an apparent increase in fluorescence intensity above control level in those perikarya that had remained large.

The effect of axotomy on the still large perikarya could possibly be explained by a stagnation of NA axonal transport under conditions of intact catecholamine synthesis. In the apparently small chromatolytic cells, the catecholamine synthesis may be assumed to be decreased. This view is in accord with a number of qualitative observations[8, 12–14].

The relationship of cell size to ACHE activity remains an open question. Histological studies of autonomic ganglia in the rat revealed that the parasympathetic (i.e. highly cholinergic) perikarya of the ganglion nodosum were on the average larger than the sympathetic (i.e. noradrenergic) perikarya of the superior cervical ganglion[15]. By using the acetylthiocholineiodide method of KARNOVSKY and ROOTS[16], we found in the rat superior cervical ganglion both small and large perikarya to exhibit a highly positive reaction for ACHE activity. In conclusion, our investigation furnished no evidence that the size of the perikaryon determines the total catecholamine content or the apparent ACHE activity of the sympathetic neuron.

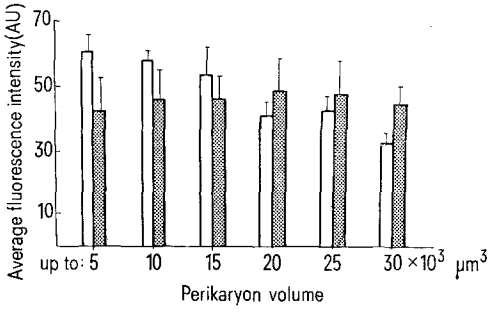

Fig. 2. 1 week after postganglionic axotomy, there is a decrease in NA fluorescence in the chromatolytic perikarya, which appear to be smaller (volume classes up to 5 and $10 \times 10^3 \mu m^3$). The large perikarya exhibit an increase in average NA fluorescence (volume class up to $30 \times 10^3 \mu m^3$).

□, control ganglia; ▨, ganglia 1 week after postganglionic nerve dissection.

Zusammenfassung. Die mikrofluorimetrische Messung der spezifischen Katecholaminfluoreszenz von sympathischen Neuren im Ganglion cervicale superius der Ratte ergab eine reziproke Beziehung zwischen apparentem Noradrenalingehalt und Perikaryonvolumen. Der totale Katecholamingehalt der Neuren sowie die qualitativ erfasste Azetylcholinesteraseaktivität erschienen von der Zellgrösse unabhängig. Diese Beziehung war nach postganglionärer Axotomie verändert.

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Recovery of Cholinesterase Activity in the Cerebrospinal Fluid, Brainstem and Plasma of the Unanesthetized Cat After Irreversible Cholinesterase Inhibition

The apparent recovery of cholinesterase (ChE) activity following administration of an irreversible ChE inhibitor, such as Soman (pinacolyl methylphosphonofluoridate), has not been reported for cerebrospinal fluid (CSF). As several studies have dealt with the effect of ChE inhibition on brain-stem centers related to basic functions such as respiration[1, 2], we were curious as to whether ChE activity in CSF might be used as a measure to reflect the level of ChE activity in brainstem. To study this, the time course of recovery of ChE activity in brainstem, CSF, and plasma were compared following Soman intoxication in cats.

Materials and methods. Samples of CSF (1.0 ml) were obtained daily from the cisterna magna of unanesthetized cats previously implanted with cisterna cannulae, as described elsewhere[3]. Blood was simultaneously collected from the brachial vein using a sterile, heparinized syringe. Plasma samples were obtained immediately after withdrawal by spinning the whole blood sample at 1000 *g* for 10 min in a refrigerated centrifuge. The total ChE activity of each sample was then measured using the radiometric method of SIAKOTOS et al.[4] and protein levels obtained using LOWRY's method[5]. These assays were performed on pure CSF while plasma was diluted 1:3 with distilled water and 1:100 for protein determinations. Brainstem samples were obtained by sacrificing other animals with an overdose of sodium pentobarbital and perfusing the brain through the descending aorta with 0.9% NaCl for 3–5 min, or until clear of blood. The brainstem, that tissue, exclusive of cerebellum, lying between the inferior colliculus and C-1, was then removed and washed in ice cold saline. An homogenate was then prepared by dispersing the tissue in a glass homogenizing tube with a teflon pestle in 10 volumes of an ice cold solution consisting of 0.1*M* sodium phosphate buffer at pH 7.8, 0.3*M* NaCl and 1% Lubrol WX (I.C.I. Organics Inc., Stamford, Connecticut). This homogenate was then diluted to 1% with additional volumes of the same buffer. Protein determinations as well as ChE assays were performed with the 1% homogenates.

After the establishment of a stable baseline for 3 days, each animal was pretreated with a peripheral antimuscarinic compound, atropine methyl nitrate (0.5 mg/kg, i.m.). 1 h later, Soman (27 µg/kg; 27 µg/ml), or an equal volume of saline, was injected s.c. in a shaved region between the scapulae. CSF and blood samples were taken and assayed in duplicate as described above. Statistical comparisons were made using the Student's *t*-test statistic.

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Recovery of cholinesterase activity[a] in cerebrospinal fluid, plasma and brainstem in cat as a function time following irreversible inhibition with Soman

	Days										
	Preinhibition				Postinhibition						$T_{1/2}$[c]
	−3	−2	−1	0[b]	1	2	3	4	7	12	
CSF[d]	8.4 (1.2)	7.6 (0.9)	8.8 (0.9)	0.3[f] (0.1)	4.5[f] (1.4)	5.6[f] (0.8)	6.5 (1.6)	6.9 (1.6)	7.4 (0.9)	7.5 (1.2)	0.9
Plasma[d]	0.62 (0.09)	0.54 (0.11)	0.68 (0.16)	0.03[f] (0.01)	0.27[f] (0.03)	0.30[f] (0.04)	0.33[f] (0.09)	0.41 (0.12)	0.42 (0.03)	0.57 (0.13)	2.2
Brainstem[e]			[2.6] [2.9]	[0.5] [0.3]	[0.7] [0.5]	[0.8] [0.6]	[1.2] [1.3]	[1.7] [1.6]	[2.2] [2.4]	— —	4.5

[a] Activity expressed in terms of μmoles of ^{14}C-ACh substrate hydrolyzed/h/mg protein; [b] Values at '0' were obtained 1 h after the administration of Soman. Mean protein levels in CSF and plasma were 0.36 ± 0.08 mg/ml and 51.7 ± 4.9 mg/ml respectively; [c] $T_{1/2}$ indicates time in days required for the recovery of 50% of enzyme activity after inhibition; [d] Values represent the mean and S.E. of ChE activity observed in the CSF and plasma taken concurrently from five animals; [e] Brainstem ChE activity was obtained from 2 animals sacrificed at each time period after inhibition. Both values are given; [f] $p \leq 0.05$ compared to control levels as determined by Students *t*-test.

Results. As can be seen in the Table, after administration of Soman, CSF-ChE fell rapidly from around 8.1 μmoles/h/mg, during the 1st h, to 3.9% of baseline and remained significantly depressed through the first 48 h after intoxication ($p \leq 0.05$). Plasma ChE fell to 5.8% of baseline, or to 0.036 ± 0.019 μmoles/h/mg, and remained below control levels for the next 3 days ($p \leq 0.05$), while brainstem-ChE activity fell to 13.5% of baseline. Calculation of the time required for the apparent recovery of 50% of baseline activity ($T_1/_2$) gave values of 0.9 days for CSF, 2.2 days for plasma; and 4.5 days for brainstem. Statistical comparison of the pre- and postintoxication levels of protein for CSF and for plasma revealed no significant difference.

To determine whether any free Soman was in the samples at the time of assay, 50 μl samples of CSF, plasma, or brain homogenate were mixed with an equal volume of the same substrate obtained from a nonintoxicated control animal; at the same time, other control samples were diluted with 50 μl of saline and measured for ChE activity. No difference was noted between the 2 sets of assays, indicating that no free inhibitor was present.

Discussion. The apparent recovery of total ChE enzyme activity following administration of an irreversible anti-ChE such as Soman has not, heretofore, been reported for CSF, but it is clear from these data that it is substantially more rapid than the recovery observed in brainstem. The value we found for the $T_1/_2$ of tissue recovery corresponds to results obtained with guinea-pig brainstem (4.5 days)[6], monkey pons (4.9 days), and medulla (5.0 days)[7].

The levels of total ChE activity in cat cisternal CSF are approximately three times those reported in human CSF[8]. The activity observed in this laboratory for cat brainstem (2.8 μmoles/h/mg) is lower than that observed by Siakotos et al.[4] for the whole brain homogenates taken from mouse, rat, guinea-pig, and rabbit (6.3–7.4 μmoles/h/mg) but higher than for bovine (2.1 μmoles/h/mg)[4].

As dephosphorylation apparently plays no role in the return of total ChE activity following Soman[9,10], recovery of ChE activity in CSF must be associated principally with the movement of newly synthesized protein into the CSF from either brain or from a plasma source. On the basis of the halftime of recovery, it is apparent that the rate of return for the ChE activity in CSF most closely corresponds with that of plasma. Preliminary investigations, however, have indicated that there may be significant regional differences in the rate of apparent recovery of enzyme activity in brain with perhaps the most rapid being observed in caudate nucleus and hypothalamus (Yaksh and Yamamura, in preparation). Thus, it is not possible to determine at this time the principal source of ChE activity in CSF. Although a likely source for this enzyme is from plasma elaborated through the choroid plexus, as are other proteins[11,12], contribution by brain cannot be completely disregarded, as it has been postulated that the CSF may serve as a 'protein sink' for neural tissues[13].

In conclusion, these studies indicate that the recovery of ChE enzyme in CSF greatly exceeds that of brainstem and thus does not reflect the relative state of recovery of brainstem cholinesterase. The data further suggest, indirectly, by similarity of the recovery rates, a role for plasma in the maintenance of ChE activity in the cerebroventricles. The action of the choroid plexus in maintaining these levels in the CSF is presently being examined. In addition, we are investigating the potential difference in the distribution and turnover rates of true and pseudo ChE in brain and CSF, as opposed to total ChE, as studied in these present experiments.

Zusammenfassung. Nachweis, dass bei Katzen die Reaktivierung der totalen Cholinesterasenaktivität nach Soman-Vergiftung am schnellsten in der Zerebrospinalflüssigkeit, dann im Blutplasma und danach erst im Hirnstamm eintrat.

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