

COMPLEMENT activation during extracorporeal membrane oxygenation (ECMO) in newborns can be caused by both the underlying disease processes and by blood contact with the ECMO circuit. We investigated the relative importance of these mechanisms by measuring C3a, C5a and sC5b-9 before, during and after neonatal ECMO in six consecutive newborn patients using enzyme-linked immunoassay. In addition complement activation during *in vitro* ECMO with repeated flow of the same blood volume was measured using blood from healthy adult donors. C3a increased significantly *in vivo* after 1 h (from 1035 ± 193 to 1865 ± 419 $\mu\text{g/l}$) and *in vitro* ECMO (from 314 ± 75 to 1962 ± 1062 $\mu\text{g/l}$). C5a increased during ECMO without significant differences between *in vivo* and *in vitro* activation. In neonatal patients, sC5b-9 rose faster than *in vitro*, but the rapid increase was also significant for *in vitro* experiments (*in vivo*: from 328 ± 63 to 1623 ± 387 $\mu\text{g/l}$ after 2 h; and *in vitro*: from 78 ± 32 to 453 ± 179 $\mu\text{g/l}$ after 8 h). After this initial peak at 1–2 h, complement activation decreased gradually until 2–3 days after the initiation of ECMO. We conclude that in newborns the rapid activation of the complement system after the start of ECMO is predominantly caused by contact with artificial surfaces rather than the patient's underlying disease.

Key words: Neonate, ECMO, Complement activation, Artificial surfaces

Complement activation by *in vivo* neonatal and *in vitro* extracorporeal membrane oxygenation

Johannes Graulich¹, Joseph Sonntag¹,
Monika Marcinkowski², Karl Bauer², Hans Kössel²,
Christoph Bühner^{1,CA}, Michael Obladen¹ and
Hans T. Versmold²

¹Department of Neonatology, Charité School of Medicine, Campus Virchow Klinikum, Humboldt Universität, D-13353 Berlin, Germany; and
²Department of Pediatrics, Universitätsklinikum Benjamin Franklin, Free University Berlin, D-12200 Berlin, Germany

^{CA}Corresponding Author

Tel: +49 30 450566413

Fax: +49 30 450566922

Email: christoph.buehrer@charite.de

Introduction

Extracorporeal membrane oxygenation (ECMO) has become standard treatment for newborn infants with severe neonatal respiratory failure not responding to conventional pulmonary support.¹ Since the first successful ECMO treatment in 1971, more than 10,000 babies have been treated world-wide with ECMO, with an improved survival and a favourable outcome compared with conventional treatment.² Additionally, ECMO is used for temporary circulatory assist in children with heart failure awaiting transplantation.³

During ECMO, circulating plasma levels of inflammatory mediators and neutrophil activation increase.^{4,5} This generalized inflammatory reaction contributes to the capillary leak syndrome and pulmonary oedema observed during ECMO therapy.^{6–9} A major mechanism triggering this inflammatory reaction is the activation of complement by contact of blood with the surface of the synthetic tubing and the oxygenator. Complement activation has also been described during cardiopulmonary bypass and the apheresis technique.^{10–12} In contrast to cardiopulmonary bypass, which lasts only for a few hours and is combined with deep hypothermia,

ECMO lasts for several days, is performed at normal temperature and the patient sometimes has systemic infection.

The aims of the present study were to investigate the relative contribution of primary disease, contact with the ECMO circuit, and infection to the complement activation during neonatal ECMO.

Patients and methods

In vivo neonatal ECMO

The study was approved by the institutional review board and written parental consent was obtained for each patient. During the study period, six newborns requiring ECMO for cardiorespiratory failure unresponsive to conventional mechanical ventilation were included in this prospective study.

The total volume of the ECMO circuit was 434 ± 8 ml. The ECMO circuit was primed by the introduction of carbon dioxide, 0.9% saline solution containing 0.5 IU of heparin/ml of solution and 5% albumin containing 0.25 of IU heparin/ml of albumin.

After the priming procedure, the circuit was filled with type-specific, cross-matched, cytomegalovirus

antibody-negative packed erythrocytes (<3 days of age). Tris buffer and calcium gluconate were added to ensure pH 7.4 and a serum Ca of 2.4 mmol/l. Then 100 U of heparin was added to the circuit. All patients were placed on veno-arterial ECMO. The right common carotid artery and jugular vein were cannulated. All patients had surveillance blood cultures daily. Anticoagulation was achieved with a continuous infusion of heparin administered into the venous tubing of the circuit. Bedside monitoring of heparinization was performed hourly by measuring the activated clotting time (ACT). The target ACT was 180–200 sec.

In vitro ECMO experiments

The same ECMO system used in the clinical situation was used for *in vitro* experiments. After priming of the ECMO circuit with carbon dioxide, saline solution and albumin (as already described), the circuit was filled with fresh human citrated phosphate dextrose (CPD) blood, collected from healthy adult donors by venipuncture. Heparin was added (300 IU/l). *In vitro* ECMO was performed at a constant temperature of 37°C and a constant flow rate of 300 ml/min. An appropriate mixture of oxygen, nitrogen and carbon dioxide was supplied to the membrane oxygenator to keep blood gases in physiologic ranges. Electrolytes, pH, and glucose were kept constant in the physiological range during the 8 h of the experiments. For further studies, cells and plasma were separated by centrifugation. A total of six independent experiments were performed.

Analytical methods

Blood samples for complement analysis were drawn after filling the ECMO circuit (0 min), and 15 min, 30 min, 1 h, 2 h, 4 h, 8 h (endpoint of *in vitro* ECMO), 16 h, 32 h, and 64 h after initiation of ECMO, and 12 h after decanulation from ECMO. They were collected in tubes containing ethylenediamine tetraacetic acid. Within 10 min of collection, the samples were centrifuged for 3 min at 3000 rpm. Plasma was immediately separated and stored at –80°C.

Measurement of complement factors C3a, C5a, sC5b-9:

The concentrations of the complement factors C3a, C5a and sC5b-9 were measured in duplicate as previously described.^{10,11,13} The bioassays were blinded for group assignment and clinical data. A C3a enzyme-linked immunoassay (EIA) (Progen Biotechnik, Inc., Heidelberg, Germany) detected the complement component C3a-desArg using highly selected monoclonal antibodies. C5a was determined with a specific sandwich EIA (Aventis-Behring, Marburg, Germany). Soluble terminal complement complex sC5b-9 was measured with an EIA (LD Labour Diagnostika, Heiden, Germany). The accuracy of all methods used was assessed. Within-run precision study ($n = 20$) of all methods used showed coefficients of variation between 4 and 9%.

Statistical analysis

All results are expressed as mean \pm standard error of the mean. Changes in the concentrations of the measured parameters with time were compared with analysis of variance (Friedman analysis). Differences between the neonatal and *in vitro* ECMO were assessed by the Mann-Whitney U test. Statistical significance was assumed at $p < 0.05$. All calculations and tests were performed with SPSS-PC software (Chicago, IL, USA).

Results

The patients' clinical data and ECMO characteristics are presented in Table 1. ECMO was started at a mean age of 147 h after birth and the mean duration of ECMO was 152 h. Using a neonatal sepsis score,¹⁴ three newborns were diagnosed with infection at the initiation of ECMO according to the clinical situation and laboratory infection values (immature:total ratio of neutrophils > 20%, thrombopenia, leukopenia, CrP > 2 mg/dl). All patients received ampicillin (150 mg/kg/day), cefotaxime (100 mg/kg/day) and tobramycin or gentamicin (5 mg/kg/day) prior to the

Table 1. Clinical data of six newborns undergoing ECMO treatment for severe respiratory failure

Patient number	Weeks of gestation	Birth weight (g)	Sex	Primary diagnosis	Secondary sepsis	Start of ECMO (h)	Time on ECMO (h)	Outcome
1	38 + 3	3260	Male	Pneumonia, PPHN	No	19	82	Survived
2	35 + 0	2760	Male	Pneumonia	Yes	56	165	Died after ECMO
3	40 + 0	3025	Female	MAS	Yes	160	120	Survived
4	41 + 6	4770	Male	MAS, group B streptococcus sepsis	No	264	179	Survived
5	38 + 0	3650	Female	PPHN	No	48	137	Survived
6	39 + 5	3130	Male	PPHN	No	334	228	Survived

MAS, Meconium aspiration syndrome; PPHN, persistent pulmonary hypertension of the newborn.

ECMO course, and antibiotic treatment was continued during the course of ECMO treatment. Blood cultures were positive only in one case (patient 4, group B streptococcus). Following deterioration of the clinical status and changes in infection values at day 3 of the course of ECMO, the antibiotic regime was changed in two out of six newborn babies to vancomycin and imipenem (patients 2 and 3), and secondary sepsis was diagnosed using the neonatal sepsis score.¹⁴ A causative agent for the secondary sepsis was not detected in blood cultures drawn daily under ECMO treatment.

All complement indices increased during *in vivo* and *in vitro* ECMO (Figs 1-3).

C3a increased significantly within the first hour of *in vivo* and *in vitro* ECMO experiments. Baseline values *in vivo* were higher than those *in vitro* ($p = 0.02$ for start and 15 min) but the C3a increase was more pronounced with *in vitro* ECMO. Between 30 min and 8 h, C3a concentrations did not differ significantly from *in vivo* and *in vitro* experiments. *In vivo* ECMO C3a peaked after 1 h (Fig. 1).

C5a concentrations also increased steadily during *in vitro* ECMO. During *in vivo* ECMO there was an increase of C5a over time, with a peak after 2 h. During the 8 h, no significant differences were found between *in vivo* and *in vitro* ECMO (Fig. 2).

The sC5b-9 complex concentration increased in both *in vitro* ($p = 0.02$) and neonatal *in vivo* ($p < 0.001$) ECMO, with higher baseline values in neonatal ECMO. *In vitro*, the terminal complement complex was lower after filling the ECMO circuit and throughout 8 h of ECMO. *In vivo*, sC5b-9 increased after only 15 min of ECMO and was maximal at 2 h of ECMO (Fig. 3).

A second increase of C5a and sC5b-9 after 32 h was mainly due to patients 2 and 3, who developed symptoms of secondary sepsis at this time (Table 1).

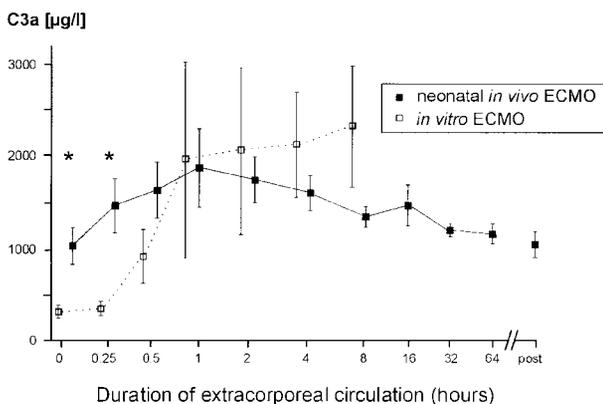


FIG. 1. C3a concentration (mean \pm SEM) in *in vivo* neonatal ECMO and *in vitro* ECMO ($n = 6$ for both groups). At 0 h and 0.25 h, C3a is different in neonatal and *in vitro* ECMO ($*p < 0.05$ by Mann-Whitney U test). Both groups show significant changes with time during the observation period (Friedman variance analysis). Post, 12 h after decanulation.

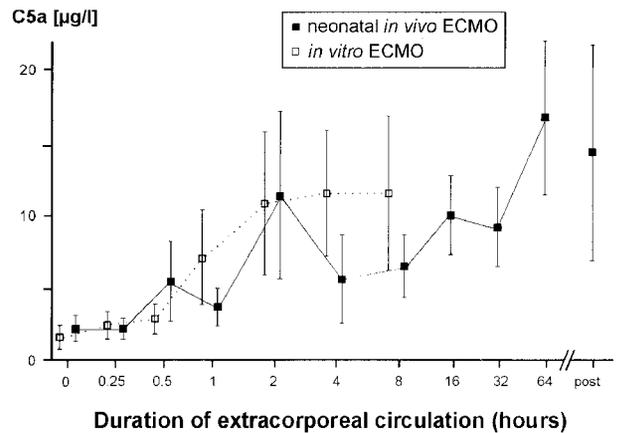


FIG. 2. C5a concentration (mean \pm SEM) in *in vivo* neonatal ECMO and *in vitro* ECMO ($n = 6$ for both groups). Both groups show significant changes with time during the observation period (Friedman variance analysis) but no difference between both groups. Post, 12 h after decanulation.

They had an increased immature to total neutrophil ratio, an increase of C-reactive protein, plus clinical deterioration with signs of an infection. Patient 2 also had leukocytosis. This was reflected by an intense increase of C5a concentration after 64 h to 33 and 33.5 $\mu\text{g/l}$ in patients 2 and 3, respectively. Twelve hours after decanulation of the ECMO circulation, C5a remained elevated in both secondary-infected patients (27.5 and 45 $\mu\text{g/l}$). The other four patients showed a decrease of C5a. This was also true for the sC5b-9 concentration, where a comparable increase in the secondary-infected patients was observed. In patient 2 (after 32 h, 892 ng/ml; after 64 h, 3510 ng/ml; 12 h after decanulation, 3660 ng/ml) and patient 3 (after 32 h, 1630 ng/ml; after 64 h, 1920 ng/ml; 12 h

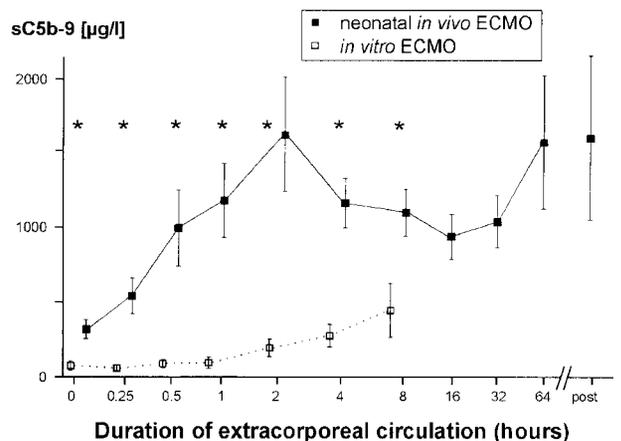


FIG. 3. sC5b-9 concentration (mean \pm SEM) of *in vivo* neonatal ECMO and *in vitro* ECMO ($n = 6$ for both groups). In neonatal *in vivo* ECMO, sC5b-9 is higher compared with the *in vitro* situation ($*p < 0.05$ by Mann-Whitney U test). Both groups demonstrate a significant increase during the observation period (Friedman variance analysis). Post, 12 h after decanulation.

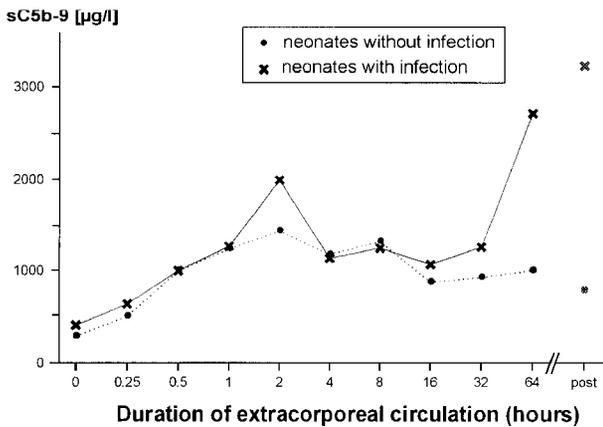


FIG. 4. Mean sC5b-9 concentration of neonates without secondary infection during ECMO (●, patients 1, 4, 5, and 6) and in neonates with secondary infection during ECMO (x, patients 2 and 3). Post, 12 h after decannulation.

after decannulation 2800 ng/ml), an increase was observed, whereas the other patients remained unchanged (Fig. 4).

Discussion

Our study demonstrates complement activation during neonatal ECMO (Figs 1–3) with a similar time course compared with adults. The complement system is similarly activated during cardiopulmonary bypass, hemodialysis, and plasmapheresis by contact with artificial membranes via the alternative pathway.¹² The peak of activation was reached for C3a after 1 h and for C5a and sC5b-9 after 2 h, in analogy to the results of Vallhonrat *et al.* in adults.¹² The stronger binding of C5a to the complement receptors could explain the delayed course of C5a activation compared with C3a activation.¹⁵ In contrast to the results of Vallhonrat *et al.* in two adults, the sC5b-9 values of the investigated six neonates did not decline to the beginning values after 2 days. Only C3a concentrations decreased after the initial peak at 1 h to values similar to the initial value at 24 h, confirming the results of Hocker *et al.*⁴ and Plötz *et al.*⁷

Concentrations of C5a and sC5b-9 showed a second increase at 64 h due to secondary infection of two newborns during ECMO, with extremely high values for sC5b-9 and C5a. Secondary infections during ECMO can be favoured by elevated endotoxin levels after 36 h of ECMO.¹⁶ But even for the four infants without secondary infection, concentrations of sC5b-9 remain elevated (Fig. 4). Obviously, the complement activation in neonates persists longer than in adults. The difference in time patterns of the activation products during neonatal ECMO corresponded to the reaction pattern of the clotting system in neonatal ECMO, where some activation products were normalized after an initial increase, such as thrombin–antithrombin complexes. Other products persisted on a higher level

during the whole course of the membrane oxygenation procedure, such as D-dimer.^{7,17}

A comparison of complement activation between neonatal and *in vitro* ECMO is especially suitable for determining which part of complement activation is caused by the extracorporeal circuit and which part by patients' underlying disease and their treatment. In neonatal *in vivo* ECMO, higher initial values of C3a and sC5b-9 indicate a mild complement activation in patients at the start of ECMO, corresponding to the results of Plötz *et al.*⁷ Suitable to this finding, Wagner *et al.*¹⁸ reported a complement activation in pre-term infants with respiratory distress syndrome. The differences between neonatal and *in vitro* ECMO were balanced for C3a and C5a at 1 h by a powerful complement activation after the start of ECMO. The simultaneous rise of anaphylatoxin concentration in neonatal and *in vitro* ECMO indicate that this part of complement activation is independent of interaction with endothelium or tissue. *In vitro* the anaphylatoxin concentration increased steadily, whereas in neonates concentrations peaked after 1 h for C3a and after 2 h for C5a. This difference could be caused by an *in vivo* suppression of the complement activation process due to increased production of complement inhibitors during ECMO or by reduced clearance of anaphylatoxins in the *in vitro* ECMO situation. *In vivo*, desArg-forms of anaphylatoxins are hepatically metabolized.

The marked quantitative difference of sC5b-9 concentrations between neonatal and *in vitro* ECMO is mainly due to the different blood used. In neonatal ECMO only heparin was added to prevent coagulation, but in *in vitro* ECMO CPD blood was used with additional heparin. This can inhibit the terminal complement pathway with a lower amount of sC5b-9 produced in *in vitro* ECMO. Beyond that, different sample characteristics of citrated and heparin blood in the EIA can disturb the determination of sC5b-9. We speculate that it is also possible that the activation of the terminal complement pathway is due to the interaction with the *in vivo* system (e.g. the production of complement proteins C6–C9 in the liver or other cells after initial complement activation).

The observed anaphylatoxins and the terminal complement complex promote neutrophil and monocyte activation, chemotaxis, the production of various cytokines, histamine release from basophils and mast cells, contraction of smooth muscle cells and aggregation of platelets, and therefore increase morbidity with capillary leakage syndrome, which was often observed in neonatal ECMO.^{8,19} The decrease of complement activation by inhibiting drugs or improved circuits with higher biocompatibility may be beneficial for newborn patients on ECMO.^{7,19,20} Our *in vitro* ECMO model showed similar activation of C3a and C5a, which resembles the *in vivo* situation.

In summary, the complement system in newborns is rapidly activated by ECMO. After an initial peak of

complement activation products at 1–2 h, a gradual decrease of complement activation products occurs until 2–3 days with ECMO. The rapid initial increase is caused predominantly by the contact with artificial surfaces and is not an effect of the patients underlying disease. The *in vitro* model closely resembles the *in vivo* situation and can therefore be used to test strategies to attenuate complement activation by improving ECMO with more inert extracorporeal material or complement activation inhibiting drugs.

ACKNOWLEDGEMENTS. The excellent technical assistance of Mrs W. Jekabsons and Mrs E. Strauss, help in statistical analysis by Mr B. Metzke and help with the English manuscript by Craig Jackson is gratefully acknowledged. Supported by Forschungsschwerpunkt Immunologie des Fachbereichs Humanmedizin, Freie Universität Berlin, Germany.

References

1. UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation. UK Collaborative ECMO Trial Group. *Lancet* 1996; **348**: 75–82.
2. Bennett CC, Johnson A, Field DJ, Elbourne D. UK Collaborative ECMO Trial Group. UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation: follow-up to age 4 years. *Lancet* 2001; **357**: 1094–1196.
3. Ishino K, Weng Y, Alexi-Meskishvili V, *et al*. Extracorporeal membrane oxygenation as a bridge to cardiac transplantation in children. *Artif Organs* 1996; **20**: 728–732.
4. Hocker JR, Wellhausen SR, Ward RA, Simpson PM, Cook LN. Effect of extracorporeal membrane oxygenation on leukocyte function in neonates. *Artif Organs* 1991; **15**: 23–28.
5. Graulich J, Walzog B, Marcinkowski M, *et al*. Leukocyte and endothelial activation in a laboratory model of extracorporeal membrane oxygenation (ECMO). *Pediatr Res* 2000; **48**: 679–684.
6. Anderson HL III, Coran AG, Drongowski RA, Ha HJ, Bartlett RH. Extracellular fluid and total body water changes in neonates undergoing extracorporeal membrane oxygenation. *J Pediatr Surg* 1992; **27**: 1003–1007.
7. Plötz FB, van Oeveren W, Bartlett RH, Wildevuur CR. Blood activation during neonatal extracorporeal life support. *J Thorac Cardiovasc Surg* 1993; **105**: 823–832.
8. Fortenberry JD, Bhardwaj V, Niemer P, Cornish JD, Wright JA, Bland L. Neutrophil and cytokine activation with neonatal extracorporeal membrane oxygenation. *J Pediatr* 1996; **128**: 670–678.
9. Kelly RE Jr, Phillips JD, Foglia RP, *et al*. Pulmonary edema and fluid mobilization as determinants of the duration of ECMO support. *J Pediatr Surg* 1991; **26**: 1016–1022.
10. Sonntag J, Dähnert I, Stiller B, Hetzer R, Lange PE. Complement and contact activation during cardiovascular operations in infants. *Ann Thorac Surg* 1998; **65**: 525–531.
11. Sonntag J, Emeis M, Vornwald A, Strauss E, Maier RE. Complement activation during plasma production depends on the apheresis technique. *Transfus Med* 1998; **8**: 205–208.
12. Vallhonrat H, Swinford RD, Ingelfinger JR, *et al*. Rapid activation of the alternative pathway of complement by extracorporeal membrane oxygenation. *ASAIO J* 1999; **45**: 113–114.
13. Sonntag J, Brandenburg U, Polzehl D, *et al*. Complement system in healthy term newborns: reference values in umbilical cord blood. *Pediatr Dev Pathol* 1998; **1**: 131–135.
14. Bühner C, Luxemburger U, Metzke B, *et al*. Diminished cord blood lymphocyte L-selectin expression in neonatal bacterial infection. *Eur J Pediatr* 1993; **152**: 519–522.
15. Hetland G, Moen O, Bergh K, *et al*. Both plasma- and leukocyte-associated C5a are essential for assessment of C5a generation in vivo. *Ann Thorac Surg* 1997; **63**: 1076–1080.
16. Hirthler M, Simoni J, Dickson M. Elevated levels of endotoxin, oxygen-derived free radicals, and cytokines during extracorporeal membrane oxygenation. *J Pediatr Surg* 1992; **27**: 1199–1202.
17. Uriesberger B, Zobel G, Zenz W, *et al*. Activation of the clotting system during extracorporeal membrane oxygenation in term newborn infants. *J Pediatr* 1996; **129**: 264–268.
18. Wagner MH, Sonntag J, Strauss E, Obladen M. Complement and contact activation related to surfactant response in respiratory distress syndrome. *Pediatr Res* 1999; **45**: 14–18.
19. Mollnes TE, Videm V, Götze O, Harboe M, Oppermann M. Formation of C5a during cardiopulmonary bypass: inhibition by precoating with heparin. *Ann Thorac Surg* 1991; **52**: 92–97.
20. Tofukuji M, Stahl GL, Agah A, Metais C, Simons M, Sellke FW. Anti-c5a monoclonal antibody reduces cardiopulmonary bypass and cardioplegia-induced coronary endothelial dysfunction. *J Thorac Cardiovasc Surg* 1998; **116**: 1060–1068.

Received 17 September 2001

Accepted 10 January 2002



Hindawi
Submit your manuscripts at
<http://www.hindawi.com>

