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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Substrates. Routine measurements of butyrylcholinesterase and arylesterase activities were carried out with butyrylcholine iodide and phenyl acetate respectively in a final concentration of 10 mM.

Inhibitor. Prostigmine bromide was used as a selective inhibitor for butyrylcholinesterase in the estimation of the two types of esterase present in swine plasma.

Blood plasma (serum). Blood samples were collected with heparin without anaesthesia from sows and boars by puncture of an ear vein and from piglets by puncture of the anterior vena cava. All animals used were of the Swedish Landrace breed. The plasma was centrifuged free from all cells including thrombocytes. In some instances, the plasma was preserved by freezing, which resulted in the formation of fibrin clots; these were removed by centrifuging (or by hand) before using the plasma. Serum was prepared by removing the clot of untreated whole blood, and was always free from signs of haemolysis; the plasma was more difficult to obtain free from some signs of haemolysis but this did not influence the results, as was shown by control experiments.

The esterase activities of plasma and milk were followed during the lactation period for three sows and their piglets (Expts. I-III); the same boar was the father of all three litters. In addition, the plasmas of a fourth litter (Expt. IV) of the same father were analysed at 22 and 70 days of age. Blood samples were collected from the sows 2-4 days before expected parturition, at parturition and 36 hr. and 3 days after parturition, and then weekly to about 56 days after parturition. The piglets were removed from the sow immediately after birth and were not allowed to suckle. Blood samples from the cord (after cutting) of each piglet were pooled. All piglets of the litter were then allowed to suckle and subsequent blood samples from individual piglets were collected at the age of 36 hr., 3 days and then weekly to the age of about 56 days.

Milk. Colostrum and milk were collected at the time when the blood samples were drawn. All colostrum and milk samples were pooled samples, obtained from several teats by manual expression. The piglets were isolated from the sow 2-3 hr. before milking. In Expt. I during the whole lactation period, and in Expts. II and III at parturition and 36 hr. after parturition, colostrum and milk were collected at the ordinary let-down when the piglets were allowed to suckle. From 3 days after parturition in Expts. II and III, the milk samples were obtained after inducing the let-down by the intravenous injection of 10 i.u. of oxytocin (Hypadrin; Astra, Södertälje, Sweden) into an ear vein. The samples were analysed without delay, or preserved by freezing before use; the latter procedure did not influence the esterase activity, nor did it produce any

significant change in the electrophoretic patterns, as has been demonstrated (Mathews & Buthala, 1956).

Electrophoresis. Electrophoretic separation of the protein components of milk and blood plasma was performed in cellulose columns, 1.5 cm. $\times$ 40 cm., in veronal buffer solution (pH 8.4, I 0.1) (Augustinsson, 1958a).

RESULTS

Butyrylcholinesterase of colostrum and milk

Activity during the course of lactation. The butyrylcholinesterase in sow's milk is responsible for the high hydrolysis rate of both choline and aromatic esters. This makes possible the use of either type of ester when studying the esterase activity during the course of lactation. Butyrylcholine was preferred, because this substrate is generally hydrolysed more rapidly. The butyrylcholinesterase activity declined during the first 2 weeks after parturition; it then varied from time to time, but was rather constant during the later part of lactation. The activity of later milk was usually lower than that of colostrum (Expts. II and III, Table 1; cf. Fig. 2), but higher activity (Expt. I) or the same activity was also observed. Hines & McCance (1953), who measured the esterase activity with acetylcholine as substrate, found colostrum and milk to have the same activity. The hydrolysis rate of phenyl acetate paralleled that of butyrylcholine, which is consistent with the previous observations that the same enzyme is responsible for the hydrolysis of the two substrates. In contrast with milk, colostrum in some cases (as in Fig. 2) hydrolysed phenyl acetate somewhat more rapidly than butyrylcholine (cf. Augustinsson & Olsson, 1959).

Changes in electrophoretic pattern during the course of lactation. The electrophoresis pattern of colostrum was characterized by two peaks of high cholinesterase activity (Fig. 1). The properties of the enzymes present in the two fractions were similar in substrate specificity and sensitivity to inhibitors. The slower-moving esterase component was identical with the active component (C1) of later milk, in which the faster-moving esterase component (C3) was missing. In addition to these

Table 1. *Butyrylcholinesterase activity of colostrum and milk from three sows during the course of lactation*

Esterase activity was measured towards 10 mM-butyrylcholine and expressed in $b_{30}/0.1$ ml. of colostrum or milk.

Expt. I		Expt. II		Expt. III	
Day of lactation	b_{30}	Day of lactation	b_{30}	Day of lactation	b_{30}
1	350	1	670	1	732
6	177	8	435	8	465
23	325	22	365	22	487
37	427	36	385	43	388
51	497	50	470	—	—

peaks of maximum esterase activity, colostrum as well as mature milk gave a peak (C2) of lower esterase activity between the two main peaks. The faster-moving component (C3), which was shown to be identical with the cholinesterase present in blood plasma, disappeared gradually during the first days of lactation and was not present in later milk from the fourth day after parturition. It is obvious that the mobility of the esterase component is significantly changed during lactation. When the esterase is formed, probably in the mammary glands, the mobility is that of the faster-

moving fraction (C3); it is then gradually lowered to reach the mobility of the slower-moving fraction (C1).

The results of protein analysis demonstrated the high relative concentration of slow-moving globulins in early colostrum, and the marked decrease in concentration of these proteins during the first 24 hr. after delivery. This significant change in protein composition of milk during lactation confirms previous results obtained with conventional free-electrophoresis technique and paper electrophoresis, and reported for both sow's milk (Deutsch, 1947; Foster, Friedell, Catron & Dieckmann, 1951; Nordbring, 1957) and milk of other animal species.

Butyrylcholinesterase of blood plasma

The butyrylcholinesterase activity of sow's blood plasma was almost constant during the whole lactation period, and the same as before delivery (Fig. 2). At birth, the cholinesterase activity was lower in the plasma of the piglets than in that of their mother. The activity rose in the first 10–15 days of life to about the same order of magnitude

Fig. 1. Electrophoresis patterns of colostrum and milk at various stages during the lactation period: (A) at parturition; (B) 2 days, (C) 4 days, and (D) 25 days after parturition. The run was performed with 3.0 ml. each of colostrum or milk 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. Relative protein contents (unbroken lines without symbols; measured by the extinction coefficient of the Folin colour) and esterase activity (b_{30} per 0.4 ml. fractions; ■, butyrylcholine; ○, phenyl acetate) are plotted against the fraction numbers. C1, C2 and C3 are active fractions referred to in the text.

Fig. 2. Butyrylcholinesterase activities of the plasmas of the sow and her seven piglets, and of sow's milk (colostrum) during the course of lactation and suckling (Expt. III; cf. Table 3). The vertical arrow indicates weaning. Esterase activity (b_{30} per 0.01 ml. of milk or 0.1 ml. of plasma) was measured with butyrylcholine (■, milk; ○, sow plasma; △, mean activity values, and ●, highest and lowest activity values, of piglet plasmas); milk-esterase activity was also tested against phenyl acetate (□). Esterase activity of piglet plasmas at birth was measured with a pooled sample collected from the cords. Butyrylcholinesterase activity in the plasma of the boar was 19.5.

as that of the mother. All piglets behaved similarly, and the values obtained for individual piglets of a litter did not show greater variation than could be expected from a normal enzyme-activity variation of a pure population (Table 3). Even between different litters no significant differences were observed.

The rise in plasma-butyrylcholinesterase activity of piglets during the first 2 weeks of suckling may well depend on the consumption of mother's colostrum, which contains the enzyme in high concentration. Experiments to test this assumption were not performed, mainly because of difficulties entailed in rearing the piglets on cholinesterase-free colostrum and milk (evaporated or synthetic).

Acetylarylesterase of blood plasma

Plasmas with high and low activity. The concentration in plasma of an arylesterase, not present in milk, varied considerably from animal to animal. In fact, various types of swine plasma exist with high (b_{30} 300–500), intermediate or low (b_{30} 20–35) activity towards phenyl acetate. There is no sex difference. The arylesterase was specific towards aromatic esters, and butyrylcholine was therefore not split by this enzyme. Aromatic esters, on the other hand, were also hydrolysed by butyryl-

cholinesterase, which was always present in low concentration (b_{30} 18–25) in swine plasma, and did not exhibit significant individual variation.

In order to calculate the contribution made by the two esterases (butyrylcholinesterase and acetylarylesterase) to the hydrolysis of phenyl acetate, the activity was determined in the presence and absence of prostigmine; the prostigmine-sensitive portion of the total hydrolysis was taken as being due to cholinesterase. Determinations were made on a series of plasmas from adult and young animals differing in their capacity to hydrolyse phenyl acetate (Fig. 3). In a plasma from an adult swine with high arylesterase activity, less than 5% of the hydrolysis rate of phenyl acetate was attributed to butyrylcholinesterase; in a plasma with low arylesterase activity this portion was about 58%. In newborn animals, which irrespective of the activities of the parents had low plasma-arylesterase activity, less than 3% of the hydrolysis rate of phenyl acetate was due to arylesterase. This enzyme may be regarded as being absent at birth in the piglets, since in the plasma from cord blood the hydrolysis of phenyl acetate could be almost completely abolished by 0.01–0.1 mM-physostigmine or prostigmine.

The low arylesterase activity of certain plasmas was probably not due to inhibition, because mixing plasmas with high and low activity respectively yielded enzyme preparations with activities equivalent to the sum of the activities of the components (Table 2). Nor did the activities of isolated enzyme preparations (by electrophoresis) from plasmas with low arylesterase activity suggest the presence of inhibitors as an explanation for the low activity.

Activity during the course of lactation and suckling period. Preliminary experiments demonstrated that the arylesterase activities in the plasmas of individual piglets from the same litter were not of the same order of magnitude. Within a litter of nine piglets, 135 days old (litter I, Table 3), four piglets had high plasma-arylesterase activity (278 ± 37.5) and five piglets low activity (26.5 ± 3.5).

Fig. 3. Contribution made by cholinesterase and arylesterase of various swine plasmas from adult and young animals to the hydrolysis of phenyl acetate. Cholinesterase activity of each plasma (1–6) (b_{30} per 0.1 ml., right abscissa) was abolished by treatment with prostigmine bromide for 45 min. before addition of substrate. Left abscissae, negative logarithm (pl) of prostigmine concentration (M) during activity determinations. The percentage contribution made by each esterase (●, butyrylcholinesterase; ○, acetylarylesterase) to the total hydrolysis of phenyl acetate is indicated in the histogram. (1) and (2): adult animals; (3)–(6): piglets of various ages; (3), cord blood; (4), 2 days; (5) and (6), 36 days old.

Table 2. Summation experiments with plasmas having high (A) and low (a) arylesterase activity

Esterase activity was measured with phenyl acetate as substrate and with mixtures of 0.1 ml. of plasma A and various amounts of plasma a.

Plasma	Volume (ml.)	b_{30}	b_{30} of mixtures of plasma A and a	
			Calc.	Found
A	0.1	61.5	—	—
a	0.1	18.5	80.0	82.5
	0.2	37.0	98.5	99.0
	0.3	54.0	115.5	116.5

This observation indicated a possible inheritance of arylesterase in swine plasma, and the activity was therefore studied in more detail with various litters and their parents. So far, four litters with the same father but with different mothers have been examined. Table 3 summarizes the results obtained with the litters of various ages. The boar had low enzyme activity, and the sows high in two experiments (II and III; the activity of the mother of litter I, most probably high, was not examined because it was slaughtered before the genetic aspects of the arylesterase were noted) and low in one experiment (IV). In the litters, the sows of which had high arylesterase activity, one group had high (three out of seven in both litters II and III) and another low (or medium) activity; there was a significant difference between the activities of the two groups in all three litters (I, II and III). In litter IV, both parents of which had low arylesterase activity, all six piglets had the same low activities 22 and 70 days after birth. We are inclined to regard these observations as evidence that the property of swine plasma to hydrolyse phenyl acetate (as a measure of arylesterase) is inherited.

In contrast with the individual variation of arylesterase activity in piglets 3 weeks or more of age, all newborn animals had the same low enzyme activity irrespective of their mothers' high activity (litters II and III). Differentiation into two groups, one with high and one with low enzyme activity, began on the second to the fourth day of life (Figs. 4, 5). It is therefore not the enzyme protein itself which is inherited, but the power to synthesize it. The activity increased steadily up to the

value found for the plasma of the sow (Expt. III) or to medium values (Expt. II) compared with sow's activity. In one of the experiments (Expt. III, Fig. 5) the activities of the second group of animals were at the same low level during the entire lactation period. In the other experiment (Expt. II, Fig. 4) there was an increase of the arylesterase activity also in the plasmas of the second group during the first 2 weeks; this increase was, however, significantly smaller than in the animals with high activity, and the final activity values were constant and at a relatively low level compared with those of high activity. It will be observed that the plasma activity of the sow in Expt. III was much lower (about 250) than that of the sow in Expt. II (about 400).

It will be noticed that the arylesterase activity of the sows was lower than normal at parturition. The activity increased rapidly after parturition (in about 4 days) to higher (normal) values.

As noted in Fig. 5, the activity in one of the animals (with high adult activity values) decreased significantly at 29 and 36 days after birth and then rose again up to the levels found in the two other animals of the same group. We are inclined to regard this disturbance in enzymic synthesis as possibly being due to a subclinical hepatitis; the esterase is most probably synthesized in the liver.

Electrophoretic patterns. Esterase activity measured towards phenyl acetate was found in two peaks. The faster-moving component moved together with the albumin fraction and represented arylesterase. The slower-moving esterase component (C3), of much lower activity than the faster-moving one, was sensitive to physostigmine and it

Table 3. *Butyrylcholinesterase and arylesterase activities in the blood plasma of piglets from four litters of various ages, with the same father*

Esterase activity (b_{30}) was measured towards butyrylcholine (BuCh) and phenyl acetate (PhAc) and with 0.1 ml. of plasma. The plasma activities of the father were 19.5 for BuCh and 33.5 for PhAc.

Litter (Expt. no.)	Age (days)	b_{30}											
		I			II			III			IV		
		135			49			22			22		
		Sex	BuCh	PhAc	Sex	BuCh	PhAc	Sex	BuCh	PhAc	Sex	BuCh	PhAc
Sow		—	—	—	—	21	389	—	23	246.5	—	23.5	31.5
Piglet													
1		F	19	251	F	20.5	271	M	19.5	137	M	17	23
2		F	24	246	M	22	230	F	21	180	M	19	22
3		F	27	389	F	24.5	263	F	17	144	F	19	28.5
4		M	21.5	224	F	29	85.5	M	21	28.5	M	20.5	24.5
5		M	26.5	30	F	24.5	90	M	20	28.5	F	20	27.5
6		F	23.5	19	F	29	103	F	17	22	F	20	32
7		F	26	27.5	F	23	99.5	M	15	14.5	—	—	—
8		F	22.5	17.5	—	—	—	—	—	—	—	—	—
9		F	28	37.5	—	—	—	—	—	—	—	—	—
Mean (BuCh)			24±1			24.5±1			18.5±1			19±0.5

Fig. 4. Acetylarylesterase activity of the plasmas of the sow and her seven piglets during the course of lactation (Expt. II). Esterase activity (b_{30} per 0.1 ml.) was measured with phenyl acetate (○, sow; △, mean activity values of one male and two female piglets; □, mean activity values of four female piglets). Arylesterase activity in the plasma of the boar was 33.5. See Fig. 2 for further details.

Fig. 5. Acetylarylesterase activity of the plasmas of the sow and her seven piglets during the course of lactation (Expt. III). Esterase activity (b_{30} per 0.1 ml.) was measured with phenyl acetate [○, sow; □, mean activity values of three male and one female piglets; △, mean activity values of two male and one female piglets, except for values obtained 29 and 36 days after birth, these referring only to the female and one of the males, since the activities of the other male piglet (▲) were significantly lower on these days]. The father of the litter was the same as in Expt. II (Fig. 4). See Fig. 2 for further details.

Fig. 6. Electrophoresis patterns of the plasmas of piglets of various ages: (A) 2 days, (B) 7 days, (C) 80 days. In each run 1.5 ml. of plasma was used. Experimental conditions were as described for Fig. 1. Relative protein contents (unbroken line; measured by the extinction coefficient of the Folin colour) and esterase activity (b_{30} per 0.4 ml. fractions, measured against phenyl acetate, ○) are plotted against the fraction numbers. The active fractions at C3 are due to butyrylcholinesterase; they have the same position as the butyrylcholinesterase peak in the colostrum electrophoresis pattern (C3 in Fig. 1). α , β and γ refer to serum α - β - and γ -globulin. ϕ refers to fibrinogen.

hydrolysed butyrylcholine. This component was actually identical with the butyrylcholinesterase present in low concentration, and was found in fractions between α - and β -globulin. The marked increase in plasma-arylesterase activity observed for some piglets in Expts. II and III (Figs. 4, 5) is also illustrated in Fig. 6, which shows the electrophoresis patterns at various stages of life for one of the three piglets of Expt. II which had high arylesterase activity at the time of weaning. It will be seen that there was no significant change in the mobilities of the two esterase components as a function of time. The marked increase in electrophoretic mobility of the butyrylcholinesterase of sow's colostrum during the first day after delivery did not occur in the blood plasma of piglets. It should be remembered that the cholinesterase activity in sow's plasma was constant during the entire lactation period.

The change in the relative protein composition of plasma is also illustrated in Fig. 6. The most

striking change was the marked decrease in γ -globulin concentration from the second day after birth, reaching normal adult level at 80 days, and the steady increase in albumin concentration during the same period. Arylesterase moved together with albumin in electrophoresis, and the concentration of both these components increased gradually during the first 2 months of life. Moreover, albumin is synthesized in the liver, which therefore is probably the organ which synthesizes arylesterase. The relative concentration of α -globulin also increased during the first week, but β -globulin was comparatively constant in relative concentration throughout the period studied. Fibrinogen was relatively high on the second day after birth and then decreased gradually. These results for protein composition confirm those obtained by Foster *et al.* (1951) with a conventional free-electrophoresis technique and by Nordbring & Olsson (1957) with paper electrophoresis.

DISCUSSION

Butyrylcholinesterase. The physiological function of butyrylcholinesterase in swine plasma is unknown, as is that of all cholinesterases in vertebrate plasmas. The enzyme is present at birth in the plasma of piglets in a concentration which is lower than that of the mother's plasma. It is not possible to decide whether the enzyme is transmitted through the placenta to the foetus. It is characteristic of swine that they have an epitheliochorial placental barrier. The transfer of immune globulins occurs entirely by the colostral route. The power to absorb immune globulins decreases rapidly during the first days after birth (Nordbring & Olsson, 1957). The increase in plasma butyrylcholinesterase activity to the levels found in the mother, on the other hand, takes place during a longer period of time (2-3 weeks). It is therefore improbable that butyrylcholinesterase is absorbed as such from colostrum and milk. The enzyme, most probably, is synthesized in the piglet itself.

The physiological function of butyrylcholinesterase of milk is as uncertain as that of the plasma. The enzyme may be produced actively by the mammary gland, which is known to contain it, and this may explain the high concentration in milk. Preliminary experiments have revealed that the mammary gland of the sow contains the same cholinesterase in relatively high concentration (b_{30} per 100 mg. of fresh gland was about 400 with butyrylcholine); the activity was the same as that of the nuclei of mammalian brains when tested against acetylcholine. The possibility that the enzyme is produced in some other organ (e.g. the liver) and is secreted into the milk via the blood is unlikely, mainly because of its much higher con-

centration in milk than in plasma. However, the fact that the enzyme is present at birth suggests that it is synthesized elsewhere, the pancreas being a possible source. The pancreatic juice of dog has high cholinesterase activity (McCance, Brown, Comline & Titchen, 1951) and the pancreas of various animal species has long been known to contain cholinesterases (see review by Augustinsson, 1948). Butyrylcholinesterase is actually present in swine pancreas (in low concentration) as was demonstrated in some preliminary experiments.

The same butyrylcholinesterase has also been found in parotid-gland saliva obtained from adult swine; the activity was 10-20% of that of milk. The presence of a cholinesterase in swine saliva has been observed previously (McCance *et al.* 1951; Hines & McCance, 1953), and the parotid gland of swine has been reported to have a high cholinesterase activity (Glick, Levin & Antopol, 1939). Gastric juice of swine does not contain cholinesterase and has no effect on milk butyrylcholinesterase.

The milk esterase may be synthesized from the amino acids of the circulating blood, from the amino acids formed from the degradation of blood plasma proteins in the mammary gland or from plasma proteins by transpeptidation; it is also possible that two or all three of these reactions take part in the synthesis. A close relation may exist between the arylesterase present in high concentration in certain swine plasmas and butyrylcholinesterase of milk; in some cases, for instance, the two enzymes were found to move together in electrophoresis. An inactive protein (proesterase) of blood plasma may be the precursor for the two esterases, a transpeptidation of the protein molecule then giving rise to either arylesterase (probably in the liver) or cholinesterase, the latter being especially actively produced in the mammary gland and less so in the pancreas. It is interesting in this connexion to refer to recent observations (Staub & Boguth, 1956; Hoerlein, 1957) which suggest that partly degraded proteins or polypeptides may be absorbed by the piglets from colostrum and milk and later used in protein synthesis.

The butyrylcholinesterase of colostrum, in contrast with later milk, was demonstrated to be present in two fractions separated by electrophoresis. Experimental evidence was presented that the mobility of the enzyme is lowered during the first days of lactation. The mobility of the faster-moving esterase fraction is the same as that of plasma butyrylcholinesterase. Because of practical difficulties, prenatal secretion has not been available for analysis. This secretion may well contain the faster-moving esterase fraction as the predominant one.

Evidence has been presented recently that esterases are localized mainly in the microsomes of animal tissues. Milk contains microsomes (Bailie & Morton, 1958), which are derived from mammary tissue and might serve as a vehicle for the butyrylcholinesterase. It may therefore well be that the slower-moving component (C1) represents microsomal esterase and the faster-moving one (C3) represents free enzyme.

Acetylarylesterase. The physiological function as well as the natural substrate of the arylesterase of swine are unknown. The occurrence of the esterase in other tissues except blood has not been investigated. The natural substrate should be present in higher concentration in animals which have a low arylesterase activity compared with those having a high esterase activity, and consequently the two groups of animals might be expected to differ also as far as the substrate concentration is concerned.

The discovery that the piglets of our four litters could be separated into two groups, one with high and the other with relatively low plasma-arylesterase activity, is considered by us to give suggestive evidence that the biosynthesis of the enzyme is gene-controlled, though as yet no complete genetic analysis has been performed. There is no sex influence upon this synthesis. All piglets studied had low activity against phenyl acetate at birth, irrespective of high or low activity of the parents; less than 3 % of this activity is due to arylesterase, the butyrylcholinesterase being mainly responsible for it. Therefore diaplacental transmission of the esterase does not occur. The increase in arylesterase activity in some groups of piglets cannot be due to absorption from colostrum or milk, because these do not contain this enzyme. It has been shown that the enzyme activity appears during the first weeks of life and electrophoresis shows that the enzyme is closely related to the albumins. Since albumin is synthesized by the liver it is probable that the liver synthesizes arylesterase also.

The suggested gene-controlled esterase activity is to be regarded as one of the first clear examples of a direct relation between genes and enzyme in higher animals. It has been reported that the ability of the blood of some rabbits to hydrolyse atropine is inherited as a partially dominant character (Sawin & Glick, 1943; Ammon & Savelsberg, 1949), and the inheritance of atypical forms of human serum cholinesterase (with respect to sensitivity to dibucaine) has been discussed recently (Kalow & Staron, 1957). In none of these cases was experimental evidence presented to show that the esterases are direct products of gene action. Experimental findings with micro-organisms and also with higher animals including man have been presented to support the idea that

a direct relation does exist between genes and enzymes, 'but in each example alternative and equally plausible interpretations are possible' (Wagner & Mitchell, 1955). We are inclined to believe that the differences in the power to synthesize arylesterase of the two groups of piglets from one and the same litter (Expts. II and III) cannot be interpreted otherwise than being due to a difference in the genotype of the two groups. The variation in enzyme activity must be directly related to genetic factors, because all piglets of the litter lived together under the same surrounding conditions.

It is important to note that the esterase activities, so far observed, can be classified into three or more groups: high (500–300), intermediate (250–100) and low (35–20) activity. These various groups of phenotypes can be produced genetically in several ways. Experiments in progress have the aim of exploring which type of gene interaction is the most likely one.

SUMMARY

1. The variation in activity of butyrylcholinesterase of sow's colostrum and milk, and that of acetylarylesterase and butyrylcholinesterase of swine blood plasma, have been studied in relation to the stage of lactation.

2. Butyrylcholinesterase activity of colostrum and milk declines during the first weeks after parturition and then rises, being rather constant during the later part of lactation.

3. The electrophoresis pattern of colostrum is characterized by two peaks of high cholinesterase activity. The faster-moving component, which is identical with the cholinesterase present in blood plasma, disappears during the first days of lactation.

4. Butyrylcholinesterase activity of sow's blood plasma is constant during the lactation. The activity of the piglets is lower at birth than that of their mother and then rises to adult levels during the first 2 weeks.

5. Three types of plasmas, with high, intermediate and low acetylarylesterase content, are found.

6. Newborn piglets have low plasma-arylesterase activity irrespective of high activities in both or one of the parents. At birth, most of the low activity towards phenyl acetate is due to butyrylcholinesterase.

7. The low plasma arylesterase activity is not due to the presence of an inhibitor.

8. Evidence is presented that the power of piglets to synthesize acetylarylesterase is gene-controlled.

9. The increase in plasma arylesterase of some piglets during the first weeks of life runs parallel with the increase of albumin concentration.

10. The implications of these observations are discussed.

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Lactic Dehydrogenase and Cytochrome b_2 of Baker's Yeast

PURIFICATION AND CRYSTALLIZATION

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Meyerhof (1919) first showed that yeast could oxidize lactic acid in the absence of any added coenzyme. Bernheim (1928) later extracted acetone-dried baker's yeast with a phosphate buffer and, after dialysis, obtained a turbid solution which reduced methylene blue in the presence of lactate, glycolate and α -hydroxybutyrate. No added coenzyme was required. Reduction of both methylene blue and heart-muscle cytochrome c by a preparation from plasmolysed Delft yeast in the presence of lactate was shown by Ogston & Green (1935). Boyland & Boyland (1934) and Adler & Michaelis (1935) both showed that there was no activation of the yeast enzyme by added diphosphopyridine nucleotide or triphosphopyridine nucleotide.

Dixon and co-workers (Dixon & Zervas, 1939; Bach, Dixon & Zervas, 1942) extensively purified the enzyme from autolysates of air-dried baker's yeast. Added methylene blue and heart-muscle cytochrome c , and yeast cytochrome c present in the extract, were reduced with lactate as substrate.

The rate of enzymic reduction of methylene blue was proportional to the concentration of a new protein haemochromogen which these workers called cytochrome b_2 (Bach, Dixon & Keilin, 1942). Bach, Dixon & Zervas (1946) therefore concluded that 'cytochrome b_2 forms an essential part of the enzyme system, either as the dehydrogenase itself or as an essential intermediate carrier between lactate and methylene blue'.

This paper describes the purification and crystallization of yeast lactic dehydrogenase. This work, together with unpublished studies of the enzymic, chemical and physical properties of the enzyme, was carried out to determine the relationship between the dehydrogenase activity and cytochrome b_2 . The results were described at the meetings of the Australian and New Zealand Association for the Advancement of Science in Canberra, 1953, and in Melbourne, 1955, and preliminary accounts have been published (Appleby & Morton, 1954; Morton, 1955*a*).

MATERIALS

DL-Lactate. A.R.-grade DL-lactic acid (British Drug Houses Ltd.) was diluted to 25% (w/v), the lactone hydrolysed by refluxing for 4 hr. and then adjusted to the required pH with 8.2N-NaOH and diluted to 2M-concentration.

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