during perfusion of SP. Removal of SP from the medium caused the gradual decrease of the firing rate. When SP was applied again together with baclofen (8×10^{-8} M) under the aforementioned circumstances, the excitatory action of SP was blocked. Introduction of the normal Krebs solution restored the firing rate to the original control level. When SP was applied together with baclofen (8×10^{-9} M) in a Ca-free medium, excitation occurred, but here the magnitude was about half of that produced by SP alone. Namely, the excitatory action of SP in the Ca-free medium was antagonized by a low dose of baclofen which exerted no depressant effect per se. In contrast to baclofen, GABA in a dose of 2×10^{-4} M consistently produced total suppression of the excitations induced by both SP and ACh in the normal Krebs solution (see fig. 3c and d).

Our findings that the excitatory action of SP persisted even in the medium containing 12 mM Mg^{2+} but no Ca^{2+} confirm that SP has a direct action on the postsynaptic membrane in neurons of the guinea-pig hypothalamus, as under these conditions, the possibility of the involvement of a synaptic event can be excluded. This is significant in view of the findings in experiments using the rat spinal cord[6,7] and the guinea-pig sympathetic ganglia[8]. In these experiments, the effect of reduced external Ca^{2+}/Mg^{2+} ratio on the SP-induced excitation was studied using media containing 0.4 mM Ca^{2+} plus 7 mM Mg^{2+}, 0.1 mM Ca^{2+} plus 1.6–3.5 mM Mg^{2+}, and 0.1 mM Ca^{2+} plus 5 mM Mg^{2+}, respectively, owing to the fact that SP-induced excitation was progressively reduced when external Mg^{2+} concentration was increased. Therefore, the possiblity has not been ruled out that the SP-induced excitation in these preparations is mostly due to other excitatory transmitters that are somehow resistant to the lowering of external Ca^{2+} concentration[7].

Baclofen (β-4-chlorophenyl-GABA) is a putative SP antagonist in the central neurons[4]. However, subsequent investigations[9–11] did not confirm the specificity of antagonism between baclofen and SP. This problem has been re-examined by Otsuka and Yanagisawa[7], and they again suggested that baclofen blocks transmission at certain primary afferent synapses by antagonizing the action of SP in the rat spinal cord. On the other hand, Hanley et al.[12] recently reported that baclofen had no effect on ^{3}H-substance P binding in the rat brain membrane. Therefore, the antagonism between SP and baclofen may not be specific. When we placed preparations of the guinea-pig hypothalamus in a Ca-free medium, the action of SP was antagonized by a low dose of baclofen whereas that of ACh was not antagonized even by much higher doses of this drug, and GABA suppressed both excitations induced by SP and ACh. In addition, the time course of the depressant action of baclofen was remarkably slower than that of GABA (fig. 2) suggesting that the action of baclofen is not due to activation of GABA receptors. These findings might indicate that there are some important functional interactions between the actions of SP and baclofen in several portions of the central nervous system, despite the findings of Hanley et al.[12].

1 Acknowledgments. We thank Prof. M. Otsuka for providing baclofen and for helpful suggestions, Prof. H. Kuriyama for valuable discussions, Dr. M. Ohara for critical reading of the manuscript, and Ciba-Geigy (Japan) for providing baclofen.

2 N. Ogata, Nature *277*, 480 (1979).
3 I. Kanazawa and T. Jessell, Brain Res. *117*, 362 (1976).
4 K. Saito, S. Konishi and M. Otsuka, Brain Res. *97*, 177 (1975).
5 N. Ogata, Experientia *35*, 1202 (1979).
6 S. Konishi and M. Otsuka, Nature *252*, 735 (1974).
7 M. Otsuka and M. Yanagisawa, J. exp. Biol., in press (1981).
8 S. Konishi, A. Tsunoo and M. Otsuka, Proc. Japan Acad. *55*, 525 (1979).
9 K.J. Fotherby, N.J. Morrish and R.W. Ryall, Brain Res. *113*, 210 (1976).
10 L.H. Henry and Y. Ben-Ari, Brain Res. *117*, 540 (1976).
11 J.W. Phillis, Experientia *32*, 593 (1976).
12 M.R. Hanley, B.E.B. Sandberg, C.M. Lee, L.L. Iversen, D.E. Brundish and R. Wade, Nature *286*, 810 (1980).

Impaired bone resorption of cultured calvaria from mice with abnormal lysosomal function (the Chediak-Higashi syndrome)[1]

U. Lerner, Maria Ransjö and G.T. Gustafson

Department of Oral Pathology, University of Umeå, S-901 87 Umeå (Sweden), 1 December 1980

Summary. Spontaneous bone resorption is reduced in cultured calvarial bones from mice with the Chediak-Higashi syndrome, as indicated by decreased mobilization of calcium from the bones to the medium. Although bone resorption in calvaria from mice with this disease can be stimulated by PGE_2 and 1 α $(OH)D_3$, the amounts of mineral released after stimulation is also decreased.

The Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disease which is clinically characterized by increased susceptibility to bacterial infection, pseudoalbinism, pancytopenia, lymphadenopathy, splenomegaly and sometimes lymphoreticular infiltrative disease[2]. The CHS has been described in man, mink, mice, cattle and killer whales and in all these species the occurrence of giant lysosomes in granular neutrophils and monocytes is the hallmark of the disease[2–9]. In the Chediak-Higashi variant of mouse, the beige mouse, the lysosomal anomaly has recently been reported to be manifested also in the osteoclasts[10]. We have shown that CHS mink has increased susceptibility to periodontitis compared to normal range mink, with more advanced periodontal bone destruction and rapid loss of teeth[11]. This observation has been confirmed and extended to the beige mouse[12,13]. Similarily, in patients with CHS, bone loss can be seen already around the teeth in the primary dentition[14].

The increased periodontal bone loss associated with CHS might be due to an inborn error of bone tissue catabolism or be secondary to the increased susceptibility to infection. Although there is a lot of evidence to favour the latter alternative, the first possibility has not been eliminated. We have therefore studied the mineral mobilizing capacity of calvarial bones from the beige mouse and from the corresponding normal wild type (C57BL) in a bone organ culture system.

Material and methods. Calvarial (frontal and parietal) bones from 6 to 7 days old mice, that had been prelabelled with 1.5 µCi ^{45}Ca 4 days prior to sacrifice, were dissected and divided into 2 halves along the sagittal suture. The half-calvaria were separately placed on grids in plastic

Comparison of cell-mediated resorption in vitro of bone explants from mice with Chediak-Higashi's syndrome (CHS) and corresponding normal littermates (C57) of the same age

Culture conditions	Release of ^{45}Ca from the living half-calvarium (% of initial ^{45}Ca)	Release of ^{45}Ca from the dead half-calvarium (% of initial ^{45}Ca)	Cell-mediated resorption (CMR) (% of initial ^{45}Ca)	Stimulation of CMR by PGE_2 and $1\alpha(OH)D_3$ respectively (%)
No test substance (spontaneous resorption)				
$C57_{(n=48)}$	28.8±0.7	14.5±0.4	14.3±0.7	-
$CHS_{(n=54)}$	25.2±0.4	14.0±0.3	11.2±0.4*	-
PGE_2 (10^{-7} M)				
$C57_{(n=8)}$	40.4±1.9	13.8±0.4	26.6±1.7	86.0
$CHS_{(n=17)}$	35.6±1.2	13.7±0.3	21.9±1.3	95.5
1 α $(OH)D_3$ (25 ng/ml)				
$C57_{(n=13)}$	46.6±1.5	13.6±0.4	33.0±1.7	130.8
$CHS_{(n=17)}$	40.0±1.9	15.3±0.5	24.7±2.0*	120.5

Results given are means ± SE. n, number of paired experiments; * statistical difference from CMR obtained with C57 $p < 0.01$.

dishes containing 5.5 ml of culture medium. The medium employed, CMRL 1066 (Flow Laboratories), was supplemented with ascorbic acid (150 mg/l), $Fe(NO_3)_3 \times 9H_2O$ (100 µg/l) and essentially fatty acid-free bovine serum albumin, fraction V(1 g/l). The cultures were gassed with 5% CO_2 in air and maintained for 48 h. 1 half of the calvarium was killed by thrice freezing and thawing prior to explantation. The release of ^{45}Ca to the culture medium (% of initial radioactivity) from both the living and dead half was determined and the cell-mediated resorption was calculated from the difference in ^{45}Ca release, living half minus dead half[13]. When resorption was stimulated by PGE_2 and 1 α $(OH)D_3$ respectively, both the living and dead halves of the calvarium were cultured in the presence of the substances.

Statistical analysis was performed with Student's t-test for unpaired samples.

PGE_2 was supplied by the Upjohn Co., Kalamazoo, Mi., through the courtesy of Dr John E. Pike and 1 α $(OH)D_3$ was given by Leo Pharmaceutical Products, Denmark. Both the substances were dissolved in ethanol. The final concentration of ethanol did not exceed 0.1%.

Results. From the table it appears that the spontaneous cell-mediated release of ^{45}Ca from calvarial bones of CHS mice was less (21.7%) than that seen in cultures with bones from normal C57BL mice. When bone explants from both CHS and normal C57BL were cultured in the presence of PGE_2(10^{-7} M) and 1 α OHD_3 (25 ng/ml) respectively, a marked stimulation of ^{45}Ca mobilization was obtained. The degree of stimulation was almost equal for CHS and normals; however, the total amounts of mineral released from CHS bones were significantly less in these experiments also (17.7% with PGE_2 and 25.1% with 1 α OHD_3) respectively.

Discussion. These results indicate that the bone resorptive capacity of bones from CHS mice is reduced in relation to that of bones from normal mice. Thus, the periodontal bone loss seen in the Chediak-Higashi syndrome can hardly be a consequence of a general tendency to increased bone resorption. It therefore seems more likely that the augmented periodontal bone destruction in mice with this trait is related to the general increased susceptibility to infection, which in turn may be due to a functional deficiency of the neutrophil leukocytes[7,9] and/or to an immunodeficiency related to natural killer cell dysfunction[16].

The basic defect responsible for the reduced bone resorption observed in vitro with calvaria from CHS mice can only be speculated on. However, the osteoclasts from CHS mice contain giant lysosomes[10] and therefore these cells may have a defective lysosomal function similar to that shown for the neutrophil leukocytes[2]. This is interesting in view of the intimate relationship between lysosomal enzyme release and bone resorption suggested by the work of Vaes[17], Eilon and Raisz[18] and Lerner[19,20]. The impaired bone resorption of the CHS calvaria may also be related to natural killer cell dysfunction in this trait[16], as promonocytes have recently been shown to have the functional characteristics of natural killer cells[21] and monocytes have the capacity to resorb bone[22]. There is also evidence that the multinucleated osteoclasts are derived from monocytes[23-25]. It should be added that the natural killer cell defect in CHS may also be due to the lysosomal dysfunction[26].

1 This investigation was supported by grants from the Swedish Association against Rheumatic Diseases, from the Royal 80 Year Fund of King Gustav V, and from the Swedish Medical Research Council (project No 14X-05426).
2 I.G. White and C.C. Clawson, Am. J. Path. *98,* 151 (1980).
3 A. Bequez-Cesar, Soc. cab. Pediat. Bol. *15,* 900 (1943).
4 W. Steinbrinck, Dt. Arch. klin. Med. *193,* 577 (1948).
5 M.M. Chédiak, Hematology *7,* 362 (1952).
6 O. Higashi, Tohuku J. exp. Med. *59,* 315 (1954).
7 D.B. Windhorst and G.J. Padgett, Invest. Derm. *60,* 529 (1973).
8 C. Oliver and E. Essner, Lab. Invest. *32,* 17 (1975).
9 G.A. Padgett, C.W. Requiam, J.R. Gorham, J.B. Henson and C.C. O'Mary, Am. J. Path. *51,* 553 (1967).
10 P. Ash, J.F. Louitit and K.M.S. Townsend, Nature *283,* 669 (1980).
11 G.T. Gustafson, Periodont. Res. *4,* 259 (1969).
12 W.S. Lavine, R.C. Page and G.A. Padgett, J. Periodont. *47,* 621 (1976).
13 G.T. Gustafson, K. Axrup, F. Gustafson and S. Wall, in preparation.
14 G.T. Gustafson, J. Killander, C.O. Norrbohm and A. Rangne, in preparation.
15 J.J. Reynolds, in: Organ Culture in Biomedical Research, p.355. Ed. M. Balls and M. Monnickendam. University Press, Cambridge 1976.
16 J.C. Roder and A.K. Duwe, Nature *278,* 451 (1979).
17 G. Vaes, J. Cell Biol. *39,* 679 (1968).
18 G. Eilon and L.G. Raisz, Endocrinology *103,* 1969 (1978).
19 U. Lerner, Acta physiol. scand. *110,* 123-130 (1980).
20 U. Lerner, Biochim. biophys. Acta *632,* 204 (1980).
21 M.L. Lohmann-Matthes, W. Domzig and J.J. Roder, Immunology *123,* 1883 (1979).
22 A.J. Kahn, C.C. Stewart and S.L. Teitelbaum, Science *199,* 988 (1978).
23 D.A. Fischman and E.D. Hay, Antat. Rec. *143,* 329 (1962).
24 G. Göthlin and J.L.E. Ericsson, Virchows Arch. B *12,* 318 (1973).
25 D.W. Walker, Science *190,* 784 (1975).
26 J.C. Roder, S. Argov, M. Klein, C. Petterson, R. Kiessling, K. Andersson and Mona Hansson, Immunology *40,* 107 (1980).