

Osteoporosis in Johannesburg Bantu Males

Its Relationship to Siderosis and Ascorbic Acid Deficiency^{1, 2}

S. R. LYNCH, M.B., B.CH.,³ I. BERELOWITZ, M.B., B.CH., D.M.R.D.,⁴
H. C. SEFTTEL, B.SC.,⁵ DIP. MED., G. B. MILLER, M.B., B.CH.,⁵ P. KRAWITZ, B.SC.,⁶
R. W. CHARLTON, M.D., B.SC., M.R.C.P.E.,⁷ AND
T. H. BOTHWELL, M.D., D.SC., M.R.C.P.⁸

AN UNUSUAL FORM of osteoporosis occurs in Johannesburg Bantu subjects. The condition is seen predominantly in male manual laborers between the ages of 40 and 60 years, and pain from vertebral collapse is the common presenting symptom (1, 2). On radiological, pathological, and biochemical grounds the disease has been shown to be osteoporosis and not osteomalacia or other rarer forms of bone disease (2). Osteoporosis also occurs in white subjects living in Johannesburg, but it is the common variety seen most frequently in females over the age of 60 years.

In searching for the cause of the osteoporosis which is found in middle-aged Bantu males, two associated conditions have been identified. Firstly, scurvy is much more common in osteoporotic individuals than in the general hospital population. In one study the incidence was found to vary with the season; in late winter and early spring it was 71%, while

during the remainder of the year it was 22% (2). In addition, the results of another investigation revealed that 19% of patients presenting with classical acute scurvy exhibited marked osteoporosis (1). The second associated condition is severe siderosis. Iron overload of varying degree is extremely common among adult Bantu subjects in Johannesburg (3, 4). The iron accumulates in the body stores as a consequence of prolonged exposure to a diet with a very high iron content (3). The major source of the excessive dietary iron is the traditional home-brewed alcoholic beverages (3, 5). These are prepared and stored in rusted metal containers, and inorganic iron is leached off the walls by the acid mixtures. Most of the drinks have a low alcohol content, and large volumes are consumed. Many Bantu ingest between 50 and 100 mg iron/day (5). The majority of adult Bantu males drink these beverages and therefore have iron stores that are increased to varying degrees. However, subjects with osteoporosis have almost invariably exhibited iron overload in its severest form (1, 2).

Osteoporosis has therefore been shown to be associated with scurvy and with siderosis. A relationship between scurvy and iron overload has also been defined. It has been shown that scurvy only occurs in severely siderotic individuals (6), and evi-

¹ From the Council for Scientific and Industrial Research, Iron and Red Cell Metabolism Group, Department of Medicine, University of the Witwatersrand Medical School, Johannesburg, South Africa.

² This work was supported in part by National Institutes of Health Grant AM 04912-06.

³ Research Fellow. ⁴ Radiologist. ⁵ Physician. ⁶ Technician. ⁷ Professor of Experimental and Clinical Pharmacology. ⁸ Senior Tutorial Physician.

dence has been presented that the heavy tissue deposits of iron play a part in the pathogenesis of the ascorbic acid deficiency (7). The three conditions are thus closely interrelated. However, the role, if any, of scurvy or iron overload, or both, in the pathogenesis of the osteoporosis has not as yet been established.

The data which have so far been collected have all been obtained from patients presenting with osteoporosis in the hospital. In the current investigation an attempt was made to define the interrelationships between siderosis, osteoporosis, and tissue ascorbic acid concentrations in apparently healthy Bantu subjects.

MATERIAL AND METHODS

Both a clinical and a necropsy study were carried out in order to investigate different aspects of the problem.

Clinical Investigation

Subjects studied. A group of 2,033 male Bantu manual laborers employed by the Johannesburg Municipality was selected for this investigation because it was a stable, accessible population, and it was known that most of the subjects adhered to the traditional dietary habits. One hundred and ten individuals between the ages of 35 and 60 years were randomly selected for study. Eighty-nine of them were bachelors who lived in a hostel where they were required to provide and prepare their own food. The remainder lived with their families in the neighborhood. The average age was 48.3 years. Each individual was interviewed and his diet established. He was asked about back pain or previous injury to the back. A clinical examination was performed, and lateral radiographs of the thoracic and lumbar spine were obtained. The radiographs revealed evidence of vertebral osteoporosis in 17 of the 110 subjects examined. It was therefore decided to determine the serum iron concentration, the iron-binding capacity and the white blood cell ascorbic acid content of as many of the members of the group as possible. Forty-eight (including 10 with radiological evidence of osteoporosis) allowed blood samples to be taken for study.

In order to establish normal values for white

cell ascorbic acid concentrations for this laboratory, Bantu and white individuals attending the outpatient department of a Johannesburg hospital were studied at the same time. In contrast to the group of laborers their diets were of the usual Western type. Subjects were randomly selected, only those with obvious severe infection or a history of a high alcohol intake being excluded. In addition, white cell ascorbic acid and serum iron estimations were carried out on six Bantu male patients who were admitted to the hospital suffering from osteoporosis during the course of the study.

Diagnosis of osteoporosis. Collapse of two or more vertebral bodies on radiography was the criterion for the diagnosis of osteoporosis, provided there was no history of trauma and no radiological evidence of callus, osteophyte formation, or other bone disease. Anterior wedging of thoracic vertebrae and biconcavity of lumbar vertebrae were accepted as evidence of vertebral body collapse. The anterior borders of thoracic vertebrae are not normally more than 1 mm shorter than the posterior borders (8). A difference of greater than 2 mm was therefore taken to indicate significant anterior wedging. In nearly all instances the wedging was, in fact, considerably more marked than this. In some cases there was also obvious anterior wedging of lumbar vertebrae. The presence of biconcavity in lumbar vertebrae was accepted if the length of the bone in its middle was less than 80% of its anterior border (9). Every individual with biconcave lumbar vertebrae also exhibited wedging of at least two thoracic vertebrae. Other radiological signs of osteoporosis, such as generalized loss of bone density, increased prominence of the primary trabeculations, and "stencilling" of the vertebral end plates, were also present in those subjects with vertebral body collapse.

Chemical methods. Serum iron concentrations were determined by the method of Bothwell and Mallett (10) and unsaturated iron-binding capacities by the method of Charlton et al. (11). Buffy coat ascorbic acid concentrations were performed as described by Denson and Bowers (12). Duplicate estimations showed close agreement (within 10%). Wet platelet counts were performed, since an abnormal number of platelets may alter the result (13). However, the numbers were within normal limits in all subjects. The buffy coat ascorbic acid con-

centration (usually termed the white blood cell ascorbic acid concentration) has been shown to reflect the tissue ascorbic acid content (14-16).

Necropsy Investigation

Specimens were obtained from 71 Bantu males aged between 20 and 55 years who had died from acute trauma and who exhibited no evidence of disease at necropsy. In each case duplicate specimens of bone were obtained from the left iliac crest between 1 inch and 2 inches behind the anterior superior iliac spine. A Williams and Nicholson-modified Sackers trephine (17) was used and the technique described by Saville (18) was followed. A specimen of liver for tissue iron estimation was also obtained in each case.

The bone specimens were washed in cold running water for 2 hr to get rid of blood. Fat was removed by passing the specimens through 70%, 90%, and absolute ethyl alcohol, allowing them to stand for 24 hr in each concentration, and then extracting the residual fat in a Soxhlet apparatus with three parts of ethyl ether to one part of absolute ethyl alcohol. The samples were dried in an oven at 110 C for 3 days and then weighed. Thereafter each sample was impregnated with wax. By carefully controlling the temperature of the wax it was possible to do this in such a way that the wax followed the contours of the bone exactly. The drop of wax which collected at the bottom of the cylinder was trimmed off with a scalpel. The volume of the waxed bone was then determined by mercury displacement. Duplicate volume determinations were made on each sample, and these varied by less than 2%. Finally, the samples were ashed in a muffle furnace at 580 C for 48 hr, the weight of the ash being determined. Mineral density was calculated as weight of ash/volume of waxed bone. The ash was then prepared for calcium and phosphorus assay by dissolving it in 0.5 N hydrochloric acid. The calcium content was determined by titration against disodium ethylenediaminetetraacetic acid (EDTA) using the method of Bachra, Dauer and Sobel (19). In the initial estimations the calcium was precipitated as oxalate before titration against EDTA, but since the values were within 3% of those obtained by direct titration, this step was omitted in the later determinations. Phosphorus was measured by the method of Fiske and Subbarow (20) as modified

by King and Wootton (21). All chemical measurements were made in duplicate and the mean of the duplicate values obtained for each of the two bone specimens was used. The concentration of iron in the liver was estimated by the thioglycolic acid method after wet digestion (22).

RESULTS

Clinical Investigation

Diet and alcohol consumption. The diet was very constant from person to person and from day to day. The major constituents were maize porridge, "mareu" (fermented porridge), bread, and small quantities of meat. Cooked vegetables were occasionally eaten. A proportion of subjects drank milk irregularly and ate fresh fruit about once a week, but 41% drank no milk at all and 40% virtually never ate fresh fruit. Eighty-five percent of the men drank alcohol every day, and this was usually the traditional Bantu beer. The beer was either home-brewed or supplied by the Municipal brewery. The 1,782 residents of the hostel were provided with 600 gal of beer each day from the brewery. Forty percent of the subjects in the study admitted to drinking 6-8 pints/day, 36%, 4-6 pints, and 9%, 2-4 pints.

Incidence of osteoporosis. Seventeen out of 110 subjects exhibited osteoporotic vertebral body collapse. They ranged in age from 36 to 60 years (mean 49.7). Eleven of them drank no milk at all. The same number ate virtually no fruit. In both cases the proportions were somewhat higher than in the group as a whole. However, the drinking habits of the osteoporotic subjects did not differ significantly from those of the other workers. Thirteen drank more than 4 pints/day, and only one claimed not to drink at all.

White blood cell ascorbic acid concentrations. The mean concentrations of white blood cell ascorbic acid in three of the four groups of control subjects (white males, white females and Bantu females)

TABLE 1
White Blood Cell Ascorbic Acid Concentrations in Different Groups

Group	No. of Subjects	Mean Age (Range)	Mean White Blood Cell Ascorbic Acid Concentration, $\mu\text{g}/10^8$ White Blood Cells (\pm SD)	Significance of the Difference (<i>P</i>)
1) Outpatient white females	13	51 (34-75)	30.4 \pm 9.4	
2) Outpatient white males	13	58.6 (32-72)	30.3 \pm 11.0	
3) Outpatient Bantu females	24	30.5 (17-47)	30.7 \pm 11.9	
4) Outpatient Bantu males	24	31.8 (19-56)	26.6 \pm 11.2	3:4 NS
5) Laborers	48	46.5 (35-60)	11.5 \pm 8.4	4:5 <0.001
6) Asymptomatic osteoporotic laborers	10	49.4 (36-60)	7.8 \pm 2.4	5:6 <0.02
7) Osteoporotic Bantu hospital patients	6	47.8 (45-52)	2.5 \pm 0.9	6:7 <0.001

were 30.3, 30.4, and 30.7 $\mu\text{g}/10^8$ leukocytes, respectively (Table 1). These figures are comparable with normal values in other populations (12, 13). The mean value in Bantu male outpatients was slightly lower, with a figure of 26.6 $\mu\text{g}/10^8$ leukocytes. In contrast, the mean concentration in the group of Municipal laborers was considerably lower (11.5 $\mu\text{g}/10^8$ leukocytes). The figures in those individuals with radiological evidence of osteoporosis were significantly lower than the group of laborers as a whole. Excluding one subject, the highest value in the remaining individuals was 11.3 $\mu\text{g}/10^8$ leukocytes and the mean was 7.8 $\mu\text{g}/10^8$ leukocytes. The concentrations in the six patients admitted to the hospital because of osteoporotic vertebral collapse were even lower, with a mean value of 2.5 $\mu\text{g}/10^8$ leukocytes. One of the group of laborers with asymptomatic osteoporosis had a leukocyte ascorbic acid concentration on repeated estimations which was much higher (36 $\mu\text{g}/10^8$ leukocytes) than any of the others. Although he was a heavy drinker and said that he seldom ate fruit, it remains possible that he had been exposed to some form of dietary ascorbic acid.

When the group of laborers was analyzed according to the intake of alcohol, it was found that the mean concentration of ascorbic acid was 7.2 $\mu\text{g}/10^8$ leukocytes (SD \pm 7.2) in subjects drinking at least 4 pints of beer/day while it was 14.9 $\mu\text{g}/10^8$ leukocytes (SD \pm 7.5) in those drinking less than 4 pints/day. This difference is significant ($P < 0.02$). The mean concentration in those individuals who virtually never ate fruit was 6.7 $\mu\text{g}/10^8$ leukocytes (SD \pm 4.6). Within this group the amount of alcohol consumed again appeared to affect the tissue ascorbic acid levels. In those who seldom ate fruit and also drank 4 or more pints/day the mean figure was 4.8 $\mu\text{g}/10^8$ leukocytes (SD \pm 3.0), while in those drinking less than 4 pints it was 12.0 $\mu\text{g}/10^8$ leukocytes (SD \pm 5.1) ($P < 0.02$).

Assessment of siderosis. The mean serum iron concentration in the 48 subjects studied was 131 $\mu\text{g}/100$ ml (range 40-254) with a mean transferrin saturation of 50% (range 17.6-96.7). In the 10 individuals with radiological osteoporosis the figures were 132 $\mu\text{g}/100$ ml (range 47-222) and 54% (range 33.1-93.3), respectively. A comparison was made between the serum iron and white cell ascorbic acid concen-

trations in the 48 manual laborers, the 6 osteoporotic patients, and 10 of the Bantu hospital outpatients (Fig. 1). All except two of the subjects with serum iron levels above 150 $\mu\text{g}/100\text{ ml}$ had ascorbic acid values below 10 $\mu\text{g}/10^8$ leukocytes. On the other hand, ascorbic acid levels varied widely in subjects with lower serum iron levels.

Necropsy Investigation

A highly significant negative correlation ($r = -0.63$, $P < 0.001$) was found to

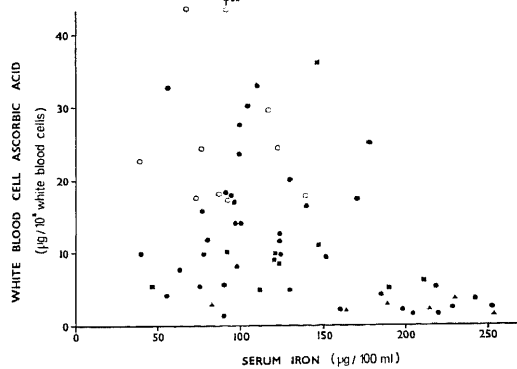


FIG. 1. Comparison between serum iron concentration and white blood cell ascorbic acid content in nonosteoporotic control subjects (open circles), nonosteoporotic manual laborers (closed circles), manual laborers with asymptomatic osteoporosis (closed squares), and patients admitted to hospital because of osteoporosis (closed triangles).

exist between the iron concentration in the liver and the mineral density of iliac crest bone (Fig. 2). Since mineral density decreases with age (23–25), and siderosis in the Bantu tends to be more marked in older age groups (4), it was possible that the observed inverse correlation was due to this alone. For this reason the mineral densities were also plotted against the ages of the subjects (Fig. 2). Although there was again a significant inverse correlation, it was not as close as that between mineral density and hepatic iron concentration ($r = -0.36$, $P < 0.01$). It was therefore concluded that the association between siderosis and osteoporosis was a real one. This conclusion was supported by a comparison between the mineral densities in subjects over the age of 35 years with hepatic iron concentrations above 2.0 mg/g wet weight and those with iron concentrations less than this. The mean mineral density in the nine siderotic individuals (mean age 45.6 years) was 230 mg/cc (sd ± 47), while that of the 25 other subjects (mean age 40.9 years) was 312 mg/cc (sd ± 45). This difference was significant at the 0.1% level.

In contrast to the mineral density, the mineral content of the bone, expressed as a percentage of the dry fat-free weight, was very constant at all iron concentrations. Mean figures (\pm sd) were as follows: ash

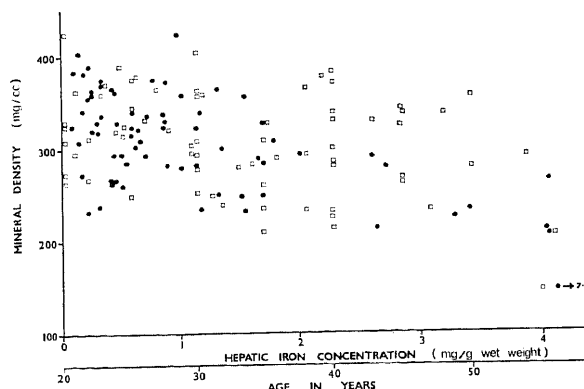


FIG. 2. Correlation between mineral density of iliac crest bone and hepatic iron concentration (closed circles, $r = -0.63$, $P < 0.001$) and mineral density of iliac crest bone and age (open squares, $r = -0.36$, $P < 0.01$).

56.0% (± 3.3); calcium 20.0% (± 0.8); and phosphorus 9.7% (± 0.3).

DISCUSSION

It has previously been established that the osteoporosis which occurs in middle-aged male Bantu laborers is associated with severe siderosis and with scurvy (1, 2). Scurvy in this population is seen almost exclusively in individuals with marked iron overload (6), and recent studies have revealed a possible reason for this association (7). It has been shown that if ascorbic acid is administered to severely siderotic individuals even in large repeated doses, only a fraction can be recovered from the urine (26). There is, however, a considerable increase in the excretion of oxalic acid, an oxidation product of ascorbic acid (7). In control subjects there is no such rise in the urinary oxalic acid, and virtually the whole administered dose of ascorbic acid can be recovered unchanged in the urine. It has therefore been postulated that the massive deposits of iron in the tissues irreversibly oxidize the ascorbic acid. Scurvy in this population thus results from the combination of a poor dietary intake plus the oxidative destruction of much of the small quantity ingested. The importance of iron overload in the pathogenesis of scurvy is underlined by the fact that neither condition occurs in children, although other deficiency diseases such as kwashiorkor and rickets are common (27).

The data obtained in the present investigation add weight to this view. The 48 laborers studied were found to have low tissue ascorbic acid concentrations and analysis of their dietary habits revealed that the intake of fresh fruit was extremely low. However, this was not the only factor contributing to ascorbic acid deficiency. The mean leukocyte ascorbic acid concentration was significantly lower in those individuals who drank 4 or more pints of beer per day than in those who drank less

heavily. This was not because those who drank excessively ate less fruit. Further analysis of the group of subjects who seldom if ever ate fruit revealed that the ascorbic acid levels were considerably lower in those among them who drank 4 or more pints of beer a day than in those who consumed less than 4 pints. On the basis of previous studies it seems valid to assume that those individuals who habitually drank more beer had larger deposits of iron in their tissues (3, 5). They would therefore have a greater potential for the oxidative catabolism of ingested ascorbic acid. Some evidence in support of this was provided by the comparison between the serum iron concentrations and the white blood cell ascorbic acid concentrations. The latter were low in the majority of individuals with serum iron levels greater than 150 $\mu\text{g}/100\text{ ml}$ (Fig. 1).

The results obtained both in previous investigations and in the present study thus indicate that the combination of a poor dietary intake of ascorbic acid together with heavy beer drinking leads to severe ascorbic acid deficiency. This deficiency may, in turn, have relevance to the development of bone changes, since there is evidence that osteoporosis develops in children and in animals suffering from scurvy (28, 29). If ascorbic acid deficiency were, in fact, the cause of the osteoporosis noted in the present Bantu subjects, affected individuals would be expected to be heavy drinkers who ate no fruit. On questioning, it was found that 11 out of 17 (65%) ate no fruit, a figure somewhat higher than that of the group as a whole (40%), while 13 (76%) drank more than 4 pints of beer a day. Although this last figure is not different from that of the rest of the subjects, the beer consumption of the whole group was so high that this is perhaps not unexpected. Only 15% of the 110 subjects studied drank less than 2 pints/day.

Although the drinking habits of these

subjects indicated that the majority were siderotic, the direct evidence of iron overload was confined to the serum iron concentrations and iron binding capacities. The serum iron level tends to reflect the degree of siderosis, although there may be considerable individual variation. In the present investigation the mean figures were similar in the group as a whole and in the osteoporotic individuals.

Since these observations cast some doubt on the validity of the previously observed association between severe siderosis and osteoporosis, a further study was undertaken to obtain more quantitative information. Hepatic iron concentrations and iliac bone mineral densities were determined in unselected male Bantu dying of trauma. An inverse relationship was discovered. Since bone density decreases (23-25) and (in this population) hepatic iron concentration increases (4) with age, it was necessary to show that the correlation was not due simply to this factor. While it was apparent that some decrease in mineral density occurred with age, this alone did not explain the current findings, and it was therefore concluded that the association between siderosis and osteoporosis was a real one.

The results of the present study are thus consistent with the hypothesis that severe siderosis plays a part in the pathogenesis of ascorbic acid deficiency, and that chronic ascorbic acid deficiency results in osteoporosis. However, this is not the only hypothesis which could account for the observed associations between severe siderosis, scurvy, and osteoporosis. For example, it is possible that both the scurvy and the iron overload simply indicate that the osteoporotic subjects are heavy drinkers. The bone densities of alcoholics have been reported to be less than those of age-matched normal subjects (18). However, it is doubtful whether this explains the findings in Bantu subjects. While many Bantu males consume large quanti-

ties of iron-containing brews, the alcohol content of most of them is very low, and the majority cannot therefore be regarded as alcoholics in the generally accepted sense. For example, the laborers investigated in the present study were in steady employment, and none showed clinical evidence of alcoholic peripheral neuritis, cirrhosis of the liver, or other stigmata of alcoholism. According to a third hypothesis the iron overload itself might produce the osteoporosis. It could theoretically impair calcium absorption, impede bone formation, or accelerate bone resorption. However, at present there is no evidence to support any of these contentions. Finally, it is possible that a dietary deficiency of calcium is a factor in the genesis of the osteoporosis, since it is known that the intake of calcium in Bantu adults is low by Western standards (30). In this context, it may be relevant that 41% of the laborers in the present study seldom if ever drank milk, and that the proportion was even higher in the subjects with osteoporosis. It is therefore conceivable that several factors may be involved in the genesis of the osteoporosis. However, the findings in the present investigation suggest that ascorbic acid deficiency occupies a central role and that this deficiency is in turn related to iron overload.

SUMMARY

An unusual variety of osteoporosis has previously been shown to occur in middle-aged male Bantu subjects in Johannesburg, and associations with severe iron overload and with scurvy have been established. In the present investigation 110 asymptomatic manual laborers whose diets contained large amounts of iron and little ascorbic acid were studied. The mean leukocyte ascorbic acid concentration of this group was significantly lower than that of control groups. Seventeen (15%) of these subjects exhibited radiological evidence of vertebral osteoporosis, and the mean leu-

kocyte ascorbic acid concentration of the osteoporotic individuals was significantly lower than that of the group as a whole. These results are consistent with the hypothesis that this unusual form of osteoporosis is the consequence of chronic ascorbic acid deficiency.

A study of 71 Bantu male subjects dying of acute trauma and exhibiting no evidence of disease at necropsy was also carried out. A highly significant inverse correlation was established between the mineral density of the iliac crest bone and the hepatic iron concentration. This association may be due to the part played by severe iron overload in the pathogenesis of ascorbic acid deficiency in this population.

REFERENCES

- GRUSIN, H., AND E. SAMUEL. A syndrome of osteoporosis in Africans and its relationship to scurvy. *Am. J. Clin. Nutr.* 5: 644, 1957.
- SEFTEL, H. C., C. MALKIN, A. SCHMAMAN, C. ABRAHAMS, S. R. LYNCH, R. W. CHARLTON AND T. H. BOTHWELL. Osteoporosis, scurvy and siderosis in Johannesburg Bantu. *Brit. Med. J.* 1: 642, 1966.
- WALKER, A. R. P., AND U. B. ARVIDSSON. Iron "overload" in the South African Bantu. *Trans. Roy. Soc. Trop. Med. Hyg.* 47: 536, 1953.
- BOTHWELL, T. H., AND B. A. BRADLOW. Siderosis in the Bantu. A combined histopathological and chemical study. *Arch. Pathol.* 70: 279, 1960.
- BOTHWELL, T. H., H. SEFTEL, P. JACOBS, J. D. TORRANCE AND N. BAUMSLAG. Iron overload in Bantu subjects. Studies on the availability of iron in Bantu beer. *Am. J. Clin. Nutr.* 14: 47, 1964.
- BOTHWELL, T. H., B. A. BRADLOW, P. JACOBS, K. KEELEY, S. KRAMER, H. C. SEFTEL AND S. ZAIL. Iron metabolism in scurvy with special reference to erythropoiesis. *Brit. J. Haematol.* 10: 50, 1964.
- LYNCH, S. R., H. C. SEFTEL, J. D. TORRANCE, R. W. CHARLTON AND T. H. BOTHWELL. Accelerated oxidative catabolism of ascorbic acid in siderotic Bantu. *Am. J. Clin. Nutr.* 20: 641, 1967.
- KÖHLER, A. *Borderlands of the Normal and Early Pathologic in Skeletal Roentgenology* (10th ed.), edited by E. A. Zimmer. English translation arranged and edited by J. T. Case, New York: Grune & Stratton, 1956, p. 332.
- BARNETT, E., AND B. E. C. NORDIN. The radiological diagnosis of osteoporosis. A new approach. *Clin. Radiol.* XI: 166, 1960.
- BOTHWELL, T. H., AND B. MALLETT. The determination of iron in plasma or serum. *Biochem. J.* 59: 599, 1955.
- CHARLTON, R. W., N. HARDIE AND T. H. BOTHWELL. A note on the measurement of the unsaturated iron-binding capacity of serum using radio-active iron. *S. African J. Med. Sci.* 30: 71, 1965.
- DENSON, K. W., AND E. F. BOWERS. The determination of ascorbic acid in white blood cells. A comparison of W.B.C. ascorbic acid and phenolic acid excretion in elderly patients. *Clin. Sci.* 21: 157, 1961.
- GIBSON, S. I. M., F. M. L. MOORE AND A. GOLDBERG. Measurement of leucocyte ascorbic acid. *Brit. Med. J.* 1: 1152, 1966.
- CRANDON, J. H., C. C. LUND AND D. B. DILL. Experimental human scurvy. *New Engl. J. Med.* 223: 353, 1940.
- LOWRY, O. H., O. A. BESSEY, M. J. BROCK AND J. A. LOPEZ. The interrelationship of dietary, serum, white blood cell and total body ascorbic acid. *J. Biol. Chem.* 166: 111, 1946.
- BARTLEY, W., H. A. KREBS AND J. R. P. O'BRIEN. Vitamin C. Requirements of human adults. *Med. Res. Council Spec. Rept. Ser.*, no. 280, 1953.
- WILLIAMS, J. A., AND G. I. NICHOLSON. A modified bone-biopsy drill for outpatient use. *Lancet* 1: 1408, 1963.
- SAVILLE, P. D. Changes in bone mass with age and alcoholism. *J. Bone Joint Surg.* 47A: 492, 1965.
- BACHRA, B. N., A. DAUER AND A. E. SOBEL. The complexometric titration of micro and ultramicro quantities of calcium in blood serum, urine and inorganic salt solutions. *Clin. Chem.* 4: 107, 1958.
- FISKE, C. H., AND Y. SUBBAROW. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375, 1925.
- KING, E. J., AND I. D. P. WOOTTON. *Micro-Analysis in Medical Biochemistry* (3rd ed.). London: Churchill, 1956, p. 75.
- BOTHWELL, T. H., AND C. A. FINCH. *Iron Metabolism*. Boston: Little, Brown, 1962, p. 26.
- TROTTER, M., G. E. BROMAN AND R. R. PETERSON. Densities of bones of white and Negro skeletons. *J. Bone Joint Surg.* 42A: 50, 1960.
- ARNOLD, J. S. The quantitation of bone mineralization as an organ and tissue in osteoporosis. In: *Dynamic Studies of Metabolic Bone Disease*,

- edited by O. H. Pearson and G. F. Joplin. Oxford: Blackwell, 1964, p. 59.
25. MUELLER, K. H., A. TRIAS AND R. D. RAY. Bone density and composition. Age-related and pathological changes in water and mineral content. *J. Bone Joint Surg.* 48A: 140, 1966.
26. SCHULZ, E. J., AND H. SWANEPOEL. Scorbutic pseudoscleroderma. An aspect of Bantu siderosis. *S. African Med. J.* 36: 367, 1962.
27. TROWELL, H. C. *Non-Infective Disease in Africa*. London: Edward Arnold, Ltd. 1960, p. 363.
28. VILTER, R. W. In: *The Vitamins*, edited by W. H. Sebrell and R. S. Harris. New York: Academic, 1954, vol. 1, p. 371.
29. HÖJER, J. A. Studies in scurvy. *Acta Paediat. Suppl.* 3: 48, 1923.
30. WALKER, A. R. P. Osteoporosis and calcium deficiency. *Am. J. Clin. Nutr.* 16: 327, 1965.