each of the phenols (a), one or (in the case of the dihydric phenols catechol and resorcinol) two new main spots appeared on the chromatograms (Fig. 2). Fig. 3A shows the electrophoretic pattern of the same series of experiments. When compared to the control (on the left), all phenols tested induced the formation of one new spot (Phe I to Phl I) in the central region of the electrophoretic strip. In addition, in the experiments on each of the dihydric phenols catechol and resorcinol and the trihydric pyrogallol and phloroglucin, a second fast-moving spot appeared (Cat II, Res II, Pyr II and Phl II). After elution of the spots denoted as Cat II and Res II on the chromatograms, these spots were found to migrate to the Cat II and Res II regions, respectively, on the electropherograms. When the eluted spot in the right lower corner of the chromatograms from the pyrogallol and phloroglucin experiments was submitted to electrophoresis, new spots (in addition to the expected PAPS and inorganic sulphate spots) were observed in the Pyr II and Phl II regions, respectively.

Figure 3B shows the formation of Cat I and II, respectively, during incubation for 2 h in the presence of various amounts of catechol in the medium (0·1–2 *mM*). At a low concentration of catechol, the S^{35} incorporation in Cat II was higher than in Cat I, whereas a high concentration produced the reverse picture.

Figure 3C illustrates the formation of Pyr I and Pyr II, respectively, during incubation of 1·0 *mM* of pyrogallol for various lengths of time (0–2 h). In both spots, sulphate incorporation increased as a function of time.

In the series of experiments (c) where the S^{35}-sulphate and the phenol solutions in the incubating media were replaced by the S^{35}-labelled compounds of Phe-Phl I considered to constitute the monosulphates of respective phenols, no indication was obtained of a formation of the aforementioned fast-moving spots (Cat II, Res II, Pyr II, Phl II).

Acid hydrolysis under various conditions of all the S^{35}-labelled spots listed above, followed by electrophoretic or chromatographic investigation of the split products, resulted in appearance of no labelled compounds, but of inorganic sulphate or of the inorganic + the unhydrolyzed compounds tested. In the case of Res II and Pyr II, a formation of Res I and Pyr I from these compounds was produced by weak acid hydrolysis.

Incubation of a series of mono-, di-, and trihydric phenols in a sulphurylating system thus resulted in formation of a number of sulphate conjugates. Each of the phenols tested induced formation of a sulphate conjugate which migrated in electrophoresis, under the conditions used, to a characteristic position in the central part of the electrophoretic strip. These compounds, which are easily hydrolyzed in hydrochloric acid, giving inorganic sulphate, are considered to represent monosulphates of the corresponding phenols. From two of the three dihydric phenols (catechol and resorcinol) and both the trihydric phenols tested (pyrogallol and phloroglucin), an additional, characteristic, fast-moving electrophoretic spot was obtained which, judging by its electrophoretic mobility, seemed to contain two negative charges. It seems reasonable to believe that these compounds, which also give inorganic sulphate in acid hydrolysates, represent disulphates of the respective phenols. Some support to this view was given by the fact that a formation of monosulphates (Res I and Pyr I) + inorganic sulphate could be demonstrated when eluted specimens of these fast-moving compounds (Res II and Pyr II) were subjected to weak acid hydrolysis. On the other hand, attempts to synthetize these compounds by sulphurylation of the corresponding S^{35}-labelled monosulphates have so far been unsuccessful.

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The Wenner-Gren Institute, Department of Metabolic Research, University of Stockholm (Sweden), December 9, 1959.

Zusammenfassung

Die Bildung der Monosulfate bzw. Disulfate in einer Reihe von Phenolen ist auf Grund der *in-vitro*-Versuche mit einem partikelfreien sulfurylierenden Enzymsystem diskutiert worden. S^{35}-markiertes Sulfat wurde als Tracer-Substanz gebraucht. Die gebildeten Sulfatester sind mit zweidimensionaler Papierchromatographie und papierelektrophoretisch getrennt und mittels Autoradiographie lokalisiert worden.

Tyrosine Phosphate on Paper Chromatograms of *Drosophila melanogaster*

Several years ago HADORN and MITCHELL[1] presented results of a study, by paper chromatography, of the ninhydrinreacting components of *Drosophila melanogaster*. In this work and in a number of subsequent investigations[2–5] several substances not corresponding to positions of known amino acids were observed. These were tentatively concluded to be peptides since they gave more than one amino acid on hydrolysis, but mixtures of amino acid derivatives were also possible. Recently, during an examination of this problem, MITCHELL and SIMMONS (unpublished) isolated from *Drosophila* larvae a substance identified as tyrosine-O-phosphate and since this is a major ninhydrin-reacting component in *Drosophila*, its position in the standard chromatographic system is of some interest.

For comparisons, synthetic tyrosine phosphate was prepared according to the method of PASTERNAK and GRAFL[6] and the natural compound was obtained by fractionation of a larval extract on a Dowex 50 column (MITCHELL and SIMMONS, unpublished). These two products had identical absorption spectra in UV light (in 0.1 *M* HCl: max., 265 mμ; inflection, 210 mμ; min., 240 mμ) and behaved as a single substance on co-chromatography in *n*-propanol: 1% ammonia (2:1; $R_f = 0.14$). In order to determine which component from a *Drosophila* extract corresponds to tyrosine phosphate in the two dimensional chromatographic system that has been used extensively in this laboratory[2–5], an extract was prepared and chromatographed alone and in combination with each the synthetic and the natural substance. The tyrosine phosphate moved in each case to the position that had been designated as peptide 1 plus peptide 2[2–5]. As a further check of identity, material from this position on a chromatogram of larval extract

[1] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci., Wash. *37*, 650 (1951).
[2] G. BENZ, Arch. Julius Klaus-Stiftung *30*, 498 (1955).
[3] P. S. CHEN and E. HADORN, Rev. suisse Zool. *62*, 338 (1955).
[4] I. FAULHABER, Z. Vererbungslehre *90*, 299 (1959).
[5] E. STUMM-ZOLLINGER, Z. Vererbungslehre *86*, 126 (1954).
[6] T. PASTERNAK and S. GRAFL, Helv. Chim. Acta *28*, 1258 (1945).
[7] P. S. CHEN and A. KÜHN, Z. Naturforschung *11b*, 305 (1956).
[8] P. S. CHEN, J. Insect. Physiol. *2*, 38 (1958).
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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.

H. K. MITCHELL[9], P. S. CHEN, and E. HADORN

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.

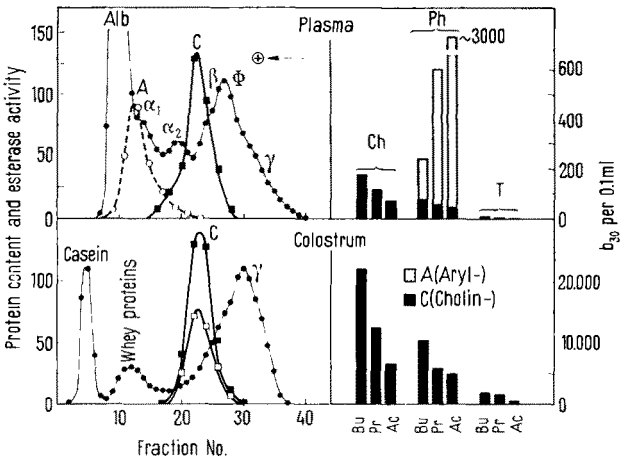
This report presents evidence that the cholinesterases present in bitch's blood plasma and colostrum are identical, and that cholinesterase is the only esterase present in colostrum of this species.

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).

[1] R. A. MCCANCE, A. O. HUTCHINSON, R. F. A. DEAN, and R. E. H. JONES, Biochem. J. *45*, 193 (1949).

[2] T. L. FORSTER, personal communication.

[3] B. E. HINES and R. A. MCCANCE, J. Physiol. *122*, 188 (1953).

[4] K.-B. AUGUSTINSSON, Acta chem. scand. *12*, 1150 (1958).

[5] K.-B. AUGUSTINSSON and B. OLSSON, Biochem. J. *71*, 477, 484 (1959).

[6] K.-B. AUGUSTINSSON, Acta physiol. scand. *15*, Suppl. 52 (1948).

[7] A. ALM and K.-B. AUGUSTINSSON, Acta physiol. scand. *39*, 203 (1957).

[8] K.-B. AUGUSTINSSON, Acta chem. scand. *13*, 571 (1959).