Investigations to elucidate the process and its connection with virus synthesis are going on.

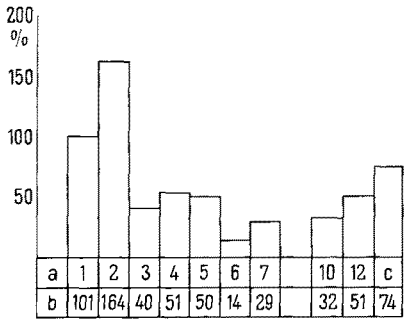

Fig. 1. Activity of transaminase on dry weight

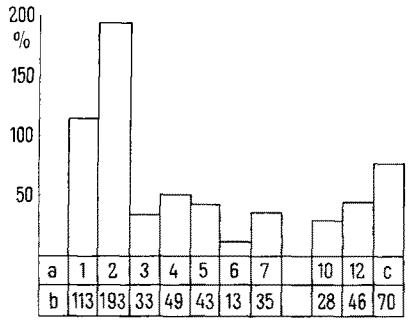

Fig. 2. Activity of transaminase on fresh weight

a) Days after infection b) Percentage of transamination in inoculated leaves in terms of the activity of sound plants (100%). c) 4 weeks after infection.

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Zusammenfassung

In den mit Mosaikvirus infizierten Tabakblättern wurden im Vergleich mit den gesunden bedeutende Veränderungen in der Glutamin-alanin-transaminase-Aktivität festgestellt. Zwei Tage nach Infektion erfolgt zunächst Zunahme, dann Abnahme der Enzymaktivität.

10. On the Sulphurylation of Mono-, Di-, and Trihydric Phenols

The sulphurylation of a large number of phenols and phenol derivatives has been studied by earlier workers. This has been done in *in vivo* experiments on various species[1], or *in vitro* in slices of various tissues[2,3], as well in particle-free media containing sulphate-activating and sulphate-transferring enzymes[4–7].

The last-mentioned technique was used in the present comparative study on the sulphurylation of a number of mono-, di-, and trihydric phenols. S^{35}-labelled sulphate was used as tracer. The formation of conjugated sulphates in the incubating medium was followed by means of two-dimensional paper chromatography and electrophoresis,

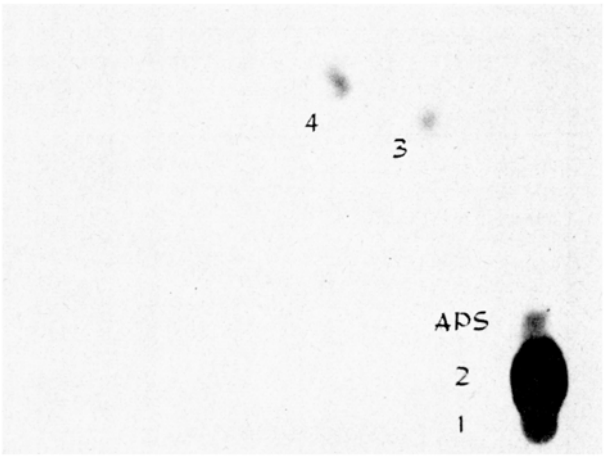

Fig. 1. Basic chromatographic pattern of the incubating medium. Spots 1 and 2 correspond to inorganic sulphate and active sulphate (PAPS) and spots 3 and 4 to unknowns derived from the liver extract.

both techniques combined with autoradiography on Gevaert X-ray film. Detailed descriptions of the methods used have been given elsewhere[8].

The following types of experiment were run:

(a) Incubation for 2 h at 37°C in the complete system (particle-free rat liver supernatant buffer[9], ATP, Mg ions and S^{35}-labelled sulphate) of various concentrations (0·1 to 2·0 *mM*) of each of the following phenols: phenol, catechol, resorcinol, hydroquinone, pyrogallol, and phloroglucin.

(b) Incubation as above of the complete system, with the phenol solutions replaced by water (control).

(c) Incubation as above of the complete system, with the S^{35}-sulphate solution replaced by water, and the phenol solutions replaced by S^{35}-labelled monosulphates of the aforementioned phenols obtained by biochemical synthesis.

(d) Acid hydrolysis in 0·1 *N* HCl for 0–2 h of eluted S^{35}-labelled spots obtained in the experiments listed above. The hydrolysates were submitted to paper chromatography and paper electrophoresis combined with autoradiography.

The results of these experiments are illustrated in Figures 1–3. The basic chromatographic pattern of the incubating medium in the control experiments (b), shown in Figure 1, was essentially the same as described earlier[8]. In addition to the inorganic + PAPS spot and the APS spot in the right lower corner, a few additional spots due to the sulphurylation of sulphate acceptors present in the rat liver supernatant are visualized. In the presence of

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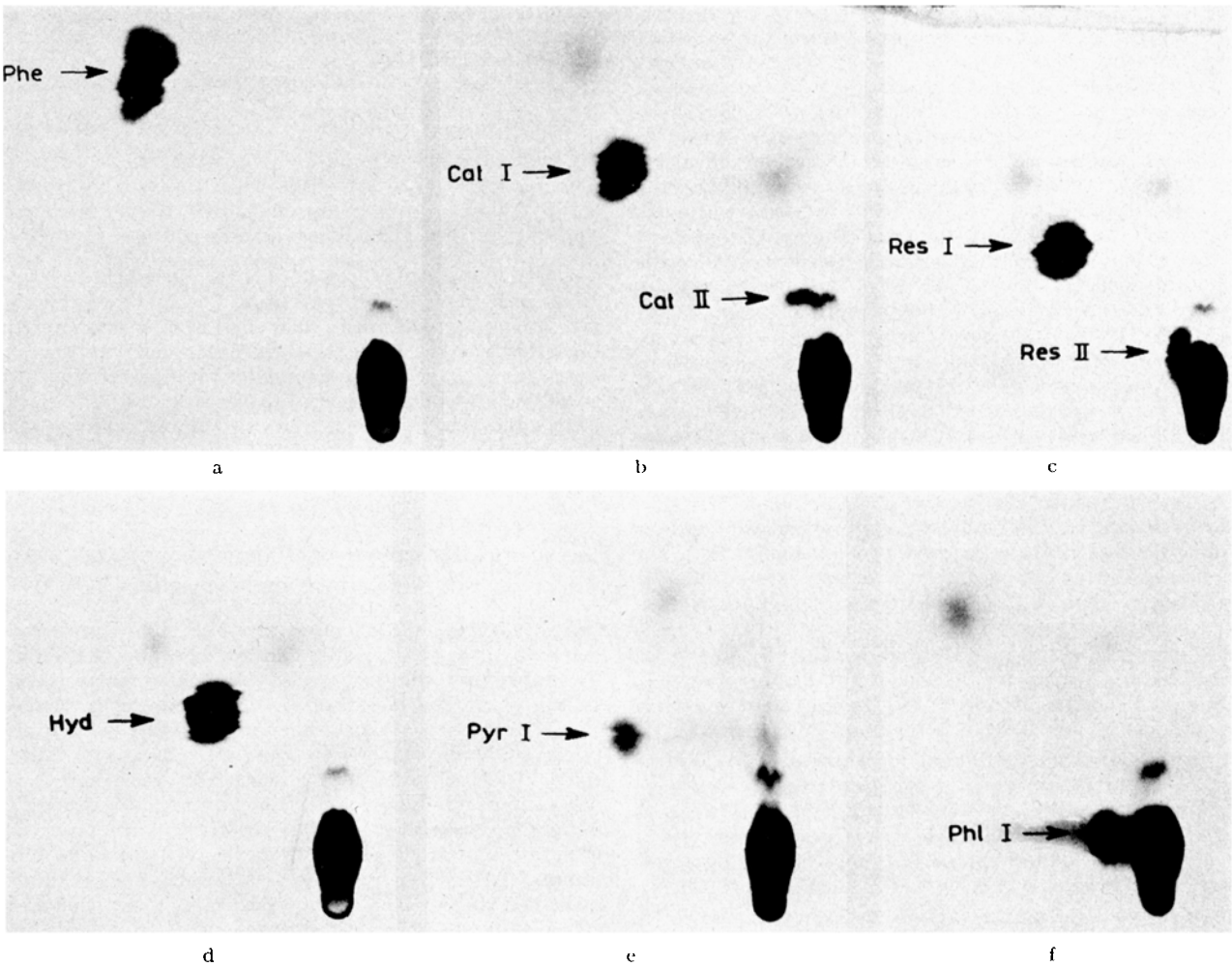

Fig. 2 Chromatographic pattern of the incubating medium in the presence of phenol (Phe), catechol (Cat), resorcinol (Res), hydroquinone (Hyd), pyrogallol (Pyr), and phloroglucin (Phl). Phenol-induced spots indicated by arrows.

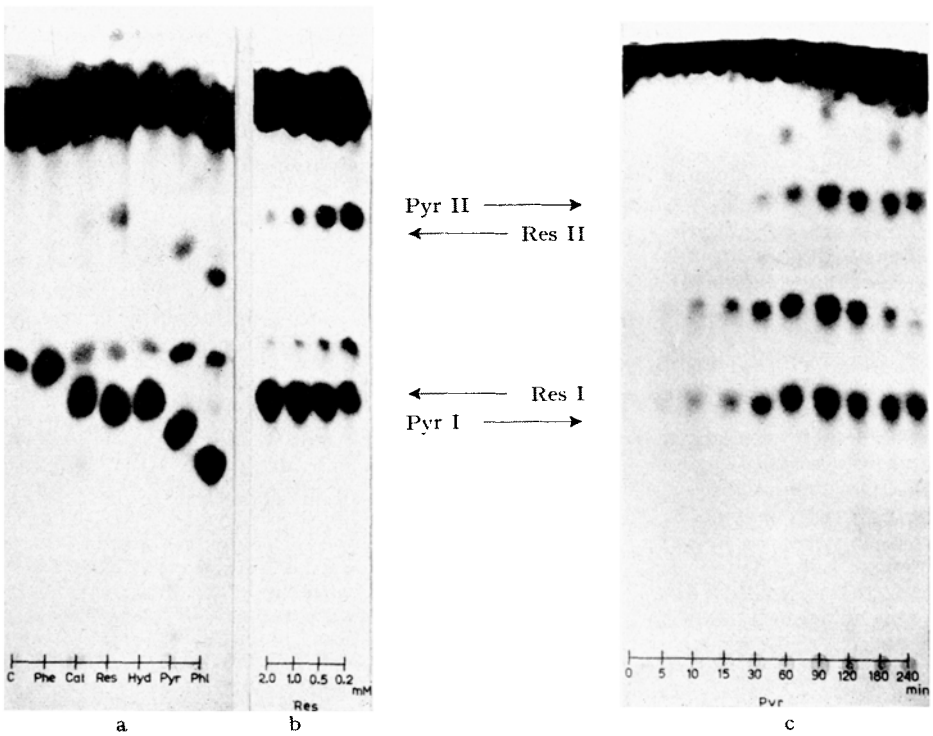

Fig. 3. a) Electrophoretic pattern of the incubating medium from a control experiment (C) and in the presence of various phenols (abbreviations as in Fig. 2). b) Patterns obtained with various amounts of resorcinol present in the medium (0·1–2 *mM*). c) Patterns obtained with pyrogallol (1·0 *mM*) in the medium during incubation for various lengths of time (0–2 h).

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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A. VESTERMARK and H. BOSTRÖM

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

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