to clarify which components of complement are crucial for Gr binding to nephritic glomeruli.

Materials and methods. Glomerulonephritis was induced by prolonged administration of bovine serum albumin to primed rats as described previously[4]. Renal sections of 4 micron thickness were made with a cryostat and placed on glass slides. The purified early components of human complement were purchased from Cordis Laboratories (Florida, USA). Each component was diluted with veronal buffered saline containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (VBS) at the concentrations listed in the table and incubated with the renal tissue at 37 °C for 30 min. Then the sections were washed in VBS with three changes of bath. Alternatively, the sections were incubated with each complement component at 37 °C for 30 min each in sequence as depicted in the table. Granulocytes were collected from the peritoneal cavity of a rat given 20 ml of 0.1% glycogen 4 h earlier and were suspended in VBS at a density of 3×10^7/ml. This Gr suspension was poured into the space between two glass slides held 0.2 mm apart, one on top of the other, by placing strips of electric insulating tape across the lower slide containing the renal section[3]. These glass slides were placed in a moist chamber and kept at 37 °C for 40 min, then washed in cold PBS. The sections were fixed with ethanol and stained with hematoxylin and eosin. The Gr adhering to a glomerulus were easily counted when viewed by light microscopy. Over 50 glomeruli of moderate diameter were evaluated.

Number of Gr adhering to glomeruli with immune complex glomerulonephritis

Complement component (U/ml)	Gr No./ Glomerulus	Complement fixation	Gr No./ Glomerulus
Cl (10,000)	3.9 ± 2.1	C14	80.2 ± 11.9
C2 (1000)	5.2 ± 4.0	C142	67.4 ± 15.1
C3 (1000)	29.0 ± 8.5	C1423	88.4 ± 12.4
C4 (1000)	23.3 ± 7.7	C23	39.7 ± 5.8
VBS control	4.8 ± 1.7	Fresh serum	108.4 ± 20.3

Cryostat sections of rat kidney with immune complex glomerulonephritis were pre-treated with a single or several components of human complement in sequence and incubated with rat granulocytes. The granulocytes adhering to a glomerulus were counted by light microscopy.

Results. In kidneys from glomerulonephritic rats, Gr adhered exclusively to nephritic glomeruli bearing immune complexes as described previously[5]. Significant increases in the number of Gr on the glomerulus were observed in sections treated with human C3 or C4, although a small number of Gr adhered to the VBS-treated glomerulus, as shown in the table. C3- and C4-treatment produced almost identical results. Gr adherence increased most significantly on sections incubated with C1 and C4 or C1, C4 and C2 or C1, C4, C2 and C3 in sequence. Moreover, the numbers of adherent Gr in the latter groups were almost equal to each other and to those on fresh serum-treated sections.

Discussion. The significant increase in numbers of Gr adhering to C4- or C3-fixed glomeruli should indicate that C1 esterase and C3 activator, respectively, were present in the immune deposits on glomerulonephritic kidneys and could activate human C4 and C3 molecules. The remarkable increase observed on C14-treated glomeruli probably resulted from the recognition of C4b by their C3b–C4b receptor, as reported previously[6]. The present study confirms that Gr recognize the activated complement components on immune complexes in nephritic glomeruli through their complement receptors and adhere to the nephritic glomeruli, which was suspected from our previous data[7].

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Quantitative cytophotometric analyses of mesenteric mast cell granulation in acute soman intoxicated rats[1]

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Summary. Effects of the organophosphate neurotoxin soman on rat mesenteric mast cell granule content were determined using scanning-integrating microdensitometric analysis of individual cell metachromasia. Mast cell degranulation was evidenced both with sublethal (0.5 LD_{50}) and lethal (1.5 LD_{50}) dosages and as early as 3–10 min post-injection. These data indicate a possible contribution of mast cell autacoids in the genesis of organophosphate-induced respiratory and circulatory collapse.
Key words. Mast cell degranulation; organophosphate neurotoxins; rat mesenteric preparations; cholinergic mechanisms.

During the course of routine histopathological analysis of peripheral tissues obtained from rats treated with the organophosphate (OP) neurotoxin soman (pinacolyl methylphosphonofluoridate), mast cell degranulation was noted both in mesenteric preparations and in the stromal compartment of lungs, liver and salivary glands[2]. Although several clinical manifestations of OP intoxication, such as bronchoconstriction with hypersecretion and circulatory collapse, constitute integral aspects of immunologic mast cell activation and anaphylactic shock, the in vivo responsiveness of mast cells during acute OP intoxication remains largely unexplored. The objectives of the current investigation were twofold: 1) to obtain quantitative data relating to mast cell granulation in soman-intoxicated rats; and 2) to investigate dose-response relationships. This entailed use of the metachromatic reaction of mast cell granules with cationic dyes and the recommended cytophotometric assay of Kelly and Bloom[3].

Male Sprague-Dawley X Wistar rats weighing 200–250 g were randomly assigned into soman and saline-control treatment groups. Soman (Analytical Chemistry Branch, Biomedical and Chemical Systems Laboratories, Edgewood, MD) was administered s.c. in physiological saline at dosages of 65, 120 or 195 μg/kg (0.5, 0.9 and 1.5 LD_{50}). Rats were sacrificed by decapitation 30, 60 or 120 min post-injection, with the exception of the

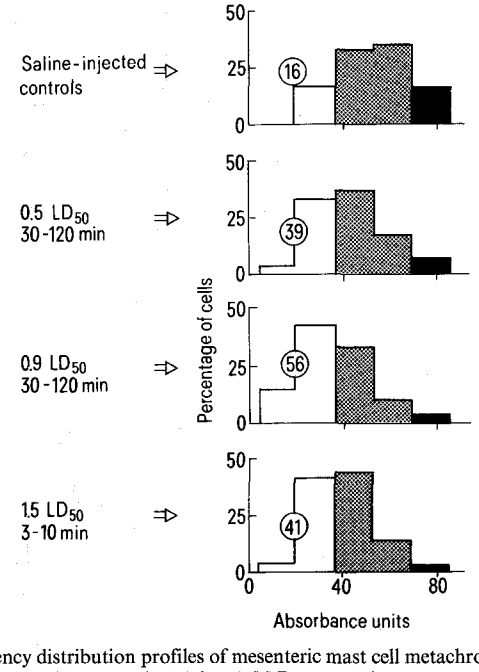
Mesenteric mast cell metachromasia and plasma cholinesterase activity in acute soman treated rats[1]

Treatment	N(n)	Mast cell metachromasia	Plasma cholinesterase
Control	4(180)	54 ± 15^{a}	0.62 ± 0.12^{a}
0.5 LD_{50} soman	16(320)	42 ± 13^{b}	0.17 ± 0.05^{b}
0.9 LD_{50} soman	17(340)	37 ± 14^{c}	$0.08 \pm 0.05^{c}x$
1.5 LD_{50} soman	6(220)	$39 \pm 12^{b,c}$	0.02 ± 0.05^{d}

[1] Soman treatments described in text. Mast cell metachromasia (absorbancy units ± SD) determined using scanning-integrating microdensitometry. Plasma cholinesterase (μmol/ml/min ± SD) determined using an automated colorimetric method with acetylthiocholine as substrate. N, number of animals per treatment group; n, number of individual mast cell measurements. Means with different superscripts are significant ($p < 0.05$), Duncan's new multiple range test. [a-d] Means ranked, the highest value designated (a).

1.5 LD_{50} group which was necropsied upon impending respiratory failure (3–10 min). Mesenteric preparations were made from the proximal jejunum, pre-fixed in Cytoprep (Fisher Scientific) and stained with azure B using the Shea[4] modification of the original Flax and Himes[5] protocol. Cytophotometric analyses of mast cell metachromasia on an individual cell basis were made using a Vickers M85a scanning-integrating microdensitometer at a wavelength setting of 580 nm. Control mesentery preparations treated with DNase and RNase revealed that azure B-nucleic acid binding provided only negligible (< 2%) contribution to total mast cell azure B uptake. Mast cell responses were compared to plasma cholinesterase levels, determined using an automated colorimetric procedure[6].

Individual cytophotometric mast cell determinations are presented in conventional frequency distribution form in the figure. Overall mean density values, and corresponding plasma cholinesterase levels, are summarized in the table. Two-way analysis of variance revealed the absence of significant contribution of the time factor in determining total sum-of-squares variability associated with the mast cell data; thus, data obtained from rats sacrificed at 30, 60 and 120 min were combined for analysis of dose-related effects. From the data it is clear that all three soman dosages significantly reduced mast cell metachromasia (granule content). Mast cell granule depletion was significantly ($p < 0.05$) more severe in rats treated with 0.9 LD_{50} soman than those receiving 0.5 LD_{50}. Rats given 1.5 LD_{50} soman and sacrificed upon impending death (3–10 min) exhibited depressed metachromasia not significantly different from 0.5 or 0.9 LD_{50} treatment groups at 30–120 min. These alterations are evident both in frequency distribution profiles as elevated proportions of lightly metachromatic cells with concomitant reduction in intensely metachromatic categories, and in declines in overall mean values.



Frequency distribution profiles of mesenteric mast cell metachromasia in rats treated with soman (0.5, 0.9 or 1.5 LD_{50}, s.c). Histograms constructed to depict the frequency occurrence of cells exhibiting light (□), moderate (▨) or intense (■) metachromasia, the moderate category representing that portion of the mast cell population where individual densitometric values fall within plus/minus 1 SD of the control mean value. Circled numbers indicate percentages of lightly metachromatic cells.

Quantitative data are presented indicating that the potent OP soman produces prominent degranulation of rat mesenteric mast cells in vivo. Degranulation, based on diminution of contained metachromatic material (heparin), is more severe with near-lethal (0.9 LD_{50}) than sublethal (0.5 LD_{50}) dosages and is present as early as 3–10 min following lethal dosages (1.5 LD_{50}). This appears to be a generalized response since previously we have reported morphological evidence of degranulation of mast cell in stromal compartments of lungs, liver and salivary glands[2]. Extensive lung hemorrhaging, one of the primary manifestations of anaphylaxis in the rat, is commonly present in soman poisoned animals (unpublished observations). These considerations suggest that an anaphylactoid-type response may be an important aspect of OP intoxication, in part mediating bronchoconstriction, bronchosecretion and circulatory collapse.

At present, mechanisms underlying soman-induced mast cell degranulation remain speculative. Toxic effects are generally attributed to acetylcholinesterase inactivation and the resultant cholinergic hyperexcitation[7]. It is now well documented that mast cells contain muscarinic-cholinergic receptors, and low concentrations of acetylcholine (10^{-10} M) or carbamylcholine (10^{-12} M) potentiate the immunologic release of histamine and slow-reacting substance of anaphylaxis from human lung tissue in vitro[8]. Thus, mast cell degranulation may be a direct effect of the cholinergic crisis. However, the possibility that direct toxin actions on the mast cell are involved cannot be dismissed. Nonspecific cell damage produces passive degranulation, and many drugs and toxic agents induce mediator release directly and independent of hypersensitization[9]. It may be noteworthy in this regard that high concentrations (10^{-2}–10^{-4} M) of the related OP diisopropylfluorophosphate (DFP) block mast cell mediator release in vitro[10]. Whether mast cell responses are related to immunosuppressant actions of OP[11] also remains to be ascertained.

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