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CHANGES IN THE LEVELS AND FORMS OF RAT PLASMA CHOLINESTERASES DURING CHRONIC DIISOPROPYLPHOSPHOROFLUORIDATE INTOXICATION

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Abstract—The effect of chronic administration of diisopropylphosphorofluoridate (DFP) on the levels and forms of plasma cholinesterase (ChE), were studied in male Wistar albino rats sacrificed at different time intervals after various schedules of treatment. In particular the inhibition and recovery rate of the enzymatic activity was evaluated for butyrylcholinesterase (BuChE), determined using butyrylthiocholine (BuThCh) as substrate and for acetylcholinesterase (AChE), measured using acetylthiocholine (AcThCh) in the presence of iso-OMPA 0.1 mM. At 1½ and 24 hr after the DFP treatments, BuChE was considerably more depressed than was the case for AChE. Moreover, the recovery of BuChE proceeded more slowly, its activity being restored only seven days after the last treatment, while the recovery of AChE was completed 72 hr after the end of the treatments. Plasma molecular forms were separated by polyacrylamide gel electrophoresis and were revealed by enzymatic reaction with BuThCh or AcThCh as substrates. By using selective inhibitors, five main molecular forms of BuChE and two of AChE were found to exist in control plasma samples. A differential inhibition and recovery rate was observed among these forms after DFP intoxication. At 1½ hr after the treatments, the BuChE activity was too low to be detected on the gels, but 24 hr thereafter, the quantitative determination of the different forms, performed by scanning densitometry, showed a significant increase of the two faster migrating ones. At the following time intervals, the electrophoretic pattern returned progressively towards normality. The faster migrating forms are therefore probably the first synthesized in the process of recovery of BuChE activity. As for AChE activity, in control plasma the most intensely coloured band accounted for about 40% of the total activity detected on the gels whereas at the time of maximal inhibition by DFP it represented about 80%. Its subsequent recovery occurred very quickly, namely to about 95% of control activity by about 48 hr after the last treatment.

It is well known that plasma cholinesterase (ChE) is an important target of the inhibitory effect of the organophosphorus (OP) compounds. Studies on the experimental model represented by treated laboratory animals [1–5] have suggested that plasma ChE may be a more sensitive parameter for the biological monitoring of persons exposed to some of these compounds than erythrocyte ChE, though the latter enzymatic test has the advantage of a slower recovery rate.

In order to gain a better insight on the significance of plasma or serum ChE levels during chronic intoxication from OP compounds and to provide further information on the turnover of this enzyme, we considered useful to study, in an animal model, the fine balance between inhibition and recovery of enzymatic activity, in terms of the relative contribution of the individual molecular forms. Up to now very little attention has been paid to the differential inhibition of the ChE molecular forms of plasma, while many studies have been carried out on this aspect in various other tissues as rat brain [6–12], retina [13] and muscle [14]; differences in susceptibility to inhibition have been shown among the molecular forms of rat brain acetylcholinesterase (AChE) after treatment with parathion [6] or diisopropylphosphorofluoridate [7–12].

As regards plasma ChE, it may be remembered here that studies using specific substrates and inhibitors have established that plasma (or serum) of rat [15, 16] and of several other animals, e.g. chicken [17], dog [18], cat [19] and rabbit [20], contain, besides butyrylcholinesterase (BuChE) or nonspecific or "pseudo" ChE (acylcholine acylhydrolase; EC 3.1.1.8.), also AChE (acetylcholine hydrolase; EC 3.1.1.7). Moreover female rats have been demonstrated to have much higher plasma ChE levels than males; this difference is linked to circulating steroidal hormones and is due entirely to BuChE [21, 22]; the latter enzyme was found to be affected by thyroid hormone [23].

As regards the characterization of rat plasma molecular forms, the information actually available is incomplete and in part contradictory. In fact Vijayan and Brownson [24] in an electrophoretic study on ChEs from different tissues, obtained in female rat plasma seven bands of BuChE and three bands of AChE; one of the latter (the heavier staining and faster migrating) had the same electrophoretic mobility as one of the three bands obtained from brain (the intermediate migrating). Illsley *et al.* [25] separated electrophoretically six molecular forms of hydrolytic activity towards AcThCh in female rat plasma, while males exhibited only three forms; alterations of these patterns were observed under different hormonal conditions. However the latter study does not differentiate between AChE and BuChE. By means of sucrose density gradient

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centrifugation, rat plasma AChE was found to have a sedimentation constant of about 10S [26], while rat erythrocytes contain a lighter form of AChE [26, 27].

Several papers deal with the alterations in the levels of total ChE in rat plasma after treatment with OP compounds, but only recently AChE and BuChE have been considered separately [28]. However information on the susceptibility of the different molecular forms of these enzymes is lacking.

In the present study the following terms will be used: "total ChE" for the hydrolytic activity towards AcThCh without the use of inhibitors; "AChE" for the hydrolytic activity towards AcThCh in the presence of iso-OMPA and "BuChE" for the hydrolytic activity towards BuThCh (for explanation see first paragraph of results).

Our purpose was to study the modification of the levels and molecular forms of rat plasma AChE and BuChE at different time intervals during and after treatment with various doses of DFP. In order to do this, our attention has been necessarily also focused on the separation and quantitative determination of the molecular forms of AChE and BuChE in normal rat plasma.

MATERIALS AND METHODS

Chemicals. Acetylthiocholine iodide (AcThCh), butyrylthiocholine iodide (BuThCh), physostigmine salicylate (eserine) and acrylamide-bis Premix 4% C, were purchased from Serva (Feinbiochemica Heidelberg); 2,2'-dinitro-5,5'-dithiodibenzoic acid (for biochemistry) from Merck, diisopropylphosphorofluoridate (DFP) from Fluka (Buchs, Switzerland), tetraisopropylphosphoramide (iso-OMPA) and 1,5-bis-(4-allyl dimethyl-ammoniumphenyl)-pentan-3-one-dibromide (BW284C51) from Sigma Chemicals.

Animals and treatment. In all experiments male Wistar albino rats, purchased from Charles River Italia S.p.A. (Calco, 22050 Como), were used. Male rats weighing 150–200 g were injected subcutaneously every second day with a first dose of 1.1 mg/kg DFP (in arachis oil) and subsequent doses of 0.7 mg/kg each until the 23rd day. Groups of 5 rats (4 DFP treated and one, used as control, injected with the vehicle alone) were sacrificed 1½ and 24 hr after the 1st, 2nd, 4th, 6th, 9th and 12th DFP administration and at various intervals (48, 72 hr, 7, 14, 28 and 35 days) after the 12th treatment. According to this schedule, the animals sacrificed on day 1 received only one dose of 1.1 mg/kg, while those killed on day 3, 7, 11, 17 and 23 received in addition to the first dose of 1.1 mg/kg, 1, 3, 5, 8 and 11 doses of 0.7 mg/kg respectively.

Enzyme analyses. Blood samples were collected in heparinized containers during animal decapitation from control and from DFP treated rats. Plasma was separated by centrifugation at 1000 *g* for 30 min, at 4° and its cholinesterase activity was determined according to the method of Ellman *et al.* [29] using either BuThCh 15 mM or AcThCh 0.9 mM as a substrate. The assay medium contained 50 mM Tris buffer (pH 7.5), 0.3 mM dithiobisdinitrobenzoic acid and the substrate BuThCh or AcThCh, in the appropriate concentration. The reaction was monitored at 30° and 412 nm, in cuvettes of 1 cm pathlength, after 20 μl of plasma samples were added to 1 ml of medium. Eserine 0.1 mM, a selective inhibitor of cholinesterase, iso-OMPA 0.1 mM, a selective inhibitor of pseudo-ChE, and BW284C51 (50 μM), a selective inhibitor of AChE, were used in the various experiments. These inhibitors were added to the reaction medium 30 min before the addition of the substrate.

Enzyme activity was expressed as mU/ml plasma (1 mU = 1 nmole of substrate hydrolysed/min at 30°, pH 7.5).

Electrophoretic analyses. Polyacrylamide gel electrophoresis was carried out according to the method of Clark (30) in 7% (w/v) polyacrylamide cylindrical gels (6 × 100 mm) with Tris–glycine buffer pH 8.1 (4.8 mM Tris and 37 mM glycine). Plasma samples containing a constant number of activity units were applied on the gels, namely 1 mU when BuThCh was used as a substrate and 2 mU with AcThCh. Electrophoresis was carried out at 0.5 mA per tube for 5 min and at 2.5 mA per tube for about 45 min (this time corresponded to the dye elimination).

ChE activity was detected on the gels by Koelle's histochemical method [31], as modified by Chubb and Smith [32], in the presence of AcThCh (0.9 mM) or BuThCh (15 mM). Inhibition assays were performed with eserine 10 μM, iso-OMPA 10 μM and BW284C51 50 μM, the inhibitors being added both in the preincubation and incubation medium. The copper thiocholine precipitate obtained was stained with dithio-oxamide and densitometrically measured at 566 nm by means of a Gilford 2400 spectrophotometer, equipped with a gel scanning system. The relative activity of the bands was evaluated by graphic integration of the peak areas using a Hewlett–Packard (9804) digitizer. Linearity of the optical density response to increasing enzymatic activity had been demonstrated in a preliminary experiment, in the range between 0.5 and 4 mU.

RESULTS

Normal plasma. Cholinesterase activity of normal rat plasma determined using AcThCh or BuThCh as substrates was completely inhibited by preincubation (for 30 min) with eserine 0.1 mM. Iso-OMPA 0.1 mM inhibited about 50% (48.9 ± 2.9%, $\bar{x}$ ± S.E. of 19 samples) of the ChE activity towards AcThCh and more than 85% (85.9 ±2.7%) towards BuThCh, while BW284C51 50 μM caused little inhibition (6.4 ± 1.2%) of the activity towards the latter substrate, and about 60% (64.3 ± 3%) towards AcThCh.

When polyacrylamide gel electrophoresis was carried out using BuThCh as substrate, nine bands (molecular forms) of ChE activity, all inhibited by eserine 10 μM, were revealed (Fig. 1). The relative activity of the bands, which were numbered from 9 to 1 (the most anionic form), measured as total activity percent, is shown in the insert of Fig. 1. When specific inhibitors were added to gel medium, none of these bands were apparent in the presence of iso-OMPA, while the five major ones were detectable in the presence of BW284C51 (Fig. 2) and could therefore be ascribed to BuChE activity.

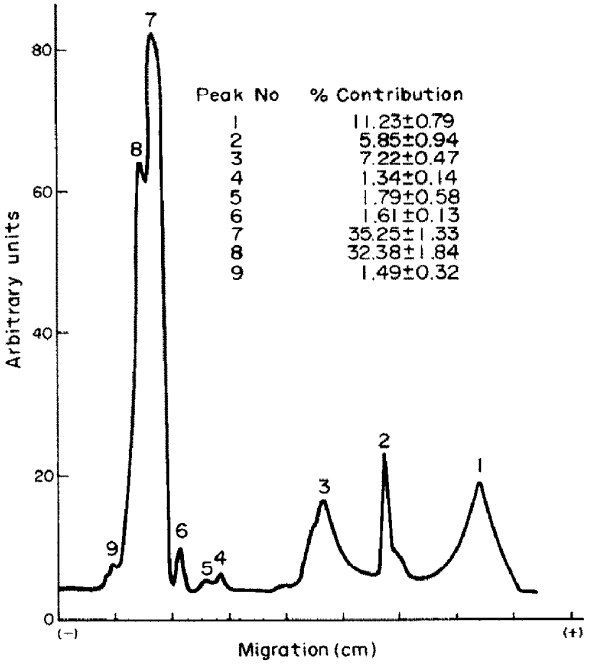

Fig. 1. Electrophoretic pattern of control rat plasma ChE determined with BuThCh as substrate. The activity peaks are numbered 9 to 1 (the most anionic form). The percentage contribution of each peak to the total enzyme activity detected on the gel (mean ± S.E. of 18 control rats) is reported in the insert.

A different electrophoretic pattern was revealed in the presence of AcThCh: an intense band, consisting of about 40% of the total activity, appeared in the same zone as forms 4 and 5 of the preceding pattern and will therefore be called form 4–5; form 6 also stained more intensely than was the case when using BuThCh as substrate (about 10% of the total activity). On the other hand, while bands 1 and 2 were often not detectable, a lower relative activity was revealed for bands 3, 7, and 8 (Fig. 3). The

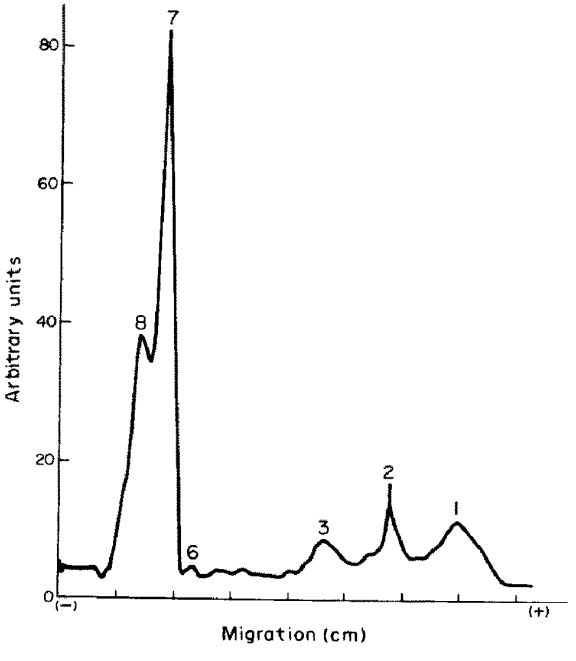

Fig. 2. Electrophoretic pattern of control rat plasma ChE determined with BuThCh as substrate in the presence of BW284C51, 50 μM.

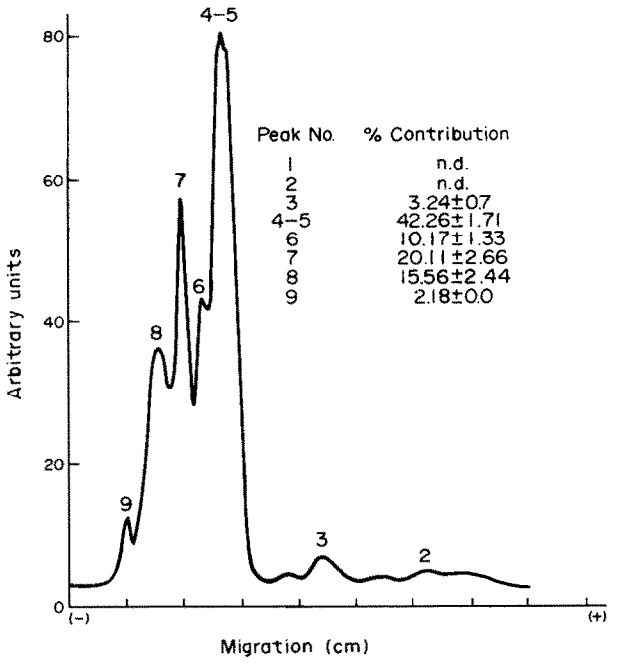

Fig. 3. Electrophoretic pattern of control rat plasma ChE determined with AcThCh as substrate. The activity peaks are numbered 9 to 1 (the most anionic form). The percentage contribution of each peak to the total enzyme activity detected on the gel (mean ± S.E. of 16 control rats) is reported in the insert.

relative contribution of these activity bands is shown in the insert of Fig. 3. Two of these bands could be attributed to AChE activity (4–5 and 6) since they were intensely stained in the presence of iso-OMPA (Fig. 4) but were completely inhibited by BW284C51.

Effects of DFP treatments on ChE levels. Table 1 reports the levels of total ChE, BuChE and AChE activities (in absolute values) for all treatment groups at 1½ and 24 hr after treatment. At 1½ hr after dosing, plasma BuChE showed a greater reduction (to about 12% of control value was the mean of all treatment

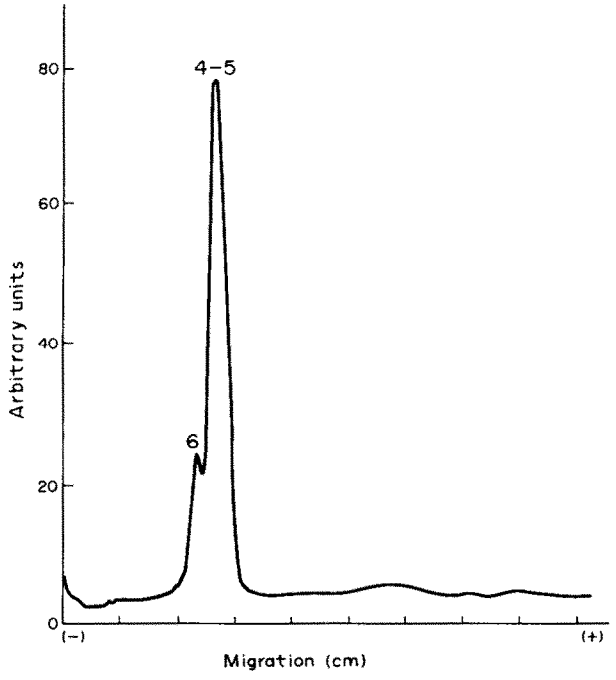

Fig. 4. Electrophoretic pattern of control rat plasma determined with AcThCh as substrate in the presence of iso-OMPA, 10 μM.

Table 1. Plasma ChE activity during chronic intoxication by DFP, determined in the presence of AcThCh (total ChE activity), BuThCh (BuChE activity), AcThCh and iso-OMPA (AChE activity)

No. of DFP doses*	Total ChE activity (mU/ml)†	BuChE activity (mU/ml)†	AChE activity (mU/ml)†
Control	452 ± 17	175 ± 12	210 ± 8
1½ hr after treatment			
1	91 ± 4	29 ± 3	96 ± 2
2	99 ± 9	25 ±4	88 ± 7
6	72 ± 13	15 ± 1	73 ± 9
9	89 ± 7	20 ± 0.3	75 ± 7
12	106 ± 14	21 ± 3	80 ± 6
24 hr after treatment			
1	193 ± 20	72 ± 11	144 ± 8
2	226 ± 14	65 ± 6	156 ± 12
4	196	66	156
6	212 ± 8	67 ± 5	169 ± 17
9	200 ± 15	59 ± 5	141 ± 9
12	218 ± 25	59 ± 2	175 ± 17

* DFP doses were injected sub-cutaneously every second day, until the 23rd day: first dose 1.1 mg/kg, subsequent doses 0.7 mg/kg.

† Mean ± S.E. of four rats for each DFP treated groups and of 18 rats for the control group. Where S.E. is not indicated, the data refer to a pool of 4 samples.

groups investigated) than was the case for total (about 20%) and AChE (about 39%) activities. The same was true at 24 hr after DFP treatment, while at this time a significant recovery of the ChE activities was already revealed (37% of the control valued for BuChE, 46% for total and 74% for AChE).

The differences in enzyme activity in relation to the number of DFP injections were not statistically significant ($P > 0.05$) either at 1½ or at 24 hr after treatment (ANOVA test 2×6 on data at 1½ and 24 hr, i.e. not considering control data).

Figure 5 indicates the recovery pattern of plasma ChE activity, at several time intervals after the administration of one dose of 1.1 mg/kg DFP and 11 subsequent doses of 0.7 mg/kg. The BuChE activity recovered completely only seven days after dosing, with a more rapid rate during the first 48 hr (to about 60% of the control value). On the other hand the AChE activity was completely recovered

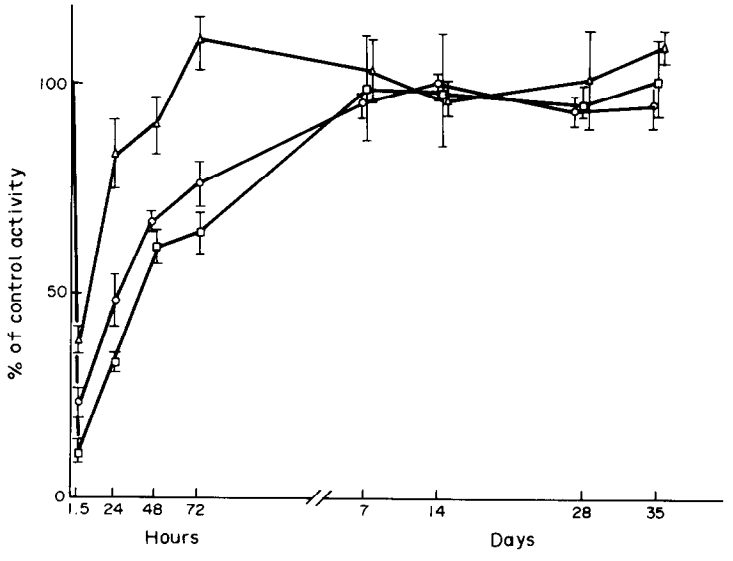

Fig. 5. Inhibition and recovery pattern of total ChE (○———○), AChE (△———△), and BuChE (□———□) activities, determined in rat plasma at several time intervals after the last administration of DFP, on day 23. The results (mean ± S.E. of 4 rats for each group) are expressed as percentage of control activity.

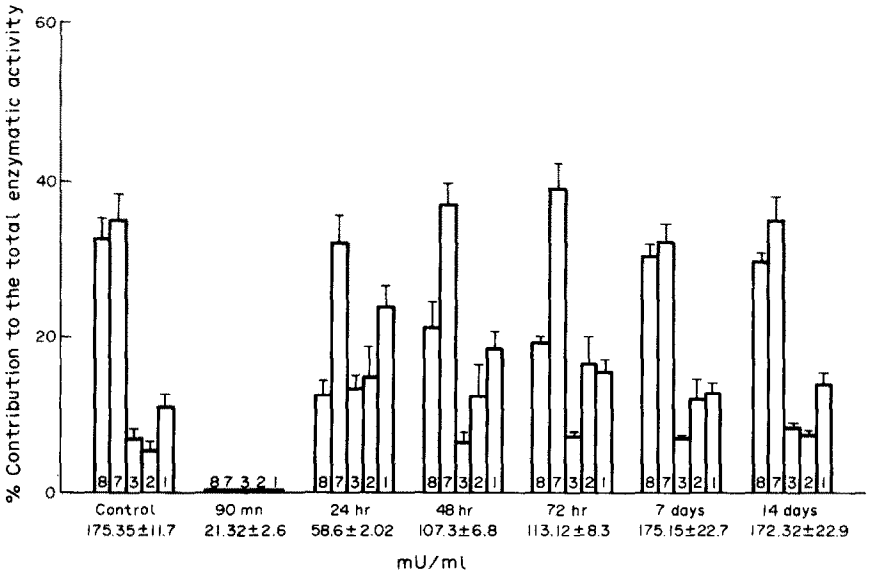

Fig. 6. Percent contribution to total activity of the five most active ChE forms, at different time intervals after the last DFP treatment, determined with BuThCh as substrate. The results are expressed as mean ± S.E. of 4 rats for each DFP treated group and as mean ± S.E. of 18 rats for the control group. Pooling together the data of the six treatment groups, statistically significant changes of the relative contribution were found at 24 hr after DFP injections, for form 1 (28.4 ± 2% instead of 11.2 ± 0.8% in controls, $P < 0.001$), 2 (16 ± 1.6% instead of 5.8 ± 0.9%, $P < 0.001$), 3 (11.8 ± 1.3% instead of 7.2 ± 0.48%, $P < 0.001$) and 8 (7.5 ± 1.2% instead of 32.4 ± 1.8%, $P < 0.001$).

by 72 hr after treatment, with a more rapid rate (about 80% of the control value) in the first 24 hr. An intermediate trend was followed by total ChE activity.

Effects of DFP treatments on ChE electrophoretic patterns. The alterations of the electrophoretic patterns caused by DFP were studied without the use of specific inhibitors.

With BuThCh as substrate, 1½ hr after treatment, none of the activity bands were detectable in the gels for any of the treatment groups, but at the following intervals quantitative changes became evident for the five more intense forms. At 24 hr, when the data for the six treatment groups were pooled, the relative contribution of the three faster moving forms to the total enzyme activity revealed on each gel was significantly increased with respect to controls; this was especially true for forms 1 (28 instead of 11%) and

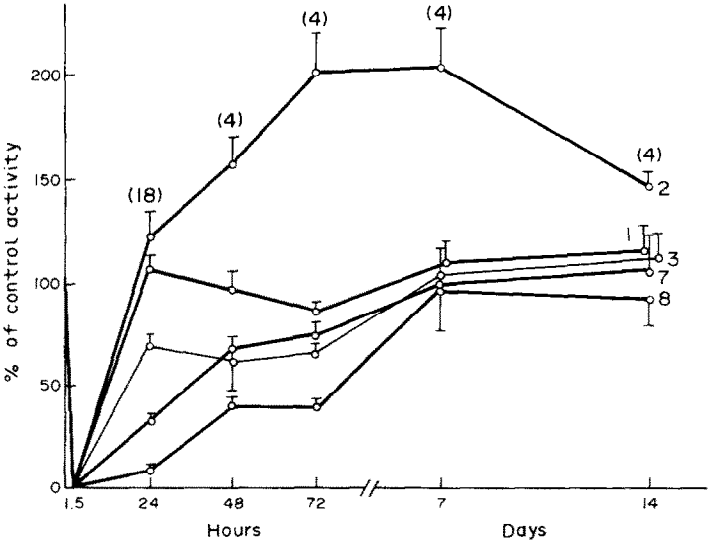

Fig. 7. Recovery of the five most active forms of BuChE at different time intervals after the last treatment, revealed with BuThCh as substrate. The activity of each individual form is expressed as percentage of its activity in control rats.

2 (15 instead of 6%). At the same time the activity of form 8 appeared markedly decrease while the contribution of form 7 was not statistically different from the control value. During the following recovery steps a progressive normalization of the electrophoretic pattern occurred, with a decrease in relative contribution of the faster moving forms and an increase of the slower ones, until at 14 days the normal pattern was restored (Fig. 6). The differential recovery pattern of the five higher activity bands, expressed as percent activity of the control values, as illustrated in Fig. 7. In particular the recovery of form 2, which already at 24 hr had reached about 100% of its control value, increased further in the following days until a maximum was obtained at seven days (about 200% of the control value) and later returned towards 100% of the control activity.

Concerning the electrophoretic pattern revealed in the presence of AcThCh, forms 4–5 and 6 were the only ones which were detectable at 1½ hr after treatment. At 24 hr the two slower moving forms (7 and 8) were again detectable, while the relative contribution of form 4–5 tended to decrease although its difference from the control value remained statistically significant. This trend towards normalization proceeded at the successive time intervals until at seven days an approximately normal distribution was restored (Fig. 8). Figure 9 shows the pattern of inhibition and recovery of each form at the different times, expressed as percent activity of the mean of control values. Form 4–5 was less inhibited than form 6 at 1½ hr after treatment and recovered more quickly, about 95% of its control activity being present at 48 hr after treatment. As concerns forms 7 and 8, a slower recovery was observed, which was completed at seven days after DFP treatments.

DISCUSSION

The presence of AChE in plasma of different animal species and its relative contribution to the total plasma ChE activity are still controversial. The experiments of Augustinsson [33] already suggested the presence of this enzyme in the plasma of goat, cow and rabbit. Chubb *et al.* [20] report that more than 90% of the ChE activity of rabbit plasma can be attributed to AChE.

As concerns rat plasma, the presence of AChE, still denied by some authors (see e.g.25), results from experiments on substrate preferences [15] and sensitivity to inhibitors of the ChE activity [16]. In substantial agreement with the inhibition curves pre-

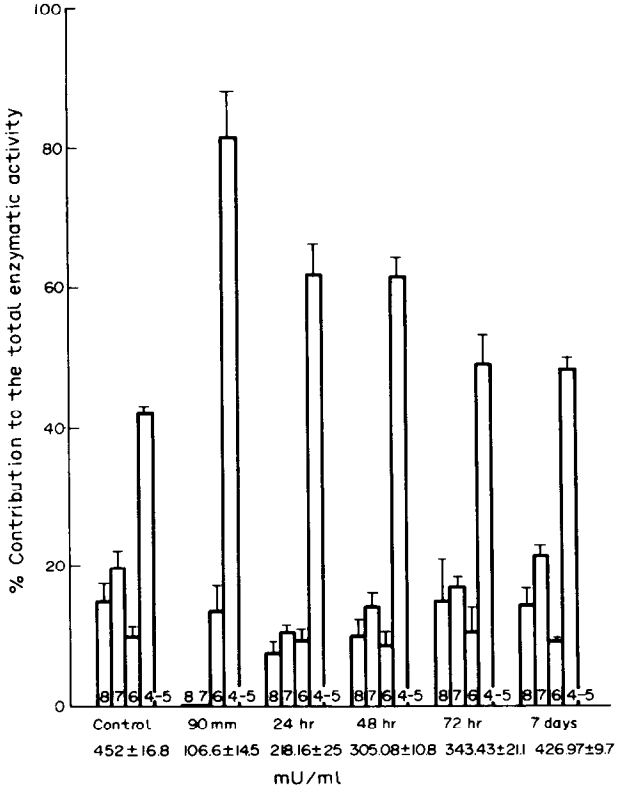

Fig. 8. Percent contribution to the total activity of the four most active ChE forms at different time intervals after the last treatment, revealed with AcThCh as substrate. The results are expressed as mean ± S.E. of 4 rats for each DFP treated group and as mean ± S.E. of 16 rats for the control group. Pooling together the data of all the treatment groups, statistically significant changes of the relative contribution were found for form 4–5 at 1½ hr after DFP injections (88 ± 1.8% instead of 42.3 ± 1.7% in the controls, $P < 0.001$) and at 24 hr (61.5 ± 1.8% instead of 42.3 ± 1.7% in the controls, $P < 0.001$) and for form 8 at 24 hr after dosing (7 ± 1.3% instead of 15.5 ± 2.4% in the controls, $P < 0.001$).

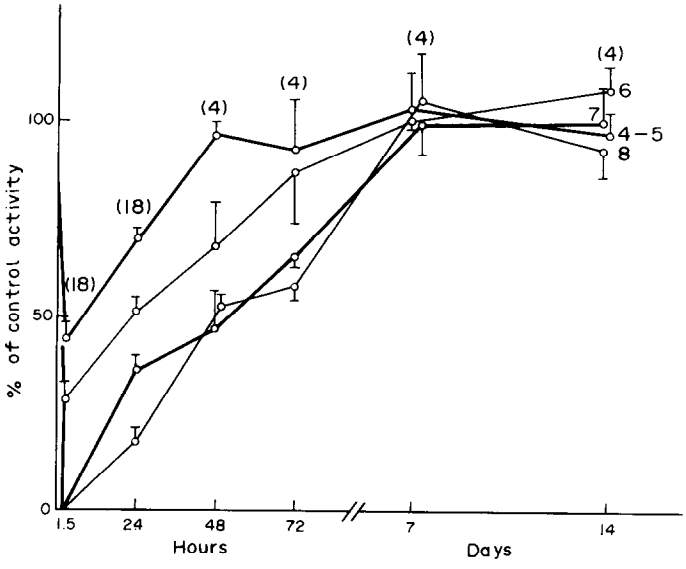

Fig. 9. Recovery of the four most active forms at different time intervals after the last DFP treatment revealed with AcThCh as substrate. The activity of each individual form is expressed as percentage of its activity in control rats.

sented by the latter authors, we found that the hydrolysis of BuThCh is strongly inhibited in rat plasma by 0.1 mM iso-OMPA (about 86% inhibition), but only slightly by 50 μM BW284C51 (6% inhibition). This type of sensitivity to inhibitors is typical of BuChE [34]. The residual hydrolysis of AcThCh in the presence of 0.1 mM iso-OMPA (51% of activity left) is thus mostly due to AChE; this is confirmed by the inhibition levels of AcThCh hydrolysis obtained in the presence of 50 μM BW284C51 (about 64%). The two residual activities account for about 90% of the total, suggesting that they reflect relative contributions of AChE and BuChE (approximately 50% each) to AcThCh hydrolysis.

The experiments on the levels and composition in molecular forms of AChE during and after chronic DFP intoxication brought additional evidence for the presence of this enzyme in rat plasma and some conclusions on its properties. 1—BuChE activity of male rat plasma was more inhibited by DFP than AChE; this was true for all the different schedules of treatment tried in this work and at all times before total recovery (Table 1). In fact DFP is known to be a more specific inhibitor of BuChE than AChE [34]. AChE activity, besides being less affected, also recovered more rapidly after DFP treatment than BuChE (see Fig. 5).

2—Upon gel electrophoresis of normal rat plasma in the presence of AcThCh, a prominent feature of the pattern was an intensely staining form (4–5) which had the following characteristics: it was not detectable when BuThCh was used as substrate nor when BW284C51 was present and it could not be inhibited by iso-OMPA 10 μM. It was still intensely stained at the time of maximal inhibition (1½ hr after DFP treatment) and quickly recovered thereafter, with 70% of the control value restored at 24 hr after treatment and 95% after 48 hr; this indicates that the ChE activity detectable in whole plasma 1½ hr after DFP treatment (Table 1) consisted practically only of AChE.

While BuChE is considered to be synthesized by the liver [35], the origin of the plasma AChE is not well elucidated at present. According to some hypotheses it could have the same origin as cerebrospinal fluid (CSF) AChE, i.e. it might be secreted by AChE containing neurons into the cerebrospinal space, as suggested by Chubb *et al.* [20] in their work on rabbits and by Scarsella *et al.* [18] on Beagle dogs. In this connection it must be pointed out that in previous studies [10, 11] on the acute and chronic effects of DFP on soluble AChE from rat brain, one of the three molecular forms revealed by polyacrylamide gel electrophoresis (the medium migrating form) was less affected (40% activity left) than the other two at 3 hr after treatment, and recovered more quickly as shown by the fact that its activity reached 80 and 95% of pretreatment levels at 18 and 48 hr respectively, while at these times the two other forms were still markedly inhibited. Moreover when a sample of rat brain soluble AChE (kindly supplied by the authors and prepared as described in ref. 10) was run on polyacrylamide gel in parallel with rat plasma using AcThCh as a substrate, this molecular form migrated with the same mobility as band 4–5 (unpublished results).

As regards BuChE of rat plasma, the trend towards recovery from OP treatment *in vivo*, which shows a more rapid rate in the first 48 hr, is in good agreement with previous studies: e.g. Fleming [5] obtained a recovery of about 50% of the plasma ChE activity level (from mallard ducklings) within two days after exposure to dicrotophos and fenthion regardless of how far below 50% the ChE activity was depressed; Ecobichon *et al.* [1] reported a return of plasma BuChE of rat to 78% of control activity values within 5 days after daily doses of fenitrothion;

also Mikalsen *et al.* [26] obtained recovery of the normal activity level of rat plasma ChE, 6 days after inhibition with soman.

Concerning the electrophoretic patterns, in our experimental conditions the BuChE activity was too low to be detected on the gels at the time of maximal inhibition after DFP injections, however a marked increase in activity of the faster moving forms with respect to their control values was already evident at 24 hr and at the following time intervals. DFP is known to be an irreversible inhibitor of ChE [34], i.e. a dephosphorilation of the enzyme with subsequent reactivation would occur at an extremely slow rate and the observed regeneration of BuChE activity would therefore largely depend on *de novo* synthesis. Bearing this in mind, it is suggested that in our experimental conditions the faster migrating forms could correspond to the first steps of the polymerization of the enzyme [36]; this would explain their earlier appearance after DFP treatments. Up to now insufficient data is available on the characterization of rat plasma BuChE molecular forms, in particular it is not known if the faster migrating forms are the lighter ones, but in this connection it should be noted that among the four main molecular forms of human plasma BuChE detected by Masson [37], the component with the highest electrophoretic mobility was the monomeric form (M_r 84,000 ± 5800) while the two slower forms were size-isomers corresponding to the dimer and the tetramer of the enzyme.

Finally, a subcutaneous dose of 1.1 mg DFP/kg caused a degree of inhibition of AChE and BuChE activity which remained approximately at the same level after subsequent injections of 0.7 mg DFP/kg every second day (Table 1); also the recovery trend was not influenced by the number of injections. This finding, which still awaits an explanation, is in agreement with the results of other studies on mammals, showing that recovery of ChE activity does not depend on the duration of exposure; e.g. Brodeur and Dubois [38] reported that the 24 hr initial rate of brain ChE recovery was similar for rats after 1 and 60 days of daily disufulton injections.

These observations on the recovery patterns of AChE and BuChE activities in rat plasma, during and after chronic DFP intoxication, suggest that BuChE activity, being more depressed by the treatments, could be considered a more sensitive indicator of the studied effect than AChE. The latter enzyme moreover showed a more rapid recovery, as confirmed also by the results obtained by gel electrophoresis: the two major bands of AChE activity were in fact the only ones which could be detected on the gel at the time of maximal DFP inhibition. However, one could expect, also on the basis of some recent experiments carried out with soman on AChE and BuChE activities of rat plasma [28] that the differential inhibition and recovery rate of the two ChE activities vary according to the different OP compounds used.

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