STUDIES IN RICKETS IN THE CAPE PENINSULA

IV. BIOCHEMISTRY AND RADIOGRAPHY

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Biochemical criteria for the diagnosis of rickets include alterations in the levels of serum inorganic phosphorus, alkaline phosphatase, and calcium. Elevated phosphatase levels have been said to be the most constant change.[1-5] More recently serum-citrate levels have been estimated and low values have been found.[6-8] These biochemical parameters have been correlated with radiological changes to assess their diagnostic value as indices of activity.

MATERIAL AND METHODS

Serial biochemical studies were made on 54 non-White children with active 'ordinary' vitamin-lack rickets. Serum alkaline phosphatase (Bodansky-Shinowara), inorganic phosphate (King), calcium (Greenblatt and Hartman) and citrate (Ettinger, Goldbaum and Smith) levels were estimated, in most cases at monthly intervals.

The rickets was classified as severe, moderate or mild according to the radiographic changes at the wrist. In severe cases both radius and ulna were involved, the osteoid extending for a centimetre or more. The ends of the bones were widely 'splayed', the shafts grossly decalcified, and the phalanges also obviously involved (Fig. 1A). In mild rickets the bony changes were usually limited to definitely irregular, poorly defined and rather woolly contours of the distal ulna or radius — in some cases only one bone was involved. A distinct osteoid region was not always seen. Cases of moderate severity had changes which fell between these two extremes. X-rays were usually repeated at monthly intervals, and bone age was estimated from X-rays of the hand and wrist, after Greulich and Pyle.[9] These

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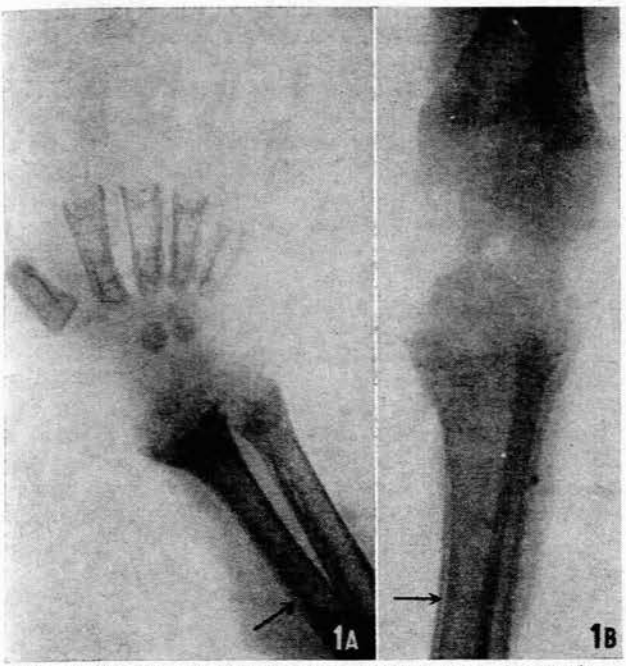

Fig. 1. X-rays of patient D.N., aged 1 year 8 months, showing gross rickets with double outline of the cortex (see arrows).
A. Wrist bones.
B. Upper end of tibia and fibula.

X-rays were also examined for evidence of secondary hyperparathyroidism.

RESULTS

Serum Chemistry in Active Rickets (Tables I and II)

Serum-phosphorus levels below 4·5 mg. per 100 ml. were present in 31 out of 45 cases, and were more frequently subnormal in severe cases.

TABLE I. SERUM-BIOCHEMISTRY FINDINGS IN CHILDREN WITH ACTIVE RICKETS*

Radiological assessment	*Calcium*		*Phosphorus*		*Alkaline phosphatase*	
	Normal	*Abnormal***	*Normal*	*Abnormal***	*Normal*	*Abnormal***
Severe ..	7	12	3	13	0	17
Moderate ..	9	9	7	10	4	16
Mild ..	7	6	4	8	5	12
Total ..	23	27 (54%)	14	31 (69%)	9	45 (83%)

* Figures represent number of children in each category.
** Abnormal levels:
Calcium below 9 mg. per 100 ml.
Phosphorus below 4·5 mg. per 100 ml.
Alkaline phosphatase above 20 Shinowara-Bodansky units.

Alkaline-phosphatase levels were markedly elevated (above 20 Shinowara-Bodansky units) in 45 out of 54 patients with active rickets. All patients with severe rickets had elevated levels.

Serum-calcium levels were below 9 mg. per 100 ml. in 27 children with active rickets, and above 9 mg. in 23. They were more frequently subnormal in children with severe rickets.

Citrate. The mean level of serum citrate was slightly lower in 21 children with active rickets (3·5 mg. per 100 ml.) than in 23 control children (mean 3·7 mg. per 100 ml.).

TABLE II. SERUM-CITRATE LEVELS IN VARIOUS GROUPS OF CHILDREN (IN MG. PER 100 ML.)

Normal children	*Children with*		
(3 mths.-3 yrs.)	*Active rickets*	*Healing rickets*	*Healed rickets*
6·4	5·4	6·8	3·2
6·0	5·2	5·8	3·0
5·3	5·2	4·7	2·5
4·7	5·0	2·9	2·4
4·7	5·0	2·8	2·1
4·6	4·6	2·8	
4·6	4·5	2·7	
4·5	4·2	2·3	
4·2	3·8	2·1	
3·9	3·7	1·6	
3·8	3·2	1·5	
3·6	3·2	1·3	
3·5	2·9		
3·3	2·7		
3·1	2·7		
3·1	2·3		
3·0	2·3		
2·6	2·2		
2·5	2·1		
2·4	2·1		
2·2	1·6		
2·1			
1·9			
Mean 3·7	3·5	3·1	2·6

There was a large overlap in the 2 groups (Table II) and the differences are not significant.

Serial Biochemical Estimations as Indices of Healing

1. *Serum phosphorus.* Progress was observed in 22 patients with rickets which healed radiologically and in whom the original serum-phosphorus level had been below 4·5 mg. per 100 ml. In 17 of these patients the serum-phosphorus level rose as radiological healing progressed. In most patients normal levels were achieved long before radiological healing was complete. In the remaining 5 patients the serum-phosphorus level did not rise, and in 2 it remained subnormal even after complete radiological healing.

Three patients with active rickets in whom initial serum-phosphorus levels were above 4·5 mg. per 100 ml. were followed-up, and in all of these the phosphorus levels rose, as healing progressed, to 6·2, 8·4 and 9·4 mg. per 100 ml. There was only 1 instance of a significant *drop* in the serum-phosphorus level in the presence of healing. (The serum-phosphorus level in this case dropped from 4·5 mg. per 100 ml. to 4·0 and later to 2·4 mg. per 100 ml. in the presence of good radiological healing. When healing was complete radiologically the level was 4·2 mg. per 100 ml.)

No radiological healing occurred in 9 patients with rickets in whom serum-phosphorus levels were below 4·5 mg. per 100 ml. In 7 of these the serum-phosphorus level did not change, but in 2 it rose to normal despite lack of bone healing.

2. *Alkaline phosphatase.* Radiological healing occurred in 27 patients with active rickets in whom serum-alkaline-phosphatase levels were initially high. In 15 of these the serum level fell; in 8 it remained unchanged; and in 4 it rose further. Abnormally high levels were still present in 6 out of 13 patients in whom healing was considered complete radiologically.

In 11 patients the rickets either regressed or was unchanged radiologically. In 8 of these the alkaline-phosphatase level rose further, and in 3 it remained the same. In no case did it fall.

3. *Serum calcium.* In 3 out of 16 patients the serum-calcium level remained persistently low, even in the presence of complete radiological healing. In the remainder it returned to normal as healing occurred. Six patients who showed no radiological improvement had low serum-calcium levels initially; in 3 of these the serum-calcium level returned to normal despite an absence of radiological healing.

The calcium × phosphorus product was calculated in 33 children with active rickets. In 5 it was *above* 40 (60, 55, 54, 48, and 46); in 12 it was between 30 and 40; in 13 it was between 20 and 30; and in 3 it was below 20. In 4 out of 17 patients showing radiological healing this product remained *below* 40, and in 2 of these it continued to remain below 40 even when healing was complete. In 8 out of 10 patients who did not improve radiologically, the calcium × phosphorus product remained below 40.

4. *Serum citrate (Table II).* Twelve children with healing rickets had a mean serum-citrate level of 3·1 mg. per 100 ml. (range 1·3 - 6·8 mg. per 100 ml.). The mean level in 21 children with active rickets was 3·5 mg. per 100 ml., and in 5 children in whom the rickets had healed it was 2·6 mg. per 100 ml. (range 2·1 - 3·2). The differences in the mean levels are not significant. In 7 individual patients whose progress was followed, the serum-citrate level became lower in 5, and remained unaltered in 2 as healing progressed.

Radiography

Bone age was found to correspond with chronological age in 17 consecutive unselected normal Coloured and Bantu children between 3 and 24 months. In 48 children with active rickets, the bone age was retarded in 18, and corresponded to the chronological age in 30. Eight children with retarded bone age had severe rickets, in 5 the rickets was moderate, and in 5 mild. Of the 30 children with normal bone age, rickets was severe in 6, moderate in 13 and mild in 11. A bone age which was grossly retarded during active rickets often became normal rapidly as the carpal bones calcified following therapy (Fig. 2).

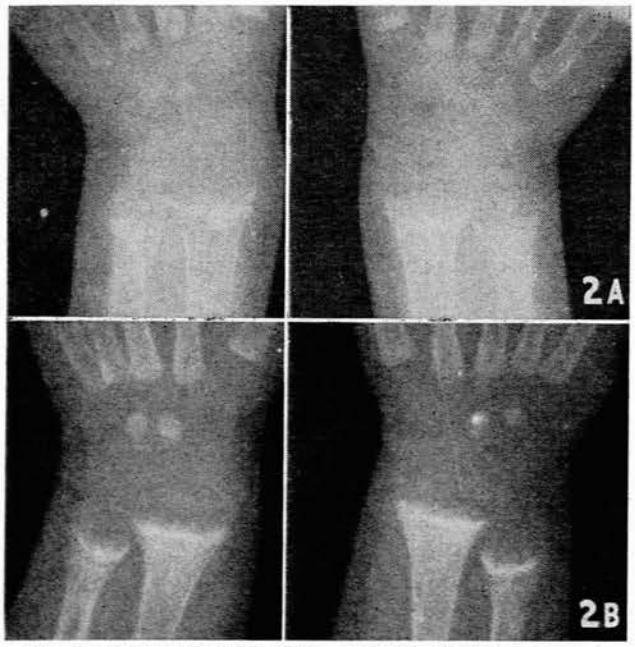

Fig. 2. X-rays of patient V.L., aged 8 months.
A. Wrist bones on 1 October 1959 — retarded bone age.
B. Wrist bones on 27 October 1959 — calcification appearing in 2 carpal bones.

No areas of subperiosteal erosion, suggesting hyperparathyroidism, were noted in the hands of any rachitic child. Generalized decalcification was often considerable — in one case it was so severe that it was impossible to obtain an adequate spinal radiograph.

Severity of demineralization did not affect the healing potential in individual patients. A frequent, and in some cases striking, sign in active rickets was the presence of a thin line of subperiosteal calcification (sometimes mistaken for scurvy). It was notable that this 'double cortical line' on the radiograph did not occur only with healing (Fig. 1A and B).

DISCUSSION

An abnormal serum-alkaline-phosphatase level was the most constant biochemical abnormality in active rickets. Abnormalities in serum calcium, phosphorus and alkaline phosphatase were more often present in the severe than in the mild cases.

In comparing radiological and biochemical changes as an index of progress, alterations in the serum-phosphorus level seemed to be the most reliable biochemical guide. The serum-phosphorus level rose in 75% of cases that healed, and, conversely, low levels persisted in 75% of cases which continued to be active radiologically. It was rare for the serum-phosphorus level to become further depressed in the presence of radiological improvement. Alteration in the serum-alkaline-phosphatase level was not such a reliable index of progress. A falling serum level indicated healing, but raised levels were found during both healing and regression.

Radiological assessment of healing did not always coincide with biochemical assessment. There were 2 patients in whom radiological healing appeared complete, yet both the serum-calcium and -phosphorus levels remained subnormal. In a further 6 patients alkaline-phosphatase levels remained high despite complete radiological healing (high levels have been reported for as long as 1 year after radiological healing[2]). Histological examination of bone might have revealed activity in these patients. In some of them the serum levels returned to normal later.

A calcium × phosphorus product of 40 mg. per 100 ml. or more has been said to exclude rickets,[10] except perhaps in premature infants[11] and uraemia.[12] When this product was below 30, rickets was always present.[10] Between 30 and 40, rickets was usually present.[10] In the present series 5 children with active rickets had a calcium × phosphorus product of above 40. Four children showed good radiological healing with a calcium × phosphorus product below 40 and in 2 of these healing became complete with the product still below 40. Thomas and his co-workers[13] induced *in vitro* calcification in rachitic rat-cartilage matrix by bathing cartilage slices in artificial media in which the

calcium level was only 4·5 mg. per 100 ml., provided calcification had previously been initiated by exposure to higher concentrations. It is possible that the patients in whom rickets healed with the calcium × phosphorus products below 40 may previously have had higher concentrations which had initiated calcification.

The ability of citrate mixtures to heal rickets has been demonstrated in both man and experimental animals.[14,15] It is therefore not unexpected that depressed serum levels have been found in active rickets, returning to normal with healing. However, in agreement with Ochse,[16] we were unable to demonstrate any significant difference between the mean citrate levels of control children, those with active rickets, and those with healing rickets.

The appearance of the carpal centres was delayed in many of the rickety children, but was 'normal' (by American standards) in the controls. This has been observed previously.[17,18] Retardation of bone age was commoner in severe rickets. In some patients it may have been apparent rather than true retardation, caused by decalcification of carpal bones as part of the rickety process. This was suggested by the rapid return to normal following therapy in these patients. In some children, however, the bone age remained retarded despite advanced healing.

Secondary hyperparathyroid bone changes have rarely been demonstrated in active vitamin-deficiency rickets.[19-21] Parathyroid overactivity may account for the normal serum-calcium levels which are frequently found in active cases. However, we did not see subperiosteal resorption in hand bones.

SUMMARY

Fifty-four non-White children with rickets were studied by means of serial serum biochemical estimations and serial radiographs, both during the active phase and following treatment. A raised serum-alkaline-phosphatase level was found to be the best chemical index of activity. All children with severe rickets had raised levels, but in 9 children with mild rickets (20%), the serum-alkaline-phosphatase level was normal. A progressively falling level indicated healing, but a rising serum level occurred with both healing and regression.

The serum-phosphorus level was depressed in nearly all children with severe rickets, but was normal in over a third of children with mild rickets. In three-quarters of the patients it was a reliable index of progress — i.e. rising with healing but remaining low with continued activity.

The serum-calcium level was depressed in half the children with active rickets. In some children the calcium × phosphorus product remained below 40 despite radiological evidence of good healing. Serum-citrate levels were normal in active rickets.

Retarded bone age was present in 18 out of 48 children with active rickets. No evidence of secondary hyperparathyroidism was seen in radiographs of the hands.

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APPENDIX

Biochemical methods used were those reported previously by us in part I of this series [this *Journal* 10 September 1960 (**34,** 776)]; in addition the method for sodium citrate was that reported in the following reference: Ettinger, R. H., Goldbaum, L. R. and Smith, L. H. (1952): J. Biol. Chem., **199,** 531.