Isolated bone cells demonstrate cell-type specific responses to glucocorticoids. Osteoclast-like (OC) cells exhibit a large decrease in basal hyaluronate synthesis at physiological doses of glucocorticoids and resistance to further inhibition by pharmacological doses up to $10^{-4}$ M. This effect is not accompanied by decreases in protein synthesis. In contrast, osteoblast-like (OB) cell metabolism is not inhibited by physiological doses of glucocorticoids. However, in OB cells both citrate decarboxylation and collagen synthesis are decreased at pharmacological doses of glucocorticoids and these effects are accompanied by a decrease in general protein synthesis. In addition to these effects on basal and general cell activities, physiological doses of glucocorticoids modulate the hormonal sensitivity of OC and OB bone cells such that lower concentrations of bovine parathyroid hormone (PTH) are necessary to elicit measurable biochemical changes.

At present the effect of glucocorticoids on osteoclast activity and bone resorption is still unclear. Steroid enhancement of activity (10) and, presumably, the effects of glucocorticoids on bone have shown that inhibition of matrix synthesis occurs after exposure of bone to high levels of adrenal glucocorticoids. In cultured bone cells, glucocorticoids have also been found to reduce the synthesis of proteins (8) and RNA (9). High affinity cytoplasmic receptors for glucocorticoids have been identified in bone cells (10) and, presumably, the effects of glucocorticoids on bone formation reflect direct inhibition of osteoblast activity.

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## EXPERIMENTAL PROCEDURES

### Materials

- Minimal essential medium and fetal calf serum were purchased from Grand Island Biological Co., NY. Polystyrene tissue culture ware was manufactured by Falcon, CA or Costar, MA. [6-C]Citric acid (4.5 mCi/mmol) was purchased from New England Nuclear, MA. [6-3H]Glucosamine (6.3 Ci/mmol) and [1-14C]Proline (225 mCi/mmol) were obtained from ICN Pharmaceuticals, CA. [1-14C]Leucine (105 Ci/mmol) was bought from Amersham Corp., IL. Bovine parathyroid hormone (1300 U.S.P. units/mg) was a gift of the National Pituitary Agency, National Institutes of Health. Prednisolone (Hydeltrasol) was obtained from Merck, Sharp and Dohme, PA. Dexamethasone was manufactured by Elkins-Sinn, Inc., NJ and methylprednisolone (Depo-Medrol) by Upjohn, MI. ACS aqueous scintillant was purchased from Amersham, IL. Hyamine-10x was manufactured by Packard, IL.

### Methods

- Osteoclast-like and osteoblast-like bone cells were prepared by sequential digestion of mouse calvaria and cultured as previously described (23, 24) in minimal essential medium containing 10% fetal calf serum (complete medium). The cells were subdivided after 6 days in primary culture, and the replicate cell cultures were exposed to various levels of glucocorticoid or parathyroid hormone, or both, for 24 h, after which the rate of citrate decarboxylation and the synthesis of citrate decarboxylase and the synthesis of hyaluronate, collagen, and protein were measured. For hyaluronate synthesis, [6-3H]glucosamine (1 μCi/ml) was added to the cultures for 4 h, after which the cell layer and growth medium were digested together at pH 5.0 with papain (4.0 μg/ml) for 24 h at 55°C. [3H]Hyaluronate was isolated by chromatography on DEAE-cellulose as previously described (25, 26). To assay citrate decarboxylation, [6-14C]citrate (0.2 μCi/ml) was added to the cultures for 2 h and the 14CO2 evolved by the cells was trapped on Hyamine-saturated filter paper fans (25, 26). The incorporation of [4,5-3H]Leucine into acid-precipitable radioactivity was used as an indication of protein synthesis. Cell cultures were exposed to 1.0 μCi/ml of [1-14C]proline for 4 h, following which the medium was aspirated off and the monolayers rinsed twice with ice cold phosphate-buffered saline. The cell layers were frozen and then extracted three times with cold 5% trichloroacetic acid. The insoluble residue was dissolved in 0.2 ml of 0.1 N NaOH at 60°C for 2 h. Collagen synthesis was measured as the

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**Basal Activities and Hormone Responsiveness of Osteoclast-like and Osteoblast-like Bone Cells Are Regulated by Glucocorticoids**

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incorporation of \(^{14}\)C]proline into acid-insoluble radioactivity that was subsequently rendered soluble by heating in 5% trichloroacetic acid (29). At the indicated time, \(^{14}\)C]proline (0.5 \(\mu\)Ci/ml) was added to the cell cultures for 75 min, after which the medium was aspirated off. Subsequently, the cell layers were washed four times over a 24-h period with cold 5% trichloroacetic acid. The radioactivity rendered soluble in hot trichloroacetic acid was used as a measure of collagen (29). The insoluble residue was solubilized in 0.1 M NaOH and used as a measure of noncollagenous protein. All samples were counted in ACS aqueous scintillant in a Searle Mark II liquid scintillation counter. Alternatively, samples were digested in chromatographically pure bacterial collagenase, as previously described (26). Results from each method varied no more than 10%.

RESULTS

In these studies, the effect of glucocorticoid on the basal activities of isolated OC and OB (23–27) bone cells was investigated. Previous studies had demonstrated that OC cells were characterized by high basal levels of hyaluronate synthesis, whereas OB cells expressed high basal rates of citrate decarboxylation and collagen synthesis (26). Fig. 1 shows that over a wide range of prednisolone doses that would be regarded as subphysiological to physiological (10⁻³ M to 10⁻¹ M) OC cell hyaluronate synthesis underwent no further inhibition. On the other hand, at physiological doses prednisolone was without effect on basal citrate decarboxylation in either cell type. However, OB but not OC cell citrate decarboxylation declined markedly at pharmacological doses of prednisolone (>10⁻⁶ M). This inhibition was paralleled by a decrease in OB cell collagen synthesis at similar pharmacological doses of prednisolone (Fig. 2).

To investigate whether the inhibitory effects of glucocorticoid on OC and OB cell metabolism arose from selective inhibition of specific cell functions or from general cell toxicity, [³H]leucine incorporation into acid-precipitable radioactivity was measured in these cells (Table I). OC cells demonstrated no decrease in the acid-precipitable radioactivity incorporated over a wide range of prednisolone doses (10⁻⁴ M to 10⁻¹ M)

although major decreases in hyaluronate synthesis were seen at these doses (Fig. 1). In contrast, inhibition of OB cell citrate decarboxylation and collagen synthesis at >10⁻⁶ M prednisolone was accompanied by a decrease in the incorporation of [³H]leucine into acid-precipitable radioactivity (Table I). It thus appeared that prednisolone inhibition of OB cell function represented a general inhibition of cellular functions, whereas the effect of this agent on OC cells was due to selective inhibition of gene expression. In Fig. 3, the ability of other glucocorticoid compounds to bring about inhibition of OC and OB cell function was investigated. It was found that dexamethasone and methyl prednisolone were also inhibitors of OC cell hyaluronate synthesis and OB cell citrate decarboxylation. Of these three compounds, dexamethasone was the most potent, followed by prednisolone, with methylprednisolone being the least effective. At equivalent doses, the mineralocorticoid aldosterone was without effect (results not shown) on these parameters.

In view of these inhibitory effects of glucocorticoids on OC cell marker activity and OB cell metabolism, a possible effect on cell hormone sensitivity was studied. PTH has previously been shown to increase hyaluronate synthesis in OC cells and to decrease citrate decarboxylation in OB cells (25), and so the effect of PTH on these activities was measured in OC and OB cells cultured in physiological doses of prednisolone. It was found (Fig. 4) that in the presence of glucocorticoids, OB

TABLE I

\[\begin{array}{lll}
\text{Prednisolone} & \text{OC cells} & \text{OB cells} \\
\hline
M & \text{Dpm/10}^5 \text{cells} & \text{Dpm/10}^5 \text{cells} \\
\text{0} & 16,600 ± 980 & 21,600 ± 1,500 \\
10^{-4} & 15,600 ± 1,740 & 15,600 ± 1,100^a \\
10^{-6} & 15,520 ± 1,600 & 10,070 ± 2,300 \\
10^{-7} & 16,010 ± 1,100 & 19,620 ± 300 \\
10^{-8} & 17,084 ± 1,700 & 20,040 ± 380 \\
10^{-9} & 16,010 ± 875 & 21,030 ± 400 \\
\end{array}\]

\(\text{a} \) Significantly different from control: \(p < 0.002.\)

Fig. 1. Effects of prednisolone on OC and OB bone cells. Bone cells were cultured for 24 h in complete medium in the presence or absence of prednisolone (10⁻³ M to 10⁻¹ M). To measure hyaluronate synthesis, [³H]glucosamine (1.0 \(\mu\)Ci/ml) was added to the cultures for 20 to 24 h and OC (⃣⃣⃣) and OB cells (○○○) were then frozen and processed as described under "Methods." Citrate decarboxylation was measured in OB (△△△) and OC (△△△) cells by adding [⁶⁰³C]citrate (0.2 \(\mu\)Ci/ml) for 24 h and absorbing the \(^{14}\)CO₂ evolved on filter paper fans saturated with Hyamine 10X.
cells responded to 1\(^{3}1\)H with a larger decrease in citrate decarboxylation than in its absence. This was seen at all hormone concentrations tested. However, this effect was especially striking at submaximal doses of PTH where hormone levels as low as 2 \( \times 10^{-13} \) M could be measured in contrast to a limit of 10\(^{-10}\) M when the glucocorticoid was omitted. At low PTH doses, therefore, a 500-fold increase in OB cell sensitivity to PTH was seen with glucocorticoids. Enhancement of PTH responsiveness was also seen when OC cells were exposed to PTH in the presence of 10\(^{-7}\) M prednisolone (Fig. 4) and the per cent increase in hyaluronate synthesis was measured. As in OB cells, the enhancement of OC cell response to PTH was most apparent at submaximal hormone doses.

Table II shows that this enhancement of OC and OB cell responsiveness to PTH was glucocorticoid dose dependent. In OC cells, glucocorticoid enhancement of PTH responsiveness became apparent at 10\(^{-8}\) M, was maximally expressed at 10\(^{-7}\) M, and decreased at higher doses. No enhancement of OC cell PTH response was detectable at glucocorticoid doses above 10\(^{-5}\) M although OC cells still retained the ability to respond to PTH at these high steroid levels. In contrast, the inhibitory effects of PTH on OB cell citrate decarboxylation were maximally enhanced at glucocorticoid levels as low as 10\(^{-9}\) M, remained maximal up to 10\(^{-7}\) M, and decreased thereafter with no OB cell response to PTH being apparent at 10\(^{-5}\) M glucocorticoid when decreases in protein synthesis were also occurring (Table I).

## DISCUSSION

These studies demonstrate that glucocorticoids modulate both the basal activities and hormonal responses of isolated bone cells. Specific and different changes are elicited in OC and OB bone cells. These include a marked reduction in basal hyaluronate synthesis in OC cells at low to normal prednisolone doses with only a slight effect on this activity in OB cells. This decrease in the marker activity used to denote osteoclast-like activity represents a specific inhibition in that it is not accompanied by a decrease in OC cell protein synthesis. In addition, OC cell marker functions appear relatively resistant to pharmacological doses of glucocorticoids, having been already inhibited by physiological doses, and OC cell hyaluronate synthesis at 10\(^{-7}\) M prednisolone is not very different from that seen at 10\(^{-7}\) M. In contrast to these findings in OC cells, prednisolone doses even at high normal levels (up to 10\(^{-4}\) M) do not inhibit OB cells. However, pharmacological doses of prednisolone produce marked inhibition of citrate decarboxylation and collagen synthesis in OB cells. This inhibition of the marker activities used to denote osteoblast-like phenotype represents nonspecific inhibition of OB cell metabolism, as protein synthesis is also decreased. These results are
consistently with those previously reported for unseparated bone cells from fetal rat calvaria (8, 9).

Taken together, these results suggest that under physiological conditions, osteoblast but not osteoclast functions may be under negative control by glucocorticoids. Decreased levels of glucocorticoid would, therefore, be expected to permit increased osteoelastic activity, which would be in accord with the reports that hypercalcemia accompanies adrenal hypofunction (16, 17). The finding that OB but not OC cells show a decrease in protein synthesis at pharmacological doses of prednisolone also suggests that OC cells are more resistant to high levels of glucocorticoids, and steroid treatment could perhaps decrease bone formation by osteoblasts without reducing bone resorption by osteoclasts. Such an uncoupling of bone formation and resorption could be the basis of the well-documented osteoporosis of steroid therapy. In addition, the secondary hyperparathyroidism often associated with steroid impairment of calcium transport by the gut (30) would further enhance osteoporosis since osteoclasts retain the ability to respond to PTH at steroid doses at which osteoblast anabolic functions are reduced.

In terms of hormone response, one of the most remarkable effects of glucocorticoids on these bone cells is the apparent increase in their sensitivity to submaximal doses of PTH. The glucocorticoid enhancement of OC and OB cell response to PTH permits detection of PTH doses up to 500 times less (2 × 10^(-13) M) than previously possible with these cells (23, 25) and brings the PTH response of isolated bone cells within the range of physiological hormone levels. These findings suggest that glucocorticoids in vivo may modulate bone cell metabolism in a manner that permits them to respond to small fluctuations in PTH secretion. However, it must be pointed out that in OC cells the enhancing effects of glucocorticoid on PTH response may be more apparent than real. Basal hyaluronate synthesis is depressed in OC cells cultured in 10^-7 M glucocorticoid so that although the per cent increase induced by PTH is greater in glucocorticoid-treated cells, the total amount of hyaluronate synthesized is no more than that seen by PTH alone (26).

The mechanism whereby prednisolone enhances PTH induction of hyaluronate synthesis in OC cells and inhibition of citrate decarboxylation in OB cells remains to be established. Glucocorticoid modulation of bone cell hormone responsiveness could occur at a number of obvious points including membrane changes that alter the cell permeability to effector molecules such as calcium (27). Conceivably, glucocorticoids could also increase the number of available receptors for PTH, promote the linking of PTH receptors to adenylate cyclase, or increase the affinity of the receptors for PTH. In addition, glucocorticoids could modulate bone cell genome expression at the transcriptional level as in other systems (21, 22). A possible mechanism for glucocorticoid action in bone cells is suggested by the work of Chen and Feldman (31) who have elegantly demonstrated that glucocorticoids inhibit phosphodiesterase and, thereby, potentiate the cyclic AMP increases induced by PTH in OC and OB bone cells. In addition, the PTH-inducible adenylate cyclase activity of OB bone cells is increased (32) with dexamethasone.

To relate these present findings with PTH to an in vivo situation, one could speculate that glucocorticoids permit both OC and OB cell types to be finely regulated by small changes in PTH and possibly calcitonin (14) secretion. During adrenal insufficiency, both osteoblasts and osteoclasts would become less sensitive to these bone active hormones. However, the basal activity of the osteoclasts would increase due to release from glucocorticoid repression, and very high levels of calcitonin would be required to control the ensuing hypercalcemia. It is at present unknown whether glucocorticoid enhancement of bone cell responsiveness is limited to PTH or includes other bone active hormones such as calcitonin and 1,25-dihydroxyvitamin D. In this regard, Raiz et al. (14) have reported enhanced calcitonin responsiveness in glucocorticoid-treated bones. Enhancement of hormone responsiveness by glucocorticoids may also not be limited to bone since glucocorticoid modulation of hormonal response has been reported in adrenal medulla responding to nerve growth factor (33). It remains to be seen whether modulation of target tissue responsiveness to peptide hormones is a general characteristic of glucocorticoids.

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