A CHROMATOGRAPHIC STUDY OF BILIRUBIN

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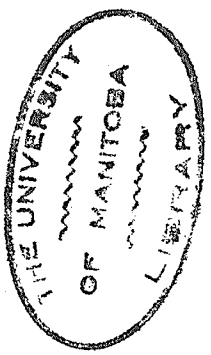

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ABSTRACT

The bilirubin pigments found in human blood serum have been studied by means of the reverse phase chromatographic technique. The technique itself was found to be effective only in the qualitative separation of the pigments. From the evidence of this study, it can be stated that there are three distinct bilirubin pigments, only one of which can be properly called bilirubin, the other two being of a "direct" acting nature as judged by their coupling reaction with diazo reagent. Evidence is given in support of the theory that direct acting bilirubin is attached to protein in vivo, and that this complex increases the ability of the pigment to resist oxidation by exposure to air. It has been demonstrated that greatly increased levels of bilirubin in the body are not alone responsible for the development of kernicterus in the newborn rat, and that other unknown factors are the toxic agents in this condition.

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INTRODUCTION

Since the year 1916, when Hijman van den Bergh and Muller (1916) discovered that the diazotization reaction could be used to indicate the presence of two different states of bilirubin in blood serum, there has been a constant search to discover the nature of these two or more fractions. The development of any new technique gives further impetus to this search, and accordingly Cole and Lathe (1953) adapted the chromatographic technique introduced by Howard and Martin (1950) in an attempt to separate serum bilirubin into its component fractions.

The solubility of bilirubin in serum at pH 7.35 has posed a major problem, since in vitro pure bilirubin is only soluble at pH 8.0 or higher. The nature of the diazo reaction in water and in alcohol, and the ability of bilirubin to remain in solution at physiological pH values seem to be closely connected. The present study was carried out with a view to discovering if the chromatographic technique was useful for separating bilirubin fractions and if it could be used to elucidate the vexed question as to how bilirubin is carried in the blood stream.

REVIEW OF THE LITERATURE

The determination of the structure of the bilirubin

molecule has been established within the last twenty years, due chiefly to the work of Fischer (1937) and of Lemberg (1949). Each has approached the problem from a different angle. Lemberg was able to synthesize bilirubin from haemin in 1935 and Fischer synthesized both bilirubin and biliverdin from simple pyrrole compounds in 1942.

The accepted formula for bilirubin as synthesized by Fischer is a tetra-pyrrole di-carboxylic acid having an open chain structure. The degradation of bilirubin by resorcinol fusion by Fischer (1931) has led to a further understanding of the mechanism by which the molecule is able to enter into coupling reactions with diazonium chloride.

This coupling reaction is of particular importance in biological chemistry because it has been used to measure the quantity of bilirubin occurring in blood. The bilirubin is capable of being split at the central carbon atom and because of its asymmetrical nature, the two resulting dipyrroles will be different. Thus one half of the tetrapyrrole forms a dipyrrole having a free alpha position, while the other half has no such free position, it being filled by a methoxy group. Because of the asymmetry of the bilirubin molecule, the molecule can split at either side of the central CH_2 group, and therefore for each of the two active dipyrroles formed, there will be an inactive form. The two possible active forms are shown in Fig. 1. These are neoxanthobilirubinic acid and isoneoxanthobilirubinic acid. The phenyl diazonium chloride can couple at either of two