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Plasma protein binding of propranolol in disease states

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Propranolol is highly bound to plasma proteins and the degree of this binding varies little among normal subjects (Evans & Shand, 1973). Alprenolol, a closely related β-adrenoceptor blocker has been shown to bind extensively to isolated α_1-acid glycoprotein (orosomucoid) (Borgå, Piafsky & Nilsen, 1977). A highly significant correlation has been demonstrated between the binding of alprenolol in normal volunteers and the measured concentration of this protein in plasma (Piafsky & Borgå, 1977). α_1-acid glycoprotein is one of the acute phase reactants known to increase in concentration in plasma in numerous diseases (Kawai, 1973). Using equilibrium dialysis at 37°C we have investigated the plasma binding of propranolol in eighty patients with various diseases and compared this to the plasma binding in a group of volunteers (Table 1). Binding of propranolol in all patient groups differed significantly from that seen in the control group.

The α_1-acid glycoprotein concentration was determined by radial immunodiffusion. There was a significant negative correlation between the plasma concentration of α_1-acid glycoprotein and the free fraction of propranolol in plasma ($r=-0.75$, $P>0.001$).

Table 1 Plasma binding of propranolol in normal volunteers and various disease states

		% free propranolol	
Disease state	n	*Mean*	*Range*
Control	25	10.7	8.3–15.4
Renal	22	9.5	3.4–16.3
Arthritis	9	6.8	4.3–8.5
Crohn's	12	6.3	3.2–11.3
Cirrhosis	12	11.9	8.6–17.7

It appears likely that the large variation in binding observed will have significant effects on the pharmacokinetics of propranolol in these patients.

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Plasma protein binding of drugs in thyroid dysfunction

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The metabolism of certain drugs is altered in patients with thyroid dysfunction (Eichelbaum, 1976; Bell, Russell, Nelson, Kelly & McDevitt, 1977). In addition, hypersensitivity to catecholamines has been said to exist in patients with hyperthyroidism (Turner, 1974). This concept has recently been questioned (Levey, 1971; McDevitt, 1977). Clinical manifestations of hyperthyroidism ascribed to catecholamine hypersensitivity might result from alterations in the plasma binding of catecholamines in thyroid disease, if these occurred. No information in man is available on this point. We have investigated plasma binding of isoprenaline and propranolol in patients with thyroid dysfunction.

Seven hyperthyroid and ten hypothyroid patients were studied before treatment and again when euthyroid. On each occasion, 20 ml of venous blood was removed and the plasma separated. Plasma protein binding of tritiated isoprenaline and propranolol was measured by equilibrium dialysis at 37°C for 18 h. Four concentrations of each drug were used and were chosen so that, after dialysis, plasma concentrations were in the range encountered in clinical practice. Drug concentrations in plasma and buffer were measured by liquid scintillation spectrometry.

After dialysis, plasma concentrations of isoprenaline were in the range 3.5–56 ng/ml and of propranolol were in the range 27–425 ng/ml. There was no variation in plasma binding of either drug with concentration in the range studied.

Mean results are shown in the Table 1. For each drug a small increase in protein binding was observed when both the hyperthyroid and hypothyroid patients became euthyroid. This was only significant for isoprenaline binding in the plasma of hypothyroid subjects ($P<0.001$). However, the small changes in binding observed for these two drugs are unlikely to result in significant alterations in free drug concentrations, and, in the case of isoprenaline, if representative of catecholamine binding, would not account for the clinical manifestations of hyperthyroidism.

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Table 1 % Plasma protein binding of propranolol and isoprenaline (mean ± s.e. mean) before and after treatment

	Isoprenaline		*Propranolol*	
Initial patient status	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*
Hyperthyroid ($n=7$)	65.1 ± 3.2	68.1 ± 1.4	86.1 ± 1.7	88.4 ± 0.8
Hypothyroid ($n=10$)	64.3 ± 1.6	68.8 ± 1.2	87.7 ± 0.8	88.6 ± 0.6

The effects of chronic alcohol ingestion and alcoholic liver disease on drug-protein binding

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We have previously reported reduced serum binding capacity for salicylate, sulphadiazine and phenylbutazone in patients with chronic hepatic disease (Wallace & Brodie, 1976), confirming the findings of others using different drugs (Reidenberg, 1974; Affrime & Reidenberg, 1975). Several of these patients had alcohol-induced liver disease and we now report further on the effects of chronic alcohol ingestion and alcoholic liver disease on drug binding.

Measurement of protein binding by ultrafiltration and assay of salicylate, sulphadiazine and phenylbutazone were carried out as previously described (Wallace & Brodie, 1976) on serum samples, from eight controls, twenty chronic alcoholics with no clinical or biochemical evidence of liver disease and twenty-three patients with alcoholic liver disease diagnosed histologically as cirrhosis ($n = 11$) or hepatitis ($n = 12$).

In the chronic alcoholic group, binding of the three test drugs did not significantly differ from control values. Binding of salicylate and phenylbutazone was reduced in the cirrhotics and that of sulphadiazine and phenylbutazone reduced in the patients with alcoholic hepatitis (Table 1). In this latter group, also, significant correlations were obtained between drug binding and plasma bilirubin and albumin levels ($P < 0.01$).

These results suggest that chronic alcohol ingestion *per se* has no effect on drug binding to serum proteins. However, patients with alcoholic liver disease show reduced binding and in the hepatic group this is apparently related to changes in serum bilirubin and albumin levels. These observations may have relevance to the distribution of drugs in the alcoholic patient with alcoholic liver disease.

Table 1 Drug binding and biochemical data in controls, chronic alcoholics and patients with alcoholic liver disease. Statistics were obtained using Student's *t* test for paired values

		% bound				
Subjects	n	*Salicylate*	*Sulphadiazine*	*Phenylbutazone*	*Bilirubin (μmol/l)*	*Albumin (g/g)*
Control	8	73 ± 2.4	54 ± 2.6	94 ± 0.4	15 ± 0.6	43 ± 0.8
Chronic alcoholism	20	73 ± 2.1 (NS)	58 ± 2.3 (NS)	93 ± 0.4 (NS)	13 ± 4.9 (NS)	41 ± 1.1 (NS)
Alcoholic cirrhosis	11	59 ± 3.6 ($P < 0.005$)	49 ± 4.6 (NS)	81 ± 2.9 ($P < 0.001$)	74 ± 40.2 (NS)	32 ± 2.2 ($P < 0.001$)
Alcoholic hepatitis	12	66 ± 3.5 (NS)	42 ± 3.5 ($P < 0.02$)	87 ± 2.9 ($P < 0.05$)	31 ± 11.3 (NS)	36 ± 2.7 ($P < 0.025$)

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Biological determinants of propranolol disposition in normal subjects and patients with cirrhosis

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It is well recognized that the hepatic elimination of a highly extracted compound is influenced by both liver blood flow and intrinsic clearance, a measure of the basic removal process (Rowland, Benet & Graham, 1973). However, investigations in man have been hampered by the lack of simple methods for measuring these parameters. Recent theoretical considerations suggest that these determinants of hepatic elimination can be assessed from a knowledge of the oral and intravenous kinetics of a highly extracted model drug which is completely absorbed and eliminated only by the liver (Perrier & Gibaldi, 1974; Wilkinson & Shand, 1975).

This approach has been tested using propranolol as a model drug possessing the necessary characteristics in fifteen normal subjects and six patients with biopsy-proven, stable cirrhosis. 4·0 μCi of [^{3}H]-propranolol was administered i.v. after steady-state plasma concentration had been attained by oral administration of the unlabelled drug (80 mg, 8 hourly). Serial blood samples were assayed for both labelled and unlabelled drug after extraction by the method of Shand, Nuckolls & Oates (1970) followed by liquid scintillation counting and fluorimetry, respectively.

In normal subjects, systemic clearance and bioavailability were consistent with a high intrinsic clearance and a steady-state hepatic extraction of 64%. Values for apparent liver blood flow agreed with previous estimates using the Fick method. Systemic clearance in cirrhosis was reduced and bioavailability increased. The increases in volume of distribution and blood to plasma ratio were related to decreased plasma binding. In cirrhosis the value for apparent liver blood flow is equivalent to the ratio of hepatic flow to the fraction of mesenteric flow shunted to the liver by any portacaval anastomoses that might be present. In conclusion, the observations of the higher concentrations of propranolol after oral administration in cirrhosis are compatible with a reduced intrinsic clearance and/or the development of extra-hepatic shunts.

Table 1 Disposition of propranolol in normal and cirrhotic individuals (mean ± s.e. mean results)

	Normal subjects (n=15)	*Cirrhotics (n=6)*
Systemic clearance (ml/min)	898 ± 67	558 ± 143*
Volume of distribution (l)	293 ± 15.3	375 ± 41.1*
Half life (h)	3.9 ± 0.22	11.8 ± 3.45*
Bioavailability	0.36 ± 0.024	0.60 ± 0.08*
% unbound in plasma	6.7 ± 0.31	9.67 ± 1.2*
Blood plasma ratio	0.78 ± 0.03	0.97 ± 0.0*[illegible]
Intrinsic clearance (ml/min)	2711 ± 277	1347 ± 388*
Apparent liver blood flow (ml/min)	1425 ± 102	1540 ± 187

* $P < 0.05$ different from normal subjects.

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Antipyrine metabolism in iron deficiency

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A number of studies have demonstrated the effect of nutritional deficiences on the rate of hepatic drug metabolism in animals. Although most deficiencies tend to impair metabolism, Catz, Juchau & Yaffe (1970) and Becking (1972) have shown that chronic iron deficiency in mice and in rats respectively can enhance the metabolism of some drugs such as aminopyrine whilst the elimination of others is unchanged. However, iron deficiency sufficient to reduce the haemoglobin to 50% of normal is required before any effect is observed.

In this study elderly people (over 65 years) with no evidence clinically or biochemically of renal, hepatic or gastrointestinal disease were screened for the presence of iron and other deficiencies. None was taking drugs known to influence hepatic microsomal enzymes. Venous blood samples were taken at intervals during the 24 h after the oral administration of antipyrine (18 mg/kg). Plasma antipyrine was measured by the method of Brodie, Axelrod, Soberman & Levy (1949) and the half-life, plasma clearance and volume of distribution calculated assuming a one compartment open model.

Table 1 shows that six patients had evidence of iron deficiency and metabolized antipyrine more rapidly than non-deficient people. In twelve people with transferrin saturations ranging from 5.1–41.7% there was a significant correlation between saturation and antipyrine clearance ($r = -0.806$, $P < 0.005$).

Four other iron deficient patients have been studied with co-existing deficiency of either vitamin C or folic acid. In these the rate of antipyrine metabolism is similar to that found in isolated iron deficiency rather than to that expected in pure vitamin C or folic acid deficiency.

This effect of iron deficiency on antipyrine metabolism may explain the sex difference in antipyrine half-lives observed in young people by O'Malley, Crooks, Duke & Stevenson (1971).

Table 1 Antipyrine handling in six iron deficient patients compared with twenty-eight non-deficient people

				Antipyrine		
Patient	*Haemoglobin (g/100 ml)*	*Microcytosis hypochromia*	*Transferrin saturation (%)*	*Plasma half-life (h)*	*Plasma clearance (ml h^{-1} kg^{-1})*	*Volume of distribution (ml kg^{-1})*
1	7.2	Yes	7.0	6.9	59.0	587
2	9.5	Yes	5.1	7.1	64.2	656
3	10.2	Yes	—	4.8	61.0	425
4	11.3	Yes	15.0	7.0	69.5	701
5	11.8	No	16.7	7.8	49.2	560
6	12.8	No	15.6	6.7	46.6	450
Mean ± s.d.	10.5 ± 2.0	—	11.9 ± 5.4	6.7 ± 1.0	58.25 ± 8.8	563 ± 110
Non-deficient people	13.0 ± 1.5	None	32.4 ± 9.0	12.4 ± 2.7	30.8 ± 9.8	522 ± 9.1
P value	<0.005		<0.005	<0.001	<0.001	NS

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Comparative evaluation of procainamide and its main metabolite, N-acetylprocainamide, in the acute treatment of ventricular arrhythmias

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Procainamide (PA) has been shown in man to be acetylated by the same polymorphic enzyme system as isoniazid and some sulphonamides (Karlsson & Molin, 1975; Reidenberg, Drayer, Levy & Warner, 1975). During PA-therapy N-acetyl-procainamide (NAPA) accumulates to particularly high plasma levels in patients who are rapid acetylators and have poor kidney function (Karlsson, Molin, Norlander & Sjöqvist, 1974; Reidenberg *et al.*, 1975). It has been demonstrated that NAPA has an antiarrhythmic activity in animals (Drayer, Reidenberg & Sevy, 1974; Karlsson, Åberg, Collste, Molin, Norlander & Sjöqvist, 1975; Refsum, Frislid, Lunde & Landmark, 1975) and in man (Lee, Strong, Keohe, Dutcher & Atkinson, Jr, 1976).

To evaluate further the antiarrhythmic and pharmacokinetic properties of NAPA five patients have been studied by means of repeated exercise tests over 40 min at a submaximal fixed work load. They all suffered from ventricular arrhythmias which were persistent and of increasing severity during work. Each patient performed three tests. In the first test no drug was given. In the other two PA and NAPA were chosen at random for i.v. administration in doses of 500 mg over 10 min. Blood samples for plasma analysis of PA and NAPA were frequently drawn and the ECG was recorded continuously and analysed minute by minute. PA and NAPA were determined by a sensitive liquid chromatographic method (Graffner, Jansson, Lagerström & Persson, 1977). Mean peak PA plasma levels of 8.2 μg/ml (range 5.5–12.2) gave a mean 81% (range 20–100) reduction of premature ventricular contractions (PVCs) whilst the corresponding figures for NAPA were 15.9 μg/ml (range 15.3–16.5) and 53% (range 32–66). The mean elimination half life was 2.4 h for PA (range 2.2–2.6) and 6.7 h for NAPA (range 4.3–8.7).

When PA was given intravenously the concomitant plasma levels of NAPA were negligible suggesting that acetylation of PA takes place during the first pass circulation through the intestinal wall and/or liver. In the doses used NAPA showed an antiarrhythmic activity inferior to that of PA but of longer duration corresponding to the longer plasma $T_{\frac{1}{2}}$. The optimal NAPA dosage and plasma level are unknown. By increasing the NAPA dose to 750 mg one patient so far studied showed an 87% reduction of PVCs at a peak plasma level of 19.6 μg/ml.

The possibility that NAPA as opposed to PA may not induce a SLE-like syndrome (Drayer *et al.*, 1974; Karlsson *et al.*, 1974), the fact that it has a longer elimination half life and that it is to a greater extent excreted unchanged by the kidneys (Graffner *et al.*, 1977; Strong, Dutcher, Lee & Atkinson, Jr, 1975a; Strong, Dutcher, Lee & Atkinson, Jr, 1975b) make it attractive as a potential antiarrhythmic drug.

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Disopyramide plasma kinetics and pharmacodynamics applied to the assessment of bioavailability

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Disopyramide, a quinidine-like antiarrhythmic agent, is available intravenously as the phosphate (Rythmodan) and orally in two forms, the base (Rythmodan) and the phosphate (Norpace). To determine the relative bioavailabilities of these oral preparations, the relation between the pharmacokinetics and pharmacodynamics of disopyramide has been investigated. The kinetics of one measure of the pharmacological effect of disopyramide, *viz.* prolongation of the QT interval (Hinderling & Garrett, 1976) have been assessed in a group of eight normal volunteers together with plasma kinetics of the parent drug after intravenous (2 mg/kg) and oral (300 mg base or base equivalent) administration of both oral forms. Twenty to thirty observations on the QT interval (corrected for heart rate) were made at specific times for 24 h after drug administration and simultaneous serum samples, together with additional samples at 32 and 48 h were obtained for determination of disopyramide concentrations by a specific g.l.c. technique using nitrogen detection (modified from Duchateau, Merkus & Schobben, 1975). A two-compartment open pharmacokinetic model was found to be consistent with the plasma data. After intravenous administration, the apparent half-lives (mean ± s.d.) of the α and β phases were 3.1 ± 1.6 min and 8.2 ± 1.8 h respectively. After oral administration, elimination was similar with β half-lives of 8.2 ± 1.7 h for Rythmodan with 8.9 ± 1.45 h for Norpace. Oral absorption patterns differed as can be seen in Table 1 which also illustrates slight but insignificant differences in bioavailabilities assessed by measurement and comparison of the total areas under the intravenous and oral concentration/time curves. Individual differences in the shape of these curves were closely paralleled by corresponding dynamic changes. The relatively rapid absorption of oral Rythmodan was associated with an earlier prolongation of the QT interval which served as a useful index of disopyramide activity and availability.

Table 1 Mean ± s.d. ($n = 8$) absorption characteristics and comparative bioavailabilities of disopyramide

	Lag time (min)	*Absorption half-life (min)*	*Time to peak concentration (h)*	*$AUC_{0-\infty}$ (% dose l^{-1} h)*	*AUC oral/ AUC i.v. ×100%*
'Rythmodan'	11 ± 7.10	19 ± 8.30	1.8 ± 0.35	13.1 ± 4.90	64.87 ± 12.57
'Norpace'	10 ± 6.60	44 ± 25.80	2.86 ± 0.66	15.65 ± 5.30	78.23 ± 16.18
P value (*t* test)	NS	<0.05	<0.01	NS	NS

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Pharmacokinetics of clonidine and its relation to the hypotensive effect in patients

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Changes in sleep pattern, blood pressure, heart rate and plasma noradrenaline after night-time administration of slow release clonidine

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Clonidine is a potent centrally-acting hypotensive agent one of whose principal side-effects is sedation. One way of minimizing the impact of this side-effect is to give a single night time dose of a centrally-acting hypotensive agent so that the peak sedative effect is declining when the patient wakes in the morning. This study was undertaken to determine the effect upon blood pressure, electroencephalogram (EEG) and plasma noradrenaline of a single night time dose of sustained release formulation of clonidine when compared with placebo.

Five healthy male volunteers were each studied for 8 h on the third and fifth of five consecutive nights. The first two nights were for adaptation to the laboratory environment. On the third and fifth study night placebo or 300 μg of slow release clonidine was administered at the beginning of the study period. The EEG, electro-oculogram, electromyogram and electrocardiogram were recorded continuously using a Grass Polygraph recorder. Blood pressure was measured at 10 min intervals by a Roche 1217 Arteriosonde. Blood was sampled at 45 min intervals from a central venous catheter and plasma noradrenaline was estimated by the method of Henry, Starman, Johnson & Williams (1975). Sleep stage was defined according to the criteria of Rechtstaffen & Kales (1968).

On the placebo night the onset of sleep (awake to Stage II) was associated with a fall in mean systolic and diastolic pressures from 115.4 ± 2.4 to 106.0 ± 3.9 mmHg systolic and from 66.3 ± 4.5 to 51.8 ± 2.0 mmHg diastolic. There was a fall in heart rate from 61.1 ± 2.2 to 54.0 ± 1.6 beats/min. A further fall in systolic blood pressure occurred with deep sleep (Stage II/IV) to 94.6 ± 3.1 mmHg.

On the clonidine night the fall in systolic pressure was greater than on placebo. On the placebo night the pressure fell to 109.0 ± 4.2 mmHg at 5.5 h whereas on the clonidine it fell to 86.8 ± 5.0 mmHg at that time. This difference was significant ($P < 0.02$). On the clonidine night the fall in diastolic pressure was only slightly greater than on the placebo night and the difference was not significant. The heart rate during sleep was unaffected by clonidine.

The duration of paradoxical rapid eye movement sleep time was markedly reduced by clonidine (10.3 ± 5.3 min) compared with placebo (107.9 ± 2.0 min) and was abolished completely in two subjects. Total sleep time was not prolonged compared to placebo but Stage II sleep time was increased on clonidine from 169.7 ± 16.7 min to 244 ± 15.7 min. Plasma noradrenaline fell on placebo from 0.21 ± 0.07 ng/ml to 0.19 ± 0.05 ng/ml at 90 min and to 0.09 ± 0.04 ng/ml at 8 h. On clonidine plasma noradrenaline fell from 0.2 ± 0.09 ng/ml to 0.09 ± 0.04 ng/ml at 90 min and had only risen to 0.09 ± 0.06 ng/ml at 8 h. Although the mean concentrations of noradrenaline in plasma were lower on the clonidine night than on placebo the differences were not statistically significant. There was no correlation between plasma noradrenaline and sleep stage.

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Concentration of debrisoquine by human platelets *in vivo*: relationship to hypotensive effect

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In vitro studies have suggested that the human platelet might be a useful pharmacological model of the adrenergic neurone, one common property being the ability actively to concentrate guanidinium antihypertensive agents (Abrams & Solomon, 1969; Boullin & O'Brien, 1968). The development of sensitive and specific assays for debrisoquine (D) and 4-hydroxy debrisoquine (HD), a principle metabolite (Lennard, Silas, Smith & Tucker, 1977; Malcolm & Marten, 1976) has allowed us to determine the *in vivo* content of these compounds in platelets taken from patients receiving oral doses of D. Furthermore, in view of our finding that plasma D concentrations during continuous therapy are highly correlated with hypotensive response (Silas, Lennard, Tucker, Smith, Malcolm & Marten, 1977) we wondered if platelet D might show an even closer relationship.

Venous blood samples were taken from patients on continuous D therapy (5–60 mg two times a day) at the end of a dosage interval ($n=10$) and 2 h after dosage ($n=7$). Plasma, prepared by spinning blood at 1000 *g* for 10 min, was analysed for D and platelet pellets were obtained from platelet-rich plasma prepared by spinning blood at 150 *g* for 12 min. Mean ± s.d. pre-dose concentrations of D and HD/ml of total platelet water (81 ± 3% wet weight) were found to be 47 ± 15 μg/ml ($n=10$) and 6 ± 2 μg/ml ($n=3$; undetectable in 7), respectively. Although, in a second group of seven patients, a correlation was found between platelet D concentration and the fall in standing diastolic blood pressure from pre-treatment values ($r=+0.59$; $P<0.05$; $n=12$), this was not as close as that between plasma D concentration and blood pressure fall ($r=+0.91$; $P<0.001$; $n=12$). Platelet/plasma concentration ratios of D were inversely related to plasma D concentration ($r=-0.84$; $P<0.01$), varying from 400:1 to 1600:1. On average, pre-dose plasma D concentration (± s.d.) was about half of that at 2 h post-dose (72 ± 45 ng/ml and 135 ± 76 ng/ml) but platelet D concentrations were relatively constant (48 ± 17 μg/ml and 52 ± 15 μg/ml). Comparison of blood pressures during treatment with pre-treatment pressures obtained at identical times of the day revealed that pre-dose and 2 h post-dose responses were similar. In a volunteer given a 20 mg oral dose of D the concentration ratio of D in platelet-rich plasma to that in platelet-poor plasma increased to 15:1 after 36 h. Half-lives determined after 12 h were 17.5 h in platelet-poor and 56 h in platelet-rich plasma.

Collectively, these data indicate an extensive, saturable accumulation of D by platelets *in vivo*, whereas uptake of HD, which does not appear to contribute to hypotensive response (Silas *et al.*, 1977) was much less. However, the platelet appears to have deficiencies as a model of the adrenergic neurone if it is assumed that hypotensive response in our patients is entirely a function of D concentration in the neuronal pool. Many reasons can be postulated to account for the relatively poor correlation between platelet D and the ultimate fall in blood pressure. Further studies of the time-dependence of platelet D concentration may nevertheless show that changes in this, rather than plasma D concentration, afford a better reflection of the kinetics of hypotensive response.

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Factors affecting anticoagulant response

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A recent study of 228 patients maintained at a prothrombin ratio of 1.8–2.5 has indicated that warfarin dosage requirements (mg/kg body weight) decrease with increasing age from the third decade ($r=0.33$, $P<0.001$), (Routledge, unpublished observations).

In order to study the factors involved in this trend, fifteen patients (seven males) in whom warfarin therapy was to be discontinued on clinical grounds, were studied. Their ages ranged from 33–78 years. None was taking any drug known to interact with warfarin and all were receiving a dose sufficient to achieve a prothrombin ratio of 1.8 to 2.5. Patients were instructed to take their daily dose of warfarin at 12.00 h throughout the study. At intervals of 3 weeks, the daily warfarin dose was decreased in 0.5–1 mg steps until a prothrombin ratio of 1.0 had been reached. Every third week a specimen of blood was taken at 10 h for estimation of plasma warfarin concentration and prothrombin ratio.

Plasma warfarin concentrations were estimated by a modification of the method of Lewis, Ilnicki, Leon & Carlstrom (1970) which had a lower limit of sensitivity of 0.05 μg/ml. Prothrombin ratio was estimated by the method of Owren & Aas (1951) using thrombokinase (Geigy).

Patients' warfarin dosage at the beginning of the study varied from 0.028 to 0.112 mg kg^{-1} day^{-1} (mean ± s.d. 0.070 ± 0.029) and correlated inversely with age ($r=-0.6$ $P<0.05$).

For each patient, the logarithm of the plasma warfarin concentration was linearly related to the anticoagulant response between a prothrombin ratio of 1.3 and 2.5. The plasma warfarin concentrations at a prothrombin ratio of 1.8 were calculated to vary from 0.19–0.93 μg/ml between individuals and were significantly inversely correlated with age ($r=-0.58$, $P<0.05$).

Individual plasma clearances were calculated at each dose level thus:

$$\text{Clearance} = \frac{\text{Dose}}{\text{Plasma concentration} \times \text{dose interval}}$$

(Wagner, Northam, Alway & Carpenter, 1965), and average clearance was observed to vary from 2.56–6.37 ml h^{-1} kg^{-1} body weight (mean ± s.d. 4.01 ± 1.13), but was unrelated to age ($r=-0.03$, $P>0.05$).

The results indicate that therapeutic effects of warfarin are associated with lower plasma concentrations as well as reduced dosage in elderly individuals.

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Comparative effects of β-adrenoceptor antagonists on airways resistance at rest and during exercise in normal subjects

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Although β-adrenoceptor antagonists may aggravate symptoms in obstructive airways disease, the majority of patients requiring treatment with these drugs have normal respiratory function and controversy still concerns the comparative efficacy of drugs with different ancillary pharmacological properties in this respect in such patients. In the majority of reported studies drugs were given either intravenously or by aerosol and the studies usually carried out at rest, the least sensitive state in which to measure airways resistance.

A study was therefore designed to compare the effects on airways resistance at rest and during submaximal exercise of propranolol, oxprenolol, practolol and metoprolol in the maximum doses usually used clinically as single oral doses (320, 320,

800, and 400 mg) to six normal male subjects (age range 22–47 years), three of whom were cigarette smokers. Drugs and placebo were given double-blind in random order and measurements of heart rate (HR), respiratory rate (RR), tidal volume (TV), forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and peak expiratory flow rate (PEFR) measured at the same time each day 2 h after ingestion of drug or placebo.

Preliminary studies established that there were no significant differences in PEFR and FEV_1, between bicycle and treadmill exercise at heart rates of 140 beats/min and that maximum changes were present at 2 min. There was no significant trend in heart rate or spirometric measurements in eight within-day and five between-day studies.

All drugs produced a significant reduction in resting and exercise heart rates which was greater for propranolol and metoprolol than practolol or oxprenolol ($P<0.01$). After placebo, exercise was associated with an increase in respiratory rate ($P<0.001$), tidal volume ($P<0.001$) and PEFR ($P<0.01$), but with no change in FEV_1 or FVC. The spirometric changes were uninfluenced by any of the four β-adrenoceptor blocking drugs compared with placebo either in the smokers or non-smokers.

These results indicate that the administration of relatively large single oral doses of β-adrenoceptor antagonists with or without relative cardioselective activity to subjects without a history of asthma or chronic bronchitis have no detectable effect on airways resistance either at rest or during submaximal exercise.

The influence of dietary methylxanthines on the metabolism and pharmacokinetics of intravenously administered theophylline

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Theophylline (1,3-dimethylxanthine) is widely used in the treatment of asthma and obstructive airways disease. Effective bronchodilation occurs when the plasma level is within a range of 10–20 μg/ml, higher levels being associated with toxicity (Jenne, Wyze, Rood & MacDonald, 1972). There occur, however, marked inter-individual variations in the plasma levels of theophylline, due to inter-subject differences in the capacity to metabolize the drug. Many genetic and environmental factors can influence the pharmacokinetic profiles of drugs by influencing the drug-metabolizing enzymes. Diet can be important, and in the case of theophylline it may be significant because of the ingestion of large amounts of chemically related methylxanthines in the diet, which might alter the disposition of theophylline. Accordingly we have investigated the metabolic fate of [^{14}C]-theophylline in a group of volunteers before and after deprivation of their normal methylxanthine intake.

[^{14}C]-Theophylline (100 mg; 10 μCi) labelled in the 8-position was administered intravenously to three male volunteers, aged 23–29 years. Urine samples were collected at regular intervals up to 48 h, the excretion of ^{14}C monitored by liquid scintillation counting and the metabolites present examined by ion-exchange chromatography, ion-exchange paper chromatography followed by radiochromatography scanning and by reverse isotope dilution. Three metabolites were excreted—3-methylxanthine, 1,3-dimethyluric acid and 1-methyluric acid, in addition to unchanged theophylline. Theophylline, 1,3-dimethyluric acid and 1-methyluric acid were excreted by apparent first order kinetics but for the first 12 h after dosage, 3-methylxanthine was excreted at a constant rate and followed an apparent first order decline after this time. Kinetic analysis of 3-methylxanthine elimination was performed with the modification of the Michaelis-Menten equation used by Levy, Tsuchiya & Amsel (1972), and K_m and V_{max} values were obtained.

In the second part of the investigation, the same volunteers abstained from the intake of dietary methylxanthines for 7 days, after which [^{14}C]-theophylline was again administered intravenously. The metabolic pattern of theophylline was not altered, but the kinetic parameters of its elimination changed markedly, notably those for theophylline (k_{el}^{T} normal diet 0.015 h^{-1}, xanthine free 0.023 h^{-1}, $P<0.05$), 1,3-dimethyluric acid (k_{el}^{DMU} normal diet 0.22 h^{-1}, xanthine free 0.032 h^{-1}, $P<0.05$) and 3-methylxanthine (k_m^{3MX} normal diet, 20.1 mg, xanthine free, 54.8 mg, $P<0.001$, V_{max}^{3MX} normal diet 0.94 mg h^{-1}, xanthine free 2.07 mg h^{-1}, $P<0.001$). The elimination $T_{\frac{1}{2}}$ of total ^{14}C was also changed, from 10.1 ± 1.6 h (mean ± s.e. mean) with normal diet to 6.9 ± 0.8 h with xanthine free diet ($P<0.05$).

These findings indicate that the metabolism and

elimination of theophylline is influenced by the dietary intake of methylxanthines. Kinetic analysis of the data showed that the elimination of theophylline follows parallel first order and Michaelis-Menten kinetics and would therefore be expected (Wagner, 1974) to be dose-dependent. The influence of deprivation of dietary methylxanthines on the elimination of theophylline is consistent with the latter being dose-dependent.

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Simultaneous pharmacokinetic analysis for comparison of absorption, distribution and elimination of some tetracycline antibiotics

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Tetracycline antibiotics, with the possible exception of doxycycline and minocycline, are incompletely absorbed following oral administration. It may, therefore, be difficult to compare their relative bio-availabilities and pharmacokinetics due to differences in absorption in the same subjects on different test occasions, even under strictly controlled conditions of administration. The difficulty may be overcome by simultaneous administration of different tetracyclines followed by selective spectrofluorimetric analysis of individual drug levels.

The pharmacokinetics of chlortetracycline (122 mg, CTC), demthylchlortetracycline (74.6 mg, DMCTC) and tetracycline (125 mg, TC) in a single oral tablet (Deteclo, Lederle), were compared in six healthy volunteers on two occasions under fasting and non-fasting conditions over a 25 h period. Individual plasma levels were assayed fluorimetrically (Hall, 1976), and concentration/time profiles analysed using a one-compartment open model (Table 1). Differences were compared statistically using the sign test (Siegel, 1956) and the following conclusions were made: in the presence of food, (a) lag times were not significantly changed; (b) times to peak concentration were reduced (DMCTC, $P=0.03$; TC, $P=0.02$; CTC, $P=0.11$); (c) apparent absorption half-lives were reduced (DMCTC, CTC, $P=0.11$; TC, $P=0.02$); (d) CTC peak concentrations were reduced ($P=0.11$). TC peak concentrations were reduced for four of the six subjects; (e) apparent elimination half-lives were not significantly changed overall; (f) areas under time/concentration curves were reduced. Although only six subjects were studied and statistical inference is, therefore, of limited value, these results indicate the potential value of this technique for comparative kinetic studies of tetracycline antibiotics.

Table 1 Mean ± s.e. mean values of derived pharmacokinetic terms

	Fasting			*Non-Fasting*		
Parameter	*CTC*	*DMCTC*	*TC*	*CTC*	*DMCTC*	*TC*
1. Lag (h)	0.54 ± 0.23	0.56 ± 0.17	0.45 ± 0.10	0.28 ± 0.18	0.28 ± 0.16	0.36 ± 0.22
2. Time to peak (h)	3.35 ± 0.64	4.43 ± 0.72	2.85 ± 0.31	1.95 ± 0.36	2.10 ± 0.44	1.58 ± 0.24
3. $T_{\frac{1}{2}}$ absorption (h)	0.86 ± 0.23	1.47 ± 0.25	0.91 ± 0.18	0.51 ± 0.17	0.53 ± 0.19	0.27 ± 0.07
4. $T_{\frac{1}{2}}$ elimination (h)	7.79 ± 0.83	10.74 ± 3.00	5.69 ± 1.08	7.22 ± 0.88	7.28 ± 1.6	7.25 ± 1.04
5. Peak concentration ($\mu g\ ml^{-1}$)	0.81 ± 0.14	0.61 ± 0.11	1.01 ± 0.19	0.54 ± 0.09	0.88 ± 0.21	0.77 ± 0.20
6. Area under curve	10.98 ± 1.95	11.03 ± 2.33	10.57 ± 1.75	6.36 ± 1.00	8.67 ± 1.92	7.88 ± 2.19

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Hepatobiliary metabolism and excretion of adriamycin (ADR) in man

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Earlier studies on human biliary metabolites of ADR have used bile samples obtained post-mortem (Bachur, Egorin, Hildebrand & Takanashi, 1973) or from a patient with an indwelling T-tube (Benjamin, Riggs, Serpick & Bachur, 1975). Separation of metabolites was achieved on silica gel TLC plates, followed by elution and fluorimetry. Experimentally, this methodology is laborious and time consuming and, because of the light-sensitive nature of the chromophore and ease of hydrolysis of the glucoside linkage, in our hands has resulted in the formation of artifactual products. Instead, we have developed a rapid and nondestructive HPLC assay system with flow fluorescence detection which allows identification and quantitation of ADR and adriamycinol in the sample of range 1–4 p mol. By means of this technique we have examined serial samples of bile and urine from two patients with choledocal T-tubes who received ADR intravenously. Unchanged ADR was found to be the major component present in bile, accounting for 98% of the total fluorescence at 1 h and 35% at 72 h. Adriamycinol, the main metabolite, was present within 0.5 h following ADR administration and increased from 2 to 30% of the total fluorescence at 72 h. The remaining fluorescent species were identified as polar conjugates. No free aglycones were detected at any time point. In one patient from whom bile collection was complete, the cumulative total biliary fluorescence (% of administered dose) was 24% at 24 h and 33% at 72 h. Urinary excretion in the two patients accounted for 4 and 7% of the total dose at 72 h; total fluorescence was accounted for by ADR (the major fraction) and adriamycinol, with no other fluorescent metabolites present. The findings (a) confirm that the liver is the major excretory pathway for ADR in man, and (b) indicate that ADR metabolism in man may be less complex than has been previously suggested (Takanashi & Bachur, 1976). In addition, this work establishes that a simple, rapid, highly sensitive and reproducible methodology now exists for metabolic studies with ADR, including studies on drug-drug interactions and the influence of liver disease (including metastases).

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The influence of therapeutic doses of spironolactone on the liver microsomal enzyme system and digoxin elimination in man

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An increased excretion of digoxin was found following high doses of spironolactone in rats (Wirth & Fröhlich, 1974) and this finding was thought to be due to enzyme induction observed in rats after spironolactone treatment (Stripp, Hamrick, Zampaglione & Gilette, 1971). As spironolactone in therapeutic doses was found to be a weak inducer in man (Taylor, Rawlins & Smith, 1972) a combined study investigating the effect of spironolactone treatment on the liver microsomal enzyme system and digoxin elimination was performed in eight patients. After an overnight fasting period the patients were given 0.1 mg [^{3}H]-digoxin (120 μCi) intravenously and 1200 mg antipyrine orally before and after spironolactone treatment (150 mg daily) for a period of 15 days. Plasma samples for the estimation of antipyrine and digoxin were taken at intervals up to 120 h, urine and faeces were collected over a period of 5 days. From the concentrations in the plasma, half-life and total body clearance of antipyrine were calculated. As additional parameters of the liver microsomal enzyme system γ-glutamyl-transpeptidase in the plasma and D-glucaric acid excretion in the 24 h urine was estimated. From the digoxin concentrations

in the plasma and urine different pharmacokinetic parameters were calculated. The total digoxin excretion in the faeces was estimated.

Following spironolactone treatment the mean antipyrine half-life was not significantly different having values of 13.5 or 14.4 h respectively, while the total body clearance was about 30.5 ml/min under both experimental conditions.

γ-glutamyl-transpeptidase and D-glucaric acid excretion showed no significant differences. In addition, based on the different calculated pharmacokinetic parameters no significant changes in digoxin elimination were found following spironolactone treatment. The overall elimination rate constant was 0.016 or 0.014 h^{-1}, reflecting a half-life of 43 ± 8 or 49 ± 13 h. The volume of distribution did not change and the total body clearance of digoxin remained unaltered having values of 109 ± 39 or 90 ± 40 ml/min before and after spironolactone treatment. The renal and nonrenal clearance were unaffected by spironolactone administration and the faecal excretion of digoxin was about 15% under both experimental conditions. Based on these results influences on the parameters of the microsomal enzyme system and digoxin elimination measured were not found following therapeutic doses of spironolactone. Therapeutic doses of spironolactone, do not influence liver microsomal enzyme activity and digoxin elimination to any significant extent.

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Individual variation in response to thiopentone

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The anaesthetic records of 540 patients operated on at the City Hospital, Aarhus, during a 3-week period from May to June 1976 were studied. The purpose was to elucidate the influence of age, sex, concentration of haemoglobin, creatinine concentration in serum, heart disease and the premedication given, on the size of intravenous induction dose of thiopentone. The dose was not significantly different from 20–60 years of age, but after 60 years significant falls in dose were seen with increasing age. The significance was increased when a weight-related dose was used for the calculations. A 6% difference indicating a smaller dose requirement in women was not significant.

When premedication with hyoscine and morphine had been used, significantly less thiopentone was given to induce anaesthesia than after atropine, diazepam and pethidine. When both sexes were considered together no correlation between haemoglobin concentration and induction dose of thiopentone was found, but in two groups of women, there may be a weak negative correlation between these two variables ($r \simeq -0.3$, $P = 0.025$). Thirty-seven patients on permanent treatment with digoxin and a diuretic required significantly less thiopentone than 37 patients, otherwise comparable, not receiving this medication. About 60% of the dose, used in patients who were to have operations in the limbs, was needed in 21 patients who were to be operated on for valvular heart disease. Eleven patients with moderately elevated serum creatinine (average 2.1 ± 1.0 mg/100 ml) required 11% (NS) less than eleven patients with normal serum creatinine (1.1 ± 0.2 mg/100 ml).

Attenuation of psychic sequelae from ketamine

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Ketamine is the only available general anaesthetic which is effective by both the intramuscular and intravenous routes, but it causes troublesome intra-operative complications (hypertonus, hypertension and tachycardia) and postoperative sequelae (emergence delirium and unpleasant 'dreams'). However the usefulness of ketamine is such as to warrant continuing attempts to attenuate these side effects. Preoperative and intraoperative diazepam (Coppel, Bovill & Dundee, 1973) or other benzodiazepines (Johnstone, 1972) and droperidol (Becsey, Malamed, Radnay & Foldes, 1972) will reduce both emergence delirium and unpleasant 'dreams'.

This is the first systematic comparison of the efficacy of various benzodiazepines, neurolept combinations and standard premedicants under constant conditions. Women of reproductive years (40–70 kg) undergoing minor gynaecological operations were anaesthetized with 2 mg/kg ketamine and increments as required for 5–10 min operations. Normal premedicant doses of the drugs were injected intravenously in random order approximately 10 min before anaesthesia. Emergence delirium was classed as prolonged if lasting for more than 10 min and severe if upsetting attendants or requiring active treatment. Six hours after anaesthesia, patients were asked if they had any unpleasant dreams and if, in similar circumstances, they would accept the same anaesthetic again.

Table 1 shows that all the medications studied reduced ketamine sequelae to some extent and increased patient acceptability of this form of anaesthesia. However, significantly beneficial effects on all three aspects of drug action studied were only found with lorazepam (4 mg), flunitrazepam (1.5 mg) and the droperidol (5 mg)–fentanyl (0.1 mg) combination. This latter caused an unacceptably high incidence of respiratory depression before and during anaesthesia.

The effects of the three benzodiazepines were further studied when given intravenously 30–40 min before anaesthesia. In 20 patients diazepam caused no reduction in emergence delirium. Flunitrazepam (20 patients) was not as effective as when given 10 min before anaesthesia. In contrast, there was no troublesome emergence delirium in 35 patients given 4 mg lorazepam, only one had unpleasant 'dreams'

Table 1 Percentage incidence of postoperative sequelae in patients anaesthetized with 2 mg/kg ketamine preceded by drugs shown, given intravenously 10 min before operation

Preliminary medication	*Number of patients*	*Severe/prolonged emergence delirium*	*Unpleasant 'dreams'*	*Anaesthesia unacceptable to patient*
Saline	50	40	35	64
Benzodiazepines				
Diazepam 15 mg	20	30	40	30*
Diazepam 0.2 mg/kg	20	20	25	15***
Flunitrazepam 1.5 mg	20	10**	0***	0***
Flunitrazepam 0.02 mg/kg	20	35	5**	15***
Lorazepam 4 mg	25	4**	4**	4***
Neurolept combinations				
Droperidol 5 mg	20	5**	30	30*
Droperidol 5 mg + Fentanyl 0.1 mg	20	0***	10*	15***
Standard premedicants				
Pentobarbitone 100 mg	10	40	20	20*
Hydroxyzine 100 mg	20	0***	35	35*
Pethidine 100 mg	10	15	20	40
Promethazine 25 mg	20	5**	20	0***

Differences from control (saline) * $P<0.050$, ** $P<0.005$, *** $P<0.001$.

and all were prepared to have the same anaesthetic on a subsequent occasion.

On the basis of this study lorazepam (4 mg), given intravenously at least 30 min before anaesthesia is a reliable method of reducing the sequelae of ketamine. This has now been given to 120 patients before a continuous ketamine infusion and no patients has disturbing sequelae or found anaesthesia unacceptable for this reason. Continuing studies suggest that oral lorazepam may be equally effective.

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In vivo and *in vitro* studies on cholinomimetic miotic drugs

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A study has been made of the action of six cholinoceptor agonists on the pupil in man, on the isolated rabbit iris sphincter (Smith, 1976) and on guinea pig ileum. For the *in vivo* study, at least four concentrations of each drug were applied dropwise to one conjunctival sac of a healthy subject and the resulting pupillary effects were measured by infra-red television pupillometry (Lowenstein & Loewenfeld, 1958) in darkness. Drug effects on pupillary diameter and on the magnitude of the light reflex were determined. All drugs causing miosis also inhibited the light reflex proportionally; log dose-response curves for both effects were constructed, from which EC_{50} values and relative potency ratios were obtained. Comparable potency ratios for iris sphincter and ileum *in vitro* (five or more experiments) were obtained from log dose-response lines by standard methods.

The mean potency ratios to carbachol for all actions are given in Table 1. No value was obtainable for pilocarpine on the isolated iris because on this tissue the drug was a partial agonist, capable of contracting it to only 20% of maximum. In contrast to the choline esters which are fully ionized, the three weak bases, arecoline, aceclidine and pilocarpine, which are only partially ionized at physiological pH, produced full miosis. Their order of potency corresponded with that found *in vitro*.

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Table 1 Mean molar potency ratios (carbachol = 1) for miotic drugs

	In vivo		In vitro	
	Miosis	*Light reflex inhibition*	*Rabbit iris sphincter*	*Guinea pig ileum*
Arecoline hydrobromide	350	565	0.49	
Aceclidine hydrochloride	87	105	0.14	0.19
Pilocarpine nitrate	61	91		0.092
Carbachol	1	1	1	1
Methacholine chloride	0	0	0.23	1.41
Acetylcholine chloride	0	0	0.014	1.93

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Arachidonic acid, PGE_2 and $PGF_{2\alpha}$ determined by GC-MS 24 h after irradiation of human skin with ultraviolet B (290–320 nm) and the effect of indomethacin

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We have previously reported raised concentrations of arachidonic acid and prostaglandin E_2 and $F_{2\alpha}$-like materials in the inflammatory exudate obtained at different times after exposure of human skin to ultraviolet B (UVB) irradiation (290–320 nm) (Black, Greaves, Hensby & Plummer, 1976a). The greatest concentrations of these compounds coincided with maximum erythema (24 h).

We have now used quantitative GC-MS to determine the concentrations of arachidonic acid, PGE_2 and $PGF_{2\alpha}$ in individual exudate samples obtained by the method of Black, Greaves, Hensby & Plummer (1976b) 24 h after exposure of human abdominal skin to three times the minimum erythema dose before and after oral and topical indomethacin (Table 1).

Irradiation significantly increased arachidonic acid ($P<0.001$) PGE_2 ($P<0.001$) and $PGF_{2\alpha}$ ($P<0.001$) compared with control values. Although both topical and oral indomethacin reduced the PGE_2 and $PGF_{2\alpha}$ below control values the erythema was only partially suppressed. The possibility that neither these prostaglandins, nor any other product of the prostaglandin cycloxygenase pathway are major mediators of UVB erythema therefore arises. However, firm conclusions must await turnover studies of arachidonic acid and its metabolites, together with simultaneous serial blood flow measurements in irradiated skin.

Table 1 Concentrations (mean ± s.e. mean) of PGE_2, $PGF_{2\alpha}$ and arachidonic acid 24 h after UVB (290–320 nm) irradiation and the effects of topical and oral indomethacin

	Arachidonic acid	*PGE_2*	*$PGF_{2\alpha}$*
		(ng ml^{-1} exudate)	
Control ($n=46$)	284.6 ± 24.6	21.9 ± 1.2	18.2 ± 1.1
Control + oral indomethacin*	723.0 ± 74.0	15.7 ± 2.9	15.2 ± 2.0
($n=5$)	$P<0.001$	$P>0.1$	$P>0.3$
24 h UVB	785.4 ± 56.8	49.4 ± 5.2	32.4 ± 2.9
($n=13$)	$P<0.001$	$P<0.001$	$P<0.001$
24 h UVB + topical indomethacin†	909.4 ± 104.5	16.9 ± 3.4	11.6 ± 1.5
($n=8$)	$P<0.001$	$P>0.1$	$P>0.02$
24 h UVB + indomethacin vehicle††	471.9 ± 52.7	24.6 ± 3.4	18.5 ± 1.5
($n=8$)	$P<0.005$	$P>0.4$	$P>0.9$
24 h UVB + oral indomethacin*	894.0 ± 125.6	16.6 ± 1.3	14.0 ± 1.4
($n=5$)	$P<0.001$	$P<0.02$	$P>0.2$

* 50 mg every 8 h for 24 h. † 2.5% indomethacin; †† Propylene glycol; ethanol; dimethylacetamide 19:19:2 by volume.

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Aryl hydroxylase activity in psoriatic skin

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Psoriasis is a chronic disorder of epidermal proliferation. The lesions are characterized by an increased mitotic rate of the basal layer, with abnormal keratinization of the stratum corneum (Mier & Cotton, 1976). The uninvolved skin of psoriatics also has abnormal structural and biochemical properties (Mier & Cotton, 1976) compared to skin from non-psoriatic individuals. Aryl hydrocarbon hydroxylase (AHH) is a microsomal mixed-function oxidase system which is present in normal epidermis (Chapman, Rawlins & Shuster, 1977). Since AHH may play a role in determining the efficacy and toxicity of drugs within the skin, we have examined its activity in epidermal skin biopsies from psoriatic patients.

Epidermal blisters were raised on forearm skin of psoriatic patients and normal volunteers with a suction pump. The blister tops were removed and immediately placed in ice cold isolation medium (20 mM tris-(hydroxymethyl) methylamide in 0.3 M mannitol, pH 7.4). The epidermis was divided into two fractions, weighed, and transferred to a tissue culture system (Nebert & Gelboin, 1968) containing either 0 μM or 50 μM benzanthracene. After preincubation for 18 h at 37°C in an oxygen atmosphere the activity of AHH in epidermal microsomes was determined as previously described (Chapman, Rawlins & Shuster, 1977).

In the absence of benzanthracene the activity of AHH was similar ($P > 0.20$, $n = 10$) in both psoriatic lesion (0.64 ± 0.19 pmol mg^{-1} h^{-1}) and noninvolved skin from psoriatic patients (0.69 ± 0.07 pmol mg^{-1} h^{-1}). However, in both instances these were significantly less ($P < 0.05$) than the activity of AHH in epidermis from normal volunteers (1.13 ± 0.17 pmol mg^{-1} h^{-1}, $n = 11$).

Preincubation with 50 μM benzathracene increased the absolute activity of AHH in epidermis in normal individuals to 2.29 ± 0.30 pmol mg^{-1} h^{-1} ($P < 0.05$) and to a lesser extent in the uninvolved skin of psoriatic patients (1.18 ± 0.24 pmol mg^{-1} h^{-1}, $P < 0.0001$) and not at all in psoriatic lesions (0.68 ± 0.19 pmol mg^{-1} h^{-1}, $P > 0.05$).

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Bumetanide and frusemide: qualitative differences

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Bumetanide has diuretic activity and therapeutic efficacy broadly similar to that of frusemide. Its potency relative to frusemide is 40:1 at doses ordinarily used in clinical practice (0.5–2 mg), but at high doses (5–10 mg) in chronic renal failure the relative potency is 20:1 (Allison, Lindsay & Kennedy, 1975). The effects of the two diuretics on renal handling of urate (Davies, Lant, Millard, Smith, Ward & Wilson, 1974) and potassium (Branch, Read, Levine, Vander Elst, Shelton, Rupp & Ramsay, 1976) may differ. We compare the diuretic properties of the two drugs in a three dose parallel line bioassay in twelve healthy males. Subjects received six oral treatments (bumetanide 0.5, 1 and 2 mg; frusemide 20, 40 and 80 mg) at intervals of at least 1 week. The order of treatments was balanced. The responses (urine volume, electrolyte excretion and changes in plasma uric acid) were measured over a 6 h period, which encompasses the full activity of each diuretic (Branch *et al.*, 1976). Variables were measured by standard methods and without knowledge of the treatment taken. The linearity, significance of slopes, and parallelism of log dose-responses were tested by analysis of variance. Urine volume responses were rendered linear by log transformation. Both diuretics produced log dose-responses for urine log-volume and sodium excretion which were linear and had significant slopes (all $P < 0.001$). The log dose-responses for the diuretics were non-parallel (for log volume, $P = 0.001$; for sodium excretion, $P < 0.025$). Ignoring non-parallelism, the relative potency (bumet-

anide:frusemide) for sodium excretion was 46:1 (95% C.L. 39:1–56:1), agreeing with previous studies in this dose range.

Extrapolating from the slopes observed, the predicted natriuretic potency, bumetanide: frusemide, falls with increasing dose from 92:1 at bumetanide 0.125 mg, to 45:1 at bumetanide 1 mg, and 21:1 at bumetanide 10 mg. For urine potassium excretion the log dose-response after frusemide was highly significant ($P < 0.001$), and that for bumetanide was not ($P > 0.1$). The relative potency (bumetanide:frusemide) for potassium excretion was 21:1, with 95% C.L. of 7:1 to 38:1. These confidence limits did not overlap those for sodium excretion in the dose range tested. The kaliuretic potency of bumetanide was therefore significantly ($P < 0.05$) less than its natriuretic potency, when related to frusemide. The diuretics produced significant ($P < 0.005$) and parallel dose-related increases in plasma uric acid over 6 hours. The relative potency (bumetanide:frusemide) was 65:1, an estimate which did not differ significantly from that for urine sodium excretion. The results for urine volume and sodium excretion suggest that bumetanide and frusemide may act on different renal receptors. The relatively low potency of bumetanide at high dosage in chronic renal failure may be a function of its pharmacological properties, and not of the disease state. The findings for potassium excretion might reflect different degrees of carbonic anhydrase inhibition by the two drugs.

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Pharmacokinetics of diflunisal elimination in patients with renal insufficiency

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To determine the elimination kinetics of the salicylate derivative diflunisal (2′,4′-difluoro-4-hydroxy-3-biphenylcarboxylic acid) doses of the drug (500 mg) were administered to five normal volunteers ($C_{Cr} > 95$ ml/min), nine patients with moderate renal insufficiency (MRI) ($C_{Cr} = 10$ to 50 ml/min), three patients with preterminal renal insufficiency (PRI)) ($C_{Cr} = 2–10$ ml/min) and five patients with terminal renal insufficiency (TRI) ($C_{Cr} < 2$ ml/min). Diflunisal concentrations in body fluids were measured fluorimetrically using the method described by Tocco, Breault, Zacchei, Steelman & Perrier (1975). Total diflunisal glucuronides were estimated using a preliminary acid hydrolysis before the fluorimetric procedure.

Diflunisal was rapidly absorbed to reach peak plasma concentrations of 60 to 80 μg/ml within 2 h after the dose both in normals and in patients with MRI and PRI. Lower values were obtained in patients with TRI (approximately 30 μg/ml within 4 h after dosing) possibly due to decreased oral availability resulting from chronic aluminium hydroxide treatment. Total diflunisal glucuronides (ester and

Table 1 Elimination kinetic data of diflunisal after single oral doses of 500 mg

Groups	n	$T_{\frac{1}{2}el}$ *(h)*	k_{el} *(h^{-1})*	*Total plasma clearance (ml/min)*	V_d *area (l)*	*Recovery in 72-h urine (% of dose)*
Normals	5	10.8 ± 8	0.065 ± 0.005	7.9 ± 0.7	7.3 ± 0.4	76.8 ± 3.5
Patients						
MRI	9	22.4 ± 2.5	0.034 ± 0.004	6.9 ± 1.0	12.7 ± 1.6	55.0 ± 5.7
PRI	3	59.6 ± 3.3	0.012 ± 0.001	2.9 ± 0.2	14.3 ± 0.7	9.5 ± 5.2
TRI	5	114.9 ± 13.9	0.006 ± 0.001	(2.8 ± 0.5)	(27.0 ± 3.6)	(2.7 ± 0.9)

ether glucuronides) rapidly appeared in plasma reaching maximal concentrations (8 to 10 μg/ml) after 2 to 4 h and in patients with TRI, approximately 6 h after dosing. The parameters of diflunisal elimination kinetics obtained in the four groups of subjects are given in Table 1. In patients, plasma elimination half-life of diflunisal ($T_{\frac{1}{2}}$) was progressively prolonged with increasing degrees of renal function impairment. This was associated with increasing retention of the conjugated metabolites in plasma. The associated $T_{\frac{1}{2}}$ values of total diflunisal glucuronides were 14.8 h in normals, 32.7 h in the MRI-group, 84.4 h in the PRI-group and 219.4 h in the TRI-group. In each group, both plasma concentration decay-curves (of the unchanged drug and of the glucuronides) tended to be parallel suggesting similar decay rates. The apparent volume of distribution (V_d) in the first two groups of patients with renal insufficiency was significantly ($P < 0.01$) increased, and almost twice that of normals.

These results demonstrate that impaired excretion of diflunisal (predominantly as glucuronides) in renal insufficiency is associated with increasing degree of diflunisal-glucuronides retention in plasma and with $T_{\frac{1}{2}}$-prolongation of the unchanged drug. This suggests that biotransformation of diflunisal to its glucuronides is either limited by the presence of the elevated glucuronide concentrations in the body or that the glucuronides retained in plasma may be conjugated to diflunisal, probably involving biliary excretion of the conjugated metabolites and enterohepatic recirculation after deconjugation.

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Pharmacokinetics of nitrazepam in young volunteers and aged patients

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In order to elucidate possible pharmacokinetic differences of nitrazepam between elderly patients and young people, both an acute and a prolonged study with the drug were carried out.

In the acute experiment a single oral dose of nitrazepam (5 mg) was given at 20.00 h to twenty-five healthy volunteers (age 21–38 years) and to twelve elderly patients (age 66–89 years). The patients suffered from various debilitating diseases (heart failure, coronary heart disease, hemiplegia, leukaemia, paralysis agitans, diabetes, etc.). Some of them were totally confined to bed and suffered from several diseases. Nitrazepam concentrations in the serum were determined up to 72 h after the ingestion of the drug by direct ^{63}Ni-ELC-g.l.c. method according to Kangas (1977).

The volume of distribution during elimination phase and other pharmacokinetic parameters were calculated by assuming a total absorption of nitrazepam (Table 1).

In the more prolonged study, nitrazepam (5 mg) was given daily at 20.00 h to healthy volunteers for 14 days and to twelve aged patients for 2 months. The concentrations of nitrazepam were measured during treatment 12 h after the ingestion of the drug.

Table 1 Some pharmacokinetic parameters of nitrazepam

	Young volunteers (n=25)	*Old patients (n=12)*	P
Peak concentrations (ng/ml)	39.9 ± 12.7	21.8 ± 9.2	<0.001
V_d (l/kg)	2.4 ± 0.8	4.8 ± 1.7	<0.001
$T_{\frac{1}{2}}$ (β) (h)	28.9 ± 7.4	40.4 ± 16.2	<0.01
Cl_{tot} (l/h)	4.1 ± 2.0	4.7 ± 1.5	NS
β (h^{-1})	−0.0257 ± 0.0074	−0.0198 ± 0.008	<0.0.5
AUC (ng ml^{-1} h^{-1})	1371 ± 398	1078 ± 295	<0.5 <0.05

Symbols: AUC = area under the serum concentration—time curve; β = slope of β-phase elimination curve; $Cl_{tot} = \beta . V_\beta$; $T_{\frac{1}{2}}$ = half-life; $V_d = (Dose/_\beta \cdot AUC)/kg$ (total absorption assumed).

The steady state concentration of nitrazepam in the serum was reached in the young volunteers about 3.5 days and in the aged patients about 7.5 days after the beginning of the treatment. The theoretically expected steady state levels calculated on the ground of the acute experiment were 57 ± 17 ng/ml in young volunteers and 45 ± 12 ng/ml in aged patients. The concentrations actually measured were 59.7 ± 21.0 ng/l and 59.8 ± 30.3 ng/ml, respectively. The serum half-life of nitrazepam after the ending of the treatment was 24.2 ± 4.9 h in young volunteers and 39.6 ± 13.8 in aged patients ($P < 0.01$).

Our study showed a marked difference in the volume of distribution of nitrazepam and in some other pharmacokinetic parameters between sick elderly patients and healthy young volunteers. Recently, in a study of Castleden, George, Marcer & Hallet (1977) no differences were found in the pharmacokinetics of nitrazepam between healthy aged and young persons. It seems thus reasonable to assume that the immobility of our patients and diseases in themselves are the main determinants of the differences. Similar pharmacokinetic differences in the old and the young have been shown earlier with another benzodiazepine, diazepam (Klotz, Avant, Hoyumpa, Schenker & Wilkinson, 1975).

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Activity of N-desmethyldiazepam (nordiazepam)

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N-desmethyldiazepam (nordiazepam) is a metabolite of many benzodiazepines, yet, though the parent compounds have been investigated in detail, little is known about the activity of nordiazepam itself, even though it may be largely responsible for their therapeutic effect. Drugs with nordiazepam as a metabolite are used mainly as anxiolytics, but nordiazepam also shortens sleep onset latencies, reduces awake activity and drowsy sleep, prolongs total sleep time and modifies the sleep of the recovery night. Indeed, behaviour may be modified for 28–30 h after ingestion of nordiazepam or drugs with nordiazepam as their principal metabolite (Nicholson, Stone, Clarke & Ferres, 1976; Nicholson, Stone & Clarke, 1976). However, though nordiazepam has persistent anxiolytic and hypnotic activity, it may have much less effect on performance than other 1,4-benzodiazepines (Borland & Nicholson, 1977; Palva & Linnoila, 1977), and it is in this context that we have studied immediate and residual effects on visuo-motor coordination (Borland & Nicholson, 1975).

Six healthy male subjects were used. Their ages ranged from 25–43 (mean 36) years and their weight ranged from 67–84 (mean 75) kg. All subjects were required to avoid alcohol within 24 h of the experiments, and they were not involved in any other form of drug therapy. There were no restrictions on the consumption of non-alcoholic beverages. Performance was measured 0.5, 2.5, 4.5 and 6.5 h after morning ingestion of 10 mg diazepam and 5 and 10 mg nordiazepam, and 10, 12, 14 and 16 h after overnight ingestion of 5 and 10 mg nordiazepam. Performance after a drug was compared with performance after ingestion of matching placebo. The experiment was double-blind, treatments were presented in random order with 7 days between treatments and change in performance was analysed by analysis of variance. With the morning ingestion of 10 mg diazepam performance was impaired at 0.5 h ($P < 0.01$) and 2.5 h ($P < 0.05$), but with the morning ingestion of 10 mg nordiazepam performance was not impaired at these times though performance diverged from that after placebo ($P < 0.05$) and was impaired at 6.5 h ($P < 0.05$). Performance was not impaired after the overnight ingestion of 5 and 10 mg nordiazepam or after the morning ingestion of 5 mg nordiazepam.

It is considered that the limited impairment of performance after nordiazepam may be due to the ability of subjects to overcome, at least in part, any effect of the drug, or the impaired ability of subjects to sustain high levels of performance for several hours. In either case the observations suggest that nordiazepam, or drugs with nordiazepam as a metabolite, may be useful in the management of sleep difficulties when a persistent daytime anxiolytic effect is required.

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The clinical effects of the isomers of flupenthixol—the consequences of dopamine receptor blockade in acute schizophrenia

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In a recent clinical trial (Crow & Johnstone, 1977) it was demonstrated that the therapeutic effects of the thiaxanthene flupenthixol are confined to the dopamine-receptor blocking α-(cis-) isomer. This trial has enabled us to examine the selectivity of the effects of this neuroleptic drug upon the characteristic changes in schizophrenia.

Forty-five patients, diagnosed as suffering from acute schizophrenic illnesses by Present State Examination criteria, were randomly and blindly allocated to treatment with α-flupenthixol, β-flupenthixol or placebo. The dose of flupenthixol was 6 mg/day for 6 days, followed by 9 mg/day for 22 days. All patients also received orphenadrine 50 mg three times daily, and doses of chlorpromazine 100 mg were administered where acute behavioural disturbance of severe distress necessitated extra medication. Progress was assessed at weekly intervals with the Krawiecka rating scales, and in terms of the additional chlorpromazine administered.

Differences attributable to medication were not apparent until the third week of the trial. In the third and fourth weeks patients on α-flupenthixol showed a significantly greater ($P < 0.05$) overall improvement than patients in the other two groups. Analysis of individual symptoms using Student's t-test revealed that the superiority of α-flupenthixol was greatest for the positive (hallucinations, delusions, and thought disorder) symptoms of the disease, but was much less apparent for the negative symptoms (psychomotor retardation, poverty of speech and flattening of affect).

An attempt to select a sub-group of patients with particularly characteristic schizophrenic illnesses was made by excluding, firstly, those patients without evidence of progressive deterioration (The 'Feighner' criteria) and secondly, those patients with evidence of mood disturbance ('schizo-affective' psychoses). Examination of the effectiveness of the two flupenthixol isomers in the remaining patients suggested that the relative superiority of the α-isomer was enhanced rather than decreased.

These findings suggest that the therapeutic effects of neuroleptics are directly related to an action upon typically schizophrenic illnesses and characteristically schizophrenic features, but may be confined to the positive symptoms of the disease. In so far as the differences in the actions of the two isomers can be related to their differing dopamine receptor blocking potencies, the results emphasize the role of dopaminergic mechanisms the response to drug treatment in acute schizophrenia.

We are grateful to Dr L.L. Iversen for drawing our attention to the feasibility of this trial, and to Lundbeck Ltd for making available preparations of the isomers of flupenthixol.

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Effects of bone of long-term lithium administration in man and the rat

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Lithium treatment results both in decreased bone calcium in rats (Birch & Jenner, 1973) and in accumulation and retention of lithium in rat and human bone (Birch, 1974). Since lithium is used over long periods in the prophylactic treatment of periodic affective disorders, it is important to determine whether or not it has long term deleterious effects on bone.

Hand radiographs were taken under standard conditions (Nordin, 1976) in a group of 74 lithium treated outpatients (Hullin, McDonald & Allsopp, 1975). Further radiographs were obtained under identical conditions from 37 of this group after approximately 2 years. Morphometric data were obtained by methods described by Nordin (1976).

One hundred weanling rats were divided into two groups, one receiving drinking fluid containing 10 mmol/l LiCl (dose approximately 1 mmol kg^{-1} day^{-1}), the other tap water. Ten control animals were killed at zero time and ten of each of the control and treated animals were killed at intervals up to 1 year. Both femora were removed and stored at −15°C. Postero-anterior contact radiographs of the rat femora were taken under standard conditions.

Of the 37 postmenopausal female patients studied only four were found to have a metacarpal area ratio (cortical area/total area) more than 2 standard deviations below the age matched controls and the mean of the treated subjects was almost identical to control values (Horsman, 1976). Measurements of the phalanges, radius and ulna gave similar results. The distribution of all other results was normal.

In 14 of the 28 female subjects with repeat radiographs there was a significant decrease in cortical width ($P < 0.05$). However, bone loss is a normal feature of ageing in postmenopausal women and the mean rate of loss in this group is closely comparable to that observed in similar, psychiatrically normal, populations (Horsman, 1976). There was only one exceptional case.

These results suggest that lithium does not generally cause acceleration of bone loss in mature humans. However, Christiansen, Baastrup & Transbøl (1976) have reported that in the forearm, the bone mineral content of lithium treated subjects was 93% of control subjects.

In contrast, the results of the morphometric study of young rats treated with lithium show that there may be an effect on growing bone. After 1 year there was a significantly reduced total femoral width (*t*-test $P < 0.0025$) in the lithium treated rats and a less significant decrease in cortical and medullary widths ($P < 0.05$).

We therefore urge caution in the use of lithium in patients of immature bone structure though neither in this study nor in biochemical investigations (Birch, Greenfield & Hullin, 1974) were we able to demonstrate any lithium effect on bone in mature subjects.

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Pharmacokinetic and concentration-effect studies with metoclopramide

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Metoclopramide (4-amino-5-chloro-2-methoxy-N-)2-diethylamino ethyl) benzamide, MCP) is a widely used anti-emetic agent. The drug is thought to have actions on the chemoreceptor trigger zone and on the gastro-intestinal tract (Robinson, 1973a). It is also a dopamine antagonist (Dougan, Mearrick & Wade, 1974) and in man elevates serum prolactin (McCallum, Sowers, Hershman & Sturdevant, 1976). Extrapyramidal side effects have been reported in man (Robinson, 1973b).

There have been no studies of the kinetics or concentration-effect relationships of MCP in man because of a lack of a suitable, sensitive assay. We have developed a sensitive specific assay based on stable isotope dilution and utilizing gas chromatography-mass spectrometry with selected ion monitoring. With this method we have studied the pharmacokinetics and plasma concentration-effect relationships in a placebo controlled study in seven normal male volunteers (age 26–36 years).

Subjects fasted overnight and refrained from alcohol for 36 h beforehand. They were given an intravenous injection of 10 mg MCP HCl or saline (S). Samples for analysis were drawn from an indwelling forearm cannula. Studies of gastro-intestinal and central sedative effects were performed immediately (Study 1) and 3 h (Study 2) after the injection. Blood samples were drawn for drug analysis at 0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 360 and 480 min after administration.

Gastric emptying was assessed by measuring the plasma concentration profile of ethanol after a drink of 500 ml of warm (37°C) orange cordial containing ethanol (70 mg/kg body weight). Sedative effects were measured by a self-scored visual analogue scale administered every 5 min during the emptying studies. Serum prolactin concentrations were measured by radioimmunoassay. Statistical analyses were performed by 3-way analysis of variance; results are expressed as mean $\pm$ s.e. mean. Gastric emptying time as measured by time to peak alcohol concentration was significantly decreased by MCP: Study 1, MCP 11.79 ± 1.79 min, S 20.71 ± 2.3 min ($P < 0.005$), Study 2, MCP 13.04 ± 1.81 min, S 20.71 ± 1.8 min ($P < 0.001$). Sedation scores after placebo were low; however in the MCP studies significant sedation occurred ($P < 0.001$) during both the alcohol absorption tests suggesting a drug-alcohol interaction. There was a linear correlation between increase in serum prolactin over mean placebo levels and MCP concentration in plasma ($r = 0.806$).

Plasma concentration time curves were fitted to a 2-compartment model by least squares regression analysis. Terminal half-lives ranged from 120 to 260 min and clearances from 556 to 1313 ml/min.

The experiments described demonstrate a relationship between plasma concentration of MCP and pharmacological effects when the drug is given intravenously.

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Serum prolactin as an index of dopamine receptor blockade in acute schizophrenia

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The actions of neuroleptic drugs on central dopaminergic mechanisms (Carlsson & Lundqvist, 1963; Miller, Horn & Iversen, 1974) are well established. There is suggestive evidence (Crow & Johnstone, 1977) that these actions are related to the therapeutic effects of these drugs in schizophrenia, and for this reason other possible indices of dopamine receptor bloackade are of clinical interest. It is likely that the tubero-infundibular dopamine system inhibits prolactin secretion (Fuxe, Hökfelt & Nilsson, 1969) and administration of neuroleptic drugs is associated with increased prolactin secretion (Wilson, Hamilton, Boyd, Forrest, Cole, Boyns & Griffiths, 1975).

In a recent study (Crow & Johnstone, 1977) we investigated the relative therapeutic efficacy in patients with acute schizophrenia of the two isomers of flupenthixol, which differ in their ability to block the dopamine-sensitive adenylate cyclase (Miller *et al.*, 1974). The effects of blockade of dopaminergic transmission in the tubero-infundibular system were assessed by estimation of prolactin in serum samples from the patients in this study. Blood samples were removed at weekly intervals, and separated sera were stored at −40°C. Prolactin was estimated by radio-immunoassay in terms of Research Standard A for human prolactin (in ampoules coded 71/222) (Cotes, 1973) using I^{125}-human prolactin (hPRL VLS 3), rabbit antiserum to human prolactin (VLS batch 3 at final dilution 1:80,000) and a double antibody separation.

In patients receiving α-flupenthixol (6 mg daily for 6 days, and 9 mg daily for 22 days) prolactin levels were increased from a mean pre-treatment level of 459 mu/l (s.d. 423 mu/l) to 376% (s.d. 302%) and 470% (s.d. 423%) of pre-treatment levels at the ends of the first and the fourth weeks of treatment respectively. The corresponding values for placebo and β-flupenthixol treated patients were, respectively, after 1 week, 75% (s.d. 45%) and 172% (s.d. 189%), and, after 4 weeks, 97% (s.d. 51%) and 151% (s.d. 37%), of pre-treatment levels. In the group of patients treated with α-flupenthixol no significant relationship was apparent between increase in prolactin concentration and clinical improvement, whether the latter was considered over the 4-week period of the trial, or over weeks 2 to 4, when the improvement attributable to medication was observable. Moreover the increase in prolactin secretion in the patients on α-flupenthixol preceded the improvement attributable to medication by approximately 2 weeks. This finding suggests that if the anti-psychotic effect, as seems likely from our earlier findings (Crow & Johnstone, 1977) is dependent upon central dopamine receptor blockade, it is a somewhat indirect effect. Perhaps dopamine receptor blockade permits other and longer-term changes to take place, and it is these changes rather than the dopamine receptor blockade itself which are reflected in the clinical improvement seen on medication.

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Neuroregulation of thyrotrophin (TSH) secretion in man

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Effects of caffeine and cyclizine alone and in combination on human performance and subjective ratings

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Cyclizine is used as an antimetic and anti-motion sickness agent and being related to histamine (H_1) antagonists, may produce drowsiness (Lederer & Putnam, 1958; Brand, Colquhoun, Gould & Perry, 1967). Caffeine usually produces increased alertness, though effects on performance are inconsistent (Weiss & Laties, 1962). No information is available on the effects of combinations of the two drugs given simultaneously though combinations of this type are administered with ergotamine for migraine. Effects of the drugs alone and together are reported here.

Two studies were performed both on twelve volunteers. All treatments were administered in identical capsules under double-blind conditions. In Trial 1 the treatments were: caffeine (base) 75, 150 and 300 mg; cyclizine (HCl) 25 and 50 mg; lactose dummy. In Trial 2 they were: caffeine 100 mg; cyclizine 50 and 100 mg; caffeine 100 mg + cyclizine 50 mg; and caffeine 100 mg + cyclizine 100 mg; lactose. Administration was at weekly intervals based on a balanced 6 x 6 Latin square design. A test battery lasting 2 h began 45 min after treatment and was repeated after a 1 h lunch. Sound-proof conditions at 21°C were used. Analysis of variance was used on all variables and $P < 0.05$ taken as significant.

In Trial 1 all doses of caffeine improved auditory vigilance tested over 1 h using the Wilkinson (1968) test except for the 300 mg 0.75 to 1.75 h post treatment. Auditory reaction time, tested over 15 min was also significantly shortened by caffeine 75 and 150 mg 1.75 to 2 h post treatment. Tapping rate, measured over 1 min increased after caffeine 150 and 300 mg. Values after cyclizine failed to differ from those after lactose in these three tests. No changes occurred in arithmetic, short memory (STM) or digit symbol substitution (DSST). Alertness, assessed using visual analogue scales, increased after all doses of caffeine and cyclizine 25 mg at 2 h 40 min and persisted to 6 h after caffeine 150 and 300 mg. Cyclizine 50 mg produced no effect.

In Trial 2 no treatment produced any changes in vigilance differing from lactose. Mean correct detections, however, were significantly higher after caffeine 100 mg than after both doses cyclizine. Both caffeine/cyclizine combinations gave intermediate values not differing from lactose. A similar pattern occurred in STM. No differences occurred in reaction time between active drugs and lactose. Mean values were higher after cyclizine 100 mg, and were significantly lower combined with caffeine 100 mg. A similar pattern occurred in arithmetic though here performance after cyclizine 100 mg was less than after lactose at 5 h. Ratings of alertness were increased after caffeine 100 mg at 2 h 40 min and decreased by cyclizine 100 mg at 6 h compared with lactose.

In conclusion caffeine improved auditory vigilance, reaction time, and tapping, but cyclizine up to 100 mg produced no significant difference from lactose except reduced arithmetic at 5 h. Cyclizine tended to impair performance, and differences between the two active drugs were frequent. Similar changes occurred in subjective ratings of alertness. Combination of the two drugs gave either intermediate values not differing from lactose, or values similar to caffeine given alone, but never to cyclizine.

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Behavioural regulation of nicotine intake in cigarette smokers presented with a 'shortened' cigarette

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Interest in the smoker's self-regulation of nicotine intake has recently been extended from experimental situations in which the nicotine delivery of cigarettes is varied (Ashton & Watson, 1970; Frith, 1971; Russell, Wilson, Patel, Feyerbend & Cole, 1975) to those in which the length of cigarette available to be smoked is altered (Gritz, Baer-Weiss & Jarvik, 1976).

In the present study fourteen volunteers (seven male, seven female; mean age 22.9 years) who regularly smoked 15–20 middle-tar cigarettes per day, attended the laboratory at the same time on two successive days, on one occasion fully smoking a cigarette, and on the other smoking only two-thirds of the length of tobacco rod normally consumed. In the 'two-thirds condition' the cigarettes were not cut, but were marked with a red line beyond which the subject was told not to smoke.

Subjects differed in the order in which they smoked the full and two-thirds cigarettes but all subjects had 24 h experience smoking the two-thirds cigarettes before smoking one in the laboratory.

Heart rate and fingertip temperature were recorded during the smoking of each laboratory cigarette and venous blood samples taken pre- and post-smoking were assayed for nicotine. Subjects were observed through a 'one-way' screen and the length of time taken to smoke each cigarette, and the duration of each puff and interpuff interval, marked on an event recorder.

Thirteen subjects adapted to the shorter length of available cigarette by increasing their puff duration or decreasing their interpuff interval. Since either method would make possible a faster rate of nicotine intake, behavioural change is best expressed as total time spent puffing/total time cigarette lit × 100. The mean percent time spent puffing increased from 5.46 in the full condition to 7.61 in the two-thirds condition ($P < 0.005$).

Since the amount of nicotine retained by the filter of a cigarette is proportional to the total amount which passes through it, the amount of nicotine inhaled by the smoker can be estimated by measuring the nicotine left in the filter. On this basis the nicotine dose obtained from the two-thirds cigarettes varied between 66% and 95% of the full cigarette dose.

Blood nicotine levels for the subjects for whom complete data is available indicate that, on average, 71.7% of the full-cigarette nicotine dose was obtained in the two-thirds cigarette condition.

There were no significant differences in heart rate increase or in subjective ratings of enjoyment/satisfaction between the full and two-thirds cigarettes, although the decrease in fingertip temperature was significantly greater in the full cigarette condition.

Comparison of two 24 h collections of butts from full length cigarettes showed that the intervening experience with the two-thirds cigarettes had led subjects to leave significantly longer butts.

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DEMONSTRATIONS

The use of event-related slow potentials of the brain as an objective method to analyse the actions of centrally-active drugs in man

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Up to now the study of centrally-acting drugs in man has been based on tests which mainly depend upon subjective methods. In the present method centrally acting drugs are studied by examining their effects on the characteristics of event-related slow potentials, the archetype of which is the contingent negative variation (CNV). The CNV, first described by Grey Walter and his colleagues in 1964, is a slow negative potential measured from scalp electrodes. The potential builds up maximally at the vertex when a warning stimulus (S_1), for example a light flash or a tone, is followed by an imperative stimulus (S_2), a second or so later, to which the subject must respond in some way, usually by pressing a button. Although a single trial is followed by a detectable slow potential, it is common practice to average a number of time-locked trials (in the present study 10) in which the S_1 and S_2 interval is constant (1.25 s) whilst the intertrial interval (S_2–S_1) is varied randomly between 5–10 s. After a suitable number of control recordings (each an average of 10 trials) the drug under study is administered and the further recordings are made at suitable intervals for an appropriate length of time. The method is sensitive and reproducible.

The basic method has been described elsewhere (Ashton, Millman, Telford & Thompson, 1974). This demonstration will show how a PDP8/E Digital computer linked to a Floppy Disk auxiliary can be used to store and recall individual trials and blocks of averaged trials, from which a number of variables can readily be calculated and in which potentials due to eye movements and blinks have been automatically compensated.

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Codeine-induced facilitation of memory functions

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Some drugs with potential for abuse induce state-dependent effects on memory processes (Overton, 1973). We have recently reported (Liljequist, Linnoila & Mattila, 1977) that diazepam, 10 mg orally, interferes with memory functions probably by impairing acquisition and facilitating recall.

The present double-blind study with single oral doses of codeine (50 mg) or chlorpromazine (25 mg) on healthy paid students, 20 for each drug, aimed to analyze the drug effects on acquisition, retention, and recall of memory by means of a paired-association learning task. The drugs were given 30 min before the learning session which in turn lasted for 40 min. The transfer of learning between four states was set up by dividing the twenty subjects to two subgroups ten subjects each. They received placebo (P) and codeine (C) on four consecutive days; group I: PPCP and Group II: PCCP. This arrangement allowed the comparison of the state effects as well as separate effects between four treatment combinations: PP, PC, CP, and CC. The first letter refers to the treatment on day 1 when learning the material, and the second to day 2 when the material was recalled.

The number of incorrect responses (mean $\pm$ s.d.) recalled from these four state groups were PP 4.6 ± 1.0, PC 3.9 ± 2.4, CP 3.2 ± 2.0, and CC 1.7 ± 1.5. The PP and CC situations differed significantly (two-way analysis of variance, $F = 12.84$,

$P < 0.001$) suggesting a positive state-dependent effect of codeine on learning. Significant differences were even for the treatments given on day 1 ($F = 6.43$, $P < 0.001$) suggesting that codeine might improve learning. The interaction checked for the change of the state in these four conditions proved non-significant ($F = 0.80$). This indicates that the state-dependent learning could not cause the differences observed. Chlorpromazine in similar circumstances did not modify learning.

Codeine thus always facilitated memory stages when present, the effect being most evident at the acquisition stage. The recall facilitating effect was seen in the situation where the learning material was acquired under placebo and recalled under codeine. Our results may justify the conclusion that codeine facilitates acquisition, retention, and recall of simple learning independently on the stage it was administered.

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The buccal absorption of atenolol and propranolol, and their physicochemical characteristics

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The influence of some physicochemical factors on the absorption of atenolol and propranolol through biological membranes was demonstrated using the Buccal Absorption Test (Beckett & Triggs, 1967) as a model.

The pKa values were taken from the titration curves as the midpoints of the buffering plateaus (9.60 for atenolol and 9.45 for propranolol, both being bases), and water solubility was measured as the pH-independent solubility of free base (Martin, Swarbrick & Cammarata, 1975), being 9.5 mg/ml and 0.07 mg/ml for atenolol and propranolol, repectively. Partition coefficients were established in a n-heptane/0.1 N NaOH system as 0.05 for atenolol and 29.85 for propranolol. Hence, with a similar pKa, atenolol is considerably more hydrophilic than propranolol.

Drugs were assayed spectrofluorimetrically after extraction into 0.01 N HCl with 30% amyl alcohol in n-heptane (Kaye, 1974; Shand, Nuckolls & Oates, 1970).

Buccal absorption experiments were controlled using phenol red (5 μg/ml) as a nonabsorbable internal marker (Schanker, Tocco, Brodie & Hogben, 1958; Schedl, 1966), which was measured spectrophotometrically at 560 nm with a second reading at 625 nm to subtract background extinction. Swallowing as calculated from marker loss was minimal at pH 8 ($1 \pm 1.8\%$) and increased with acidity of the test solution to $14 \pm 7\%$ at pH 5, probably because of acid stimulated salivation (1.8 ml/min at pH 5).

Drug absorption was calculated as the difference between the percentages of marker and drug loss. In a preliminary trial it was shown to be constant, i.e. independent of the initial drug concentration, over a 32-fold concentration range (10–320 μg/ml atenolol, 0.5–16 μg/ml propranolol). Whereas the absorption of propranolol increased with rising pH in an S-shaped curve, from 3.2% at pH 5, 9.3% (pH 6), 26.0% (pH 7), 55.8% (pH 8), 76.9% (pH 9) to 89.0% at pH 10 (confirming Hicks, 1973), that of atenolol was pH independent and low throughout (< 5% in 5 min). Time dependence of absorption showed biexponential decay suggestive of membrane storage (Doluisio, Crouthamel, Tan, Swintosky & Dittert, 1970).

Between subject variation was the same as within one subject at pH 5, 6 and 7. At points of higher absorption the scatter in untrained subjects increased (variance ratios 12.5, 15.2 and 319 at pH 8, 9 and 10; $n = 4$), which suggests that for kinetic studies single subject trials may be advantageous.

The parallelism between physicochemical parameters and absorption data is further evidence for the integrative model character of the oral mucous membrane.

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Power curves in clinical trial reports

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Clinical trials are designed to detect a specified difference between two or more treatments. Usually this difference is the smallest that can be associated with clinical significance, although in some cases smaller differences may be of pharmacological interest. Occasionally trials set out to test the hypothesis that there is nothing to choose between the treatments (Sackett, Spitzer, Gent & Roberts, 1974). The number of patients needed for a trial is usually calculated in terms of the probability (α) of accepting the specified difference as real when no such difference exists (a type 1 error). Larger numbers of patients are needed to also reduce the probability (β) of failing to detect a difference when it is truly present (a type 2 error) (Feinstein, 1975). Calculation of the risk of these errors during the planning stage involves assumptions which may turn out to be unjustified, and they are usually re-examined when the trial has finished. Published trial reports emphasize statistically significant differences between the treatments with their attendant α probabilities. Analysis of results showing no difference is frequently minimal, possibly because it is assumed that this outcome reflects the insensitivity of the trial. An alternative explanation is that no useful difference exists between the treatments. The likelihood that a trial has missed such a difference can be expressed in a power curve relating the β probability to the size of the difference. The publication of this type of curve allows readers to estimate the probability that the trial has failed to detect what they consider to be an important difference.

Curves can also be used at the completion of a trial to relate treatment differences to the number of patients that would be needed to detect them with a given α probability. This would help readers appreciate the feasibility of searching for small differences using the trial design described. This demonstration illustrates the use of power curves in the context of a recent trial of oral and intravenous iron in patients receiving renal dialysis treatment. (Strickland, Chaput de Saintonge, Boulton, Francis, Roubikova & Waters, 1977).

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Subclasses of histamine receptors on human skin blood vessels and their possible clinical significance

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The existence of two subclasses of histamine receptors, now called H_1 and H_2, was established by Ash & Schild (1966). Their distribution and properties have now been defined for a number of different organs and tissues. That human skin blood vessels bear both classes of receptor can be concluded from two sources of evidence: characterization of the responses of skin to drugs with specific H_1 and H_2 agonist activity, and study of the effects of specific H_1 and H_2 receptor blocking agents on histamine reactions in skin.

synthetic histamine analogues with highly specific H_1 and H_2 agonist activity respectively (Black, Duncan, Durant, Ganellin & Parsons, 1972). Intradermal injection of both drugs causes dose-related erythema and wealing. Erythema due to the H_1 agonist is at least partly the result of an axon reflex flare. In contrast, the H_2 agonist causes erythema by a direct vasodilator action. Both drugs cause variable itching and pain.

The specific H_2 receptor blocking agent cimetidine, given systemically, causes parallel displacement of the erythema dose-response curve for the H_2 but not the H_1 agonist. However, chlorpheniramine, an H_1 receptor blocking agent, causes significant inhibition of erythema due to both agonists. Neither blocking agent suppresses wealing due to either agonist.

The possible clinical significance of subclasses of histamine receptors on human skin blood vessels is suggested by studies of the effects of cimetidine alone, chlorpheniramine alone, and both drugs in combination on dose-response curves for erythema and wealing due to histamine and the liberator of endogenous histamine compound 48/80. Both cimetidine and chlorpheniramine separately caused displacement of the dose response curves for histamine erythema. Together, the two antagonists caused significantly greater suppression than either alone. Similar, though less clear-cut suppression could be demonstrated on histamine wealing and on erythema and wealing due to compound 48/80.

These findings suggest that combination therapy with H_1 and H_2 receptor blocking agents may offer significant advantages over conventional H_1 receptor blocking agents alone in the drug treatment of urticaria and other histamine-mediated skin disorders. Clinical trials on this basis are in progress.

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Attenuation of the cardiostimulatory effects of ketamine

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Many attempts have been made to attenuate the cardiovascular stimulant effects of ketamine, which occur invariably with doses above 1 mg/kg in unpremedicated patients. Their severity is not related to the rate or route of administration, nor to dosage. Animal experiments suggest that ketamine may interfere with the reuptake of noradrenaline by adrenergic nerve terminals, but desensitization of arterial baroreceptors and stimulation of the central cardiovascular regulatory mechanism have been suggested as alternative explanations for this action.

In this study, carried out in fit adults having body surface operations and premedicated with intravenous 4 mg lorazepam, anaesthesia was induced with 1 mg/kg ketamine and continued with an an infusion of 1 mg/ml at a rate sufficient to obtund movement (average 2.2 mg/kg for 10 min, 3.5 mg/kg for 20 min). All observations were completed prior to onset of surgery. In twenty control patients the average peak increase in heart rate was 17.5 ± 2.7 beats and in mean arterial pressure 23.3 ± 2.4 mmHg (29.3/19.8). The stimulant effect began to wane after 20 min despite continuing infusion.

Ethical considerations precluded the planning of a definitive study involving predetermined number of patients, but six potentially suitable drugs were given intravenously before or during anaesthesia in recommended clinical doses. The number of patients in each series depended on the response and side effects.

Labetalol, in an initial dose of 1 mg/kg, was the most promising of the drugs studied. In the doses required to control the cardiovascular effects of ketamine it is not free from troublesome side effects as it made patients unduly sensitive to blood loss and caused bronchospasm in an asthmatic. Further studies are needed to assess the risk of these in relation to the dangers of unattenuated ketamine effects.

Table 1 Summary of effect of six drugs on cardiostimulatory effects of 1 mg/kg ketamine followed by infusion of 1 mg/ml

Drug	n	*Dose and time*	*Effect*
Procainamide	2	100 mg repeated in 2–5 min to total of 600 mg	Nil
Hexamethonium	4	25 mg 5 min prior to ketamine	Blood pressure rise controlled in 3: severe tachycardia. Prolonged postoperative hypotension
Promethazine	2	25 mg 5 min prior to ketamine	Nil
Phentolamine	2	2.5–5 mg prior to and during ketamine	Unacceptable tachycardia
Practolol	2	5 + 5 mg prior to and during ketamine	Nil
Practolol/ phentolamine	3	5 + 5 mg practolol, 5 mg phentolamine	No benefit even when order reversed
Labetalol	6	Pretreatment with 0.5 + 0.5 mg/kg	Nil
	10	0.5 mg/kg with ketamine, further increments during infusion	Good control of heart rate: unreliable blood pressure control. Transitory effects (5–10 min)
	10	1 mg/kg with ketamine, increments of 0.5 mg/kg to total of 2.5 mg/kg	Good control of heart rate. Inconsistent control of blood pressure

An EEG method to monitor drug effects on the stimulus, response and motor components of reaction time

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The measurement of the EEG while a subject performs a standard behavioural task provides direct evidence of the brain processes related to that behaviour and a means of studying the effect of drugs on such processes (Ashton, Millman, Telford & Thompson, 1974; 1976). A basic paradigm of this type incorporates a simple reaction time task during which a slow negative potential (CNV) arises between a warning signal and a following 'imperative' stimulus to which a rapid response must be made. This potential reflects subject alertness and cortical or subcortical excitation (Rebert, 1972).

The changes in slow potentials which occur after the imperative stimulus and before the motor response, that is, during the reaction time interval, may also reflect meaningful brain processes. However, these later processes and the effects of drugs upon them have been less studied by such methods. Reaction time has two major components: decision time and motor time. Decision time can be further divided into stimulus analysis time and response selection time. In a simple reaction time task, the two decision time components are reduced to a minimum and the concurrent EEG reflects potentials associated mainly with motor activity. To investigate the possible locus of action of drugs which may affect such cognitive processes during the reaction time interval (e.g. nicotine, cannabis or alcohol) by the analysis of concurrent brain potentials, a choice reaction time (information processing) task is more appropriate. This psychological paradigm provides longer and more accurately analyzed decision time components (Marshall, 1973) and the present report describes a method to incorporate this procedure within a CNV paradigm.

In place of a single imperative stimulus, such as a tone, repeated throughout a series at a pre-set rate, subjects present themselves, at their own rate, with a series of different coloured shapes projected onto a screen. They know the type and proportions of the possible stimuli which can appear but not their particular (random) order. Reaction time is measured between the appearance of the stimulus and the time at which the subject presses the appropriate one of several buttons which indicate different response categories. Stimulus analysis time and response selection time increase with the uncertainty associated with increasing stimulus and response possibilities, respectively. A range of such possibilities is given over different series to provide unit increases in uncertainty. This allows the two decision time segments to be identified with precision. The motor time is calculated graphically by extrapolation or measured directly. The slow potentials measured concurrently are averaged and examined for evidence of drug-induced changes during these three component phases after subtracting from such traces (by computer) the fast potentials evoked by the imperative stimulus which otherwise would mask the brain activity associated with the decision phase. Slow potentials associated with any one phase can be further isolated and given greater definition by computing the difference between two series which differ in that phase only.

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