Thus, since, as this study showed, evoked potentials in both LGBs were suppressed during saccades insignificantly as compared to their suppression on gaze holding (which agrees with the reported findings), it may be stated with reasonable confidence that the LGB contralateral to the direction of SEM is virtually not implicated in visual suppression when the background is homogeneous as well as in darkness. Our findings provide first-time evidence that the ipsilateral LGB can convey extraretinal influences eliciting visual suppression.

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"Medium Molecules" as Nonspecific Regulators of Phagocytic Activity

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Three identical oligopeptide-containing fractions of so-called "medium molecules," isolated by sequential ultrafiltration and gel chromatography from the blood of 8 intact dogs and 15 dogs with an extensive thermal burn, were examined for their impact on phagocytic cells (neutrophil granulocytes and macrophages) in relation to the molecular-weight distribution of the molecules. The relatively high-polymer fractions of medium molecules, unlike oligomeric fractions, stimulated the phagocytic activity of these cells. Because of their increased polymerism, the fractions of medium molecules from dogs with thermal trauma stimulated phagocytosis to a greater extent than did those from intact animals.

Key Words: *medium molecules; molecular-weight distribution; phagocytic cells*

The early 1920s saw the discovery of a nonspecific phenomenon of accumulation of heterogeneous oligopeptides in body fluids in disease states [10]. Subsequently, these compounds became known under the general name of "medium molecules" (300-5000 D), whose elevated levels in the systemic circulation came to be regarded as a major factor in the development of a universal endogenous intoxication syndrome [6]. This view was based on the observed nonselective membrane-damaging effect of medium molecules (MM) [6]; one of the purported manifestations of this effect

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is MM-induced dysfunction of the immunobiological surveillance system, and in particular dysfunction of phagocytic cells [13,14]. It has been shown that the quality of biological effects produced by MM is determined by their elution characteristics on gel chromatography, i.e., by their molecular-weight distribution [5], and that changes in this distribution are associated with a transformation of MM with "toxic" properties into molecules with antioxidant, antinociceptive, and stress-protecting actions [2-5]. Previously, associations of this kind with special reference to the impact of MM on phagocytic cells had not been addressed, and this gap in knowledge prompted the present study.

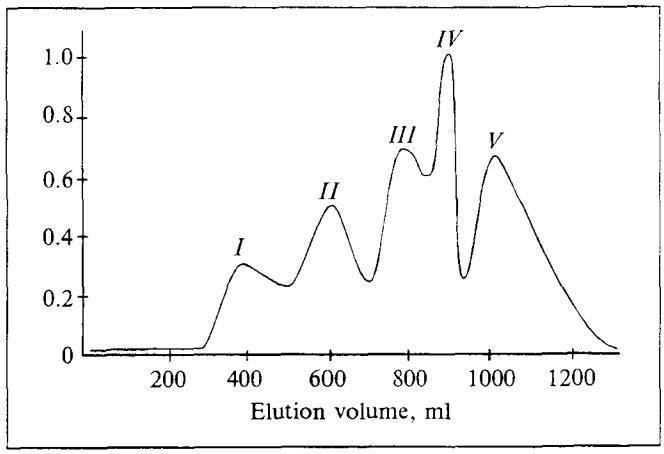

Fig. 1. Elution profile of MM from dog blood. Ordinate: optical density of the eluate at 206 nm. *I–V*: *MM* fractions in order of recovery from the column.

MATERIALS AND METHODS

MM were isolated from the blood of 23 dogs by sequential ultrafiltration on UAM-500M, UAM-150M, and UAM-50M Vladipor membranes and Sephadex G-15 chromatography [2-5]. In order to effect a stress-induced shift in the molecular-weight distribution of oligopeptides such that high-polymer forms would predominate [11], a thermal burn of degree IIIA-IIIB involving 20-25% of the body surface was produced 24 h before blood sampling in 15 of the 23 dogs under thiopental sodium anesthesia (30-40 mg/kg). Substances of peptide nature in the MM fractions were determined quantitatively by a microbiuret method and also using Folin's reagent [2]. The fractions were numbered in the order of their elution.

The isolated MM were assayed for activity on neutrophil granulocytes and macrophages by recording changes in spontaneous chemiluminescence, in the color reaction with nitro blue tetrazolium (NBT test), and in the phagocytosis of monodisperse polystyrene latex [8,9]. Sources of phagocytic cells were a pooled karyocyte suspension from peritoneal flushes of 14 random-bred mice and a leukocyte suspension from the blood of 8 healthy donors. To each of these suspensions, which contained 5×10^6 karyocytes/ml, MM were added in an amount equivalent to their content per ml of dog blood (a dose designated "absolute dose 1"). In tests with spontaneous chemiluminescence, the proportion of MM in the leukocyte suspension was five times that in all other *in vitro* tests.

The modulating effect of MM on the local inflammatory response was studied using a murine model of formalin-induced edema [1]. MM were administered to mice in a dose equal to 10 absolute doses 30 min prior to edema induction.

For statistical treatment of the results, Student's *t* test, Wilcoxon-Mann-Whitney's *U* test, Wilcoxon's paired *T* test, and the sign test were used.

RESULTS

From the blood of each intact and each burnt dog five MM fractions with identical elution profiles were obtained (Fig. 1). The shape of the profiles coincided virtually completely with the configura-

TABLE 1. Effect of MM on Latex Phagocytosis by Human Blood Neutrophils from Healthy Donors ($n=8$) *in Vitro*

Parameter of phagocytosis	Control (0.9% NaCl)	Fraction					
		2:normal	2:burn	3:normal	3:burn	4:normal	4:burn
Activity:	75.75	75.13	83.25*°°	74.50	76.38	74.38	78.13°
% of phagocytic cells	(67–82)	(70–84)	(77–95)	(67–85)	(66–83)	(66–82)	(72–81)
% of control		99.2	109.9	98.4	100.8	98.2	103.1
Intensity:	596.75	613.75	767.88**°°°	656.00**	591.88	556.63**	650.00**°°
№ of phagocytized latex particles per 100 cells	(440–776)	(474–896)	(545–1035)	(363–880)	(408–758)	(424–712)	(468–816)
% of control		102.9	128.7	109.9	99.2	93.3	108.9

Note. Significant differences by the sign test and Wilcoxon's paired test: *$p<0.05$, **$p=0.05$ in comparison with control; °$p<0.05$, °°$p=0.01$, °°°$p=0.05$ in comparison with normal values.

TABLE 2. Effects of MM from the Blood of Burnt Dogs on Latex Phagocytosis and on NBT Test Results Using Murine Peritoneal Macrophages ($M \pm m$)

Fraction	NBT test				Phagocytosis	
	spontaneous		latex-induced			
	activity	intensity	activity	intensity	activity	intensity
Control (medium 199)	36.00±2.69	0.46±0.04	33.63±1.88	0.45±0.03	50.57±2.82	256.81±19.9
2:burn	32.50±1.82 (90.3)	0.41±0.04 (89.7)	33.31±1.58 (99.1)	0.42±0.03 (94.2)	54.76±3.96 (108.3)	266.12±25.54 (103.6)
3:burn	65.00±2.45*** (180.6)	0.80±0.04*** (176.1)	60.38±2.04*** (179.5)	0.73±0.03*** (164.7)	54.87±3.99 (108.5)	249.81±32.11 (97.3)
4:burn	30.25±2.60 (84.0)	0.38±0.03 (82.9)	27.25±2.31* (81.0)	0.35±0.03* (78.2)	42.70±5.26 (84.4)	155.63±20.25** (60.6)

Note. A pooled suspension of karyocytes obtained from peritoneal flushes from 14 mice was used. MM fractions were diluted in medium 199. Each value is the mean of eight replicate determinations. Figures in parentheses are percentages of control values. Significant differences from control by Student's and Wilcoxon–Mann–Whitney's tests: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

tion of MM elution curves recorded by other authorities [7,12] using Sephadex G-15 for the isolation of these molecules. In view of this, fractions 1 and 5 were excluded from further analysis because they were known to be contaminated with protein and low-molecular nitrogenous waste [7,12]. In the remaining fractions (2, 3, and 4), the molecular weights of their components decreased from 1355 to 630 D with an increase in elution volume [12]. As found in our previous study [2], relatively high-polymer MM fractions such as 2 and 3 have the largest content of biuret-positive substances, while the level of Folin-positive substances is highest in oligomeric fractions. Therefore, the ratio between biuret-positive and Folin-positive substances well reflects the molecular-weight distribution of MM. The thermal burn led to an increase in this ratio in all the fractions tested [2], which indicates that the fractions had undergone redistribution resulting in the predominance of relatively high-polymer forms. As shown in Table 1, such a redistribution incurred a qualitative change in the influence exerted by the medium-molecular fractions on the phagocytic reaction of neutrophil granulocytes. The highest phagocytosis-stimulating activity was exhibited by the high-polymer fraction of MM from the blood of burnt dogs (fraction 2:burn in Table 1), while fraction 4, which reduced the intensity of phagocytosis if derived from normal (intact) dogs (fraction 4:normal in Table 1), produced a strongly marked opposite effect if obtained from burnt dogs 24 h after the burn (fraction 4:burn). In sharp contrast, fraction 3 stimulated phagocytosis when obtained from intact dogs but failed to do so after the burn. On the other hand, fraction 3:burn was much more potent than fraction 3:normal in stimulating the production of biooxidants by phagocytic cells. Thus, the magnitude of spontaneous chemiluminescence was 1.30 (range O.14-3.64)$\times 10^3$ cpm in the control tests, 2.14 (0.15-7.02)$\times 10^3$ cpm in the presence of fraction 3:normal ($p>0.05$), and 4.49 (0.40-17.92)$\times 10^3$ cpm in the presence of fraction 3:burn ($p=0.01$). In addition, fraction 3:burn increased the parameter values of the NBT test with peritoneal macrophages (Table 2). It is important to note that, as indicated by the parameters of the NBT test, fraction 2:normal and fraction 3:normal also significantly stimulated oxygen metabolism in resting neutrophil granulocytes, raising the proportion of diformazan-positive cells from 29.38 (range 14-55)% (control tests) to 37.88 (22-56)% ($p=0.05$) and 36.25 (21-52)% ($p<0.05$), respectively.

Thus, as the results presented above indicate, relatively high-polymer (rapidly eluted) MM fractions (fractions 2 and 3) have beneficial effects on the functions of neutrophil granulocytes and macrophages, whereas oligomeric fractions such as fraction 4 produce the opposite effects on these phagocytic cells. As can be seen in Tables 1 and 2, although fraction 4:burn stimulated the phagocytic activity of granulocytes, MM of this category lowered the parameter values of the NBT test with latex-stimulated macrophages and reduced the intensity of phagocytosis by these cells. The macrophage-inhibiting effect of fraction 4:burn appears to exceed its granulocyte-stimulating effect, given that after an intravenous injection of corresponding MM the index of formalin-induced edema was only 50.57±3.11% ($n=20$) *vs.* 56.4±1.74% in the control tests ($n=44$; $p<$O.O5 by the U test).

The results of this study make it possible to regard MM as nonspecific modulators of the activity of phagocytic cells.

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