only from fractions 30—50. They were combined and evaporated.

The residue weighed 5.6 mg and was hydrolyzed in 0.56 ml N sulfuric acid for 5 h at 100°C. The digest was neutralized with $Ba(OH)_2$ to pH 5.8. The supernatant was evaporated, the residue dissolved in 0.05 ml of water and chromatographed with butanol:pyridine:water (5:3:2). No distinct carbohydrate spots were obtained with aniline phthalate, but with ninhydrin several spots, one coinciding with that of glucosamine. The test with aniline for furfurol [7] was negative. 2,4-Dinitrophenylhydrazine (dissolved into 2 N H_2SO_4) gave a precipitate, which was collected and submitted to spectroscopic examination (Fig. 1). The sharp change to violet in alkali seems worthy of mention.

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Studies on Ester Sulfates

1. Application of Two-Dimensional Paper Chromatography in Studies on the Biosynthesis of Ester Sulfates

ANDERS VESTERMARK and HARRY BOSTRÖM

Department of Metabolic Research, the Wenner-Gren Institute of Experimental Biology, Stockholm, Sweden

In several recent biochemical studies on ester sulfate compounds, great advantage has been taken of the selective incorporation of ^{35}S-sulfate in these compounds. Mainly on the grounds of their high sensitivity, different isotope methods, such as autoradiography of electropherograms on body fluids and tissue extracts, have been used successfully in this field.

A somewhat more detailed picture of the sulfatation of different compounds in various *in vitro* systems could be expected to result from the use of two-dimensional paper chromatography combined with autoradiography. For this reason, a screening study along these lines was undertaken. Some observations made during this work will be briefly reported in the following.

In one type of experiment, 2-mg slices of liver from albino rats were incubated in small test tubes in 100 µl of Krebs-Ringer bicarbonate solution and 10 µl of a solution of ^{35}S-labeled sulfate containing 10 mc/ml. A gas mixture consisting of 93.5 % of oxygen and 6.5 % of carbon dioxide was insufflated into each test tube during incubation.

In another type of experiment, 40 µl of a cell-free supernatant of liver homogenate prepared according to Roy [1] was mixed with 50 µl of a buffer solution, containing equal parts of 0.3 M KH_2PO_4/K_2HPO_4 buffer, pH 6.8, 0.03 M K_2SO_4 and 0.005 M $MgCl_2$. This mixture was combined with 10 µl of 0.04 M ATP and 10 µl of the ^{35}S-labeled sodium sulfate solution.

When these types of sulfatation system were used for studies on sulfatation of different substances, 10 µl of a 10 mM solution of the substance in alcohol or water was added to an empty test tube. The solvent was evaporated *in vacuo*. The slices or supernatant systems were added to this tube.

In each series of experiments, one tube with 10 µl of water and another with 10 µl of alcohol, to which the relevant sulfatation system was added, were run as controls.

In all experiments, 5 µl-samples of the medium were taken 45 and 120 min after starting incubation, for two-dimensional ascending chromatography according to Datta, Dent and Harris [2]. Solvent I: Phenol-water (400 g + 100 g). A beaker containing concentrated H_3N was placed in the chromatographic tank. Solvent II: Butanol — 2 N H_3N (250 ml + 250 ml).

After drying the papers in a stream of hot air from an electric hair-drying fan, the chromatograms were subjected to autoradiography on Gevaert Curix X-ray film, exposed for one week in a wooden screw press and then developed with Gevaert G 150.

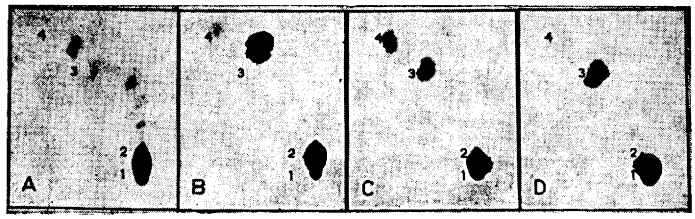

Fig. 1. Chromato-autoradiogram on 5-μl samples of the incubation mixtures after 2 h incubation. A. Slices system (see text) + 10 μl of water. B. Slices system + 10 μl of ethanol. C. Cell-free system + 10 μl of water. D. Cell-free system + 10 μl of ethanol.

Paper electrophoresis (0.075 M sodium acetate-acetic acid buffer, pH 5.5, 30 V/cm, 1 h) was also performed on 5-μl samples of the incubation medium.

In the control experiments with the slices system (Fig. 1, chromatograms A and B), the following basic pattern was obtained. In addition to a spot, No. 1, obtained where the sample was placed on the paper, and the inorganic sulfate spot, No. 2, two other spots, Nos. 3 and 4, were consistently present. The control with water (A) showed a weak No. 3 spot and a distinct No. 4. The control with ethanol (B) showed the reverse picture, a weak No. 4 and a very strong No. 3.

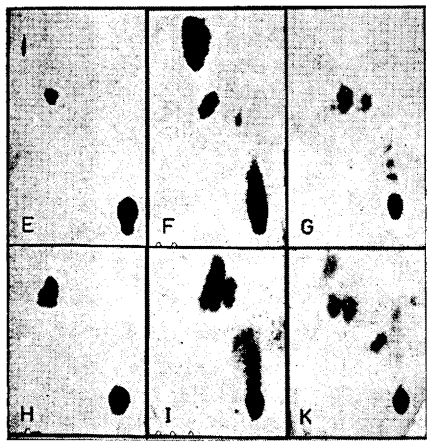

Fig. 2. Ester sulfate pattern of the incubation media after addition to the slices system of androsterone (E), α-naphthylamine (G) and β-naphthylamine (I) as compared to the corresponding pattern obtained with the same substances in the cell-free system (F, H, K), respectively.

In the supernatant system as well, the presence of small amounts of ethanol (D) resulted in increased strength of No. 3 and a decrease in concentration of No. 4, as compared to the water controls (C).

Addition of amines, steroids or phenols to either of the sulfatation systems used was generally followed by the appearance of one or several new spots in the chromatograms, as exemplified in Figs. 2 and 3 (chromatograms E—S).

Thus, both in the slices system (Fig. 2 E) and in the cell-free medium (F), one new distinct spot appeared when androsterone was added to the incubation mixtures. In similar studies, ester sulfates of several other steroids as well have been obtained [3].

Of the two isomeric amines tested, α-naphthylamine gave one new single spot in the cell-free medium (G), whereas at least two ester sulfates were formed in the presence of liver slices. More complex patterns were obtained in both systems with β-naphthylamine.

A series of new unidentified ester sulfates (Fig. 3) was also obtained when adrenaline (L) or noradrenaline (N) was added to the slices. Somewhat less complicated patterns were produced by these substances in the cell-free system (M and O).

A marked difference between the two systems was also observed in the presence of thymol, which gave two well-marked ester sulfates in the slices system (P), but only one in the cell-free medium (Q).

No evidence was obtained of the formation of tyrosine-O-sulfate, known to occur in human urine [4], in either of the sulfatation systems used (R and S).

When high voltage electrophoresis was applied to samples of all the incubation media mentioned above, separation of some of the

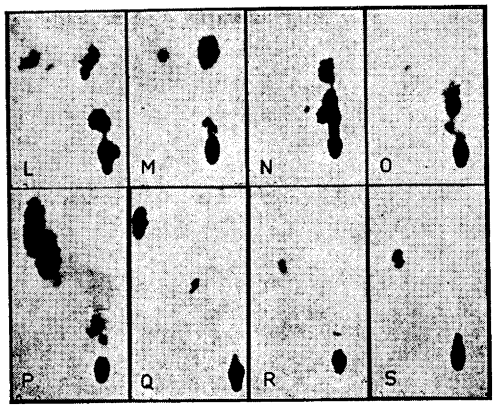

Fig. 3. Ester sulfate pattern found in the presence of adrenaline (L), noradrenaline (N), thymol (P) and tyrosine (R), in the slices system and the cell-free system (M, O, Q and S), respectively.

Fig. 4. Formation of different ^{35}S-labeled compounds *in vitro* in a cell-free or a slices system of rat liver, in the presence of various possible sulfate acceptors, as studied by high voltage electrophoresis and autoradiography. The black zone at the top of the picture corresponds to the position of inorganic sulfate. The various experiments are marked as in Figs. 1—3.

labeled compounds was also obtained. Thus, in the basic liver supernatant pattern, three distinct spots (C) were visualized, as compared to only two in the chromatographic system (Fig. 1 C). In the presence of ethanol (D), a shift occurred, consisting of a decrease in concentration of the slowest moving fraction, and an increase in the fastest moving compound. A similar phenomenon was observed in the slices system (A and B).

When adrenaline was present in the supernatant system (M), one radioactive spot, probably corresponding to a monosulfate of adrenaline, moved very slowly. Other compounds, which might have been derivatives of adrenaline containing two or three sulfate groups, moved faster. In the slices system (L), the slow moving spot was found, in addition to two other ^{35}S-labeled compounds.

With noradrenaline as well, a slow moving compound was synthesized in both systems (O:cell-free, N:slices). A few other labeled compounds were also recognized in these cases.

The results of this screening study on the ability of a system of liver slices, or of a cell-free supernatant of liver homogenates, to activate sulfate groups and transfer them to different types of acceptors are in good agreement with those reported by other authors on phenols [5-7], steroids [1,8,9] and amines [10].

Features of special interest in our study were the complicated ester sulfate pattern found in the presence of catechol amines, the failure of sulfatation of free tyrosine in either of the systems, and the interesting shift in the basic ester sulfate pattern in both systems in the presence of small amounts of ethanol (Fig. 1).

The particular value of the chromato-autoradiographic method used in the present study seems to be that a good overall picture of the occurrence of different types of transferring enzymes and sulfate acceptors in different sulfatation systems is obtained in small-scale experiments.

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Mass Transfer by Forced Laminar Convection at Plane Plate Electrodes

GÖSTA WRANGLÉN

Division of Applied Electrochemistry, Royal Institute of Technology, Stockholm 70, Sweden

The investigation of the influence of liquid flow on electrode processes is of great interest for industrial electrolysis and for the theory of metallic corrosion in flowing liquids. Heat and mass transfer at a plane plate in longitudinal flow under laminar conditions have been treated by various authors [1-3] under the assumption of a uniform surface temperature and concentration, respectively. As far as electrolysis is concerned, this postulate probably applies only for limiting current conditions at the electrodes. In normal electrolysis with a uniform current density, the actual boundary condition is a uniform concentration gradient at the electrode surface. This case or the corresponding heat transfer problem does not seem to have been considered earlier.

The notation used in an earlier communication [4] is employed, except that x is taken to mean the distance in the flow direction from the leading edge of the electrodes and u denotes the flow velocity in the x-direction in the boundary layer. Furthermore, l is the length of the electrode in the flow direction and $\mathrm{Re}_x = \frac{u_b x}{\nu}$ is the local Reynolds number. The relevant boundary layer equations in integrated form are