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Research Article

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Effect of Osteoclast Activating Factor from Human Leukocytes on Bone Metabolism

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ABSTRACT The effects of osteoclast activating factor (OAF) released by normal human peripheral blood leukocytes cultured with phytohemagglutinin have been examined in organ culture. Like parathyroid hormone (PTH), OAF causes a rapid increase in the release of previously incorporated ^{45}Ca from fetal rat bone after brief or continuous exposure; the bones also lose stable calcium and collagen content. The resorption response to OAF also resembles that of PTH in having a steep dose response curve and being only transiently inhibited by calcitonin and partially inhibited by increasing medium phosphate concentration. OAF-stimulated resorption was inhibited more effectively by cortisol than was PTH stimulation. The response to maximally effective doses of OAF was not enhanced by PTH or prostaglandin E_2, but submaximal doses gave additive effects. Both OAF and PTH inhibit collagen synthesis in fetal rat calvaria at the concentrations that stimulate bone resorption.

INTRODUCTION

Osteoclast activating factor (OAF)[1] has been found in the supernatant fluid of cultures of normal human peripheral blood leukocytes that have been activated by an antigen or plant mitogen such as phytohemagglutinin (PHA) (2). OAF is detected by its ability to stimulate resorption of fetal rat bone in organ culture. Chemically it is a protein-containing macromolecule which can be differentiated from other known stimulators of bone resorption such as parathyroid hormone (PTH), active metabolites of vitamin D, and prostaglandins (3). In the present studies OAF has been compared with these agents for its effects on bone resorption and bone collagen synthesis in culture. OAF and PTH had similar effects in vitro, except that OAF appeared more susceptible to inhibition by cortisol.

METHODS

OAF was obtained from supernates of cultures of normal human leukocytes activated with PHA as described elsewhere (2, 3). Supernates were harvested at 1–4 days. The following preparations were used for biological studies: (*a*) crude OAF, the supernate was diluted with BGJ (chemically defined medium developed by Biggers, Gwatkin, and Heyner [1]) and assayed either directly or after concentration by ultrafiltration on an Amicon PM10 membrane (Amicon Corp., Lexington, Mass.) with a nominal mol wt cutoff of 10,000. (*b*) Fraction IV OAF was obtained by chromatography of concentrated crude OAF on Sephadex G-100 (2.5 × 40 cm, 0.02 M phosphate in 0.15 M NaCl at pH 7.2) and was that fraction eluting between the molecular weight markers, chymotrypsinogen (25,000 daltons), and ribonuclease A (13,700 daltons). Fraction IV was dialyzed against BGJ and concentrated by ultrafiltration and then diluted with fresh BGJ for assay. (*c*) DEAE-OAF was fraction IV material further purified on a DEAE cellulose column. If the column was equilibrated with 0.05 M Tris buffer at pH 7.2, OAF was eluted with the original buffer, but im-

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[1] *Abbreviations used in this paper:* BGJ, a chemically defined medium developed by Biggers, Gwatkin, and Heyner (1); OAF, osteoclast activating factor; 25-OH D_3, 25-hydroxy-vitamin D_3; 1,25-$(OH)_2D_3$, 1,25-dihydroxy-vitamin D_3; PGE_2, prostoglandin E_2; PHA, phytohemagglutinin; PTH, parathyroid hormone; SCT, salmon calcitonin; T/C ratio, treated/control ratio.

purities were retained on the column and the potency relative to protein content was increased at least twofold. When DEAE cellulose was equilibrated with 0.05 M Tris at pH 7.5, OAF could be eluted by a linear NaCl gradient at approximately 0.1 M and was purified approximately 10-fold relative to Fraction IV OAF.

The PTH used for comparison with OAF was either partially purified on Sephadex G-100 with a potency of approximately 300 U/mg (kindly provided by Dr. William Y. W. Au) or purified on carboxymethyl cellulose with a potency of approximately 2,000 U/mg (kindly provided by Dr. G. D. Aurbach). Synthetic salmon calcitonin (SCT, 1,500 U/mg, kindly provided by Dr. John Lesh, Armour Pharmaceutical Co., Chicago, Ill.), prostaglandin E_2 (PGE_2, kindly provided by Dr. John Pike, Upjohn Co., Kalamazoo, Mich.), cortisol (Calbiochem, San Diego, Calif.), dexamethasone (Sigma Chemical Co., St. Louis, Mo.), Indomethacin (Kindly provided by Dr. Michael Aylotte, Pfizer Inc., Groton, Conn.), and 1,25-dihydroxy-vitamin D_3 (1,25-$(OH)_2D_3$, kindly provided by Dr. Hector DeLuca) were also used. The last five agents were added to the medium in ethanol to a final ethanol concentration less than 1%; a similar concentration of ethanol was added to the control medium.

Bone resorption assays. Bone resorption was measured in organ cultures of the shafts of the radius and ulna of 19-day fetal rats (4). The bones were labeled by injection of the mother with 0.2–0.4 mCi of ^{45}Ca 1 day before sacrifice. Bones were cultured in BGJ supplemented with 1 or 4 mg/ml of bovine serum albumin Fraction V (BSA, Armour Pharmaceutical Co.). After 18–24 h of preculture bones were transferred to paired treated and control cultures for an additional 48 h, and ^{45}Ca was measured in the medium by liquid scintillation counting. In other experiments the bones were not paired and the percentage of ^{45}Ca released was calculated. In a few experiments, stable calcium content of the bone was measured by atomic absorption spectrophotometry and collagen content by analysis of hydroxyproline (5).

Bone collagen synthesis studies. Effects on bone collagen synthesis were assessed using short-term cultures of half calvaria (the central thin portions of the frontal and parietal bone) from 21-day fetal rats. Two to four calvaria were incubated in 5 ml of BGJ with BSA for 24 h at 37°C in a humidified atmosphere of 5% CO_2 in air. The cultures were placed on a rocking platform (Belco Glass Inc., Vineland, N. J.) which tipped them to an angle of 15° at 6 cycle/min. During the last 2 h of incubation 5 μCi/ml of [2,3-^{3}H]proline (New England Nuclear, Boston, Mass., 30 Ci/mole) was added to the medium. At the end of culture the bones were either dialyzed exhaustively against 0.5 M acetic acid or washed in 5% trichloroacetic acid, acetone, and ether, and weighed on a Cahn electrobalance (Cahn Div., Ventron Instruments Corp., Cerritos, Calif.). The weighed bones were homogenized in 0.5 M acetic acid, and aliquots were analyzed for radioactivity in collagenase digestible and noncollagen protein using purified bacterial collagenase (6). The relative proportion of collagen to total protein synthesis (percent collagen synthesized) was calculated using a factor of 5.4 to correct for the greater abundance of proline in collagen than in noncollagen protein.

RESULTS

Supernates of normal human peripheral blood leukocytes cultured with PHA always contained bone-resorbing activity. The amount of crude OAF that produced maximum bone resorption ranged from a twofold concentrate of the original supernatant fluid to a 1:4 dilution in different samples. Whatever the potency of the original supernate, the dose-response curves always showed a sharp loss of activity from the maximum effective dose on a two- or fourfold dilution. Supramaximal concentrations obtained by concentrating crude OAF generally produced less than the maximal response. Some highly concentrated OAF preparations inhibited ^{45}Ca release below control values. More purified preparations sometimes also showed this decrease in effect but more often showed a plateau at the maximal response (Fig. 1). The steep portion of the dose-response curve was similar for all OAF preparations and for PTH. The mean change in T/C ratio (treated/control) for the twofold dilution representing the steepest portion of the dose-response curve for crude OAF was 0.61± 0.06 ($n = 14$). For Fraction IV OAF the mean change was 0.62±0.04 ($n = 11$) and for DEAE OAF 0.62± 0.10 ($n = 6$). The change in T/C ratios for twofold dilution of PTH was 0.65±0.09 ($n = 7$).

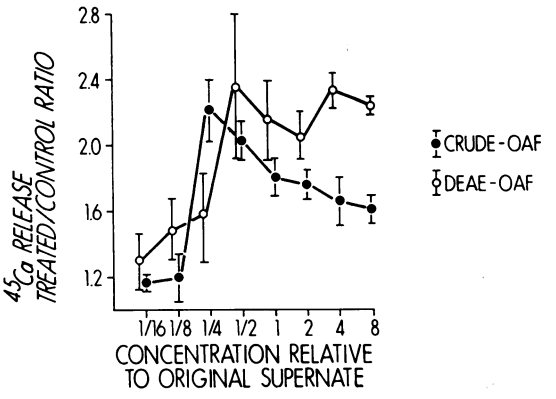

FIGURE 1 Dose response curves for crude and purified OAF. The crude OAF was concentrated on an Amicon PM-10 filter and diluted with fresh BGJ as indicated. The DEAE-OAF represents the active fraction isolated by chromatography at pH 7.5 which eluted at approximately 0.1 M NaCl. This material contained 0.7 μg/ml of protein (Lowry) at the maximally active dilution. The points are means and the vertical lines SE for four bone cultures.

OAF caused loss of stable calcium and of collagen from the bone. For example in one experiment maximally effective concentrations of OAF decreased stable bone calcium content by 46±4% ($n = 4$) and collagen content by 51±15% compared with untreated control. The time-course for increasing ^{45}Ca release after OAF and PTH were also similar, and continued resorption was observed after brief exposure to either agent (Fig. 2). At supramaximal concentration, PGE_2, 1,25-$(OH)_2D_3$ and OAF could all induce prolonged resorption after brief exposure, but OAF showed a greater effect than PGE_2 (Table I). In other induction experiments (not shown) exposure to supramaximal concentrations of

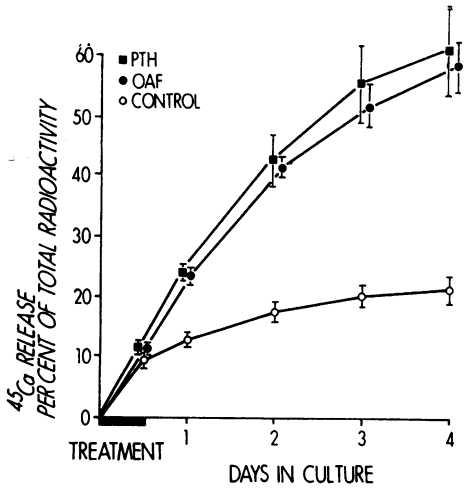

FIGURE 2 Time-course of ^{45}Ca release after 12-h exposure to PTH or OAF. The bones were changed to vessels containing fresh culture medium at the times indicated by the points. The crude OAF used was a supernate concentrated 20 × on an Amicon filter and then diluted to 4 × with fresh BGJ. This preparation produced maximal stimulation of bone resorption at 4 × and 2 ×. Partially purified PTH (1.6 μg/ml) was used at a concentration that gave maximal bone resorption in continuous culture. Points are means and vertical lines are SE for four cultures for the cumulative percent of total bone ^{45}Ca released at each time point.

OAF for 6–12 h produced as great an increase in 48-h ^{45}Ca release as did continuous exposure; however, when the concentration was below maximal, the response to brief exposure was much decreased. Addition of PTH or PGE_2 to maximally effective concentrations of OAF did not stimulate bone resorption further; however, submaximal concentrations of these agents could produce additive effects (Table II). In other experiments (data not shown) addition of OAF to maximally effective doses of PTH or PGE_2 did not significantly increase ^{45}Ca release further.

As observed previously with PTH (4), the effect of OAF on percent of total bone ^{45}Ca released could be reduced by increasing the phosphate concentration in the medium from 1 to 3 mM; however, ^{45}Ca release from control cultures was also reduced so that T/C ratios were unchanged. Pretreatment of the bone with 3 mM phosphate did not alter the subsequent response to OAF (Table III). Calcitonin blocked the initial effect of OAF on ^{45}Ca release; but, as observed previously with PTH and 25-hydroxy-vitamin D_3 (25-OHD_3) (7), ^{45}Ca release increased during continued adminstration of supramaximal doses of SCT and OAF (Fig. 3). OAF was more sensitive to inhibition by cortisol than PTH (Ta-

TABLE I

Effect of 12 h or Continuous Treatment with PGE_2, 10^{-4} M, 1,25-$(OH)_2D_3$, 1 μg/ml, or Fraction IV OAF, 4 × Concentrate, on ^{45}Ca Release from Cultured Fetal Rat Bones

Treatment	^{45}Ca release, T/C ratio		
	12-h treatment		Continuous treatment
	0–12 h	12–48 h	0–48 h
PGE_2	1.06±0.03	1.72±0.22*	2.46±0.07*
1,25-$(OH)_2D_3$	1.12±0.06	2.17±0.46*	2.75±0.17*
OAF	1.17±0.06*	2.24±0.24*	2.65±0.37*

Values are means±SE for four paired cultures either incubated for 12 h in treated or control medium and then washed and transferred to control medium, or incubated continuously in treated or control medium.
* Significantly greater than 1.0 ($P < 0.05$).

TABLE II

Effects of Partially Purified Fraction IV OAF, PTH, and PGE_2 Simply and in Combination on ^{45}Ca Release from Cultured Fetal Rat Bones

Agent	Dose	^{45}Ca release*
Experiment one		
OAF	2×	2.45±0.25‡
PTH	12.5 ng/ml	1.32±0.07‡
PGE_2	10^{-8} M	1.40±0.14‡
OAF plus PTH	2× + 12.5 ng/ml	2.82±0.29‡
OAF plus PGE_2	2× + 10^{-8} M	2.44±0.24‡
Experiment two		
OAF	0.5×	1.48±0.13‡
PTH	12.5 ng/ml	1.01±0.06
PGE_2	10^{-8} M	1.35±0.12‡
OAF plus PTH	0.5× + 12.5 ng/ml	2.03±0.20‡
OAF plus PGE_2	0.5× + 10^{-8} M	1.82±0.20‡

Bones were treated for 48 h after a 24-h preculture. Values are means±SE for six to eight cultures. Pure bovine PTH was used.
* T/C ratio.
‡ Significantly greater than 1.0 ($P < 0.05$).

TABLE III

Effect of Increasing Phosphate before or during Treatment with Fraction IV OAF, 2× Concentrate, on ^{45}Ca Release from Cultured Fetal Rat Bone

PO_4 concentration		48-h ^{45}Ca release	
24-h preculture	48-h treatment	OAF	Control
mM		*% of total bone radioactivity*	
1	1	72±5	32±1
1	3	44±4*	20±2*
3	1	74±7	30±2

Values are means±SE for four cultures or pairs.
* Significantly different from values at 1 mM phosphate, $P < 0.05$.

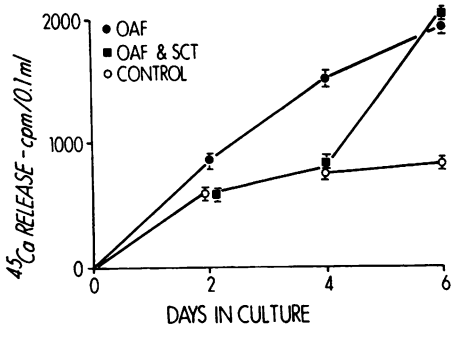

FIGURE 3 Effect of calcitonin on OAF-stimulated bone resorption. Fraction IV OAF (concentrated 10 × by filtration and diluted to 1 × with fresh BGJ) and SCT (100 mU/ml) were used. The bones were transferred to fresh culture medium containing these agents at each time point. Points are means and vertical lines are SE for four cultures for cumulative ^{45}Ca release.

ble IV). Sustained inhibition was seen with as little as 10^{-7} M cortisol. Dexamethasone at 10^{-7} also blocked OAF when given simultaneously, but neither cortisol nor dexamethasone was effective at this concentration when given after a 24 h pretreatment with OAF (data not shown). The effect of OAF was not blocked by indomethacin at 10^{-4} to 10^{-6} M (Table V).

Bone collagen synthesis assays. After 24-h culture of fetal rat calvaria with high concentration of either PTH or OAF, the incorporation of a 2-h pulse of [^{3}H]-proline into collagenase-digestible protein was inhibited by more than 50% (Table VI). The incorporation of proline into noncollagen protein was less affected, so that collagen synthesis represented a smaller percentage of newly synthesized protein. This occurred with both crude and purified OAF preparations. In this system PTH and OAF were more effective both as stimulators of ^{45}Ca release and inhibitors of collagen synthesis than 1,25-$(OH)_2D_3$ (Table VI, experiment B). The results were similar with purified DEAE-OAF; only inhibition of collagen synthesis was observed at a low concentration, whereas a higher concentration also inhibited noncollagen protein synthesis (data not shown).

TABLE IV

Effect of Cortisol on Stimulation of ^{45}Ca Release by PTH, 1.6 μg/ml, or Crude OAF, 1× Concentrate, from Cultured Fetal Rat Bones

	48-h ^{45}Ca release		T/C ratio
	Treated	Control	
	% total bone radioactivity		
PTH/control	80±9	28±1	2.87±0.31*
PTH + cortisol 10^{-7}/cortisol 10^{-7}	75±4	20±1	3.67±0.15*
PTH + cortisol 10^{-6}/cortisol 10^{-6}	59±8	18±1	3.31±0.30*
PTH + cortisol 10^{-5}/cortisol 10^{-5}	26±5	17±1	1.51±0.17
OAF/control	82±3	28±1	2.94±0.10*
OAF + cortisol 10^{-7}/cortisol 10^{-7}	24±2	20±1	1.19±0.08
OAF + cortisol 10^{-6}/cortisol 10^{-6}	20±1	18±1	1.15±0.04
OAF + cortisol 10^{-5}/cortisol 10^{-5}	23±2	17±1	1.36±0.08*

Values are means±SE for four to eight cultures.
* Significantly greater than 1.0, $P < 0.01$.

TABLE V

Effect of Indomethacin on Stimulation of ^{45}Ca Release from Cultured Fetal Rat Bone by Crude OAF, One-half Dilution

Indomethacin	48-h ^{45}Ca release,* OAF
M	
None	1.60±0.19‡
10^{-4}	1.67±0.11‡
10^{-5}	1.96±0.18‡
10^{-6}	1.96±0.17‡

Values are means±SE for four cultures.
* T/C ratio.
‡ Significantly greater than 1.0, $P < 0.01$.

DISCUSSION

The present studies indicate that the bone resorbing factor secreted by human leukocytes, which we have named OAF, acts much like PTH in organ cultures of fetal rat bone. Crude and partially purified preparations of OAF gave similar effects except that the cruder preparations more often showed loss of effect at supramaximal concentrations. This could have been due to toxic contaminants since at high concentrations certain OAF preparations inhibited ^{45}Ca release below controls. The ascending portions of the dose-response curves were steep and had similar slopes at all steps of purification. The similarity of slope, together with the chromatographic results (2), suggest that the stimulation of bone resorption by OAF could be ascribed to the effects of a single material. The slope of the dose-response curve for PTH was not significantly different from that for OAF while that for vitamin D metabolites is less steep and for PGE_2 much less steep (8). The first three agents all act rapidly and have similar effects on removal of mineral and matrix.

The interactions of OAF, PTH, and PGE_2 were examined largely because all three agents are potential mediators of neoplastic and inflammatory bone loss. Effects were additive at low concentrations, but PTH and PGE_2 did not increase resorption further in the presence of high concentrations of OAF. This probably reflects the limited capacity of the tissue to respond and need not indicate a common receptor or mechanism of action. In preliminary studies treatment with OAF, unlike PTH and PGE_2, has not increased cyclic 3′,5′-adenosine monophosphate concentrations in cultured bone (L. G. Raisz, and W. A. Peck, unpublished observations).

OAF resembles PTH in being less effective at high medium phosphate concentrations but still stimulates re-

TABLE VI

Effect of PTH, 1 μg/ml, 1,25-$(OH)_2D_3$, or OAF on [^3H]Proline Incorporation into Collagenase Digestible (CDP) and Noncollagen Protein (NCP) in Fetal Rat Calvaria

Treatment	Specific activity		Collagen synthesized	^{45}Ca release
	CDP	NCP		
	dpm/μg		%	*% of total bone radioactivity*
Experiment A				
Control	270±20	280±10	15.0±8	4.9±0.1
PTH, 1 μg/ml	130±10*	290±20	7.7±0.3*	7.6±0.1*
Crude OAF, 1× concentration	70±10*	240±10*	5.2±0.5*	7.2±0.1*
Experiment B				
Control	182±25	496±74	6.4±0.1	10.0±0.5
1,25-$(OH)_2D_3$, 10 ng/ml	120±23	386±78	5.6±0.3*	11.8±1.0
PTH, 2 μg/ml	62±7*	260±24*	4.1±0.1*	12.4±1.0
Fraction IV OAF, 4× concentration	60±6*	243±13*	4.4±0.2*	16.2±1.1*

Values are means±SE for six to eight half calvaria. Bones were cultured for 24 h and pulsed for last 2 h with 5 μCi/ml [^3H]proline.

* Significantly different from control, $P < 0.05$.

sorption in high phosphate medium. In fact, OAF activity was first detected using medium RPMI 1640 which has a phosphate concentration over 5 mM (2). OAF showed the characteristic initial inhibition by calcitonin followed by escape (7). Failure to inhibit OAF or PTH with indomethacin suggests that prostaglandin synthesis does not mediate the resorptive effects of these two agents. The concentration of indomethacin used has been shown to inhibit complement dependent stimulation of prostaglandin synthesis and bone resorption (9) in our culture system. The only difference we detected between OAF and PTH effects on bone resorption was in their susceptibility to inhibition by cortisol. The reason for this difference is not known; however, it might explain the clinical effectiveness of glucocorticoids in some forms of hypercalcemia and their ineffectiveness in hyperparathyroidism.

In short-term cultures of fetal rat calvaria, PTH and OAF both inhibited the incorporation of [^3H]proline into collagenase-digestible protein. Noncollagen protein labeling was less inhibited. This could represent a specific inhibition of osteoblastic activity which has been postulated for PTH on the basis of autoradiographic studies (10). PTH and OAF appeared to be more potent inhibitors than 1,25-$(OH)_2D_3$ even when the vitamin D metabolite was added in a dose 10 times that required to produce maximal stimulation of bone resorption. We have found that prostaglandins also inhibit collagen synthesis in calvaria, but only at concentrations (10^{-6} to 10^{-4} M) which are high relative to those that stimulate resorption (10^{-9} to 10^{-5} M) (11). The rough reciprocal relation between inhibition of osteoblastic collagen synthesis and stimulation of osteoclastic bone resorption in culture need not indicate any connection between the two phenomena at the cellular level. Moreover, in the case of OAF, it is not certain that these two effects are mediated by the same substance, although the persistence of both effects through two stages of purification supports this possibility. DEAE-OAF is not pure and may still contain toxic contaminants. This seems unlikely since OAF inhibited collagen labeling at concentrations which did not affect noncollagen protein labeling. These results do not bear on the specificity of OAF for bone. We are currently testing whether these preparations influence metabolism of other tissues.

Despite its powerful effects on bone in vitro, we have been unable to increase serum calcium concentrations by injection of large quantities of OAF in intact or thyroparathyroidectomized rats (Raisz, unpublished observations). A similar dissociation between in vitro and in vivo effects has been observed with prostaglandins (12). This could have been due to rapid inactivation of OAF in the circulation which prevented its reaching bone in sufficient concentrations. Direct measurements of OAF in blood will be required to assess this possibility. There is now some evidence that OAF production may be clinically important. OAF-like factors have been found in the supernates of cultured lymphoid cell lines as well as short-term cultures of bone marrow cells aspirated from patients with myeloma (8, 13). OAF could also be released in chronic inflammation by activated lymphocytes (14). The effects of OAF we observed in vitro are consistent with the nature of the bone lesions in periodontal disease and in myeloma. In both disorders, bone destruction is osteoclastic, local, and not regularly associated with an increase in bone formation. It is even more difficult to

ascribe a physiologic function to OAF. If bone marrow lymphocytes produced OAF during their proliferation, this might enhance endosteal bone resorption and facilitate the rapid development of the medullary cavity. If so, some forms of osteopetrosis in animals and man in which bone marrow formation and bone resorption are both decreased and medullary bone formation appears to be excessive could be related to deficient OAF production.

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REFERENCES

1. Biggers, J. D., R. B. L. Gwatkin, and S. Heyner. 1961. Growth of embryonic avian and mammalian tibiae on a relatively simple chemically defined medium. *Exp. Cell Res.* **25**: 41–58.
2. Horton, J. E., L. G. Raisz, H. A. Simmons, J. J. Oppenheim, and S. E. Mergenhagen. 1972. Bone resorbing activity in supernatant fluid from cultured human peripheral blood leukocytes. *Science (Wash. D. C.)*. **177**: 793–795.
3. Luben, R. A., G. R. Mundy, C. L. Trummel, and L. G. Raisz. 1974. Partial purification of osteoclast-activating factor from photohemagglutinin-stimulated human leukocytes. *J. Clin. Invest.* **53**: 1473–1480.
4. Raisz, L. G., and I. Niemann. 1969. Effect of phosphate, calcium, and magnesium on bone resorption and hormonal response in tissue culture. *Endocrinology.* **85**: 446–452.
5. Cheng, P-T. 1969. An improved method for the determination of hydroxyproline in rat skin. *J. Invest. Dermatol.* **53**: 112–115.
6. Peterkofsky, B., and R. Diegelmann. 1971. Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry.* **10**: 988–994.
7. Wener, J. A., S. J. Gorton, and L. G. Raisz. 1972. Escape from inhibition of resorption in cultures of fetal bone treated with calcitonin and parathyroid hormone. *Endocrinology.* **90**: 752–759.
8. Mundy, G. R., R. A. Luben, L. G. Raisz, J. J. Oppenheim, and D. N. Buell. 1974. Bone-resorbing activity in supernatants from lymphoid cell lines. *N. Engl. J. Med.* **290**: 867–871.
9. Raisz, L. G., A. L. Sandberg, J. M. Goodson, H. A. Simmons, and S. E. Mergenhagen. 1974. Complement-dependent stimulation of prostaglandin synthesis and bone resorption. *Science (Wash. D. C.)*. **185**: 789–791.
10. Bingham, P. J., I. A. Brazell, and M. Owen. 1969. The effect of parathyroid extract on cellular activity and plasma calcium levels *in vivo*. *J. Endocrinol.* **45**: 387–400.
11. Raisz, L. G., and A. R. Koolemans-Beynen. 1974. Inhibition of bone collagen synthesis by Prostaglandin E_2 in organ culture. *Prostaglandins.* **8**: 377–385.
12. Klein, D. C., and L. G. Raisz. 1970. Prostaglandins; Stimulation of Bone Resorption in Tissue Culture. *Endocrinology.* **86**: 1436–1440.
13. Mundy, G. R., L. G. Raisz, R. A. Cooper, G. P. Schechter, and S. E. Salmon. 1974. Evidence for the secretion of an osteoclast stimulating factor in myeloma. *N. Engl. J. Med.* **291**: 1041–1046.
14. Horton, J. E., J. J. Oppenheim, S. E. Mergenhagen, and L. G. Raisz. 1974. Macrophage-lymphocyte synergy in the production of osteoclast activating factor. *J. Immunol.* **113**: 1278–1287.