SYNOVIAL FLUID DRUG LEVELS: THE RECOVERY OF PENICILLINS AND PREDNISOLONE AND THEIR ESTIMATION BY HPLC

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Many studies have defined conditions for the estimation of drugs in blood, its fractions and urine but little systematic data is available for estimating drugs in synovial fluid. Although synovial fluid contains many of the electrolytes and proteins found in plasma it also contains a few unique proteins and a very high content of hyaluronate. The polyanionic structure of hyaluronate confers a high viscosity on synovial fluid although this viscosity may be reduced in arthritic conditions. The high viscosity compared to plasma and the small, variable amounts of synovial fluid available makes the extraction of drugs more difficult.

H.P.L.C. was carried out on an Altex 100A pump with a Hitachi 100-10 variable wavelength UV-Vis detector. A 25 x 0.5 cm Spherisorb 5-ODS (5μ microspherical) reverse phase column was used for H.P.L.C. of both steroids and penicillins. Steroids were eluted with methanol:acetic acid:water (53:5:42) at a flow rate of 1 ml/min. Good sensitivity and low noise was achieved at a detector setting of 250 nm and at 0.005 A.U.F.S. precise measurements of 0.1 μg/ml of prednisolone in water resulted. Penicillins were eluted with methanol:ammonium carbonate solution (0.05M in water) 30:70 at a flow rate of 1 ml/min. The low A(1%, 1cm) of penicillins (V and G) at 240nm and the high noise, even at 0.02 A.U.F.S., resulted in a minimum practicable measurement level of about 9 μg/ml in synovial fluid.

The different hydrophilic/hydrophobic characteristics of the two groups of drugs required different recovery procedures. The penicillins could be recovered readily by an ethanol precipitation procedure which formed no emulsions and which was much more rapid and simple than extraction methods (Dell 1976). The same method could not be used for the extraction of steroids when the concentration was less than about 10μg/ml because there was an average loss of drug of 14%. It is thought that the loss of steroids in this precipitation method may have been due to binding to the precipitated protein. A more complex extraction method using ether:dichloromethane (60:40) (Dell 1976) and high speed centrifugation to break the emulsion resulted in good recovery of prednisolone down to 125 ng/ml in synovial fluid.

A rise in pH occurred on storage of precipitated and unprecipitated fluids which caused degradation of penicillins over 1 to 3 days with the appearance of degradation products as H.P.L.C. peaks (mainly penicilloic acids).

H.P.L.C. of extracts for steroids gave several small peaks, which were not identified, one large peak probably due to hydrocortisone administered systemically to the patient and another which may have been due to the non-steroidal anti-inflammatory drug Froben.

Dell, D. (1976) in "Methodological Developments in Biochemistry 5", "Assays of drugs and other trace organics in biological fluids". North Holland P.C., Amsterdam Ed. E. Reid. 131-134.

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