described. Effects of $PGF_{2\alpha}$, $PGF_{1\alpha}$, PGE_1 and PGE_2 on the cardiovascular and respiratory systems of anaesthetized calves and on isolated pulmonary tissues have been examined in order to clarify the role of prostaglandins in bovine anaphylaxis.

Calves aged 3-4 months, anaesthetized with pentobarbitone, received prostaglandins intravenously. $PGF_{2\alpha}$ (10-60 μg/kg) increased systemic arterial B.P. by 20-85%, pulmonary arterial pressure by 20-200% and heart rate by 3-23%. Respiratory minute volume was reduced by 13-90%. $PGF_{1\alpha}$ (35-140 μg/kg) produced similar increases in systemic pressure, but had no effect on respiration. PGE_1 (1-8 μg/kg) and PGE_2 (2-8 μg/kg) reduced systemic arterial pressure (17-44%), pulmonary arterial pressure (12-37%) and heart rate (5-23%). Respiratory minute volume was reduced by PGE_1 (7-23%) and PGE_2 (26-42%). Tachyphylaxis occurred to $PGF_{2\alpha}$, $PGF_{1\alpha}$, and PGE_2.

Segments of bronchiole and spiral strips of pulmonary artery and vein were suspended in Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 at 37° C in organ baths of 80 ml capacity. Contractions were recorded isotonically. Drugs remained in contact with artery and vein for 5 min and with bronchiole for 15 minutes.

PGF_2 contracted bronchiole (0.2-0.3 μg/ml threshold dose), pulmonary artery (0.3 μg/ml) and vein (0.1 μg/ml). All three tissues showed tachyphylaxis. $PGF_{1\alpha}$ (0.5-2 μg/ml) contracted pulmonary artery but concentrations up to 20 μg/ml had no effect on bronchiole. PGE_1 relaxed bronchiole (6-12 μg/ml) and pulmonary artery (0.5-2 μg/ml) but contracted pulmonary vein (1 μg/ml). PGE_2 had variable effects on bronchiole causing contraction (2-5 μg/ml) or, at higher concentrations (6-12 μg/ml), relaxation of muscle contracted by acetylcholine. Pulmonary artery was contracted by PGE_2 (0.4-2 μg/ml), but, when submaximally contracted by serotonin, PGE_2 (5-10 μg/ml) caused relaxation. PGE_2 (1 μg/ml) contracted vein.

Unlike Lewis & Eyre (1972), we found that $PGF_{2\alpha}$ contracted bovine bronchiole as did PGE_2. Human bronchial muscle is contracted by $PGF_{2\alpha}$ but relaxed by PGE_2 (Sweatman & Collier, 1968). Pulmonary hypertensive effects of PGE_1 and PGE_2 have been described in calves (Lewis & Eyre, 1972). The differences in our findings *in vivo* might be related to differences in depths of anaesthesia and smooth muscle tone.

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Teratogenic effects of primidone in mice

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Clinical studies have indicated that there is an increased incidence of congenital malformations, particularly cleft palate, in the infants of mothers exposed to anticonvulsant drugs during pregnancy (Speidel & Meadow, 1972). Since the majority of epileptics in these studies were treated with more than one drug it is not possible to determine exactly which anticonvulsant drugs may possess this teratogenic potential. Of the major anticonvulsants, phenytoin and phenobarbitone have both been implicated as teratogens in animals (Massey, 1969; McColl, Robinson & Globus, 1967), but we are not aware of any reports on the teratogenic effects of primidone in animals.

Primidone was administered to mice derived from the I.C.I. pathogen free strain, either in the diet or by gastric intubation for varying periods from days 6-16 of pregnancy which covers the period of embryogenesis and palatal closure in the mouse. Doses of primidone of 500, 1250, 2000 and 2500 mg/kg administered in the diet on days 6-16 of pregnancy established that primidone was teratogenic; the incidence of cleft palate increased with dose from a control incidence of 0.3% to 29.4% at the highest dose level.

As it has been suggested that low blood folate levels following anticonvulsant therapy might be related to the tetratogenic effects (Elshove, 1969), folic acid (25 mg/kg orally) was administered with primidone (1250 mg/kg). Although folic acid itself was not teratogenic, a significant ($P = 0.0004$) increase in cleft palate was seen with combined treatment compared with primidone alone. However, when folinic acid (3.75 mg/kg s.c.) was administered with primidone (1250 mg/kg) there

was a significant decrease in the incidence of cleft palate ($P = 0.03$).

The doses of primidone used in the dietary experiments were far in excess of those used clinically, so much lower doses (100, 150, 250 mg/kg) were given by gastric intubation. All three doses of primidone produced a similar incidence of cleft palate (9-10%) in 50-60% of the litters.

Blood levels of primidone after 100 mg/kg by gastric intubation were measured in pregnant (day 14) mice. A peak level of 42.7 ± 2.8 μg/ml was attained after 30 min and it was virtually cleared from the blood by eight hours. Thus, although the dose of primidone which was teratogenic in mice was about 10 times the therapeutic dose for humans the blood levels obtained were only three to four times higher than those commonly found in patients on primidone (Kutt, 1974).

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A new approach to the evaluation of the safety of flavouring esters

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Experiments have been performed which are designed to determine whether it is possible to reduce the amount of animal experimentation that is needed to assess the safety of flavouring materials. Many of the flavours are esters which are readily hydrolysed *in vivo* to their component acids and alcohols. It is postulated that if the toxicity of each component is known then it might be possible to extrapolate the results to other esters of a particular series, with a consequent economy in the number of animals used and in the experimental time involved.

A series of allyl esters with a straight, branched or a cyclic chain has been investigated. This series was chosen because many pure allyl esters are available and because allyl alcohol produces an unusual effect on the liver—namely periportal necrosis. Allyl alcohol, acetate, propionate, hexanoate, isobutyrate, isovalerate and 2-ethyl hexoate were administered by daily oral intubation in equimolar doses (based on doses of 5, 25 or 60 mg kg^{-1} day^{-1} of the alcohol) to groups of ten male rats. After 21 days the animals were killed and comparisons of the hepatic effects were made. The lesions were classified into three types according to severity; namely periportal cell enlargement, followed by necrosis, and subsequent fibrosis with bile duct hyperplasia.

The severities of the hepatic effects from the straight chain esters were similar to those produced by allyl alcohol and were more marked than those from the branched chain ones. In terms of the number of animals affected, there was a positive relationship between the dose of ester administered and the degree of periportal damage. The animals given the high dose of the straight chain esters exhibited the most extreme lesions and in many instances the effects were so marked as to obscure any signs of early damage. The results of these *in vivo* experiments are in agreement with the *in vitro* hydrolysis studies. The latter indicate that hydrolysis proceeds about 100 times more slowly with the branched chain than with the straight chain esters.

From the data with this series of model compounds it is suggested that it may be possible to evaluate the toxicity of such a series of esters, if the following information is available: (1) the rate and degree of hydrolysis in the alimentary tract, (2) well established data on the parent alcohols and acids, and (3) results of short-term animal experiments, so that it is possible to correlate the *in vitro* hydrolysis studies with the *in vivo* results.