Chapter 8

Cellular Labeling with ^{99m}Tc Chelates: Relevance of In Vitro and In Vivo Viability Testing

8

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8.1 Introduction

Labeling of blood cells has gained importance as routine procedure in nuclear medicine. A variety of different blood cells such as red blood cells (RBC), platelets, and white blood cells ([WBC] neutrophils, lymphocytes and monocytes and recently stem cells) can be radiolabeled and applied for diagnosis and therapeutic monitoring in specific disease states.

In order to label specific cell types, the separation of that particular type of cell must be carefully performed.

Two main categories of cell labeling have been used, cohort and random population labeling. In cohort labeling, the label binds to marrow precursors, which appear after a few days as a labeled cell population of uniform age in the circulation, enabling the study of the cells during their life span. Cohort labeling is useful for the study of cell production rates and survival. In random labeling, the circulating cell population (of all ages) is uniformly labeled, allowing mean life span determination. Random labeling is carried out usually in vitro on a small sample of venous blood. Random labeling methods have been more successfully used than cohort labeling techniques.

Accuracy in diagnosis depends largely on the labeling efficiency (LE) of blood cells, which can be affected by many factors. The specificity of the labeling, maintenance of viability, and normal physiological function of the labeled cells, together with sterility, apyrogenicity and radiopharmaceutical purity, and stability have to be carefully controlled.

The methods involved in the labeling of blood cells with ^{99m}Tc are reviewed and discussed in this overview.

8.2 Red Blood Cells

Labeling of RBC is easily facilitated as they are relatively abundant in the blood, easily separated and handled in vitro, not very susceptible to damage from physical or chemical manipulations, not as dependent on energy and nutritional requirements as the other cellular elements in vitro, and have a variety of cellular transport mechanisms and hemoglobin within that is rich in active metal-binding sites (Srivastava and Rao Chervu 1984).

Random labeling methods of RBC have been more widely utilized than have cohort labeling techniques.

Besides ^{99m}Tc, several radionuclides such as ^{3}H, ^{11}C, ^{14}C, ^{15}N, ^{32}P, ^{51}Cr, ^{55}Fe, ^{59}Fe, ^{67}Ga, ^{68}Ga, and ^{111}In have been evaluated for labeling of RBC. In vivo, in vitro, and in vivo/in vitro techniques have been developed and are presently available for routine use in nuclear medicine (for imaging as well as kinetic studies).

Technetium as pertechnetate is in the +7 valency state and as such, is not bound firmly to RBC, and moves in and out of the RBC rather freely. In contrast, reduced technetium cannot readily cross the cell membrane (Srivastava and Rao Chervu 1984) and leave the cell, and binds rapidly and irreversibly mainly to the β-chain of the globin part of hemoglobin (Eckleman et al. 1979). Reducing agents thus have to be used for labeling RBC in vivo and in vitro.

For in vivo labeling, stannous salts are injected intravenously approximately 15–20 min before injection of ^{99m}Tc-pertechnetate. Stannous ions diffuse into the cell, become bound to a cellular component, have a relatively slow clearance (Srivastava and Rao Chervu 1984), and reduce ^{99m}Tc when it enters the RBC. The labeling of RBC thus occurs within the intravenous space. Using the in vitro technique, the labeling of RBC is performed in a sterile vial. Most methodologies contact RBC, generally in whole blood, with stannous ions, using a suitable tin (II) preparation (Srivastava and Rao Chervu 1984). In the in vitro technique, stannous ions are injected into the patient, and labeling with ^{99m}Tc-pertechnetate of a smaller number of RBC is performed in a closed system (e.g., butterfly needle placed in a peripheral vein). Thereafter, the labeled RBC (in plasma) are reinjected into the patient (Berger and Zaret 1984; Winzelberg et al. 1982). In vivo labeling is more widely used. However, RBC compete for intravenous ^{99m}Tc-pertechnetate with the stomach, thyroid, and kidney. In vivo methods thus have frequently variable and irreproducible LE, which is generally lower than that of the in vitro technique. In the in vitro techniques, quality control in order to avoid free ^{99m}Tc-pertechnetate and thus, unnecessary radiation exposure to the patient, is performed prior to the injection of ^{99m}Tc-RBC. In vitro techniques are therefore the methods of choice when high LE is required as there is almost no free pertechnetate in the patient (Berger and Zaret 1984; Winzelberg et al. 1982).

Several parameters are influencing RBC labeling and thus, LE (which is determined by measuring the cell-bound radioactivity as compared with the total radioactivity in percentage) (Table 8.1). Carrying out an in vitro technique the type of anticoagulant used can modify the ^{99m}Tc labeling of RBC as well as the ^{99m}Tc-RBC distribution. Controversy exists about the effect of the type of anticoagulants that have been used (ethylenediaminetetraacetic acid [EDTA], heparin, acid-citrate-dextrose [ACD], sodium citrate, sodium oxalate and others) for RBC labeling in vitro. LE, in vitro and in vivo stability of the label with EDTA are higher as compared with saline. EDTA reduces the stannous tin content of plasma to a minimum level and assures good labeling yields, which is particularly important with samples of blood with very low hematocrit (Srivastava and Rao Chervu 1984). The use of ACD results in superior RBC labeling relative to heparin (the most utilized methods) (Porter et al. 1983) and ^{99m}Tc-RBC/ACD images are superior to those of ^{99m}Tc-RBC/heparin (Wilson and Hung 1992). Heparin in the catheter was shown to affect RBC labeling (Hegge et al. 1978). In addition, ACD preserves the cellular function much better than does heparin (Srivastava and Straub 1990). ACD is thus the anticoagulant preferred for preparing in vivo/in vitro ^{99m}Tc labeling of RBC. The optimal conditions for RBC labeling, both in vitro and in vivo, evaluated with ^{99m}Tc-oxine (1 mCi/ml) and ACD or sodium citrate as anticoagulants, showed that the LE is also dependent on the density of RBC (Fig. 8.1), time (Fig. 8.2), pH (Fig. 8.3), and temperature of incubation (Figs. 8.1–8.4), as well as on the amount of the tracer (Fig. 8.4) (optimal conditions, LE >95%: 1×10^9 RBC/ml, 1 min, pH 5.0–8.0, 37 °C incubation, and 5 μg oxine/ml) (Reiter et al. 1984).

The LE, as well as the localization, of RBC can be markedly influenced by drugs circulating in the plasma and/or diseases, which can affect RBC (the membrane potential, among others) (Table 5.1). In patients who have recently received iodinated contrast

Table 8.1. Variables influencing red blood cell (*RBC*) labeling with ^{99m}Tc-pertechnetate

Collection injury
Density of RBC
Disease (e.g., leukemia, high fibrinogen level, sickle cell disease, other abnormalities of hemoglobin (Riordon and Nelp 1982)
RBC antibodies (Leitl et al. 1980)
Intravenous canulae
Anticoagulant
Quantity of stannous ions (II) (Rao et al. 1986)
Time delay between "tinning" of RBC and pertechnetate administration
Incubation Medium (e.g., dextrose in water) (Berger and Zaret 1984) pH Temperature Time Speed of centrifugation
Drugs or pharmacological interventions (heparin (Berger and Zaret 1984), antimicrobials or antibiotics, anticonvulsants, tranquilizers, anti-inflammatory agents (Chervu et al. 1981; Hladik et al. 1982), nifedipine (Sampson 1993), prazosin, digoxin (Lee 1983), propanolol, hydralazine, methyldopa, verapamil, chlorothiazide, furosemide, ranitidine, iron therapy, etoposide+ doxontbicin (Sampson 1993, 1995), others)
Aluminum (Sampson 1995)
Iodinated contrast media (Tatum et al. 1983)

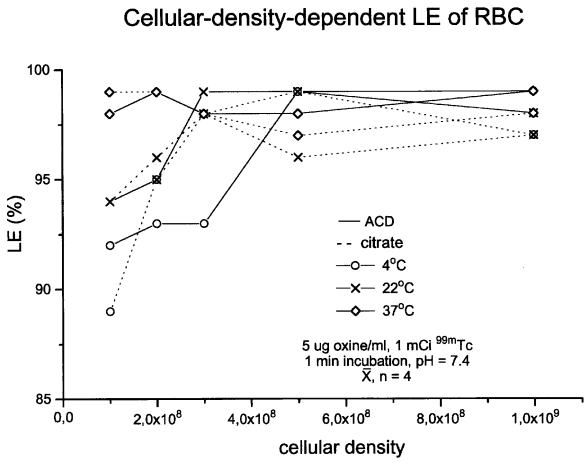

Fig. 8.1. Cellular density–dependent labeling efficiency (*LE*) of red blood cells (*RBC*)

media, a great reduction of LE (from the normal value of 90% to as low as 30%) can occur, probably due to a change in either stannous ion distribution or redox potential (Tatum et al. 1983). Stannous ions should be injected directly and not through an intravenous canulae, as synthetic material also diminishes LE (Sampson 1995).

For evaluation the function of labeled RBC, in vitro and in vivo viability testing have been used. No evidence of morphological alteration of RBC (histological and/or electron microscopic examinations (Fig. 8.5) after ^{99m}Tc-labeling was seen.

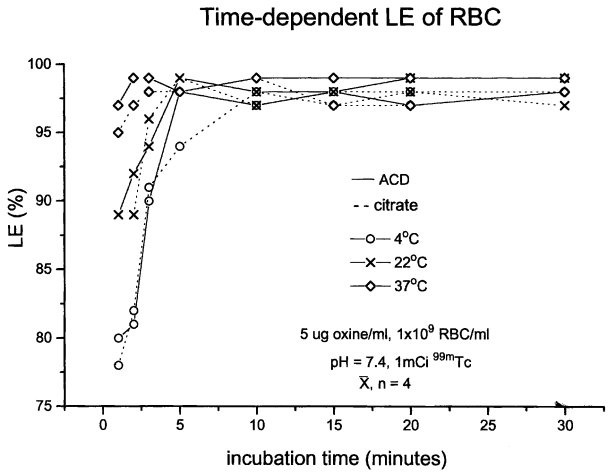

Fig. 8.2. Time-dependent LE of RBC

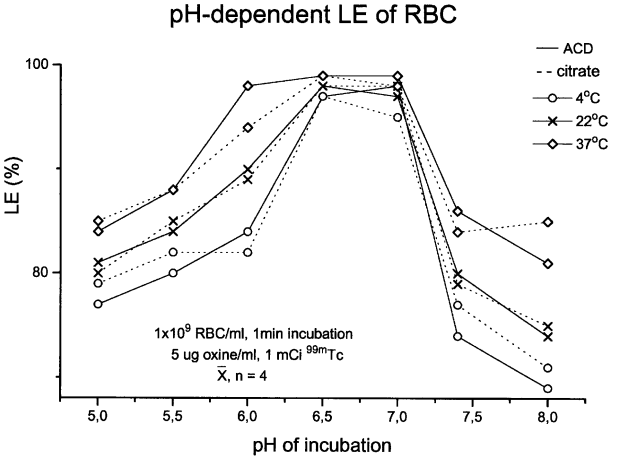

Fig. 8.3. pH-dependent LE of RBC

Recovery ([REC] i.e., the percentage of injected dose remaining cell bound in the circulation for 60 min) and in vivo biodistribution provide key information about cell integrity and function. Even after several isolation and incubation steps and being exposed to radioactive material, RBC have been shown not to alter their in vivo behavior significantly. Elution of ^{99m}Tc from RBC and/or its poor LE is reflected by gastric secretion of ^{99m}Tc-pertechnetate and accumulation of free pertechnetate in the thyroid, as well as increased urinary radioactivity.

^{99m}Tc labeling of RBC allows imaging with low-dose radiation to the patient. Applications of ^{99m}Tc-RBC are in widespread use clinically (Table 8.2). In particular, ^{99m}Tc-RBC have greatly contributed to the rapid growth of nuclear cardiology. However, due to the short half-life of ^{99m}Tc, serial imaging should be performed up to 12 h after the

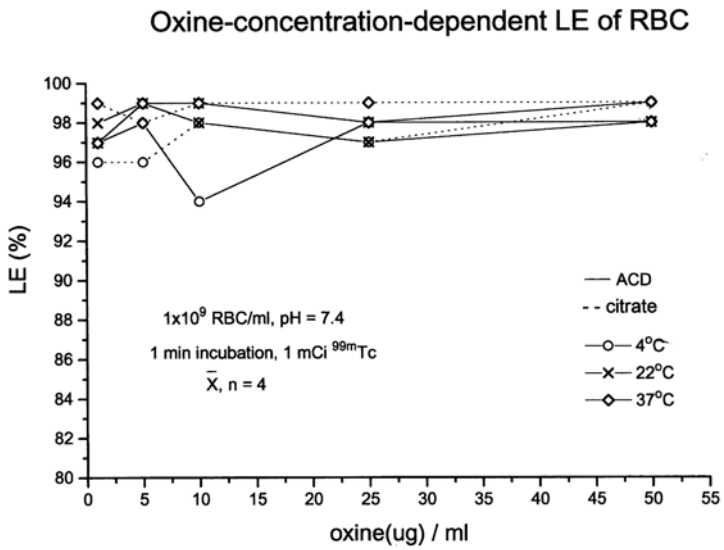

Fig. 8.4. Oxine concentration–dependent LE of RBC

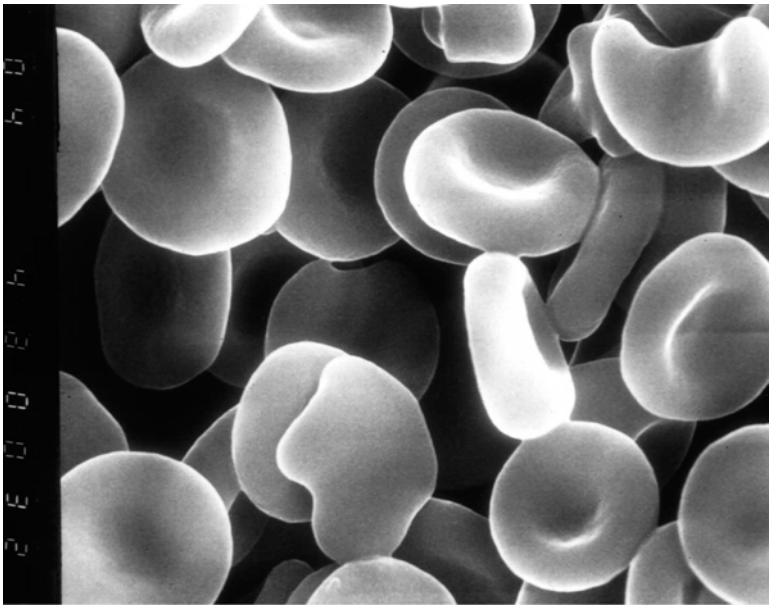

Fig. 8.5. Human RBC after radiolabeling, examined by scanning electron microscopy. No sign of morphological alteration is visible. Perfusion fixation, critical point drying, ×1500

Table 8.2. Clinical applications of ^{99m}Tc-RBC

Normal RBC	Cardiovascular procedures hemangiomas Blood pool imaging Vascular malformations Bleeding (GI, cerebral, others), cerebral blood flow stroke Deep vein thrombosis
Denaturated RBC	Spleen imaging Splenic infarcts Accessory spleen(s) localization Assessment of splenic function and size Space-occupying disease GI bleeding

GI gastrointestinal

radiolabeling. In addition, a spontaneous elution of the label in vivo of 4–10% per hour occurs (Ferrant et al. 1974). ^{99m}Tc-RBC is thus not applicable for studying RBC mass determination and lifespan measurement. For these and other clinical procedures, longer-lived labels (such as ^{51}Cr) are required.

For splenic studies (Table 8.2), heat-damaged ^{99m}Tc-RBC have to be used, the in vitro technique being the method of choice. High splenic uptake (~70%) allows the administration of low activities of the radiotracer (~1 mCi), thus reducing the radiation dose to patients considerably (Srivastava and Rao Chervu 1984).

8.3 Platelets

Random labeling of platelets has been more successfully employed than cohort labeling. Only autologous platelets are used nowadays. Platelets have to be isolated prior to labeling, as the radiopharmaceuticals presently available for platelet-labeling are nonspecific, labeling other blood cells and some plasma proteins as well (Najean 1986). The centrifugation steps and washing procedures are potentially damaging to platelets, and platelet isolation must thus be carefully performed in order to avoid cellular activation and in vitro damage. Great varieties of methods have been reported for platelet isolation (Bunting et al. 1982; Hawker et al. 1987; Sinzinger et al. 1984). In practice, however, the platelets are isolated from anticoagulated blood by simple differential centrifugation (Najean 1986; Sinzinger et al. 1984). This method allows obtaining platelets in high concentration, maintaining their functional qualities (in terms of viability, cell kinetics, survival, and sequestration sites). Complete separation of platelets from the other blood cells is, however, difficult to obtain. Recently (Sweeney et al. 1995), relatively rapid (average filtration time of 6.6 min) preparation of platelet concentrates with good platelet yields (total platelet concentrates platelet counts averaging $7.8 \pm 1.8 \times 10^{10}$) that are WBC-reduced (WBC count to levels below 7×10^5) before storage has been made possible by the introduction of filtration (using a large-capacity filter) of the platelet-rich-plasma (PRP). Prestorage filtration of PRP and the preparation of filtered platelets did not result in any significant beneficial or adverse effect on subsequent platelet quality. This approach seems thus to be a new and easy means of producing a PRP-derived WBC-reduced platelet concentrate (Sweeney et al. 1995).

Various factors influence platelet labeling (Rodrigues and Sinzinger 1994; Table 8.3). Platelet function depends on glycogen as an energy source, glucose being a key sub-

Table 8.3. Variables influencing platelet labeling with ^{99m}Tc-pertechnetate

Collection injury
Density of platelets
Anticoagulant
Amount of the complex
Incubation
Medium
pH
Temperature
Time
Speed of centrifugation
Type of plastic used for bags and tubes
Aging (Baldini and Myers 1980)
Calcium ions (Becker et al. 1988)
Lipids
Cholesterol (Granegger et al. 1988; Sinzinger 1987 a)
Low-density lipoprotein (LDL) (Granegger et al. 1988; Sinzinger 1987 a)
Additives
Prostaglandins (Sinzinger 1987 a)
Nitric oxide (Wagner et al. 1989)
Others

strate for platelet function (Schneider and Gear 1994). The medium used for resuspending the platelet pellet is thus particularly important for the maintenance of the functional properties of platelets. The choice of the anticoagulant is also crucial. Several anticoagulants have been used: heparin (may induce platelet activation and shortens the platelet survival), citrate (lacks dextrose for platelet nutrition), EDTA (may damage platelets), and ACD (pH of 6.5, contains dextrose, increases thus cellular viability). LE of platelets is by far higher with ACD and sodium citrate as compared with EDTA and heparin (Fig. 8.6).

Due to the absence of proteins and calcium, incubation of platelets in ACD-saline increases the membrane permeability and further the incorporation of tracers and LE. ACD is thus nowadays the anticoagulant of choice for platelet labeling. During pelleting, platelets may become activated and may become difficult to resuspend, unless activation is inhibited. The simplest way of performing such inhibition is to acidify (to pH 6.2–6.5) the platelet-rich plasma (Lötter et al. 1986). In addition, antiaggregatory prostaglandins (PG), either PGE_1 (Hawker et al. 1987) or prostacyclin ([PGI_2] 3.33 μg) (Sinzinger et al. 1981), which are protecting platelets from the activating effects of centrifugation and other damage by elevation of cAMP, among others, can be added and are commonly used.

The addition of PG affects labeling parameters and in vitro viability testing favorably (Sinzinger et al. 1987a), being especially useful in thrombocytopenia or other rare clinical conditions, to avoid damage of particularly sensitive platelets. PG stabilizes the platelet membrane (Gorman et al. 1977), minimizes artificial damage (Sinzinger et al. 1981, 1987 b), and reduces platelet trapping on the reinjection site (Sinzinger et al. 1981), thereby increasing REC significantly (Sinzinger et al. 1987 a). The duration of the effect of PG on platelets is prolonged, and PG can thus be added to the whole blood once, only at the start of the labeling procedure, without the need to further add the compounds at subsequent steps (of the procedure), even though these may involve platelet washing (Peters et al. 1986). The platelet pellet can then be easily resuspended

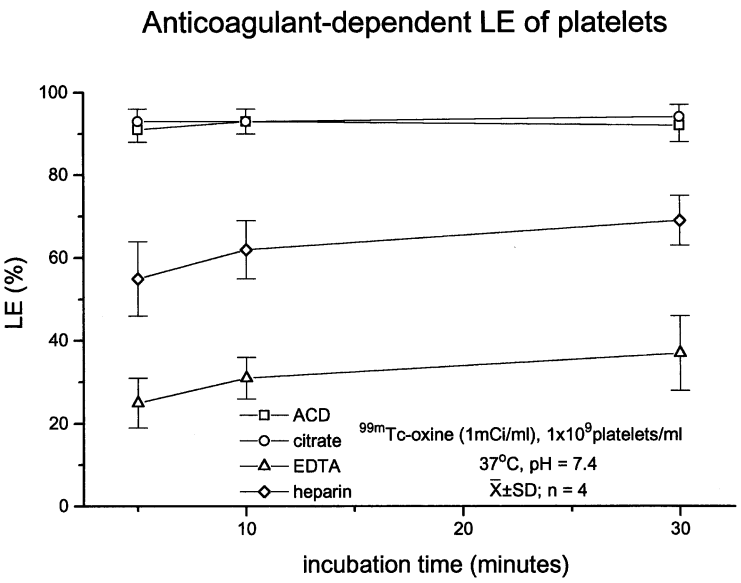

Fig. 8.6. Anticoagulant-dependent LE of platelets

in a small volume of buffer or plasma for labeling (Peters 1988). Nitric oxide (NO), the active compound of the endothelium derived relaxing factor (EDRF), improves the platelet metabolic activity by increasing cGMP, and may increase the REC and facilitate platelet handling (Wagner et al. 1989). It has to be added at a quite late stage after the preparation of the platelet pellet, because hemoglobin inactivates NO. Therefore, extremely high amounts of NO or NO donors (e.g., doses of more than 5–10 mg of molsidomine) per preparation added immediately and repeatedly would be necessary, which are rather expensive and thus unrealistic. Platelet function is better preserved in plasmatic environment and thus viability is superior, and a higher REC is seen when platelets are labeled in plasma as compared with buffer (Thakur et al. 1981). Use of plasma, however, requires longer incubation times to achieve acceptable LE as compared with buffered salt solutions (Scheffel et al. 1979). Keeping the time of incubation of platelets as short as possible is fundamental, as platelet viability due to damaging decreases with time (Mathias and Welch 1979). A closed Monovette technique (Sinzinger et al. 1984) made the techniques of labeling platelets accessible for wide clinical use, even in smaller units, at low costs. This method combines the requirements currently considered as optimal for platelet labeling (i.e., lowest amount of blood [16 ml], shortest ex vivo period of platelets (less than 60 min), and optimal labeling conditions (37 °C, 5 min).

Several techniques, radionuclides and tracers (such as ^{51}Cr, ^{111}In [-oxine, -oxine-sulfate, -tropolone, -mercaptopyridine-*N*-oxide, -acetylacetone, and -chlorotetraphenylporphyrin], ^{99m}Tc [-oxine, -hexamethylpropyleneamine oxime {HMPAO} and -phytate] and ^{123}I-metaiodobenzylguanidine [MIBG]) have been communicated in an attempt to achieve the requirements for optimal in vitro radiolabeling and subsequent in vivo studies. The labeling of platelets with ^{99m}Tc is still in its infancy. ^{99m}Tc has the advantage of easy storage, low cost, and it shows almost no reutilization. However, it has no specificity of labeling, and the elution rate (for platelet labeling) is high (8% per hour) (Becker et al. 1988 a). Various tracers have been evaluated.

^{99m}Tc-oxine has proved to be a good platelet label. The mean LE (91%) and REC (71%) data of ^{99m}Tc-oxine were found to be comparable to ^{99m}Tc-oxine-sulfate and ^{111}In-oxine (Angelberger et al. 1981). An LE of >90% was found after an incubation time of only 1 min at 37 °C (Fig. 8.2). ^{99m}Tc-oxine and ^{99m}Tc-oxine-sulfate, however, are not commercially available.

^{99m}Tc-HMPAO is an interesting platelet-labeling compound, mainly due to its lipophilicity and the good quality images of platelet accumulation over the lifetime of ^{99m}Tc (Peters 1988). However, ^{99m}Tc-HMPAO has an LE by far lower, and requires a much longer incubation time than do ^{99m}Tc- and ^{111}In-oxine, which favors platelet functional damage. Due to the relatively high elution rate from platelets, a significant gastrointestinal and renal excretion of free ^{99m}Tc-pertechnetate occurs (Becker et al. 1988a), which significantly impairs abdominal and pelvic imaging.

^{99m}Tc-phytate again requires a long incubation time (30 min), shows a low LE (20–50%) and a high elution rate. In addition, divalent cations, such as Ca^{2+} and Mg^{2+}, in the anticoagulant and the incubation buffer interfere negatively with the labeling (H. Sinzinger, unpublished data). Thus, ^{99m}Tc-phytate did not succeed as a platelet label, nor is it commercially available. The viability of platelets following radiolabeling has been evaluated by in vitro tests and by their in vivo distribution. The most commonly used in vitro methods have been testing the response of radiolabeled platelets to aggregating agents (e.g., adenosine diphosphate [ADP] (Sinzinger et al. 1987a) [Fig. 8.7], collagen, ristocetin, arachidonic acid, PAF, epinephrine (Mathias and Welch 1984; and others), their adhesion to foreign (e.g., vascular) surfaces (Peters 1988), their migration

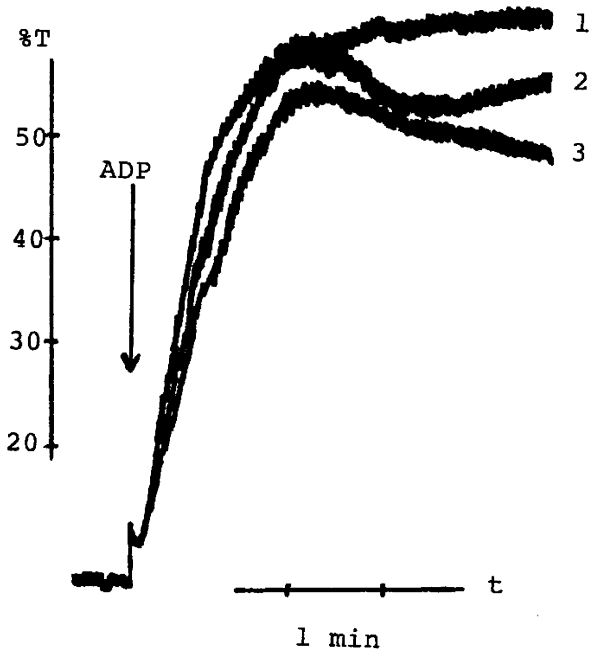

Fig. 8.7. Aggregation response before (*1*), during (*2*) and after (*3*) the handling of platelets, showing that the platelet function was not impaired as measured by the adenosine diphosphate (*ADP*)-induced aggregation. Inducing agent: ADP (10 μ*M*, 100 μl); Born-type aggregometer; 0.6-ml, platelet-rich plasma samples; platelet concentration: 250×10^3/μl. *T* light transmission (percentage)

(Sinzinger et al. 1987 a) or histological and/or electron microscopic examination, before the reinjection of the radiolabeled platelets.

Other parameters include the release of markers of platelet activation, such as β-thromboglobulin (Towler 1985). Electron microscopy is a sensitive indicator of platelet activation, manifested as pseudopodia formation (Badenhorst et al. 1982; Figs. 8.8 and 8.9). However, all the available in vitro tests failed to predict correctly collection and labeling injury, showing their limited practical value, and take a long time, which results in functional platelet deterioration and excludes their routine application (Sinzinger et al. 1987 a).

Labeling-induced injury is also reflected by the in vivo behavior of platelets. Platelets activated and/or damaged during the labeling procedure show an abnormal biodistribution, such as sequestration of radiolabeled platelets in the liver, spleen, and lungs, which results in lower REC in blood and shortened survival. REC is therefore the only reliable viability parameter, as morphologically damaged cells may recover completely after reinjection, while apparently intact ones, in contrast, may not. The normal values for platelet REC range from 55–72% of the injected dose (Baldini and Myers 1980; International Committee for Standardization in Hematology 1988; Mortelmans et al. 1986) due to pooling of about one third of the platelets in the liver and spleen (Baldini and Myers 1980). ^{99m}Tc in none of the tracers used until now caused by its own any sign of morphological or functional alteration due to radiation injury. Conclusive microdosimetry data are not yet available.

The too-short half-life of ^{99m}Tc for platelet survival studies and the high elution rate (instability of cell binding) with the tracers so far examined have limited considerably the clinical use, although with various applications (Table 8.4), of ^{99m}Tc-labeled platelets. ^{111}In-oxine is still nowadays the tracer of choice for the studies with radiolabeled platelets, which are long established and have attracted considerable interest.

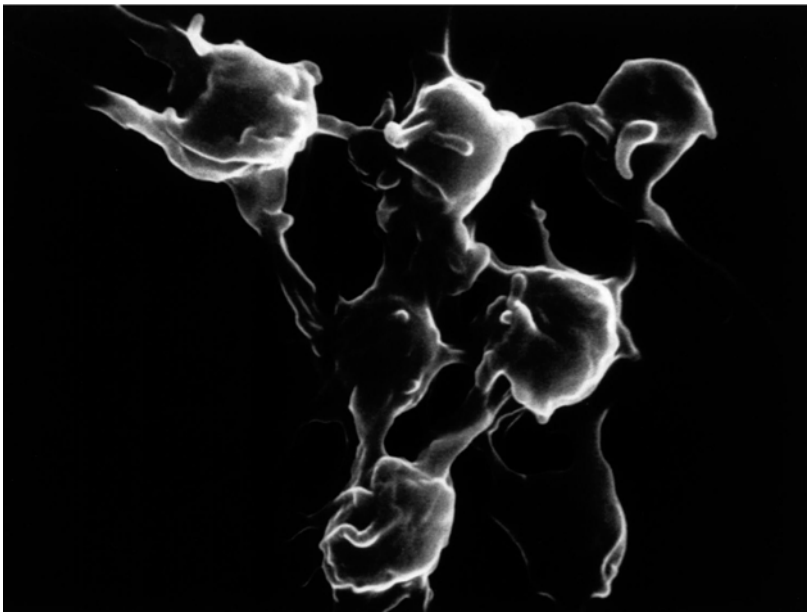

Fig. 8.8. Human platelets after radiolabeling, examined by scanning electron microscopy. No sign of morphological alteration is visible. Perfusion fixation, critical point drying, ×8000

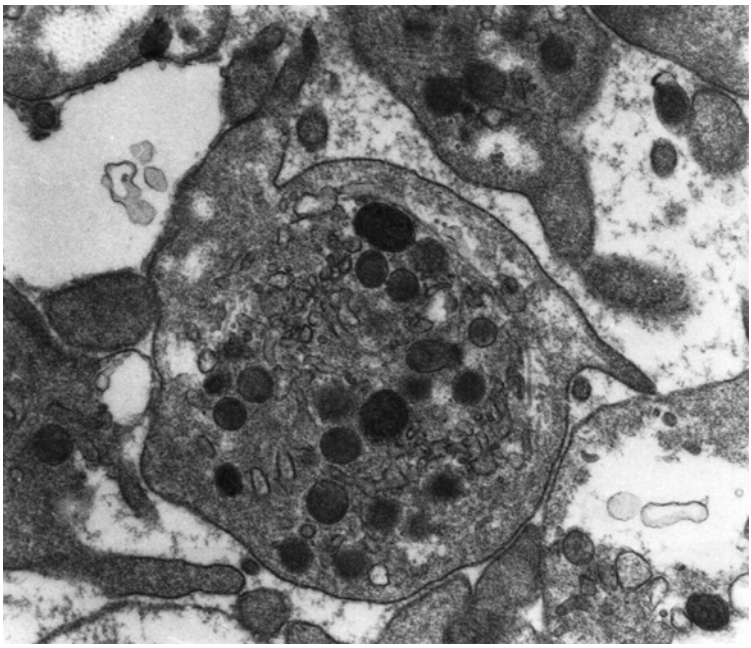

Fig. 8.9. Human platelets after the radiolabeling procedure, examined by transmission electron microscopy. Almost no signs of platelet activation, manifested as pseudopodia formation. Perfusion fixation, critical point drying, ×40 500

Table 8.4. Indications for studies with radiolabeled platelets

Kinetic studies
Evaluation of the normal platelet kinetics and biodistribution
Understanding of the role of platelets, platelet activation and consumption in diseases characterized by thromboembolic phenomena or thrombocytopenia (Baldini and Myers 1980; Lötter et al. 1986; International Committee for Standardization in Hematology 1988; Vallabhajosula et al. 1986) as well as in vascular disorders or endothelial (surface) abnormalities (Baldini and Myers 1980; Vallabhajosula et al. 1986; Harker et al. 1977)
Differentiation of the cause of thrombocytopenia
Evaluation of the effects of platelet-inhibitor drugs
Detection of prethrombotic states (Lötter et al. 1986)
Evaluation of the effects of risk factors (Rodrigues and Sinzinger 1884)
Evaluation of different cell separation techniques, storage or transfusion of platelets procedures, in transfusion medicine (International Committee for Standardization in Hematology 1988; De Vries et al. 1993; Mollison et al. 1987)
Imaging (Rodrigues and Sinzinger 1994)
Atherosclerosis
Surgical interventions
Synthetic material
Thrombosis
Transplantation

8.4 White Blood Cells

Several methods are available for labeling WBC. Their underlying principles are, however, similar and include isolation of WBC, prevention of cell activation and/or damage, and choice of ligand (Peters et al. 1986). Diverse factors influence the LE of WBC (Table 8.5). Apart from maintenance in plasma (a major factor in the promotion of granulocyte activation is their isolation from plasma), no further additives have been used (Peters et al. 1986) to inhibit granulocytes activation during their isolation and/or labeling procedure.

As an anticoagulant, ACD solution is preferred to heparin, because WBC show less tendency to adhere to plasticware with ACD (McAfee et al. 1984).

Antibiotics seem to be very unlikely to have a negative influence on LE (Sinzinger and Granegger 1988). WBC cannot be isolated from platelets and RBC by differential centrifugation alone. Random labels are the ones in common use for WBC. Either a mixed WBC suspension or a specific WBC type population, which requires more-sophisticated approaches, can be isolated, depending on the circulating WBC count, and the purpose of the study, while preserving cell viability. Homologous WBC can be used in leukopenic patients and have proven successful (Sinzinger and Granegger 1988). Monocytes are particularly difficult to separate from whole blood because they are normally very few in number, and their viability is affected by the dose of radiation (Fig. 8.10).

Lymphocytes are exquisitely sensitive and damaged by radiation (Chisholm and Peters 1980; Chisholm et al. 1979), and very few clinical studies using radiolabeled lymphocytes thus exist. After labeling WBC with 740 MBq of ^{99m}Tc-HMPAO, the radiation damage of the lymphocytes due to self-irradiation was estimated to be equivalent to 26 Gy of x-rays. Due to almost complete inhibition of the proliferative capacity at this high dose level, the increased risk for a lymphoid malignancy after administration of isolated lymphocytes or mixed WBC labeled with ^{99m}Tc-HMPAO activities sufficient for scintigraphy can, however, be regarded as small (Thierens et al. 1982). An injection of 100 MBq ^{99m}Tc-labeled autologous human granulocytes was found to give them an absorbed radiation dose of 1.8 Gy after 25 min and 8.4 Gy after 4 h. In vitro tests revealed no signs of radiation damage to the cells (Skretting et al. 1988).

Table 8.5. Variables influencing white blood cells (*WBC*) labeling with 99mTc- hexamethylpropyleneamine oxime (*HMPAO*)

Collection injury
Density of WBC
Anticoagulant
Sedimenting agents (type and volume) (Webber et al. 1994)
Amount of the complex
Time delay between labeling of WBC and administration (HMPAO)
Incubation
Medium
pH
Temperature
Time
Speed of centrifugation
Drugs: corticosteroids (Sampson 1995; McAfee et al. 1984), ethanol (McAfee et al. 1984), cyclosporin, azathioprine, ranitidine, nifedipine, procainamide (Sampson 1995), others

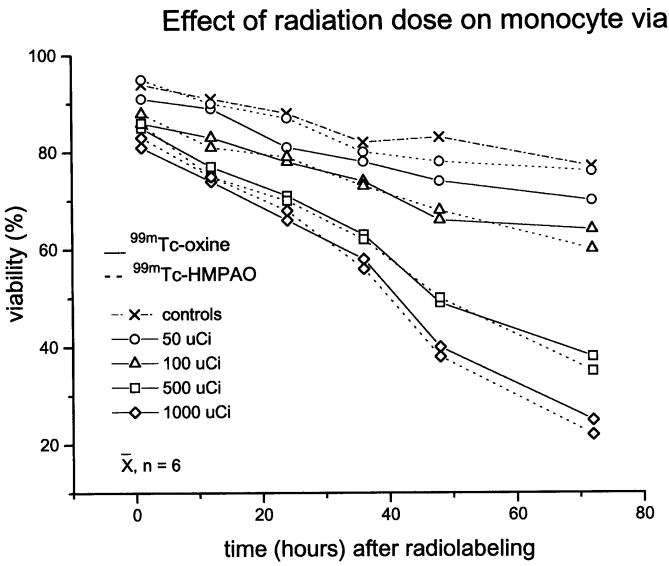

Fig. 8.10. Effect of radiation dose on monocyte viability

Separation of WBC involves the addition of a sedimenting agent (e.g., dextran, methylcellulose, and hydroxyethylstarch) to whole blood (Peters et al. 1986). Laborious cell harvesting techniques include newer density gradients for isopycnic centrifugation (nonionic contrast media), centrifugal elutriation, and flow cytometry, among others (McAfee et al. 1984). Relatively pure populations of granulocytes, lymphocytes, or monocytes can be harvested by elutriation (Berger and Ederson 1979; McAfee et al. 1984), which is, however, rather expensive. Granulocytes or lymphocyte–monocyte isolation can also be performed, achieving a high yield or high purity by isopycnic density centrifugation, such as Percoll-plasma and metrizamide-plasma density gradients, because of slight differences in cell density (Peters et al. 1986). With the surface adherence method, pure lymphocyte or monocyte populations can also be obtained (McAfee et al. 1984). However, some cell functions are altered by this manipulation (Fisher and Koren 1981). Monocytes and granulocytes may also be isolated by phagocytosis of radioactive particles, followed by density gradient centrifugation or flow through a magnetic field for in vitro experiments, with an LE generally only of 30–40%. However, this approach activates the cells metabolically, resulting in poor in vivo survival (McAfee et al. 1984), and thus is not acceptable for in vivo studies.

Several radiotracers such as ^{32}P-, ^{51}Cr-, ^{125}I-, ^{67}Ga-, ^{68}Ga-, and ^{111}In-lipophilic chelates, among others, have been developed for the purpose of labeling WBC. ^{111}In-oxine has become the standard for radiolabeling WBC. However, ^{99m}Tc has more favorable dosimetry and energy and many attempts have been made to replace ^{111}In with ^{99m}Tc, the use of ^{99m}Tc-compounds for WBC labeling having attracted considerable interest becoming an area of research undergoing much experimentation. Several ^{99m}Tc radiopharmaceuticals, such as ^{99m}Tc-HMPAO, -albumin colloid, -sulfur colloid, -sestamibi, -teboroxime, -mebrofenin, -disofenin, -gluceptate, -dimercaptosuccinic acid (DMSA) (Segall et al. 1994), -oxine, -phytate (H. Sinzinger, unpublished data) and -pyrophosphate (Uchida et al. 1979) have been tested. HMPAO showed the highest mean LE, 41–56% (Arndt et al.

1993; Becker et al. 1988b; Danpure et al. 1988; Lantto et al. 1991; Mountford et al. 1990; Roddie et al. 1988) and 79.4% (Segall et al. 1994), as compared with albumin colloid (12.1%, Segall et al. 1994), sulfur colloid (11.7%, McAfee et al. 1984; Segall et al. 1994), sestamibi (11.1%), and teboroxime (5.3–14.2%, Segall et al. 1994). Mebrofenin, disofenin, gluceptate, and DMSA do not label WBC (Segall et al. 1994). Pyrophosphate had a LE of 40–60% and showed poor in vivo recovery of the labeled WBC and poor abscess localization (Uchida et al. 1979). All the other agents have high labeling stability (ranging from 80 to >95% at 4 h and >80% at 24 h), except sestamibi, which washed out very rapidly (remaining 52% at 4 h and 5% at 24 h, Segall et al. 1994). ^{99m}Tc-oxine had an LE ranging from 50 to >80%, with 5 min of incubation, while ^{99m}Tc-phytate required a long incubation time (30 min), had low LE (20–50 %), and a high elution rate (H. Sinzinger, unpublished data). Attempts were recently made to use ^{99m}Tc-ethylcysteinate dimer (ECD) to label WBC (Kao et al. 1994). ECD is a neutral, lipophilic agent, introduced as a new brain perfusion agent. It has a higher radiochemical stability than HMPAO, being stable in vitro for at least 24 h, but it has lower WBC LE, worse stability of labeled WBC, and more rapid elimination than ^{99m}Tc- HMPAO from most tissues, thus being not a good choice as a WBC-labeling agent to replace ^{99m}Tc-HMPAO (Kao et al. 1994).

For assessing radiolabeled WBC viability, in vitro testing before administration to the patient and in vivo distribution of WBC can be performed. The adherence characteristics of WBC are measured in vitro and performed easily, but suffer from a lack of specificity and sensitivity (McAfee et al. 1984). Adherence of WBC is not altered by labeling with HMPAO, while it is decreased by albumin colloid, sestamibi, and teboroxime and increased by sulfur colloid (probably as a result of activation) (Segall et al. 1994). Routine tests of granulocyte function, namely random migration, chemotaxis, *Candida* killing, phagocytosis, and trypan blue exclusion test, are not sensitive enough to detect abnormalities on the status of the cell function before administration, which profoundly change further in vivo behavior (Peters et al. 1986) and are of limited value, only showing a difference in rather extreme situations. Besides, results of these tests generally are not available until several hours later. Labeled WBC smeared and observed with light microscopy provide a reliable, inexpensive, and quick insight into their purity, structural integrity, and their dispersal (McAfee et al. 1984), but do not replace the other functional tests. Transmission electron microscopy (Fig. 8.11) is expensive and rather time consuming.

The most valuable parameter remains the in vivo function of reinjecting the labeled WBC and measuring recovery and in vivo distribution, although too late for consequences if negative. Following injection, normal distribution of labeled WBC includes an initial transitory uptake in the lungs and, later, in the spleen, liver, and bone marrow. The relative amounts of the distribution of each of these sites vary with the labeling procedure used. A disadvantage of ^{99m}Tc complexes in general is nonspecific bowel accumulation, which makes their late abdominal imaging difficult. Damage to WBC or cell clumping during and/or because of the labeling procedure produces an abnormal distribution of the labeled WBC. A prolonged and delayed lung transit time and/or an abnormal high liver uptake are ominous signs that some damage of the labeled WBC occurred (Bowring 1986). Damage of lymphocytes by ^{111}In was detected as a failure of recirculation between blood and lymph (Peters et al. 1986). To minimize damage of the specific lymphocyte, activity must be restricted, requiring harvesting of a high number of cells for imaging.

HMPAO thus has become the best agent for labeling WBC. It was a radiopharmaceutical introduced for evaluating regional cerebral blood flow and is currently the only ^{99m}Tc-labeling agent for WBC commercially available in most countries. HMPAO is a li-

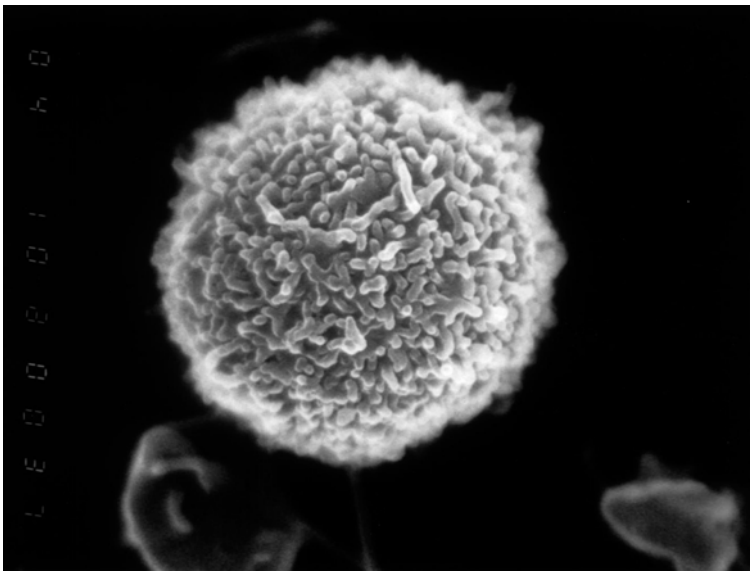

Fig. 8.11. Human lymphocyte after radiolabeling, examined by scanning electron microscopy. No sign of morphological alteration is visible. Perfusion fixation, critical point drying, ×2000

Table 8.6. Clinical Applications of ^{99m}Tc-WBC

Inflammatory bowel disease
Ulcerative colitis
Crohn's disease
Localization of sepsis
Localization of abscesses
Infections after surgery (e.g., grafts, prostheses)
Osteomyelitis (Mortelmans et al. 1989)
Pneumonia (Raptopoulos et al. 1982)
Endocarditis (Sfakianakis et al. 1982 a)
WBC accumulation around tumor infiltration (Sfakianakis et al. 1982 b)
Response to splenectomy (ratio of splenic to hepatic uptake) (Birnie et al. 1982)
WBC kinetics (e.g., quantification of fecal WBC excretion) (Saverymuttu et al. 1983), others

pophilic compound that diffuses through the cell membrane and converts with time to a secondary complex that is less lipophilic. The conversion to a less lipophilic form inhibits back diffusion across the cell membrane (Segall et al. 1994). However, HMPAO is limited in that its rapid decomposition in vitro requires its usage within 30 min of preparation (Neirinckx 1987). In addition, elution of ^{99m}Tc is a major problem, 20% (Mortelmans et al. 1989) to 25% (Segall et al. 1994) of the label eluting from WBC by 24 h. The feasibility of using other chelating agents, such as the neutral lipophilic complex teboroxime, still needs further investigation.

The labeling of WBC with ^{99m}Tc chelates, in particular HMPAO dramatically increased the widespread availability and use of these studies in nuclear medicine. Several clinical applications of radiolabeled WBC exist (Table 8.6), but the main clinical indication of imaging WBC distribution has been the detection of inflammatory bowel disease.

Peptides, proteins, and monoclonal antibodies or its fragments, with the potential for labeling specific subpopulations of WBC by binding to specific surface receptors or antigens of WBC, may be challenging and exciting in the future for selective cell labeling and in vivo studies.

8.5 Stem Cells

Stem cell therapy is gaining considerable interest, mainly in cardiovascular medicine and oncology (Barbash et al. 2003, Bengel et al. 2005). Visualization and monitoring of the therapeutically administered cells could provide a better understanding of the underlying mechanisms and open a new field of research for clinical nuclear medicine. Preliminary attempts to label stem cells in vitro have been successful (Brenner et al. 2004, Chin et al. 2003). However, while labeling with In-111-oxine is above 80%, not affecting cell viability, a rather high elution rate was observed when labeling was performed with either ^{99m}Tc-HMPAO or ^{99m}Tc-oxine. Together with its shorter half-life, Tc-99m therefore does not allow longterm in vivo monitoring. Since the clinical use of labeled stem cells in patients is in a rather early stage, not enough information on the in vitro and in vivo viability is as yet available, in particular, the number of labeled cells required to correctly target the few percent of accumulating cells is not known. Other tracers, such as Cu-64 and ^{18}F-labeled deoxyglucose (FDG) do not offer any advantage over In-111-oxine. Like with platelets, the labeling efficiency of stem cells is negatively affected by risk factors of cardiovascular disease (Sinzinger et al. 2006), such as cigarette smoking, diabetes mellitus, and hyperlipidemia.

8.6 Conclusions

Developments in both instrumentation and cell labeling have enabled a great expansion of their use in both clinical practice and research.

Studies with ^{99m}Tc-RBC are in widespread use in clinical practice. In contrast, ^{99m}Tc has not been successfully applied to platelet labeling. In contrast to ^{99m}Tc-RBC-research, there have been more methodological reports with ^{99m}Tc-WBC than clinical applications at the present. Much progress has been achieved in the techniques of harvesting and labeling WBC, but many challenges remain. HMPAO is currently the best ^{99m}Tc agent for labeling WBC, and played a particularly important role for the successful expansion of diagnostic imaging with radiolabeled WBC in nuclear medicine.

New approaches, such as the use of peptides, proteins, antibodies, and molecular recognition unit technologies, may result in substantial improvements in the labeling methodology and could yield labeled cells with the least damage and high in vivo stability in the future.

Acknowledgments

The authors gratefully acknowledge the collaboration and assistance by Judith Bednar, Susanne Granegger, Ulli Horvarth, Brigitte Jankovic, J. Kaliman, G. Karanikas, H.

Kolbe, H. Kritz, W. Linkesch, H. Ludwig, Ch. Müller, Irmgard Neumann, J. O'Grady, Adelaide Osei-Bonsu, H. Pesl, Cornelia Pones, Sonja Reiter, Eva Strobl-Jäger, H. Vinazzer, K. Widhalm, and Ch. Zielinski.

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