5 min, the samples were cooled at the same rate as heating and the OD changes continuously monitored.

Results and discussion. Significant age-dependent alterations in the thermal profiles of ribosomes were observed. As shown in the figure and the table there is an 8 °C decrease in the mean temperature of thermal denaturation between ribosomes extracted from young versus old flies. There is also a marked and reproducible inability of the ribosomes from older animals to reassociate upon cooling. Thermal analysis of ribosomes co-extracted from young and old animals exhibit melting and cooling profiles which one would expect based on the arithmetic mean of young and old samples alone (data not shown). The observed alterations are therefore apparently not an artifact of the extraction procedures nor due to age-related increases in ribonuclease or protease activities but rather must reflect an age-dependent alteration in the structural integrity of the rRNA-r protein complex. Ribosomal proteins have been reported to turn over with a half-life of approximately 10 days in adult *Drosophila*[23]. Thus, the observed changes could reflect altered synthetic processes in either ribosomal proteins or presumably rRNA as both synthetic processes are apparently closely coordinated within eukaryotic cells[24,25].

Wallach and Gershon[18] reported a similar decrease in the Tm's of ribosomal monomers from aging nematodes. These authors also observed a 5-6 °C earlier commencement of the hyperchromic effect in melts of ribosomes from older animals which compares well with our results.

These results collaborate and extend earlier studies on ribosomal monomers from young and old *Drosophila* which indicated a loss in the structural integrity of the rRNA-r protein complex with advancing age. The age-dependent alterations in the structural stability of ribosomes are apparently not due to changes in the ribosomal proteins as analysis by 2-dimensional polyacrylamide gel electrophoresis of the total ribosomal proteins from young and old flies revealed no major quantitative or qualitative differences[26]. It is therefore suspected that the primary lesion(s) responsible for the altered physicochemical stability of the ribosomal monomers resides within the rRNA. Indeed, preliminary analysis of the melting profiles of the rRNA from young and old animals supports this hypothesis[27]. In conclusion, it is suggested that the observed loss of structural integrity within the ribosomal complex may contribute to the diminished capacity for net protein synthesis reported for this species[9] with advancing age.

Thermal analysis of ribosomes from young and old male *Drosophila*

	4-day-old	30-day-old
Purity OD_{260}/OD_{280}	1.88±0.06 (N=15)	1.87±0.08 (N=18)
Mean temperature of thermal denaturation	55.17±2.60 (N=9)	47.45±3.20 (N=6)**
%Δ Absolute hyperchromicity	15.21±2.40 (N=7)	15.44±2.30 (N=6)
% Renaturation	99.00±2.50 (N=7)	79.30±9.00 (N=5)*

Mean ±SD (N=sample size); probability: Student's t-test; *$p < 0.01$, **$p < 0.001$.

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Dependence on the lipophilicity of maleimide derivatives in their inhibitory action upon chemotaxis in neutrophils[1]

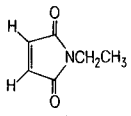
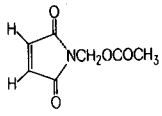
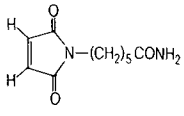
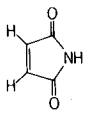
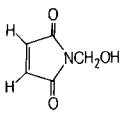
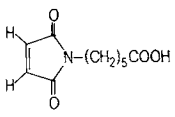
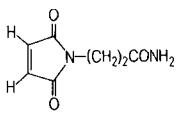
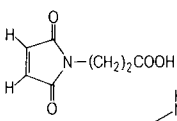
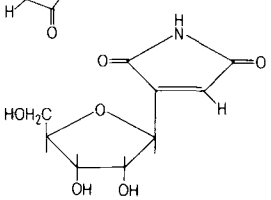
T. Yamashita, Y. Tanaka, T. Horigome and E. Fujikawa

Laboratory of Physiological Chemistry, School of Medicine, Juntendo University, Hongo, Bunkyo-ku, Tokyo 113 (Japan), 24 October 1978

Summary. Modification of polymorphonuclear neutrophils by a series of maleimide derivatives with various degrees of lipophilicity and hydrophilicity indicated that hydrophilic reagents had little effect on chemotaxis, whereas the degree of the inhibitory effect of lipophilic reagents on the chemotaxis was parallel to the degree of their lipophilicity.

Parachloromercuribenzoate (PCMB) can penetrate the cell membrane while parachloromercuribenzenesulfonate (PCMBS) may penetrate the membrane at a much slower rate than PCMB due to its marked hydrophilicity, reacting only with sulfhydryl groups on the outer surface of the lipid membrane of red blood cells[2,3]. N-ethylmaleimide (NEM) is also said to be a rapidly penetrating sulfhydryl reagent although it is very water-soluble. Recent observations on the effect of NEM and PCMBS on the functions of human polymorphonuclear neutrophils (PMNs)[4] were of interest

Table 1. Chemical structures and physical properties of maleimide derivatives

Compounds		Mol. wt	ε	λ_{max}	K
	NEM N-Ethylmaleimide	125.1	620	300	69
	NAMM N-Acetoxymethylmaleimide	169.1	384	277	38
	NCAPM N-(5-Carbamido-1-pentyl)maleimide	210.2	588	302	1.8
	Maleimide	97.1	550	277	0.43
	NMM N-Methylolmaleimide	127.1	488	283	0.21
	NCPM N-(5-Carboxy-1-pentyl)-maleimide	211.2	584	302	0.09
	NCAEM N-(2-Carbamidoethyl)-maleimide	168.2	533	297	0.05
	NCEM N-(2-Carboxyethyl)-maleimide	169.1	543	297	0.01
	Showdomycin 2-(β-D-Ribofuranosyl)-maleimide	229.2	1.01×10^4	220	<0.01

to us because we have studied the role of sulfhydryl groups in muscular contraction[5,6], to which the locomotion of PMNs is thought to be effectuated by an analogous mechanism. Therefore, to stain separately sulfhydryl groups concerned with neutrophil functions, if possible, we synthesized a series of maleimide derivatives with various degrees of hydrophilicity and lipophilicity, and therefore probably with different penetrating rates, and examined the effects of these derivatives on PMN migration as reported in this preliminary communication.

Materials and methods. Maleimide and NEM were purchased from Nakarai Chemicals Ltd and Wako Pure Chemical Industries Ltd respectively. Showdomycin was donated by the Shionogi Research Laboratory[7]. Other maleimide derivatives were synthesized[8-11]. The lipophilicity of sulfhydryl reagents was examined by measuring the partitioning of them between chloroform and water layers. Approximately 10 mM reagent was dissolved in 0.1 M phosphate buffer (pH 7.0) and an equal volume of chloroform was added to the reagent solution and the mixture shaken vigorously to reach equilibrium at room temperature (25 °C). After centrifugation, an aliquot of the chloroform layer was evaporated and then a 6-fold volume of 0.1 M phosphate buffer was added. The water layer was diluted 6 times with the phosphate buffer. The amount of the sulfhydryl reagent in the 2 phases was determined spectrophotometrically by using the molar extinction coefficients (ε) at the wavelength with the maximum absorption (λ_{max}), see table 1. The partition coefficient (K) is expressed as follows: the concentration of sulfhydryl reagent in the chloroform layer/the concentration of sulfhydryl reagent in the water layer. PMNs were obtained from glycogen-induced peritoneal exudates of guinea-pigs. The viability of PMNs was checked by the trypan blue exclusion test. Chemical modification of PMNs was carried out by incubating 2×10^7 cells/ml with maleimide derivatives at 0 °C for 5 and 60 min. The reaction was stopped by addition of a 2-fold molar excess of cysteine. Then the reaction mixture was diluted 4 times with PBS for use for the motility assays. PMN migration was assayed in a modified Boyden chamber[12] using a Sartorius membranefilter (pore size 3 µm) in the presence (chemotaxis) and absence (random movement) of a chemoattractant (the supernatant of sonicated suspensions of *Escherichia coli*). Chemotactic and random

Table 2. Effects of maleimide derivatives on chemotaxis of polymorphonuclear neutrophils

Compounds	Modification time (min)	Chemotaxis of neutrophils (% of control) modified at 1 mM	100 µM	50 µM	Viability (%)
NEM	5	4±1 (31±8)	11±3 (37±8)	29±9 (61±2)	-
	60	2±1 (25±3)	5±1 (26±2)	12±3 (32±7)	94–97
NAMM	5	6±3 (38±8)	16±2 (65±4)	43±9 (74±7)	-
	60	3±2 (20±2)	7±2 (32±7)	23±8 (42±3)	93–97
NCAPM	5	94±8 (91±8)	97±5 (91±9)		-
	60	34±9 (92±6)	89±7 (92±7)		94–96
Maleimide	5	8±2 (37±5)	31±8 (78±4)	73±9 (75±9)	-
	60	4±3 (26±7)	9±2 (42±6)	24±7 (65±9)	93–98
NMM	5	23±9 (61±9)	94±7 (88±9)	99±2 (88±9)	-
	60	8±4 (27±8)	42±9 (58±9)	90±9 (87±2)	95–98
NCPM	5	92±4 (89±6)	98±1 (96±4)		-
	60	89±9 (91±9)	94±6 (98±3)		97–98
NCAEM	5	86±9 (99±3)	95±7 (95±7)		-
	60	94±9 (92±8)	96±5 (96±3)		96–99
NCEM	5	90±1 (90±3)	97±5 (94±9)		-
	60	86±5 (95±6)	99±2 (99±2)		96–98
Showdomycin	5	80±8 (84±8)	92±1 (98±4)		-
	60	82±4 (97±4)	91±3 (98±3)		95–98

Chemotaxis values indicate the mean±SD of 4–5 experiments and figures in parentheses represent the random movement. Positive controls (not modified and containing a chemoattractant at the bottom of the chamber) migrated 69–99 µm, whereas negative controls (not modified and lacking an attractant at the bottom of the chamber) moved 11–18 µm in 60 min at 37°C. Viability is shown on PMNs modified by 1 mM maleimide derivatives.

activity is 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[13].

Results and discussion. Chemical structures and physical properties of maleimide derivatives are shown in table 1. The arrangement of derivatives in the table is in order of decreasing lipophilicity from top to bottom. NEM was found to be the most lipophilic among the reagents examined, whereas showdomycin was the most hydrophilic, which may be due to the hydroxyl group of the ribofuranose moiety. Next, the effects of these maleimide derivatives on the chemotaxis of PMNs were examined. As can be seen in table 2, sulfhydryl reagents whose partition coefficients were 0.09 and below barely affected the PMN migration even during a 60-min modification period at 1 mM, whereas maleimide derivatives with partition coefficients of 0.21 and above had already caused almost complete inhibition in 5 min with the exception of NCAPM that caused no effect on the PMN migration in 5 min but approximately 50% inhibition of the chemotaxis without suppression of the random movement in 60 min. However, at 100 µM, NCAPM was not effective even after 60-min modification. Neither was NMM in 5 min, although more than 60% inhibition was observed on 60-min incubation. Maleimide still showed marked inhibition and NEM and NAMM with a higher lipophilicity caused almost complete chemotactic inhibition on 5-min modification. At 50 µM, NMM became ineffective but the degree of the inhibitory effects of the last 3 derivatives on the chemotaxis ran parallel with increasing lipophilicity of the reagents at least in 5 min. The viability of PMNs was not affected in any case by chemical modification, suggesting that the inhibition of the chemotaxis is not due to death of cells. On the other hand, highly hydrophilic showdomycin and highly lipophilic NEM reacted in an identical fashion with sulfhydryl groups on a molecular basis, e.g. ATPase activities of myosin A, skeletal muscle contractile protein, were similarly modified by both reagents (unpublished results).

The facts, that lipophilic sulfhydryl reagents can react rapidly to inhibit the chemotaxis, while hydrophilic reagents cannot react even at a higher concentration under our conditions, and that the initial rate of chemotactic inhibition (5-min modification) is proportional to the initial concentration of the reagents, moreover the positive interrelation existing between the degree of inhibition of chemotaxis and the degree of lipophilicity of reagents, suggest that sulfhydryl groups whose modification appears to be associated with the inhibition of chemotaxis, are not located on the outer surface of the plasma membrane but within the membrane or cytoplasm, so that the diffusion of the reagents into the cell is rate-limiting. The result that NCAPM blocks the chemotactic response without interference with the basic mechanism of locomotion is of interest because it suggests that separate modification of the cell functions would be possible by the choice of a reagent with an appropriate penetrating rate, i.e. with suitable lipophilicity. Studies on the subcellular sites of the action of reagents remain to be carried out, although effects of these reagents on other functions of neutrophils are now being examined.

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