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Radioimmunoassay of Procollagen in Serum of Patients with Paget's Disease of Bone (39380)

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Tropocollagen is the triple helical molecule which aggregates to form the insoluble collagen fibers of the body. Tropocollagen is generated by the enzymatic conversion of procollagen, a soluble precursor molecule secreted into the extracellular space. The enzymatic conversion of procollagen entails the excision of nonhelical polypeptides from the ends of the molecule (1, for review). At least one of these excisions occurs *en bloc* to release a disulfide-linked, three chain fragment (2) from the carboxyterminus of the precursor (3-5). In contrast to triple helical tropocollagen, this excised fragment lacks hydroxyproline and hydroxylysine, has much less glycine and proline, and uniquely contains tryptophan and half-cystine residues (6).

The disulfide-linked carboxyterminal fragment of human procollagen has been isolated and purified and antibodies specific for it developed in rabbits (6, 7). A radioimmunoassay (RIA) for human procollagen has been developed which has demonstrated the antigen to be present in human sera (8). We now report that the RIA demonstrates abnormally high concentrations of procollagen protein in the sera of patients with Paget's disease of bone. Moreover, the serum procollagen concentrations decrease when the patients are treated with disodium etidronate (EHDP), a drug known to produce biochemical improvement in this disorder (9, 10).

Materials and methods. Patients. Thirty-one male and 19 female patients, ages 44-85, with Paget's disease of bone were included in this study. The diagnosis was based on characteristic roentgenographic findings, increased uptake of radioisotope into the affected bones, and elevated serum alkaline phosphatase values. None of the patients had received any therapy for Paget's disease for at least 3 months prior to the study. None of the patients had recent bone fractures or significant complicating illnesses. Sera were obtained prior to and after 3 months of therapy with 5-20 mg/kg/day of disodium etidronate (EHDP), supplied by Proctor and Gamble, Co., Cincinnati, Ohio. A detailed clinical analysis of this group of patients will be reported elsewhere. Informed consent for the study was given by all patients.

The patients were on a low gelatin diet when serum and urine samples were collected within the same 24-hr period. Serum alkaline phosphatase activity was measured by a modification of the thymolphthalein monophosphate method of Roy (11; normal range 13-41 units per liter). Total urinary hydroxyproline was measured by the method of Kivirikko *et al.* (12; normal range, 9-45 mg/24 hr).

Radioimmunoassay. Assays were performed as reported (8) except that radioactivity in the immune precipitates was measured in a gamma spectrometer. Sera were tested without knowing if they had been drawn before or after drug therapy. Purified, unlabeled antigen was used to generate a standard calibration curve for each group of sera tested. Percent displacements of radioactivity from immune precipitates were plotted against the log of the nanograms of standard antigen or microliters of test serum added. Concentrations of procollagen antigen in sera were calculated from points in the linear portion of the displacement curves (20-80% range; see Fig. 1). Independent assays of the same serum sample gave values which did not differ by more than 10%. The normal range for procollagen protein in adult serum is 5-15 μg/100 ml.

Results. Figure 1 shows the typical com-

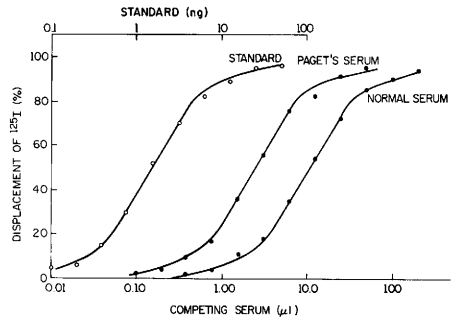

FIG. 1. Radioimmunoassay: binding displacement curves for standard antigen, a Paget's serum, and a normal serum.

petition curves obtained when increasing amounts of unlabeled standard antigen, a pretreatment Paget's serum, and a normal adult serum, compete for antibody sites int the radioimmunoassay. Complete displacement of radioactivity is achieved in all three instances and the slopes of the three curves are identical in the 20–80% displacement range. These data support the view that normal and Paget's sera contain a molecule antigenically identical to the purified procollagen standard.

Samples of serum from 44 patients were tested in the assay before and after treatment with EHDP, and these values were correlated with simultaneous determinations of urinary total hydroxyproline (Fig. 2) and serum alkaline phosphatase activity (Fig. 3). All but four of the patients had elevated levels of procollagen before treatment (Figs. 2a, 3a); the average value for the group was 52 μg/100 ml, a greater than threefold elevation above normal. Figures 2 and 3 show that prior to treatment the increases in urinary hydroxyproline and serum alkaline phosphatase activity were generally coordinate with the increases in serum procollagen.

All elevated serum procollagen concentrations decreased significantly after treatment with EHDP, and they returned to the normal range in 33 of the 40 patients. The average procollagen concentration calculated for these 40 patients receiving the drug was 12 μg/100 ml. The decrease in procollagen concentration was accompanied by a decrease in urinary total hydroxyproline (Fig. 2b), and 66% of the patients achieved normal levels of both parameters. The drug lowered the serum alkaline phosphatase levels in all patients (Fig. 3b), but in only seven instances did the values return to the normal range. On the average, the drug caused the pretreatment values for procollagen, urinary hydroxyproline, and alkaline phosphatase activity to decrease by 74, 79, and 67% respectively.

The average procollagen levels determined for six patients before and after administration of a placebo for 3 months were 58 and

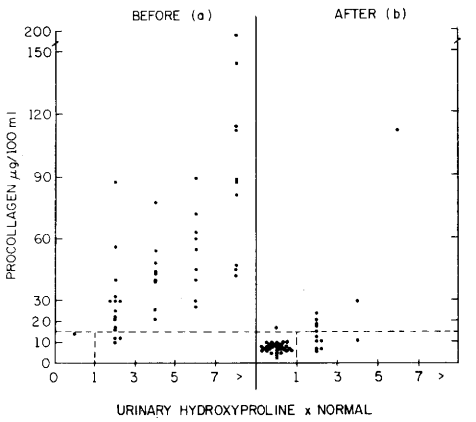

FIG. 2. Levels of serum procollagen and urinary total hydroxyproline before (a) and after (b) treatment with disodium etidronate. Hydroxyproline data plotted as multiples of the upper limit of the normal value. Results for 44 patients are given. Dotted lines define upper limits of normal for each variable.

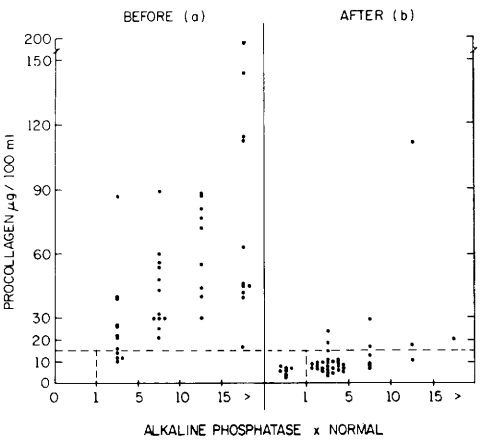

FIG. 3. Levels of serum procollagen and serum alkaline phosphatase before (a) and after (b) treatment with disodium etidronate. Alkaline phosphatase data plotted as multiples of the upper limit of the normal value. Same patient group as in Fig. 2. Dotted lines define upper limits of normal for each variable.

53 μg/100 ml, respectively (data not included in Figs. 2-3).

The RIA of normal serum was unaffected when EHDP was added to the assay tubes in concentrations greatly in excess of those occurring in the sera of patients taking the drug. We conclude that the levels of EHDP in the serum of treated patients did not interfere in the assay to produce artefactually low procollagen values.

Discussion. When the procollagen concentrations determined for 123 serum samples were matched with the record of treatment and the other clinical chemistries, the following correlations attested to the reliability and usefulness of the assay: (i) the procollagen concentrations in multiple pretreatment sera from a given patient were in good agreement, and 92% of such sera had significantly elevated concentrations of procollagen protein; (ii) significant decreases in procollagen concentrations were recorded for patients receiving EHDP, whereas the levels remained high in patients given a placebo; and (iii) the procollagen levels before and after treatment with EHDP were coordinate with the values for urinary total hydroxyproline and serum alkaline phosphatase activity.

Appropriate measurements demonstrate that bone resorption and synthesis are positively correlated in Paget's disease but that synthesis may lag behind resorption (13). Urinary hydroxyproline as measured in our study includes contributions from both bone resorption and synthesis (14–16). We therefore cannot decide from these data whether the procollagen levels of serum are only a function of collagen synthesis, or reflect collagen resorption as well. Although the metabolism of procollagen is by no means completely defined, we favor the view that neither the intact precursor nor the antigenic fragment excised from it become structural components of the insoluble matrix of bone. By this assumption, resorption of the matrix of bone in Paget's disease would not increase the soluble pool of procollagen protein, and the concentration of the latter would be primarily a function of its rate of synthesis. Studies are in progress to clarify this point. Contributions from tissues other than bone to the elevated procollagen levels in Paget's disease, while unlikely, cannot be excluded. However, the data as reported show that the RIA for procollagen in serum reliably reflects the changes in collagen metabolism occurring in Paget's disease of bone. We anticipate that the assay may be of value in other disorders affecting collagenous tissues.

Summary. A new radioimmunoassay for human procollagen showed that the sera of 46 of 50 untreated patients with Paget's disease of bone contained increased concentrations of procollagen protein as compared to normal adults. After therapy with disodium etidronate, all the elevated serum procollagen concentrations decreased significantly, falling to normal levels in 33 of 40 patients. The procollagen levels before and after treatment were coordinate with the values for urinary total hydroxyproline and serum alkaline phosphatase activity. The data show that the radioimmunoassay for procollagen is a dependable and useful adjunct to the study of Paget's disease of bone.

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