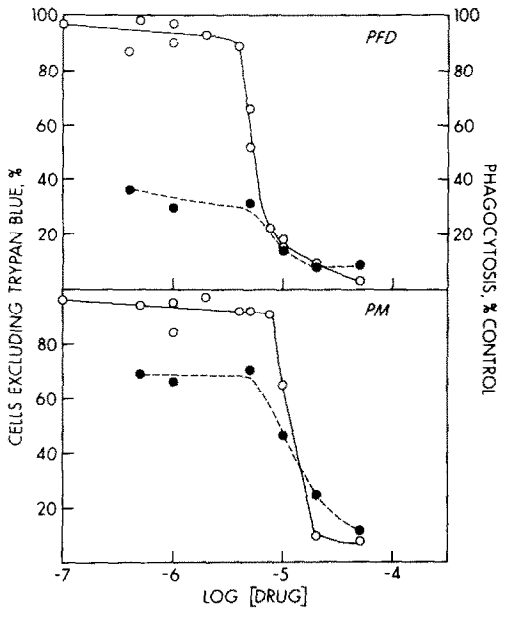

Fig. 1. Effects of pimozide and penfluridol on trypan blue exclusion and erythrophagocytosis by macrophages. Macrophage monolayers were incubated for 2 hr at 37° with various concentrations of penfluridol (PFD) or pimozide (PM). Key: (○) percentage of cells excluding trypan blue; and (●) erythrophagocytosis expressed as a percentage of control. Control is defined as monolayers incubated in the presence of DMSO but without PM or PFD.

cytosis of IgG coated sheep red blood cells was inhibited30% by 1 μM pimozide and 70% by 1 μM penfluridol, but 90% of the cells excluded trypan blue at these concentrations. The binding and endocytosis of α_2M-CH_3NH_2 were unaffected by 10 μM pimozide or 3 μM penfluridol.

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The effect of soman poisoning on phosphorylating capability and adenylate cyclase activity of isolated synaptosomal membranes

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In the preceding works Stitcher *et al.* [1] and Sevaljević *et al.* [2] have reported a significant increase of plasma cAMP in rats poisoned with soman (pinacolyl methylphosphonofluoridate) and discussed the possibility that it arose from an activation of adenylate cyclase via an ACh-induced release of humoral and pharmacologically active substances. This suggested that soman might affect protein phosphorylation which is a process involved in the regulation of various nervous tissue functions, particularly those related to synaptic transmission [3]. Along this line we have examined the effect of soman poisoning on protein kinase and adenylate cyclase activities in isolated synaptosomal membranes.

Male albino rats weighing 300–350 g were poisoned by a subcutaneous administration of 0.75 or 1.3 LD_{50} of soman (corresponding to 0.3–0.5 μmol/kg, respectively) and decapitated at the onset of convulsions. The cerebellum and medula oblongata were removed and the remaining tissue

was processed for isolation of synaptosomes as described by Gray and Whittaker [4]. The purified synaptosomes were lysed in ice-cold redistilled water and material sedimenting at 14,000 *g* was submitted to fraction in a sucrose gradient as described by Whittaker [5]. The 0.8 M sucrose fractions were collected and the membranes pelleted at 15,000 *g* were used for protein kinase assays. The activity of adenylate cyclase was assayed in a crude membrane preparation obtained by sedimentation of purified and lysed synaptosomes at 20,000 *g* [6].

Figure 1 shows that incorporation of radioactive phosphate into membrane proteins of both the control and poisoned rats increased with the concentration of proteins in the reaction mixture up to 400 μg of proteins when a plateau was reached. Within the 50–800 μg range of protein concentrations the phosphorylating capability of membrane from poisoned rats was higher than that of the control. Although the difference increased with the concentration of proteins, neither of the registered increases was statistically significant ($P = 0.40$–0.20). Nevertheless, the labelling of all protein fractions and particularly of the 60 kd protein was heavier in the samples from poisoned than in those from control rats (Fig. 2, lane 2). An examination of

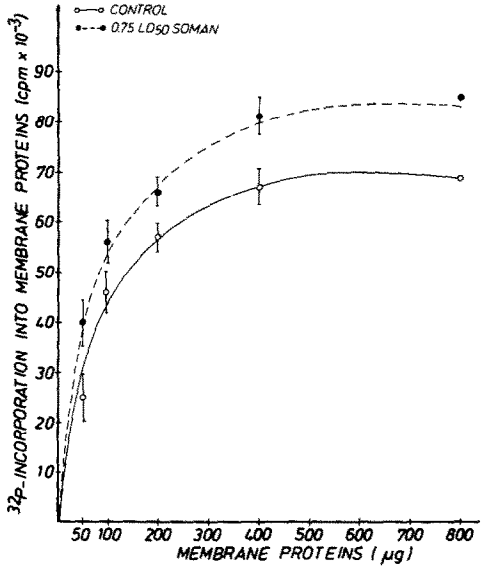

Fig. 1. The relationship between the rate of protein phosphorylation by ^{32}P-ATP and the concentration of membrane proteins in the mixture. The activity of protein kinases was assayed in 0.2 ml of 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$ as described by Sieghart *et al.* [7]. The mixture was equilibrated for 60 sec at 30° and then incubated with 5 μM of ^{32}P-ATP (NEN, lot 1300-294, 1 Ci/mmole) for 15 sec. The reaction was stopped by addition of ice-cold TCA to a final concentration of 10%. The precipitate was collected on Sartorius membrane filters and the radioactivity of the proteins was monitored in a toluol scintillation cocktail in Packard Scintillation Spectrometer. The presented values are averages ± S.E.

Table 1. The effect of poisoning with soman on the activity of adenylate cyclase in isolated synaptosomal membranes

	pmol cAMP/mg protein	
Adenylate cyclase	Control	1.3 LD_{50} soman
(A) Stimulated by incubation of sample at 30° for 5 min	27.1 ± 1.5 (11)	31.8 ± 2.4 (6)
(B) Inhibited by boiling the sample for 3 min at 100°	18.8 ± 2.1 (10)	16.0 ± 1.4 (9)
A–B	8.7 ± 1.4 (11)	15.8 ± 2.1 (6)

The aliquots containing 100 μg of membrane proteins were suspended in 400 μl of 10 mM theophylline, 8 mM $MgSO_4$, 0.6 mM EDTA, 0.02% ascorbic acid, 80 mM Tris–HCl, pH 7.4 and the enzyme was activated by addition of 100 μl of 2 mM ATP and 50 μM GTP at 30°, as described by Sano *et al.* [6]. The reaction was allowed to proceed for 5 min when the enzyme was inactivated by boiling the probe at 100° for 3 min. The mixture was cooled, centrifuged at 3000 *g* and the concentration of cAMP in the supernate was estimated by a protein binding assay [9]. To obtain an exact value for the cAMP produced during the period of incubation, this estimate was corrected for the contribution of preexisting membrane-associated cAMP and the molecules competing with cAMP for binding protein. This value was determined in a parallel 'blank' probe (B) where adenylate cyclase was inhibited prior to incubation of the mixture with ATP and GTP and substracted from total (Šoškic *et al.*, manuscript in preparation). Protein concentration was determined by the method of Lowry *et al.* [10]. The presented values are averages (± S.E.) calculated from the number of determinations indicated in the brackets.

adenylate cyclase activity demonstrated that synaptosomal membranes prepared from poisoned rats were capable of producing 16 pmol of cAMP per mg protein which was a significantly higher ($P = 0.01$) amount than that registered in the controls (Table 1).

These results show that poisoning with soman rendered the synaptosomal membrane-associated adenylate cyclase more active in producing cAMP in an *in vitro* system which probably enhanced the activity of protein kinases and the extent of membrane protein phosphorylation. When integral synaptosomes were tested for protein kinase activity in the presence of ^{32}P-orthophosphate, a slight decrease of the specific radioactivity of total synaptosomal proteins was registered (unpublished data). Also, Grisk *et al.* [11] have found a decrease in the total cAMP content and an inhibition of adenylate cyclase activity in hearts and brains of rats poisoned with diisopropylfluorophosphate (DFP). This suggests that a feature of poisoning with organophosphates, when examined at the level of adenylate cyclase and protein kinase activities in isolated tissues and subcellular structures, might depend on the influence of various factors present in the isolates.

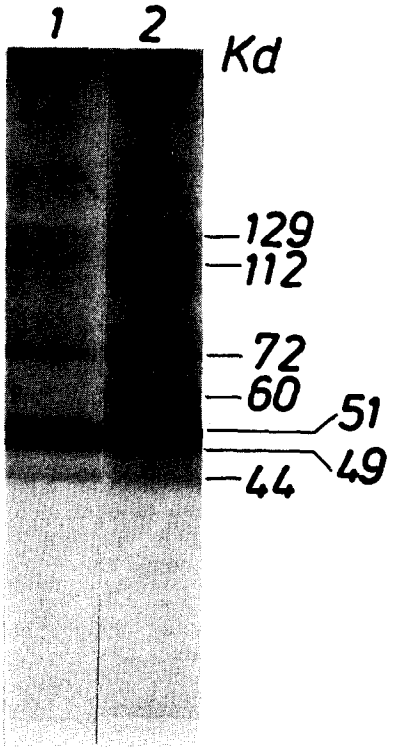

Fig. 2. Autoradiography of ^{32}P-labelled membrane proteins from synaptosomes of control (1) and rats poisoned with 0.75 LD^{50} soman (2). Aliquots containing 80–100 μg of *in vitro* phosphorylated membrane proteins were submitted to electrophoretic separation in the system described by O'Farrell [8]. The gel was dried and exposed to Kodak X-omat film for 4–8 days at −20° in the presence of a screen intensifier.

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