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Pharmacokinetics of digoxin in the elderly

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Pharmacokinetics of a number of drugs are altered in the elderly (Crooks, O'Malley & Stevenson, 1976). A reduction in digoxin excretion related to decreased renal function has been shown in the elderly (Ewy, Kapadia, Yao, Lullin & Marcus, 1969) and is an important determinant of added susceptibility to unwanted effects of this drug. Alterations in absorption or distribution of digoxin would also affect dose requirements in these patients. In this study we compared digoxin pharmacokinetics in elderly and younger patients with particular emphasis on absorption and distribution. Observations were made in seven elderly (aged 72–91 years) and six younger (aged 34–61 years) patients, in whom digoxin was indicated for its inotropic effect. Elderly patients received digoxin 0.25 mg and younger patients digoxin 0.5 mg

both intravenously and orally with at least 2 weeks between doses. Blood samples for estimation of plasma digoxin concentrations by radioimmunoassay were withdrawn before, at 15, 30, 45 and 60 min and at 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 36 and 48 h after administration of digoxin.

The results are shown in Table 1. Peak digoxin concentrations were attained earlier in the younger subjects ($P < 0.05$, Wilcoxon's rank sum test). Digoxin half-life after both routes was significantly longer in the elderly. The extent of absorption calculated as the ratio of area under the plasma concentration-time curves after oral and intravenous administration was not significantly different in the young and old subjects. The elderly had a significantly smaller apparent volume of distribution but after correction for weight the difference was not significant. Plasma clearance of digoxin (both absolute and weight corrected) was significantly lower in the older patients.

Thus the rate of absorption of digoxin is slowed in elderly patients but the extent of absorption is unchanged. Older patients have a smaller volume of distribution of digoxin which probably reflects their decreased lean body mass.

Table 1 Digoxin pharmacokinetics in young and elderly patients

	$T_{1/2}$ oral (h)	$T_{1/2}$ i.v. (h)	AUC oral (nmol ml ⁻¹ h ⁻¹)	AUC i.v. (nmol ml ⁻¹ h ⁻¹)	% Absorbed	V_d Area (l)	V_d Area (l kg ⁻¹)	Cl (ml min ⁻¹)	Cl (ml min ⁻¹ kg ⁻¹)
Young	36.8 ± 4.5	38.2 ± 3.5	89.7 ± 9.0	109.8 ± 15.5	84.3 ± 6.5	338.7 ± 35.0	5.3 ± 0.6	106.2 ± 13.6	1.7 ± 0.2
Elderly	69.6 ± 13.1	68.8 ± 12.3	144.6 ± 39.8	171.7 ± 32.8	76.0 ± 10.0	193.8 ± 24.0	4.1 ± 0.9	37.4 ± 5.9	0.8 ± 0.2
P*	<0.05	<0.05			NS	<0.01	NS	<0.001	<0.01

Results are expressed as mean ± s.e. mean.

 $T_{1/2}$ = half-life; AUC = area under the plasma concentration/time curve; V_d Area = Apparent volume of distribution; Cl = Plasma clearance.* Young v Old, Student's *t*-test.

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Non-invasive assessment of the haemodynamic and electrophysiological effects of medigoxin

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The effect of cardiac glycosides on systolic time intervals has been extensively reported, but previous studies have been performed either without placebo control or in a single-blind or open fashion. Although the electrocardiographic changes induced by the chronic administration of these drugs are well known, the acute effects of single doses are not. The present study compares the effects on the systolic time intervals (STI) and high-speed surface electrocardiogram (ECG) of a single dose of medigoxin with those of placebo, administered in a double-blind, randomised, balanced, crossover design.

Six healthy young adult volunteers consented to participate in the study. They fasted overnight and rested supine for 30 min before three control recordings of STI, 100 mm/s surface ECG, and systemic arterial blood pressure were made (Burgess, Turner & Wadsworth, 1978). Medigoxin 12 µg/kg, or an equivalent volume of saline, was then injected over 5 min via an indwelling intravenous cannula. Recordings were made at 5, 10, 20, 30 and 40 min after the start of the injection. A blood sample for medigoxin assay was obtained at the end of the study. Half the volunteers received medigoxin first, and 1

week elapsed between treatments. The STI and ECG tracings were shuffled and analysed 'blind'. STI were corrected for heart rate using regression equations derived from experiments in a group of subjects of similar age to those in the present study. Rate-corrected QT interval (QTc) was calculated using Bazett's formula. The significance of differences between treatments was determined using Student's *t*-test for paired data; the null hypothesis was rejected when $P < 0.05$.

Intravenous saline caused negligible changes in all the variables examined. Medigoxin reduced heart rate and QTc at all times after administration; QS₂ index (QS₂I), pre-ejection period (PEP), the ratio PEP/left ventricular ejection time, and T wave amplitude were all reduced significantly at 20, 30, and 40 min. Left ventricular ejection time index (LVETI), PR interval, QRS duration and diastolic blood pressure showed no significant changes, but systolic blood pressure tended to be higher after medigoxin treatment.

In this acute experiment, QS₂I, PEP, and PEP/LVET ratio were sensitive indicators of inotropic activity, but LVETI was not. Changes in QTc paralleled changes in heart rate, whereas T wave amplitude fell as the STI were shortened. There was temporal dissociation between chronotropic and inotropic actions of medigoxin.

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Can lignocaine kinetics be predicted?

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The analysis of bolus data from normal subjects and from patients with heart failure yielded plasma elimination half-lives for lignocaine of 1.3–1.8 h (Benowitz & Meister, 1978) suggesting that during a continuous i.v. infusion steady-state levels would be attained in about 7–9 h. However, plasma lignocaine concentrations have been shown to rise throughout infusions

lasting 24 h or more in patients with and without heart failure (Prescott, Adjepon-Yamoah & Talbot, 1976; LeLorier, Grenon, Latour, Caillé, Dumont, Brosseau & Solignac, 1977). After stopping the infusion lignocaine half-lives in these patients were greater than 3 h.

We have also seen plasma lignocaine concentrations rising beyond the time at which bolus kinetics would have anticipated a steady-state and have observed prolonged post-infusion half-lives ($6.8 \text{ h} \pm 2.5 \text{ s.d.}$; $n = 7$). These findings are consistent with product-inhibition of the metabolism of lignocaine (LeLorier, Moisan, Gagné & Caillé, 1977; Lennard, Bax, Tucker & Woods, 1978), possibly associ-

ated with its distribution into a previously unrecognised deep tissue compartment. However, in 16 post-myocardial infarct patients receiving lignocaine by infusion for the treatment of ventricular dysrhythmias, we observed periods of time during which the s.d. of the plasma lignocaine level was no greater than 10% of the mean (mean number of samples = 6, range 3–9; mean sampling time = 7.4h, range 1.7–25.4h). During these periods indocyanine green (ICG) 0.5 mg/kg was given intravenously as a bolus and its clearance and half-life were calculated from venous plasma concentrations between 6 and 14 min after injection.

Although there was a significant difference between mean lignocaine clearances (ml whole blood $\text{min}^{-1}\text{kg}^{-1}$) in patients with and without heart failure (7.0 ± 1.0 s.d. v. 12.0 ± 1.7 s.d.; $P < 0.01$), ICG clearances (ml whole blood $\text{min}^{-1}\text{kg}^{-1}$) did not discriminate between these two groups (11.0 ± 3.0 s.d. v. 12.1 ± 3.7 s.d.; $P > 0.05$). Furthermore, there was no significant linear correlation between lignocaine and ICG clearances ($r_s = -0.114$; $P > 0.05$).

These results contrast with the recent findings reported by Zito & Reid (1978) who suggest that ICG can be used to predict lignocaine dosage requirements in the clinical setting. Our data question the reliability

of ICG clearance as an index of hepatic blood flow in post-myocardial infarct patients and underline the complexity of lignocaine kinetics.

N.D.S.B. is an MRC Research fellow.

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The systemic availability of a slow release rectal preparation of lignocaine

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Lignocaine has proved valuable in the treatment of cardiac dysrhythmias and may have a further application in the prophylaxis of post-operative deep venous thrombosis, but its use is restricted by the need for parenteral administration. The short elimination half-life of lignocaine means that an i.v. infusion is required to keep plasma concentrations within the therapeutic range when the drug is given for longer than a few hours. Bioavailability of lignocaine given p.o. is low because of extensive first pass metabolism in the liver, and the large doses needed cause high concentrations of toxic metabolites. Rectal administration may enhance availability, since venous blood in the lower rectum and anal canal drains at least partly into the systemic rather than the hepatic portal

system. This study was designed to show whether a slow release preparation of lignocaine given per rectum would both reduce hepatic metabolism and sustain plasma concentrations of the drug.

Six healthy volunteers, four male and two female aged from 20–30 years and weighing from 56–65 kg, gave their informed consent to participate in the study. Lignocaine hydrochloride 200 mg (Xylocard® 2% solution, Astra) was given i.v. over 15 min and venous blood samples were taken during the 6 h after administration. A gelatin capsule containing 300 mg lignocaine hydrochloride in a wax/cellulose matrix was given per rectum, followed immediately by 10 ml 2% w/v aqueous solution of sodium dihydrogen phosphate injected through a quill cannula; venous blood samples were taken during the 8 h after administration. Half the volunteers received the intravenous dose first. Plasma lignocaine was estimated using a modified EMIT procedure (Syva Corporation). Computer-assisted curve fitting was used in the analysis of the data.

The plasma elimination half-life of lignocaine after i.v. administration was 116 min (range 80 to 143). After rectal administration of the slow release preparation, plasma concentration of lignocaine reached a peak of 600 to 1300 ng ml^{-1} (mean 790) at 120

to 160 min and then declined biexponentially with a final apparent elimination half-life of 216 min (range 115 to 633). Systemic availability of rectal lignocaine

was 38 to 78% (mean 57%) during the first 8 h after dosing.

The effects of propranolol and metoprolol on exercise responses in normal man

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The effects of single oral doses of 80 mg propranolol or 100 mg metoprolol on the cardiovascular and respiratory responses to bicycle ergometer exercise have been measured in nine normal men in a double-blind, placebo-controlled experiment. Each subject attended on three separate occasions 2 h after taking a drug or placebo on an empty stomach. The experiments were separated by a minimum of 48 h and the drugs were administered in random order. In each experiment the subject first exercised at two modest steady-state work loads of 25 W and 75 W and then performed a progressive exercise test to exhaustion with load increments of 25 W every 2 min. Alveolar and mixed-expired gases were collected and analysed using an infra-red CO₂ analyser and a paramagnetic oxygen analyser previously calibrated with standard gases analysed volumetrically. Oxygenated mixed venous P_{CO₂} was measured during the steady-state periods of exercise using a rebreathing method (Jones, Campbell, McHardy, Higgs & Clode, 1967). An ECG was recorded continuously. Perceived exertion at the higher exercise levels (>100 W) was assessed using a twenty-point integer scale (Borg, 1970). FEV₁, FVC and PEFR were measured before and after exercise.

At the two steady-state work levels propranolol and metoprolol both produced the same reduction of about 22% in exercise heart rate. There was a significant 12% increase in stroke volume which was not

sufficient to compensate for the reduction in heart rate so that cardiac output fell by 12% ($P < 0.05$).

Endurance was assessed as the total work done during the progressive test to exhaustion and was 18% (26 KJ, s.e. mean = 9) less on propranolol than on placebo, a significant reduction. The effect of metoprolol on endurance was less (16 ± 14 KJ) and not significant at the 0.05 level.

Both drugs produced a significant ($P < 0.01$) reduction of 3.5% in oxygen consumption over the whole work range corresponding to a fall of 84 ± 30 ml O₂/min at a work load of 200 W. CO₂ production and ventilation were unchanged. The respiratory exchange ratio was raised by 0.056 units, s.e. mean = 0.012, over the whole range of work loads ($P < 0.001$) and the two drugs had similar effects. FEV₁, FVC and PEFR were unaffected.

Both drugs produced a significant increase in the perceived exertion rating over the work range above 100 W (mean increase 0.74 Borg scale units, s.e. mean = 0.20). That is, any given work load was associated with a greater sense of fatigue on the β -adrenoreceptor blocking drugs than on placebo (Ekblom & Goldbarg, 1971). This was also true if perceived exertion was related to oxygen consumption rather than work load. There was no significant difference between the two drugs although metoprolol had the greater effect.

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The relative potency of amiloride and spironolactone in bendrofluazide-treated hypertensive patients

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Fifteen hypertensive patients taking 10 mg bendrofluazide daily received, in addition, amiloride 5, 10 and 20 mg daily; spironolactone 25, 50, 100 and 200 mg daily; and placebo in a crossover design. Treatment phases lasted 4 weeks and treatment order was varied systematically. During placebo treatment the mean ($\pm$ s.d.) plasma potassium was 3.06 ± 0.43 mmol/l; plasma aldosterone 12.5 ± 6.2 pmol/l; and lying BP $160 \pm 18/92 \pm 9$ mmHg.

Plasma aldosterone and plasma potassium did not correlate ($r = -0.23$). Amiloride and spironolactone each produced significant dose response curves for plasma potassium, angiotensin II, aldosterone (all positive), sodium and bicarbonate (each negative). There were small reductions in systolic, but not diastolic, BP. The relative potency amiloride: spironolactone in correcting hypokalaemia was 2.8:1 (95% C.L. 1.3:1 – 4.8:1). In individuals, plasma potassium responses to amiloride and spironolactone correlated with each other ($r = +0.71$, $P < 0.01$), with placebo plasma potassium ($r = -0.62$ and -0.71), but not with placebo plasma aldosterone. For a given plasma angiotensin II, plasma aldosterone was less with spironolactone than with amiloride treatment. This was consistent with a partial, but weak, inhibition of aldosterone production by spironolactone. The potency of amiloride:spironolactone in correcting diuretic-induced hypokalaemia was significantly lower than the accepted 5:1 ratio.

Fludrocortisone in the treatment of postural hypotension: altered sensitivity to pressor agents

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The benefit from fludrocortisone (9 α -fluorohydrocorti-

tisone) in postural hypotension resulting from sympathetic nervous degeneration may be due partially to fluid retention (Bannister, Ardill & Fentem, 1969). However, fludrocortisone may sensitise vascular receptors to pressor amines (Schmid, Eckstein & Abboud, 1966), to which patients with autonomic failure are supersensitive (Wilcox & Aminoff, 1974; Davies, Bannister & Sever, 1978). We have studied changes in pressor sensitivity after fludrocortisone in three patients with sympathetic degeneration.

Table 1 Supersensitivity to pressor agents

Drug	Dose to give blood pressure rise ≥ 20 m Hg		P*	Blockade of pressor response by phenolamine (2–5 μ g/min)
	Before 9 α -F	After 9 α -F		
NA (μ g min ⁻¹)	0.83 ± 0.58	0.19 ± 0.27	<0.05	—
A II (ng kg ⁻¹ min ⁻¹)	0.57 ± 0.4	0.3 ± 0.17	0.2	Partial
Sar (μ g kg ⁻¹ min ⁻¹)	3.0 ± 1.73	1.03 ± 0.95	<0.05	Complete

Values are means $\pm$ s.d.

* Student's *t*-test (paired).

9 α -F Fludrocortisone.

NA noradrenaline acid tartate (Levophed, Winthrop Laboratories).

A II angiotensin II (Hypertensin, CIBA).

Sar saralasin (Eaton Laboratories).

Phentolamine (Rogitine, CIBA).

Patients ate a diet providing 90 mmol Na day⁻¹. After 7 days without any drugs, the intravenous dose of noradrenaline, angiotensin II and saralasin (1-sarcosyl-8-alanyl-angiotensin II, an angiotensin receptor blocker), (Pals, Masucci, Denning, Sipos, Fessler, 1971) necessary to change the systolic pressure at least 20 mm Hg, was determined. The effect of phentolamine infused intravenously with the latter two drugs was also assessed. After a loading dose (0.2 mg), fludrocortisone (0.1 mg day⁻¹, orally) was given for 7 days. Then the changes in blood pressure in response to noradrenaline, angiotensin and saralasin, with and without phentolamine, were re-determined.

Compared with normals (Wilcox & Aminoff, 1976; Streeten, Anderson, Freiberg & Dalakos, 1975) our patients were supersensitive to noradrenaline and angiotensin II (Table 1) and after fludrocortisone the dose of noradrenaline, but not angiotensin II, necessary for a pressor response was markedly decreased. Saralasin produced a paradoxical, marked, rise in pressure in all our patients; the dose necessary for this was decreased after fludrocortisone therapy. Phentolamine blocked the pressor response to saralasin completely, but only partially to angiotensin II.

In essential hypertensives, saralasin can increase blood pressure. This can be blocked by phentolamine and is associated with a rise in plasma noradrenaline (McGrath, Ledingham & Benedict, 1977; Davies, unpublished observations). Body-weight, plasma and urinary urea and electrolytes, plasma proteins and haematocrit, showed no evidence of significant fluid retention with fludrocortisone. These results show that in chronic autonomic failure, fludrocortisone can increase the degree of sensitivity to noradrenaline.

Therefore, fludrocortisone therapy may allow some restoration of reflex function in partially denervated patients.

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The pharmacokinetics of labetalol in chronic liver disease

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The effect of age and hyperthyroidism on plasma propranolol steady state concentration

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The hepatic elimination of a number of drugs appears to be impaired in the elderly (Crooks, O'Malley & Stevenson, 1976). For the most part data has been obtained from single dose studies but there is evidence that, in patients given the same dose of drug chronically, the plasma drug steady state concentration (Css) may increase with age e.g. phenytoin (Houghton, Richens & Leighton, 1975) imipramine and amitriptyline (Nies, Robinson, Friedman, Green, Cooper, Ravaris & Ives, 1977).

We have determined the plasma propranolol C_{ss} (4 h post-dosage) in 20 hyperthyroid patients, aged 16–75 years during continuous treatment with propranolol (160 mg/day) and again in 17 of these patients when euthyroid. Propranolol compliance, as assessed by direct questioning and tablet-counting, was satisfactory.

There was a wide inter-individual variation in the plasma propranolol C_{ss} both when hyperthyroid (range 5 to 205 ng/ml) and when euthyroid (35 to

235 ng/ml). As noted previously (Feely, Crooks & Stevenson, 1978) there was a significant rise in the plasma propranolol C_{ss} following treatment of hyperthyroidism. There was a significant correlation between plasma propranolol C_{ss} and age, which was more marked in the hyperthyroid ($r = 0.74$, $P < 0.001$) than in the euthyroid ($r = 0.58$, $P < 0.05$) state.

These results suggest that ageing may, either through an effect on liver blood flow or intrinsic liver cell extraction of propranolol, reduce the elimination of propranolol during chronic therapy and that hyperthyroidism accentuates this age effect.

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Protein binding of atenolol and propranolol to human serum albumin and in human plasma

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The binding of atenolol to human serum albumin (HSA) and in human plasma has been compared with that of propranolol using equilibrium dialysis.

[¹⁴C]-Atenolol, 0.5 – 10 µg/ml (1.7 – 33.1 µM, sp.act. = 2.2 µCi/µmol) and [¹⁴C]-propranolol, 0.5 – 10 µg/ml (1.7 – 33.8 µM, sp.act. = 3.6 µCi/µmol) were dissolved in phosphate buffer (pH 7.4, 0.1 M) and

dialysed against HSA (Sigma: 4% in phosphate buffer pH 7.4, 0.1 M) or blood-bank plasma (3.2% albumin, 6.3% total protein) at 37°C for 3 h in teflon half cells (volume 1.36 ml) separated by a single thickness membrane (Visking 36/32). The total concentration and the concentration at equilibrium of the drug on each side of the membrane were measured by scintillation counting using 10 ml of an homogenous Triton X-100 scintillant (6 g PPO in 667 ml toluene, 333 ml Triton X-100). Quench corrections were made by the external standard channels ratio method.

The results shown in Table 1 indicate that atenolol is bound in the plasma solution or to HSA to a limited extent and to a lesser degree than propranolol. Analysis of variance indicated that the binding of atenolol to HSA was not concentration dependent within the range studied allowing the calculation of a mean. Also, as previously reported (Evans, Nies & Shand, 1973; Borga, Odar-Cederlöf, Piafsky &

Table 1 Binding of atenolol and propranolol to 4% human serum albumin

Concentration ($\mu\text{g/ml}$)	% Bound	
	Atenolol	Propranolol
0.5	3.9 ± 1.1 (6)	43.9 ± 1.9 (4)
1.0	3.4 ± 1.2 (6)	44.1 ± 0.7 (4)
5.0	2.7 ± 0.7 (9)	50.5 ± 0.6 (4)
10.0	2.8 ± 0.8 (10)	51.6 ± 1.4 (5)
Mean	3.1 ± 0.4 (31)	

*Binding of atenolol and propranolol in human plasma**

Drug	Concentration	
	($\mu\text{g/ml}$)	% Bound
Atenolol	1.0 – 5.0	3.0 ± 1.4 (5)
Propranolol	0.5 – 10.0	82.3 ± 1.0 (8)

All values are given as mean $\pm$ s.e. mean with the number of determinations in parentheses.

Sjöqvist, 1977) the binding of propranolol in human plasma cannot be accounted for by albumin alone. In contrast, the binding of atenolol in plasma is essentially to the albumin fraction.

The tabulated results were calculated directly from the equilibrium concentration of the drug on each

side of the membrane. Indirect calculation, by reference to the total concentration of the drug before dialysis is also possible. For non-radiolabelled drugs the latter method may be used if the equilibrium concentration of the drug cannot be measured on the side of the membrane containing the proteinaceous material. In an experiment not subject to error both methods of calculation give identical results. However, in actual experiments subject to variation and possible systematic errors the second method when used with non-radiolabelled drugs may give inaccurate, imprecise and often erroneously high results, particularly with lowly bound drugs like atenolol.

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CSF/plasma ratios of propranolol in man

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Although some β -adrenoceptor blocking drugs such as propranolol have been shown to be effective in the treatment of a variety of central nervous system disorders such as schizophrenia, anxiety, tremor (Conway, Greenwood & Middlemiss, 1978), whether their mechanism of action is centrally or peripherally mediated is not resolved. Propranolol is known to penetrate the blood-brain barrier in animals and man (Day, Hemsworth & Street, 1977; Myers, Lewis, Reid & Dollery, 1975). Myers *et al.* (1975) reported that the concentration of propranolol in the human brain is 15 times greater than in plasma after i.v. infusion.

We have measured propranolol concentrations in samples of plasma and cerebrospinal fluid (CSF) obtained simultaneously from patients who had received the drug orally in single doses of 80 or 160 mg or sequential doses of 40 mg 8 hourly as an anxiolytic before routine diagnostic lumbar puncture. A mean CSF/plasma ratio of 0.07 was obtained for propranolol.

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Plasma protein binding interaction between valproic acid and palmitic acid *in vitro*

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Valproic acid (VPA), an antiepileptic drug, is an eight carbon, branched chain, simply fatty acid which is highly bound to plasma proteins (80–90%). VPA has a low volume of distribution (V_d) (5–13 l) and exhibits restricted hepatic extraction. Therefore, alterations in VPA protein binding may change the clearance of the drug.

The binding of $350 \mu\text{mol l}^{-1}$ VPA was investigated in both human serum (35 g/l albumin) and human crystallised albumin solutions (35 g/l), containing varying concentrations of palmitic acid (PA). PA was used as a model for endogenous free fatty acids (FFA), which are more strongly bound to plasma proteins than most drugs, and being of similar structure

may displace VPA by competing for the same binding sites.

The protein binding of $350 \mu\text{mol l}^{-1}$ VPA in human serum was directly related to the concentration of PA present in the serum. Concentrations of PA ($n = 22$) ranging from $50\text{--}1700 \mu\text{mol l}^{-1}$ (0.1–3.3 molar ratio PA to albumin) caused a linear decrease ($r = 0.95$) in VPA binding from 95 to 65%. Under physiological conditions the molar ratio FFA to albumin in human plasma can vary between 0.5–1.5 ($250\text{--}750 \mu\text{mol l}^{-1}$) (Court, Dunlop & Leonard, 1971). Within this range VPA binding decreased from 91% at $250 \mu\text{mol l}^{-1}$ PA to 82% at $750 \mu\text{mol l}^{-1}$ PA resulting in a two-fold increase in the free VPA concentration.

The binding of $350 \mu\text{mol l}^{-1}$ VPA in a crystallised human albumin solution containing similar PA concentrations to above, was decreased in a linear fashion ($r = 0.96$) from 95 to 54%. The VPA binding at $0 \mu\text{mol l}^{-1}$ PA in serum was not significantly different from that in the albumin solution, but the fall in binding of VPA with increasing PA concentrations was significantly greater in pure albumin than in serum. This indicates that at low PA concentrations VPA is bound almost exclusively to the albumin fraction in human serum. However, with increasing displacement of VPA from albumin by PA, the VPA bound to protein sites other than on the albumin molecule.

As K_1 and K_2 decreased with increasing PA concentrations (Table 1) and the number of primary binding sites was unchanged, VPA and PA probably compete for the same albumin binding sites.

Table 1 Number of binding sites, n_1 , n_2 and apparent association constants K_1 and K_2 for valproic acid binding in human serum with varying concentrations of palmitic acid (PA). Analysis was performed by the method of Scatchard (1949)

Concentration of PA ($\mu\text{mol l}^{-1}$)	n_1	$K_1 \times 10^4$ (l mol^{-1})	n_2	$K_2 \times 10^3$ (l mol^{-1})
327	1.6	0.941	4.6	0.124
2800	1.9	0.201	9.7	0.017
4500	1.3	0.112	18.9	0.011

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Plasma protein binding of maprotiline in geriatric patients—influence of α_1 -acid glycoprotein

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Recent interest has focussed on the plasma protein binding of basic (cationic) type drugs and the role of proteins other than albumin. One of the 'acute-

phase' reactant proteins, α_1 -acid glycoprotein (AGP) has been singled out for particular investigation (Fremstad, Bergerud, Haffner & Lund, 1976; Borgå, Piafsky & Nilsen, 1977). In a study carried out by Piafsky & Borgå (1977) individual differences in plasma protein binding of imipramine and alprenolol in a group of healthy volunteers were related to the plasma concentrations of AGP. There are several investigations showing that a number of disease states gives rise to greatly increased levels of AGP, these include: local inflammation, surgery, bacterial infection and neoplastic growth. It is, therefore possible

Table 1 Serum protein binding of maprotiline in young and old subjects

Group	Age (years)		% Free maprotiline		α_1 -acid glycoprotein (g l ⁻¹)	
	mean	range	mean	range	mean	range
Young healthy volunteers <i>n</i> = 14	22.6	20–32	10.5	9.1–12.4	0.73	0.56–1.03
Geriatric patients <i>n</i> = 25	82.3	74–95	11.0*	8.6–15.0	1.37**	0.48–3.10

* *P* = 0.254 (NS).** *P* = 0.0004.Significance values refer to difference between corresponding results in volunteers and geriatric patients using an independent *t*-test.

that in patients with serious or multiple disease states, the plasma concentration of AGP may vary considerably and so exert a major influence on the plasma protein binding of basic drugs.

To investigate this possibility, we have carried out a preliminary study of the plasma protein binding of maprotiline, a 'basic' type tetracyclic antidepressant, in a group of geriatric in-patients suffering from various acute disease states. Clotted blood samples were obtained from a group of 25 female geriatric in-patients and 14 young healthy volunteers (7 male and 7 female). Serum protein binding of maprotiline was carried out in duplicate at 37°C by equilibrium dialysis using ¹⁴C-labelled drug and pH 7.4 phosphate buffer according to the technique described by Ehrnebo, Agurell, Jalling & Boréus (1971). [¹⁴C]-maprotiline (Ciba-Geigy, Basle) 4.5 µCi/mg was used at a concentration of 600 ng/ml. Measurement of serum AGP concentrations were carried out using a radial immunodiffusion technique (M – Partigen, Behring/Hoechst). The results obtained for maprotiline binding (free fraction) and AGP concentrations in both groups are shown in Table 1. Serum maprotiline binding in geriatric patients was not significantly different from that obtained in healthy volun-

teers despite the presence of significantly higher AGP concentrations. No correlation was found between the free fraction of maprotiline and concentration of AGP in healthy controls (*r* = -0.16) geriatric patients (*r* = 0.07) or the combined groups (*r* = 0.00). These results suggest that the general role of α_1 -acid glycoprotein in determining the plasma protein binding of basic drugs may be uncertain.

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Mixed function oxidase activity in microsomal fractions of human liver biopsy specimens

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The hepatic mixed function oxidase system is respon-

sible for oxidation of many endogenous and exogenous substrates. The terminal electron acceptor is the haemoprotein cytochrome P450. In animals there are multiple forms of P450 in the hepatic endoplasmic reticulum each with differing relative substrate and site specificity. The presence of similar multiplicity of P450 in man is likely and has considerable toxicological and therapeutic implications. Methods have been developed to isolate the microsomal fraction of needle biopsies of human liver and to assay this for components of the mixed function oxidase system

Table 1 Mixed function oxidase activity in human and rat liver microsomes

Sample units	n	Protein (mg/g liver)	Cytochrome P-450		NADPH-cytochrome c reductase (nmol reduced mg ⁻¹ microsomal protein min ⁻¹)	AHH (pmol mg ⁻¹ min)
			(nmol/mg microsomal protein)	(nmol/g liver)		
Human liver needle biopsies	11	13.6 ± 0.9 (8.6–18.3)	0.29 ± 0.03 (0.14–0.54)	4.2 ± 0.7 (1.5–9.0)	147.4 ± 8.8 (102.1–214.3)	26.2 ± 3.0 (10.8–46.3)
Human liver wedge biopsies	15	13.5 ± 1.3 (10.5–22.7)	0.48 ± 0.04 (0.29–0.77)	5.8 ± 1.5 (2.2–10.0)	170.7 ± 20.7 (100.0–366.5)	29.9 ± 2.8 (20.0–44.1)
Rat liver	6	17.5 ± 1.1 (15.1–20.2)	0.63 ± 0.04 (0.59–0.72)	10.8 ± 1.5 (9.1–14.4)	110.3 ± 3.4 (101.1–120.2)	270 ± 19.4 (210–300)

Values are mean ± s.e. mean (range).

together with aryl hydrocarbon hydroxylase (AHH) activity as an indicator of mono-oxygenase function.

Needle biopsy specimens of liver were obtained using the Menghini technique and were surplus to that required for routine histological examination. Wedge biopsy specimens were obtained from patients undergoing abdominal surgery when liver biopsy was indicated for diagnostic purposes. Immediately after biopsy the tissue was placed in buffer and left on ice. The microsomal fraction was obtained by differential centrifugation. The microsomal pellet was resuspended using a glass–glass microhomogeniser to give a protein concentration of 10–15 mg/ml. The microsomal suspension was assayed fresh or frozen at –80°C.

Cytochrome P450 content and NADPH-cyto-

chrome c reductase activity were assayed spectrophotometrically using 400 µg and 20 µg microsomal protein respectively. AHH activity was measured using a fluorometric assay following incubation with 50 µg microsomal protein at 37°C for 15 min.

The protein yield, P450 content, NADPH-cytochrome c reductase and AHH activities in needle and wedge biopsies of human liver together with accompanying rat controls are listed in Table 1.

In this report data are presented on mixed function oxidase activity in microsomal fractions of needle biopsies of human liver and compared with similar data for wedge biopsy samples. This technique will allow *in vitro* study of the substrate specificity of the cytochrome P450 present in needle biopsies of human liver.

Liver function studies after ketamine infusions

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Because of its analgesic action, infusions of ketamine have been used to produce anaesthesia without the hazards of theatre pollution. This may involve doses of 3–4 mg kg⁻¹ and it was necessary to study liver function in patients in whom this was unlikely to be affected by the operation and who had no blood transfusions. A preliminary study in minor operations showed significant enzymatic changes in 2/20 patients given ketamine, compared with 0/30 controls. Continuous ketamine is not indicated in these procedures

and a more detailed study was carried out in 68 fit women undergoing intermediate gynaecological operations (e.g. sterilisation) lasting on the average 30 ± 2 min. All were premedicated with 2.5–4.0 mg lorazepam to make the study ethically acceptable. Patients were allocated to techniques at random. Half were anaesthetised with ketamine infusions and half with standard techniques. Since ketamine is usually given with 100% O₂, this was also given to about half of the controls; likewise N₂O–O₂ was used in half the ketamine cases.

Blood samples were drawn immediately before induction and on the 3rd–4th and 13th–15th postoperative days and subjected to 22 biochemical tests. From these, four serum enzyme levels, known to be raised in toxic hepatitis, were used to monitor liver function; alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). Significant

Table 1 Number of patients with abnormality in 1 or more (1+) enzyme tests

	3–4 days		13–15 days		3–15 days	
Abnormal tests	1	1 +	1	1 +	1	1 +
Ketamine (34)	8	5	7	1	8	6
Controls (34)	4	0	5	0	7	0
<i>P</i>	<0.025		<0.40		<0.10	

P value calculated on any abnormal results.

elevations were considered to have occurred in those patients in whom the post-anaesthetic levels were outside the normal range and had increased by more than $3 \times \text{s.d.}$ from the preanaesthetic levels. (s.d. of each test was calculated from the coefficient of variation

of several thousand repeat estimations on control sera carried out over the period of the study and was expressed at the upper limit of the normal range).

Significantly more patients had enzymatic changes suggestive of liver dysfunction on the 3rd–4th post-operative days after ketamine than in the control series. Furthermore Table 1 shows that no patient in the non-ketamine series had more than one abnormal liver function test at any time during the period of study as compared with 6 in the ketamine series ($\chi^2 = 4.57$; $P < 0.05$). Applying a logarithmic transformation to correct the skew distribution of enzyme readings, the average 3–4 day levels with all four tests was significantly higher than their preoperative levels in those patients given ketamine ($P < 0.05$) but not in the controls.

Although this study shows enzyme changes which can be attributed to ketamine, no patient became jaundiced and their clinical significance is not known.

Assessment of the onset and magnitude of neuromuscular blockade in anaesthetized man

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Although the single twitch response of the adductor pollicis muscle has been widely used to assess the onset and degree of neuromuscular blockade we have demonstrated that the measurement of the tetanic response is a more sensitive index (Sugai, Hughes &

Payne, 1976). Nevertheless, it has been claimed that 95% depression of the single twitch response is necessary to provide good intubating conditions (Blackburn & Morgan, 1978).

Accordingly, a comparative assessment of the two methods was undertaken in 19 anaesthetised patients using techniques described previously (Hughes, Ingram & Payne, 1976). Simultaneous recordings of tetanic and single twitch contractions of the adductor pollicis muscle were obtained by stimulating one ulnar nerve at 50 Hz for 1 s and the other with single shocks every 12 s. Five groups of 3 to 5 patients were studied, one group for each neuromuscular blocking drug tested; the drugs administered were tubocurarine, dimethyl tubocurarine, gallamine, pancuronium and fazadinium.

Table 1 Mean results with ranges

Drug	No. of patients	Dose (mg/kg)	Tetanus		Twitch	
			Maximum depression (%)	Onset time (s)	Maximum depression (%)	Onset time (s)
Tubocurarine	3	0.1–0.15	94 (90–97)	236 (109–312)	54 (33–59)	560 (436–696)
Dimethyl tubocurarine	5	0.075–0.15	94 (87–97)	193 (96–300)	27 (20–45)	524 (192–648)
Gallamine	5	0.75–1.0	97 (93–99)	86 (68–120)	40 (10–60)	329 (180–540)
Pancuronium	3	0.05	97 (94–99)	176 (145–204)	63 (37–90)	596 (504–684)
Fazadinium	3	0.4	98 (96–99)	108 (60–120)	83 (71–89)	408 (240–540)

From Table 1 it can be seen that blockade of the tetanic response was more rapid in onset and greater in magnitude than that of the single twitch with every drug tested. Intubating conditions were good when blockade of the tetanic response had achieved 94–98% despite the fact that the single twitch was much less affected. Maximum block of the single twitch response was reached 4 to 5 min after tetanic block and was of shorter duration. The patients continued to breathe spontaneously although some respiratory depression was evident. These results indicate that intubation can be achieved more rapidly and with a lower dose of the neuromuscular blocking agent than that estimated from the single twitch response.

Plasma levels of pancuronium as a determinant of neuromuscular response in man

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A number of pharmacokinetic techniques have been applied to plasma level and muscle twitch response data previously reported in the literature (Somogyi, Shanks & Triggs, 1976; 1977a, b; 1978). Using a modification of an equation proposed by Wagner (1968) the relationship between plasma level and response data for pancuronium in 9 patients was examined. For each patient's data the fit to the equation was good for both onset and offset of action. The mean value for the ratio of the plasma concentration to elicit 50% of the response at onset to that at offset (1.18) indicated that a slightly higher plasma level of the drug is necessary to achieve paralysis as compared to that required to maintain paralysis.

The procedure of Galeazzi, Benet & Sheiner (1976) was used to determine whether one set of data (plasma level) lags behind another (response) by plotting the simultaneous values for the quantities against each other. For two patients in whom responses over the entire range of offset and onset of paralysis were available there was no evidence of hysteresis in the plot, indicating that the kinetics of effect are indistinguishable from those of the drug in plasma.

The relationship noted by Gibaldi & Levy (1972) that the response versus logarithm of *d*-tubocurarine

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plasma levels was linear in the 20 to 80% recovery range was investigated for pancuronium in 22 patients. A plot of the decline of paralysis against the apparent rate of decline of plasma level showed a strong, positive correlation ($P < 0.001$, $r^2 = 0.85$).

An attempt was made to predict the duration of action of a second dose of pancuronium based on the decline of plasma levels. Computer simulations were performed using averaged data from 15 normal patients. Plasma levels of pancuronium were simulated following an initial dose of either 0.05 or 0.10 mg/kg followed by a second variable dose administered when the plasma level had declined to 0.21 µg/ml; a plasma level corresponding to 80% paralysis. The second dose was expressed as a ratio (DR) relative to the first dose, and the time interval for the plasma level to decline to 0.21 µg/ml following each dose calculated and expressed as a ratio (TR) to that for the first dose. Least squares regression analysis gave the following equations relating to initial doses of 0.05 and 0.10 mg/kg respectively:

$$\begin{aligned} \text{TR} &= 0.19 + 1.67 \text{ DR} \\ \text{TR} &= 0.23 + 1.77 \text{ DR} \end{aligned}$$

To determine the validity of these predictions, comparisons were made with data obtained from normal patients who received multiple doses of pancuronium. In each case the predicted duration of effect of the second dose was in good agreement with that observed. It is suggested that the major determinant of the termination of action and subsequent recovery from pancuronium paralysis is the plasma concentration of the drug and its rate of decline.

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Concentrations of ibuprofen in serum and synovial fluid from patients with arthritis

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Concurrent concentrations of ibuprofen in the serum and knee joint synovial fluid of patients with rheumatoid arthritis have been measured by a gas chromatographic method.

Nineteen patients suffering from classical or definite rheumatoid arthritis and two with osteoarthritis were admitted to the study and some patients participated more than once. All routine drug therapy, including anti-rheumatic drugs, was continued. The knee aspirations, which were required for diagnostic or therapeutic reasons, were performed at varying times up to 7 h after administration of a single 400 mg oral dose of ibuprofen. A blood sample was taken within minutes of the aspiration. The composite serum profile obtained from the individual patient data shown in Figure 1 indicated that peak serum concentrations of ibuprofen occurred between 60 to 140 min following administration of the drug, mean value $25.8 \mu\text{g ml}^{-1}$, and thereafter the concentrations fell rapidly. The concentration of ibuprofen in synovial fluid reached a mean plateau value of $8.2 \mu\text{g ml}^{-1}$ which was sustained over the period 80 to 285 min and then declined a little. Up to 150 min after administration of the dose, the concentration of ibuprofen was higher in serum than in synovial fluid but thereafter a majority of the patients had a higher concentration of the drug in the synovial fluid. Thus the pharmacokinetics

Figure 1 Concentrations of ibuprofen in serum (●---) and synovial fluid (○—) after a single 400 mg oral dose (26 samples from 21 patients).

of ibuprofen in both fluids are qualitatively similar to those reported for acetylsalicylic acid (Sholkoff, Eyring, Rowland & Riegelman, 1967), alclofenac (Thomas, Dippy & Maddock, 1975), ketoprofen (Mitchell, Scott, Kennedy, Brooks, Templeton & Jefferies, 1975) and indomethacin (Emori, Champion, Bluestone & Paulus, 1973). They differ from those of total salicylate (Rosenthal, Bayles & Frement-Smith, 1964) and gold (Gerber, Paulus, Bluestone & Lederer, 1972) for which the drug concentration in synovial fluid was substantially lower than that in serum following equilibration.

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Rowachol, a proprietary terpene preparation, dissolves cholesterol gallstones

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Chenodeoxycholic acid (CDCA) is now known to be an effective treatment for cholesterol gallstones. It acts by reducing biliary cholesterol secretion and thereby converting saturated bile to bile that is unsaturated with cholesterol. The decreased cholesterol secretion is ascribed to a direct effect of the bile acid on hepatic microsomal 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMGCoA reductase)—the rate limiting enzyme for cholesterol biosynthesis (Coyne, Bonorris, Goldstein & Schoenfield, 1976). The major disadvantage of CDCA therapy is the time taken to dissolve the patient's stones. In the case of larger stones (>1 cm in diameter) CDCA treatment may have to be continued for *several years* before complete dissolution is observed radiologically. Other agents capable of reducing biliary cholesterol saturation could prove to be either a useful adjuvant to CDCA therapy or even cholelitholytic agents in their own right.

Rowachol (Rowa Limited, Bantry), a proprietary choleretic contains 32% menthol, 17% pinene, 6% menthone, 5% borneol, 5% camphene and 2% cineol dissolved in olive oil. Each capsule contains 0.1 ml of liquid. We have previously shown that rowachol significantly lowers the cholesterol saturation index of human bile when given to gallstone patients in

a dose of 2 capsules three times daily for 48 h prior to cholecystectomy (Doran, Keighley & Bell, 1977). Evidence will be presented to show that, rowachol is a potent inducer of hepatic microsomal drug metabolising enzymes but in the rat, unlike phenobarbitone, it markedly *inhibited* microsomal HMGCoA reductase levels.

We have now treated a total of 24 patients with radiolucent gallstones with rowachol (2 capsules three times daily) for periods in excess of 6 months. Repeat oral or intravenous cholangiograms have been performed on all patients. At 6 months two patients' gallstones have disappeared while in a further three cases the stones are significantly smaller and/or reduced in number. The remaining 19 patients' stones were unchanged after 6 months but one of these has shown evidence of reduction in stone size after 1 year's treatment. The drug is well tolerated and no evidence of hepatotoxicity has emerged. Urinary D-glucuronic acid excretion, an indirect marker of enzyme induction, rose significantly ($P < 0.01$) from 12.5 ± 1.6 $\mu\text{mol/g}$ creatinine before rowachol to 29.5 ± 4.2 $\mu\text{mol/g}$ creatinine after 6 weeks of therapy. Urinary glucuronide excretion rose significantly ($P < 0.001$) from a mean of 449.9 ± 25.6 mg/24 h before rowachol to 988.8 ± 52.5 mg/24 h after 4 days treatment.

Prolonged oral administration of rowachol appears to be safe. This compound and possibly other terpene substances merit more detailed consideration as possible cholelitholytic agents.

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Antibacterial activity in serum and urine following oral administration in man of DL473 (a cyclopentyl derivative of rifampicin)

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A cyclopentyl derivative of rifampicin, DL473, (3-[4 cyclopentyl-1-piperazinyl]-imino-methyl] rifamycin SV) has an antibacterial spectrum similar to that of rifampicin and about ten times its potency against *Myco. tuberculosis*. In animals DL473 is absorbed after oral administration, has a lower toxicity and longer serum half-life than rifampicin (data from Lepetit Pharmaceuticals Ltd).

We have given DL473 orally to twelve healthy male volunteers (age range 26-47 years) on an empty stomach in a single-dose study (4, 6 or 8 mg/kg, four

subjects each dose) and a repeated-dose study (4 or 8 mg/kg, six subjects each group, four doses at 48 h intervals). Blood and urine were collected at intervals up to 120 h after the dose. Serum and urine were assayed against DL473 standards in a microbiological assay.

For six subjects, serum antipyrine concentrations were measured (Chang, Wood, Dixon, Conney, Anderson, Eiseman & Alvares, 1976) at intervals for 24 h following an oral dose of 600 mg antipyrine about 1 week before the first dose of 4 or 8 mg/kg DL473 and 48 h after the 4th dose. For all subjects daily urinary outputs of 6- β -hydroxycortisol (Park, 1978) and 17-hydroxycorticosteroids (Silber & Porter, 1954) were measured for 2 days before the first and 4 days after the fourth dose of DL473 respectively.

A summary of some of the multiple-dose results is given in Table 1.

Urinary peaks of DL473 activity ranged from 2.5-18.8 μ g/ml at 3-9 h; the time course of activity corresponded to the observed serum elimination. Under 10% of the dose was found in the urine as antibacterial activity.

Antipyrine half-life was significantly ($P < 0.01$, paired t -test, $n = 6$) reduced from 10.9 ± 2.0 to 5.5 ± 0.9 h, after the fourth dose of DL473.

The serum half-lives were longer than those

Table 1 Means of peak serum levels of antibacterial activity in terms of DL473 activity, elimination constants, half-lives and ratios of urinary corticosteroid metabolites for four repeated oral doses of DL473 in man

Serum DL473	4 × 4 mg/kg (n = 6)		4 × 8 mg/kg (n = 6)	
	First dose	Fourth dose	First dose	Fourth dose
Peak concentration (µg/ml)				
mean	5.13	6.83	8.52	13.28
s.e. mean	0.65	1.09	1.35	3.16
Time of peak activity (h) range	3–9			
Half-life (h)				
mean	14.1	9.8	19.9	12.5
95% confidence interval	10.9–17.9	7.8–12.1	15.1–22.9	10.7–15.1
Elimination constant (h ⁻¹)				
mean	0.0510	0.0730	0.0347	0.0554
s.e. mean	0.0048	0.0061	0.0044	0.0037
Paired <i>t</i> -test	0.01 > <i>P</i> > 0.001		0.02 > <i>P</i> > 0.01	
	<i>Before</i>	<i>After</i>	<i>Before</i>	<i>After</i>
<i>Urinary ratio</i>	<i>first dose</i>	<i>fourth dose</i>	<i>first dose</i>	<i>fourth dose</i>
6-β-OH-cortisol				
17-OH-corticoid				
mean	4.12	11.33	4.19	12.15
s.e. mean	0.72	1.91	0.84	2.15
Paired <i>t</i> -test	0.01 > <i>P</i> > 0.001		<i>P</i> < 0.001	

reported (2.6–3.9 h) for rifampicin (Keberle, Krüger-Thiemer, Sackmann, Schmid & Seydel, 1967). The changes in antipyrine metabolism and urinary ratios of corticosteroid metabolites indicate hepatic enzyme induction which could account for the enhanced elimination of DL473 on repeated dosage.

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Plasma-saliva ratio of theophylline following oral theophylline derivatives

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Theophylline (1,3-dimethylxanthine) is widely used as a bronchodilator in the management of patients with asthma. Lack of compliance of these patients with oral theophylline therapy has been reported (Eney & Goldstein, 1976), and in a group of fifteen outpatients receiving oral sustained-release theophylline derivatives we have found that over 50% had plasma levels below the recommended range of 56–112 $\mu\text{mol l}^{-1}$ (10–20 $\mu\text{g ml}^{-1}$). In the process of assessing the value of measurement of plasma concentrations of theophylline in the management of these patients, we have also compared theophylline levels in plasma and saliva, and report here data which indicate a need for caution in using salivary levels as an index of plasma levels by means of a single conversion factor.

Plasma and saliva samples were collected simultaneously from twenty-four patients on long-term theophylline therapy and from four healthy volunteers who were given oral theophylline derivatives, and analysed by gas chromatography (Dusci, Hackett & McDonald, 1975) or EMIT (Syva, England).

Figure 1 shows the relationship between plasma and saliva theophylline concentrations in the subjects studied. The correlation coefficient was 0.87, and intercept 15.40, which differs significantly from zero ($P < 0.01$). Within the range of 56–112 $\mu\text{mol l}^{-1}$, salivary levels were approximately 67% of plasma levels. However, at plasma levels of less than 30 $\mu\text{mol l}^{-1}$, salivary concentrations fell to less than 40% of plasma theophylline concentrations. A ranked corre-

lation of plasma-saliva ratios with plasma concentrations was significant ($P < 0.01$), suggesting a true increase in plasma-saliva ratio as plasma concentration decreases.

Figure 1 Relationship between plasma and saliva theophylline concentrations in four healthy subjects and twenty-four patients. ($r = 0.87$, intercept 15.40).

Several possible explanations for these findings have been considered. As theophylline is a weak acid ($\text{pK}_a = 8.6$) it is unlikely that changes in salivary pH will affect its concentration in saliva (Danhof & Breimer, 1978). Changes in protein binding with plasma concentration, or the existence of an active or 'facilitated' transport mechanism for movement of theophylline from plasma to saliva may be involved.

These results suggest that it may not be possible to extrapolate plasma theophylline levels from those in saliva by means of a single conversion factor.

PWT is an NH & MRC (Australia) Clinical Sciences Fellow.

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The effect of cigarette smoking on finger tremor

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There is still controversy about the effect of nicotine and smoking on tremor in man (Brimblecombe & Pinder, 1972). We have measured finger tremor in 35 normal subjects in the age range 17 to 63 years, under controlled conditions, during cigarette smoking, and during 'sham' smoking (in which the subject was asked to mimic the actions of smoking on an unlit

cigarette). Eleven more subjects acted as non-smoker controls. For thirteen of the smokers, urine samples were collected before and 2 h after smoking and analysed for nicotine and cotinine content using the gas liquid chromatography method of Feyerabend, Levitt & Russel (1975).

Tremor was recorded using the strain gauge method described by Lippold (1970); 5 and 10 s epochs of the tape recorded signal were submitted to frequency analysis using HP 5451B and Nicolet digital Fourier analysers in the range 0–25 Hz. The frequency resolution was 0.4 Hz. The amount of tremor was expressed as the amplitude or the power of the components at the specified frequencies.

An increase in tremor over the range 1 Hz to 12 Hz was seen in 30 of the 35 subjects during cigarette smoking. The five subjects who showed no change, described themselves as 'non-inhalers'. The eleven

Figure 1 The effect of smoking on finger tremor. The graph shows a frequency spectrum of tremor amplitude for one subject (a) before and (b) for 1 min after beginning to smoke a cigarette. The histograms are drawn from pooled results from eight subjects; (c) control observations and (d) during cigarette smoking. Each column represents the mean power distribution obtained from 160 analyses of 10 s epochs. Column 1, 0.01–4.00 Hz; 2, 4.01–8.00 Hz; 3, 8.01–12.00 Hz and 4, 12.01–16.00 Hz. Bars $\pm$ s.e. mean. The increases of mean values in column (d) compared with column (c) were all statistically significant ($P = 0.001$, paired t -tests).

controls showed no significant increase in tremor during 'sham' smoking; two of them showed a small decrease. Figure 1 shows a frequency spectrum of a record from one subject together with the pooled results of eight of the thirty subjects which were subjected to further analysis within sequential 4 Hz bandwidths.

There was no apparent correlation between the amounts of nicotine or cotinine in the post-smoking 2 h urine and the magnitude of the increases in tremor produced by smoking.

Human endometrial renin levels during the menstrual cycle

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In 1968 an enzyme with identical biochemical properties to human renal renin was found in the plasma of anephric human females. This renin appears to come from the uterus (Capelli, Wesson, Aponte, Faraldo & Jaffe, 1968). Tissue culture studies of human myometrium and fetal membranes (Symonds, Stanley & Skinner, 1968) demonstrated that these tissues were capable of synthesizing renin.

In the present study plasma and endometrial samples were taken from 86 healthy women undergoing curettage during sterilization procedures. Twenty-six of the patients were taking oral contraceptive preparations containing oestrogens. The samples were chilled and the endometrium homogenised in distilled water (10 µl/mg). Renin concentration was determined by incubating the samples with excess sheep renin substrate and measuring the angiotensin I produced by radioimmunoassay, a modification of the method of Poulsen & Jorgensen (1974). Renin substrate was measured by incubating the samples with excess human renin and estimating the angiotensin produced as above.

In the 60 patients not taking oral contraceptives the plasma renin concentration increased progressively from a level of 6.08 ± 1.20 ng angiotensin I produced $\text{ml}^{-1} \text{h}^{-1}$ (mean $\pm$ s.e. mean) in the early proliferative phase of the cycle ($n = 10$) to 14.38 ± 1.60 ng angiotensin I produced $\text{ml}^{-1} \text{h}^{-1}$ in the late secretory phase ($n = 11$), a significant rise ($P < 0.001$). Endometrial renin concentration was 15 to 100 times greater than that in the plasma, but did

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not alter significantly during the cycle. Neither plasma nor endometrial renin substrate concentrations altered significantly; the concentrations in the endometrium were five times higher than those in the plasma.

The plasma renin of patients taking oral contraceptives did not rise in the secretory phase of the cycle. The renin substrate concentration was significantly higher at all stages than that of those not taking oral contraceptives ($P < 0.001$). The endometrial results were not significantly different from those of normal endometrium.

These results for plasma confirm earlier work (Skinner, Lumbers & Symonds, 1968). Investigation of endometrial renin has not previously been reported. Capelli and his co-workers (1968) demonstrated that their renin did not respond to changes in salt and water balance, and whilst the endometrium may be source of plasma renin there is no evidence to support this as a physiological role. The role of the endometrial renin remains uncertain. The potential to produce angiotensin in the endometrium may be of importance in the local vascular changes associated with menstruation, but the lack of fluctuation in the levels of endometrial renin and renin substrate does not support this. Other factors, such as the angiotensinases, may alter the local angiotensin II levels without affecting the renin concentration. It is possible that the capability of human endometrium to produce angiotensin may be of more importance in pregnancy. The effects of oral contraceptives on the endometrial renin-angiotensin system are not yet clear.

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POSTER COMMUNICATIONS

Failure of 2 h [^{14}C]-aminopyrine breath test to demonstrate microsomal enzyme induction by glutethimide

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The specific activity of $^{14}\text{CO}_2$ exhaled in the breath in the 2 h following an oral dose of [^{14}C]-labelled aminopyrine, [^{14}C]-aminopyrine breath test (ABT), is a convenient measure of hepatic microsomal demethylating capacity (Hepner & Vesell, 1976). Values for the ABT are reduced in the presence of liver disease (Galizzi, Long, Billing & Sherlock, 1978) and are increased in patients on chronic anticonvulsant therapy probably indicating hepatic microsomal induction (Hepner, Vesell, Lipton, Harvey, Wilkinson & Schenker, 1977). In our study an ABT was performed on five healthy volunteers before and after a 14 day course of glutethimide (500 mg/day), a drug known to cause enzyme induction (Corn, 1966). A tracer dose (1.5 μCi) of [^{14}C]-aminopyrine was given orally in a fasting state. $^{14}\text{CO}_2$, produced by hepatic demethylation of the drug, was collected by blowing into vials employing hyamine as a trapping agent. The vials were counted in a liquid scintillation counter and the specific activity of the 2 h breath sample was expressed as a percentage of the administered dose excreted in 2 h (ABT). The half life ($T_{1/2}$) of the decay of specific activity was calculated from at least four values obtained 2–6 h after administration of the drug. The following results were obtained:

ABT	Range (mean $\pm$ s.d.)
(i) before glutethimide	7.5–12.6% (10.8 $\pm$ 2.1)
(ii) after glutethimide	9.4–13.2% (10.5 $\pm$ 1.5)

$T_{1/2}$ specific activity breath $^{14}\text{CO}_2$.

(i) before glutethimide 106.9–137.8 min (126.6 $\pm$ 11.6)
(ii) after glutethimide 86.3–111.7 min (100.7* $\pm$ 9.3)
* $P < 0.01$ paired t -test.

All five individuals demonstrated shortening of the $T_{1/2}$ values following glutethimide treatment and in three cases studied there was also marked increase in urinary D-glucaric acid excretion. Thus there was clear evidence of induction of microsomal enzyme activity which was not reflected in a corresponding increase in the value for the ABT.

We conclude that if the [^{14}C]-aminopyrine breath test is to be used as a screening test for drug induced microsomal induction, it is necessary to calculate the $T_{1/2}$ and not just simply take one sample at 2 h as has been previously suggested (Hepner & Vesell, 1976).

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Continuous infusion of frusemide in refractory oedema

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It is well known that patients may be resistant to 'conventional' doses of frusemide, and may require a dose of up to 500 mg or more to achieve a therapeutic response (Allison & Kennedy, 1971). In our study ten patients with severe congestive cardiac failure which had proven resistant to single doses of 120 mg were treated with a continuous infusion of frusemide delivered by a constant rate infusion pump. The patients' urine volume and urine and plasma electrolyte concentrations were monitored in detail during therapy. The patients received frusemide at a mean delivery rate of 8.9 mg/h (range 4–16 mg/h). On this regime all patients experienced a vigorous diuresis: 24 h urine output 5.05 ± 3.7 l (mean $\pm$ s.d.). The mean 12 h urinary sodium excretion rose from 58.2 ± 28 mmol before therapy to 327.2 ± 202.4 mmol during the infusion. There was a similar rise in the mean 12 h potassium excretion from 29 ± 14.7 to 79.8 ± 56.6 mmol. Patients did not receive potas-

sium supplementation during treatment, and four became hypokalaemic (plasma $K^+ < 3.5$ mmol/l). No other adverse reactions were experienced. Steady state plasma frusemide concentrations were measured during therapy in eight patients by the method of Forrey, Kimpel, Blair & Cutler (1974). In all cases the levels were low: mean 0.68 μ g/ml, range 0.4–0.9 μ g/ml. There was a significant correlation between the delivery rate and the obtained plasma frusemide concentration ($r = 0.79$, $P < 0.02$). Thus, these patients exhibited a satisfactory and controllable diuresis with plasma frusemide concentrations well below those thought to be necessary for a therapeutic response following administration of frusemide by a single dose (Branch, Roberts, Homeida & Levine, 1977). This proved to be a safe and effective treatment, and provides an alternative to the use of massive single doses of frusemide without the associated toxicity.

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A domiciliary study of urinary circadian rhythms in rheumatoid patients with particular reference to the effects of the time of administration of corticosteroid drugs

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There is a circadian rhythm in renal function which results in low urine flow at night and maximal urine flow in the forenoon. The loss or reversal of the normal pattern of urinary excretion often results in noc-

turia. This may be especially troublesome in rheumatoid arthritis patients who suffer from impaired mobility.

Many drugs alter the normal circadian rhythm of urine production and it seemed likely from a hospital based study of asthmatics that nocturia was a side-effect of corticosteroid therapy (Thomas, Coles & El-Shaboury, 1970).

Prednisolone is frequently prescribed in rheumatoid arthritis. The circadian rhythmicity of urine flow rate and electrolyte excretion has been investigated in rheumatoid arthritis patients taking prednisolone at various times of the day, particularly to determine whether nocturia was more likely with a single evening dose. Patients were studied over a 3 day period on three separate occasions in the sequence, (i) after taking prednisolone as a single morning dose for at least 1 month, (ii) 4 to 6 days after changing to a

single evening dose of prednisolone and (ii) 28 days after this change.

The domiciliary collection of urine (Hillier & Knapp, 1974) was used as this avoids many of the disturbances of physiological rhythms associated with hospital admission. The subjects were asked to record the clock-time of micturition and to measure the volume of urine passed. An aliquot was retained and later analysed for sodium, potassium, calcium, creatinine and osmolality. The subjects also recorded their body temperature, weight, and assessed their joint stiffness. Six out of ten patients showed nocturia 4–6 days after the change to a single evening dose. Analysis of the urinary data (Fort & Mills, 1970) showed

changes in the timing of peak electrolyte excretion rate and urine flow rate when prednisolone was taken as a single dose in the evening.

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The effect of diazepam on the threshold of the ventilatory response to CO₂

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The ventilatory response to CO₂ ($\dot{V}$,P_{CO₂} response) is measured as the linear relation between ventilation and P_{CO₂}. The slope of the line describes the sensitivity to CO₂ and the intercept on the CO₂ axis represents the threshold of the response. Recent investigations into the onset of electromyogram activity in respiratory muscles during breath-holding (Sempik, 1978) have indicated that it may be governed by the

threshold of the $\dot{V}$,P_{CO₂} response and to test this hypothesis some means of changing the threshold would be useful.

There is some discrepancy in the literature as to the depressant effect of diazepam on respiration assessed by a shift of the $\dot{V}$,P_{CO₂} response. No significant respiratory depression was found by Cohen, Finn & Steen (1969) using intravenous doses up to 0.266 mg kg⁻¹. However, respiratory depression was reported by Catchlove & Kafer (1971) using intravenous doses of 0.14 mg kg⁻¹ and by Utting & Pleuvry (1975) using oral doses of 5 mg.

In view of this disagreement it was decided to examine the effects of single oral doses of 5 and 10 mg diazepam by measuring the $\dot{V}$,P_{CO₂} response of ten healthy subjects immediately before, and 1 h after the drug, using the CO₂-rebreathing method of Read (1967). Table 1 shows the results obtained. In a paired *t*-test, doses of 5 mg caused an insignificant

Table 1 The mean $\pm$ s.e. mean slopes and thresholds of the $\dot{V}$,P_{CO₂} responses obtained before and after 5 or 10 mg diazepam

	Slope ($l\ min^{-1}\ torr^{-1}$)		Threshold (torr)	
	Mean	Differences	Mean	Differences
Control	4.49 $\pm$ 0.62		45.4 $\pm$ 1.44	
Diazepam 5 mg <i>n</i> = 7*	3.67 $\pm$ 0.51	-0.82 $\pm$ 0.26 (<i>P</i> < 0.025)	45.3 $\pm$ 1.26	-0.1 $\pm$ 0.78 (NS)
Control	3.35 $\pm$ 0.48		42.7 $\pm$ 2.17	
Diazepam 10 mg <i>n</i> = 10	3.34 $\pm$ 0.53	-0.01 $\pm$ 0.26 (NS)	45.4 $\pm$ 2.36	+2.76 $\pm$ 0.76 (<i>P</i> < 0.01)

* Three subjects were excluded from the 5 mg study because of non-linearity of the $\dot{V}$,P_{CO₂} responses.

fall in threshold of 0.1 torr, whereas 10 mg caused a significant increase of threshold of 2.76 torr ($P < 0.01$), i.e. a respiratory depression. Doses of 5 mg caused a small, significant depression of slope of the $\dot{V}_P\text{CO}_2$ response line of $0.82 \text{ l min}^{-1} \text{ torr}^{-1}$ ($P < 0.025$) which is also indicative of respiratory depression. Doses of 10 mg did not cause a significant change of slope.

These results confirm that diazepam at commonly prescribed doses causes significant respiratory depression by raising the threshold or depressing the slope of the $\dot{V}_P\text{CO}_2$ response, and will provide a basis for further work on e.m.g activity in respiratory muscles.

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A simple microassay for human tissue renin

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Renin in tissue homogenates is measured by its ability to form angiotensin I from renin substrate. The rate at which angiotensin I is generated is taken as a measure of the quantity of renin present. In currently used methods the generation and estimation steps are performed in separate tubes, aliquots of the incubation mixture being removed and assayed for angiotensin I. This approach introduces an unnecessary complication. A simple two step assay for the measurement of renin is described, using a single tube and conventional inhibitors. In this method all of the angiotensin I generated is estimated, enabling the sample volume to be kept to a minimum. The assay has high sensitivity and renin can be measured adequately when the tissue sample available is very small.

Renin was mobilised from human endometrium by homogenisation in distilled water (10 $\mu\text{l/ml}$). The homogenate was freeze-thawed three times and the supernatant clarified by centrifugation and stored at -20°C until assayed. A mixture of 5 μl sample, 5 μl buffered inhibitor (5-OH-quinoline, EDTA, dimercaprol buffered in TRIS pH 7.2) and 20 μl sheep renin substrate (11.6 μM) (Skinner, 1967) was incubated at

37°C for 2 h. After incubation the reaction was stopped by cooling the mixture to 1°C . Blanks were prepared as samples, but were incubated at 0°C for 2 h. Angiotensin I formed was measured by radioimmunoassay. The standard curve was prepared by adding unlabelled asp-ileu-angiotensin I in 5 μl TRIS albumen buffer (pH 7.4) to 20 μl of sheep renin substrate and 5 μl inhibitor. Five hundred microlitres of a specific angiotensin I antiserum, diluted 1 in 10,000 in barbital (pH 8.6) was added to each tube, together with 500 μl of monoiodinated asp-ileu-angiotensin I (10,000 counts $\text{min}^{-1} \text{ ml}^{-1}$).

This mixture was left to equilibrate for 16 h at 4°C . Bound angiotensin I was separated from free angiotensin I with charcoal. Both the supernatant and the pellet were counted separately for 2000 counts and the results expressed as percentage bound.

Using this method angiotensin I generation was linear for 3 h and the rate of generation was directly dependent upon the quantity of enzyme present. During the incubation step less than 0.2% of the substrate was hydrolyzed. Measurement of the Michaelis constant, K_m , (0.27 μM) indicated that the substrate concentration used was ten times greater than K_m and therefore the generation of angiotensin I followed zero order kinetics. The use of the inhibitors allowed complete recovery of generated angiotensin I. The sensitivity of the radioimmunoassay enabled the detection of as little as 150 nU of human renin/ml of sample (i.e. 2.5 ng angiotensin I $\text{ml}^{-1} \text{ h}^{-1}$). The inter-assay variability was 9.3% (in 6 separate assays) and the intra-assay variability was 10.8% ($n = 12$).

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Plasma sex hormone binding globulin and oestrogen therapy

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Oestrogens increase sex hormone binding globulin (SHBG) in human plasma and SHBG levels are a sensitive indication of oestrogenisation.

Using the method of Rudd, Duignan & London (1974), with ^{14}C -labelled testosterone as a ligand, SHBG levels were measured in groups of women on oestrogen or oestrogen-progestogen therapy and the levels compared with those seen in comparable groups of untreated women. The levels of SHBG are expressed as μg of bound testosterone per 100 ml plasma (± 1 s.e. mean).

The first group were twenty-two pre-menopausal women on a combined oral contraceptive. Of these, eleven were taking a Pill containing 50 μg ethinyl oestradiol and eleven a Pill containing 35 μg or less ethinyl oestradiol. Plasma SHBG levels in these women were compared with those of fifty-two normal, ovulating women who had not received hormone therapy over the previous 6 months. The mean SHBG levels in women on (a) a 50 μg Pill was 2.73 ± 0.13 , (b) a 35 μg or less Pill was 2.2 ± 0.12 and (c) in the untreated women was 2.31 ± 0.05 μg testosterone/100 ml. Statistical comparison between these three groups of premenopausal women showed that the levels in women on the 50 μg Pill were significantly elevated over the levels in the women taking a 35 μg or less Pill as well as over the levels seen in the untreated ovulating group ($P < 0.01$ and $P < 0.005$).

The second group consisted of nine women with hypergonadotrophic ovarian failure eight of whom

were post menopausal and all of whom had been receiving a variety of different hormone replacement regimes over the previous 3 months. The SHBG levels in the treated women were compared with those seen in twenty-six untreated post-menopausal women who had never received any therapy. The mean SHBG level in the treated group was 2.75 ± 0.16 μg testosterone/100 ml while in the untreated group the mean level was 1.84 ± 0.05 μg testosterone/100 ml. The levels of SHBG in the treated patients were significantly elevated ($P < 0.001$) over those in the untreated group.

A further comparison showed a significant elevation of SHBG levels in the hormone-treated post menopausal women over those seen in the normal ovulating women ($P < 0.02$).

These results show that the combined oral contraceptive containing 35 μg or less of ethinyl oestradiol more closely mimics the SHBG levels in the normal menstrual cycle than those seen in women on a 50 μg combined Pill. Standard hormone replacement therapy substantially elevates the SHBG to levels higher than those seen in normal ovulating women and suggests an element of overtreatment rather than true replacement therapy in the post menopausal women. These results also suggest that oral contraceptives containing 35 μg or less of ethinyl oestradiol would more closely resemble, in terms of SHBG levels, true replacement therapy than do the preparations more commonly used for this purpose.

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The time of steroid therapy

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Physicians have become aware that evening doses of corticosteroids cause more adreno-pituitary suppression (Di Riamando & Forsham, 1956). There is an increasing trend for physicians to give corticosteroid therapy as a once daily morning dose, or as alternate day therapy.

Recent investigations by our group have demonstrated circadian variations in cell-mediated immune responses in the rat (Knapp & Pownall, 1977). We have analysed the time of onset of change in renal function, due to acute rejection, in fifty patients following a renal transplant, and find this is most frequently at night. This is also the time when humans show the maximum cell-mediated immune response to Heaf testing (Cove-Smith, Kabler, Pownall & Knapp, 1978).

Analysis of rejection episodes in the fifty transplanted patients shows that a rejection episode most commonly affects renal function 6–8 days after the onset of graft function (twenty of thirty rejections occurring in the first 10 days), and that there is an increased probability of rejection around 7 days after a previous episode (the interval between rejections being 5–7 days in thirty-five pairs of rejection episodes out of fifty-six pairs separated by less than 14 days). These results confirm those of de Vecchi, Halberg, Sothorn, Cantaluppi & Ponticelli (1978).

These studies suggest that more thought should be given to the time when a treatment will favourably influence a pathological process. Timing of therapy to match the biological rhythmicity of a pathological process may result in more effective prophylaxis or treatment.

Prescribing patterns for immunosuppressive therapy in fourteen British Transplant Units have been studied, and show a wide range of policy, for example—prednisolone is given twice daily by six, three times daily in one, once daily in the morning by two, once daily in the evening by two, on alternate days in another and in a variable way in two. Azathioprine may be taken once, twice or three times daily depending on the unit. This range of policy reflects medical uncertainty about the optimum time for administration.

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Figure 1 Typical records showing the effects before and after of lorazepam (a, b) and diazepam (c) on ventilatory patterns. Vertical bars represent 1 l.

Ventilatory patterns in surgical patients premedicated with lorazepam or diazepam

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The benzodiazepines are widely used in anxiety states including dental phobia (Healy, Robinson & Vickers, 1970), and to allay fear and apprehension prior to surgery. However, respiratory side-effects have been observed.

Dalen, Evans, Banas, Brooks, Paraskos & Dexter (1969) reported ventilatory depression after the intravenous injection of 5-10 mg diazepam in fifteen patients before cardiac catheterization. Denaut, Yernault, & De Coster (1975) reported periodic breathing in two out of twenty patients with chronic obstructive lung disease after an i.v. injection of 2.5 mg lorazepam. Cormack, Milledge & Hanning (1977) also reported periodic breathing in one normal subject after an i.m. injection of 4 mg lorazepam. Any such alteration in respiratory control may be a potential hazard in the use of such drugs.

Using the trans-thoracic impedance method of Boyd (1976) we have recorded the effects on ventilatory pattern of intravenous lorazepam or diazepam administered slowly in premedication doses. Continuous recordings were made before and after 2 mg lorazepam in ten awake patients and after 10 mg diazepam in a second group of ten patients. All patients were without any apparent respiratory or cardiovascular disease and were receiving no other drugs. The group give diazepam found it difficult to stay awake.

Periodic breathing was recorded in nine out of ten patients following lorazepam but no periodic breathing was observed in the group receiving diazepam whether they were awake or asleep except in one patient in whom partial airway obstruction occurred. Typical records made before and after the administration of either drug are shown in Figure 1.

These results indicate that despite the rather similar structures of the drugs the incidence of periodic breathing is likely to be higher with lorazepam than with diazepam when given intravenously for premedication of recumbent patients awaiting general surgery.

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DEMONSTRATIONS

Ambulatory monitoring of ECG and footfall in the investigation of arrhythmias and the assessment of anti-arrhythmic therapy

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It is not yet established that drug treatment of ventricular arrhythmias is effective in reducing the risk of ventricular fibrillation. Attempts to define the circumstances in which ventricular ectopic activity is most likely to precipitate sudden cardiac death have met with only limited success. Ambulatory monitoring can be used to investigate both problems further. With this technique the ECG waveform and an index of physical activity can be recorded simultaneously on body borne cassette recorders for periods up to 24 h. Thus the relationship between arrhythmias and activity can be examined at various times including those during which patients are receiving anti-arrhythmic drugs.

Oxford Instrument Medilog Tape Recorders are used to record ECG, from chest electrodes, and footfall from a simple pressure transducer worn under one heel (Barber, Evans, Fentem & Wilson, 1973). Each recorder is fitted with an event marker and a clock signal is also recorded. Abnormalities of ECG waveform and rhythm are examined visually using an Oxford Instruments Medilog ECG Analysis System which replays the cassette at 60 times real time. The relationship between abnormalities and walking is established by a simultaneous display and U.V. record of the ECG and footfall signals on a Medelec FOR-4 oscilloscope and by a computer analysis of parts or all of the 24 h recording.

A PDP 8 computer with analogue to digital converter measures each R-R interval and stores it, with

the footfall signal in binary form, on DEC magnetic tape. These are subsequently read by an IBM 1906A computer and ectopic beats identified from analysis of successive R-R intervals using simple arithmetic algorithms (Fentem, Fitton, Hampton, Hayward & Willmott, 1977).

The data collected from each cassette will therefore include

(1) The frequency of occurrence of different grades of ectopic beats (isolated, frequent multiform, repetitive and early coupled).

(2) The frequency of occurrence of such arrhythmias during activity and during inactivity.

(3) A histogram and mean of the coupling intervals of the ectopics.

(4) A histogram and mean of the heart rate at which the ectopics were occurring.

(5) A histogram, mean, maximum, minimum and variance of the heart rate. The heart rate analysis can be extended to include the additional analyses used by Bassey, Fitton, McDonald, Patrick & Scriven (1978).

From this information it is proving possible to classify patients according to the heart rate and activity dependence of their arrhythmias. It is hoped that this will eventually lead to a better selection of patients for anti-arrhythmic drug treatment.

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The effect of drug therapy on circadian variations in blood pressure, and the influence of the timing of therapy

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In 1961 hospital-made automatic blood pressure measuring equipment was used to demonstrate the changes in blood pressure during sleep in patients with hypertension (Shaw, Knapp & Davies, 1963). Measurements were made of the hypotensive effect, during the 24 hour period, of the drug therapy then in use (McInness, Shaw, Knapp & Davies, 1964 unpublished observations).

In 1976 a commercially available automatic machine, the Roche Arteriosonde, was used to record blood pressures over 24 h during studies into the pharmacokinetics and biological effects of atenolol therapy during both acute and chronic administration (Woolfson & Knapp, 1977).

The domiciliary approach to the study of biological rhythms (Knapp & Hillier, 1974) has been adapted to investigate patients on treatment with hypotensive medication using simple electronic 'do it yourself' sphygmomanometers. Data has been collected on the control of early morning blood pressure and on the effect of the timing of drug therapy on the control of blood pressure. The equipment used in these studies will be demonstrated, together with illustrative results and their analysis.

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Menstrual fluctuations in hearing—suggestions for hormone or drug monitoring methods in man

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The many delicate mechanical, metabolic and electrophysiological stages of the peripheral hearing mechanism lead to ambiguities in specifying causes of a hearing loss but justify considering hearing as a method of drug monitoring. As the salicylates and some diuretics and antibiotics are ototoxic, (Hawkins, 1976) there are specific reasons for such auditory monitoring. Modern psychoacoustics offers a wide range of perceptual phenomena with associated methods of stimulus control and task organisation; these can stress and hence reveal the underlying

mechanisms more critically than absolute threshold tasks do. An example is in the human menstrual cycle where no absolute threshold effects are reported. A task involving the angular localisation of sounds by interaural time differences in the region of 100 μ s showed large and significant menstrual variations, while other tasks did not, rendering a mood explanation implausible (Haggard & Gaston, 1978). The oestrogen/progesterone ratio may be involved. Auditory demonstrations of localisation of clicks and the central detection of 'beating' in tones delivered to different ears will be given.

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