Glutamine in the Biosynthesis of Mammalian Mucopolysaccharides

Harry Boström, Lennart Rodén and Anders Vestermark

Chemistry Department II, Karolinska Institutet, Stockholm, Sweden

Boström and Månsson [1,2] studied the factors governing the incorporation of ^{35}S-labelled sulphate into the chondroitin sulphuric acid of surviving calf cartilage. It was found that small amounts of a liver homogenate added to the incubation medium greatly increased the ^{35}S incorporation [3]. The active principle (named sulphate exchange stimulating factor: S.E.S. factor) could be purified from calf liver by means of fractional precipitation of a boiled liver extract with mercuric acetate and subsequent preparative paper electrophoresis [4]. When subjected to paper chromatography, the electrophoretically purified fraction showed 7 ninhydrin-positive spots, one of which contained the whole S.E.S. activity. The chemical nature of the S.E.S. factor has not yet been determined, but strong evidence points towards the identity of the factor with glutamine [5]. Some facts pertinent to this supposition are presented below.

1) The S.E.S.-active ninhydrin-positive spot and L-glutamine have the same R_F value in the solvents used for two-dimensional paper chromatography (80 % ethanol in water; *sec*-butanol-90 % formic acid-water: 75+15+10 parts, respectively).

2) In chromatography on the cation exchange resin Dowex 50, L-glutamine and the S.E.S. factor have the same effluent volume, both when tested separately and in mixed chromatography. The recovery of glutamine is not quantitative; this also applies to the S.E.S. factor.

3) L-Glutamine has a five times greater S.E.S. activity per mg of dry substance than that of the electrophoretically purified S.E.S. factor from calf liver.

Glutamine could not be replaced by asparagine, aspartic acid, glutamic acid, glutathione, ammonium chloride or glutamic acid + ammonium chloride.

Lowther and Rogers [6] have shown that glutamine increases the quantity of bound glucosamine formed by streptococci, and Leloir and Cardini [7] have studied the role of glutamine in the biosynthesis of glucosamine in cell-free extracts of *Neurospora crassa*.

On the basis of the observations reported above, we can now assume that glutamine also plays an important part in the biosynthesis of animal mucopolysaccharides.

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Quantitative Determination of Bile Acids in Bile

Jan Sjövall

Department of Physiological Chemistry, University of Lund, Lund, Sweden

A method for the quantitative determination of mixtures of synthetic bile acids has recently been published [1]. The bile acids are separated by paper chromatography, eluted from the chromatograms and then determined by spectrophotometric assay in sulfuric acid.

This method has now been adapted for the determination of bile acids in bile. A suitable volume of bile, usually in the order of 10—20 µl, is applied on the starting line as a spot. The chromatogram is developed and the bile acids are localized as earlier described [1]. The spots are then cut out and eluted with ethanol. A corresponding filter paper blank is eluted in the same way. The bile acid content then can be determined as earlier described for synthetic bile acids. Other substances present in the bile do not interfere with the quantitative determination. A blank value which depends on the bile pigment concentration is satisfactorily corrected for by measuring the optical density of one bile acid sample in ethanol and another in 65 % sulfuric acid as described. The bile acid content can be calculated from the value obtained by subtracting the value in ethanol from that in the sulfuric acid. Added amounts of known bile acids are quantitatively recovered. Some results obtained by this method will be described.

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