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Short Communication

An 'in vitro' system for the correlation of drug/protein binding with variable plasma protein profile

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SUMMARY

Drug binding to plasma proteins is an important determinant on the pharmacological fate of that drug. With the rapid evolution of dosage regimen compilations, more and more pharmacokinetic parameters are being considered. The present study describes a simple system which enables the correlation of drug protein binding with variable plasma protein profile, and suggests how such information may be of value in the regulation of drug dosage.

Many drugs in clinical usage are bound to plasma proteins, mainly albumin. Only free, unbound drug is therapeutically active and so any change in binding will put the patient at risk of either under-control of his disease symptoms, or overactivity of his medicament. Binding of drugs to plasma proteins is, therefore, an important pharmacokinetic parameter.

It is well known that in myocardial infarction rapid changes occur in the plasma protein profile in the first week, returning to normal in three to five weeks (Johansson et al., 1972). This is also true for surgical trauma (Aronsen et al., 1972).

In uraemic patients the plasma protein binding of pentobarbital, diphenylhydantoin (Ehrnebo and Odar-Cederlöf, 1975), antimicrobial agents (Craig and Wagnild, 1974), digitoxin (Shoeman and Azarnoff, 1972), digoxin (Storstein, 1976), phenylbutazone and thiopentone (Andreasen, 1973) is decreased.

A routine system is, therefore, required for the correlation of protein binding of drugs with changing protein profile in order that dosage regimens may be altered accordingly.

(a) The plasma protein profile is found using established procedures. Total protein is measured by an Auto Analyser technique and albumin by the bromcresol green technique (Nisbet et al., 1973). Electrophoretic separation is then carried out on cellulose acetate plates and the plates mounted on glass slides. Densitometric scanning of these slides gives rise to a trace (Fig. 1). The integrated area under each curve on the trace is proportional to the amount of each protein fraction present. These proportions are related back to

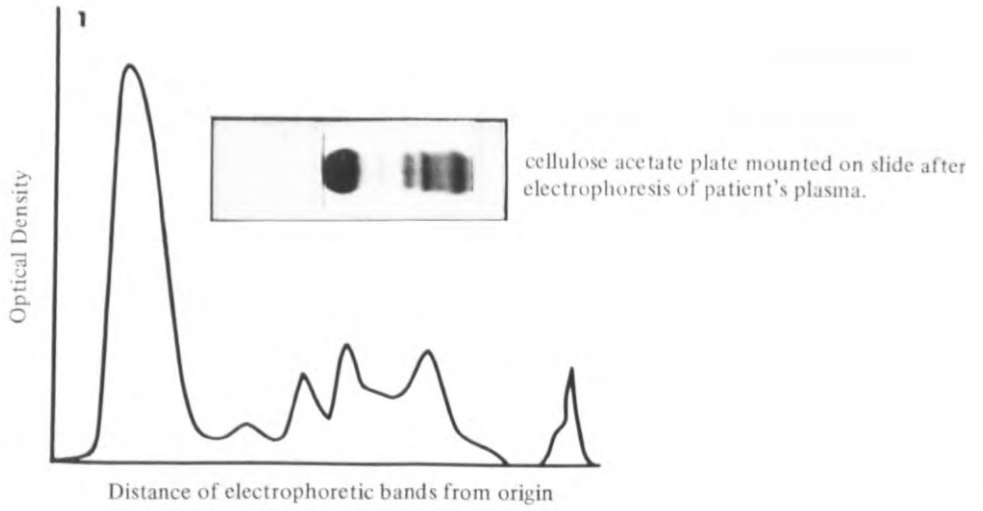

Fig. 1. Densitometer absorption curve; the peaks from left to right represent albumin, alpha-1, alpha-2, beta and gamma globulins.

total protein concentration and the amount in each protein fraction is then calculated.

(b) Plasma protein binding parameters are found using a custom built multicavity dialysis cell of dimensions 7 X 9 X 4 cm (Fig. 2). The cell consists of two machined slabs of perspex into which cavities have been cut. Pre-soaked visking, which acts as the semi-permeable membrane, is placed between the slabs. The slabs are held together with 6 steel bolts to give a watertight seal. Perspex stoppers of a diameter large enough to enable sample removal from individual dialysis chambers are used.

Each cavity is of 1.2 ml capacity. Each of the cavities on one side of the visking are filled with 1 ml of a drug solution in isotonic phosphate buffer at pH 7.4; 1.0 ml of

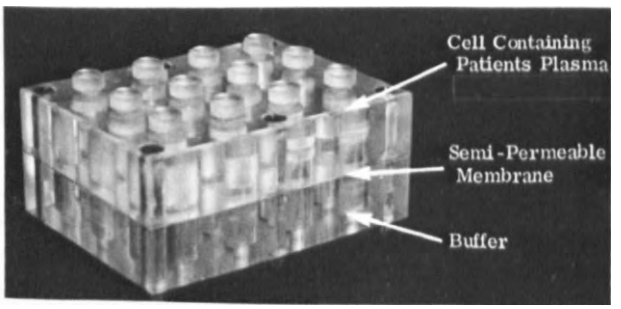

Fig. 2. The Multi-Cavity Equilibrium Dialysis Cell is placed in a shaking water-bath at 37°C until equilibrium is attained.

patient plasma is placed in each cavity on the opposite side of the membrane. A range of drug concentrations is used in each experimental run so that 12 data points can be obtained. The complete cell is then shaken in a controlled temperature (37°C) water bath until equilibrium is attained; samples are then withdrawn from either side of the membrane. By assaying these samples, concentrations of the drug in buffer and in plasma are found and the percentage bound values are then calculated for the drug within the concentration range used. In more specific work, when a drug is known to be bound to a specific protein, e.g. albumin, more specific binding parameters n (numbers of binding sites per mole of albumin) and k (affinity coefficient or association constants) may be found, as molar albumin concentration of the plasma and a range of bound and free drug molar concentrations at equilibrium is known. To obtain n and k values, these data can be processed using the non-linear regression analysis programme, NONLIN (Metzler, 1969), with the subroutines shown in Figs. 3 and 4.

Changes in percentage bound and in n and k values will alter the distribution and free plasma levels of a drug. Explanation of such deviations from the norm may be found by the examination of the patient's plasma protein profile, e.g. a decrease in the binding of a certain drug may be due to plasma albumin deficiency.

Variations in binding may not be entirely due to changes in plasma protein profile, but may be due to more subtle parameters, e.g. competition for binding sites by endogenous substances whose titre has become elevated due to a diseased state (Reidenberg, 1976).

Further biochemical screening of plasma will be needed in these cases in order that a more complete pharmacokinetic picture can be built up.

However, with routine use of the described system, useful correlations between plasma protein binding and plasma protein profile may become apparent. Such parameters would prove useful in the clinical setting in the calculation of dosage regimens.

```
      SUBROUTINE DFUNC(F,P,CON,VAL,X,I,J,ISPEC,XVEC,Y,W,NOBS)
      DIMENSION ISPEC(ID13),NOBS(ID18)
      DOUBLE PRECISION P(ID1),VAL(ID6),F,CON(ID2),Y(ID4),W(ID5),X,
     1XVEC(ID3)
      COMMON/B1/ID1,ID2,ID3,ID4,ID5,ID6,ID7,ID8,ID9,ID10,ID11,ID12,
     1ID13,ID14,ID15,ID16,ID17,ID18
C
C     PROTEIN BINDING, ONE SITE : C/R VS. C
C      SELLERS AND KOCK-WESER TYPE PLOT
C****     IF ORIGINAL DATA NOT C/R AND C, TRANSFORM WHEN ISPEC(8)=-1
C****     THIS MODEL COULD BE ENTIRELY LINEARIZED
C     TWO PARAMETERS, NO CONSTANTS
C     P(1) : N
C     P(2) : K
C
      F=X/P(1)+1.0/P(1)/(P(2)+1.0E4)
      RETURN
      END
      FINISH
```

Fig. 3. Function to be fitted to the non-linear regression analysis computer programme, NONLIN (Metzler, 1969), where only primary binding sites are indicated.

```
      SUBROUTINE DFUNC(F,P,CON,VAL,X,I,J,ISPEC,XVEC,Y,W,NOBS)
      DIMENSION ISPEC(ID13),NOBS(ID18)
      DOUBLE PRECISION P(ID1),VAL(ID6),F,CON(ID2),Y(ID4),W(ID5),X,
     1XVEC(ID3)
      COMMON/B1/ID1,ID2,ID3,ID4,ID5,ID6,ID7,ID8,ID9,ID10,ID11,ID12,
     1ID13,ID14,ID15,ID16,ID17,ID18
C
C     TWO BINDING SITES     R  VS  C
C****    IF ORIGINAL DATA NOT R/C and R, TRANSFORM WHEN ISPEC(8)=-1
C     FOUR PARAMETERS, NO CONSTANTS
C     P(1) : N1
C     P(2) : K1
C     P(3) : N2
C     P(4) : K2
C
      IF (ISPEC(8).NE.-1) GO TO 1
      DO 2 II=1,ISPEC(2)
      XVEC(II)=XVEC(II)/Y(II)
      Y(II)=XVEC(II)*Y(II)
2     CONTINUE
1     CONTINUE
      F=P(1)*P(2)*1.0E4*X/(1.0+P(2)*1.0E4*X)
     *+P(3)*P(4)*1.0E4*X/(1.0+P(4)*1.0E4*X)
      RETURN
      END
      FINISH
```

Fig. 4. Function to be fitted to the non-linear regression analysis computer programme, NONLIN (Metzler, 1969), where primary and secondary binding sites are indicated.

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