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Postsynaptic Neuromuscular Dysfunction in Organophosphate Induced Intermediate Syndrome*

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Summary. A 65-year-old Caucasian female developed an intermediate syndrome seven days after an acute cholinergic crisis, caused by the ingestion of fenthion.

Cholinesterase activity in the blood, plasma and red cells was monitored daily by the method according to Nenner and serial serum fenthion levels were measured by capillary gas chromatography.

Electromyographic studies showed fade on tetanic stimulation by means of surface electrodes at 20 Hz of the left M. abductor digiti quinti at day 7, which could no longer be observed at day 19.

Fade on low-frequency stimulation and post-tetanic facilitation were both absent.

A biopsy of the N. suralis was normal. A biopsy of the M. tibialis anterior revealed a limited rhabdomyolysis with a very weak staining for cholinesterase.

It is hypothesized that the pathophysiologic process underlying the syndrome is the result of a time-confined phenomenon, which includes both changes in the postsynaptic structures by a desensitization process and a gradually restoring ratio of acetylcholine to acetylcholinesterase. This hypothesis is suggested by the similarity in the EMG-findings of this patient and those in myasthenia gravis, which is known to be characterized by a postsynaptic transmission defect.

Key words: Acetylcholinesterase – Cholinesterase inhibitors – Organophosphorus compounds

Acute neurotoxic effects during the cholinergic phase of organophosphorus-intoxication as well as the organophosphor-induced delayed polyneuropathy are well recognized. In 1987 an intermediate syndrome was first described as a separate clinical entity in patients of Asian origin [8].

We observed a Caucasian female in whom an intermediate syndrome developed 7 days after ingestion of fenthion with suicidal intent.

Case Report

A 65-year-old farmer's widow with a negative medical history was urgently referred to the Intensive Care Unit with symptoms of coma, bilateral miosis, diffuse sweating, hypersecretion, faeco-urinary incontinence and respiratory failure.

The initial treatment consisted of endotracheal intubation and ventilation, and gastric lavage, followed by a dose of activated charcoal. The intravenous administration of 2 mg of atropine because of sinus bradycardia was followed by a marked improvement of the level of consciousness and adequate motor response of all limbs, enabling detubation. The symptoms and the reaction to atropine suggested the diagnosis of organophosphate poisoning.

At that moment clinical examination showed a symmetrical hyperreflexia. The pupils remained miotic and symmetric. Fasciculations were not observed. Coarse rales were audible over the lungs with prolonged expiration. The blood pressure was 120/95 mm Hg. The pulse was regular with a

Abbreviations: AchE = Acetylcholinesterase; EMG = Electromyography; M. = musculus; N. = nervus; NTE = Neuropathy Targed Esterase; OPIDP = Organo phosphor induced delayed polyneuropathy; SGE = Fused silica needle; TSD = Thermionic Specific Detection

* Data of this paper were reported at the XIII International Congress of the European Association of Poison Control Centres in Edinburgh – September 1988.

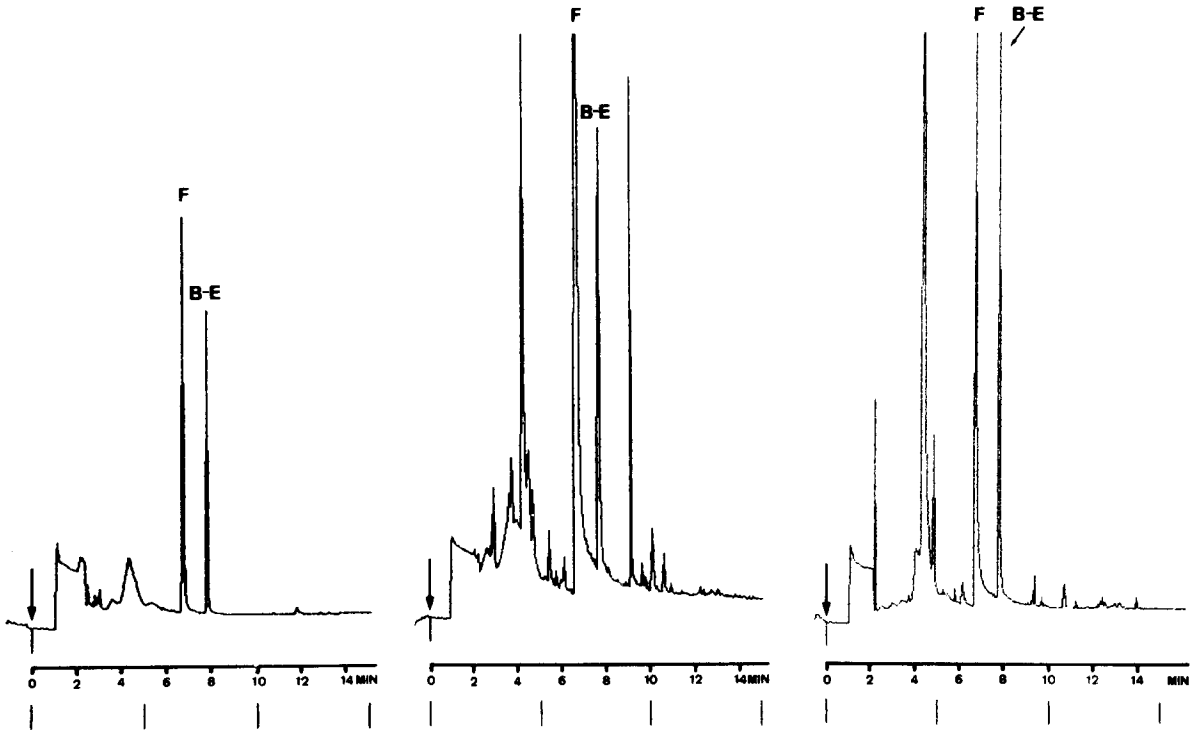


Fig. 1. Chromatograms of (**a**) a standard mixture (F: Fenthion; B-E: Bromophos-ethyl), (**b**) an extract of the first gastric lavage fluid and (**c**) an extract of a plasma sample taken shortly after admission to the hospital

rhythm of 90 beats per minute. The body temperature was 37° C. Further physical findings were normal.

After the initial dose, 1 mg of atropine was administered every thirty minutes IV until atropinisation and pralidoxime (Contrathion®) 250 mg IV every four hours for three days. Gastric lavage was performed every four hours, followed by the administration of activated charcoal (Fig. 2).

A toxicological screening revealed a decrease in the acetylcholinesterase-activity both in whole blood (2.0 micromole/ml/min; reference values: 5.11–8.75, plasma (0.27 micromole/ml/min; 1.26–2.66) and in red blood cells (3.54 micromole/ml/min; 9.85–16.41).

With the exception of a leucocytosis of 15.900/µl and a hyperglycemia (10.8 mmol per liter) the laboratory data showed no abnormalities.

Eight hours after arrival the patient admitted the ingestion in a depressive mood of an unknown volume of a commercial preparation of fenthion (550 g/l in xylol). At home the family found a real armoury of insecticides. After a 36 hours interval without problems there was a sudden relapse of the cholinergic crisis with fasciculations, obvious transpiration, bronchial hypersecretion, respiratory insufficiency and a decrease in consciousness. The patient was reintubated and ventilated 43 hours after admission. The atropine dosage, which had been decreased to 1 mg every two hours was doubled again (Fig. 2).

The acetylcholinesterase-activity stayed very low and there remained evidence of circulating fenthion in the plasma, which could no longer be detected at day 11 (detection limit 0.10 µg/ml) (Table 1).

An EMG at day 4 showed low-voltage intermediary contraction contours with very short contraction periods. Muscle fibers of the four limbs showed signs of fibrillation at rest. A follow-up EMG 3 days later was suggestive of both a motor and sensory axonal polyneuropathy without clear signs of demyelinisation.

However, tetanic stimulation at 20 Hz of the left M. abductor digiti quinti by means of surface electrodes showed a marked decrement of 40%, decreasing to 25% after a one minute rest, which is consistent with a "myasthenia-like syndrome" (Fig. 3a).

At that time the patient suffered from general pareses, but fasciculations or rest activities could not be observed. Gradually, muscular strength increased again. An attempt at detubation on day 9 however rapidly led to a third episode of respiratory insufficiency as a result of pneumonia in the left lower lobe. Intubation had to be continued until day 19 (Fig. 2).

At that moment a fourth EMG study was per-

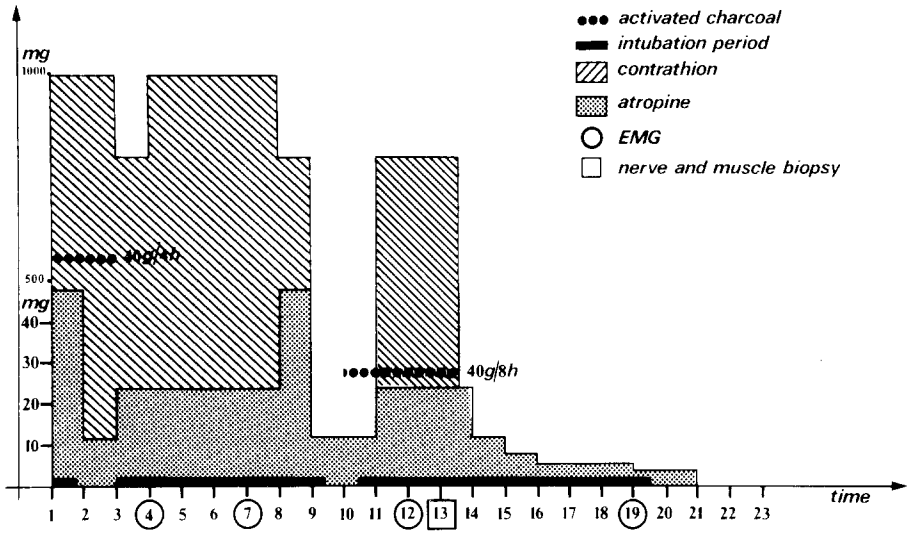


Fig. 2. Diagram of the hospitalisation course

Table 1. Fenthion concentrations in blood and acetylcholinesterase activity in red cells, whole blood and plasma

Days	Fenthion concentration in blood (ng/ml)	AchE-activity (μmol/ml/min)		
		in red cells	in whole blood	in plasma
	Ref:	9.85–16.41	5.11–8.75	1.26–2.66
1	27.05	3.54	1.70	0.27
2	N.A.*	1.60	0.96	0.00
3	4.82	0.30	0.43	0.03
4	3.32	2.40	0.95	0.06
5	1.75	1.18	0.45	0.00
6	0.16	0.79	0.30	0.00
7	N.A.*	N.A.*	N.A.*	N.A.*
9	0.19	2.15	0.80	0.03
10	N.A.*	0.81	0.35	0.00
11	–**	0.96	0.40	0.18
12	N.A.*	2.40	0.82	0.10
21	N.A.*	5.85	2.60	0.69

* not analysed
** below the detection limit (0.10 ng/ml)

formed. The axonal polyneuropathy still persisted, though to a lesser degree, but the decrement upon repetitive stimulation at 20 Hz was no longer present.

An earlier EMG on day 12 had already shown a regression of the fade with a decrement of 10% on 20 Hz stimulation after a one minute rest (Fig. 3b).

A quadriceps muscle biopsy was performed. On light microscopy, a moderate variation in muscle fiber diameter was observed. Some fibres had excentric, swollen sarcolemmal nuclei; a few fibers were more basophilic and had central nuclei (Fig. 4). Only in 2 fibers, vacuolisation and proliferation of myoblasts was found. On histochemistry, a mosaic distribution of fiber types was present. The neuromuscular endplates were only poorly stained by Karnovsky's method for cholinesterase detection (Fig. 5). A N. suralis biopsy revealed no light microscopical abnormalities.

The ensuing recuperation was uneventful and finally the patient could be discharged after 23 days and referred to the psychiatric department for further treatment of her depression.

Materials and Methods

Determination of Cholinesterase Activity

The cholinesterase activity in the blood, plasma and red cells was determined by the method according to Nenner [1].

Determination of Fenthion

Standards and Reagents

Fenthion and bromophos-ethyl (Pestanal R) were supplied by Riedel-De Haën AG, Seelze-Hannover.

Sodium sulphate, anhydrous, and n-hexane GR were from Merck (Darmstadt, Germany).

Sample Preparation

The blood samples were centrifuged at 5000 rpm during 15 minutes. A 2.0-ml aliquot of gastric lavage fluid, plasma or urine was transferred to a glass-stoppered tube and extracted with 10 ml n-hexane containing the internal standard (bromophos-ethyl: 1 μg/liter n-hexane).

After centrifugation, the organic layer was dried with anhydrous sodium sulfate, filtered and evaporated to dryness under a nitrogen stream.

The residue was redissolved in 50 μl of metha-

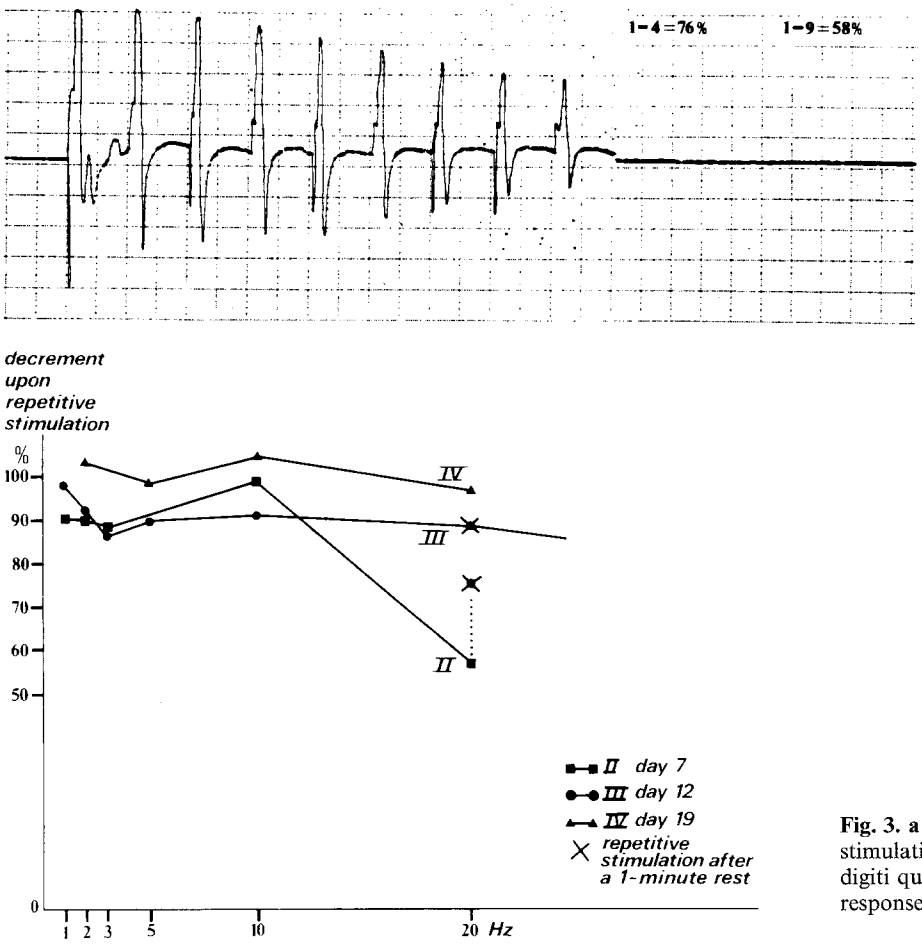


Fig. 3. a Electromyographic fade on tetanic stimulation at 20 Hz of the left M. abductor digiti quinti (day 7). **b** Evolution of motor response upon repetitive stimulation

nol. A 1.0-µl aliquot was deposited directly onto the head of the capillary column by means of a fused silica needle (SGE).

Gas Chromatographic Conditions

A Model 3400 gas chromatograph (Varian) was used with capillary on-column injection, and Thermionic Specific Detection (TSD).

Chromatography was performed on a 25 m × 0.32 mm i.d. fused silica column coated with a 0.25-µm-thick film of methyl phenyl polysiloxane (Sil 8 CB, Chrompack).

The carrier gas (helium) was passed through a moisture trap (RSL N.V.-Alltech Europe) and an oxygen remover (Pierce Europe B.V.). The column head pressure was monitored at 0.7 bar.

The temperature of the TSD was maintained at 300° C. Before chromatography the column oven and injector were cooled down to 100 and 50° C, respectively.

After sample introduction the injector was immediately heated at 125° C/min to 300° C.

The oven temperature was first increased at a rate of 40° C/min with a maximum heating to 210° C, where it was held for 2 minutes. The temperature was further programmed at 5° C/min to 260° C, where it was held for 1 minute, and then at 10° C/min to 300° C, where it was maintained for another 6 minutes.

Data handling was performed using a 601 Data System (Varian) and a Think Jet printer (Hewlett Packard).

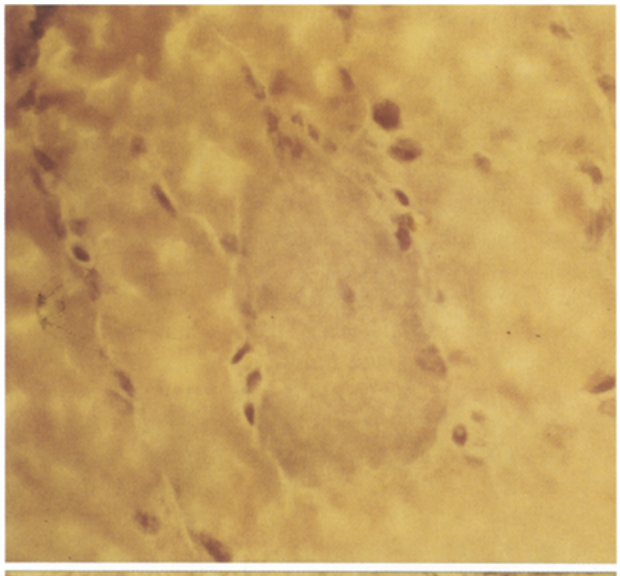
Quantitative information was obtained from comparison with an internal standard (the ratio of the peak area of Fenthion to that of Bromophos-ethyl, which was considered as a suitable internal standard, because its retention time was different from those of co-extracted substances) (Fig. 1).

Results

Cholinesterase Activity Determination

See Table 1.

Longterm depression of acetylcholinesterase activity in red cells, whole blood and plasma in conjunction with detectable serum fenthion concentrations.



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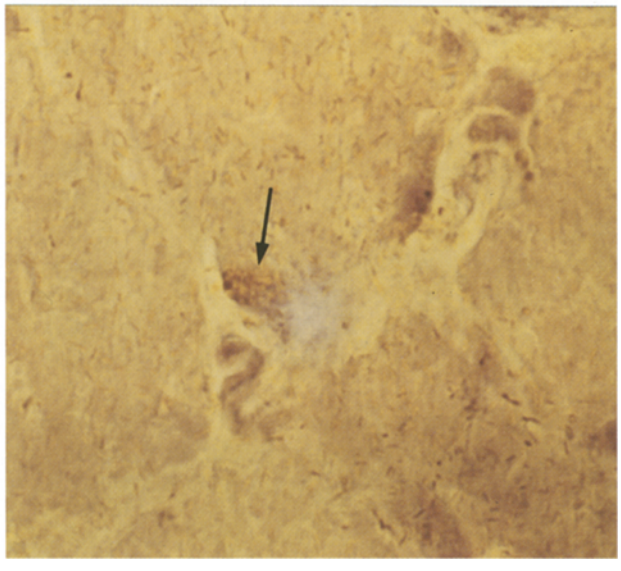

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Fig. 4. Basophilic muscle fiber with central nucleus. (Frozen section, hematoxylin-eosin, ×400)

Fig. 5. Poor cholinesterase enzyme activity at the motor endplates (arrow). (Frozen section, Karnovsky's method, ×400)

Fenthion Determination

See Table 1.
Evidence of circulating serum fenthion, which could no longer be detected at day 11.

Discussion

Acute neurotoxic effects during the cholinergic phase of organophosphorus intoxication are caused by the inhibition of the enzyme acetylcholinesterase, resulting in a depolarizing neuromuscular block [2]. Nerve damage of a delayed type, organophosphor-induced delayed polyneuropathy [OPIDP], is also well recognized. OPIDP is triggered by phosphorylation and subsequent inhibition of the neuropathy target esterase (NTE), followed by an irreversible biochemical step, called aging. The delayed polyneuropathy is characterized by a distal and mainly motor polyneuropathy with muscle atrophy. Electromyographic examination and nerve biopsy indicate chronic axonal degeneration with secondary demyelinisation and remyelinisation. The cranial nerves and respiratory muscles remain intact. The delayed polyneuropathy occurs 1 to 3 weeks after the acute intoxication [3, 4].

Recently an intermediate syndrome was described as a distinct clinical entity in 10 Asian dark skinned patients with organophosphorus intoxication [5], including fenthion in 4 cases. Nine were male. The neurotoxic effects generally ocurred 24 to 96 hours after poisoning and after a well defined acute cholinergic phase. In each case patients were conscious when developing the intermediate syndrome. Recovery generally ocurred 4 to 18 days after onset. Characteristically an acute muscular weakness was observed, predominantly affecting the neck flexors, the proximal limb muscles and the respiratory muscles, leading to respiratory insufficiency. Cranial nerve palsies were also not uncommon. Fasciculations or other cholinergic symptoms did not occur. Neither the clinical picture during the cholinergic crisis nor its treatment made it possible to predict the development of the intermediate syndrome or to change it. The causing agent belonged in at least 9 out of 10 cases to the dimethoxy compounds, which includes fenthion. Electromyographic studies indicated a decrementing response or fade on tetanic stimulation, absence of fade on low frequency stimulation and absence of post-tectanic facilitation. Nerve conduction remained normal in all cases.

In retrospect, the type II paralysis after organophosphorus-intoxication, as described by Wadia et al. in 1974, seems identical to this intermediate syndrome [6].

Type II paralysis signs appeared from 12–72 hours after poisoning, and lasted up to 5–6 days. These signs were seen more frequently with some organophosphates than with others, even with similar acute treatment schedules, and lasted longer with fenthion than with other organophosphates. In 1987 Wadia et al. reported EMG and nerve conduction studies in 66 suicide attempts with organophosphate compounds [7]. During muscle paralysis nerve conduction velocity and distal latencies were normal in even severely paralysed patients. On repetitive stimulation there was a decrement at three

stimuli per second in only two cases, who went into respiratory paralysis 24 hours later, and only an occasional decrement at 10 per second. At 30 Hz several cases showed a decrement even in the absence of paralysis. They found that a significant decrement may last for 4–11 days after onset of paralysis.

These neurophysiologic findings were seen to demonstrate a reversible lesion at the myoneural junction and perhaps also at the anterior horn cell. A personal communication by Mishra mentioned 24 subjects with chronic occupational exposure to fenthion and with normal nerve conduction velocities [7].

A similar pattern of electromyographic abnormalities was thus found in both clinical series, respectively in the intermediate syndrome [5] and in type II paralysis [7].

In our patient a "typical" clinical-neurological picture of intermediate syndrome with acute weakness of the neck flexors, proximal muscles of the limbs and respiratory muscles was largely masked because of the necessity to reintubate the patient as early as 43 hours after admission because of a recurrence of cholinergic crisis.

Such recurrences have not been described before. In this case the recurrence is to be considered as iatrogenic due to a too early reduction of oxime and atropine dosage, when serum fenthion concentrations remained high and no significant acetylcholinesterase activity could yet be detected (Fig. 2). Phosphoric-acidic esters such as fenthion are known to have a high distribution volume and can be released from the tissues for a long period [8]. Moreover fenthion causes an often lengthy respiratory depression and in two cases a relapse has been described in spite of an apparently adequate therapy [9, 10, 11]. Unfortunately no assay of acetylcholinesterase-activity was available for the series of 10 patients described as intermediate syndrome [5].

In fact, our patient had one cholinergic crisis with an iatrogenic relapse under high circulating serum fenthion.

The persisting muscle weakness and ventilator dependency with electromyographic findings including a decrement on high-frequency stimulation at 20 Hz 7 days after the intoxication are consistent with the interval described by Senanayake et al. [5]. Fasciculations, rest activity and other cholinergic symptoms were absent at this stage. Serum fenthion levels had fallen to near the detection limit. Finally, the recovery with concomitant disappearence of the decrement on high-frequency stimulation occurred within the range of the same series of Senanayake. This evolution in the later stage after organophosphorus-intoxication confirms the diagnosis of intermediate syndrome.

The fact that de novo axonal polyneuropathy – which was first observed in the second EMG, and showed a clear regression after only two weeks – is inconsistent with both the intermediate syndrome and the delayed polyneuropathy after organophosphorus intoxication does not invalidate the diagnosis of an intermediate syndrome. Indeed it should be remembered that a polyneuropathy of unclear origin frequently develops in critically ill and ventilated patients in the intensive care unit [12].

The etiopathogenesis of the intermediate syndrome is still obscure. However, in our opinion the neurotransmission at the synaptic cleft and the concept of the safety margin or transmission surplus in particular play a central role. In normal circumstances depolarisation of the presynaptic nerve terminal is followed by the release of acetylcholine molecules into the synaptic cleft. Because of the acetylcholine surplus, there is a high probability of interaction with the postsynaptic receptor and only a small fraction of the molecules released is necessary to cause an end-plate potential that surpasses the treshold for muscular response. After 2 msec acetylcholine detaches from the receptor and is hydrolysed by the enzyme acetylcholinesterase into acetate and choline, the latter being reused for the production of acetylcholine.

In myasthenia gravis a shortage of acetylcholine receptors decreases the probability of interaction. This lower safety margin leads to lower endplate potential amplitudes of the muscle fibers involved, even to the point where the treshold is no longer surpassed. This causes general decrease in muscle strength. Since the presynaptic nerve terminal releases less and less acetylcholine in repeated nerve stimulations, even if neuromuscular transmission is intact, the safety margin will decrease even further with the treshold potential being reached in even fewer muscle fibers. Electromyographically this leads to a pathological decline in response or fade on both low- and high-frequency stimulation. The aim of a treatment with carbamates in myasthenia gravis is to increase the safety margin by limiting acetylcholine hydrolysis.

In organophosphorus intoxications phosphorylation and inactivation of the enzyme acetylcholinesterase leads to an enormous surplus of acetylcholine, inducing a cholinergic syndrome. The aim of the early administration of an oxime is to remove the organophosphate from the hydrolase in order to restore the enzyme activity [2]. This prevents

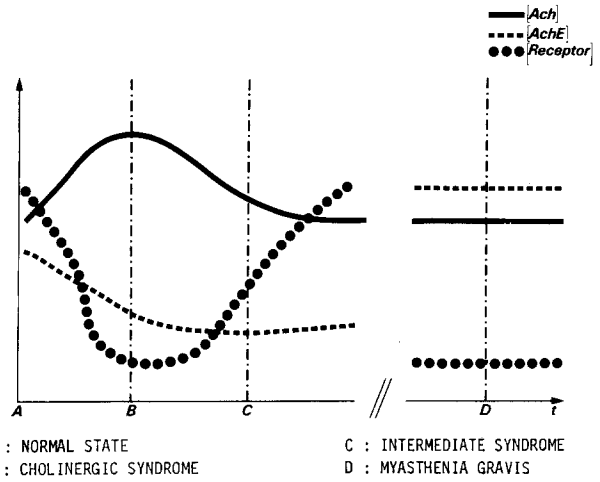

Fig. 6. Evolution of acetylcholine, acetylcholinesterase, and acetylcholine-receptor concentrations in the course of severe organophosphorus intoxication

aging, the irreversible loss of enzyme activity after metabolic cleavage of a group from the organophosphorus-acetylcholinesterase-complex. There are marked differences in degree and time course of aging between different organophosphorus compounds. Aging seems to occur less frequently in the dimethoxy compounds, which turn to be closely associated with the intermediate syndrome [5]. The electromyographic findings (fade on tetanic stimulation, absence of fade on low-frequency stimulation and absence of post-tetanic facilitation) are comparable to those occurring in myasthenia gravis, with the exception of the decrement on low-frequency stimulation, which is absent in the intermediate syndrome. So the neuromuscular dysfunction in the intermediate syndrome is post-synaptic, as in myasthenia gravis, but is probably characterized by a larger safety margin.

In the case of the correctly treated cholinergic crisis the caricatural imbalance between the excessively high amount of acetylcholine in the synaptic cleft and the inhibition of acetylcholinesterase is progressively reverted. At the same time a desensitisation process occurs with a decrease in the number of acetylcholine receptors under a protracted and abundant presence of acetylcholine [13]. This leads at a particular stage of the intoxication to a situation similar to myasthenia gravis, but with a greater availability of acetylcholine and possibly also of receptors (Fig. 6). This suggests the hypothesis that the morphopathologic changes in the postsynaptic device structures and the time-confined phenomenon described play a role in the etiopathogenesis of the intermediate syndrome. The possible involvement of the solvent used, of the toxico-kinetics of the organophosphoric-acidic ester and of possible interactions at the molecular level distally of the post-synaptic membrane remain to be studied more thoroughly.

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