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Forensic Significance of Acetylcholine Esterase Histochemistry in Organophosphate Intoxication*

Original Investigations and Review of the Literature

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Summary. The reduction of acetylcholine esterase (AChE) activity or the complete blocking of AChE to be observed by histochemical demonstration of AChE in tissue after experimental and spontaneous (human) organophosphate intoxication (especially paraoxone = E 600 and parathion = E 605) should be interpreted as an indication of an in vivo inhibition of the cholinergic system. In animal experiments, a relationship was demonstrated between AChE activity and the applied dose of organophosphorous compounds. In addition, enzyme inhibition was observed in in vitro systems using AChE-containing mouse tissue sections pretreated with organophosphate solutions or with body fluids containing organophosphates. Examination of the concentration dependency indicated that the inhibiting solution must contain at least 0.15 µg/ml paraoxone or 5 mg/ml parathion to block AChE in the section. Using the same in vitro system, a half-life of 6–7 min was established for the paraoxone inactivating enzyme in blood. The in vivo and in vitro inhibited AChE was reactivated by consecutive treatment of blocked sections with toxogonin. This possibility of reactivation therefore allows qualitative classifications of the AChE-inhibiting toxin to the alkylphosphates. The postmortem persistence of the AChE inhibitory effect was demonstrable for about a 2-month interval. Since the histochemically demonstrable activity of the enzyme AChE is more or less constant during a postmortem interval of at least 70 h, the model of histochemical demonstration is a method which provides a morphological equivalent for acute organophosphate intoxication.

Key words: Organophosphate – Paraoxone – Parathion – Acetylcholine esterase

Zusammenfassung. Die Reduktion oder vollständige Hemmung der histochemisch nachweisbaren Acetylcholinesterase (AChE)-Aktivität nach experimentellen und spontanen (menschlichen) Organophosphatvergiftungen (besonders mittels Paraoxon = E 600 und Parathion = E 605) muß als Zeichen einer in vivo-Hemmung des cholinergen Systems interpretiert werden. Im Tierversuch

* Dedicated to Prof. Dr. J. Peiffer on occasion of his 60th birthday

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wurde eine Beziehung zwischen der AChE-Aktivität und applizierten Dosis der Organophosphate nachgewiesen. In einem in vitro-System konnte die Enzymhemmung nach Exposition von AChE-enthaltenden histologischen Schnitten mit Organophosphat-enthaltenden Körperflüssigkeiten bzw. angesetzten Lösungen festgestellt werden. Bei Untersuchung der Dosisabhängigkeit wurde ferner festgestellt, daß mindestens 0.15 µg/ml Paraoxon oder 5 mg/ml Parathion notwendig sind, um in vitro die AChE zu blockieren. Bei Anwendung desselben in vitro-Systems konnte eine Halbwertzeit für das Paraoxon-inaktivierende Enzym im Blut von 6–7 min nachgewiesen werden. Die in vivo und in vitro gehemmte AChE war bei anschließender Behandlung der Schnitte mit Toxogonin reaktivierbar; die Möglichkeit der Reaktivation erlaubt eine qualitative Zuordnung des AChE-hemmenden Toxins zu den Alkylphosphaten. Eine postmortale Persistenz der Enzymhemmung war am menschlichen Material für ca. 2 Monate nachweisbar. Da auch die Acetylcholinesterase in weitgehend unveränderter Aktivität nach einem postmortalen Intervall von wenigstens 70 h nachweisbar ist, muß der histochemische Nachweis als morphologisches Äquivalent einer akuten Organophosphatintoxikation angesehen werden.

Schlüsselwörter: Organophosphate – Paraoxon – Parathion – Acetylcholinesterase

Introduction

Esterases are found in almost all body cells and in the blood of mammals. The activity of acetylcholine esterase (AChE) is extremely strong, especially at the site of its action, i.e., cholinergic junctional site of the vegetative nervous system (preganglionic and postganglionic synapses of the parasympathetic system, preganglionic synapses of the sympathetic systems), the striated muscle system (motor endplates), and the central nervous system (particularly within the striatum). In synapses, AChE inactivates the acetylcholine (ACh) released by the presynaptic membrane. ACh induces depolarization at the postsynaptic membrane. The inactivation of ACh by AChE usually frees the ACh receptor on the postsynaptic membrane for redepolarization.

Organophosphorous compounds block specific and, sometimes, nonspecific esterases. Since AChE is blocked in the case of organophosphate intoxication, only small amounts of AChE, if any, may be demonstrated histochemically at the junctional sites. These considerations prompted us to carry out the experiments described in the present study.

Material and Methods

Experimental Animals

Male and female mice (body weight 30–35 g) of the inbred breed NMRI (Ivanovas, Kißlegg, Allgäu/FRG) were fed ad libitum with Altromin and water.

Active Substances

The following active substances were used for the in vivo and in vitro experiments:

a) Parathion (E 605; mol.wt., 291.27) at least 99% (0,0-diethyl-0-[4-nitrophenyl]-monothiophosphoric acid ester—Riedel de Haen AG, Selze-Hannover/FRG; Code: 35,747);
b) Paraoxone (E 600; mol.wt., 275.19), at least 99% (0,0-diethyl-0-[4-nitrophenyl]-phosphoric acid ester—Riedel de Haen AG, Selze-Hannover/FRG; Code: 35,840).

The active substances were diluted with glycerin solution for all concentrations.

c) Toxogonin (mol. wt., 359; bis-[4-hydroxyiminoethyl-pyridinium-(1)-methyl]-ether dichloride) (Merck, Darmstadt/FRG). The toxogonin was dissolved in isotonic saline.

Histochemical Demonstration of AChE

The histochemical demonstration of AChE was carried out with the thiocholine method, which was originally described by Karnovsky and Roots (1964) and later modified by El Badawi and Schenk (1967). In our laboratory, the following techniques provided the best and most easily reproducible results: Untreated tissue blocks were stored under the exclusion of air at −70° C. The frozen blocks were cut into 5 µm sections with a cryostat microtome. The sections were then fixed in an ice-cold 4% solution of neutral paraformaldehyde for 10 min, washed briefly, and incubated for 45 min at 37° C. The final incubation medium must be prepared 30 min prior to use adding the following solutions to the substrate (acetylthiocholine iodide) in the order in which they are listed. The solution is clear; pH = 5.5–5.6. The individual solutions can be stored for months in the frozen state.

Acetylcholine iodide (Serva, Heidelberg/FRG)		12.5 mg
0.06 *N* (0.82%)	sodium acetate	15.8 ml
0.1 *N* (0.6%)	acetate acid	0.5 ml
0.1 *M* (2.94%)	sodium citrate	1.2 ml
30.0 m*M* (0.75%)	cupric sulfate (Merck, Darmstadt/FRG)	2.5 ml
4.0 m*M* (0.137%)	iso-OMPA (= tetraisopropylpyrophosphamide; Sigma, Munich/FRG)	0.5 ml
5.0 m*M* (0.165%)	potassium ferricyanide	2.5 ml

The sections were rinsed in distilled water and counterstained for 30 s with freshly filtered Harris hematoxylin, rinsed in distilled water for 1 min, blued in solution of lithium carbonate, and rinsed again in distilled water. The sections were dehydrated, cleared, and then mounted in permount.

The described methods were applied under constant conditions to avoid method-related variations in the quantity of CuS precipitates. Sites of AChE activity are characterized by a sharply defined, granular, reddish-brown CuS precipitate.

AChE Blocking on the Section

Briefly fixed cryostat sections from otherwise untreated mouse intestine were exposed to organophosphate-containing solutions prior to AChE demonstration to investigate the blocking effect of different organophosphates. The sections were regularly exposed to different concentrations of organophosphate for 5 min at room temperature, if not described otherwise in Results. Prior to the histochemical demonstration of AChE, the sections were rinsed with distilled water.

Organophosphates are soluble in both glycerin and blood; high concentrations of parathion, however, do not dissolve well in glycerin. Pure glycerin, like urine, blood, or gastric juice from nonintoxicated humans, does not quantitatively or qualitatively alter AChE activity on sections.

AChE Distribution and Localization in the Examined Organs under Normal Conditions

The same human and mouse organs were studied in each case. The brain, particularly the striatum (caudato-putamen), was examined, since AChE is demonstrable in high concentrations in many of the nerve fibers and synapses (Fig. 2a). Moreover, the striated muscle of the diaphragm was studied because AChE activity at the motor endplates is high (Fig. 3). Finally, transverse sections of the small intestine, especially the jejunum, were examined (Fig. 4a). AChE activity was high in

this organ, particularly in the intramural and submucosal plexus. Activity in the cholinergic nerve fibers of the ring muscle and in the submucosal lamina of the intestinal wall was also impressively high.

Quantification of Histochemical Findings

Rough quantification of the CuS precipitate was possible in brain and in muscle:

− = no CuS precipitate demonstrable;
+ = CuS precipitate demonstrable in small amounts;
++ = normal AChE activity with normal quantity of CuS precipitates.

The differentiation was easiest in the intestine.

Additional quantification was also possible.

When AChE-positive nerve fibers were no longer demonstrable in the ring muscle and the CuS precipitates in the intramural plexus were almost normal, AChE was only moderately blocked (normal activity, +++; moderately blocked activity, ++). When, in addition, AChE activity was decreased in the intramural plexus, pronounced AChE blocking (+) was documented (Fig. 1). Since quantification is simple and unequivocal on intestinal tissue, the in vitro results were quantified primarily on sections of intestine.

Blood

Paraoxone in the blood is inactivated in vitro and therefore loses its capacity for blocking AChE. The blood of only one test subject was used for all in vitro experiments described in the present study to eliminate the possibility of quantitative differences in the findings as a result of the polymorphism of paraoxonase, the paraoxone-inactivating enzyme (Goedde et al. 1967; Krisch 1968; Flügel and Geldmacher-von Mallinckrodt 1978). In addition, blood was partly defibrinate with glass beads, herein after referred to as "blood", or rendered anticoagulable by EDTA, herein after referred to as "EDTA blood".

Chemical-analytic Method for the Identification of Alkylphosphates in Organ Tissue

The initial solution was 1 ml of serum, plasma, or blood diluted in 10 ml water or 10 ml urine. Extraction was carried out by shaking the solutions with 30 ml freshly distilled diethyl ether. The upper phase was dried and evaporated, and the residue resolved in 100 µl n-hexane; 1 µl of this solution was analyzed with gas-liquid chromatography (GLC). The external standard method (3380 A Integrator, Hewlett-Packard) was used to quantify the solution. The GLC conditions were as follows:
Model 5710 Hewlett-Packard;
Column: 2-foot glass column filled with 5% Apiezone L on a Hewlett-Packard Chromosorbe W AW-DMCS, 80–100 mesh, 200° C isotherme;
Carrier gas: He 30 ml/min;
Injection port: 250° C;
Detector: 350° C; NP-FID.

Results

Series 1: In vivo Studies on the Relationship Between AChE Blocking and Organophosphate Dose

Different paraoxone and parathion doses were applied s.c. to mice. Some of the animals were killed by decapitation 15, 30 or 50 min later, some died spontaneously. The jejunum, diaphragm muscle, and brain were removed from each mouse, immediately frozen, and stored under the exclusion of air until histochemical demonstration of AChE activity. The semiquantitative results are presented in

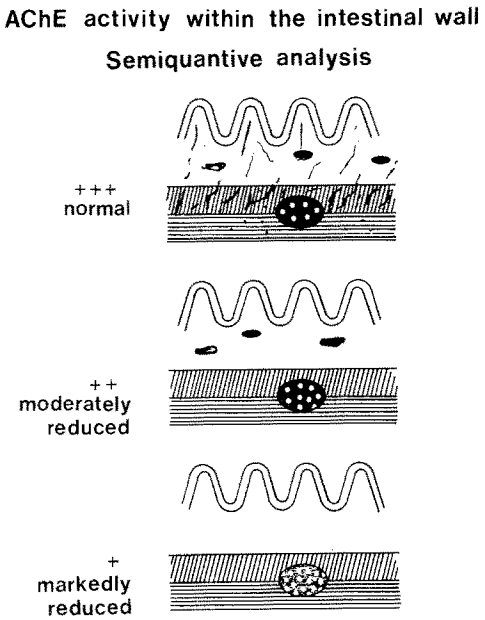

Fig. 1. Schematic presentation of different degrees of AChE blocking in the intestine as used in semiquantitative analysis

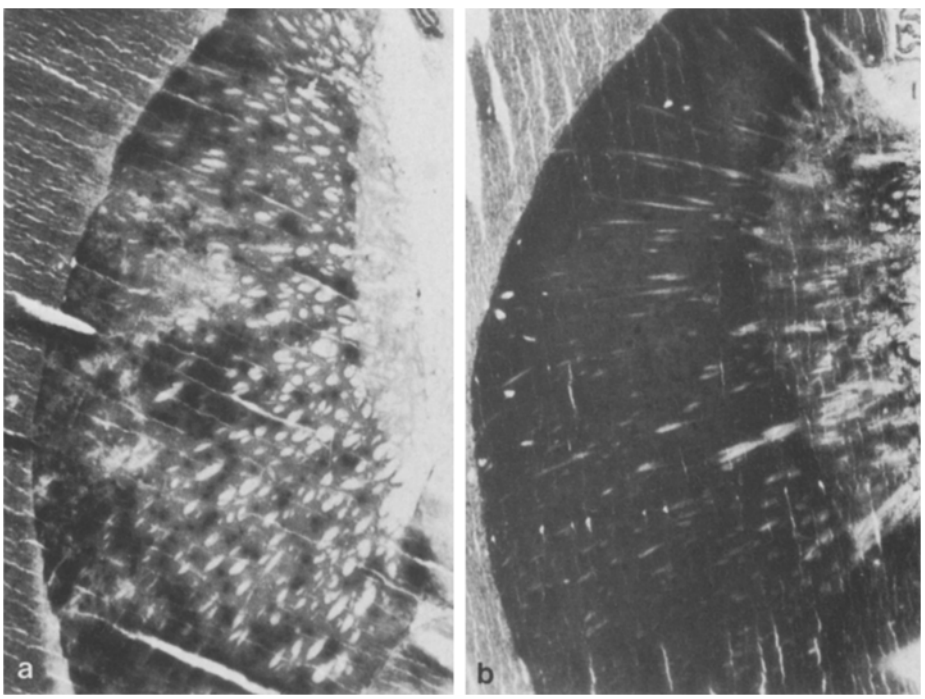

Fig. 2a, b. Demonstration of AChE activity in brain tissue of mouse (putamen). **a** Normal AChE activity. **b** Markedly decreased AChE activity after in vivo alkylphosphate intoxication. (AChE; hemalun; **a, b** ×37)

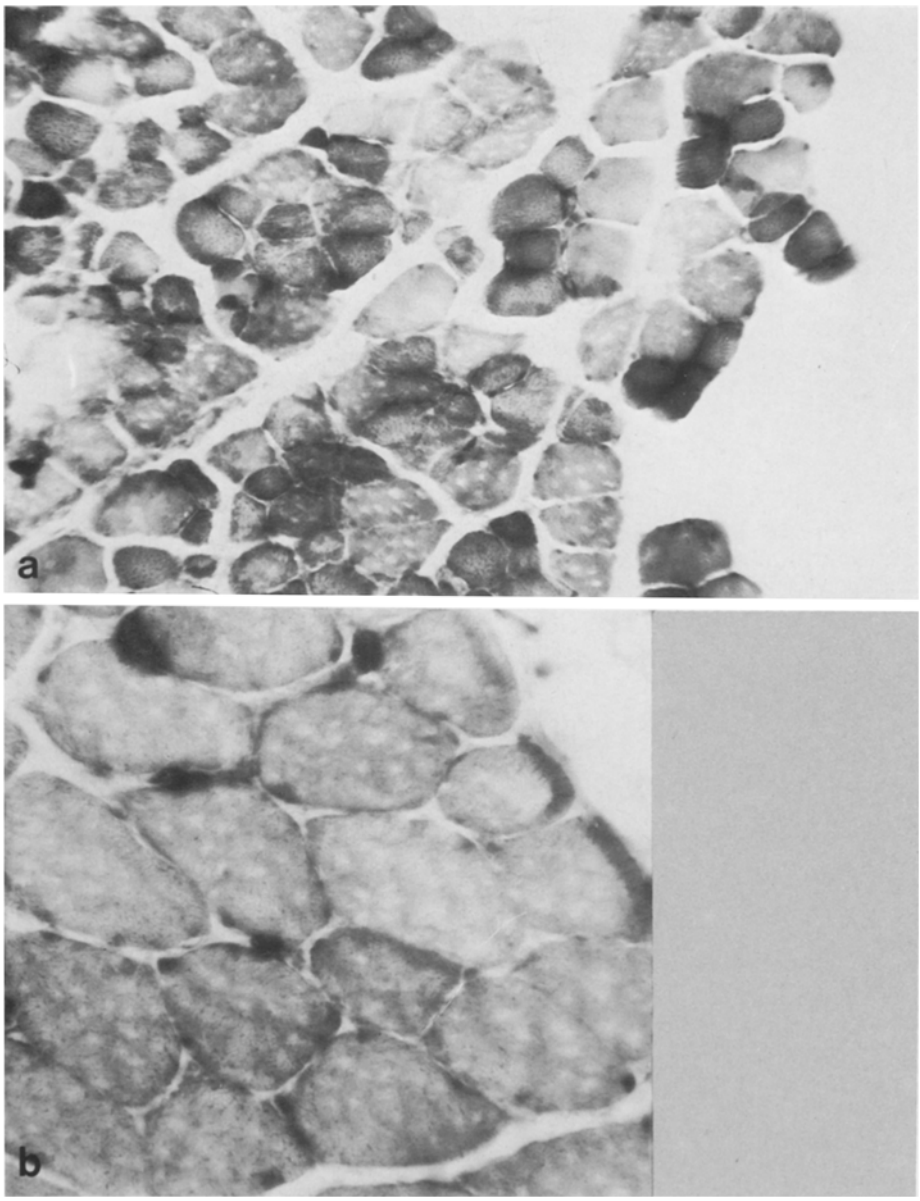

Fig. 3a, b. Demonstration of normal AChE activity in neuromuscular junctions of diaphragm muscle of the mouse (AChE; hemalun; **a** ×250, **b** ×500)

Table 1; some results are based on two experiments. The reduction of histochemically demonstrable AChE in the brain and intestine after organophosphate intoxication is shown in Figs. 2b and 4b, c.

The most important findings are as follows:

1. AChE activity in all examined organs depends apparently on the dose of parathion or paraoxone.

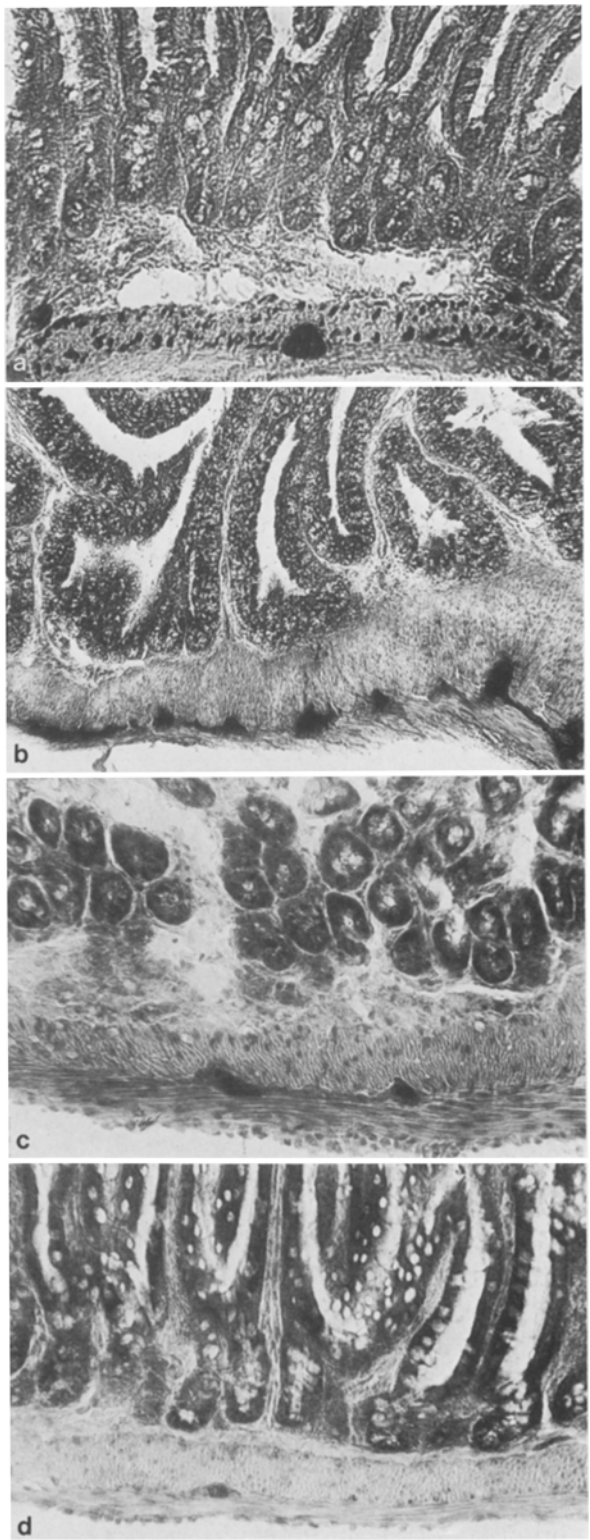

Fig. 4a–c. Quantification of AChE activity in mouse intestine. **a** Normal AChE activity (quantification = +++). **b** Moderately reduced AChE activity after alkylphosphate intoxication characterized by the absence of AChE-positive intramural nerve fibers (quantification = ++). **c** Markedly reduced AChE activity characterized by an additional decrease of CuS deposits in intramural ganglia (quantification = +). **d** Total lack of enzyme activity. (AChE; hemalun; **a–c** × 63)

Table 1. Relationship between paraoxone and parathion dose (s.c. application) and acetylcholine esterase activity in different mouse organs

Dose: mouse	Survival time (min)	AChE activity in		
		intestine	muscle	brain
Paraoxone				
0.2 mg/kg	15[a]	+++	++	++
	30[a]	+++	++	++
0.7 mg/kg	15[a]	+	+	+
	30[a]	+/−	++	+
	60[a]	+	+	−
1.4 mg/kg	15[b]	−	−	−
2.1 mg/kg	13[b]	+/−	+	−
Parathion				
1 g/kg	43[b]	−	++	+/−
5 g/kg	50[a]	−	+	−
10 g/kg	30[a]	−	+	−
20 g/kg	20[b]	−	+	−

[a] Results checked in two decapitated mice
[b] Died spontaneously

2. Pure paraoxone is considerably more toxic in mice than pure parathion.
3. Blocking is weakest in the striated muscles as compared to the blocking in the brain and intestines.

Series 2: AChE Activity in Normal Human Tissue During the Postmortem Interval

Postmortem intestine from cases in which death was due to different causes was examined to determine the relationships between AChE activity and the postmortem interval. We expected to find a decrease or diffusion of enzyme activity in the postmortem interval (cp. Oehmichen 1980). AChE activity in the intestine did not fall markedly in a postmortem interval of 51 h (Table 2). Activity was clearly demonstrable at the junctional sites up to a postmortem interval of 76 h, although activity was decreased in the cholinergic nerve fibers. The findings in the intestine of a person who was discovered mummified in a room (probable postmortem interval: approximately 5 months, according to the statement of a witness) was impressive: Marked activity was still observable at the intramural plexus, although the cell structures barely stained. The localization of the CuS precipitates clearly indicated that the enzyme in the intramural plexus was specific esterase.

Series 3: AChE Activity in Human Tissue after Fatal Alkylphosphate Intoxication

Tissue specimens from the brain, diaphragm, and intestine of cases with suspected organophosphate intoxication were preserved. AChE activity was demonstrated

Table 2. Demonstration of acetylcholine esterase activity in human intestine (autopsy material) in different postmortem intervals and different causes of death

Code	Postmortem interval	Cooled	Postmortem diagnosis	AChE activity in intestine
136/81	6 h	No	Stab wound in heart Exsanguination	+++
147/81	7 h	No	Sudden infant death	+++
132/81	14 h	No	Embolism	+++
139/81	20 h	No	Acute craniocerebral trauma	+++
148/81	24 h	No	Hypovolemic shock	+++
131/81	30 h	Partly	Acute craniocerebral trauma	+++
137/81	38 h	No	Hypovolemic shock	+++
130/81	41 h	Partly	Pneumonia	+++
143/81	41 h	Yes	Acute craniocerebral trauma	+++
135/81	51 h	No	Drowning	+++
122/81	64 h	No	Subdural hemorrhage	++
105/81	76 h	Partly	Pancreatic shock	++
168/81	5 months	No	Myocardial infarction (mummification)	+

after the chemical-toxicologic analysis had confirmed organophosphate intoxication. While all cases without intoxication showed activity comparable to that of mouse tissue, most specimens from cases with organophosphate intoxication showed neither activity nor markedly decreased activity. AChE blocking was observed in human material as well, particularly in intestine and brain tissue. Inhibition of AChE activity was least pronounced after fatal intoxication with parathion (Folidol-Öl).

Series 4: AChE-inhibiting Effect of Body Fluids from Humans with Alkylphosphate Intoxication

Prior to the histochemical demonstration of AChE activity, sections of intestine from nonintoxicated mice were exposed to body fluids from intoxicated human beings. AChE activity was normal after incubation of sections with blood, urine, and stomach contents from nonintoxicated individuals. The body fluids from the cases with organophosphate intoxication, however, sometimes contained active substances which blocked AChE (Table 3). The following two phenomena were especially impressive:

1. In individual cases, AChE was blocked even by urine, insofar as it was available, although parathion or paraoxone was demonstrable in very low concentrations with chemical-analytical methods.

2. After E 605 intoxication, inhibition of AChE activity is demonstrable only by blood from a living, intoxicated individual to which EDTA has been added

Table 3. In vitro inhibition of acetylcholine esterase activity with body fluids from individuals with organophosphate intoxication. Only parathion was estimated in E 605 + systox intoxication; only phosdrine in mevinphos intoxication

Case No.	Post-mortem interval	Post-mortem examination (+)	Incorporated active substance	Concentration of active substance/kg			AChE activity after incubation of body fluids with sections of mouse intestine			
				Blood	Urine	Stomach contents	Blood without EDTA	Blood with EDTA	Urine	Stomach contents
1	3 days	+	Mevinphos	15.2 mg	0.9 mg	46.25 mg	–	o	+	–
2	24 h	+	Parathion	1.3 µg	0	2.6 g	+++	o	+++	–
3			Parathion	10.0 µg	0.2 µg	o	+++	o	++	o
4	7 days	+	Parathion	80.0 µg	0	654.70 mg	+++	o	o	++
5			Parathion	450.0 µg	0	560.00 µg	+++	–	–	–
6	64 h	+	Parathion	10.0 µg	0	o	+++	o	+++	o
7	5 days	+	Parathion + demeton-0-methylsulfoxide	0.3 mg	8.0 µg	37.25 mg	–	o	–	–
8	still living	o	Parathion + demeton-0-methylsulfoxide	275.0 µg	57.0 µg	o	+	–	–	o
9	2 months	+	Mevinphos	20.5 mg	10.0 µg	382.00 mg	–	o	o	o

o = not examined
– = AChE activity not demonstrable, quantification otherwise as described in Material and Methods

Table 4. In vitro studies on the relationship between the incubation time, the different amounts of paraoxone dissolved in glycerin and the AChE activity on sections of mouse intestine

Incubation time (min)	AChE activity in intestine after incubation of the section with different amounts of paraoxone dissolved in glycerin				
	10 ng/ml	1 ng/ml	100 ng/ml	10 pg/ml	Pure glycerin without paraoxone
5	–	++	+++	+++	+++
25	–	+	+++	+++	+++
45	–	+	++	+++	+++

Table 5. In vitro studies on the relationship between the incubation time, the different amounts of paraoxone dissolved in EDTA blood and the AChE activity on sections of mouse intestine

Incubation time (min)	AChE activity in the intestine after incubation of the section with different amounts of paraoxone dissolved in EDTA blood					
	2 µg/ml	1 µg/ml	150 ng/ml	10 ng/ml	1 ng/ml	pure EDTA blood without paraoxone
5	–	–	+	+++	+++	+++
25	–	–	–	++	+++	+++
45	–	–	–	++	+++	+++

immediately after collection. EDTA apparently has an inhibiting effect on the paraoxone-inactivating enzyme.

Series 5: In vitro Blocking Effect of Different Concentrations of Organophosphate on Histochemically Demonstrable AChE Activity

Although parathion does not dissolves completely in glycerin, marked AChE blocking was observed at parathion concentrations of 50 mg/ml. The blocking effect was already questionable at parathion concentrations of 5–15 mg/ml. A paraoxone concentration of 10 ng/ml, however, completely inhibits AChE activity; the blocking effect was already cancelled at a concentration of 1.0 ng/ml.

The duration of the effect of paraoxone on AChE activity on the section was arbitrarily limited to 5 min (see Material and Methods). A longer incubation time is possible if the sections are treated with extreme care. Sections, therefore, can be stored in glycerin solution without any relevant change occurring in AChE distribution and activity; an incubation time of 60 min at 37° C proved critical in our experiments. As demonstrated in Table 4 the blocking effect, the concentration, and the duration of paraoxone exposition are clearly interrelated.

Sections of mouse intestine were exposed to these different amounts of paraoxone added to EDTA blood, and AChE activity was demonstrated. A

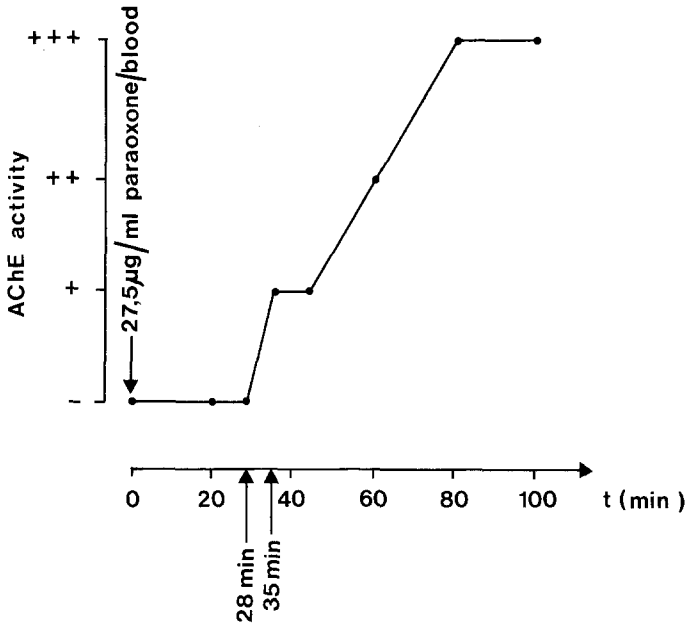

Fig. 5. Semiquantitative analysis of AChE activity in sections of mouse intestine after their exposure to paraoxone dissolved in defibrinated blood (without EDTA) for different time intervals. Total blocking of AChE is identifiable within the first 28 min. The blocking effect declines thereafter, thus indicating increasing paraoxone inactivation by the paraoxone-inactivating enzyme in the blood

Table 6. Limited reactivation of alkylphosphate-inhibited AChE activity with toxogonin in in vitro and in vivo experiments

Type of AChE inhibition	Incubation solution (incubation, 30 min at 20° C)	Results
Section of mouse intestine, in vitro inhibition with 1 µg paraoxone/ml glycerin	NaCl	–
	Toxogonin	+
Inhibition by in vivo application of 1.4 mg paraoxone/kg mouse	NaCl	–
	Toxogonin	+

paraoxone concentration of 0.15 µg/ml is sufficient to almost completely block AChE in mouse intestine (Table 5).

Series 6: In vitro Experiments on the Time Dependency of Paraoxone Inactivation in Whole Blood

Since blood contains an enzyme which degrades paraoxone, the AChE-blocking acticity of paraoxone must be time-dependent. 27.5 µg/ml paraoxone was added to blood defibrinated with glass beads, and the suspension was stored for various

lengths of time at 20°–22° C prior to incubation with intestine sections from untreated mice. After exposition of the section to the paraoxone-blood mixture, AChE activity was histochemically demonstrated.

The total blocking effect of the paraoxone-blood mixture was still present as long as 28 min after addition of paraoxone to blood; no inhibitory effect of the blood mixture could be observed after 80 min (Fig. 5).

Assuming that the paraoxone concentration still active in the blood at 28–35 min corresponds to the dose limits established above (0.15 µg/ml), the half-life of the paraoxone is approximately 6–7 min.

Series 7: Reactivation of Alkylphosphate-induced AChE Inhibition with Toxogonin

Since many other substances are known to inhibit AChE activity, the problem of the qualitative classification of the AChE inhibitor arises. Reactivation of AChE with toxogonin is possible only after alkylphosphate intoxication; the next logical step therefore seemed to be to set up reactivation experiments.

AChE activity was inhibited both on the section (in vitro via 1 µg/ml paraoxone in glycerin) and in the living animal (in vivo application of 1.4 mg/kg mouse). Sections of the intestine were then treated with 10^{-5} *M* toxogonin for 30 min at room temperature. Sections of intestine incubated for the same length of time in isotonic saline served as the control. Table 6 shows that distinct, but limited, reactivation is present after in vitro and in vivo AChE blocking.

Discussion

Morphological alterations were described after chronic and/or after subacute, nonfatal, alkylphosphate intoxication. On the one hand, these alterations are (secondary) nonspecific, hypoxemic alterations of the heart (Jääskeläinen and Alha 1969) and the CNS (Oehmichen M, Schlote W, Mallach HJ (1983) Hirnveränderungen bei Parathion-Vergiftungen. Beobachtungen in 42 Fällen (in prep)), and on the other, toxic alterations of the peripheral nerves (Olajos et al. 1978; Baker et al. 1977), muscle (Wecker and Dettbarn 1976), and the neuromuscular junctions (Baker et al. 1977). Moreover, several studies are available on pathomorphological findings after acute, fatal alkylphosphate intoxication (Pribilla 1954; Böhmer 1955; Maresch 1957; Holmstedt et al. 1957; Adebahr 1960): the non-specific findings are basically characterized by cerebral and pulmonary edema. The lack of a specific morphological equivalent in the CNS prompted the present histochemical experiments.

The possibility of histochemical identification of alkylphosphate intoxication through the demonstration of AChE activity was considered relatively early (Bergner and Durlacher 1951; Bergner and Bayliss 1952). The idea was investigated again by Petty and Moore (1958) and Petty et al. (1958) and later by De la Mancha et al. (1979). Between 1958 and 1979, the only studies on this question were published in Italy in the case of spontaneous human intoxications (Barsotti et al. 1954; Graev and Fabioni 1960; Frada et al. 1964; Caruso and Tessitore 1968) and experimental intoxications (Graev and Fabioni 1960; Frada et al. 1964; Caruso and Tessitore 1968; De la Mancha et al. 1979). The authors sometimes observed total

AChE blocking. Bergner and Bayliss (1952) and De la Mancha et al. (1979) carried out additional in vitro experiments similar to those described by Pearse (1968) in the context of a histochemical method for specification of the demonstrated enzyme. The in vitro method differed slightly in terms of the quantity of inhibitor and the length of the exposure time. Most authors, however, obtained very similar results. The demonstrated dose dependency in our in vivo and in vitro experiments indicates that the histochemical demonstration of AChE activity is a morphological equivalent for the inhibition of cholinergic transference by alkylphosphate. The demonstration of the AChE inhibitory effect in autoptic human material of unambiguous organophosphate intoxication revealed the persistence of enzyme inhibition for a postmortal interval of at least 2 months.

Our own investigations indicate that this conclusion can be applied to both the motor endplates and the central and vegetative nervous system. Our findings also indicate that a diagnoses of alkylphosphate intoxication can be established by examining organs from intoxicated individuals, on the one hand, and determining the blocking effect of body fluids after alkylphosphate intoxication, on the other. Therefore, a diagnosis can be made by examining body fluids from living individuals with alkylphosphate intoxication.

It has been established that the in vitro toxicity of highly purified parathion is relatively low (Diggle and Gage 1951) and that parathion is oxidized in vivo to paraoxone via metabolism in the liver (Davison 1957; Neal 1967a, b), a process in which parathion becomes toxic (for review see O'Brien 1960; Koelle 1963; Schrader 1963; Geldmacher-von Mallinckrodt 1975, 1978). Our in vitro experiments demonstrating very weak blocking activity for parathion dissolved in glycerin and in blood confirmed these observations.

Paraoxone is inactivated in the blood, like in the liver and other organs (Kubistova 1959), by the hydrolysis of arylesterase and/or "paraoxonase" (E.C. 3.3.3.2) (Aldridge 1953a, b). Inactivation in the in vitro system, i.e., in blood specimens, can be prevented by the addition of EDTA (Erdös and Boggs 1961; Neal 1967b), as series 4, 5, and 6 demonstrate. In our experiments, a half-life of 6–7 min was calculated for the enzyme paraoxonase (series 6); this finding is compatible with chemical-analytic estimations in which the half-life appeared to range between 8.6 and 60.4 min (Geldmacher-von Mallinckrodt et al. 1978).

The experiments in series 2 were carried out to study the possibility of demonstrating AChE activity at different intervals after death. The results agree with the findings reported by Bergner and Durlacher (1951) and Bergner and Bayliss (1952) in mice. These authors found only a slight decrease in AChE activity within 48 h, even though severe postmortem putrefaction was present AChE activity in human muscle persists for at least 26 h at 20° C to 26° C and in refrigerated corpses up to 180 h (Petty and Moore 1958). Friedberg and Sakai (1958), in their studies of serum esterase, confirmed the stability of AChE activity during the postmortal level. The significance of a still demonstrable enzyme activity and the lack of function is not clear.

Experiments were carried out with toxogonin to determine the specifications of the toxic substance. It has already been established that the phenomenon of AChE inhibition is not specific for organophosphate intoxication and can be induced by different toxins (for survey see Augustinsson 1948; Aldridge 1980; Alsen et al.

1973/tabun; Maayani and Weinstein 1979/phencyclidine derivatives). Only one part of organophosphate, however, can be reactivated with toxogonin and PAM, as is known by clinical studies (for review: Moeschlin 1980). This reactivation therefore can serve an indication of toxin specificity when AChE is blocked (Friedberg and Sakai 1958). Similar experiments in histochemical (in vitro) models were carried out by De la Mancha et al. (1979); their results are in agreement with our findings.

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