Biochemical Pharmacology, Vol. 38, No. 1, pp. 17–22, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
© 1989. Pergamon Press plc

PULMONARY DISPOSITION OF THE POTENT GLUCOCORTICOID BUDESONIDE, EVALUATED IN AN ISOLATED PERFUSED RAT LUNG MODEL

ÅKE RYRFELDT,* GUNILLA PERSSON† and ELISABETH NILSSON†

* AB Astra, Safety Assessment, S-151 85 Södertälje, and Department of Toxicology, Karolinska Institutet, S-104 01 Stockholm, and † AB Draco (subsidiary of AB Astra, Sweden), Research and Development Laboratories, Box 34, S-221 00 Lund, Sweden

(*Received* 11 *December* 1987; *accepted* 6 *July* 1988)

Abstract—Budesonide is a potent glucocorticoid developed for the local treatment of respiratory disorders such as bronchial asthma and allergic rhinitis. We now report the lung disposition of ^{3}H-budesonide administered either via the air passages or via the pulmonary circulation using isolated perfused and ventilated rat lungs. A rapid initial absorption was found after intratracheal administration of a clinically relevant dose of the drug. However, about half of the given dose was slowly released into the lung perfusate. The lung uptake of budesonide from the pulmonary circulation was relatively high (lung extraction ratio about 0.12). These data points to a high lung affinity for budesonide. The drug was not biotransformed in the lung. The high lung affinity and absence of lung metabolism can be important factors to explain the good therapeutic effects seen with budesonide in the clinic.

The clinical effectiveness of inhaled glucocorticoids in the treatment of asthma is clearly proven. However, limited information is available about the disposition of glucocorticosteroids in the respiratory tract [1–5]. Knowledge about lung absorption and affinity and local metabolism of glucocorticoids can have important implications on, e.g. dosing frequency, new formulations and clinical indications. Clinical studies to elucidate the fate of inhaled glucocorticoids in the lung are difficult to perform both from a practical and an ethical point of view. Therefore, experimental pharmacological models such as the isolated perfused lung preparation can be a complementary approach to elucidate basic processes of lung pharmacokinetics. To address all these aspects, the fate of the drug should be studied both after administration via the air passages and via the pulmonary circulation.

In the present investigation the pulmonary disposition of ^{3}H-budesonide was studied after intratracheal instillation and after infusion into the pulmonary circulation using isolated perfused and ventilated rat lungs. The doses used in our experimental studies correspond to clinically relevant doses. Budesonide is a potent glucocorticoid, which is effective in the local treatment of bronchial asthma and allergic rhinitis, with a very low risk to induce systemic side-effects [6–8]. The compound consists of a one-to-one mixture of the epimers with 22*R* and 22*S* configuration (Fig. 1). Both epimers seem to have the same qualitative pharmacological effects. However, epimer 22*R* is two to three times as potent as epimer 22*S* [9].

MATERIALS AND METHODS

Compounds

1,2-^{3}H-budesonide (spec. activity 94 mCi/mg) was obtained from the Radiochemical Centre, Amersham, U.K.. The epimeric ratio (22*R*/22*S*) was 53/47. High performance liquid chromatography (HPLC) analysis revealed that the radiochemical purity was 96.8% (see system below). Non-labelled budesonide was obtained from Dr. A. Thalén, AB Draco.

Lung experiments

Male Sprague–Dawley rats, weighing 300–400 g, were anesthetized with pentobarbital (about 40 mg/kg i.p.). The surgical procedure and the perfusion system has been described earlier [10]. The perfusion medium consisted of Krebs–Ringer bicarbonate solution (pH 7.4) containing 0.1% glucose and 4.5% bovine albumin (fraction V, Sigma). The lungs were perfused at a constant perfusion pressure of about

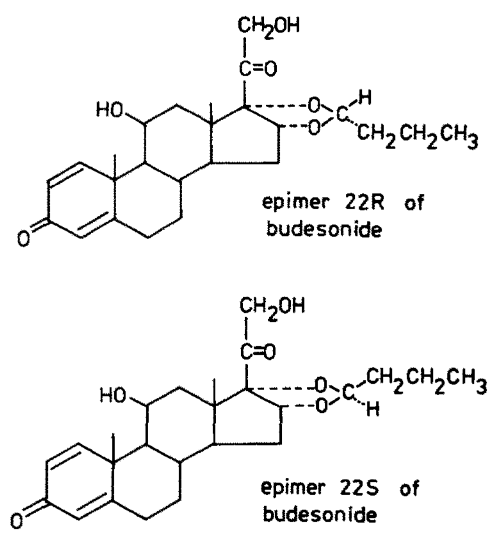

Fig. 1. Molecular structures of budesonide epimers.

10 cm H_2O. The variation in perfusion pressure throughout each separate experiment was never larger than ±5%. The perfusion flow was measured by an extracorporal flow transducer (model 2000-C, Biotronex Lab.) and a blood flow meter (model BL-610, Biotronex Lab.) and recorded on a Grass model 7D polygraph. The pO_2 and pH in the effluent from the lungs were monitored (acid-base analyzer, model pH M72 MK2, Radiometer) and recorded. The lungs were ventilated by creating an alternating negative pressure inside the lung chamber (−1 to −11 cm H_2O) relative to the ambient atmosphere by using a vacuum source and an animal respirator (model 681, Harvard Apparatus). Respiration rate was about 70 cycles/min. The viability of the preparation has previously been documented [10].

The lungs were perfused either in a single-pass or a recirculating system. The single-pass perfusion mode was used for kinetic studies and the recirculating for metabolism studies. In the recirculating system the volume of the perfusion medium was 40 ml. Using these modes of perfusion, ^{3}H-budesonide was administered either via intratracheal installation or via the pulmonary circulation. The intratracheally administered volume ranged from 50 to 100 μl. In these studies ^{3}H-budesonide was dissolved in 0.9% NaCl solution containing 10% ethanol. When budesonide was added to the perfusate in the recirculating studies, the compound was administered as a bolus dose to the reservoir. Experiments were also performed with ^{3}H-budesonide added to the perfusion medium without lung present in the system. This was done to investigate whether non-specific binding of ^{3}H-budesonide to the perfusion system occurred. No such binding was observed. In the single-pass studies, perfusion started with buffer for about 15 min. After that, the perfusion was switched over to a reservoir with buffer containing ^{3}H-budesonide for 10 min, followed by perfusion with buffer without drug for another 10–15 min. The dead time in the tubings of the single-pass system was about 10 sec for the perfusion flow used. Doses for all routes of administration and modes of perfusion are given in Table 1.

Samples (0.5 ml) were taken from the lung effluent as well as from the reservoir and saved for analysis of total radioactivity. In the recirculating experiments the lungs as well as perfusate samples were taken for analysis of unchanged budesonide. The samples were stored at −20° before analysis.

Extraction procedures of perfusion medium and lung tissue

The extraction of unchanged budesonide in the perfusion medium and the addition of an internal standard were performed according to Ryrfeldt *et al.* [11]. To each sample, 1.5 ml of an internal standard solution (40 μg budesonide/ml ethanol) and 4 ml of dichloromethane were added. The extraction was made by gentle shaking for 30 min followed by centrifugation for 15 min at 2000 rpm. The aqueous phase was analyzed for total radioactivity. The organic phase (3.0 ml) was collected and dried under a gentle stream of nitrogen. The residue was then dissolved in 200 μl of the mobile phase used in HPLC. The extraction of simulated samples gave a recovery of 103.8 ± 1.6 ($\bar{\chi}$ ± SEM; N = 5).

The lungs from an experiment were homogenized in 6 ml homogenizing buffer (500 μl internal standard budesonide solution added to 50 ml MCJ Waine buffer: 0.028 M citric acid, 0.025 M phosphoric acid, pH adjusted to 8.0 with 5 M sodium hydroxide) with a polytron PT20 homogenizer (Kinematica, Lucerne, Switzerland) under cooling. After rinsing with 6 ml homogenizing buffer, added to the original homogenate, the pooled sample was rapidly heated to 80° to denaturate enzymes. After cooling of the pooled homogenate, 6 ml of a Subtilisin A solution (Subtilisin, Novo, Batch A 9001-75, dissolved in homogenizing buffer to a concentration of 0.5 mg/ml) was added and incubation performed at 50° for 2 hr. Samples were withdrawn and analyzed for total radioactivity. Unchanged budesonide was extracted as described above. The recovery of radioactivity extracted into the organic phase, from separate lung experiments, was 93.6 ± 5.3% ($\bar{\chi}$ ± SEM, N = 5).

High-performance liquid chromatography (HPLC)

The liquid chromatograph consisted of a Waters M 6000 A pump, a Valco injector equipped with a 30 μl loop, and a Waters M 440 v.v. detector (254 nm) connected to a Tarkan W + W 600 recorder. The outlet of the detector was interconnected with a LKB Redirac fraction collector. The epimers of budesonide separate in a reversed phase system which has been described by Wikby *et al.* [12]. The analysis of ^{3}H-budesonide was performed on a Corasil C18 precolumn (5 × 50 mm) connected to a Nucleosil C18 (5 μm) column (5 × 200 mm) with ethanol/H_2O (45/55) as mobile phase. The flow rate was 1 ml/

Table 1. Experimental design, dosages and lung perfusion flows

Mode of perfusion and route of drug administered	No. of experiments	Total dose administered (g/lung)	Perfusion flow (ml/min)
Single-pass			
intratracheal	4	0.91 ± 0.08	7.2 ± 0.6
intravascular*	4	0.27 ± 0.03	7.8 ± 0.6
Recirculating			
intratracheal	5	0.67 ± 0.07	11.4 ± 1.2
intravascular	4	1.77 ± 0.10	9.0 ± 1.2

* Infusion for 10 min.
Mean lung weight 1.37 ± 0.10 g. Given values are mean ± SEM.

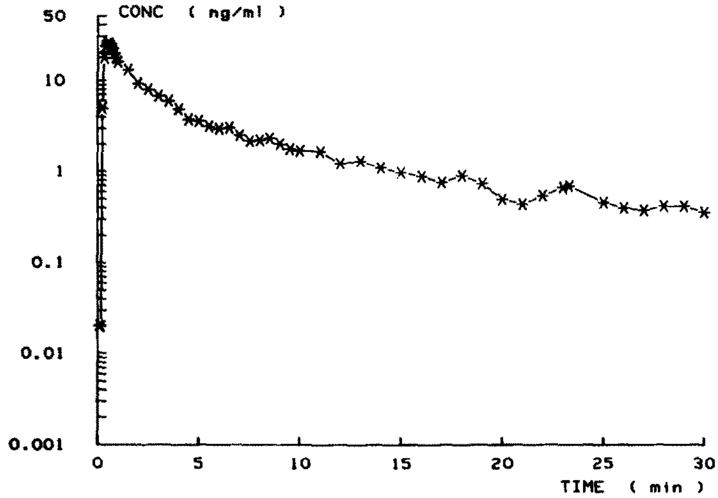

Fig. 2. Effluent concentration of drug after intratracheal instillation of ^{3}H-budesonide (dose: $0.91 \pm 0.08\ \mu g$/lung) to an isolated rat lung, perfused in the single-pass mode.

min. The effluent was collected into scintillation vials, and the radioactivity was measured. The UV-peak of the reference compound (non-labelled budesonide) was recorded.

Measurements of radioactivity

Radioactivity was measured by liquid scintillation counting. The measurements were performed in Instagel (Packard), using a Packard liquid scintillation counter. The counting efficiency was estimated by means of the external standard channel ratio procedure.

Calculations

Pharmacokinetics.
it: % dose unabsorbed = 100 × (^{q}admin − ^{q}absorbed/^{q}admin)

$$^q\text{absorbed} = \int_0^t F \times C_{out}\,dt$$

q = amount drug, F = perfusion flow, C_{out} = conc. of drug in venous effluent

$$iv: E = \frac{C_{in} - C_{out}}{C_{in}}$$

Accumulated lung uptake =

$$\int_0^t F(C_{in} - C_{out})\,dt$$

E = lung extraction ratio, C_{in} = conc. of drug in the inflowing medium

$t_{1/2}$-values were obtained by linear regression analysis, using the method of least-square

Statistics. Results are given as means ± SEM.

RESULTS

The concentration profile of budesonide in the venous effluent obtained after intracheal instillation, using the single-pass perfusion mode, is illustrated in Fig. 2. The peak level of budesonide was obtained in less than 1 min after instillation in all experiments (N = 4), suggesting a rapid initial absorption. After the peak, the effluent concentration declined rapidly ($t_{1/2} = 0.68 \pm 0.12$ min) during the first 5 min, followed by a slower decline ($t_{1/2} = 18.9 \pm 3.7$ min).

In Fig. 3 is shown the decline in the fraction of unabsorbed drug in the lung (drug remaining in

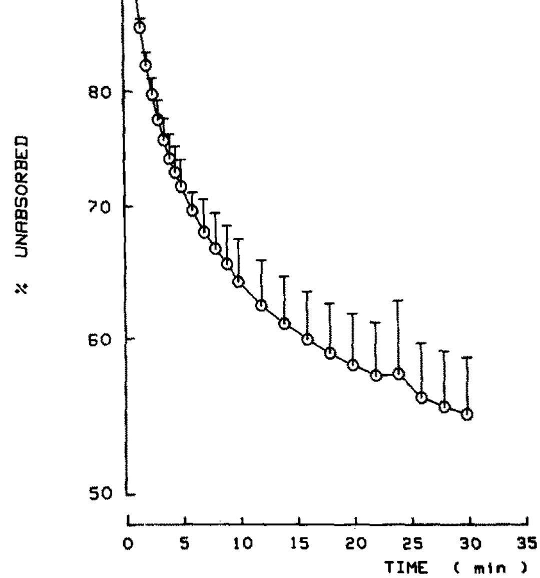

Fig. 3. Fraction (expressed as % of administered dose) of unabsorbed drug after intratracheal instillation of ^{3}H-budesonide (dose: $0.91 \pm 0.08\ \mu g$/lung) to isolated rat lung, perfused in the single-pass mode. Given values are mean ± SEM (N = 4).

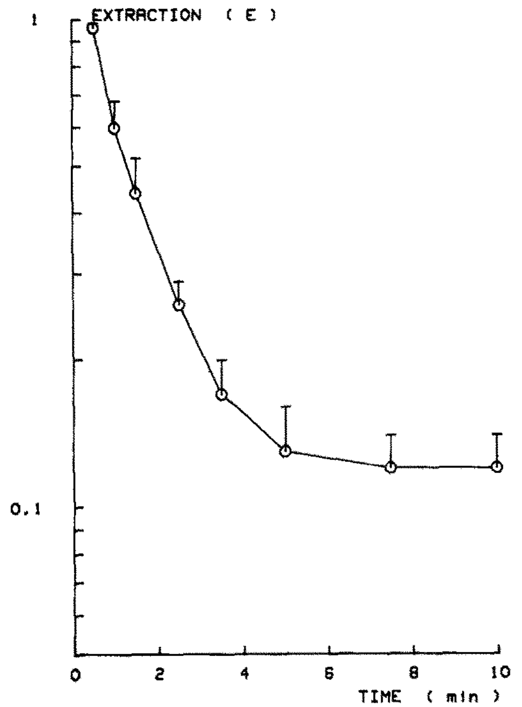

Fig. 4. Lung extraction ratio (E) of budesonide, with time, when the drug was infused into the pulmonary circulation of isolated rat lungs using single-pass perfusion. Inflowing concentration was 3.6 ± 0.2 ng/ml. Given values are means ± SEM (N = 4).

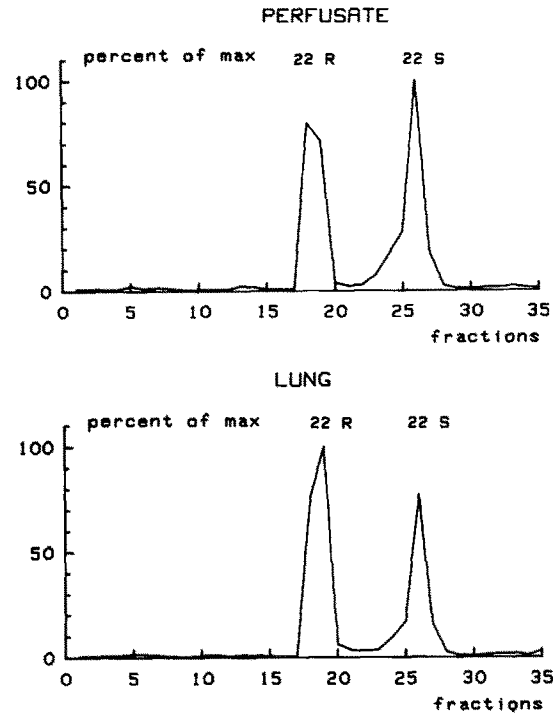

Fig. 6. HPLC-elution profiles of a perfusate and a lung sample extract obtained from a lung perfused with ^{3}H-budesonide. 22*R*: Epimer 22*R* of budesonide (retention time 20.35 min). 22*S*: Epimer 22*S* of budesonide (retention time 23.30 min).

the lung) with time. At 5 min after administration $71.6 \pm 4.6\%$ of the administered dose remained in the lungs. The corresponding value after 30 min was $54.8 \pm 7.6\%$. The calculated value for the concentration of budesonide in the lungs at 30 min was 0.36 ± 0.02 μg/g tissue.

The lung extraction ratio (E) of budesonide was determined when the drug was infused into the pulmonary circulation, using the single-pass system (Fig. 4). Still 10 min after start of infusion the lung extraction was as high as 0.12 ± 0.07. The lung clearance ($F \times E$) was calculated to be 0.94 ml/min at 30 min ($F = 7.8$ ml/min).

After the end of drug infusion into the pulmonary circulation, at least two phases in the decline of the budesonide concentration in the venous outflow could be discerned (Fig. 5), one very rapid ($t_{1/2} = 0.64 \pm 0.18$ min) followed by a slow ($t_{1/2} =$

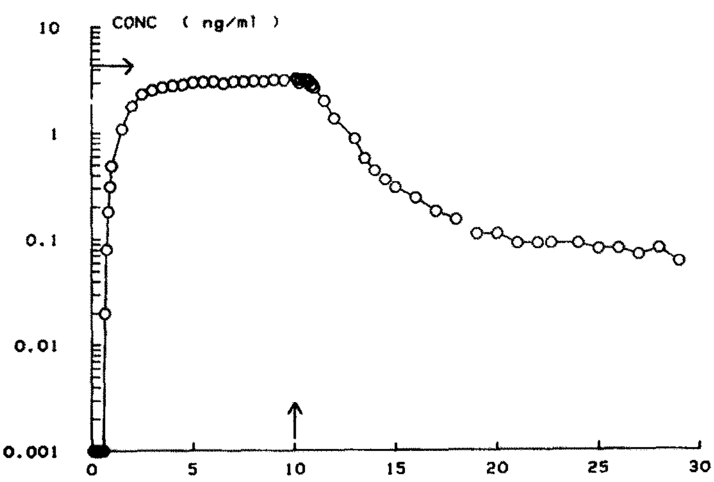

Fig. 5. Effluent concentration of drug after infusion of ^{3}H-budesonide during 10 min into the pulmonary circulation of an isolated rat lung, perfused in the single-pass mode. Inflowing concentration was 3.4 ng/ml.

14.8 ± 7.0 min). In these studies, the maximal fraction of the administered dose, taken up by the lungs, was calculated to be 0.246 ± 0.024. This was achieved at the end of the drug infusion period of 10 min, giving a lung concentration of 0.054 ± 0.015 μg/g tissue. At the end of the experiment, after a wash-out period of 20 min, the concentration of the drug in the lungs was 0.022 ± 0.009 μg/g tissue.

In the recirculating experiments (60-min perfusion period), HPLC analysis of lung tissue and perfusate samples were performed to investigate possible metabolism of budesonide. No clear evidence of formation of metabolites of budesonide could be detected in either lung or perfusate samples (Fig. 6). However, the results showed the epimer 22*R* of budesonide had a higher lung uptake (1.41 ± 0.06 times) than that found for epimer 22*S*.

DISCUSSION

In our study with budesonide administered via the air passages to isolated rat lungs, about 45% of the administered dose had been absorbed into the lung perfusate after 30 min perfusion. During the actual perfusion period at least two absorption phases, one rapid and one slow, could be discerned. The rapid absorption phase can describe a direct passage of the drug through the lung parenchyma into the pulmonary circulation. The slow phase can indicate that a fraction of the administered dose is sequestered by the lung tissue and then slowly released into the pulmonary circulation. This indicates that budesonide has lung affinity. The lung affinity was also demonstrated by a relatively high lung extraction ratio ($E > 0.1$) shown when budesonide was infused into the pulmonary circulation. This lung extraction ratio could be compared with that found for β-adrenoceptor agonists such as terbutaline ($E =$ 0.013–0.021), obtained in isolated perfused rat and guinea-pig lungs using similar lung perfusion flows as in the present study [13]. The lung extraction ratio of xanthines such as theophylline and enprofylline is comparable to that of β-adrenoceptor stimulants [14]. If other perfusion flows have been used this might have influenced the extraction ratio.

This lung tissue binding of budesonide was confirmed in the experiments to extract the compound from perfused lungs. After extraction of lung homogenate obtained from lungs perfused with ^{3}H-budesonide for 60 min, only 61.7 ± 14.7% (N = 7) of the tissue radioactivity could be recovered in the organic phase, using the extraction procedure for the perfusate. First after enzymatic digestion of the lung homogenate (see Materials and Methods) an almost quantitative recovery (93.6 ± 5.3%; N = 5) of radioactivity into the organic phase was obtained. HPLC analysis showed that only unchanged budesonide could be detected in this extract. Also, only unchanged budesonide could be detected in the lung perfusate. These experiments demonstrate that a substantial fraction of the drug present in the lungs is bound to tissue components and that budesonide is not biotransformed in the lungs. Incubation experiments with human lung homogenate disclosed no biotransformation of budesonide [15]. However, in the liver budesonide is rapidly and extensively biotransformed to low/non-active metabolites in all species investigated (mouse, rat and man) [15, 16]. The cellular localization of this tissue-bound fraction of budesonide is not known. One can speculate that the bound fraction or part of it may be of importance for the pharmacodynamic activity of the drug in the lung. It has been reported that ^{3}H-dexamethasone incubated with lung tissue from mice (*in vitro*) showed radioactivity localized to alveolar type II cells, bronchiolar and arteriolar smooth muscle cells, fibroblasts and endothelial cells of the pulmonary vasculature [17]. Whether the same distribution, as described for dexamethasone, prevails with budesonide is not known.

A difference in the distribution between lung tissue and perfusion medium for the two epimers of budesonide was found. Interestingly, the pharmacodynamically more potent epimer 22*R* showed a 1.4 times higher lung uptake than epimer 22*S*. This may be due to the fact that epimer 22*R* is less water-soluble than epimer 22*S*.

To conclude, these data clearly indicate that budesonide has lung affinity. This lung affinity of budesonide in combination with metabolic stability in the lung can be important factors for the good therapeutic effects seen with the compound in the clinic. Also, the extensive systemic inactivation of budesonide contributes to the lung selectivity seen with inhaled budesonide.

REFERENCES

1. Burton JA and Schanker LS, Absorption of corticosteroids from the rat lung. *Steroids* **23**: 617–624, 1974.
2. Hartiala J, Uotila P and Nienstedt W, Absorption and metabolism of intratracheally instilled cortisol and beclomethasone dipropionate in the isolated perfused rat lungs. *Med Biol* **57**: 294–297, 1979.
3. Chaplin MD, Cooper WC, Segre EJ, Oren J, Jones RE and Nerenberg C, Correlation of flunisolide plasma levels to eosinopenic response in humans. *J Allergy Clin Immunol* **65**: 445–453, 1980.
4. Brattsand R, Källström L, Nilsson E, Ryrfeldt Å and Tönnesson M, The lung disposition of budesonide in guinea-pig and rat. *Eur J Respir Dis* **63**(Suppl 122): 263–265, 1982.
5. Ryrfeldt Å, Andersson P, Edsbäcker S, Tönnesson M, Davies D and Pauwels R, Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur J Respir Dis* **63**(Suppl. 122): 86–95, 1982.
6. Pipkorn U, Rundcrantz H and Lindqvist N, Budesonide—a new nasal steroid. *Rhinology* **18**: 171–175, 1980.
7. Rosenhall L, Lundqvist G, Ädelroth E and Glennow C, Comparison between inhaled oral corticosteroids in patients with chronic asthma. *Eur J Respir Dis* **63**(Suppl. 122): 154–162, 1982.
8. Willey R, Godden D, Carmichael J, Preston P, Frame M and Cromptom G, Twice daily inhalation of a new corticosteroid, budesonide, in the treatment of chronic asthma. *Eur J Respir Dis* **63**(Suppl. 122): 138–142, 1982.
9. Brattsand R, Thalén A, Roempke K, Källström L and Gruvstad E, Influence of 16α, 17α-acetal substitution and steroid nuclear fluorination on the topical to systemic activity of glucocorticoids. *J Steroid Biochem* **16**: 779–786, 1982.

10. Ryrfeldt Å and Nilsson E, Lung uptake and anti-anaphylactic effects of KWD2131 (α, β-adrenergic agonist) in perfused guinea-pig lungs. *Acta Pharmacol Toxicol* **48**: 390–396, 1981.
11. Ryrfeldt Å, Tönnesson M, Nilsson E and Wikby A, Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by high-performance liquid chromatography. *J Steroid Biochem* **10**: 317–327, 1979.
12. Wikby A, Nilsson L and Hällsås G, Separation of epimers of budesonide and related corticosteroids by reversed bonded phase liquid chromatography. *J Chromatogr* **157**: 51–64, 1978.
13. Ryrfeldt Å and Nilsson E, Uptake and biotransformation of ibuterol and terbutaline in isolated perfused rat and guinea-pig lungs. *Biochem Pharmacol* **27**: 301–305, 1978.
14. Ryrfeldt Å *et al.*, in preparation.
15. Andersson P and Ryrfeldt Å, Biotransformation of the topical glucocorticoids budesonide and beclomethasone 17α, 21-diproprionate in human liver and lung homogenate. *J Pharm Pharmacol* **36**: 763–765, 1984.
16. Edsbäcker S, Andersson P, Lindberg C, Paulson J, Ryrfeldt Å and Thalén A, Liver metabolism of budesonide in rat, mouse and man-comparative aspects. *Drug Metab Dispos* **15**: 403–411, 1987.
17. Beer DG, Chuna GR and Malkinson AM, Autoradiographic demonstration of the specific binding and nuclear localization of ^{3}H-dexamethasone in adult mouse lung. *Lab Invest* **49**: 725–734, 1983.