High cathepsin B activity in arthroplasty interface membranes

A histochemical study of 9 loose cemented total hip prostheses

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We studied biopsies of interface membranes of 9 aseptically loosened total hip prostheses. The morphologic resemblance of the cement-facing surface of the membranes to synovial tissue of arthritic joints, as noticed by others, was confirmed by histochemical techniques. High cathepsin B activity was found in the bone-facing surface of the membranes. Since this enzyme also plays an important role in tissue destruction of arthritic joints, further similarities in the mechanisms of tissue breakdown in arthritis and aseptic loosening of cemented hip prostheses may be conjectured.

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Submitted 92-09-13. Accepted 93-06-28

Usually a soft tissue membrane is present at the cement-bone interface of both well-fixed and loose components of hip arthroplasties. The cement-facing side of this interface membrane resembles a synovial membrane; the opposite side is known to be capable of inducing bone resorption (Goldring et al. 1983, Linder et al. 1983, Goldring et al. 1986, Linder and Carlsson 1986, Johanson et al. 1987, Lennox et al. 1987). Histochemical studies have demonstrated high activity of various catabolic enzymes in cells present in the interface membrane. In particular, acid phosphatase activity in macrophages, giant cells and osteoclasts was used as a parameter to study resorption processes (Linder and Carlsson 1986, Pazzaglia et al. 1987). Furthermore, the activity of lysosomal proteases, such as cathepsin B and DPP II activity, has been demonstrated in the cells at the cement surface of the membrane (Lennox et al. 1987).

A possible key role of cathepsin B in destruction of cartilage and bone in arthritic joints has been noticed in studies of rheumatoid arthritis (Mort et al. 1984) and experimentally-induced arthritis (Van Noorden and Vogels 1986, Van Noorden et al. 1988, 1989). The present study is a histochemical investigation of interface membrane biopsies of aseptically loosened total hip prostheses to establish the involvement of cathepsin B in bone resorption.

Patients and methods

Biopsies of the interface membrane around loosened stems of cemented total hip arthroplasties of 9 patients were taken during revision. There were 6 women and 3 men aged 74 (62–84) years. The primary arthroplasty had been for arthrosis in all patients and the mean time between the initial arthroplasty and the revision was 8 (4–14) years. In the operating room, the biopsies were divided into 2 parts. 1 part was fixed and decalcified in an aqueous medium saturated with EDTA containing 2% (w/v) freshly prepared paraformaldehyde and 100 mM cacodylate buffer (pH 7.2). The other part was immediately frozen in liquid nitrogen and stored at –80 °C until further use.

For morphological analysis and orientation in the interface membrane, biopsies were fixed and decalcified for 1 week at room temperature, rinsed overnight in 20 mM phosphate buffer containing 6% (w/v) sucrose, dehydrated in an alcohol series and embedded in Histoplast (Shandon, Runcorn, England). Sections of 5 μm were cut and stained with the polychrome methods of Shoobridge (1983) and Giemsa (Merck, Darmstadt, Germany; see Van Noorden and Frederiks 1992). Sirius Red F3BA (Chroma AG, Stuttgart, Germany) in saturated picric acid which was used for collagen staining (James et al. 1986). Sections were mounted in Euparal.

For immunochemistry, 8-μm-thick cryostat sections were cut at 25 °C from the frozen biopsies. Sections were dried for 30 min at room temperature, fixed in acetone for 10 min at 4 °C and dried for 30 min. Monocyte/macrophage antigen (Leu-M3 (CD14)) was detected with a monoclonal antibody (Becton Dickinson, Mountain View, CA, U.S.A.). First the incubation step was performed for 60 min at room temperature with a 1:20 dilution of the antibody solution in 10 mM phosphat-buffered saline (PBS, pH 7.4) containing 0.2% (w/v) bovine serum albumin. Sections were rinsed thrice for 5 min each in PBS and the second incubation was performed for 30 min using horseradish-peroxidase labeled rabbit-anti-mouse IgG (Dakopatts, Glastrup, Denmark), diluted 1:200 with PBS containing 0.2% bovine serum albumin and 1% human serum. After rinsing thrice for 5 min each in PBS, peroxidase activity was detected by incubation in a solution containing 1 mM 3-amino-9-ethylcarbazole (AEC; Sigma, St. Louis, MI, U.S.A.), 5% (v/v) dimethylformamide and 50 mM acetate buffer (pH 4.9). AEC was dissolved first in dimethylformamide. Immediately before use, 0.05% (v/v) hydrogen peroxide was added to the medium. Incubation lasted for 10 min at room temperature; then sections were rinsed in distilled water and counterstained with diluted hematoxylin for 1 min and mounted in glycerin-gelatin. The immunocytochemical method was based on the methods of Stet et al. (1986) and Dijkstra et al. (1987) and optimized for the present conditions. Control incubations were performed by a first-step incubation with PBS and 0.2% bovine serum albumin alone (Dijkstra et al. 1987).

Metabolic analysis was performed using enzyme histochemical methods and unfixed cryostat sections (8 µm thick), as described by Van Noorden and Frederiks (1992). The activity of alkaline phosphatase was detected by incubating the sections for 10 min at 37 °C, tartrate-resistant acid phosphatase was detected by incubation for 15 min at 37 °C in the presence of 40 mM sodium, potassium L-tartrate (Merck), b-hydroxybutyrate dehydrogenase activity was detected by incubation for 30 min at 37 °C. Cathepsin B activity was analyzed after 20 min incubation at 20 °C, using a kinetic fluorescence method and CBZ-Ala-Arg-Arg-4-methoxy-2-naphthylamide (MNA; Enzyme Systems Products, Dublin, CA, U.S.A.) as substrate and 5'-nitrosalicylaldehyde as coupling agent. Elastase activity was detected after 60 min incubation at 20 °C, using the same fluorescence method and Suc-Ala-Ala-Ala-MNA (Enzyme Systems Products; Rudolphus et al. 1992). Control incubations were performed, as described by Van Noorden and Frederiks (1992) for alkaline phosphatase in the presence of 10 mM levamisole, for tartrate-resistant acid phosphatase and b-hydroxybutyrate dehydrogenase in the absence of substrate, for cathepsin B in the presence of 10 µM Leupeptin (Sigma) and for elastase in the absence of substrate. Photomicrographs were taken using white light with an Olympus Vanox-T photomicroscope with ×1 and ×10 Olympus objectives. Fluorescence photomicrographs were taken using excitation between 440 and 500 nm and a barrier filter of 515 nm and a ×25 Leitz NPI Fluotar objective.

Results

Sections of interface membrane stained with Shoobridge, Giemsa and Sirius Red showed similar morphology as described by Goldring et al. (1983, 1986) and Lennox et al. (1987). A synovial membrane-like cell layer was present at the cement side. Underneath this layer a fibrous connective tissue was found sometimes infiltrated with mononuclear leukocytes and round particles, probably cement particles. In this layer, areas of necrosis frequently occurred. At the other side of the membrane (bone surface) the fibrous tissue was infiltrated more frequently with mononuclear leukocytes than at the cement surface. Figures 1 and 2 demonstrate that many of these infiltrating cells were macrophages detected immunocytochemically. In contrast, elastase activity which is particularly present in neutrophils (Rudolphus et al. 1992) was found only in a few cells scattered over the whole of the membranes. The bone surface showed, in all membranes, areas with a high density of multinuclear giant cells with osteoclast features (Figure 1). In some cases, bone was included in the section, and lacunae in the bone containing these giant cells were found frequently. This bone surface contained a high alkaline phosphatase activity (Figures 3 and 4) as well as tartrate-resistant acid phosphatase activity (Figure 5). Figures 3 and 5 show that these activities were not very pronounced in other areas of the interface membranes. Figure 6 shows that the giant cells contained a very high b-hydroxybutyrate dehydrogenase activity, suggesting that these cells were osteoclasts (Van Noorden and Vogels 1986, Van Noorden et al. 1989). In areas of the interface membrane where high amounts of osteoclast-like cells were found, high cathepsin B activity was also present (Figure 7). Other areas, particularly at the cement surface, did not show significant amounts of cathepsin B activity (Figure 8).

Discussion

Besides similarities, there are also differences between the microscopic appearance of an active interface

Figure 1-8: Serial sections of interface membrane. B bone-side of membrane; C cement-side of membrane; gc osteoclast-like multinuclear giant cell; m macrophage; sm synovial membrane-like surface of interface membrane at the cement-side

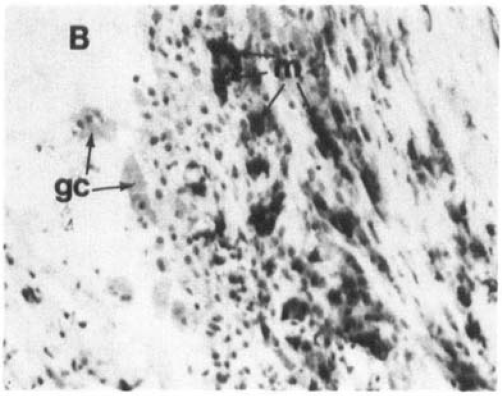

Figure 1. Bone surface of interface membrane stained with CD14 antibody to demonstrate macrophages, ×100.

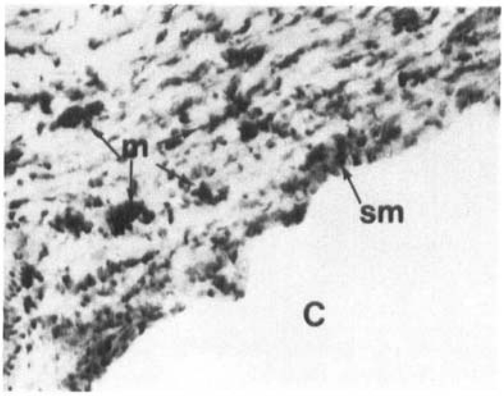

Figure 2. Cement surface of interface membrane stained with CD14 antibody to demonstrate macrophages, ×100.

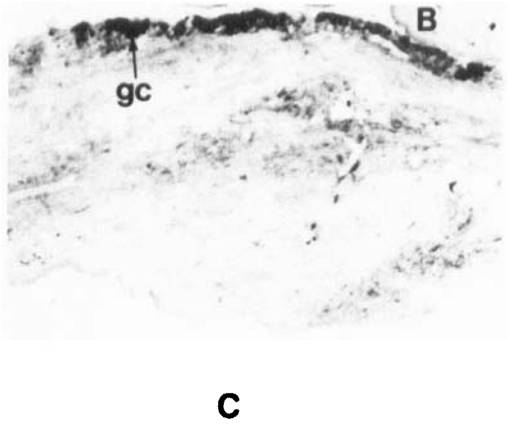

Figure 3. Cross-section of interface membrane incubated for alkaline phosphatase activity, ×20.

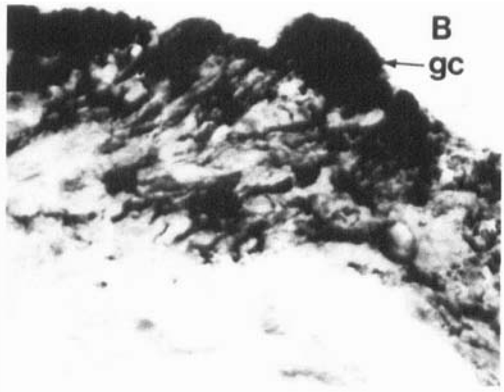

Figure 4. Detail of distribution of alkaline phosphatase activity at bone surface, as shown in Figure 3 at low power, ×150.

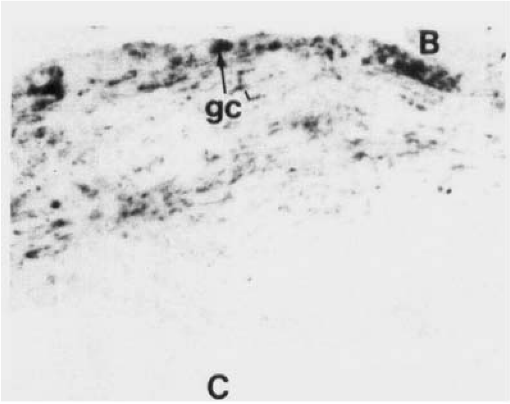

Figure 5. Cross-section of interface membrane incubated for tartrate-resistant acid phosphatase activity. Parallel section of section shown in Figure 3, ×20.

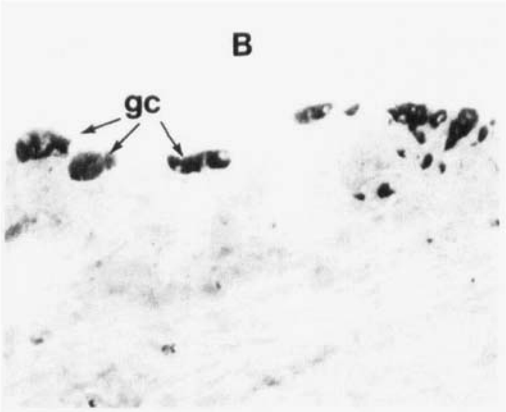

Figure 6. b-Hydroxybutyrate dehydrogenase activity in bone surface of interface membrane. Osteoclast-like multinuclear giant cells have a high activity, ×120.

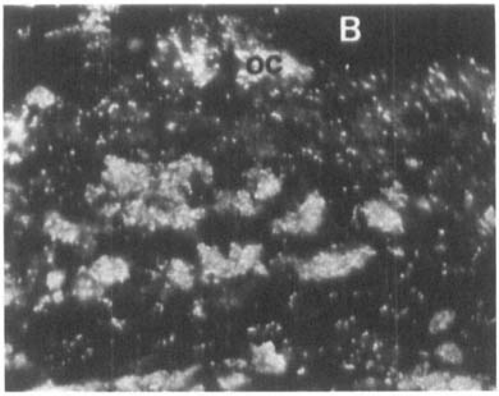

Figure 7. Cathepsin B activity in bone surface of interface membrane, as demonstrated with a fluorescence method. White dots represent cathepsin B activity, x150.

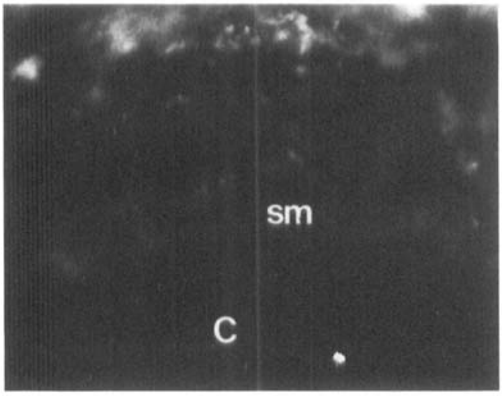

Figure 8. Cathepsin B activity in synovia-like cement surface of interface membrane, as demonstrated with a fluorescence method. Hardly any activity is present, ×150.

membrane and that of synovial and subsynovial tissues, as seen in non-septic arthritis. One important feature of the interface membrane is the presence of foreign body giant cells around wear particles, sometimes leading to granulomata; this is not found in arthritic lesions. However, in both pathological conditions, macrophages are the predominant infiltrating cells and it is likely that macrophage-mediated responses finally cause tissue destruction. Apparently, in the loosening process of hip prostheses, activation of macrophages is caused by particles of polyethylene, cement or metal (Howie et al. 1988, Howie and Vernon-Roberts 1988, Murray and Rushton 1990, Goodman and Lidgren 1992) and by prosthetic micromotion (Santavirta et al. 1992), whereas in arthritis the macrophages are triggered by cytokines or immunological and/or chemical agents.

The biopsies in the present study confirmed the microscopical resemblance of the interface membrane to synovial tissue of arthritic joints. This, however, accounts for the cement-facing side of the membrane; the bone resorption occurs at the opposite, bone-facing side. The present histochemical study of unfixed, undecalcified cryostat sections of interface membranes offered the possibility to evaluate the biological behavior of this membrane without the distorting effect of fixation and decalcification on the biopsies (Van Noorden and Frederiks 1992). High cathepsin B activity was found in macrophages, fibroblasts and osteoclast-like cells along the bone surface of interface membranes, particularly in those areas where active bone resorption was found; other parts of the membrane did not show significant amounts of cathepsin B activity. The same observations were made in experimentally-induced arthritis by Van Noorden et al. (1988, 1989).

On the basis of these observations, further similarities, especially between mechanisms leading to tissue destruction in arthritis and mechanisms leading to bone resorption around the interface membrane, may be conjectured. In clinical and experimental studies on arthritis, the significance of cathepsin B in the breakdown of cartilage has been demonstrated. In the normal, non-arthritic joint the cysteine proteinases cathepsin B, D and L participate in cartilage remodeling by intracellular breakdown of proteoglycans and collagen type II. In histochemical studies of experimentally-induced arthritis, cathepsin B has been identified as a possible key factor in cartilage and bone destruction; high cathepsin B activity has been found in osteoclasts, chondrocytes, fibroblasts, synovial cells and bone marrow cells after induction of arthritis in rat knee joints (Van Noorden et al. 1988, 1989). Immunocytochemical studies revealed extracellular cathepsin B and D in rheumatoid articular cartilage (Poole and Mort 1981).

Cathepsin B is involved in active bone resorption by osteoclasts by breaking down the collagen (type 1) of the ground substance of demineralized bone (Van Noorden and Everts 1991, Everts et al. 1992); it is active in normal bone metabolism as well as in pathological resorption (Vaes 1988). Lennox et al. (1987) found cathepsin B activity in the implant facing the pseudo-synovial cells of the interface membrane of loosened total hip components. Spector et al. (1990) have noticed that many of the morphological features of the cells constituting the pseudomembranes which they analyzed resembled those of fibroblasts rather than of macrophages.

The similarity between arthritic joints and the interface membranes of loose cemented hip prostheses is probably explained by the fact that we are looking at common pathways in tissue destruction; cathepsin B is one of the important catabolic enzymes involved in this destruction. Inhibition of the trigger mechanisms which initiate the chain of events finally causing osteolysis would be the most effective way to improve longevity of hip prostheses; as long as we are not able to achieve this, the inhibition or alteration of other steps later in the chain must be studied.

The observations in these studies justify further investigation of the role of fibroblasts and cathepsin B in the process of aseptic loosening. One of the areas of interest could be the selective inhibition of cathepsin B by Z-Phe-Ala-fluormethyl ketone (CH_2F). In experimentally-induced arthritis of rat knees, this compound has been shown to suppress the inflammation with decreased intracellular and extracellular cathepsin B activity, and less cartilage damage as a result (Van Noorden et al. 1988, Van Noorden and Everts 1991). The possible inhibition by Z-Phe-AlaCH_2F on the bone-resorbing effects of cathepsin B in the interface membrane of total hip arthroplasties by either local or systemic treatment needs further study.

Acknowledgements

The technical assistance of Klazien S Bosch and Ilse M C Vogels, as well as the preparation of the figures by Jan Peeters, are gratefully acknowledged.

References

Dijkstra C D, Döpp E A, Vogels I M, Van Noorden C J. Macrophages and dendritic cells in antigen-induced arthritis. An immunohistochemical study using cryostat sections of the whole knee joint of rat. Scand J Immunol 1987; 26 (5): 513–23.

Everts V, Delaissé J M, Korper W, Niehof A, Vaes G, Beertsen W. Degradation of collagen in the bone-resorbing compartment underlying the osteoclast involves both cysteine proteinases and matrix metalloproteinases. J Cell Physiol 1992; 150 (2): 221–31.

Goldring S R, Schiller A L, Roelke M, Rourke C M, O'Neil D A, Harris W H. The synovial-like membrane at the bone cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg (Am) 1983; 65 (5): 575–84

Goldring S R, Jasty M, Roelke M S, Rourke C M, Bringhurst F R, Harris W H. Formation of a synovial-like membrane at the bone cement interface. Its role in bone resorption and implant loosening after total hip replacement. Arthritis Rheum 1986; 29 (7): 836–42.

Goodman S, Lidgren L. Polyethylene wear in knee arthroplasty. A review. Acta Orthop Scand 1992; 63 (3): 358–64.

Howie D W, Vernon-Roberts B. The synovial response to intraarticular cobalt-chrome wear particles. Clin Orthop 1988; 232: 244–54.

Howie D W, Vernon-Roberts B, Oakeshott R, Manthey B. A rat model of resorption of bone at the cement bone interface in the presence of polyethylene wear particles. J Bone Joint Surg (Am) 1988; 70 (2): 257–63.

James J, Bosch K S, Zuyderhoudt F M, Houtkooper J M, van Gool J. Histophotometric estimation of volume density of collagen as an indication of fibrosis in rat liver. Histochemistry 1986; 85 (2): 129–33.

Johanson N A, Bullough P G, Wilson P D Jr, Salvati E A, Ranawat C S. The microscopic anatomy of the bone cement interface in failed total hip arthroplasties. Clin Orthop 1987; 218: 123–35.

Lennox D W, Schofield B H, McDonald D F, Riley L H Jr. A histologic comparison of aseptic loosening of cemented, press-fit, and biologic ingrowth prostheses. Clin Orthop 1987; 225: 171–91.

Linder L, Lindberg L, Carlsson A. Aseptic loosening of hip prostheses. A histologic and enzyme histochemical study. Clin Orthop 1983; 175: 93–104.

Linder L, Carlsson A S. The bone cement interface in hip arthroplasty. A histologic and enzyme study of stable components. Acta Orthop Scand 1986; 57 (6): 495–500.

Mort J S, Recklies A D, Poole A R. Extracellular presence of the lysosomal proteinase cathepsin B in rheumatoid synovium and its activity at neutral pH. Arthritis Rheum 1984; 27 (5): 509–15.

Murray D W, Rushton N. Macrophages stimulate bone resorption when they phagocytose particles. J Bone Joint Surg (Br) 1990; 72 (6): 988–92.

Pazzaglia U E, Dell Orbo C, Wilkinson M J. The foreign body reaction in total hip arthroplasties. A correlated light microscopy, SEM, and TEM study. Arch Orthop Trauma Surg 1987; 106 (4): 209–19.

Poole A R, Mort J S. Biochemical and immunological studies of lysosomal and related proteinases in health and disease. J Histochem Cytochem (3A Suppl) 1981; 29: 494–502.

Rudolphus A, Stolk J, van Twisk C, van Noorden C J, Dijkman J H, Kramps J A. Detection of extracellular neutrophil elastase in hamster lungs after intratracheal instillation of E. coli lipopolysaccharide, using a fluorogenic, elastase-specific, synthetic substrate. Am J Pathol 1992; 141 (1): 153–60.

Santavirta S, Gristina A, Konttinen Y T. Cemented versus cementless hip arthroplasty. A review of prosthetic biocompatibility. Acta Orthop Scand 1992; 63 (2): 225–32.

Shoobridge M P. A new principle in polychrome staining: a system of automated staining, complementary to hematoxylin and eosin, and usable as a research tool. Stain Technol 1983; 58 (5): 245–58.

Spector M, Shortkroff S, Hsu H P, Lane N, Sledge C B, Thornhill T S. Tissue changes around loose prostheses. A canine model to investigate the effects of an antiinflammatory agent. Clin Orthop 1990; 261: 140–52.

Stet R J, Thomas C, Koudstaal J, Hardonk M J, Hulstaert C E, Nieuwenhuis P. Graft versus host disease in the rat: cellular changes and major histocompatibility complex antigen expression in the liver (published erratum appears in Scand J Immunol 1986 Apr; 23 (4): 521). Scand J Immunol 1986; 23 (1): 81–9.

Vaes G. Cellular biology and biochemical mechanism of bone resorption. A review of recent developments on the formation, activation, and mode of action of osteoclasts. Clin Orthop 1988; 231: 239–71.

Van Noorden C J, Vogels I M. Enzyme histochemical reactions in unfixed and undecalcified cryostat sections of mouse knee joints, with special reference to arthritic lesions. Histochemistry 1986; 86 (2): 127–33.

Van Noorden C J, Smith R E, Rasnick D. Cysteine proteinase activity in arthritic rat knee joints and the effects of a selective systemic inhibitor, Z Phe AlaCH2F. J Rheumatol 1988; 15 (10): 1525–35.

Van Noorden C J, Vogels I M, Smith R E. Localization and cytophotometric analysis of cathepsin B activity in unfixed and undecalcified cryostat sections of whole rat knee joints. J Histochem Cytochem 1989; 37 (5): 617–24.

Van Noorden C J, Everts V. Selective inhibition of cysteine proteinases by Z Phe AlaCH2F suppresses digestion of collagen by fibroblasts and osteoclasts. Biochem Biophys Res Commun 1991; 178 (1): 178–84.

Van Noorden C J, Frederiks W M. Enzyme histochemistry: a laboratory manual of current methods. Oxford Univ Press, Oxford 1992.