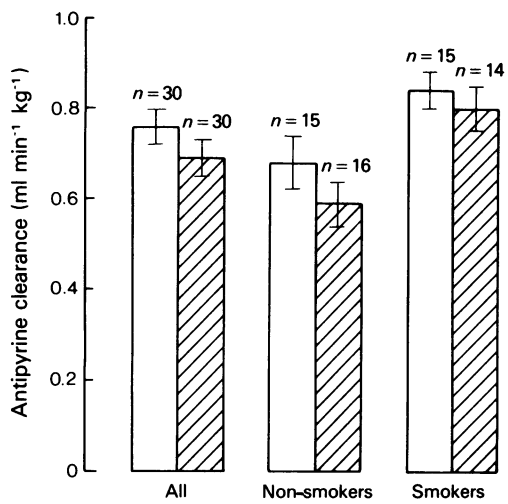

Figure 2 Antipyrine clearance (mean ±s.e. mean) in non-smokers, smokers and all subjects together to show difference between subjects aged under 40 years (□) and subjects aged 40 years and over (▨).

any analysis with the exception of women who did not smoke ($r=0.63$, $P<0.05$).

These findings do not support the hypothesis of Wood *et al.* (1979). Only three of their subjects were aged over 60 years and exclusion of these three from the analysis would not have affected their conclusions. The overall age range is therefore similar in the two studies. Methodological differences between the two studies are unlikely to be responsible since antipyrine clearance can be estimated satisfactorily after either oral or intravenous administration (Andreasen & Vesell, 1974) and equally reliably from saliva samples as from plasma samples (Fraser, Mucklow, Murray & Davies, 1976.

We submit that more evidence is required before it can be concluded that the stimulating effect of smoking upon antipyrine metabolism diminishes with advancing years.

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Received October 27, 1979

References

ANDREASEN, P.B. & VESELL, E.S. (1974). Comparison of plasma levels of antipyrine, tolbutamide and warfarin after oral and intravenous administration. *Clin. Pharmac. Ther.*, **16**, 1059–1065.

FRASER, H.S., MUCKLOW, J.C. BULPITT, C.J., KAHN, C., MOULD, G. & DOLLERY, C.T. (1979). Environmental factors affecting antipyrine metabolism in London factory and office workers. *Br. J. clin. Pharmac.*, **7**, 237–243.

FRASER, H.S., MUCKLOW, J.C., MURRAY, S. & DAVIES, D.S. (1976). Assessment of antipyrine kinetics by measurement in saliva. *Br. J. clin. Pharmac.*, **3**, 321–325.

O'MALLEY, K., CROOKS, J., DUKE, E. & STEVENSON, I.H. (1971). Effect of age and sex on human drug metabolism. *Br. med. J.*, **3**, 607–609.

STEVENSON, I.H. (1977). Factors influencing antipyrine elimination. *Br. J. clin. Pharmac.*, **4**, 261–265.

VESTAL, R.E., NORRIS, A.H., TOBIN, J.D., COHEN, B.H., SHOCK, N.W. & ANDRES, R. (1975). Antipyrine metabolism in man: Influence of age, alcohol, caffeine, and smoking. *Clin. Pharmac. Ther.*, **18**, 425–432.

WOOD, A.J.J., VESTAL, R.E., WILKINSON, G.R., BRANCH, R.A. & SHAND, D.G. (1979). Effect of aging and cigarette smoking on antipyrine and indocyanine green elimination. *Clin. Pharmac. Ther.*, **26**, 16–20.

PLASMA BINDING OF DISOPYRAMIDE

Disopyramide is an antiarrhythmic drug with pharmacological properties qualitatively similar to quinidine and procainamide which shows concentration dependent plasma binding within the manufacturer's recommended therapeutic range of 3.3–7.5 mg/l (Heel, Brogden, Speight & Avery, 1978). Disopyramide kinetics are altered with cardiovascular disease (Ward & Kinghorn, 1976) and non-linear behaviour has been reported, with some authors maintaining that dose-dependency can be explained by variations in plasma protein binding (Hinderling & Garrett, 1976; Meffin, Robert, Winkle, Harapat, Peters & Harrison, 1979), while others consider this aspect to be insufficient to explain the evidence (Cunningham, Shen, Shudo & Azarnoff, 1977 & 1978; Ilett, Madsen & Woods, 1979). Adding to the difficulty of answering the latter feature, there may be considerable variation in the extent of plasma

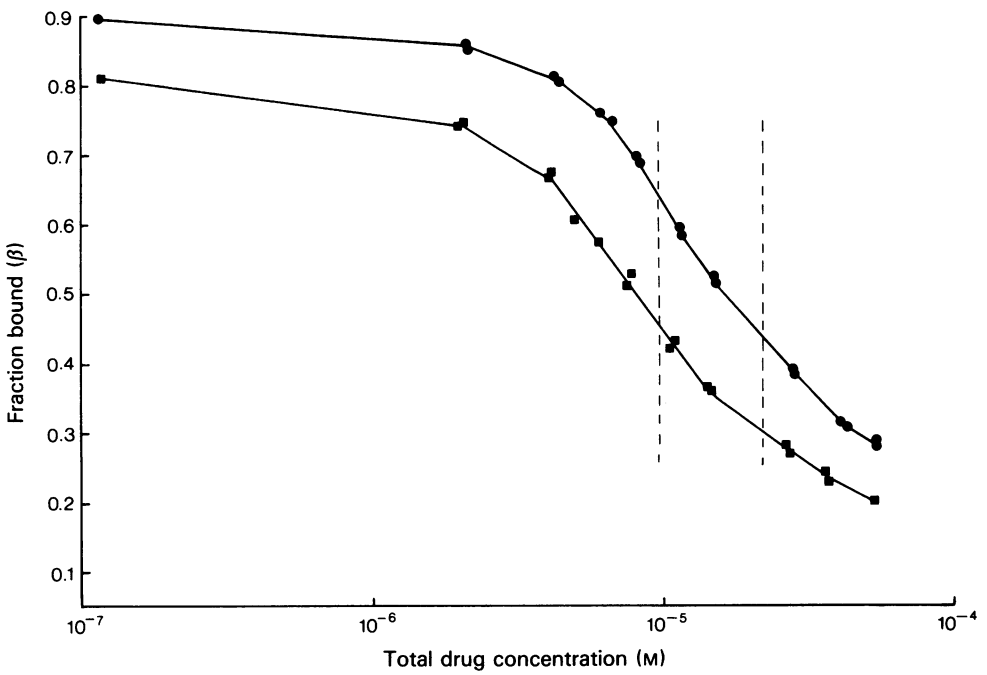

Figure 1 Plasma binding of disopyramide. The two curves represent the extremes in binding for eight subjects. The manufacturer's recommended plasma therapeutic range is shown as dotted lines. (●) subject M.V., (■) G.E.

binding between patients (Meffin *et al.*, 1979). While there is superficial agreement regarding *in vitro* disopyramide binding properties in the literature, closer inspection reveals differences between laboratories, and it is perhaps this lack of agreement which hinders a clear resolution of the importance of plasma binding in disopyramide kinetics, and the effect of disease thereon. The goal of the present study, therefore, was to establish as unequivocally as possible, the binding characteristics of disopyramide in normal plasma.

The interaction was studied by equilibrium dialysis. [^{14}C]-disopyramide phosphate (20 mCi/mM) was supplied by Roussel Pharmaceuticals and purified to greater than 98% by thin layer chromatography (silica gel, ethyl acetate-methanol-concentrated ammonia solution (78:20:2)). Appropriate concentrations of unlabelled drug were added to isotope in 0.1 M phosphate buffer containing 0.05 M NaCl, pH 7.4, to give standard solutions of 0.05–32 mg/l calculated as disopyramide base. Blood (60 ml) was collected by venepuncture from eight healthy volunteers aged 22–41 years (4 male, 4 female). Plasma and standard solutions were placed in 1 ml teflon dialysis cells with a separating cellulose membrane (Spectrapor 2) and dialysed for 5 h at 37°C. In initial experiments over 8 h it was shown that thermodynamic equilibrium was established in 3 h. After equilibration, aliquots of both sides of the cells were assayed by liquid scintillation (Packard Tricarb Model B2450) and converted to molar concentrations through known specific activities. Binding parameters according to the following model (Brand & Toribara, 1975) were estimated by nonlinear least squares regression (Metzler, 1969).

$$D_b = \frac{nP_t K D_f}{1 + K D_f} + K_{o/w} D_f \qquad (1)$$

where D_b = bound drug concentration, nP_t = capacity of binding sites in plasma for disopyramide, K = affinity constant, D_f = free drug concentration and $K_{o/w}$ = partition coefficient.

Figure 1 shows the form of binding isotherms for disopyramide in freshly drawn normal human plasma, and also the small degree of inter-subject variation, the two curves shown being the extremes for eight subjects. It is seen that the therapeutic range lies across the region where variation in fraction of drug bound is greatest. At lower disopyramide concentrations the data fitted a one-class binding site model extremely well. However, at higher drug concentrations the curves did not steadily approach zero fraction drug bound as predicted with such a

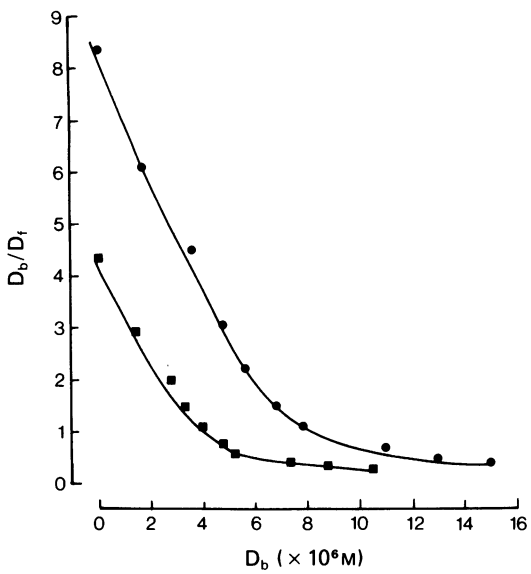

Figure 2 Modified Scatchard plot for the binding of disopyramide to plasma at 37°C for subjects (●) M.V. and (■) G.E.

model, and this gives an indication that more than one type of binding site is involved. In Figure 2, this feature can be clearly seen with the curves at highest drug concentration being almost horizontal. This suggests that addition of a partitioning component to the one-class model would be more appropriate than postulating a second class (Equation 1), and in all data sets this model proved to be a good representation. Table 1 shows the three binding parameters nP_t, K and $K_{o/w}$ for all eight subjects; these were not dependent on sex or age. The fraction of drug bound (β) decreased from 0.862 ± 0.0063 to 0.259 ± 0.008 (mean ± s.e. mean) over the total drug concentration range 1.16×10^{-7} – 5.25×10^{-5}M; in the recommended therapeutic range (9.7×10^{-6} – 2.2×10^{-5}M or 3.3–7.5 mg/l) β decreased from 0.56 to 0.36 (Figure 3).

Figure 3 contrasts earlier literature data on disopyramide binding with mean data from the present study. The large variation in binding behaviour between the earlier studies is readily apparent. Some of the reasons for these variations might include: the ultrafiltration method is quicker and more convenient than equilibrium dialysis but is not without attendant problems which must be carefully controlled (Steinhardt & Reynolds, 1969); use of blood bank rather than fresh plasma; corrections for membrane and cell binding; and adequate demonstration of sufficient time for true equilibrium.

In the present study, the capacity (nP_t) and affinity (K) parameters with pooled blood bank plasma were significantly ($P<0.05$) decreased from 5.58 ± 0.33 × 10^{-6}M and 1.14 ± 0.05 × $10^{6}M^{-1}$ to 4.26 ± 0.97 × 10^{-6}M and 0.60 ± 0.08 × $10^{6}M^{-1}$ (mean ± s.e. mean) respectively. A change in binding could have been predicted since it is known that plasticisers can be leached out of plastic containers and may cause displacement (Borga, Piafsky & Nilsen, 1977). Other additives (e.g. citrate, dextrose, phosphate and adenine) may also compete for plasma disopyramide binding sites, together with any drugs present in the plasma of blood bank donors. Membrane and cell binding is never insignificant, although it usually

Table 1 Binding parameters (mean ± s.e. mean) of disopyramide in plasma from healthy volunteers

Subject	*Binding capacity* nP_t ($\times 10^{-6}$ M)	*Affinity constant* K ($\times 10^{6}$ M^{-1})	*Partition coefficient* $K_{o/w}$
B.D.	5.14 ± 0.116	1.12 ± 0.069	0.187 ± 0.0047
B.M.	5.72 ± 0.227	1.07 ± 0.109	0.203 ± 0.0093
R.M.	4.87 ± 0.256	1.08 ± 0.148	0.264 ± 0.0111
G.E.	4.38 ± 0.114	0.96 ± 0.065	0.155 ± 0.0044
J.P.	6.94 ± 0.184	1.17 ± 0.078	0.27 ± 0.0078
M.V.	6.96 ± 0.148	1.17 ± 0.063	0.22 ± 0.0061
E.K.	5.07 ± 0.165	1.43 ± 0.133	0.177 ± 0.0071
M.M.	5.54 ± 0.158	1.11 ± 0.084	0.203 ± 0.0065
Mean ± s.e. mean	5.58 ± 0.332	1.14 ± 0.048	0.21 ± 0.014

Binding capacities, affinity constants and oil/water partition coefficients were determined by nonlinear least squares regression of bound drug concentrations (D_b) on free drug concentrations (D_f) assuming the model:
$D_b = nP_t\ K\ D_f/(1+K\ D_f) + K_{o/w}\ D_f$ (Equation 1).

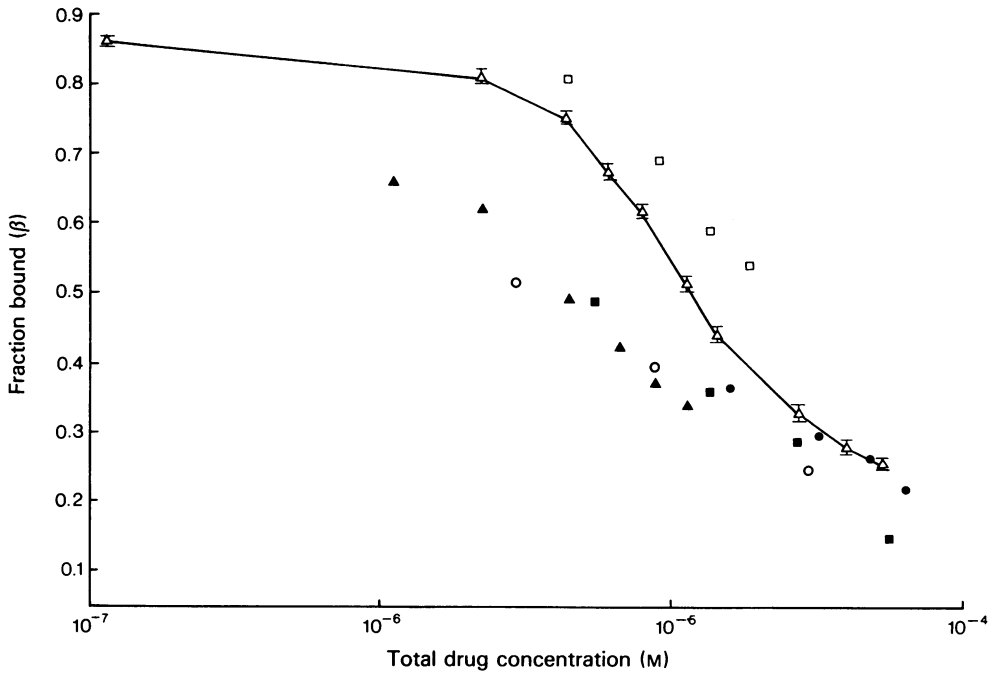

Figure 3 Published *in vitro* data for disopyramide-plasma binding showing the variability in reported fraction bound over the plasma concentration range $1.1 \times 10^{-7} - 1 \times 10^{-4}$M. Data points are as quoted in the individual papers (●) Chien *et al.* (1974), (■) Hinderling *et al.* (1974), (▲) Cunningham *et al.* (1977), (○) Karim *et al.* (1978), (□) Meffin *et al.* (1979), (△) mean (± s.e. mean) data from present study.

decreases with decreasing hydrophobocity of the test ligand, and must be actively allowed for in the experimental design and calculations. The commonly reported method of establishing equilibration time in the clinical literature is to measure drug concentration on one side of the membrane (usually the protein-free side) as a function of time, and to assume equilibrium when this is invariant. Steinhardt & Reynolds (1969) have described the problems with this approach and have stated that the only reliable way of assuring true equilibrium is to obtain the same bound and free drug concentrations when the drug is initially placed on either side.

Meffin *et al.* (1979) have stated that there is large inter-patient variability of disopyramide binding in patients with cardiac disease. Our results do not support this contention in healthy volunteers; indeed, for a biological phenomenon, there is surprising conformity (Figure 3). However, preliminary work in our laboratory provides some support for increased variability of binding in myocardial infarct patients. In four patients with confirmed myocardial infarction, plasma was taken within 24 h of onset of symptoms and the binding parameters were $3.91 \pm 0.72 \times 10^{-6}$M, $0.71 \pm 0.19 \times 10^{6}$M^{-1} and 0.42 ± 0.05 for nP_t, K & $K_{o/w}$ respectively. Compared to the data for normals, the following changes were significant ($P < 0.05$): decreased capacity and affinity, increased partitioning from aqueous to non-aqueous phase, and increased variability in affinity and partitioning. Whether the partial increase in variability is sufficient to jeopardise general binding predictions is a separate question of importance if it is established that plasma protein binding of disopyramide is the key to understanding kinetics; as mentioned previously, this is by no means agreed upon.

Myocardial infarction appears to decrease disopyramide binding in the therapeutic range, but this would not have been inferred when comparing the data of Meffin *et al.* (1979) with previous workers (Figure 3). Decreased binding might be expected in view of the well known decrease in plasma albumin concentrations after infarction (Johansson, Kindmark, Trell & Wollheim, 1972; Smith, Bos, Esseveld, Van Eijk & Gerbrandy, 1977), and the finding by Chien, Lambert & Karim (1974) that in plasma, disopyramide binds mainly to albumin. At higher disopyramide concentrations there was an increase in the magnitude and variability of the non-specific partitioning component of binding ($K_{o/w}$). This increase could be related to the transient

increases in acute phase reactants (e.g. α_1-acid glycoprotein and fibrinogen) and serum enzymes following infarction (Johansson *et al.*, 1972; Smith *et al.*, 1977), with variability tied to extent of changes in a given patient.

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Received December 31, 1979

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References

BORGA, O., PIAFSKY, K.M. & NILSEN, O.G. (1977). Plasma protein binding of basic drugs. I. Selective displacement from α_1-acid glycoprotein by trist(2-butoxyethyl)phosphate. *Clin. Pharmac. Ther.*, **22**, 539–544.

BRAND, J.G. & TORIBARA, T.Y. (1975). Equilibrium dialysis and circular dichroic analysis of the novobiocin-human serum albumin complex. *Arch. Biochem. Biophys.*, **167**, 91–98.

CHIEN, Y.W., LAMBERT, H.J. & KARIM, A. (1974). Comparative binding of disopyramide phosphate and quinidine sulfate to human plasma proteins. *J. pharm. Sci.*, **63**, 1877–1879.

CUNNINGHAM, J.L., SHEN, D.D., SHUDO, I. & AZARNOFF, D.L. (1977). The effects of urine pH and plasma protein binding on the renal clearance of disopyramide. *Clin. Pharmacokin.*, **2**, 373–383.

CUNNINGHAM, J.L., SHEN, D.D., SHUDO, I. & AZARNOFF, D.L. (1978). The effect of non-linear disposition kinetics on the systemic availability of disopyramide. *Br. J. clin. Pharmac.*, **5**, 343–346.

HEEL, R.C., BROGDEN, R.N., SPEIGHT, T.M. & AVERY, G.S. (1978). Disopyramide: a review of its pharmacological properties and therapeutic use in treating cardiac arrhythmias. *Drugs*, **15**, 331–368.

HINDERLING, P.H., BRES, J. & GARRETT, E.R. (1974). Protein binding and erythrocyte partitioning of disopyramide and its monodealkylated metabolite. *J. pharm. Sci.*, **63**, 1684–1690.

HINDERLING, P.H. & GARRETT, E.R. (1976). Parmacokinetics of the antiarrhythmic disopyramide in healthy humans. *J. Pharmacokin. Biopharm.*, **4**, 199–230.

ILETT, K.F., MADSEN, B.W. & WOODS, J.D. (1979). Pharmacokinetics of disopyramide in patients with acute myocardial infarction. *Clin. Pharmac. Ther.*, **26**, 1–7.

JOHANSSON, B.G., KINDMARK, C.O., TRELL, E.Y. & WOLLHEIM, F.A. (1972). Sequential changes of plasma proteins after myocardial infarction. *Scand. J. clin. lab. Invest.*, **29**, suppl. 124, 117–126.

KARIM, S., KOOK, C., NOVOTNEY, R.L., ZAGARELLA, J. & CAMPION, J. (1978). Pharmacokinetics and steady-state myocardial uptake of disopyramide in the dog. *Drug Metab. Dispos.*, **6**, 338–345.

MEFFIN, P.J., ROBERT, E.W., WINKLE, R.A., HARAPAT, S., PETERS, F.A. & HARRISON, D.C. (1979). Role of concentration-dependent plasma protein binding in disopyramide disposition. *J. Pharmacokin. Biopharm.*, **7**, 29–46.

METZLER, G.M. (1969). *A users' manual for NONLIN.* The Upjohn Company.

SMITH, S.J., BOS, G., ESSEVELD, M.R., VAN EIJK, H.G. & GERBRANDY, J. (1977). Acute-phase proteins from the liver and enzymes from myocardial infarction; a quantitative relationship. *Clin. Chim. Acta*, **81**, 75–85.

STEINHARDT, J. & REYNOLDS, J.A. (1969). The measurement of complex formation. In *Multiple Equilibria in Proteins*, pp. 34–52. New York: Academic Press, Inc.

WARD, J.M. & KINGHORN, G.R. (1976). The pharmacokinetics of disopyramide following myocardial infarction with special reference to oral and intravenous dose regimens. *J. int. med. Res.*, **4**, suppl. 1, 49–53.

HYPNOTIC AND RESIDUAL EFFECTS OF TEMAZEPAM IN VOLUNTEERS

A rapid and predictable response in inducing normal sleep without producing residual morning-after effects is a fundamental requirement of the ideal hypnotic (Lader, 1979). The 1–4 benzodiazepine, temazepam, which is presented as a solution in polyethylene glycol contained in a soft gelatin capsule (Normison®), is rapidly absorbed, has a short elimination half-life due to its rapid conjugation and excretion and should not, therefore, accumulate on repeated administration (Fuccella, Bolcioni, Tamassia, Ferrario & Tognoni, 1977; Bittencourt, Richens, Toseland, Wicks & Latham, 1979). In addition, temazepam has been demonstrated to be an effective hypnotic (Nicholson & Stone, 1976; Hindmarch, 1976). From a multicentre study in a large population of insomniacs Harry & Johnson (1978) confirmed the efficacy of temazepam as an hypnotic free of morning after residual effects at a dose of 20 mg. Hindmarch (1976) indicated mild but significant residual effects on the morning after the use of a 30 mg dose of temazepam. Clarke & Nicholson (1978) could not confirm this finding