dary to fluid secretion[8,9]. During perfusion with a high K^+ solution, there was a sustained decrease in amylase release in the experiments on control glands[8] (figure 2). The parotid segments from the reserpine treated rats, however, showed an inability to maintain a reduced level of amylase release when perfused with a high K^+ solution. Decreasing the transmembrane K^+ gradient reduces ACh-evoked K^+ release in normal salivary glands[10]. The much diminished and shortlasting inhibition by high K^+ of ACh-evoked amylase release in the glands from reserpinized animals is probably related to the enhanced stimulant-evoked K^+ release in these conditions[5].

The results showing that the reserpine-induced impairment of stimulant-evoked enzyme secretion is much more marked during excitation of cholinergic or α-adrenergic receptors than during activation of β-adrenergic receptors (table) again points to an anomalous K^+ metabolism in the glands from reserpinized animals, since cholinergic or α-adrenergic stimulants, in contrast to β-adrenergic stimulants, cause a marked K^+ release[8,11].

Glands from reserpinized animals contain more Ca than controls[5,6] and since stimulant-evoked K release seems to be initiated by an increase in internal Ca^{2+} concentration[11], the results presented here may at least in part be explained by an abnormal cellular Ca metabolism.

Since the salivary glands of reserpinized animals have defects similar to those seen in cystic fibrosis[4,5] the present results would indicate that an abnormal K transport is an important element in the cellular disorder underlying this disease.

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A possible preferential inhibition of chemotaxis of polymorphonuclear neutrophils by a chemical modification

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Summary. The modification of neutrophils with amino group blocking reagents of different chemical specificities showed that dansyl chloride caused inhibition of chemotaxis without suppression of random movement. Dansylated neutrophils, like control cells, ingested bacteria. Neither the stimulated cyanide-insensitive respiration, nor lactate production during phagocytosis, was affected significantly by dansylation as compared with the inhibition of directed movement.

The movement of polymorphonuclear neutrophils (PMNs) towards a site of infection or injury is directed by a chemical gradient produced at the site. Leukocyte chemotaxis has been extensively investigated in vitro using the Boyden technique[1,2]. However, the mechanisms involved are still almost entirely unknown; how the attractant is detected and how the sensed information is translated into action. It would be very useful as a means of answering these questions to inhibit separately locomotion and chemotactic response by a chemical modification. Therefore, we tried to stain separately the directional and random movements, using amino group blocking reagents of different chemical specificities, and obtained the result that dansyl chloride inhibited chemotaxis more specifically than random movement, phagocytic ability and lactate formation in PMNs.

Materials and methods. Fluorescamine was from Roche; dansyl chloride from Sigma; sodium 2,4,6-trinitrobenzene sulfonate (TNBS) from Tokyo Chemical Industry Co. PMNs were obtained from glycogen-induced peritoneal exudates of guinea-pig[3,4]. A chemical modification of PMNs was carried out by incubating 2×10^7 cells/ml with reagents at 0 °C for a defined time. When acetone was used as a solvent for blocking reagents, its final concentration was 1% in a modification mixture. The reaction was stopped by the addition of a 2-fold molar excess of glycine. The reaction mixture was diluted 4 times with PBS for the motility assay. PMN migration was assayed as described previously[3,4] in a modified Boyden chamber in the presence (chemotaxis) and absence (random movement) of the chemoattractant (the supernatant from a sonicated suspension of *Escherichia coli* grown for 20 h at 37 °C in a polypeptone medium): 2 ml of a bacterial attractant in PBS, or PBS alone, were placed in the bottom of a

Table 1. Effects of amino group blocking reagents, glycine and acetone on chemotaxis of neutrophils

Compounds		Neutrophil migration (percent of control)	Viability (%)
Fluorescamine	100 μM	2	90
Dansyl chloride	100 μM	4	90
TNBS	1 mM	102	91
Glycine	10 mM	100	93
Acetone	4%	102	96
	8%	95	93
	12%	85	94

A chemical modification of PMNs with reagents and a treatment of PMNs with glycine or acetone were carried out at 0 °C for 60 min. After 4-fold dilution with PBS, chemotaxis was assayed. Migration values indicate the mean of 2 experiments in which the control PMN migrated 70 and 86 μm in 40 min at 37 °C, respectively.

chamber, and 1 ml of modified PMN suspension (5×10^6 cells/ml in PBS) was placed in the top of the chamber, separated from the bottom by Sartorius Membranfilter (pore size 3 µm). The chamber was incubated at 37 °C for 40–60 min. Chemotaxis and random movement are expressed as the distance from the top of the filter to the furthest 2 cells at the same focal plane, according to the method of Zigmond and Hirsch[5]. Viability was checked by trypan blue exclusion. The ability of PMNs to phagocytize bacteria was determined by the microscopic examination of Gram stained smears of PMNs incubated with heat-killed bacteria[3]. Oxygen consumption was measured polarograph-

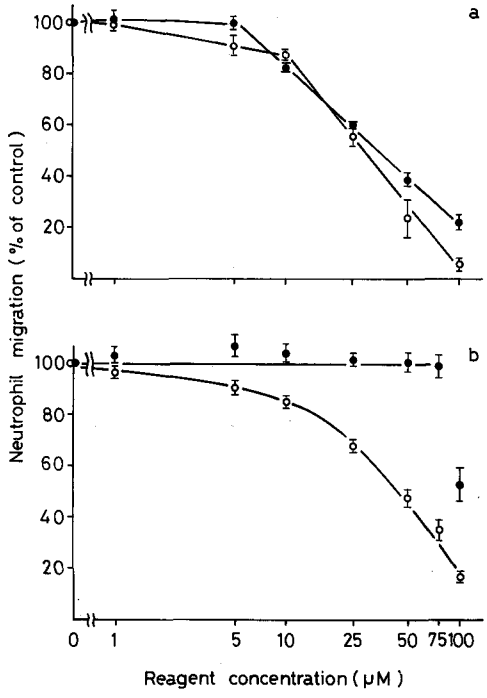

Fig. 1. Effect of amino group blocking reagent concentration on the directed and random movements of neutrophils. PMN suspension containing 2×10^7 cells/ml were incubated with varying concentration of fluorescamine (a) or dansyl chloride (b) in a total volume of 1.01 ml at 0 °C. After 2-min modification, 0.01 ml of 20 mM glycine solution was added to each PMN suspension to stop the reaction. Modified PMN suspension was diluted 4 times with PBS and subjected to the motility assays in the presence (○) and absence (●) of the bacterial attractant. Positive controls (not modified and containing an attractant in the bottom of a Boyden chamber) migrated 68–97 µm, and negative controls (not modified and lacking an attractant in the bottom of chamber) moved 12–16 µm in 40 min at 37 °C. Migration values represent the mean ± SD of 3 experiments.

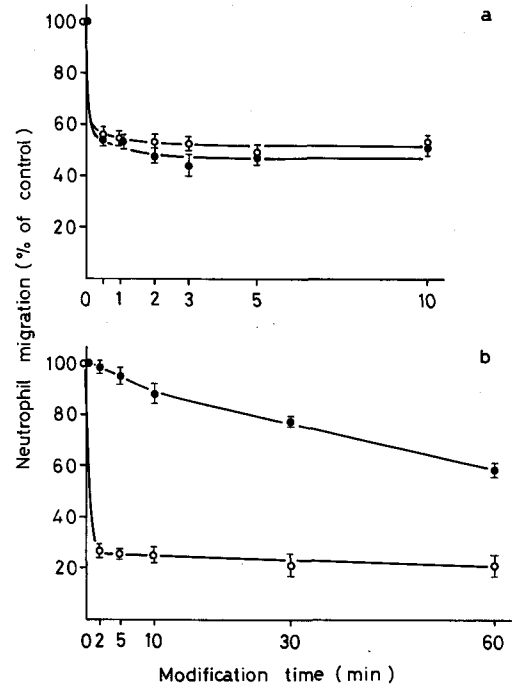

Fig. 2. Changes in the motility of PMNs during the modification with reagent. A chemical modification was performed by the treatment of 2×10^7 cells/ml with 25 µM fluorescamine (a) or 75 µM dansyl chloride (b) at 0 °C. At indicated time intervals, the reaction was stopped by an addition of glycine. After 4-fold dilution of the reaction mixture with PBS, the migration of modified PMNs was tested in the presence (○) and absence (●) of an attractant in the lower compartment of a chemotaxis chamber. Positive and negative controls migrated 63–100 and 12–14 µm in 40 min, respectively. Migration values indicate the mean ± SD of 3 experiments.

Table 2. Effect of dansylation on phagocytosis, cyanide-insensitive oxygen uptake and lactate production

		Migration[a] (percent of control)	Phagocytosis[b] 5 min	15 min	30 min	O_2 consumption[c]	Lactate[d] produced
1	Control	100 (100)	15	21	41	–	–
	Modified PMN	27 (85)	12	25	41	–	–
2	Control	100 (100)	12	32	43	244	–
	Modified PMN	40 (98)	15	36	44	209	–
3	Control	100 (100)	14	32	49	145	–
	Modified PMN	35 (93)	12	27	44	124	–
4	Control	100 (100)		–		–	6.1
	Modified PMN	51 (86)		–		–	5.5
5	Control	100 (100)		–		–	3.7
	Modified PMN	47 (91)		–		–	2.9
6	Control	100 (100)		–		–	6.5
	Modified PMN	44 (90)		–		–	4.9

[a] Values in the migration column represent chemotaxis and random movement (in parentheses), respectively. [b] To the mixture containing 0.25 ml of PMN suspension (2×10^7 cells/ml), 0.45 ml PBS and 0.1 ml fresh guinea-pig serum was added 0.1 ml of a saline suspension containing 5×10^7 *Staphylococcus aureus* per ml. The tubes were constantly shaken at 37 °C. Aliquots of the mixture were removed at 5, 15 and 30 min, smeared on the glass slides, fixed with methanol and stained with Gram stain. Ingestive ability was represented as the ratio of PMNs with intracellular bacteria to total PMNs enumerated (300–400 cells). [c] Atoms $0/10^6$ cells/h. [d] µmoles/5×10^7 cells/h.

ically with a Clarke oxygen electrode (Rank Brothers, England) during magnetic stirring at 37 °C. The period chosen for the measurement of oxygen consumption was the first 4 min following a brief period (30–60 sec). Assay medium contained Krebs-Ringer phosphate buffer without Ca^{2+} (pH 7.4, KRPB), 2 mM KCN, 4×10^7 leukocytes and 10^9 heat-killed *Staphylococcus aureus* in a final volume of 2.12 ml. Lactate was assayed with the Biochemica test combination from Boehringer GmbH, Mannheim (Federal Republic of Germany). PMNs were incubated with bacteria (bacterium to cell ratio of 1:1) at 37 °C in 1 ml KRPB containing 10 µmoles glucose. After the addition of 1.5 ml of ice-cold KRPB in an hour, the incubation mixtures were centrifuged at 4 °C and the supernatant fluids were used for lactate assay.

Results and discussion. Since fluorescamine and dansyl chloride can be dissolved only in an organic solvent, the effect of acetone on the chemotaxis was studied. As seen in table 1, acetone did not affect chemotaxis at the concentration used for the chemical modification. Glycine, used to stop the reaction of modification reagents, produced no effect on chemotaxis even at 10 mM. Among modification reagents, TNBS, which is markedly hydrophilic due to the highly polar sulfonic acid moiety, and consequently may penetrate cells at a much slower rate, produced little effect on the chemotaxis even at 1 mM. On the other hand, fluorescamine and dansyl chloride, a hydrophobic reagent which may penetrate the membrane at a faster rate, strongly inhibited chemotaxis at 100 µM but no detectable changes were observed in viability between unmodified and modified PMNs. Neither glycine adducts nor hydrolysates of fluorescamine and dansyl chloride had any detectable effects on leukocyte motility. These results seem to indicate that the functional groups whose modification appears to be associated with the inhibition of chemotaxis are not located on the outside surface of the plasma membrane but within the membrane or cytoplasm. Next, we examined the inhibitory effect of 2 reagents on directed and random movements. As shown in figure 1, no remarkable alterations in directed and random movements were observed at a low concentration with either reagent. When the concentration was further increased the 2 reagents showed different inhibitory patterns. Namely, with fluorescamine 50% inhibition of chemotaxis was observed at 25 µM, and nearly complete inhibition occurred at 50 µM where modified PMNs were microscopically fairly rounded-up. Random movement was also inhibited almost in parallel with the decrease of the directed migration. On the other hand, dansyl chloride produced 50% inhibition of chemotaxis at 50 µM but did not exhibit any appreciable inhibitory effects on random movement until 75 µM. As can be deduced from the rapidity of the reaction of fluorescamine[6], the inhibition of random and directed movements by fluorescamine was completed within 30 sec (figure 2). As for dansyl chloride, the inhibition of chemotaxis was completed within 2 min, whereas the random movement (contrary to that seen with fluorescamine) was not appreciably inhibited until at least 5 min. The difference in the inhibitory effects shown by 2 reagents would depend on differences in the penetrating rate and specificity of the reagents, i.e. fluorescamine seems to penetrate the cell very rapidly and label nonspecifically functional groups concerned not only with chemotaxis but also with random movement, whereas dansyl chloride does not seem to modify functional groups associated with random movement, at least in a short modification time. We then studied the effect of dansylation on some functions other than motility. As can be seen in table 2, modified PMNs retained the same ingestive ability as unmodified PMNs. There were no remarkable differences found in the initial rate of the stimulated oxygen consumption during phagocytosis between modified and unmodified PMNs, although the rate was inhibited to a certain degree by dansylation. Lactate production, one of the glycolytic parameters, was inhibited to 90–75% of the control by a chemical modification, suggesting that the aerobic glycolytic process is not so much affected as the chemotaxis. Comparison of the degree of inhibition of the above parameters would indicate that dansyl chloride modified preferentially directed movement rather than the basic contractile, phagocytic and glycolytic processes.

As our attractant also had chemokinetic activity, preliminary studies were made of the effect of dansylation on the chemokinetic response. Chemokinesis of neutrophils was inhibited by dansylation in a parallel fashion to the inhibition of directed movement. At this stage, therefore, it seems possible that chemokinetic inhibition is at least one factor involved in the inhibition of directed movement by dansylation. Further detailed studies are in progress using serum albumin and formyl-methionyl peptide.

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Acid-base equilibrium in the blood of sheep

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Summary. The acid-base equilibrium in the blood of sheep is different from that of human blood mainly because of a lower concentration of 2,3-DPG. A nomogram relating pH, pCO_2, total CO_2 content and base excess has been developed.

Recently, we described the oxygen affinity pattern in the blood of sheep[3], which was found to be quite different from that of human blood. Some differences between the 2 species should be expected as regards the acid-base status also, mainly because of different concentrations of 2,3-diphosphoglyceric acid (2,3-DPG), which is known to be lacking in sheep[4]. The aim of this work is to investigate the in vitro relationship between pH, pCO_2, total CO_2 content and base excess (BE) and to provide a tool for computing the acid-base equilibrium in the blood of sheep.