alone was eluted with water and subjected to paper electrophoresis (Veronal-Acetate buffer, pH 8.6; 200 V, 3.8 ma) The major portion of the ninhydrin-reacting material (more than 80%) migrated identically with the synthetic and natural tyrosine phosphate preparations (5.0 cm in 3 h).

These results show clearly that the component from *Drosophila* that has been designated $P_1 + P_2$[2-5] consists mainly of tyrosine-O-phosphate.

Ninhydrin-positive components of similar R_f-values have also been found on chromatograms of *Ephestia*- and *Culex*-larvae[7,8]. Studies are now in progress to see if tyrosine-O-phosphate is really present in these insects.

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Zoologisches Institut der Universität Zürich (Switzerland), July 7, 1960.

Zusammenfassung

Es wird gezeigt, dass die auf zweidimensionalen Chromatogrammen von *Drosophila melanogaster* auftretenden Ninhydrin-positiven Flecken, die als $P_1 + P_2$ (Peptide) bezeichnet wurden, sehr viel freies Tyrosin-O-phosphat enthalten.

The Esterase Activity of Dog's Colostrum

Milk and colostrum from most mammals have low esterase activity. In a few instances, however, milk was found to exhibit high esterase activity, i.e., the colostrum of dog[1] and cow[2], and the colostrum and milk of swine[3-5]. Evidence was presented that the enzyme responsible for this activity is cholinesterase, which is lacking in these secretions of other mammals studied[6,1,7]. No biological explanation is known so far for this species difference in biochemical behaviour, and the question is still open whether esterase-active colostrum contains other esterases in addition to cholinesterase.

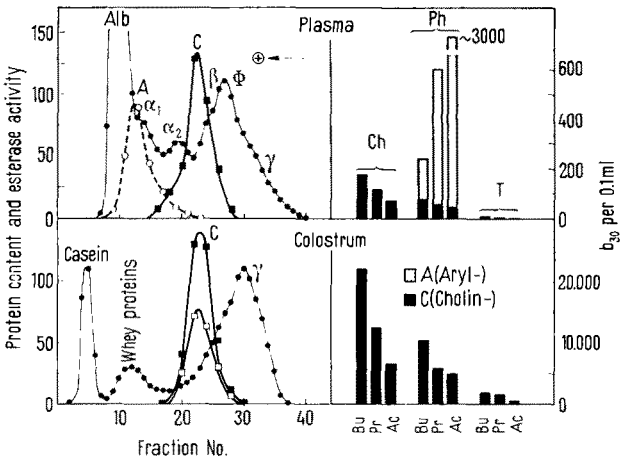
This report presents evidence that the cholinesterases present in bitch's blood plasma and colostrum are identical, and that cholinesterase is the only esterase present in colostrum of this species.

Colostrum was obtained by manual expression very soon after delivery, and its esterase activity measured by the Warburg technique using the acetyl, propionyl, and butyryl esters of choline, phenol, and glycerol (triglycerides). Preparative electrophoretic separation was performed in cellulose columns[8]. For comparison, blood plasma from the same bitch was analysed similarly.

Colostrum was 100 times more active in hydrolysing choline esters than was blood plasma from the same bitch (Fig.). In both cases, butyrylcholine was hydrolysed at the highest rate The triglycerides behaved similarly, but the hydrolysis rates were approximately 12 times lower for colostrum and plasma. As was previously demonstrated for dog plasma[8], cholinesterase was alone responsible for the hydrolysis of tributyrin. The activity of bitch's colostrum in the hydrolysis of tributyrin was completely abolished by 10^{-5} *M* physostigmine, suggesting that cholinesterase of this secretion is the only esterase present which hydrolyses tributyrin.

Phenyl acetate was hydrolysed at about the same rate by colostrum and plasma, but phenyl butyrate was split at a 50 times higher rate by colostrum than by plasma. These differences in specificity of the two esterase sources towards phenyl esters were explained when active proteins were electrophoretically separated (Fig.). Dog plasma contained, in addition to a butyrylcholinesterase, an acetylarylesterase which was absent in colostrum. Evidence was obtained by using seperated enzyme fractions and selective esterase inhibitors that the hydrolysis of phenyl esters by dog plasma was due partly to butyrylcholinesterase. Colostrum gave on electrophoresis only one esterase peak which was due to butyrylcholinesterase, and had the same electrophoretic mobility as the plasma cholinesterase. This enzyme was alone responsible for the hydrolysis of all esters studied as substrates with colostrum.

The butyrylcholinesterase of bitch's colostrum probably originates from blood plasma. Prenatal colostrum, obtained one of the last days of pregnancy, had the same, or slightly lower, cholinesterase activity as postnatal colostrum. In constrast to plasma arylesterase, cholinesterase can pass unchanged into the secretion of bitch's mammary glands. A similar observation was reported previously for sow's colostrum[5], which contains a butyrylcholinesterase in high concentration, but no arylesterase. In contrast to sow's milk, which has the same activity as colostrum during the whole lactation period, the activity of bitch's milk is several hundred times lower than



Electropherograms and specificity of esterases of bitch's colostrum and blood plasma. Distribution of protein and esterase activity (to the left) after electrophoresis of 2.0 ml of diluted (1 : 4) fat-free colostrum and 1.0 ml of plasma, performed over a period of 16 h in a 1.5 cm × 40 cm cellulose column in veronal buffer (pH 8.4, *I* 0.1) at 10°C with an applied voltage of 300 V. Displacement from the column in 1.5 ml fractions. Thin line, ●—●: relative protein contents (optical density of the Folin colour). Heavy lines: esterase activity, b_{30}; ○- - -○, phenyl acetate (0.015 ml fractions of plasma); ■—■, butyrylcholine (0.6 ml, plasma; 0.01 ml, colostrum); □—□, tributyrin (0.1 ml, colostrum). In the case of colostrum, one peak only was obtained with phenyl butyrate (0.02 ml fractions) and this peak was identical with that obtained with butyrylcholine. Esterase activities of original material (to the right) are expressed in b_{30} values per 0.1 ml and were obtained with the butyrate (Bu), propionate (Pr), and acetate (Ac) of choline (Ch), phenol (Ph) and glycerol (T, triglycerides).

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bitch's colostrum. It was also demonstrated that during the first days of suckling, the plasma cholinesterase of puppies increases[1], but this is not the case for the plasma cholinesterase of piglets[5]. This species difference, as well as the functional role of colostrum (and milk) cholinesterase, are still unsolved problems.

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Zusammenfassung

Es wird gezeigt, dass die Cholinesterasen im Blutplasma und Colostrum von Hündinnen identisch sind, und dass Cholinesterase die einzige im Colostrum vorhandene Esterase dieser Spezies ist.

Rifomycin IX[1]
Two New Antibiotics of Rifomycin Family: Rifomycin S and Rifomycin SV
Preliminary Report

It has been previously reported[2,3] that the antibacterial activity of aqueous solutions of rifomycin B increases with aging when the samples are exposed to air at room temperature. This property of rifomycin B is shown also by rifomycin O, a product which can be obtained by oxidation of rifomycin B and can be reduced again into rifomycin B[4].

The products found in activated solutions of both rifomycin B and rifomycin O appear to be the same substance[4].

Preliminary data are reported here concerning some properties of this activation product which was named by us rifomycin S. Rifomycin S cristallizes from methanol as yellow-orange crystals, m. p. 179–181°C (dec.). $[\alpha]_D^{20} = +476°C$ ($c = 0.1$, methanol). Rifomycin S has a weakly acidic nature (pH $1/2 = 7.2$; equivalent weight 685); its sodium salt is violet. Rifomycin S in buffer phosphate solution pH 7.3 shows absorption maxima at 317 mμ ($E_{1\,cm}^{1\%} = 426$) and at 525 mμ ($E_{1\,cm}^{1\%} = 62$).

Analysis: C 63.34; H 6.79; N 2.18; O 27.80; OCH_3 4.50; $COCH_3$ 6.10. Calc. for $C_{37}H_{47}NO_{12}$ (m.w. 697.75) [proposed]: C 63.68; H 6.79; N 2.01; O 27.51; 1 OCH_3 4.45; 1 $COCH_3$ 6.16.

Rifomycin S can be reduced by ascorbic acid into another antimicrobial substance, rifomycin SV. Rifomycin SV is a yellow-orange crystalline substance, decomposes at 140°C and does not melt until 300°C. $[\alpha]_D^{20} = -4°$ ($c = 1.0$, methanol). Rifomycin SV has an acidic nature (pH $1/2 = 2.7$; equivalent weight 685); its sodium salt is orange-red. Rifomycin SV in buffer phosphate solution pH 7.3 shows absorption maxima at 223 mμ ($E_{1\,cm}^{1\%} = 565$), 314 mμ ($E_{1\,cm}^{1\%} = 309$) and 445 mμ ($E_{1\,cm}^{1\%} = 188$).

Rifomycin SV can be transformed again, by oxidation, into rifomycin S. Analysis: C 62.85; H 7.15; N 2.08; O 28.01; OCH_3 4.40; $COCH_3$ 6.15. Calc. for $C_{37}H_{49}NO_{12}$ (m. w. 699.77) [proposed]: C 63.50; H 7.06; N 2.00; O 27.44; 1 OCH_3 4.43; 1 $COCH_3$ 6.15.

Both rifomycin S and rifomycin SV show high activity against Gram-positive bacteria and mycobacteria. In the Table I, the minimal inhibitory concentrations of the two antibiotics against a limited number of strains is reported.

While rifomycin S and rifomycin SV show practically identical antibacterial spectra, they have different acute toxicities. The LD_{50} values in mice by different administration routes are reported in Table II. Mice infected with lethal doses of virulent strains of *Streptococcus haemolyticus*, *Diplococcus pneumoniae*, or *Staphylococcus aureus* are protected by subcutaneous or oral administration of rifomycin SV.

Tab. I. Antibacterial activity of rifomycin S and rifomycin SV

Microorganism	Minimal inhibitory concentration γ/ml	
	Rifomycin S	Rifomycin SV
Micrococcus aureus	0.005	0.005
Streptococcus faecalis	0.09	0.05
Streptococcus haemolyticus	0.0025	0.0025
Bacillus subtilis	0.075	0.075
Proteus vulgaris	25	25
Escherichia coli	12	25
Pseudomonas aeruginosa	25	50
Klebsiella pneumoniae	25	25
Mycobacterium tuberculosis H 37 Rv	0.05	0.05
Candida albicans	> 100	> 100

Tab. II. Acute toxicity of rifomycin S and rifomycin SV in mice

	Administration route	LD_{50} (mg/kg) values and confidence limits ($p = 0.05$)
Rifomycin S	i. v.	122 (108–138)
	i. p.	258 (243–273)
	os	>3000
Rifomycin SV	i. v.	550 (482–627)
	i. p.	625 (579–675)
	os	2120 (1876–2395)

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Research Laboratories, Lepetit S. p. A., Milano (Italy), April 8, 1960.

Riassunto

Da soluzioni di rifomicina B e rifomicina O, che presentano il caratteristico comportamento di un incremento del titolo microbiologico con il tempo, è stato isolato un nuovo antibiotico chiamato rifomicina S. La rifomicina S, per blanda riduzione con acido ascorbico, viene trasformata in rifomicina SV.

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