PROCEEDINGS OF THE British Pharmacological Society

CLINICAL PHARMACOLOGY SECTION

30th–31st March, 1977

UNIVERSITY OF LIVERPOOL

COMMUNICATIONS

In communications with more than one author, an asterisk (*) denotes the one who presented the work.

The evaluation of the anticholinergic activity of glycopyrronium

J.W. DUNDEE, C.J. JONES &
R.K. MIRAKHUR*

Department of Anaesthetics, Queen's University of Belfast and A.H. Robins Company Limited.

Despite their widespread use, the popular anticholinergic drugs atropine and hyoscine have shortcomings such as a short duration of action (Eger, 1962) and passage across the blood-brain barrier, with resulting central effects. In the case of hyoscine there is also a weak cardiac vagolytic action (Orkin, Bergman & Nathanson, 1956).

A long acting quaternary anticholinergic agent, glycopyrronium (Franko & Lunsford, 1960) has been evaluated as a possible alternative. Its effects on various aspects of cholinergic function were studied in six healthy adult volunteers (mean $\pm$ s.e. mean age 32 ± 2 years, weight 62 ± 6 kg). These included pulse rate, blood pressure, temperature, pupil size (in standard room light and in bright light), the visual near point, the active sweat gland count and salivary secretion.

The drug was administered by intramuscular,

intravenous and oral routes in the following doses selected after initial pilot studies: 0.1 mg, 0.2 mg and 0.4 mg intramuscularly; 0.1 mg, 0.14 mg and 0.2 mg intravenously; 2.0 mg, 4.0 mg and 8.0 mg orally.

Observations were made at 30 min, 60 min and thereafter at hourly intervals for 6 hours. There was an interval of at least 1 week between repeat studies in the same subject.

The lowest doses given by the three routes did not produce any significant effects. The two higher doses given by mouth produced significant reduction in salivary secretion starting at about 3 to 4 h after administration. By the parenteral route a significant effect was noted after 30 minutes. The changes in the count of active sweat glands were generally parallel to the changes in salivary secretion. There were however, minimal changes in the rest of the parameters tested. In some doses the drug had a significant effect on salivary secretion even at 6 h particularly when given by mouth or with highest dose by other routes. Subjectively dryness was experienced by volunteers for periods extending beyond 12 h when the drug was taken orally.

Glycopyrronium (0.5 mg i.v.) was given to 20 patients in a mixture with neostigmine (2.5 mg) for the reversal of neuromuscular block. It prevented the bradycardia due to neostigmine but without causing any tachycardia.

Glycopyrronium thus appears to be an effective anticholinergic agent with antisialogogue activity of prolonged duration. The optimum intramuscular dose appears to be 0.2 mg for routine premedication. Little is to be gained clinically by increasing the dose except perhaps subjective discomfort of excessive and prolonged dryness. By oral route the effect comes rather late and persists for too long. The drug can be given safely intravenously without the fear of causing profound cardiovascular changes.

References

- EGER, E.I. (1962). Atropine, scopolamine and related compounds. *Anaesthesiology*, **23**, 365–383.
- FRANKO, B.V. & LUNSFORD, C.D. (1960). Derivatives of 3-pyrrolidinols III. The chemistry, pharmacology and toxicology of some N-substituted-3-pyrrolidyl γ -substituted phenylacetates. *J. med. pharm. Chem.*, **2**, 523–540.
- ORKIN, L.R., BERGMAN, P.S. & NATHANSON, M. (1956). Effect of atropine scopolamine and meperidine on man. *Anesthesiology*, **17**, 30–37.

Interaction of halothane with non-depolarizing neuromuscular blocking drugs in man

R. HUGHES* & J.P. PAYNE

Research Department of Anaesthetics, Royal College of Surgeons of England, London WC2, and St. Peter's Hospital, London WC2

Experimental work in animals and clinical experience in man have indicated that halothane interacts with non-depolarizing neuromuscular blocking drugs to enhance their effects. However, no quantitative assessment of this interaction in man has yet been published and accordingly the studies to be reported were undertaken in six anaesthetized patients using techniques described previously (Hughes, Ingram & Payne, 1976). Simultaneous recordings of tetanic and single twitch contractions of the adductor pollicis muscles were obtained by stimulating one ulnar nerve at 50 Hz for 1 s and the other with single shocks every 12 s. Concentrations of 1–2% halothane were administered for periods of 10 min during recovery from neuromuscular blockade when tetanic fade had almost disappeared. Six studies with halothane were carried out in three patients given 0.25 or 0.5 mg/kg tubocurarine i.v., seven studies in two patients given 0.1 mg/kg dimethyl tubocurarine and three studies in one patient given 0.8 mg/kg gallamine.

In all six patients studied, halothane had no measurable effect on the recovery of the single twitch response but it caused an almost immediate increase in fade with a reduction in the size of the tetanic contraction as shown in Figure 1. At the end of the exposure to halothane the peak height of the tetanic contraction was reduced on average to about 77% of the control but the response was not significantly

Figure 1 Tracings from three anaesthetized patients showing the responses of the ulnar nerve at 50 Hz for 1 s every 12 seconds. Administration of halothane (2%) during recovery from neuromuscular blockade by (a) tubocurarine (0.5 mg/kg), (b) dimethyl tubocurarine (0.1 mg/kg) and (c) gallamine (0.8 mg/kg) reduced the peak height of the tetanic contraction and enhanced the degree of fade (see as darker lines).

affected by the concentration used. In the case of fade however its extent was greater and more closely related to the concentration of halothane. After 10 min exposure to 1% halothane 26% fade was achieved and after the same exposure to 2% the fade had increased

to 66%. This difference was highly significant ($P < 0.001$).

These effects were readily reversed by intravenous neostigmine (2.5–5.0 mg) preceded by intravenous atropine (1.2 mg).

Reference

HUGHES, R., INGRAM, G.S. & PAYNE, J.P. (1976). Studies on dimethyl tubocurarine in anaesthetised man. *Br. J. Anaesth.*, **48**, 969–974.

Induction of bronchial β -adrenergic receptor resistance in normal man

S.T. HOLGATE* & A.E. TATTERSFIELD
(Introduced by C.F. GEORGE)

Department of Medicine, University of Southampton, Southampton

During the last decade, there has been controversy over whether β -adrenoceptor bronchodilator drugs, when taken by asthmatic patients in excess, could lead to a state of diminished airway responsiveness to β -adrenoceptor agonists (resistance). Clinical observations on some asthmatic patients and studies in animals have lent support to this concept. However, prospective studies on asthmatic patients taking large regular doses of β -agonists have been unable to demonstrate development of adrenergic resistance in human airways. In view of these conflicting findings we have studied a group of normal subjects before and after prolonged administration of a selective β_2 -agonist (salbutamol).

Specific airway conductance (sGaw), measured in the whole body plethysmograph, was used to follow the bronchodilator response to inhaled salbutamol delivered by pressurized aerosol in six normal subjects. Changes in sGaw were plotted in response to cumulative doses of salbutamol up to a total dose of 400 μ g. For each subject three baseline dose response studies were carried out on separate days. Subjects were then asked to take regular inhaled salbutamol, 100 μ g four times daily. At weekly intervals for 4 weeks the dose was increased by 100 μ g four times daily. At

the end of each week salbutamol was discontinued and 12 h later a dose response was repeated.

The mean ($\pm$ s.d.) baseline sGaw for six subjects was $1.53 \pm 0.27 \text{ s}^{-1} \text{ kPa}^{-1}$. The mean value of sGaw after salbutamol (400 μ g) was $2.37 \pm 0.37 \text{ s}^{-1} \text{ kPa}^{-1}$. In all subjects, after the first week of taking regular salbutamol, there was a progressive reduction in broncho dilator response to the drug. Thus by the end of week 4 the response to salbutamol (400 μ g) had fallen to 46% of the control responses ($P < 0.0025$). We interpret this to indicate development of resistance to the bronchodilator effects of salbutamol. In four of these subjects the bronchodilator response to i.v. salbutamol was studied before and after becoming resistant to the inhaled drug. Changes in sGaw were measured in response to i.v. boli of salbutamol sulphate (25 μ g) up to a cumulative dose of 300 μ g. When resistant, subjects showed a highly significant reduction in bronchodilator response to i.v. salbutamol. (20% of control response to salbutamol (300 μ g), $P < 0.002$). Two subjects given large doses of inhaled salbutamol (1600 μ g/day) from the start developed resistance in 9–10 days and on stopping the regular drug full sensitivity was regained in 7–10 days.

Hydrocortisone hemisuccinate (200 mg i.v.) given to four resistant subjects restored the salbutamol response to normal after a latent period of 4–6 hours. Aminophylline (250 mg i.v.) given to three resistant subjects restored the salbutamol response within 5 min of administration.

Our studies indicate that human bronchi can be made resistant to β -adrenoceptor agonists and that this can be overcome by hydrocortisone and aminophylline.

Dynamic and kinetic comparison of penbutolol and propranolol in healthy volunteers

I. McLEAN BAIRD, H. HILL, R.M.J. INGS,
K.I. JOHNSON & J. McEWEN*

*Hoechst Pharmaceuticals Research, Walton Manor,
Milton Keynes, Bucks and Clinical Pharmacology Unit,
West Middlesex Hospital, Isleworth, Middlesex*

Penbutolol is a new non-selective β -adrenoceptor blocker. The influence of single oral doses of penbutolol on exercise-induced tachycardia was compared with that of propranolol and placebo over 24 h in three separate double-blind balanced crossover studies. At each dose level penbutolol (10 mg) v propranolol (160 mg) and placebo: penbutolol (20 mg) v propranolol (80 mg) and placebo: penbutolol (40 mg) v propranolol (160 mg) and placebo, six healthy volunteers, who gave their signed informed consent received each of the three treatments at intervals of 1 week according to a Latin square design. Matching capsules were administered under standard conditions 1 h before a light breakfast.

Pulse rates were recorded at 15 s intervals by means of an oscilloscope ratemeter before and during exercise on a stationary bicycle ergometer. At each assessment point, volunteers exercised for 2 min at a work load which resulted in a final pulse rate as close as possible to 130 beats/min under control conditions. Exercise assessment, followed by venepuncture was carried out immediately before treatment then at 1, 2, 3, 5, 7, 9, 12 and 24 h following treatment. Propranolol was measured in plasma according to the fluorimetric method of Shand, Nuckolls & Oates (1970), and penbutolol was measured by a fluorimetric procedure, involving ether extraction of alkalinized

plasma followed by back extraction into dilute acid.

It was therefore possible from this series of experiments to construct dose-response curves for propranolol and penbutolol at time points up to 24 h after treatment. The maximum effect of propranolol was at 2 h: in some individuals the maximum effect of penbutolol occurred at 1 hour. In comparison with placebo, significant inhibition of exercise-induced tachycardia could still be detected 24 h after each drug: however apart from onset of action no difference could be shown between active treatments at any dose level: in other words the dose response curves of propranolol and penbutolol were parallel at each time point.

It was also possible to construct plasma level response curves for each group and each individual. Both drugs showed inter-individual variation, but for most individuals there was a clear linear relationship between the effect and the logarithm of the plasma level of each drug.

In addition we were able to compare the dynamic area under the curve of pulse rate versus time for each exercise period (total pulse beats during exercise) with the more traditional measurement of final pulse rate, to determine whether the former was a more sensitive method of assessing the effect of β -adrenoceptor-blocking drugs. We were unable to demonstrate any significant advantage of the dynamic area under the curve, although it remains an attractive concept in the evaluation of cardiac responses.

Reference

- SHAND, D.G., NUCKOLLS, E.M. & OATES, J.A. (1970). Plasma propranolol levels in adults with observations in four children. *Clin. Pharmac. Ther.*, 11, 112–120.

Slow release oxprenolol in angina pectoris

P.W. GOLDSTRAW, S.H. TAYLOR,*
U. THADANI & S.J. WATT

*University Department of Cardiovascular Studies and
Department of Medical Cardiology, The General
Infirmary, Leeds*

Oxprenolol is effective in increasing exercise capacity and reducing electrocardiographic signs of myocardial ischaemia in the majority of patients with exercise-induced angina pectoris (Thadani, Sharma, Meeran, Majid, Whitaker & Taylor, 1973; Taylor & Thadani, 1976). However, as blockade of cardiac β -adrenoceptors (as measured by the reduction in maximum exercise heart rate) is related to the logarithm of its plasma concentration, and as the pharmacodynamic activity of the normal formulation of oxprenolol can be significantly extended without loss of peak activity by incorporation into a slow-release matrix (Davidson, Thadani, Taylor, Hess & Riess, 1976), it is reasonable to expect that such a formulation may extend the therapeutic effectiveness of the drug. This thesis was tested in a double-blind randomized cross-over study, in six previously untreated male patients with angiographically-proven, uncomplicated, stable, exercise-induced angina pectoris.

Compared with placebo, both normal (N) and slow-release (SR) formulations of oxprenolol significantly increased the time walked on the treadmill before the

onset of anginal pain, both significantly reduced ECG evidence of myocardial ischaemia and both significantly attenuated the tachycardia and increase in systolic pressure during exercise. Compared with the N formulation, SR oxprenolol significantly extended the duration of action of the drug without reducing the magnitude of its antianginal efficiency.

In twelve similar patients under sustained treatment with N oxprenolol 160 mg morning and evening, its single-blind substitution with the SR formulation as a single morning dose of 320 mg resulted in increase in treadmill walking distance throughout the subsequent 24 h except for a brief period after the evening dose of the normal formulation.

SR formulation offers advantages over the normal formulation of oxprenolol in the treatment of patients with exercise-induced angina pectoris.

References

- DAVIDSON, C., THADANI, U., TAYLOR, S.H., HESS, H. & RIESS, W. (1976). Pharmacological studies with slow-release formulations of oxprenolol in man. *Eur. J. Clin. Pharmac.*, **10**, 189–195.
TAYLOR, S.H. & THADANI, U. (1976). Oxprenolol in Angina Pectoris. *Br. J. Pharmac.*, **58**, 412.
THADANI, U., SHARMA, B., MEERAN, M.K., MAJID, P. A., WHITAKER, W. & TAYLOR, S.H. (1973). Comparison of adrenergic beta-receptor antagonists in angina pectoris. *Br. med. J.*, **1**, 138–142.

The duration of action of atenolol in man

J.D. HARRY* & J. YOUNG

*Clinical Pharmacology Unit, ICI Pharmaceuticals
Division, Alderley Park, Macclesfield, Cheshire*

Atenolol, given as a single daily dose, has been shown to be effective in reducing the blood pressure of patients with hypertension (Douglas-Jones & Cruickshank, 1976). It becomes of interest, consequently, to determine the duration of action of atenolol as a β -adrenoceptor blocking drug in man. This communication presents the results of a double-blind, randomized within volunteer comparison of the effects of atenolol with placebo on the heart rate increase induced by exercise in which the effects were monitored for 31 hours.

The subjects were five healthy male volunteers. The exercise was carried out using a bicycle ergometer and the load on this ergometer was selected so that the

heart rate after 4 min of exercise was between 125–135 beats/min when the subjects were not taking tablets. This load, although different for each volunteer, remained constant for each individual throughout the investigation. Heart rate was recorded for 0.5 min at 4 min of exercise from an ECG. The drugs used were atenolol (25, 50, 100 and 200 mg) and matching placebo tablets. The drug or placebo was given as a single dose, each dose being given on a separate day with at least one week between dosing. For each dose, exercise was performed immediately before giving the drug and again at 3, 5, 7, 25, 27, 29 and 31 h afterwards.

Three hours after each dose of atenolol the heart rate response to exercise when compared to placebo was decreased (mean $\pm$ s.e. mean beats/min 129 ± 5.3 after placebo, 110 ± 2.7 after atenolol (25 mg), 109 ± 4.1 after 50 mg, 103 ± 2.6 after 100 mg, and 103 ± 1.5 after 200 mg). The heart rate response to exercise returned towards values obtained after placebo most rapidly after 25 mg, next for 50 mg and most slowly for 100 and 200 mg, which were not

different from each other. At 27 h after ingestion the heart rates achieved after atenolol (100 and 200 mg) were statistically significantly lower than the values after placebo ($P < 0.05$; mean differences 18 beats/min for 100 mg and 19 beats/min for 200 mg) and those values achieved after 25 and 50 mg of atenolol ($P < 0.05$), which had returned to near placebo values. Some degree of blockade was present at 31 h after ingestion (mean value for heart rate after placebo 132 beats/min, after 25 mg atenolol 122, after 50 mg 118, after 100 mg 115 and after 200 mg 109). Maximal effects on the systolic blood pressure response to exercise were seen 5 h after dosing (mean value on placebo 133 ± 6.4 mm Hg, on 25 mg atenolol 122 ± 7.2 , on 50 mg 119 ± 4.0 , on 100 mg 121 ± 3.3

and on 200 mg 118 ± 5.2). The duration of the effect was longest with the 100 and 200 mg doses, but after all doses the effect was over at 25 h. There was no effect on the diastolic blood pressure response to exercise.

We conclude that atenolol in the doses used can produce blockade of cardiac β -adrenoceptors in man for at least 31 h, but that this is less than the level achieved at 3 hours.

References

- DOUGLAS JONES, A.P. & CRUICKSHANK, J.M. (1976). Once daily dosing with atenolol in patients with mild or moderate hypertension. *Br. med. J.*, 2, 990–991.

Rate of onset of hypotensive response with oral labetalol

A.M. BRECKENRIDGE, CHRISTINE
M. MACNEE, N.L'E. ORME,
D.A. RICHARDS & M.J. STERLIN*

*Department of Pharmacology and Therapeutics,
University of Liverpool, and Allen & Hanburys Ltd, Ware,
Hertfordshire*

Labetalol (AH 5158) is a novel hypotensive agent with both α - and β -adrenergic receptor blocking properties (Farmer, Kennedy, Levy & Marshall, 1972). We have studied the rate of onset of the hypotensive effect of oral labetalol in five patients with mild to moderate hypertension.

All patients were studied in hospital, and, after a 3-day period on placebo, each patient received, on consecutive days, 100 mg, 200 mg and 400 mg labetalol, each dose being given three times daily. Placebo tablets were then substituted until the blood pressure (BP) returned to control levels. Each patient then received labetalol (100 mg three times a day) for 3 days, and, after a further placebo period, labetalol (200 mg three times a day) for a further 3 days. Blood pressures were recorded both lying and standing with a random zero sphygmomanometer. Pulse rates and expiratory peak flow rates were measured throughout the study. Blood was taken for measurement of both the plasma labetalol concentration and the plasma renin activity (PRA) 2–3 h after the morning dose. Labetalol concentrations in plasma were measured by

spectrofluorimetric analysis and the PRA by radio-immunoassay.

The hypotensive effect of labetalol was apparent within 2 h of drug administration and was maximal by 4 h. The lying BP during the first 24 h on labetalol (100 mg three times a day) fell from a mean value of 185.4/107.8 to 159.4/93.8 and the standing BP fell from 183.2/113.2 to 156.4/97.6. In three of the five patients the hypotensive response clearly increased with increasing doses of labetalol. There were no significant changes in either pulse rate, expiratory peak flow rate or in PRA.

Plasma concentrations of labetalol, obtained at a mean of 2.6 h after the morning dose during the single drug treatment periods were: on 100 mg 0.29 ± 0.08 mg/l (mean $\pm$ s.e. mean); on 200 mg 0.81 ± 0.14 mg/l and on 400 mg 2.15 ± 0.3 mg/l. During the administration of labetalol (200 mg three times a day) for 3 days, the plasma labetalol concentration on the third day, 2.6 h after the morning dose was 1.42 ± 0.18 mg/l. In one patient, labetalol was given as a single 100 mg dose by both oral and intravenous routes and blood samples were taken at intervals up to 4 h after dosing. The area under plasma concentration versus time curve (AUC) was 4.41 h mg/l after i.v. dosing and 1.79 h mg/l after oral dosing, giving a bioavailability of 40.7%.

We conclude that labetalol has a rapid onset of hypotensive action, and a first pass effect.

References

- FARMER, J.B., KENNEDY, I. & MARSHALL, R.J. (1972). Pharmacology of AH 5158, a drug which blocks both α - and β -adrenoceptors. *Br. J. Pharmac.*, 45, 660–675.

Modification of pressor responses induced by 'cold'

J.G. MACONOCHE, D.A. RICHARDS* & E.P. WOODINGS

Allen & Hanburys Research Limited, Ware, Hertfordshire

In normotensive subjects, hand immersion in cold water increases blood pressure (Hines & Brown, 1936). Two studies were performed to see if adrenoceptor blockade with propranolol, phentolamine and labetalol might influence cold-induced pressor responses.

In study 1, six normal males aged 20–40 years weighing 61–87 kg received under double-blind crossover conditions, oral propranolol (160 mg), labetalol (400 mg) and placebo. Each remained semi-recumbent and after 2 h immersed a hand into ice and water at 0°C for 1 min. Blood pressure was measured with an Elag sphygmomanometer at 30 min intervals throughout, and at 30 and 60 s during hand immersion. Heart rate was measured from a Narco biosystems biotachometer at the same times.

In the second study, five subjects from the first plus two others, received under similar circumstances, and double-blind crossover conditions, intravenous phentolamine (0.1 mg/kg), labetalol (1.0 mg/kg) and sterile water. In addition to measurements of blood pressure and heart rate, circulatory changes during the next 20 min were recorded using an IMF impedance cardiograph. The hand was then immersed in cold water and circulatory changes were measured over the 60 s of hand immersion.

Two hours after propranolol mean ($\pm$ s.e. mean) heart rate was 52 (1.5) beats/min which was

significantly lower ($P < 0.05$) than after labetalol 63 (3.0) beats/min and placebo 63 (2.6) beats/min; mean arterial pressure ($\pm$ s.e. mean) (MAP) after labetalol 83 (3.0) mmHg was significantly lower ($P < 0.05$) than after propranolol 92 (3.0) mmHg and placebo 94 (3.0) mmHg. Hand immersion in cold water caused increases in MAP of 19 (3.0) mmHg after placebo, 23 (3.0) mmHg after propranolol and 14 (3.0) mmHg after labetalol; the latter being significantly lower ($P < 0.05$) than after placebo and propranolol.

Immediately following intravenous phentolamine, diastolic pressure decreased (–13%) whereas increases occurred in heart rate (+23%) and cardiac output (+17%). Each of these changes differed significantly from placebo ($P < 0.001$). Circulatory changes were small after labetalol and sterile water. Irrespective of prior drug administration, hand immersion provoked marked increases in MAP and peripheral resistance (range 17–32%). No significant changes occurred in heart rate, stroke volume or cardiac output. After both phentolamine and sterile water MAP increased after hand immersion by 21 mmHg and after labetalol by 16 mmHg.

It is concluded that increases in MAP and peripheral resistance are the major circulatory changes induced by cold and that prior treatment with neither oral propranolol (160 mg) nor intravenous phentolamine (0.1 mg/kg) modifies the pressor responses. However, oral and intravenous labetalol inhibit the pressor response presumably due to α -adrenoceptor blockade.

References

- HINES, E.A. & BROWN, G.E.A. (1936). The cold pressor test for measuring the reactivity of the blood pressure: data concerning 571 normal and hypertensive subjects. *Am. Heart J.*, 11, 1–9.

The effect of noradrenaline infusion on blood pressure and plasma noradrenaline following clonidine administration in man

H.J. DARGIE, D.S. DAVIES, C.R. DEAN,* C.T. DOLLERY, T.J.B. MALING & J.L. REID

Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W6

Clonidine is a centrally acting antihypertensive agent which causes a rapid decrease in the spontaneous

electrical activity of sympathetic nerves when injected directly into the cisterna magna (Kobinger, 1975). In man the maximum effect on blood pressure is seen 2–4 h following oral administration and the fall in blood pressure is accompanied by a decrease in plasma noradrenaline (Wing, Reid, Hamilton, Davies & Dollery, 1976). Following intravenous administration of clonidine, although penetration of the CNS is rapid as indicated by the immediate onset of sedation (Davies, Wing, Reid, Neill, Tippet & Dollery, 1976), there is a delay in achieving the maximal fall in blood pressure. A possible explanation for the delayed hypotensive effect is that plasma noradrenaline which declined slowly after clonidine, contributes to the

maintenance of blood pressure. We report the effect of noradrenaline infusion on blood pressure and plasma noradrenaline concentration following oral administration of clonidine (300 µg) to four normal subjects.

Intravenous cannulae were inserted into each antecubital fossae; a 3 in catheter on the left and a 12 in catheter on the right. Blood pressure was measured using a Roche Arteriosonde 1217 with the pressure cuff on the right arm. Blood for plasma noradrenaline measurements was drawn from the left arm and noradrenaline was infused through the right arm cannula. Noradrenaline infusion was started 4 h after clonidine administration and the rate of infusion was increased in a stepwise fashion. Rates of 0.01, 0.03, 0.05, 0.075, 0.10 and 0.200 µg kg⁻¹ min⁻¹ were used and infusion at each rate was continued for 10 min. Noradrenaline administration was stopped when the systolic blood pressure reached a value 30% greater than the pre-dose value. Pulse rate was measured continuously, blood pressure was measured every 2 min during the noradrenaline infusion and blood for noradrenaline was withdrawn before clonidine, 4 h after clonidine and at the eighth and tenth min of each infusion.

The mean control blood pressure was $102 \pm 2.9/71.2 \pm 1.8$ which fell to $82.7 \pm 2.3/55.6 \pm 3.3$ at 4 h after clonidine. Plasma noradrenaline fell from 0.24 ± 0.06 to 0.16 ± 0.05 4 h after clonidine. Infusion of noradrenaline at $0.01 \mu\text{g kg}^{-1} \text{min}^{-1}$ raised plasma noradrenaline to 0.30 ± 0.08 ng/ml whereas the mean blood pressure during the last 4 min of the infusion was $87.1 \pm 3.2/58.8 \pm 2.0$. Blood pressure did not

return to control values or greater until plasma noradrenaline had reached a mean of 1.44 ± 0.54 ng/ml in the four subjects. There were marked differences between subjects in the plasma noradrenaline levels achieved at any given infusion rate; at $0.10 \mu\text{g kg}^{-1} \text{min}^{-1}$ the mean value was 2.62 ± 0.72 with a range of from 0.85 ng/ml to 4.35 ng/ml. The sensitivity of blood pressure to changes in plasma noradrenaline also varied markedly between subjects.

These results show that restoration of the concentration of noradrenaline in plasma to control levels does not reverse the hypotensive effect of clonidine. Following oral or intravenous administration of this agent, circulating noradrenaline does not appear to be responsible for the delayed fall in blood pressure.

References

- DAVIES, D.S., WING, L.M.H., REID, J.L., NEILL, E., TIPPETT, P. & DOLLERY, C.T. (1977). Pharmacokinetics and concentration-effect relationships of intravenous and oral clonidine. *Clin. Pharm. Ther.* (in press).
- KOBINGER, W. (1975). Central cardiovascular actions of clonidine. In *Central action of drugs in blood pressure regulation*. ed. Davies, D.S. & Reid, J.L., pp. 181-193. London: Pitman Medical.
- WING, L.M.H., REID, J.L., HAMILTON, C.A., DAVIES, D.S. & DOLLERY, C.T. (1976). The effect of clonidine on biochemical indices of sympathetic function in normotensive subjects. *Clin. Sci. Mol. Med.*, 51, 15P.

Absence of correlation between the oral pharmacokinetics and response to bethanidine

L.G. DRING, G.H. KAMANYIRO,*
R. LANCASTER & R.L. SMITH

Department of Biochemical and Experimental Pharmacology and Department of Clinical Pharmacology, St. Mary's Hospital Medical School, London W2 1PG

Previous studies have shown that inter-individual differences in response to the hypotensive adrenergic blocking drug debrisoquine are due to individual variations in the rates of metabolism of the drug (Angelo, Dring, Lancaster, Latham & Smith, 1975; Angelo, Dring, Lancaster & Smith, 1976). Marked inter-individual differences in hypotensive responses to the related guanidine based drug bethanidine also

occur and to investigate these we have studied the absorption, metabolism and elimination of the drug in human subjects and at the same time monitored the blood pressure response.

The extent to which bethanidine is metabolized was determined in three normotensive and a single hypertensive subject who each received [¹⁴C]-bethanidine (5 µCi; 30 mg) orally and whose urine and faeces were collected for up to 5 days. Chromatography and reverse isotope dilution studies showed the drug to be little metabolized; $95\% \pm 1.7$ (mean $\pm$ s.d.) of the urinary radioactivity consisted of the unchanged drug together with traces of hippuric acid (<1%) as the only detectable metabolite.

For absorption studies, ten subjects received bethanidine (30 mg orally) and the amount of unchanged drug excreted in the 24 h urine was analysed by g.l.c. using an adaption of the method described by Hengstmann, Falkner, Watson & Oates (1974). The amount of unchanged bethanidine recovered in the

urine ranged from 48–79% of the dose ($62.3 \pm 9.9\%$: mean $\pm$ s.d.) There was, however, no relationship between hypotensive response and the amount of unchanged drug eliminated in the urine.

The relationship between the pharmacokinetics of the drug and its hypotensive effect was investigated in five normotensive subjects in an open study. Each subject received the same dose of [^{14}C]-bethanidine as above; the blood pressure (lying, standing and post-exercise) was initially measured at 1, 2, 3, 4, 6, 8, and 24 h and then blood and urine samples taken. Peak plasma concentrations occurred between 1–4 h and the plasma disappearance of the drug was biphasic. The average $T_{1/2\alpha}$ was 2.0 h for plasma and 2.4 h for urine and the $T_{1/2\beta}$ (urine) was 13.5 h (range 8.6–15 h). The renal clearance rate was 397 ± 130 ml/min (mean $\pm$ s.d.) over 0–8 h and the apparent volume of distribution 369 ± 701 (mean $\pm$ s.d.). The range in peak plasma concentrations, $T_{1/2\beta}$ and area under the curve (AUC) was less than two-fold in the five subjects studied. Compared with a control day the blood pressure in all subjects fell but there was no correlation between the hypotensive response and either the peak plasma concentration, AUC or $T_{1/2\beta}$.

It is concluded from these studies that the inter-individual differences in the pharmacokinetics of bethanidine are much smaller than those found for debrisoquine and that these are not of crucial importance in determining inter-individual differences in response to the drug.

G.H.K. was supported by a W.H.O. Fellowship. We thank the Wellcome Trust for a grant for interdisciplinary research.

References

- ANGELO, M., DRING, L.G., LANCASTER, R., LATHAM, A. & SMITH, R.L. (1975). A correlation between the response to debrisoquine and the amount of unchanged drug excreted in the urine. *Br. J. Pharmac.*, **55**, 264P.
- ANGELO, M., DRING, L.G., LANCASTER, R. & SMITH, R.L. (1976). The metabolism of debrisoquine in rat and man. *Biochem. Soc. Trans.*, **4**, 704–706.
- HENGSTMANN, J.H., FALKNER, F.C., WATSON, J.T. & OATES, J. (1974). Quantitative determination of guanethidine and other guanido-containing drugs in biological fluids by gas chromatography with flame ionization detection and multiple ion detection. *Analyt. Chem.*, **46**, 34–39.

Digoxin compliance in patients from general practice

G.D. JOHNSTON* & D.G. McDEVITT

Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Northern Ireland

In a recent study, plasma digoxin concentrations on admission to hospital were compared with levels on day 8 in a group of 50 patients who were maintained on their pre-admission digoxin doses for 7 days (Johnston, Kelly & McDevitt, 1976). Patients in whom plasma digoxin concentrations had changed by more than 0.2 ng/ml between days 1 and 8 were considered to be non-compliant. In the absence of altered renal function, 18 patients (36%) had higher levels on day 8 and were considered to be non-compliant: a further 7 patients (14%) had lower levels on day 8 suggesting that they were taking more than their prescribed dose before admission. Fifty per cent were, therefore, taking their digoxin improperly.

To investigate whether a similar pattern of compliance occurred in patients, receiving digoxin at home but without emergency illness requiring admission to hospital, we have now studied 30 patients from general practice who had no recent contact with hospital. Patients known to be receiving digoxin from their general practitioner were visited at home on three separate occasions. A history of

digoxin therapy was taken, including dose, number of tablets, and possible symptoms of toxicity. An electrocardiography was performed on each patient and a blood sample taken for estimation of urea, creatinine and plasma digoxin concentration. Plasma digoxin levels were sampled at least 6 h after the last digoxin dose. One week later, on the second visit, the tests were all repeated. On this occasion, the patients were given a specific number of digoxin tablets to cover a 10-day period and asked to record the time when the tablets were taken. The dose was that previously prescribed. After 10 days, the tests were repeated and the remaining tablets counted.

The mean $\pm$ s.e. mean plasma digoxin concentrations were 1.43 ± 0.7 ng/ml on day 1, 1.32 ± 0.6 ng/ml on day 8 and 1.65 ± 0.7 ng/ml on day 18. Using a paired *t* test, no significant difference was found between the levels on day 1 and day 8, the levels rose significantly from day 8 to day 18 ($P < 0.01$), suggesting that digoxin intake had been influenced by the measured consumption.

Between day 1 and day 8, no changes in renal function occurred whilst plasma digoxin concentrations remained unchanged in 21 patients (70%), increased by more than 0.2 ng/ml in 5 patients (17%) and decreased in the remaining 4 patients (13%). Using the same criteria to compare day 8 with day 18 values, 14 patients (47%) showed no change in plasma digoxin concentrations but 14 patients (47%) had increased digoxin levels suggesting that they were

previously non-compliant. In one other patient a rise in digoxin was paralleled by a rise in serum creatinine, and in the remaining patient, a fall in digoxin concentration occurred. Possible alterations in bioavailability due to changes in digoxin tablet brand did not contribute to the estimations of patient compliance.

Thus poor compliance seems to be a significant problem in this type of patient and does not necessarily equate with deterioration in clinical status and consequent admission to hospital.

We are grateful to Drs Loughridge, Brown and Green for allowing us to study their patients.

Reference

- JOHNSTON, G.D., KELLY, J.G. & McDEVITT, D.G. (1976). Serial plasma digoxin concentration measurement as an indicator of patient compliance and adequate digoxin prescribing. *Br. J. clin. Pharmacol.*, 3, 953P.

Relationship between urate retention and electrolyte excretion during treatment with loop diuretics

W. BOGIE, M. HOMEIDA, C.J.C. ROBERTS* & FIONA ROBERTS

Departments of Medicine & Pharmacology, University of Bristol and Pharmaceutical Division, Hoechst U.K. Ltd, Hounslow, Middlesex

Two mechanisms have been postulated to account for the hyperuricaemia produced by thiazide and loop diuretics. Firstly, the drugs may inhibit tubular urate secretion by competition for an active transport process (Mudge, 1975). Alternatively urate retention may be more fundamentally related to sodium depletion and diminished extracellular fluid volume (Steele, 1969) and may be affected by the physiological homeostatic mechanisms which modify the diuretic response to repeated administration (Nicholls, Espiner, Donald & Hughes, 1974; Branch, Homeida & Roberts, 1976). A study in which the actions of a new loop diuretic, piretanide (Merkel, Bormann, Maria, Murchaweck & Hropot, 1976), was compared to those of frusemide and bumetanide has provided results which distinguish between these alternatives.

The study consisted of 3 trial periods lasting 10 days and separated by 11 days. Two control days were followed by either piretanide (6 mg), frusemide (40 mg) or bumetanide (1 mg) administered at 08 h 30 min for 7 days. Each of the nine healthy male volunteers aged between 20 and 30 years received each diuretic using a balanced design. The trial was conducted open. Urine was collected between 08 h 30 min and 14 h 30 min (6 h collection) and separately between 14 h 30 min and 08 h 30 min the following morning (18 h collection) on control days and on the first two and last two treatment days. Volume, sodium, potassium and urate content of each collection were measured. Venous blood for plasma uric acid estimation was taken at 08 h 30 min twice before, twice during and at the end of treatment. Non-parametric statistical analysis was applied and results judged as significant at a level $\alpha < 0.05$.

Piretanide was found to have a potency similar to

that of frusemide at the doses used. The patterns of the diuretic response during the 7 days' treatment were similar for the three diuretics.

In the 6 h following first administration there was a slight increase in urate excretion as well as in urine volume, sodium and potassium. After repeated administrations, these increases in sodium and urate excretion became smaller but neither fell below control values. Thus urate excretion was increased when competition for an active transport process might be expected to be greatest.

In the following 18 h collections the effects tended to be reversed. Urine volume, electrolyte and urate excretion were all decreased. With repeated administrations, sodium and urate excretion remained below control values whilst potassium excretion returned to control levels. Thus decreased urate excretion occurred at the time when homeostatic sodium retaining mechanisms were operating.

Net 24 h urate excretion was below that on control days. Consistent with this there was a gradual fall in urate clearance accompanied by a rise in plasma uric acid.

It is concluded that hyperuricaemia produced by loop diuretics is not due to competition for a transport process but might be related to homeostatic sodium retaining mechanisms.

References

- BRANCH, R.A., HOMEIDA, M. & ROBERTS, C.J.C. (1976). Pharmacokinetics of frusemide related to diuretic response. *Br. J. Pharmacol.*, 57, 442P.
- MERKEL, W., BORMANN, D., MARIA, D., MURCHAWECK, R. & HROPOT, M. (1976). Piretanide (Hoe 118), a new high ceiling salidiuretic. *Eur. J. med. clin. chim. Ther.*, 11, 399–406.
- MUDGE, G.H. (1975). Diuretics and other agents employed in the mobilization of oedema fluid. In *The Pharmacological Basis of Therapeutics*, eds Goodman, L.S. & Gilman, A. pp. 817–847. New York: Macmillan.
- NICHOLLS, M.G., ESPINER, E.A., DONALD, R.A. & HUGHES, H. (1974). Aldosterone and its regulation during diuresis in patients with gross congestive heart failure. *Clin. Sci. mol. Med.*, 47, 301–305.
- STEELE, T.H. (1969). Evidence for altered renal urate reabsorption during changes in volume of the extracellular fluid. *J. lab. clin. Med.*, 74, 288–299.

Activity of aryl hydroxylase in adult human skin

P.H. CHAPMAN,* M.D. RAWLINS & S. SHUSTER

Departments of Pharmacological Sciences (Clinical Pharmacology) and Dermatology, University of Newcastle upon Tyne, England

Aryl hydrocarbon hydroxylase (AHH), a microsomal mixed-function oxidase system has been identified in liver, lung, small intestine, kidney and placenta (Nebert & Gelboin, 1969). It has also been found in human neonatal foreskin (Alvares, Kappas, Levin & Conney, 1973). Since this AHH could play an important role in determining the activity and toxicity of drugs within skin, we have examined some of its properties in adult skin.

Skin was obtained from surgical specimens and immediately placed in ice cold isolation medium (20 mM Tris-(hydroxymethyl)-methylamide in 0.3 M mannitol, pH 7.4). After removal of subcutaneous fat, the skin was finely chopped and homogenized in ice cold isolation medium (15 ml). The homogenate was centrifuged at 10,000 g and the supernatant removed and centrifuged at 100,000 g. The microsomal pellet was resuspended in isolation medium (1 ml) and the activity of AHH determined according to the method of Nebert & Gelboin (1968) using benz[a]pyrene as substrate. The activity was expressed as pM of 3-hydroxybenz[a]pyrene formed mg^{-1} microsomal protein h^{-1} .

AHH activity was found in skin from all the sites examined (breast 0.815 ± 0.036 , $n=22$; foreskin 0.906 ± 0.086 , $n=7$; neck 0.867 , $n=1$; forearm 0.985 ± 0.0178 , $n=8$ pM mg^{-1} h^{-1}). Although there was no difference in activity between sites, individuals differed markedly from one another in activity at any

one site: thus, for breast skin, activity of AHH ranged from 0.123 – 1.887 pM mg^{-1} h^{-1} .

Separation of dermis from epidermis by stretching, showed that $96.45 \pm 0.97\%$ ($n=4$) of skin AHH activity was located in the epidermis. Since the dermal preparation contained approximately 3–5% epidermis, AHH activity in human skin appears to be almost exclusively located within the epidermis. Subcellular fractionation of epidermis indicated that 91.4% of AHH activity was present in the 100,000 g pellet.

Pre-incubation of chopped skin with benzantracene ($5 \mu\text{M}$ – $100 \mu\text{M}$) using the cell culture system of Nebert & Gelboin (1968) was accompanied by an increase in AHH activity of 111%–470% dependent on benzantracene concentration. Pre-incubation with dithranol (anthracene 1, 8, 9 triol) at concentrations of $10 \mu\text{M}$ – $200 \mu\text{M}$ also increased AHH activity in a dose-dependent manner. No increase in activity was observed after pre-incubation with salicylate ($50 \mu\text{M}$ – $350 \mu\text{M}$).

This study is supported by a Medical Research Council project grant No. 419121.

References

- ALVARES, A.P., KAPPAS, A., LEVIN, W. & CONNEY, A.H. (1973). Inducibility of benzo[a]pyrene hydroxylase in human skin by polycyclic hydrocarbons. *Clin. Pharmac. Ther.*, **14**, 30–40.
- NEBERT, D.W. & GELBOIN, H.V. (1968). Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. 1. Assay and properties of induced enzymes. *J. biol. Chem.*, **243**, 6242–6249.
- NEBERT, D.W. & GELBOIN, H.V. (1969). The *in vivo* and *in vitro* induction of aryl hydrocarbon hydroxylase in mammalian cells of different species, tissues, strains, and developmental and hormonal states. *Arch. Biochem. Biophys.*, **134**, 76–89.

Value of liver volume measurement in the study of antipyrine kinetics in liver disease

M. HALLIWELL, M. HOMEIDA* & C.J.C. ROBERTS

Departments of Medicine and Pharmacology, The Medical School, University of Bristol, Bristol BS8 1TD

Plasma half-life and clearance of antipyrine have been used widely as indices of drug metabolizing capacity. However, wide inter-individual variation makes these parameters relatively insensitive in the detection of small differences between patients (Andreassen, Ranek,

Statland & Tygstrup, 1974). The finding of a correlation between antipyrine clearance and liver volume in healthy volunteers before and after enzyme induction suggested that hepatic parenchymal mass accounted for part of the variability (Roberts, Jackson, Halliwell & Branch, 1976). This study has examined the relationship between antipyrine clearance and liver size in the disease state and has assessed the contribution of liver volume measurement in discriminating between groups of patients.

Three populations were studied as follows: 25 normal volunteers (aged 16–61 years), 13 patients with severe chronic liver disease (aged 33–75 years) and 7 with milder chronic liver disease (aged 16–69 years). None was taking drugs known to influence hepatic

microsomal enzyme systems. Venous blood samples were taken at 3, 6, 9, 12, 24, 48 and 72 h after the oral administration of a freshly prepared solution of antipyrine (1200 mg). Plasma antipyrine was measured by the method of Brodie, Axelrod, Soberman & Levy (1949). Liver volume was estimated using the ultrasonic scanning technique previously described (Roberts *et al.*, 1976).

There was a strong correlation between liver volume and body weight in the normal subjects ($r = +.84$; $P < 0.001$) but no such relationship was found in the patients with liver disease. The correlation between antipyrine clearance and liver volume in the normals was confirmed ($r = +0.54$; $P < 0.01$). A similar correlation was found in the severe liver disease group ($r = +0.76$; $P < 0.01$).

Plasma half-life of antipyrine was significantly longer in the severe liver disease group than in both the normal ($P < 0.0005$) and the mild liver disease groups ($P < 0.05$). There was no difference in plasma half-life between the normals and the mild liver disease group.

Mean antipyrine clearance was significantly lower than normal in both the severe and mild liver disease groups ($P < 0.001$) and the difference between the disease groups was significant ($P < 0.05$). However, there was considerable overlap of the 95% confidence limits of the three groups.

Comparison of the index, antipyrine clearance per unit volume of liver, also revealed significant differences between all three groups. The overlap of the 95% confidence limits of the three groups was considerably reduced, so that there was no overlap between the severe liver disease group and the normal group.

It is concluded that liver size is a source of variability in antipyrine clearance both in normality and in chronic liver disease. Antipyrine clearance per unit volume of liver provides a sensitive index of drug metabolizing capacity.

References

- ANDREASEN, P.B., RANEK, L., STATLAND, B.E. & TYGSTRUP, N. (1974). Clearance of antipyrine – Dependence of quantitative liver function. *Eur. J. clin. Invest.*, **4**, 129–134.
- BRODIE, B.B., AXELROD, J., SOBERMAN, R. & LEVY, B.B. (1949). The estimation of antipyrine in biological materials. *J. biol. Chem.*, **179**, 25–29.
- ROBERTS, C.J.C., JACKSON, L., HALLIWELL, M. & BRANCH, R.A. (1976). The relationship between liver volume, antipyrine clearance and indocyanine green clearance before and after phenobarbitone administration in man. *Br. J. clin. Pharmac.*, **3**, 907–913.

Factors influencing the therapeutic response to gentamicin treatment in children

A. ASHURST, I.B. HOUSTON, G.E. MAWER & L. SAMBO-DONGA*

Royal Manchester Children's Hospital, Pendlebury, M27 1HA and Department of Pharmacology, Materia Medica and Therapeutics, The University, Manchester M13 9PT

The response of patients receiving gentamicin for Gram-negative infections has been related to the serum antibiotic concentration 1 h after dosage (Noone, Parsons, Pattison, Slack, Garfield-Davies & Hughes, 1974).

One hundred children (24 h–15 years-old) receiving gentamicin for a wide variety of bacterial infections were studied prospectively. Seventy-two children simultaneously received a second antibacterial agent. The number of days of treatment required either before fever subsided or bacterial

culture became negative, was chosen as an inverse measure of therapeutic response. Potentially relevant non-parametric attributes recorded for each child related to the site, accessibility and nature of the bacterial infection. Each of these was recorded as present (1) or absent (0). Weight, height and age were also recorded.

The serum antibiotic concentrations were measured by a plate diffusion method using *Klebsiella pneumoniae*. Steady-state values for 1 h, trough and mean concentrations were calculated. Gentamicin doses (5–360 mg/day) were selected by the prescribing physician and were modified only if the antibiotic concentrations were considered inadequate ($1 \text{ h} < 3 \text{ mg/l}$) or excessive (trough $> 2 \text{ mg/l}$).

The results were analysed using stepwise multiple linear regression (SLR1, Table 1). The duration of fever was longer in children with septicaemia, chest infection, abscess formation and infection by *Bacteriodes* or *Streptococci*. Surgical drainage of pus shortened the duration of fever but none of the parameters of serum gentamicin concentration showed a significant correlation.

Table 1 Relationship between the duration of fever and clinical attributes in 100 children treated with gentamicin

Attribute	Correlation coefficient	Coefficient of multiple correlation	P*
Septicaemia	0.31	0.31	<0.005
Chest infection	0.18	0.40	<0.01
Poorly accessible site	0.23	0.47	<0.01
Surgical drainage	-0.24	0.50	<0.05
Highly resistant organism	0.19	0.53	<0.05

* Significance associated with the addition of each succeeding item.

Thus within the limits imposed by ethical considerations the serum gentamicin concentration appeared not to be a major determinant of the duration of fever. Further investigation probably requires a comparison of alternative gentamicin dosage regimens in patients with specific bacterial infections exposed to no other antibiotic.

Reference

NOONE, P., PARSONS, T.M.C., PATTISON, J.R., SLACK, R.C.B., GARFIELD-DAVIES, D. & HUGHES, K. (1974). Experience in monitoring gentamicin therapy during treatment of serious gram-negative sepsis. *Br. med. J.*, 1, 447–481.

Reduction of human cord serum bilirubin binding capacity by different antibiotics

L.A. FRIEDMAN* & P.J. LEWIS

Clinical Pharmacology Unit, Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London

Neonates conjugate bilirubin poorly and jaundice is common in the first week after birth, especially in prematures. As bilirubin is bound by serum albumin, uptake into the brain can only occur in severe jaundice when the bilirubin binding capacity (BBC) of albumin is exceeded. Drugs may interfere with bilirubin binding (Bratlid, 1972) and precipitate kernicterus at low bilirubin concentrations. Sulphafurazole has precipitated kernicterus in preterm infants (Silverman, Andersen, Blanc & Crozier, 1956).

The effect of ampicillin, cloxacillin and flucloxacillin (Beecham) on the binding of bilirubin by human cord serum has been compared with that of sulphafurazole (Sigma). Cord blood was collected at unselected deliveries and the serum pooled. Apparent BBC, the maximum serum concentration of bilirubin at which no unbound bilirubin could be detected, was measured by filtering artificially jaundiced sera through G-25 M Sephadex (Kaufmann, Kapitulnik & Blondheim, 1969). Free bilirubin retained by the Sephadex is eluted and determined by spectrophotometry. Free

Table 1 Effect of antibiotics on apparent bilirubin binding capacity (μM) of pooled cord serum

Drug	Concentration (mM)				
	0	1	2	4	8
Sulphafurazole	411	393	368	351	—
Cloxacillin	487	411	385	359	351
Flucloxacillin	487	445	411	376	342

bilirubin is plotted against total bilirubin for each sample and serum BBC determined by extrapolation.

Ampicillin had no effect. Cloxacillin, flucloxacillin, and sulphafurazole decreased BBC (Table 1). This suggests that cloxacillin and flucloxacillin displace bound bilirubin although the present method investigates bilirubin binding at concentrations where the first high affinity binding site is saturated and significance of bilirubin displacement from the second binding site is uncertain. The expected concentration of these penicillins in neonatal serum is around 0.05 mM whereas that for sulphafurazole is 0.5 mM.

In premature neonates treated with cloxacillin free bilirubin has been reported at low serum bilirubin concentrations (Satge, Colin, Voyer, Crumiere & Charlas, 1974). Furthermore, in newborn Gunn rats with unconjugated hyperbilirubinaemia flucloxacillin

precipitated kernicterus (Hanefeld & Ballowitz, 1976). These observations, together with our own, suggest caution in the use of cloxacillin and flucloxacillin in jaundiced neonates.

This work was supported by the Wellcome Trust.

References

BRATLID, D. (1972). The effect of antimicrobial agents on bilirubin binding by human erythrocytes. *Scand. J. clin. lab. Invest.*, **30**, 331–337.

HANEFELD, F. & BALLOWITZ, L. (1976). Flucloxacillin and bilirubin binding. *Lancet*, **i**, 433–434.

KAUFMANN, N.A., KAPITULNIK, M.S. & BLONDHEIM, M.D. (1969). The absorption of bilirubin by sephadex and its relationship to the criteria for exchange transfusion. *Pediatrics*, **44**, 543–548.

SATGE, P., COLIN, J., VOYER, M., CRUMIERE, C. & CHARLAS, J. (1974). The place of free bilirubin estimations and of serum albumin in the case of jaundice in the premature child. *Arch. Franç. Pédi.*, **31**, 201–213.

SILVERMAN, W.A., ANDERSEN, D.H., BLANC, W.A. & CROZIER, D.A. (1956). A difference in mortality rate and incidence of kernicterus among premature infants allotted to two prophylactic antibacterial regimens. *Pediatrics*, **18**, 614–624.

Does ampicillin affect practolol kinetics?

J.G. KELLY, W.J. LEAHEY, D.G. McDEVITT & J.G. RIDDELL*

Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Northern Ireland

It has been suggested that practolol may have an enterohepatic recirculation (Aellig, Prichard & Scales, 1970; Carruthers, Kelly, McDevitt & Shanks, 1974). In patients following cholecystectomy with T-tubes in the common bile duct given practolol orally, Carruthers, Kelly, Johnson & McDevitt (1976) found wide intersubject variation in the concentration of practolol excreted in the bile. Patients excreting least practolol in the bile were also taking other drugs and it was considered possible that competition was occurring between drugs of biliary excretion.

To investigate this possibility further we have studied the effect of ampicillin on the pharmacokinetics of practolol in normal subjects. Eight healthy male volunteers, aged 24–34 years, took practolol (400 mg orally) as a single dose on two occasions separated by at least 1 week. On one of these occasions, randomly chosen, ampicillin (500 mg four times a day orally) was commenced 24 h prior to the administration of practolol and continued throughout the study period. Venous blood samples were obtained before and at 3, 5, 7, 12, 24 and 31 h after the practolol dose and the blood practolol concentrations determined by high pressure liquid chromatography. The mean $\pm$ s.e. mean elimination half-life of practolol when given alone was 8.8 ± 1.4 h (range 6.0–15.0) and when given with ampicillin was 7.2 ± 0.7 h (range 4.8–9.8). The difference was not significant ($P > 0.1$) but, because the

trend of the change was as anticipated, a more detailed study was undertaken.

Four healthy male volunteers, aged 24–34 years, took part. On two occasions, following an overnight fast, they received practolol either 400 mg orally or a rapid intravenous infusion of 1.5 mg/kg. On two further occasions, ampicillin (500 mg four times a day orally) was commenced 24 h prior to practolol administration and continued throughout the study as before.

The mean $\pm$ s.e. mean elimination half-life for oral practolol alone was 8.6 ± 1.0 h (range 7.0–11.5) and for oral practolol plus ampicillin was 6.3 ± 0.3 h (range 5.8–7.3). For intravenous practolol, the mean $\pm$ s.e. mean elimination half-life was 5.9 ± 0.5 h (range 5.0–6.9) for drug alone and 6.6 ± 0.3 h (range 5.8–7.1) for practolol and ampicillin together, the half-life of practolol after oral administration being significantly shorter when given in the presence of ampicillin than when given alone ($P < 0.05$). A similar change was not seen when the practolol was given intravenously and it seems unlikely, therefore, that competitive interference with biliary excretion is the explanation for the observed effect.

References

AELIG, W.H., PRICHARD, B.N.C. & SCALES, B. (1970). Blood levels of practolol following intravenous administrations. *Br. J. Pharmacol.*, **40**, 573P.

CARRUTHERS, S.G., KELLY, J.G., McDEVITT, D.G. & SHANKS, R.G. (1974). Blood levels of practolol after oral and parenteral administration and their relationship to exercise heart rate. *Clin. Pharmacol. Ther.*, **14**, 497–509.

CARRUTHERS, S.G., KELLY, J.G., JOHNSON, G.W. & McDEVITT, D.G. (1976). Biliary excretion and enterohepatic recirculation of practolol in man. *Ir. J. Med. Sci.*, **145**, 187–194.

Absorption kinetics of aspirin and quinine in elderly subjects

S.A.M. SALEM*¹ & I.H. STEVENSON

Department of Pharmacology & Therapeutics, University of Dundee, Ninewells Hospital & Medical School, DD1 9SY

A number of studies have compared pharmacokinetic parameters in young and elderly subjects (see Crooks, O'Malley & Stevenson, 1976), and there is good evidence that, in old age, the renal excretion and extent of metabolism of some drugs may be reduced. Drug absorption has not yet been systematically examined in this age group, but it is suggested, however, that this process may also be reduced in the elderly (Bender, 1968).

Table 1 compares pharmacokinetic parameters for aspirin and quinine in the two age groups. There is no evidence of impaired absorption of either drug in the elderly subjects and indeed from the increased area under the plasma concentration versus time curve it might be inferred that the extent of absorption was increased. With neither drug, however, was the absorption rate constant significantly altered with ageing.

Some of the parameters measured may be influenced by change in drug distribution or in rate of elimination. The apparent volume of distribution (calculated from the extrapolated zero time concentration and assuming complete absorption) of aspirin was similar in the two age groups (0.11 ± 0.03 l/kg elderly, 0.08 ± 0.01 l/kg young subjects). In the case of quinine, however, the value in the elderly subjects (1.74 ± 0.68 l/kg) was approximately half that in the

Table 1 Absorption kinetics of aspirin and quinine in young and elderly subjects

Drug	Subjects	Peak plasma concentration ($\mu\text{g ml}^{-1}$)	Time to peak plasma concentration (min)	Area under the plasma concentration time curve ($\mu\text{g ml}^{-1} \text{h}^{-1}$)	Absorption rate constant (h^{-1})
Aspirin	Young (n=6)	35.0 ± 3.0	48.0 ± 32.0	136.0 ± 36.1	13.8 ± 2.5
	Elderly (n=5)	40.5 ± 11.7	69.0 ± 29.9	$287.0 \pm 141.6^*$	12.5 ± 6.2
Quinine	Young (n=6)	1.1 ± 0.4	77.2 ± 28.8	10.0 ± 3.2	2.1 ± 1.6
	Elderly (n=5)	$2.3 \pm 0.74^{**}$	110.0 ± 75.0	$25.7 \pm 13.5^*$	6.0 ± 4.6

* $P < 0.05$, ** $P < 0.01$.

Results are expressed as means $\pm$ s.d. and significance values refer to the difference between the corresponding results in elderly and young subjects.

In this study, the absorption kinetics of aspirin and quinine were determined in two groups of five geriatric inpatients (>65 years) and compared with those in healthy volunteers (20–40 years). All subjects were confined to bed for the first 3 h. Each drug was given on an empty stomach in aqueous solution in a dose of 4 mg/kg, with six blood samples being taken through an intravenous cannula in the first 2 h (absorption phase) and a further four over the following 7 h (elimination phase). Aspirin absorption was determined from the appearance of salicylic acid in plasma as estimated by the method of Trinder (1954) and quinine was determined by a gas-liquid chromatographic procedure (Salem & Stevenson, unpublished observations).

younger subjects (3.2 ± 0.75 l/kg). The plasma clearance values were, in the case of aspirin 0.017 ± 0.011 l $\text{kg}^{-1} \text{h}^{-1}$ (elderly subjects), and 0.024 ± 0.004 l $\text{kg}^{-1} \text{h}^{-1}$ (young subjects) and in the case of quinine 0.193 ± 0.079 l $\text{kg}^{-1} \text{h}^{-1}$ and 0.397 ± 0.115 l $\text{kg}^{-1} \text{h}^{-1}$ respectively.

References

- BENDER, A.D. (1968). The effect of age on intestinal absorption. Implication for drug absorption in the elderly. *J. Am. Geriatr. Soc.*, **13**, 1331–1339.
- CROOKS, J., O'MALLEY, K. & STEVENSON, I.H. (1976). Pharmacokinetics in the elderly. *Clin. Pharmacokin.*, **1**, 280–296.
- TRINDER, P.C. (1954). Rapid determination of salicylate in biological fluids. *Biochem. J.*, **57**, 301–303.

¹ Department of Therapeutics & Pharmacology, Medical Biology Centre, Queen's University, Belfast.

A comparison of three different enzyme inducing agents and their influence on plasma lipids in man

E.E. OHNHAUS

Department of Medicine, University of Berne, Switzerland

Besides its involvement in drug metabolism the liver microsomal enzyme system participates also in cholesterol and triglyceride synthesis. Elevated cholesterol and triglycerides were found following ethanol (Lefevre, de Carli & Lieber, 1972), phenobarbitone (Jones & Armstrong, 1965) and in patients having increased γ -glutamyltranspeptidase (Martin, Martin & Goldberg, 1975). These findings were thought to be due to enzyme induction, and therefore plasma lipids and lipoproteins were investigated in the present study following three different enzyme inducing drugs. In addition, we were interested in the differences in microsomal enzyme inducing capacity of these drugs in man.

Seven healthy volunteers were randomly allocated in a crossover design to three different treatments known to induce liver microsomal enzyme activity in man: antipyrine (1200 mg), phenobarbitone (100 mg) and rifampicin (1200 mg), daily for 14 days. The time interval between each drug administration was at least 4 weeks. Before and after each treatment the following parameters of enzyme induction were measured: antipyrine half-life and total body clearance, γ -glutamyltranspeptidase in the plasma and D-glucaric acid excretion in the urine. Plasma cholesterol, triglycerides and phosphatides were measured and lipid electrophoresis was performed.

All the parameters of enzyme induction and the plasma lipids measured were similar before starting the different drug administrations. However, after cessation of the three inducing agents different results were obtained. After antipyrine administration its half-

life decreased significantly from 12.5 ± 2.8 to 7.7 ± 1.2 h ($P < 0.001$), while the total body clearance of antipyrine significantly increased from 43.3 ± 14.1 up to 64.7 ± 12.4 ml/min ($P < 0.001$). Following phenobarbitone, the antipyrine half-life decreased from 13.6 ± 2.8 to 8.3 ± 2.5 h ($P < 0.02$) and the total body clearance increased from 38.8 ± 8.9 to 61.6 ± 11.4 ml/min ($P < 0.02$). The greatest change was observed following rifampicin, when antipyrine half-life decreased from 11.6 ± 2.6 to 5.4 ± 0.8 h ($P < 0.001$) and the total body clearance increased from 42.6 ± 9.2 to 95.5 ± 12.0 ml/min ($P < 0.001$). γ -glutamyltranspeptidase and D-glucaric acid excretion were also significantly increased following the three treatments ($P < 0.02$). In contrast, no changes were found in plasma cholesterol, triglyceride and phosphatide levels. Furthermore no changes were seen in the lipid electrophoresis following the three different treatments.

Based on the results of the present study antipyrine and phenobarbitone given in the dose mentioned for 14 days showed the same degree of microsomal enzyme inducing capacity, while rifampicin was found to alter the parameters measured to a much greater extent. Changes in plasma lipids and lipid electrophoresis were not observed, and the plasma concentrations seem not to be influenced by any of the drugs.

References

- JONES, A.L. & ARMSTRONG, D.T. (1965). Increased cholesterol biosynthesis following phenobarbital-induced hypertrophy of agranular endoplasmic reticulum in liver. *Proc. Soc. exp. Biol. Med.*, **119**, 1136-1139.
- LEFEVRE, A.F., de CARLI, L.M. & LIEBER, C.S. (1972). Effect of ethanol on cholesterol and bile acid metabolism. *J. Lip. Res.*, **13**, 48-55.
- MARTIN, P.J., MARTIN, J.V. & GOLDBERG, D.M. (1975). γ -Glutamyltranspeptidase, triglycerides and enzyme introduction. *Br. med. J.*, **1**, 17-18.

Induction of imipramine metabolism following barbiturate administration

D.S. HEWICK, R.G. SPARKS,*¹

I.H. STEVENSON & I.D. WATSON

Departments of Pharmacology & Therapeutics and Biochemical Medicine, University of Dundee, Ninewells Hospital, Dundee

It has been suggested that the metabolism of tricyclic antidepressants is not readily inducible *in vivo* (Sjoqvist & von Barr, 1973), although Ballinger, Presly, Reid & Stevenson (1974) have reported slightly lower 'total tricyclic' plasma levels (imipramine plus desmethylinipramine) in patients receiving imipramine plus amylobarbitone than in those receiving imipramine alone. The situation *in vitro* seems clearer and increased hepatic microsomal metabolism of imipramine has been reported in rats pretreated with phenobarbitone (Nakazawa, 1970; Bickel & Gigon, 1971; Gigon & Bickel, 1974). The objectives of the present study were firstly to confirm that the metabolism of imipramine was inducible using rat hepatic microsomal preparations and secondly to ascertain whether this induction could be detected *in vivo* both in the rat and man.

Following phenobarbitone treatment of rats (80 mg/kg i.p. for 3 days) the conversion of [¹⁴C]-imipramine to desmethylinipramine and 2-hydroxy-imipramine in hepatic microsomal preparations was increased by 5.0 and 2.5 fold respectively. This induction of imipramine metabolism by phenobarbitone was also reflected *in vivo*, the elimination half-lives for total radioactivity in plasma, brain, liver and kidney being decreased by 24.6%, 26.9%, 43.6% and 43.7% respectively. In addition, phenobarbitone treatment tended to lower levels of unchanged imipramine and raise those of desmethylinipramine in these tissues.

In the human studies, four volunteers received imipramine (25 mg three times daily) for a total of 3 weeks, with amylobarbitone (200 mg nightly) being given in addition for the last 10 days of this period. Plasma levels of imipramine and desmethylinipramine

were measured using the methods of Watson & Stewart (1977) and Hammer & Brodie (1967) respectively. Plasma steady-state levels (ng/ml, mean $\pm$ s.e. mean of imipramine fell from 31.5 ± 6.3 to 6.3 ± 3.4 ($P < 0.02$: paired *t*-test) with this treatment, while those of desmethylinipramine rose from 19.2 ± 5.1 to 23.0 ± 5.4 ($P < 0.02$). The total imipramine and desmethylinipramine levels after amylobarbitone treatment fell by about 40%.

These data suggest that the metabolism of imipramine is inducible *in vivo* both in the rat and man. Since there is a considerable reduction in total tricyclic levels in plasma the antidepressant effect may be reduced in patients taking barbiturate hypnotics in addition. The slightly higher levels of the supposedly active derivative desmethylinipramine are unlikely to compensate to any significant degree.

This work was carried out while R.G.S. held an MRC Studentship.

References

- BALLINGER, B.R., PRESLY, A., REID, A.H. & STEVENSON, I.H. (1974). The effects of hypnotics on imipramine treatment. *Psychopharmacologia*, **39**, 267–274.
- BICKEL, M.H. & GIGON, P.L. (1971). Metabolic interconversions and binding of imipramine, imipramine N-oxide and desmethylinipramine in rat liver slices. *Xenobiotica*, **1**, 531–641.
- GIGON, P.L. & BICKEL, M.H. (1971). N-Demethylation and N-oxidation of imipramine by rat and pig liver microsomes. *Biochem. Pharmacol.*, **20**, 1921–1931.
- HAMMER, W.M. & BRODIE, B.B. (1967). Application of isotope derivative technique to assay of secondary amines: estimation of desipramine by acetylation with [³H]-acetic anhydride. *J. Pharmac. exp. Ther.*, **157**, 503–508.
- NAKAZAWA, K. (1970). Studies on the N-demethylation, hydroxylation and N-oxidation of imipramine in rat liver. *Biochem. Pharmacol.*, **19**, 1363–1369.
- SJOQVIST F. & von BARR, C. (1973). Interindividual differences in drug oxidation: clinical importance. *Drug Metab. Dispos.*, **1**, 469–481.
- WATSON, I.D. & STEWART, M.J. (1977). Quantitative determination of amitriptyline and nortriptyline in plasma by H.P.L.C. *J. Chromatog.*, **132**, 155–159.

¹ Inveresk Research International, Musselburgh, Edinburgh.

Some clinical pharmacological studies with ciclazindol hydrochloride

RAIHANA S.B. EHSANULLAH, MARILYN J. KIRBY, MONICA LEIGHTON & V.M.S. OH*

Department of Clinical Pharmacology & Computing Unit for Medical Sciences, St. Bartholomew's Hospital, London, EC1A 7BE and Department of Pharmacology School of Pharmacy & Pharmacology, University of Bath, Bath, BA2 7AY

Ciclazindol (10-(*m*-chlorophenyl)-2,3,4,10-tetrahydropyrimido [1, 2 α] indol-10-ol) hydrochloride has pharmacological properties in animals that suggest that it may have antidepressant activity in man (Southgate, unpublished observations). Ciclazindol 10^{-4} M (33.52 μ g/ml) caused 27% inhibition of [3 H]-5HT and 55% inhibition of [3 H]-dopamine uptake into human platelets. At 10^{-5} M and 10^{-6} M the drug has no apparent effect on the uptake of [3 H]-5HT, but caused 29% and 15% inhibition respectively of [3 H]-dopamine uptake (Trenchard, Turner, Pare & Hills, 1975). Ciclazindol (50 to 1000 ng/ml) produced a significant increase in glucose uptake into human isolated skeletal muscle which was independent of the presence of insulin (Kirby & Turner, 1977).

A single dose oral study of ciclazindol (50 mg), tandamine (50 mg) (Ehsanullah, Ghose, Kirby & Witts, 1976) and placebo was carried out on nine healthy volunteers of both sexes aged 20–23 years. Compared with a placebo under double-blind conditions, ciclazindol was found (a) not to reduce salivary volume (Kingsley & Turner, 1974) but (b) to reduce

appetite significantly at 2 h using a linear analogue rating scale. No significant changes were found on rating scales for sedation or concentration. Under double-blind conditions, tyramine pressor responses (Ghose, Turner & Coppen, 1975) were compared in six subjects at 2.5 h after oral ciclazindol 50 mg, desipramine (DMI) 50 mg and placebo. Both drugs produced significant shifts in the dose-response curves. The inhibitory effect of ciclazindol was significantly less than that of DMI.

These results suggest that ciclazindol may have antidepressant and anorectic properties in man.

We thank Wyeth Laboratories Ltd for supplying ciclazindol and for financial support.

References

- EHSANULLAH, R.S.B., GHOSE, K., KIRBY, M.J. & WITTS, D. (1976). Some clinical pharmacological studies with tandarine hydrochloride, a potential antidepressive agent. *Br. J. clin. Pharmacol.*, **3**, 950P.
- GHOSE, K., TURNER, P. & COPPEN, A. (1975). Intravenous tyramine pressor response in depression. *Lancet*, **i**, 1317–1318.
- KINGSLEY, P.J. & TURNER, P. (1974). Class experiment in clinical pharmacology with benzolium bromide, an anticholinergic drug. *Eur. J. clin. Pharmacol.*, **1**, 141–143.
- KIRBY, M.J. & TURNER, P. (1977). Ciclazindol and mazindol on glucose uptake into human isolated skeletal muscle: no interaction of mazindol with methysergide. *Br. J. clin. Pharmacol.*, **4**, (in press).
- TRENCHARD, A., TURNER, P., PARE, C.M.B. & HILLS, M. (1975). The effects of protriptyline and clomipramine *in vitro* on the uptake of 5-hydroxytryptamine and dopamine in human platelet-rich plasma. *Psychopharmacologia (Berl.)*, **43**, 89–93.

Immediate and residual effects on human performance of the hydroxylated metabolites of diazepam

CORAL H. CLARKE & A.N. NICHOLSON*

Royal Air Force Institute of Aviation Medicine, Farnborough, Hampshire

The residual effects of the benzodiazepines most commonly used as hypnotics have been described by several workers (Malpas, Rowan, Joyce & Scott, 1970; Bond & Lader, 1972, 1973, 1975; Borland & Nicholson, 1975a), but such sequelae are less persistent with diazepam (Borland & Nicholson, 1975b; Linnoila, 1973). Indeed, diazepam, or closely

related drugs, may be particularly useful as hypnotics when impaired performance the next day would be unacceptable, and it is in this context that we have studied the immediate and residual effects of diazepam and of its hydroxylated metabolites, 3-hydroxy-diazepam (temazepam) and 3-hydroxy-N-desmethyl-diazepam (oxazepam).

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 experiment, and they were not involved in any other form of drug therapy. There were no restrictions on the consumption of non-alcoholic beverages. Performance on a visuo-motor co-ordination task (Borland & Nicholson, 1975a) was measured 0.5, 2.5, 4.5 and 6.5 h after morning ingestion and 10, 12, 14

and 16 h after overnight ingestion of each drug. Performance after a drug was compared with performance after ingestion of matching placebos. The experiment was double-blind, treatments were presented in random order and change in performance was analysed by analysis of variance.

With 10 mg diazepam performance was impaired at 0.5 h ($P < 0.01$) and 2.5 h ($P < 0.05$), but there were no residual effects with overnight ingestion. With the morning ingestion of 10 mg temazepam performance was not impaired, but with 20 mg performance was impaired at 0.5 h ($P < 0.001$) and 2.5 h ($P < 0.05$). With 10, 20 and 30 mg temazepam overnight there was no impairment of performance, but 10 h after ingestion there was a trend toward impaired performance over the dose range. With the morning ingestion of 30 mg oxazepam performance was impaired at 2.5 h ($P < 0.001$). There were no residual effects of 15 and 30 mg oxazepam, but with 45 mg performance was impaired at 10 h ($P < 0.01$), and at this time there was a trend of increasingly impaired performance over the dose range 15–45 mg.

Diazepam and temazepam are likely to prove acceptable hypnotics if impaired performance the next day is to be avoided. Diazepam (5–10 mg) is a useful hypnotic (Nicholson, Stone & Clarke, 1976) and would appear to be without residual effects, though it is possible that repeated use may be complicated by the activity of its long acting metabolite, N-desmethyldiazepam, which is known to have persistent effects on sleep (Nicholson, Stone, Clarke & Ferres, 1976). Temazepam is free of the possible complications of a long acting metabolite, but in view of the trend with dose toward residual impairment of performance (Hindmarch, 1975) it is suggested that the maximum dose should be limited to 20 mg which, in any case, provides a satisfactory hypnotic effect (Nicholson & Stone, 1976). However, though oxazepam (15 and 30 mg) is also without residual sequelae, the performance data suggest that the onset of the effect of this drug may be slow. This would be consistent with the observation that sleep onset latencies are not shortened by this metabolite (Nicholson & Stone,

unpublished observations), and so oxazepam may be of limited use as an hypnotic.

References

- BOND, A.J. & LADER, M.H. (1972). Residual effects of hypnotics. *Psychopharmacologia* (Berl.), **25**, 117–132.
- BOND, A.J. & LADER, M.H. (1973). The residual effects of hypnotics. *Psychopharmacologia* (Berl.), **25**, 117–132.
- BOND, A.J. & LADER, M.H. (1975). Residual effects of flunitrazepam. *Br. J. clin. Pharmacol.*, **2**, 143–150.
- BORLAND, R.G. & NICHOLSON, A.N. (1975a). Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. *Br. J. clin. Pharmacol.*, **2**, 9–17.
- BORLAND, R.G. & NICHOLSON, A.N. (1975b). Immediate effects on human performance of a 1,5-benzodiazepine (clobazam) compared with the 1,4-benzodiazepines, chlordiazepoxide hydrochloride and diazepam. *Br. J. clin. Pharmacol.*, **2**, 215–221.
- HINDMARCH, I. (1975). A 1,4-benzodiazepine, temazepam (K 3917), its effect on some psychological parameters of sleep and behaviour. *Arzneim. Forsch. (Drug Res.)*, **25**, 1836–1839.
- LINNOILA, M. (1973). Drug interaction on psychomotor skills related to driving: Hypnotics and alcohol. *Ann. Med. exp. Biol. Fenn.*, **51**, 118–124.
- MALPAS, A., ROWAN, A.J., JOYCE, C.R.B. & SCOTT, D.F. (1970). Persistent behavioural and electroencephalographic changes after single doses of nitrazepam and amylobarbitone sodium. *Br. med. J.*, **2**, 762–764.
- NICHOLSON, A.N. & STONE, B.M. (1976). Effect of a metabolite of diazepam, 3-hydroxydiazepam (temazepam), on sleep in man. *Br. J. clin. Pharmacol.*, **3**, 543–550.
- NICHOLSON, A.N., STONE, B.M. & CLARKE, C.H. (1976). Effect of diazepam and fosazepam (a soluble derivative of diazepam) on the sleep in man. *Br. J. clin. Pharmacol.*, **3**, 533–541.
- NICHOLSON, A.N., STONE, B.M., CLARKE, C.H. & FERRES, H.M. (1976). Effect of N-desmethyldiazepam (nordiazepam) and a precursor, potassium clorazepate, on sleep in man. *Br. J. clin. Pharmacol.*, **3**, 429–438.

Controlled comparison of a single high dose of oxprenolol with lorazepam on stress-induced physiological changes and on some psychophysiological variables

N. FARHOUMAND*, JEAN HARRISON & JANE WADSWORTH (Introduced by P. TURNER)

Departments of Psychiatry, Clinical Pharmacology and the Computing Unit for Medical Sciences, St. Bartholomew's Hospital, London EC1A 7BE

β -adrenoceptor blocking drugs are now used in the treatment of some patients with anxiety states but the relative importance of central and peripheral components in this anxiolytic action are still uncertain (Turner, 1976). Several investigators (Lader & Tyrer, 1972; Ogle, Turner & Markomihelakis, 1976) have failed to demonstrate consistent sedative effects with doses of propranolol or oxprenolol up to 320 mg. This paper describes a single dose double-blind study with oxprenolol (480 mg), the benzodiazepine lorazepam (2 mg) and placebo using a double-dummy technique in six healthy male volunteers (17-42 years), each treatment being given at weekly intervals with a randomization based on two latin squares. Each of the following procedures which included a stressful task, was carried out at 0 h, 45 min-1 h 45 min, and 4-5 h, following treatment: measurement of (a) heart rate and blood pressure, during 5 min of physical and mental relaxation, a period of stress in which the subject maintained an outstretched straight leg against gravity for as long as possible (Eysenck, 1947) and 5 min of relaxation; (b) single and multiple choice

visual reaction times and critical flicker frequency (CFF) (Ogle *et al.*, 1976); (c) self rating on the Salkind's Morbid Anxiety Inventory, Eysenck's Personality Inventory, and visual analogue scales for anxiety, sedation and concentration. Oxprenolol produced a fall in pulse rate and blood pressure compared with lorazepam and placebo, this effect being significant in the stress test. CFF fell significantly after lorazepam compared with placebo. Reaction time was prolonged after both lorazepam and oxprenolol compared with placebo, but the differences were not statistically significant for oxprenolol. The analogue rating scale for alertness showed a significant decrease following both oxprenolol and lorazepam. Concentration also decreased after both drugs but not to statistical significance. Length of performance in the leg raising task was not affected by either drug.

We thank our colleagues who volunteered for these studies and Ciba Laboratories Ltd for financial support.

References

- EYSENCK, H.J. (1947). *Dimensions of Personality*. London: Routledge & Kegan Paul.
- LADER, M.H. & TYRER, P.J. (1972). Central and peripheral effects of propranolol and sotalol in normal human subjects. *Br. J. Pharmac.*, **45**, 557-560.
- OGLE, C.W., TURNER, P. & MARKOMIHELAKIS, H. (1976). The effects of high doses of oxprenolol and of propranolol on pursuit rotor performance, reaction time and critical flicker frequency. *Psychopharmacologia (Berl.)*, **46**, 295-299.
- TURNER, P. (1976). Beta-adrenoceptor blockade in hyperthyroidism and anxiety. *Proc. Roy. Soc. Med.*, **69**, 375-377.

5-Hydroxytryptophan-induced behaviour in guinea-pigs: its relevance to human myoclonus

D. CHADWICK*, M. HALLETT, P. JENNER & C.D. MARSDEN

University Department of Neurology, Institute of Psychiatry and King's College Hospital Medical School, Denmark Hill, London SE5 8AF

The administration of L-5-hydroxytryptophan (5HTP) (60 mg/kg s.c. following α -methyl-dopahydrazine; 25 mg/kg) causes a behavioural syndrome characterized by piloerection, increased locomotor activity (gnawing, chewing, grooming and wet-dog shakes) and jerking of the whole body in guinea-pigs. This

muscle jerking has been termed 'myoclonus' and has been used as a model for human myoclonus and tricyclic-induced convulsions (Klawans, Goetz & Weiner, 1973; Westheimer & Klawans, 1974). We have studied this animal model physiologically and pharmacologically in order to compare it with human myoclonus.

Jerking occurs synchronously in forelimbs and hindlimbs and displays a stereotyped electromyographical (EMG) pattern of a burst of activity lasting 40-50 ms, followed by silence lasting 50-70 ms, and then by a further variable period of muscle activity. Electroencephalographic (EEG) recordings in animals with chronically implanted recording electrodes show no alteration of background activity preceding muscle jerks. 5-HTP-induced jerks are abolished below the level of a spinal cord

transection but persist in decerebrate animals. They cannot be involved by cutaneous, visual or auditory stimuli. Jerking identical to 5-HTP-induced behaviour may also be produced by the administration of L-tryptophan (200 mg/kg s.c.) following pretreatment with a monoamine oxidase inhibitor (pargyline 75 mg/kg i.p.), but not by either of these agents alone. Presumed 5-HT receptor agonists 5-methoxy-N,N-dimethyltryptamine (5 mg/kg i.p., quipazine (200 mg/kg i.p.) and lysergic acid diethylamide (0.1–3.2 mg/kg i.p.) produced head and body jerks but were less effective than 5-HTP.

5-HTP-induced jerking is abolished by pretreatment with a central decarboxylase inhibitor NSD 1034 (N-(3-hydroxybenzyl)-N-methylhydrazine dihydrogen-phosphate: 50 mg/kg i.p.) and by presumed 5-HT-receptor blockers cyproheptadine (5–10 mg i.p.) and methysergide (5–10 mg/kg i.p.), but not by agent blocking central catecholamine or cholinergic receptors.

Thus this syndrome of muscle jerking appears dependent on increased activity at 5-HT post-synaptic receptors and is mediated by brain-stem and spinal cord pathways. It differs physiologically in many respects from human myoclonus, some forms of

which may be related to hypoactivity of the central serotonergic neurones and may respond dramatically to L-5-HTP (Chadwick, Hallett, Harris, Jenner, Reynolds & Marsden, 1977). Whilst the syndrome of 5-HTP-induced muscle jerking in guinea-pigs represents an interesting model for the investigation of drugs acting on central 5-HT systems, it does not resemble most forms of human myoclonus.

References

- CHADWICK, D., HALLETT, M., HARRIS, R., JENNER, P., REYNOLDS, E.H. & MARSDEN, C.D. (1977). Clinical, biochemical and physiological factors distinguishing myoclonus responsive to 5-hydroxytryptophan, tryptophan plus a monoamine oxidase inhibitor and clonazepam. *Brain* (in press).
- KLAWANS, H.L., GOETZ, B.A. & WEINER, W.J. (1973). 5-Hydroxytryptophan-induced myoclonus in guinea-pigs and the possible role of serotonin in infantile myoclonus. *Neurology* (Minneapolis), **23**, 1234–1240.
- WEISTHEIMER, R. & KLAWANS, H.L. (1974). The role of serotonin in the pathophysiology of myoclonic seizures associated with acute imipramine toxicity. *Neurology* (Minneapolis), **24**, 1175–1177.

DEMONSTRATIONS

The differential effects of non-depolarizing muscle relaxants on neuromuscular transmission in man

T.N. CALVEY, SANDRA N. WEBB &
N.E. WILLIAMS

*Department of Pharmacology and Therapeutics,
University of Liverpool, Liverpool*

Plasma concentration and pharmacological effects of pyridostigmine in patients with myasthenia gravis

T.N. CALVEY & K. CHAN

*Department of Pharmacology and Therapeutics,
University of Liverpool, Liverpool*

Pyridostigmine bromide (Mestinon) is one of the main anticholinesterase drugs currently used in the treatment of myasthenia gravis. Differences in the daily dosage requirement of oral pyridostigmine in myasthenic patients can vary widely from approximately 30–2000 mg day⁻¹; these variations are usually ascribed to individual differences in the severity of the disease. Little is known of the possible role of individual differences in drug disposition in myasthenic patients.

We have recently developed a sensitive gas-liquid chromatographic method that can be applied to the measurement of pyridostigmine in human plasma (Chan, Williams, Baty & Calvey, 1976). The present work is concerned with the plasma concentration of pyridostigmine and its relationship to electromyographic decrement in subjects with myasthenia gravis. The patients were all stabilized on widely different doses of pyridostigmine bromide (60–660 mg day⁻¹). Despite this 11-fold difference in daily dosage, the plasma concentration of

pyridostigmine was usually maintained between 20 and 60 ng ml⁻¹. Similarly, the area under the plasma concentration-time curve within 4 h of oral administration only varied over a two-fold range (from 5755–10,320 ng ml⁻¹ min) irrespective of the dose.

The plasma concentration of pyridostigmine was invariably correlated with the improvement in neuromuscular transmission or the regression of ptosis. In patients with electromyographic evidence of myasthenia in the thenar muscles, the concentration of pyridostigmine required to restore synaptic transmission to normal (as calculated from the regression line relating plasma concentration to neuromuscular function) ranged from 27.8–125.7 ng ml⁻¹.

It was concluded from these studies that the differential dosage requirements of pyridostigmine in myasthenia gravis were related to both individual differences in drug disposition and the variable severity of the disease.

The financial assistance of the Muscular Dystrophy Group of Great Britain is gratefully acknowledged.

Reference

- CHAN, K., WILLIAMS, N.E., BATY, J.D. & CALVEY, T.N. (1976). A quantitative gas-liquid chromatographic method for the determination of neostigmine and pyridostigmine in human plasma. *J. Chromat.*, **120**, 349–358.

A simple, small-volume ultrafiltration apparatus applicable to protein binding studies

D.L. BLOXAM, G. CURZON & P.H. HUTSON

Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG

Available methods for separating free from protein bound substances, by dialysis or ultrafiltration, all involve disadvantages or errors, e.g. slowness, complexity, inefficiency, unsuitability for small volumes or large batches, lack of pH control. The ultrafiltration cells demonstrated are made cheaply and easily from readily available components and are reliable and simple to use. Large batches of 200 μ l samples can be rapidly ultrafiltered at controlled pH. The cells should have wide applicability to protein binding studies and to the determination of concentrations of free and protein bound drugs and other substances in plasma.

The ultrafiltration cell (Figure 1) consists of a sample reservoir closed at the bottom by a membrane disc, sealed with an 'O' ring and held together with a receiver by a connector. The ultrafiltrate is forced through the membrane by centrifugation.

The cell was developed for determination of free and albumin bound tryptophan in plasma. The pH was controlled by gassing with 5% CO₂, 95% O₂ and shaking before centrifugation. Using PM10 membranes (Amicon) 100 μ l of ultrafiltrate was obtained from 200 μ l of plasma after 20 min centrifugation at 1500 g. Only 0.1% of ¹²⁵I-labelled albumin in human and rat plasma was present in the ultrafiltrate. This would give about 0.5% error for a normal free tryptophan value. Replication was similar to that found for non-ultrafiltered tryptophan standards.

A Scatchard (1949) analysis of tryptophan binding to human serum albumin in Krebs-Ringer bicarbonate buffer at pH 7.35 and 20°C gave 0.98 binding sites per albumin molecule and a binding constant of 6.7×10^{-3} M which is consistent with the results of McMenamy & Oncley (1958). A Scatchard plot can be obtained from the plasma of a single rat.

References

McMENAMY, R.H. & ONCLEY, J.L. (1958). The specific binding of L-tryptophan to serum albumin. *J. biol. Chem.*, **233**, 1436–1447.

Figure 1 Ultrafiltration cell. Overall dimensions, 1.7 × 11.0 cm. (a) Base tube (10 ml Trident glass vial, tall form, series S-N-B); (b) connector (two screw caps for above vial glued back to back); (c) membrane disc (PM10, Amicon); (d) rubber stopper (type 3A 8–36, Freeman, Staincross, Yorks.); (e) gassing channel (from 16 gauge hypodermic needle, Yale); (f) sample reservoir (specimen tube—see (a)—cut 26 mm from end); (g) rubber 'O' ring ($\frac{1}{8}$ in o.d., $\frac{1}{16}$ in thick); (h) receiver (1.5 ml polypropylene tube, No. 39/10, Sarstedt, U.K.).

SCATCHARD, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, **51**, 660–672.

The effect of a Lundh meal on the plasma concentration/time curve of ampicillin, amoxycillin, pivampicillin and cephalixin in normal subjects

R.L. PARSONS, GILLIAN A. HOSSACK,
GILLIAN M. PADDOCK & J.R. TROUNCE

*Department of Clinical Pharmacology, Guy's Hospital
Medical School, London SE1 9RT*

Food impairs the absorption of many drugs including ampicillin (Magni & Sjoval, 1972), cephalixin (Griffith & Black, 1971) and pivampicillin (Magni & Sjoval, 1972; Fernandez, Menezes & Ximenes, 1973). We wondered whether this change in the shape of the plasma concentration/time curve after oral administration of ampicillin, amoxycillin, pivampicillin and cephalixin with food was related to the physical form of the meal. The plasma concentration/time curve of these drugs in the fasting state was compared with that obtained in the same individuals when these antibiotics were given with a liquid Lundh meal (Lundh, 1962) that contains similar proportions of carbohydrate, fat and protein to a standardized breakfast.

Thirteen normal subjects took each drug on one occasion with water and on another occasion with the Lundh meal. Blood samples were collected prior to dosing and at the times indicated in the table. Urine was collected for 6 (cephalixin) or 8 (the penicillins) h after dosing. The antibiotic concentration in plasma and urine was measured by small plate microbiological assay (Grove & Randall, 1955) and the pharmacokinetic parameters obtained by the curve-fitting programme of Engberg-Pedersen (1974).

The Lundh meal did not reduce the peak or mean plasma concentration at any sampling time of any of the four drugs studied. After the Lundh meal, the area under the curve and urinary excretion of ampicillin and amoxycillin were significantly *greater* than when fasting. These findings suggest that the changes in antibiotic absorption after food may depend on the physical form of the meal.

References

- ENGBERG-PEDERSEN, H. (1974). Empirical equation for pharmacokinetic analysis of drug serum levels after oral application. *Antimicrob Agents Chemother*, **6**, 554-562.
FERNANDEZ, C.A., MENEZES, J.P. & XIMENES, J. (1973). The effect of food on the absorption of pivampicillin and a comparison with the absorption of ampicillin potassium. *J. int. med. Res.*, **1**, 530-533.

GRIFFITH, R.S. & BLACK, H.R. (1971). Blood, urine and

Table 1 Mean $\pm$ s.e. mean plasma concentration and urinary excretion of ampicillin, amoxycillin, pivampicillin and cephalixin when fasting and after the meal Lundh

Drug	Dose (mg)	Sampling time	Ampicillin		Amoxycillin		Pivampicillin		Cephalixin	
			500		250		350		500	
			Starved	Meal	Starved	Meal	Starved	Meal	Starved	Meal
		30 min	1.23 $\pm$ 0.26	0.63 $\pm$ 0.24	1.56 $\pm$ 0.37	0.60 $\pm$ 0.26**	2.27 $\pm$ 0.49	1.66 $\pm$ 0.44	7.65 $\pm$ 1.77	2.06 $\pm$ 0.78
		1 h	2.76 $\pm$ 0.39	2.12 $\pm$ 0.43	2.67 $\pm$ 0.46	3.71 $\pm$ 0.50	4.23 $\pm$ 0.42	5.42 $\pm$ 0.95	13.31 $\pm$ 1.64	12.76 $\pm$ 1.55
		2 h	3.39 $\pm$ 0.31	4.30 $\pm$ 0.61	2.87 $\pm$ 0.39	4.17 $\pm$ 0.54*	3.45 $\pm$ 0.48	3.90 $\pm$ 0.40	6.66 $\pm$ 1.10	12.79 $\pm$ 2.18***
		4 h	1.57 $\pm$ 0.25	1.95 $\pm$ 0.25	1.06 $\pm$ 0.14	1.40 $\pm$ 0.18	0.61 $\pm$ 0.07	1.10 $\pm$ 0.19**	1.36 $\pm$ 0.27	3.33 $\pm$ 0.67***
		6 h	0.42 $\pm$ 0.08	0.62 $\pm$ 0.12	0.24 $\pm$ 0.05	0.33 $\pm$ 0.07	0.13 $\pm$ 0.02	0.30 $\pm$ 0.11	0.36 $\pm$ 0.12	0.59 $\pm$ 0.13
		8 h	0.11 $\pm$ 0.02	0.26 $\pm$ 0.12	0.06 $\pm$ 0.01	0.15 $\pm$ 0.04**	0.05 $\pm$ 0.01	0.12 $\pm$ 0.06	—	—
		Urinary excretion (mg)	187.96 $\pm$ 28.31	288.81 $\pm$ 19.23***	113.86 $\pm$ 19.10	230.05 $\pm$ 22.83***	183.74 $\pm$ 25.88	234.17 $\pm$ 18.91	148.17 $\pm$ 25.53	271.00 $\pm$ 73.66
		C _{max} (μ g/ml)	4.56 $\pm$ 0.32	5.27 $\pm$ 0.52	4.07 $\pm$ 0.45	5.78 $\pm$ 0.46***	5.40 $\pm$ 0.41	5.99 $\pm$ 1.02	19.74 $\pm$ 2.76	16.92 $\pm$ 1.92
		T _{max} (h)	1.73 $\pm$ 0.10	1.97 $\pm$ 0.12	1.66 $\pm$ 0.16	1.49 $\pm$ 0.46	1.23 $\pm$ 0.10	1.39 $\pm$ 0.16	0.86 $\pm$ 0.07	1.41 $\pm$ 0.10***
		T _{asc} (h)	0.88 $\pm$ 0.07	1.07 $\pm$ 0.10	0.88 $\pm$ 0.08	0.90 $\pm$ 0.07	0.71 $\pm$ 0.10	0.81 $\pm$ 0.11	0.56 $\pm$ 0.04	0.79 $\pm$ 0.06***
		T _{desc} (h)	3.42 $\pm$ 0.15	3.63 $\pm$ 0.15	3.23 $\pm$ 0.25	2.91 $\pm$ 0.16	2.45 $\pm$ 0.15	2.69 $\pm$ 0.21	1.74 $\pm$ 0.15	2.80 $\pm$ 0.13***
		AUC (μ g ml ⁻¹ h)	12.84 $\pm$ 1.09	15.04 $\pm$ 1.47*	10.72 $\pm$ 0.82	13.30 $\pm$ 1.07*	11.00 $\pm$ 0.88	12.36 $\pm$ 1.29	28.01 $\pm$ 3.89	37.93 $\pm$ 5.55

P = * < 0.05; ** < 0.02; *** < 0.01; **** < 0.002.

tissue concentrations of the cephalosporin antibiotics in normal subjects. *Postgrad. med. J.*, **47**, 32–40.
 GROVE, D.C. & RANDALL, W.R. (1955). *Assay methods of antibiotics: a laboratory manual*. New York: Medical Encyclopedia Inc.
 LUNDH, G. (1962). Pancreatic exocrine function in

neoplastic and inflammatory disease: A simple and reliable new test. *Gastroenterology*, **44**, 588–597.
 MAGNI, L. & SJOVALL, J. (1972). Absorption of ampicillin and pivampicillin in relation to food intake. *Farmaceutisk Tidende*, **32**, 645–648.

A direct radioimmunoassay for flupenthixol in plasma

D. RISBY & J.D. ROBINSON

Division of Psychiatry, Clinical Research Centre, Watford Road, Harrow HA1 3UJ

Flupenthixol is a potent neuroleptic drug used in the treatment of schizophrenia. It is administered daily in tablet form or at 2–4-week intervals by intramuscular depot injections. Owing to the lack of a sufficiently sensitive assay technique little information is available regarding the blood levels of these drugs during the course of treatment. A radioimmunoassay has now been developed capable of detecting the low therapeutic plasma concentrations of the drug without the need for prior extraction of the drug.

Antibodies for the radioimmunoassay were raised in a sheep against a conjugate of flupenthixol to ovalbumin. The immunogen was prepared by condensing a 3-carboxylic acid derivative of flupenthixol to ovalbumin in the presence of water soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Goodfriend, Levine & Fasman, 1964). Unconjugated material was removed by dialysis against distilled water and the purified conjugate solution freeze-dried. Ultraviolet analysis of the dialysate enabled the concentration of free drug to be determined and hence the molar ratio of drug to carrier could be calculated (Robinson, 1975); the ratio was found to be 45:1.

A female sheep was immunized with 2.5 mg conjugate dissolved in 0.5 ml distilled water and emulsified with two volumes Freund's 'complete' adjuvant. Booster immunization of similar amounts of conjugate in a Marcol 52/10% Arlacel A adjuvant (Robinson, Morris & Marks, 1975) were given at frequent intervals over the following year. Venous

blood samples were collected from the external jugular vein seven days after each booster.

Using [³H]-flupenthixol (Lundbeck; S.A. = 0.5 Ci/mmol), 0.1 M phosphate buffered saline, pH 7.4 with 1.0% EDTA and 1.0% gelatin, and serum collected after the tenth booster at a final dilution of 1:120, an assay was developed that is capable of detecting as little as 0.2 ng flupenthixol directly in a 100 µl plasma sample.

The avidity constant, K_a , of the antiserum, determined from a modified Scatchard plot, was $5.24 \times 10^7 \text{ l mol}^{-1}$ at a binding site concentration of $n = 46.50 \text{ mol l}^{-1}$. The assay sensitivity (Albano & Ekins, 1970) for α -flupenthixol was 2.06 ng/ml. The antiserum showed 77.0% cross-reactivity with the β -isomer of flupenthixol and 60.0% cross-reactivity with the closely related drug fluphenazine. Cross-reaction with the carboxylated derivative used in the immunogen preparation was 77.5% while that with amitriptyline, chlorpromazine, thioridazine and orphenadrenine was very much less than 4.0%. The mean intra-assay coefficient of variation ($n = 20$) was 6.8% and the mean inter-assay coefficient of variation ($n = 5$) was 10.4%.

References

- ALBANO, J. & EKINS, R.P. (1970). The attainment of high sensitivity and precision in radioimmunoassay techniques as exemplified in a simple assay of serum insulin. In *In Vitro Procedures with Radioisotopes in Medicine*, p. 491. Vienna: I.A.E.A.
- GOODFRIEND, T.L., LEVINE, L. & FASMAN, G.D. (1964). Antibodies to bradykinin and angiotensin; a use of carbodiimides in immunology. *Science*, **144**, 1344.
- ROBINSON, J.D. (1975). The development of radioimmunoassays for therapeutic drugs. *Ph.D. Thesis, University of Surrey*.
- ROBINSON, J.D., MORRIS, B.A. & MARKS, V. (1975). The development of a radioimmunoassay for etorphine. *Res. Comm. Chem. Path. Pharmac.*, **10**, 1.

Measurement of buccal potential difference in the assessment of mucosal damaging agents

G.J. HUSTON (introduced by P. TURNER)

Department of Clinical Pharmacology, St. Bartholomew's Hospital, London EC1A 7BE

Potential difference measurements have been made between a saline injected reference area of skin, and three areas of buccal mucosa, on the lower lip between lip and gum, at the entrance of the parotid duct and on the sublingual papilla. The buccal mucosa was found to be negatively charged with respect to the skin electrode, thus demonstrating a polarity similar to other gastrointestinal mucosal surfaces (Andersson & Grossman, 1965; Geall, Phillips & Summerskill, 1970; Gustke, Whalen, Geenán & Soergel, 1967; Edmonds & Godfrey, 1969). The buccal potential difference (b.p.d.) was reduced over areas of aphthous ulceration and after treatment of the mucosa with deionized water at 65°C, no change being produced by deionized water at 20°C. This decrease in p.d. is typical of the response of gastrointestinal mucosa to damage (Edmonds & Pilcher, 1973; Smith, Skillman, Edwards & Silen, 1971). A characteristic pressure artefact was demonstrated in response to an increased force applied to the mucosa through the electrode.

In contrast to the considerable increase in transmural rectal potential difference in response to treatment with mineralocorticoids (Edmonds & Godfrey, 1970), no change in b.p.d. was seen 4 and 8 h after ingestion of 1 mg 9 α -fluorohydrocortisone. A significant reduction in b.p.d. ($P=0.016$) was seen after treatment of the mucosa with aspirin (900 mg in 15 ml

of water), and ethanol (30% w/v), their effects being additive. No change was seen after local treatment with 50 mg of indomethacin in 15 ml water.

These potential difference changes in response to agents generally regarded as damaging to mucosa are similar to the responses of gastric mucosa (Murray, Strottman & Cooke, 1974). Measurement of b.p.d. may provide an assay of functional mucosal damage caused by the local effects of pharmacological agents.

References

- ANDERSSON, S. & GROSSMAN, M.I. (1965). Profile of pH pressure and potential difference at gastroduodenal junction in man. *Gastroenterology*, **49**, 364–371.
- EDMONDS, C.J. & GODFREY, R.C. (1969). Transmucosal electrical potentials of human rectum and pelvic colon. *Gut*, **10**, 1044.
- EDMONDS, C.J. & GODFREY, R.C. (1970). Measurement of electrical potentials of the human rectum and pelvic colon in normal and aldosterone treated patients. *Gut*, **11**, 330–337.
- EDMONDS, C.J. & PILCHER, D. (1973). Electrical potential difference and sodium and potassium fluxes across rectal mucosa in ulcerative colitis. *Gut*, **14**, 784–789.
- GEALL, M.G., PHILLIPS, S.F. & SUMMERSKILL, W.H.J. (1970). Profile of gastric potential difference in man. Effects of aspirin, alcohol, bile and endogenous acid. *Gastroenterology*, **58**, 437–443.
- GUSTKE, R., WHALEN, G.E., GEENAN, J.E. & SOERGEL, K.H. (1967). Mucosal potential difference in the intact small intestine. *Gastroenterology*, **52**, 1134.
- MURRAY, H.S., STROTTMAN, M.P. & COOKE, A.R. (1974). Effect of several drugs on gastric potential difference in man. *Br. med. J.*, **1**, 19–21.
- SMITH, B.M., SKILLMAN, J.J., EDWARDS, B.G. & SILEN, W. (1971). Permeability of the human gastric mucosa. *New Engl. J. Med.*, **28**, 716–721.