were compared with the values given in group II (see table). As a result, the uterine glycogen content showed a decrease in response to the conjoint treatment with estradiol dipropionate and progesterone, while an increase in the vaginal glycogen content was observed.

Discussion. It is well established that the glycogen metabolism in normal mammalian tissues is regulated by the changes in glycogen synthase and glycogen phosphorylase activities due to alteration in the phosphorylation state of these two enzymes. Estradiol brings about the conversion of glycogen synthase b to a, so that the synthase a/b ratio is elevated and concomitantly glycogenesis is stimulated[10]. It has been reported that the accumulation of glycogen proceeds at a greater pace than its consumption in uterine tissue[11]. Thus the estrogen-induced glycogen deposition in the uterus is a result of both the effects. However, the estrogen-induced decline in vaginal glycogen may perhaps be due to high glycogen consumption to meet the physiological demand of heavy vaginal keratinization[12]. Progesterone alone caused a decrease in uterine glycogen; this is in agreement with the previous report in the rat[4]. On the other hand, progesterone produced only an insignificant decrease in vaginal glycogen content when compared with the control value. This indirectly strengthens the fact that the content of vaginal glycogen largely depends on its consumption for cellular proliferation in vagina. It is shown in the table, that during combined administration of the two female sex-hormones (estradiol dipropionate and progesterone), progesterone abolished the estrogenic-effect on uterine (increasing-effect) and vaginal (reducing-effect) glycogen content in P-mice. This is similar to a previous observation in albino rats[5]. Ultimately, due to progesterone antagonism to an estradiol ester a reduced level of uterine glycogen, well below the control value, was noticed. The vaginal glycogen content in group IV approached a value equivalent to the control. So, unlike the conditions reported in the albino rat[5], it appears that the antagonizing effect of progesterone is more pronounced in the uterus than in the vagina of P-mice.

Antagonistic effects of estradiol dipropionate and progesterone on uterine and vaginal histology of the mouse have been documented[13]. Similarly, in the present investigation, the progesterone antagonism to an estradiol ester is demonstrated in relation to uterine and vaginal glycogen content of P-mice. Various workers have reported the modulation of estradiol activity by progesterone[14–16]. On the question of estrogen-progesterone interactions in relation to glycogen content, it is more reliable to speculate that the antagonistic action of progesterone on an estradiol receptor[17,18] evokes progesterone antagonism to estradiol dipropionate in uterus and vagina. But the dissimilarity in the degree of antagonism in both the two organs (uterus and vagina) could be attributed to the differences in the rate of absorption and/or retention of sex hormones in the target organs[19].

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Granulocyte adhesion to nephritic glomeruli through recognition of activated C4 and C3 in immune deposits

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Summary. Sections of rat kidney with bovine serum albumin nephritis were incubated either with a single component of complement or with several components in sequence and then reacted with granulocytes. The average number of granulocytes bound to a nephritic glomerulus was elevated in sections incubated with C4 or C3 and increases were most significant when C14, C142 or C1423 were incubated.

Key words. Granulocyte; complement; immune adherence; immune deposit; glomerulonephritis.

Most types of glomerulonephritis in humans are considered to be induced by immune complexes accumulated in glomeruli. Although leukocytic accumulation is also characteristic of glomerulonephritis, the precise mechanism is not clear. Results derived from experiments with the Arthus reaction in vivo and related systems[2] have led to speculation that chemotactic factors play an important role in the accumulation of leukocytes within glomeruli. However, any extrapolation of evidence derived from studies of the Arthus reaction must include awareness of differences between inflammation in glomeruli and skin. The sites of leukocyte accumulation in glomeruli are essentially the capillary lumen and the mesangium, whereas leukocytes infiltrate into the extravascular space of the skin in the Arthus reaction. Moreover, glomerular function is to filter low molecular substances from plasma into the urinary space. This should make it difficult for chemotactic factors to maintain the concentration gradient essential for their activity, because they are usually small in molecular size and are filtered easily through the glomerular basement membrane. However, activation of the complement system on immune complexes in glomeruli can generate these chemotactic factors. We report here the essential role of immune adherence through complement receptors on granulocytes (Gr) in the adhesion of Gr to glomerular immune complexes, according to a Gr binding assay in vitro[3]. The purpose of this study was

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