Br. J. clin. Pharmac. (1982), **14**, 201–206

A COMPARISON OF THE PHARMACOKINETICS OF METRONIDAZOLE IN MAN AFTER ORAL ADMINISTRATION OF SINGLE DOSES OF BENZOYLMETRONIDAZOLE AND METRONIDAZOLE

G.W. HOUGHTON[1], H.K.L. HUNDT[2], F.O. MULLER[2] & R. TEMPLETON[1]

[1]Pharmacokinetic Section, Biopharmaceutical Research, May & Baker Ltd, Dagenham, Essex and [2]Department of Clinical Pharmacology, University of the Orange Free State, South Africa.

1 Three healthy male volunteers were treated with benzoylmetronidazole suspension (3.2 g equivalent to 2 g metronidazole) in a pilot study to investigate the absorption of benzoylmetronidazole into the systemic circulation. A further ten healthy male volunteers took part in a crossover study to compare the pharmacokinetics of metronidazole and its principal oxidative metabolites after administration of benzoylmetronidazole (equivalent to 2 g or 400 mg of metronidazole) or metronidazole (400 mg).

2 The plasma pharmacokinetics of metronidazole and metabolite I[1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] and plasma and urinary concentrations of these, plus benzoylmetronidazole and metabolite II [2-methyl-5-nitroimidazole-1-acetic acid], were determined using specific and sensitive high performance liquid chromatographic assay procedures.

3 No benzoylmetronidazole was observed in any plasma or urine sample assayed. The values for and times of the highest observed plasma metronidazole concentrations after a single oral dose of benzoylmetronidazole, equivalent to 2 g and 400 mg metronidazole, were 17 μg/ml at 5.1 h after dosing and 4.6 μg/ml at 3.2 h after dosing, respectively. Following oral administration of metronidazole (400 mg), the comparable values were 8.5 μg/ml at 0.8 h after dosing. Peak plasma concentrations of metabolite I after each dose were comparable with each other when corrected for the amount of metronidazole reaching the systemic circulation. The peak concentrations of this metabolite were markedly lower than the peak metronidazole concentrations in the same volunteer. Metabolite II was observed in low concentrations (0.8 μg/ml or less) in plasma at a few time intervals after administration of the higher dose of benzoylmetrinidazole and was not detected at any time interval after administration of benzoylmetronidazole (640 mg, equivalent to 400 mg metronidazole) or metronidazole (400 mg).

4 Pharmacokinetic parameters of metronidazole absorption are markedly different after administration of benzoylmetronidazole than after dosing with metronidazole, but the pharmacokinetic parameters of metronidazole and metabolite I elimination are essentially identical after equimolar doses of each form of the drug. The systemic availability of metronidazole derived from benzoylmetronidazole is approximately 80% of that from metronidazole and is independent of dose over the range studied.

5 The mean value for minimum plasma metronidazole concentration at steady-state during the o.d. administration of benzoylmetronidazole (3.2 g equivalent to 2 g metronidazole) was predicted (from these single dose data) to be 6.2 μg/ml. Thus, these predictions suggest that the majority of patients will maintain therapeutic plasma metronidazole concentrations for the whole of the dosing interval during a once-daily dosing regimen. This oral liquid formulation of metronidazole may thus be regarded as a suitable alternative to other presentations of the drug.

Introduction

Benzoylmetronidazole ('Flagyl'-S ®) is the benzoyl ester of metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], an antibacterial agent used systemically against a wide variety of anaerobic bacteria (Ingham *et al.*, 1975; Galgiani *et al.*, 1978), and in the treatment of trichomoniasis (McGill & Black, 1962), giardiasis (Khan & Muazzam, 1964), amoebiasis (Powell, 1969) and other conditions.

0306-5251/82/080201-06 $01.00

Benzoylmetronidazole has been formulated to hydrolyse in the gastrointestinal tract to release therapeutic doses of metronidazole over a period of several hours. In addition, benzoylmetronidazole is presented as liquid oral presentation (stable suspension), in contrast to the available oral tablet or capsule formulations of metronidazole. Metronidazole itself is rapidly, and almost completely, absorbed when given by the oral route (Houghton *et al.*, 1979a, b), and the release of metronidazole from the benzoyl ester is intended to be at a slower rate than the absorption rate of metronidazole from conventional metronidazole tablets.

The purpose of this study was to show the systemic availability and comparative pharmacokinetics of metronidazole derived from the benzoyl ester, and to determine the systemic availability of unhydrolysed benzoylmetronidazole, using commercially available metronidazole tablets ('Flagyl' 200 mg) as a standard.

Methods

Test subjects

Three healthy Caucasian male volunteers (aged 21 to 33 years, weight range 77 to 87 kg) participated in the first part of the study. A further ten healthy Caucasian male volunteers (mean ± s.d. for age 29 ± 6.0 years and weight 76 ± 7.8 kg) were invited to participate in the second part of the study. All volunteers received a briefing on the purpose and nature of the study so that they could give written, informed, consent. The volunteers were found to be healthy by medical examination and by biochemical and haematological tests prior to receiving the first dose of drug. All volunteers fasted overnight prior to dosing and none received any other medication for at least 2 weeks prior to dosing.

The three volunteers in the first part of study received a single oral dose of benzoylmetronidazole (3.2 g equivalent to 2 g metronidazole). The ten volunteers in the second part of the study received, at different times, one dose of metronidazole (400 mg, 2 × 200 mg 'Flagyl' tablets), one dose of benzoylmetronidazole (640 mg, equivalent to 400 mg of metronidazole), and one dose of benzoylmetronidazole, (3.2 g, equivalent to 2 g of metronidazole), according to an incomplete Latin square design. At least 14 days was allowed between each single dose administration. Benzoylmetronidazole was administered as 10 ml or 50 ml doses of 'Flagyl'-S, containing 6.4% w/v benzoylmetronidazole in suspension.

Blood and urine sampling

A total of seven blood samples were taken between 1 and 8 h after dosing in the first part of the study. A minimum of 16 blood samples were taken from each volunteer participating in the second part of the study during the first 48 h after administration of each dose. Additionally, after administration of the larger dose of benzoylmetronidazole in the second part of the study, further blood samples were taken at 58 and 72 h after dosing. Plasma, separated by centrifugation, was stored at −20°C until analysed.

Urine collections were made at eight time intervals up to 71 h after dosing in the first part of the study and at 0–24, 24–48 and 48–72 h after each dose in the second part of the study. The volume of each collection was recorded and aliquots stored at −20°C until analysed. Metronidazole and its principal oxidative metabolites—1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (metabolite I) and 2-methyl-5-nitroimidazole-1-acetic acid (metabolite II) have been shown, in the May & Baker laboratories, to be stable on storage in plasma and urine for up to 1 year.

Assay procedure

Metronidazole and metabolites I and II were assayed using a specific high performance liquid chromatographic assay procedure with u.v. detection (Gulaid *et al.*, 1978). The h.p.l.c. equipment used comprised a constant flow reciprocating pump (Applied Chromatography Systems, model HPRP 750/03), Waters Intelligent Sample Processor, variable wavelength u.v. detector (Cecil, model 212) and dual-pen chart recorder (Philips model 8222). Benzoylmetronidazole was assayed in plasma and urine in three volunteers, and thereafter in plasma only. Heparinised whole blood to be assayed for benzoylmetronidazole was treated with sodium fluoride (5 mg/ml) immediately after sampling to prevent *in vitro* hydrolysis of benzoylmetronidazole. The addition of sodium fluoride immediately following sampling was found to almost totally inhibit hydrolysis of benzoylmetronidazole (<2.5% hydrolysis in 1 h). Benzoylmetronidazole stored at −20°C for 11 days in plasma underwent approximately 11% hydrolysis in the presence of sodium fluoride. All samples collected in this study were assayed for benzoylmetronidazole within 12 days of collection. After addition of known amounts of internal standard [1-(2-benzoyloxyethyl)-2-ethyl-5-nitroimidazole], plasma or urine (5 ml) was extracted using diethyl ether (25 ml), the organic phase was separated, evaporated to dryness and the residue dissolved in acetone (3 ml) and transferred to a small conical tube. The acetone was evaporated to dryness and the residue redissolved in h.p.l.c. mobile phase (diammonium hydrogen phosphate: acetonitrile mixture; 3:2 v/v) and injected onto a 25 cm h.p.l.c. column packed with Spherisorb S5 ODS 5μ. The packing material was obtained from Phase Separations Ltd. and the column prepared using a slurry packing pump (Magnus Scientific). Chromato-

graphy was carried out at room temperature with a flow rate of 1.0 to 1.5 ml/min of solvent. Detection was by u.v. absorption at 320 nm. Under these conditions, with a column of 9,000 theoretical plates, the retention times of benzoylmetronidazole and internal standard were 6 and 8 min, respectively.

All reagents used were Merck reagent grade chemicals. Drug, metabolite and internal standards were produced at May & Baker, Dagenham or Rhone-Poulenc, Vitry, France, and the purity of each was confirmed by h.p.l.c., t.l.c. and physiochemical tests before use.

Data analysis

Pharmacokinetic analyses were carried out only on data from volunteers in the second part of the study. Absorption and elimination half-lives were calculated using the method of residuals. Area under the plasma concentration against time curve (AUC) was calculated using the trapezoidal rule with the terminal portion of the curve estimated by dividing the last observed plasma concentration by the elimination rate constant. The ratio of AUC values was used to calculate relative systemic availability. 'Steady - state' plasma concentrations were predicted using conventional model-dependent pharmacokinetic methods (Gibaldi & Perrier, 1972), assuming a one-compartment open model with first-order absorption and elimination. This model has previously been shown to accurately predict 'steady-state' metronidazole pharmacokinetics from single dose data (Houghton *et al.*, 1979c).

Two-way, cross-classification analysis of variance (Davies & Goldsmith, 1972) was used to compare the variances due to differences between volunteers, between treatments and the residual error not accounted for by either of these parameters.

Results

Plasma pharmacokinetics of benzoylmetronidazole, metronidazole and their metabolites

The plasma concentrations of metronidazole and metabolite I in each volunteer in the second part of the study were meaned (Figure 1). However, all pharmacokinetic parameters were calculated using the individual data. The highest observed plasma metronidazole and metabolite and metabolite I concentrations are given in Table 1. No metabolite II was observed in plasma at any time interval following administration of the lower doses, although, with the assay procedures used, plasma concentrations equal to greater than 200 ng/ml would have been quantified. Following the administration of benzoylmetronidazole equivalent to 2 g metronidazole, low but measurable concentrations of metabolite II were observed in plasma. These concentrations did not exceed 0.8 μg/ml in any volunteer at any time interval. In nine volunteers, measurable metabolite II concentrations were observed between 3 and 14 h after dosing. In the tenth, they persisted until 36 h after dosing. Because of the limited nature of these data, no pharmacokinetic analyses were carried out.

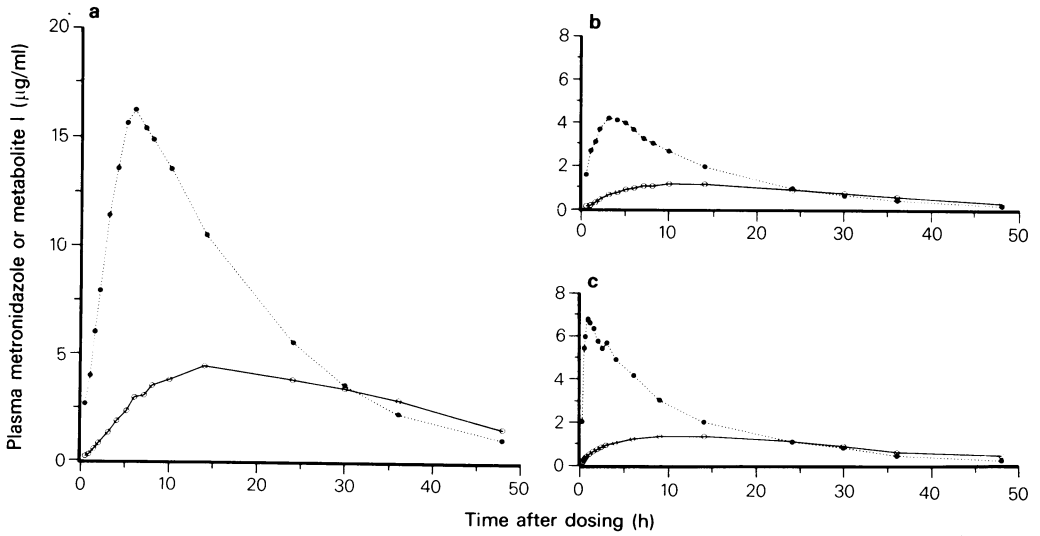

Figure 1 Mean plasma metronidazole (●) and metabolite I (○) concentrations after oral dosing with metronidazole or benzoylmetronidazole.(a) Benzoylmetronidazole equivalent to 2 g metronidazole, (b) benzoylmetronidazole equivalent to 400 mg metronidazole and (c) metronidazole (400 mg)

Table 1 Plasma metronidazole and metabolite I concentrations after oral administration of metronidazole or benzoylmetronidaxole

Parameter observed (units)	*Mean (± s.d.) value for observed parameter after administration of a single oral dose of:*		
	benzoylmetronidazole equivalent to 2 g metronidazole	*benzoylmetronidazle equivalent to 400 mg metronidazole*	*metronidazole 400 mg*
Highest observed plasma metronidazole concentration (μg/ml)	17 ± 4.8	4.6 ± 0.8	8.5 ± 2.3
Time of highest observed metronidazole concentration (h after dosing)	5.1 ± 2.0	3.2 ± 1.5	0.84 ± 0.55
Highest observed plasma metabolite I concentration (μg/ml)	4.4 ± 1.5	1.2 ± 0.46	1.4 ± 0.32
Time of highest observed metabolite I concentration (h after dosing)	16 ± 5.3	14 ± 4.3	13 ± 4.7

The plasma from the three volunteers receiving benzoylmetronidazole, equivalent to 2 g metronidazole, in the first part of the study, was assayed for benzoylmetronidazole at seven time intervals between 1 and 8 h after dosing. No benzoylmetronidazole was observed in any plasma sample, although, with the methodology used, plasma benzoylmetronidazole concentrations equal to or greater than 14 ng/ml would have been detected. Thereafter, in the second part of the study, only 1 h and 6 h plasma samples were assayed for benzoylmetronidazole, which was not detected in any sample.

The pharmacokinetic parameters calculated for each volunteer are meaned (Table 2) and the individual results were used to predict (Table 3) the peak, average and minimum plasma metronidazole concentrations which would would be obtained at steady-rate during administration of three potentially therapeutic dosing regimens, namely: benzoylmetro-

Table 2 Pharmacokinetic parameters after oral dosing with metronidazole or benzoylmetronidazole

Pharmacokinetic parameter (units)	*Mean (± s.d.) value for pharmacokinetic parameter after administration of a single oral dose of:*		
	benzoylmetronidazole equivalent to 2 g metronidazole	*benzoylmetronidazole equivalent to 400 mg metronidazole*	*metronidazole 400 mg*
Elimination half-life of metronidazole (h)	9.7 ± 1.9	8.6 ± 1.1	8.6 ± 1.2
Absorption half-life of metronidazole (h)	1.8 ± 0.58	0.91 ± 0.53	0.18 ± 0.29
Area under plasma metronidazole concentration against time curve ($\mu g\ ml^{-1}\ h$)	294 ± 124	66 ± 13	82 ± 17
AUC, corrected for dose ($\mu g\ ml^{-1}\ g^{-1}$)	147 ± 62	165 ± 33	205 ± 43
Relative systemic availability (%)	79 ± 16	82 ± 15	100*
Apparent elimination half-life of metabolite I (h)	12 ± 2.8	16 ± 12	15 ± 10
Area under the plasma metabolite I concentrations against time curve ($\mu g\ ml^{-1}\ h$)	166 ± 59	42 ± 14	50 ± 20
AUC, corrected for dose of metabolite I ($\mu g\ ml^{-1}\ h\ g^{-1}$)	83 ± 30	105 ± 35	125 ± 50

* Defined value, no s.d.

Table 3 Predicted steady-state plasma metronidazole concentrations

Parameter predicted from single dose data	*Predicted mean (± s.d.) steady-state plasma metronidazole concentrations during repeated oral dosing with:*		
	benzoylmetronidazole equivalent to 2 g metronidazole once daily	*benzoylmetronidazole equivalent to 400 mg metronidazole three times daily*	*metronidazole 400 mg three times daily*
Maximum plasma metronidazole concentration (μg/ml)	21 ± 4.3	9.6 ± 1.5	13 ± 2.8
Time of maximum (h after dosing)	4.7 ± 0.9	2.0 ± 0.9	0.61 ± 0.76
Average plasma metronidazole concentration (μg/ml)	14 ± 3.0	8.3 ± 1.6	10 ± 2.0
Minimum plasma metronidazole concentration (μg/ml)	6.2 ± 2.0	6.6 ± 1.7	7.5 ± 1.8

nidazole (equivalent to 2 g metronidazole), once daily, benzoylmetronidazole (equivalent to 400 mg metronidazole) three times daily, or metronidazole (400 mg), three times daily.

Analysis of variance suggests that the elimination half-life of metronidazole is statistically significantly longer (variance ratio 6.8 with 2 and 18 degrees of freedom; probability by chance = 0.6%) after oral administration of benzoylmetronidazole (equivalent to 2 g metronidazole), than after the lower dose administered as either formulation. However, the mean observed differences were probably not of sufficient magnitude to have any clinical significance.

The systemic availabilities of metronidazole derived from the two benzoylmetronidazole dose levels were statistically significantly less (variance ratio 6.9 with 2 and 18 degrees of freedom; probability by chance = 0.6%) than that from metronidazole. However, the availability of metronidazole from two benzoylmetronidazole dose levels did not differ significantly from each other (variance ratio <1).

Urinary excretion of benzoylmetronidazole, metronidazole and its metabolites

No benzoylmetronidazole was observed in any urine sample from the three volunteers participating in the first part of the study. Thereafter, in the second part of the study, determination of only metronidazole and its metabolites was carried out in urine. The mean values for the cumulative urinary excretion of metronidazole and metabolites I and II are given in Table 4.

The majority of the urinary excretion of these three products occurred within the first 24 h after dosing. No systematic difference due to formulation or dose size was observed in the proportion of metronidazole

Table 4 Mean urinary excretion of metronidazole and metabolites I and II after oral administration of metronidazole or benzoylmetronidazole

Compound	*Mean (± s.d.) urinary excretion of metronidazole and metabolites after administration of:*					
	benzoylmetronidazole equivalent to 2 g metronidazole		*benzoylmetronidazole equivalent to 400 mg metronidazole*		*metronidazole 400 mg*	
	% dose	*% total**	*% dose*	*% total**	*% dose*	*% total**
Metronidazole	6.8 ± 1.0	31 ± 4.6	6.5 ± 2.3	26 ± 1.2	9.3 ± 4.6	28 ± 14
Metabolite I	9.2 ± 3.6	40 ± 16	11 ± 3.2	44 ± 13	14 ± 3.0	43 ± 9.2
Metabolite II	6.6 ± 1.0	30 ± 4.5	7.4 ± 1.5	31 ± 6.3	9.6 ± 2.6	29 ± 7.9
Total*	23 ± 4.4	—	25 ± 5.7	—	33 ± 7.3	—

* Total is the total of metronidazole and metabolites I and II

and its metabolites in urine. The mean relative systemic availabilities (± s.d.) of metronidazole derived from doses of benzoylmetronidazole equivalent to 2 g or 400 mg metronidazole were calculated, from these urinary data, to be 87 ± 35% and 79 ± 29%, respectively. These results were in agreement, within the limits of experimental error, to those obtained from plasma concentration data.

Discussion

The plasma and urinary concentrations of metronidazole and its oxidative metabolites have been determined and compared following oral administration of benzoylmetronidazole (equivalent to 2 g or 400 mg metronidazole) or metronidazole (400 mg). The systemic availability of metronidazole derived from benzoylmetronidazole was estimated to be approximately 80% of that observed after administration of metronidazole. Benzoylmetronidazole itself was not systemically available under the conditions of this study.

The absorption of metronidazole into the systemic circulation was markedly slower following treatment with benzoylmetronidazole than after dosing with metronidazole, but the pharmacokinetic parameters of elimination were unchanged after administration of equimolar doses of benzoylmetronidazole and metronidazole. The predicted peak plasma metronidazole concentration at 'steady-state' during once-daily dosing with benzoylmetronidazole equivalent to 2 g metronidazole was 21 μg/ml. This maximum value was similar to that observed immediately after an infusion of 500 mg metronidazole (Houghton *et al.*, 1979b). The mean value for the minimum metronidazole concentration was predicted to be equal to the minimum inhibitory concentration for most susceptible anaerobes (6.2 μg/ml; Finegold & Sutter, 1972). Since there is evidence that the pharmacokinetics of metronidazole in volunteers and patients are essentially identical (Houghton *et al.*, 1979c), these data suggest that the majority of patients dosed once-daily with benzoylmetronidazole (3.2 g equivalent to 2 g metronidazole) will maintain therapeutic concentrations of metronidazole for the majority or whole of the dosing interval. The average exposure of perfused tissues to metronidazole is greater after once-daily administration of benzoylmetronidazole, equivalent to 2 g metronidazole, than after three times daily dosing with metronidazole 400 mg tablets.

We conclude that once daily dosing with benzoylmetronidazole (3.2 g) will provide an alternative oral liquid dosing regimen to that of other metronidazole presentations for those therapeutic indications for which the drug is prescribed.

The authors should like to acknowledge the assistance of the late Peter S. Thorne in the analyses of the data reported in this study.

References

DAVIES, O.L. & GOLDSMITH, P.L. (1972). Analysis of variance. In *Statistical methods in research and production*, pp. 121–177. London: Oliver & Boyd.

FINEGOLD, S.M. & SUTTER, V.L. (1972). In *Host resistance to commensal bacteria*, ed. Mac Phee, T., pp. 275–297. London: Churchill–Livingstone.

GALGIANI, J.N., BUSCH, D.F., BRASS, C., RUMANS, L.W., MANGELS, J.I. & STEVENS, D.A. (1978). Bacteroides fragilis endocarditis, bacteremia and other infections treated with oral or intravenous metronidazole. *Am. J. Med.*, **65**, 284–289.

GIBALDI, M. & PERRIER, D. (1975). Pharmacokinetics. In *Drugs and the Pharmaceutical Sciences*, Vol. 1. ed. Swarbrick, J. New York: Marcel Dekker.

GULAID, A., HOUGHTON, G.W., LEWELLEN, O.R.W., SMITH, J. & THORNE, P.S. (1978). Determination of metronidazole and its major metabolites in biological fluids by high pressure liquid chromatography. *Br. J. clin. Pharmac.*, **6**, 430–432.

HOUGHTON, G.W., SMITH, J., THORNE, P.S. & TEMPLETON, R. (1979a). The pharmacokinetics of oral and intravenous metronidazole in man. *J. Antimicrob. Chemother.*, **5**, 621–623.

HOUGHTON, G.W., THORNE, P.S., SMITH, J., TEMPLETON, R. & COLLIER, J. (1979b). Comparison of the pharmacokinetics of metronidazole in healthy female volunteers following either a single oral or intravenous dose. *Br. J. clin. Pharmac.*, **8**, 337–341.

HOUGHTON, G.W., THORNE, P.S., SMITH, J., TEMPLETON, R., COLLIER, J., MOESGAARD, F. & LUKKEGAARD-NEILSEN, M. (1979c). The pharmacokinetics of intravenous metronidazole (single and multiple dosing). In *Metronidazole: Proceedings of the 2nd International Symposium on Anaerobic Infections*, Geneva, 25–27 April, 1979, International Congress and Symposium Series No. 18, pp. 35–40, eds Philips, I. & Collier, J. London: Royal Society of Medicine.

INGHAM, H.R., RICH, G.E., SELKON, J.B., HALE, J.H., ROXBY, C.M., BETTY, M.J., JOHNSON, R.W.G. & ULDHALL, P.R. (1975). Treatment with metronidazole of three patients with serious infections due to *Bacteroides fragilis*. *J. Antimicrob. Chemother.*, **1**, 235–242.

KHAN, A.K. & MUAZZAM, M.G. (1964). Diarrhoeal disease (Intestinal flagellates). *East Pakistan Med. J.*, **8**, 18–20.

McGILL, M.I. & BLACK, M.D. (1962). Observations on the use of metronidazole in the treatment of trichomoniasis. *Am. J. Obstet, Gynec.*, **83**, 1280–1283.

POWELL, S.J. (1969). Metronidazole in the treatment of amoebic dysentery. *Med. Today*, **3**, 48–53.

(Received December 12, 1981, accepted February 18, 1982)