BIOCHEMISTRY

Measurement of Chenodeoxycholic Acid and Deoxycholic Acid or their Derivatives in a Mixture

The measurement of the dihydroxycholanic acids found in human bile (chenodeoxycholic acid, 3α,7α-dihydroxycholanic acid and deoxycholic acid, 3α,12α-dihydroxycholanic acid) or of their ester derivatives or conjugates with glycine or taurine has always presented difficulties where the original material to be analysed contains both acids or derivatives of them. Before 1953, the only method available depended on the repeated precipitation of the barium salt of chenodeoxycholic acid. Its value may be gauged from the fact that until this time chenodeoxycholic acid was considered to be a very minor constituent of human bile, a belief shown to be erroneous by Wootton[1] in 1953 when he separated methyl cholate from the two dihydroxy methyl esters by a column chromatographic procedure which utilized silica gel as the supporting medium and pentane/hexane and petroleum ether as the developing solvents. The proportions of methyl chenodeoxycholate and methyl deoxycholate in a mixture could be readily estimated by an investigation of the infra-red spectra of solutions in carbon disulphide. By these means, Wootton demonstrated that chenodeoxycholic acid was, in fact, a major constituent of human bile. The method is relatively insensitive compared with later methods of bile acid measurement which have been devised, requiring about a milligram of the mixed dihydroxy compounds to ensure reasonable accuracy of estimation of the proportion of the two esters.

Later, Sjövall[2] found that by using *iso*-propyl ether : heptane (20 : 80) as the mobile phase and 70 per cent acetic acid as the stationary phase, separation of the two dihydroxy acids on paper was obtained after the system had operated for 18 h. Pure heptane was also found to be suitable as the mobile phase, effective separation being obtained after 72 h. Such a system was found to be capable of handling quantities of the order of several micrograms. The glycine conjugates of chenodeoxycholic and of deoxycholic acids were also reported by Sjövall to have been separated by paper chromatography using *iso*-propyl ether : heptane (80 : 20 or 85 : 15) as the mobile phase and 70 per cent acetic acid as the stationary phase. However, no separation could be achieved between the taurine conjugates of the two dihydroxy bile acids. In the work reported by Sjövall, estimation of the different bile acids was carried out by measurement of the ultra-violet adsorption in 65 per cent aqueous H_2SO_4 of the acids eluted from the paper.

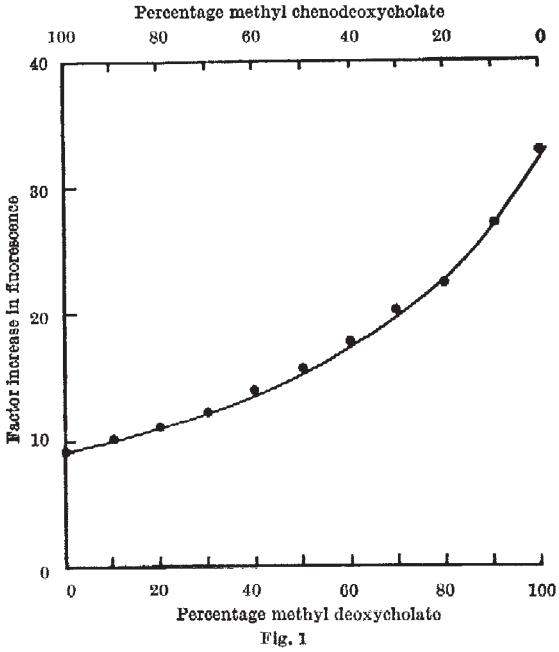

Fig. 1

The fluorescence of the bile acids and their derivatives in concentrated H_2SO_4 has been known for many years, the fluorescence of cholic acid in H_2SO_4 being reported by von Hammarsten[3] in 1922. However, it was not until many years later that Turner *et al.*[4], Wootton and Osborn[5] and Osborn[6] reported in detail the fluorescent properties of the major human bile acids and their derivatives in H_2SO_4 under various conditions of development of fluorescence with respect to temperature and time.

A method based on the fluorescence properties of methyl chenodeoxycholate and methyl deoxycholate, involving the measurement of the fluorescence of a mixture in H_2SO_4 at 4° C and 100° C, enables the proportions of each in a mixture to be accurately determined at concentrations as low as 0·5 μg/ml. H_2SO_4. The factor increase in fluorescence from 4° C, after standing at this temperature for 1 h, to that obtained after subsequent heating at 100° C for 20 min, is about 9 for methyl chenodeoxycholate, whereas with methyl deoxycholate the factor increase is about 33. Intermediate rises in fluorescence are shown by mixtures of the two compounds in varying proportions, when similarly treated (Fig. 1). When this method is used for analysing a mixture of the two dihydroxy compounds, it is unnecessary to determine the factor increase in fluorescence for other than the pure substances, since the necessary intermediate points on the graph may be obtained by assuming the fluorescences are additive.

It is necessary when working over such a wide range of fluorescence to use two quinine sulphate reference standards. The fluorescence developed at 4° C, 1 h, is measured against the lower standard and that at 100° C, 20 min, against the second standard which is ten times as concentrated as the lower one. Once the relative proportions of chenodeoxycholate and deoxycholate in a mixture have been determined, the absolute amounts of each may be estimated by preparing a mixed standard of the two in the same ratio, fluorescence being induced by heating an H_2SO_4 solution at 100° for 20 min.

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Amino-acids on Hands

Recent methods for the determination of amino-acids by ion-exchange chromatography at the 10^{-6} and 10^{-9} mole levels[1,2] are now able to give a quantitative measure of observations that have hitherto been of a qualitative nature[3,4]. For example, it is well known that finger-marks on paper chromatography are to be avoided because they are deeply coloured on treatment with ninhydrin reagent. It is probably less well appreciated that amino-acids might be introduced, some considerably in excess of the 10^{-6} mole level, at any or all steps of preparative manipulation prior to chromatography, and these contaminants would then be unwittingly measured along with the material of analytical interest.

Work that necessitated very rigid exclusion of accidental contamination led to the following experiments. (1) A