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# MULTIPLE MOLECULAR FORMS AND LECTIN INTERACTIONS OF ORGANOPHOSPHATE-SENSITIVE PLASMA AND LIVER ESTERASES DURING DEVELOPMENT OF THE CHICK*

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**Abstract**—Liver and plasma acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and carboxylesterase activities of the chick embryo and adult chickens were separated by sucrose density gradient sedimentation and further differentiated by their lectin affinities and organophosphate sensitivites. Changes in plasma cholinesterases during development indicated a characteristic shift in tetrameric ($G_4$) isoforms from a slightly larger $G_4$ AChE in the embryo to $G_4$ BChE in the adult. These changes were not reflected in isoform patterns of liver homogenates, however. Interestingly, the time course of an increase in plasma BChE activity corresponded to the time course of a decrease in liver BChE activity, as if this enzyme was being mobilized and released. The distribution of liver esterases included both monomeric ($G_1$) and $G_4$ BChE and a large *p*-nitrophenylacetate (p-NPA) esterase activity that was separated into two main peaks by density gradient ultracentrifugation. The effects of organophosphate inhibitors indicated that the two liver p-NPA esterase activities may be regarded as carboxylesterases; however, these enzymes showed very different sensitivities to paraoxon and diisopropylfluorophosphate (DFP), with $IC_{50}$ values differing by 3 and 4 orders of magnitude. Lectin affinity studies with multiple esterase forms suggested a heterogeneous group of glycoproteins that were packaged at different sites in the liver cell and were consistent with the presence of an intracellular precursor form to plasma BChE.

Cholinesterases and carboxylesterases (EC 3.1.1.1) in plasma and liver are pharmacologically important organosphosphate-sensitive esterases (B-type esterases [1]). These esterases hydrolyze drugs, pesticides, and other chemical agents [2–5], as well as scavenge organophosphorus compounds (OPs [6, 7] that might otherwise be available to inhibit acetylcholinesterase (AChE, EC 3.1.1.7) in nerve and muscle. Because of the role of esterases in the detoxication of numerous compounds, alterations of their activities have been implicated in both drug-induced toxicity [8] and in the potentiation of pesticides and nerve agents [9, 10]. The extraordinary sensitivity of these enzymes to the OP-ester poisons in pesticides, plasticizers and nerve gas has fostered a continued interest in the diversity of esterases and their roles and regulations.

Previously, we identified changes in plasma cholinesterase activities during development of the chick using selective inhibitors and substrates [11]. Separation of the multiple molecular forms by gel electrophoresis indicated changes in plasma cholinesterase patterns from AChE bands that are typical of the embryo to butyrylcholinesterase (BChE, EC 3.1.1.8) bands typical of the adult. Since plasma cholinesterases and other serum esterases are believed to be formed in the liver, we compared plasma and liver esterase molecular forms and changes taking place during development. Multiple esterase activities were separated by sucrose density gradient centrifugation, and different metabolic and catalytic species were investigated with sugar-specific lectins and OP inhibitors.

## MATERIALS AND METHODS

*Chemicals*. Diethyl *p*-nitrophenyl phosphate (paraoxon) was obtained from the Aldrich Chemical Co. and was purified further (97%) by fraction distillation performed by M. McChesney, Department of Environmental Toxicology, University of California at Davis. Lectins and all other chemicals were purchased from the Sigma Chemical Co. and were of the highest purity commercially available.

*Animals*. White Leghorn eggs were obtained from Hyline, and incubated at 37.5° and 85% humidity, with rotations every hour. Eggs were selected for fertility and normal embryonic development by candling. Chicks were maintained at 37° with a 16:8 hr light:dark cycle and fed chick starter ration.

*Tissue preparations*. Heparinized blood samples, for determination of plasma cholinesterase activity, were obtained by cardiac puncture and were centrifuged at 800 *g* for 5 min to remove blood cells. As much as 1 mL of blood could be collected from 18-day embryos, while blood from 14-day embryos was

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pooled. Livers were perfused via the heart with ice-cold 0.9% sodium chloride and were further rinsed three times and blotted dry after removal from the embryo. Livers were homogenized with 10% (w/v) ice-cold extraction buffer [Triton X-100 (0.5%, v/v), 1.0 M NaCl, 0.2 mM EDTA, and 50 mM Tris–HCl], using a close-fitting Kontes/glass homogenizer, and centrifuged in an SM-24 rotor for 30 min at 28,000 *g* and 4° using a Sorvall RC2-B centrifuge. Supernatants were collected and further diluted for assays and sucrose gradients.

*Enzyme assays.* Cholinesterase activity was determined spectrophotometrically by acetylthiocholine (4 mM) hydrolysis with dithiobisnitrobenzoate (DTNB), according to Ellman *et al.* [12], scaled down for a microplate assay with a final volume of 250 μL of phosphate-buffered medium (0.1 M, pH 8). BChE was distinguished from AChE activity by incubations with a 1 mM concentration of the selective BChE inhibitor iso-OMPA (tetraisopropylpyrophosphoramide) for 5 min, at 22–25°. Carboxylesterase activities were determined in sucrose gradient fractions with *p*-nitrophenylacetate (p-NPA) hydrolysis according to Ljungquist and Augustinsson [13] adapted for a microplate assay using a final reaction volume of 300 μL of Tris–HCl buffer, pH 7.5, and 10 mM substrate. A substrate blank (without enzyme) was run in the same manner for all enzyme assays. To determine $IC_{50}$ values (concentration of inhibitor producing 50% decrease in activity), carboxylesterase fractions were incubated with increasing concentrations of inhibitors, DFP (diisopropylfluorophosphate), paraoxon, physostigmine, or bis(*p*-nitrophenyl)-phosphate (BPNP) for 5 min at 22–25°. The $IC_{50}$ values were calculated by a log concentration-probit inhibition linear regression. For cholinesterase and carboxylesterase assays, changes in absorbance at 410 nm were determined for 6 min in an automated microplate reader (Bio-Tek Instruments Inc.), the data transferred to a microcomputer, and the rates of hydrolysis calculated by software written in the laboratory. Specific activities were based on protein content determined according to Lowry *et al.* [14].

*Sedimentation analysis.* Esterase multiple molecular forms were determined by 5–20% sucrose density gradient centrifugation for 26–28 hr at 202,000 *g* using a Beckman SW 40 rotor and a Sorvall OTD-50 ultracentrifuge [15]. Mean sedimentation coefficients ($S_{20,w}$) for three separate determinations were obtained with sedimentation markers: horse liver alcohol dehydrogenase (4.8 S), *Escherichia coli* alkaline phosphatase (6.1 S), yeast alcohol dehydrogenase (7.4 S), and horse liver catalase (11.4 S) assayed according to standard techniques [16]. Cholinesterase molecular forms, $G_1$, $G_2$ and $G_4$, were identified by their $S_{20,w}$ value [17] and confirmed by the behavior of individual forms under reducing conditions with 20 mM dithiothreitol (DTT) for 1–2 hr at 22–25°.

*Lectin interactions.* Glycosylations of cholinesterases and carboxylesterases were studied with concanavalin A (Con A), wheat germ agglutinin (WGA), coral tree lectin (*Erythrina cristagelli*), and horseshoe crab lectin. Liver (1:19 and 1:39, w/v) and plasma (1:3, v/v) were incubated with lectins (1 mg/mL) for 0.5 hr in an EDTA-free, Tris–Triton extraction buffer containing high calcium (20 mM $CaCl_2$) to facilitate lectin interactions. Samples (200 μL) were loaded onto sucrose gradients and interactions of the lectins with individual cholinesterase and carboxylesterase forms were detected by changes in the sedimentation rate of these activities during ultracentrifugation. In some runs, lectin-specific interactions with esterases were validated by the ability of lectin specific sugars (100 mM) to compete with these interactions and the failure of non-specific sugars to compete.

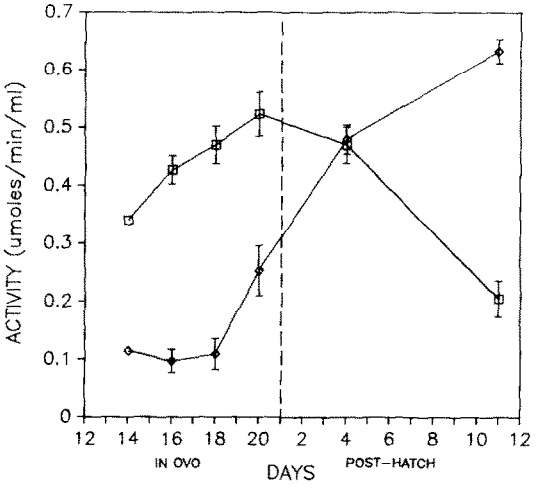


Fig. 1. Changes in plasma AChE and BChE activities during development of the chicken. Key: (□) AChE and (◇) BChE. The broken vertical line represents the day of hatching. The points are mean ± SE values of three to four animals. (Adult plasma BChE and AChE values, respectively, were 0.725 ± 0.0788 μmol/min/mL and 0.0201 ± 0.00804 μmol/min/mL, mean ± SD for ten animals.)

## RESULTS

*Plasma and liver cholinesterase levels and multiple molecular forms during development.* Figure 1 depicts changes in plasma cholinesterase levels from days 14 *in ovo* to 11 days post-hatch. AChE activity reached a peak around hatching and then declined, while BChE activity rose from low levels (10–20% that of adult).

Distribution of cholinesterase activities in embryonic and adult plasma and liver were obtained on sucrose density gradients. Figure 2 shows a typical pattern of plasma cholinesterase forms in embryonic (18-day-old) and adult chicken.

Embryonic plasma contained mostly $G_4$ AChE ($S_{20,w}$, 11.7 ± 0.3) and little $G_4$ BChE, in contrast to adult plasma which contained mostly $G_4$ BChE ($S_{20,w}$, 11.1 ± 0.3) and little $G_4$ AChE. $G_1$ BChE ($S_{20,w}$, 3.9 ± 0.2), $G_2$ BChE ($S_{20,w}$, 5.9 ± 0.3) and $G_2$ AChE ($S_{20,w}$, 6.5 ± 0.2) were other forms that were identified in the plasma. Changes in plasma cholinesterase molecular form patterns did not reflect changes in the liver. Liver homogenates contained two BChE forms (Fig. 3) for both 18-day embryo

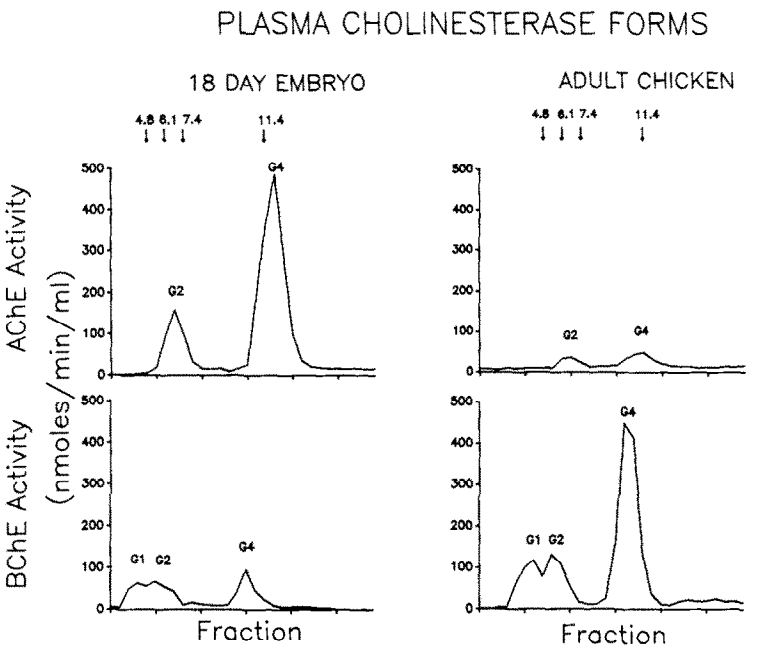


Fig. 2. Developmental changes in the distribution of plasma cholinesterase multiple molecular forms. AChE (top) and BChE (bottom) forms in the plasma of 18-day embryo and adult chicken were separated on 5–20% sucrose density gradients and identified by methods described in Materials and Methods. Arrows indicate the positions of sedimentation markers.

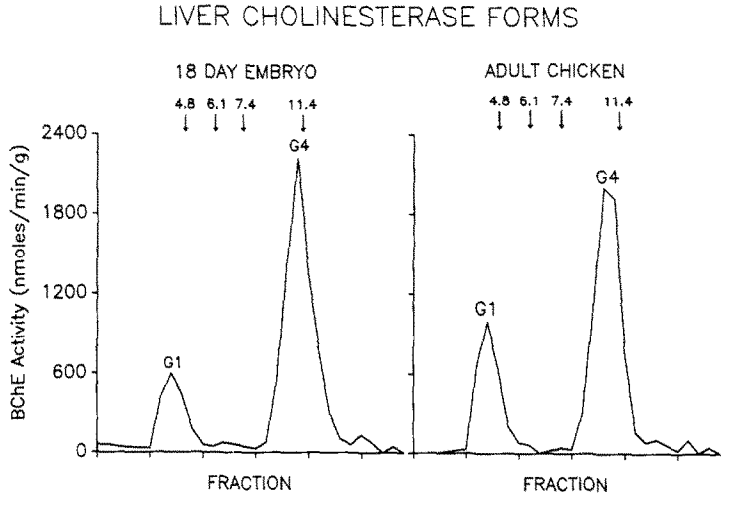


Fig. 3. Cholinesterase molecular forms of a liver extract of 18-day embryo (left) and adult chicken (right). AChE activity in embryo and adult liver homogenates was too low for detection of individual AChE forms by these methods. Arrows indicate the positions of sedimentation markers.

and adult: $G_1$ BChE ($S_{20,w}$, 3.9 ± 0.3) and $G_4$ BChE ($S_{20,w}$, 11.0 ± 0.2).

Eighteen-day embryo and adult livers contained similar cholinesterase activities, with AChE accounting for less than 5% of the total liver cholinesterase activity. We compared changes in BChE levels in the plasma to those occurring in the liver around hatching. There was a reproducible decrease in liver activity. (I.U. per gram wet weight and per milligram protein) corresponding to the time course of increase of plasma BChE; by day 10 however, liver activity had returned to adult levels (Fig. 4).

*Lectin interactions with cholinesterases and non-specific esterases.* Lectin affinities of esterases formed in the liver and present in the plasma suggested that there was a heterogeneous group of glycoproteins formed by an orderly sequence of glycosylations. Although lectins did not inhibit esterase activity

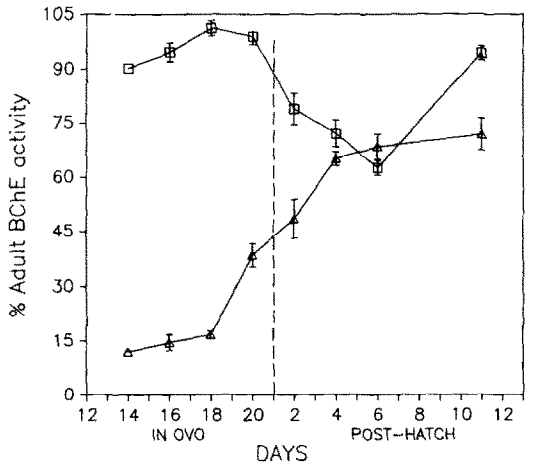


Fig. 4. Changes in BChE activity in the liver and plasma of the developing chick. The broken vertical line represents the day of hatching. Liver (□) and plasma (△) activities (mean ± SEM for three to four animals) are expressed as a percentage of adult. (Adult liver BChE values were 2.99 ± 0.488 μmol/min/g, mean ± SD for five animals).

(even at the highest concentrations tested, 5 mg/mL lectin), incubation of samples with sugar-specific lectins, followed by centrifugation, resulted in reduced esterase activities in gradient fractions. Apparently the reduced activity was not due to an inhibitory effect of the lectins, but to the ability of these sugar-specific proteins to bind to and precipitate esterase glycoproteins found at the bottom of the centrifuge tube. Increased activity at the bottom of the tube following ultracentrifugation was observed when there was a lectin interaction. However, activity was never fully recoverable due to the loss of activity in the high-density (60%) sucrose.

Esterases in chick embryo liver and plasma were mostly glycoproteins (Table 1), as indicated by their affinity for Con A, a general marker for glycoproteins that recognized mannose [18] added in the early steps of the glycosylation pathway [19]. Cholinesterases released to the plasma contained additional glycosylations that were identified by their affinities for WGA and coral tree lectin. BChEs had a greater affinity for galactose-specific coral tree lectin than did AChE, and both enzyme species had a strong affinity for WGA, which recognizes *N*-acetylglucosamine.

Liver $G_1$ and $G_4$ BChE demonstrated different affinities for lectins (Fig. 5). $G_1$ BChE had no observable affinity for WGA and coral tree lectins, unlike $G_4$ BChE, which completely interacted with these lectins in a manner similar to released BChE forms. Horseshoe crab lectin did not interact with any BChE forms, which was expected, because hepatic secretory glycoproteins in the chicken are usually terminated by galactose and not sialic acid [20].

*Organophosphate-sensitive esterases of liver and plasma.* p-NPA esterase activities in embryo and adult chicken liver were partially resolved into two peaks on sucrose density gradients (Fig. 6). Incubations of esterase fractions with $10^{-5}$ M or $5 \times 10^{-5}$ M DFP allowed for complete separation of two p-NPA esterase activities ($S_{20,w}$, 2.8 ± 0.1 and 4.3 ± 0.3).

The $IC_{50}$ values for OP inhibitors and physostigmine were determined by collecting the peak esterase activities from chick embryo liver gradients and examining their p-NPA hydrolyses over a wide range of inhibitor concentrations (experiments summarized in Table 2). The $IC_{50}$ values shown here were estimated for 18-day embryos. Similar results were obtained for adult chickens. Plasma p-NPA esterase had a sedimentation coefficient, $S_{20,w}$ 4.2 ± 0.3, similar to that of $G_1$ BChE. However, it is unlikely that this activity was due to a plasma cholinesterase because p-NPA esterase activity was undetectable in gradient fractions containing $G_4$

Table 1. Lectin affinities of cholinesterase and nonspecific esterase molecular forms in chicken plasma and liver

| Molecular forms | % Lectin binding Con A | WGA | Coral tree |
|---|---|---|---|
| Plasma | | | |
| AChE | | | |
| $G_2$ | 89–99 | 82–93 | 20–33 |
| $G_4$ | 91–99 | 82–94 | 18–33 |
| BChE | | | |
| $G_1$, $G_2$ | 95–101 | 86–97 | 83–91 |
| $G_4$ | 96–100 | 94–99 | 87–95 |
| Liver | | | |
| BChE | | | |
| $G_1$ | 89–98 | 0–3 | 1–6 |
| $G_4$ | 92–99 | 89–98 | 86–97 |
| Nonspecific esterases (1–5 S range) | 67–72 | 0–5 | 0–4 |

Percent lectin binding was determined as follows: gradient fractions containing activities for individual esterase peaks were pooled and the difference in activity between control (peak activity = 100%) and lectin-treated samples (peak activity = percent activity remaining) was defined as percent lectin binding. Numbers are the range of percent values obtained with two separate experiments. G designations are according to Bon *et al.* [17].

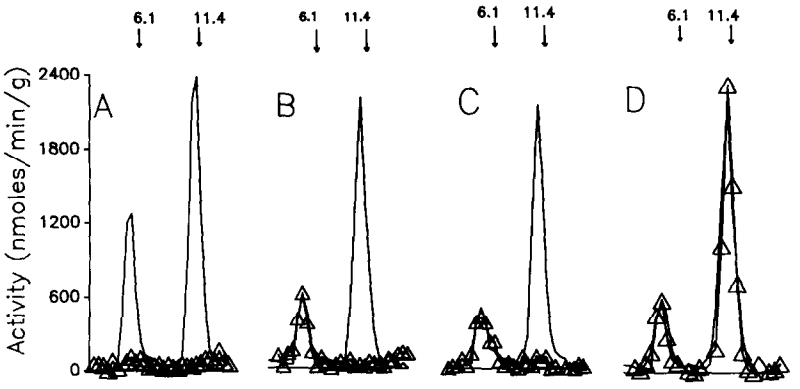


Fig. 5. Lectin interactions with BChE molecular forms ($G_1$ and $G_4$) of an extract of 18-day chick embryo liver (see text). Control activities (——) and activities in the presence of lectins (△): (A) Con A, (B) WGA, (C) coral tree lectin and (D) horseshoe crab lectin. Arrows indicate the position of sedimentation markers.

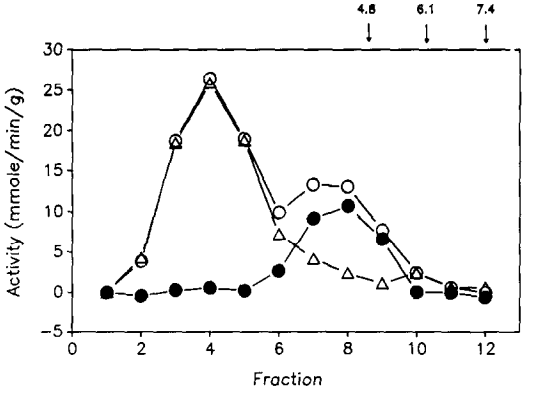


Fig. 6. Distribution of p-NPA esterases of an extract of 18-day chick embryo liver. Key: (○) total hydrolysis of p-NPA, (△) activity after preincubation with 50 μM DFP, and (●) activity that was inhibited by DFP. No significant activities were detected above the 7.4 S range.

cholinesterases. Plasma p-NPA esterase activity (0.0420 ± 0.0108 μmol/min/mL for ten adult chickens) was close to the lower limits of detection following fractionation in sucrose gradients. For this reason, the variance in activity from sample to sample made it difficult to obtain an accurate estimate of $IC_{50}$ values for this enzyme.

## DISCUSSION

The purposes of this study were to extend the present knowledge of the diversity of liver and plasma esterase activities and to determine whether changes in plasma cholinesterase isoforms were related to changes in the liver during development of the chick.

The liver is widely recognized as the source of plasma BChE. Since there is relatively little AChE activity in liver homogenates, the source of plasma AChE is still unknown. Avian erythrocytes contain little or no acetylcholinesterase activity [21], so that the presence of AChE in plasma is unlikely to be due to contamination with red blood cells. It has

Table 2. OP sensitivity of two *p*-nitrophenylacetate (p-NPA) esterase activities in embryo liver gradients

| Peak ($S_{20,w}$) | $IC_{50}$* (M) | | | |
|---|---|---|---|---|
| | Paraoxon | DFP | BPNP | Physostigmine |
| 2.8 | $2.0 \times 10^{-9}$ | $7.9 \times 10^{-4}$ | $1.0 \times 10^{-3}$ | $2.9 \times 10^{-4}$ |
| 4.3 | $3.2 \times 10^{-6}$ | $5.0 \times 10^{-8}$ | $2.5 \times 10^{-5}$ | $1.3 \times 10^{-2}$ |

* The $IC_{50}$ values for physostigmine and OP inhibitors were determined by collecting peak activities from chick embryo liver gradients and examining p-NPA hydrolyses over a wide range of inhibitor concentrations. Values are the means of three separate determinations and in all cases yielded a correlation coefficient ($r$) greater than 0.98. BPNP refers to bis(*p*-nitrophenyl)-phosphate.

been shown previously that, in dystrophic chickens, a highly increased level of AChE occurs in the plasma that corresponds to abnormally high activity in the muscle [11–22]. We have noted that developing muscle and brain contain high AChE activity (over 100 times more AChE activity per gram tissue than the liver) and that muscle and neurons in culture release a sizeable fraction of AChE [23, 24], raising the possibility of extrahepatic sources for this enzyme.

Our findings with plasma BChE activity, and studies of other species, with the possible exception of the rat [7], indicate that plasma BChE activity is low in embryo and newborn in comparison with adults [11, 22, 25–27]. In the experiments presented here, there was a sharp rise in plasma BChE activity during development of the chicken, accompanied by a transient decrease in liver BChE activity as if liver BChE had been mobilized and released at this time. Grieninger and Granick have observed that chick embryo liver cells begin to release adult plasma proteins in culture that were not present in embryonic plasma [28], and we have observed that there was little BChE released from newly isolated liver cells; however, after 24 hr of culture, the enzyme steadily accumulated in the medium (unpublished results).

The approach using sugar-specific lectins, introduced by Rotundo [29], suggested that there are different sites of packaging of cholinesterases before their release to the plasma. Con A identifies an early step in the glycosylation pathway presumably taking place in the endoplasmic reticulum (ER), whereas WGA and coral tree lectin have high affinities for sugars acquired in the Golgi complex [18, 19]. One of the most interesting findings was the difference in lectin affinities for the $G_1$ and $G_4$ BChEs in the liver, consistent with an intracellular $G_1$ precursor to $G_4$ BChE [30]. Lectin interactions suggested that $G_4$ BChE was localized in the Golgi; however, the assembly of $G_4$ AChE and BChE is thought to take place in the ER [31]. The inability to detect an intermediate species of $G_4$ or, for that matter, liver $G_2$ BChE could be due to their short half-life.

Kinetic studies with different substrates and inhibitors provide evidence for more than one carboxylesterase in the chicken liver [32]. In the present investigation, liver p-NPA esterase activities were separated into two peaks (2.8 and 4.3 S) by sucrose density gradient sedimentation, and it was demonstrated that these activities showed very different sensitivities to OPs. Of the OPs tested, paraoxon was the most potent inhibitor of the 2.8 S activity, whereas DFP was the most potent inhibitor of the 4.3 S activity. We were unable to detect any p-NPA activity in gradient fractions, indicating the presence of a DF-hydrolyzing esterase (A-esterase [1]). It is possible that the relative insensitivity of the 2.8 S esterase to DFP is due to alternative inter- or intramolecular DFP-binding sites competing with the catalytic center. The extreme sensitivity of the 2.8 S activity to paraoxon (and not DFP) deserves further attention.

In conclusion, changes in plasma cholinesterase isoforms during development of the chick did not reflect changes in liver cholinesterase forms. However, an intracellular form suggestive of a precursor to plasma BChE was detected, along with two carboxylesterase activities with very different sensitivities to OPs. The approach taken in this study could be useful in investigating the roles of specific esterase isoforms in the detoxication of xenobiotics and in identifying distinct esterase metabolites in the glycosylation pathway.

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## REFERENCES

1. Aldridge WN, 1. Two types of esterase (A and B) hydrolysing *p*-nitrophenyl acetate, propionate and butyrate, and a method for their determination. *Biochem J* **53**: 110–117, 1953.
2. Stewart DJ, Inaba T, Lucassen M and Kalow W, Cocaine metabolism: Cocaine and norcocaine hydrolysis by liver and serum esterases. *Clin Pharmacol Ther* **25**: 464–468, 1979.
3. Masakawa M, Inoue M, Tsuboi M and Sugiura M, Studies of aspirin esterase of human serum. *Jpn J Pharmacol* **29**: 581–586, 1979.
4. Lockridge O, Mottershaw-Jackson N, Eckerson HW and La Du BN, Hydrolysis of diacetylmorphine (heroin) by human serum cholinesterase. *J Pharmacol Exp Ther* **215**: 1–8, 1980.
5. Nichol AJ, Shebuski RJ and Ruffolo RR, Inhibition of plasma cholinesterase prevents the dopamine DA-1 receptor mediated renal vasodilation produced by ibopamine. *Eur J Pharmacol* **141**: 515–518, 1987.
6. Bisschop HC, Meerleer WA and Willems JL, Stereoselective phosphonylation of human serum proteins by soman. *Biochem Pharmacol* **36**: 3587–3591, 1987.
7. Sterri SH, Berge G and Fonnum F, Esterase activities and soman toxicity in developing rat. *Acta Pharmacol Toxicol* **57**: 136–140, 1985.
8. Kalow W, Dose–response relationships and genetic variation. *Ann NY Acad Sci* **123**: 212–218, 1965.
9. Murphy SD, Mechanisms of pesticide interactions in vertebrates. *Residue Rev* **25**: 201–221, 1969.
10. Clement JG, Importance of aliesterase as a detoxification mechanism for soman (pinacolyl methylphophofluouridate) in mice. *Biochem Pharmacol* **33**: 3807–3811, 1984.
11. Wilson BW, Linkhart TA, Walker CR and Nieberg PS, Tissue acetylcholinesterase in plasma of chick embryos and dystrophic chickens. *J Neurol Sci* **18**: 333–350, 1973.
12. Ellman GL, Courtney KD, Andres V and Featherstone RM, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**: 88–95, 1961.
13. Ljungquist A and Augustinsson K-B, Purification and properties of two carboxylesterases from rat-liver microsomes. *Eur J Biochem* **23**: 303–313, 1971.
14. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
15. Martin RG and Ames BN, A method for determining the sedimentation behavior of enzymes: Application to protein mixtures. *J Biol Chem* **236**: 1372–1379, 1961.
16. Decker LA, *Worthington Enzymes and Enzyme Reagents*. Worthington Biochemical Inc., Freehold, NJ, 1968.

17. Bon S, Vigny M and Massoulie J, Asymmetric and globular forms of acetylcholinesterase in mammals and birds. *Proc Natl Acad Sci USA* **76**: 2546–2550, 1979.
18. Sharon N and Lis H, Lectins: Cell-agglutinating and sugar-specific proteins. *Science* **177**: 949–959, 1972.
19. Kornfeld R and Kornfeld S, Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem* **54**: 631–664, 1985.
20. Lunney J and Ashwell G, A hepatic receptor of avian origin capable of binding specifically modified glycoproteins. *Proc Natl Acad Sci USA* **73**: 341–343, 1976.
21. Pickering CE and Pickering RG, The interference by erythrocyte "acetylcholinesterase" in the estimation of the blood cholinesterase activity of the chicken. *Toxicol Appl Pharmacol* **39**: 229–237, 1977.
22. Lyles JM, Barnard EA and Silman I, Changes in levels and forms of cholinesterases in the blood plasma of normal and dystrophic chickens. *J Neurochem* **34**: 978–987, 1980.
23. Wilson BW, Nieberg PS, Walker CR, Linkhart TA and Fry DM, Production and release of AChE by cultured chick embryo muscle. *Dev Biol* **33**: 285–299, 1973.
24. Walker KB and Wilson BW, Regulation of AChE in cultured chick embryo spinal cord neurons. *FEBS Lett* **93**: 81–85, 1978.
25. Holmes RS and Masters CJ, The ontogeny of pig and duck esterases. *Biochim Biophys Acta* **159**: 81–93, 1968.
26. Zsigmond EK and Downs JR, Plasma cholinesterase activity in newborns and infants. *Can Anaesth Soc J* **18**: 278–285, 1971.
27. McCance RA, Hutchinson AO, Dean RFA and Jones PEH, The cholinesterase activity of the serum of newborn animals, and of colostrum. *Biochem J* **45**: 493–496, 1949.
28. Grieninger G and Granick S, Synthesis and differentiation of plasma proteins in cultured embryonic chicken liver cells: A system for study of regulation of protein synthesis. *Proc Natl Acad Sci USA* **72**: 5007–5011, 1975.
29. Rotundo RL, Asymmetric acetylcholinesterase is assembled in the Golgi apparatus. *Proc Natl Acad Sci USA* **81**: 479–483, 1984.
30. Svensmark O, Enzymatic and molecular properties of cholinesterases in human liver. *Acta Physiol Scand* **59**: 378–389, 1963.
31. Rose JK and Doms RW, Regulation of protein export from the endoplasmic reticulum. *Annu Rev Cell Biol* **4**: 257–288, 1988.
32. Inkerman PA, Scott K, Runnegar MTC, Hamilton SE, Bennett EA and Zerner B, Carboxylesterases (EC 3.1.1). Purification and titration of chicken, sheep, and horse liver carboxylesterases. *Can J Biochem* **53**: 536–546, 1975.