bladder whereas other organs showed a slowly rising concentration. After an i.p. dose the liver was the target organ: 35% of the dose (0.29 mmol/kg, $n = 3$) was excreted as CO_2 by the lungs in the first 8 h after injection, 40% in the first 72 hours.

After local application no thioether excretion was found in the urine in the first 24 h, 2% of the dose in the second 24 hours. Systemic administration resulted in a urinary thioetherexcretion of 6% of the dose in the first 24 h, 1% in the second 24 hours. Metabolism was studied further in the rat and in isolated rat liver.

A decreasing thioether excretion and glutathione depletion in the liver was found in the application of acrylic esters of alcohols with increasing chain lengths. A simultaneous administration of the esterase inhibitor TOTP showed a dramatic rise in thioether excretion both with methyl acrylate (0.7 mmol/kg, from 4.3% to 27.4% of the dose) and methyl methacrylate (0.85 mmol/kg, from 0.55% to 12.1% of the dose).

Mercapturic acids were isolated and identified as β-S-(*N*-acetylcysteinyl)propionic acid from acrylate and β-S-(*N*-acetylcysteinyl)isobutyric acid from methacrylate.

The quantitative analysis of 6-keto $PGF_{1\alpha}$ in biological fluids by stable isotope dilution utilizing gas chromatography–mass spectrometry (GC–MS)

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Prostacyclin (PGI_2) a potent anti-aggregatory and vasodilator prostanoid is chemically hydrolysed to 6-keto $PGF_{1\alpha}$, a substance which has been isolated from numerous biological systems (Moncada & Vane, 1979). We have developed a GC–MS assay for 6-keto $PGF_{1\alpha}$ to quantify its synthesis in cardiovascular and pulmonary systems.

Plasma (15–20 ml) or lung perfusate (50 ml) is equilibrated with 3,3,4,4 tetradeutero 6-keto $PGF_{1\alpha}$ (25 ng). After acidification to pH 3 samples are applied to 10 ml volume XAD-2 columns and successively eluted with 15 ml of distilled water and 5 ml of *n*-heptane. Prostanoids are eluted with 10 ml methanol, collected in test tubes and the methanol evaporated under nitrogen. The vacuum dried residues are subjected to thin layer chromatography using the FVI solvent (Anderson, 1969). Zones corresponding to authentic 6-keto $PGF_{1\alpha}$ are scraped from the plates, eluted twice with methanol which is evaporated to dryness. Organic residues are redissolved in 1.0 ml borate buffer (pH 8.5) and extracted with ethyl acetate (2.5 ml) which is discarded. The aqueous phase is reacidified to pH 3 and extracted with fresh ethyl acetate (2.5 ml). The ethyl acetate is evaporated under nitrogen and the residues converted to *O*-methyloxime, methyl ester, trimethylsilyl ether derivatives as described previously (Black, *et al.*, 1978). From the final solution (25 µl) 2 × 10 µl aliquots are assayed using a Finnigan 4000 GC–MS equipped with a glass column (1.5 m × 2 mm) packed with 3% OV-1 on Supelcoport. Column temperature was 255°C and helium carrier gas flow rate 20 ml/minute. Ion fragments in the protium form at m/e 418 M-[(2 × 90) + 31] and 508[M-(90 + 31)] and the corresponding deuterium ions at m/e 422 and 512 are monitored. Calibration lines of the do/d4 ratio versus do concentration injected were constructed for quantitation.

Table 1 The concentrations of 6-keto $PGF_{1\alpha}$ (picograms/ml) in dog lung perfusates at different times after perfusion began

Perfusion time (minutes)	*DOG* 1	2	3	*Mean ± s.e. mean*
0	100	95	140	112 ± 14
5	250	530	460	413 ± 84
10	360	730	490	527 ± 108
15	390	890	580	620 ± 146
20	410	960	650	673 ± 159
30	490	1150	790	810 ± 191
45	520	1250	890	887 ± 211
60	590	1430	930	983 ± 244

Using this procedure a precision of 14.9% (coefficient of variation) was obtained at 250 picograms injected on column. The detection limit was set at 500 pg/injection for plasma samples, corresponding to a value of 62.5 pg/ml of plasma (for a 20 ml sample). Over the range 0 to 10,000 picograms a linear and quantitative recovery of 6-keto $PGE_{1\alpha}$ was observed. The assay has been applied to the determination of circulating levels of 6-keto $PGF_{1\alpha}$ during PGI_2 infusions in man and to the production of 6-keto $PGF_{1\alpha}$ by perfused dog lung. The results obtained when isolated left lung (wet weight 147 ± 7.3 g) of male greyhound dogs are perfused *in vitro* (volume of perfusion system 1.5 litres) with tyrode solution containing 4% w/v bovine serum albumin are shown in Table 1. It can be seen that the circulating level of 6-keto $PGF_{1\alpha}$ increases with time, indicating that the lung preparation is continuously producing 6-keto $PGF_{1\alpha}$, an indicator of PGI_2 production.

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A model of transient neutropenia in the rat

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The severe but transient neutropenia occurring in man, for example during haemodialysis and nylon fibre leukapheresis, has been attributed to neutrophil aggregation and margination caused by C5a released during complement activation (Nusbacher, Rosenfeld, Macpherson, Thiem & Leddy, 1978; Hammerschmidt, Craddock, McCullough, Kronenberg, Dalmasso & Jacob, 1978). We have shown this effect to occur in the rat following the intravenous administration of either zymosan activated serum (ZAS) or the synthetic tripeptide N.formyl methionyl-leucyl-phenylalanine (FMLP). The effect of some conventional anti-inflammatory drugs on this response have also been studied.

Female albino rats (150–200 g) were anaesthetized with a mixture of urethane and pentobarbitone (i.p.). One h later a 0.5 ml blood sample was obtained by cardiac puncture. After 5 min the animals were given an intravenous injection of either ZAS or FMLP, further blood samples being taken 1 min and 5 min after injection. Dilutions of citrated blood were made in ammonium oxalate (1:10). Total counts and differential cell counts on 500 cells were performed on each sample by standard techniques, and the results calculated as percentage changes in neutrophil cell numbers.

The i.v. injection of ZAS or FMLP resulted in a dose related transient neutropenia which was maximal 1 min after injection. Neutrophil counts normally reached pre-injection values after 5 minutes. The reduction in the number of circulating neutrophils was shown not to be due to the experimental procedures. The total white cell counts did not change significantly during the response.

The degree of neutropenia obtained in control animals was not significantly altered in experimental groups pretreated with either colchicine (2 mg/kg p.o.) or hydrocortisone sodium succinate (10 and 30 mg/kg i.m.) 1 h prior to the experiment, or indomethacin (3 mg/kg p.o.) daily for 5 days and 1 h prior to the experiment. The addition of hydrocortisone sodium succinate to serum (3–12 mg/ml) prior to activation with zymosan resulted in a dose related inhibition of the neutropenia. This inhibition was not observed if the steroid was added to the serum after zymosan activation.

The results suggest that the animal model used in the present study may simulate the cellular response observed in man during haemodialysis, nylon fibre leukapheresis and shock lung. It has not been established whether the neutropenia is an aspect of the inflammatory process in general and the model appears to be of little use in investigating possible mechanisms of action of anti-inflammatory drugs *in vivo*.

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