SERUM PHOSPHATASE IN LEPROSY[1]

By Sister Hilary Ross, B.S.
Medical Technician, National Leprosarium
Carville, Louisiana

During the past twenty years considerable attention has been given to chemical determinations on the blood of lepers, because of the bone changes that occur. From our own observations (9), which confirmed the findings of others, it appears that bone changes in leprosy are not dependent on total serum calcium. However, investigations of the diffusible and colloidal calcium content revealed that the amount of active or diffusible calcium was reduced, while the nondiffusible or colloidal calcium was high, although the total calcium was within physiological limits. Radiographs of 48 of the cases studied showed atrophy of some degree, either in the form of decalcification or of bone absorption, in 44 instances. From a subsequent study (10) it was thought that possibly the bone changes were due in part to a lack of transference to the tissues of the diffusible and functionally available calcium, and it was suggested that the disturbance in the protein balance noted may in some way affect the degree of diffusibility.

In recent years much significance has been attached to the role of phosphatase in the physiology of the bone. Villela (8), in an excellent review of the biochemistry of leprosy, suggests a study of the phosphatase content of the blood plasma. He states: "Bone changes being found in advanced leprosy, the power to hydrolyze phosphate esters by plasma is possibly disturbed."

The phosphatase of the blood and plasma had been investigated relatively little until Kay (7) found that its activity in plasma was increased in various diseases, particularly in those of bone. He states, in substance, that it would not perhaps be surprising if in diseases of phosphatase-rich tissues (intestinal mucosa, kidneys[2] and bone), particularly those conditions involving a progressive and widespread breakdown of such tis-

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[2] The kidney of an adult contains 300 times the amount of phosphatase found in the blood.

sues with possible leakage of intracellular materials into the blood, there should occur an increase above normal in the relatively low phosphatase content of the plasma. In considering possible sources from which this enzyme might leak into the blood under pathological conditions, it is unlikely that there are any other tissues than those mentioned above which have any quantitative significance. Since Kay's work, this enzyme has assumed significance as a laboratory diagnostic aid (4).

Harrow and Sherman (6) give a brief description of the relation of phosphatase activity to the synthesis of bone. The action of phosphatase is believed to be one of a number of factors in that process. This enzyme, which is present in the osteoblasts, the hypertrophic cartilage cells and certain cells of the periosteum, hydrolyzes the salts of phosphoric esters brought to the ossifying zone by the blood, and thus causes a local increase in phosphate ions. A deposition of calcium phosphate is brought about in the vicinity of these cells. It is maintained by some that the action of the bone esterase (phosphatase) is to react with the organic phosphorus compounds in the blood plasma, thus increasing the concentration of the phosphate ions. The latter then react with calcium ions, depositing calcium phosphate as $CaHPO_4.H_20$. In the ossifying tissue this compound is rapidly converted into $Ca_3(PO_4)_2.Ca(OH)_2$.

PRESENT REPORT

Since there is no literature available on the determination of phosphatase or its activity in leprosy, it seemed desirable to undertake the study, as suggested by Villela. For this purpose 102 patients were selected, representing the various types of the disease, periods of duration and stages of progression, and their sera were analyzed for inorganic phosphorus and the phosphatase activity. The plasmas of 70 of these cases were examined for their ascorbic acid content. Control sera and plasmas were collected from 15 young men and women, employees of this institution, and analyzed coincidentally with the materials from patients.

Analytical methods.—Approximately 15 cc. of blood was collected from a cubital vein. The phosphatase activity was determined by the method of Bodansky (2, 3), which depends on the determination of the inorganic phosphorus liberated from a phosphoric acid ester by the action of an enzymatic material. Ten cc. of a buffered substrate, consisting of sodium beta-glycero-phosphate and monosodium diethyl-barbiturate, and 2 cc. of serum were incubated at 37°C. for two hours. At the end of this time the tubes

were cooled, 9 cc. of 10 percent trichloracetic acid was added, and the mixture was shaken and filtered. To 5 cc. of this filtrate were added 4 cc. of molybdate reagent and 1 cc. of stannous chloride solution. The blue color developed was read in a colorimeter against the color developed on similar treatment with a standard phosphate solution. The difference in milligrams of phosphorus found after incubation and that initially present in the serum was taken as the measure of phosphatase activity. Bodansky defines a unit of activity as "equivalent to the actual or calculated liberation of 1 mgm. of phosphorus as the phosphate ion during the first hour of incubation at 37°C. and pH 8.6, with the substrate containing sodium beta-glycero-phosphate, hydrolysis not exceeding 10 percent of the substrate." Employing this procedure, the range of normal values for plasma or serum phosphatase activity in adults is 1.5 to 4.0 units per 100 cc.

All determinations were completed within three hours. Bodansky found increased activity (about 10 percent) in serum after twenty-four hours' refrigeration and increase (from 16 to 20 percent) after incubation for four to six hours at 37°C.

RESULTS

Normal persons.—The phosphatase activity determined in the sera of 15 normal individuals is shown in Table 1. These figures average slightly higher than those reported by Bodansky (1), which range from 1.5 to 4 units per 100 cc.

TABLE 1.—*Control determinations, 15 cases, per 100 cc. serum.*

	Serum inorganic phosphorus*	Phosphatase, Bodansky units
Minimum	3.2	1.5
Average	3.8	3.5
Maximum	4.1	5.6

*Expressed in milligrams.

Leprous persons.—The results obtained with the lepers' sera are presented in Table 2. Although the arithmetical average is within the normal range of the controls, the distribution curve varies somewhat. Of the 102 cases, 89 showed normal values; 3 cases showed a value slightly exceeding 5.6 Bodansky units, the upper limit of normal; and 10 cases had values slightly

TABLE 2.—*Serum phosphatase in lepers, 102 cases, per 100 cc. serum.*

	Serum inorganic phosphorus*	Phosphatase, Bodansky units
Minimum	2.1	0.5
Average	3.5	3.9
Maximum	6.7	10.9

*Expressed in milligrams.

below normal, four of them below 1.0 unit. The distribution of these cases is as follows:

Phosphatase units	*Number of cases*
0.5 to 0.9	4
1.0 to 1.4	6
1.5 to 1.9	18
2.0 to 2.9	31
3.0 to 3.9	22
4.0 to 4.9	15
5.0 to 5.5	3
5.6 to 10.9	3

Roentgenologic data on 20 of the cases show the presence of atrophy of some degree, either in the form of decalcification or of bone absorption. In all of these cases the Bodansky unit was within the normal range.

Clinical data on 73 of the cases show common leprotic changes, as atrophy of the interosseous muscles of the hands and feet, contraction of fingers, bone absorption or bone necrosis. Of these cases, two had values slightly above 5.6 units (6.9 and 8.9 units respectively), and ten were slightly below 1.5 units. Of nine cases without evidence of bone pathology or anesthesia, eight had normal values and one (a paroled patient) had a value of 10.9 units.

It has been found that serum phosphatase is activated by a variety of substances, among which are iron and ascorbic acid (5). In this study the ascorbic acid content of the plasma was determined in parallel with the phosphatase in 70 of the cases. It was found that, although the ascorbic acid was below normal in amount in 46 instances, no definite relationship existed between its concentration and the phosphatase activity. Those cases having a high phosphatase activity had low ascorbic acid values, and in some of the cases with low phosphatase activity those values were normal.

Kay explained high plasma phosphatase in diseases of bone by leakage "at more than normal rate, possibly because it is produced in excessive amount in the bones in attempted compensation for the lesion, or possibly because owing to weakness of the bones there is a greater amount of bending and crushing which mechanically squeezes out some of the cell contents." Bodansky and Jaffe (1) consider the serum phosphatase activity in bone disease to be an expression of the "specific reactivity of the bone," or its capacity for cellular activities. In

the chemistry of bone formation the production of the enzyme phosphatase constitutes a part of the cellular activities leading to that end.

The above hypotheses fail to explain in a consistent manner the differences between serum phosphatase in normal and pathological states. The serum phosphatase activity in leprosy appears to be within the normal limits, as found in the majority of the cases studied whether they showed bone atrophy, decalcification or necrosis.

SUMMARY

Sera from 102 lepers, representing various types, stages and degrees of activity of the disease, were examined for inorganic phosphorus and the phosphatase activity. The plasma ascorbic acid was determined in parallel with the phosphatase activity in 70 of the cases. Sera and plasma from 15 normal men and women were similarly examined as controls.

The serum phosphatase, calculated on the bases of Bodansky units, averaged within the normal range. Of the 102 leprosy cases, 89 showed normal values, three were slightly above normal, and ten had values below the normal range.

There was no definite correlation between the ascorbic acid in the plasma and the phosphatase activity, though the concentration of ascorbic acid was below normal in 46 instances.

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