Esterases in the Milk and Blood Plasma of Swine

1. SUBSTRATE SPECIFICITY AND ELECTROPHORESIS STUDIES

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Except for the well-known capacity of milk to hydrolyse fat and certain triglycerides (e.g. tributyrin; see review by Herrington, 1954), used as a measure of hydrolytic rancidity, its esterase activity has been little studied. Among aromatic esters, phenyl salicylate (salol) attracted some interest in the beginning of this century because it was found to be susceptible to enzymic hydrolysis by milk and mammary glands of various species (Grimmer, 1913; Takai, 1936). The hydrolysis of naphthyl acetate by milk has been used as a colorimetric method for 'lipase' determination (Greenbank, 1950).

Choline esters are generally not hydrolysed by milk (man, cow, cat, goat, horse) (Augustinsson, 1948; McCance, Hutchinson, Dean & Jones, 1949; Alm & Augustinsson, 1957). The absence of cholinesterase in the milk of various species was also demonstrated by a biological technique (Kocić-Mitrović, 1955). In two instances, however, milk was shown to exhibit high or moderate cholinesterase activity, i.e. the colostrum of dog (McCance *et al.* 1949; McCance & Brown, 1953) and the colostrum and milk of swine (Hines & McCance, 1953). These two sources of cholinesterases are examples of interesting species differences in biochemical behaviour for which no biological explanation is known so far.

During studies on the species variation in esterase patterns of vertebrate blood plasmas (Augustinsson, 1958), swine plasma was found to contain, in addition to an aromatic esterase (arylesterase), a cholinesterase possessing remarkable properties. Sow's milk was also found to contain an esterase, the properties of which did not resemble those of any other esterase hitherto described. Very little is known about the esterase or lipase activity of swine milk, and the specificity of the enzyme responsible for the lipase activity is unknown.

The present investigation was undertaken in order to study the specificity and other properties of milk and plasma esterases of swine. A second paper (Augustinsson & Olsson, 1959) reports the variation of the esterase activities during the course of lactation and suckling.

METHODS

Assay of esterase activity. The esterase activity was determined manometrically by the Warburg technique at 25° in a bicarbonate-CO₂ buffer, pH 7.4 (Augustinsson, 1957), and was expressed in b_{50} values, i.e. μ l. of CO₂ evolved from a total volume of 2.0 ml. during 30 min., corrections being made for non-enzymic hydrolysis of the substrates. For practical reasons, the amount of enzyme present in each determination was selected to give b_{50} between 50 and 150.

Esterase preparations. Milk and blood were collected from adult swine of the Swedish Landrace breed (Augustinsson & Olsson, 1959). Fat was removed from the milk by centrifuging. Plasma was prepared from heparinized blood, and was free from all cells including thrombocytes. Serum, instead of plasma, was used in some cases; both had the same esterase activity when tested with various substrates.

Substrates. Aliphatic, aromatic and choline esters were tested as substrates. The substrates used were the acetyl, propionyl and butyryl esters of the following radicals: isoamyl, glycerol, phenyl and choline, also acetyl- β -methylcholine (β -acetoxypentyltrimethylammonium iodide), benzoylcholine, succinylcholine, the carbon analogue of acetylcholine (3,3'-dimethylbutyl acetate), and Tween 20 (polyoxyethylenesorbitan monolaurate). Choline esters were all employed in the form of iodides. The substrate concentration in the reaction mixture was 0.01 M.

Esterase inhibitors. Eight compounds were selected as inhibitors from those frequently employed as selective enzyme inhibitors in recent esterase studies (Augustinsson, 1957): physostigmine salicylate, tetraisopropylpyrophosphoramide, bismonoisopropylaminofluorophosphine oxide (mipafox), 10-(1-diethylaminopropionyl)phenothiazine hydrochloride (Astra 1397), 1:5-bis(4-trimethylammoniumphenyl)pentane-3-one di-iodide (62C47), bis-(piperidinomethylcoumaranyl-5) ketone (3318CT), sodium arsenilate (Atoxyl) and quinine sulphate. The enzyme preparations were incubated for 50 min. with inhibitor in bicarbonate-CO₂ buffer before addition of substrate. The esterase inhibition was expressed in pI_{50} values, i.e. the negative logarithm of the molar inhibitor concentration producing 50% enzyme inhibition. The active fractions obtained in electrophoresis experiments were dialysed against the bicarbonate buffer before testing against the inhibitor.

Electrophoresis. Preparative electrophoretic separation was performed in cellulose columns (1.5 cm. $\times$ 40 cm., veronal buffer, pH 8.4, I 0.1). Details of the procedure have been presented elsewhere (Augustinsson, 1958).

Protein concentration was estimated by a modified Folin procedure (Lowry, Rosebrough, Farr & Randall, 1951); extinction coefficient is given in arbitrary units.

RESULTS

Esterase specificity pattern of sow's milk and plasma

Sow's milk. The substrate-specificity pattern of sow's milk esterase compared with those of the esterases of swine plasma and of the milk and plasma of cow is shown in Fig. 1. The most characteristic feature of the esterase activity of sow's milk was the rapid hydrolysis of choline esters, butyrylcholine being hydrolysed at the highest rate. The milk enzyme responsible for this reaction exhibited exceptional substrate specificity; both acetylcholine and propionylcholine were hydrolysed more slowly than butyrylcholine, and acetylcholine more rapidly than propionylcholine. This specificity pattern differs from those of all other cholinesterases (except that of swine-plasma cholinesterase, see below). The difference in the

hydrolysis rates of acetyl- and propionyl-choline was small, but significant, and the same when tested at various substrate concentrations. The same enzyme was responsible for the hydrolysis of the three choline esters, as could be demonstrated when the enzyme was isolated by electrophoresis. Benzoylcholine and succinylcholine were also split by this enzyme; the hydrolysis rates were lower than for butyrylcholine (13.3 and 11.3 % respectively as compared with the rate for butyrylcholine), but the hydrolysis of succinylcholine was exceptional since no other animal tissue so far studied hydrolyses this ester at such a relatively high rate. The rate of hydrolysis of the carbon analogue of acetylcholine (3:3'-dimethylbutyl acetate) was 10 % of the hydrolysis rate of the choline ester. Acetyl- β -methylcholine was not split by sow's milk.

Sow's milk also hydrolysed phenyl esters; the acetate was split more rapidly than either the propionate or butyrate. The substitution of an NO₂ group in the *para* position of phenyl acetate lowered the enzymic hydrolysis rate by about 90 %. As will be demonstrated below, a single enzyme is responsible for the hydrolysis of both choline and aromatic esters. It is characteristic of this enzyme (classified as a butyrylcholinesterase) that phenyl acetate is hydrolysed at about the same rate as butyrylcholine.

Among the aliphatic esters used as substrates, the *iso*amyl esters were hydrolysed at very low rates. Triglycerides were also split slowly, the propionate being hydrolysed more rapidly than the acetate and butyrate; evidence will be given below to show that the triglycerides are also hydrolysed by the butyrylcholinesterase present in sow's milk. Among other esters tested as substrates the following were not hydrolysed: procaine hydrochloride, atropine sulphate, cocaine hydrochloride and heroin. Tween 20 was hydrolysed as rapidly as tributyrin.

Swine plasma. Sow's milk esterase may well originate from the blood plasma, the esterase pattern of which is therefore of interest in this connexion (Fig. 1). A recent paper summarizing the results obtained with swine and other plasmas (Augustinsson, 1958) reports that three types of esterases are present in most vertebrate plasmas: arylesterase (aromatic or A-esterase), ali-esterase (aliphatic or B-esterase) and cholinesterase. The cholinesterase present in low concentration in swine plasma was reported previously to have an exceptional substrate specificity (Levine & Suran, 1950, 1951). The present results for the plasma cholinesterase of swine confirm those reported by Myers (1953), including the hydrolysis of benzoylcholine (13 % of the hydrolysis rate of butyrylcholine), which has been a subject of discussion.

Fig. 1. Enzymic hydrolysis of various esters by milk and plasma of swine and cow. Esterase activities were determined with 0.01 M-substrate and suitable amounts of the enzyme solution; all substrates were tested and some were found to give zero values. Activity values, b_{30} , refer to 0.1 ml. of milk or plasma (see Methods). Note different scales for swine and cow diagrams. The effect of NO₂-substitution in the *para* position of phenyl acetate is illustrated by different shading (▨, phenyl acetate; ▩, *p*-nitrophenyl acetate). T, Glycerol; iAm, *iso*amyl; Ch, choline; Ph, phenyl; Ac, acetyl; Pr, propionyl; Bu, butyryl; Bz, benzoyl; Su, succinyl.

The specificity pattern of the plasma cholinesterase was similar to that of the butyrylcholinesterase of sow's milk, but the activity of the plasma towards butyrylcholine was only 4% of the activity possessed by milk (Fig. 1).

In contrast with choline esters, the phenyl esters were hydrolysed by the plasma of certain swine at about the same rate as by the milk. *para*-Substitution of phenyl acetate with NO₂ decreased the enzymic hydrolysis with milk, but increased the hydrolysis rate about eight times with plasma. Swine plasma contains, in addition to a butyrylcholinesterase, an arylesterase of the acetyl type, resistant to physostigmine and organophosphorus compounds (Augustinsson, 1958). Such an esterase is not present in milk, and it is also absent, or present in only very low concentration, in certain swine plasma. It was found that the arylesterase concentration of swine plasma exhibits considerable individual variability. For convenience of presentation, two types of plasmas will be distinguished: plasma *A* with high activity (b_{50} 300–500) and plasma *a* with low activity (b_{50} 20–35) towards phenyl acetate. This individual variability will be discussed further in the next paper (Augustinsson & Olsson, 1959).

Most vertebrate plasmas were found to contain a third esterase, ali-esterase, which was sensitive to toxic organophosphorus compounds but resistant to physostigmine (or prostigmine). There is no evidence that an ali-esterase is present in swine plasma. Aliphatic esters were hydrolysed at negligible rates, and electrophoretic and inhibitory studies revealed that the butyrylcholinesterase was responsible for the hydrolysis of these esters. The activity against phenyl acetate was reduced to an equal extent by both an organophosphorus compound (tetra α -isopropylpyrophosphoramidate) and

physostigmine (or prostigmine) (cf. Augustinsson & Olsson, 1959), suggesting that no other enzymes were responsible for the hydrolysis of phenyl acetate except cholinesterase and arylesterase.

Comparison with enzymes of the cow. Cow's milk hydrolysed all the esters studied at very low rates. The hydrolysis rates both of choline esters and phenyl esters were particularly low. The triglycerides were also hydrolysed at low rates (10% of the rates of hydrolysis by sow's milk). Cow plasma has long been known to have low cholinesterase activity, and the enzyme was recently shown to have the specificity of a propionylcholinesterase (Augustinsson, 1958). The only esterase present in significant amounts in cow plasma was an acetyl-arylesterase which hydrolysed phenyl acetate at a very high rate.

Sensitivity to selective esterase inhibitors

The activities of sow's milk towards butyrylcholine, phenyl acetate and tripropionin were inhibited to the same degree by a number of compounds which have been used previously as selective esterase inhibitors (Table 1). This is a strong indication that one single enzyme hydrolyses all three esters. The esterase is a typical cholinesterase, being sensitive to both physostigmine and organophosphorus compounds ($pI_{50} > 5$). The phenothiazine derivative (Astra 1397) was a comparatively strong inhibitor, indicating that the enzyme could be classified as a group II cholinesterase (pseudocholinesterases) (Augustinsson, 1957), to which group the butyrylcholinesterases belong. The sensitivity to compounds 62C47 and 3318CT (see Methods) is in favour of this suggestion. The results obtained with sodium arsanilate and quinine, substances which are used in clinical work as selective esterase inhibitors for

Table 1. *Susceptibility of milk and plasma esterases to various inhibitors*

Esterase activity was measured with butyrylcholine, phenyl acetate and tripropionin as substrates. The enzyme preparation was incubated for 50 min. with the inhibitor before addition of substrate. Inhibition was expressed in pI_{50} values, i.e. negative logarithm of molar inhibitor concentration producing 50% esterase inhibition. The concentration of milk during the incubation with inhibitor was 5% for butyrylcholine and phenyl acetate, and 50% for tripropionin. The concentration of plasma was 50%, except that of plasma *A* which was 5% for phenyl acetate. *iso*-OMPA, tetra α -isopropylpyrophosphoramidate. The other inhibitors listed are identified in the Methods section.

Inhibitor	Milk			Plasma		
	Butyrylcholine	Phenyl acetate	Tripropionin	Phenyl acetate		
				Butyrylcholine	Plasma <i>A</i>	Plasma <i>a</i>
Physostigmine	7.0	7.2	6.2	7.0	< 3.5	6.25
<i>iso</i> -OMPA	6.7	7.2	6.4	6.5	< 3	5.5
Mipafox	6.25	6.55	5.75	6.5	< 3.5	4.5
Astra 1397	5.4	5.4	5.8	4.1	< 3	4.4
62C47	< 3.5	< 3.5	< 3.5	3.4	< 3	2.7
3318CT	5.3	4.6	5.3	4.0	< 3.5	4.0
Sodium arsanilate	< 3	< 3	< 3.5	3.3	< 3.5	3.1
Quinine	4.5	4.3	4	4.5	< 4	3.85

liver and pancreatic esterases, indicated that the milk enzyme was similar to an esterase resistant to sodium arsenite thought to be produced in the pancreas. The similarity of cow's milk lipase and pancreatic lipase has been demonstrated previously (Rice & Markley, 1922).

The butyrylcholinesterase of swine plasma showed an inhibitory pattern which resembled closely that of the milk esterase. This evidence, in combination with results reported above, strongly suggests that the plasma and milk cholinesterases are identical.

The enzyme which hydrolysed aromatic esters and was present in high concentration in the plasma of some swine (plasma A, Table 1) was resistant to all inhibitors tested. It therefore resembled arylesterase present in mammalian plasmas. By contrast, the hydrolysis of phenyl acetate by a plasma exhibiting low arylesterase activity (plasma α , Table 1) was strongly inhibited by all those compounds which were strong inhibitors of the plasma butyrylcholinesterase; the inhibitory pattern of such plasmas was, on the whole, similar to that characteristic of cholinesterase. This suggests that the hydrolysis of phenyl acetate by this type of plasma was due mainly to the plasma cholinesterase. The less active the plasma was in hydrolysing phenyl acetate the more closely did the pattern of inhibition resemble that of cholinesterase.

Fig. 2. Distribution of protein and esterase activity after column electrophoresis of 3.0 ml. of sow's milk (fat-free). The run was performed at 30 mA over a period of 13.5 hr. in a 1.5 cm. $\times$ 40 cm. cellulose column in veronal buffer (pH 8.4, I 0.1) at 5°. The material was displaced from the column in 1.5 ml. fractions. The protein contents (●) (measured by the extinction coefficient of the Folin colour) and esterase activity (b_{30} per 0.4 ml. fractions; ■, butyrylcholine; ○, phenyl acetate; △, benzoylcholine; □, succinylcholine) are plotted as ordinates against the fraction numbers as abscissae. Peaks indicated by C1, etc. refer to fractions with butyrylcholinesterase activity. γ refers to immune globulins.

Electrophoretic fractionation

Milk and colostrum. Typical electrophoresis patterns obtained with sow's milk and colostrum are illustrated in Figs. 2 and 3. The milk electrophoresis pattern was characterized by a sharp, but non-symmetrical, esterase peak (C1), this being due to the highly active butyrylcholinesterase, which moved slower than all major milk-protein components, including the slow-moving globulins. A much smaller, faster-moving peak (C3) was also usually seen, especially with milk collected during the first days of lactation. The esterase activities as measured with butyrylcholine and phenyl acetate paralleled each other in all active fractions; this is suggestive evidence, together with the data presented above, for the presence in sow's milk of only one esterase.

In contrast with mature milk, colostrum gave an electrophoresis pattern in which the second, fast-moving peak (C3) was much more prominent, and the slow-moving peak (C1) correspondingly reduced. The faster-moving peak now accounted for over half the cholinesterase of the colostrum, and the main component in it could be seen to be accompanied by a more slowly moving second component revealed by the shoulder C2. The mobility of the main component was the same as that of the main cholinesterase fraction of plasma (see below), and the substrate specificity and other properties (e.g. sensitivity to inhibitors) were also the same for the two fractions. It is therefore suggested that the faster-moving esterase component of colostrum and mature milk and the cholinesterase in plasma are identical, that they originate from the same (unknown) source, and that the electrophoretic mobility of the enzyme present in colostrum is lowered during the course of

Fig. 3. Distribution of protein and esterase activity of 3.0 ml. of sow's colostrum (fat-free) after column electrophoresis. Experimental conditions and symbols were as described for Fig. 2.

lactation. This may be due to the loss of some anionic groups not essential for esterase activity, perhaps by combination with another constituent of milk which appears in increasing amounts. Except for the electrophoretic mobility, the properties of the esterases found in the two fractions (C3 and C1) of colostrum were identical. The differences in electrophoretic patterns of colostrum and milk samples collected at various stages of lactation, in relation to both protein and esterase composition, will be discussed in more detail in the next paper (Augustinsson & Olsson, 1959).

Blood serum. The arylesterase of serum (plasma) (Fig. 4) moved together with the albumin components, as do all vertebrate-plasma esterases of the arylesterase type (Augustinsson, 1958). Butyrylcholinesterase activity was at a maximum in two distinct fractions. The two peaks (C3 and C4) of the electrophoresis pattern were due to the same enzyme, as could be verified by specificity and inhibitor experiments. The esterase activity of fractions with the highest electrophoretic mobility and having the same position as the arylesterase active fractions was sensitive to physostigmine when tested with butyrylcholine as substrate but not when tested with phenyl acetate. Arylesterase and cholinesterase seemed to be able to move together in electrophoresis. Thus, as in milk, cholinesterase in plasma did not move as a single entity, but was found in two separate fractions.

Mixtures of esterases from milk and blood serum. Evidence was presented above that milk and blood plasma contain the same butyrylcholinesterase. This fact, and the interaction observed between this enzyme and the arylesterase present in plasma (but not present in milk), led to more detailed electrophoretic studies of the two types of esterases in mixtures. The electrophoresis pattern of a mixture of milk and blood serum is illustrated in Fig. 5. It will be observed that maximum activity against both phenyl acetate and butyrylcholine was obtained in one and the same peak with the same, or almost the same, electrophoretic mobility as that of the arylesterase peak of the serum electrophoresis pattern. That the esterase peak in Fig. 5 represented both the serum-arylesterase and milk-cholinesterase activity was revealed by substrate specificity and inhibitory studies of the most active fractions. Thus the enzymic hydrolysis of phenyl acetate by these fractions was partially sensitive to physostigmine (pI_{50} 6.5), suggesting that cholinesterase (from the milk) was actually present in the fractions tested and was contributing to the total hydrolysis of phenyl acetate. These results suggest that the two enzyme proteins form a complex with an electrophoretic mobility which is the same as that of the plasma arylesterase. Active fractions of lower mobility showed a

Fig. 4. Distribution of protein and esterase activity of 1.5 ml. of swine-blood serum after column electrophoresis. Experimental conditions and symbols were as described for Fig. 2. α , β and γ refer to serum α -, β - and γ -globulin.

Fig. 5. Electrophoresis patterns of mixtures of milk- and plasma-esterase preparations. (A) 2.0 ml. of milk and 1.0 ml. of blood serum (protein curve not inserted). (B) 2.0 ml. of isolated milk butyrylcholinesterase and 1.0 ml. of blood plasma. (C) 2.0 ml. of milk and 1.0 ml. of isolated plasma acetylarylesterase. Isolated esterases used were obtained by electrophoretic separation. Esterase activities (b_{50} per 0.4 ml. of each fraction) were measured against phenyl acetate (○) and butyrylcholine (■). Unbroken lines (without symbols) are protein curves. Other experimental conditions were as described for Fig. 2.

different substrate specificity from the highly active fractions (C4). These slower-moving fractions were represented by two esterase peaks (C3 and C2), although not clearly separated. Specificity and inhibition studies with these slower-moving fractions revealed that they contained cholinesterase only, one fraction (C3) corresponding to the original cholinesterase peak of the serum electrophoresis pattern, and the other (C2) corresponding to one of the slower-running cholinesterase complexes seen in colostrum.

Further evidence for interaction between the two esterase types was obtained when the isolated esterases were mixed together or with crude enzyme preparations before electrophoresis. It will be seen from Fig. 5 (B) that the electrophoresis pattern of a mixture of separated milk cholinesterase (peak C1) and blood plasma had lost the original milk-esterase peak, the cholinesterase activity being found in three other fractions instead, all with higher electrophoretic mobilities than the original fraction. These three peaks were all close to the three peaks demonstrated in Fig. 5 (A): C4 moving together with arylesterase, C3 identical with the cholinesterase peak of original plasma and C2 originating from the milk esterase. These three cholinesterase peaks, in addition to the original milk-esterase peak (C1), were also observed in an electrophoresis pattern (Fig. 5C) of milk which was mixed with separated plasma arylesterase before electrophoresis.

DISCUSSION

Unique substrate specificity of swine butyrylcholinesterase. Owing to species variation in the properties of enzymes, there are usually difficulties in classifying them on a strict enzymological basis. However, the enzymes hydrolysing choline esters can no doubt be regarded as a group of esterases differing from other esterases in being sensitive to $10\text{ }\mu\text{M}$ or lower concentrations of physostigmine. It is probably justifiable to retain the classification into the two types of cholinesterases which has been established during the last few years, and the use of more specific terms, such as acetylcholinesterase, propionylcholinesterase and butyrylcholinesterase is still recommended. The problem of classification, however, remains a complicated one, and the group of butyrylcholinesterases, for instance, is represented by a variety of esterase types which are best designated according to their sources.

The cholinesterase described in the present paper differs, as far as the substrate specificity is concerned, from all other cholinesterases known. The presence in swine serum of a cholinesterase which hydrolyses acetylcholine at a relatively high rate,

but which has no measurable activity when tested with benzoylcholine or acetyl- β -methylcholine, was reported by Levine & Suran (1950, 1951). Myers (1953), on the other hand, found benzoylcholine to be hydrolysed by swine serum. He reported also that both acetylcholine and butyrylcholine are hydrolysed more rapidly than propionylcholine, but unfortunately absolute activity values were not given. The experimental data presented by Myers strongly suggested that all choline esters tested were hydrolysed by the same enzyme. The results obtained in the present investigation with swine plasma confirm those reported by Myers. It is characteristic of the cholinesterase present in low concentration in swine plasma that acetylcholine is hydrolysed at about 58 % of the hydrolysis rate of butyrylcholine, and at a higher rate (1–2 %) than that of propionylcholine, the substrate concentration (10 mM) being the same for all esters. This is an exceptional substrate-specificity pattern for a cholinesterase, since all enzymes of this type described so far either split acetylcholine at a higher rate than propionyl- and butyrylcholine (group I cholinesterases), or at a lower rate than both these esters (group II cholinesterases; see review by Augustinsson, 1957).

It seems to be of great significance that the butyrylcholinesterase is present in 25 times higher concentration in sow's colostrum and milk than in plasma. All the results presented above strongly suggest that the enzyme present in milk is identical with that of plasma. The high concentration in milk and the ease with which the enzyme can be isolated (by electrophoresis) from this secretion have made it possible to study the properties of the enzyme in greater detail. Except for the properties already mentioned, the capacities for hydrolysing succinylcholine at a relatively high rate, and phenyl acetate at a rate almost the same as that of butyrylcholine, are most significant. In this respect, also, the enzyme is unique among cholinesterases.

It has been emphasized by others (e.g. Whittaker, 1951) that the effect of variation in the acyl group of the ester substrate on the rate of enzymic hydrolysis is not the same for all series of esters being hydrolysed by the same enzyme. It will be noted that the effect of changes in the acyl group on the rate of hydrolysis of aromatic and choline esters by sow's milk is not the same for the two series of esters, the acetate being hydrolysed more rapidly than both the propionate and butyrate in the aromatic series. The hydrolysis rate of phenyl acetate relative to that of butyrylcholine is high, and in some instances, especially when high concentration of the milk is tested, the rate of hydrolysis of the aromatic ester may exceed that of the choline ester, when the activities are measured at

the same substrate concentration. This high rate of enzymic hydrolysis of the aromatic ester relative to that of the choline ester may be due to an increase in the capacity of the substrate to become adsorbed on proteins, perhaps because of the presence of the aromatic ring (cf. Klotz, 1953).

Significance of arylesterase in swine plasma. In contrast with milk, the plasma of certain swine contains an arylesterase which hydrolyses neither aliphatic nor choline esters. The presence of such esterases in the plasmas of a great variety of vertebrates has recently been reported (Augustinsson, 1958); they are identical with the A-esterases reported by Aldridge (1953). The arylesterases are resistant to organophosphorus compounds and to physostigmine and have, in most cases, greater electrophoretic mobility than the other two esterase groups (aliesterases and cholinesterases) present in vertebrate plasmas. They are generally found in the albumin fraction, this being true also for the swine-plasma enzyme. The swine arylesterase is a typical acetylarylesterase, and is easily separated from the butyrylcholinesterase by electrophoresis.

The interesting feature of the arylesterase is the great variation in concentration from animal to animal. It has been demonstrated (Augustinsson & Olsson, 1959) that it is almost absent in the plasma of piglets at birth, that the capacity to synthesize it, most probably residing in the liver, is inherited, and that its concentration rises during the course of lactation to an extent which is dependent on the genotype of each animal.

The presence of the acetylarylesterase in other swine organs has not been examined, except for mammary glands in which it is present together with butyrylcholinesterase. In contrast with the latter enzyme, the arylesterase cannot be secreted actively by the glands.

Electrophoresis patterns of swine esterases. Electrophoretic separation of plasma esterases has been described previously in a few cases only (Goutier, 1956; Skofepa, 1956). The technique using electrophoresis in cellulose columns was employed recently in a comparative study of esterases present in vertebrate plasmas (Augustinsson, 1958). The electrophoretic esterase pattern of swine plasma resembles those of other mammalian plasmas. The arylesterase is located in the albumin fraction, and the cholinesterase migrates between the α_2 - and β -globulin. In addition, the butyrylcholinesterase of swine plasma is normally found also in a fraction which moves together with arylesterase; this is considered to be due to complex formation between the two enzyme proteins.

Milk (or colostrum) has not been previously used in any electrophoretic studies like those described in the present paper. Electrophoresis patterns of

sow's colostrum and milk, dealing with protein composition and being obtained with conventional free-electrophoresis technique (Deutsch, 1947; Foster, Friedell, Catron & Dieckmann, 1951) or paper electrophoresis (Nordbring, 1957), have been published previously; they are equivalent to those obtained by electrophoresis in cellulose columns under otherwise similar experimental conditions (Figs. 2 and 3). In a single electrophoretic run, the butyrylcholinesterase of mature milk is easily separated from other milk proteins owing to the very low mobility of the enzyme. Except for the mobility in electrophoresis, all properties of the milk esterase so far examined are similar to those of the plasma butyrylcholinesterase. It is therefore of significance that the esterase of colostrum is present not only in the slow-moving fraction (C1) but also in a faster-moving one (C3), the mobility of which is the same as that of the plasma enzyme. This observation strongly suggests that the two esterases are identical. It will be noted that the milk enzyme is found, not in its original position when mixed with plasma, but in the two positions characteristic for plasma butyrylcholinesterase.

The faster-moving esterase fraction of colostrum probably represents the enzyme uncombined with other components. The mobility of the enzyme protein may be lowered during the first days of lactation. Experimental evidence to show this has been obtained (Augustinsson & Olsson, 1959). The mechanism by which the enzyme changes its electrophoretic mobility will not be further investigated until the two esterase fractions have been isolated in a pure state. Similarly, further investigations must be carried out with isolated esterases from milk and plasma before any suggestions are made about the mechanism by which the butyrylcholinesterase and the plasma arylesterase interact. Changes in electrophoretic mobility of proteins due to interaction with other proteins have been observed previously (see review by Klotz, 1953).

SUMMARY

1. Sow's milk contains in high concentration a butyrylcholinesterase with a substrate-specificity pattern differing from all other cholinesterases. This enzyme hydrolyses phenyl acetate at a rate which is only slightly lower than that of butyrylcholine. No other esterase is present in important amounts in sow's milk.

2. Swine plasma contains the same butyrylcholinesterase, although in 20–25 times lower concentration.

3. An acetylarylesterase (aromatic esterase) is present in high concentration in the plasma of some

swine, and in low or medium concentration in that of others.

4. The sensitivity of the milk and plasma esterases to various inhibitors has been examined.

5. Electrophoresis in cellulose columns has been performed with milk, colostrum and blood serum of swine, and also with mixtures of separated and crude enzyme preparations.

6. The milk electrophoresis pattern is characterized by one peak of maximum butyrylcholinesterase activity. At pH 8.4, the enzyme moves more slowly than all major milk-protein components.

7. In addition to the slow-moving esterase fraction of mature milk, a second faster-moving fraction of high esterase activity is characteristic of the electrophoresis pattern of colostrum. The properties of the esterase present in the two fractions are similar.

8. Arylesterase of plasma moves together with the albumin components. Plasma butyrylcholinesterase is concentrated in two separated fractions, one moving together with arylesterase, the other having the same mobility as the faster-moving enzyme of colostrum.

9. The implications of these observations are discussed.

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Esterases in the Milk and Blood Plasma of Swine

2. ACTIVITIES AT DIFFERENT STAGES DURING THE LACTATION AND SUCKLING PERIODS, AND PLASMA ARYLESTERASE AS A GENE-CONTROLLED ENZYME

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A butyrylcholinesterase of substrate specificity different from that of all other cholinesterases is present in high concentration in sow's milk; no other esterases are present (Augustinsson, 1958*b*; Augustinsson & Olsson, 1959). Swine plasma contains the same esterase, but in much lower concentration; in addition, an acetylarylesterase is present in high concentration in some plasmas and in very low concentration in others. There is no evidence so far that other types of esterases (aliesterases, lipases) are present in the plasma of swine. The present investigation was designed to study the

esterase activities in colostrum, milk and plasma of the sow during the course of lactation and the activities in the plasmas of her piglets during their suckling period. Special attention was directed to the frequency of high and low plasma arylesterase activity in the litter.

METHODS

Assay of esterase activity. The esterase activity was measured by the Warburg technique and expressed in b_{30} /0.1 ml. (unless otherwise stated) of colostrum, milk, plasma or serum (Augustinsson, 1957).

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