Stimulation of bone resorption by the kallikrein-kinin system and the coagulation cascade

Ulf H Lerner

Department of Oral Cell Biology, Umeå University, S-901 87 Umeå, Sweden. Tel +46 90 176290. Fax +46 90 139289.

The activities of bone forming osteoblasts and bone resorbing osteoclasts are regulated in physiological bone metabolism by several endocrine and paracrine factors, including peptide and steroid hormones as well as a variety of cytokines and growth factors (reviewed by Vaes 1988, Suda et al. 1992). Recently it has been demonstrated that also proto-oncogenes (c-fos and c-src) are involved in the regulation of bone cell activities (reviewed by Suda et al. 1995). Several pathological conditions, e.g. inflammation, tumors, Paget disease, intraosseous cysts, hyperparathyroidism, osteoporosis, also influence the activity of bone cells by the release of endocrine and paracrine factors. This may give rise to loss of bone tissue or to new bone formation. Inflammatory processes in the vicinity of the skeleton, e.g. rheumatoid arthritis, periodontitis and osteomyelitis, frequently result in loss of bone. The disappearance and changes in the architecture of bone tissue in these diseases are mostly a result of locally activated bone resorbing osteoclasts, although inhibition of bone forming osteoblasts also may contribute.

The pathogenesis of inflammation induced osteoclast activation is not well understood, but the common view is that factors produced in the inflammatory reaction enhance osteoclastic bone resorption by mechanisms involving both activation of latent multinucleated osteoclasts and stimulation of late mononuclear osteoclast progenitor cells present within or in the vicinity of the periosteum. This leads to more actively bone resorbing osteoclasts and to enhanced number of these multinucleated cells. Much efforts have been made to clarify which factor(s) in the inflammatory processes that are responsible for the local activation of osteoclasts. Current knowledge is summarized in Figure 1. Several cytokines produced by lymphocytes and monocytes have been shown to be able to stimulate or inhibit bone resorption and bone formation. This list includes interleukin-1 (IL-1α, IL-1ß), IL-3, IL-4, IL-6, IL-11, tumor necrosis factors (TNF-α, TNF-ß), granulocyte-macrophage colony stimulating factor (GM-CSF), M-CSF, leukemia inhibitory factor (LIF), as well as growth factors such as transforming growth factor ß (TGF-ß) and platelet-derived growth factor (PDGF; Gowen 1992). In inflammatory processes not only cytokines and growth factors are produced, but also several inflammatory mediators. We have recently shown that factors produced during inflammation by the kallikrein-kinin system and the coagulation cascade can stimulate bone resorption. The kinins generated in the kallikrein-kinin system—bradykinin and kallidin—were previously most well known for their effects on vascular dilatation, vessel permeability and pain. The end product of the coagulation cascade—thrombin—was known mainly for its capacity to release fibrin from fibrinogen. During the last decade, however, it has been reported that kinins and thrombin, in a hormone-like manner, affect the activity of a variety of cells, similar to the cytokines and growth factors. This review will briefly summarize the activation of the kallikrein-kinin system and the coagulation cascade and effects of kinins and thrombin on bone metabolism. For more extensive reviews, see Lerner 1992, 1994.

Activation of the kallikrein-kinin system and the coagulation cascade

The key factor in the activation of the kallikrein-kinin system and the coagulation cascade is the Hageman factor (factor XII), which by unknown mechanisms is activated in inflammatory processes. The activated Hageman factor has enzymatic activity which can use both prekallikrein and coagulation factor XI as substrates.

Prekallikrein is a single-chain plasma protein synthesized by the liver and which is cleaved by activated Hageman factor into one heavy and one light chain

Copyright © Scandinavian University Press *1995. ISSN 0001–6470. Printed in Sweden – all rights reserved.*

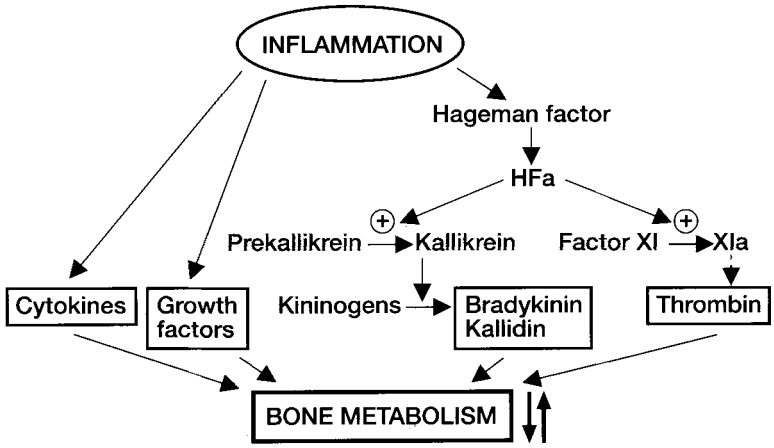

Figure 1. In Inflammatory processes several cytokines and growth factors, as well as proinflammatory mediators produced in the kallikrein-kinin system and the coagulation cascade, are present which can affect the bone forming osteoblasts and the bone resorbing osteoclasts. The interactions between these factors and the bone cells most frequently leads to bone loss, but in some cases can result in new bone formation.

linked together by a disulfide bond. The enzymatic activity in the light chain preferentially uses high molecular weight kininogen (HMW-kininogen) as substrate. The kininogens, HMW-kininogen (molecular weight 120,000) and low molecular weight kininogen (LMW-kininogen; molecular weight 70,000) are liver proteins circulating in serum. The plasma prekallikrein releases the biologically active nine amino acid peptide bradykinin (H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) from HMW-kininogen. In addition to this circulating prekallikrein, several tissues contain a related enzyme called tissue kallikrein. This enzyme releases the decapeptide kallidin (Lys-bradykinin) from both HMW- and LMW-kininogens. Kallidin and bradykinin have overlapping biological effects which may by due to either that kallidin has affinity to the bradykinin receptor or that kallidin is converted to bradykinin by arginine aminopeptidase. It has been suggested that tissue kallikrein is more important for kinin generation in inflammatory processes since this enzyme can use both kininogens as substrate and since tissue kallikrein is less susceptible than plasma kallikrein to inactivation by protease inhibitors.

The half-life of kinins is short since these peptides are locally degraded by several protesases. The carboxyterminal Arg is cleaved off by kininase I leading to generation of des-Arg^9-bradykinin and des-Arg^{10}-kallidin. In many tissues, these peptides are biologically inactive, but in some they cause effects similar to those of bradykinin and kallidin. Kininase II, also known as angiotensin-converting enzyme, further degrades des-Arg^9-bradykinin or des-Arg^{10}-kallidin to inactive penta- and tripeptides.

Activation of the Hageman factor in inflammatory processes thus leads to generation of kinins and thrombin, factors which cause several characteristic features of inflammation including edema, pain and extravascular fibrin deposition. In periodontal disease increased kallikrein activity and kinin levels have been demonstrated in gingival exudates, similar to findings in synovial fluid from patients with rheumatoid arthritis, gout and psoriasis-associated arthritis. Extravasacular fibrin deposition has been observed both in synovial tissue from patients with rheumatoid arthritis and in gingival tissue from patients with periodontal disease. Studies during recent years have shown that kinins and thrombin not only act as proinflammatory mediators, but also can affect the activity of connective tissue cells in the vicinity of inflammatory processes. Thus, kinins and thrombin initiate cell proliferation and prostaglandin biosynthesis in a variety of different fibroblasts. As will be discussed in detail below, we have reported that kinins and thrombin can stimulate bone resorption.

Effects of kinins on bone metabolism

Bone resorption

Bradykinin and kallidin, at concentrations of 3 nM and above, time- and dose-dependently stimulate bone resorption in neonatal mouse calvarial bones.

The effect of bradykinin in this system is due to enhanced recruitment of multinucleated osteoclasts and is inhibited by the specific osteoclast inhibitor calcitonin (Gustafson et al. 1986, Lerner et al. 1987). Whether or not bradykinin, in addition, directly or indirectly stimulate preexisting multinucleated osteoclasts or if kinins can stimulate osteoclasts formation in bone marrow cultures have not been assessed hitherto. The degree of stimulation by bradykinin and kallidin is less than that induced by parathyroid hormone (PTH) or $1,25(OH)_2$-vitamin D3, probably because bradykinin is degraded to inactive peptides in the long-term organ cultures used in the bone resorption bioassay. In line with this view, we have shown that kininase inhibitors potentiate the bone resorptive effect of bradykinin, but not that of PTH. Interestingly, des-Arg^9-bradykinin also is a potent stimulator of bone resorption in vitro (Ljunggren and Lerner 1990). Since des-Arg^9-bradykinin can be formed in the bone organ cultures used for assessment of bone resorbing activity, the relative importance of bradykinin and des-Arg^9-bradykinin in the bone resorptive effect of bradykinin is an open question. This point is particularly interesting since the two peptides use different receptors.

In the mouse calvarial assay, most stimulators of bone resorption also enhance the formation of prostaglandins. The stimulatory effect of bradykinin and kallidin on bone resorption is abolished by several non-steroidal antiinflammatory drugs, including indomethacin, meclofenamic acid, flurbiprofen and naproxen. In contrast, the stimulatory actions of PTH, $1,25(OH)_2$-vitamin D3 and TGF-ß are unaffected, whereas those of IL-1 and TNF are partially reduced by these drugs (Lerner et al. 1987, 1991, Lerner and Ohlin 1993, Lerner 1995). These observations show that activation of endogenous prostaglandin production is a prerequisite for kinin-induced resorption.

Prostaglandin biosynthesis

In line with the observation that inhibitors of prostaglandin biosynthesis abolish the bone resorptive effect of bradykinin and kallidin, we have found that these peptides stimulate the formation of PGE_2 and PGI_2 in intact bones, in enzymatically isolated primary osteoblasts from mouse calvariae, in a cloned non-transformed mouse calvarial osteoblastic cell line (MC3T3-E1), in a human osteoblastic osteosarcoma cell line (MG-63) and in non-enzymatically isolated human bone cells (Lerner et al. 1989, Ljunggren et al. 1990). In the rat osteosarcoma cell lines ROS 17/2.8 and UMR 106-01, no effect by the kinins on prostaglandin production can be observed probably because the spontaneous release of PGE^2 in ROS 17/2.8 is very high and the capacity of UMR 106-01 to synthesize prostaglandins is very low. Kinins cause a burst of prostaglandin production in all these cell types with an optimal effect after 5–10 min, whereas IL-1, TNF and TGF-ß have no effect at these early time points and cause a maximal effect after 18–24 h. Similarly, the stimulatory effect of des-Arg^9-bradykinin on prostaglandin formation in bone cells is substantially delayed. These findings indicate that bradykinin and kallidin activate prostaglandin biosynthesis by a molecular mechanism different from that induced by cytokines and des-Arg^9-bradykinin.

Receptors and signal transducing mechanisms

Using pharmacological methods and different kinins and kinin analogues with agonistic and antagonistic properties, at least two separate kinin receptors have been demonstrated, designated B1 and B2 receptors. The B2 receptors have affinity to bradykinin and kallidin and are the receptor subtype most frequently distributed in different cell types. The B1 receptors preferentially recognize des-Arg^9-bradykinin and des-Arg^{10}-kallidin. The cDNAs for the rat and human B2 receptors have been cloned and encode receptor proteins belonging to the superfamily of rhodopsin-like receptors with seven putative transmembrane spanning domains (McEachern et al. 1991).

The receptors for hormones and cytokines stimulating bone resorption are, surprisingly, located in the bone forming osteoblasts and not in mature osteoclasts or their mononuclear precursor cells (Vaes 1988). The activation of bone resorption is mediated by an hitherto not fully understood interaction between osteoblasts and osteoclasts. We have shown that receptors for bradykinin also are located on osteoblasts. Thus, mouse, rat and human osteoblasts have B2 bradykinin receptors coupled to a burst of prostaglandin formation (Ljunggren et al. 1991 a). Occupancy of these receptors gives rise to a rapid transient increase of intracellular calcium independent of extracellular calcium, to enhancement of inositol phosphate formation and increased activity of protein kinase C (Ljunggren et al. 1991 b, Ljunggren et al. 1993). These observations suggest that B2 bradykinin receptors use phospolipase C mediated turnover of phosphatidylinositol-4,5-bisphosphate as signal transducing mechanism. Using bradykinin analogues with agonistic and antagonistic properties, the existence of B1 receptors on osteoblasts has also been demonstrated. These receptors are linked to a delayed stimulation of prostaglandin biosynthesis and are not associated with any changes in phosphatidyl-4,5-bisphosphate

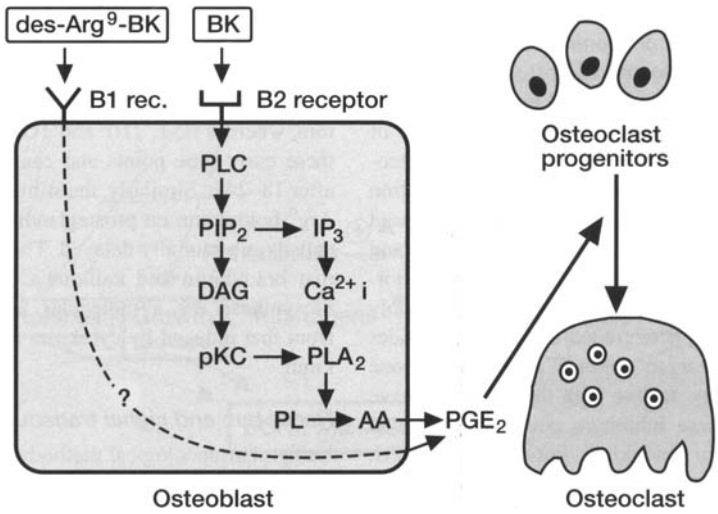

Figure 2. Schematical drawing of hypothetical mechanisms involved in bradykinin-induced bone resorption via B2 and B1 receptors. Occupancy of bradykinin B2 receptors in osteoblasts is linked to phospholipase C (PLC) mediated turnover of phosphatidylinositol-4,5-bisphosphate (PIP_2) with the formation of the two intracellular second messengers inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP_3 then causes a rapid transient increase of intracellular calcium ($Ca^{2+}i$) by releasing Ca^{2+} from intracellular stores and DAG activates protein kinase C (PKC). $Ca^{2+}i$ and PKC, in a synergistic manner, activates phospholipase A_2 (PLA_2) which releases arachidonic acid (AA) from membrane bound phospholipids (PL) with subsequent burst of prostaglandin E_2 (PGE_2) formation. PGE_2 finally stimulates the formation of mature osteoclasts from a precursor pool of osteoclast progenitor cells. Occupancy of bradykinin B1 receptors leads by an unknown mechanism to a delayed stimulation of PGE_2 biosynthesis and then to osteoclast activation.

metabolism. These findings suggest that the molecular basis for B1 and B2 receptor activation is quite different and involves different signal transducing mechanisms. The existence of B1 and B2 receptor subtypes on osteoblasts has also been demonstrated with the use of specific radiolabelled ligands (Santora 1989, Ljunggren et al. 1991 a). In Figure 2 is schematically shown our hypothesis by which bradykinin activates osteoblasts and the mechanism by which this receptor activation leads to osteoclast activation and bone resorption.

Effects of thrombin on bone metabolism

Bone resorption

Thrombin, both bovine and human, was first shown to stimulate bone resorption in cultured neonatal mouse calvariae (Gustafson and Lerner 1983). Later, Hoffmann et al. reported that bovine thrombin stimulates bone resorption also in cultured fetal rat long bones (Hoffmann et al. 1986). Similar to bradykinin, the effect is delayed for 24 hours and is inhibited by calcitonin, suggesting that the mechanism involves recruitment of new osteoclasts. The stimulatory effect of thrombin is partially inhibited by several non-steroidal cyclooxygenase inhibitors (Lerner and Gustafson 1988). Glucocorticoids are more potent inhibitors of thrombin-induced resorption probably by interacting with a mechanism of action unrelated to prostaglandin biosynthesis. These findings suggest that prostaglandins contribute, but are not a prerequisite for the bone resorptive effect of thrombin, similarly to IL-1 and TNF.

Thrombin is a multifunctional protein with both enzymatic and non-enzymatic activities, including procoagulant activity and pleiotropic, hormone-like, cellular effects such as cell proliferation and chemotaxis of monocytes (Fenton 1986). Stern et al. (1990) have shown that catalytically inactivated γ-thrombin does not stimulate bone resorption but that γ-thrombin possess such an activity, showing that the site of thrombin molecule responsible for bone resorption is different from that involved in chemotaxis.

Thrombin has not only effects on bone resorption, but has also been shown to stimulate proliferation of a variety of different osteoblasts (Tatakis et al. 1989) and the biosynthesis of type I collagen in the human osteosarcoma cell line MG-63 (Lerner et al. unpublished), indicating that thrombin may be an anabolic agonist in bone.

Prostaglandin biosynthesis

Thrombin stimulates prostaglandin formation in chicken, rat and mouse osteoblasts (Feyen et al. 1984, Partridge et al. 1985, Ljunggren et al. 1991 c). Similar to bradykinin acting via the B2 receptors, but at variance with IL-1, TNF and TGF-ß, thrombin causes a burst of PGE_2 and PGI_2 biosynthesis with maximal effect at 5–10 minutes.

Receptors and signal transducing mechanisms

Although radioligand studies have shown that different cells express specific binding sites for thrombin, very little is known on the interaction between thrombin and cellular receptors. Recently, however, it was demonstrated that platelets express a thrombin binding receptor with an aminoterminal extracellular domain, seven transmembrane spanning loops and a carboxyterminal intracellular end (Coughlin et al. 1992). The thrombin receptor is unique since after binding to the aminoterminal end, thrombin cleaves the receptor at a specific site and releases an inactive peptide and thereby exposes a new aminoterminal extension, which then is responsible for cell activation. It is not known if bone cells express this or another thrombin receptor. It was, however, recently reported that a pentapeptide corresponding to a small region of the new aminoterminal end of the thrombin receptor with capacity to activate platelets, stimulates plasminogen activator inhibitor-1 production in rat osteoblasts (Allan and Martin 1995).

The receptor for thrombin seems to be located in osteoblasts, in agreement with findings for other stimulators of bone resorption. This receptor is likely to be linked to phosphatidylinositol-4,5-bisphosphate as signal transducing mechanism since thrombin causes an acute, transient rise of intracellular calcium independently of extracellular calcium in human osteosarcoma cell lines and in the mouse osteoblastic cell line MC3T3-E1 (Ljunggren et al. 1991 c).

Acknowledgements

Studies performed in the author's laboratory have been supported by The Swedish Medical Research Council (project no 7525), The Swedish Association Against Rheumatic Diseases, the Royal 80 Year Fund of King Gustav V, and A-G Craaford Foundation.

References

Allan E H, Martin T J. Receptor-mediated effect of thrombin on plasminogen activator inhibitor-1 production in rat osteoblasts. Bone 1995; 16 (suppl): 549 (abstr.)

Coughlin S R, Vu T-K H, Hung D T, Wheaton V I. Characterization of a functional thrombin receptor: issues and opportunities. J Clin Invest 1992; 89: 351–5.

Fenton J W II. Thrombin. Ann NY Acad Sci 1986; 485: 5–15.

Feyen J H M, van der Welt G, Moonen P, DiBon A, Nijweide P. Stimulation of arachidonic acid metabolism in primary cultures of osteoblast-like cells by hormones and drugs. Prostaglandins 1984; 28: 769–81.

Gowen M. Cytokines and bone metabolism. Boca Raton, CRC Press, 1992.

Gustafson G T, Lerner U H. Thrombin: a stimulator of bone resorption. Biosci Rep 1983; 3: 225–31.

Gustafson G T, Ljunggren Ö, Boonekamp P, Lerner U H. Stimulation of bone resorption in cultured mouse calvariae by Lys-bradykinin (kallidin): a potential mediator of bone resorption linking anaphylaxis processes to rarefying osteitis. Bone Miner 1986; 1: 267–77.

Hoffmann O, Klaushofer K, Peterlik M, Mavreas T, Stern P. Indomethacin inhibits thrombin-, but not thyroxin-stimulated resorption of fetal rat limb bones. Prostaglandins 1986; 31: 601–8.

Lerner U H. Effects of kinins, thrombin and neuropeptides on bone. In: Cytokines and bone metabolism (Ed. Gowen M). Boca Raton: CRC Press, 1992: 267–98.

Lerner U H. Regulation of bone metabolism by the kallikrein-kinin system, the coagulation cascade, and the acute-phase reactants. Oral Surg, Oral Med, Oral Pathol 1994; 78: 481–93.

Lerner U H. Transforming growth factor-ß stimulates bone resorption in neonatal mouse calvariae by a prostaglandin-unrelated but cell proliferation dependent pathway. J Bone Miner Res 1995: accepted for publication.

Lerner U H, Gustafson G T. Blood coagulation and bone metabolism: some characteristics of the bone resorptive effect of thrombin in mouse calvarial bones in vitro. Biochim Biophys Acta 1988; 964: 309–318.

Lerner U H, Ohlin A. Tumor necrosis factors α and ß can stimulate bone resorption in cultured mouse calvariae by a prostaglandin-independent mechanism. J Bone Miner Res 1993; 8: 147–55.

Lerner U H, Jones I L, Gustafson G T. Bradykinin: a new potential mediator of inflammation-induced bone resorption. Arthr Rheum 1987; 30: 530–40.

Lerner U H, Ransjö M, Ljunggren Ö. Bradykinin stimulates production of prostaglandin E_2 and prostacyclin in murine osteoblasts. Bone Miner 1989; 5: 139–54.

Lerner U H, Ljunggren Ö, Dewhirst F, Boraschi D. Comparison of interleukin-1 ß and its 163-171 peptide in bone resorption and the immune response. Cytokine 1991; 3: 141–8.

Ljunggren Ö, Lerner U H. Evidence for BK1 bradykinin-receptor-mediated prostaglandin formation in osteoblasts and subsequent enhancement of bone resorption. Br J Pharmacol 1990; 101: 382–6.

Ljunggren Ö, Rosenquist J, Ransjö M, Lerner U H. Bradykinin stimulates prostaglandin E_2 formation in isolated human osteoblast-like cells. Biosci Rep 1990; 10: 121–6.

Ljunggren Ö, Vavrek R, Stewart J M, Lerner U H. Bradykinin induced burst of prostaglandin formation in osteoblasts is mediated via B2 bradykinin receptors. J Bone Miner Res 1991a; 6: 807–15.

Ljunggren Ö, Johansson H, Ljunghall S, Fredholm B B, Lerner U H. Bradykinin induces formation of inositol phosphates and causes an increase in cytoplasmic Ca^{2+} in the osteoblastic cell lineage MC3T3-E1. J Bone Miner Res 1991b; 6: 443–52.

Ljunggren Ö, Johansson H, Ljunghall S, Lerner U H. Thrombin increases cytoplasmic Ca^{2+} and stimulates formation of prostglandin E_2 in the osteoblastic cell line MC3T3-E1. Bone Miner 1991c; 12: 81–90.

Ljunggren Ö, Fredholm B B, Nordstedt C, Ljunghall S, Lerner U H. Role of protein kinase C in bradykinin-induced prostaglandin formation in osteoblasts. Eur J Pharmacol 1993; 244: 111–7.

McEachern A E, Shelton E R, Bhakta S, Obernolte R, Bach C, Zuppan P, Fujisake J, Aldrich R W, Jarnagin K. Expression cloning of a rat B2 bradykinin receptor. Proc Natl Acad Sci USA 1991; 88: 7724–8.

Partridge N C, Hillyard C J, Nolan R D, Martin T J. Regulation of prostaglandin production by osteoblast-rich calvarial cells. Prostaglandins 1985; 30: 527–39.

Santora A C II. Des-Arg^9-bradykinin stimulation of ROS 17/2.8 rat osteosarcoma adenosine 3', 5' -cyclic monophosphate content: receptor specificity. J Bone Miner Res 1989; 4 (suppl): S332 (abstract).

Stern P H, Stathopoulos V M, Shankar G, Fenton J W II. Second messengers in thrombin-stimulated bone resorption. J Bone Miner Res 1990; 5: 443–9.

Suda T, Takahashi N, Martin T J. Modulation of osteoclast differentiation. Endocr Rev 1992; 13: 66–80.

Suda T, Takahashi N, Martin T J. Modulation of osteoclast differentiation: update 1995. Endocr Rev 1995, in press.

Tatakis D N, Dolce C, Dziak R. Thrombin's effects on osteoblastic cells: I. Cytosolic calcium and phosphoinositides. Biochim Biophys Res Commun 1989; 164: 119–27.

Vaes G. Cellular biology and biochemical mechanism of bone resorption. Clin Orthop 1988; 231: 239–71.