A LINK BETWEEN LIVER MICROSOMAL ENZYME ACTIVITY AND THYROID HORMONE METABOLISM IN MAN

E.E. OHNHAUS* & H. STUDER

Department of Medicine, University of Berne, Switzerland

1 The effect of different combinations of liver microsomal enzyme inducing drugs on thyroidal hormone steady state concentrations was investigated. Three groups of healthy volunteers were given daily either antipyrine 1200 mg together with phenobarbitone 100 mg, antipyrine 1200 mg combined with rifampicin 600 mg or rifampicin 600 mg alone for a period of 14 days.

2 Before and after each treatment the total body clearance of antipyrine, γ -glutamyl transferase (γ -GT), 6 β -OH-cortisol as *in vivo* parameters of liver microsomal enzyme activity were measured. In addition, thyroxine (T_4), free thyroxine (FT_4), T_3 resin uptake, tri-iodothyronine (T_3) reverse T_3 (rT_3) and TSH were estimated.

3 After rifampicin administration there was a 60% increase in antipyrine clearance while following combinations of antipyrine–phenobarbitone or antipyrine–rifampicin an 80% and 128% increase respectively occurred.

4 A marked decrease of T_4 , FT_4 and rT_3 was seen in all groups while T_3 remained stable in all groups investigated. This effect may be partly due to an increase in extrathyroidal metabolism of T_4 as found previously by a kinetic turnover study using ^{125}I - T_4 . It also depends on the extent of the liver microsomal enzyme inducing capacity rather than on the nature of the drugs used. The striking disparity of liver enzyme induction of T_4 and rT_3 disposal on the one hand and T_3 metabolism on the other is a unique phenomenon whose pathogenesis is not clear at the present time.

Introduction

In a previous study the effect of antipyrine, phenobarbitone and rifampicin on the liver microsomal enzyme system and on thyroid hormone metabolism in man was investigated (Ohnhaus *et al.*, 1981). The three agents studied produced different changes in the *in vivo* parameters of liver microsomal enzyme activity. Thyroxin-binding globulin (TBG), T_3 resin uptake, thyroxine (T_4), tri-iodothyronine (T_3), free thyroxine (FT_4) and reverse T_3 (rT_3) did not change in its plasma concentrations following antipyrine and phenobarbitone. In contrast, rifampicin, the most potent inducer of liver microsomal enzyme activity, lowered T_4 , FT_4 and rT_3 by about 10–15%, while T_3 increased by about 25%; ^{125}I - T_4 $t_{1/2}$ decreased from 155 to 106 h and its clearance increased from 36 to 50 ml/h. These results indicated that rifampicin increased hepatic clearance of T_4 and rT_3 and enhanced the generation of metabolically active T_3 .

The major finding of our previous study was the enhanced non-deiodinative T_4 clearance in the liver. Similar results were found following chronic treatment with diphenylhydantoin, phenobarbitone and

primidone, mainly metabolised into phenobarbitone (Cavalieri *et al.*, 1973; Cavalieri *et al.*, 1979; Chin & Schussler, 1968; Cullen & Burger, 1972; Gallager *et al.*, 1970; Hansen *et al.*, 1974; Larsen *et al.*, 1970, 1979; Liewendahl & Majuri, 1976; Oppenheimer *et al.*, 1960, 1968; Mendoza *et al.*, 1966; Schönberger *et al.*, 1979). In addition, on long term administration of both diphenylhydantoin and phenobarbitone changes in liver microsomal enzyme activity were reported (Ohnhaus & Park, 1979; Roots *et al.*, 1979).

Based on these findings drugs capable of increasing liver microsomal activity may simultaneously enhance T_4 turnover in man. However, changes in T_4 metabolism probably do not appear before a certain extent of enzyme induction is achieved. Therefore, in the present study different drug combinations having a more potent enzyme inducing capacity (Ohnhaus *et al.*, 1982) than antipyrine and phenobarbitone given alone were administered and their effect on liver microsomal activity and thyroid hormone metabolism was simultaneously investigated. In addition, because of the known dose-dependent enzyme

inducing capacity of rifampicin (Ohnhaus & Park, 1979) a smaller dose of this drug was also tested for its efficacy to change thyroid hormone metabolism.

Methods

Volunteers

Eighteen healthy volunteers, 13 males and five females, ranging in age from 22 to 39 years were investigated. All volunteers were informed about the study and gave their written consent. Their health before and after each study was checked by clinical and laboratory investigations as previously published (Ohnhaus *et al.*, 1977).

Design of the study

Three different studies in three different groups of volunteers were performed under normal living conditions. In the first group six volunteers took daily doses of 600 mg rifampicin as a single dose in the morning (Rimactan[®], Ciba) for a period of 14 days. In the second group 1200 mg of antipyrine together with phenobarbitone 100 mg (Luminal[®], Merck, Darmstadt) were administered daily in the evening for the same period of time. In the third group antipyrine 1200 mg combined with 600 mg rifampicin was given daily in the morning for 14 days.

Evaluation of liver microsomal enzyme function

As parameters of liver microsomal enzyme activity antipyrine elimination, γ -glutamyl transferase (γ -GT) in plasma and 6 β -hydroxycortisol urinary excretion were estimated before and after each drug administration. After an overnight fast antipyrine 1200 mg was given orally and 1 h later breakfast was allowed. Blood for measuring antipyrine concentrations according to the method of Brodie *et al.* (1949) was taken at the following time intervals: 0, 3, 6, 9, 12 and 24 h. From these plasma concentrations, the overall elimination rate constant was calculated by means of linear regression using the method of the least squares of errors (Sachs, 1978). From the overall elimination rate constant the antipyrine half-life, the apparent volume of distribution (V_D) and the total body clearance (CL_A) was calculated as published previously (Ohnhaus *et al.*, 1977; Ohnhaus & Park, 1979). The evaluation after each induction period was performed 36 h after the last dose given in order to avoid interference with the measurements performed (Prescott, 1969; Miguet *et al.*, 1977; Ohnhaus *et al.*, 1981).

γ -GT in the plasma was estimated according to the method of Szasz (1969). Urine for the estimation of 6 β -hydroxycortisol was collected twice during 24 h

at the time when antipyrine elimination was performed. 6 β -hydroxycortisol was measured by a radioimmunological method (Park, 1978) and corrected by the 17-hydroxycorticosteroids according to the method of Sanghvi *et al.* (1973).

Evaluation of thyroidal function

Concentration of total T_4 , T_3 and reverse tri-iodothyronine (rT_3) were measured by radioimmunoassays (Burger, 1974; Burger *et al.*, 1975; Nicod *et al.*, 1976). The fraction of free thyroxine (FT_4) was determined by radioimmunoassay (Corning IMMO PhaseTM Free T_4 Assays), a method yielding practically identical results to those obtained by equilibrium dialysis in euthyroid, hypothyroid and hyperthyroid patients (Bürki *et al.*, 1974; Chopra *et al.*, 1980; Hermann, 1979). The thyroxine binding globulin (TBG) capacity was measured according to Roberts & Nicolai (1969), TSH by a radioimmunological method (Lemarchand-Beraud *et al.*, 1972) and the T_3 resin uptake using the Triosorb test kit by Abbot Ltd. The normal values and 95% confidence limits for the assays used range for total T_4 from 55.0 to 139 nmol/l, for FT_4 from 10.0 to 27.0 pmol/l, for total T_3 from 1.23 to 3.1 nmol/l, for rT_3 from 0.15 to 9.2 nmol/l and for TSH from 0.5 to 7.2 μ U/ml.

Statistical analysis

For each parameter measured the mean value and its standard deviation was calculated. The variances of the means were tested for homogeneity of the distribution by the *F*-Test (Sachs, 1978). If the variances were found to be homogeneously distributed, all values were compared by a *t*-test for paired observation. In the case of inhomogeneity the Wilcoxon's rank test was performed.

Results

Administration of the different enzyme inducing agents over a period of 14 days did not produce any side effects in the volunteers. In addition, none of the clinical or laboratory investigations showed any unwanted changes.

Measurements of liver microsomal enzyme function

The results of the relevant measurements are listed in Table 1. The volume of distribution for antipyrine was not altered by any of the drugs administered, whereas the antipyrine clearance showed different changes. Following 600 mg rifampicin an increase of about 60%, following the combination antipyrine/phenobarbitone of about 80% and following the

Table 1 Calculated pharmacokinetic parameters of antipyrine, values of γ -GT in plasma, 6 β -hydroxycortisol (6 β -OHC) and 17-hydroxycorticosteroids (17-OHCS) urinary excretion and their calculated ratio before and after the administration of various enzyme inducing agents (mean $\pm$ s.d.).

		Rifampicin 600 mg (n = 6)	Antipyrine 1200 mg + phenobarbitone 100 mg (n = 6)	Antipyrine 1200 mg + rifampicin 600 mg (n = 6)
Antipyrine				
V_D (l)	before	53.2 $\pm$ 14.1	39.8 $\pm$ 6.3	41.2 $\pm$ 7.0
	after	50.9 $\pm$ 9.5	49.4 $\pm$ 12.3	36.3 $\pm$ 11.3
$t_{1/2}$ (h)	before	11.7 $\pm$ 2.6	10.4 $\pm$ 2.3	12.5 $\pm$ 2.9
	after	7.1 $\pm$ 1.2**	7.0 $\pm$ 1.0***	4.6 $\pm$ 1.4****
CL	before	52.9 $\pm$ 8.5	45.6 $\pm$ 8.8	40.5 $\pm$ 12.9
(ml/min)	after	84.3 $\pm$ 19.8**	82.5 $\pm$ 23.7***	92.5 $\pm$ 20.7***
γ -GT	before	14.0 $\pm$ 6.5	14.3 $\pm$ 10.3	8.7 $\pm$ 1.9
(μ mol/ min $^{-1}$ l $^{-1}$)	after	15.4 $\pm$ 6.2	26.5 $\pm$ 18.1	15.0 $\pm$ 6.3*
6 β -OHC	before	342 $\pm$ 102	217 $\pm$ 42	297 $\pm$ 56
(μ g/day)	after	1260 $\pm$ 266****	621 $\pm$ 154 ***	1946 $\pm$ 643****
17-OHCS	before	7.1 $\pm$ 2.6	9.7 $\pm$ 4.1	7.6 $\pm$ 2.1
(mg/day)	after	7.3 $\pm$ 2.7	9.5 $\pm$ 3.9	7.5 $\pm$ 2.1
Ratio	before	5.2 $\pm$ 1.8	2.6 $\pm$ 0.9	4.4 $\pm$ 2.1
6 β -OHC/ 17-OHCS	after	19.3 $\pm$ 7.3***	8.0 $\pm$ 5.0**	27.9 $\pm$ 14.5***

* $P < 0.025$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$.

combined administration of antipyrine and rifampicin of 128% was observed. The γ -GT values were significantly increased following the drug combinations, but remained constant following rifampicin 600 mg given alone for a period of 14 days. All inducing agents raised the urinary excretion of 6 β -hydroxycortisol. Since the urinary excretion of 17-hydroxycorticosteroids remained constant the ratio of 6 β -hydroxycortisol to 17-hydroxycorticosteroids rose from values between 2.6% and 5.2% to levels between 8% to 27.9% after the different drugs.

Measurement of thyroid hormone concentrations

The thyroid hormone steady state levels before and after administration of the different enzyme inducing agents are given in Table 2. Following all three different drug administrations similar changes occurred in each study. T_4 levels decreased simultaneously with FT_4 and rT_3 concentrations, while T_3 , TBG and TSH baseline levels remained unaltered following the different drugs administered. The relative changes in percentage of the original value were similar in the three different groups and gave an identical fall in T_4 of about 18% and 33% in rT_3 respectively.

Discussion

Using rifampicin and two different drug combina-

tions a significant enhancement of the three *in vivo* indices of liver microsomal enzyme activity used was observed. Taking the increase in metabolic clearance of antipyrine as an indirect index of enzyme induction *in vivo* (Huffman *et al.*, 1974; Vessell *et al.*, 1975) the inducing capacities of the drug combinations are higher compared to single doses of antipyrine, phenobarbitone and rifampicin (Davis *et al.*, 1974; Hildebrandt *et al.*, 1975; Miguët *et al.*, 1977; Ohnhaus *et al.*, 1977; Ohnhaus & Park, 1979; Roberts *et al.*, 1976; Vesell & Page, 1969). Following the combined administration of antipyrine/rifampicin the enhancement in antipyrine clearance was much more pronounced compared to rifampicin given alone or the combination antipyrine/phenobarbitone. This increase was similar to that observed with a daily dose of 1200 mg rifampicin given for a period of 14 days (Ohnhaus & Park, 1979). Similar changes between the individual drug treatments were seen using 6 β -hydroxycortisol urinary excretion corrected by the 17-hydroxycorticosteroids, whereby increases between 8 and 28% were observed following the different drugs administered (Table 1).

Plotting antipyrine clearance as a ratio after and before the induction period vs the same ratio in 6 β -hydroxycortisol excretion corrected by the 17-hydroxycorticosteroids, a distinction between the enzyme inducing potency of the drugs in the different groups investigated is possible (Ohnhaus & Park, 1979). In Figure 1 the results of the present and our previous study are seen (Ohnhaus *et al.*, 1981). Rifampicin and

Table 2 Thyroid hormone steady state concentrations before and after administration of various enzyme inducing agents (mean $\pm$ s.d.).

	Rifampicin 600 mg daily		Antipyrine 1200 mg + phenobarbitone 100 mg daily		Antipyrine 1200 mg + rifampicin 600 mg daily	
	Before	After	Before	After	Before	After
TBG (μ g/ml)	15.6 $\pm$ 2.4	16.4 $\pm$ 2.4	17.7 $\pm$ 2.4	16.4 $\pm$ 3.0	20.6 $\pm$ 3.6	23.9 $\pm$ 4.2
T ₃ resin uptake (%)	30.2 $\pm$ 1.7	30.0 $\pm$ 1.9	26.7 $\pm$ 1.3	26.3 $\pm$ 2.1	25.1 $\pm$ 6.0	23.9 $\pm$ 3.5
FT ₄ (pmol/l)	22.9 $\pm$ 3.6	* 18.4 $\pm$ 1.8	19.2 $\pm$ 3.2	** 15.7 $\pm$ 2.8	19.6 $\pm$ 4.2	*** 14.3 $\pm$ 3.2
T ₄ (nmol/l)	102.6 $\pm$ 11.5	** 84.3 $\pm$ 16.8	104.4 $\pm$ 27.3	** 86.0 $\pm$ 22.3	124.5 $\pm$ 11.1	*** 101.7 $\pm$ 12.4
T ₃ (nmol/l)	2.0 $\pm$ 0.4	1.9 $\pm$ 0.5	2.8 $\pm$ 0.3	2.9 $\pm$ 0.6	3.6 $\pm$ 0.5	3.4 $\pm$ 0.7
rT ₃ (nmol/l)	0.50 $\pm$ 0.07	*** 0.34 $\pm$ 0.09	0.40 $\pm$ 0.16	** 0.29 $\pm$ 0.10	0.50 $\pm$ 0.13	* 0.32 $\pm$ 0.07
TSH (μ U/ml)	3.8 $\pm$ 0.6	3.6 $\pm$ 1.0	3.2 $\pm$ 1.2	2.9 $\pm$ 1.3	3.3 $\pm$ 1.7	3.1 $\pm$ 1.6

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **Figure 1** The ratio of 6 β -hydroxycortisol/17-hydroxycorticosteroids before and after induction by various drugs and drug combinations used is plotted against the ratio of antipyrine clearance obtained. Each data point represents the mean of seven different groups of volunteers showing a good correlation between both parameters ($r = 0.92$). From this figure the enzyme inducing capacity of each drug administered can be seen. ● antipyrine 1200 mg, ▲ phenobarbitone 100 mg, △ phenobarbitone + antipyrine, * rifampicin 600 mg, ○ rifampicin + antipyrine, □ rifampicin 1200 mg for 8 days, ■ rifampicin 1200 mg for 14 days.

the drug combinations chosen here have a more potent enzyme inducing capacity compared to antipyrine and phenobarbitone given alone, while a dose of 1200 mg rifampicin showed a similar inducing capacity.

Following 1200 mg rifampicin a fall in T₄, FT₄ and rT₃ was observed previously, while no changes in thyroid hormone concentrations were seen after antipyrine and phenobarbitone administration (Ohnhaus *et al.*, 1981). Using the latter drugs and rifampicin in combination over the same period of time a fall in T₄, FT₄ and rT₃ plasma levels occurred which was connected to increases in antipyrine clearance between 60% to 120%. This might be the extent of liver microsomal enzyme induction at which the accelerated T₄ and rT₃ disposal cannot be longer compensated for by increased thyroid secretion or increased conversion of T₄ to rT₃. Below this level of activation a higher turnover of T₄ may still exist but the increased production of hormones in the thyroid may compensate the increased hepatic elimination. On the other hand, no increase in T₃ concentrations was found in the present study while T₃ clearly rose after the administration of 1200 mg rifampicin. Thereby the thyroidal origin of this additional T₃ had clearly

been demonstrated by additional studies with iodide blocked thyroid (Ohnhaus *et al.*, 1981).

In conclusion, in the present study again a striking effect of liver microsomal enzyme induction on thyroid hormone secretion and disposal was observed. While an increase of T_4 and rT_3 metabolism beyond the compensatory capability of the thyroid gland invariably occurs, depending on the degree of induction achieved rather than on the kind of inducing drug used, no such effect is demonstrable for T_3 . Since thyroid activation through the TSH mechanism has not been demonstrated using the most sensitive methods available today (Ohnhaus *et al.*, 1981), the pathway of thyroid compensation remains speculative.

References

- BRODIE, B.B., AXELROD, J., SOBERMAN, R. & LEVY, B.B. (1949). The estimation of antipyrine in biological materials. *J. biol. Chem.*, **179**, 25–29.
- BURGER, A. (1974). Thyroxine radioimmunoassay. In *Clinical Biochemistry: Principles and Methods*, ed. Curtius, H.C. & Roth, M., pp. 881–884. Berlin: Walter de Gruyter & Co.
- BURGER, A., SAKOLOFF, C., STAEHELI, V., VALLOTTON, M.B. & INGBAR, S.H. (1975). Radioimmunoassays of 3,5,3'-triiodothyronine with and without a prior extraction step. *Acta Endocrinol., Suppl.* **80**, 58–69.
- BÜRGI, H., ZUPPINGER, K., KOECHLI, H.P. & BURGER, A. (1974). Familiäre Störungen des thyroxinbindenden Globulins als Ursache irreführender Serumthyroxinwerte. *Schweiz. Med. Wschr.*, **104**, 1141–1147.
- CAVALIERI, R.R., SUNG, L.C. & BECKER, C.E. (1973). Effects of phenobarbital on thyroxine and triiodothyronine kinetics in Graves' disease. *J. clin. Endocrinol.*, **37**, 308–316.
- CAVALIERI, R.R., GAVIN, L.A., WALLACE, A., HAMMOND, M.E. & CRUSE, K. (1979). Serumthyroxine, free T_4 , triiodothyronine and reverse- T_3 in diphenylhydantoin-treated patients. *Metabolism*, **28**, 1161–1165.
- CHIN, W. & SCHUSSLER, G.C. (1968). Decreased thyroxine concentration in patients treated with diphenylhydantoin. *J. clin. Endocrinol.*, **28**, 181–186.
- CHOPRA, I.J., VAN HERLE, A.J., CHUA TECO, G.N. & NGUYEN, A.H. (1980). Serum free thyroxine in thyroidal and nonthyroidal illnesses: a comparison of measurements by radioimmunoassay, equilibrium dialysis and free thyroxine index. *J. clin. Endocrinol. Metab.*, **51**, 135–143.
- CULLEN, M.J. & BURGER, A. (1972). Effects of diphenylhydantoin on peripheral thyroid hormone economy and the conversion of thyroxine to triiodothyronine. 48th Meeting. *American Thyroid Association*, Abstract Volume, p. 21.
- DAVIS, M., SIMMONS, C.J., DORDONI, B. & WILLIAMS, R. (1974). Urinary D-glucaric acid excretion and plasma antipyrine kinetics during enzyme induction. *Br. J. clin. Pharmacol.*, **1**, 253–257.
- GALLAGHER, B.B., SMITH, D.B. & MATTSO, R.H. (1970). The relationship of the anticonvulsant properties of primidone to phenobarbital. *Epilepsia*, **11**, 293–301.
- HANSEN, J.M., SKOVSTED, L., LAURIDSEN, U.B., KIRKEGAARD, C. & SIERSBAEK-NIELSEN, K. (1974). The effect of diphenylhydantoin on thyroid function. *J. clin. Endocrinol.*, **39**, 785–789.
- HERMANN, J. (1979). Diagnostische Bedeutung der freien Schilddrüsenhormone in Serum. *Int. Welt.*, **4**, 136–144.
- HILDEBRANDT, A.G., ROOTS, I., SPECK, M., SAALFRANK, K. & KEWITZ, H. (1975). Evaluation of *in vivo* parameters of drug metabolizing enzyme activity in man after administration of clemastine, phenobarbital or placebo. *Eur. J. clin. Pharmacol.*, **8**, 327–336.
- HUFFMAN, D.H., SHOEMAN, D.W. & AZARNOFF, D.L. (1974). Correlation of the plasma elimination of antipyrine and the appearance of 4-hydroxy-antipyrine in the urine of man. *Biochem. Pharmacol.*, **23**, 197–201.
- LARSEN, P.R., ATKINSON, A.J., WELLMANN, H.N. & GOLDSMITH, R.E. (1970). The effect of diphenylhydantoin on thyroxine metabolism in man. *J. clin. Invest.*, **49**, 1266–1279.
- LARSEN, P.R., DICK, T.E., MARKOVITZ, B.P., KAPLAN, M.M. & GARD, T.C. (1979). Inhibition of intrapituitary thyroxine to 3,5,3'-triiodothyronine conversion prevents the acute suppression of thyrotropin release by thyroxine in hypothyroid rats. *J. clin. Invest.*, **64**, 117–128.
- LEMARCHAND-BERAUD, T., GRIESSEN, M. & SCAZZIGA, B.R. (1972). Clinical significance of LATs and TSH in the blood. *Ann. clin. Res.*, **4**, 121–137.
- LIEWENDAHL, K. & MAJURI, H. (1976). Thyroxine, triiodothyronine and thyrotropin in serum during long-term diphenylhydantoin therapy. *Scand. J. clin. lab. Invest.*, **36**, 141–144.
- MENDOZA, D.M., FLOCK, E.V., OWEN, C.A. & PARIS, J. (1966). Effect of 5,5'-diphenylhydantoin on the metabolism of L-thyroxine ^{131}I in the rat. *Endocrinology*, **79**, 106–108.
- MIGUET, J.P., MAVIER, P., SOUSSY, C.J. & DUHAMEAUX, D. (1977). Induction of hepatic microsomal enzymes after brief administration of rifampicin in man. *Gastroenterology*, **72**, 924–926.
- NICOD, P., BURGER, A., STAEHELI, V. & VALLOTTON, B. (1976). A radioimmunoassay for 3,3',5'-triiodothyronine in unextracted serum: method and clinical results. *J. clin. Endocrinol. Metab.*, **42**, 823–829.

No experimental or naturally occurring event is known to produce identical changes of thyroid hormone metabolism. However, it should be noted that marked alterations of thyroid hormone steady state concentration only occur after a liver microsomal enzyme activation which is strong enough to produce an increase of antipyrine clearance by about 60%.

This work was generously supported by the Swiss National Science Foundation, Grant No. 3.981/0.78.

We are indebted to Miss C. von Grünigen and Dr. E. Gerber-Taras for excellent technical assistance.

*Address for reprints: Medizinische Universitäts-Klinik, D-4300 Essen, Hufelandstrasse 55, West Germany

- OHNHAUS, E.E., MARTIN, J., KINSER, J. & COLOMBO, J.P. (1977). Enzyme induction and renal function in man. *Br. J. clin. Pharmac.*, **4**, 33–37.
- OHNHAUS, E.E. & PARK, B.K. (1979). Measurement of urinary 6 β -hydroxycortisol excretion as an *in vivo* parameter in the clinical assessment of the microsomal enzyme-inducing capacity of antipyrine, phenobarbitone and rifampicin. *Eur. J. clin. Pharmac.*, **15**, 139–145.
- OHNHAUS, E.E., BÜRGI, H., BURGER, A. & STUDER, H. (1981). The effect of antipyrine, phenobarbital and rifampicin on thyroid hormone metabolism in man. *Eur. J. clin. Invest.*, **11**, 381–387.
- OHNHAUS, E.E., GERBER-TARAS, E. & PARK, B.K. (1982). Enzyme inducing drug combinations and their effects on liver microsomal enzyme activity in man. *Eur. J. clin. Pharmac.* (in press).
- OPPENHEIMER, J.H., FISHER, L.V., NELSON, K.M. & JAILER, J.W. (1961). Depression of the serum protein-bound iodine level by diphenylhydantoin. *J. clin. Endocrinol.*, **21**, 252–262.
- OPPENHEIMER, J.H., BERNSTEIN, G. & SURKS, M.I. (1968). Increased thyroxine turn-over and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J. clin. Invest.*, **47**, 1399–1406.
- PARK, B.K. (1978). A direct radioimmunoassay for 6 β -hydroxycortisol in human urine. *J. steroid Biochem.*, **9**, 963–966.
- PRESCOTT, L.F. (1969). Pharmacokinetic drug interactions. *Lancet*, **ii**, 1239–1243.
- ROBERTS, R.C. & NICOLAI, T.F. (1969). Determination thyroxine-binding globulin. A simplified procedure utilizing dextran-coated charcoal. *Clin. Chem.*, **15**, 1132–1140.
- ROBERTS, C.J., JACKSON, C., 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.
- ROOTS, I., HOLBE, R., HÖVERMANN, W., NIGAMS, S., HEINEMEYER, G. & HILDEBRANDT, A.G. (1979). Quantitative determination by HPLC of urinary 6 β -hydroxycortisol, an indicator of enzyme induction by rifampicin and antiepileptic drugs. *Eur. J. clin. Pharmac.*, **16**, 63–71.
- SACHS, L. (1978). *Angewandte Statistik*. Berlin: Springer.
- SANGHVI, A., WIGHT, C., PARIKH, B. & DESAI, H. (1973). Urinary 17-hydroxycorticosteroid determination with *p*-hydrazinobenzene-sulfonic-acid-phosphoric-acid. *Am. J. clin. Path.*, **60**, 684–690.
- SCHÖNBERGER, W., GRIMM, W., SCHÖNBERGER, G., SINTERHAUF, K., SCHEIDT, E. & ZIEGLER, R. (1979). Der Einfluß von Primidon auf die Schilddrüsenfunktion. *Dtsch. med. Wschr.*, **104**, 915–917.
- SZASZ, G. (1969). A kinetic photometric method for serum γ -glutamyltranspeptidase. *Clin. Chem.*, **15**, 124–136.
- VESELL, E.S. & PAGE, J.G. (1969). Genetic of the phenobarbital induced shortening of plasma antipyrine half-lives in man. *J. clin. Invest.*, **48**, 2202–2209.
- VESELL, E.S., PASSANANTI, G.T. & GLENWRIGHT, P.A. (1975). Studies on the disposition of antipyrine, aminopyrine and phenacetin using plasma, saliva and urine. *Clin. Pharmac. Ther.*, **18**, 259–272.

(Received May 26, 1982
accepted September 6, 1982)