Neutrophils from patients undergoing hip surgery exhibit enhanced movement under spinal anaesthesia compared with general anaesthesia

Richard Erskine MBChB FFA(SA), Piotr K. Janicki MD PhD,*
Pat Ellis Nat. Dip. (BT) and (Haem),
Michael F.M. James MBChB PhD FFARCS

The purpose of this research was to investigate whether the effects of regional anaesthesia on neutrophil migration differ from those due to general anaesthesia during major orthopaedic surgery in human patients. Eighteen patients underwent spinal or general anaesthesia (halothane or isoflurane) for surgery (six patients in each group). Blood samples were taken prior to induction of anaesthesia and after surgery was in progress for one hour. The movement of isolated neutrophils was measured in both samples in the chemotactic chamber toward lipopolysaccharide activated pooled serum. In addition plasma concentrations of catecholamines were determined in the blood samples. Neutrophils extracted from peripheral blood during spinal anaesthesia and surgery moved further towards a complement-derived attractant than neutrophils obtained from patients undergoing surgery under general anaesthesia with halothane or isoflurane and surgery ($156.4 \pm 7.6 \mu\text{m}$ vs $114.3 \pm 6.1 \mu\text{m}$ or $119 \pm 8.4 \mu\text{m}$ respectively, $P < 0.05$). Increased concentrations of adrenaline were present in both general anaesthetic groups whereas the spinal group had lower concentrations than those

prior to anaesthesia and surgery. It is considered unlikely that these differences in neutrophil reactivity are due to the direct effects of anaesthetic agents employed. The effects are likely to be the result of differing effects of spinal anaesthesia on the stress response or immunological mediators.

Cette recherche avait pour but d'étudier les effets de l'anesthésie régionale et générale sur la migration des neutrophiles durant la chirurgie orthopédique majeure. Dix-huit patients furent soumis à une anesthésie rachidienne (R) ou générale à l'halothane (H) ou à l'isoflurane (I) (six patients dans chaque groupe). Des échantillons de sang périphérique furent prélevés avant l'induction de l'anesthésie et une heure après le début de la chirurgie afin d'étudier le mouvement des neutrophiles isolés et de mesurer la concentration sérique des catécholamines. Le mouvement des neutrophiles isolés fut mesuré dans une chambre chimiotactique à l'aide de sérums mélangés activés par des lipopolysaccharides. Le déplacement des neutrophiles vers l'activateur dérivé du complément fut plus important dans le groupe R ($156,4 \pm 7,6 \mu\text{m}$) que dans les groupes H ($114,3 \pm 6,1 \mu\text{m}$) ou I ($119 \pm 8,4 \mu\text{m}$) ($P < 0.05$). La concentration d'adrénaline s'est élevée en per-opératoire dans les groupes H et I alors qu'elle s'est abaissée dans le groupe R (par comparaison avec le prélèvement pré-induction). On considère peu probable que les différences observées sur la réactivité des neutrophiles découlent de l'action directe des agents anesthésiques employés. Ces différences résultent plus vraisemblablement de l'action de l'anesthésie rachidienne sur la réponse au stress ou sur des médiateurs immunologiques.

Key words

ANAESTHETIC TECHNIQUES: spinal;
BLOOD: leucocytes.

From the Anglo-American Corp. Anaesthetic Research Laboratory, Department of Anaesthetics and Department of Physiology,* Medical School, University of Cape Town.

Address correspondence to: Dr. Richard Erskine, Hinchingsbrooke Hospital, Huntingdon, PE18 8NT, United Kingdom.

Supported in part by grants from the Medical Research Council of South Africa, Anglo-American Corporation and Abbott Laboratories.

Accepted for publication 4th May, 1992.

Infection remains a major cause of morbidity and mortality in patients undergoing surgery. Innate immunological mechanisms, in essence complement-activated neutrophils, constitute the first line of defence against invading organisms in the early perioperative period.¹ In surgical patients

various aspects of neutrophil function are depressed resulting in inhibited postoperative killing ability,²⁻⁷ but the mechanism of impaired neutrophil killing is unclear. Phagocytosis is not depressed^{2,8} especially if regional anaesthesia is employed,⁹ and no inhibition of neutrophil movement was observed in one study in which ascorbate, a free radical scavenger, was used to premedicate children prior to surgery.¹⁰ Others have studied the effects of anaesthetic agents *in vitro* on neutrophils isolated from volunteers. Nitrous oxide has a marked depressant effect on neutrophil movement in the two studies in which it has been examined,^{11,12} but with the volatile agents, these experiments have yielded conflicting results which may reflect different methodologies as much as the effects of volatile anaesthetics on neutrophil movement. We have summarized these results in a previous paper.¹³

Recently, Mealy *et al.*¹⁴ have demonstrated a factor present in the serum of patients anaesthetized with enflurane and nitrous oxide which inhibited the ability of neutrophils from normal volunteers to generate active killing substances. It has been suggested that these effects may be less apparent when surgery is performed under regional anaesthesia.^{5,9}

In a previous study we showed that neutrophils activated by elicitation into the peritoneal cavity of rabbits showed greater stimulation of the chemotactic responses during isoflurane than halothane anaesthesia which had no effect.¹³ This study was designed to investigate whether the effects of regional anaesthesia on neutrophil migration differed from those due to general anaesthesia during surgery in human patients.

Methods

The protocol was approved by the Ethical and Research Committee of the University of Cape Town. Eighteen patients undergoing hip arthroplasty were randomised into three groups ($n = \text{six}$ in each group). One group received general anaesthesia with halothane (GA-HALO), the second received general anaesthesia with isoflurane (GA-ISO), and the third group received spinal anaesthesia (SPINAL).

All patients were of ASA physical status 1 or 2 and were receiving nonsteroidal analgesic drugs but not aspirin, up to the time of surgery. All patients received premedication with temazepam 20 mg two hours before surgery.

In the general anaesthesia groups, anaesthesia was induced with morphine $0.15 \text{ mg} \cdot \text{kg}^{-1}$ followed by a sleep dose of thiopentone ($4\text{--}6 \text{ mg} \cdot \text{kg}^{-1}$). Tracheal intubation was facilitated by alcuronium $0.25 \text{ mg} \cdot \text{kg}^{-1}$ and anaesthesia was continued with IPPV to PETCO_2 5 kPa utilizing nitrous oxide 70% in oxygen. In the GA-HALO group, halothane 1% was administered from a previously cali-

brated vaporiser for the duration of the study period. The GA-ISO group received isoflurane at an equivalent MAC concentration (1.5%) instead of halothane.

Spinal group

After a preload of Plasmalyte B (Ringer's lactate without calcium in which the lactate is replaced by bicarbonate) $10 \text{ ml} \cdot \text{kg}^{-1}$ had been administered, anaesthesia was induced by subarachnoid injection of bupivacaine 20 mg in 0.5% isobaric solution into the L_{3-4} or L_{4-5} interspace. This produced a block to a median level of T_6 (range $\text{T}_4\text{--}\text{T}_{10}$). Patients were lightly sedated by the administration of propofol $0.2\text{--}0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ after a loading dose of $0.5 \text{ mg} \cdot \text{kg}^{-1}$ to maintain a sedation score of 3.¹⁵

Systolic blood pressure was maintained above 100 mmHg in all groups by the judicious infusion of Plasmalyte B crystalloid throughout the study period. No other intravenous fluids or blood were administered during the study. No bone cement was utilized by the surgeons during the study period.

Neutrophil preparation

Blood samples (35 ml) were drawn on two occasions, the first prior to induction of anaesthesia and the second after surgery had been in progress for one hour. Blood samples (30 ml in heparinised tubes) were kept on ice until the neutrophil extraction began 30 min after the second sample was collected. Blood was taken under similar conditions from five volunteers (reference samples) to ensure that no changes occurred due to storage of the first sample for 1.5 hr longer than the second. Five millilitres of blood were taken into EDTA tubes, the plasma was separated immediately by centrifugation and frozen at -20°C until catecholamine estimations were performed.

Neutrophils were obtained by layering on Ficoll (Histo-paque-1077 from Sigma Diagnostics, Mo, USA) and spinning down for 30 min. The supernatant containing mononuclear cells was discarded, the neutrophils and red blood cells remaining. Pellets were diluted 1:1 with phosphate buffered saline (PBS). The PBS used throughout was standard phosphate to which was added MgCl_2 , CaCl_2 and glucose. Three millilitres of dextran 6% (mw 150 kD, Sigma Diagnostics, Mo, USA) were added to the cell mixture, well mixed and allowed to settle for 40 min. The supernatant containing polymorph neutrophils was resuspended in PBS. The few remaining red cells were removed by lysis in water for 40 sec. Isotonicity was restored with NaCl 3.6%. This procedure yielded a harvest of $2\text{--}5 \times 10^6 \text{ cells} \cdot \text{ml}^{-1}$ in 10 ml suspension of which 91–99% were neutrophils. Viability >95% was ensured in each case by a trypan blue dye exclusion test. The cell suspensions were made up to a final concentration of $2 \times 10^6 \cdot \text{ml}^{-1}$ in PBS and kept on ice until the experiments

TABLE Total white blood cells count (WBC, $10^3 \cdot \mu\text{L}^{-1}$) and the raw data indicating the distance (in μm) migrated by neutrophils in chemotactic chamber (Movement). The results are expressed as mean and SEM for six patients in each group

Experimental group	Preoperative samples		Intraoperative samples	
	WBC	Movement	WBC	Movement
GA-HALO	7.1 ± 0.5	105.2 ± 9.7	6.9 ± 0.8	114.3 ± 6.1
GA-ISO	8.1 ± 0.8	113 ± 11.4	8.4 ± 0.7	119 ± 8.4
Spinal	7.3 ± 0.6	102.1 ± 7.2	7.9 ± 0.7	156.4 ± 7.6
Healthy volunteers ($n = 5$)	First sample		Second sample (90 min)	
	7.8 ± 0.3	133.3 ± 4.3	7.7 ± 0.4	104 ± 10.1

commenced. Movement was assessed through $3.0 \mu\text{m}$ nitrocellulose membranes (Sartorius, Germany) towards E. Coli lipopolysaccharide (Difco WE E Coli 3123-25-7) activated pooled serum. This was prepared by incubating $500 \mu\text{g}$ in 1 ml PBS with 1 ml pooled serum and 2 ml PBS for 30 minutes at 37°C . The number of cells per chemotactic chamber was 0.4×10^6 . Movement was assessed after 40 min incubation in the presence of pooled serum above the membrane using the raft system as described by Addison and Babbage.¹⁶ Migration was measured utilizing the moving front technique of Zigmond¹⁷ with five fields examined for each cell population. Distances migrated by cells obtained intraoperatively were compared to the movement of cells sampled preoperatively in each patient.

In order to establish the possible influence of propofol, we compared the movement of neutrophils from four volunteers in the presence and absence of propofol $10 \text{ mg} \cdot \text{L}^{-1}$ using the same experimental methodology.

Catecholamine concentrations in plasma were determined by electrochemical detection after separation with HPLC using dihydroxybenzylamine as internal standard.¹⁸ The coefficient of variation was 7.9% and 8.7% for noradrenaline ($500 \text{ pg} \cdot \text{mL}^{-1}$) and adrenaline ($300 \text{ pg} \cdot \text{mL}^{-1}$) and the percentage recovery was 75% and 69% respectively for the above-mentioned concentrations of standards of catecholamines extracted using an aluminium oxide absorption technique from catecholamine-free plasma. The limit of sensitivity was $10 \text{ pg} \cdot \text{mL}^{-1}$ for both measured catecholamines.

As the data were assumed not to be normally distributed, the statistical analysis was performed using the non-parametric Kruskal-Wallis test followed by the Mann-Whitney U test for intergroup comparisons. Differences were assumed to be significant when $P < 0.05$.

Results

The ages of the patients in years expressed as mean (range) of the study groups were: HALO-GA 55 (35–70) yr, ISO-GA 53 (31–73), Spinal 65 (56–73). Weights in kg also expressed as mean (range) were: HALO-GA 65

(56–80), ISO-GA 67 (54–74) and Spinal group 63 (56–74). No differences among the study groups were noted with respect to age or weight except the group of healthy volunteers from which the reference samples were taken for the reassessment of the influence of keeping them on ice for 90 min (age 24–45, mean 36 yr; weight 55–75, mean 65 kg). The total white blood cell count in the samples taken both pre- and intraoperatively did not show differences among experimental groups (Table).

Neutrophils from patients undergoing surgery under spinal anaesthesia demonstrated enhanced movement towards C5a compared with both groups receiving general anaesthesia (Figure 1, Table). In addition, the movement in the spinal group during surgery was enhanced ($P < 0.05$) compared with the preoperative movement in this group. Neutrophils from four volunteers exposed to propofol at $10 \text{ mg} \cdot \text{L}^{-1}$ showed no differences in migration compared with migration in the absence of the drug (mean $71.0 \mu\text{m}$ vs $64.7 \mu\text{m}$, results not shown).

Increased concentrations of adrenaline were present in both general anaesthetic groups whereas the spinal group had lower concentrations than those prior to anaesthesia and surgery (Figure 2). No time-related differences or differences among groups were seen in the levels of noradrenaline. This may reflect the large scatter of the noradrenaline data.

Discussion

This study demonstrated that neutrophils from patients undergoing surgery with spinal anaesthesia exhibit increased movement towards a complement-associated chemotactic factor compared with neutrophils from similar patients undergoing surgery with general anaesthesia. In addition, the neutrophil movement in the spinal group during surgery was considerably enhanced compared with the movement in this group prior to surgery. The wide scatter of the data in the spinal group probably reflects different subpopulations of primed neutrophils with large differences in their chemotactic responses. Bass *et al.*¹⁹ demonstrated recently, in neutrophils extracted from

FIGURE 1 Changes in movement of neutrophils between pre-operative and intraoperative samples are presented as box and whisker plots. The central box covers the middle 50% of the data values between the lower and upper quartiles. The central line is the median. The whiskers extend out 1.5 times the inter-quartile range. Crosses indicate outliers. The asterisk indicates an increase of movement of the neutrophils during surgery in the spinal anaesthesia group ($P < 0.05$). Control group indicates samples taken from five healthy volunteers and kept on ice for 90 min.

FIGURE 2 Results of determination of plasma catecholamine concentrations expressed as the mean of percentage changes in intra-/preoperative levels. Vertical bars indicate SEM ($n = 6$). Asterisk indicates changes ($P < 0.05$) compared with reference samples taken from healthy volunteers 90 min apart (black column).

patients with acute bacterial infections, similar variations among patients and different response patterns of subpopulations of neutrophils within the same patient to a phorbol ester membrane stimulant.¹⁹

During surgery, neutrophils activated and attracted by inflammatory mediators at the surgical site attach to the endothelium at the site of surgery and then migrate through spaces induced by cytokines and inflammatory mediators between the endothelial cells to enter the interstitial space where they take part in host defence.^{20,21} Such cells activated into the active site probably represent a different subpopulation of neutrophils from those obtained by sampling peripheral blood at the same time.¹⁹ Experiments such as ours in which samples of the circulating neutrophils are taken during surgery represent data which may not reflect accurately the function of the neutrophils attracted into the inflammatory area but should reflect changes in activity state of the cells in the same direction. It is our hypothesis that neutrophils even in peripheral blood are primed during surgery and exhibit an enhanced response to a chemotactic stimulus for at least the first hour of anaesthesia and surgery.

Explanations that have to be considered include firstly that volatile general anaesthetics or intravenous agents in the general anaesthetic groups directly depressed a surgically induced enhancement of neutrophil motility for a period of time exceeding the presence of anaesthetics in the blood. Although possible there are no data available which suggest that the changes in the function of cells can persist after discontinuation of anaesthesia. Only one study has examined the movement of neutrophils sampled during anaesthesia and surgery. Stanley *et al.*³ demonstrated diminished chemotactic responses towards a complement-associated attractant of neutrophils from patients undergoing an hour of general anaesthesia prior to an hour of surgery. We feel it is unlikely that anaesthetic agents depressed directly the motility of neutrophils because they were no longer present during the chemotactic experiments. In any case, the effects of volatile agents on activated neutrophils in a protein-containing solution at clinical concentrations are probably negligible. Nitrous oxide, although not present after the extraction of the cells, may have contributed to the lack of enhanced movement in both the general anaesthesia groups.

Thiopentone and morphine in the 10^{-6} – 10^{-3} M range have been shown to depress chemotaxis in protein-free experiments.²² The plasma concentrations of these agents in our groups must have been extremely low after the cells were isolated from plasma, resuspended in PBS and the movement experiments conducted in the presence of pooled serum which would bind nearly all the drug residuals.

Secondly, the differences in migration between the

general anaesthesia groups and the spinal group may be due to differences in circulating stress hormones observed between general and spinal anaesthesia. Hill *et al.*²³ showed that adrenaline at 10^{-5} – 10^{-3} M concentration inhibited chemotaxis by 30% compared with the movement in the absence of the drug. This would indicate a maximal depressant affect of adrenaline at all these concentrations. Our patients revealed mean concentrations of 1.3×10^{-9} , 0.76×10^{-9} and 0.17×10^{-9} M adrenaline in the GA-HALO, GA-ISO and spinal groups respectively. The preoperative concentrations for these groups were in the range of 0.4 – 0.6×10^{-9} M. These reflect an inter-group range of about 1.5×10^{-9} M in the 10^{-9} M range. Differing levels of catecholamines may well have contributed to our findings but the magnitude of catecholamine-induced changes in the neutrophil movement are probably small. Interestingly, in the same study they demonstrated that noradrenaline in the 10^{-3} to 10^{-5} M range stimulated chemotaxis in a dose-dependant fashion. After one hour of surgery patients anaesthetized by subarachnoid blockade to T₈ would be expected to experience 2–3 times higher plasma cortisol level than patients under general anaesthesia.^{24–26} Cortisol, although a direct depressant of neutrophil chemotaxis,²⁷ is a far more potent inhibitor of the synthesis of leukotriene B₄, a potent chemoattractant liberated at sites of inflammation.²⁸ This effect takes two hours to develop.^{29–32} It is possible that a cortisol depressant effect may have influenced the findings of Stanley *et al.*, as at that sample time a neutrophilia was present in their patients. After 90 min of anaesthesia and surgery, our patients did not show any alterations in total white counts compared with preoperative levels in any group probably due to a shorter duration of anaesthesia prior to the commencement of surgery in all groups. Dale demonstrated that a period of at least two hours is necessary for the cortisol-mediated recruitment of neutrophils from the bone marrow into the peripheral pool.³³ We feel that differences in cortisol at this time are unlikely to have been responsible although they may have been contributory. Thirdly, drugs utilized in the spinal group may have stimulated the response of the cells. Our results in these experiments have shown that propofol at $10 \text{ mg} \cdot \text{L}^{-1}$ has no effect on the migration of quiescent neutrophils. Moudgil *et al.* demonstrated that bupivacaine at 10^{-4} M concentration had no effect on the migration of volunteer neutrophils towards casein.²² Our levels of drug in the plasma after spinal anaesthesia would have been much lower than this.

Recently Mealy *et al.* have demonstrated that a factor present in the serum of patients anaesthetized with enflurane and nitrous oxide inhibits luminol-enhanced chemiluminescence of normal volunteer neutrophils.¹⁴ They postulated that it might be a degradation product of

complement. We are currently investigating this as a possible explanation for our findings.

This study and others suggest that anaesthetic techniques may modify the response of neutrophils during surgery. Further understanding of these mechanisms and the recent advances in our understanding of factors responsible for neutrophil adherence, migration and activation coupled to increasing understanding of cytokine-mediated neutrophil kinetics may enable anaesthetists to achieve biological advantages for host defense not necessarily at the expense of host damage.³⁴ We are reluctant to make clinical recommendations from our findings. However, it is certain that more research needs to investigate the function of innate mechanisms of host resistance in different anaesthetic conditions.

Acknowledgement

We would like to acknowledge the help we received from Prof. Ronnie Anderson of the Medical Research Council Phagocytic Cell Research Unit at the University of Pretoria for much useful advice in the design of these experiments.

References

- 1 Easmon C. The nature of host defenses. In: Pollock A (Ed.). *Surgical Infections*, 1st ed., Baltimore: Edward Arnold, 1987.
- 2 El-Maalllem H, Fletcher J. Effects of surgery on neutrophil granulocyte function. *Infect Immun* 1981; 32: 38–41.
- 3 Hill GE, Stanley TH, Lunn JK, *et al.* Neutrophil chemotaxis during halothane and halothane-N₂O anaesthesia in man. *Anesth Analg* 1977; 56: 696–702.
- 4 Edwards AE, Gemmell LW, Mawkin PP, Smith CJ, Allen JC, Hunter A. The effects of three differing anaesthetics on the immune response. *Anaesthesia* 1984; 39: 1071–8.
- 5 Busoni P, Sarti A, De Martino M, Graziani E, Santoro S. The effect of general and regional anesthesia on oxygen-dependent microbiocidal mechanisms of polymorphonuclear leukocytes in children. *Anesth Analg* 1988; 67: 453–6.
- 6 Moudgil GC, Pandya AR, Ludlow DJ. Influence of anaesthesia and surgery on neutrophil chemotaxis. *Can J Anaesth* 1981; 28: 232–8.
- 7 Solomkin JS, Bauman MP, Nelson RD, Simmons RL. Neutrophils dysfunction during the course of intra-abdominal infection. *Ann Surg* 1981; 194: 9–17.
- 8 Pertilla J, Salo M, Rajamaki A. Granulocyte microbial function in patients undergoing major abdominal surgery under balanced anaesthesia. *Acta Anaesthesiol Scand* 1987; 31: 100–3.
- 9 Bardosi L, Tekeres M. Impaired metabolic activity of phagocytic cells after anaesthesia and surgery. *Br J Anaesth* 1985; 57: 520–3.

- 10 Charlton AJ, Harvey BA, Hatch D, Soothill. Neutrophil mobility during anaesthesia in children. A trial of ascorbate premedication. *Acta Anaesthesiol Scand* 1976; 31: 343-6.
- 11 Nunn JF, O'Morain C. Nitrous oxide decreases motility of human neutrophils *in vitro*. *Anaesthesiology* 1982; 56: 45-8.
- 12 Moudgil GC, Gordon J, Forrest JB. Comparative effects of volatile anaesthetic agents and nitrous oxide on human leucocyte chemotaxis *in vitro*. *Can J Anaesth* 1984; 31: 631-7.
- 13 Erskine R, James MFM. Isoflurane but not halothane stimulates neutrophil chemotaxis. *Br J Anaesth* 1990; 64: 723-7.
- 14 Mealy K, O'Farrelly C, Stephens R, Feighery C. Impaired neutrophil function during anaesthesia and surgery is due to serum factors. *J Surg Res* 1987; 43: 393-7.
- 15 Mackenzie N, Grant IS. Propofol for intravenous sedation. *Anaesthesia* 1987; 42: 3-6.
- 16 Addison IE, Babbage JW. A raft technique for chemotaxis: a versatile method suitable for clinical studies. *J Immunol Methods* 1976; 10: 385-8.
- 17 Zigmond SN, Hirsch JG. Leucocyte locomotion and chemotaxis. New methods for evaluation and demonstration of a cell-derived chemotactic factor. *J Exp Med* 1973; 137: 387-410.
- 18 Weiker H, Feraudi N, Haegele H, Pluto R. Electrochemical detection of catecholamines in urine and plasma after separation with HPLC. *Clin Chim Acta*, 1984; 141, 17-25.
- 19 Bass DA, Obrantz P, Szejda P, Seeds MC, McCall CE. Subpopulations of neutrophils with increased oxidative product formation in blood of patients with infection. *J Immunol* 1986; 136: 860-6.
- 20 Prober JS. Cytokine-mediated activation of vascular endothelium: physiology and pathology. *Am J Pathol* 1988; 133: 426-33.
- 21 Wayne Smith C, Kishimoto TK, Abbass O, et al. Chemotactic factors regulate lectin adhesion molecule 1 (LECAM-1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells *in vitro*. *J. Clin Invest* 1991; 87: 609-18.
- 22 Moudgil GC. Effect of premedicants, intravenous anaesthetic agents and local anaesthetics on phagocytosis *in vitro*. *Can J Anaesth* 1981; 25: 591-602.
- 23 Hill HR, Estensen RD, Quie PG, Hogan NA, Goldberg ND. Modulation of neutrophil chemotactic responses by cyclic 3',5'-guanosine monophosphate and cyclic 3',5'-adenosine monophosphate. *Metabolism* 1975; 24: 447-56.
- 24 Brandt M, Kehlet H, Binder C, Hagen C, McNeilly AS. Effect of epidural analgesia on the glycoregulatory endocrine response to surgery. *Clin Endocrinol* 1976; 5: 107-14.
- 25 Cooper GM, Holdcroft A, Hall GM, Alaghband-Zadeh J. Epidural analgesia and the metabolic response to surgery. *Can J Anaesth* 1979; 26: 381-5.
- 26 Brandt MR, Olgaard K, Kehlet H. Epidural anaesthesia inhibits the renin and aldosterone response to surgery. *Acta Anaesthesiol Scand* 1979; 23: 267-72.
- 27 Ward PA. The chemosuppression of chemotaxis. *J Exp Med* 1966; 1966: 209-25.
- 28 Tsurufuji S, Kurihara A, Kiso S, Suzuki Y, Ohuchi K. Dexamethasone inhibits generation in inflammatory sites the chemotactic activity attributable to leukotriene B₄. *Biochem Biophys Res Commun* 1984; 119: 884-90.
- 29 Bowen DL, Fauci AS. Adrenal corticosteroids. In: Gallin JI, Goldstein IM, Snyderman R (Eds.). *Inflammation*. New York: Raven Press 1988: 877-95.
- 30 Hirata F, Schiffmann E, Venkotasubramanian K, Salomon D, Axelrod J. A phospholipase A₂-inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc Natl Acad Sci USA* 1980; 5: 2533-6.
- 31 Blackwell GJ, Carnuccio R, Di Rosa M, Flower RJ, Parente L, Persico P. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature* 1980; 287: 147-9.
- 32 Wallner BP, Mattaliano RJ, Hession C, Gate RL, et al. Cloning and expression of human lipocortin, a phospholipase A₂ inhibitor with potential anti-inflammatory activity. *Nature* 1986; 320: 77-81.
- 33 Dale DC, Fauci AS, Guerry DuP, Wolff SM. Comparison of agents producing a neutrophil leucocytosis in man. *J. Clin Invest* 1975; 57: 808-13.
- 34 Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989; 320: 365-76.