The optimum pH range of the extraction varies for different barbiturates, but a pH of 8.0 is suitable for most of the compounds.

Chloroform can be used for the extraction of the N-substituted compounds and of 5-*iso*amyl-5-ethyl- and 5-allyl-5-phenyl-barbituric acids. Chloroform with 20 % dioxane is suitable for 5-ethyl-5-phenyl-barbituric acid. Chloroform with 20 % of benzyl alcohol extracts 5,5-diallyl-, 5,5-diethyl-, 5-allyl-5-*iso*propyl-, and 5-ethyl-5-*cyclo*hexenyl-barbituric acids.

The infrared spectra of some barbituric acids and their compounds with sodium, barium, and mercuric ions indicate that the mercury forms complexes rather than salts with the barbituric acids.

Of compounds containing nitrogen and having a structure similar to barbituric acid certain hydantoins and cyclic imides of dicarboxylic acids will be extracted and interfere.

The method has been applied to the determination of barbituric acids in blood. A full report will be published elsewhere.

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A New Type of Cholinesterase in Sow's Milk

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Choline esters are generally not hydrolysed by milk. In two instances, however, milk was shown to exhibit high or moderate cholinesterase activity (acetylcholine used as substrate), *i.e.*, the colostrum of dog [1] and the colostrum and milk of swine [2]. These two sources of cholinesterases are interesting species differences in biochemical behaviour for which no biological explanation is known so far.

Table 1. Enzymic hydrolysis of various choline esters and phenyl esters by the milk of sow and cow. Substrate concentration, 0.01 M. Activity expressed in b_{30} per 0.1 ml of milk.

Acyl radical	Choline esters		Phenyl esters	
	Sow	Cow	Sow	Cow
Acetyl	290	0	465	1
Propionyl	280	1	255	1.5
Butyryl	500	1	205	0
Benzoyl	65	1	—	—
Succinyl	55	0	—	—

The properties of cholinesterase present in sow's colostrum and milk were investigated in connection with studies on the variation of esterase activities of milk and plasma of swine during the course of lactation and suckling [3]. The most characteristic feature of this esterase (Table 1) is the rapid hydrolysis of choline esters and phenyl esters; in the first group of esters butyrylcholine is hydrolysed at the highest rate, in the second group the acetate is hydrolysed more rapidly than both the propionate and butyrate. The specificity pattern with choline esters as substrates differs from those of all other cholinesterases known, except that of swine plasma cholinesterase which contains the same enzyme in 20—25 times lower concentration; the exceptional substrate specificity of the plasma enzyme as far as choline esters are concerned was reported previously [4,5]. Both acetylcholine and propionylcholine are hydrolysed more slowly than butyrylcholine, and acetylcholine more rapidly than propionylcholine. Benzoylcholine and succinylcholine are also split by the milk esterase; the hydrolysis of the latter ester is exceptional since no other animal tissue so far studied hydrolyses this ester at such a relatively high rate.

It was demonstrated in summation experiments, as well as in inhibition experiments with selective esterase inhibitors, that a single enzyme in sow's milk is responsible for the hydrolysis of both the choline and aromatic esters tested. It is characteristic of this esterase that butyrylcholine and phenyl acetate are hydrolysed at about the same high rate when tested at the same substrate concentration ($[S] > 5 \times 10^{-3}$ M); in this concentration range K_S is the same for the two reactions (2.8×10^{-3}). Triglycerides are also

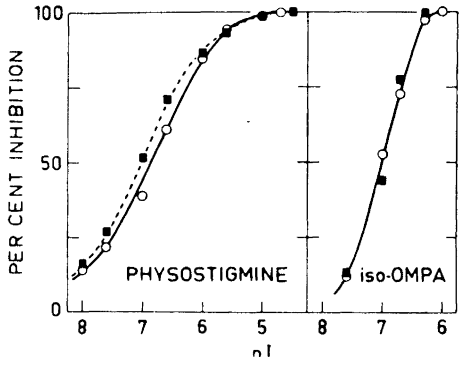

Fig. 1. Inhibition of sow's milk esterase by physostigmine salicylate and tetra-*iso*propyl-pyrophosphoramide (iso-OMPA). Substrates (0.01 M): butyrylcholine iodide (■) and phenyl acetate (○). pI = — log molar inhibitor concentration in the reaction mixture. Enzyme incubated 50 min with the inhibitor prior to addition of substrate.

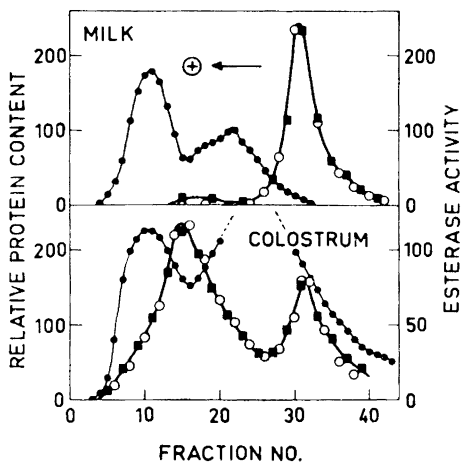

Fig. 2. Electropherograms of sow's milk and colostrum obtained with 3.0 ml material in cellulose columns (1.5 cm. × 40 cm.; veronal buffer, pH 8.4, $I = 0.1$). Displacement from the column in 1.5 ml fractions. Thin line (●): relative protein contents (optical density of the Folin colour). Heavy line: esterase activity (b_{30}/0.4 ml fractions); ■, butyrylcholine; ○, phenyl acetate.

hydrolysed by this enzyme, but the hydrolysis rate is only 1—3 % of those obtained with butyrylcholine and phenyl acetate.

The esterase is a cholinesterase, because it is sensitive to both physostigmine and organophosphorus compounds ($pI_{50} > 5$). Phenothiazine derivatives (*e.g.*, 10-[-diethylamine-propionyl] phenothiazine hydrochloride) are comparatively strong inhibitors, indicating that the enzyme is classified as a Group II cholinesterase [6], to which the butyrylcholinesterases of other sources belong. The sensitivity to 62C47 (1,5-bis[4-trimethyl-ammoniumphenyl] pentane-3-one diiodide) is in favour of this suggestion. Fig. 1 shows the inhibition curves obtained with butyrylcholine and phenyl acetate as substrates and physostigmine and iso-OMPA (tetra-*iso*propylpyrophosphoramide) as inhibitors; these results are indicative evidence that the same enzyme hydrolyses the two substrates. The inihibition by physostigmine differs from that of other cholinesterases by not being of the true competitive type.

Additional proof for the existence of one esterase in sow's milk was performed by electrophoresis analysis in cellulose columns [7]. Typical electrophoresis patterns of sow's milk and colostrum are illustrated in Fig. 2. The milk electropherogram is characteristic by a sharp esterase peak, being due to the highly active butyrylcholinesterase, which moves slower than all major milk protein components. In contrast to mature milk, colostrum gives an electropherogram in which a second, faster-moving peak is much more prominent, and the slow-moving peak correspondingly reduced. The two esterase fractions of colostrum have identical properties, as far as enzyme specificity and sensitivity to inhibitors are concerned. The mobility of the faster-moving cholinesterase fraction of colostrum is identical with the cholinesterase fraction of swine plasma, and the substrate specificity and other properties are also the same for the two fractions. It is therefore suggested that the faster-moving esterase component of sow's colostrum and swine plasma cholinesterase are identical, and that the electrophoretic mobility of the esterase present in colostrum is previously lowered during the course of lactation.

Evidence has been presented recently that esterases are localized mainly in the microsomes of animal tissues. Milk con-

tains microsomes [8] which are derived from mammary tissue and might serve as a vehicle for its butyrylcholinesterase. It may therefore well be that the slow-moving component represents microsomal esterase and the fast-moving one, free enzyme.

A full account of this work including studies on the esterases present in swine plasma will be published in *Biochem. J.*

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