the pathological changes was suppressed by the effects of forphenicine. Also, the findings in cerebral tissues were compatible with these results (figs 4–6). Namely, the demyelination of the central nerves occurred in the diseased animals but not in the forphenicine-treated ones, although meningeal infiltration of mononuclear cells persisted in some of the latter animals. The findings in the animals saved by the other inhibitors were similar to these.

Discussion. Previously, Brosnan et al. reported that inhibitors of plasminogen activators and other neutral proteinases gave significant protection against the clinical expression of EAE in Lewis rats[14]. The present study, using other inhibitors, clearly demonstrated their suppressive effects on pathological changes in the central nervous tissues and muscles. Our previous studies suggested that this disease model presents unexpectedly extensive changes involving multiple organs[7]. Accordingly, the beneficial effects of these inhibitors may have a broader significance, encompassing even systemic autoimmune diseases. The effects of the inhibitors against alkaline phosphatase, aminopeptidase B and esterase were remarkable.

Judging from our observations, as well as those of others, it seems difficult to relate the pathogenic mechanisms of this disease to any specific enzymes. It seems more reasonable to explain the effects of these inhibitors by their actions on immune-responsive cells. In this respect, it is noteworthy that 500 μg of forphenicine saved 80% of the experimental animals. This agent resembles levamisole[15] in that it is an inhibitor of alkaline phosphatase. Also, both of these substances are known to be immunopotentiators rather than immunosuppressors. The reason why the immunopotentiators favorably affect the autoallergic state is not known yet. If autoimmunity were a manifestation of immunodeficiency it would not be surprising if drugs stimulating immune responsiveness produced improvement in autoimmune diseases[3]. These paradoxical phenomena concerning the so-called immunomodifiers should be reanalyzed in the light of an improved understanding of the antigens, the cellular interactions and the suppressor cell system[16]. Another question arising from this work is that of the optimal dosage of each enzyme inhibitor. Since it is possible that some of the immunomodifiers have dual effects depending on the doses administered, a more detailed study should be done to decide their optimum dosage precisely. Such a study, using the most promising agent, is being planned now.

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Chemotactic and random movement of cord-blood granulocytes

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Summary. Chemotactic responsiveness and random movement of cord-blood granulocytes were studied with a modified Boyden's method. Cord-blood granulocytes were less active chemotactically than granulocytes from healthy children and adults, whereas the random filter movement of the cells from all three sources was about the same.

In cord sera, concentrations of cell directed chemotaxis inhibitors were equal to those in sera from other age groups. Compared with the situation in healthy children and adults, the generation of chemotactic factors in cord-blood sera was impaired. This impairment was not related to an increased activity of chemotactic factor inactivators.

Measurement of the cyclic nucleotide levels in granulocytes from cord-blood and from children belonging to various age groups revealed that the cord granulocytes have significantly lower concentrations of cAMP and cGMP, which could have been responsible for the decreased chemotactic responsiveness.

Key words. Granulocytes, cord-blood; random movement; chemotactic responsiveness; chemotaxis inhibitors; cyclic nucleotide levels.

Introduction. Rapid migration of neutrophils into tissues invaded by bacteria is essential for host defense. An association between depressed neutrophil chemotaxis and susceptibility to infections in patients with recurrent bacterial infection has been established[1–5]. Chemotactic disorders can be divided into cellular and humoral defects, and can be caused by specific

inhibitors. Newborn infants are known to develop severe bacterial infections very easily and to respond poorly to invasion by micro-organisms[1,6]. The increased susceptibility of neonates to infections can be attributed to functional abnormalities of their neutrophils[2,4,5]. The present study was undertaken to investigate some cellular and humoral aspects of neonatal granulocyte chemotaxis and to evaluate various parameters of these processes.

Since cyclic nucleotide metabolism plays an important role in the chemotactic movement of cells[7,8], experiments were performed to whether there is any correlation between the intracellular levels of cyclic nucleotides (cyclic 3′,-5′-adenosine monophosphate (cAMP), and cyclic 3′-5′-guanosine monophosphate (cGMP)) on the one hand and on the other the chemotactic activity of granulocytes from cord-blood and blood of children of different ages.

Materials and methods. Blood samples. Blood was taken from the placental end of the cut umbilical cord, using 20 U/ml heparin as anticoagulant, at the delivery of healthy full-term babies. For control studies, blood from the cubital veins of healthy fasting children (aged 2–7 years) and adults was obtained by venipuncture.

Isolation of granulocytes. Granulocytes were obtained by dextran sedimentation of the granulocyte sediment from a Ficoll-Hypaque gradient after reconstitution of the pellet to the original volume with phosphate-buffered saline (PBS)[9–11]. Finally, 5×10^6 granulocytes/ml were suspended in Gey's solution.

Serum. Serum was obtained form cord-blood and from blood of healthy children and adults. The blood was allowed to clot for 1 h at room temperature before being centrifuged for 20 min at 1200 × g, and the supernatant serum was collected and stored in 0.5 ml aliquots at −70 °C.

Chemotactic attractants. Chemotactic substance was obtained by activating 10% (v/v) serum with *E. coli* endotoxin. The dose of endotoxin used for this purpose was one-third of the LD_{50}/24 h value assessed in mice. In some experiments activation was performed with 1 ml of a suspension of 10^9/ml frozen-dried *Staphylococcus aureus* (5814 S)[12]. In control experiments 1 mg casein/ml (Merck, Darmstadt, W. Germany) in Gey's solution was used as a chemo-attractant.

Chemotactic assay. The chemotactic and random mobility of granulocytes was measured in a modified Boyden's chamber, as described elsewhere[13]. Filters (Millipore Filter Corp., Bedford, Mass., USA) with a pore size of 3 µm were used to separate the two compartments, one containing 5×10^6 granulocytes/ml and the other containing chemo-attractant, i.e. 10% activated serum. Chemotactic responsiveness was expressed as the average number of cells appearing on the lower surface of the filter after 2.5 h of incubation a 37 °C. Cells were counted by light microscopy (magnification ×400) after staining with Ehrlich's hematoxilin.

Cyclic nucleotides. After sedimentation, 5×10^6 granulocytes were washed twice with PBS and the sediment was homogenized in cold 5% trichloro-acetic-acid (TCA). Next, the TCA was removed by extraction with diethylether. The aqueous phase was freeze-dried and stored at −20 °C until use. The cAMP and cGMP contents were measured by using assay-kits (Amersham International, formerly Radiochemical Center Ltd, Buckinghamshire, UK), according to the manufacturer's instructions.

Statistical evaluations. For the statistical evaluation of the differences use was made of Student's t-test. The standard errors of the means (SEM) were calculated for each group.

Results. Chemotactic activity and random movement of cord-blood granulocytes. The chemotactic activity of cord-blood granulocytes was measured with endotoxin- or *S. aureus*-activated serum from healthy adults as attractant, and the results were compared with the chemotactic activity of granulocytes from healthy children and adults. Compared with the controls, fewer cord-blood granulocytes reached the lower surface of the filter ($p < 0.01$) (fig.). When casein was used as a chemoattractant, the results were similar, i.e. the response of cord-blood granulocytes was lower than that of the granulocytes of healthy adult controls (data not shown). No significant differences were found between the chemotactic responses in the various control groups of children and adults ($p > 0.1$), or between the movement of granulocytes from neonates, children, and adults in the absence of chemoattractants (fig.).

Taken together, these results indicate that the stimulated movement of cord-blood granulocytes towards chemotactic factors is strongly depressed. The random mobility of cells from neonates is similar to that of granulocytes from children older than two years and from adults.

Chemotactic inhibitors in cord-blood serum. To find out whether the defective chemotaxis of cord-blood phagocytes is due to inhibitors still adhering to granulocytes after the isolation procedure, granulocytes from healthy donors were pre-incubated for 30 min with 10% serum form newborns, children, or adults and then washed three times with Gey's solution to remove any free serum. The controls were adults' granulocytes

Table 1. Inhibitor activity of cord blood sera on the chemotactic movement of adult granulocytes*

Pre-incubation of granulocytes with	Number of granulocytes per high powerfield**	
Gey's solution	21.5 ± 4.9	p
10% cord-blood serum	14.8 ± 4.2	< 0.001
10% serum of children aged 1–2 yr	15.3 ± 5.3	< 0.01
10% serum of children aged 3–5 yr	17.2 ± 6.4	< 0.05
10% serum of children aged 6–8 yr	14.2 ± 5.1	< 0.001
10% serum of adults	15.4 ± 6.7	< 0.01

* Granulocytes were incubated with 10% sera for 30 min at 37 °C and then washed 3 times to remove any free serum. ** Chemo-attractant: 10% (v/v) adult sera activated with E. coli endotoxin. Values represent the mean ± SD of 15 experiments. p, compared to chemotaxis after pre-incubation with Gey's solution.

Table 2. Chemotaxis of adults cells towards endotoxin activated serum

Chemoattractant	Number of cells per high power field (± SD)	p_1	p_2	(n)
Activated cord-blood serum	9.1 ± 3			(17)
Activated serum of children (ca. 2½ yr)	19.4 ± 4.1	< 0.01		(17)
Activated serum of children (ca. 4½ yr)	24.5 ± 5.3	< 0.01		(20)
Activated serum of children (ca. 7½ yr)	22.7 ± 4.4	< 0.01		(15)
Activated serum of adults	23.0 ± 5.2	< 0.01		(19)
Activated serum of adults + 10% Gey's solution	21.4 ± 4.9	–	NS	(9)
Activated serum of adults + 10% serum of newborns	20.3 ± 5.2	–	NS	(11)
Activated serum of adults + 10% serum of adults	23.0 ± 5.8	–	NS	(14)

p_1, compared to the chemotaxis towards activated cord-blood serum; p_2, compared to the chemotaxis towards activated serum of adults; NS, $p > 0.1$; (n), number of experiments.

Table 3. Cyclic nucleotide levels of granulocytes

Age	cAMP* pM/mg protein	%	cGMP pM/mg protein	%	(n)
Newborn	4.15 ± 0.31	100	0.50 ± 0.03	100	(11)
2 ± ½ yr	11.40 ± 0.87**	275	1.90 ± 0.04**	380	(9)
7 ± ½ yr	10.60 ± 0.55**	255	1.80 ± 0.03**	360	(9)

* mean value ± SEM; **$p < 0.001$ compared to the newborns; (n) number of experiments.

not pre-incubated with serum. Pre-incubation of adults' blood granulocytes with 10% serum from various sources led to a similar decrease of the chemotactic activity (table 1). This indicates that the decreased chemotactic activity of cord granulocytes cannot be explained by a higher concentration of chemotactic inhibitors present in cord sera compared to that in sera form children and adults.

Generation of chemotactic factors in cord-blood serum. To investigate the generation of chemotactic factors in cord-blood serum, serum from neonates, children of different ages, and adults, was activated by endotoxin and the chemotactic activity measured from the response of normal adults' granulocytes. The results show that such granulocytes migrate significantly less toward activated cord-blood sera than toward activated sera than toward activated sera of other age groups (table 2). These results indicate that under identical conditions cord-blood serum generates fewer chemotactic factors than does serum from children or adults.

Chemotactic factor inactivators in cord-blood sera. To find out whether the impaired generation of chemotactic factors in cord-blood serum is due to inactivators interacting with these factors, 10% fresh serum from newborns or adults was added to the lower compartment of the chamber containing 10% endotoxin-activated adult serum as chemo-attractant. The results show no difference in the chemotactic activity of these two groups of granulocytes (table 2). This indicates that serum from newborns does not contain specific factors that inactivates chemo-attractrants generated in adult serum.

Cyclic nucleotide levels of granulocytes and chemotactic activity. Concentrations of cAMP and cGMP were considerably lower in cord-blood granulocytes than in granulocytes of children of various age groups and did not differ significantly between the two groups of children (table 3).

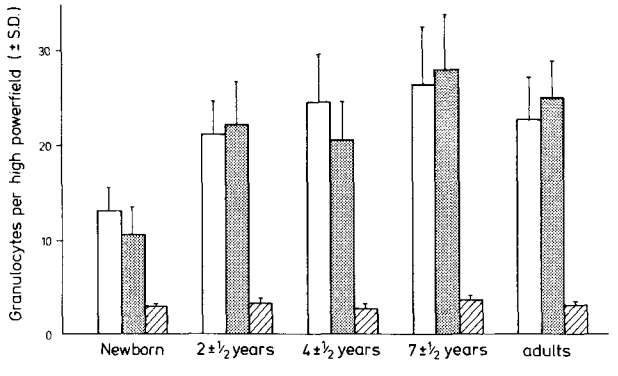
To investigate whether agents known to increase cellular cAMP also alter the chemotactic movement of cord blood granulocytes, various prostaglandins were added to the cell compartment of the chemotaxis chamber. Chemotaxis of cord granulocytes in the presence of 10^{-8} and 10^{-7} M prostaglandin $F2_\alpha$ significantly increased the chemotactic activity of cord-blood granulocytes from 9.6 ± 3.2 ($n = 9$) to 13.9 ± 3.2 ($p < 0.01$, $n = 10$) and 17.3 ± 4.2 ($p < 0.001$, $n = 9$) granulocytes/high power field, respectively. However, incubation of cord granulocytes in the presence of 10^{-6} and 10^{-5} M prostaglandin E2, i.e., concentrations which increase intracellular cAMP[14,15], did not increase the chemotactic capacity of these cells.

Discussion. The results of the present study show that the chemotactic motility of cord-blood granulocytes towards endotoxin-activated adults' serum as well as to casein is severely impaired. This finding is in agreement with previous reports[2,4,16,17]. It was suggested that the lower chemotactic activity of newborn granulocytes might be attributable to the decreased deformability of cell membrane[5] and maybe correlated with the functional state of the cytoskeleton at that stage[18,19]. Since the organization and activity of the microtubular and microfilament systems are strongly influenced by Ca^{2+} and cyclic nucleotides[7,8], cAMP and cGMP concentrations in neonatal granulocytes were measured; they were found to be lower than in granulocytes from older children. These findings suggest a relationship between the lower chemotactic activity of cord-blood granulocytes ad the lower nucleotide concentrations of these cells. Further investigation of this relationship by increasing the cyclic nucleotide levels of cord blood granulocytes by incubation with prostaglandin E2 and $F2_\alpha$[14,15] revealed increased chemotaxis in the presence of prostaglandin $F2_\alpha$, but prostaglandin E2 had no effect.

The random mobility of the granulocytes showed no correlation with the age-dependent changes of the cyclic nucleotide concentrations, which is in agreement with the observations of Sandler[20], and Hill[14], as well as with the observation of the latter that incubation of granulocytes with cAMP and cGMP does not effect the random motility of these cells.

An alternative explanation for the decreased neonatal granulocyte chemotaxis could be that inhibitors influencing the chemotactic responsiveness of the granulocytes which are normally present in human plasma[21] are present in greater amounts on cord-blood granulocytes or in cord-blood serum, compared to their presence in serum from adults. However, the present results revealed that granulocytes are not coated with inhibitors present in cord-blood serum or chemotactic factors inactivated by factors present in cord-blood serum. Recently it was shown for rats that granulocyte chemotaxis in vitro is inhibited by α-macrofoetoprotein[22]. However, the concentration of α-macrofoetoprotein was not determined in our cord sera. Since chemotactic factors can regulate the emigration of granulocytes during an inflammatory reaction, we investigated the capacity of cord-blood serum to generate chemoattractants. The results showed diminished generation of chemotactic factors in cord sera after activation by either whole bacteria or bacterial endotoxins. This decrease might have been attributed to the inactivation of chemotactic factors by humoral inactivators, but, when cord serum was added to activated adult serum the chemotactic activity did not decline. Thus, a decrease in the production of chemotactic factors in cord-blood serum offers the most likely explanation of this phenomenon.

In sum, the results of the present study show that cord-blood granulocytes are less chemotactically active than are the granulocytes of older children and adults, and that this defect is probably related to the decrease in cAMP/cGMP levels in neonatal granulocytes. Furthermore, cord-blood serum was found to have a defective mechanism for the generation of chemotactically active products, independent of the presence of inhibitors in this serum.



Chemotactic and random movement of granulocytes from patients in the various age groups. Chemotaxis towards serum activated by *E. coli* endotoxin (□), and by *S. aureus* (▨) was measured; normal Gey's solution (▨) was used in the study of random migration.

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Properties of bovine interferons[1]

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Summary. This study was done in an attempt to elucidate some of the properties of bovine IFNs. Maximum levels of both fibroblast and leukocyte IFNs occurred prior to 24 h whereas maximum levels of immune IFN were not reached until after 72 h. The latter species of IFN was unstable at either pH 2 or 56 °C whereas both the fibroblast and leukocyte IFNs were more stable under these conditions. Studies of cross-species protection between fibroblast and leukocyte IFNs indicate that the former was more protective for other species than the latter.
Key words. Interferon; bovine interferon.

A number of studies have been published on the production of bovine interferons (Bov IFN). These reports have described some of the properties of these IFNs, however, no definitive effort has been made to separate and classify them. Because studies have not been published on the molecular species we will use the 'classical' terms of leukocyte, fibroblast and immune to describe the properties of these IFNs. This paper is an attempt to elucidate some of the properties of Bov IFNS.

Materials and methods. Media used for growth and maintenance of fibroblast cells were Eagles MEM with 10 and 2% fetal calf serum (FCS), respectively[3]. RPMI with 10% FCS was used for leucocyte cultures. All media contained 0.25% gentamicin. Viurses used were Sendai, Newcastle disease virus (NDV), and vesicular stomatitis virus, Indiana strain (VSV). Sendai virus and NDV were grown in embryonated eggs. VSV was grown in cultures of bovine cells and titrated by plaque formation (PFU) in MDBK cells. Confluent cultures of bovine kidney cells (MDBK) were the primary source of fibroblast IFN and were used in the IFN assays. Other bovine cells employed included bovine embryonic kidney (BEK), embryonic bovine trachael cells (EBTr), and bovine turbinate (BT). All of which were purchased from the American Type Culture Collection, Rockville, MD. Nonlactating dairy Holstein cows were used as the source of bovine leukocytes. Leukocytes were separated from whole blood by means of a selective hypotonic lysis procedure developed in this laboratory[4]. Briefly, bovine blood was collected by jugular venipuncture and mixed with distilled water containing 0.3% galactose in a 1:1.5 ratio of blood to galactose solution respectively. After 3 min, 1.5 parts of cold buffered saline gelatin (BSG) at pH 7.2 was added. Leucocyte suspensions were centrifuged, washed in BSG and resuspended in RPMI-1640 to a final concentration of 5×10^6 cells per ml. When only lymphocytes were needed, aliquots of leukocytes, obtained by the 'lysis' procedure described above, were centrifuged on Ficoll-Paque (Pharmacia, Piscataway, NJ).

For the production of fibroblast IFN four bovine cell types were employed: BEK, MDBK, EBTr, and BT. Triplicate cultures of each cell type were established on plastic tissue culture flasks. The virus (NDV or Sendai) was added to cell monolayers as previously described[1]. After adsorption the virus was decanted, the monolayers washed and 10 ml of MEM were added. After 24 h incubation, the fluids were collected and the virus inactivated by irradiation for 15 min at 12 cm from an UV light (15 W) source. Poly I:C was used at a concentration of 0.01 mg/ml along with DEAE-dextran at 0.1 mg/ml. IFN production during the assay was prevented by one of the following: removing the inducers, UV inactivation or RNAase treatment.

For the production of leukocyte IFN the virus was added to the leukocyte suspensions and allowed to adsorb for 1 h. The cells were then washed, resuspended in RPMI-1640, and incubated for 24 h at 37 °C under 5% CO_2. The fluids were collected and frozen for subsequent assay.

Immune type IFN was obtained from lymphocytes induced with con A. The lymphocyte population was obtained as previously described. The mitogen was added at a concentration of 0.01–0.05 mg/ml. Tissue culture fluids, collected at various intervals post-induction, were frozen at −20 °C until assayed. The pH stability of the different IFNs was evaluated by dialyzing preparations against 100 volumes of pH 2 buffer. After dialysis for 24 h, the samples were redialyzed against pH 7.2 buffer for 24 h. IFN preparations were also subjected to 45 °C and 56 °C for different time periods, then assayed along with control preparations. The activity of IFN on both homologous and heterologous cells was determined using infection by VSV. This protection was compared with that obtained using MDBK cells. Prior to the assays, the stock VSV was titered on each cell type to determine the amount of virus required for the production of 30–50 PFU per well.

IFN was assayed using MDBK cells in a microplaque reduction assay using VSV. IFN preparations were serially diluted directly in the microtiter dishes. MDBK cells were then added, and the plates were incubated overnight at 37 °C under 5% CO_2. The cells were challenged with 30–50 PFU of VSV which