Table 2 includes classified children from incompletely studied sibships as well. It can be seen that the hypophosphatemic and normal sons and daughters of affected parents occur in an approximate 1:1:1:1 ratio in both tables. The ratio of affected males: normal males: affected females: normal females is 14:20:24:20 in completely studied sibships (Table 1) and 20:29:31:25 if the incompletely studied sibships are also included (Table 2).

The evidence that this undoubtedly dominant trait is not autosomal is seen in Line 6 of either Table 1 or Table 2. Affected fathers in all 4 kindreds produced only normal sons and affected daughters. The probability that this might have occurred by chance with an autosomal dominant is very slight. The χ^2 for Table 1 equals 25, and for Table 2 equals 37. (The exact probabilities are $(\frac{1}{2})^{25}$ and $(\frac{1}{2})^{37}$ respectively.) Such a distribution of children is, of course, expected with a sex-linked dominant. It should be emphasized that no instance of male-male transmission of hypophosphatemia was observed in any of the kindreds.

Penetrance: It was noted in the "E" kindred that while hypophosphatemia appeared to be fully penetrant, the *bone lesions* "skipped" in 4 of 7 instances, a penetrance rate of 43%. Five additional "chains" of three generations of hypophosphatemics were provided by the "B", "H" and "L" kindreds, giving a total of 12. In 9 of 12 such "chains" the intervening transmitter did not have evidence of bone disease, penetrance for bone disease thus appearing to be about 25%. If penetrance

for bone lesions was calculated separately by the sex of the "middle" parent, it would appear to have been 100% for males (2 in 2) and 10% for females (1 in 10).

Penetrance for hypophosphatemia was examined in two ways. The living parents of hypophosphatemic persons were examined, and one of them invariably proved to be hypophosphatemic. This suggested that hypophosphatemia is fully penetrant. When, however, the classified children of affected persons were tabulated, hypophosphatemia appeared highly but not completely penetrant. The observation suggesting some reduction in penetrance was a significant deficiency of affected and an excess of normal daughters (but not sons) among the progeny of hypophosphatemic women.

The discrepancy can be seen best in Line 12 of Tables 1 and 2. If hypophosphatemia is inherited as a fully penetrant sex-linked dominant, affected females should have affected and normal sons and daughters in a 1:1:1:1 ratio. But the hypophosphatemic mothers in Table 1 (all of whose children were studied) form the ratio, 14:10:9:20, and the larger group of hypophosphatemic mothers in Table 2 form the ratio 20:13:10:25. The heterogeneity χ^2 with 1 d.f. (sum of χ^2 for each sex separately minus total χ^2) for the completely studied sibships of Table 1 was 3.914, $P < 5\%$. For the larger group in Table 2, heterogeneity χ^2 was 6.43, significant at the 2% point.

The total male:total female ratios 24:29 (Line 12 Table 1) and 33:35 (Table 2) are clearly not significant deviations from unity. This suggests that the deficiency of affected females did not result from loss through decreased viability. The disturbance can be seen to have resulted largely from an abnormal ratio of affected:normal females. In the completely studied sibships of Table 1 the ratio of affected:normal females is 9:20 ($\chi^2 = 4.172$, $P < 5\%$) while with all classified females, Table 2, it is 10:25 ($\chi^2 = 6.971$, $P < 1\%$).

Looking further into reasons for the excess of normal daughters from affected mothers, the deficit appears to have been produced by misclassification of young girls. Figure 6 shows that the largest disproportion between normal and affected daughters occurs in the first decade. Table F and Figure 2 show four young daughters of affected mothers in the "H" kindred whose serum phosphorus values are low with 95% confidence but not 99% confidence. Transfer of this group from the normal into the hypophosphatemic category would abolish the discrepancy and make it appear that hypophosphatemia were fully penetrant.

Sex Difference in Expression of the Abnormal Gene: It was pointed out in connection with the "E" kindred that the abnormal gene had a more striking effect in males than in females. This sex difference was manifested in three ways: 1. By a larger *proportion* of hypophosphatemic males than females who showed clinical bone disease, 2. By bone lesions of *greater severity* in males with bone disease than females with bone disease, and 3. By slightly, but significantly, lower serum phosphorus values in hypophosphatemic males than hypophosphatemic females, the sex difference in phosphorus levels being greatest between boys and girls under 16 years.

The hypophosphatemic persons are listed in Table 3, by sexes and by presence or absence of rachitic bone disease. It is apparent that most hypophosphatemic males have some evidence of bone disease (28 in 30 = 93%) while only a minority of hypophosphatemic females do (12 in 48 = 25%).

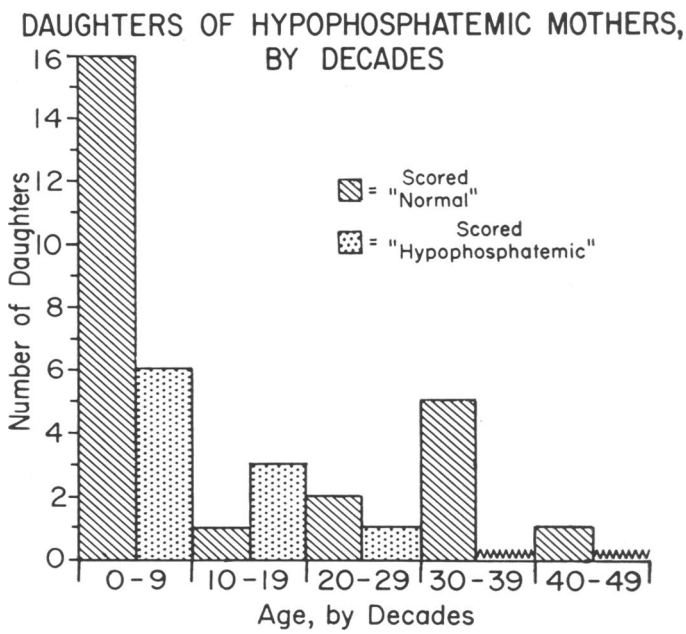

FIG. 6. A frequency distribution, by decades, of all normal and hypophosphatemic daughters of hypophosphatemic mothers from "E," "B," "H" and "L" kindreds.

The sex difference in severity of bone involvement is greater than the rough fourfold difference in thresholds suggested by the ratio 93:25. Males with bone lesions generally have severe, unequivocal disease while females scored as having bone disease rarely have even moderately severe lesions.

The third sex difference between the hypophosphatemic persons in the "E" kindred was a statistically significant difference between mean levels of serum phosphorus. The greatest mean difference was found between 6 boys and 5 girls and averaged 0.47 mg.%. The three new kindreds afford the opportunity of retesting this finding with larger groups. By pooling data on all hypophosphatemic children of the four kindreds, groups of 12 boys and 13 girls under 16 years of age can be compared. Their serum phosphorus values were adjusted to age 2, separately by sexes, using the normal regression curves of age on phosphorus published earlier (14). Means and standard errors of these adjusted values were computed and are shown in Table 4. It will be seen that the difference in means is 0.46 mg.%, essentially the same as for the "E" kindred alone. The standard errors are small, and the "t" test is highly significant. This test suggests that hypophosphatemic boys hemizygous for the abnormal gene have significantly lower serum phosphorus values than hypophosphatemic girls who are heterozygous.

A mathematically more complex, but possibly less sensitive, test of this sex difference was performed for us by Professor Bernard Greenberg. He used for his test portions of the regression curves fitted to the *abnormal* populations separately by

TABLE 3. HYPOPHOSPHATEMIC PERSONS WITH AND WITHOUT CLINICAL EVIDENCE OF BONE DISEASE, BY SEXES

Kindred	Males		Females		Total
	With bone disease	Without bone disease	With bone disease	Without bone disease	
"E"	13	2	6	16	37
"B"	6	0	5	9	20
"H"	6	0	0	7	13
"L"	3	0	1	4	8
Totals	28	2	12	36	78

TABLE 4. AGE ADJUSTED SERUM PHOSPHORUS VALUES OF 12 HYPOPHOSPHATEMIC BOYS AND 13 HYPOPHOSPHATEMIC GIRLS LESS THAN 16 YEARS OF AGE

Sex	Average Age	No.	d.f.	Serum Phosphorus, mg.%		
				Mean, mg.%	Std. error	Sum of squares
Boys	5.5 yrs.	12	11	2.92	.082	.8968
Girls	6.6 yrs.	13	12	3.38	.093	1.3572
Total		25	23	.46		2.2540

difference of means = .46 mg.%; t = 3.67, 23 d.f., $P < .01$

sexes and described elsewhere (14). The difference between the curves for affected boys and affected girls proved not to be statistically significant.

Linkage

The four kindreds were examined systematically for presence of colorblindness. Only one colorblind person was found, a woman who had recently married into the family. Although no tests were made for any of the rarer sex-linked conditions, there is no reason to think that any were segregating in the kindreds. For example, no "bleeders" or familial eye diseases were reported or encountered.

Environmental Factors Influencing Expression of Hypophosphatemia

In the report on the "E" kindred it was pointed out that the members of the family had no peculiar dietary habits which might lead to rickets. On the contrary, most of them were out-of-doors workers eating diets rich in dairy products. The dietary histories of the members of the "B", "H" and "L" kindreds were similar to those of the "E" kindred, and most of the persons had occupations which kept them out-of-doors much of the time.

Maternal age and parity did not appear to affect the expression of the abnormal gene in the original "E" kindred. An effect was sought in the combined kindreds by the method of Haldane and Smith (6). The raw birth rank data from the three new kindreds are listed in Table J. The data from the 15 completely studied sibships of the "E" kindred, plus 8 completely studied new sibships from the "B", "H" and "L" kindreds, plus 3 sibships in which the missing information concerned only first or last children (2 from "E", 1 from "H" kindred) are combined for the test shown in Table 5. The fact that expected and observed mean birth ranks (6A and M)

TABLE 5. BIRTH RANK TEST (HALDANE AND SMITH)

Sibship Size	Number of Sibships	Birth Rank of Affected Persons							Total Affected	Total Siblings
		1	2	3	4	5	6	7		
2	11	5	6						11	22
3	7	2	4	5					11	21
4	4	2	0	5	2				9	16
5	3	1	1	1	1	2			6	15
6	0	0	0	0	0	0	0		0	0
7	1	1	0	0	0	1	1	0	3	7
Totals	26	11	11	11	3	3	1	0	40	81

$6A = 594$, $M = 558$, $V = 1128$, $S = 33.59$

differ by less than two standard deviations (2S) means that there was not a significant birth rank effect in these 26 sibships.

DISCUSSION

The material contained in this paper confirms all but one of the conclusions reached in our study of the "E" kindred. The "B", "H" and "L" kindreds add 29 new sibships containing 15 affected males and 26 affected females to the literature. We have, therefore, diagnosed in North Carolina a total of 78 cases of familial hypophosphatemia in a population of 4.5 million persons. This does not represent complete ascertainment, because others have reported similar families (9). We suggest that sex-linked dominant familial hypophosphatemia should not be considered a rare and exotic entity. This concentration of cases in a limited geographical area raises the question whether the four kindreds are related. Three of the kindreds ("E", "B" and "L") live within a relatively small sector of the Coastal Plain of North Carolina. The fourth kindred ("H") is largely located in a community 100 miles to the west. No connection as recent as the last 6 generations has been established between any of the kindreds. Since the state was settled by emigration largely from the British Isles 200–250 years ago, however, it is quite possible that such a relationship exists.

In all three of the new kindreds, hypophosphatemia is inherited as a sex-linked dominant trait. Seven additional affected fathers had had 17 children, 15 of whom could be classified. These children consisted of 6 normal sons and 9 affected daughters. Thus, in our 4 kindreds, 14 affected fathers have produced 37 children, 16 normal sons and 21 affected daughters, without a single instance of father-to-son transmission of hypophosphatemia or a single normophosphatemic daughter. The possibility is very remote that this distribution is merely an abnormal sample from a population segregating for an autosomal dominant trait.

The characteristics of the homozygous state still remain unknown. We have not yet encountered a marriage of two affected persons, apparently because the rate of inbreeding has been low in the native white people of the Coastal Plain.

The new kindreds, like the original one, demonstrate that penetrance for hypophosphatemia is high in both sexes. A new observation, however, is that penetrance for hypophosphatemia is not *complete* in females (see below). Penetrance for overt bone disease is clearly high among males but low among females. Among mothers

with affected fathers and affected children, penetrance for the bone disease is only 10%. If hypophosphatemic females of all ages are considered, however, bone lesions can be detected in about 25%.

The three new kindreds showed again the sex difference with respect to severity of bone lesions. The bone lesions of the males in the "E", "H" and "L" kindreds were more severe than the bone lesions of the females. The "B" kindred is exceptional, because the proband was a woman, and the bone lesions in her and her sisters were definitely more severe than those of the hypophosphatemic women of the other kindreds. Reference to Table A, will emphasize this. All of these women, IV-44 through IV-48, had had osteotomies between ages 3 and 6. Furthermore, 3 of the 5 women had had at least one Caesarian section because of rachitic pelvic deformity. The lesions in these women appear to be as severe as those of the males in this and the other kindreds. Yet these women were not homozygous, because their mother was normophosphatemic as were some of their children.

Another finding in the "E" kindred had been a significantly lower serum inorganic phosphorus level in affected males than affected females. The greatest difference had been found between 6 affected boys and 5 affected girls and had amounted to 0.47 mg.%. The new kindreds provided 6 additional affected boys and 8 affected girls. When these were added to the previous examples, giving groups of 12 and 13, essentially the same mean difference was observed (0.46 mg.%), the males again being lower. The means of the two groups were found to be significantly different when tested in the manner previously described. When the sex difference was tested by comparing the curvilinear functions fitted separately by sexes to the abnormal populations, however, the difference was not significant. The last result may imply that there is no real sex difference for serum inorganic phosphorus among affected persons. On the other hand, it may mean that the complex functions fitted to the small abnormal populations had excessively large variances. If a real difference in serum phosphorus exists between hypophosphatemic boys and girls, it is slight. One is tempted to suggest that the accompanying normal allele in the heterozygous female accounts for the difference. How this might operate is not clear. We were unable, for example, in the "E" kindred to show any difference in phosphate re-absorption in the kidneys of affected males and females (14).

The last confirmation of our original findings was with respect to lack of an effect of maternal age or parity on expression of the gene. The Haldane and Smith test applied to 40 affected persons among a total of 81 in 26 sibships showed no effect of birth rank (Table 5).

A new and at first puzzling finding was the distribution of children of affected females (Line 12, Tables 1 and 2). There was a slight but not significant excess of affected sons and a significant deficiency of affected daughters. The reason for the discrepancy with the daughters can be clearly deduced from the operating characteristics of the discriminant devised for defining the normal limits for serum phosphorus (5). We pointed out in this connection that the discriminant is highly efficient in correctly categorizing the hypophosphatemics, both males and females, i.e. not more than 0.5% of the normals will be incorrectly diagnosed as abnormals. Furthermore, it was pointed out that while an *average* of not more than about 6% of abnormal

persons should be incorrectly diagnosed as normal, the rate of false negatives is expected to be higher among young females. It is among the young "normal" daughters, therefore, that most of the incorrectly classified persons are to be expected. We are of the opinion that this diagnostic error has occurred and explains the apparent excess of normal daughters from affected mothers. It is presumed that the four young girls of the "H" kindred whose phosphorus values are low with 95% but not 99% confidence will be scored as abnormal when they become older. We anticipate that their phosphorus values will slip below the confidence limit as its discriminatory power becomes greater (lower rate of false negatives at older ages).

It is of some interest that the presumed false negative diagnoses (lack of penetrance) involves daughters of affected mothers but not daughters of affected fathers. All 21 daughters of affected fathers who were studied were clearly abnormal. The low rate of false negatives in this circumstance may be related to the fact that 17 of the 21 daughters of affected fathers were more than 15 years of age. It is also possible that the study was unconsciously biased in another manner, i.e. that we examined the daughters of affected fathers more carefully because of their importance to the genetic argument.

Finally, it is interesting to note how an arbitrarily set serum phosphorus discriminant produces a reduction in penetrance among affected females. The phosphorus discriminant was deliberately set at the 99% point to minimize false positive diagnoses. This in turn increased the probable number of false negatives. The ratios can be shown to come into balance if the 95% confidence limit rather than the 99% confidence limit is used. It will be very interesting to follow the girls thought to have been incorrectly scored as normal to see whether their phosphorus values become clearly abnormal as they become older. If this does not occur and they can be shown to be genetically abnormal through their progeny, it may be wise to adopt the 95% confidence limit as the discriminant. It is for this reason that both limits will be published in our definitive paper on the normal range of serum phosphorus (5).

SUMMARY

1. Three additional kindreds of sex-linked dominant familial hypophosphatemia and vitamin D resistant rickets including 41 new patients are described.

2. It is shown that the new families conform in most respects to the previously described "E" kindred, i.e.

a) Hypophosphatemia appears highly penetrant. Only in young girls does there appear to be any reduction in penetrance.

b) Penetrance for bone lesions is clearly and greatly reduced in females.

c) In hypophosphatemic mothers, penetrance for bone lesions is about 10%. Some types of bone lesions can be found, however, in 25% of hypophosphatemic females of all ages.

d) Bone lesions are more severe in males than in females.

e) Hypophosphatemic girls appear to have a slightly higher level of serum inorganic phosphorus than hypophosphatemic boys.

f) Maternal age and parity appear *not* to affect expression of the gene.

3. The observation of an excess of apparently normal females from affected mothers

(incomplete penetrance for hypophosphatemia in females) is probably an error in falsely diagnosing genetically affected girls as normal. This error is an empirical confirmation of the expected higher rate of false negative diagnoses in young females than older females or males, an expectation previously arrived at on theoretical grounds.

REFERENCES

1. ALBRIGHT, F., BUTLER, A. M., AND BLOOMBERG, E. 1937. Rickets resistant to vitamin D therapy. *Am. J. Dis. Child.* 54: 529–547.
2. ALBRIGHT, F., CONSALAZIO, W. V., COOMBS, F. S., SULKOWITCH, H. W., AND TALBOTT, J. H. 1940. Metabolic studies and therapy in a case of nephrocalcinosis with rickets and dwarfism. *Bull. Johns Hopkins Hosp.* 66: 7–33.
3. FANCONI, G. 1936. Der frühinfantile nephrotische-glykosurische Zwergwuchs mit hypophosphatämischer Rachitis. *Jahrb. Kinderh.* 147: 299–338.
4. GERSTENBERGER, H. J. 1933. Rachitis hepatica. *Monatsschr. Kinderh.* 56: 217–226.
5. GREENBERG, B. G., WINTERS, R. W., AND GRAHAM, J. B. The normal range of serum inorganic phosphorus and its use as a discriminant in the diagnosis of congenital hypophosphatemia. *J. Clin. Endocr. and Metab.* (in press).
6. HALDANE, J. B. S., AND SMITH, C. A. B. 1948. A simple exact test for birth-order effect. *Ann. Eugen.* 14: 117–124.
7. LOWE, C. V., TERREY, M., AND MACLACHLAN, E. A. 1952. Organic aciduria, decreased renal ammonia production, hydrophthalmos, and mental retardation. A clinical entity. *Am. J. Dis. Child.* 83: 164–184.
8. MCCANCE, R. A. 1947. Osteomalacia with Looser's nodes (Milkman's Syndrome) due to a raised resistance to vitamin D acquired about the age of 15 years. *Q. J. Med.*, 16: 33–45.
9. MITCHELL, F. N., AND MITCHELL, J. E. 1957. Vitamin D-resistant rickets. *Am. J. Dis. Child.* 93: 385–390.
10. PARSONS, L. G. 1927. The bone changes occurring in renal and coeliac infantilism, and their relationship to rickets. Part I. Renal rickets. *Arch. Dis. Child.* 2: 1–25. Part II. Coeliac rickets. *Arch. Dis. Child.* 2: 198–211.
11. RATHBUN, J. C. 1948. 'Hypophosphatasia', a new developmental anomaly. *Am. J. Dis. Child* 75: 822–831.
12. SWANN, G. F. 1954. Pathogenesis of bone lesions in neurofibromatosis. *Brit. J. Radiol.* 27: 623–629.
13. WINTERS, R. W., GRAHAM, J. B., WILLIAMS, T. F., MCFALLS, V. W., AND BURNETT, C. H. 1957. A genetic study of familial hypophosphatemia and vitamin D resistant rickets. *Tr. Ass. Am. Physicians* 70: 234–242.
14. WINTERS, R. W., GRAHAM, J. B., WILLIAMS, T. F., MCFALLS, V. W., AND BURNETT, C. H. 1958. A genetic study of familial hypophosphatemia and vitamin D resistant rickets with a review of the literature. *Medicine* 37: 97–142.
15. WINTERS, R. W., MCFALLS, V. W., AND GRAHAM, J. B. 1960. A girl with sporadic hypophosphatemia and vitamin D resistant rickets. *Pediatrics* (in press).

APPENDIX

TABLE A. CLINICAL DATA ON HYPOPHOSPHATEMIC MEMBERS OF "B" KINDRED

Generation and Number	Age When Studied	Sex	History	Height[1]	Physical and Radiologic Findings[2]
			Children		
V-1	14	M (Male)	+	25	Moderately severe rickets during early childhood, successful osteotomy at age 6 years, slight residual *genu varum* deformity.
V-18	4	M	+	10	Active rickets of moderate severity with slight *genu varum*.
V-81	3	M	+	<3	Severe active rickets, partly treated; moderate *genu varum*.
V-82	11	M	+	<3	Active rickets of moderate severity with moderate *genu varum*.
V-2	11	F (Female)	−	10	Negative
V-66	9 mos.	F	−	25	Negative
V-83	12	F	−	50	Negative
V-87	3	F	−	25	Negative
			Adults		
III-1	60	M	+	62	Severe *genu varum*; moderate anterior bowing of tibiae. Degenerative joint disease at knees.
III-10	56	M	+	63	Moderate *genu varum*; degenerative joint disease of knees.
IV-1	37	F	−	59	Negative
IV-7	26	F	−	58	Negative
IV-8	24	F	−	59	Negative
IV-37	26	F	−	63	Negative
IV-38	31	F	−	60	Negative
IV-44	18	F	+	61	Moderately severe deformities during childhood; successful corrective osteotomy at age 6 years. Slight residual *genu varum* deformity.
IV-45 (Proband)	25	F	+	57	Moderately severe rickets during childhood;[3] successful corrective osteotomy at age 5 years. Moderate residual *genu varum* deformity. Caesarian section both pregnancies.
IV-46	27	F	+	58	Active rickets of moderate severity during childhood;[3] successful osteotomy at age 3 years. Slight residual *genu varum* deformity. Caesarian section one time.
IV-47	34	F	+	59	Active rickets of moderate severity during childhood,[3] successful osteotomy at age 4 years. Slight residual *genu varum* deformity. Caesarian section three times.
IV-48	30	F	+	58	Active rickets of moderate severity during childhood;[3] successful osteotomy at age 3 years. Slight residual *genu varum* deformity.

[1] Height of children in percentiles; height of adults in inches.

[2] Based upon physical examination and x-rays of long bones.

[3] Verified by old roentgenograms taken at the time of osteotomies.

TABLE B. BLOOD CHEMICAL DATA ON HYPOPHOSPHATEMIC MEMBERS OF "B" KINDRED*

Generation and Number	Age When Studied	Year of Birth	Sex	Ca^{++} mg %	T.P. gm %	P mg %	Alkaline Phosphatase B.U.	Na^{+} mEq/L	K^{+} mEq/L	Cl^{-} mEq/L	BUN mg %
					Children						
V-1	14	1943	M (Male)	9.1	6.8	2.8	17.4	137	5.0	103	9
V-18	4	1953	M	10.5	6.5	2.4	16.5	—	—	—	—
V-81	3	1955	M	10.3	6.7	3.1	11.1	136	5.4	102	10
V-82	11	1946	M	9.5	7.0	2.4	11.3	141	3.8	103	16
V-2	11	1946	F (Female)	9.8	6.3	3.0	14.1	—	—	—	—
V-66	9 mos.	1957	F	—	—	3.2	17.3	—	—	—	—
V-83	12	1945	F	9.3	6.7	3.0	5.7	139	4.2	106	13
V-87	3	1955	F	—	—	3.2	—	—	—	—	—
					Adults						
III-1	60	1897	M	9.0	6.8	2.0	4.4	139	4.3	103	14
III-10	56	1901	M	9.3	7.1	2.1	5.1	141	3.8	103	11
IV-1	37	1920	F	9.8	6.6	1.8	3.9	138	4.2	103	8
IV-7	26	1931	F	9.2	6.5	2.4	4.4	140	3.9	105	10
IV-8	24	1933	F	9.2	7.1	2.2	5.3	142	4.4	106	11
IV-37	26	1931	F	9.2	7.0	2.0	5.7	139	4.0	105	16
IV-38	31	1927	F	—	—	1.9	—	—	—	—	—
IV-44	18	1939	F	9.8	7.4	2.7	4.3	137	4.6	104	12
IV-45 (Proband)	25	1932	F	9.0	7.1	2.6	4.4	138	3.9	101	11
IV-46	27	1930	F	9.5	6.9	2.4	3.6	140	4.3	104	11
IV-47	34	1923	F	9.8	6.8	2.0	5.1	140	4.5	103	10
IV-48	30	1928	F	9.9	7.0	2.4	—	142	4.3	105	12

* T.P. = total protein; P = serum inorganic phosphorus; BUN = blood urea nitrogen.

TABLE C. SERUM PHOSPHORUS LEVELS OF NORMAL MEMBERS OF "B" KINDRED*

Generation and Number	Sex	Age When Studied	Year of Birth	P (mg %)
		Children		
IV-42	M (Male)	7	1950	4.1
V-69	M	4	1953	5.3
V-70	M	10	1947	4.8
V-71	M	8	1949	4.6
V-79	M	8	1949	4.3
V-85	M	9	1948	4.6
V-19	F (Female)	4	1953	5.5
V-20	F	5	1952	5.2
V-21	F	1	1956	5.4
V-65	F	6	1951	4.6
V-72	F	11	1946	5.3
V-80	F	8	1949	4.3

TABLE C (*Continued*)

Generation and Number	Sex	Age When Studied	Year of Birth	P (mg%)
		Adults		
III-4	M	66	1891	3.1
III-8	M	64	1892	2.9
III-9	M	60	1897	2.9
III-18	M	56	1901	2.8
III-19	M	57	1900	4.0
III-20	M	42	1915	2.7
III-21	M	49	1908	3.5
IV-2	M	35	1922	3.3
IV-3	M	33	1924	3.9
IV-4	M	28	1929	4.1
IV-5	M	25	1932	3.7
IV-9	M	27	1930	2.9
IV-10	M	22	1935	4.2
IV-39	M	38	1919	4.3
IV-40	M	36	1921	3.4
IV-41	M	29	1928	4.0
V-3	M	17	1940	3.5
II-4	F	74	1883	4.0
III-12	F	57	1900	4.0
III-22	F	53	1904	4.0
V-84	F	16	1941	3.5

* P = serum inorganic phosphorus.

TABLE D. CLINICAL DATA ON HYPOPHOSPHATEMIC MEMBERS OF "H" KINDRED

Generation and Number	Age When Studied	Year of Birth	Sex	History	Height[1]	Physical Findings[2]
			Children			
IV-2 (Proband)	4	1954	M (Male)	+	<3	Moderately severe active rickets with *genu varum*; slight anterior gowing of tibiae; pigeon breast.
V-8	6	1951	M	+	25	Moderate *genu varum*
V-6	5	1952	F (Female)	−	25	Negative
			Adults			
III-11	48	1909	M	+	60	Marked *genu varum*
III-12	41	1916	M	+	60	Moderate *genu varum*
III-13	33	1924	M	+	62	Moderate *genu varum*
III-25	50	1907	M	+	59	Moderate *genu varum*
II-3	67	1890	F	−	62	Negative
III-1	32	1925	F	−	63	Negative
III-8	24	1933	F	−	60	Negative
III-9	22	1935	F	−	61	Negative
IV-35	26	1931	F	−	61	Negative
IV-38	21	1936	F	−	59	Negative

[1] Height of children as percentile; height of adults in inches.

[2] Based on physical examination.

TABLE E. BLOOD CHEMICAL DATA ON HYPOPHOSPHATEMIC MEMBERS OF "H" KINDRED*

Generation and Number	Age When Studied	Year of Birth	Sex	Ca^{++} mg %	T.P. gm %	P mg %	Alkaline Phosphatase B.U.	Na^{+} mEq/L	K^{+} mEq/L	Cl^{-} mEq/L	BUN mg %
Children											
IV-2 (Proband)	3	1954	M (Male)	9.7	6.1	2.7	12.9	138	4.2	102	9
V-8	6	1951	M	11.1	7.4	2.9	16.8	139	3.8	105	14
V-6	5	1952	F (Female)	9.1	6.8	3.0	9.4	141	3.9	106	—
Adults											
III-11	48	1909	M	10.9	6.9	2.2	2.4	141	4.1	105	—
III-12	41	1916	M	11.0	7.0	2.2	3.1	141	4.0	100	—
III-13	33	1924	M	10.1	6.9	2.4	4.4	142	3.8	108	19
III-25	50	1907	M	10.7	6.6	2.2	2.3	144	4.8	107	17
II-3	67	1890	F	10.9	7.2	2.6	4.5	142	4.3	104	—
III-1	32	1925	F	—	—	2.4	2.8	—	—	—	—
III-8	24	1933	F	—	—	2.4	—	—	—	—	—
III-9	22	1935	F	—	—	2.3	—	—	—	—	—
IV-35	26	1931	F	11.6	8.4	2.5	2.3	137	4.7	103	17
IV-38	21	1936	F	10.8	7.0	2.7	4.1	141	3.7	107	6

* T.P. = total protein; P = serum inorganic phosphorus; BUN = blood urea nitrogen.

TABLE F. BLOOD CHEMICAL DATA ON NORMAL MEMBERS "H" KINDRED[1]

Generation and Number	Sex	Age When Studied	Year of Birth	P (mg %)
		Children		
IV-1	M (Male)	7	1950	5.0
IV-19	M	6	1951	4.7
IV-36	M	9	1948	4.5
V-3	M	8	1949	4.3
V-4	F (Female)	6* (7)†	1952	4.3* (3.7)†
V-5	F	6* (7)†	1952	4.4* (3.7)†
V-7	F	2* (3)†	1956	5.5* (3.8)†
V-9	F	4* (5)†	1953	5.2* (3.9)†
V-10	F	2†	1957	5.2†
		Adults		
II-7	M	64	1893	4.4
III-4	M	30	1927	4.0
III-5	M	28	1929	4.3
III-6	M	26	1930	4.1
III-7	M	22	1935	4.2

TABLE F (*Continued*)

Generation and Number	Sex	Age When Studied	Year of Birth	P (mg %)
		Adults		
III-14	M	45	1911	3.4
III-15	M	24	1933	3.8
III-18	F	43	1914	3.8
III-19	F	36	1921	3.4
III-20	F	31	1926	3.7
III-31	F	64	1893	4.5

* Determinations in 1958, not fasting. Clearly normal.

† Determinations in 1959, fasting. The values within parentheses are significantly low at the 95% point, but not at 99% point.

[1] *P* = serum inorganic phosphorus.

TABLE G. CLINICAL DATA ON HYPOPHOSPHATEMIC MEMBERS OF "L" KINDRED

Generation and Number	Age When Studied	Sex	History	Height[1]	Physical Findings[2]
			Children		
V-2	15	F (Female)	–	59	Negative
V-6	4	F	–	25	Negative
			Adults		
IV-1 (Proband)	37	M (Male)	+	63	Moderate *genu varum*
IV-2	35	M	+	62	Moderate *genu varum*
IV-6	23	M	+	64	Moderate *genu varum*
III-4	64	F	–	63	Negative
III-5	51	F	+	60	Moderate *genu varum*
V-1	16	F	–	61	Negative

[1] Height of children in percentiles. Height of adults in inches.

[2] Based on physical examination.

TABLE H. BLOOD CHEMICAL DATA ON HYPOPHOSPHATEMIC MEMBERS OF "L" KINDRED*

Generation and Number	Age When Studied	Year of Birth	Sex	Ca^{++} (mg %)	T.P. (gm %)	P (mg %)
			Children			
V-2	15	1942	F (Female)	—	—	2.8
V-6	4	1952	F	—	—	3.1
			Adults			
IV-1 (Proband)	37	1919	M (Male)	9.8	6.9	2.4
IV-2	35	1921	M	9.6	6.7	2.3
IV-6	23	1933	M	10.3	7.0	2.5
III-4	64	1891	F	—	—	1.7
III-5	51	1905	F	—	—	2.5
V-1	16	1939	F	—	—	2.6

* T.P. = total protein; P = serum inorganic phosphorus.

TABLE I. BLOOD CHEMICAL DATA ON NORMAL MEMBERS OF "L" KINDRED*

Generation and Number	Sex	Age When Studied	Year of Birth	P (mg %)
		Children		
IV-9	M (Male)	9	1946	3.4
V-7	M	12	1944	3.8
V-8	M	10	1946	4.4
IV-8	F (Female)	15	1941	4.0
V-4	F	7	1948	4.8
V-5	F	5	1951	4.9
		Adults		
III-11	M	46	1909	4.4
III-10	F	51	1904	4.5
IV-3	F	31	1925	3.8
IV-4	F	29	1927	4.5
IV-7	F	31	1924	3.4

* P = serum inorganic phosphorus.

TABLE J. BIRTH ORDER OF CLASSIFIED CHILDREN OF HYPOPHOSPHATEMIC PARENTS, OF MEMBERS OF SIBSHIPS SEGREGATING FOR HYPOPHOSPHATEMIA OR BOTH

"B" Kindred			"H" Kindred			"L" Kindred		
Sib-ship size	Affected parent	Birth order*	Sib-ship size	Affected parent	Birth order*	Sib-ship size	Affected parent	Birth order*
Phenotype of Parent Established—*All* Progeny Classified								
1	Mother	N	1	Father	N	1	Father	A
1	Mother	A	2	Mother	NA	1	Father	A
2	Mother	NN	3	Mother	ANN	1	Father	A
2	Mother	NA	5	Mother	N(NN)AN			
3	Mother	NAA						
3	Mother	NNA						
3	Mother	AAN						
2	Father	AA						
Phenotype of Parent Established—*Not All* Progeny Classified								
2	Mother	--	4	Father	AAN-	6	Mother	AANN-A
3	Mother	N-A	10	Mother	ANNA-NAN-N			
7	Father	ANN-NAN						
Phenotype of Parent *Not* Established—*All* Progeny Classified								
3	—	NAN						
Phenotype of Parent *Not* Established—*Not All* Progeny Classified								
4	—	----	7	—	A--N---	5	—	A--A-
7	—	-------	8	—	---A--N-			
10	—	NNAANAA-AN	10	—	AAN-NAN-N-			

* A = affected, i.e. hypophosphatemic; N = normophosphatemic.