Biochemical Pharmacology, Vol. 28, pp. 3373–3378

0006-2952/79/1201-3373 $02.00/0

ATYPICAL INDUCTIVE PROPERTIES OF RIFAMPICIN

FRIEDRICH HEUBEL and KARL JOACHIM NETTER
Institut für Pharmakologie und Toxikologie, Lahnberge, Philipps-Universität, D-3550 Marburg/L., Deutschland

(*Received* 16 *January* 1979; *accepted* 1 *June* 1979)

Abstract—Hepatic and microsomal parameters, metabolism of cytochrome P-450-dependent substrates and spectral properties of cytochrome P-450 have been used in mice in order to classify rifampicin as an inducer by comparing it to phenobarbitone and 3-methylcholanthrene. Rifampicin significantly enhanced relative liver weight, cytochrome P-450 content, microsomal protein, weight of the 100,000 *g* pellet and shortened the zoxazolamine paralysis time. Compared on the basis of microsomal protein, of five substrate reactions only the ethylmorphine demethylation was enhanced; ethoxycoumarin de-ethylation and biphenyl 1-2-hydroxylation were unaltered; ethoxyresorufin de-ethylation and biphenyl-4-hydroxylation were decreased. The CO–cytochrome P-450 absorption maximum showed a blue shift of about 0.5 nm after only 1 day of rifampicin pretreatment, while there was no significant blue shift after 1 day of 3-methylcholanthrene pretreatment. Rifampicin has been shown to be an inducer in NMRI mice. Although resembling phenobarbitone it seems, however, to be a similarly atypical inducer as it is in man.

Rifampicin, called rifampin in the U.S.A. [1], has attracted the attention of clinical pharmacologists because of a series of clinical drug interactions which is listed in Table 1.

Not only does rifampicin, when given for a week or longer, shorten its own plasma half life and decrease its serum levels [12, 13, 14, 15, 16], but rifampicin serum levels seemed to be decreased also by phenobarbitone pretreatment [17]. Under the reasonable assumption that rifampicin is an enzyme inducer in man, its effects on substances which are known to be substrates of the microsomal drug oxidizing system have been investigated and conflicting data have been reported. Antipyrin metabolism was found to be increased [16, 18] and unchanged [11]. D-Glucaric acid excretion in urine, which is a somewhat controversial sign of enzyme induction, equally was found to be elevated [6, 11] as well as unchanged [19]. Morphologically a "salt and pepper"-like augmentation of smooth endoplasmic reticulum in human liver cells after 2 days of rifampicin treatment has been described [20]. *In vitro*, cytochrome P-450 levels were found to be doubled in liver homogenates and liver microsomes, respectively [21, 22].

With respect to animals, findings are less uniform. Induction of cytochrome P-450 in *guinea pigs* has been tried unsuccessfully [23], although diffuse augmentation of smooth endoplasmic reticulum, similar to that after induction by phenobarbitone, is known [20]. *Rats* were not inducible [21, 23, 24]. In *mice*, an increase of cytochrome P-450 was found; consequently, a number of cytochrome P-450 dependent oxidation reactions exhibited increased activities [23, 24]. An inbred strain of mice developed hepatomas with a higher incidence on long term rifampicin treatment, whereas rats remained unaffected [25]. In *hens*, impairment of egg production was reported [26].

Thus, there is little doubt that rifampicin induces the foreign compound metabolizing system in man in spite of the fact that the main metabolic route of rifampicin itself seems to be desacetylation [27, 28]. The data on animals confirm the assessment of rifampicin as an inducer with uncommon features: Inducibility as well as induced metabolic pathways

Table 1. Clinical interactions of rifampicin with other drugs

Drug concerned	Year	Literature	Result of interaction
Anticoagulants	1970	[2]	Higher acenocoumarol doses necessary
	1974	[3]	Severe impairment of blood coagulation after discontinuation of rifampicin
Oral contraceptives	1971	[4]	Menstruation disorders
	1973	[5]	Pregnancies
	1977	[37]	Shortened serum half life of ethinyloestradiol
Corticosteroids	1974	[6]	Failure of common corticoid doses in tuberculosis with Addison's disease
Digitoxin	1974	[7]	Lowered plasma levels of digitoxin
Tolbutamide	1974	[8]	Shortened serum half life of tolbutamide
	1975	[9]	Shortened serum half life of tolbutamide
Methadone	1976	[10]	Withdrawal symptoms
Hexobarbitone	1975	[9]	Shortened serum half life of hexobarbitone
	1977	[11]	Shortened serum half life of hexobarbitone

are largely different between species; in spite of increased liver weight microsomal protein content was found to be unchanged [24]; in spite of non-inducibility of guinea pigs their endoplasmic reticulum is augmented.

Therefore we tried to classify rifampicin as an inducer by comparing it to the well known inducers phenobarbitone and 3-methylcholanthrene in the mouse. Efforts were made in three directions: first, further completion of the list of investigated substrates of the microsomal mixed function oxygenase; secondly, exploration of changes in microsomal protein; and thirdly, characterization of the kind of induced cytochrome P-450.

METHODS

Maintenance and treatment of animals. Male NMRI mice (Zentralinstitut für Versuchstiere, Hannover, 25–35 g) were used because their relatively low initial liver microsomal cytochrome P-450 content would make probable inductive effects more prominent. Each trial was carried out using six treated animals and six controls. Groups were housed separately in plastic cages with sawdust bedding. Darkness was timed from 18 hr to 6 hr; the mice were fed the conventional pellet diet. Tap water was allowed *ad lib.* Rifampicin (4 mg/ml 1% methylcellulose) was given orally, 40 mg/kg at 9 hr and 40 mg/kg at 18 hr. Phenobarbitone (5 mg/ml water) was given i.p., 50 mg/kg at 9 hr and 50 mg/kg at 18 hr. 3-Methylcholanthrene (3 mg/ml peanut oil) was given i.p., 30 mg/kg once a day at 9 hr. Controls received 1% methylcellulose orally at 9 hr and 18 hr. All volumes were 0.1 ml per 10 g body weight. Food was removed at 19 hr in the evening before the day of experiment.

Sleeping time experiments. Experiments were started at 9 hr, the distance between the last dose of rifampicin and the application of hexobarbitone/zoxazolamine being 15 hr. Animals received 100 mg hexobarbitone/kg (0.1 ml per 10 g body weight of a solution of 10 mg hexobarbitone/ml) or 200 mg zoxazolamine/kg, respectively (0.2 ml per 10 g body weight; 10 mg zoxazolamine/ml were suspended by ultrasonication, Tween 80 and permanent stirring). The time between loss and reappearance of righting reflexes was recorded. The same group of animals was used repeatedly for hexobarbitone experiments (pretreatment for 1 day, 3 days and 10 days) and another group for zoxazolamine experiments (pretreatment for 1 day, 3 days and 10 days).

Preparation. Experiments were started at 8.30 hr, the distance between the last dose of inducer and killing of animals being 14.5 hr for rifampicin and phenobarbitone and 23.5 hr for 3-methylcholanthrene. Animals were killed by cervical dislocation. Livers were immediately perfused *in situ* with ice cold 1.15% KCl (38.4 ml/hr for 2 min), in order to obtain rapid cooling, less evaporation, equal fluid filling and less haemoglobin content. They were then removed and immediately weighed on aluminium foil. After the 100,000 *g* supernatant was discarded tubes were kept on blotting paper in an inverse position for 20–30 sec, returned to the ice bath and wiped off before weighing. Pellets were equally resuspended in a 5 ml volume of pH 7.4 buffer, containing 20 mM Tris and 0.25 M saccharose.

Chemicals. Rifampicin was kindly donated by Chemie Grünenthal, Stolberg. Zoxazolamine was purchased from Aldrich-Europe, Beerse, Belgium. Hexobarbitone (Evipan®) was purchased from Bayer, Leverkusen. Ethoxycoumarin was synthesized in this laboratory (W. Legrum) according to Ullrich and Weber [21] from 7-hydroxycoumarin purchased from Janssen, Düsseldorf. Biphenyl and 3-methylcholanthrene were purchased from Serva Feinbiochemica, Heidelberg. Ethoxyresorufin was a generous gift of Dr. D. Burke, University of Surrey, who synthesized the material according to Burke and Mayer [31]. Phenobarbitone and ethylmorphine were purchased from Merck, Darmstadt.

Assays. Cytochrome P-450 was measured according to Omura and Sato [29], using an Aminco DW-2 spectrophotometer. The exact position of the maximal absorption peak was determined graphically on the original recordings, the spectra being recorded from 400 to 500 nm. Rifampicin (0.05 mM) added to microsomes of untreated mice in the sample as well as in the reference cuvette did not interfere with the assay. Ethoxycoumarin-de-ethylation was measured according to Aitio [30]. Ethoxyresorufin-de-ethylation was measured according to Burke and Mayer [31] (60 μl microsomal suspension; 0.06–0.4 mg protein/ml in the cuvette; readings of increasing fluorescence were followed during 3 or 6 min respectively at 37° in a Perkin-Elmer 204-A spectrofluorometer, 560/590 nm excitation/emission wavelengths). Biphenyl 4- and 2-hydroxylation were measured according to Creaven *et al.* [32]. Fluorescence intensity was determined at 290/415 and 275/338 nm excitation/emission wavelengths and hydroxybiphenyl concentrations calculated by a programmable desk calculator. Ethylmorphine demethylation was measured by a modified method according to Mazel [33]. The homogenate was prepared in 20 mM Tris buffer pH 7.4. One millilitre of diluted microsomal suspension, equivalent to 1/50 of the individual liver, was incubated with a NADPH regenerating system of glucose-6-phosphate, glucose-6-phosphate-dehydrogenase and NADP, $MgCl_2$ (5 μmol per sample), 0.1 M phosphate buffer pH 7.4 and 0.1 ml 1 mM ethylmorphine (without nicotinamide) for 10 min and 37°. Reaction was stopped by adding TCA. Colour was assayed after mixing a 1 ml aliquot with 1 ml Nash reagent.

Statistical evaluations. Where no special mention is made, statistical evaluation was achieved by the usual *t*-tests. In peak shift experiments (see Results section) an additional two factor analysis of variance was used. Factor I was "treatment" (rifampicin–methylcellulose), factor II was "time" (date of experiment, 10.5.78–30.5.78–28.9.78). Calculations were performed according to Bruning and Kintz [38].

RESULTS

"Sleeping time" experiments

Duration of anesthesia after 100 mg hexobarbitone/kg and of paralysis after 200 mg zoxazolamine/kg following different pretreatment periods with

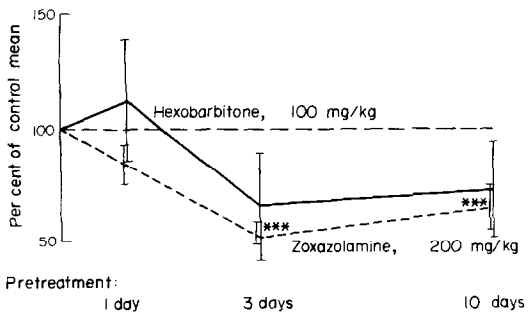

Fig. 1. Average duration of hexobarbitone anesthesia and zoxazolamine paralysis in mice treated with 2 × 40 mg rifampicin/kg/day for 1, 3 and 10 days. Values are expressed as per cent of the control mean of the same day. Bars indicate standard deviations calculated in the same way. Standard deviations of controls are not given, because two of them would have to be drawn on each time point, one for hexobarbitone and one for zoxazolamine controls. Group size ranged from 5 (zoxazolamine, 10 days) to 13 (hexobarbitone, 1 and 3 days). *** Indicates statistical significance of difference between average sleeping times of control and treated animals in minutes, $P < 0.01$.

2 × 40 mg rifampicin/kg/day are shown in Fig. 1. Absolute average sleeping times ranged from 14.9 min in pretreated animals to 113.9 min in controls. As can be seen, only zoxazolamine paralysis times after 3 and 10 days of rifampicin pretreatment are significantly shortened while those for hexobarbitone are not significantly different due to their large variance. This outcome would suggest a polycyclic hydrocarbon type of induction rather than a phenobarbitone type [34].

Hepatic and microsomal parameters

Animals were pretreated 1, 3 and 10 days with phenobarbitone (2 × 50 mg/kg/day i.p.) and rifampicin (2 × 40 mg/kg/day p.o.), and 1 and 3 days with 3-methylcholanthrene (30 mg/kg/day i.p.). Relative liver weight (liver weight in per cent of body weight), cytochrome P-450 (nmol per mg microsomal protein), microsomal protein (mg per g liver), relative pellet weight (weight of pellet after 100,000 *g* centrifugation in per cent of liver weight), and protein content of pellet (per cent of pellet weight) were estimated. The results are shown in Fig. 2. The increase in relative liver weight is similar to that under phenobarbitone pretreatment, although less marked. Cytochrome P-450 also increased in a manner similar to that after phenobarbitone pretreatment, but the increase was less steep and seemed to persist, when the phenobarbitone effect already decreased. The increase of microsomal protein under rifampicin again was similar to that under phenobarbitone, but distinctly higher. The course of the relative pellet weight seemed interesting to us. It differed from the phenobarbitone course as well as from the 3-methylcholanthrene one with a sharp decrease after day 1 and a sharp increase after day 3. The curve for the protein content of the pellets is the resultant of the preceding plots and marks the striking role of rifampicin.

Substrate metabolism

Liver microsomes of the animals treated as described above were incubated with ethoxycoumarin, ethoxyresorufin, biphenyl and ethylmorphine as substrates and the emerging metabolites hydroxycoumarin, resorufin (7-hydroxyphenoxazone), 4-hydroxybiphenyl, 2-hydroxybiphenyl and formaldehyde, respectively, were measured. The bulk of data is shown in Fig. 3. As can be seen, rifampicin plays an individual role among the common inducers: it behaves differently from phenobarbitone and 3-methylcholanthrene in respect to induction of ethoxycoumarin-de-ethylation and biphenyl-hydroxylation in that metabolism of these two substrates remains unaltered or is depressed; but it resembles phenobarbitone in decreasing ethoxyresorufin de-ethylation and increasing ethylmorphine-demethylation.

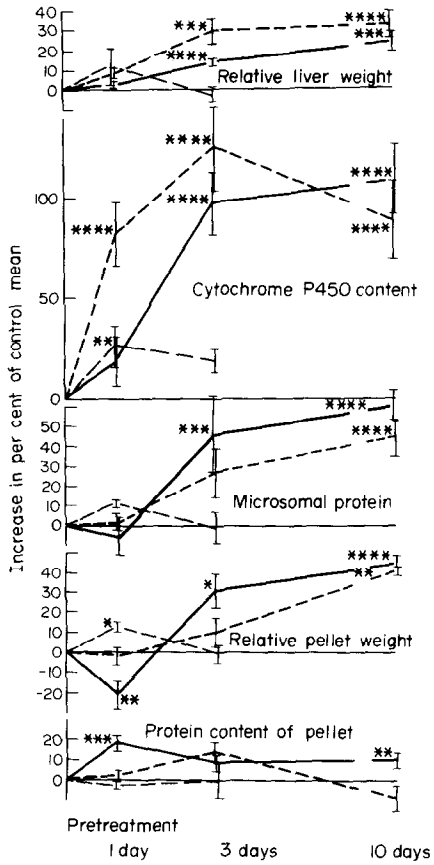

Fig. 2. Liver and microsomal parameters of mice treated 1, 3 and 10 days with phenobarbitone (2 × 50 mg/kg/day i.p., – – – –), rifampicin (2 × 40 mg/kg/day p.o., ———), or 3-methylcholanthrene (30 mg/kg/day i.p., — — —), respectively. Values are expressed as increase or decrease in per cent of the control mean. Bars indicate standard deviations. Cytochrome P-450 had been calculated in nmol/mg microsomal protein; microsomal protein had been calculated in mg/g liver. Pellet weight had been calculated as per cent of liver weight; protein content of pellet had been calculated as per cent of pellet weight. Statistical significances refer to differences from control means. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$. Average of all absolute control means: ~ 5.4% liver weight of body weight; ~ 0.6 nmol cytochrome P-450 per mg liver; ~ 19 mg protein per g liver; ~ 18 per cent pellet weight of liver weight.

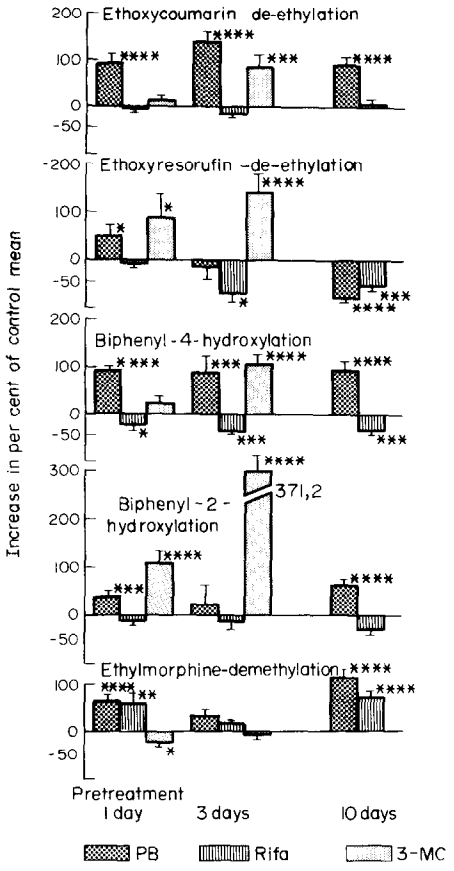

Fig. 3. Metabolism of substrates by liver microsomes of mice treated 1, 3 and 10 days with phenobarbitone (2 × 50 mg/kg/day i.p.), rifampicin (2 × 40 mg/kg/day p.o.), or 3-methylcholanthrene (30 mg/kg/day i.p.), respectively. Fluorescence readings have been calculated per mg microsomal protein and expressed as increase or decrease in per cent of the control mean. Bars indicate standard deviation. * Indicates statistical significance of difference from control mean. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$. Average of all absolute control means: ~ 0.5 nmol 2-OH-biphenyl per mg protein in 10 min; ~ 1.6 nmol 4-OH-biphenyl per mg protein in 10 min; ~ 4, 5 μg coumarine per mg protein in 10 min; ~ 20 ng resorufin per mg protein in 2 min (3rd minus 1st min); ~ 0.5 μg formaldehyde per mg protein in 10 min. Variance of control substrate metabolism was considerably high, but only controls of the same day were used.

Like Pessayre and Mazel [24] for ethylmorphine, we found an inhibition of ethoxyresorufin metabolism after addition of 0.05–0.6 M rifampicin *in vitro*. Concentrations of this order of magnitude may be reached in our experiments *in vivo* (as a mouse of 30 g would receive 2.4 mg rifampicin, which, equally distributed in a 20 ml compartment, without excretion, would cause a 0.15 mM concentration).

Shift of cytochrome P-450 absorption peak

It was not possible to elicit a rifampicin–cytochrome P-450 binding spectrum. On the other hand, the maximum absorption peak of CO-cytochrome P-450 was shifted to shorter wavelengths after all rifampicin pretreatment periods. The shift was significant at least at the 2 per cent level (Table 2). It

Table 2. Shift of CO-cytochrome P-450 complex absorption peak after different "inducers"

1 day pretreatment				3 days pretreatment				10 days pretreatment			
Control	Treated	Difference	Significance	Control	Treated	Difference	Significance	Control	Treated	Difference	Significance
					Phenobarbitone						
450.13 ± 0.28	449.91 ± 0.32	−0.22	n.s.	450.44 ± 0.25	450.30 ± 0.11	−0.14	n.s.	450.37 ± 0.14	450.57 ± 0.21	+0.20	n.s
449.97 ± 0.31	449.92 ± 0.23	−0.05	n.s.								
					Rifampicin						
450.45 ± 0.23	449.97 ± 0.28	−0.45	< 0.02	450.37 ± 0.12	449.72 ± 0.33	−0.65	< 0.01	450.40 ± 0.09	450.02 ± 0.22	−0.38	< 0.01
450.32 ± 0.15	449.67 ± 0.08	−0.65	< 0.001								
450.28 ± 0.07	449.87 ± 0.23	−0.41	< 0.01								
					3-Methylcholanthrene						
450.33 ± 0.15	450.38 ± 0.17	+0.05	n.s.	450.38 ± 0.12	450.07 ± 0.27	−0.31	< 0.05				
449.97 ± 0.41	450.08 ± 0.21	+0.11	n.s.								

Mean wavelengths of absorption maxima of each trial are given in nm ± S.D. Each trial consisted of 6 treated and 6 control animals.

already showed a considerable magnitude after 1 day of pretreatment (all 1-day-rifampicin experiments proved to be significant as well). As far as we know a similarly marked "peak shifting" after 1 day has not yet been described for any of the inducing agents. The difference found is only 2–3 times as large as the tolerance of the recording equipment. Therefore we tried to rule out a possible variance which might be generated by the different dates of the experiments. All data obtained after 1 day rifampicin pretreatment were combined and subjected to a two factor analysis of variance, the first factor being "treatment" and the second factor being "time" (date of investigation). The factor "treatment" retained its high significance ($P < 0.001$).

Our results on the NMRI mice did not show the anticipated peak shift after 3-methylcholanthrene. One day pretreatment caused no significant shift and 3 days pretreatment caused only a small blue shift (Table 2). This finding is obviously due to some kind of non-responsiveness of our NMRI mouse strain: Comparative pretreatment of male C57 B1/6 mice with the same dose of 3-methylcholanthrene for 3 days resulted in a highly significant 1.1 nm blue shift. Interestingly, although our NMRI mice did not respond to 3-methylcholanthrene in the expected amount as far as the peak shift is concerned, the metabolism of ethoxycoumarin, ethoxyresorufin and biphenyl was markedly enhanced (Fig. 3).

DISCUSSION

As discussed in the introduction of this paper, the uncommon broadness of its clinical interactions and the species specificity of its inductive properties render rifampicin a rather atypical inducer. Therefore, our objective was to classify rifampicin by comparing it to phenobarbitone and 3-methylcholanthrene. The outcome is that it plays an atypical role even if consideration is restricted to mice. Although such classic parameters as relative liver weight, cytochrome P-450 content and microsomal protein increased, only one of the offered substrates was metabolized faster (ethylmorphine). Amount and time course of relative liver weight, cytochrome P-450 content and microsomal protein after rifampicin induction resemble those after phenobarbitone, but in the metabolic field, similarity to phenobarbitone is restricted to ethylmorpine-demethylation and perhaps ethoxyresorufin-de-ethylation. With regard to ethoxycoumarin-de-ethylation and both biphenyl hydroxylations there is neither a phenobarbitone-like nor a 3-methylcholanthrene-like action. On the other hand, the shortening of zoxazolamine paralysis time suggests a hydrocarbon type of induction.

A phenobarbitone-like inducing activity favours the expectation of increased microsomal protein content. Our results confirm this expectation, whereas, interestingly, Pessayre and Mazel [24], working on the same species, found no alteration. Perhaps this discrepancy is simply due to some strain specificity. However, the time course within the first 3 days of induction of microsomal protein content, pellet weight and resulting protein content of pellet (Fig. 2) rather suggests major reconstruction processes in the endoplasmic reticulum. Evidence for such processes has been provided by De Matteis [35] for cytochrome P-450, cytochrome b_5 and total protoheme, and by Pansegrouw and Netter (unpublished) for cytochrome P-450.

According to the literature there is no doubt about the "normal" absorption spectrum of the reduced cytochrome P-450 CO complex under rifampicin induction. In contrast to this fact we found a small but distinct shift—about 0.5 nm—of the maximum absorption to shorter wavelengths. Since we could neither demonstrate a binding spectrum nor a "metabolic intermediate complex" as described by Franklin [36], the most probable explanation is the appearance of a new species of cytochrome P-450. On the other hand, the spectral phenomenon of peak shifting is fully developed already after 1 day excluding a considerable *de novo* synthesis of a different apoprotein in that time; furthermore, the amount of cytochrome P-450 continues to increase after that time point, while the blue shift is not further intensified. Perhaps the separation of these assumed multiple species of cytochrome P-450 by other methods will shed light on the mechanisms of induction, and, probably, atypical inducers promise more success in that way than do typical ones.

Acknowledgement—Uschi Ihls has given skilful and circumspect technical assistance, for which the authors are very grateful.

REFERENCES

1. L. Weinstein, in *The Pharmacological Basis of Therapeutics* (Eds. S. Goodman and A. Gilman), p. 1333. Macmillan, New York (1970).
2. F. Michot, M. Bürgi and J. Büttner, *Schweiz. med. Wschr.* **100**, 583 (1970).
3. M. J. Boekhout-Mussert, R. Bieger, P. Van Brummelen and H. H. P. J. Lenkes, *J. Am. med. Ass.* **229**, 1903 (1974).
4. D. Reimer and A. Jezek, *Prax. Pneumol.* **25**, 255 (1971).
5. L. Nocke-Finck, H. Breuer and D. Reimers, *Dtsch. med. Wschr.* **98**, 1521 (1973).
6. O. M. Edwards, R. J. Courtenay-Evans, J. M. Galley, J. Hunter and A. D. Tait, *Lancet* **ii**, 549 (1974).
7. U. Peters, T. U. Hausamen and F. Grosse-Brockhoff, *Verh. Dtsch. Ges. Inn. Med.* **80**, 1533 (1974).
8. E. K. Syvälahti, K. K. Pihlajamäki and E. J. Iisalo, *Lancet* **ii**, 232 (1974).
9. W. Zilly, D. D. Breimer and E. Richter, *Eur. J. Clin. Pharmac.* **9**, 219 (1975).
10. M. J. Kreek, J. W. Garfield, C. L. Gutjahr and L. M. Ginsti, *N. Engl. J. Med.* **294**, 1104 (1976).
11. D. D. Breimer, W. Zilly and E. Richter, *Clin. Pharmac. Ther.* **21**, 470 (1977).
12. S. Furesz, *Antibiotica Chemother.* **16**, 316 (1970).
13. G. Acocella, L. Bonollo, M. Garimoldi, M. Mainardi, T. Tenconi and F. B. Nicolis, *Gut* **13**, 47 (1972).
14. G. Curci, N. Bergamini, F. DelliVeneri, A. Ninni and V. Nitti, *Chemotherapia (Basel)* **17**, 373 (1972).
15. S. Virtanen and E. Tala, *Clin. Pharmac. Ther.* **16**, 817 (1974).
16. J. P. Miguet, P. Mavier, C. J. Soussy and D. Dhumaux, *Gastro-Enterology* **72**, 924 (1977).
17. V. Nitti, A. Ninni, G. Meola, A. Iuliano and G. Curci, *Chemotherapy* **19**, 206 (1973).
18. M. McIver, D. J. Back, A. Breckenridge, F. E. Crawford, M. L'E. Orme, B. K. Park, P. H. Rowe and E. Smith, 7th Internat. Congress of Pharmacology, Paris, 16–21 July 1978; Abstract, p. 650, Pergamon Press, Oxford (1978).

19. B. K. Park and E. E. Ohnhaus, 7th Internat. Congress of Pharmacology, Paris, 16–21 July 1978; Abstract, p. 298, Pergamon Press, Oxford (1978).
20. A. M. Jezequel, F. Orlandi and L. T. Tenconi, *Gut* **12**, 984 (1971).
21. H. Remmer, B. Schoene and R. A. Fleischmann, *Drug Metab. Disp.* **1**, 224 (1973).
22. H. M. Bolt, H. Kappus and M. Bolt, *Eur. J. Clin. Pharmac.* **8**, 301 (1975).
23. E. Beretta, D. Barone and L. T. Tenconi, Riunione monotematica: Induzione enzimatica a livello dei microsomi epatici, Padova, 20 maggio 1972, Associazione italiana per lo studio del fegato; Cooperativa libraria editrice degli student i dell'università di Padova (1972).
24. D. Pessayre and P. Mazel, *Biochem. Pharmac.* **25**, 943 (1976).
25. P. G. Della, J. R. Cabral and L. Ross, *Toxicol. appl. Pharmac.* **43**, 293 (1978).
26. E. Freerksen and J. Kazda, *Arzneimittelforsch.* **24**, 89 (1974).
27. G. Binda, E. Domenichini, A. Gottardi, B. Orlandi, E. Ortelli, B. Pacini and G. Fowst, *Arzneimittelforsch.* **21**, 1908 (1971).
28. T. Komatsu, *Chemotherapy (Tokyo)* **25**, 565 (1977).
29. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
30. A. Aitio, *Analyt. Biochem.* **85**, 488 (1978).
31. M. D. Burke and R. T. Mayer, *Drug Met. Disp.* **2**, 583 (1974).
32. P. J. Creaven, D. V. Parke and R. T. Williams, *Biochem. J.* **96**, 879 (1965).
33. P. Mazel, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. La Du, H. G. Mandel and E. L. Way), p. 562. Williams & Wilkins, Baltimore (1971).
34. A. H. Conney, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. La Du, H. G. Mandel and E. L. Way), p. 260. Williams & Wilkins, Baltimore (1971).
35. F. De Matteis, *Drug Metab. Disp.* **1**, 267 (1973).
36. M. R. Franklin, *Pharmac. Ther. A.* **2**, 227 (1977).
37. H. M. Bolt, M. Bolt and H. Kappus, *Acta endocrinol. (Copenhagen)* **85**, 189 (1977).
38. J. L. Bruning and B. L. Kintz, *Computational Handbook of Statistics*. Glenview, Illinois (1968).
39. V. Ullrich and P. Weber, *Hoppe-Seyler's Z. physiol. Chem.* **353**, 1171 (1972).