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UPTAKE AND BIOTRANSFORMATION OF IBUTEROL AND TERBUTALINE IN ISOLATED PERFUSED RAT AND GUINEA PIG LUNGS

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Abstract—The lung uptake and biotransformation of [^{3}H]terbutaline and [^{3}H]ibuterol (diisobutyrate ester of terbutaline) was studied using isolated perfused and ventilated rat and guinea pig lungs. The lung extraction ratio, as calculated from the concentration of drug in the inflowing and outflowing medium, in single pass studies of ibuterol ranged from 0.32 to 0.41 at inflowing concentrations 1×10^{-5} and 1×10^{-7} M and that of terbutaline ranged from 0.013 to 0.021. Ibuterol was hydrolyzed to terbutaline but no further biotransformation of terbutaline was found. The lung clearance of ibuterol (1×10^{-7} M) was estimated to 0.092 ml/sec. When ibuterol (1×10^{-7} M) was infused together with eserine, an esterase inhibitor, the clearance of ibuterol decreased to 0.088 ml/sec (eserine 1×10^{-5} M) and 0.045 ml/sec (eserine 1×10^{-4} M). The latter value was significantly lower ($P < 0.001$) than control.

Recent investigations have shown that many organic amines, e.g. amphetamine, imipramine and methadone are accumulated in the lung [1, 2]. It is also known that many endogenous as well as exogenous compounds are biotransformed in the lung by e.g. oxidative, hydrolytic and conjugation processes. Examples of exogenous compounds which are subjected to oxidative processes are methadone [3], nicotine [4] and nortriptyline [5]. Hydrolytic degradation has been noted for carbaryl [6], beclomethasone dipropionate [7] and ibuterol [8] and glucuronic acid conjugation for 4-methylumbelliferone [9].

Of special interest is the pulmonary fate of drugs used in the treatment of obstructive lung diseases, because the lung is the target organ for the drug therapy. Few reports describe lung uptake and biotransformation of β-adrenoceptor stimulating agents, used in the treatment of bronchial asthma [8, 10–13].

This study describes the single-pass effect of the lung on terbutaline and ibuterol (diisobutyrate ester of terbutaline). Terbutaline is a β_2-adrenoceptor stimulating agent. Isolated perfused and ventilated rat and guinea pig lungs were used as the biological model.

MATERIALS AND METHODS

Materials. DL-[^{3}H]Ibuterol hydrochloride [1-(3,5-diisobutyroxyphenyl)-2-(*t*-butylamino)-(1-^{3}H) ethanol hydrochloride], 2.6 Ci/m-mole, and DL-[^{3}H]terbutaline sulfate [1-(3,5-dihydroxyphenyl)-2-(*t*-butylamino)-(1-^{3}H) ethanol sulfate], 0.4 Ci/m-mole, were synthetized in the Research laboratories, Astra Läkemedel AB, Södertälje, Sweden. The radiochemical purity of the compounds was checked by reverse isotope dilution and ion-exchange chromatography [12] and found to be better than 95 per cent. Non-labelled ibuterol hydrochloride and terbutaline sulfate were synthetized at the Research and Development Laboratories, AB Draco, Lund. Eserine (free base) was obtained from Sigma.

Experimental procedures. Male Sprague–Dawley rats and male Dunkin–Hartley guinea pigs, weighing about 200 g, were anesthetized with pentobarbital (~40 mg/kg i.p.) and tracheostomized. The chest was opened and the pulmonary artery cannulated via the right ventricle of the heart. The left auricle was cut. The lungs were excised, and suspended in an artificial thoracic chamber and ventilated with a humidified and warmed atmosphere of carbogen (95% O_2 + 5% CO_2) by creating an alternating negative pressure. This was created by a vacuum source and an animal respirator (Model 681, Harvard Apparatus). Respiratory rate was about 70 cycles/min. The pressure inside the chamber was monitored with a water manometer (−0.5 to −8.0 cm H_2O). A schematic drawing of the perfusion apparatus is shown in Fig. 1. The lungs were perfused with Krebs–Ringer bicarbonate buffer (pH ~7.35) containing 4% bovine albumin (fraction V, Sigma) and 0.1% glucose at about 37°. The initial perfusion flow was about 0.22 ml/sec (~ 13 ml/min) in both species at a perfusion pressure of about 15 cm H_2O. Perfusion flow was checked by measuring the time for the perfusion fluid in the reservoir to decrease 5 or 10 mm and then calculating the volume.

The relative water content of the lungs, expressed as per cent of wet weight, was estimated by drying one of the lungs at 100° in an oven over night and then weighing. Increase in relative water concentration was taken as a sign of edema formation.

Perfused lungs as well as non-perfused lungs were subjected to histological examination. Tissue pieces from central and peripheral parts of lung lobes were fixed in buffered formalin solution (10%), paraffin-embedded, sectioned and stained with haematoxylin and eosin.

In the drug-perfusion experiments the lungs were perfused and ventilated for 10–15 min to establish constant perfusion flow and ventilation, before the lungs were perfused with buffer containing dissolved drug. Care was taken to avoid mixing with the control buffer. The dead time in tubings leading to the

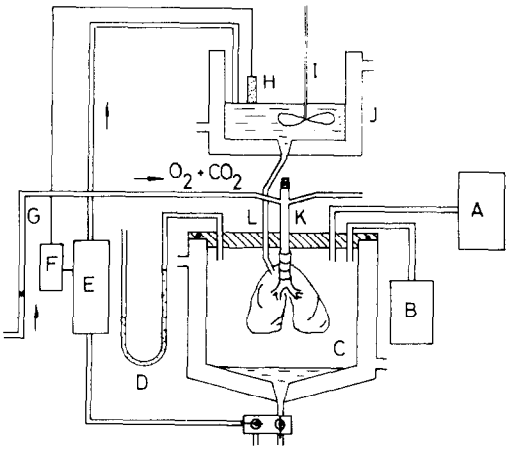

Fig. 1. Schematic drawing of the perfusion apparatus. A, animal respirator; B, vacuum pump; C, lung chamber; D, water manometer; E, peristaltic pump; F, relay; G, gas flow meter; H, termistor; I, stirrer; J, reservoir; K, tracheal cannula; L, pulmonary artery cannula.

lungs was less than 8 sec at actual perfusion flows. No correction for this dead time was made. Perfusion with eserine started from a separate bottle containing eserine in buffer about 5 min before perfusion with buffer containing both eserine and ibuterol was started.

Samples were taken from the effluent, at selected times, as well as from the reservoir, and saved for analysis. In some studies the lungs were taken out and analysed for total radioactivity.

Analytical procedures. Ibuterol was analyzed by extracting 100 μl of the perfusion medium with 500 μl chloroform and the organic phase was after separation assayed for radioactivity by liquid scintillation counting. The recovery was 91.5 $\pm$ 2.5 per cent (n = 13). Correction for the uncomplete recovery was made. To check the specificity of the method organic phases from some separate experiments were taken to near dryness and spotted on precoated silica gel plates (E Merk AG). The plates were developed with $CHCl_3$–EtOH–HAc (5:1:1). After developing and drying the plates, eleven zones of 1 cm each were scraped off and the scrapings transferred into counting vials containing 1.0 ml EtOH. The vials were shaken for 30 min, after this scintillation solution was added and the samples assayed. Ibuterol, monoisobutyrate ester of terbutaline and terbutaline were used as reference substances. The R_f-values of these compounds were 0.60, 0.35 and 0.20, respectively. In Fig. 2 is shown a chromatogram of a chloroform extract from a perfusion experiment. No detectable co-extraction of the monoester and terbutaline was found.

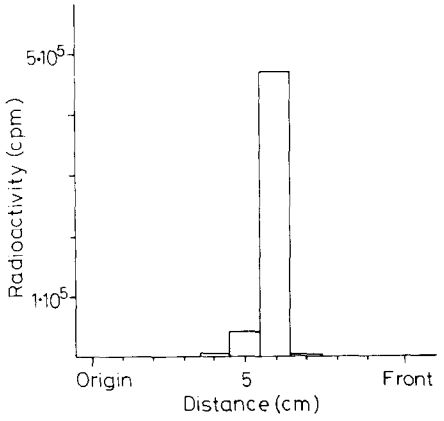

Fig. 2. Thin-layer chromatography (silica gel—$CHCl_3$–EtOH–HAc (5:1:1)) of a concentrated chloroform extract from a perfusion experiment with ibuterol. The R_f-values of ibuterol, monoisobutyrate ester of terbutaline and terbutaline are 0.60, 0.35 and 0.20, respectively.

Terbutaline was assayed by ion-pair extraction [12, 14].

Kinetic and statistical calculations. Lung extraction (E) = $C_{in} - C_{out}/C_{in}$ (C_{in} and C_{out}; concentration of drug in the inflowing and the outflowing medium).

Lung clearance (Cl) = $E \times F$ (F; perfusion flow) Accumulated uptake = $_0\int^T F \times (C_{in} - C_{out})\, dt$ calculation was performed by numerical integration using the trapezoid rule.

Results are given as mean values $\pm$ S.E.M. The number of estimations were never less than 4. Significance analyses were performed using Student's t-test.

RESULTS

The viability of the preparation was evaluated as changes in perfusion flow, edema formation and histological examination. At a perfusion pressure of about 15 cm H_2O the initial flow was 0.22 $\pm$ 0.01 ml/sec and 0.20 $\pm$ 0.01 after about 30 min perfusion time. Edema formation was estimated as increase in relative water content of perfused lungs compared with control (not perfused) lungs. The relative water content of control lungs was 80.48 $\pm$ 0.42 per cent and that of perfused lungs 80.15 $\pm$ 0.67 (30 min perfusion time) indicating no edema formation during actual perfusion times. Histological examination revealed no abnormalities.

The lung extractions (E) of terbutaline and ibuterol were plotted as a function of perfusion time in Fig. 3. The inflowing concentration of the drugs was 1×10^{-7} M. The results showed a pronounced difference in lung extraction of terbutaline and ibuterol. When steady-state was obtained the lung extraction of ibuterol was 0.41 $\pm$ 0.01 and 0.36 $\pm$ 0.01 in rat and guinea pig, respectively. The extraction of total radioactivity was somewhat lower and decreased at a constant rate during the perfusion. With terbutaline lung extration amounted to 0.013 $\pm$ 0.003 in the rat studies and 0.021 $\pm$ 0.005 in the guinea pig studies. The lung extraction of total radioactivity and unchanged drug was the same. No or negligible biotransformation of terbutaline was found. The lung extraction in rat lungs was also studied using a high concentration of the drugs (1×10^{-5} M). The same extraction profiles were obtained as at the low concentration. The extraction of ibuterol was 0.32 $\pm$ 0.01 and that of terbutaline 0.013 $\pm$ 0.005.

The efflux of unchanged drug and total radioactivity into the perfusion medium after discontinuation of ibuterol infusion is shown in Fig. 4. No such experiments were performed with terbutaline because of the low lung extraction of this compound. For the efflux of unchanged ibuterol two phases, one slow and one rapid, may be discernible with both rat and

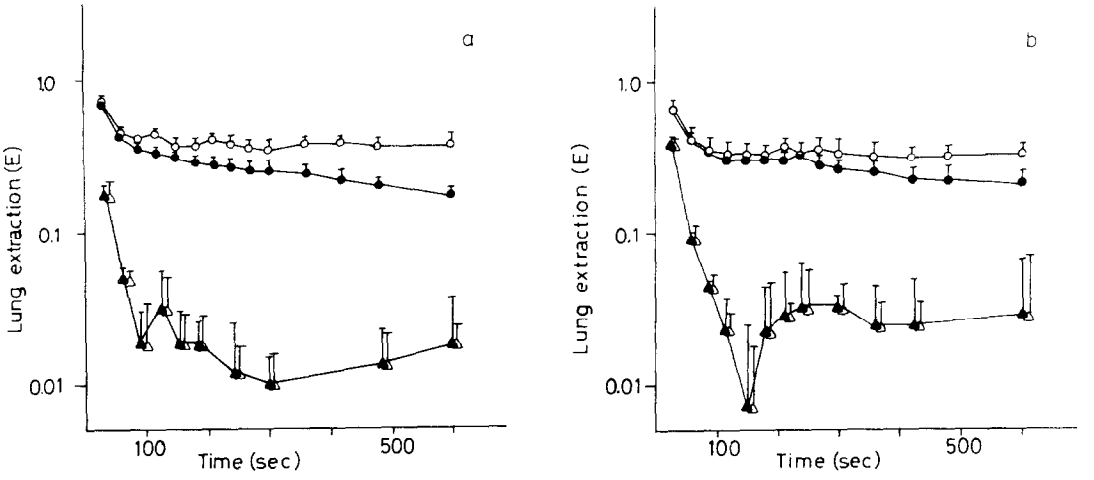

Fig. 3. Variation in lung extraction (E) of ibuterol (O——O) and terbutaline (△——△) with time using rat (a) and guinea pig lungs (b). Inflowing concentration was 1×10^{-7} M. Given values are means ± S.E.M. (n = 4–8). Open symbols represent unchanged drug and filled symbols total radioactivity.

guinea pig lungs. Regarding total radioactivity a more complex picture was obtained with at least two efflux phases. Values for efflux constants are given in Table 1. It was not possible to calculate accurate figures for the rapid phases because of too few experimental values.

In these efflux studies the total amounts of radioactivity (ibuterol + formed metabolites) accumulated in the lungs were measured at the end of the perfusion and compared with calculated values. In the rat experiments the experimental values were 2.23 ± 0.41 and 134 ± 16 nmoles/lung at the low and high inflowing concentrations and corresponding calculated values were 2.05 ± 0.46 and 125 ± 11 nmoles/lung. In the guinea pig studies (1×10^{-7} M) experimental and calculated values were 2.53 ± 0.20 and 2.70 ± 0.05 nmoles/lung, respectively.

The effect of eserine on the lung clearance of ibuterol was investigated using rat lungs (Fig. 5). In these studies the inflowing concentration of ibuterol was 1×10^{-7} M while that of eserine was either 1×10^{-5} or 1×10^{-4} M. At the low eserine concentration no effect on lung extraction (0.39 ± 0.02; control 0.42 ± 0.03) and lung clearance of ibuterol was found. At the high eserine concentration (1×10^{-4} M) a decrease in perfusion flow (0.18 ± 0.01; control 0.22 ± 0.01; $P < 0.05$) was found as well as a significant decrease in the E-value (0.24 ± 0.02; control 0.42 ± 0.03; $P < 0.001$). Consequently the lung clearance was significantly decreased. The clearances of unchanged ibuterol at steady-state were 0.092 ± 0.002 (control), 0.088 ± 0.001 (eserine 1×10^{-5} M) and 0.045 ± 0.002 (eserine 1×10^{-4} M) ml/sec. The total amounts of radioactivity accumulated in the

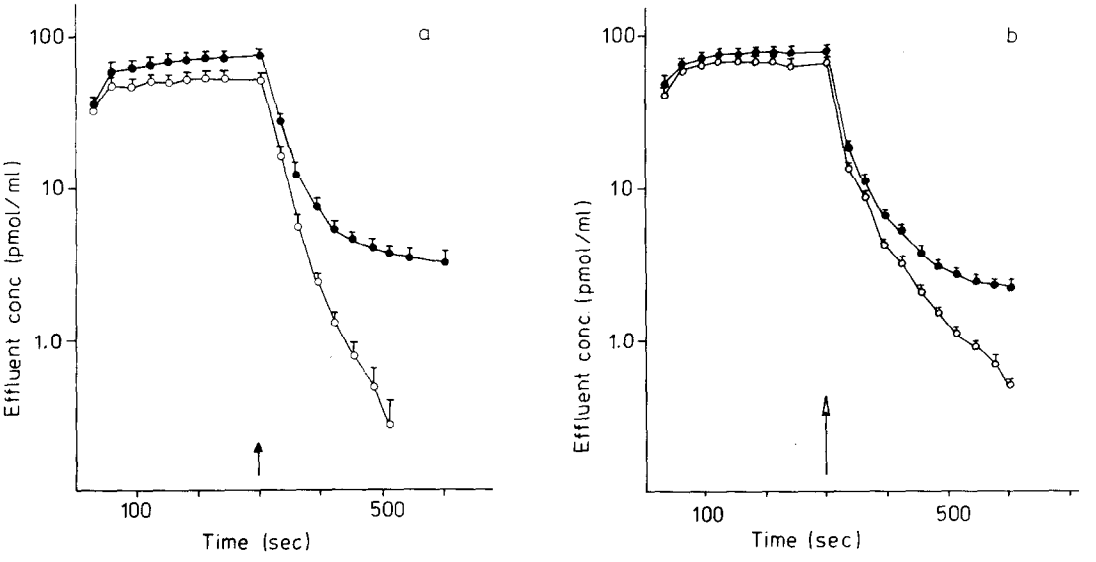

Fig. 4. Variation in effluent concentration of ibuterol with time in rat (a) and guinea pig lungs (b). Inflowing concentration was 1×10^{-7} M. Arrow indicates stop of infusion of drug. Given values are means ± S.E.M. (n = 4–6). Open symbols represent unchanged drug and filled symbols total radioactivity.

Table 1. Values for efflux constants (sec^{-1}) for unchanged ibuterol and total radioactivity from isolated perfused lungs after discontinuation of ibuterol infusion (for 300 sec)*

Species	Inflowing conc. of ibuterol (M)	Unchanged ibuterol rapid phase	Unchanged ibuterol slow phase	Total radioactivity rapid phase	Total radioactivity slow phase
Rat	1×10^{-7}	≥ 0.046	0.021 ± 0.001	≥ 0.020	0.0012 ± 0.0002
	1×10^{-5}	≥ 0.055	0.023 ± 0.001	≥ 0.028	0.0012 ± 0.0004
Guinea pig	1×10^{-7}	≥ 0.046	0.014 ± 0.003	≥ 0.023	0.0016 ± 0.0010

* Given values are means ± S.E.M. ($n = 4$–6).

lungs at the end of the experiments were also measured and calculated as described above. In these studies the experimental and calculated values were 4.48 ± 0.69 and 4.05 ± 0.36 nmoles/lung (eserine 1×10^{-5} M) and 2.80 ± 0.37 and 2.86 ± 0.22 (eserine 1×10^{-4} M). Control lungs are not included since they were only perfused for 300 sec with drug.

DISCUSSION

With the central position of the lungs in the circulation receiving total cardiac output lung clearance of endogenous as well as of exogenous compounds can be considerable. During exercise lung blood flow can increase nearly 5-fold. For comparison, liver blood flow is about 1/3 of cardiac output at rest. During exercise liver blood flow is about the same as that at rest. Lung clearance of a compound equals lung extraction (E) times lung blood flow. The extraction term is an expression for lung uptake, binding and/or biotransformation.

These studies showed that at steady-state lung extraction of unchanged ibuterol ranged from 0.41 to 0.32 at actual inflowing concentrations and was constant. Previous studies with ibuterol indicated extensive lung extraction [8]. Ibuterol is an amine (pK_a 9.1) and very lipophilic (log k_D 4.3, where log k_D is the log of the partition coefficient between chloroform and water). The lipophilic ibuterol can easily pass cell membranes. Ibuterol was found to be hydrolyzed to terbutaline in the lung. During actual perfusion times there is no detectable hydrolysis in the perfusion medium and minimal (less than 2 per cent) adsorption of ibuterol to the perfusion apparatus. This was shown in perfusion experiments without lung in the system. Other compounds which are known to be hydrolyzed in the lung are e.g. carbaryl [6] and beclomethasone dipropionate [7]. This shows the presence of extensive esterase activity in the lung capable to hydrolyze various ester compounds. The lung extraction of total radioactivity decreased at a constant rate during the perfusion. The reason for this is that terbutaline formed by hydrolysis in the lungs diffuses out into the effluent. The total radioactivity in the effluent represents both unchanged ibuterol and formed terbutaline. Slight amounts of monoester may also be present.

With terbutaline a very low lung extraction was found ($E \leq 0.02$) at both concentrations studied and with both rat and guinea pig lung. No detectable biotransformation was noted. Terbutaline is an ampholyte and very hydrophilic (log k_D-3.9) and consequently passes cell membranes more slowly than ibuterol. Isoproterenol which has physico-chemical properties similar to terbutaline, also shows very low lung uptake [10].

Previous studies in rats with terbutaline revealed that the only metabolite formed was the glucuronic acid conjugate [14, 15]. In the present study no clear indications of glucuronidation was found. In per-

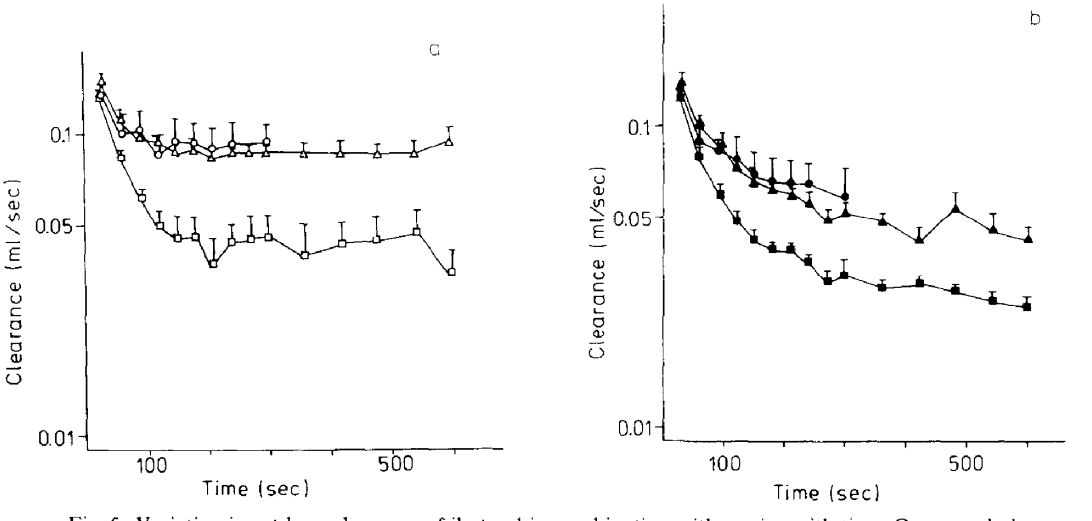

Fig. 5. Variation in rat lung clearance of ibuterol in combination with eserine with time. Open symbols represent data for unchanged ibuterol (a) and filled symbols data for total radioactivity (b). Given values are means ± S.E.M. ($n = 5$). ○: ibuterol 1×10^{-7} M; △: ibuterol 1×10^{-7} M + eserine 1×10^{-5} M; □: ibuterol 1×10^{-7} M + eserine 1×10^{-4} M.

fusion experiments for 60 min using a recirculating system no or minor biotransformation of terbutaline was noted as judged from analysis of perfusate samples by ion-pair extraction and ion-exchange chromatography [12, 14]. This indicates that the lungs must be of minor importance concerning the biotransformation of terbutaline in the intact animal. It has been shown that pulmonary microsomes are capable of glucuronide synthesis [16]. In the effluent from perfused rat lungs the glucuronide of 4-methylumbelliferone was found after a lag period of about 1 hour [9]. It was suggested that the lag period was due to accumulation of the substrate and of the conjugate in the lung before the latter compound begun to appear in the perfusate. It must also be pointed out that after long perfusion times the preparation starts to deteriorate. The substrate can then come in contact with enzymes not accessible to the substrate in an intact preparation.

The efflux studies revealed one slow and one rapid phase for unchanged ibuterol and at least two for total radioactivity. For ibuterol the more rapid phase may describe wash-out from the vascular compartment and the slower efflux from e.g. intracellular pools. Concerning total radioactivity the rapid phase may describe wash-out of ibuterol (and terbutaline) from the vascular compartment as well as efflux of ibuterol from intracellular sites while the slow phase may describe efflux of terbutaline from intracellular sites.

The effect of eserine, here used as an inhibitor of lung esterase activity, on the lung clearance of ibuterol was studied. At the low eserine concentration (1×10^{-5} M) no effect on ibuterol clearance was found but at the high eserine concentration (1×10^{-4} M) a statistically significant ($P < 0.001$) decrease was noticed. In this study eserine decreased both perfusion flow and lung extraction (E). In isolated perfused guinea pig lungs eserine has been reported to produce vasoconstriction [17] and in the anesthetized dog an increase in pulmonary arterial pressure was found following the intravenous injection of eserine [18]. A decrease of the lung extraction was also found. This effect is considered to be due to esterase inhibition leading to a decrease in the hydrolysis of ibuterol. There may also be the possibility that eserine competes with eventual storage sites for ibuterol in the lung since both compounds are amines [1, 2]. These results with eserine stress the importance of investigating both pharmacodynamic as well as metabolic effects in drug interaction studies.

REFERENCES

1. T. C. Orton, M. W. Anderson, R. D. Pickett, T. E. Eling and J. R. Fouts, *J. Pharmac. exp. Ther.* **186,** 482 (1973).
2. M. W. Anderson, T. C. Orton, R. D. Pickett and T. E. Eling, *J. Pharmac. exp. Ther.* **189,** 456 (1974).
3. F. C. P. Law, T. E. Eling, J. R. Bend and J. R. Fouts, *Drug Metab. Dispos.* **2,** 433 (1974).
4. D. M. Turner, A. K. Armitage, R. H. Briant and C. T. Dollery, *Xenobiotica* **5,** 539 (1975).
5. E. Whitnack, D. R. Knapp, J. C. Holmes, N. O. Fowler and T. E. Gaffney, *J. Pharmac. exp. Ther.* **181,** 288 (1972).
6. B. W. Blase and T. A. Loomis, *Toxic. appl. Pharmac.* **37,** 481 (1976).
7. J. Hartiala, P. Uotila and W. Nienstedt, *Br. J. Pharmac.* **57,** 422P (1976).
8. Å. Ryrfeldt and E. Nilsson, *Acta pharmac. toxic.* **39,** 39 (1976).
9. A. Aitio, J. Hartiala and P. Uotila, *Biochem. Pharmac.* **25,** 1919 (1976).
10. E. H. Butler, K. M. Moser and P. T. Kot, *J. Lab. clin. Med.* **74,** 129 (1969).
11. R. H. Briant, E. W. Blackwell, F. M. Williams, D. S. Davies and C. T. Dollery, *Xenobiotica* **3,** 787 (1973).
12. Å. Ryrfeldt and N. O. Bodin, *Xenobiotica* **5,** 521 (1975).
13. G. M. Shenfield, M. E. Evans and J. W. Paterson, *Br. J. clin. Pharmac.* **3,** 583 (1976).
14. H. T. Nilsson, C. G. A. Persson, K. Persson, K. Tegnér and Å. Ryrfeldt, *Xenobiotica* **3,** 615 (1973).
15. W. D. Conway, S. M. Singhvi, M. Gibaldi and R. N. Boyes, *Xenobiotica* **3,** 813 (1973).
16. A. Aitio, *Xenobiotica* **3,** 13 (1973).
17. A. S. Sinha, Indian J. med. Res. **30,** 123 (1942).
18. G. Morin and V. Donnet, *Archs. int. Physiol.* **54,** 161 (1946).