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THE ONTOGENY OF PIG AND DUCK ESTERASES

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SUMMARY

1. Sixteen multiple forms of soluble esterase activity have been resolved from pig tissue extracts.

2. Duck tissue esterases have also been shown to exist in multiple forms and exhibit nineteen bands of activity.

3. By means of substrate and inhibitor studies, these heteromorphs have been characterized into four main classes: carboxylesterase, cholinesterase, arylesterase and acetylesterase

	Pig esterase multiplicity	*Duck esterase multiplicity*
Carboxylesterase	6	5
Cholinesterase	unresolved	2
Acetylesterase	5	4
Arylesterase	4	8

4. Further differentiation of the multiple forms in some of the classes has been achieved from an investigation into the developmental and physicochemical properties of these enzymes

INTRODUCTION

Previous publications from this laboratory[1-3] have demonstrated the complexity of the inter-relationships which exist between the multiple forms of esterase activity, and the considerable value of developmental parameters in the elucidation of the isoenzyme status of these heteromorphs Furthermore, although both the rat and the guinea pig had been found to display distinct ontogenetic progressions of esterase activities, the nature of these developmental changes had proved to be significantly different between these two rodents. Such divergent characteristics

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between closely related species provided a distinct contrast to the known developmental behaviour of other isoenzyme systems[4–8].

These facts, then, emphasized the desirability of additional ontogenetic studies in order to extend an understanding of these phenomena, and in this publication, the developmental progressions of esterases are detailed for two further species To widen the available representation, an avian species has been included (duck), as well as a further commonly studied source of mammalian esterase (pig).

METHODS

Tissue extracts

A number of pigs (*Sus scrota*) and ducks (*Carina moschata* or domestic white muscovy) were selected to cover a representative range of intervals during gestation, infancy and maturity. The required tissues were exised from the freshly slaughtered animals and stored at −10° until required.

Electrophoresis

Homogenates were prepared in cold, glass-distilled water and were centrifuged (100 000 × *g* for 30 min) Electrophoresis of the supernatant was carried out on vertical columns of polyarylamide gel (7.5%, pH 8.6) which were subsequently histochemically treated (for details see previous papers[1–3])

Physicochemical properties of esterases

The urea (10 M) and heat (60°) lability of the esterase multiple forms was investigated as previously described[1]

Esterase and protein assay

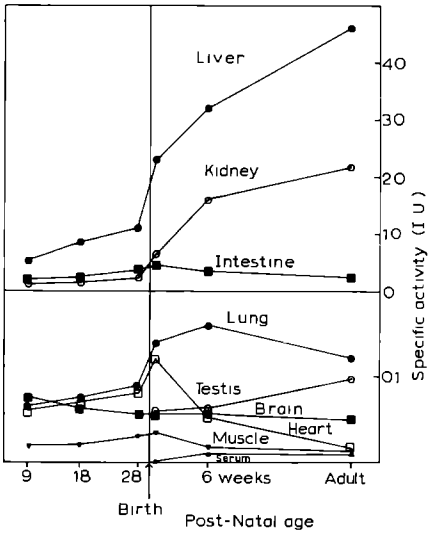
Total esterase activity was determined using α-naphthyl acetate (Sigma Chemical Company) as substrate, Fast Blue RR salt (Sigma Chemical Company) as coupling agent, and the absorption peak at 500 mμ. Measurements were made on a recording Unicam SP 800 spectrophotometer[1–3] Protein determination was performed by the biuret method[9] with bovine serum albumin for standards

Enzyme activity was calculated as μmoles α-naphthol released (37°) per min per mg protein

RESULTS

Developmental alterations of esterase specific activities in the various pig tissue extracts are detailed in Fig 1 In the period immediately following birth, general increases in activity are evident in some tissues, but the subsequent postnatal sequences show considerable individual variation between tissues The esterase activity increases during postnatal maturation of liver, kidney, and testis, however, that of intestine, heart, muscle and lung decrease in this period.

Zymograms of pig tissue esterases are represented diagramatically in Fig. 2. The highest concentrations of activity in the adult occur in liver, kidney and intestine, but activity was present in all tissues examined. The distribution of the esterase



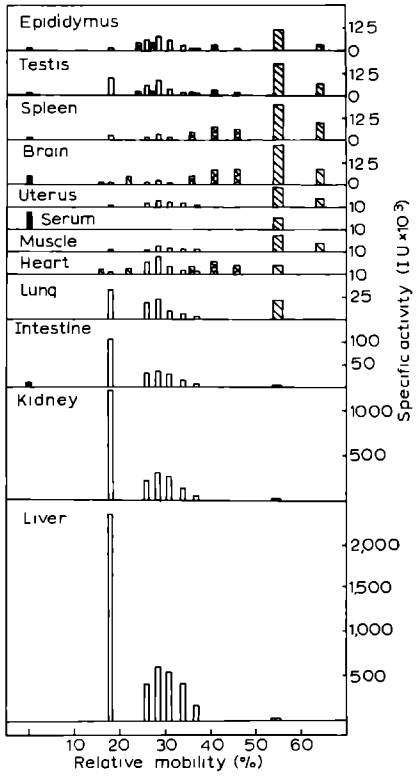


Fig 1 Developmental alterations of total esterase activities in pig tissues and serum

Fig 2 The distribution of esterase multiple forms in adult pig tissues and serum Carboxylesterase activity is represented by open histograms, arylesterase by diagonal shadings, acetylesterase by crosshatching and cholinesterase by complete shading

multiple forms is not entirely tissue specific since liver, kidney, intestine and lung have the same group of carboxylesterases predominating.

Figs. 3–11 illustrate the developmental progression of multiple esterase forms in different pig tissues. Pig liver (Fig 3) and kidney (Fig 4) exhibit 6 forms of carboxylesterase and one diffuse region of arylesterase activity throughout development. The basic developmental change in activity is a large postnatal increase in carboxylesterase, which reaches a maximum in the adult animal, while the arylesterase component remains constant throughout Other sources of esterase activity, intestine (Fig 5) and lung (Fig. 6) show similar patterns, but maximal activity is observed at birth and at 6 weeks, respectively

Adult pig heart esterase patterns (Fig. 7) reveal 6 carboxylesterases, 3 arylesterases, and 3 acetylesterases On development, the carboxylesterases increase to a maximum at birth, the acetylestereases appear in the adult animal, and the arylesterases undergo a decrease in activity The carboxyl and arylesterases of developing pig skeletal muscle (Fig. 8) undergo similar changes to that of heart Pig brain esterase patterns (Fig 9) reveal 4 forms of arylesterase and 3 forms of acetylesterase which are

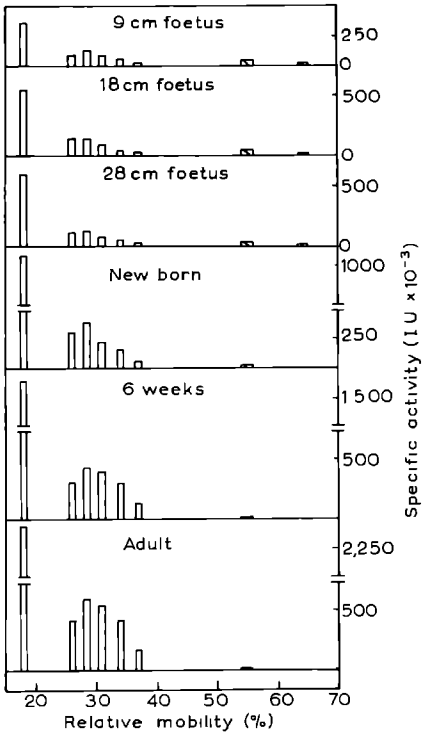
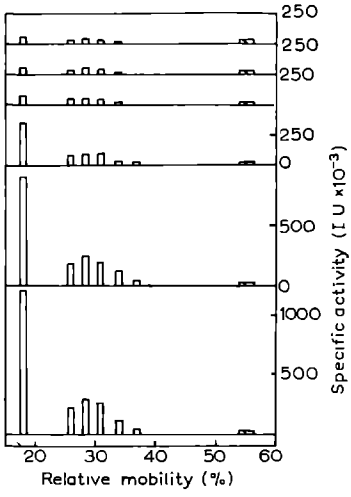


Fig 3 The developmental progression of esterase forms in pig liver. Representations of the type of esterase activity are the same as in Fig 2



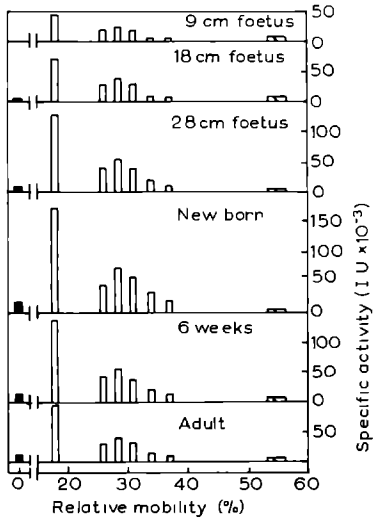
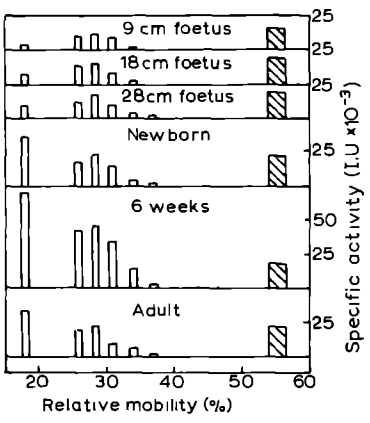


Fig 4 The developmental progression of esterase forms in pig kidney Representations of the type of esterase activity are the same as in Fig. 2

Fig 5 The developmental progression of esterase forms in pig intestine Representations of the type of esterase activity are the same as in Fig. 2



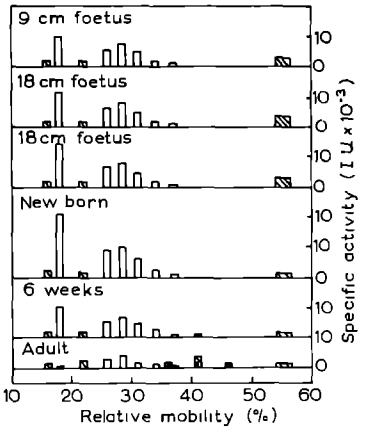
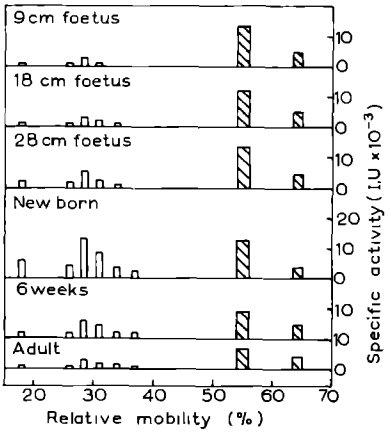


Fig 6 The developmental progression of esterase forms in pig lung Representations of the type of esterase activity are the same as in Fig 2.

Fig 7 The developmental progression of esterase forms in pig heart Representations of the type of esterase activity are the same as in Fig 2

stable throughout the developmental period studied Minor regions of carboxylesterase activity appear during postnatal development

As the pig testis matures (Fig. 10), minor bands of acetylesterase appear, the carboxylesterases increase in activity, and the arylesterases undergo a slight decrease in activity. Adult pig serum (Fig 11) reveals a simple electrophoretic pattern, consisting of a diffuse arylesterase region and cholinesterase, which is localized at the origin and is not resolved under the conditions used in this study. During postnatal growth, pig serum develops all of the arylesterase, and most of the cholinesterase activity



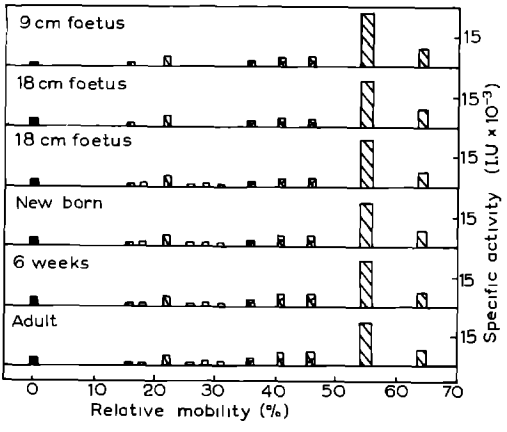


Fig 8 The developmental progression of esterase forms in pig skeletal muscle Representations of the type of esterase activity are the same as in Fig 2

Fig 9 The developmental progression of esterase forms in pig brain Representations of the type of esterase activity are the same as in Fig 2.

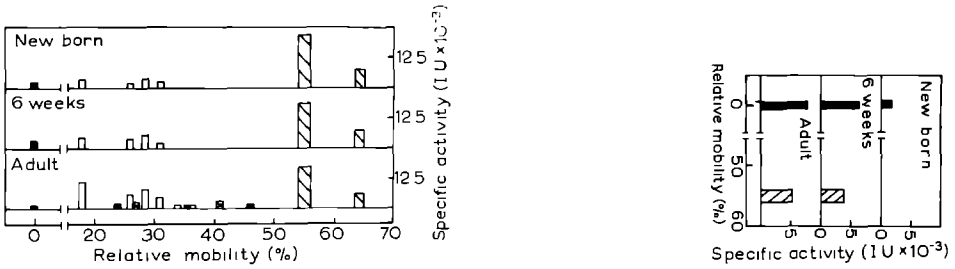


Fig 10 The developmental progression of esterase forms in pig testis Representations of the type of esterase activity are the same as in Fig 2

Fig 11 The developmental progression of esterase forms in pig serum Representations of the type of esterase activity are the same as in Fig 2

Developmental changes in esterase specific activities in various duck tissues are detailed in Fig 12. Maximal activity occurs at one week post-hatching in all tissues except brain, which exhibited constant activity throughout the developmental period studied. Embryo esterase is low in most tissues, however, liver and kindney embryo tissue extracts reveal high activity

Zymograms of duck tissue esterases are represented diagramatically in Fig 13. Highest concentrations of activity in the adult occur in liver, kidney, intestine and serum, but activity was present in all tissues A group of carboxylesterases occurred

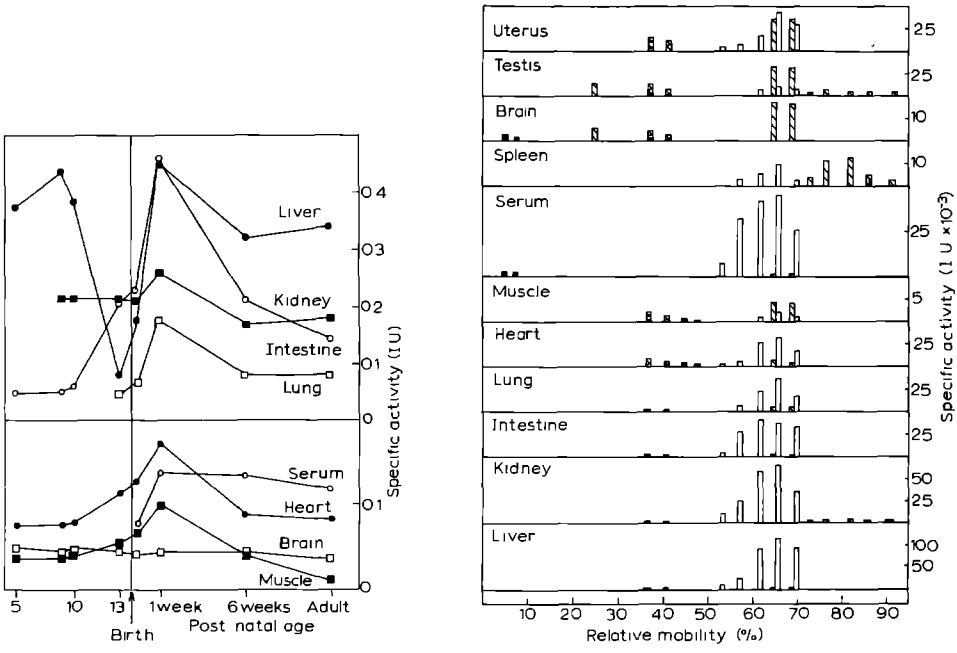


Fig 12 Developmental alterations of toal esterase activities in duck tissues and serum.

Fig 13 The distribution of esterase multiple forms in adult duck tissues and serum Representations of the type of esterase activity are the same as in Fig 2

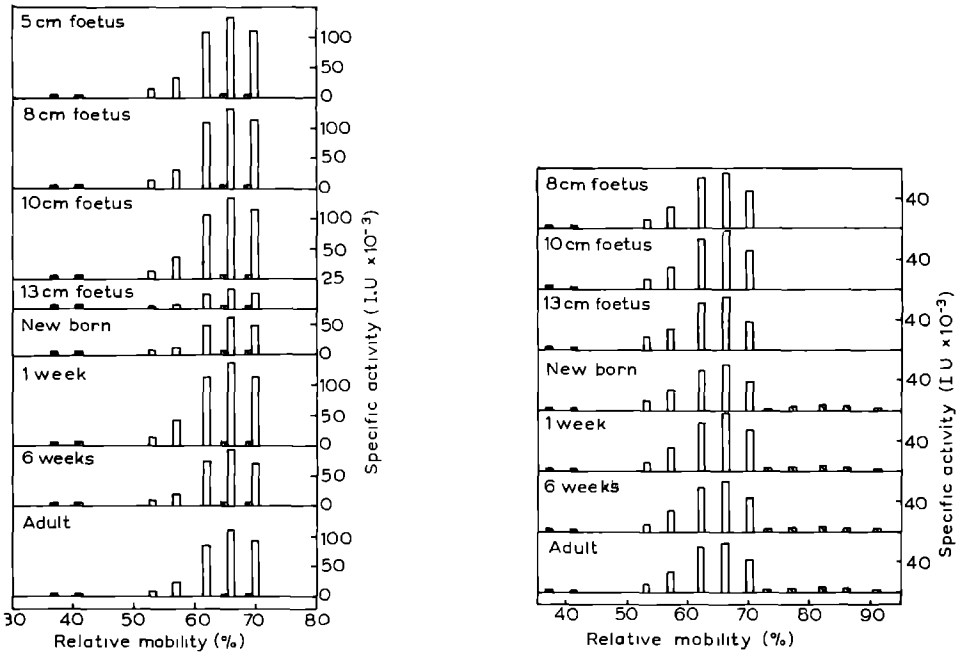


Fig 14 The developmental progression of esterase forms in duck liver Representations of the type of esterase activity are the same as in Fig 2.

Fig 15 The developmental progression of esterase forms in duck kidney Representations of the type of esterase activity are the same as in Fig 2

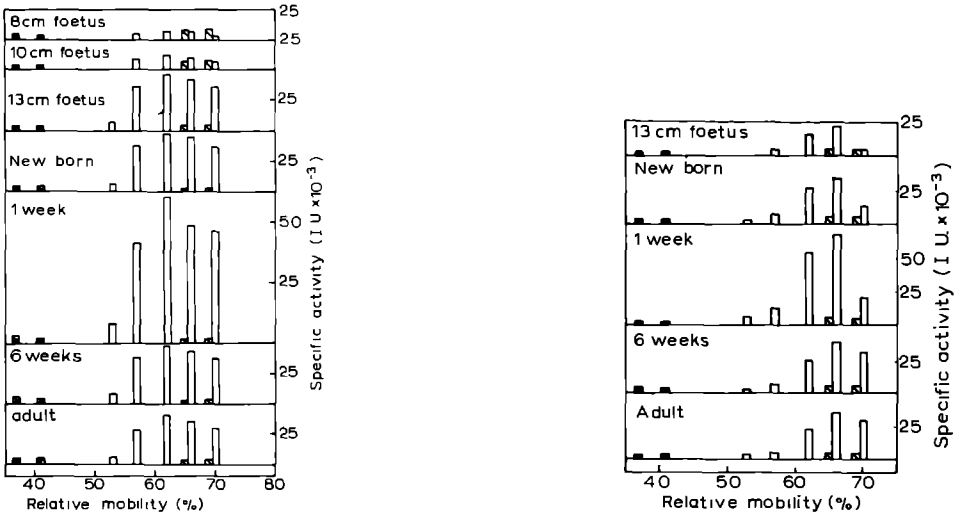
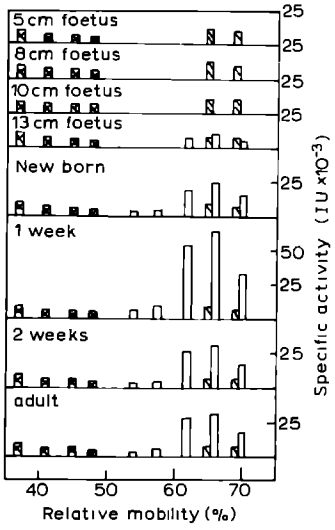


Fig 16 The developmental progression of esterase forms in duck intestine Representations of the type of esterase activity are the same as in Fig 2

Fig 17 The developmental progression of esterase forms in duck lung Representations of the type of esterase activity are the same as in Fig 2



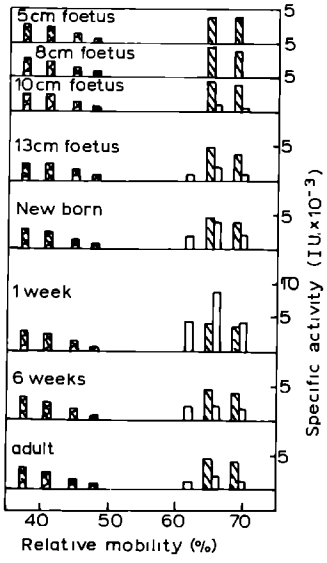
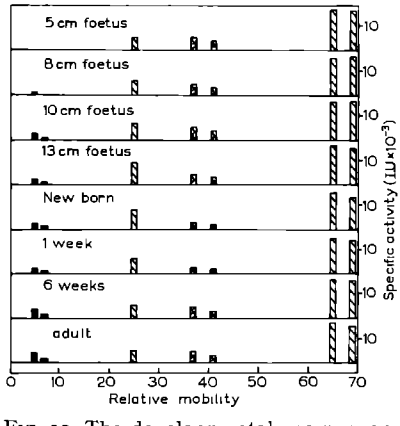


Fig 18 The developmental progression of esterase forms in duck heart Representations of the type of esterase activity are the same as in Fig 2

Fig 19 The developmental progression of esterase forms in duck skeletal muscle Representations of the type of esterase activity are the same as in Fig 2

in every tissue except brain, and accounted for most of the activity in liver, kidney, intestine, lung, heart and serum. Arylesterase predominated in the other tissue extracts.

Figs. 14–21 illustrate the developmental progression of multiple esterase forms in duck tissues. Duck liver (Fig 14) exhibits 5 forms of carboxylesterase and 2 forms of both acetyl and arylesterase activity The carboxylesterases show high activity in



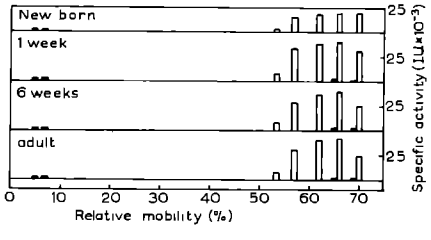


Fig 20 The developmental progression of esterase forms in duck brain Representations of the type of esterase activity are the same as in Fig 2

Fig 21 The developmental progression of esterase forms in duck serum Representations of the type of esterase activity are the same as in Fig 2

TABLE I

PROPERTIES OF PORCINE ESTERASES

Ac, acetate ester, Bu, butyrate ester

Relative mobility	*Classification*	*Substrate specificity*	*Half life (60°) (min)*	*Half life (10 M urea) (min)*
0	Cholinesterase	Ac < Bu	< 2	<5
16, 22	Arylesterase	Ac > Bu	< 2	<5
18	Carboxylesterase	Ac < Bu	>20	8
26, 28, 5, 31, 34, 37	Carboxylesterase	Ac < Bu	>20	17
24, 27	Acetylesterase	Ac ≫ Bu	4	>40
36, 41, 46	Acetylesterase	Ac ≫ Bu	9	30
55, 64	Arylesterase	Ac > Bu	<2	5

the early embryo and postnatal animals, but low activity around birth Duck kidney esterase (Fig. 15) is divisible into 3 groups of activity including 5 carboxylesterases, 5 arylesterases, and 2 acetylesterase components. The carboxylesterases and the acetylesterases showed little variation throughout development; the arylesterases, however, were undetected in the embryo kidney and were observed only after hatching

The developmental changes of esterases in the other duck tissues (Figs 16–21) may be summarized as follows The carboxylesterases exhibit low activity in the embryonic tissues but develop maximum activity within one week after hatching The activity subsequently decreases with maturity Cholinesterase has low activity in the embryo and develops maximum activity in the adult animal, while arylesterase and acetylesterase do not vary significantly during the period studied.

Tables I and II summarize the properties of porcine and duck tissue esterases, respectively, and generally support the overall division of esterase activity into 4 classes.

TABLE II

PROPERTIES OF DUCK ESTERASES

Ac, acetate ester, Bu, butyrate ester

Relative mobility	*Classification*	*Substrate specificity*	*Half life (60°) (min)*	*Half life (10 M urea) (min)*
5, 7	Cholinesterase	Ac < Bu	<2	<5
25	Arylesterase	Ac > Bu	<2	<5
37, 41, 45, 48	Acetylesterase	Ac > Bu	20	35
53 5, 57, 62, 66, 70	Carboxylesterase	Ac < Bu	8	35
65, 69	Arylesterase	Ac > Bu	<2	8
73, 77, 82, 86, 91	Arylesterase	Ac > Bu	<2	10

DISCUSSION

Pig tissues, in particular liver and kidney, are excellent sources of esterase activity[10], and have been used for many years as the starting material for esterase preparations · carboxylesterase[11–16], arylesterase[17] and acetylesterase[18] Although there

are some reports in the literature on the multiplicity of pig arylesterase[19], and cholinesterase[20], the present study is the first to extensively investigate the distribution of pig esterase multiple forms The predominant feature about the various pig tissue esterase zymograms is the very high carboxylesterase activity in liver, kidney, intestine and lung extracts and the apparent lack of the carboxyl- and cholinesterases of higher electrophoretic mobility (and perhaps low molecular weight) which occur in other species[1–3]

No references to duck tissue esterase multiplicity appear in the literature. They have been resolved in this study into four groups of activity; carboxylesterases, cholinesterases, arylesterases and acetylesterases. Carboxylesterase activity is again predominant, occurring in all tissues except brain, with the highest activity being observed in liver, kidney, intestine and serum

Previous studies of esterases in animal tissues and plasma had reported that increases in total enzymatic activity occurred with ageing[21,22], but more detailed and precise analyses have become available in recent years Developmental progression of esterase multiple forms have now been established for the mouse[23,24], human[25], guinea pig[1] and rat[2,26] In addition, the present studies establish that ontogenetic variations occur in the pig and duck as well

The latter result is of special interest in view of previous reports that seemed to indicate an absence of an ontogenetic change in avian species Using an immunoelectrophoretic technique, CROISILLE[27] had found no changes in the esterase composition of chicken liver during development, and this observation has recently been supported by histochemical techniques[28]. The present results reveal that a developmental alteration in avian liver esterase activity does occur, and that this change is associated with a large decrease in activity at birth (Fig 14) Duck kidney esterase (Fig. 15), however, undergoes no significant changes during development, while other tissues, *e g* , intestine (Fig. 16), lung (Fig 17) and heart (Fig 18) exhibit low embryo esterase activity and develop maximum enzyme levels within one week of hatching. Thus, the distinction between the developmental characteristics of mammalian and avian livers (Figs 3 and 14) is the higher activity of avian hepatic esterase during early development, and this, in turn, is probably a reflection of the difference in embryonic environment between birds and mammals The avian embryo is isolated from maternal influence and cannot delegate renal or hepatic function; the mammalian fetus, however, relies largely on the parent for these essential processes, and does not develop the esterase levels which are characteristic of functional adult tissue until after parturition

The arylesterase activity of pig tissue extracts has been resolved into 4 components in these studies, and these multiple forms may be divided into 3 groups on the basis of their tissue distribution and electrophoretic mobility· (*Rm** 16), (*Rm* 55) and (*Rm* 64) The individual occurrence and variation of activity in different tissues suggests separate genetic control

The genetic control and the endocrinological influence on the synthesis of arylesterases have been recently investigated by AUGUSTINSSON and co-workers[20,29–32]. They concluded that the postnatal biosynthesis of pig plasma arylesterase is genetically controlled by a set of multiple alleles and may be influenced by sex hormones Pig

* *Rm* refers to the electrophoretic mobility with respect to bromophenol blue at a gel concentration of 7 5%

tissue arylesterase activity in these studies, however, does not vary significantly during development although some tissues decrease in activity with the onset of maturation It would seem, therefore, that the postnatal increase in pig plasma arylesterase, observed by AUGUSTINSSON and co-workers as well as ourselves (Fig. 11), may be due to other factors as well as the suggested increase in synthesis, *e g*, the increased release of the enzyme from tissues. In this context, though, the limitations of specific substrates should be kept in mind. Arylesterase does not catalyse the hydrolysis of α-naphthol acetate as efficiently as with some other substrates, *e g*, phenylacetate Hence, more appreciable developmental changes, and even a more extensive heterogeneity, may be indicated by reference to alternative ester substrates.

Duck tissue arylesterase activity has been separated, in this study, into eight components, which are again divisible into three groups on the basis of their differential distribution and activities in various tissues (*Rm* 25), (*Rm* 65, 69) and (*Rm* 73, 77, 82, 86, 91) The distinctive feature is the high degree of multiplicity of the latter group which exists as only 1 or 2 multiple forms in mammalian tissue extracts

Acetylesterase activity of pig tissues has been resolved into 5 components and can be divided into 2 groups (*Rm* 24, 27) and (*Rm* 36, 41, 46) They are distinguished from the other esterase activities not only by their substrate and inhibitor specificity, but also by their considerable stability to urea treatment and their association with the mature male sex tissue extracts In this regard, they exhibit similar properties to guinea pig acetylesterases[1]. Duck acetylesterase activity is widely distributed throughout the various tissues and is separated into four multiple forms. They are stable to both heat and urea denaturation treatments and undergo no visible changes during the developmental period studied.

There is a distinctive lack of the cholinesterases of higher electrophoretic mobility in porcine and duck tissue extracts which have been observed previously in a variety of mammalian tissue extracts[1-3]. "Serum type" cholinesterase activity however, is present, and exists in 2 multiple forms in duck tissues, while pig tissue cholinesterase was localized at the origin and was unresolved in these studies. During development, these enzymes are found to exhibit low activity in the prenatal tissues, and increase to a maximum in the adult animal

Pig liver carboxylesterase has recently been purified and crystallized and its properties extensively investigated[33,34]. It has a molecular weight of 163 000 and contains 2 active sites per molecule[34]. This enzyme activity has been resolved in this study into 6 multiple forms which are divided into 2 groups on the basis of their differential tissue distribution and activity the major liver carboxylesterase (*Rm* 18) and a secondary group (*Rm*, 26, 28 5, 31, 37). Extreme care is therefore required in order to ensure that observed characteristics of esterases are typical of a single enzyme form. Pig carboxylesterases have been found to occur in a wide variety of tissues and undergo large postnatal increases in general. They may be differentiated from the other types of esterase activity not only by their inhibitor specificity, but also by their stability to heat (60°) treatment

Noticeable features of duck tissue carboxylesterases are their much higher relative mobilities than the corresponding pig enzymes on gel electrophoresis and their appearance in serum These observations and unpublished work in this department on chicken liver carboxylesterase (P INKERMAN, E C WEBB AND B. ZERNER, unpublished results) suggest that avian tissue carboxylesterases have much lower

molecular weights than the corresponding mammalian enzymes Five multiple forms of duck carboxylesterase activity are observed in this study and their activity is found to be present in a variety of tissues and to change significantly throughout development

In summary the soluble porcine and duck tissue esterases have been separated into their multiple forms and divided into 4 main groups carboxylesterase, cholinesterase, arylesterase and acetylesterase. Each of these groups may be considered as an isoenzyme system[35] The developmental, tissue distribution, and physicochemical studies have confirmed these broad groupings of esterase types, but also allow further differentiation within the carboxyl and acetylesterase groups of the pig, and the arylesterase group in both the pig and the duck These observations support the concept of a complex genetic control for these enzymes in both mammalian and avian tissues.

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