the precise nature of the polysaccharides remained unclear because of the use of limited experimental methods.

In Rhaphidophycean algal cells, the presence of a glycocalyx was first demonstrated at EM level in the present study. In addition, the histochemical nature of this extracellular structure was made clear for the first time by the use of a wide range of current methods for LM carbohydrate histochemistry. The staining reactions of the glycocalyx imply that the structure contains acidic complex carbohydrates. In line with this, chemical modification of the reactive components clearly indicates that ester sulfate and carboxyl groups are responsible for the above reactions. Digestion experiments with amylase and sialidase show that the glycocalyx contains a neutral carbohydrate-protein complex besides acidic complex carbohydrates. Further, reaction with Con A-PO-DAB-PA-AP-FBK demonstrates the existence of 1,2-glycol and α-*D*-mannosyl and α-D-glucosyl residues[15]. The substrate specificities of hyaluronidases[17,18] and chondroitinases[19] and the staining selectivities of the AB (pH 2.5) reactions indicate that a particular moiety involved in the acidic complex carbohydrates of the glycocalyx may be hyaluronic acid or at least a closely related substance.

Demonstration of functional roles for complex carbohydrates involved in the extracellular structure of phytoplankton has long been lacking. However, recent knowledge on the subject in animal and plant cells[4,5,23] suggests that these substances may perform physiologically important functions in plankton. Our present findings may provide a histochemical basis for assigning a valid function for the flagellate glycocalyx.

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Adrenocortical metabolism and plasma corticosterone in soman intoxicated rabbits[1]

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Summary. The organophosphate neurotoxin soman produced impairments in adrenocortical RNA and protein metabolism. Fasciculate and reticular cell RNA and protein contents were suppressed with sublethal to acutely lethal dosages (20, 30 and 40 µg/kg, s.c.) during the acute excitatory phase of intoxication and at 6–8 h post injection. All three dosages produced ca 90% inactivation of plasma cholinesterase. A transient elevation of plasma corticosterone occurred with 20 µg/kg soman whereas there was a protracted increase with 30 µg/kg. Corticosterone was not significantly elevated with 40 µg/kg, but death occurred at 13 ± 4 min. Thus, the magnitude and/or nature of soman-induced metabolic impairments does not appear to prevent adrenal activation.
Key words. Adrenocortical RNA and protein; plasma corticosterone levels; organophosphate-intoxicated rabbits; quantitative cytophotometry.

The role of adrenal glucocorticoids in eliciting adaptive and homeostatic adjustments occurring with diverse forms of stress, including exposure to toxic chemicals, is well documented. It might therefore be expected that organophosphates (OP) cause adrenal activation and enhanced corticoid secretion. This has indeed been shown following the administration of sublethal dosages of OP insecticides[2,3]. However, several OP insecticides have also been shown to impair adrenal steroidogenesis both in vitro and in vivo. Impairments are evident as: 1) reduced endogenous corticoid synthesis with diminished in vitro corticosteroidogenesis in response to ACTH or cAMP stimulation[4]; and 2) reduced in vivo capacity for steroid output in response to subsequent cold stress[2]. Presently, alterations in adrenal metabolism and function occurring with related but more highly toxic OP, generally classified as chemical warfare agents, have not been ascertained.

The current investigation was conducted to determine dose- and time-dependent effects of the potent OP-neurotoxin, soman (pinacolyl methylphosphonofluoridate), on rabbit adrenocortical metabolism and accompanying alterations in plasma corticosterone levels. Dosages used were previously determined to be: sublethal but producing overt toxic symptoms (20 µg/kg); lethal producing delayed death (30 µg/kg); and lethal during the acute excitatory period (40 µg/kg)[5]. Quantitative azure B-RNA and mercuric bromophenol blue-protein cytophotometry was used to monitor responses of individual fasciculate and reticular

Table 1. Adrenocortical cellular RNA and protein in soman intoxicated rabbits

Treatment group	N	Cellular RNA Z. fasciculata	Z. reticularis	Cellular protein Z. fasciculata	Z. reticularis
Control	7	72 ± 6^{a}	76 ± 7^{a}	83 ± 8^{a}	97 ± 8^{a}
20 µg/kg Acute	6	66 ± 5^{b}	74 ± 6^{a}	66 ± 8^{b}	86 ± 8^{b}
Subacute	7	66 ± 7^{b}	$66 \pm 6^{b,c}$	67 ± 8^{b}	84 ± 7^{b}
30 µg/kg Acute	5	67 ± 5^{b}	71 ± 6^{b}	60 ± 10^{c}	70 ± 9^{c}
Subacute	6	64 ± 5^{b}	65 ± 6^{c}	59 ± 9^{c}	64 ± 7^{d}
40 µg/kg	11	63 ± 4^{b}	$69 \pm 4^{b,c}$	57 ± 10^{c}	72 ± 10^{c}

Azure B-RNA and bromophenol blue-protein contents in absorbancy units. N, number of rabbits per treatment group. Each value represents mean ± SEM with 20 microdensitometric determinations per cortical zone per animal. Means with different superscripts are significant, $p < 0.05$, Duncan's new multiple range test. [a–d] Values ranked ([a] highest value).

cells. Plasma cholinesterase activity was also measured, since inactivation of cholinesterases is generally acknowledged to be the primary biochemical lesion of OP intoxication.

New Zealand White rabbits (2.5–3.6 kg) were randomly assigned to soman and saline-control treatment groups. Soman (Analytical Chemistry Branch, Biomedical and Chemical Systems Laboratories, Edgewood, MD, USA) was administered s.c. in physiological saline at a final volume of 0.1 ml/kg. Representative animals of 20 and 30 µg/kg groups and controls were sacrificed by cervical dislocation 60 min post-injection; the others were killed upon impending respiratory collapse (6–8 h) or at 8 h. Rabbits given 40 µg/kg were also sacrificed upon impending death, which occurred at 5–20 (13 ± 4) min. Immediately upon sacrifice, blood was collected in EDTA-vacutainer tubes and the right adrenal removed, sliced transversely, and fixed in 10% neutral-buffered formalin (24 h at 4 °C). Plasma corticosterone assays were performed in duplicate using the methodology of Gross et al.[6] and antibody raised in female sheep against corticosterone-21-hemisuccinate-BSA (Steraloids, Inc, NH, USA). Plasma cholinesterase was assayed using the automated colorimetric procedure of Groff et al.[7] with acetylthiocholine iodide as substrate. Procedures used in cytophotometric RNA and protein analyses were as described previously[8,9]. Briefly, 8-µm paraffin sections were stained with azure B-RNA[10] or mercuric bromophenol blue-protein[11]. Specificity of RNA staining was confirmed using control sections treated with DNase with and without RNase prior to azure B-RNA or Feulgen-DNA staining. Cytophotometric analyses of adrenocortical cell RNA and protein contents were made using a Vickers M85a scanning-integrating microdensitometer. Total (nuclear plus cytoplasmic) cellular measurements were made on randomly selected cells of the mid-fasciculate and mid-reticular zones using a field-delimiting mask (113 μm^2) approximating the size of one cell. Measurements were made at a magnification of × 1250 at the absorption maximum of RNA-azure B (580 nm) or protein-bromophenol blue (610 nm) dye complexes. The general protocol entailed making 20 individual measures in each cortical zone on sections prepared from each adrenal. All data were examined using analysis of variance, and when the ANOVA F-test revealed significant differences between group means, Duncan's new multiple range test.

Data obtained relating to fasciculate and reticular cell RNA and protein contents are summarized in table 1. From these data it is clear that soman produced RNA and protein depletion in both cortical zones with all three dosages. The only RNA value not significantly ($p > 0.05$) lower than respective control means was that of the reticular zone 60 min post 20 µg/kg soman. There was little difference in the magnitude of RNA depletion among the soman treatment groups, the extent of RNA suppression ranging 7–15%. Adrenocortical cellular protein levels were reduced by a somewhat greater extent (11–34%). Unlike RNA, protein depletion was significantly greater with 30 and 40 µg/kg than with 20 µg/kg.

Corresponding alterations in plasma corticosterone levels and cholinesterase activity are depicted in table 2. The following pattern of alterations in circulating corticosterone levels is apparent. With 20 µg/kg soman, hormone levels were significantly ($p < 0.05$) elevated during the acute phase of intoxication but restored to control values at 8 h post-injection; 30 µg/kg produced further elevations in corticosterone levels during the acute phase which were maintained until death 6–8 h post-soman. With 40 µg/kg, corticosterone was not significantly higher than the control value, but death occurred at 5–20 (13 ± 4) min post-injection. All three dosages were associated with marked (circa 90%) inactivation of plasma cholinesterase. The absence of any differences in plasma enzyme activity among acute 20, 30 or 40 µg/kg treatment groups may be related to the large dosages of soman employed and the severity of the cholinesterase impairment. In subacute groups (6–8 h post-injection), there was partial restoration of plasma enzyme, values being significantly ($p < 0.05$) higher than in acute treatment groups.

The current results demonstrate that the potent OP-neurotoxin soman produces impairments in adrenal metabolism, manifested as cellular RNA and protein depletion and present both in fasciculate and reticular cortical zones. Since the predominant form of RNA is ribosomal, with messenger RNA comprising 1–2% and transfer RNA 10–15% of cellular RNA[12], it is generally acknowledged that alterations in total RNA stem from an increase or diminution of ribosomal RNA and thus closely parallel cellular protein synthetic capacity. It has been amply documented that RNA and protein levels provide sensitive markers for differentiating metabolically active cells from those with diminished function in brain[8], liver[13], and in steroidogenic tissues such as in the ovary[9] and adrenal[14]. It is also known that RNA and protein accumulation is closely linked to an active proliferation of endoplasmic reticulum occurring in response to increased demand for protein synthesis[15]. Conversely, RNA loss and proteolysis are among the earliest events associated with incipient cellular injury[13]. Thus, these responses occurring within adrenocortical cells of soman-intoxicated rabbits are probably related to a diminution in proteo-synthetic capacity and may signify toxin-induced cellular injury.

Despite this adrenocortical RNA and protein depletion, the corticosterone data afford little evidence of an impairment in

Table 2. Plasma corticosterone levels and cholinesterase activity in soman toxicated rabbits

Treatment group	N	Corticosterone	Cholinesterase
Control	7	4.9 ± 2.9^{c}	0.65 ± 0.04^{a}
20 µg/kg Acute	6	11.3 ± 5.1^{b}	0.07 ± 0.01^{c}
Subacute	7	3.8 ± 0.7^{c}	0.21 ± 0.07^{b}
30 µg/kg Acute	5	24.6 ± 4.2^{a}	0.05 ± 0.01^{c}
Subacute	6	22.7 ± 7.3^{a}	0.22 ± 0.03^{b}
40 µg/kg	11	$9.0 \pm 1.6^{b,c}$	0.05 ± 0.02^{c}

N, number of rabbits per treatment group. Corticosterone, mean ± SEM in µg/dl. Cholinesterase, mean ± SEM in µmoles/ml/min. Means with different superscripts are significant, $p < 0.05$, Duncan's new multiple range test. [a–c] Values ranked ([a] highest value).

adrenal activation as long as 6–8 h post-injection. The absence of significant elevations in corticosterone with 40 μg/kg soman may be attributable to the early occurrence of death and time required for stimulation of the hypothalamo-pituitary-adrenal axis. It is significant in this regard that the augmentation of plasma corticosterone evidenced with 30 μg/kg is of approximately equal magnitude (about 5-fold) to that occurring with exposure to OP-insecticides or various other forms of stress[2]. Successful adrenal activation in the presence of diminished proteo-synthetic capacity may be a manifestation of the magnitude and/or nature of specific metabolic impairments. Adrenocortical cell RNA and protein depletion was not more severe with 40 μg/kg than with 30 μg/kg (table 1), although the latter produced near-maximal adrenal activation. Both RNA and protein synthesis are required for continued expression of the ACTH-induced secretory response, but severe inhibition of macromolecular synthesis is required to diminish actual corticoid secretion; moreover, the acute steroidogenic response does not appear to require newly synthesized RNA or protein[16,17]. Additional studies are required to determine whether soman-induced impairments in adrenal metabolism translate into diminished corticoid secretion in vivo during later stages of intoxication.

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Inhibition of sarcolemmal Na, K, Mg ATPase from the guinea pig heart is not compatible with a homogeneous population of non-interacting ouabain receptors[1]

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Summary. The inhibition of sarcolemmal Na, K, Mg ATPase from the guinea pig heart by ouabain was evaluated with a coupled enzyme assay. Models of negative cooperativity and of two independent receptors fitted the inhibition data equally well. The analysis was not compatible with a homogeneous population of non-interacting ouabain receptors.
Key words. Na, K, Mg ATPase; ouabain; negative cooperativity; multi-site ouabain binding.

In various preparations exhibiting Na, K, Mg ATPase activity two ouabain binding sites were shown repeatedly[2–6]. However, it has been questioned whether both sites contribute to the inhibition of the Na pump, since in the rat heart only the site with the low affinity correlated with inhibition of ^{86}Rb uptake[6,7]. Recently we described two ouabain binding sites in the intact guinea pig papillary muscle[8]. Occupation of either site with ouabain elicited a positive inotropic effect. The common cause of the inotropic effects was proposed to be the inhibition of the Na pump. It was, therefore, of interest to evaluate whether inhibition of Na, K, Mg ATPase from the guinea pig heart by ouabain would be compatible with different ouabain binding sites of the enzyme.

Materials and methods. Sarcolemmal vesicles were prepared from the hearts of five guinea pigs (circa 150 g) by a procedure similar to that of Jones et al.[9]. After excision the hearts were cooled immediately in a buffer containing 0.75 moles/l KCl and 5 mmoles/l histidine, pH 7.4, 4 °C. After the connective tissue had been removed, the ventricles (circa 3 g) were minced in 10 volumes of the buffer with a Waring Blendor 8011 C at full speed. The disrupted tissue was further homogenized with a Potter Elvehjem S (three times for 10 s). This homogenate was then passed through four layers of gauze. The filtrate was at first centrifuged at 500 × g for 5 min to remove the large fragments, then at 14,000 × g for 20 min. The resulting pellet was resuspended in 80 ml of the above medium, and resedimented at the same speed. The pellet was washed similarly at first in 80 ml of a buffer containing 10 mmoles/l $NaHCO_3$ and 5 mmoles/l histidine, pH 7.4, then in 80 ml of 3 mmoles/l Tris buffer, pH 7.0. Thereafter the contractile proteins were extracted with 0.4 moles/l LiBr in 10 mmoles/l Tris, pH 8.2 at 0 °C for 60 min, maintaining a ratio of 2 mg protein/mmole LiBr. After a threefold wash at 500 g for 20 min the last pellet with the Na, K, Mg ATPase activity (0.21–0.47 μmoles/min/mg protein) was stored in Tris buffer at a concentration of 2.5 mg protein/ml. The yield of sarcolemmal vesicles was 2 mg per g wet wt. of cardiac tissue. Cytochrome-c-oxidase and glucose-6-phosphatase activity of the preparation were 3- and 10-fold lower than in a mitochondrial or microsomal preparation. These enzyme activities indicate low contamination of the sarcolemmal membranes with subcellular organelles. Protein was determined by the method of Lowry et al.[10].

Na, K, Mg ATPase activity was determined by a method similar to the coupled enzyme assay of Schwartz et al.[11]. The reaction medium contained, in a final volume of 2.6 ml, 5.9 mmoles/l KCl, 140 mmoles/l NaCl, 5 mmoles/l $MgCl_2$, 5 mmoles/l NaN_3,