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Characterization of Cells Isolated and Cultured from Human Bone (41843)

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Abstract. Cells isolated from samples of human iliac crest and human femoral heads by collagenase digestion have been successfully cultured in Fitton–Jackson modified BGJ_b culture medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and fetal calf serum (10%). Although only a low proportion of the cells survived the initial plating (<1%), cells established in culture were readily passaged. Examination of cells obtained at intervals during the collagenase digestion showed that the percentage of cells that attached increased with time of digestion. Rapid sample preparation of rat bone did not substantially increase the number of cells attaching. Thus, it seems unlikely that the low survival was due to loss of viability during sample transportation and preparation. Of several media tested BGJ_b supplemented with 10% fetal calf serum supported the best growth. Population doubling time averaged 104 hr. Cultured human bone cells were assayed for alkaline phosphatase activity using the azo dye method with naphthol ASTR phosphate as the substrate. A portion of the cells (19%) demonstrated high activity in all cultures examined regardless of the passage number of the culture. Autoradiography of cells exposed to [^{3}H]thymidine showed incorporation of the label into both alkaline phosphate-positive and -negative cells. The stimulation of cell proliferation by growth factors was studied by determining the incorporation of [^{3}H]thymidine into DNA. The specific skeletal growth factor from human bone stimulated cell proliferation several-fold with a half-maximal effect at 5 μg/ml. Insulin, epidermal growth factor, and a crude preparation of somatomedin C also stimulated cell proliferation.

The study of bone diseases has been largely focused on systemic causes. Local bone cell defects are less well known and more difficult to study. One way to examine the bone cell activity is to isolate and culture the cells *in vitro*. Methods for isolating and culturing bone cells have been developed and applied to a number of species (1–6). Work on human bone cells has been largely confined to isolation of tumor cell lines. However, Singer and co-workers have grown human bone cells from explants of bone biopsies of normal subjects and from patients with Paget's disease (5, 6). They demonstrated that human bone cells could be maintained in long-term cultures.

An initial report from this laboratory demonstrated the successful isolation and culture of human bone cells from samples of iliac crest (7). These cells have the expected characteristics of bone cells including alkaline phosphatase activity and 1α-hydroxylase activity for 25-hydroxyvitamin D. The availability of a method of preparation and culture of human bone cells opens up the possibility of many different types of direct studies on the cells under carefully controlled conditions. For example the proliferation and response to mitogens can be compared in cells from normal and diseased subjects.

The present study examines the conditions for isolating and culturing human bone cells from samples of femoral heads and iliac crests. In addition the cultured cells have been characterized by examining their proliferation response to various systemic and local growth factors.

Materials and Methods. *Bone samples.* Human bone cells were obtained from needle biopsies of the iliac crest and from femoral heads removed during hip replacement operations. The entire iliac crest biopsy was used, but only the trabecular bone from the femoral head was used. The data in the tables and figures was obtained mainly from cells isolated from two femoral heads from female subjects and two iliac crest biopsies. Iliac 1 cells were from a female osteoporotic and Iliac 2 cells were from a male hyperparathyroid subject. Rat bone cells were isolated from bone samples obtained by dissection of the distal femoral metaphysis of 100-g rats. Chick bone cells were

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obtained from fetal chick calvariae as previously described (1).

Bone cell isolation. Bone samples were rinsed briefly in Fitton–Jackson modified BGJ_b culture medium (from Gibco), diced finely with a scalpel, and placed in a sterile tube with 5 ml of crude collagenase (2 mg/ml) in BGJ_b culture medium. The samples were incubated for 2 hr at 37°C with shaking. The cell suspension was drawn off and the residual bone chips rinsed three times with culture medium or Hank's buffered salt solution. The combined cell suspension and rinse solution were centrifuged at 150*g* for 12 min in a refrigerated centrifuge. The cell pellet was washed by resuspending in fresh medium and centrifuging again. The cell pellet was resuspended in culture medium containing 10% fetal calf serum and plated at a density of 10^3–10^4 cells/mm^2.

Cell culture. The culture medium was Fitton-Jackson modified BGJ_b culture medium and contained penicillin (100 units/ml), streptomycin (100 μg/ml) and 10% heat inactivated fetal calf serum unless otherwise noted. The culture medium was changed every 2–3 days. Cultured cells were freed for passaging by exposure to trypsin (0.05%)–EDTA (0.02%) for 5 to 15 min following a rinse with serum free medium.

Population doubling. To determine the population doubling time cells were plated in microwell plates. For most determinations 1.5×10^3 cells were plated in the 30 mm^2 wells. After cultures were established cell density measurements were made at the beginning and end of a 3–7 day period. To determine the cell density the medium was removed, the cells were rinsed once with serum free medium and 0.05% trypsin, 0.02% EDTA was added. When the cells had rounded up, but were still attached, they were counted in an inverted microscope using a calibrated grid eye piece and phase contrast optics. Five grid areas (0.3 mm^2) were counted in each well and 3 to 6 wells were analyzed per assay. Different wells were counted for initial and final values and the cells discarded after counting. Doubling time was calculated from the equation: doubling time = number of days/3.32 (log final cell count − log initial cell count).

Thymidine incorporation. The incorporation of [^{3}H]thymidine into DNA was determined using a method developed by Gospodarowicz *et al.* (8) and adapted for chick calvarial cells in our laboratory (1). The bone cells were plated in microwell (30 mm^2/well) or multiwell (200 mm^2/well) plates in BGJ_b culture medium containing 10% fetal calf serum at a density of 100 cells/mm^2. After several days, the cells were rinsed twice with serum-free medium and incubated for 4–24 hr in serum-free medium. The medium was drawn off and replaced with medium containing the test agents. After 16 hr [^{3}H]thymidine was added (2 μCi/ml for multiwell plates, 5 μCi/ml for microwell plates). The medium was drawn off 4 hr later. The cells were then rinsed twice with phosphate-buffered saline and frozen to kill the cells. The cells were removed from the plate with a Q-tip swab dipped in 12.5% trichloroacetic acid. The Q-tips were rinsed twice with trichloroacetic acid and once with 95% alcohol for 10 min each. The end of the Q-tip was clipped off, placed in a scintillation vial with 5 ml of aquasol (New England Nuclear), and the level of radioactivity determined in a scintillation counter. This Q-tip method has been previously shown to give comparable values to other methods for determining uptake of [^{3}H]thymidine into DNA (1).

Alkaline phosphatase. The histochemical localization of alkaline phosphatase in cells was determined by the azo dye method of Burstone (9). The substrate was naphthol ASTR phosphate (3-hydroxy-2-naphthoic acid 4-chloro-2-methylanilide, sodium salt) (80 mg/100 ml). The diazonium was red violet LB diazonium salt (5-chloro-4-benzamido-2-methylbenzene-diazonium chloride, hemizinc chloride salt) (60 mg/100 ml). The reagents were dissolved in Tris buffer (50 m*M*) containing NaCl (100 m*M*), KCl (5 m*M*), $CaCl_2$ (1 m*M*), and $MgCl_2$ (1 m*M*) and adjusted to pH 8.6. For the determination the medium was removed and the cell cultures rinsed once with buffer before incubating for 20 min at 37°C with the substrate.

Materials. Crude collagenase and the culture media were obtained from Gibco. Somatomedin was a crude preparation provided by Dr. M. Spencer, University of California, San Francisco. Epidermal growth factor, fibroblast growth factor, multiplication stimulatory factor, platelet-derived growth factor,

and insulin were obtained from Collaborative Research. Transferrin was obtained from Sigma Chemical Company. Human skeletal growth factor was prepared as previously described (10).

Results. *Primary cultures.* Preparation of cells by a standard approach of freeing the cells by crude collagenase digestion and plating the cells in Fitton–Jackson modified BGJ_b culture medium supplemented with 10% heat-inactivated fetal calf serum resulted in cell cultures with a fibroblast-like morphology. Cells have been successfully isolated and established in culture from more than 10 different bone specimens including both iliac crest and femoral head specimens. Cells attaching to the culture dish were initially bipolar and then became more stellate similar to what has been reported for bone cells from human bone explants (5, 6) and from other species (1, 2). Some marrow cell types were evident initially but disappeared during continued culture. As the bone cells approached high density there was frequent cell to cell contact through cell processes. Cells did not form confluent epitheleoid-like monolayers but tended to remain separated and began to form multilayers before all areas of the dish were covered.

Although cells were successfully cultured from a number of samples, the percentage of isolated cells that attached and spread out on the bottom of the dish was very low (<1%). Initially some cultures of iliac crest needle biopsy specimens were unsuccessful probably because of the small sample size and low cell survival. In more recent work, cultures have been more consistently obtained (seven out of eight specimens).

Investigation of low survival. Several experiments were carried out to determine if the low cell survival was due to loss of viability during sample preparation. Cells from a human femoral head specimen were drawn off at different times during the collagenase digestion procedure. Aliquots of the cells were taken for counting and Giemsa staining of cell smears. Cell samples obtained by rinsing the bone samples contained mainly red blood cells but also contained some cells that attached (Table I). A high proportion of the cells isolated during the first 20 min of collagenase digestion could be identified as marrow cells.

TABLE I. EFFECT OF TIME OF TREATMENT WITH CRUDE COLLAGENASE (2 mg/ml) ON CELL RELEASE (NOT INCLUDING RED BLOOD CELLS) FROM SAMPLES OF HUMAN FEMORAL BONE (FEMUR 1) AND ON THE PERCENTAGE OF CELLS ATTACHING IN PRIMARY CULTURE

Treatment period (min)	Cells released[a] (% of total)	Cells surviving (% of cells)
Initial rinse	5	0.075
0–20	55	0.083
20–60	21	0.29
60–120	18	1.1

[a] Mean of two determinations except for only one value for cell survival at 60–120 min. The precision of the determination of cell survival (SD/mean × 100) estimated from the difference between duplicates was 56%.

A greater percentage of the cells released during the 20- to 60-min period and the 60- to 120-min period attached and spread in the culture dishes than those released earlier in the digestion. Thus, prolonged digestion with collagenase does not appear to be responsible for the low survival.

There was usually a significant and unavoidable lapse of time between the removal of the bone sample from the patient and the start of the collagenase digestion. To determine if this delay could be responsible for the low percentage of cells attaching in primary culture, cells were isolated from rat femoral metaphysis at different times after removal of the bone from the rat. A rapid sample preparation (less than 30 min between killing the rat and the start of digestion) was compared with a slow preparation (samples stood for 1 additional hr at room temperature in culture medium after removal from the rat). The rapid sample preparation did not substantially increase the percentage of isolated cells surviving (0.083% vs 0.051%, average of two samples). Of the cells isolated from rat femoral metaphysis, a relatively low percentage attached and spread out similar to the cell isolates from human trabecular bone. Isolation of rat bone cells using the protease inhibitor tosyl-lysine-chloromethyl ketone in the isolation medium also failed to increase the number of rat bone cells attaching (0.018% vs 0.028% for controls). From the above observations it seems unlikely that the low survival of the human bone cells is due to a rapid loss of viability during sample transportation and preparation.

In primary cultures of bone cells it was

noted that any large fragments of bone, i.e., those that contained several trabeculae, became covered by a layer or layers of cells after about a week in culture. No cells were evident on the surfaces of bone chips immediately following collagenase digestion, nor did small bone chips become covered by layers of cells in primary cultures until late in the cultures when cells also covered the bottom of the dish. Thus, the cells did not appear to preferentially colonize the bone surfaces. Cells were first evident in the large pores within the large bone chips. Thus, they probably arose from cells trapped within the pores of the large chips.

Passaging cells. Cultured human bone cells were readily passaged with either trypsin (0.05% trypsin, 0.02% EDTA in phosphate-buffered saline) or pancreatin (25%). Cultured cells were also passaged with crude collagenase (1 to 2 hr in 2 mg/ml crude collagenase in BGJ_b culture medium at 37°C). Collagenase treatment appeared to give slightly better cell dispersions than trypsin treatment when cultures with high cell densities were passaged. Passaged cells plated well (60–90%) even at relatively low plating densities (Fig. 4).

Human bone cells were successfully plated in primary culture in either BGJ_b or Ham's F12 containing 10% fetal calf serum. Of several media tested for their ability to support cell growth BGJ_b appeared to support the best growth. Cell density after 2 weeks of culture was 321 cells/mm^2 in BGJ_b but 87 and 159 for medium 199 and CMRL 1060, respectively (mean of two determinations). Little cell proliferation occurred in the absence of fetal calf serum. The optimum concentration appears to be 10% or above (Fig. 1).

Alkaline phosphatase activity. One of the characteristics of osteoblasts is the presence of high alkaline phosphatase activity. When assayed cytochemically for alkaline phosphatase activity between 10 and 40% (average 19%) of the cells in a given preparation (femur or ilium) demonstrated intense staining (Fig. 2, Table II). Staining was evident over the entire cell. This is consistent with the activity being located in the cell membrane. These results demonstrate that there are high alkaline phosphatase levels in the cell membranes of a significant portion of the cultured human bone cells. There was some gradation in the levels of staining intensity between cells. In cultures

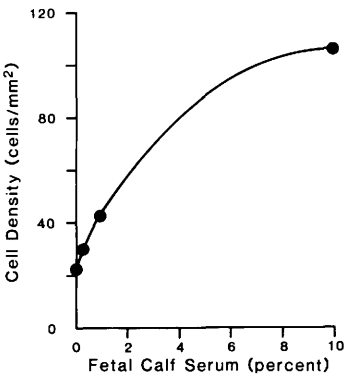

FIG. 1. Increase in cell density with increasing levels of fetal calf serum. Human bone cells (Femur 1) were incubated in BGJ_b culture medium containing varying levels of fetal calf serum for 8 days. Each point is the mean of three determinations.

of human foreskin cells few cells (<1%) were positive for alkaline phosphatase under the same staining conditions. The percentage of positive cells did not appear to decrease with age of the culture.

High alkaline phosphatase activity is a characteristic of osteoblasts or osteoblast precursors. The presence of substantial activity (cells clearly stained after 20 min at 37°C) in some of the cultured cells indicates their osteoblastic nature. The total number of alkaline phosphatase-positive cells increased with time in the bone cell cultures. Because mature osteoblasts do not divide *in vivo* the increase in alkaline phosphatase-positive cells in culture could be due to differentiation from inactive precursors rather than division of the alkaline phosphatase-positive cells. When cultures of human bone cells were exposed to [^{3}H]thymidine for 24 hr, uptake of thymidine was evident in both alkaline phosphatase-positive and -negative cells by autoradiography (Fig. 2). Thus the alkaline phosphatase-positive cells continue to synthesize DNA and presumably divide as well.

Cell proliferation assays. To determine the rate of population growth the change in cell density with time was determined (Fig. 3). Population growth closely approximated a logarithmic rate between cell densities of 50 to 400 cells/mm^2 and decreased at higher densities. For cells isolated from three different femoral heads the population growth rate in BGJ_b medium supplemented with 10% fetal

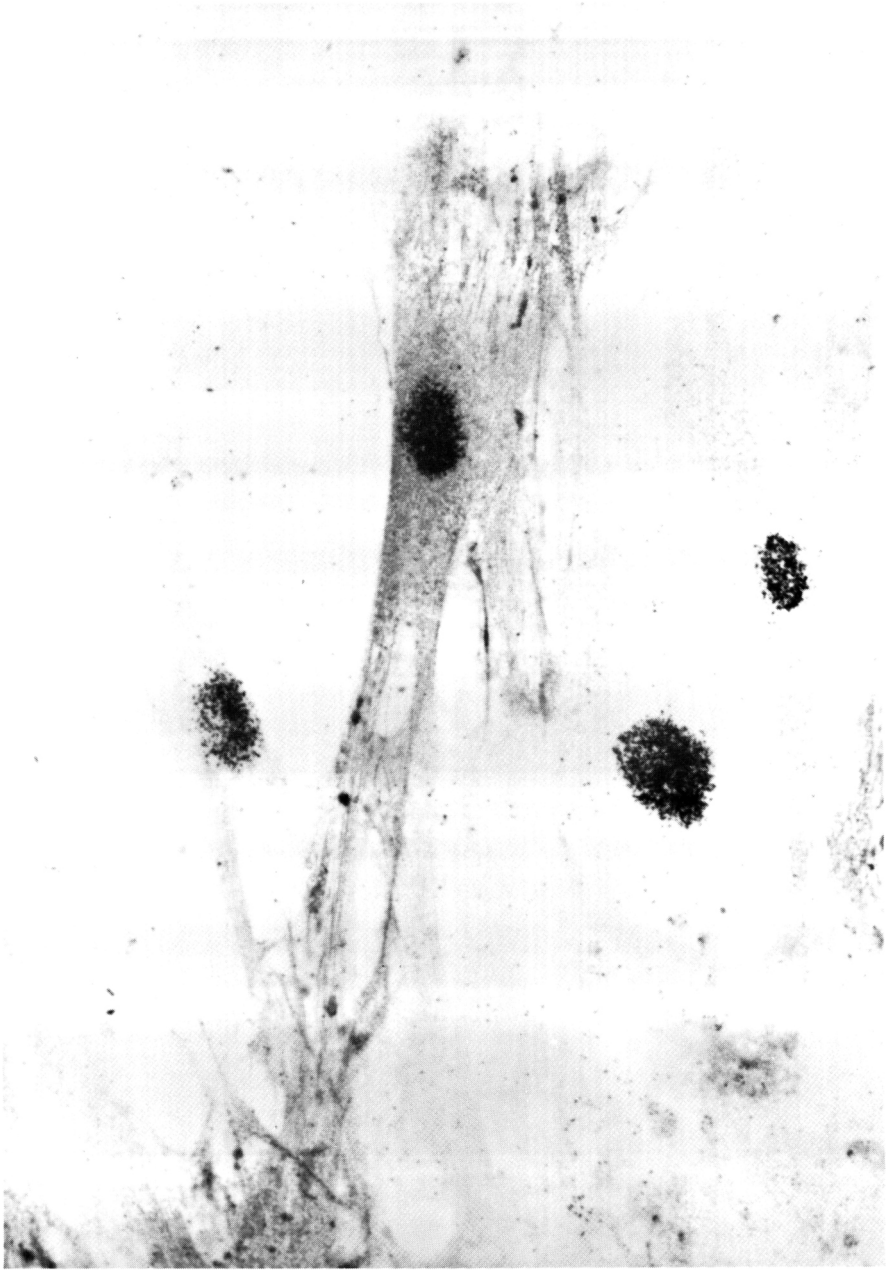

FIG. 2. Autoradiographs of human bone cells (Femur 1) exposed to [^{3}H]thymidine and stained for alkaline phosphatase. Round clusters of grains indicate nuclei which have incorporated [^{3}H]thymidine into DNA. Labeled nuclei are evident in some cells which stained because they contain alkaline phosphatase and in some which were unstained. Cells cultured in chamber slides were exposed to 2 μCi/ml [^{3}H]thymidine for 24 hr, rinsed twice with fresh medium, and stained for alkaline phosphatase activity. The chamber was removed and autoradiography performed as previously described (19) except that the scintillator was not used and the slides were exposed for 10 days.

calf serum averaged 0.23 doublings per day for a generation time of 104 hr. Cells isolated from the trabecular bone of the femoral head gave similar growth rates to those cells isolated from the iliac crest (Table III). The population growth rate was determined at several different

TABLE II. PERCENTAGE OF ALKALINE PHOSPHATASE-POSITIVE CELLS IN HUMAN, CHICK, OR RAT BONE CELL CULTURES

Bone sample[a]	Passage	Percentage of total
Human bone		
Femur 1	6	13
Femur 2	3	29
Iliac crest 1	4	10
Iliac crest 2	4	14
Chick calvaria[a]		10
Rat femur[a]		10

[a] Chick and rat bone cells were from primary cultures, Human bone cells were isolated from four different bone specimens and analyzed after several passages.

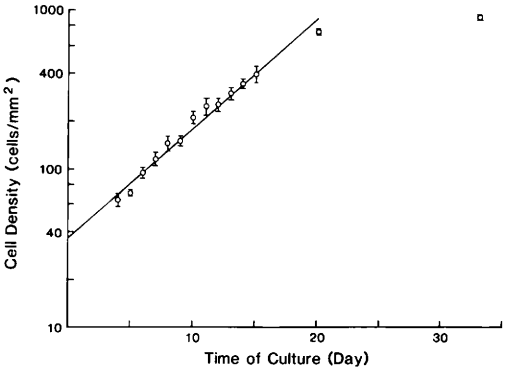

FIG. 3. Increase in cell density with time in culture. Human bone cells incubated in BGJ_b + 10% fetal calf serum. The line was drawn from the regression equation from the correlation (r = 0.990) between the log of the cell density and time of culture between 4 and 20 days. Each point is the mean ± SE of eight determinations.

passages for a few samples. Although a firm conclusion cannot be drawn because of the limited amount of data, an analysis of the data indicated that there was no effect of passage number on the population growth rate (Tables III, IV).

Cell proliferation was also assayed by determining the incorporation of [^{3}H]thymidine into DNA. Several parameters were investigated to determine the best conditions for cell proliferation assays. Passaged human bone cells plated well even at low cell concentrations (Fig. 4). The cell proliferation rate was highly dependent upon the density of the cell cultures. Figure 5 gives the results of two experiments in which passaged cells were plated over a range of different cell densities with the incorporation of [^{3}H]thymidine expressed as a function of cell density. The incorporation rate reached a maximum at a density of 100 cells/mm^2 and decreased at higher cell densities.

Effects of growth factors. A specific skeletal growth factor (SGF) has been isolated from human bone in our laboratory (10, 11). SGF stimulates the proliferation of cultured human bone cells (Fig. 6). The estimated concentration for half-maximal stimulation was 5 μg/ml of the human SGF. This is similar to the concentration required to stimulate cell proliferation by fetal chick calvarial cells (11).

Several agents known to increase growth of cultured cells were tested under serum-free conditions using the [^{3}H]thymidine incorporation assay. Insulin at 1 μg/ml consistently stimulated cell proliferation (Femur 1, 230, Iliac 1, 260, and Iliac 2, 304% of control, mean of four to six determinations). Cells isolated from femoral heads responded similarly to those isolated from the iliac crest. Transferrin has been shown to stimulate proliferation of cells from a number of tissues (12). However, neither purified human transferrin nor transferrin plus Fe were stimulatory to the bone cells (Table V). BGJ_b medium does not con-

TABLE III. POPULATION GROWTH RATE IN GENERATIONS (CELL DOUBLINGS) PER DAY OF HUMAN BONE CELLS FROM THREE DIFFERENT SAMPLES AT DIFFERENT PASSAGES

	Passage						
Bone sample[a]	1	2	3	4	5	6	7
Femur 1	0.195	0.160	—	—	—	0.141	0.153
Iliac crest 1	—	—	0.182	0.101	—	0.155	—
Iliac crest 2	—	—	—	0.248	—	0.191	—

[a] There were three to six wells counted per assay.

TABLE IV. ANALYSIS OF VARIANCE OF THE DETERMINATION OF POPULATION GROWTH RATE (GENERATIONS/DAY) OF HUMAN BONE CELLS[a]

Source of variance	Degrees of freedom	Variance	F	Significance
Between samples	2	0.01107	3.77	$P < 0.05$
Between passages	6	0.00326	1.11	ns
Within assay[b]	19	0.00293		

[a] The change in cell density with time was determined for samples from three different subjects assayed at two to four different passages with three to six wells counted per assay (see Table V).

[b] Within assay deviation, 0.054. Precision of assay (deviation/mean × 100) 32%.

tain several of the trace metals. The addition of a mixture of Fe (1 μm), Cu (1 μm), Zn (2.5 μm), Mn (0.5 μm), and Co (2.5 μm) also failed to stimulate thymidine incorporation (84 ± 26% of control, mean ± SD).

In addition to insulin several other growth factors were tested for their effect on proliferation of cultured human bone cells (Table V). Epidermal growth factor and a crude preparation of somatomedin C stimulated cell proliferation. Platelet-derived growth factor was inhibitory in the presence of insulin. Cells isolated from fetal chick calvarial cells were also inhibited by platelet-derived growth factor

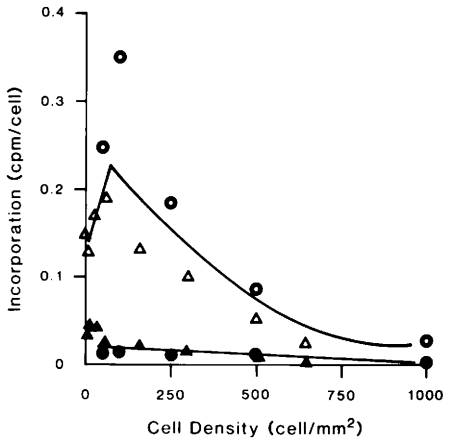

FIG. 5. Incorporation of [³H]thymidine into DNA of human bone cells (Iliac 2) as a function of cell density in the presence (open symbols) or absence (closed symbols) of 10% fetal calf serum. Cells were exposed to fetal calf serum for 20 hr and to [³H]thymidine for 4 hr. Triangles represent one experiment and circles represent a second. There were six determinations per point.

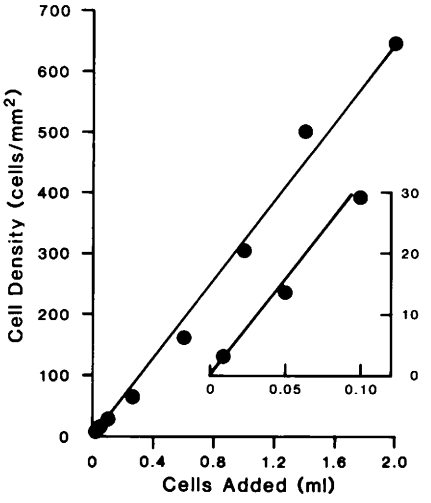

FIG. 4. Number of passaged human bone cells (Femur 1) attaching as a function of the amount of cell suspension added 24 hr after plating in BGJ_b culture medium containing 10% fetal calf serum. The inset shows a magnified portion of the graph at the smallest amounts of cell suspension added. Each point is the mean of two determinations.

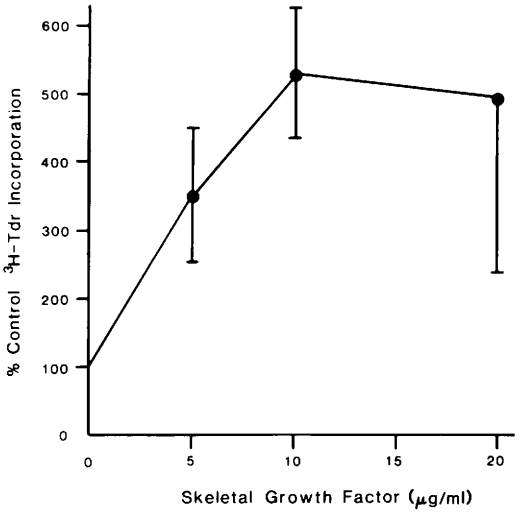

FIG. 6. Incorporation of [³H]thymidine into DNA of human bone cells (Femur 1) as a function of the concentration of human skeletal growth factor. Cells were exposed to the factor for 20 hr and to the thymidine for 4 hr. Symbols represent the means and SD of six samples.

TABLE V. EFFECT OF MITOGENS ON [^{3}H]THYMIDINE INCORPORATION INTO HUMAN BONE CELLS AND FETAL CHICK CALVARIAL CELLS

Mitogens[a]	Concentration (μg/ml)	Uptake as percentage of control[b]	
		Human cells	Chick cells
Insulin	1	199 ± 76[c]	313 ± 219[c]
Somatomedin	1	161 ± 35[c]	337 ± 75[d]
Epidermal growth factor	0.5	153 ± 22[c]	207 ± 16[d,e]
Fibroblast growth factor	0.5	127 ± 66	—
Multiplication stimulatory activity	5	78 ± 66	—
Platelet derived growth factor	1	—	157 ± 27[c]
Platelet derived growth factor[f]	1	49 ± 29[c]	45 ± 45[c]
Transferrin + Fe (4 μ*M*)	5	34 ± 18[d]	—

[a] Factors were tested in separate experiments. Human cells were from passages three to six of Femur 1.
[b] Mean ± S.D., six samples per assay.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] Concentration of the factor was 1 μg/μl.
[f] Assayed in the presence of 1 μg/ml insulin.

in the presence of insulin but were stimulated by this factor in its absence.

Discussion. Our studies show that cells can be isolated and cultured from samples of human bone using the same collagenase digestion methods used for studying bone cells from other species. The cells obtained by these methods appear to have the same characteristics as those isolated from explants of normal or Pagetic human bone by Mills *et al.* (5, 6). The percentage of alkaline phosphatase-positive cells (19 vs 25%), the population doubling time (4.4 vs 4–10 days), and the general morphology of the cells in culture appear to be similar for cells obtained by collagenase digestion of the bone matrix in our studies or by culture of explants by Mills and co-workers. The ability to obtain cultures of human bone cells allows the study of those cells from individual patients under the controlled conditions of *in vitro* culture. This provides a different avenue of approach to the investigation of bone diseases in which the defect is suspected to reside within the bone cells themselves. In addition, the mechanisms of action of various factors controlling bone metabolism can be examined at the cellular level with normal human bone cells and can be compared with results obtained with bone cells from other species.

The low percentage of cells that survive the isolation procedure and attach and spread out on the bottom of the culture dish makes it difficult to carry out any studies on primary cultures. Additional passages are necessary to obtain sufficient cells to study. However, once established, cultures can be readily passaged and appear to retain their characteristics in later passages. The reason for the low survival in primary culture remains unclear. Cells released from the bone late in the collagenase digestion period (after most marrow and red blood cells have been released) still had a low percentage of attachment. Therefore, the high number of other cell types present was not responsible for the low survival values. No evidence could be found for a loss in viability during sample preparation. The low survival appears to be a characteristic of the bone cells themselves.

Cell cultures started from the iliac crest samples and femoral head samples had similar proliferation rates, similar levels of alkaline phosphatase-positive cells and similar proliferation responses to addition of insulin. The agreement in these responses indicates that similar cell populations were being isolated and cultured from the two types of bone samples. Therefore, the parameters of cell culture study established with the larger femoral head samples should be applicable to the iliac crest specimens.

In a number of other bone cell culture studies it has been found that the characteristics

of the cultured bone cells changed with time (13–15). The levels of cell proliferation and alkaline phosphatase activity were maintained through a number of passages in our study. In addition the ability of the cultured human bone cells to convert 25-hydroxyvitamin D to 1α,25-dihydroxyvitamin D was previously shown to be maintained through at least five passages (7). Although a more detailed study may still show that some changes in the levels of these activities are occurring with time in culture, it is evident that these activities are not greatly changed with time in culture over 5 to 10 passages. Thus the cultured cells provide a reasonably stable population to carry out studies with appropriate controls for passage number.

The low percentage of cells that attach and grow in primary culture raises the question of how representative these cells are of bone cells *in vivo*. The measured characteristics of the cultured bone cells are those to be expected if the cells are truly bone cells. These characteristics include:

(i) A significant portion of the cells have high alkaline phosphatase activity, which is a characteristic of osteoblastic cells.

(ii) The presence of 1α-hydroxylase activity for 25-hydroxyvitamin D, which is found in only a few tissues including bone (16).

(iii) Responsiveness to the skeletal growth factor, which appears to be specific for skeletal tissue (11).

The cultured human bone cells also appear to be similar to cultured bone cells from other species with respect to the above characteristics, their appearance in culture and their response to growth factors. The responses of the human cells to various systemic and local growth factors, although not identical, were very similar to the responses of fetal chick bone cells (Table V). Some of the differences between the responses of the two species may be related to differences between fetal and adult tissue. Fetal chick bone cell preparations have a much higher percentage of cells that attach in primary culture (25%) than do the human cell preparations and are therefore less likely to be a selected subpopulation of the bone cell population. The fetal chick bone cell preparations are also essentially free of marrow cells in contrast to the initial human cell preparations. The similarities between the chick cell cultures and the human bone cultures lend some credence to the idea that these cultures will prove to be representative of bone cells *in vivo*.

The isolated bone cell preparations initially contain marrow cells as well as bone cells. Other workers have reported that marrow cells do not survive under the culture conditions used in this study (17), and differentiated marrow cells were not evident after several days in culture in our studies. The cultured bone cells thus do not appear to contain cells of the hemopoietic cell line although precursor cells may still be present. One cell line that ought to be present is the determined osteogenic precursor cells that Freidenstein and coworkers (17) as well as others (18) have shown to be present in low concentration (10^{-5}) in marrow from a number of species including man.

While the evidence indicates that the cell cultures contain "bone" cells, it is evident from the alkaline phosphatase cytochemistry that the cell population is not homogeneous. Bone cell cultures from other species have been shown to have considerable heterogeneity (4, 13, 15). Therefore it is not surprising to find heterogeneity in the human cell cultures. The low alkaline phosphatase-positive cells are presumably still osteogenic cells since the presence of alkaline phosphatase-positive cells is not necessary for the demonstration of osteogenic potential (18). We do not know to what extent the cell cultures may be contaminated with "non-bone" cells, except that it is clear that fibroblasts or other "non-bone" cells are not overrunning the culture.

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